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Feeding and Autoimmunity in Children with Down's Syndrome Evaluation Study (FADES)

Feeding and Autoimmunity in Children with Down's Syndrome Evaluation Study (FADES)

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Abstract

Children with Down's syndrome (DS) are at increased risk of autoimmune conditions including diabetes, coeliac and thyroid disease. Autoimmune diabetes occurs earlier in children with DS compared with the general population despite decreased levels of the typical genetic susceptibility factors. During this PhD, FADES (Feeding and Autoimmunity in Down Syndrome Evaluation Study) was established to examine early life and the development of autoimmunity. This was a UK wide feasibility study for which infants with DS were recruited under eight months of age. Parents completed detailed feeding and medical questionnaires at recruitment, seven months, 12 months and yearly thereafter. Sampling protocols were optimized for national collection by post. A DNA sample was collected as well as longitudinal samples for urine C-peptide, stool for gut microbiome and blood for antibodies. As part of the overall feasibility a qualitative study was undertaken to determine the potential barriers to recruitment of young infants with DS into research.

Between September 2014 and September 2017, 70 participants were recruited. At two years, 61% of participants had completed all the requested questionnaires and samples. Initial analysis of clinical samples proved their adequacy for HLA genotyping and antibody testing. The questionnaires revealed that exclusive breastfeeding rates at six months in the cohort was comparable to rates in the general population (4% vs 1%). Issues with feeding were explored revealing at least half of the babies had received naso-gastric tube feeds.

This study has established one of the largest longitudinal birth cohorts of children with DS. The bank of samples and data to explore early life and autoimmunity in DS is unique. Findings will be used to inform parents and professionals on early feeding. Ongoing expansion of this cohort will aim to increase understanding of the mechanisms and pathogenesis of autoimmune-mediated conditions and may provide important insights for research.

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Dedication and Acknowledgements

Firstly, I would like to thank all the families who have taken part in this study, I appreciate all the time and effort that they have given. Hearing their stories has been a constant motivation and I only hope that this work does justice to their inspiration.

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I am particularly grateful to Sofia Leadbetter and Shirley Jenkins for all their hard work with FADES and for all the personal support they have given me. Thanks go to Stu Toms for being amazing at creating all things digital and to Georgina Mortimer and Sian Grace who have provided me with so much help and advice.

My two incredible daughters Livinia and Rosalie were born during my PhD. I am so proud of you, thank you both for all your smiles and cuddles. Thank you to all my family especially my inspiring parents for their love and all that they do for us. And most importantly thank you to my wonderful husband Mike for being there at every step providing wisdom, love and happiness.

Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Abbreviations

AIRE	Auto-immune Regulator
APECED	Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy
AVSD	Atrioventricular Septal Defect
BA	Bland Altman
BRC	Biomedical Research Centre
BOX Study	The Bart's – Oxford Family Study
BRU	Biomedical Research Unit
BSA	Bovine Serum Albumin
CD	Coeliac disease
CDSS	The Children with Down's Syndrome Study
CCRN	Comprehensive clinical research network
CI	Chief Investigator
CLRN	Comprehensive Local Research Networks
CONSORT	Consolidated Standards of Reporting Trials
CPMS	Central Portfolio Management System
CRN	Clinical Research Network
C-Section	Caesarean Section
CSP	Co-ordinated system for gaining NHS permission
DAISY	Diabetes Autoimmunity Study in the Young
DELFIA	Disassociation Enhanced Lanthanide Fluroimmunoassay
DiPP	Diabetes Prediction and Prevention Study
DS	Down's Syndrome
DSA	Down's Syndrome Association
DSMIG	Down's Syndrome Medical Interest Group
DSS	Down's Syndrome Scotland
EMA	Anti-Endomysial Antibodies
EOI	Expression of Interest

FADES	Feeding and Autoimmunity in Down's Syndrome Evaluation Study
FOX P3	Forkhead Box P3
GADA	Glutamic Acid Decarboxylase Antibodies
GCP	Good Clinical Practice
GWAS	Genome wide association study
HLA	Human Leukocyte Antigen
HRA	Health Research Authority
HSCT	Health and Social Care Trusts
HTA	Human Tissue Act
IA2-A	Tyrosine Phosphatase Antibodies
IAA	Insulin Antibodies
IQR	Inter-Quartile Range
IRAS	Integrated Research Application System
ISRCTN	International Standard Randomised Controlled Trial Number
LCRN	Local Clinical Research Networks
MHC	Major Histocompatibility Complex
MMT	Mixed Meal Tolerance Test
MS	Multiple sclerosis
NDSCR	National Down's Syndrome Cytogenetic Register
NG tube	Naso-gastric tube
NICU	Neonatal Intensive Care Unit
NIHR	National Institute for Health Research
NISCHR	National Institute for Social Care and Health Research
NRES	National Research Ethics Service
NRS CC	NHS Research Scotland Coordinating Centre
OUT	Operational Taxonomic Units
PCHR	Personal Child Health Record
PCU	Permissions Coordinating Unit
PI	Principal Investigator

PPI	Patient and Public Involvement
RA	Rheumatoid Arthritis
RED	Research Enterprise and Development
RCPCH	Royal College of Paediatrics and Child Health
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
REDCap	Research Electronic Data Capture
R and D	Research and Development
SCBU	Special Care Baby Unit
SLE	Systemic Lupus Erythematosus
SOP	Standard Operating Procedure
SSI	Site Specific Information
T1D	Type 1 Diabetes
TAM	Transient Abnormal Myelopoiesis
TEDDY	The Environmental Determinants of Diabetes in the Young Study
TPO	Thyroid Peroxidase Antibodies
Tregs	T Regulatory Cells
tTG	Tissue Transglutaminase
UBASH3A	Ubiquitin associated and SH3 domain containing A
UAT	User Acceptance Testing
UC	Ulcerative colitis
UCPCR	Urine C-peptide Creatinine Ratio
UK	United Kingdom
USA	United States of America
WHO	World Health Organisation
ZnT8A	Zinc Transporter 8 Antibodies

Papers published during this PhD and presentations at conferences:

Published Papers:

Williams, Georgina M., Patricia Neville, Kathleen M. Gillespie, Sam D. Leary, Julian P. Hamilton-Shield, and Aidan J. Searle. "What factors influence recruitment to a birth cohort of infants with Down's syndrome?" Archives of disease in childhood103, no. 8 (2018): 763-766.

Williams, Georgina M., Sam D. Leary, Nadim J. Ajami, Saranna Chipper Keating, Joseph F. Petrosin, Julian P. Hamilton-Shield, and Kathleen M. Gillespie. "Gut microbiome analysis by post: Evaluation of the optimal method to collect stool samples from infants within a national cohort study." *PloS one* 14, no. 6 (2019): e0216557.

Conferences:

Why are children with Down's Syndrome at increased risk of autoimmunity? Symposia at World Down Syndrome Congress 2018 (Glasgow).

Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES): Poster at British Society of Paediatrics Endocrinology and Diabetes (BSPED) 2018 Annual Meeting in Birmingham.

FADES: A longitudinal birth cohort study to understand why children with Down's syndrome are at increased risk of autoimmunity: Poster at Immunology of Diabetes Symposium (IDS) 2018.

Establishing breast feeding in infants with Down's Syndrome; parental experiences from a UK wide birth cohort: Oral Presentation: Royal College of Paediatrics and Child Health (RCPCH) Conference 2017.

Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES): Poster at 10th Annual NIHR Trainee Meeting: Research leaders of the future. December 2016.

Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES): Poster presentation at the Royal College of Paediatrics and Child Health (RCPCH) Conference April 2016.

Future Conferences:

Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES): Oral presentation at European Society of Paediatric Endocrinology (ESPE) Conference 2019.

Additional published papers during the duration of PhD:

Williams, G.M., Long, A.E., Wilson, I.V., Aitken, R.J., Wyatt, R.C., McDonald, T.J., Wong, F.S., Hattersley, A.T., Williams, A.J., Bingley, P.J. and Gillespie, K.M., 2016. Beta cell function and ongoing autoimmunity in long-standing, childhood onset type 1 diabetes. Diabetologia, pp.1-5.

Arya R, Williams G, Kilonback A, Toward M, Griffin M, Blair PS, Fleming P. Is the infant car seat challenge useful? A pilot study in a simulated moving vehicle. Archives of Disease in Childhood-Fetal and Neonatal Edition. 2016 Sep 30:fetalneonatal-2016. (High impact paper)

Chapter 1

Introduction and Background

Chapter 1 – Introduction and Background

1.1 Introduction

Individuals with Down's syndrome (DS) are at increased risk of autoimmune conditions including Type 1 diabetes (T1D), coeliac disease (CD) and autoimmune thyroid conditions (Gillespie et al. 2006, Sánchez-Albisua et al. 2002). This is despite children with DS and T1D having a lower prevalence of high risk human leukocyte antigen (HLA) genotypes than those with T1D alone (Aitken et al. 2013). Children with DS have inherent defects in their immune system but mechanisms underlying the increased risks of autoimmunity are poorly understood.

The Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES) has been designed to determine the feasibility of a UK wide birth cohort of babies with DS, and to explore potential influences in the development of autoimmunity in DS. In time this may also lead to a better understanding of autoimmunity in the general population.

1.2 Overview of Chapter 1

Chapter 1 includes the clinical and immunological features of Down's Syndrome and the epidemiological data for the UK population of people with DS. Autoimmunity, TID, CD and thyroid conditions are described both within the general population and in people with DS. An introduction to how the gut microbiome, infections, breastfeeding and weaning might influence the development of autoimmunity is given. Chapter 1 also provides background on feasibility studies and how they should be conducted.

The aims, feasibility objectives and secondary objectives of this PhD are given at the end of the chapter.

1.3 Down's Syndrome and Immunity

1.3.1 Definition and Description

Down's Syndrome (DS) is caused by an additional copy of chromosome 21 (Trisomy21). The phenotypical features of DS combined with learning difficulties were first described by Dr John Langdon Down in 1867 (Down 1867), and the discovery that individuals with DS had 3 copies of chromosome 21 was made in 1959 (Lejeune, Gautier, and Turpin 1959).

DS is usually diagnosed within the first few days of life with typical features including: epicanthic folds, single palmar crease, hypotonia, an enlarged or protruding tongue and wide spacing of the first and second toe. Approximately 44% will have cardiac abnormalities, the most common being atrioventricular septal defect (AVSD) (Freeman et al. 1998). DS carries an increased risk of autoimmune conditions including T1D, autoimmune thyroid disease and CD (Karlsson et al. 1998, Sánchez-Albisua et al. 2002, Gillespie et al. 2006). Hearing loss is also a feature of many DS individuals (Balkany et al. 1979), as are a variety of ophthalmological problems including cataract, strabismus, refractive errors and nystagmus (Da Cunha and Moreira 1996). There is also an increased risk of haematological disorders and malignancies particularly leukemia and transient myeloproliferative disorder (Hasle, Clemmensen, and Mikkelsen 2000). Other conditions that are more common in people with DS include arthritis, atlanto-axial subluxation, obstructive sleep apnoea, seizures and early dementia particularly Alzheimer's Disease. Life expectancy in people with DS has significantly improved in the last

few decades with average life expectancy now around 50 years for a person with DS living in the USA (Yang, Rasmussen, and Friedman 2002).

People with DS have inherent defects in their immune system but mechanisms underlying the increased risk of autoimmunity are under-investigated. A study of the potential influences in the development of autoimmunity in DS may lead to a better understanding of autoimmunity in the general population.

1.3.2 Epidemiology of DS in the United Kingdom.

The availability of epidemiological data for diagnoses and live births of infants with DS for each of the individual countries in Great Britain varies.

England and Wales

Statistics on the number of diagnoses of DS in England and Wales are collected by the National Down's Syndrome Cytogenetic Register (NDSCR) (Morris and Springett 2014b). Since 1989 the NDSCR has collected notifications from all the cytogenetic laboratories across England and Wales of cases diagnosed with Patau's syndrome (Trisomy 13), Edwards' syndrome (Trisomy 18) and Down's Syndrome (Trisomy 21). The NDSCR does not have contact with any of the parents and is therefore unable to provide any contact details for recruitment to research studies. The executive summary from the NDSCR 2013 annual reported (Morris and Springett 2014a):

- in 2013 there were 1,886 diagnoses of Down syndrome, 65% of which were made prenatally, a rate of 2.7 per 1,000 births; and

- in 2013 there were an estimated 728 DS live births, a live birth rate of 1.0 per 1,000 live births.

Of those women who received a prenatal diagnosis of DS, 90% terminated the pregnancy. Of those continuing with their pregnancy, a proportion had natural miscarriages or still births and approximately six percent resulted in a live birth (Table 1).

		Number	Percentage (95% CI)
Prenatal	Termination of pregnancy	925	49 (47 - 51)
	Live Birth	82	4 (4 - 5)
	Still Birth / Miscarriage	20	1 (1 - 2)
	Unknown outcome†	205	11 (10 - 12)
		1,232	65 (63 - 67)
Postnatal	Live Birth	634	34 (32 - 36)
	Still Birth / Miscarriage	20	1 (1 - 2)
		654	35 (33 - 37)
Total		1,886	100

* 2013 data are provisional due to late reporting of cases. † About 6% of those with unknown outcomes are likely to result in a live birth.

Table 1: DS cases diagnosed in England and Wales in 2013* according to time of diagnosis and outcome. Taken from *(Morris and Springett 2014a)*

Northern Ireland

There is currently no formal register of DS diagnoses in Northern Ireland and few studies which describe the epidemiology of DS within Northern Ireland. Although abortions are legal in Northern Ireland, they are only permitted in circumstances where the mother's life is directly at risk if the pregnancy continues (1945 Criminal Justice (Northern Ireland) Act). The 1967 Abortions Act which includes the legality of abortions in the case of foetal abnormality

within the UK, does not extend to Northern Ireland. It may therefore be expected that rates of live births of babies with DS within Northern Ireland would be higher than in other parts of the UK. From January 1997 until December 2001 there were 208 cases of Down's syndrome diagnosed postnatally in Northern Ireland giving a prevalence of 167.9 per 100 000 (or 1 in 595 births) (Devlin and Morrison 2004).

Scotland

In Scotland there is only one congenital anomaly register based in Glasgow and therefore there are few data available for the number of DS diagnoses for Scotland as a whole. The Glasgow Register of Congenital anomalies reported 33 diagnoses of DS during the period from 1st April 2013 until 1st March 2014 an incidence of 1:403 maternities. Over a more extended period of time they reported a DS pregnancy prevalence of 1.24 per 1000 total births from 1980 – 1996 (Iliyasu, Gilmour, and Stone 2002). Down's Syndrome Scotland stated in 2014 that 50 - 60 babies a year are born with DS in Scotland (personal communication).

1.3.3 Immunity and Down's syndrome

Individuals with DS have defects in their immune system with increased risk of developing haematological malignancies (Øster and Nielsen 1975) and increased incidence of autoimmunity. The identified abnormalities are summarised in Table 2.

Clinical Findings
Increased incidence of infections, particularly respiratory (Øster and Nielsen 1975)
Increased incidence of lymphatic leukaemia (Hasle, Clemmensen, and Mikkelsen 2000)
High incidence of Hep B surface antigen positivity (Ugazio et al. 1977)
High incidence of thyroid autoantibodies (Ugazio et al. 1977, Karlsson et al. 1998)
Immunologic Findings
Diminished number of blood lymphocytes (Kusters et al. 2009)
Diminished phagocytic activity (Rosner, Kozinn, and Jervis 1973)
B cells
Normal number (Levin et al. 1979)
Immunoglobulin production (Burgio et al. 1975, Stiehm and Fudenberg 1966, Sutnick, London, and Blumberg 1969)
IgG level, normal or increased
IgA level, normal or increased
IgM level, normal or decreased
Defective antibody response to bacteriophage ΦX174 (Lopez et al. 1975)
T cells
Diminished number (Kusters et al. 2009)
Diminished blast transformation with phytohemaglutinin (PHA) (Sasaki and Obara 1969, Burgio et al. 1975)
Diminished leukocyte migration inhibition factor with PHA (Hahn, Levin, and Handzel 1976)
Pathology
Thymus
Small with severe lymphocyte depletion
Contracted, depleted cortex
Giant and cystic Hassall's corpuscles
Increased cellularity around some Hassall's corpuscles
Spleen
T-zone lymphocytes depleted.

Table 2: Immune defects in Down's Syndrome, adapted from Levin (Levin et al. 1979)

Various aspects of the immune system have been studied in those with DS. Levin found no differences in the percentages of B cells or immunoglobulin levels in children and newborns with DS compared with examining those without DS (Levin et al. 1979). The function of B cells has also been studied by the phagocytic function of leukocytes and the adhesiveness of neutrophils, both of which are reduced in DS (Rosner, Kozinn, and Jervis 1973, Costello and Webber 1976). These differences in function are however small and are not consistently found in other studies.

Significantly reduced numbers of T cells have been reported in people with DS and there is also evidence of reduced function. T cells do not undergo the normal pattern of massive expansion in the first year of life, suggesting that they are not responding normally to the multiple antigens to which they are exposed at this stage (Kusters et al. 2009). The number of T lymphocytes does improve with age but although normal numbers might be reached, their function is poor.

The thymus is smaller in people with DS, (Levin et al. 1979), there are fewer mature thymocytes (Murphy, Lempert, and Epstein 1990), lymphocyte numbers are depleted, and the cortex is poorly demarcated and contains large Hassall's corpuscles. Hassall's corpuscles are made up of epithelial cells and are normally present, but these very large Hassall's corpuscles are not seen in any other condition (Figure 1).

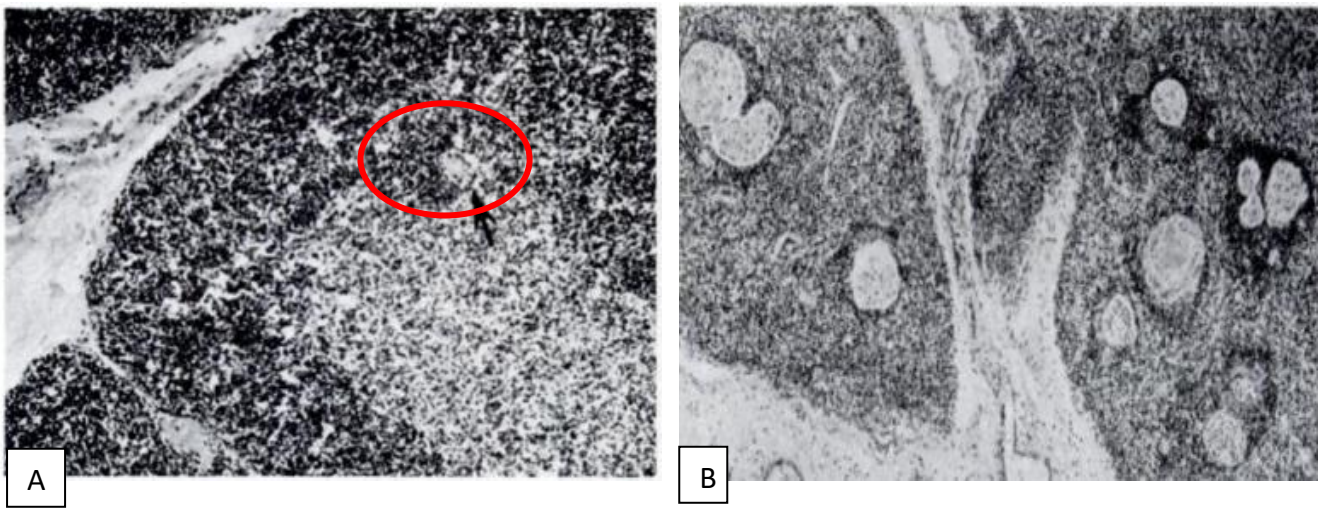


Figure 1: Comparison of Hassall's Corpuscle in the normal thymus and in an individual with DS. Adapted from (Levin et al. 1979) A - "Normal thymus (hematoxylin-eosin x 120) showing lymphocyte concentration in cortex, good corticomedullary demarcation, and a single Hassall's corpuscle (arrow)." B - "Thymus (hematoxylin-eosin x 120) of patient with DS showing lymphocyte depletion and contraction of cortex, loss of corticomedullary demarcation, and large cystic Hassall's corpuscles, many surrounded by sheath of lymphocytes."

Hassall's corpuscles play an important role in the generation of regulatory T cells within the thymus. T regulatory (Treg) cells which express the transcription factor FOXP3 (Forkhead Box P3) suppress immune response to self antigens (see Figure 2). Norihiko Watanabe described the ability of the dendritic cells within the corpuscles to "induce the proliferation and differentiation of CD4+ CD8- CD25- thymic T cells into CD4+ CD25+ FOXP3+ regulatory T cells" (Watanabe et al. 2005). Tregs are increased in the peripheral blood in DS, but their capacity to suppress T effector cells is reduced (Pellegrini et al. 2012).

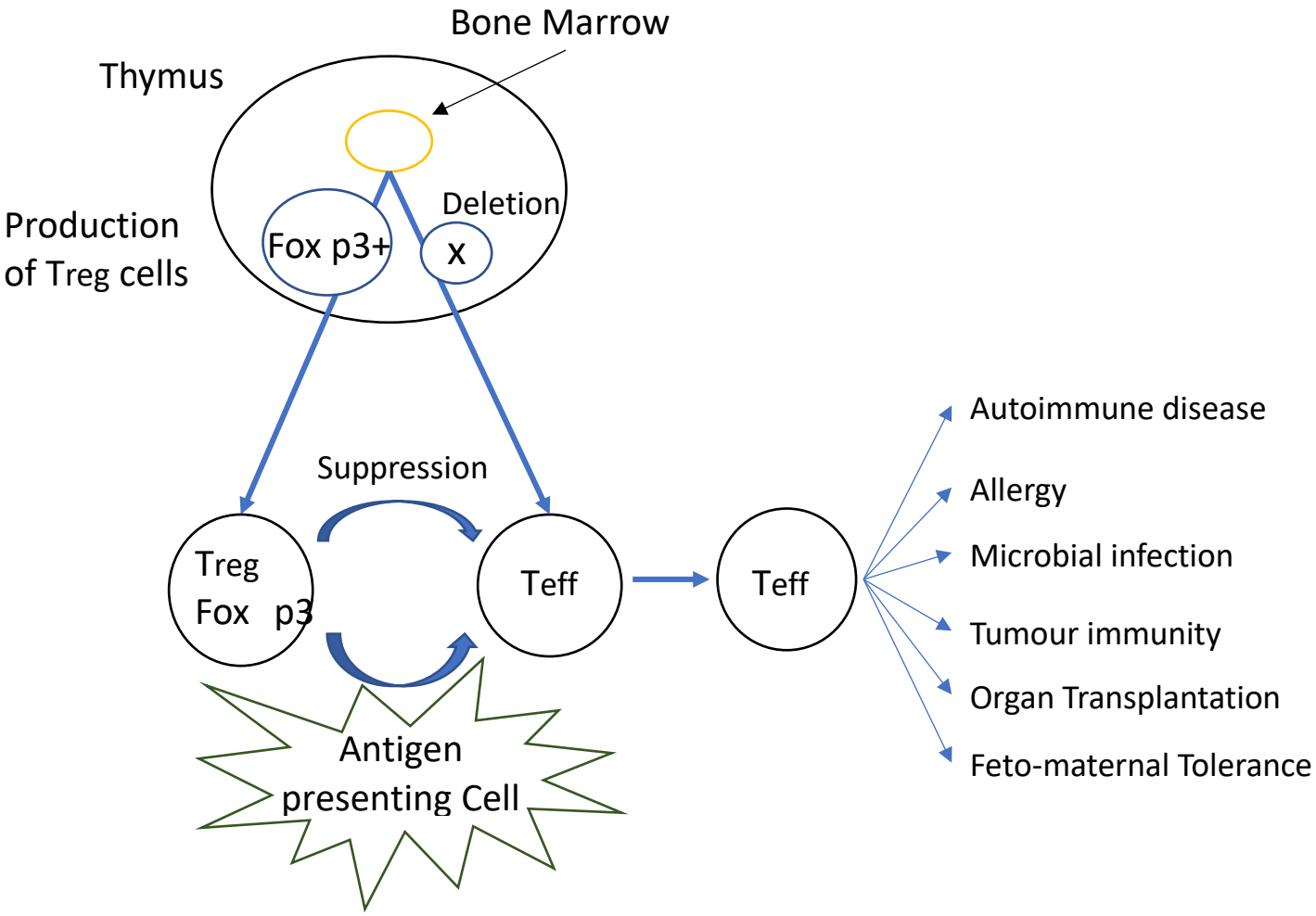


Figure 2: The role of Treg cells. Adapted from (Sakaguchi 2005) Treg cells which express FoxP3 are vital for self-tolerance. They are produced in the thymus, work in the periphery and alterations in either in their quantity or functional ability may lead to autoimmunity, rejection of organ transplantation and allergy. Treg cells can suppress T effector cells either directly or indirectly.

1.4 Autoimmunity

1.4.1 Background and epidemiology

Autoimmune conditions cause significant morbidity and mortality. The total estimated prevalence of autoimmune disease in the general population is approximately three percent (Cooper and Stroehla 2003).

Autoimmune conditions are thought to be caused by a combination of genetic predisposition and environmental triggers or risk factors. They involve a defect in immune regulation where self-antigens are not recognised, leading to damage and destruction of specific tissues. The damage occurs in the pancreas in T1D, in the joints in rheumatoid arthritis, the myelin sheath which covers nerves in multiple sclerosis (MS) and the uvea of the eye in uveitis. In some autoimmune conditions, several systems can be damaged as autoantibodies are directed against proteins found in many cells. For example, in systemic lupus erythematosus, antibodies are found against proteins in the cell's nucleus, and damage to the heart, kidney, lungs, liver, blood vessels, skin and joints is observed.

1.4.2 Incidence and prevalence

As well as the variety observed in the clinical manifestations of autoimmunity, there are variations in the prevalence of certain conditions between ethnic groups and geographical areas. The variation in incidence and prevalence in T1D will be discussed in more detail later but interestingly there are similar differences in T1D and multiple sclerosis (MS). Higher incidence rates in both T1D and MS are seen in Northern Europeans and lower rates amongst Japanese, Chinese and Black Africans (Rosati 2001). There are increased frequencies of HLA susceptibility genes amongst Northern European populations (DR15 haplotype for MS

(Schmidt, Williamson, and Ashley-Koch 2007) and HLA *DQ2-DQ8* and HLA *DQ4-DQ8* in T1D (Rönningen, Keiding, and Green 2001). As with T1D, the incidence of MS appeared to be affected in part by latitude, but this does not explain the significant variations in incidence rates that are seen within some individual countries. Ethnicity clearly plays a role, but migratory studies show that incidence rates change when ethnic groups migrate from areas of high to low incidence, highlighting the importance of environmental determinants (this is covered in further detail in Section 1.4.5.2 Epidemiology of Type 1 Diabetes).

The risk of childhood T1D is similar amongst females and males (Cooper and Stroehla 2003) but in nearly all other autoimmune conditions females are much more likely to be affected than males with an estimated 65% of patients being female (Cooper and Stroehla 2003). Other differences between these pathogenically similar diseases are in age of onset with T1D presenting in childhood and adulthood with the peak ages at onset occurring around puberty (Atkinson, Eisenbarth, and Michels 2014) and other conditions presenting at older ages such as myasthenia gravis presenting at the age of 30 to 50 years. Incidence rates over time vary, the incidence of T1D had been rising over the past couple of decades (Patterson et al. 2009) whereas the rate of rheumatoid arthritis (RA) both juvenile and adult onset has reduced (Cooper and Stroehla 2003).

1.4.3 Genetic prediction

There is evidence of genetic predisposition for many of the autoimmune diseases. For most, this genetic predisposition lies within the human leukocyte antigen (HLA) genes of the major histo-compatibility complex (MHC) on the short arm of chromosome 6 (Fernando et al. 2008) (see Figure 3). MHC class I presents antigens derived from pathogens to T cells in order to

mount an immune response. In the thymus, MHC class II presents self-peptides to the immune system to ensure that they are recognized as "self". This process is however defective in autoimmunity and autoreactive T cells are released into the periphery where they are not sufficiently influenced by Tregs. In autoimmune disease, it is thought that the particular autoantigen that is presented may determine the type of autoimmune disease (Fernando et al. 2008).

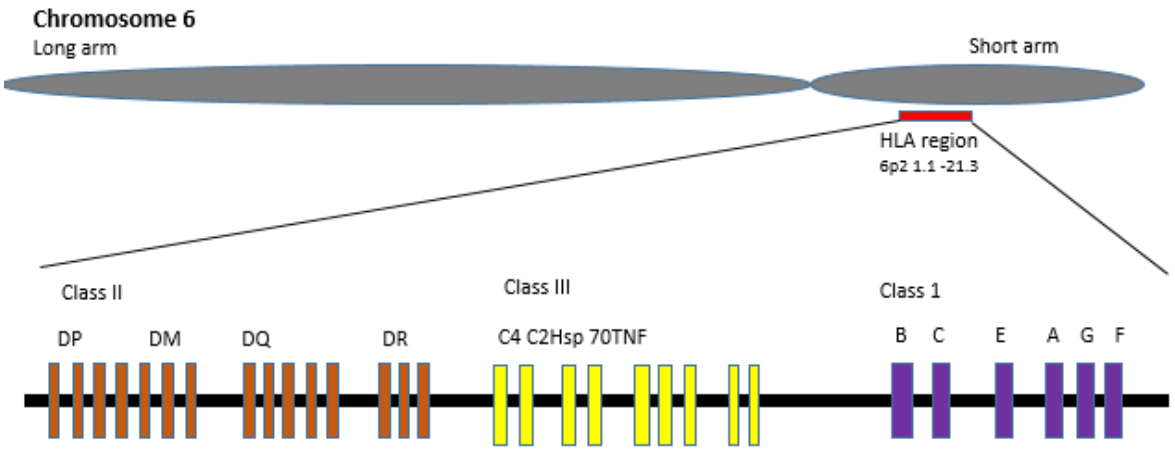


Figure 3: HLA genes of the major histo-compatibility complex (MHC) on the short arm of chromosome 6. The MHC on the short arm of chromosome 6 is comprised of class I, II and III. Risk haplotypes for TID are *DRB1*03 - DQA1*0501 – DQB1 0201* (*DR3-DQ2*) and *DRB1*04 – DQA1*0301 – DQB1 0302* (*DR4 – DQ8*) (Adapted from Todd (Todd 2010))

Fernando et al carried out a pooled analysis of the studies determining the MHC genes associated with six autoimmune, infectious and inflammatory diseases: MS, T1D, SLE, ulcerative colitis (UC), Crohn's disease and RA. HLA *DR4* haplotypes were found to be a risk allele in all conditions apart from UC. HLA *DR3* haplotypes predispose to SLE, MS and T1D while *DR9* is associated with both T1D and RA. Genome wide association studies (GWAS) have identified other risk loci for autoimmune conditions, not just within the MHC genes, and

many of these overlap between autoimmune conditions. This has provided evidence for the role of particular genes in autoimmunity and has also given targets for research into casual mechanisms and potential future therapies (Lettre and Rioux 2008). See Appendix 1 for table of common autoimmune conditions and associated HLA genotypes and autoantibodies.

1.4.4 Autoimmunity and Down's syndrome

Autoimmunity occurs at an earlier age and is more exaggerated in DS children (Aitken et al. 2013). CD associated antibodies are present in 10% of DS children, thyroid autoantibodies are found in between 13% and 34% and islet autoantibodies in 8% (Karlsson et al. 1998, Sánchez-Albisua et al. 2002, Gillespie et al. 2006). The cause of this increase in risk is not yet understood. However, intrinsic defects in the immune system particularly with abnormalities in the thymus are likely to play a significant role.

A study to measure gene expression in DS showed that there is not a 50% increase in expression in chromosome 21 genes as might be expected. Some genes are expressed by more than 150% (Li et al. 2006) while others are not overexpressed.

There are several candidate genes located on chromosome 21 that could contribute to increased risk of autoimmunity. Ubiquitin associated and SH3 domain containing A (*UBASH3A*) gene which is located on chromosome 21 (21q22.3) has been found to be associated with T1D in those that already carry the at risk HLA genotype *HLA DR3/4, DQB1*0302* (Johnson et al. 2012). *UBASH3A* has also been shown to be linked to CD through GWAS (Zhernakova et al. 2011). *UBASH3A* is also known as Suppressor of T cell signaling 2 (*Sts-2*) and it has a role in suppressing T cell receptor signaling which is important in regulating T cell activation. Therefore, it is a good candidate gene.

Another candidate gene is *AIRE* (autoimmune regulator) which is also located on chromosome 21(21q22.3). Mutations in *AIRE* are already known to be responsible for autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a condition which has several overlaps with DS. Both conditions involve an increased risk of pancreatic autoimmunity, thyroid autoimmunity, alopecia, hepatitis, mucocutaneous candidiasis and dental and nail dystrophy. In APECED, they are also at risk of autoimmune disease of the parathyroid and adrenal cortex. The possibility of the presence of two APECED at risk alleles being inherited twice from a single parent "disomic homozygosity" leading to an increased risk of T1D in DS was investigated by Shield et al (Shield et al. 1999). Their results showed no evidence of increased disomic homozygosity in the APECED locus when comparing DS patients with T1D with DS alone. Lima et al examined the expression of AIRE within 42 DS thymuses and found that it was significantly reduced together with a number of other genes also involved in the immune system and cell proliferation (Lima et al. 2011). Giménez-Barcons et al also showed reduced expression of *AIRE* and peripheral restricted Ag genes within the thymus of individuals with DS. It therefore appears that *AIRE* does play a role in autoimmunity in DS but this needs more detailed studies to understand the underlying mechanisms (Giménez-Barcons et al. 2014). *AIRE* has also been shown to be associated with autoimmune Addison's disease (Eriksson et al. 2018) and with rheumatoid arthritis (Feng et al. 2015). Interestingly GWAS have not highlighted AIRE as a susceptibility gene for organ specific autoimmune diseases such as T1D and CD (Colobran et al. 2016).

Interferon signalling is increased in DS with the overexpression of interferon-related factors in the lymphocytes of people with DS (Sullivan et al. 2016). Increased interferon signalling has been implicated in T1D and other autoimmune conditions (Ferreira et al. 2014, Lombardi et

al. 2018). There is a gene cluster on Chromosome 21 which includes four interferon receptors (IFNAR1, IFNAR2, IFNGR2 and IL10RB). These genes may also therefore be candidates for explaining the mechanisms underlying autoimmunity in this population.

It is known that environmental factors influence the development of autoimmunity in the general population and these effects will be discussed in more detail later in this chapter. Children with DS may experience different levels of exposure to these environmental factors early in life compared to the rest of the population as a result of their physical, cognitive and medical needs. For example, babies with DS are more at risk of infections as previously described, which may have an effect in themselves or due to the use of frequent antibiotics. Antibiotic use may alter the gut microbiome which in turn may influence the developing immune system. An understanding of the early life environmental influences of these children may help to explain why they are at increased risk of certain autoimmune conditions.

1.4.5 Type 1 diabetes

Definition and Description

T1D is an autoimmune condition in which the insulin producing beta cells in the islets of Langerhans within the pancreas are destroyed. When approximately 70% to 80% of beta cells have been killed, the body is no longer able to maintain glucose homeostasis. Instead of glucose being metabolized and stored, it is lost in the urine and the body enters a catabolic state which eventually results in diabetic ketoacidosis. As the amount of insulin being produced declines, the classical symptoms of polyuria, polydipsia, weight loss and lethargy ensue, and it is hoped that the diagnosis of T1D is made before the patient becomes critically unwell. T1D is managed by giving exogenous insulin which needs to be given in a way that

mimics natural insulin excretion from the pancreas in response to meals, activities, illness and rest. This means that insulin injections need to be given throughout the day or alternatively the person with T1D needs to use an insulin pump.

Epidemiology of Type 1 Diabetes

As with other autoimmune and atopic conditions, the causes of T1D are multifactorial with a combination of genetic susceptibility and environmental influences. There is a geographical and temporal variation in the incidence of T1D.

The variation between different populations is huge with incidence in China and Venezuela of 0.1/100 000 per year compared to 36.8/100 000 per year in Sardinia and 36.5/100 000 per year in Finland (Karvonen et al. 2000). This variation is not simply a North / South divide as previously thought and large differences have been noted in different ethnic groups living within the same geographical area. For example, Jewish and Arabic populations living in Israel have markedly different incidence rates. The difference in incidence therefore appears more likely to represent genetic variation between ethnic groups compounded by environmental exposures which act as triggers. A study of changing incidence in immigrant populations over time demonstrates the importance of environmental determinants. The incidence of diabetes in children of Asian immigrants born in the United Kingdom and living in Bradford, increased to match that of the general population in Bradford (Bodansky et al. 1992). The differences seen in the seasonality of diagnosis also supports environmental triggers (Durruty, Ruiz, and de los Rios 1979, Green, Patterson, and Group 2001).

The incidence of T1D is increasing, with the number of new cases in children aged 0-5yrs predicted to double from 2005 to 2020 (Patterson et al. 2009). Genetic studies have shown

that the increase cannot be explained by the proportion of people with high risk HLA genotypes, as this has decreased over time in people with newly diagnosed T1D. There has however been an increase in those with intermediate risk genotypes (Gillespie et al. 2004, Knip and Simell 2012). There have been a number of hypotheses to try and explain this rise in incidence (Knip and Simell 2012), and a reduction in early exposure to microbial antigens (Rook 2009) and increased exposure to complex proteins early in life is thought to affect the development of the immune system. These exposures take place mostly in the gut associated lymphoid tissue which is exposed to luminal contents providing protection against pathogenic antigens but also has a role to play in developing tolerance to non-harmful antigens. Viruses have been considered as environmental triggers that could also explain the geographical and temporal variation including rotavirus and enterovirus (Knip and Simell 2012). Dietary exposures which may also act as "triggers" are discussed in detail in "breastfeeding and weaning" (Sections 1.5.3 and 1.5.4).

The concordance of T1D has been reported to be between 23% and 50% in monozygotic twins (Kaprio et al. 1992, Barnett et al. 1981) with one long term study quoting up to 70% (Redondo et al. 2008). This relatively low percentage reflects the significant contribution that factors other than genetics must have on progression to disease. Between dizygotic twins the concordance was found to be between 5% and 10%. In a study of risk within families, a child who has one parent with T1D has a 3% risk of developing multiple autoimmunity by the age of 5 years. If both parents have T1D the risk increases to 23.3% (Bonifacio et al. 2004).

Genetics of Type 1 Diabetes and HLA Genotyping

As with other autoimmune conditions, genes located in the MHC on chromosome 6 play a significant role. The MHC class II genes which confer the strongest susceptibility to diabetes are *HLA- DQB1 and HLA-DRB1* (Nejentsev et al. 2007). 90% of children with T1D will have one of the predisposing HLA class II haplotypes *DRB1*04-DQB1*0302 and DRB1*03-DQB1*02* (Todd 2010). The HLA class II genotype is the strongest indicator of predisposition to developing T1D and this has been confirmed by GWAS (Nejentsev et al. 2007) which have resulted in more than 60 genetic associations with T1D. A child with the highest risk HLA genotype, *HLA DRB1*03-DQB1*02/*04-DQB1*0302* where parents both have T1D will have a risk of up to 50% (Bonifacio et al. 2004). There are also HLA haplotypes which have been found to be protective, particularly *DQA1*0102- DQB1*0602 (DQ6)* haplotype (Thorsby and Rønningen 1993). In addition, the HLA class 1 gene *HLA A*24* is associated with rapid progression and early onset of diabetes (Nakanishi et al. 1993).

Islet autoantibodies

Risk of future T1D is most accurately predicted from islet autoantibody status. Antibodies to combinations of insulin (IAA), glutamic acid decarboxylase antibodies (GADA), the tyrosine phosphatase antibodies (IA2-A) and zinc transporter 8 (ZnT8A) are present in 90% of childhood cases of T1D (Ziegler et al. 1999). The development of autoantibodies marks the pre diabetic phase and the presence of two or more islet autoantibodies is associated with increased risk of future diabetes (Ziegler et al. 2013). De novo autoantibodies are usually are not detected before the age of six months (Ziegler et al. 1999). In The Environmental Determinants of Diabetes in the Young (TEDDY) Study however, autoantibodies were found

in ten babies (0.1%) at the age of three months (Ziegler et al. 1999, Krischer et al. 2015) it is important to note that these were not maternal antibodies. Maternal antibodies can be transferred to the neonate across the placenta and have been found to be present up to 9 months of age (Ziegler et al. 1999, E. Naserke, Bonifacio, and Ziegler 2001). GAD antibodies are more likely to be acquired from the mother across the placenta but by nine months insulin auto antibodies (IAA) are not normally maternal (E. Naserke, Bonifacio, and Ziegler 2001). There are some rare exceptions when neonates may develop autoantibodies at a very young age including in immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) (Rubio-Cabezas et al. 2009).

Down's Syndrome and Type 1 Diabetes

Children with DS have approximately a three to four-fold increased risk of developing T1D compared to the general population. Bergholdt et al observed an odds ratio of 4.12 for T1D in a DS group compared to a non-DS group (Van Goor, Massa, and Hirasing 1997, Bergholdt et al. 2006). T1D in people with DS is diagnosed earlier than in those without DS (Bergholdt et al. 2006). A larger proportion are diagnosed before the age of two years (22% vs 4% $p < 0.0001$ (Aitken et al. 2013)) suggesting a more aggressive autoimmune response (Shield et al. 1999, Aitken et al. 2013, Rohrer et al. 2010). As shown in Figure 4, the HLA genotypes of children with DS and diabetes do not show a high frequency of the highest risk genotype *DR3-DQ2 / DR4-DQ8* when compared to those with T1D without DS, including in those diagnosed under the age of two years (Aitken et al. 2013).

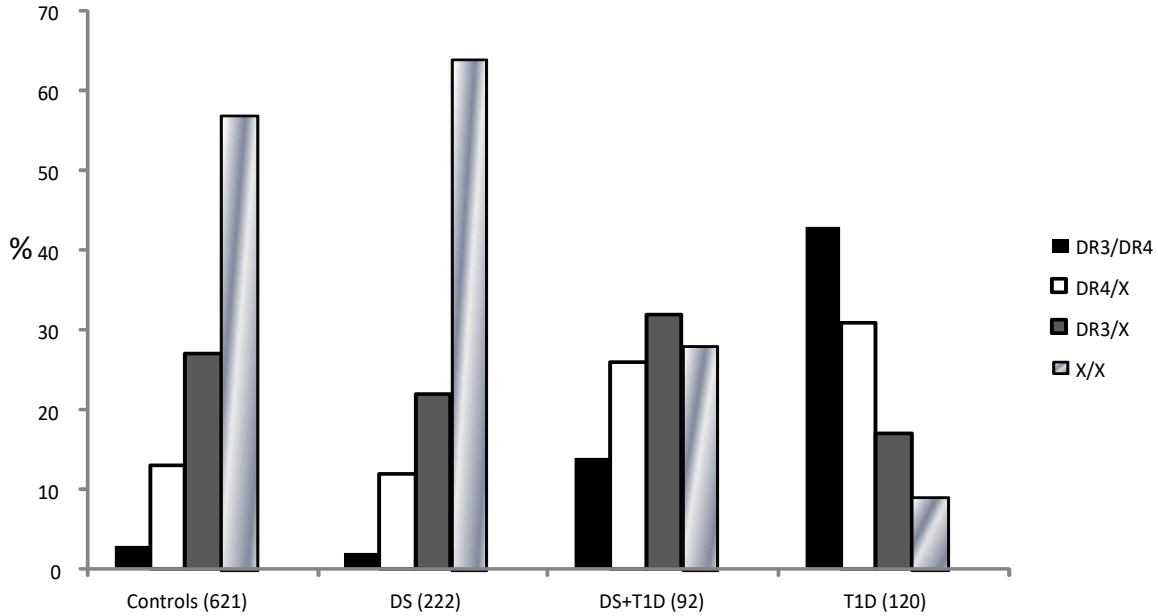


Figure 4: The frequency of T1D associated HLA class II haplotypes in DS and Diabetes. Taken from Aitken et al (Aitken et al. 2013). The proportion of people with the highest risk HLA genotype *DR3/DR4* is similar in the DS and control population. Although an increase is seen in the DSD population it is not as high as the proportion with *DR3/DR4* seen in those with TID.

1.4.6 Coeliac Disease

Definition and Description

CD is an autoimmune condition in which innate and adaptive immune responses are triggered by the presence of proline rich or glutamine rich proteins in the diet (gliadin and glutenins which come from wheat, hordeins which come from barley and secalins which come from rye). In susceptible people, peptides from these proteins pass into the lamina propria of the small intestine where they can then be presented to CD4+ cells by *DQ2* or *DQ8* bearing antigen presenting cells. If these peptides have been deamidated first by tissue transglutaminase, they bind more strongly. Once T helper cells are activated, they induce cytotoxic T cells which cause tissue damage including villous atrophy and crypt hyperplasia. Activation of the T helper cells also leads to stimulation of plasma cells and antibody

production against tissue transglutaminase and gliadin. Gluten also causes a non T cell dependent immune reaction by triggering the release of cytokines within the mucosa particularly interleukin 15 which causes several inflammatory responses including the maturation and proliferation of dendritic cells and activation of cytotoxic lymphocytes causing epithelial damage (Di Sabatino and Corazza 2009). CD has a genetic predisposition determined by the HLA genotype. CD is linked to HLA *DQ2* and *DQ8* with more than 95% of people with CD having one or the other (Kaukinen et al. 2002).

The worldwide prevalence of CD is 1 in 266 (Fasano and Catassi 2001). There has been a recently reported rise in incidence of CD although this may be partly due to an increased recognition of the disease in those with atypical symptoms and better diagnostic tests (Rewers 2005). CD causes intestinal and non-intestinal symptoms. Intestinal symptoms do not need to be present for the diagnosis of CD to be made. Intestinal symptoms include diarrhoea and a bloated abdomen. The non-intestinal manifestations are diverse and can include weight loss, lethargy, metabolic bone disease, infertility and anaemia (Green and Jabri 2003). CD is associated with an increased risk of developing intestinal lymphoma and other malignancies. This risk is reduced by maintaining a gluten free diet. It is therefore extremely important that CD is diagnosed and treated with a gluten free diet that excludes wheat, barley and rye.

The diagnosis of CD may be made by a combination of clinical suspicion, serological testing for antibodies to endomysium (EMA), tissue transglutaminase (tTG) and gliadin (in the presence of IgA deficiency, IgG antibodies should be measured) and the presence of typical histological features on jejenul/ duodenal biopsy. The presence of villous atrophy is typical with lymphocytic infiltrates. There are guidelines which also include genotyping for HLA-

DQ2/DQ8 as an alternative to having a biopsy performed (Kaukinen et al. 2002, Murch et al. 2013) but this is unsuitable for those with established T1D given shared susceptibility haplotypes.

Down's Syndrome and Coeliac disease

CD is present in five to seven percent of individuals with DS (Carnicer et al. 2001, Book et al. 2001, Bonamico et al. 2001). The symptoms of CD in DS can be silent, making it difficult to diagnose. Although screening for CD is recommended for individuals with DS by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (Husby et al. 2012), it is not included in the routine screening recommended by the DSMIG or NICE (DSMIG , Downey et al. 2015). Both do state however that clinicians should be vigilant in looking out for any symptoms or signs that may be suggestive. The reason for the increased prevalence of CD in DS is not known. Early weaning and potentially cessation of breastfeeding that may happen in children with DS could be a contributing factor. Norris et al showed that there was an increased risk of CD if gluten is introduced into the diet before the age of three months (Norris et al. 2005). Ivarsson et al showed that in children under the age of two years old, if gluten was introduced whilst children were still breastfeeding, the risk of coeliac disease was reduced (Ivarsson et al. 2002).

1.4.7 Thyroid Autoimmunity

Definition and Description

Thyroid disease is the most common of the autoimmune conditions affecting two to five percent of the general population (Simmonds and Gough 2004). Thyroid autoimmunity can lead to both hypothyroidism (Hashimoto's thyroiditis) and hyperthyroidism (Graves' disease).

Thyroid autoimmunity is much more common in women than in men (McLeod and Cooper 2012, Manji et al. 2006). Thyroid autoimmunity is also caused by a combination of genetic risk and environmental influences. There is a HLA associated risk genotype, and for autoimmune hypothyroidism this association is with *DR3* (Simmonds and Gough 2004). Thyroid autoantibodies can be against thyroglobulin, thyroid peroxidase and the thyroid stimulating hormone (TSH) receptor. When they are directed against the TSH receptor, they lead to hyperthyroidism (Weetman 2004).

Down's Syndrome and Thyroid Autoimmunity

The prevalence of thyroid autoimmunity in people with DS is reported to be between 13% and 34% (Karlsson et al. 1998). The symptoms of hypothyroidism may not be recognised in patients with DS as poor growth, hypotonia and intellectual impairment are features of both. It is extremely important that hypothyroidism is recognised as early treatment is known to be beneficial to long term outcomes. In a study of people with DS less than 20 years old, they were found to have a higher prevalence of thyroid autoantibodies. Antibodies to tyroxine peroxidase (TPO) and antithyroglobulin were two fold higher in DS participants with T1D than in participants with T1D only (Rohrer et al. 2010). In another study of children with DS, although a third developed hypothyroidism, TPO antibodies were only found in one child (n=85) under the age of eight and none had antithyroglobulin antibodies until they were older than eight. Over the age of eight, autoimmune hypothyroidism became more common, as is also seen in the general population where the onset of autoimmune thyroid disease is usually seen in early to mid-puberty (Karlsson et al. 1998, Hunter et al. 2000). From these studies autoimmunity alone cannot explain why a third of people with DS have thyroid disease. The

thyroid gland in children with DS appears to be normal on ultrasound scan suggesting that the problem is not structural, and dyshormonogenesis is less likely in the absence of a goitre (Kennedy, Jones, and Cuckle 1992).

1.5 Autoimmunity and Early Life Influences

1.5.1 Background

The rising incidence of autoimmune diseases is best explained by environmental risk factors. The increase particularly in the younger age groups with T1D, together with the knowledge that in those children who are diagnosed with T1D under the age of ten, they produce autoantibodies before the age of two, suggest that this is likely to be an exposure during pregnancy or early life (Kimpimaki et al. 2001). Various factors have been postulated including, the increase in caesarean sections and concomitant alterations in the gut microbiome, reduction in rates of breastfeeding, early introduction of formula feeds and more sanitized living conditions potentially leading to a more self-reactive immune system.

1.5.2 Microbiome

The human gut microbiome consists of trillions of bacterial cells. As evidence for its underlying role in health and disease emerges, interest in its constituents and function are increasing (Turnbaugh et al. 2007, Vatanen et al. 2016). The initial microbial colonization of the gastrointestinal tract during the first few years of life appears to be key to the development of the host's immune system regulation. Perturbations in the function or composition of the microbiota has been associated with inflammatory and autoimmune conditions (Kostic et al. 2015, Vatanen et al. 2016, Giongo et al. 2011, Morgan et al. 2012). Although studies of the gut microbiome are in their infancy, initial studies suggest that the gut microbiome of young

children at risk of autoimmunity is different from the general population with, less diverse and less stable colonisation. In contrast, in children who do not develop autoimmunity, the gut microbiome changes and stabilises in the first two years with diverse colonies of bacteria (Giongo et al. 2011, de Goffau et al. 2013, Vaarala 2013). Children from the Diabetes Prediction and Prevention study (DiPP) in Finland, who were genetically at risk of developing T1D provided stool samples every three months from birth until they developed autoantibodies for T1D, these stool samples were compared to control samples taken from non- autoimmune children. Although there were only a small number who developed two autoantibodies for T1D (n=8), their gut microbiome showed a much higher ratio of Firmicutes to Bacteroidetes compared with controls (Giongo et al. 2011).

Differences in the gut microbiome between those born via caesarean section and those born via normal vaginal delivery is thought to explain the 20% increased risk of T1D in those born via C section (Norris et al. 2003). Further research to understand how the microbiome affects autoimmunity mechanistically and to what degree small alterations in composition lead to changes in disease progression is required.

1.5.3 Breastfeeding

Prolonged exclusive breastfeeding may be protective against some autoimmune conditions including T1D and CD (Malcova et al. 2006, Sollid 2002, Akobeng et al. 2006). When formula feeds are introduced the baby is exposed to cow's milk proteins for the first time and antibodies against bovine serum albumin (BSA) will be produced. This immune response to the cow's milk proteins has been suggested as one of the triggers for pancreatic autoimmunity in T1D although this has been controversial and unproven. The levels of antibodies against

BSA have been shown in one study to be much higher in patients with T1D (Karjalainen et al. 1992). Another study found that this is not just specific to T1D but is seen in other autoimmune and inflammatory conditions (Atkinson et al. 1993). T cells in T1D however are not specific for BSA. From this it was concluded that the increase in antibodies to BSA in T1D may represent a general immune defect (Atkinson et al. 1993). Recent data from our laboratory demonstrates that levels of antibodies to BSA are elevated in children with DS (manuscript in preparation) and these antibodies are of low affinity, perhaps reflecting immature B cell responses.

1.5.4 Weaning

The current WHO recommendation is that babies should be exclusively breast fed until the age of 6 months, this is based on the health benefits that breast feeding brings to the mother and baby. It is generally recommended that babies should then be introduced to solid food around the age of 6 months (Norris et al. 2003). There is an association between the development of CD and the development of T1D, with an increased risk of developing islet autoantibodies in those with CD. As gluten is known to be a trigger for CD, it has also been postulated to be involved in the development of T1D. Introduction of foods containing cereals either before the age of four months or after the age of seven months has been associated with an increased risk of developing islet autoantibodies (Norris et al. 2003). This study in contrast with other studies (Virtanen et al. 1993, Malcova et al. 2006) did not however find a link between islet autoimmunity and the introduction of cow's milk. Interestingly breastfeeding during the introduction of gluten into the diet appears to protect against CD in babies who are started on gluten at an early age (Akobeng et al. 2006, Sollid 2002).

1.5.5 Infections

The hygiene hypothesis suggests that a reduction in exposure to pathogens, due to the more sanitized conditions that infants develop in, has led to the immune system becoming more disordered and self-reactive (Rook 2009). Viral infections however have also been postulated as the environmental trigger for autoimmune conditions in those in at risk groups.

In the Diabetes Autoimmunity Study in the Young (DAISY), the development of islet autoantibodies in relation to childhood infections was examined. DAISY found that gastrointestinal infections were related to an increased risk of developing islet autoantibodies but only in children who were exposed to barley or wheat for the first time either before four months of age or after seven months (Snell-Bergeon et al. 2012). Children who went to daycare during the day got more infections in general but that this was not related to an increase in islet autoimmunity amongst these children.

1.6 Feeding and Down's syndrome

Babies and children with DS may have several oral, anatomical and physiological abnormalities which may affect their ability to feed normally, as with other features of DS there is phenotypic variability. The following features have previously been described (Desai 1997, Kumin and Bahr 1999):

- palate abnormalities (Hard palate reduced in length, depth and height. Soft palate insufficiency);
- hypotonia in the lip, tongue, soft palate and jaw areas;
- small oral cavity (Incomplete development of midface);

- malocclusion (Malalignment between the dentition of the lower and the upper jaw);
- lax ligaments in tempero-mandibular joint;
- relative macroglossia (tongue is large relative to small oral cavity) or true macroglossia (enlarged tongue);

scalloped and fissured tongue, which affects latch;

- open mouth posture at rest leading to desiccated tongue;
- poor neuromotor control of the tongue;
- mouth breathing;
- chewing difficulties;
- bruxism (i.e. teeth grinding);
- abnormal dentition including hypoplasia and hypocalcification and partial anodontia.

To breastfeed effectively, a tight seal needs to be made. This is achieved by the tongue making a groove and the lips sealing around the nipple. For a baby with DS, the flattened shape of the tongue and low tone in the oral structures means that establishing breastfeeding may be challenging and take longer to establish.

Weaning may also be complicated with babies with DS finding it difficult to manage different textures due to poor tongue control and oral hyper or hyposensitivity. Babies with DS may experience choking and gagging during weaning on to solid feeds and may reject certain textures. Feeding is also affected by generalized hypotonia with poor posture affecting ability to feed. Kumin et al describe the importance of good positioning for feeding (Kumin, Von Hagel, and Bahr 2001).

These potential oro-motor complications may lead to babies with DS being exposed to cow's milk protein-based formulas earlier than their peers. Gluten might also be introduced earlier due to the introduction of solids at a younger age.

1.7 Cohorts of people with Down's Syndrome

Many countries have registers including cytogenetic registries which provide data on the diagnosis of people with DS. These registers have facilitated retrospective studies providing statistics on aspects of DS including birth and death rates and congenital anomalies. However longitudinal prospective birth cohort studies of infants with Downs's Syndrome are rare. A summary of the largest and most recent cohorts is given below.

The LonDownS Consortium

A current cohort recruiting in the UK, the LonDownS Consortium ((LonDownS Consortium), is a group of research studies aiming to understand dementia in people with DS. The consortium includes a longitudinal cohort of 150 babies, age six to forty months with DS. Low and high functioning subgroups within this cohort will be used to explore the neurocognitive phenotypes of infants with DS to determine the potential to predict protective/risk markers for Alzheimer's disease.

Children with Down's Syndrome Study

Another recent birth cohort in the UK was the Children with Down Syndrome Study (CDSS). This study was set up in May 2006 by paediatricians and haematologists from St James University Hospital in Leeds and the Epidemiology and Genetics Unit at the University of York (CDSS 2018). CDSS aimed to study all aspects of the health of children with DS with a more

specific aim of characterizing the haematology in neonates with DS (James 2011). The study used a two-stage approach when recruiting families. Initially new-borns were recruited to the study after birth but before they went home by neonatologists from six regional neonatal networks. If parents consented at this stage, a blood sample could be taken, the mother's maternity notes could be reviewed, and it also permitted the study team to contact the parents again once they were home to ask if they were willing to take part further in the study. If parents consented to the second stage, the child's records could be accessed. Parents were asked to complete questionnaires about the child's health and background, further blood samples collected and a buccal mouth swab for DNA. Between 2006 and 2011, 479 children were recruited to the CDSS providing 234 neonatal blood samples (49%). However, during follow up only ten blood samples were available from children aged two years old (2%) and five at four years of age (1%).

Down Syndrome: A Novel Risk Factor for Respiratory Syncytial Virus Bronchiolitis— A Prospective Birth-Cohort Study

Bloemers et al recruited 219 infants with DS into a nationwide, Dutch prospective birth cohort to study the incidence of hospitalizations associated with respiratory syncytial virus lower respiratory tract infections amongst this population (Bloemers et al. 2007). These babies were identified through the Dutch Paediatric Surveillance Unit and were followed up until they were two years old. The researchers also did a retrospective observational study of 276 children with DS. The study concluded that DS is an independent risk factor for respiratory syncytial virus bronchiolitis.

The Manchester Downs Syndrome Study and Dr Janet Carr's Cohort

There have also been two well established longitudinal cohorts of children with DS in England studying the educational, social, psychological and psychiatric aspects of having Down's syndrome. The Manchester Downs Syndrome Study recruited 181 families between 1973 and 1980 (85% to 90%) of all the infants born with DS in the Manchester area)(Cunningham 1996). Although there had been some attrition over the years, 100 participants were followed until early adulthood. The other cohort was established by Dr Janet Carr who recruited 54 babies born with DS between December 1963 and November 1964 in Surrey and the London boroughs of Camberwell and Lewisham. The participants in this cohort were followed up from the age of six weeks until they were 21 years old, with 41 participants with DS remaining in the study at the age of 21 years old (Carr 1988).

1.7.1 The need for a new cohort of babies with DS

To study the clinical / pathological features of DS, there needs to be more longitudinal birth cohorts in which serial biological samples are collected together with clinical data. Each of the studies above were designed to focus on an aspect of DS. Two of the studies (LonDownS Consortium and The Manchester Downs Syndrome Study) explore the neuro cognitive and psychological aspects of DS including the development of Alzheimer's. The CDSS study studied early haematological findings and Bloemer's study on respiratory syncytial virus lower respiratory tract infections. Excluding Bloemer's retrospective study, the challenges for each of these cohorts lie in recruiting and maintaining participants and reducing attrition rates. The Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES) developed in this PhD is a feasibility study. It was established to understand and overcome issues around

recruitment and retention for cohorts of babies with DS whilst uniquely focusing on early feeding and the development of autoimmunity. Unlike the other studies, it is UK wide. It will build a longitudinal biobank of samples including urine, stool and blood samples which can be linked to well characterised feeding and medical data (including infections and antibiotic use).

1.8 Feasibility Studies

The distinction between pilot and feasibility studies is often blurred and the definitions given have altered over the last few years, including those given by funding bodies (Arain et al. 2010, Thabane et al. 2010). In 2016, a conceptual framework was developed and validated (through a Delphi study, international expert consensus meeting and systematic review) for defining feasibility studies for randomised controlled trials (RCTs) (Eldridge, Lancaster, et al. 2016). Feasibility studies may be iterative and can be adjusted and improved if aspects of the study design are not adequate (Bowen et al. 2009, Hagen et al. 2011, Orsmond and Cohn 2015). A well-designed feasibility study will set objectives to determine whether the main study will work as shown in Figure 5.

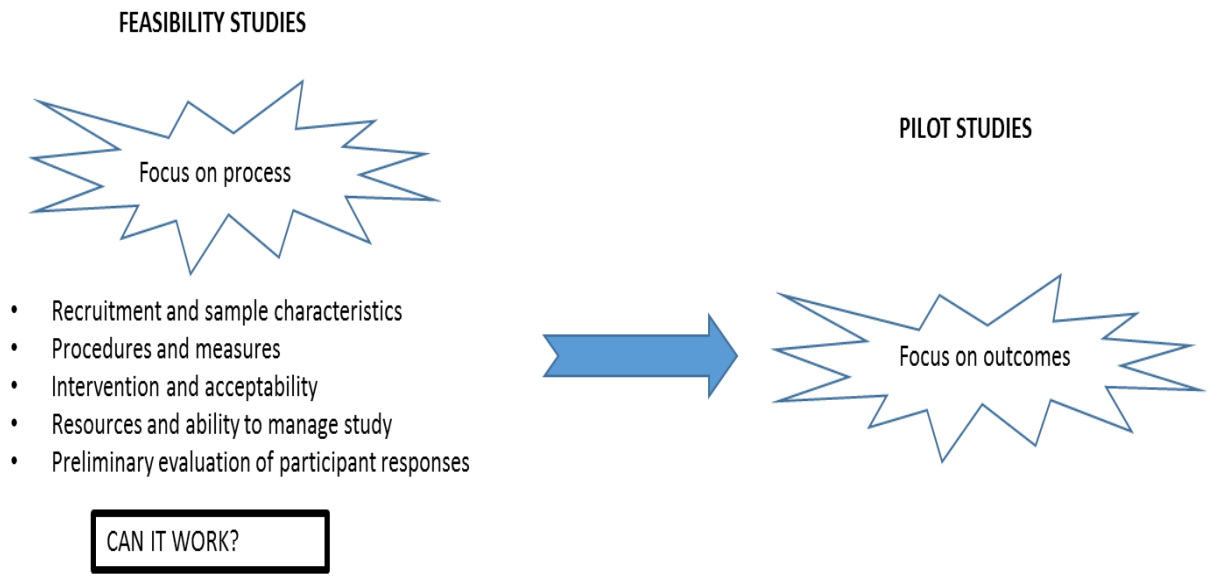


Figure 5: The features of a feasibility study. Adapted from "The distinctive features of a feasibility study:objectives and guiding questions" (Orsmond and Cohn 2015)

The following examples are parameters and areas that can be assessed from a feasibility study (NIHR Evaluation, Trials and Studies Coordinating Centre (Bowen et al. 2009, Thabane et al. 2010, Orsmond and Cohn 2015, NIHR 2019):

- acceptability, how do the target population and the researchers view the study,
- number of eligible patients,
- recruitment and consent, assessing the ability to recruit at the desired recruitment rate using the proposed methods,
- willingness of clinicians to recruit participants,
- implementation and practicality, whether a study can be conducted as planned and whether there are any constraints such as time and resources,
- usability and understanding of data collection methods and questionnaires,

- sample characteristics and generalizability,
- characteristics of the proposed outcome measure and in some cases feasibility studies might involve designing a suitable outcome measure,
- standard deviation of the outcome measure (may be needed to estimate sample size in the main study),
- follow-up rates, response rates to questionnaires, adherence/compliance rates.

The feasibility objectives can also test whether the research team has the appropriate skills and resources to run the main study. A feasibility study is not designed to test a hypothesis and therefore a power calculation is not required. However the sample size should be large enough to determine the feasibility objectives (Orsmond and Cohn 2015).

The Consolidated Standards of Reporting Trials (CONSORT) guidelines are used to standardise and improve the reporting of randomised controlled trials. In 2010 there was an extension to the guidelines to include randomised pilot and feasibility studies (Eldridge, Chan, et al. 2016). Although not all feasibility studies are randomised, the guidelines make relevant points which should be considered in planning and reporting. A review of 54 pilot and feasibility studies published between 2007 and 2008 found that many studies incorporated hypotheses testing (Arain et al. 2010). This is despite recommendations that statistical analysis should be mostly descriptive or should be focused on sample size calculation for a future study (Lancaster, Dodd, and Williamson 2004).

Qualitative research which is used to explore certain aspects of a study design is recognised as an important feature of feasibility, and can be used alongside quantitative methods (O'Cathain et al. 2015, Eldridge, Lancaster, et al. 2016). Qualitative research may be

particularly important when assessing the acceptability of a study protocol for the participants. In the extended CONSORT statement the need to link published qualitative work with the other aspects of the feasibility study is highlighted (Eldridge, Chan, et al. 2016).

This present study was a feasibility study with clearly defined feasibility objectives (see Section 1.12). An iterative approach was taken throughout, with changes made to improve aspects of the study design.

1.9 Qualitative Research

As part of the feasibility of FADES, a qualitative study was undertaken to understand how to maximise recruitment of young infants with DS into research, through qualitative interviews with parents and care providers (This qualitative research study was published (Williams et al. 2018)).The diagnosis of DS is relatively rare, an individual's experience of being a parent of a child with DS varies and there is little available prior research into recruiting this population. Qualitative research allowed in depth social inquiry which enabled a better understanding of the participants' perspectives and experiences. This is presented in Chapter 3 with a more detailed introduction to qualitative research and thematic analysis.

1.10 Aim of PhD:

The aim of this project was to develop a family acceptable study protocol and establish the ***feasibility*** of creating a national cohort of infants with DS to study the longitudinal associations between early infant feeding, infections, antibiotic usage and the development of autoimmunity in DS. The study was entitled Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES).

Aims:

- Establish the feasibility of creating a UK wide cohort of infants with DS (See section 1.12 for feasibility objectives)
- Determine the acceptability of the study protocol for families
- Determine the ability to collect appropriate questionnaire data and samples for the exploration of factors associated with the development of autoimmune conditions, specifically (T1D, autoimmune thyroid disease and coeliac disease) (See section 1.12).

As a feasibility study this PhD was not designed to test a hypothesis. However, the potential factors involved in the development of autoimmunity in this population which underlie the data collected are:

- differences in early feeding due to oromotor difficulties and hypotonia which may lead to cow's milk protein being introduced earlier,
- timing of weaning and introduction of gluten,
- Infections and use of antibiotics
- alterations in the gut microbiome,
- HLA genotype

1.11 Summary of initial protocol

The initial study protocol is summarized below (the full protocol is in Appendix 2). This is included here to provide a basic overview prior to outlining the feasibility and secondary objectives. The protocol was modified over the course of the last three years and further details of the protocol and the changes are described in Chapter 2 Materials and Methods.

1.11.1 Recruitment

The initial protocol planned for participants to be recruited through flyers sent out by the Down's Syndrome Association (DSA) and Down's Syndrome Scotland (DSS). Families would register their interest through the online expression of interest (EOI) form and the consent forms were sent out to them by post. (Changes were made to this recruitment strategy Material and Methods Chapter 2).

1.11.2 Eligibility Criteria

Inclusion Criteria:

- Babies recruited antenatally or in the first eight months of life born with DS (three copies of chromosome 21) as confirmed by karyotype after birth.

Exclusion Criteria

- Babies with DS who have a child protection plan or who are no longer with birth mother.
- Babies with DS over eight months of age.
- Babies with DS in whom the parents do not speak English. The study recruits families who are trying to cope with a potentially difficult diagnosis and may wish to have conversations with the research staff to establish what the implications of recruitment would involve for them. Participants are also required to fill out seven questionnaires over five years in English.

1.11.3 Sample Collection

Parents collected the samples from their infant/ child at home using kits sent through the post. The samples included a mouth swab sample for DNA, a baby stool sample for gut

microbiome analysis (a maternal stool sample was later added to the protocol see Chapter 2) and a urine sample for C-peptide after a feed as close to birth as possible, at 12 months and yearly until the age of five. Families were then asked for further stool samples from the child at six months, 12 months and yearly thereafter until the age of five.

The parents were provided with a kit for the blood sample and sampling was then arranged to coincide with already existing appointments if possible. The aim was for the blood sample to be collected as close to birth as possible, at six months, 12 months and yearly until the age of five years.

1.11.4 Feeding and Medical Online Questionnaires

Parents were asked to complete detailed feeding and medical questionnaires, close to birth, at seven months (when most babies will have weaned onto solids) and at a year. They then completed medical questionnaires annually until the age of five years old. The questionnaires were available online through the University of Bristol's REDCap system (University of Bristol REDCap) (Questionnaires are in Appendices 14 to 17).

1.12 Feasibility objectives

A summary of the feasibility objectives for the study are set out below (Lancaster, Dodd, and Williamson 2004, Eldridge, Chan, et al. 2016). More detailed objectives and how they will be assessed are described in the relevant sections in the Methods Chapter 2.

Objectives relating to feasibility of recruitment:

- to assess the rate of recruitment;
- to consider the feasibility of novel recruitment methods; and

- to understand the reasons why participants consented to take part in the study.

Objectives in relation to feasibility of sample collection:

- to test the validity of the sample collection methods;
- to assess the ability of families to collect all the samples within the desired timeline; and
- to assess the suitability and quality of the samples which are received.

Objectives relating to medical and feeding questionnaires

- the feasibility of the questionnaires to collect the required data within the desired timeline.

Objective relating to retention of participants and overall acceptability

- the feasibility of retaining participants in the study, which in parallel also determines the overall acceptability of the study.

The main objective of the qualitative research was to

- explore the views and opinions of healthcare professionals and support workers about potential barriers to families taking part in research and how these may be overcome.

1.13 Secondary objectives

The following were secondary objectives;

1. to characterise the cohort in relation to:
    a) maternal age, socio-economic background, ethnicity;
    b) medical conditions related to a diagnosis of DS;

2. to determine the numbers and types of infections experienced during early life and antibiotic usage;
3. to describe early feeding and weaning;
4. to measure levels of urine C-peptide levels longitudinally;
5. to measure anti-BSA antibodies longitudinally; and
6. to profile the gut microbiome during early childhood.

Characterisation of the cohort is important to show that study population is representative of the general population of children with DS (Orsmond and Cohn 2015).

CHAPTER 2

Materials and Methods

Chapter 2 Materials and Methods

2.1 Overview of Chapter 2

Key steps for determining the feasibility of a potential study protocol are the planning and consultation phases. These phases and the patient and public involvement (PPI) throughout this study are covered in this chapter. The processes involved in setting up a UK wide birth cohort of infants with DS (FADES) to meet the standards of Good Clinical Practice (GCP) and ethics, and the administrative and organisational infrastructure for the study are explained. Recruitment methods and the changes that were made to maximise recruitment, reflecting the iterative nature of this feasibility study, are detailed. Description of the design of the feeding and medical questionnaires, and their web-based functionality is highlighted.

The methods for collecting, storing and analysing the samples are included in this chapter. Two mini feasibility studies were completed within this PhD to refine the sample collection methodologies. One was for the collection of the gut microbiome samples and the other for the collection of the urine samples. Both feasibility studies are presented in full in this chapter.

The qualitative research methods used for the interviews exploring the potential barriers and motivations to parents of a new baby with DS to taking part in research are given. The design and processes for analysis of participant and non-participant questionnaires, which were used to study why parents decided to enrol in FADES or decided not to enrol in FADES, are also included at the end of the chapter.

Details of the statistical analysis and the descriptive analysis for the feasibility outcomes, questionnaire data, samples and qualitative research are all described in the relevant sections.

2.2 The research team

The study research team were multi-disciplinary covering a broad range of skills, knowledge and expertise. The study was based at the NIHR Bristol Nutrition Biomedical Research Unit (BRU) (now the NIHR Biomedical Research Centre (BRC) Nutrition Theme) allowing access to experienced health researchers outside of the core team. The processing, storage and analysis of samples were undertaken by the Diabetes and Metabolism team (Bristol Medical School, Translational Health Sciences). Table 3 shows the different roles and involvement of the different members of the research team. I was involved in all stages of the study.

Name	Initials	Job Title / Role / Expertise	Activity
Professor Julian Hamilton- Shield	JHS	PhD supervisor / Professor of diabetes and metabolic endocrinology / Paediatrician	PhD Supervision Advised on study protocol Expert review of study documentation / questionnaires
Dr Kathleen Gillespie	KG	PhD supervisor / Reader in molecular medicine	PhD Supervision Advised on study protocol Expert review of study documentation / questionnaires
Dr Sam D. Leary	SDL	PhD supervisor / Senior lecturer in statistics	PhD Supervision Expert review of study questionnaires User testing of questionnaires Advised on the statistical analysis
Sofia Leadbetter / Katie Berryman	SL KB	Admin assistant	FADES emails* Sent out participant consent forms and questionnaires (online links)* Updated database / CPMS* Sent participant reminders* User testing of questionnaires – KB
Stu Toms	ST	Database manager	Development of database, online forms (EOI, REDCap) and online questionnaires*
Georgina Mortimer Saranna Chipper-Keating / Rachel Aitken	GM SCK RA	Laboratory technician	Sent out sample packs to families* Received, logged, processed and stored samples* Updated database* HLA genotyping on DNA samples (GM and SCK)
Sian Grace	SG	Laboratory technician	LPS analysis of anti-BSA antibodies from blood samples
Shirley Jenkins	SJ	Management assistant	Set up webpage
Dr Patricia Neville	PN	Sociologist / Lecturer	Advised on design of qualitative study Reviewed topic guide for qualitative study
Dr Aidan Searle	AS	Senior research associate in qualitative studies	Advised on analysis of qualitative research Dual coded qualitative interviews

Table 3: The research team, roles and activity within the study.

Footnote: *These activities were also completed by GW during the course of the stud

2.3 Planning and consultations

2.3.1 Patient and Public Involvement (PPI)

There has been patient and public involvement (PPI) during the design stages, the piloting of questionnaires, in study promotion and in the assessment of the feasibility of the study. PPI has been in the form of consultations (with parents and charitable organisations) and collaboration (with charitable/ voluntary sector organisations including the DSA, DSS and DHG). The consultations were organised and conducted between me and members of the charities / voluntary sector organisations. The valuable role of PPI in clinical research studies has become established and is now a requirement of many funding bodies (Robinson and Otology 2014). PPI improves the success and quality of studies, by providing key insights. These include the values and priorities of the population of interest and how the research might fit into their lives (Bate et al. 2016). It is also important for producing an ethically sound protocol.

2.3.2 Consultation with the Down's Syndrome Association (DSA) and Down's Syndrome Scotland (DSS)

The DSA and DSS are both members led organisations with many committee members being parents of a child or adult with DS. They were consulted throughout the planning stages of FADES as well as during the study (organisational approvals from DSA and DSS Appendix 9 and 10). The DSA and DSS initially provided statistics on their members and the families that they were in contact with and would be able to target for recruitment.

The DSA reported that they send out approximately 500 information packs per year and could promote the study in the packs. The DSA offered to include the FADES flyer in their "continuing pregnancy booklet" which is given to parents who have had a prenatal diagnosis of DS for their baby. DSS were also consulted and they reported that approximately 50 to 60 babies are born each year with DS in Scotland. In 2012 they visited or provided information to 30 parents. Of these parents, less than 10% had received a prenatal diagnosis of DS for their baby. DSS provided information packs to every maternity unit in Scotland and the families then contacted them. They did not agree to include the FADES study information within this pack but were happy to let families know about the study once they had registered with DSS. They visited at their home all families who registered with them and took the information about the study on these home visits. They were also happy to promote the study on their website, in their newsletter and e-bulletins.

The DSA and DSS were both consulted regarding the acceptability of the study protocol and the documentation that would be sent to participants. DSS asked one of their mothers to review the FADES study documentation. The feedback from the parent was that the flyer and information sheet were "straight forward and clear although not engaging" they suggested using photos of babies or babies feeding to engage more parents. The mother and other members of the DSS suggested a simplified diary that they could use once enrolled in the study to keep information that would be useful when the time came to complete the questionnaires. The final comment from the mother was that although it is a lot of documentation, they probably would have been willing to complete the paperwork if their child was still the correct age for the study. They also highlighted the need for reminders. The DSA committee approved the study information sheet and the flyer (Appendix 11 and 12).

Both the DSA and DSS provided feedback on the questionnaire which is described in Section 2.13.1. Ongoing collaboration and consultations between myself the DSA and DSS continued throughout the conduct of the study and will be discussed further through this thesis.

2.3.3 Consultation with Bristol Area Down's Syndrome Support

The DSA's local Bristol branch, Bristol Area Down's Syndrome Support (BADSS) kindly agreed for me to attend their committee meeting on the 10th February 2014. The committee members were all parents of children with DS and included new members who were parents of young infants with DS. The study background and outline were presented, and committee members had the opportunity to review the study documentation that would be provided to families. There was then time for everyone to discuss the study in an open forum. The committee members were asked how acceptable they felt the study was and the best way to improve recruitment and work with families. Overall the feedback was very positive. They felt that it would be difficult recruiting families when they are coming to terms with the diagnosis of DS for their baby but that engaging in research may offer an opportunity for families to get extra support. Attending the meeting was extremely beneficial providing an insight into the experiences of families of children with DS and the role that research may play.

2.4 Standards, Study Ethics and R&D approval

Good Clinical Practice (GCP) are internationally recognised standards for conducting research. GCP includes study design, study conduct, monitoring, audit and reporting of trials. Compliance with GCP is a UK/European legal requirement for clinical studies investigating medicinal products (Gill 2004). Although this study did not include any medicinal products, adherence to the basic standards of GCP provided reassurance that the study results were

accurate and credible and that the rights of the participants and their families had been respected. To comply with the standards of GCP, all clinical research studies are required to have the correct approvals from the Research Ethics Committee (REC) and Research and Development (R&D).

The study also complied with the Human Tissue Act (HTA) 2004 (The Human Tissue Act 2004) with respect to the use, storage and disposal of human tissue. The study protocol and the consent process ensured that the requirements of the HTA were met.

2.4.1 Integrated Research Application System (IRAS)

Research studies conducted in the UK use the Integrated Research Application System (IRAS) which enables researchers to enter online all the required information for seeking approvals from a number of organisations avoiding duplication (IRAS). The online form uses filter questions to ensure the data entered is appropriate for the study type and meets the necessary requirements of the regulatory bodies. For this study IRAS was used to apply for NHS REC approval, adoption onto the NIHR Portfolio (see Section 2.4.3) and NHS R&D permissions.

2.4.2 Ethics

The study was presented to the National Research Ethics Service (NRES) Committee South West – Central Bristol by me and JHS on the 28th February 2014. The ethics committee had several queries and clarifications, these included assessment of inclusion criteria, clarification of samples, estimation of time taken to complete questionnaires and option for storage of data and samples for future research clarified (further details are in Appendix 6). The ethics

committee were happy with the changes made in response to their queries and approved the study on 23rd April 2014 reference 14/SW/0030 (Appendix 7).

2.4.3 The NIHR Clinical Research Network (NIHR CRN) Portfolio of Studies

The NIHR Clinical Research Network's Portfolio of Studies (CRN portfolio) are high-quality clinical research studies which have met the eligibility criteria to be approved for NIHR Clinical Research Network Support (CRN)(NIHR CRN Portfolio 2014). The NIHR Comprehensive Clinical Research Network (CCRN) is made up of 25 Comprehensive Local Research Networks (CLRN) which cover the whole of England. The infrastructure support that is afforded to studies on the portfolio includes access to experts for help in setting up studies within NHS organisations, particularly information on capability and capacity within research sites, an important aspect in the feasibility of a research study. There is also access to NHS service support resources including research nurses and data managers through the CRN. However, the availability of these resources for this study varied by geographical area, and in many instances was not accessible. The study was adopted as an NIHR portfolio study on the 14th May 2014 (UKCRN study ID is 16735).

As an NIHR CRN portfolio study, monthly recruitment data were entered by me, KB and SL onto the Central Portfolio Management System (CPMS) (a cloud-based management system). This allows research sites to claim their accruals and monitor their performance against targets set by the Department of Health.

2.4.4 NIHR Coordinated System for gaining NHS Permission (NIHR NHS CSP)

Recruitment of participants into FADES was initially through the DSA information packs and through DSS family support workers. Participants could be recruited from anywhere in the

UK. Following a consultation between myself and the study sponsor (University of Bristol, Research Enterprise and Development (RED), Dr Rachel Davies), to comply with GCP, R&D permissions were sought from all primary and secondary care sites. This meant that wherever a potential family was located they would have a local NHS organisation/ trust who would be engaged in the study and could hopefully assist with the taking of the blood samples.

The structure of the NHS varies between the devolved nations. There are around 400 NHS organisations (primary and secondary care sites) in England. In Wales primary and secondary care organisations are coordinated by seven health boards, in Scotland there are 14 regional NHS Boards and there are six Health and Social Care (HSCT) trusts in Northern Ireland. To request approval from these sites, the NIHR NHS Co-ordinated system for gaining NHS permission was used (NIHR NHS CSP was decommissioned in 2016). In order to access NIHR NHS CSP, studies needed to be eligible for the NIHR portfolio. In the devolved nations, the local NIHR NHS CSP Coordinating Centre for that country was used. For Wales this was the National Institute for Social Care and Health Research (NISCHR) Permissions Coordinating Unit (PCU) and for Scotland was the NHS Research Scotland Coordinating Centre (NRS CC). The processes that were involved in gaining R&D approvals through CSP for this study are illustrated in Figure 6.

The system for gaining R&D and ethical approval was changed in March 2016 to Health Research Authority (HRA) approval (HRA 2019) this will be discussed further in Chapter 7 Discussion.

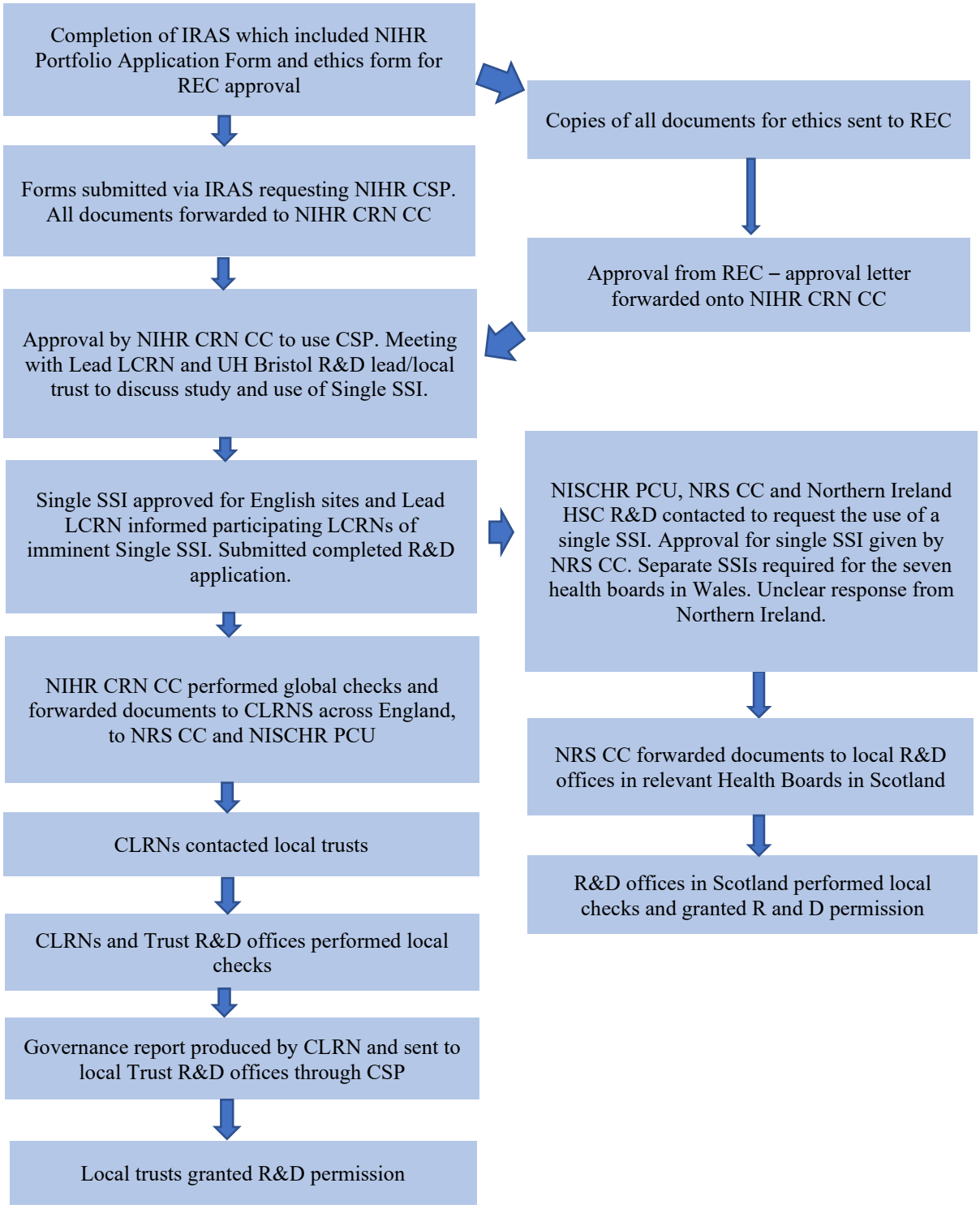


Figure 6: The approximate steps that were taken in gaining REC and R&D permission from primary and secondary care sites across the UK.

Footnote: As some of the steps were completed by the NIHR CRN organisations (which have now been decommissioned) rather than me, this is an approximate schematic.

R&D permissions in England

Approvals were sought from all primary secondary care organisations in England via a single generic Site-Specific Information form (SSI). Most research studies seeking approval prior to March 2016, required a separate SSI form for each research site, detailing the activities, resources required and the personnel that will be involved. A study which used NHS CSP might be identified as being suitable for a single generic SSI which could be used for all sites (generic SSI's were introduced to expedite gaining R&D permissions during the Swine Flu outbreak). For a generic SSI the activities taking place in each site needed to be the same, needed to be considered "low risk" and needed the central research team to be undertaking the research procedures without the need for a local research team. The lead CLRN, which for this study was West of England CRN, identified that the study was suitable, and agreed the process with me (as the chief investigator (CI)) and with the NIHR CRN CC. Authorisation for the SSI form was given by me as the CI taking on the responsibility for the activity at each of the sites negating the necessity for local Principal Investigators (PIs). During the approvals process this was a contentious issue with many sites (this is explored more in Chapter 4).

The lead CLRN and the lead NHS Site for this study was University Hospitals Bristol NHS Foundation Trust. They assessed the documents submitted via IRAS and completed global checks, validating the application form. Once signed as approved on the CSP system on the (20th May 2014) trusts within England were able to approve or reject the study. Once the R&D application had completed checks by local CLRN and R&D offices, they had a target set by the Department of Health to respond within 21 days (the time taken to receive approvals is described in the Chapter 4 Results).

R&D permissions in Wales

The NISCHR Permissions Co-ordinating Unit (PCU) were contacted by me to discuss FADES and gaining R&D approvals in Wales. They advised that a generic SSI was not accepted for R&D permissions in Wales. Each of the seven health boards in Wales needed to be contacted separately and a named person for each Health Board was needed for the IRAS form which I did. A proof of the scientific review was also requested (scientific review letter Appendix 8) (See Chapter 4 Results for permissions granted).

R&D permissions in Scotland

NHS research Scotland Coordinating Centre (NRS CC) were contacted and they accepted a generic SSI which covered all the 14 regional health boards (See Chapter 4 Results for permissions granted).

R&D permissions in Northern Ireland

Northern Ireland Health and Social Care (HSC) R&D initially said that they did not accept generic SSIs and that a PI would be needed at each site. They provided contact details for the primary care sites coordinator, NI CRN adoption. There was then a query as to whether they would accept a single SSI, which led to delays in requesting permission in Northern Ireland and in May 2014, clarification was still awaited. A NI CRN adoption form was completed. (See Chapter 4 Results for permissions granted).

Liaison and discussions with the coordinating centres, Welsh Health Boards and R&D sites were all through me. Listed on the IRAS form were fourteen research sites, University Hospitals Bristol NHS Foundation Trust for England, a single generic SSI for the Scottish Health Boards covering primary and secondary care sites, the seven Health Boards in Wales (primary

and secondary care) and five Health and Social Care Trusts in Northern Ireland covering secondary care sites only. GW was listed as the investigator at each site on the R&D form.

2.4.5 International Standard Randomised Controlled Trial Number (ISRCTN) Registration

The study was registered as International Standard Randomised Controlled Trial Number (ISRCTN) registration number (ISRCTN12415856). Although not a randomised control trial, registration with an ISRCTN may be required by ethics and registration is standard practice for studies within the NIHR portfolio.

2.5 Amendments

During the period from the start of the study in September 2014 until the end of September 2017 seven amendments were made to the study. Amendments may be changes to the study protocol, documentation or personnel. Amendments were classified as either substantial or non-substantial, the study sponsor (for this study the University of Bristol) classified the amendment once they have received the relevant documentation. GW discussed each amendment for this study with a member of the RED at the University of Bristol. Substantial amendments required REC and R&D approvals before they could be implemented whereas non- substantial amendments merely required the researchers to inform the REC and R&D sites of the amendment. A summary of the amendments is given in Table 4 the details and reasoning behind the amendments will be provided in the relevant sections of this thesis.

Amendment Number	Substantial / Non-substantial	Date of amendment	Summary
1	Substantial	30th March 2015	• Addition of a maternal stool sample collection / consent for maternal stool sample • Recruitment by local collaborators • Letter to community paediatricians • Process for reminding participants
2	Substantial	9th June 2015	• Sample collection method for gut microbiome feasibility study changed • Addition of photos to the FADES flyer
3	Non-substantial	5th August 2015	• FADES consent form typographical error corrected
4	Substantial	8th September 2015	• Addition of Qualitative Research Study • Addition of feasibility objectives to study protocol • Modification of EOI/ registration form
5	Substantial	4th August 2016	• Anonymised participant / non-participant questionnaires • Change to methodology for the collection of stool samples to include using OMNIgene GUT collection kits. • Addition of quarterly newsletters • Modification of study Flyer
6	Non- substantial	13th March 2017	• Extension to recruitment deadline until September 2017
7	Substantial	25th July 2017 (Approved through HRA)	• Extension of recruitment until January 2020 – tapered follow-up until 2022 • Re-consent participants for follow-up until 2022 • Birthday cards with annual packs • Modified all documents to say NIHR Bristol Biomedical Research Centre Nutrition Theme. • Addition of DS Ireland and collaborators in the Republic of Ireland. • Letter to repeat inadequate mouth-swab samples. • Modification of urine sample collection sheet – addition of “how long after feed.”

Table 4: Summary of study amendments.

The change to HRA approvals was partly designed to speed up and streamline the amendment process for studies. The timescales involved in gaining approval for amendments are discussed in Chapter 4.

2.6 Study Management

As the CI for the study, I organised and chaired regular FADES management meetings. These were held quarterly and agendas and minutes for the meetings were produced. The meetings were attended by all members of the research team depending on availability. At each meeting, a study update was presented, and issues were discussed. These meetings were also used to plan amendments, review participant facing documentation and for expert opinions on study protocol.

Training new members of the research team including local collaborators was completed by me. For local members of the research team this was done face to face, for local collaborators this was completed over the phone or via email.

2.7 Communication and access to study

The complete study protocol is in Appendix 2 and the eligibility criteria and brief overview were given in Chapter 1 Section 1.11. In the following sections the processes involved in the setup and running of recruitment and data collection (questionnaire and samples) are described.

As this was a UK wide research study, potential participants needed a secure route to be able to contact the study team (based in Bristol) and to provide their personal details. A dedicated

FADES email was therefore setup and a FADES webpage was created within the NIHR Bristol Nutrition BRU's website as described in the sections below.

2.7.1 FADES Website

The FADES webpage (NIHR Bristol BRU) was designed by me and set up by ST and SJ. The website contained a link to a web-based expression of interest (EOI) form where potential participants could register their details (see Section 2.13.2 on REDCap). Figure 7 is a screen shot of the FADES webpage with the link for the EOI is marked by a red arrow. They were also able to download a copy of the PIS (green arrow).

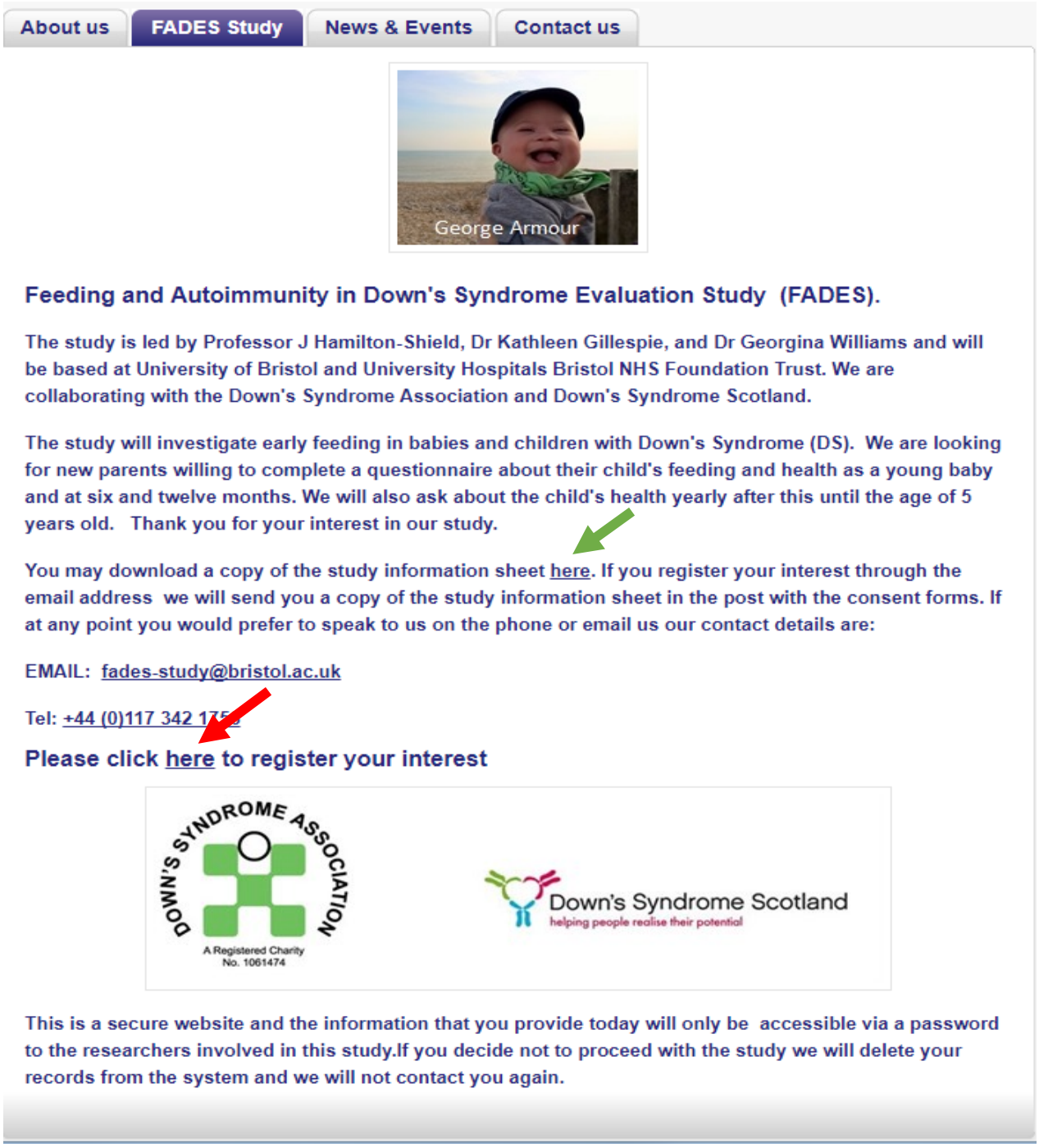


Figure 7: Screenshot of FADES webpage with link to Expression of Interest (EOI) shown by red arrow and link to the participant information sheet shown by the green arrow.

2.7.2 FADES email

The FADES mailbox was setup by me through the University of Bristol IT services. This was a shared mailbox which could only be accessed by members of the research study team to whom I had granted access. Participants, potential participants and local collaborators were able to communicate with the study team via email and the study mailbox was checked at least twice a week. Emails from this mailbox were used to:

- send links to questionnaires;
- inform participants that sample packs were being sent out;
- determine dates when blood tests might be coordinated;
- send reminders;
- send newsletters;
- send links to participant / non-participant questionnaires; and
- answer queries, for example on sample collection or completion of questionnaires.

The use of a shared mailbox also enabled members of the research team to access the contact history for participants wherever they were based. This was particularly relevant when trying to organize samples and liaison between the admin team and the laboratory technicians. The mailbox also provided an audit trail and was used in the assessment of feasibility.

2.8 Development of Feasibility Objectives

Clear feasibility objectives were developed to assess key aspects of the study design, set-up and processes as required by the CONSORT guidelines on reporting pilot and feasibility studies

(Eldridge, Chan, et al. 2016). These are described below together with an explanation of how they were developed.

2.8.1 Feasibility objectives for recruitment

Rate of Recruitment - Objective: *to assess the rate of recruitment, with an initial target of 100 participants per year over a two-year period (this represents 20% of the DS families who are in contact with the Down's Syndrome Association (DSA) per year).*

The target of 100 participants per year was based on the initial information that had been provided by the DSA. The initial study protocol planned for recruitment to take place through the DSA and DSS. The figure of 20% was based around other studies which included families of children with DS. These showed recruitment at around 30% of the eligible population (Fortnum et al. 2014) or similar rates in a multi-centre study of around one hundred participants per year (The CDSS study recruited around this number of new-born per year (CDSS 2018)). As this study was similarly recruiting babies shortly after birth and required considerable input from families, the figure of 20% was deemed appropriate.

Recruitment Methods - Objective: to consider the feasibility of novel recruitment methods, including recruitment through websites and social media.

As relatively novel methods of recruitment were used in the study it was important to assess these (Orsmond and Cohn 2015). Participants were asked on their initial registration form how they heard about the study, so that the success of different recruitment approaches could be compared. Any issues relating to online systems which prevented or delayed participants being recruited were recorded on the study database. Timelines between registering an interest in the study and then consenting were recorded.

Motivations and Barriers to joining the study - Objective: to understand the reasons why participants consented to take part in the study.

Participants were asked to complete a short survey exploring why they joined the study and their overall opinion of FADES. Families who had registered an interest in the study but who did not then go on to take part (non-participant), were also asked why they felt unable to participate in the study, and to comment on the study design. These objectives aimed to assess the 'demand' and 'acceptability' of the study as described by Bowen at al. (Bowen et al. 2009), the use of surveys and qualitative methods can be used to understand who in the target population is interested in the study and whether the study design is acceptable to this population. This also provides information regarding the validity and suitability of the study.

2.8.2 Feasibility objectives for sample collection

Sample Collection - Objective: to assess the ability of families to collect all the samples within the desired timeline, determined by the percentage of participants who return the requested samples in the time requested.

The collection methodology would be deemed feasible if:

- *at least 75% of participants provide the requested initial samples before eight months of age;*
- *at least 75% of participants provide the requested 12-month samples before 14 months of age;*
- *at least 50% of participants to provide all samples up to the age of five years; and*
- *at least 90% of samples provided are adequate for analysis.*

The figure of 75% for both the initial and 12 month samples were based on other birth cohort studies which collect longitudinal markers of autoimmunity at similar time points and had either obtained or predicted similar or better adherence (Ziegler et al. 2013, TEDDY Study Group 2007, Group 2008, Lönnrot et al. 2000). Studies collecting biological samples from babies with DS were however rare, especially ones in which parents had collected samples at home. Therefore, there was limited pre-published data from which these targets could be derived. The target of 50% at five years was decided as an appropriately sufficient proportion for a birth cohort. Compared to other cohorts of babies with DS, longitudinal data for five years would make the study compelling. The CDSS collected longitudinal blood samples but published longitudinal studies which include the collection of biomarkers are rare (CDSS 2018).

The sample collection methods had either been successfully used before or had been tested in the internal feasibility studies (Section 2.15). Excluding unavoidable incidences (e.g. spillage) the samples received were expected to be adequate for analysis. A feasibility target of 90% of samples received being adequate for analysis was therefore set. As part of the feasibility assessment, any issues or problems that occurred with sample collection were recorded.

2.8.3 Feasibility objectives for medical and feeding questionnaires

Feeding and Medical Questionnaires - Objective: to assess the ability of the online questionnaires and paper questionnaires to collect the required data:

- *for at least 75% of recruited participants to complete initial questionnaires, the questionnaires at seven months and to be completing questionnaires at a year;*

- *for at least 50% of participants to complete the annual questionnaires up until the age of five years;*
- *for at least 60% of participants to opt to use the online questionnaire rather than the paper questionnaire; and*
- *for the data produced to be valid and easily converted into a format, which is ready for analysis.*

The target of 75% was based upon the return rate seen in other studies which also required detailed responses from parents when their babies were young. The most relevant being the Infant Feeding survey (IFS) which had a response rate of 51% for the initial questionnaire and a response rate of 80% for the second questionnaire at age four to six months (McAndrew et al. 2012). The FADES study has families who likely have self-identified to be recruited and may therefore be already motivated, thus 75% seemed appropriate for these initial questionnaires. As for the collection of the samples, the target of 50% completing at five years of age was set as the level that would be acceptable to provide compelling findings.

A feasibility target of at least 60% was set for the web-based questionnaire as it was a novel aspect of this study. The use of web-based questionnaires in clinical research is relatively new and therefore there are no publications studying this or similar populations. It was hoped that over two-thirds of participants would select the web-based questionnaires to prove its value.

2.8.4 Feasibility objectives for retention of participants

*Retention of Participants - Objective**: The feasibility of retaining participants in the study, which in parallel also determines the overall acceptability of the study questionnaires was set by the following objective:*

1. *for compliance with the study protocol up until the age of five years old with a target of retaining at least 50% of participants.*

The objective target above was set in line with the '50% at five years targets' for the data collection (samples and questionnaires see sections above). As a longitudinal cohort study of children with DS, to retain 50% of participants would be a considerable achievement making this an important cohort providing contemporaneous data and a bank of longitudinal samples for this population.

2.8.5 Analysis of Feasibility objectives

The main access database recorded the dates at which samples were received and questionnaires completed. For the feasibility targets relating to the samples and questionnaires, a query was created to show the age of the participant for each of the samples and questionnaires. These data were then converted into an Excel spreadsheet from which proportions (percentage), median ages and interquartile ranges were calculated. The proportion of participants who provided longitudinal samples could also be determined. These were then compared to the targets set in the feasibility objectives. Binomial exact 95% confidence intervals were calculated for proportions using the cii proportion command in Stata. This is an immediate command and only requires the number of observations and number of successes to be inputted. As the study had not been running for five years completion of samples and questionnaires for five years could not be determined but completion of longitudinal samples was used as a proxy for this. Determining retention of participants at five years was also not possible. However, proportions of participants who

were fully active (completing all questionnaires and samples) or partially active (completing at least some of the requested questionnaires or samples) was determined as an indication.

2.8.6 Secondary Objectives

Characterisation of the cohort - Objective:

1. *Ability to characterise the cohort in relation to:*
    a) *maternal age, socio-economic background, ethnicity;*
    b) *medical conditions related to a diagnosis of DS;*
2. *to determine the numbers and types of infections experienced during early life and antibiotic usage; and*
3. *to describe early feeding and weaning in the cohort.*

The characterisation of the cohort was undertaken in order to determine whether the cohort was representative of the general population in the UK and particularly the general population of children with DS. Maternal characteristics were compared to those reported by the Office of National Statistics and other published reports for mother's in the UK (Office for National Statistics (ONS) 2015, NHS Digital 2017). Published papers describing the epidemiology of medical conditions within the population of people with DS were used to compare the proportion of babies seen with conditions known to be associated with DS (Freeman et al. 1998, Freeman et al. 2009, Roizen and Patterson 2003, Yumura-Yagi et al. 1992).

Sample Analysis – Objective

1. *to measure levels of urine C-peptide levels longitudinally;*
2. *to measure anti- BSA antibodies longitudinally; and*

3. *to profile the gut microbiome during early childhood.*

Samples were tested to show the adequacy of the samples having been collected by the families at home (blood samples were collected by health care professionals), posted in the standard post and then stored frozen at -80^{0}C.

2.9 Recruitment

The initial protocol stated that participants would be recruited through the DSA and DSS as described previously. Once the study started, it was promoted through a variety of media as listed below. These promotional activities were organised by myself in collaboration with the DSA, DSS, DHG and DSMIG. Promotional materials were written by me with feedback from the study team and the relevant organisation. Figures 8 to 10 are screen shots of just some of the web-based promotional activities.

- Flyers in DSA new parent packs (throughout the study)
- Flyers given to parents in contact with DSS (throughout the study)
- DSS e- bulletin article (July 2014)
- Study featured on DSA website on their research page (Aug 2014) (www.downs-syndrome.org.uk/about/research-campaigns/current-research-projects/feeding-and-autoimmunity-in-downs-syndrome-evaluation-study-fades/)
- Study featured within the newsfeed on the first webpage of the DSA website (August 2014)
- Article in the DSS magazine (September 2014)

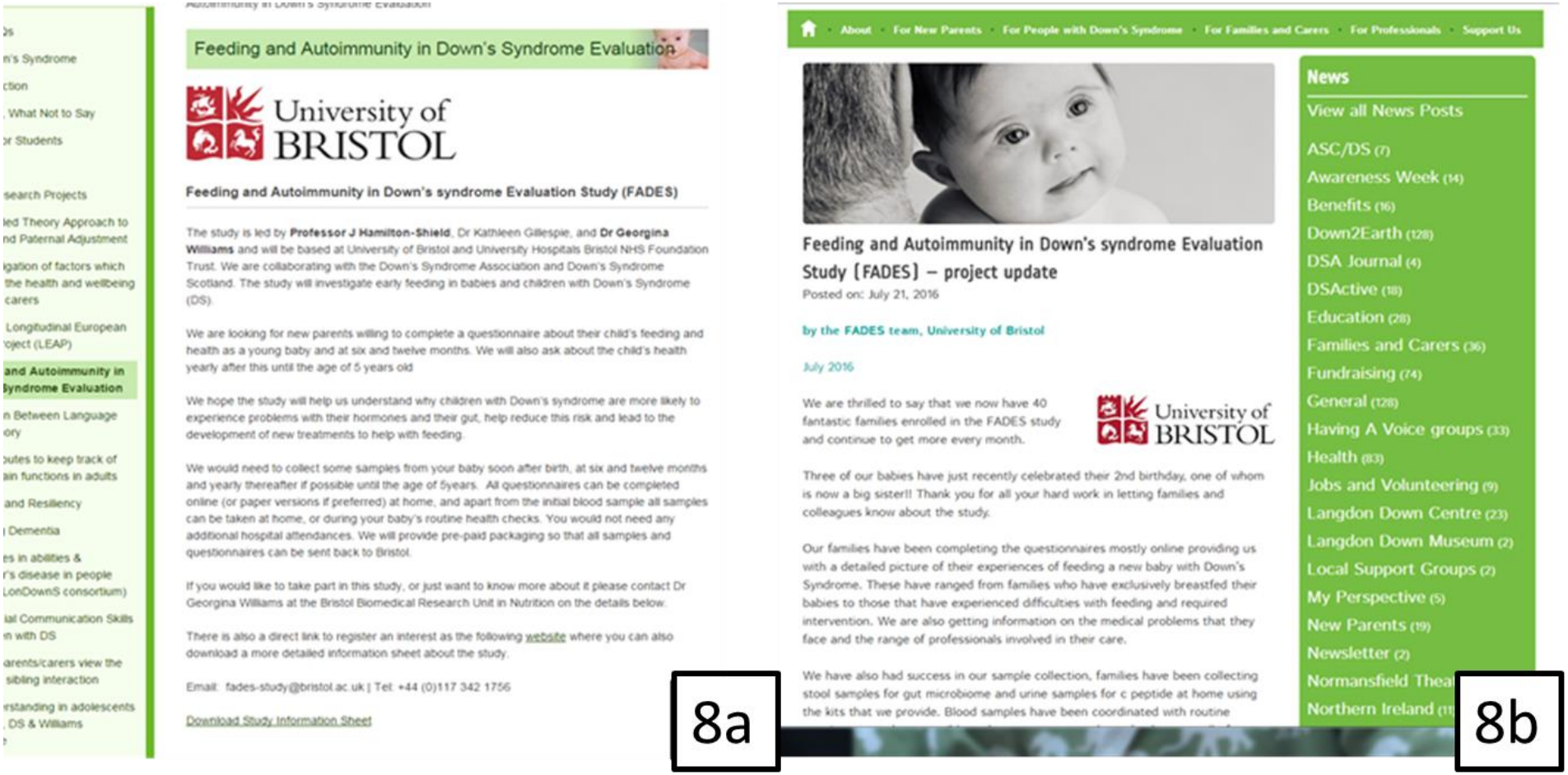


Figure 8a: Screen shot from DSA website research page advertising FADES. Figure 8b: Study update on the DSA website

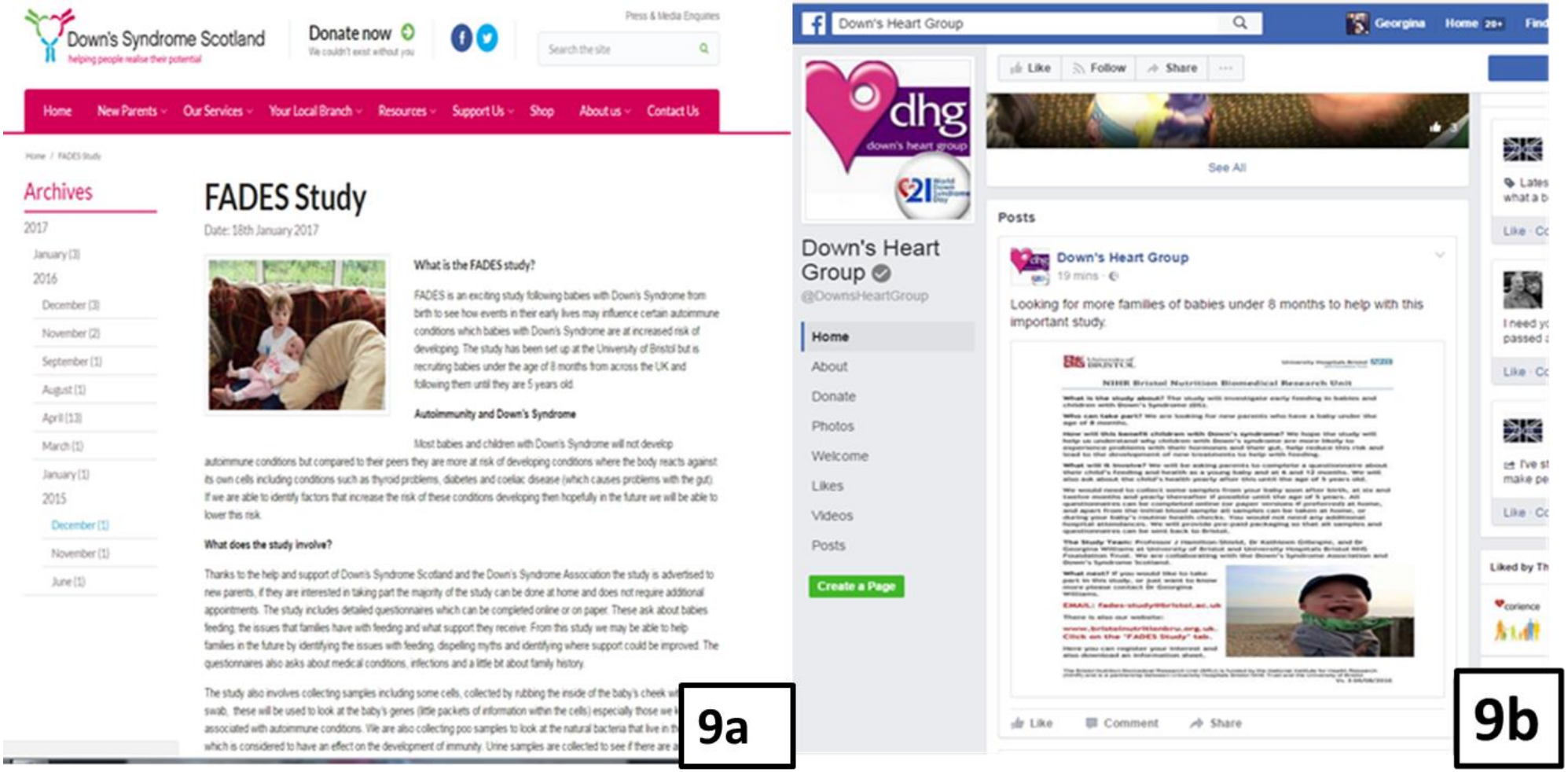


Figure 9a: Screenshot of the DSS Website advertising the study. 9b: Screenshot of the study featuring on the Down's Heart Group (DHG) Facebook page

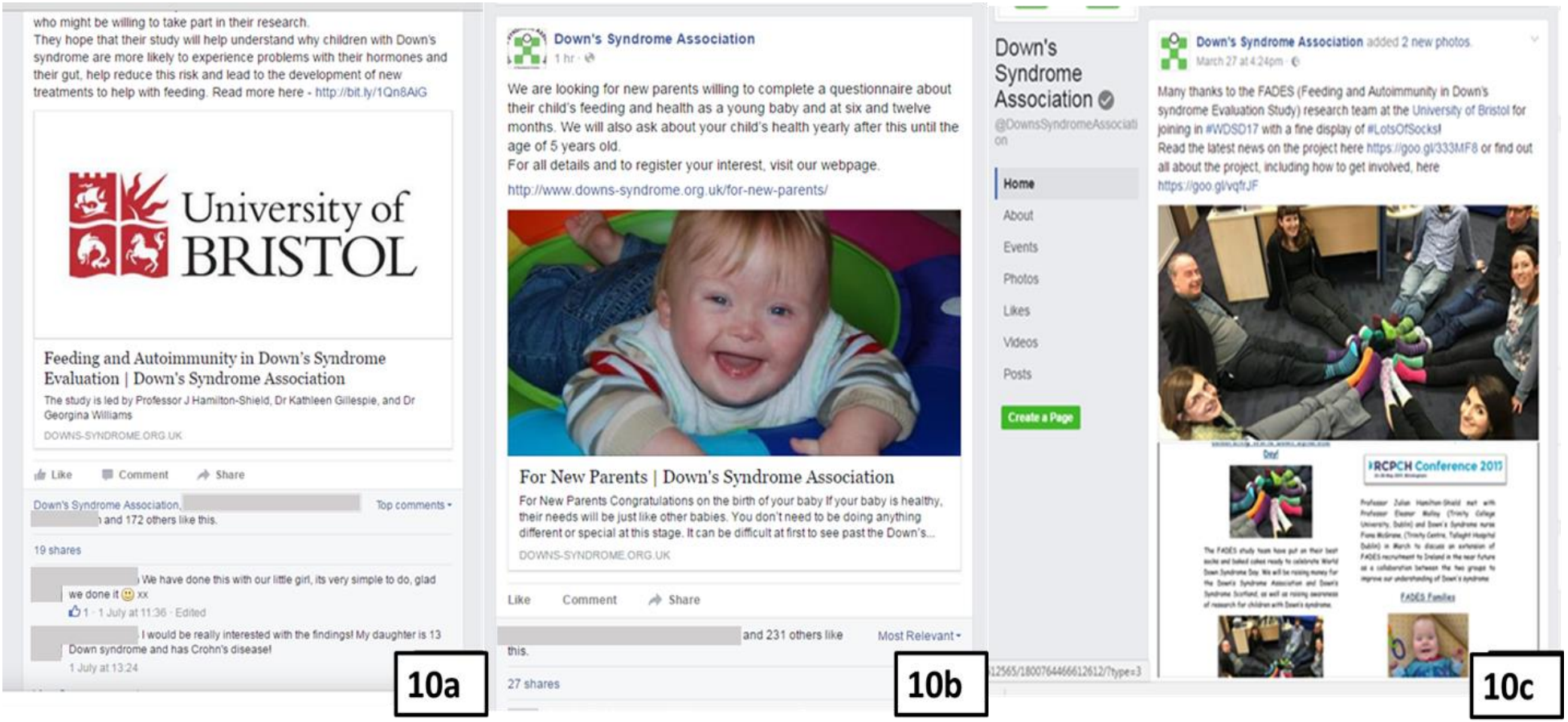


Figure 10a and 10b: Screenshots from DSA Facebook page showing "Likes" of up to 231 people and shared by 27. Figure 10c: Screenshot of post from DSA website thanking the FADES team for supporting World Down's Syndrome Day. Footnote: Identifiable names have been blocked out in grey.

Recruitment began in August 2014 but within a couple of months it was clear that recruitment was slow. The Down's Syndrome Medical Interest Group (DSMIG) is a network of around 160 doctors from across the UK and Republic of Ireland who all have a special interest in the medical care of people with DS. The DSMIG hold bi-annual scientific meetings. FADES had been presented to the DSMIG meeting in June 2014 by KG. At this meeting, members of the DSMIG had highlighted how they might help with recruitment by providing their expertise and connections. A teleconference was organised with the DSMIG, KG and myself. They advised that their members would be interested in promoting the study and providing further support. The director of research for the DSMIG Dr Jill Ellis agreed to send a letter to all members of the DSMIG informing them about the study.

A decision was made to include the use of local collaborators to actively engage in participant identification and recruitment. Local collaborators would include community paediatricians, neonatologists and research nurses. The necessary substantial amendment was made on the 30th March 2015 to approve this. Local collaborators were recruited throughout the course of the study through a variety of approaches summarised below:

- Letters were sent separately to all members of the DSMIG requesting their involvement and informing them that a substantial amendment was being submitted to approve the use of local collaborators in recruitment (June 2014)
- Flyers to recruit local collaborators at DSMIG meeting (June 2015)
- Oral presentation by myself at DSMIG meeting (November 2014)

- Royal College of Paediatrics and Child Health (RCPCH) annual conference, poster (April 2016) and oral presentation (May 2017). The DSMIG held their scientific meeting at the RCPCH conference April 2016 and May 2017.

The two routes via which a participant could enter the study and the administrative steps involved are shown in Figure 11 and 12. Figure 11 describes how participants who were recruited through social media, websites or flyers were consented and enrolled into the study and Figure 12 shows the route taken by participants who were recruited by local collaborators / clinicians.

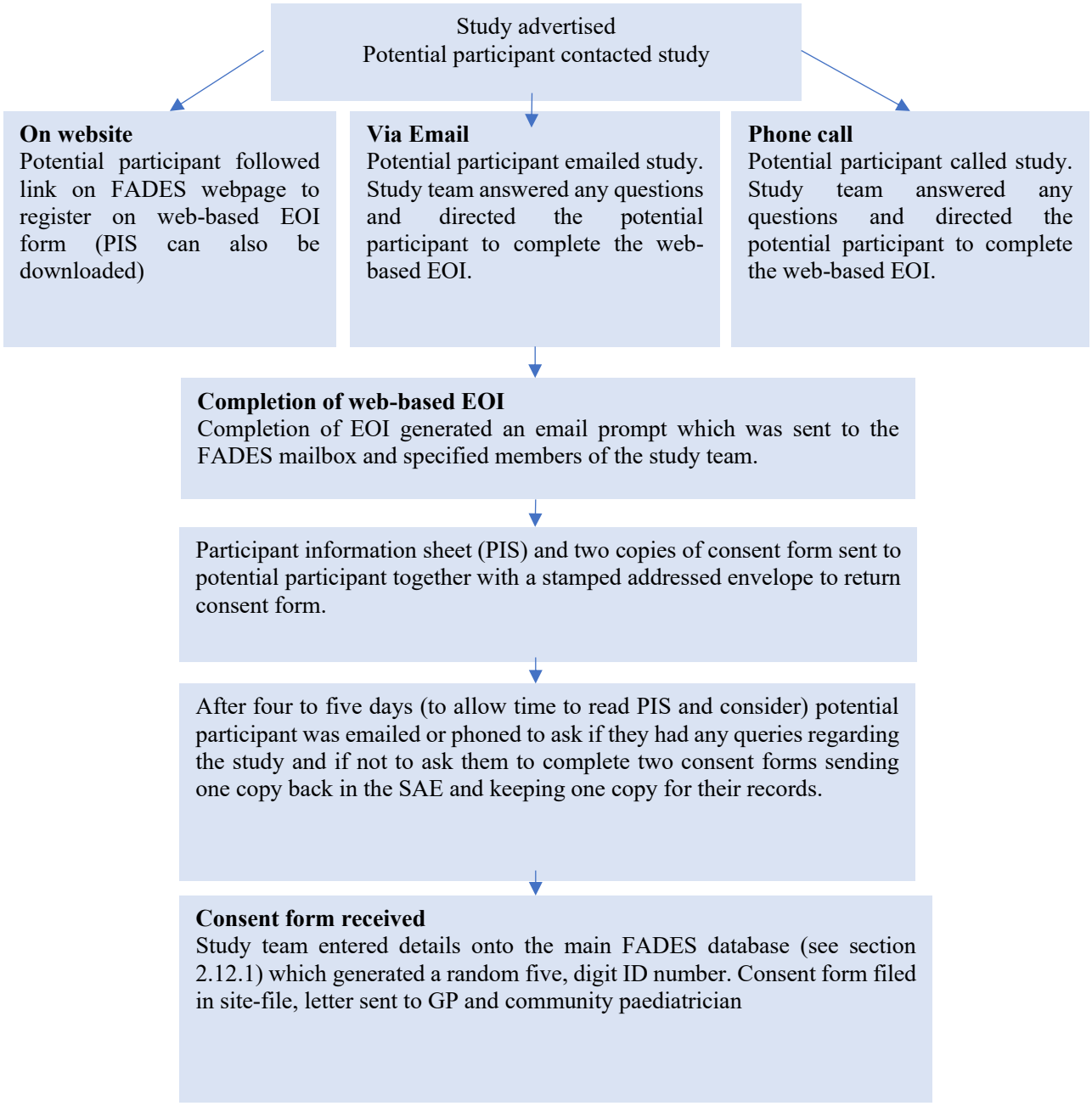


Figure 11: Summary of the administrative / management processes from the point when a potential participant first contacts the study to being fully enrolled in the study

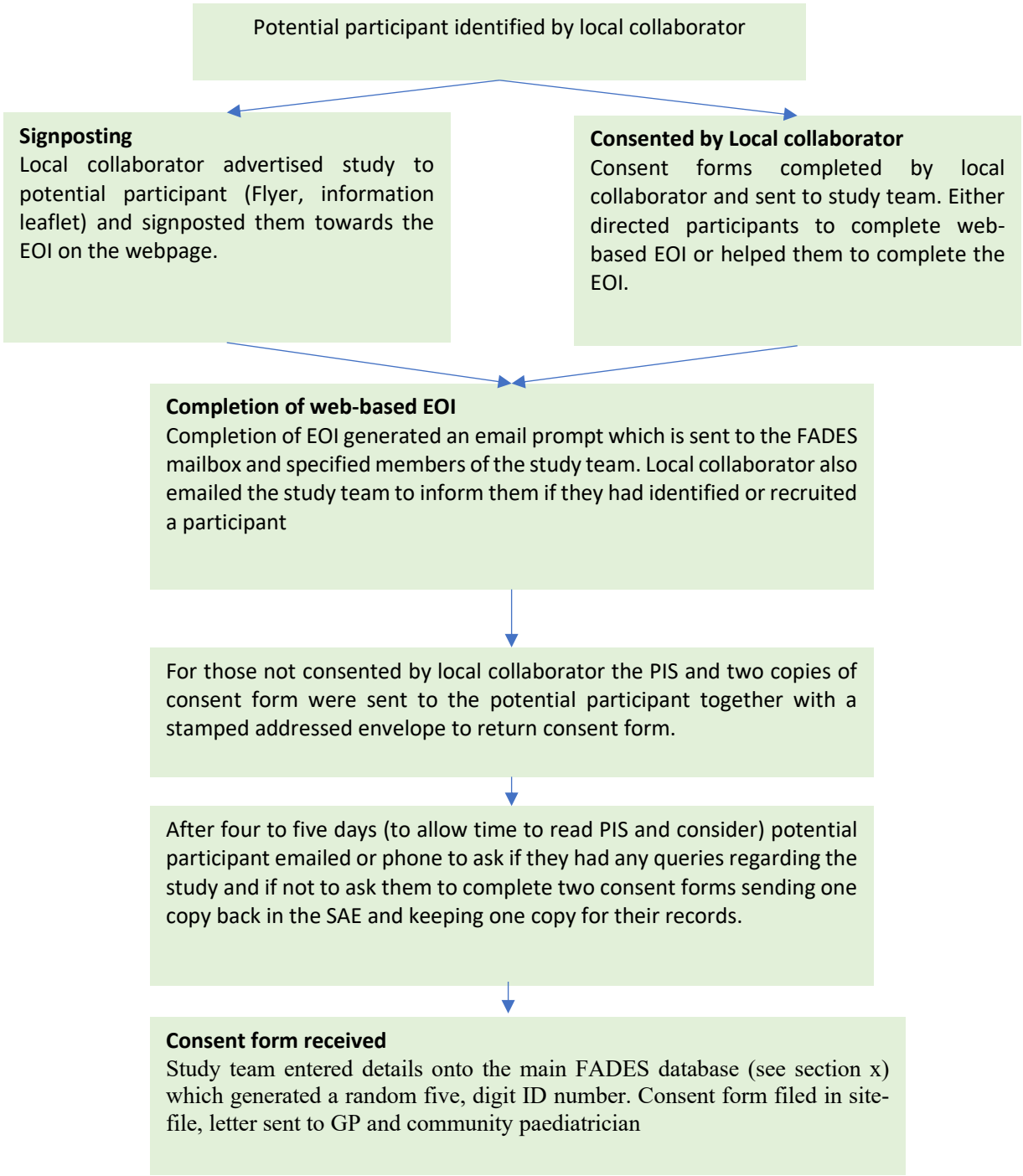


Figure 12: Summary of the administrative / management processes for participants identified and potentially recruited through local collaborators.

A variety of promotional activities continued throughout this study. To reach a cross section of families from across the UK, multiple recruitment methodologies were adopted. These included:

- Blog on DSA website (May 2015) written in collaboration with Dr Marder from the DSMIG and Vanda Ridley from the DSA.
- New flyers which included photos, packs sent to DSA and DSS and local collaborators (July 2015).
- FADES on DSA Facebook and Twitter feeds – 'liked' and 'shared' by contacts, collaborators and families (Aug 2015). Discussed with DSA who agreed to put the study on their social media feeds every other month (Jan 2017).
- FADES featured in DSA e-newsletter (September 2016) and DSS e bulletin (Jan 2017)
- Study updates provided to charities families and local collaborators. Updates added to the DSA research webpage (October 2015, Aug 2016).
- FADES featured in the newsletter for the Down's Heart Group (DHG) (November 2016).
- FADES promoted as part of an article written by me on autoimmunity in DSS newsletter (Feb 2017).
- FADES advertised on DHG website (Feb 2017).
- Study promoted and discussed at NIHR CRN Champions meetings (NIHR CRN Eastern research champions meeting, conference call with me Feb 2017) (Kent, Surrey and Sussex oral presentation by myself and open questions March 2017).

- FADES quarterly newsletter sent to all participating families and collaborators (December 2016, March 2017, July 2017).
- World Down's Syndrome Day March 2017: FADES study team raised money for the DSA and DSS. Photos of the research team on the day posted on social media by the DSA.

One local participant who met with me kindly offered to write a piece about the study for use in any promotional material for the study or in any study publications. She consented for her and her son's name to be included. This was added to the research page of the DSA website and put in the March 2017 newsletter. The quote from the mother is below:

"We found out about the FADES study not long after Alexander was born, through our local Down syndrome support group. After I found out more about it, we were very keen to take part. The research being undertaken could be crucial to understanding the nutritional needs of children with Down syndrome.

It is very easy to take part in the study. Every six months, we fill in a questionnaire and they have a blood, stool and urine sample test.

The extra support we have had with questions and queries around other areas of Alexander's health have been wonderful, and we feel privileged to be part of improving the lives of children with Down syndrome. We would encourage anyone thinking about joining the study to take the plunge!"

The success of these methods in recruiting participants to the study was assessed by monitoring participant numbers. Participants were also asked to state how they heard about

the study on the EOI form, the question asked, “how did you hear about the FADES study?” the drop down box for the answers gave the options of:

- Through the DSA website
- From the flyer in the DSA new parent pack
- From a support worker from DSS
- From the flyer in the DSS new parent pack
- From a community paediatric doctor
- From a neonatal doctor
- Through a friend
- Through social media
- Other

Recruitment methods were also assessed in the Participant and Non-participant questionnaires (see Section 2.11).

2.10 Qualitative Research Methods

The methods for the qualitative study “What factors influence recruitment to a birth cohort, of infants with Down’s Syndrome” are covered in Chapter 3. The qualitative study was published in Archives of Disease in Childhood (Williams et al. 2018). The methods are not presented here as they do not apply to the main study participants. The qualitative study is presented as introduction, method, results and discussion in Chapter 3.

2.11 Participant and non-participant questionnaires

The qualitative interviews provided information on the experiences and opinions of professionals and support workers about recruiting families into research studies, but not on the experiences of the families themselves. Therefore, participants who engaged and non-engagement participants (parents who contacted the study but then did not go on to join the study (non-participants)) were asked to complete a short questionnaire to explore motives for joining or declining the study.

The questionnaires were designed to be short and included a combination of open questions and ones with Likert scale options (copies of the questionnaires are Appendix 19 and 20). The questionnaires were designed by GW following consultation with members of the research study team. Draft questionnaires were checked for face validity by members the research team including SD (statistician), JPH (consultant paediatrician) and ST (database manager). The web-based versions of the questionnaires were hosted via the REDCap system as described in Section 2.13.2. Families were asked how they heard about the study, what interested them in taking part and to comment on the study website and information that was provided about the study. For the non-participant questionnaire, they were asked why they chose not to take part.

The questionnaires were anonymised and could be completed on the web-based format or as a paper version if requested. The questionnaire links were emailed to the engaged participants and the non-engaged participants in bulk so that the responses would be unidentifiable. No one requested a paper version. Participant questionnaires were sent out to 28 participants who were actively participating in the study. Participants who had only

recently joined the study were not included as they had not had time to demonstrate that they were actively taking part or not. Non-participant questionnaires were sent out to 26 families who had contacted the study either via an email or by completing the EOI form but that who had not then gone on to consent to being in the study.

2.11.1 Analysis of participant and non-participant questionnaires

Proportions (percentages) were calculated for closed questions and those where a Likert scale was used in the participant and non-participant questionnaires. For open questions, common themes were found, and illustrative quotes were used. Unusual answers were also noted.

2.12 Data collection, storage and data protection

All the data collected in the study were maintained and stored in strict accordance with data protection regulations. All participant identifiable information (i.e. names, addresses, dates etc.) was stored in a dedicated password protected Access database which is referred to as the Main Database in this thesis. Once a web-based EOI (see section 2.13.2 on REDCap for data storage and data protection within REDCap) was completed by a potential participant, an automatic email alert (which did not contain any participant data) was sent to the study mailbox and specified members of the research team including myself. Consent forms would then either be sent out to the participant and returned to the study team, or if the participant had been recruited by a local collaborator the consent form would be sent in by them. Once the consent form had been received, the personal data could be entered manually onto the Main Database. By entering this information, a five-digit ID would be created by a random number generator, the main database therefore acted as the "key" (Figure 13). A separate database was generated for autoantibody analysis, immune cell analysis, stool microbiome

and urine analysis. Therefore, unauthorised persons would need to circumvent the security of both databases, to obtain and link to both patient details and results of investigations. All databases used in the study were password protected and the university network itself is firewalled, IP and password authenticated. Questionnaires did not contain participant identifiable information, the five-digit study ID number was given to the participants and could only be linked to their personal details by authorised members of the research team. Any paper versions of the questionnaire and the consent forms were locked in a secure filing cabinet within the NIHR Bristol Nutrition BRU which was only accessible to authorised persons.

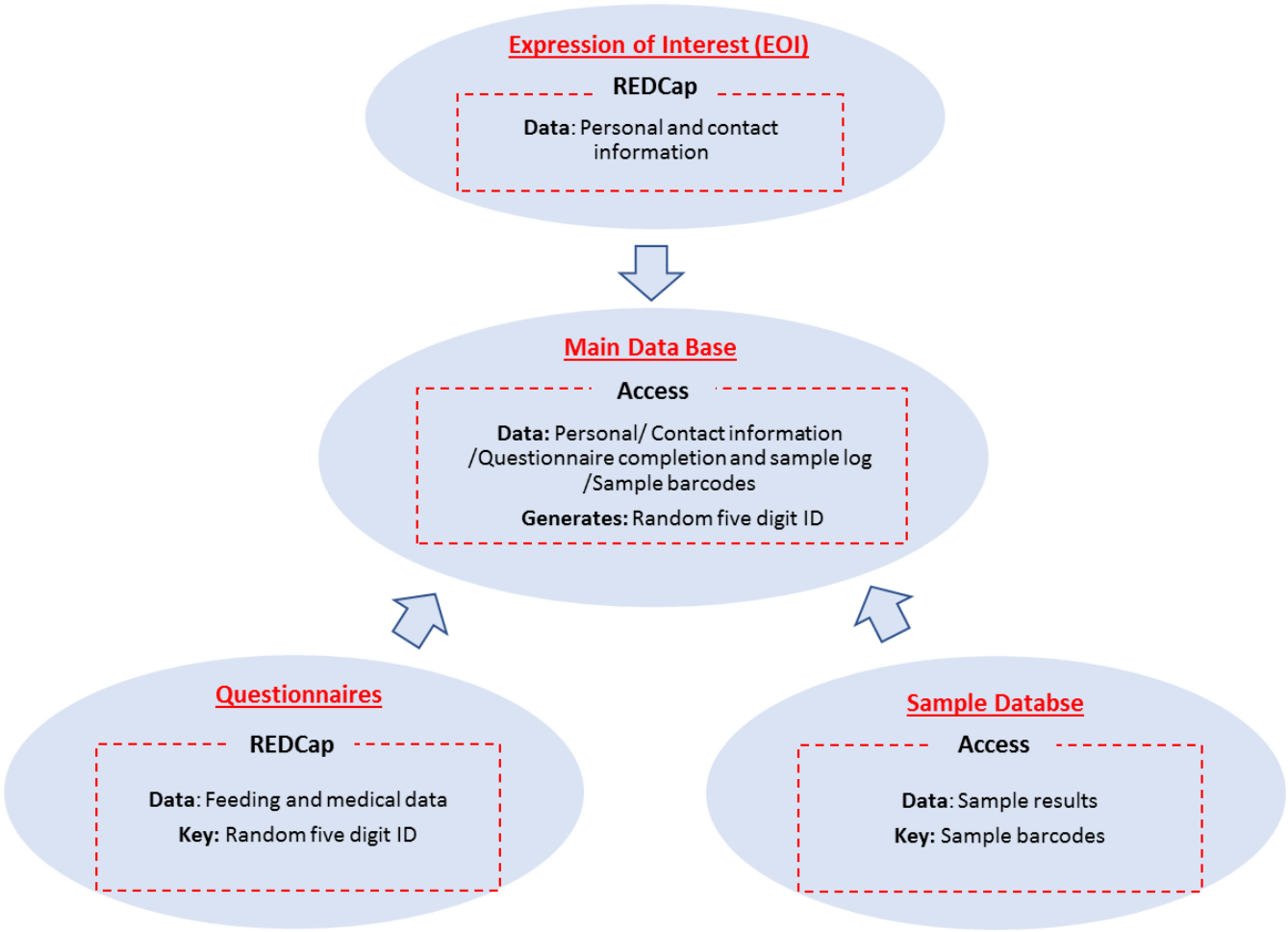


Figure 13: Illustration of the systems for secure data storage.

2.12.1 Main Database and Alert system

The organisation of the study was complex, with multiple samples being collected and questionnaire data being recorded at different time points. It was not possible to predict when a participant might join the study and therefore each participant had their own individual timeline. The need for an alert system was identified and discussed at the FADES management meeting. ST and I developed the Main Database together to include a "traffic

light" system (as illustrated in Figure 14). Conditional formatting was used so that once a study participant was entered onto the database their date of birth provided the "condition" for the other cells. When a participant was within two weeks of a data collection time point a red box would appear in the column for that data, either samples and /or questionnaires (A in Figure 14), alerting the study team to send out a sample pack and/or a questionnaire. The only caveat to this would be if a sample of the same type had been received late and was within two months of the next data time point. In this case the box would turn grey (B Figure 14). Once the sample collection pack and/or questionnaire had been sent out to the participant and logged, the box would turn to yellow (C Figure 14). Finally, once the sample collection and/or questionnaire had been completed, samples received at Southmead, and / or the questionnaire completed and logged on the main database the box would turn green (D Figure 14).

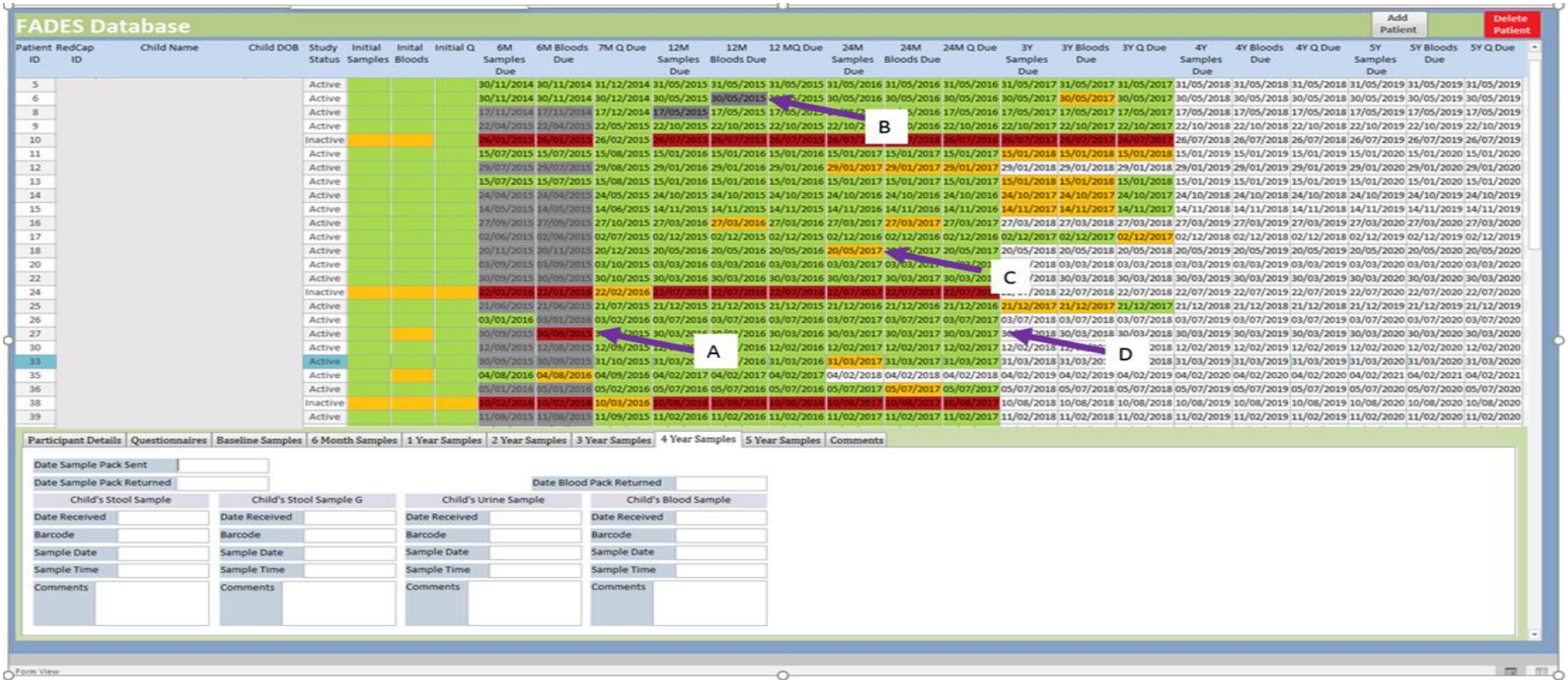


Figure 14: Screenshot of Main Database with participant identifiable data removed. A red box indicated that a participant was due a sample pack or questionnaire (A), a grey box indicated that the previous sample had been returned less than two months prior to the next due date (B), a yellow box indicated that a sample pack or questionnaire had been sent to the participant but not yet returned (C) and a green box meant that the sample or questionnaire were complete (D).

2.13 Feeding and medical questionnaires

The next sections describe how the questionnaires were designed and put into a web-based format. The piloting of the questionnaires both paper and web-based, is explained and the analysis methods are given.

Participants were given the option on the EOI of completing the questionnaire on paper or using the web-based questionnaire. Both versions asked the same questions however, the web-based questionnaire automatically bypassed irrelevant questions depending on the answers that were provided. Certain questions on the web-based form had limits set on the responses to reduce errors. Parents were asked to complete the initial medical and feeding questionnaire as close to birth as possible. Depending on the preference they expressed on the EOI, parents were either sent a paper version of the initial questionnaire (together with a stamped addressed envelope) or a link to the web-based questionnaire. They were then asked to complete further medical and feeding questionnaires at seven months and 12 months. After the first year they completed annual medical questionnaire around the time of their baby's birthday. Blank versions of the questionnaires are in Appendix 13 - 16.

2.13.1 Questionnaire design and piloting

The feeding and medical questionnaires were carefully designed and tested to produce data that were valid. It was important that questions measured what they were planned to measure. The questionnaires set out to characterise the cohort in terms of their birth history, medical background, infection rate, antibiotic use and early feeding history. As will be discussed in other sections of this thesis, individual families had very different experiences

both of feeding their baby and of their child's medical needs. Any questionnaire therefore needed to be reliable and sensitive to change. The use of different question types (both open and closed) and repeated questions facilitated this.

The steps taken in the initial design of the questionnaire are shown in Figure 15. Following a literature review of feeding surveys and other cohort studies designed to explore the development of autoimmune conditions key questions were identified (Snell-Bergeon et al. 2012, TEDDY Study Group 2007), and sources of previously validated questions were sought. The feeding aspect of the questionnaires were designed based on the questions used in the 2010 IFSs from the Health and Social Care Information Centre (McAndrew et al. 2012). The IFSs aimed to provide statistics on the incidence, prevalence and duration of breastfeeding and other infant feeding methods during the first eight to ten months of life for infants in the UK. The IFSs were conducted every five years from 1975 until 2010, they have since been discontinued. IFF Research carried out the 2010 survey in partnership with Professor Mary Renfrew, Professor of Mother and Infant Health, College of Medicine, Dentistry and Nursing, University of Dundee. The use of questions from the IFS provides construct validity and the findings from the FADES questionnaires should therefore be comparable. Questionnaires were completed at three time points for the 2010 IFS, initially when the babies were six to eight weeks old, again when they were six months old and finally when the infants were aged eight to ten months old. The FADES questionnaires were designed to also be completed early in life, at six to eight months and then again at 12 months.

The FADES questionnaires contain a combination of some questions from the 2010 IFS and additional questions designed to ask more specifically about medical conditions associated

with DS, feeding issues that are specific to DS and questions specific to the development of autoimmune conditions of interest.

To check for face and content validity, draft questionnaires were initially discussed in detail with members of the FADES research team which included a statistician, dietician and consultant paediatrician. The resulting second draft was then sent to and discussed with Dr Jenny Ingram (a senior researcher at the University of Bristol with experience in infant feeding research). Dr Ingram made several suggestions to reduce the number of questions. The questionnaire was again modified, and the resulting version was sent to the DSA.

The DSA gave feedback on the documentation for the FADES study including the questionnaires. The draft versions of the paper questionnaires were reviewed by mothers (members of the DSA) and they were asked what they thought of the questionnaires and any suggestions for improvements. They commented that there were a lot of questions but that they agreed a web-based system for the questionnaires which automatically skips irrelevant questions would help considerably in making the questionnaires feel shorter. They suggested that more information was required to explain why certain questions were being asked, and the questionnaires were therefore modified accordingly. By making these changes, the face validity was improved. They also asked that we remove the question “Is your baby no longer with you?”. This question was originally included to check eligibility as babies who had been taken into care or adopted were not eligible to be in the study due to parents being unable to provide details regarding ongoing feeding and medical care. This question was removed and instead the consent form was changed to ask, “are you the birth mother?”. It was also noted that a few questions referred to “Down’s” and it was pointed out that this term should

never be used. In all circumstances, this was changed to “a baby with Down’s Syndrome”. Overall, they felt there was a bias towards breast feeding and were concerned that the questions regarding antibiotics would make parents think that taking antibiotics is a bad thing. They also found the question about whether the mother was currently in work judgmental. The revised questionnaires were then sent back to the DSA for approval.

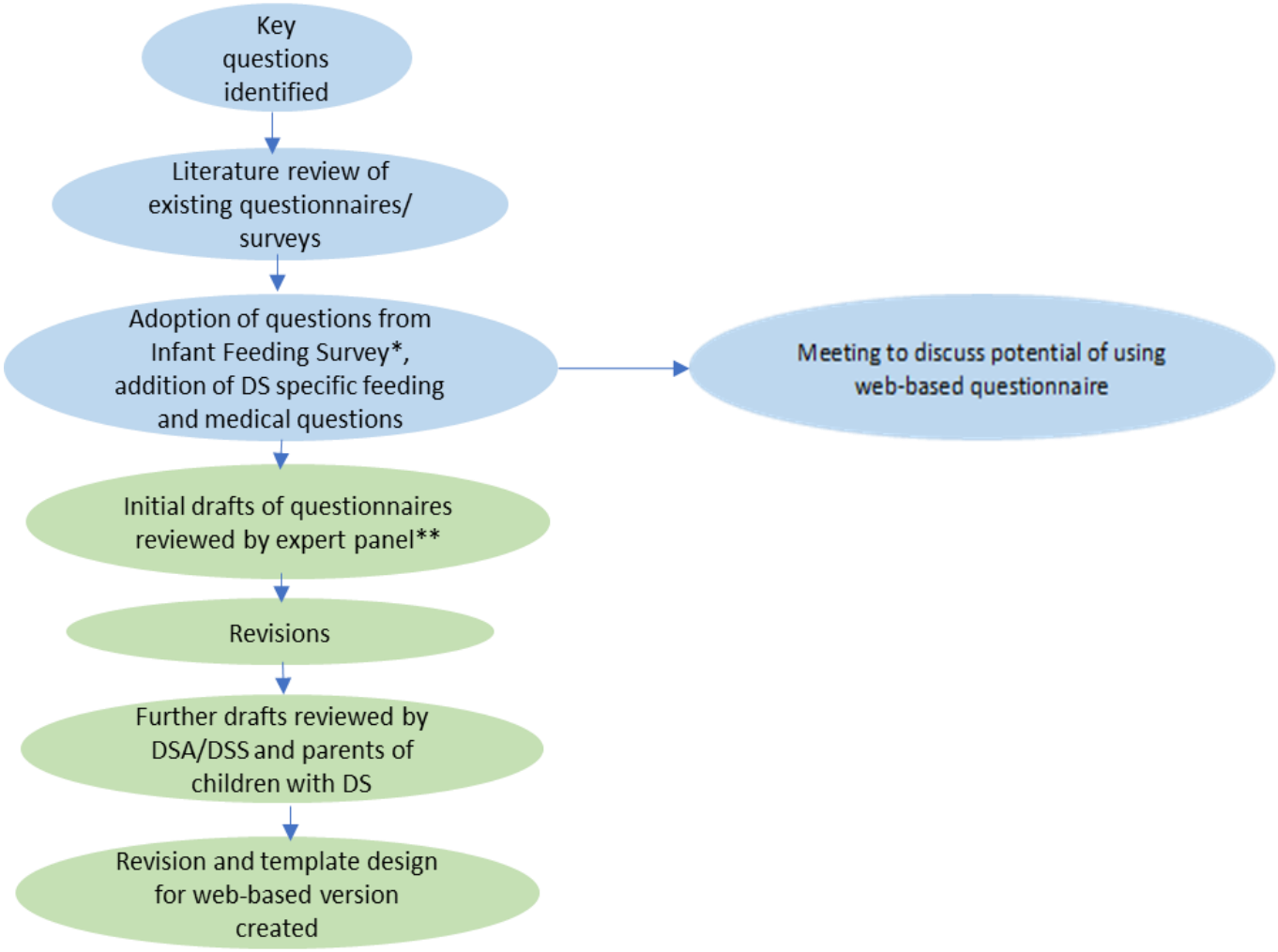


Figure 15: Initial steps in the design of medical and feeding questionnaire.

Footnote:*(McAndrew et al. 2012) **As described a statistician, consultant paediatrician, dietician, members of the DSA and DSS.

Further validation and piloting of the questionnaires (both paper and web-based was completed once the web-based version of the questionnaire had been constructed (see sections below)

2.13.2 REDCap and web-based questionnaires

The use of apps and the internet for data collection in research studies is increasing and the potential benefits are being recognised;

- accessibility is improved as questionnaires can be accessed from mobile devices wherever the participant might be located,
- questionnaires are customisable allowing the addition of branching questions which reduce the amount of content seen by participants making them potentially less overwhelming,
- intrinsic validation – limits can be set on the numerical value of responses for some questions,
- questionnaires are less easily lost than paper,
- no issues with postage,
- and reduction in human error as no manual transfer of data.

This study recruited new parents, who are a demographic recognised as having a preference for web-based communication methods (Bernhardt and Felter 2004, Plantin and Daneback 2009). Trialling the use of web-based questionnaires in this study as part of the feasibility seemed wholly appropriate.

For FADES it was decided that the REDCap system (Harris et al. 2009) would provide the features required both for potential participants to register with the study and for the medical

and feeding questionnaires. REDCap is a web based electronic data capture system designed and developed at Vanderbilt University. These features were:

- Security – user authentication through username and passwords. University of Bristol Central IT Services manages REDCap for research taking place at the university and it therefore meets its policies regarding security, resilience and backups (University of Bristol)
- Access to data across departments (laboratory, researcher and admin access)
- Electronic questionnaires / case report forms which were intuitive
- Different field types, filtering and branching logic
- Audit and validation capabilities
- Data storage and backups
- Data export functions to allow data to be exported to statistical packages including Stata

Separate "projects" were created on REDCap by ST, these were:

1. collecting the personal information on the EOI form,
2. the initial, seven-month and 12-month questionnaires (which contained both medical and feeding questions),
3. the annual questionnaires (completed at age two years and then annually until 2022, these contained only medical questions),
4. and the participant/non-participant questionnaires.

The separate projects ensured that personal identification data were kept separately from questionnaire data. Access was limited so that members of the research team only had access

to the projects they required (as previously illustrated in Figure 13, Section 2.12). Permission to access projects on REDCap required two stages: The REDCap admin team created a user and then with my permission, ST would allow access for that user to the required projects. The EOI project and data storage were held on a different university server from the other REDCap projects.

The required fields and branching for the feeding and medical questionnaires were discussed with ST. The questionnaires were setup on REDCap with fields designed to limit input errors. The web-based questionnaires underwent User Acceptance Testing (UAT) by three members of the research team see Figure 16. Once the final versions went live, the responses for each of the completed questionnaires were reviewed for content validity and reliability. In August 2015 the data that had been collected were exported and reviewed by SLD (statistician) to check the quality that the system was working correctly to enable the data to be exported directly into Stata. Due to the iterative nature of this feasibility study, the data collected by the web-based and paper questionnaires was constantly reviewed together with feedback from participants. Where errors or inconsistencies were found appropriate changes were made two examples of which are given in Appendix 21.

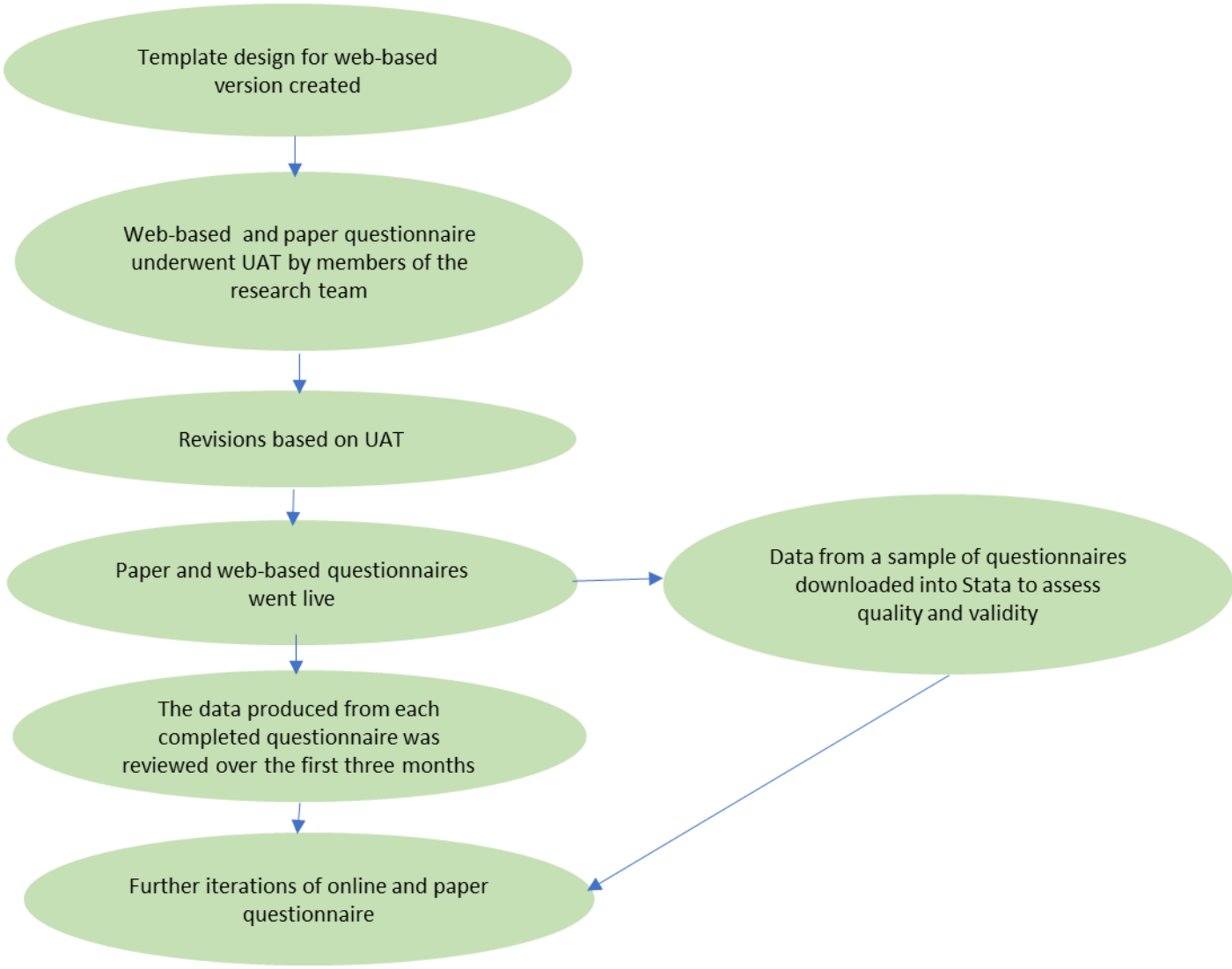


Figure 16: Process for piloting and launch of paper and web-based questionnaire.

2.13.3 Feeding and medical questionnaire data preparation

For both the paper and web-based questionnaires, participants did not enter any personal details, their five-digit ID number was written on every page of the paper questionnaire by the research team prior to sending. Once the paper questionnaire was received in the post, the data were transferred verbatim onto the web-based version by me or a member of the study team.

The data captured on the REDCap system from the feeding and medical questionnaires was exported to Stata. Data were then cleaned by me, and throughout this process the original data were maintained with changes only made within Stata. All data cleaning and coding steps were recorded within Stata do files providing an audit trail. For closed questions, the cleaning of data largely involved examining the longitudinal data to remove discrepancies in the responses given by individual participants. For example, where a participant said that they had a medical condition known to be permanent in the initial questionnaire but failed to include it in a subsequent questionnaire this was changed. For the majority of questions where there were discrepancies, it was assumed that the initial questionnaire was correct. This avoided missing data. For open questions common answers were coded and a standard coding framework was applied, this is described in the following section. When exporting the questionnaire data into Stata the variable names for many of the questions were too long and therefore new variable names needed to be created. A spreadsheet (Master Sheet) of all the variable names old and new were kept (see Appendix 22).

Open Questions

Data from free text and open responses to questions were coded to enable descriptive analysis. Depending on the question type, a standard coding framework was applied, this was important as some questions were repeated at different time points and could then be easily compared when looking at longitudinal data. The codes and the new variables that were made are in the Master Sheets Appendix 22 and 23. An example of the standard coding framework that was applied: there are a number of questions in the initial, seven month and 12-month questionnaires which ask about antibiotic use. These questions ask which antibiotics were

given and for what infection with free text boxes for the responses. For consistency and to aid longitudinal analysis of antibiotic use, answers were coded for the antibiotic name and infection type across all the questionnaires.

Thematic analysis with the use of direct quotes was used for some questions to accurately represent what the mothers were trying to portray. This was particularly pertinent to questions relating to feeding where responses were common to more than one participant. For example, in the 12-month questionnaire, question 2-7 in relation to breastfeeding "What were your reasons for stopping?", the theme of "expressing being too time consuming" emerged. This was therefore made into a binary variable. More than one theme was sometimes applicable to a given response, therefore the use of binary variables allowed for each of these themes to be captured. Selected quotes are used in the results to illustrate. Quotes are also used to show unusual or unexpected responses which were actively sought out. All coding was completed by me to prevent any concerns with inter-rater reliability.

Missing values

Within the exported data there were two distinct types of "missing data". Values that were missing because participants had bypassed those questions due to branching and values that were missing because no response had been supplied despite the question being relevant for the participant. During analysis if the true value for the missing data could be deduced this was inputted through Stata otherwise it remained as missing and the denominator for frequencies calculated in the analysis reflect this.

2.13.4 Statistical Methods

The analysis of the medical and feeding data were descriptive as this was a feasibility study the numbers of participants was too small for more analytical statistical methods. Frequencies were derived and given as a percentage. Where numerical values existed for example for birth weight and gestation interquartile ranges were calculated together with mean and standard deviations (if data followed a normal distribution) or median and interquartile ranges (where data did not follow a normal distribution).

2.14 Sample collection, Storage and Analysis

The schedule for the collection of biological samples from participants is shown in Table 5 and the instructions that were provided to the families are in Appendix 17 and 18. The samples included DNA (from a mouth-swab) for HLA genotyping, stool for microbiome analysis, urine for Urine C-peptide and blood for measurement of autoantibodies and anti BSA antibodies. A maternal stool sample was also collected for maternal gut microbiome analysis. The initial baseline samples were collected as close to birth as possible, so sample packs were sent out to families as soon as they had been enrolled into the study. If a sample had been received within two months of the next time point, a further sample was not requested until the subsequent time point.

Once collected, all samples were returned by post to the Diabetes and Metabolism Laboratory at Southmead Hospital. Once received, the samples were logged on the main database (the barcodes for the samples were entered into the database when the sample packs were sent out to the families). The barcodes were also then entered into the Sample database ready for sample analysis. The collection methods, the methods used for sample storage and analysis

for each of these sample types are described in the relevant sections (Section 2.16). Two studies were completed to refine the collection methodology for the collection of urine samples and for the collection of the stool samples. These studies are within Section 2.15 Refinement of Collection Methodologies.

The timings of the sample collection were initially designed to fit around the screening schedule recommended by the DSMIG (from the PCHR insert for babies born with DS third edition (DSMIG 2019b)). Annual thyroid screening is recommended from one year of age and the aim was to have blood samples taken at or around the same time to avoid participants having to attend additional appointments and ideally avoid them needing extra blood tests. The American Academy of Paediatrics recommends that a thyroid function test at six months of age and then every 12 months (Fergeson et al. 2009) but anecdotally this is poorly adhered to in the UK.

Months of age	**0* Baseline**	**6**	**12**	**24**	**36**	**48**	**60**
Mouth swab for DNA	X						
Maternal Stool sample for microbiome	X						
Stool for microbiome	X	X	X	X	X	X	X
Heel or finger prick blood sample for autoantibodies and BSA** antibodies.	X	X	X	X	X	X	X
Urine for C-peptide analysis	X		X	X	X	X	X

Table 5: Schedule for sample collection during FADES.

Footnote: *participants are recruited as close to birth as possible with a maximum age at recruitment of eight months ** Bovine serum albumin (BSA))

2.15 Refinement of collection methodology

Two small, internal feasibility studies were completed within this PhD. One was to establish the optimal method to preserve the integrity of gut microbiome samples during a national collection returned to the laboratory by post. The other was to determine whether the collection methods for the collection of urinary C-peptide from nappies provides a reliable

result comparable to standard testing on older people able to provide sample directly into the specimen tube.

These internal feasibility studies are presented in this section in full together with results and discussion. The results and discussion are included here rather than Chapter 6 as they determine the overall methods that were used for the main study.

2.15.1 Feasibility study for the collection of gut microbiome samples

This feasibility study has been published (Williams et al. 2019) so a brief description is given here.

Introduction and aims,

In FADES, parents were asked to collect a stool sample from their baby's nappy and send it to the laboratory at Southmead by standard post. Faecal samples have been shown to be representative of the distal intestinal microbiome (Ley et al. 2005). In order to identify distinguishing features of the gut microbiome that vary between the participants and in different conditions (e.g. breastfed versus formula fed), accurate sampling methods were essential. Extracting fresh samples or freezing the stool sample immediately preserves the sample for gut microbiome analysis (Flores et al. 2015) but this was impractical and too expensive for this study which covers a large geographical area. The aim of this internal, feasibility study was to determine the optimal method to preserve the integrity of gut microbiome samples during collection and transport to the laboratory by post.

Methods

Initially, ten participants were recruited to participate in the study. The participants were under the age of one year (six males and four females; age range six to 38 weeks) admitted to Bristol Royal Hospital for Children without diarrhoeal disease. Ethical approval was obtained from the South West Central Bristol Research Ethics Committee (14/SW/0030). Parents were asked to check their child's nappy regularly and let the researchers know as soon as they identified their infant had a dirty (soiled) nappy to ensure samples were fresh.

As shown in Figure 17, from a single stool (one "dirty" nappy) from each participant, samples were collected as follows: 1) a stool sample into an empty sterile tube (plain tube), 2) a swab, both of which were immediately frozen on dry ice. Then samples were collected into 3) a plain tube 4) onto a swab and finally 5) into the OMNIgene•GUT stool stabilisation fluid. The OMNIgene•GUT kit (commercially available from DNA Genotek Inc. Ottawa, ON, Canada) consists of a tube of stabilisation liquid and a ball bearing. The faeces are placed into the tube lid which is designed to break up the faeces and the ball homogenises the sample when it is shaken. Samples 3) -5) were posted to the Diabetes and Metabolism Unit at Southmead Hospital, Bristol, UK.

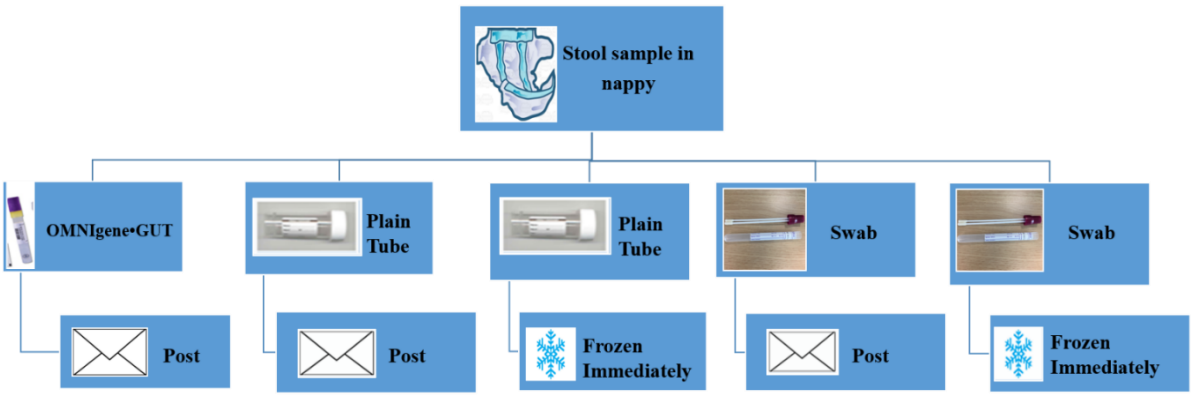


Figure 17: Process for collecting stool samples from nappies for the feasibility study. From a single stool, samples were collected as follows: 1) into a plain sterile tube, 2) onto a swab, both of which were immediately frozen, 3) into a plain tube and 4) onto a swab, both of which were sent to the laboratory through the post and 5) a sample into the OMNIgene•GUT stabilisation fluid which was also sent in the post.

All samples returned in the post (ambient conditions) arrived within three days of sampling. On arrival, the samples were immediately frozen at -80^{o}C. Samples (n=48) were shipped frozen by courier to The Alkek Center for Metagenomics and Microbiome Research (CMMR), at Baylor College of Medicine, Houston, Texas. There were nine participants who provided sufficient stool for all five sample types and one child who only provided sufficient sample for the OMNIgene Gut kit and two swabs (one at room temperature and one frozen).

The samples were subjected to extraction for 16S rRNA gene profiling using gene sequencing methods adapted from those developed for the Earth Microbiome Project (Caporaso et al. 2011, Caporaso et al. 2012) and the NIH-Human Microbiome Project (Consortium 2012b, a) (Details of extraction and sequencing techniques can be found in the published paper (Williams et al. 2019).

16Sv4 rRNA gene sequences were clustered into operational taxonomic units (OTUs) at a similarity cut-off value of 97%. OTUs were mapped to a database (Edgar 2013, Quast et al. 2013) containing only the 16S v4 region to determine taxonomies. A custom script constructed a rarefied OTU table for analyses of taxonomic relative abundance, alpha-diversity (number of OTUs - richness and Shannon's diversity index), beta-diversity (including UniFrac) (Lozupone and Knight 2005), and phylogenetic trends. The ten most abundant Phyla, Classes, Order, Families and Genera observed in the 48 samples analysed are shown in (Appendix 24).

Statistical Methods

To assess the changes seen in diversity of the samples, comparisons were made of the number of OTUs which represent the array of species observed. To compare whether changes were related to the richness and evenness of the microbes within samples, the Shannon diversity index (Shannon index) was used. For each stool sample, the difference in the measurement (number of OTUs or Shannon index) between the frozen standard and each of the methods of sample collection were plotted against the mean of the two measurements (a Bland-Altman (BA) plot) (Bland and Altman 1986). This test was used because we wished to measure the agreement between the frozen standard and the practical sample collection alternatives. The differences and the means were unrelated and therefore 95% limits of agreement could be calculated (mean difference +/- 2 standard deviations of the difference) and added to the plot. This gave the range of disagreement between the frozen standard and each of the sampling methods (Bland and Altman 1986).

The relative abundance of each of the four major phyla (*Actinobacteria, Bacteroidetes, Firmicutes* and *Proteobacteria;* (the other phyla were rare in these samples) and the ten main

genera (*Acinetobacter, Bifidobacterium, Enterobacter*, *Esherichia_Shigella, Lactobacillus, Peptoclostridium, Staphylococcus, Streptococcus, Subdoligranulum* and *Veillonella*) were calculated and plotted on a stacked bar chart (Microsoft Excel) to allow a visual comparison. Limits of agreement were calculated and used to compare the abundance of each of the four main phlya between the frozen standard and the other sampling methods. The same analysis was also completed for *Enterobacter*, *Escherichia Shigella, Bifidobacterium, Streptococcus, Staphylococcus, Veillonella, Lactobacillus* and *Rothia* (Williams et al. 2019). These are all genera which were present in the samples and in the microbiota have been associated with T1D auto-antibody seropositivity, breastfeeding or other dietary components (Vatanen et al. 2016).

Results

Recruitment: The initial recruitment target was for ten participants. One of the infants recruited did not produce an adequate stool volume to test all sampling methods (sufficient sample was available only for three tests; the Genotek method and frozen/posted swab. Analysis of initial profiling (Appendix 24) showed that one child had a significant Clostridium infection and the samples were removed from further analysis. The BA analysis is therefore presented on data from eight infants (six males and two females; age range 6 to 38 weeks) with five different sampling methods.

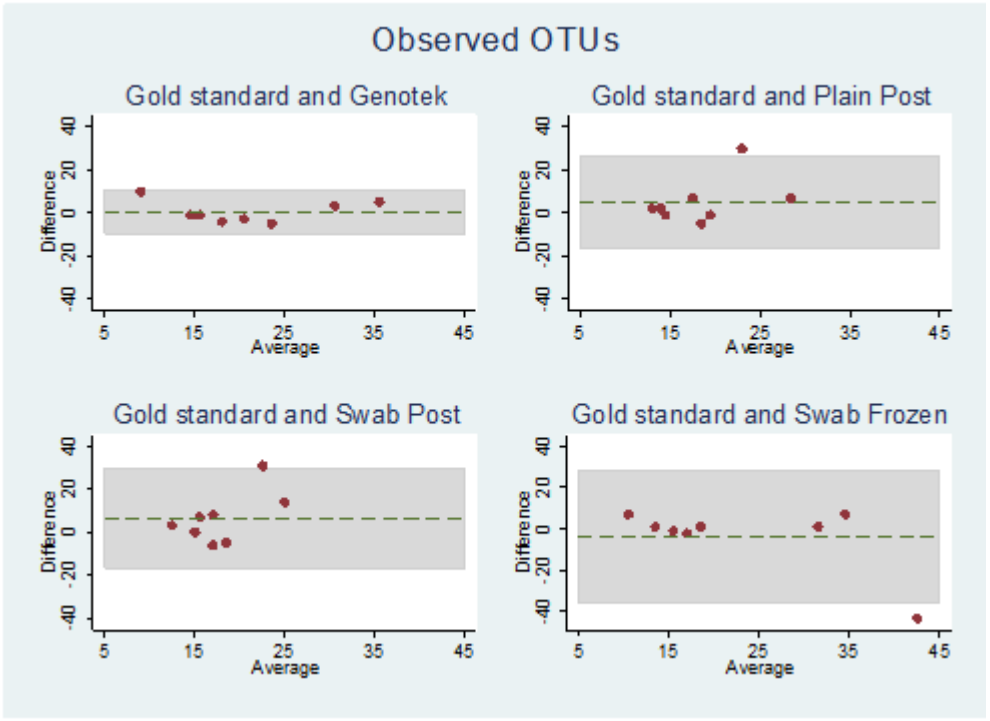
Impact of sampling method on community diversity: The BA analysis of the observed OTUs (Table 6, Figure 18a) showed the OMNIgene•GUT kit to have the narrowest limits of agreement (-9.55, 10.55) with the frozen standard. The limits of agreement for the samples sent in ambient conditions (plain post and swab post) compared with the frozen standard,

were similar to each other as expected (-16.13, 26.38 and -16.95, 29.95), and showed less agreement than the OMNIgene•GUT kit. The frozen swab compared to the frozen standard showed less agreement than would be expected (-35.49, 28.24) due to a single outlier.

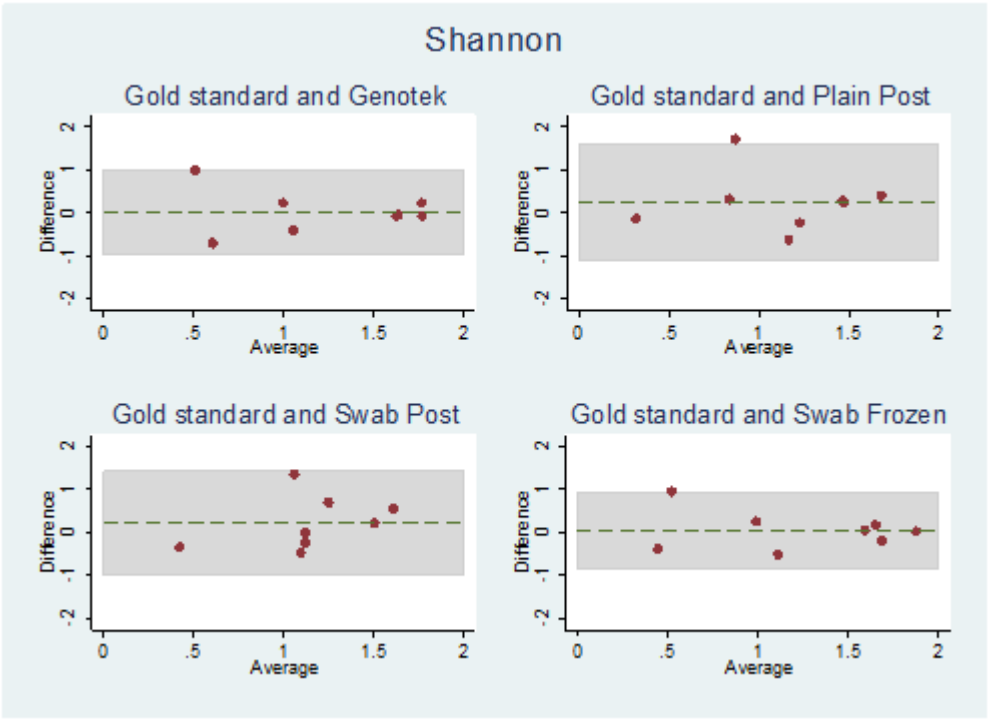
The BA analysis comparing the Shannon index results (Table 6, Figure 18b) showed that for all methods, the limits of agreement were narrow suggesting that Shannon index is not affected by collection method or by being exposed to ambient temperature. The closest agreement with the frozen standard was seen in the samples which were collected using a swab and frozen immediately (-0.85,0.93). For samples taken and exposed to ambient conditions in the post the OMNIgene•GUT kit showed the closest agreement to the frozen standard method (-0.96, 1.0), with the plain post and swab post samples showing the least agreement as seen with analysis of OTUs (-1.1, 1.59 and -0.99, 1.42)

	Methodology compared to Frozen standard (Average difference, (95% Limits of Agreement))			
	OMNIgene•GUT	Plain post	Swab post	Swab frozen
OTU	0.50 (-9.55, 10.55)	5.13 (-16.13, 26.38)	6.50 (-16.95, 29.95)	-3.63 (-35.49, 28.24)
Shannon Index	0.02 (-0.96, 1.00)	0.25 (-1.10, 1.59)	0.21 (-0.99, 1.42)	0.04 (-0.85, 0.93)

Table 6: Bland Altman analysis of OTU and Shannon index for the four different methods of sample collection compared to the standard of immediate freezing.



a)



b)

Figure 18a) and b): BA plots of observed OTUs a) and Shannon index b) for the four different methods of sample collection compared to the frozen standard of immediate freezing.

Footnote: The dashed line shows the average difference with the shaded area representing the 95% limits of agreement

Impact of sampling methods on community composition: As would be expected when samples are subjected to different conditions during sampling and transport, the relative abundance of the phyla changed as the conditions either encouraged the proliferation or suppressed the growth of microbes. The results presented are for the four phyla which were the most abundant within the infant gut microbiome, these are *Firmicutes, Proteobacteria, Actinobacteria* and *Bacteroidete*s. Figure 19 a) shows the variation in the relative abundance of different Phyla between individuals. Each of these infants appears to have a distinct gut microbiome. For five of the participants (ID 11, 14, 15, 18 and 19) the profile seen in the samples collected using the OMNIgene•GUT kit resembles the frozen standard sample method of collecting into a plain tube and freezing immediately. There has been an overgrowth of *Proteobacteria* within the samples exposed to ambient temperatures with a relative suppression of *Firmicutes.*

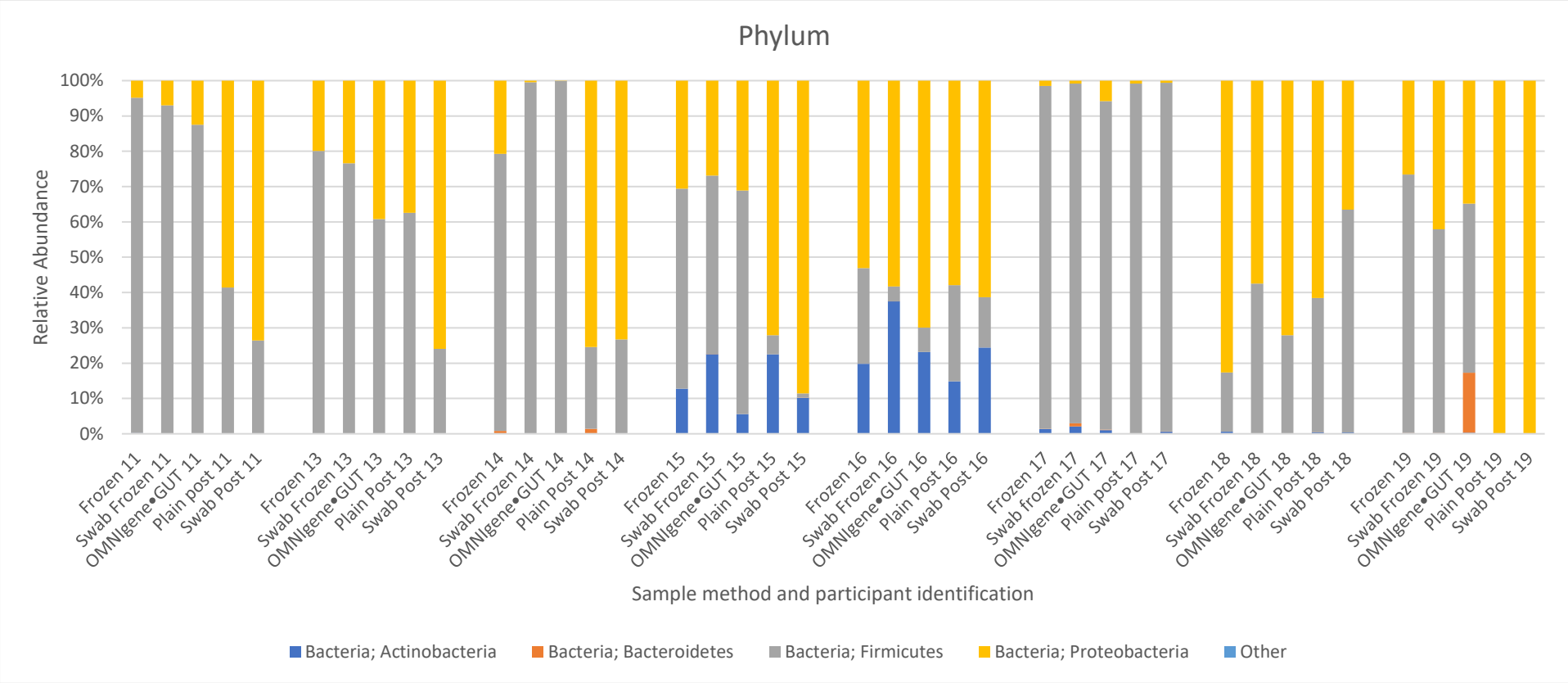


Figure 19 a) Relative abundance of the four main phyla for each sampling method (immediate freezing in a plain sterile tube (frozen), immediate freezing on a swab (swab frozen), using the OMNIgene•GUT kit (OMNIgene•GUT), collecting into a plain sterile tube in the post (plain post) and collecting onto a swab which is sent in the post (swab post).

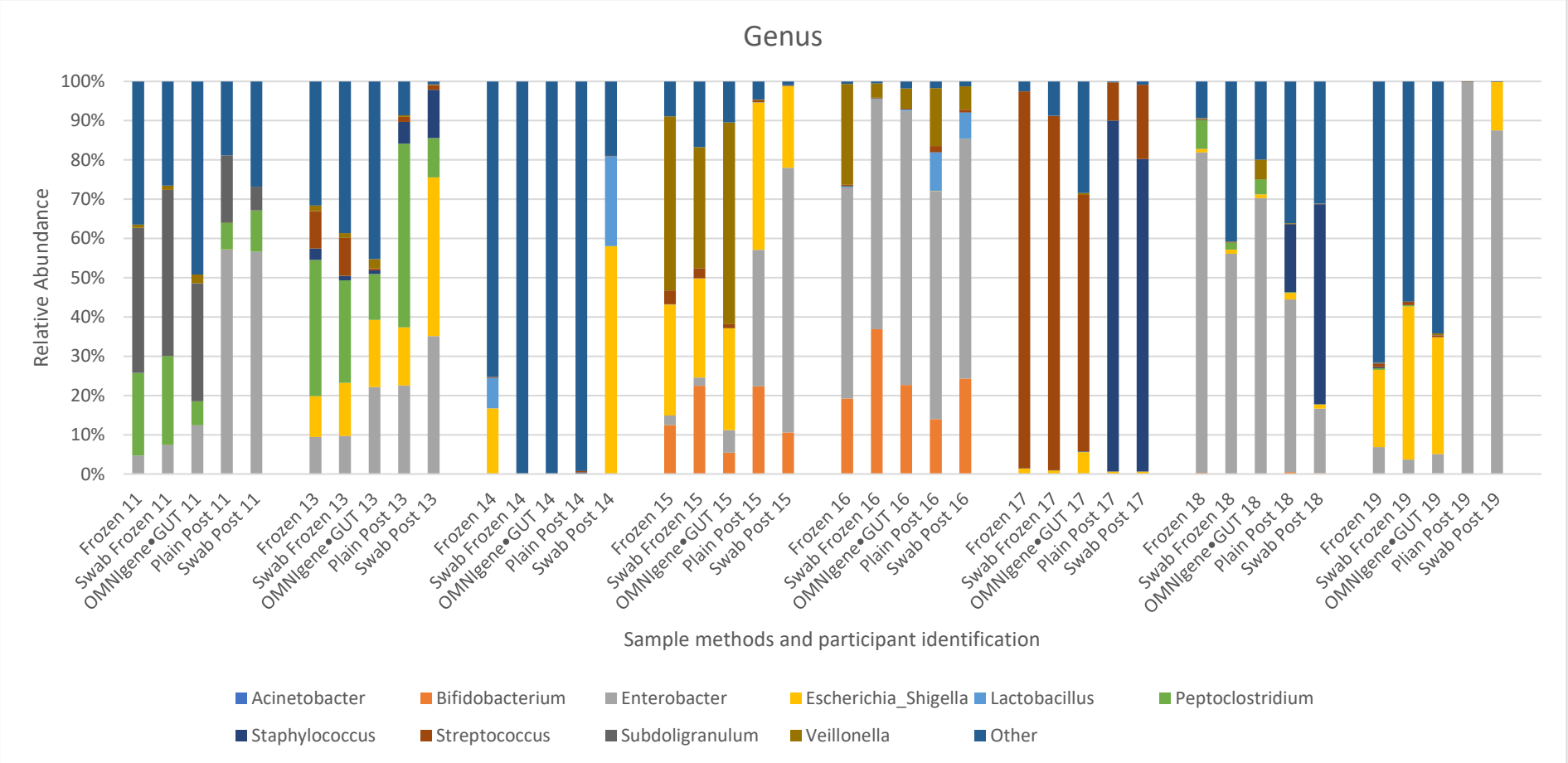


Figure 19b) Relative Abundance of the ten main genera for each sampling method.

The limits of agreement (Appendix 25) for the relative abundance of *Firmicutes* and *Proteobacteria* showed the OMNIgene•GUT kit and the frozen swab to show the closest agreement with the frozen standard method. The plain tube and the swab sent in the post showed that changes had occurred in the abundances of these phyla.

The BA plots for *Acinetobacteria* (Appendix 25) show that the growth of *Acinetobacteria* is relatively unaffected by collecting samples into a plain tube or swab and transporting in ambient conditions than from using the OMNIgene•GUT kit. The frozen swab samples showed a large disagreement from the frozen standard method which is unexpected. The agreement in the amount of *Bacteroidetes* seen between the frozen standard and the other sampling methods was comparable for all apart from the OMNIgene•GUT kit (Appendix 25). This had a single outlier affecting the result, if this were to be removed, all of the sampling methods would show a similar agreement with the frozen standard method.

The variation in the relative abundance of the different genera within the gut microbiome is much greater between individual infants than it is between the sampling methods (Figure 19b and Appendix 24). The method of collection and exposure to ambient temperatures does affect, to a variable degree, the growth or suppression of different genera (Williams et al. 2019) (Appendix 26). *Bifidobacterium* and *Lactobacillus* are both linked to autoimmunity and are affected by feeding methods and therefore are particularly relevant for the collection of samples for FADES. For *Enterobacter*, *Staphylococcus* and *lactobacillus* the samples exposed to ambient temperatures showed a distinct abundance of these bacteria from the frozen standard (Appendix 26). The closest agreement with the frozen standard was between the samples collected using the OMNIgene•GUT kit and the frozen swab. For *Bifidobacterium*

(Appendix 26) the limits of agreement were similar to those observed for the phyla *Actinobacteria* as *Bifidobacterium* is a member of the *Actinobacteria* phylum and the differences seen in both are due to differences in growth of *Bifidobacterium* between the sampling methods.

Discussion of the feasibility study for the collection of gut microbiome samples

We have shown that there is variation in microbiome results from four different collection methods compared with the frozen standard of immediate freezing. This study demonstrates that the OMNIgene•GUT kit preserves samples taken from nappies and exposed to ambient temperatures. Parents were asked to collect the samples and found the method straightforward (data collected by questionnaire). The OMNIgene•GUT kit can be considered the best option for distance sample collection in general population studies and this method was added to the FADES protocol through Amendment 5 August 2016.

Although parents were asked to check the baby's nappy regularly, we do not know how fresh the stool samples were although they were all likely to have been collected within 2 hours of having been passed. Samples sent through the post took three days to arrive in the laboratory. Sample collection at home for population-based studies may result in samples being exposed to ambient temperatures for longer periods of time although our laboratory protocols try to overcome this by asking participants to post samples on Monday or Tuesday to arrive in the laboratory before the weekend. The OMNIgene•GUT kit has recently been shown to preserve samples at ambient temperatures for up to 60 days (Hill et al. 2016, Song et al. 2016).

This is the first methodological study defining the method of choice for infants less than one year of age. Although a relatively small study, all sampling methods were consistent, each method was tested within all eight participants samples and the genus data demonstrated large inter-individual variation. The infants analysed in this study appeared to each have distinctive microbiomes.

The amount of faecal material that it is possible to collect from nappies depends on the baby's age and feeding methods. Very liquid stools are rapidly absorbed into the nappy. For each of the methods described in this paper very little faecal matter was required. A previous study to compare collection methods from young children and the elderly also showed the OMNIgene•GUT to perform well at ambient temperatures for children with a median age of 2 years old (Hill et al. 2016) and for collection of samples from adults (Choo, Leong, and Rogers 2015).

Previous studies comparing sampling and storage methods for microbiome samples have used measures of dissimilarity and correlation such as Bray-Curtis, Wilcoxon signed rank test, paired DESeq test, Man-Whitney-Wilcoxon test and Kruskal-Wallis test (Hill et al. 2016, Dominianni et al. 2014, Anderson et al. 2016) these show degrees of association between the sampling methods rather than an agreement. In this study, we conducted a Bland Altman analysis allowing analysis of agreement between each collection method with the frozen standard, this is a novel (but more appropriate) application of this statistical method for comparison of microbiome methodologies.

This study as with others, shows that for all collection methods there is a change in the gut microbiome samples once they are exposed to ambient temperatures. There was an

overgrowth of Proteobacteria and a relative reduction in abundance of Firmicutes in those samples exposed to ambient conditions. However, it also demonstrates that the OMNIgene•GUT kit preserves the samples with the least change to the diversity of the species seen (number of OTUs) or the richness and evenness of the samples (Shannon index).

This approach is now being used to collect all gut microbiome samples from FADES participants (Amendment 5 in August 2016) and other cohorts from the general population.

2.15.2 Feasibility study for the collection of urinary c- peptide samples from nappies

Introduction and aims

Collecting urine samples from infants is usually undertaken as a "clean catch" whereby the nappy is removed, and the urine is caught into a sterile pot as it is passed. Alternative methods include an adhesive bag which can cause skin irritation or by catheter insertion although this is invasive and carries the risk of introducing infection. An alternative method is to place sterile cotton wool in the nappy onto which any urine that is passed is captured. Although this latter method is perhaps less reliable for microbiological samples where the urine needs to be uncontaminated it is used for biochemical analysis of urine samples and has been shown to be an effective approach (Fell et al. 1997, Ahmad et al. 1991, Smith and Taylor 1992).

In the FADES protocol, the recommendation was for parents to collect the urine sample for C-peptide by either trying to catch a urine sample in a pot (clean catch), or by placing cotton wool in their child's nappy and once they have urinated into the nappy removing the cotton wool and squeezing the urine out. Fibres in the cotton wool balls may absorb some proteins making the measurement of proteinuria by this method unreliable (Fell et al. 1997, Smith and Taylor 1992) and this may also be affected by the contact time of the urine on the nappy

(Ahmad et al. 1991). This has been reported when measuring proteins of a higher molecular weight than urine C-peptide (molecular weight is 3600 Daltons) (Fell et al. 1997). This internal feasibility study was to determine the reliability of this method when measuring urine C - peptide.

Method

A pilot study was set up with 21 adult participants and five babies (neither adults or babies had Diabetes or DS). The adult participants were asked to provide two urine samples. One sample was collected into a sterile pot on waking and before having anything to eat or drink. This was then placed in their fridge until they had collected the second sample. They then ate a breakfast and collected a further urine sample into a second pot two hours after the meal. Pre and post prandial samples were requested in order to provide a range of urine C-peptide levels. Both the samples were transferred to the laboratory within 2 hours. For each urine sample, half was put straight into a boric acid containing collection tube in the usual way. The other half of the urine sample was poured onto cotton wool balls, and then the urine collected back off the cotton wool balls after a couple of minutes. This was done by squeezing excess fluid into a sterile pot and pipetting into a normal urine collection tube containing boric acid.

For the samples collected from babies, the urine was collected using a clean catch method (the optimal method) pouring half of the urine collected into a boric acid tube and pouring the other half, back onto cotton wool placed in the baby's nappy. After an hour in the nappy, the urine was collected off the cotton wool and poured into a boric acid tube.

All samples were labelled with an anonymised barcode and no personal data were recorded so samples could not be linked to the donor. The only information recorded was whether the donor was an adult or baby and for the adults whether the sample was pre or post prandial. The samples were frozen and sent by courier to the Biochemistry Department, Royal Devon and Exeter Hospital (Exeter, UK) where they were analysed. Electrochemiluminescence immunoassay was used to measure urine C-peptide as previously described (McDonald et al. 2009). A BA analysis was completed for the adult samples to show agreement between the two collection methods (Bland and Altman 1986).

Results

For the adult pre-prandial samples, the average difference in the UCPCR measurement between the clean catch method and cotton wool method was -0.122 nmol/mmol with the 95% limits of agreement being between -1.405 and 1.162 nmol/mmol. The majority of the participants appeared to have reasonable agreement when visually inspecting the BA plot (Figure 20) but there were four participants for whom the agreement was less with three being outside of the 95% limits of agreement.

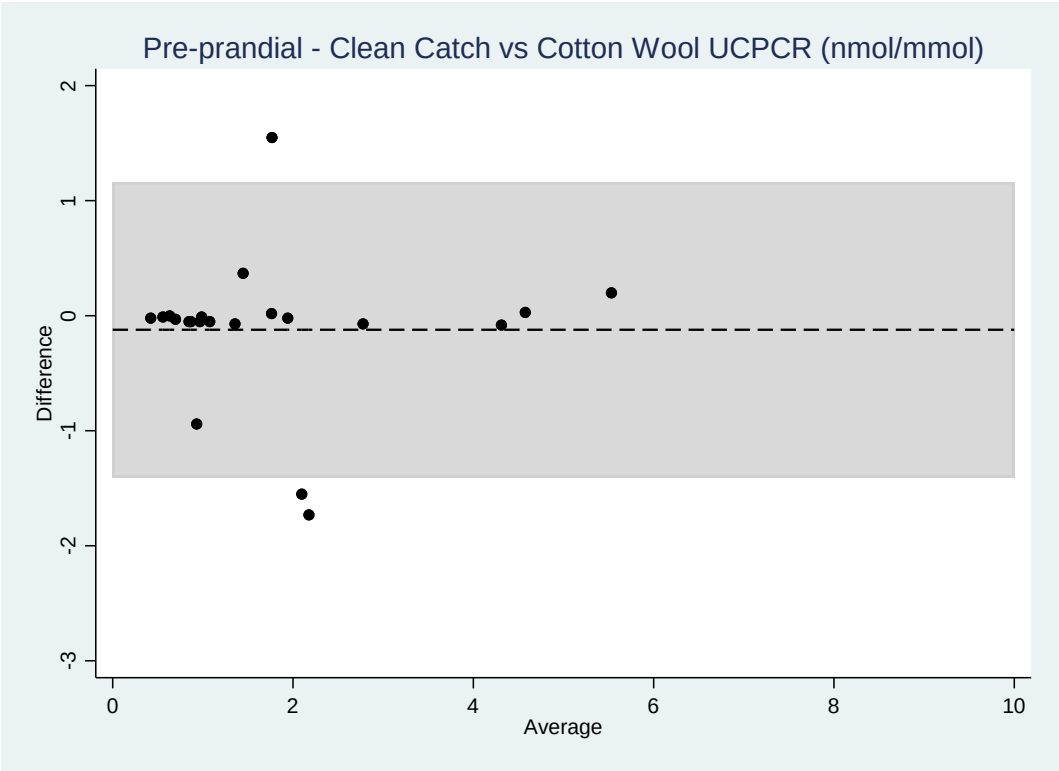


Figure 20: BA plot showing the differences and averages in the pre-prandial UCPCR between the clean catch and cotton wool methods.

Footnote: The dashed line shows the average difference with the shaded area representing the 95% limits of agreement

The BA analysis for that adult post-prandial samples shows an average difference in clean-catch UCPCR to cotton-wool UCPCR of -0.038 nmol/mmol the 95% limits of agreement were -1.267 to 1.190 nmol/mmol. Overall the BA plot (Figure 21) shows that most samples show close agreement but that for three participants with two outside of the 95% limits of agreement. There was one participant who had high UCPCR measurements, but the difference between the sample methods was within the limits of agreement.

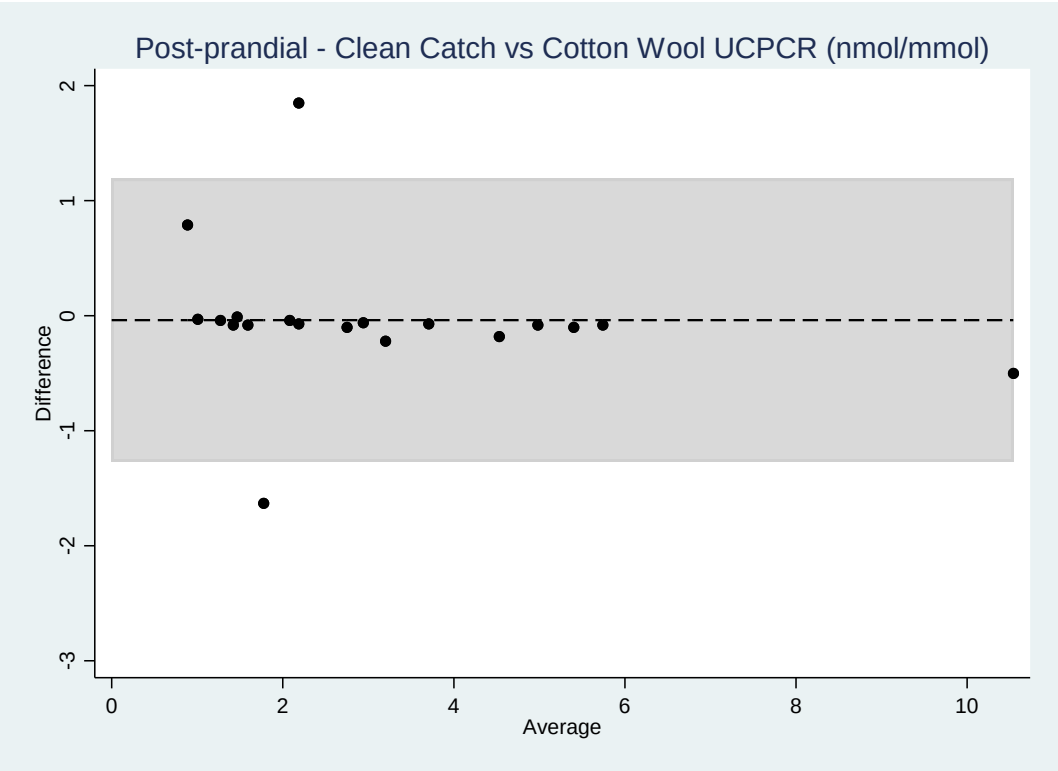


Figure 21: BA plot showing the differences and averages in the post - prandial UCPCR between the clean catch and cotton wool methods.

Footnote: The dashed line shows the average difference with the shaded area representing the 95% limits of agreement

The number of samples from babies was too small for statistical analysis but the values are presented in Table 7.

Baby	Clean Catch UCPCR (nmol/mmol)	Cotton Wool UCPCR (nmol/mmol)
1	5.02	5.24
2	6.89	6.36
3	3.12	5.79
4	0.29	0.31
5	3.3	2.16

Table 7: UCPCR measurements from samples from five babies. For each baby a single urine was tested following clean catch and having been exposed to cotton wool placed in a nappy. (All values were above the minimal value of 0.2 nmol/mmol).

Discussion of the feasibility study for the collection of urinary C-peptide samples from nappies

Papers have suggested that the fibres in the cotton wool balls may absorb some proteins making the measurement of proteinuria by this method unreliable (Smith and Taylor 1992) and that this may also be affected by the contact time of the urine on the nappy (Smith and Taylor 1992) however this has been when measuring proteins of a higher molecular weight than urine C-peptide (molecular weight is 3600 daltons (Chowta et al. 2010)). Our small internal feasibility study demonstrated that differences are seen in UCPCR with different sampling methods. The BA plots suggest that for the majority of the participants the agreement between the methods was close. However, for those participants where the differences were outside of the limits of agreement this may have implications for research studies relying on this method. Studies look for evidence of remaining beta cell function measure very small amounts of urine C-peptide (with detectable UCPCR of ≥0.03nmol/L, minimal less than 0.2 nmol/mmol) at these very low levels the effects of the collection method might alter results. The participants who were outside of the limits of agreement did not show consistent under-measurement with the cotton wool method as might be expected. The reason why these differences were observed was unclear. This internal feasibility study was small and the number of samples from babies that we were able to collect was restricted. Therefore, no conclusion can be drawn from the samples collected in nappies and this would need to be repeated with a larger group of babies.

Overall this study showed that both collection methods would be acceptable for this cohort where the practicalities for families collecting urine samples at home need to be considered.

However, if unexpected results are obtained, it would be advisable to ask for a repeat clean catch urine sample particularly when looking at low levels of urinary C-peptide.

2.16 Sample collection and laboratory methods for FADES

The following sections describe the sample collection methods for DNA, urine C-peptide and gut microbiome for the participants. The processing, storage and initial testing of the samples.

2.16.1 DNA Sample collection and analysis

DNA samples were collected by parents at home using the mouths-swab kit that was sent to them in the post together with the other sample kits and prepaid packaging. DNA samples were collected once in the initial sample collection; this was requested at recruitment. This was returned in the standard post to the Diabetes and Metabolism Unit at Southmead Hospital, Bristol, UK using the prepaid packaging together with the other samples (the full instructions for this are given in Appendix 17 and 18). Once the samples arrived in the laboratory they were logged in the main database and sample database and were frozen and stored at -20^{o}C. The samples were defrosted for DNA extraction and HLA genotyping.

DNA extraction and HLA genotyping

DNA extraction was completed by GM and SCK and the samples were genotyped using the methods described by Gillespie et al (Gillespie et al. 2000). The HLA genotypes for the study population were compared with previously published populations for DS, DS and diabetes and T1D alone (Aitken et al. 2013).

2.16.2 Stool Sample collection

Stool samples were collected for gut microbiome from the baby close to birth and then longitudinally around six months of age, one year and then annually. Maternal stool samples were also collected at the same time as the baby's initial sample collection. The maternal sample was collected following Amendment 1 (30th March 2015), this was added as the maternal gut microbiome is a key determinant of the infant's microbiome (Ferretti et al. 2018). Stool samples were collected at home by the families and then returned together with the other samples in the pre-paid package. Parents were asked to collect two samples from a single stool one using a swab as described below and one using the OMNIgene GUT collection tube. The two methods were used in order to maintain a standard collection method (i.e. swab) throughout the cohort despite the addition OMNIgene GUT collection tube methods (Amendment 5 (4th August 2016)). This amendment followed from the internal feasibility study (Williams et al. 2019).

Parents were asked to collect the sample as soon as they noticed that their baby had defecated. They used a BD™ CultureSwab™, rotating the swab within the stool in order to cover the heads of the swab. A spatula provided in the kit was then used to collect a sample from the same stool which could be placed into the OMNIgene GUT collection tube. This was placed in the return packaging together with the swab. Once the samples arrived, they were logged and stored at -80^{0}C.

Stool samples are batched ready for analysis once a significant proportion of the children have reached two years of age. By the age of two the gut microbiome is relatively established and

the differences which are seen between those who develop autoimmunity and those who do not, start to be identifiable (Giongo et al. 2011).

2.16.3 Urine Sample Collection

Urine samples for Urine C-peptide were collected shortly after recruitment (initial/ baseline sample), at onr year and then annually. A sample was not requested at six months as it was unlikely that any significant change in Urine C-peptide from the baseline sample would be noted before at least one year of age. Parents were asked to collect a sample after a feed, they were given the option of collecting a sample as a 'clean catch' or placing cotton wool into the nappy to absorb the urine and collect it this way. These two methods were compared in the internal feasibility study. The urine sample was then transferred into a sample tube containing boric acid, packaged and returned with the other samples. A study by McDonald et al showed that UCPCR was not altered when urine samples in boric acid were stored at room temperature for up to three days (McDonald et al. 2009). Once samples arrived in the laboratory, they were aliquoted and stored at -80^{0}C.

Following Amendment 7 (25th July 2017) parents were asked to document how long after the feed the sample was taken. As discussed in the feasibility study Section 2.15.2 and in Chapter 6 Section 6.5, there is not a standard method for collecting samples for urine C-peptide in this age group. It is not possible to give a standard feed and babies cannot void on demand but urine C-peptide creatinine ratio replaces the mixed meal tolerance test in this study (Besser et al. 2011).

Measuring Urine C-peptide

Samples were shipped frozen by courier to the Biochemistry Department, Royal Devon and Exeter Hospital (Exeter, UK) where the urine C-peptide analysis was completed. An Electrochemiluminescence immunoassay was used to measure urine C-peptide as previously described (McDonald et al. 2009). The threshold of UCPCR ≥0.37nmol/mmol was used to describe significant endogenous insulin secretion in this study. Besser et al found that a 120-minute post evening meal UCPCR correlated significantly with a 90 minute stimulated serum C-peptide result in children. A threshold of UCPCR ≥0.37nmol/mmol was highly specific for significant endogenous secretion (Besser et al. 2011). Although this was a small study, there are no standard cut-offs for UCPCR in children. UCPCR values were also plotted against the adult thresholds for detectable but minimal UCPCR of ≥0.03nmol/L and for intermediate insulin secretion 0.2 nmol/mmol (the cut off for intermediate insulin secretion (Oram et al. 2014).

2.17 Blood

The initial blood sample was collected as close to birth as possible, so once the participant had been recruited the parents were contacted via email to find out when they were next due to see a healthcare professional. The details of the relevant healthcare professional were provided or had to be determined and a request was made to them by GW to help facilitate the sample collection. The blood samples were taken by a variety of people, health visitors, GP practice nurses (at the time of immunisation), phlebotomist (in clinics and occasionally organised by the families themselves), neonatologists, community paediatricians, cardiologists and anaesthetists at the time of surgery for some children. Families took the

blood sample pack provided with them to the appointment. The pack contained pre-labelled tubes, instructions and all necessary equipment for a heel or finger prick sample. Some parents and families collected the annual samples themselves at home, this was mostly families who themselves had clinical experience. The Sarstedt Microvette tube that was provided for the sample allowed 300µl of blood to be collected. Two tubes were provided although only one tube was requested both tubes were filled for some participants when the blood was easy to collect. The blood sample collected was given to the family to post in the prepaid packaging. There was no involvement of local hospital NHS laboratories.

Once the blood sample arrived in the laboratory, if the sample was less than ~20µL it was not separated but was frozen whole at -80^{0}C. Larger samples were spun down using the Microfuge at a maximum speed of 13.3 revolutions per minute for fifteen minutes. The serum was pipetted off into 500µL Starstedt tube, with an O-ring lid. The tube was labelled, and the serum volume recorded. The sample together with the remaining blood clot was then frozen and stored at -80^{0}C.

2.17.1 Analysing the blood samples for anti-bovine serum albumin antibody (BSA)

Blood samples collected up until the 1st September 2017 were tested for anti-BSA antibodies by SG. These samples came from 53 participants, 30 of whom had longitudinal samples. A control group of 18 children under the age of two years from the BOX study were used (Bingley and Gale 1989, Kozhakhmetova et al. 2018).

The laboratory methods for the anti-BSA Antibody Dissociation Enhanced Lanthanide Fluoroimmunoassay (DELFIA) were provided by and with permission from SG prior to publication. 2µl of serum (in duplicate) was added to a 96-deep well microtitre plate. The BSA

was labelled using an Eu-N1 ITC chelate, with an aromatic isothiocyanato group as the reactive arm. To each well, 28.8ng/ml of labelled BSA (approximately 95,000 Europium (Eu) counts) was added in 25µl 50 mmol/l Tris 0.9% NaCl <0.5% Tween Buffer (DELFIA Assay Buffer). Glycine-blocked Protein A Sepharose was used to precipitate bound immunocomplexes. Following a centrifuge-based wash system (six times), bound Eu counts were counted on a Victor plate reader after the addition of DELFIA Enhancement Solution (Perkin Elmer). Samples were measured against a standard curve, constructed using dilutions of an anti-BSA antibody positive serum, obtained from a FDR, in an anti-BSA antibody negative serum from a healthy volunteer. Quality control sera with varying antibody titres to anti-BSA antibodies were also selected. Results were indexed by subtracting the cpm of the negative control and dividing by the positive control minus the negative control. The positive threshold was set at the 90th percentile of 228 healthy school children (Bingley et al. 1993) (113 Male, 115 Female Median age: 12.1 years, Age Range: 9.2-13.6 years) which was 0.34 Indexed Units.

CHAPTER 3

Qualitative Study: What factors influence recruitment to a birth cohort of infants with Down's syndrome?

Chapter 3 Qualitative Study: What factors influence recruitment to a birth cohort of infants with Down's syndrome?

3.1 Overview of Chapter 3

This chapter presents the qualitative study that was completed to understand the potential barriers to recruitment for the FADES study. Data pertaining to the qualitative study has been published in an international peer reviewed journal (Williams et al. 2018). However, the present chapter fully describes the methods and evaluates the findings in the wider context of the thesis.

3.2 Introduction

Birth cohorts targeting DS or other genetic conditions are rare. However, prospective cohort studies are essential for understanding the natural history of these conditions. Recruiting any family with a new-born into a research study is challenging, but the potential 'setback' of a diagnosis of DS compounds the difficulty. The majority of diagnoses are made at birth, or shortly afterwards (Morris and Alberman 2009): around 92% of prenatal diagnoses lead to a termination (Morris and Alberman 2009). As described previously babies with DS have associated conditions often diagnosed in the neonatal period (Roizen and Patterson 2003), these may have a significant impact on the baby, family and early bonding opportunities. Studies exploring how parents cope and adapt to receiving the diagnosis of DS, have described the increased stress compared to parents of a typical newborn (Van Riper 2007). Some families will respond with shock and upset whilst others will positively thrive (Van Riper 2007, Hedov, Wikblad, and Annerén 2002). The present qualitative study explored ways to improve

recruitment during the feasibility phase and to inform future birth cohorts recruiting chromosomal/genetic anomalies or complex neonatal conditions.

Qualitative methods in comparison to quantitative methods allow in-depth social inquiry enabling the researcher to gain a better understanding of the participant's perspectives and experiences. It is interpretivist rather than positivist which is important in a context such as this where the diagnosis of DS is relatively rare, an individual's reality of being a parent of a child with DS varies and there is little available prior research into recruiting this population. There are multiple qualitative analytic methods including grounded theory (Glaser 1992), interpretative phenomological analysis (Smith and Osborn 2004) and narrative analysis (Riessman 1993). However, thematic analysis was used in this study because it allows theoretical flexibility whilst being methodologically sound. Thematic analysis enables the data set to be organised into themes, a theme encompasses something important in relation to the research question and may show a response that has a degree of commonality between participants and a pattern of ideas or meaning.

Thematic analysis can be inductive or deductive. Inductive is data driven and described as a 'bottom-up' approach (Patton 1990) whereas deductive analysis is 'top-down' and is usually associated more with the researcher's topics of interest.and / or guided by pre-conceived theoretical framework. Although taking a deductive approach leads to less rich evaluation of the data it provides more detail in the desired area. Analysis occurs at different "levels", semantic being the most basic with latent analysis starting to uncover ideas, conceptualisations and ideologies (Braun, Clarke, and Terry 2014). The interviews in this study were conducted with health care professionals and family support workers thus a latent

analysis allowed an understanding of how their opinions were formed. This method acknowledges the influence of the researcher's theoretical and epistemological position (Braun, Clarke, and Terry 2014) this will be discussed in relation to this study in the discussion at the end of this chapter (Section 3.5.1.1).

3.3 Methods

A copy of the study protocol is in Appendix 4.

3.3.1 Participants

A purposive sampling strategy was used to recruit paediatricians, research nurses and family support workers. This strategy enabled participant selection based on their experience in supporting and caring for infants with DS and their families, during the months after birth, or recruiting new parents to research studies. This study was advertised amongst members of the Down's Syndrome Medical Interest Group (DSMIG). Family support workers and charity workers from the Down's Syndrome Association (DSA) and Down's Syndrome Scotland (DSS) were approached via contacts already known to the FADES team. FADES local collaborators, community paediatricians, neonatologists and research nurses were also invited to take part.

Of the 18 interviewees; two-thirds were clinical (nine paediatricians and three research nurses) and a third were family support workers. Of those interviewed, five had a child with DS. Further demographics are not given as the relatively small professional community that works with these families means that the anonymity of the interviewees may be risked.

3.3.2 Interviews

A topic guide for the interviews was developed and informed by a literature review of parental adjustment to the diagnosis of DS and recruitment issues for neonatal studies. The guide included items which explored the interviewee's experience of working with families having a child with DS and their views on recruiting this group into research studies (Appendix 5). In keeping with the iterative nature of qualitative methods, the topic guide was revised following initial interviews. In addition, the topic guide questions were tailored to the profession or background of the interviewee. For example, clinicians were asked "What are your particular areas of interest / specialism?". The data were collected by GW through conducting semi structured telephone interviews with the participants. Recruitment ended when the interviews were no longer revealing any new information and data saturation had been reached. The interviews were between twenty-five minutes to one-hour duration.

Ethical approval

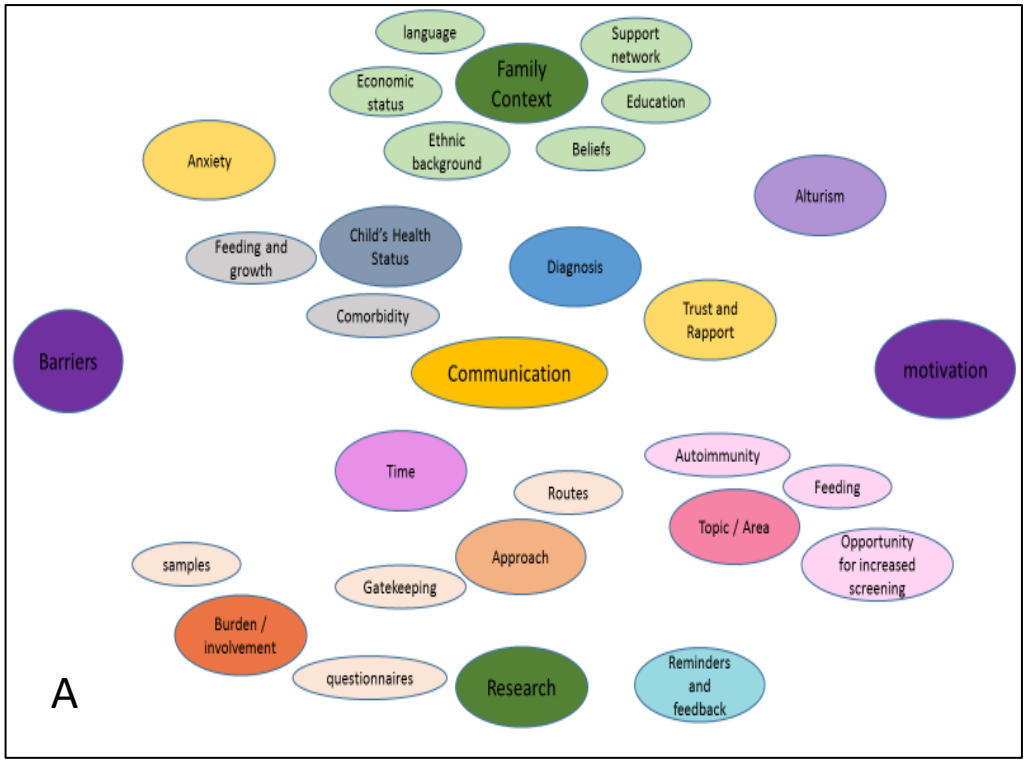
The qualitative study was approved by the South West Central Bristol Research Ethics Committee (14/SW/0030).

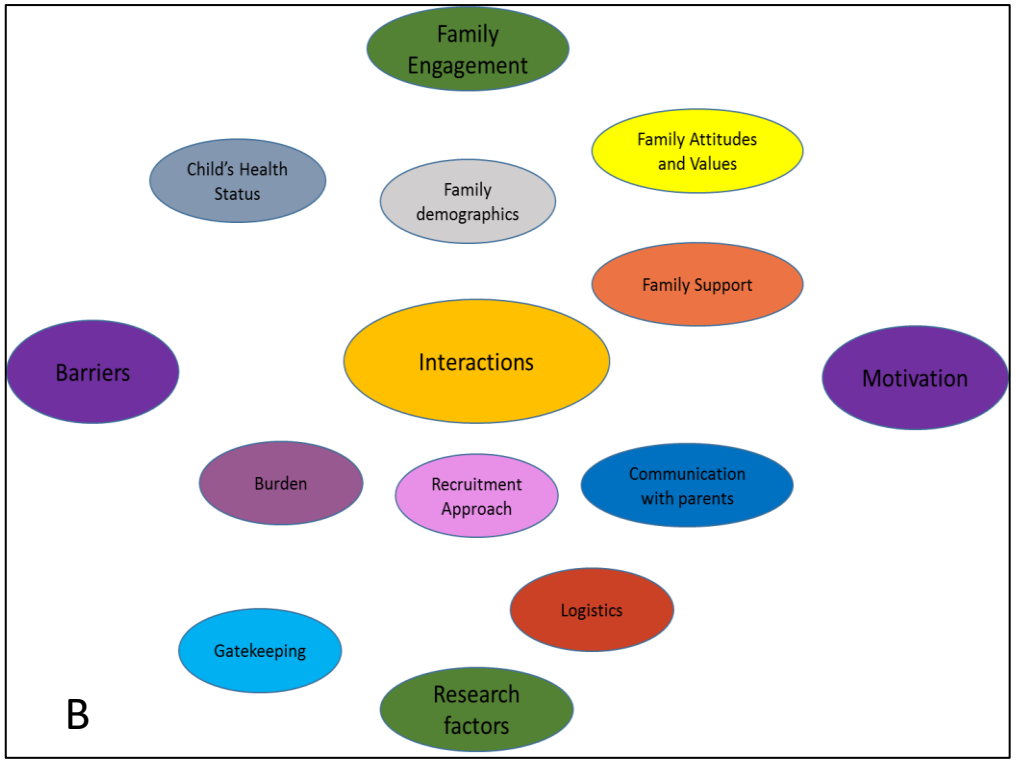
3.3.3 Analysis

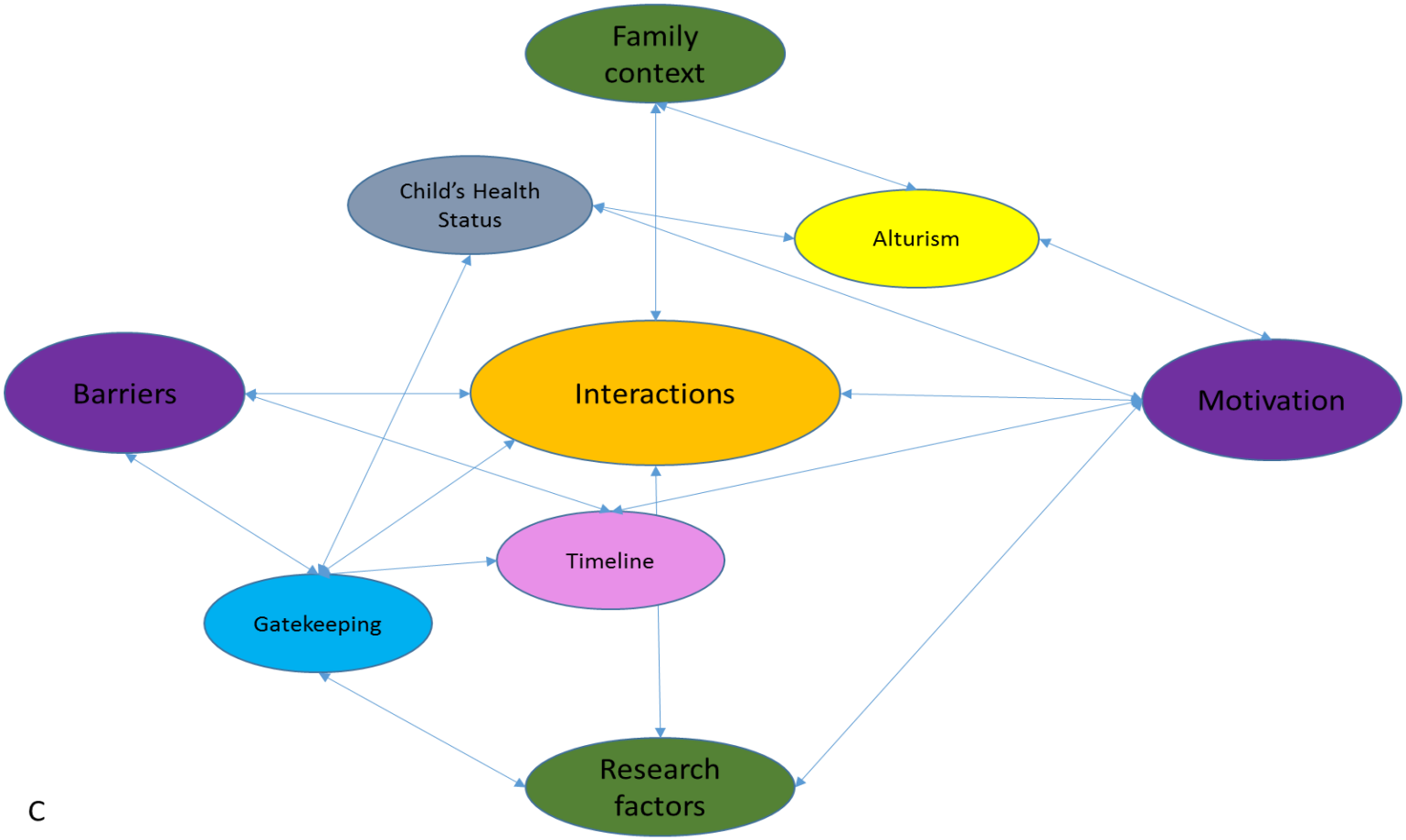
Interviews were digitally audio recorded and transcribed verbatim by a university approved transcription service. Thematic analysis was then undertaken following the guidelines stipulated by Braun & Clarke (Braun, Clarke, and Terry 2014). Thematic analysis is described as a flexible and useful research tool which can potentially provide a rich and detailed, yet complex account of data (Braun, Clarke, and Terry 2014). The interview dataset was managed in Nvivo and the data coded and analysed thematically. The coding process began with GW

and AS familiarising themselves with the dataset by reading and re-reading the transcripts. A sub-sample of transcripts (two consultants, one research nurse and one support worker) were then open coded independently by GW and AS. The initial codes were reviewed by AS and GW and disparities in codes were discussed until consensus was achieved. New codes were identified prior to coding the complete dataset. In the process of coding the dataset further refinement to codes were made before a definitive coding framework was established. Once the definitive framework was agreed GW coded all the transcripts in Nvivo V.10.

The final coding frame included the following codes: respondent's role and experience, communication, comorbidity and health, social and developmental needs, diagnosis, family needs and guidance, family response and reaction, recruitment, feeding experience and opinion, general research, potential factors impacting on engagement, study promotion and study protocol, study specific opinions, and timeline. Descriptive summaries of codes were reviewed by GW and AS which led to the development of mind maps and the identification of key themes from the dataset (Figure 22a) b) and c).


language
Support network
Economic status
Family Context
Education
Anxiety
Ethnic background
Beliefs
Alturism
Feeding and growth
Child's Health Status
Diagnosis
Comorbidity
Trust and Rapport
Barriers
Communication
motivation
Time
Autoimmunity
Routes
Feeding
Topic / Area
Approach
Opportunity for increased screening
samples
Gatekeeping
Burden / involvement
questionnaires
Research
Reminders and feedback
A


Family Engagement
Family Attitudes and Values
Child's Health Status
Family demographics
Family Support
Barriers
Interactions
Motivation
Burden
Recruitment Approach
Communication with parents
Logistics
Gatekeeping
Research factors
B



C

Figure 22a), b) and c): Mind maps used to explore emerging them

3.4 Findings

It was clear from the interview data that parents' motivation to enrol their new baby with DS into a birth cohort study depends on the family context, the parents' background, the health of the baby, and on the focus of the research being undertaken. These contextual factors contributed to forming the two main themes emerging from the dataset; these are 'Family context' and 'Research factors'. In addition,' Interactions' emerged as an overarching theme which crosses both other main themes and was associated with parents' extent of motivation to engage in research and engaging with health professionals. So thematic analysis led to the identification of three key themes; 1. 'Family context', 2. 'Interactions', and 3. 'Research factors'.

1. 'Family context' included the sub themes; 1.1'Family demographics', 1.2'Family attitudes and values' and 1.3'Child health status'.
2. 'Interactions' included the following sub-themes; 2.1'Communication with parents' and 2.2'Recruitment approach'
3. 'Research Factors' included the following sub-themes; 3.1'Burden', 3.2'Gatekeeping' and 3.3'Logistics'

1. Family context

1.1 Family demographics

Demographic factors such as the educational background, socio-economic status, ethnicity and the beliefs of families were described by health professionals to be potential barriers to engaging in research.

"The group of families that I work with in……, a lot of the time it's getting bread on the table and food and a roof over their head, and with immigration and a lot of social issues. Housing and immigration are very big issues for families where I work…. I think the more educated parents are more likely to consider it. I think a lot of the families that I work with come from cultures and communities where participation in research is almost unknown" (Community paediatrician)

"Certainly, we have some families who live in incredibly deprived circumstances and life being what it is these are also often the ones who may not be English first language speakers, so they may have difficulties" (Community paediatrician)

1.2 Family attitudes and values

Health professionals suggested that some parents are more likely to consent to a study given their "natural stance" and values.

"Having a fairly altruistic view of what's happening. That's what made me think about this mum. Because a mum being diagnosed with a Down's syndrome baby at 20 weeks is a bit of a shock; to be recruited at that time, and agree, and wasn't going to have that baby terminated. Yes, that type of stance gets selective, doesn't it? But that sort of a mum; a mum that says, "This baby has Down's syndrome. so, what?" (Research nurse who also runs a support group)

Altruism also featured as a big motivator for parents in taking part in research such that families wanted to give something back for the benefit of other families:

"Very few parents don't come back to me when I talk about a study saying, "Well even if it doesn't help my child if it helps with other babies in the future", then they want to do that. A lot of parents are altruistic from that point of view" (Paediatrician / Neonatologist)

"I think a lot of families just want to help, you know, they want to help any families that may be coming up in the future, and again it's usually the more positive people that want to do that, and the confident ones I suppose." (Family support worker who runs a support group)

There was a degree of concern about exacerbating any anxieties for families by inviting them to participate in research.

"I think maybe they might be concerned that you might be asking questions that might unearth some things that they're not ready to address." (Research nurse)

"Also the wish that the child is the same as everybody else and they don't want them to be medicalised at all" (Community paediatrician)

One health professional described parents falling into one of two groups: those that are either very involved or not psychologically prepared.

"Well in a way you have got more extremes there because you will have those, "I have got a child with a disability. I want to do everything I can for them or for others" and they are more likely to agree. Equally, "This is a shock to me. I am not used to this. I don't know what I am doing. There is so much to learn. There is so much to get my head around. I can't take that on board as well". (Research nurse)

1.3 Child health status

A significant factor for parents considering participation in a research study is their child's health. Around fifty percent of infants with DS have congenital heart disease (Roizen and Patterson 2003), potentially life threatening and requiring surgery in the neonatal period

which becomes a parental and clinical priority. Clinicians emphasised that although an important consideration, it should not preclude research engagement.

"With 54% of kids being born with some kind of cardiac condition, they may be the ones that are still in hospital further down the line, or kids who may have some kind of gut malformation will still be in the hospital for longer. For those parents, it may be that they haven't really come to terms or thought about the Down's syndrome. It's really more the getting the health issues seen to". (Family support worker)

"You are involved with so many things when the baby is first born. You have got your health visitor, your specialist health visitor, your midwife and then you have got your paediatrician, your dietician, your physiotherapist if you are lucky and your speech therapist if you are extremely lucky and portage. The eye clinic, the ear, nose and throat clinic, the general clinic, there is everything as well as trying to do your normal stuff like your normal baby clinic.

You sometimes think, "this baby is not mine everybody else has got a share." I suppose you can get quite overwhelmed with the amount of other people being involved with your baby and you might just think, "No, I can't cope with any more at the moment" (Family support worker and mother of a child with DS)

"Our children are often having heart operations in that early stage….that's the major, major thing isn't it?

Interviewer: Yes, yes.

Respondent: They're just trying to keep them alive." (Family support worker and mother of a child with DS)

2. Interactions

2.1 Communicating with parents

Health professionals emphasised the importance of effective communication when sharing the diagnosis of DS and recruiting families. Engaging parents in a study requires the involvement of someone with an established trust and rapport with that family. The interviewees identified these people as doctors, allied health care professionals, support workers and other parents.

"I guess there are multiple ways. The impact of the parent groups cannot be underestimated, as I've just said. So, through the Down's Syndrome Association, local parent groups, and making them aware. Then, of course, people who have regular contact with the families ... if you've got somebody who the parent trusts introducing the idea of the research, they're more likely to be receptive to it, and think, "Yes, that sounds like a genuine thing that we should consider."(Community paediatrician)

Poor communication around the time of diagnosis and when discussing the outcomes for children with DS makes a lasting impression on families. This may affect future relations with medical professionals and researchers.

"I mean if you've had a bad experience, and the whole thing about being told about your child's diagnosis, well it's always going to be a difficult memory, I would imagine, but I do think whether or not people are going to want to engage with their clinicians in research has got to be affected by the way that their relationship started off. I'm sure it's related." (Family support worker and mother)

Explanations given for inadequate communication with parents were: lack of time, inappropriate timing, language barriers and the limited ability of some families to read and understand participant information sheets.

"I was with a speech therapist yesterday and she said that you're so busy and you're trying to get things right, but actually you say the wrong things because of your busyness. You know that these parents are going to remember exactly what you say years later…" (Family support worker, mother and health visitor)

"the length and the complexity makes probably the biggest difference and also we never actually check how well parents can read …I think sometimes their ability to read and understand what they've got to do is sometimes a challenge for some parents." (Paediatrician / neonatologist)

2.2 Recruitment Approach

Interviewees discussed different recruitment methods, most preferring face to face but also acknowledging the roles of social media, websites and invitation letters. Interviewees regarded the communication network between families (local parent baby groups and DS specific social media groups) as an important way to disseminate information regarding research.

"But if you had a paediatrician that you didn't feel was specifically interested and engaged with your child, particularly one of those who talks over the child like they don't exist or something then you are probably not going to be engaged with it…Whereas you might actually take it on board from a support group, your local Down's Syndrome group, other parents, even social media." (Family support worker and mother)

When a family might be most receptive to being approached for research recruitment varies. Each family has their own experience and response to having a new baby with DS. Participants suggested that greater awareness of timelines might help researchers interact with the families. For example, families who received an antenatal diagnosis of DS deciding to continue with the pregnancy may be more receptive to participating in research early.

"I think the adjustments again varies. And I think, to be perfectly honest some of those with a prenatal diagnosis probably get there quicker. Because if they are say, maybe finding out at 18/20 weeks or something by the time the baby actually arrives, a lot of them now – particularly with social media and things like that – have really got into the community and

feeling part of it And I think some of them probably would get on board quicker than those people who don't have a pre-natal diagnosis, you know, have to start from scratch and getting their heads around it." (Family support worker and mother child with DS)

The importance of parental bonding with their new baby and recovering from birth means that many felt there should be a period before introducing the idea of a study. How long this period should be was unclear but could be guided by the response of the parents and the expertise of the staff caring for them.

"I think the most important is to try make sure that mum, parents have some time with the child before you actually rush in and upset everything" (Consultant neonatologist)

"I think in a sense, you kind of have to let the SCBU staff judge it, perhaps, because I'm really not sure when would be… I think that you could capture that sense that certainly a lot of dads seem to have of, "We've got to do something about it; we need to find out absolutely everything there is," and that might be an opportunity.

But on the other hand, I suspect that you've also got to ensure that all that normal bonding stuff is happening as well, and that the whole talking to people about being a part of a research study when they're still getting their heads around questions like, "Can I love this child?" and, "Is this child going to be part of our family? You know what I mean? All those real difficult bits.

I would be guided by what the staff that are looking after the babies are saying, or if you're in touch with support groups, what they're telling you as well, because we do get quite early contact from families sometimes." (Family support worker and parent)

The timing of recruitment will depend on aspects of the diagnosis. One community paediatrician referred to parents who were unwilling to engage with them until the results of the karyotype had been given.

"What I know is that practically speaking when it is an unexpected diagnosis however much you tell them you are certain that is Down's syndrome or as certain as you can be a lot of families will say, "Okay we will wait for the blood test result thank you very much. Come back and have a conversation with us in three days' time or whenever we have got the karyotype back." Very often clinically we have to do that. Some families won't agree to have an echocardiogram until they have got the blood test result back because we might have made a mistake and the baby wouldn't need it. I think with some you won't really be able to get any further until you have got that result back." (Community paediatrician)

3. Research Factors

3.1 Burden

For any parent with a new baby, life can be chaotic. Thus, involving them in research requires the design to fit around other demands. Interviewees talked about the increased

challenges which parents of a DS baby may have and how research might accommodate these.

"Well it is the time – both the time taken to do it but also the timeframe and how it fits around where they are … if it is invasive tests or something that having the flexibility to fit that around what they are already doing, if they are already doing lots of hospital visits and things like that. So yes I think that is the biggest barriers …sometimes it is things like childcare for other children because they are quite happy to go along with the child with Down's Syndrome and participate in something. But if it is supported…" (Family support worker)

"It depends on how onerous it is for them. Things that involve a lot of hospital visits are not good. Things that involve lots of blood tests are not great for families with children. There are those sorts of practical obstacles, "We would like to do it, but we don't think we can cope with all the demands made of us." (Community paediatrician)

Some health professionals felt that being part of a research study may be a supportive experience helping parents at a difficult time.

"Sometimes they probably just need someone who is there special for them that they can talk to who if they can't find an answer they can go off and find an answer for them and come

back to them …That is where the research nurse can come in and do the extra. "You are special to me and I am going to help you." (Research nurse)

3.2 Gatekeeping

Before eligible families are even approached there were several barriers highlighted by the interviewees. Institutional approvals are required and once approved, "recruiters" place restrictions on whom they will target either consciously or subconsciously. To be able to contact all potential families and minimise gatekeeping both, health services and voluntary organisations / charities should be used for recruitment:

"I do think there is something to be said about a sort of two-pronged approach. … within the hospitals and SCBU staff, there is this kind of gate-keeping thing going on. … people have to find us via the DSA website; despite the fact we've left bits and bobs that tends to be the way people find us. So part of me worries that there is a bit of overzealous gate-keeping going on sometimes….." (Family support worker and parent)

Many of the interviewees were concerned about asking families to be involved in studies where blood tests need to be taken. One however described how this might be misguided gatekeeping.

"I think people are much, much, much more willing to have blood tests done on their children than they used to be. Much less worried about it. So I think me saying

them being worried about an extra blood test at six months, I think that's probably more in my mind than it is in theirs." (Community paediatrician)

Some of the paediatricians described how all eligible participants should be informed about research that they could be involved in.

"Generally, parents are very keen to be offered it even if, and I always stress that they don't have to take part at the end of the day, but it's important to offer it to all parents even if they don't want to." (Paediatrician / Neonatologist)

"I haven't had any very negative experiences of people just saying, "How dare you approach me about research, I don't want to do it." Even they if they say no it is usually, "I am really sorry I can't help you." (Community paediatrician)

3.3 Logistics

Opportunities to interact with families and recruit into studies may be infrequent. Some babies will have a period in a neonatal unit and this was described as a good time to introduce research. Those with cardiac and bowel problems may have multiple appointments but this was perceived as a more unsettled time when parents may not be able to think about enrolling into a study

"They may be in the special care unit for an extended period of time in which case the neonatal consultant would be, you know, most suitable to do that." (Community paediatrician)

"I think one of the biggest factors that I would say from our point of view certainly is the health of the child. I mean most of them these days are having surgery if they need it at three to six months. And probably depending on what you are looking for, you are probably not going to get as many of them engaged until you have got through that post-op bit if you know what I mean. Because they have got a lot of hospital visits and check-ups and everything… So, I think they are probably less likely to engage until they have got through that surgery bit." (Family support worker and mother)

If a baby with DS has no additional medical issues the community paediatrician follow-up appointments may vary. Most health trusts adhere to current guidelines: following up at three and six months then annually until five years.

"The problem you will have is that everywhere does it differently. Some places like us: the baby is born and then they become the responsibility of a Community Paediatrician. Some areas: the hospital doctors keep them until one or two, and so it's very hard to know which group of doctors to approach. You have to find out, in each area, how they manage children with Down's syndrome and make sure your message is getting to the right people." (Community paediatrician)

"Having made the initial contact postnatally hopefully we normally get them back at three months. Then in the first year we will see them a couple more times. Up to the age of five we routinely see them every six months. Once they have got to five and they are in school we see them every year unless there is anything going on medically which requires us to see them more often, which sometimes there is but very often there isn't" (Community paediatrician)

Finally, there was some consensus that three months may be a good age to approach families. Parents were described as having "got over" the new-born period and adjusted or adapted to the diagnosis of DS. This is also the time when paediatricians are seeing the babies for their follow up appointments in the community. Some however felt that families were not ready until a year or even two years if they had a bad experience at the time of diagnosis.

"If you don't get them in the new-born period then I think there is a sort of sense in which perhaps you wait until they come back to hospital for the first visit. In our case it would be at three months, but clearly other areas might have different policies. By then I think that is as good a time as any to do it. From then on I think you could do it at any time, but there is no problem with doing it at three months" (Community paediatrician)

"I don't think there is any general rule. We get those who are ringing when the child is probably two or three days old and they are there and ready. They are full of life of this new life and undertaking they have got and they are ready to throw everything into it from day one. Others

it can be years before they really… I have got friends now with young adults the same age as my daughter who still haven't really come to terms with it all 25 years later". (Family support worker)

"I wouldn't have contacted her just after the baby was born. I've left her alone. I haven't made any contact whatsoever for two-and-a-half months, so the baby is nearly three months now. That was when I felt it was appropriate to make the contact……

….. Well, no, not really. I think it just with working with disability for such a long time, and knowing that time is needed, really." (Research nurse)

3.5 Discussion of Chapter 3

This qualitative study aimed to understand factors involved in recruiting new families to birth cohort studies of babies with DS, to optimise recruitment to the FADES study. The main findings suggest that successful recruitment requires a variety of approaches. Although parents often have a good relationship with their medical team and like being recruited by this traditional route, trust can be marred by difficult experiences at diagnosis and poor communication. Health professionals may act as gatekeepers and alternative routes may circumvent this. Using social media, websites and parent groups were suggested as alternative trusted sources. Understanding the dynamics around the time of diagnosis and following months helps in planning study logistics. Making the timings of recruitment and data collection flexible, particularly when babies have other complications is important.

Interviewees did not discuss how patient and public involvement (PPI) could inform this type of research however PPI advice was sought for FADES.

This study has benefitted from the variety of opinions gained, particularly those of affected parents. The interviewees recruited included a diverse group providing a wide range of perspectives and all demonstrated great candour in their responses. The family support workers receive training in family support, but this is unlikely to include research recruitment. Thus, the responses they gave in relation to research likely represent their own experiences and opinions. The paediatricians were all members of the DSMIG or involved in FADES which may have caused bias. However, this group has a wealth of experience with DS families and research.

Discussion of reflexivity

It was important to be aware of reflexivity throughout this study. Reflexivity takes into account the researchers' demographic background, professional role, beliefs and experiences that may consciously or unconsciously influence the study. This influence may affect the manner in which the study is conducted, the participant responses, interpretation of data and the final conclusions made from the research (Sword 1999). As Berger wrote in his article (Berger 2015) reflexivity:

> "*means turning of the researcher lens back onto oneself to recognize and take responsibility for one's own situatedness within the research and the effect that it may have on the setting and people being studied, questions being asked, data being collected and its interpretation*"

A positive bias towards research may have been introduced as the interviews were conducted by myself a paediatric registrar and CI of FADES. Thus, the researchers' background, training and own experience of working with families of children with DS should be acknowledged. My epistemological position will undoubtedly have shaped emerging themes. Working with researchers who were not part of the FADES research team and had not previously worked with families of a child with DS meant that my position and views were challenged and as such reduced the potential for bias.

Context of interviews

Interviews were conducted by telephone rather than face to face, enabling participation from a geographically wide area. Although occasionally viewed as inferior to face to face interviews, Sturges et al (Sturges and Hanrahan 2004) found no significant differences and suggested there may be notable benefits; particularly allowing participants a degree of anonymity which may have increased the candour of our participant's responses.

Previous studies

Previously published research involving infants with DS is limited and details regarding recruitment is lacking. An HTA study exploring the feasibility of recruiting one to eleven year olds with DS into an otitis media, treatment study, interviewed clinicians and parents regarding recruitment. (Fortnum et al. 2014). Their conclusions were similar: any research needs to account for both the shared experiences of parents with a baby with DS and the variety of personal experiences. They also commented on timings noting that when a family might be able to participate will vary. However, alternative recruitment strategies were not

explored and more novel recruitment methods may allow some of the variation in personal perspectives and timings to be navigated.

Specific Issues in recruiting a cohort of infants with DS

This study has explored recruitment issues that are pertinent both to the recruitment of typical new-borns and those with DS (these include family demographics, family attitudes and values and research factors). The issues which are more specific to the recruitment of a birth cohort of babies with DS are summarised in the table below:

Sub theme	Factors relating to DS	Effect
Family attitudes and values	Increased stress compared to a parent of a typical newborn. Desire not to medicalise their child.	Potentially exacerbate anxiety. Careful and understanding approach.
Child Health Status	Associated medical conditions including congenital heart disease and gastrointestinal disorders. These conditions can be potentially life threatening and may require surgery in newborn period	Timing
Communicating with parents	Communication at the time of sharing the diagnosis of DS with the families	Affects interactions and trust Alternative recruitment methods.
Recruitment Approach	Interaction with many different organisations including medical, charitable and the voluntary sector.	Affects who establishes rapport and trust. Alternative recruitment methods.
Recruitment Approach	Diagnosis of DS is frequently unexpected. Time to adjust to diagnosis varies.	Timing
Burden	Children with DS often have multiple hospital appointments.	Flexibility and timing
Logistics	Health of the child. Timing of medical treatment for cardiac or bowel abnormalities. Variations in when these babies have follow-up appointments and with whom.	Flexibility and timing

Table 8: Summary of specific issues in recruiting to a birth cohort of babies with DS

Conclusion of qualitative study

This qualitative study provides insight into the issues surrounding recruitment of babies with DS. Recruitment should include the use of clinicians and alternative methods including, social media, parent groups, charities and websites. From the knowledge provided by those interviewed, families will have different experiences in the first few months of their child's life depending on whether they had an antenatal diagnosis, how they adapt and adjust to the diagnosis, related medical conditions and the support they receive. This information helped develop the FADES protocol which has the flexibility to allow families and their babies to fully participate dependent on their own individual and medical circumstances.

CHAPTER 4

Establishing the FADES Cohort

Chapter 4 Establishing the FADES cohort

4.1 Overview of Chapter 4

In this chapter, the processes involved in setting up the cohort are summarised. The time taken to gain approvals and the ease or difficulties experienced in establishing a national cohort study are described. The results of the feasibility of the study are given in terms of whether they met the feasibility objectives (these objectives are described in more detail in the relevant sections; recruitment, sample collection, questionnaires and retention of participants). These objectives were analysed over a three-year period from September 2014 until September 2017. The initial protocol had described that recruitment would be assessed over two years, but initial recruitment was very slow. An amendment was submitted to change recruitment methods and the assessment period was therefore extended to three years.

4.2 Setting up the study

The study recruited participants from across the United Kingdom. The need to recruit babies as close to birth as possible and before eight months of age with a relatively rare condition required a wide geographical area. The study was therefore designed so that wherever a potential participant was identified within the UK, NHS Trust Research and Development (R and D) permissions would already be in place. This allowed participants to be consented immediately into the study (the processes are explained in more detail in Chapter 2). The overall timeline for setting up the study is illustrated in Figure 23.

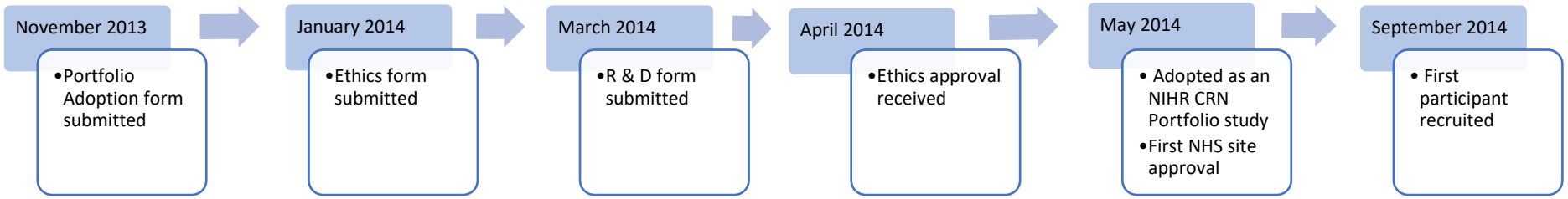


Figure 23: Timeline for setting up the study.

Footnote: Research and Development (R&D), NIHR CRN (National Institute for Health Research Clinical Research Network)

4.2.1 Approvals for FADES in England

In total, R & D permissions were sought from 318 primary and secondary care sites in England. Of these 68% approved, 17% abandoned the study and the remaining 15% neither approved or abandoned the study (25 of these sites never acknowledged receiving a valid SSI submission, and 23 validated the SSI submission but did not go on to complete local checks). Table 9 shows the time to approve or abandon the study. Of note, one site gave permission on the 2nd July 2014 and then withdrew from the study on the 19th September 2014 (they have been counted in the numbers that granted permission). Furthermore, Figure 24 illustrates that the majority of sites had approved the study within two months.

Time to:	Number of sites	Median (IQR) days
Valid *SSI submission received	293	5 (5, 5)
SSI submission validated	293	6 (6, 8)
Complete local checks	270	15 (13, 25)
NHS permission	216	18 (13, 26)
Abandon Study	54	18 (9, 48)

Table 9: Median time to approve or abandon the study in England.

Footnote: All dates were calculated from the 15th May 2014, the date that the first site, University Hospitals Bristol NHS Foundation Trust received the submission.
*Site Specific Information form (SSI)

To achieve this number of approvals, multiple emails, responses to queries and resubmission of forms were required. Points raised by some of the sites are illustrated in Table 10. Attempts were made to try and persuade sites to give approval. Some sites which initially abandoned the study or had not responded, did go on to approve the study in later years. This was

particularly true for sites where potential participants had seen the study advertised on the internet or social media and were keen to be enrolled but where the site had not approved the study. In these cases, the relevant site was contacted to advise them that there was a family in their area who wanted to join but site approval was needed. Similarly, some sites who had initially approved the study later abandoned the study usually due to poor local recruitment.

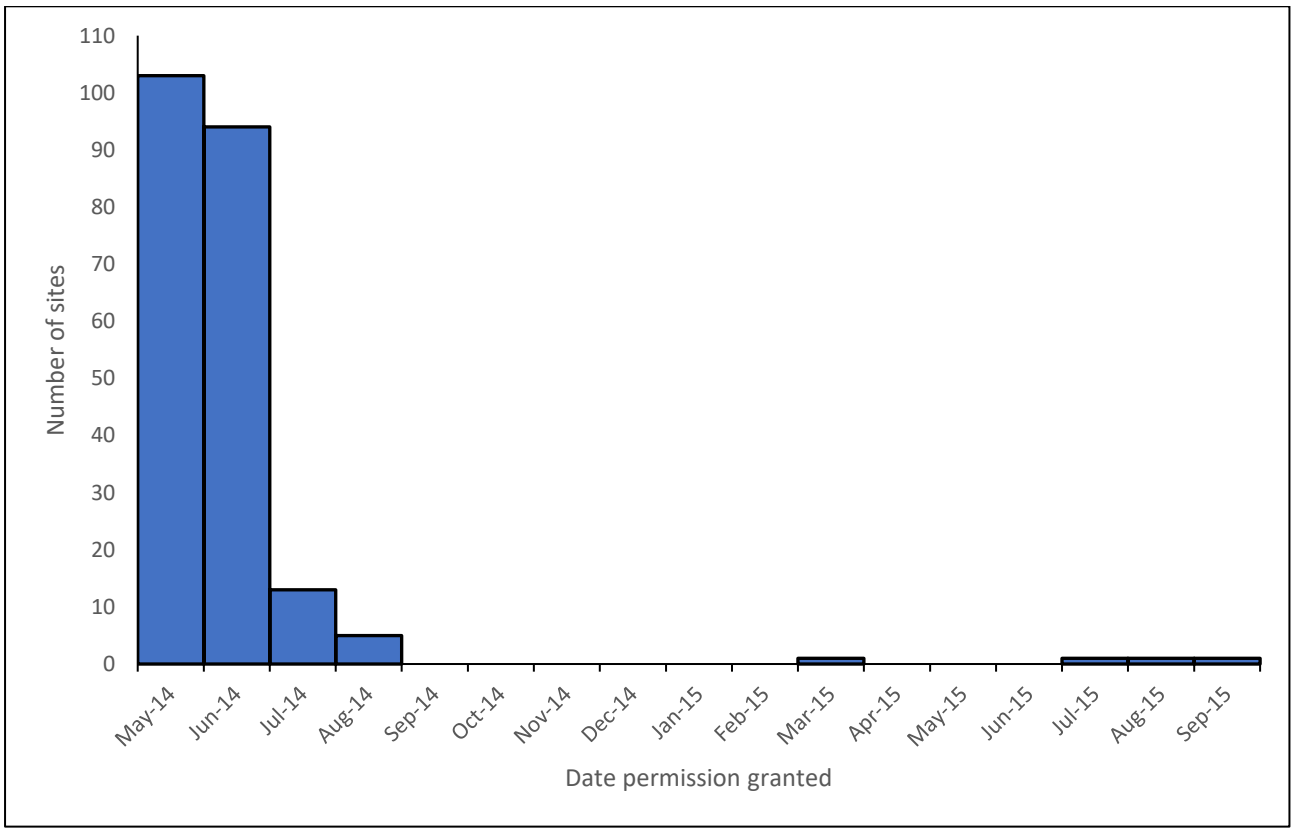


Figure 24: Dates of approvals for NHS sites in England. In total 216 sites gave approval the majority in May and June 2014.

Points raised by sites during the approvals process	Illustrative quote
Concerns over funding	"….. failure to meet this benchmark will affect the funding our site receives …… alternative choice to reject your study on the CSP system….."
Concerns regarding study setup	"Is this a secure website and will their data be protected and their ID anonymised?"
Requesting changes to study protocol	"The group suggests that clause 4 of the Consent form should also include informing community Paediatricians as these usually lead in the care of the child" "The group suggested the study title should say 'Feeding and autoimmunity in children with Downs Syndrome Evaluation Study'"
Concerns regarding the collection of blood samples	"What if the blood clots?" "Phlebotomy for small children involves 3 or 4 members of staff – a phlebotomist, someone to support the arm, a play specialist and admin support…I do not wish to appear unhelpful, but I consider that more thought was needed into these procedures and issues at the planning stage."
No concerns	"I needed to make a couple of checks that relied on other people, however they did not reply so I am going ahead with this and have attached the signed documents" "I am a Trials Manager at…… I've had a read through the study documents and protocol and it seems there is very little, if any, involvement locally so I just wanted to check I hadn't missed anything. Are you only requesting R&D approval in case a family is recruited from our Trust who may need to have their heel/ finger prick blood test done here?"

Table 10: Points and concerns raised by sites during the approvals process.

Footnote: Coordinated System for gaining NHS Permission (CSP), Research and Development (R & D).

4.2.2 Approval for FADES in Scotland

Permission was obtained from 12 of the 14 health boards in Scotland. NHS Orkney and NHS Western Isles did not give approval and no reasons were given for this. The median time to approval (from 15th May 2014) was 20 days (IQR 11, 41) (this was based on approval dates from eleven sites, the date was missing for NHS Ayrshire and Aran).

4.2.3 Approval for FADES in Wales

Permission was given from four of the seven Welsh Health Boards; Betsi Cadwaladr University Health Board, Powys Teaching Health Board, Hywel Dda University Health Board and Cwm Taf Health Board. The median time to approval from these sites was 19 days (IQR 17, 117) but it is worth noting that it took over 13 months to get approval from Cwm Taf Health Board. Aneurin Bevan Health board requested approval from local GP practices, and paediatric clinics to ensure they were happy to provide services. These local approvals were not sought due to time constraints and therefore approval from this health board was not gained. Abertawe Bro Morgannwg University Health Board did not receive an SSI despite it going through NISCHR and therefore did not approve the study. Cardiff and Vale University Health Board sent emails clarifying whether a local PI would be needed, they agreed a local PI was not required (following emails with Paediatric haematologist) but an approval was not subsequently received.

4.2.4 Approvals for FADES in Northern Ireland

Permission was gained from three out of the five Health and Social Care Trusts (the sixth HSCT is Northern Ireland Ambulance Service and is not relevant for this study). Southern Health and

Social Care Trust, South Eastern Health and Social Care Trust and Western Health and Social Care Trust gave permission. Median time to approval was 132 days (IQR 128, 168). Northern Health and Social Care Trust required a local PI, a local PI was not identified, and approval was not given. Belfast Health and Social Care Trust did not approve the study and no reason was given.

4.2.5 Study website and email

No technical difficulties were experienced with participants accessing the EOI registration form via the study webpage (NIHR Bristol BRU 2016). Emails from families, local collaborators and the research team were managed through the study email account (fades-study@bristol.ac.uk) and were checked at least twice weekly. The ease of communication via email allowed issues relating to consent or sample collection to be dealt with quickly and efficiently.

4.3 Feasibility outcomes:

4.3.1 Summary of Feasibility Results

Table 11 summarises the feasibility results in relation to the feasibility objectives. More detailed results are given in the following subsections.

Feasibility Objective	Target	Results	Confidence interval	Achieved
Rate of recruitment	100 infants per year	23 per year		No
Collection of mouth swab / DNA	75% to return initial sample before age 8 months	77.1% (54/70) median age of 5 months (IQR 4.0, 7.5)	(65.5, 86.3) %	Yes
	90% to be suitable for analysis	98.1% (52/53)	(90.0, 100.0) %	Yes
Collection of urine samples	75% to return initial sample before age 8 months	77.1% (54/70) median age 5 months (IQR 3.9, 7.5)	(65.5, 86.3) %	Yes
	75% to return 12-month sample before age 14-months	61.5% (32/52*) median age 12.7 months (IQR 12.3, 14.1)	(47.1, 74.7) %	No
	90% to be suitable for analysis	99.1% (108/109)	(95.0, 100.0) %	Yes
Collection of stool samples	75% to return initial sample before age 8 months	72.9% (51/70) median age 5 months (3.9, 7.5)	(60.9, 82.8) %	Almost achieved
	75% to return 12-month sample before age 14-months	60.4% (32/53**) median age 12.7 months (12.3, 13.5)	(46.0, 73.5) %	No
	90% to be suitable for analysis	Not yet tested so unknown***		Unknown
Collection of blood samples	75% to return initial sample before age 8 months	65.7% (46/70) median age 5.7 months (4.3, 8.5)	(53.4, 76.7) %	No
	75% to return 12-month sample before age 14-months	53.8% (28/52****) median age 12.8 months (12.4, 14.8)	(39.4, 67.8) %	No
	90% to be suitable for analysis	88.5% (92/104)	(80.7, 93.9) %	No (nearly)
Medical and Feeding questionnaires	75% to complete initial questionnaire	87.1% (61/70)	(77.0, 93.9) %	Yes
	75% to complete 12-month questionnaire	74.1% (40/54)	(60.3, 85.0) %	No
	60% of participants to opt to use the online questionnaire rather than the paper questionnaire	82% (58/70)	(72.0, 90.8) %	Yes

Table 11: Summary of feasibility objectives for recruitment, sample collection and medical and feeding questionnaires

Footnote: *16 participants were under the age of 12 months, two had provided samples after the age of ten months. **16 participants were below the age of 12 months; one had provided the six-month sample after the age of ten months. *** but feasibility study to determine the optimal method suggests that the samples will work****16 participants were below the age of 12 months and two had provided samples after the age of ten months .

4.3.2 Recruitment

The feasibility of recruitment was assessed against the following feasibility objectives

Rate of recruitment:

Objective: *to assess the rate of recruitment, with an initial target of 100 participants per year over a two-year period (this represents 20% of the DS families who are in contact with the Down's Syndrome Association (DSA) per year);*

By September 2017, 70 families had consented to the study. The overall recruitment rate for the study was 23 participants per year over three years. This objective was assessed over three years (September 2014 to September 2017) as recruitment in the first six months was slow and recruitment methods were changed in March 2015 (Amendment 1 see Chapter 2 Section 2.9). The recruitment rate increased from 1.5 participants a month in the first year up to 2.2 participants a month in the last year. The recruitment rate has remained below the feasibility target.

EOIs were completed by 100 people as shown in Table 12 between 1st September 2014 and the 1st September 2017. Of these, 11 were ineligible as their babies were too old (over the age of 8 months), one could not take part as they lived in the United States and one potential participant was ineligible because the child was in care and no longer with the birth mother. Of those that were too old to take part, many expressed how important they felt the study was. EOIs were completed by two sets of parents after they had received a prenatal diagnosis of DS for their baby and prior to their baby's birth. Both families were emailed to thank them for their interest and advised that the study team would be back in touch with them once their baby had been born. One of these families went on to consent to being in the study, the

other did not and no further contact was made. Reminders were sent to all (n=17) of those who were eligible and did not go on to consent after completing an EOI. The reasons why parents decided to take part or not were explored in the participant/ non-participant questionnaires (see Section 4.3.3). One local collaborator informed the study team that a potential participant who had completed an EOI did not go on to consent as the baby required cardiac surgery.

Number of EOIs completed	Number of EOI completed by potential participants that were eligible	Number of participants consented
100	87	70

Table 12: Number of people registering an interest in the study and the number who went on to consent.

Footnote: Expression of Interest (EOI)

Recruitment Methods

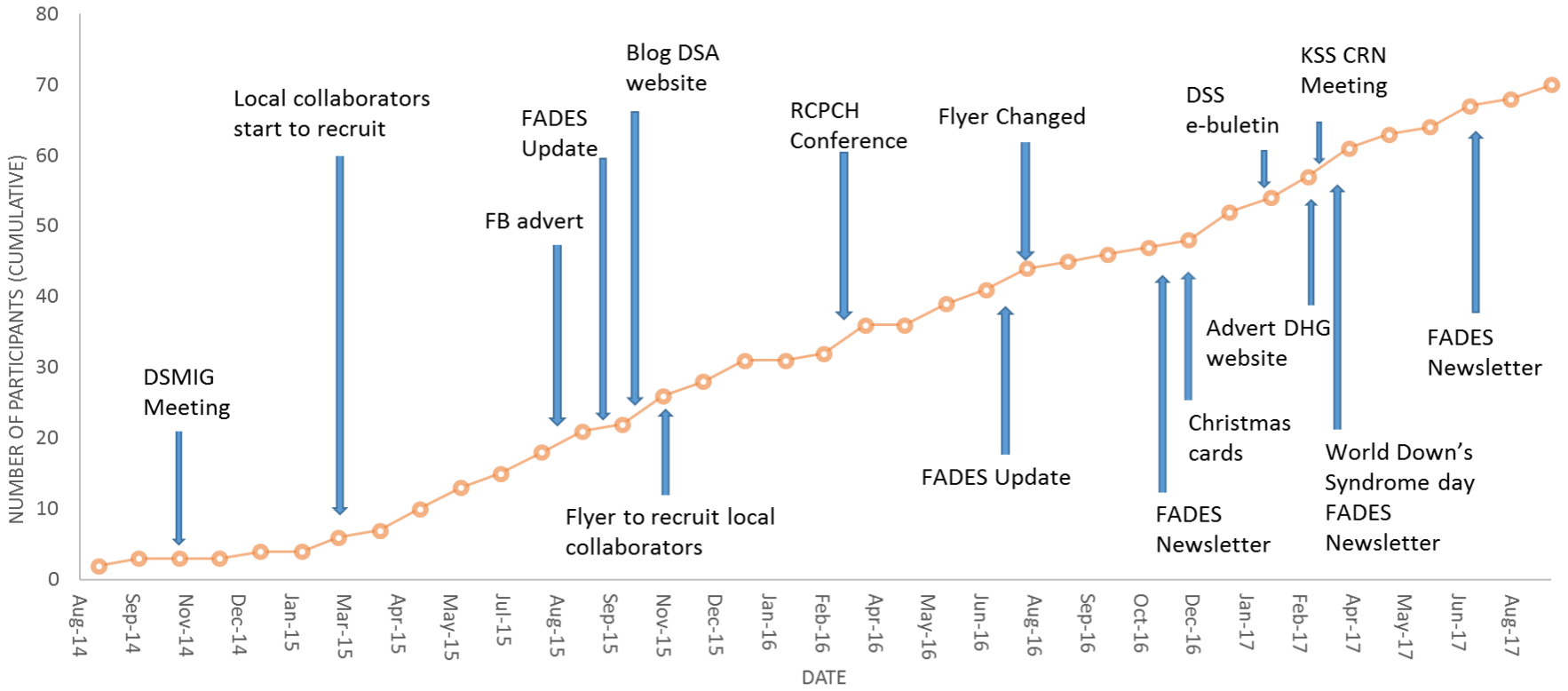
Objective: *to consider the feasibility of novel recruitment methods, including recruitment through websites and social media.*

Initial recruitment strategies solely via 'flyers' in new parent packs sent out by the DSA or given out by the DSS resulted in slow recruitment with only five participants recruited in the first six months. Alternative methodologies were instigated, and a large number of promotional activities took place as previously described in Chapter 2 Section 2.9. A

substantial amendment (Amendment 1) to allow local collaborators to actively recruit and consent participants was approved in March 2015. Figure 25 illustrates recruitment rates in context of the dates when promotional activities took place.

The time between completing an EOI form and consenting was a median of 13 days (IQR 7, 21). For many participants, reminders had to be sent to ask whether they had received the consent forms and if they were still interested and this often prompted them to consent. No families contacted the research team with questions regarding the study and no major concerns were raised by parents regarding the protocol prior to consenting. For those recruited by local collaborators consent forms were sometimes received prior to the EOI being completed. In these situations, the participants were unable to be fully enrolled into the study. For four participants consented by local collaborators, consent forms were received but the EOI was never completed and the participants were therefore not included in the study.

Parents were asked on the EOI "How did you hear about the study?" and were given options as well as a free text box for "other". This question was added to the EOI September 2015 (Amendment 4); participants who had registered with the study prior to this date were emailed to ask how they had heard about the study. Figure 26 shows the method of recruitment for the 70 participants who consented to the study. Recruitment from online sources (the combination of recruitment from the DSA website and social media) accounted for over half of the families. Recruitment by local collaborators did not begin until May 2015 and thus the number of participants recruited via this method as a proportion may be underrepresented.


NUMBER OF PARTICIPANTS (CUMULATIVE)
0
10
20
30
40
50
60
70
80
DATE
Aug-14
Sep-14
Nov-14
Dec-14
Jan-15
Mar-15
Apr-15
May-15
Jul-15
Aug-15
Sep-15
Nov-15
Dec-15
Jan-16
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May-16
Jun-16
Aug-16
Sep-16
Oct-16
Dec-16
Jan-17
Feb-17
Apr-17
May-17
Jun-17
Aug-17
DSMIG Meeting
Local collaborators start to recruit
FB advert
FADES Update
Blog DSA website
Flyer to recruit local collaborators
RCPCH Conference
FADES Update
Flyer Changed
FADES Newsletter
Christmas cards
Advert DHG website
DSS e-buletin
World Down's Syndrome day
FADES Newsletter
KSS CRN Meeting
FADES Newsletter

Figure 25: Recruitment of participants and promotional activities from September 2014 – September 2017.

Footnote: Down's Syndrome Medical Interest Group (DSMIG), Facebook (FB), Down's Syndrome Association (DSA), Royal College of Paediatrics and Child Health (RCPCH), Down's Syndrome Scotland (DSS), Down's Heart Group (DHG), Kent, Surrey and Sussex Clinical Research Network.

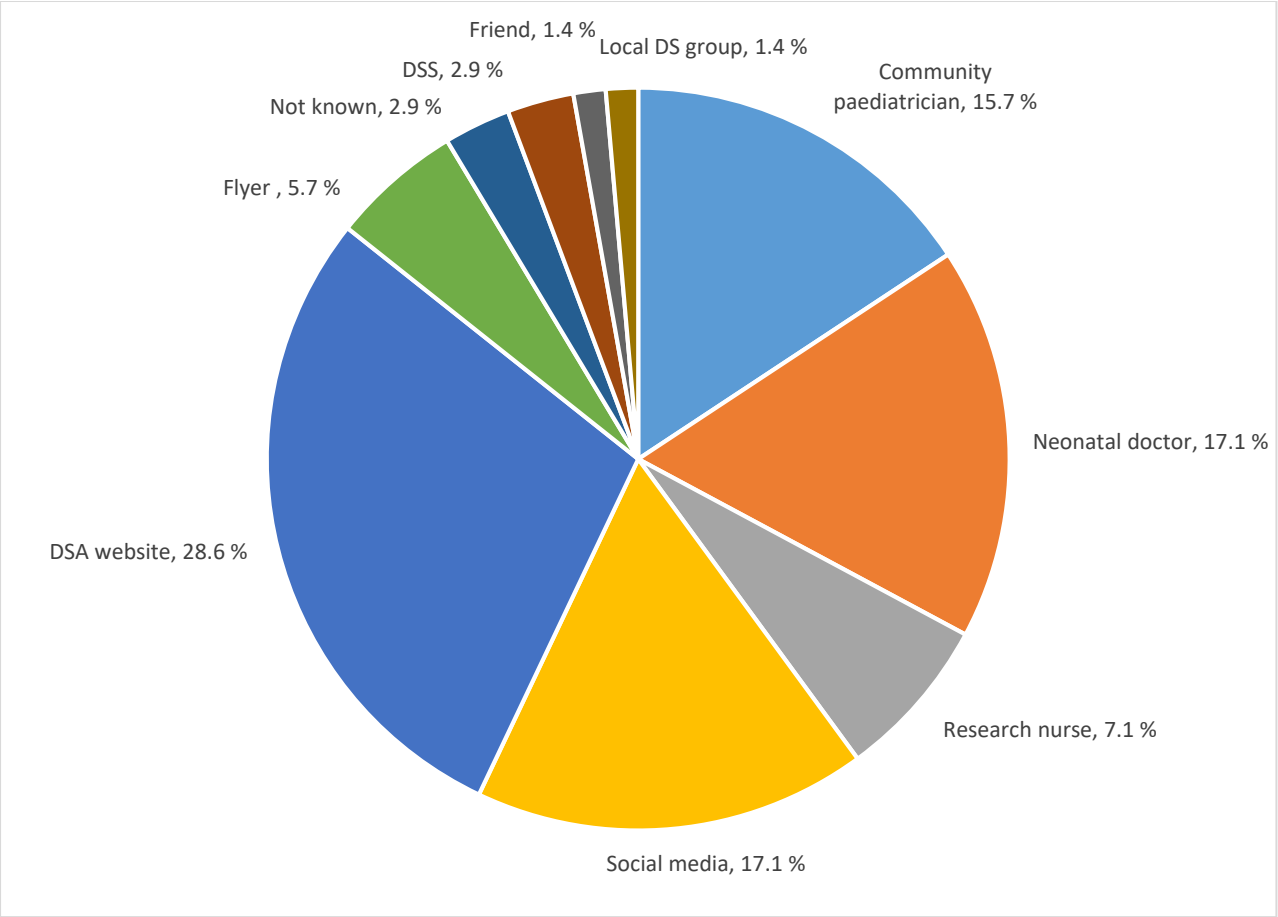


Figure 26: Recruitment Methods: proportion of participants who heard about the study from different recruitment sources.

Footnote: Down's Syndrome Association (DSA), Down's Syndrome Scotland (DSS)

Geographical distribution of participants

Participants were recruited from England, Scotland, Wales and Northern Ireland as illustrated in Figure 27. There is clustering of participants in some geographical areas and the reasons for this are explored in the Discussion Section 4.9 at the end of this chapter. The only problem encountered with web recruitment was that consent forms were occasionally incorrectly completed (in these cases the families were asked to complete another form correctly).

There was variation in the success of recruitment by different local collaborators and sites. Just under half of our participants 46% were recruited from 32 sites who only recruited one participant. Two or three participants were recruited from each of eight sites, several of which had research nurses involved in identifying and recruiting participants. There were three sites who recruited multiple participants. Queen Alexandra's Hospital, Portsmouth Hospitals NHS Foundation Trust recruited eight participants all through one enthusiastic neonatologist, Gloucestershire Royal Hospital NHS Foundation Trust recruited six participants through a very active research nurse and Norfolk and Norwich University Hospital NHS foundation trust recruited five participants. Recruitment at Norfolk and Norwich University Hospital NHS foundation trust had occurred after GW was invited to speak at the CRN East teleconference and promoted the study there, the study was then advertised amongst the CRN East Paediatric 'Research Champions'.

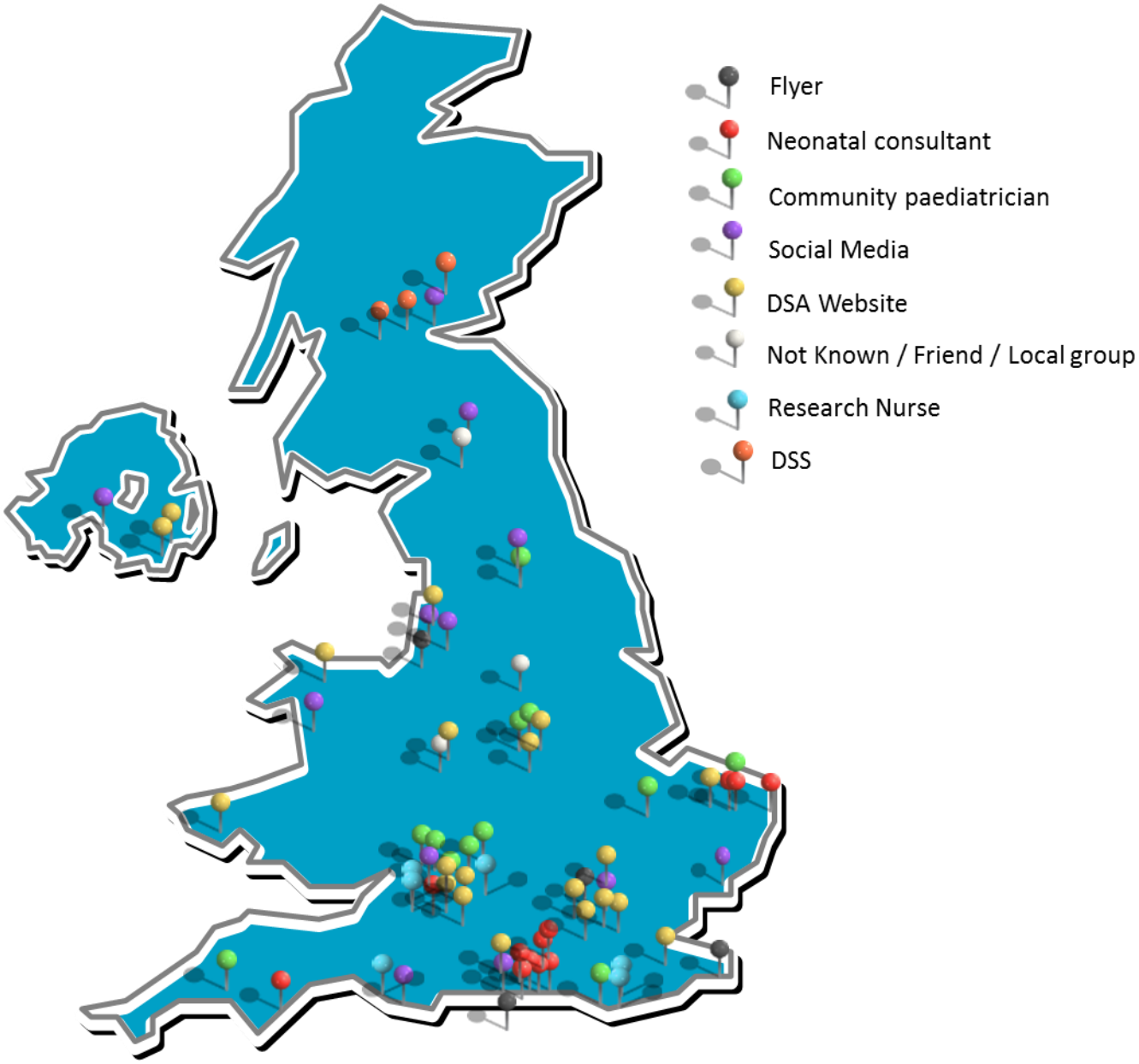


Figure 27: Geographical spread of participants recruited to FADES.

Footnote: Down's Syndrome Association (DSA), Down's Syndrome Scotland (DSS)

4.3.3 Motivations and barriers to joining the study

Objective: *to understand the reasons why participants consented to take part in the study.*

Engagement questionnaires (completed by active participants) were completed by 89%, 25 out of 28 that had been sent the questionnaires. Non-participant questionnaires (completed by families who had registered an interest in the study but who did not then go on to take part) were completed by 31%, eight of the 26 families who had been sent the non-participant questionnaire.

Engagement (Participant) questionnaire responses

Participants were asked in the engagement questionnaire "What initially interested you in the FADES study?", the responses to the question are summarised in Table 13. Half of the participants described altruistic reasons for participating. They also talked about the importance of research and how their own interests and experiences influenced their desire to take part.

Theme (the responses were not exclusive to only one theme)	Number of participants with this response	Illustrative quote
Research is important	32% (8/25)	"Interested in outcome, I think it is **important to participate in research**" "**Research is important** and wanted to explore if we could help"
Helping the community and children in the future with DS	52% (13/25)	"**Helping other families** with Children with DS" "Wanting to **help other people** if possible, in the future"
Contributing and being pro active	36% (9/25)	"We found out in pregnancy that ..had DS and were **keen to be as proactive** as possible" "We always **try and participate** in any research that will help with the DS community"
Feeding	12% (3/25)	"The new knowledge that can be produced to help people with DS and **my own interest in the role of nutrition** in health" "As it was **about feeding issues** in DS, we have had lots of issues in the early days"
Related to their own child's diagnosis and issues	16% (4/25)	"**My child** was born with DS" "**My son** has feeding difficulties
Parents own job / interests/experience	12% (3/25)	"**I work in health** and appreciate the value of research" "**Being a scientist** myself it was an opportunity to directly help with research that could benefit my daughter with Down's and future children"

Table 13: Participant's responses to "What initially interested you in the FADES study?" open responses were summarised into themes with illustrative quotes.

The responses to the question "Why did you decide to take part in the FADES study?" were similar but revealed some further information as shown in Table 14. This included the ease of participating in this particular study due to its design and the potential for participating in research to increase the health surveillance for their own child.

Theme (the responses were not exclusive to only one theme)	Number of participants with this response	Illustrative quote
Contributing and being proactive	36% (9/25)	"I find research interesting. I'm happy and proud that … and I do something which will help people with DS. Also, **it was something positive that we could do while coming to terms with the diagnosis"** "We always try and participate in any research that will help the DS community"
Ease and suitability	20% (5/25)	"It's an **easy, non-invasive**, research study which will help future generations" "Seemed very professional, **could be done from home with ease**"
Helping the community and children in the future with DS	68% (17/25)	"**Anything to help children and parents in the future**" "Great progress in caring for people with DS has been made in recent years. Our son will benefit from this, but it has been made possible by the research and volunteers that have gone before him. **We wanted to contribute to this and hope for even more improvements in the future**."
Health screening and benefit to their individual child	20% (5/25)	"felt that it could bring **benefits for my child** or others" "**I view it as additional health screening for my daughter** ... My daughter will benefit from research that others have done previously so it is only fair that we contribute to future research."
Feeding	8% (2/25)	"Might help prevent what happened to our baby happening with others. She developed NEC after being given **formula in NICU**. Also, so better advice can be given to new parents **regarding feeding**" "I have had a very successful exclusive **breast-feeding experience** with my daughter and wanted to be part of the study to show it can be done. I received lots of negative comments early on that said she wouldn't. Also want more the study to benefit future families"

Table 14: Participant's responses to "Why did you decide to take part in the FADES study?" open responses were summarised into themes with illustrative quotes.

In response to “How would you recommend we improve the information that is provided about the study?” 44% (11/25) responded positively about the information that is provided. One participant responded, “It’s perfect” another “It was rather clear to me and I’m not English”. Three participants commented that they liked the newsletters. Those who made recommendations suggested providing more information on the timings of the sample collection and the ease of collecting the samples. A couple of responses commented on the importance of having information that is easy to understand. One participant wanted to see more information on the scientific background and rationale for the study. Some participants had been unaware of the website or had not accessed it, and one participant said that they had had difficulty with the link to the online questionnaire.

Tables 15 and 16 show the opinions on the study website of those that took part with the majority finding the website attractive and not difficult to understand, although it is of note that a third found it only ‘somewhat’ attractive and two participants found the website ‘a little’ or ‘somewhat’ difficult to understand.

What did you think about the attractiveness of the study website?	
Responses	Number of respondents
Not at all attractive	0%
Somewhat attractive	33.3% (8/24)
Quite attractive	58.3% (14/24)
Very attractive	8.3% (2/24)

Table 15: Participant’s opinions on the attractiveness of the study website as rated on a Likert scale

Footnote: *missing data for one respondent on this question

How difficult did you find the study website to understand?	
Responses	Number of respondents
Very difficult to Understand	0%
Somewhat difficult to understand	4.2% (1/24)
A little difficult to understand	4.2% (1/24)
Not at all difficult to understand	91.7% (22/24)

Table 16: Participant's opinions on the comprehensibility of the study website as rated on a Likert scale.

Footnote: *missing data for one respondent on this question

Participants were asked "What improvements to the study do you think we could make to encourage families to take part?". As with the previous question, some participants commented that they felt no change was needed. Comments were made regarding issues around sample collection by seven participants, some highlighting that the need for blood samples may put some potential participants off. One parent suggested producing a credit card sized information leaflet that could be given/shown to health professionals when blood samples collection was needed. Another suggested that a doctor should go through the sample kit to demonstrate how to collect the samples. Further advertising of the study through social media was recommended by five participants and one participant suggested having an information sharing day to promote the study.

The following comment was made in the "any further comments" by one parent: *"Our hospital had a lead research promoter consultant who just happened to be doing his rounds on the first morning …child's name…was in NICU. He said as we knew about …child's name… diagnosis he felt he could mention the study to us but if we hadn't known he probably wouldn't have said anything as it would have been a delicate time. Although we knew about the study*

and signed up as interested, we might not have followed it up as we were in the throes of new baby land. I wonder how parents who find out after their child is born are approached?"

This comment illustrates potential gatekeeping that may occur.

Non-participant questionnaire responses

In response to why they were first interested in the study, six had said that they were interested in doing it to help with research for their own child or for people in the future. Two specifically said that they had been drawn to the study due to the area that this study was exploring.

Table 17 shows the reasons why people felt unable to participate or continue in the study, the most common reason being that families simply did not get around to it despite intending to. Parents found the website attractive as shown in Table 18.

Options given for "Why did you feel unable to participate or continue in the study?" (respondents could tick all that applied to them)	Number of respondents
Our baby was too unwell	0%
We meant to take part but did not get around to it	37.5% (3/8)
We decided not to take part because of the time that the study would involve	12.5% (1/8)
We did not want to take the stool, urine and mouth-swab samples	12.5% (1/8)
We did not want to take the blood samples	12.5% (1/8)*
We were worried about completing the questionnaires	12.5% (1/8)
Other	37.5% (3/8) **

Table 17: Reasons for not taking part in the study given by non-participants

Footnote: *this was not the same respondent who answered that they did not want to take the stool, urine and mouth-swab. This respondent added to the other/comments box "My son had been badly jaundiced after birth and was having bloods drawn twice daily from his feet. When they squeezed the foot to collect blood, the older holes would open up. Couldn't bear to choose to have more blood taken from him when it wasn't necessary"

**two said that they were told that their child was too old, one said that their doctor had not wanted to take the bloods as he had had trouble getting enough for routine blood sample.

What did you think about the attractiveness of the study website?	
Responses	Number of respondents
Not at all attractive	0
Somewhat attractive	28.5% (2/7)
Quite attractive	57.1% (4/7)
Very attractive	14.3% (1/7)

Table 18: Non-participant's opinions on the attractiveness of the study website as rated on a Likert scale

In response to the question "How difficult did you find the study website to understand?"

(they were given four options on a Likert scale as for the engagement questionnaire) all ticked

the response “Not at all difficult to understand” apart from one respondent who did not complete this question.

When asked “How would you recommend we improve the information that is provided about the study?” only one person made a recommendation and that was *“make it clear upfront about the level of commitment required”*. In response to the question “What improvements in the study do you think we could make to encourage families to take part?” two respondents suggested involving more health professionals (local community paediatricians and health visitors). Three asked if there was any way to make the sampling easier but did not have a recommendation for how to do this and one suggested that parents should be able to choose when to take part in the study.

4.3.4 Sample collection from participants

In this section, assessment of the feasibility objectives for the collection of samples from the participants is given. The feasibility of collecting each of the sample types, DNA, urine, stool and blood are assessed separately. The validity of the methods for the sample collection for urine C-peptide and gut microbiome was presented in Chapter 2 Section 2.15.

Objective: *to assess the ability of families to collect all the samples within the desired timeline, determined by the percentage of participants who return the requested samples in the time requested.*

The collection methodology would be deemed feasible if;

- *at least 75% of participants provide the requested initial samples before eight months of age;*

- *at least 75% of participants provide the requested 12-month samples before 14 months of age; and*
- *at least 50% of participants to provide all samples up to the age of five years.*
- *at least 90% of samples provided are adequate for analysis;*

As part of the feasibility assessment any issues or problems that occurred with sample collection were recorded. Families fell into different groups and which became clear through email correspondence when reminding them about samples. Below is a summary of those groups.

- Families who provided all samples in a timely fashion without any issues.
- Families who wanted to provide the samples but struggled with finding the time to collect them, needed reminders sent and occasionally lost the sample packs. Usually these families did eventually provide the samples.
- Families who had intended to take the samples but were daunted by the sample pack when it arrived, some managed to collect some of the samples but not all. These families tended to either fail to provide samples despite lots of messages to say they were going to or provided the initial samples but none or only sporadic samples after this.
- Families who always wanted to be in the study but had never really wanted to collect samples and didn't. These families usually did not respond to reminders or gave reasons as to why it would not be possible to collect the samples.

There were also families whose children had periods of poor health when there was a gap when they were unable (either due to lack of time or clinical reasons) to provide samples. Of note, there was one baby who initially spent a long period of time in the neonatal unit. He

provided samples during this time (presumably when the clinical staff were helping) but when he was discharged the family no longer collected samples.

Feasibility of collecting mouth-swab samples

Mouth-swab / DNA samples were received for 77.1% (54/70) of participants at a median age of five months (IQR 4.0, 7.5). This is above the feasibility target of 75% of initial samples to be received by eight months. The median age at which the samples were received is within target. DNA samples were only collected once.

DNA was extracted from 53 mouth-swab samples to date (see Chapter 2 Section 2.16.1.1) providing a mean concentration of 0.051µg/µl of DNA (range 0.003µg/µl to 0.142µg/µl)). The concentration of the samples was adequate for HLA testing but 9.4% (5/53) had a low concentration, of equal to or less than 0.01µg/µl and this level would limit what further genetic testing might be possible. Overall 98.1% (52/53) of samples were adequate for analysis which is over the feasibility target and HLA class II genotyping was completed on 52 samples. Although one sample had a detectable concentration of DNA, it was too weak for testing even after whole genome amplification. An additional sample will be requested from this child.

There were no issues raised by the participants regarding the collection of the DNA samples. Although the reasons for not returning a sample were unknown for the majority, two samples were reported lost in the post and further samples were not returned from these participants. There were also two samples for which the lysis buffer had leaked out of the bottle because the lid had not been secured properly after sampling, one of which could be used, the other could not and a repeat was requested but not returned.

Feasibility of collecting urine samples

Table 19 shows that the target of 75% for initial urine samples was achieved but not at 12-months. The median ages and IQR showed the samples were provided by the families close to the requested age. At least one urine sample was provided by 77.1% (54/70) of participants. Longitudinal samples at repeat time points were provided by 45.7% (32/70). This is below the target of 50% but only a proportion of the 70 participants will have been old enough to provide samples at subsequent time points.

	Initial	12 months	24 months
Number of samples received*	77.1% (54/70)	61.5% (32/52) *[1]	57.1% (20/35) *[2]
Median age at which samples taken in months (IQR in months)	5 (3.9, 7.5)	12.7 (12.3, 14.1)	24.9 (24.6, 26.4)

Table 19: Number of urine samples received at the requested time points and median age of the children at the time of sampling

Footnote: *The denominator for these is calculated from those that were the correct age for sampling at each stage i.e. from those that had been requested to collect a sample (and those that had not already provided a sample within two months of that time point). *[1] 16 participants were under the age of 12 months, two had provided samples after the age of ten months. *[2] 35 participants were below the age of two years.

To estimate a true reflection of post-prandial C-peptide secretion, information is also required regarding the timing of the sample in relation to feeds. Although the instructions provided to parents clearly stated that samples should be collected post feed, the actual timing of the sample post feeding, had not been requested initially. A request to collect this information was added to the sample sheet in Amendment 7 (July 2017). The time of last feed prior to the sample collection was available for 28.8% (30/109) of the samples (this includes three samples collected at three years of age). From the information that was given with these samples, the

median time post feed was 45 minutes (IQR 30, 60). This included three samples collected first thing in the morning, twelve hours after the last feed. For one sample, the parent had written “a few hours after feed” rather than an exact time.

The collection method used for urine collection to estimate C-peptide was recorded by 46% (49/106) of parents. The clean catch method was used for 36.7% (18/49) of these samples and 61.2% (30/49) used cotton wool (for one sample the parents said that they had used a combination of methods, and one sample leaked). Both methods obtained decent volumes for analysis (median 4mls). In total, 99% (108/109) samples were adequate, above the feasibility target of 90%.

A few parents commented that sample collection was difficult using either method. With the cotton wool there were issues with trying to get an uncontaminated sample as often the babies would defecate at the same time. Parents also experienced issues with getting enough urine from the cotton wool as per the quotes from parents below

“I got the stool one on Saturday and put it straight in the fridge, but the first set of cotton balls for the urine got pooed on, and I tried twice more on Sunday with flat cotton pads I had but only got a tiny drop out. I don't know if she is dehydrated in the hot weather (the poo wasn't particularly hard this time) but the nappies aren't very heavy even first thing in the morning when I haven't put the cotton pads in.”

“However, I'm really struggling to get a urine sample from child’s name. No luck yet with waiting poised with the specimen pot for him to pee. Cotton wool in his nappies hasn't worked either as the nappies are so absorbent.”

Some parents commented that using the clean catch method became more difficult once their babies were older and more active.

4.3.5 Feasibility of collecting stool samples

Whilst both the initial and 12-month stool sample collections did not meet the threshold for the feasibility study, the median ages when collected were acceptable as shown in Table 20. OMNIgene GUT stool sampling started after the feasibility project was completed in August 2016. These samples were not considered in the feasibility assessment. In 74.3% (52/70) of cases at least one stool sample was received and longitudinal, repeat samples from 52.9% (37/70) which is above the set standard.

	Initial	6 months	12 months	24 months
Number of stool swab samples received*	72.9% (51/70)	48.2% (14/29) **	60.4% (32/53) ***	54.2% (19/35) ****
Age at which samples taken in months Median (IQR in months)	5.0 (3.9, 7.5)	6.8 (6.3,7.4)	12.7 (12.3, 13.5)	25.0 (24.7, 26.2)
Number of OMNIgene GUT samples	18	5	6	14

Table 20: Number of stool samples received at the requested time points, median age of child at the time of sampling and interquartile range.

Footnote * OMNIgene GUT samples were only collected after Amendment 5 date August 2016, the ages at which these samples were collected were comparable to the ages at which the swabs were collected. ** Six participants were below the age of six months, 35 had provided the initial sample after the age of four months. ***16 participants were below the age of 12 months, one had provided the six-month sample after the age of ten months. ****35 participants were below the age of two years

Near recruitment, maternal samples were collected after Amendment 1, March 2015 so the numbers were not formerly assessed as part of the feasibility. However, there were maternal

stool samples from 60% of the participants and maternal stool samples collected using OMNIgene GUT from 24.3% of participants.

Repeat samples were requested for five samples. One sample taken into the OMNIgene GUT kit had separated into two layers and would not mix for an unknown reason, a further sample was therefore requested. Three samples were lost in the post and one sample was '*eaten by a pet'*. A few parents overfilled the OMNIgene GUT kit tube. Once received in the laboratory, the samples were frozen at – 80^{o}C for future analysis.

There were no major issues highlighted by the parents with collecting the stool samples either using swab method or OMNIgene GUT kit. Any issues expressed were around the child being constipated or having loose stool rather than problems with the kit as illustrated in the quotes below:

"The thing I'm struggling with is a stool sample. Child's name is teething at the moment and so her stools are particularly loose and just get absorbed into the nappy. I'm going to start weaning a bit early...starting tomorrow. So hopefully that will help a bit...she may just be constipated initially. I hope I'm not holding things up!"

"so far child's name has only had his bowels open twice a week with the help of lactulose. Usually catches us out in the middle of the night or whilst we are out."

Feasibility of Collecting Blood Samples

Blood sample returns did not achieve the feasibility target as demonstrated in Table 21 and the reasons for this are discussed in detail in the discussion (Section 4.11.4). At least one blood sample was provided by 71.4% (50/70) of participants, and longitudinal samples at repeat

time points were provided by 47.1% (33/70) of participants, which is below the target. Median ages were acceptable although the upper limits of the IQR were over the target. Not all of the participants will have been old enough to provide longitudinal samples.

	Initial	6 months	12 months	24 months
Number of samples received	65.7% (46/70)	31.3% (10/32)*	53.8% (28/52)**	57.1% (20/35)***
Median age at which samples taken in months (IQR in months)	5.7 (4.3, 8.5)	7.6 (6.4, 8.2)	12.8 (12.4, 14.8)	25.1 (24.6, 26.3)

Table 21: Number of blood samples received at the requested time points and median age of the children at the time of sampling.

Footnote :*Six participants were below the age of 6 months and 32 had provided their initial sample after the age of 4 months. **16 participants were below the age of 12 months and two had provided samples after the age of ten months. ***35 participants were below the age of two years.

Serum volumes (approximately half of the whole blood volume) greater than 30µl can be used for testing a panel of autoantibodies associated with T1D, thyroid and coeliac disease. Below this volume samples can be used for limited testing. The estimated mean sample volume was 107.5µl with 82.6% (86/104) of samples having a volume greater than 30 µl. Nine samples were less than 1µl, these were frozen whole and stored. Twenty-six blood samples were recorded as haemolysed and one sample had been incorrectly collected into an EDTA tube. Whilst these samples are suitable for antibody testing and thus do not affect the feasibility of this study, they might prove less useful in future studies. Of the samples received 88.5% (92/104) were adequate for analysis of anti-BSA anibodies, just below the set target of 90%.

Analysis of anti-BSA antibodies was therefore performed on samples from 53 participants (30 with longitudinal samples (see Chapter 6 Section 6.4).

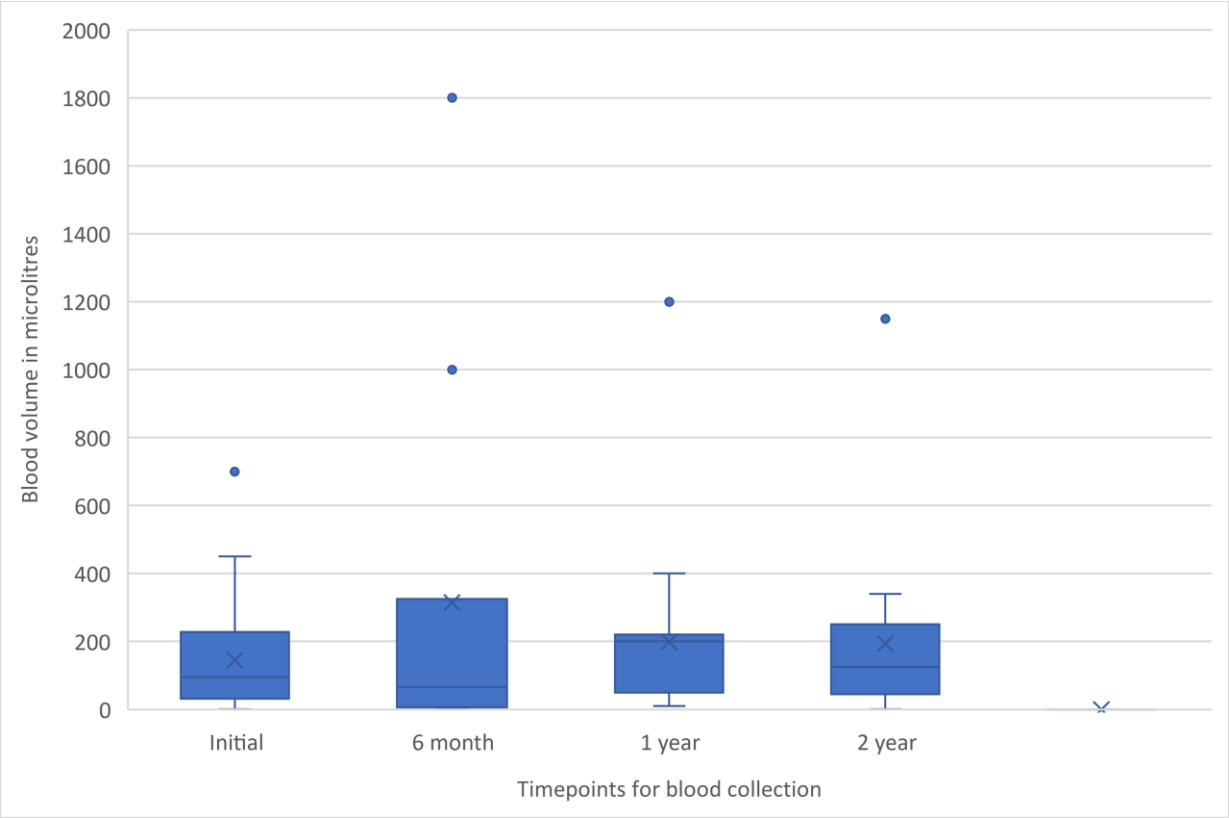


Figure 28: Sample volumes by collection time point, showing median and interquartile range. Outliers are also shown.

Repeat samples were requested only where it was felt appropriate to do so, for example when a parent emailed to say that it had been difficult to collect the sample and that they were happy to try again. Repeat samples were collected from seven participants, four due to difficulties obtaining the samples and three where the samples had been lost in the post.

The timing of blood sampling was challenging as the babies and children did not have blood tests taken at predictable time points. Parents were emailed to ask when their next appointment would be but sometimes they would have no upcoming appointments. Some

sites were unwilling to assist with sample collection. However, many sites were extremely helpful and had no issues with facilitating blood sample collection at their appointment. The list below summarise the routes via which participants had their bloods collected (GW collected five of the samples where the participants lived locally):

- families arranged the blood sample themselves with the health professional that they were seeing, or booked to have bloods taken by a phlebotomist or by their practice nurse at the GP without involving the research team;
- as previously described, families informed us of the date when they were due to see a health professional, and we then contacted the health professional and requested their assistance with collecting the blood sample (this usually required several phone calls and emails). Most were happy to help, but some cited a variety of reasons for not taking the samples including, that they would not have enough blood for the patient's routine samples, that they would not have sufficient time, that they were not setup for collecting blood samples, or that they were unclear about how they would be reimbursed;
- local collaborators helped either by arranging the samples or by research nurses meeting the participants at their appointments and collecting the samples; and
- parents took the samples themselves or the samples were taken by a family member who had clinical training (this only accounted for three participants, several families had tried to take the samples themselves but were unsuccessful).

Some participants and health care professionals experienced issues with the blood collection tubes. The tubes came in two parts - an inner tube which had a small end for capillary action

and a larger outer tube. Initially there were issues with samples leaking out of the inner tube, which was partly resolved by changing the way in which the tubes were prepared before sending them to the participants, with the small tube being sent already capped.

4.3.6 Retention of participants

The feasibility of retaining participants within the cohort so that longitudinal questionnaire data and samples could be obtained was assessed by the following feasibility objective.

Objective: *The feasibility of retaining participants in the study, which in parallel also determines the overall acceptability of the study was set by the following objective:*

1. *for compliance with the study protocol up until the age of five years old with a target of retaining at least 50% of participants.*

The target of maintaining participants in this longitudinal study was set at five years. For the purpose of this thesis however, retention of participants has been assessed up until September 2017 (none of the participants were aged over five years old at this stage). Of the 70 participants who were recruited within the three-year period there were six participants who did not complete any questionnaires or provide any samples at any time point. These included the very first participant enrolled onto the study, with no reason being given as to why they did not take part. The paediatrician of one child who was very unwell in the new-born period with cardiac complications in a paediatric intensive care unit, had advised that they would not be likely to be actively taking part in the study. Two parents emailed to say although they had wanted to take part, work and family meant that they were unable to

commit the time. For the other two participants, despite reminders and phone conversations they did not take part and no reason was given.

Retention of participants at a year was judged by those who were inactive, partially active (completing any of the requested questionnaires or samples at a year) or fully active (completing all requested samples and questionnaires). Table 22 shows over half of the participants who had ever been active in the study remained fully active and a further ~36% remained partially active. At two years 60.7% of the children (who were of the correct age) were fully active and therefore at that point on target to meet the feasibility objective of at least 50% remaining compliant with the study protocol. Figure 29 is a flow diagram showing the activity of the participants over the three years.

Compliance with Study	At 12 months	At 24 months
Fully active	53.3% (24/45*)	60.7% (17/28**)
Partially Active	35.5% (16/45)	17.8% (5/28)
Inactive	11.1% (5/45)	21.4% (6/28)

Table 22: Compliance of participants with study protocol at 12 months and 24 months. Where 'fully active' is completing all requested samples and questionnaires, 'partially active' is completing any of the requested questionnaires or samples and 'inactive' is not completed any questionnaires or samples beyond the previous timepoint.

Footnote:*six participants never completed any initial samples or questionnaires and have not been counted in this assessment. A further 19 participants were below the age of 12 months on the 1st September 2017. **36 participants were below the age of 24 months on the 1st September 2017.

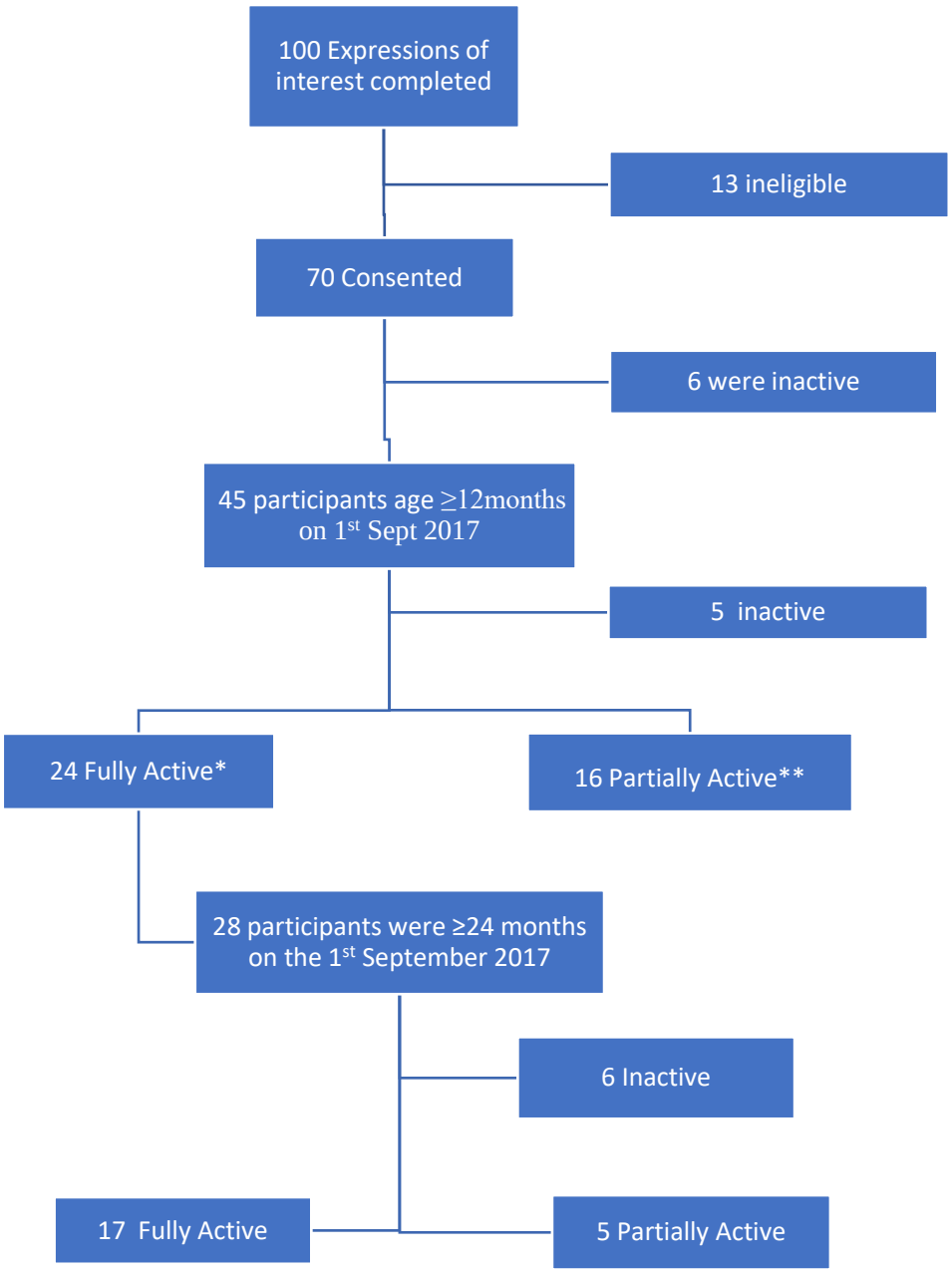


Figure 29: Flow diagram showing activity of participants over three years from September 2014 to September 2017.

Footnote * fully active meant that they were completing all of the requested questionnaires and samples. **Partially active meant that they continued to complete some of the questionnaires and/or samples.

4.3.7 Medical and feeding questionnaires

The feasibility for using the medical and feeding questionnaire was assessed, based on the following criteria.

Objective: *to assess the ability of the online questionnaires and paper questionnaires to collect the required data;*

- *for at least 75% of participants to complete initial, seven and 12-month questionnaires;*
- *for at least 60% of participants to opt to use the online questionnaire rather than the paper questionnaire;*
- *for at least 50% of participants to complete the annual questionnaires up until the age of five years; and*
- *for the data produced to be valid and easily converted into a form, which is ready for analysis.*

Table 23 shows that the feasibility target of 75% completion rate is being exceeded for the initial and 7-month questionnaires. Of nine participants who did not complete the initial questionnaire, six were inactive throughout the study and the remaining three completed the questionnaires after the cut-off date of 1st September 2017 (two had only enrolled within days of this cut-off). For the 12-month questionnaire, one family did not complete the questionnaire until after this deadline. If they had contributed in time, a completion of ~76% would have been achieved. Of note, 15 participants completed the initial and 7-month questionnaires contemporaneously given mean age of recruitment. Two participants completed their 7 and 12-month questionnaires in parallel. All participants who completed

questionnaires at more than one time point completed them in temporal order, so the previous questionnaire data was available during the analysis.

	Initial questionnaire	7-month questionnaire	12-month questionnaire
Number of completed questionnaires	87.1% (61/70)	84.4% (54/64)*	74.1% (40/54) **
Age of child at time of completion in weeks Median (IQR)	20 (13 - 29)	31 (29 - 34)	52 (51 – 55)

Table 23: Number of completed questionnaires and actual age of child at the time of completion.

Footnote: *six participants were below the age of seven months. **16 participants were below the age of 12 months

Online questionnaires were requested by 80% (56/70) of participants in their EOI form. Due to an administrative error, four participants who requested a paper questionnaire were sent the online link and completed the questionnaires online. Paper questionnaires were sent out when online questionnaires had not been completed to prompt a response or if a participant was struggling to complete the online questionnaire. In total 82% of the initial questionnaires were completed online which is above the feasibility target.

Importantly, the most frequent issue with the online questionnaires arose from there being no 'save and come back' feature within the web-based questionnaire, so the questionnaire had to be completed in one sitting. Therefore, some questionnaires were received online that were only partially completed or duplicated.

The data obtained were easily exported into Excel files and into Stata (as described in Chapter 2). An issue experienced with exporting the data was that many of the variable names were too long for Stata and new variable names had to be created. Several errors were identified during assessment of the data which were related to the branching of the questions. For some participants, they were incorrectly branched away from questions that they should have answered. These errors are described in more detail in Chapter 5.

The results of the secondary objectives are given in Chapter 5 “Analysis of Medical and Feeding Questionnaires” and in Chapter 6 “Initial analysis of Clinical Samples from FADES cohort”.

4.4 Discussion of Chapter 4

In this section the results presented in this chapter will be discussed particularly in relation to the feasibility of the study. A feasibility study should address the important overarching question as to whether the planned protocol will successfully lead to the collection of the desired data. Importantly this study successfully recruited participants from across the UK who completed all requested longitudinal questionnaires and provided all the desired samples. Although recruitment was below target, 70 participants were recruited which makes this one of the largest cohorts of babies with DS in the UK. The study met the feasibility targets for the collection and analysis of DNA (mouth-swab samples) and collection and analysis of initial urine samples for urine C-peptide. The target was almost met for the collection of stool samples for microbiome and despite not achieving the feasibility target, collected blood samples from 66% of participants, 89% of which were suitable for analysis. The retention of participants in this cohort was a strength as higher rates of attrition has been observed in

previous cohorts of babies with DS. The paper by Orsmond et al describes "the distinctive features of a feasibility study" (Orsmond and Cohn 2015). The table in Appendix 27 summarises this study in relation to these.

The study protocol evolved and adapted over the three years in which feasibility was assessed. This is particularly true in relation to recruitment methodology. Assessing feasibility has its own inherent issues, by setting a date at which feasibility will be assessed, some participants were counted as not having completed a questionnaire or a sample which may then arrive the next day. In a study designed for flexibility, built in delay of a few weeks or even months is pragmatic but in terms of achieving feasibility targets may not be acceptable.

Setting up a UK wide cohort of babies with DS was feasible but required considerable time and effort to complete all the required processes and obtain all the necessary permission. The individual aspects of the study design and set up are discussed in the sections below with the strengths and weaknesses highlighted.

4.5 Discussion of Study Set-up

Ideally a feasibility study should include a focus group or steering committee with patient or participant representatives (in this study new parents of children with DS). Not having a pre-arranged focus group for this study could be considered a weakness but this was not possible due to time constraints within the PhD program. The study was however designed with consultation from professionals and families through the DSA, DSS and a local DS group.

The eligibility criteria led to the recruitment of appropriate participants which was a strength. Although the study is a birth cohort, there was a cut-off age of 8 months. This gave time for

families who needed to adjust to their baby's diagnosis and where necessary urgent medical needs to be addressed.

Ethics and R and D

Rigorous governance is necessary to ensure that research is ethical and valid. The processes required in order to initiate this study were slow and prohibitive and this has similarly been noted in other studies (Thompson and France 2010, Snooks et al. 2012, Elwyn et al. 2005). It took six months from completing the initial Portfolio Adoption Form to receiving the first NHS site approval and then a further three months for sufficient NHS sites to be in place to start recruitment. Despite these delays however, 68% of the 318 primary and secondary care sites across the UK gave permission for the study which is testament to the study design.

With this breadth of national sites, as soon as a baby was identified or self-identified by parents, they could be enrolled immediately wherever they were born in the UK. By gaining permissions across the UK ahead of recruitment, delays as a result of lack of permissions were minimised. Although this involved a significant amount of time, effort and multiple emails only a couple of participants were born in areas where permission was not already in place.

Amendments

Amendments during the three years improved the study and corrected omissions in the original protocol which were revealed during the study. For example, Amendment 1 which allowed local collaborators to recruit helped increase recruitment rates. Amendment 7 in which the sample sheets were changed to obtain information on the timing of feeds in relation to the collection of urine samples for urine C-peptide helped interpretation of data. The process of seeking amendments was time consuming and required the necessary

approvals from ethics and local R and D sites. It may have been advisable to have included more flexibility into the original ethics and R and D forms as suggested by Hagen et al. in the quote below.

"We suggest that a Formal Feasibility Study should allow investigators to improve the conduct of the trial based on what they learn as it is implemented. Investigators should include in the original study design their explicit intention to change the patient assessment process if required, and have these changes preapproved as part of the initial submission for scientific and ethical review." (Hagen et al. 2011)

Site Approval

The concerns raised by sites unwilling to be involved varied and highlighted that internal processes were not standardised from one NHS trust to another. Full reviews of the study akin to an ethics committee meeting were undertaken by some sites whereas at other sites it was more of an administrative exercise. Some of these issues may be improved for future studies with the changes made by the introduction of HRA approvals (Salman et al. 2014, HRA 2019).

NIHR Support

Help and support was provided from the NIHR CSP team in disseminating information about the study to all of the primary and secondary care trusts and in completing the complex steps described in Chapter 2 Methods. Having access to CSP was the most valuable resource for this study and without it, involving so many sites across the UK would have been impossible (CSP was changed over to HRA approval in March 2016).

Unfortunately, it was not always clear what help was available from the NIHR Portfolio as it appeared to differ from one clinical network to another. This was also true between different NHS trusts some offering the support of research nurses to help with recruitment and blood sampling while others did not. This was partly due to available resources in the different trusts but also to the way that sites managed their accruals. This variation between networks has also been noted in other studies (Stock et al. 2016).

Approval from Scotland, Wales and Northern Ireland

Approval from the devolved nations again varied greatly, gaining approval from the Scottish Health Boards was straightforward and worked smoothly through the central NHS Research Scotland Coordinating Centre (NRS CC). For Wales each of the seven Health Boards had to be contacted separately and they had different requirements with regards to the need for named clinicians responsible for the study. There were delays in getting permission in Northern Ireland as they were themselves unclear of the best way to proceed with such a study covering many sites and it took some time to decide whether they would accept a single SSI.

4.6 Study Website and email

The study website and email system proved invaluable with the study covering a wide geographical area and participants being able to self-identify. The use of websites in research studies provides easily accessible information for participants and other research sites. In this study, the website enabled participants and researchers to download the participant information sheet and to register their interest in the study. Other studies have included protocols, contact information, FAQs sections and even secure password protected areas for

confidential information (Paul, Seib, and Prescott 2005). Feedback on the attractiveness and understanding level of the website from the Engagement (Participant) / Non-participant questionnaires was positive as discussed in more detail (Section 4.3.3). Expanding the website to include more information for families, links to study newsletters and further information for local collaborators would be beneficial. Despite the internet being increasingly available it must be recognised that some families are unable to access a study website and this will introduce selection bias (Paul, Seib, and Prescott 2005, Topolovec-Vranic and Natarajan 2016). Due to the relatively small number of participants, email enabled easy contact between families, local collaborators and the research team who could respond quickly to any queries.

4.7 Study Database

The study database had many useful features whilst being secure and complying with ethical requirements for confidentiality. The database was a strength of this study and essential when collecting longitudinal data thus ensuring protocol adherence. The information collected on the database was also key to assessing whether feasibility targets were met.

4.8 Recruitment

The study achieved a recruitment rate of 23 participants/year, far fewer than the 100 participants/year target. However, on reflection we consider recruitment as successful as between 2014 and 2017, seventy families had consented to the study making this one of the largest cohorts of babies with DS in the world recruited and still recruiting for a longitudinal

study. Although 11 children were too old to take part, 100 families completed EOIs showing the level of interest in the study.

Initial recruitment was very slow. The strategies involved having flyers describing the study in the DSA parent packs and through DSS support workers. However, following further discussions with the DSA, not all of these packs were going directly to new families. A proportion go to maternity hospitals to give out in the event of a new baby with DS being born. Some of the packs go to people who have come across the DSA when trawling for information, but this may be friends or other family members rather than parents themselves. The DSA provided information on the demographics of those who decide to become members of the DSA, if the babies are under a year of age when their parent's become members a new pack is sent out to them. For the year end 2012, only three packs were sent out to parents before they had their baby (i.e. prenatal diagnosis). For those parents who had a baby under the age of six months, 196 packs were sent out, 167 of these babies were under three months of age. The number of packs sent did reduce during the three years (2014 -2017). This may have been due to parents accessing more information through the internet likely making flyers less useful in future studies.

The recruitment rate more than doubled after the amendment (Amendment 1) to allow local collaborators to recruit and with an increase in activity on social media (0.9 participants a month increased to 2.3 participants a month). Although this increase cannot be purely attributed to the involvement of local collaborators, very active local collaborators contributed considerably to the overall numbers.

The barriers to recruitment that were experienced during this PhD and the actions taken to try and overcome these are summarised in the table below:

Barriers to recruitment	Action
Flyer provided in DSA new parent pack and through the DSS only reached a limited number of families.	Amendment to allow local collaborators (neonatologists, community paediatricians, research nurses) to recruit participants.
Initial recruitment methods only approached those families who were information seeking or whose children were actively being managed by medical professionals.	Recruitment via social media and websites.
Lack of local collaborators at all sites	Increased recruitment of local collaborators through the DSMIG, presentations given by GW at CRN meetings.
Attrition of collaborators and maintaining profile of study	Quarterly newsletters and regular updates.

Table 24: Barriers to recruitment and remedial actions.

4.9 Recruitment methods

The feasibility objective for recruitment methods was met. Participants were recruited successfully through a variety of recruitment methods. Recruitment via social media and the DSA website accounted for 46% of the participants which was comparable to the 40% recruited via local collaborators but they were only able to recruit from March 2015 when the amendment was approved (Amendment 1).

4.9.1 Strengths and weaknesses of recruitment methods

Recruitment via flyers, website and social media where participants self-identified had many advantages including allowing recruitment of participants from a wide geographical area and those who were born where there was no active local collaborator recruiting.

4.9.2 Recruitment via websites and social media

Recruitment via websites and social media took a median of almost two weeks from the time of EOI completion to consent and often required reminders to be sent by the team in Bristol. Web recruitment led to the need for some re-consenting, due to incorrectly filled forms. It should be remembered however that consent form errors happen in studies where participants are recruited directly (Smith, Moore, and Tunstall-Pedoe 1997). Consent forms completed with local collaborators were promptly filled and returned. However, reminders then had to be sent to complete the EOIs so that all the necessary participant details were obtained. System updates and repairs had little to no impact on overall recruitment.

When participants self-identify through the internet and social media, selection bias naturally occurs as demographic factors influence use and understanding of these media. A study by Topolovec-Vranic et al. (Topolovec-Vranic and Natarajan 2016) suggested that this method of recruitment leads to participants with a higher educational level and higher socioeconomic status when compared to studies recruiting via more traditional routes. The same study also commented that social media was a good way to recruit difficult to reach populations, particularly participants with specific conditions. By using both internet and traditional methods of recruitment these potential biases might be mitigated. However, as noted in the

qualitative research study, health professionals sometimes take on gatekeeping roles limiting which families are approached for research (Chapter 3).

4.9.3 Local collaborators

Local collaborators increased recruitment considerably. The role of the local collaborator at different sites varied. Some sites approved the study as “no local collaborator”, some named me as their “Principal Investigator” and others had a named local principal investigator. The status of the site did not reflect the activity. For example, some had a local PI by name, but the PI did not actively recruit. Local collaborators were recruited through the DSMIG, CRNs and R & D at some sites (where recruitment was low) tried to engage local collaborators. Some active collaborators not only recruited participants but also informed colleagues at other sites who in turn became local collaborators. GW was invited to talk at CRN meetings to recruit more local collaborators (NIHR CRN Eastern research champions meeting and NIHR CRN Kent, Surrey and Sussex).

Due to the use of the coordinated system for gaining NHS permission (CSP) and variations in the approval processes at each of the sites, information on whether a local collaborator existed could not be established with ease. This was a weakness in the study setup and caused issues when sometimes neither the site’s R&D team nor the FADES research team knew if there was a local collaborator. Importantly if local collaborators had been included in the initial protocol, it may have been slower to set up the study due to the rules around generic/single SSIs. The research activities would not have been considered as all taking place from the central research site.

Geographical distribution of participants and local collaborators

The distribution of the participants recruited reflects local activity, areas which had the highest recruitment rates were those with very active local collaborators. The reasons why some collaborators were more successful than others are unclear. Many babies with DS are admitted to neonatal units where trust and rapport is gained between the clinician and the parents as found in the qualitative research study. This is different for community paediatricians where they may only briefly meet the family post-delivery and might not see them again until the baby is a few months old. The schedule for following up with a community paediatrician varies across NHS Trusts and not all families meet their community paediatrician until their first outpatient appointment. Interestingly though, the study managed to recruit more community paediatricians as local collaborators than neonatologists, this was largely due to the study's link with the DSMIG.

4.10 Motivations and Barriers to joining the Study

Most of participants who were actively participating in the study completed the engagement questionnaire (89%) to explore why they joined the study and their opinion on the study. Not unexpectedly, only eight of the 26 non-participants with contact details (31%) completed the non–participant questionnaire exploring why they did not take part and their opinion on the study. These questionnaires only provided limited information, we know nothing of families who saw the study but did not complete an EOI or who were approached by local collaborators but declined involvement. However, it is a strength of the study that some non-participants did respond and did not raise any serious faults with the study.

Engagement (Participant) questionnaire

The engagement (participant) questionnaire showed that half of the families took part for altruistic reasons as noted in other studies (Fortnum et al. 2014, Caldwell, Butow, and Craig 2003). Parents also wanted to be proactive: by taking part in research it gave them a way to contribute and one parent said that it gave them something positive to do whilst "coming to terms with the diagnosis". The topic of feeding was of interest to some families. It is a strength of the study respondents found the study professional and that it appeared "easy and non-invasive". Previous studies (Caldwell, Butow, and Craig 2003, Nabulsi, Khalil, and Makhoul 2011) have suggested that families are more likely to engage in studies that would provide additional medical input for their child. A fifth of the participants thought the study would provide additional health screening and would be of benefit to their individual child.

Overall comments about FADES were very positive, stating that the information provided is clear and easy to understand. Participants suggestions for improvement were mostly around information on sample collection and this is an area for future development. The recommendation for improving recruitment was to increase presence on social media. One participant highlighted the potential issue of gatekeeping by clinicians.

Non-participant questionnaire

As the questionnaire was only completed by eight families, these findings can only be regarded as suggestive and conclusions should not be drawn. As for the engagement questionnaire, initial interest in the study was due to altruistic reasons or to potential benefit for their own child. The reason why many did not go on to actively participate was because they simply did not get around to it. Previous research and the qualitative study show that

this is a very busy time for families coping with a new baby who may have complex medical needs, adjusting to a new diagnosis and having multiple appointments (Van Riper 2007, Fortnum et al. 2014). However only one family said that they did not take part due to the time commitment which reflects that families did not see the study as overly burdensome. One family indicated an unwillingness to take stool, urine and mouth-swab samples and another were against taking blood samples. Collecting samples was not a barrier for most families although other families may not have completed an EOI because the collection of biological samples was unacceptable. An unwillingness by a clinician to take samples of sufficient volume was interesting as a perhaps surprising reason for one family not taking part.

Non-participants also found the website 'quite' attractive and easy to understand. Recommendations from this group had included providing clearer information on the level of commitment required, involving more health professionals in recruitment and three had asked about making the sampling easier. Overall these questionnaires suggest improvements could be made to the information provided about collecting the samples and the website could be enhanced with advice from web designers.

4.11 Sample Collection from Participants

Overall sample collection was successful, meeting the feasibility target for DNA samples, initial urine samples and nearly meeting the target for initial stool samples. Initial blood samples were collected for 65% of participants and over half of all 12 month requested samples were provided. The median ages at which samples were provided all met the targets with IQRs which were relatively narrow. Although the proportion of samples received were not all above target, this cohort has proven the ability to build a biobank of clinical samples.

There had been many questions raised during the initial approvals stage of the study as to whether families would be able or willing to provide these samples. It was suggested that the study was overly burdensome.

There were some issues with samples getting lost in the post, standard post (Royal Mail) was used and the samples were not tracked. This method was convenient for participants and cost effective but loss of samples enroute was a risk.

4.11.1 Mouth-swab samples

The feasibility target was exceeded for the collection of mouth-swab samples and the median age of the baby at the time of collecting the sample was acceptable although for this particular sample, age is not crucial. Collecting mouth-swab samples is relatively simple and non-invasive and the only issue was lysis buffer leaking when the lid was not tightly secured for a couple of samples. The concentration of DNA that was extracted from the samples was adequate and 98% of samples were analysed successfully for HLA Class II genotyping.

4.11.2 Urine Samples

The target was met for the collection of the initial urine samples but not for the 12-month samples. It is challenging to collect a clean catch urine sample from a 12-month old as they become more mobile but are not toilet trained. For urine C-peptide analysis, longitudinal samples are of importance; 46% of participants provided longitudinal samples and as 16 participants were under the age of 12 months, it suggests that at least half of participants would provide longitudinal samples as the study progresses.

Ideally for valid urinary C-peptide results, the collection needs to be a timed sample following a set meal: the mixed meal tolerance test (MMT) (Greenbaum et al. 2008). However, it is not possible to get a small child to void on demand or even to guarantee that they will take a fixed volume of feed and thus even in experimental immune-modulation trials, MMTs are not used under seven years of age. However, information on when samples were collected in relation to the time of the last feed does allow some comparisons of post-prandial urinary C-peptide measurements. Instructions were provided to parents advising them to collect urine samples post feed, but they were not required to record the time post feed until an amendment was added to change this (Amendment 7 July 2017). Time post feed was available for just under a third of participants. This is a limitation of the study. Besser et al measured UCPCR in children following an evening meal but the children had a median age of 14 years (IQR 11 – 16 years range 5 -19 years) (Besser et al. 2011). There have not been any studies measuring C-peptide in younger children which provide a normal range for a post prandial C-peptide.

Parents tried both suggested collection methods with a slight preference for the cotton wool method over the clean catch method. From the emails and comments received from the families, both methods had positives and negatives. Both methods provided good urine volumes and 99% of samples were adequate for analysis. However as discussed in Chapter 2 Section 2.15.2, there may be differences in the results related to using cotton wool and when the UCPCR result obtained is very low this should be repeated with a clean catch sample to ensure reliability.

4.11.3 Stool Samples

The number of initial stool samples collected was close to the feasibility target and longitudinal samples were collected by over half of participants. The gut microbiome changes during infancy becoming more stable and established around the age of three years (Yatsunenko et al. 2012, Lozupone et al. 2012). When considering why children with DS might be at increased risk of autoimmunity one hypothesis is that their gut microbiome may be different. This difference might be due to early feeding, infections and/or use of antibiotics. This can only be assessed when longitudinal stool samples can be linked to feeding and medical data. As the composition of the neonatal gut microbiome is also determined by the maternal gut microbiome, maternal stool samples were collected and 60% of mother's provided samples. Ideally these samples should be collected immediately at the time of birth as the mother's gut microbiome will alter with time but this is an inherent limitation of this study.

Once the pilot study for the OMNIgene GUT kit had been completed samples were collected using both methods. There were no major issues highlighted for either method and for future studies the OMNIgene GUT kit would be recommended.

4.11.4 Blood Samples

The feasibility target for the collection of blood samples was not achieved. To have at least one sample from over 70% of participants nevertheless suggests a degree of success. Of the samples received, over 88% were suitable for analysis of anti-BSA antibodies.

Collecting blood samples from babies and young children is always challenging and can be distressing for parents and the blood taker. With babies and children with DS there are added

challenges, they tend to have puffy hands and feet and can be peripherally poorly perfused. Some children also have cutis marmorata, livedo reticularis and acrocyanosis which are vascular cutaneous manifestations seen in DS which make visualising veins difficult (Madan, Williams, and Lear 2006). The volumes of blood that were collected in this study were not large, but over 80% of the samples obtained resulted in serum volumes of greater than 30µl and a mean sample volume of over 100µl. It might be thought that as the children got older, it would be easier to get a larger blood sample. Surprisingly, when volumes collected were compared for the different ages at sample collection they were similar. Repeat samples were requested if inadequate or if lost in the post only where parents had suggested that they were happy to do so.

Coordinating the collection of the bloods samples was very time consuming, although the design was for the samples to be done at the same time as a routine local appointment, the health professional needed to be contacted. The recommendation from the DSMIG is for children with DS to have thyroid function checked annually (TSH annually and TFTs every two years) (DSMIG 2019a). Protocols vary across trusts and it is sometimes unclear who organises this screening test. Certainly, in some areas of the country there are dedicated clinics for children with DS usually run by community paediatricians but usually these are in densely populated cities. Even where thyroid function is checked annually, it is not always collected close to the child's birthday. The study team contacted families close to the participants' birthdays to arrange the sample collection, but appointment dates were sometimes several months off. In these cases, the families were advised that the sample could wait, but this usually then required reminders. Participants were also seen by other health professionals or

for other interventions for example cardiac surgery which provided opportunities for blood collection.

The advantages of this proposed setup was that it avoided participants having to attend additional appointments solely for the study. It enabled participants to be recruited from a wide geographical area as dedicated trained research staff were not required. This also reduced costs in setting up and paying for clinic space or phlebotomy services. The disadvantages were that it was not possible to time the sample collection and a degree of flexibility was required. Written instructions were provided but there were a couple of errors with the use of the bottles which if used incorrectly leaked.

4.12 Retention of participants

The majority of active participants are engaged and committed. For the children who had reached the age of two by September 2017, just over 60% of participants were fully active having provided all of the requested samples and completed all of the questionnaires and nearly 80% of participants were either fully or partially active (still completing some samples and/or questionnaires at two years of age). Higher attrition levels have been observed in other studies (Golding and Birmingham 2009, Zook et al. 2010, CDSS 2018). This study requires significant family input within many cases, very little contact with the research team hence the online newsletters were employed to improve engagement.

Assessing the feasibility target of retaining 50% of participants until five years of age will be assessed in 2020 but from these data there appears to be a fall out of around 20% over two years. As discussed, some information as to why participants were unable to continue in the study were given in the non-participant questionnaire; usually because although families

intended to take part, they then felt unable to commit the time. There were also some participants who became seriously unwell and therefore the families no longer engaged. Many of the families in this study had self-identified, finding the study through their own research on websites and social media. This suggests that these are already very proactive parents who are less likely to drop out. A quarterly newsletter is sent out to all the current participants and local collaborators, it includes updates on the study progress, useful information about aspects of the study such as the sample collection and parents send in photos and news of their children (see Appendix 28). These newsletters have had positive feedback from participants and has been a good way of keeping families engaged, other studies have also found newsletters have helped with retention of participants (Abshire et al. 2017).

4.13 Medical and Feeding Questionnaires

The target of 75% for completing the initial and seven-month questionnaires was exceeded and for the 12-month questionnaire, it was almost achieved. These are long and thorough questionnaires; the ethics committee had raised a concern that they would be too burdensome for the participants. Mothers however, filled in the questionnaire promptly and in detail, providing significant extra information in the free text boxes. The detail provided in these suggests that at least for some of the respondents, response fatigue was not an issue. Birth cohorts of babies with DS are unusual and it is important for families to have the opportunity to tell their individual stories and share their experience of the care that they received. The proportion of families in the study completing the questionnaires supports this theory.

4.14 Questionnaire Design

The use of a web-based questionnaire allowing completion wherever internet access was available by phone, tablet or computer was a definite strength with 82% of participants completing questionnaires online. Many issues that frequently occur with paper questionnaires were removed; for instance, participants losing paper versions before they are completed, completing the paper questionnaire but not getting around to posting it. There were also no postage costs. As previously described, branching on the web-based questionnaire meant that the mothers saw only the relevant sections making it quicker and easier to complete. Compared with face to face or telephone interviews there is no risk of interviewer bias. Interviewer bias can be reduced with training but is often subtle and interviewers are unaware that they are being encouraging or positive, negative or interrupting. Mothers may have felt able to answer more freely as online questionnaires offer relative anonymity. There are frequently feelings of guilt around whether a mother can breastfeed or if they have decided not to breastfeed (Hegney, Fallon, and O'Brien 2008, Thomson, Ebisch-Burton, and Flacking 2015); being able to answer questions regarding early feeding without the presence of an interviewer may have provided more open responses and reduced social desirability bias (Bowling 2005). Using web-based questionnaires increases data completeness and removes input errors as the data could be transferred directly into Stata.

The strength of the questionnaires is that they are longitudinal and prospective. The initial questionnaire asks the mother to recall a significant amount about their baby's initial feeds. The median age for completing the questionnaire was 20 weeks and therefore recall bias may

be present in the data collection for early feeding. One could hypothesise that mothers coping with very young babies, often sleep deprived with the additional worries of an often-unexpected diagnosis for their baby may be poor historians. However, mothers said that receiving the diagnosis caused them to remember every detail of those early days of their baby's life. Although the seven-month and 12-month questionnaires both asked about the preceding six months, the recall bias for these questionnaires is minimal with median age at completion being seven months and 12 months respectively.

Open questions with space for participants to provide free text aimed to reduce researcher bias and allow new knowledge to be gained. Closed questions with limited responses made it easier to compare the answers given by different participants with fewer confused or irrelevant responses given.

4.15 Overall feasibility of data collection

Overall the study successfully collected the desired samples and data. The information provided to participants including the sample instruction sheets were comprehensible. Although members of the research team did not meet the families directly, this must be inferred by the number of usable samples that were returned and lack of calls or emails to the team suggesting the contrary. The SOPs for the handling of data and samples worked with no adverse events. Initial testing of samples is complete and these results are given in Chapter 6. The study team had the skills and capacity to manage the study. The lessons learnt in setting up this cohort and future directions for the cohort will be discussed in Chapter 7.

CHAPTER 5

Analysis of Medical and feeding questionnaires

Chapter 5 Analysis of Medical and feeding questionnaires

5.1 Overview of Chapter 5

This chapter provides data from the medical and feeding questionnaires. The ability to use these results to characterise the cohort is used to help determine feasibility. The results describe the demographics, medical conditions, infections and antibiotic usage, early feeding and weaning some comparisons are made with the general population.

5.2 Results of Medical and feeding questionnaires

The ability of questionnaires to obtain the required data was assessed by:

Objective: The ability to characterise the cohort in relation to;

- ***ethnicity, maternal age, socio-economic background***
- ***medical conditions related to a diagnosis of Down's syndrome***
- ***to determine the numbers and types of infections experienced during early life and antibiotic usage;***
- ***to describe early feeding and weaning in the cohort;***

The characterisation of the cohort was undertaken in order to determine whether the cohort was representative of the general population of children with DS. In the following analyses, data from the initial, seven-month and 12-month questionnaire were used. Data from the 24 and 36-month questionnaires were not analysed as the number of families reaching these time points are insufficient for meaningful results. Descriptions are given for the 61 participants who completed the initial questionnaire at a median child age of 4.6 months.

5.2.1 Maternal Characteristics

Table 25 shows that within the study population, over 80% of mothers were at least 30 years old with the majority being at least age 35 years. Mothers were largely white British, married / in a civil partnership or living together. At 12 months, half of mothers were in active employment or on paid maternity leave. Although seven mothers had returned to work by the time their child was seven months, the majority did not return until their baby was over the age of nine months.

Background Characteristics			
Maternal age (years) at time of completing initial questionnaire	20 to 24	8.2%	(5/61)
	25 to 29	9.8%	(6/61)
	30 to 34	24.6%	(15/61)
	35 to 39	37.7%	(23/61)
	40 or over	19.7%	(12/61)
Mother's marital status	Living together	27.9%	(17/61)
	Married or in a civil partnership	65.6%	(40/61)
	Single	4.9%	(3/61)
	Widowed, divorced or separated	1.6%	(1/61)
Mother's ethnic group	Other	3.3%	(2/61)
	White British	86.9%	(53/61)
	White Irish	4.9%	(3/61)
	White Other	4.9%	(3/61)
Maternal employment status at 7-months	In active employment	13.2%	(7/53)
	On paid maternity leave	64.2%	(34/53)
	On unpaid maternity leave	3.8%	(2/53)
	No	18.9%	(10/53)
Maternal employment status at 12-months	In active employment	45.0%	(18/40)
	On paid maternity leave	5.0%	(2/40)
	On unpaid maternity leave	15.0%	(6/40)
	No	35.0%	(14/40)
Age of child when mum returned to work	6 months, less than 9 months	11.1%	(2/18)
	9 months or older	88.9%	(16/18)

Table 25: Background Characteristics

5.2.2 Participant characteristics and birth details

This sub-section describes the perinatal history, birth details and any admission to Special Care Baby Unit (SCBU). Parents informed the study that their child had a diagnosis of DS. Genetic information regarding the full karyotype was not obtained. No notification was received from doctors or allied health professionals that the karyotype had altered the diagnosis, but this was not explicitly asked of them.

The median age of completion of the seven-month questionnaire was 7.2 months and 12 months for the 12-month questionnaire; the IQR for both questionnaires is relatively narrow as shown in Chapter 4 Table 22. Of the 61 participants, 54.1% were female (33/61).

Table 26 shows that a quarter of the participants were diagnosed antenatally with Down's Syndrome. Over half were born via a normal vaginal delivery with a mean birthweight of 3kg. Most of the babies were born in consultant or midwife led hospital units, three were delivered at home. The median gestation was slightly early at 38 weeks but still within the definition of "Term". Thirteen babies who were born pre-term (gestations of between 32 and 37 weeks). One baby had a sibling with DS, but no other family history of DS was given. Two participants were non-identical twins (not with each other), with siblings who did not have DS. The median length of hospital stay after birth was five days.

Only 72.3% (34/47) of participants had the correct 'DS insert' in their Red book, the personal child health record that is given to every baby. The 'DS insert' contains the correct growth charts for babies and children with DS, this is important to ensure that they are growing as would be expected for a child with DS rather than the general population.

Some babies required prolonged stays in hospital following birth. Parents were asked about hospital admissions between questionnaire time points (see Appendix 29).

Birth History			
First baby		31.2%	(19/61)
Prenatal diagnosis of DS		24.6%	(15/61)
Twin Birth		3.3%	(2/61)
Type of delivery	Normal Vaginal delivery	63.3%	(38/60)
	C-Section	28.3%	(17/60)
	Forceps	3.3%	(2/60)
	Ventouse	5.0%	(3/60)
Gestation (weeks)		Median 38 (IQR 37 – 39)	
Pre-term 32-37 weeks		21.3%	(13/61)
Birthweight	Birthweight	Mean 3.0kg (0.5 SD)	
Location of birth	At home	5.0%	(3/59)
	In hospital (consultant led)	49.2%	(29/59)
	In hospital (midwife led)	42.4%	(25/59)
	Birth centre (midwife led)	3.4%	(2/59)
Length of initial hospital stay (hours)		Median 120 (IQR 72 – 216)	

Table 26: Birth Details

Footnote: C-section (Caesarean Section)

Table 27 shows that over half of the participants were admitted to SCBU for a median admission duration of two weeks. About a third were admitted due to problems related to feeding. Respiratory problems/diagnosis were the most common medical cause for SCBU admission.

SCBU Admission		
Admitted to SCBU	55.7%	(34/61)
Admitted to SCBU due to problems with feeding	29.4%	(10/34)
Admitted to SCBU for any other reason*	70.6%	(24/34)
Hypoxia / respiratory distress / PPHN	50.0%	(17/34)
Jaundice	20.6%	(7/34)
Cardiac / cardiac monitoring	11.8%	(4/34)
Infection	11.8%	(4/34)
Prematurity	8.8%	(3/34)
Polycythaemia	5.9%	(2/34)
Other	20.6%	(7/34)
Number of different reasons given for admission to SCBU (excluding feeding as a reason)		
1	41.7%	(10/24)
2	37.5%	(9/24)
3	16.7%	(4/24)
4	4.2%	(1/24)
Length of SCBU stay (weeks)	Median 2 (IQR 2 – 4)	

Table 27: Admissions to Special Care Baby Unit

Footnote: SCBU (Special Care Baby Unit), Persistent Pulmonary Hypertension (PPHN)
*Due to the branching of the questions relating to SCBU, once the parents answered "Yes" to "After the birth, were you told that your baby needed to go to special care because of problems specifically with feeding?", they were then branched away so that they could not also answer "Yes" to "Was your baby put into special care for any other reason?". Some babies may therefore have had feeding and other issues which are not represented here.

5.2.3 Medical conditions and input of professionals

This section includes the assessment of the presence of medical conditions associated with DS, including cardiac defects and details of other common conditions in the study population. Information on the involvement of professionals in the child's care in also presented here.

Over half of the participants in the study were diagnosed with a cardiac condition with about 10% having more than one cardiac diagnosis as shown in Table 28. The most common defects were atrioventricular septal defect, atrial septal defect and ventricular septal defect.

Cardiac		
Diagnosed with a heart condition	61%	(37/61)
Atrioventricular septal defect	13.1%	(8/61)
Atrial septal defect	13.1%	(8/61)
Ventricular septal defect	13.1%	(8/61)
Patent ductus arteriosus	9.8%	(6/61)
Patent foramen ovale	6.6%	(4/61)
Unspecified hole in the heart	4.9%	(3/61)
Tetralogy of Fallot	3.3%	(2/61)
Aberrant right subclavian artery	1.6%	(1/61)
Bicuspid aortic valve	1.6%	(1/61)
Pulmonary hypertension	1.6%	(1/61)
Number cardiac diagnoses per individual		
0	39.3%	(24/61)
1	50.8%	(3161)
2	8.2%	(5/61)
3	1.6%	(1/61)

Table 28: Cardiac conditions

Table 29 shows medical diagnoses that are either known to occur more frequently in DS or which have been coded from free text and frequencies given where there was commonality between participants. The most commonly reported diagnosis was reflux. Other significant conditions reported were Transient Abnormal Myelopoiesis in three participants and gut abnormalities, Hirschsprung's and Duodenal atresia in four participants. Other diagnoses mentioned by families included for example glue ear, undescended testes, eczema, nystagmus and craniosynostosis. None of the participants were reported to have developed diabetes or coeliac disease in any of the questionnaires. Of the two hypothyroid children, one was aged less than three months at diagnosis and the other aged 9-12 months. Both children's

blood samples were tested as part of this study for autoantibodies to thyroid peroxidase and were negative suggesting that their hypothyroidism was not due to autoimmunity.

Medical diagnoses	Initial questionnaire	New diagnosis (from 7-month or 12-month questionnaire)	Total number
Hirschsprungs***	3.3% (2/61)		3.3% (2/61)
Hypothyroid	n/a**	3.3 % (2/61)	3.3% (2/61)
Transient abnormal* myelopoesis	4.9% (3/61)		4.9% (3/61)
Duodenal atresia***	3.3% (2/61)		3.3% (2/61)
Reflux*	8.2% (5/61)	3.3% (2/61)	13.1% (8/61)
Cow's milk protein* allergy age <3months		3.3% (2/61)	3.3% (2/61)
Chest / Bronchiolitis*		4.9% (3/61)	4.9% (3/61)
Laryngomalacia*		3.3% (2/61)	3.3% (2/61)
Sleep apnoea*		4.9% (3/61)	4.9% (3/61)
Number of other medical conditions not listed in the conditions above	0 65.6% (40/61) 1 26.2% (16/61) 2 4.9% (3/61) 3 1.6% (1/61) 5 1.6% (1/61)	0 77.8% (42/54) 1 18.5% (10/54) 2 1.9% (1/54) 4 1.9% (1/54)	

Table 29: Medical diagnoses excluding cardiac.

Footnote: Missing values have been counted as "no" as some diagnosis have been created from free text and therefore the denominator for these was 61 already. (Percentages were manually calculated where the denominator needed to be changed).
*New variables were created for diagnoses which were coded from free text and frequencies have been given where there was commonality between participants (see master sheet of new variables in Appendix)
**Participants were not asked in the initial questionnaire about autoimmune conditions as autoimmunity at birth is rare but were asked in the subsequent questionnaires.
***The initial questionnaire specifically asked about duodenal atresia and Hirschsprung disease, as there is an increased risk of having these malformations in babies with DS.

The variety of health professionals involved in a child with DS's care is shown in Table 30. Although nearly 80% reported having a community paediatrician before seven months of age, this decreased to only a quarter between the ages of 7-12 months. Around 60% had a

cardiologist and over half had a speech and language therapist involved in their care by 12 months. The other professional who played a role in the care of many of the participants was a physiotherapist.

Specialists involved in baby's care	0 – 7 months	7-12 months
Community Paediatrician	79.6% (43/54)	25% (10/40)
Cardiologist	61.1% (33/54)	57.5% (23/40)
Speech and Language Therapist (SALT)	44.4% (24/54)	57.5% (23/40)
Dietician	24.1% (13/54)	25% (10/40)
Gastroenterologist	7.4% (4/54)	12.5% (5/40)
Endocrinologist	3.7% (2/54)	12.5% (5/40)
Other (from open question)	55.6% (30/54)	60.0% (24/40)
Physiotherapist	42.6% (23/54)	40.0% (16/40)
Portage / Early Years Teacher	11.1% (6/54)	12.5% (5/40)
Ophthalmology	9.3% (5/54)	10.0% (4/40)
Occupational Therapist	9.3% (5/54	17.5% (7/40)
Community Nurse / Health Visitor / Paediatric Nurse	7.4% (4/54)	2.5% (1/40)
Audiologist	3.7% (2/54)	2.5% (1/40)
Cranio-facial / Head Specialist	3.7% (2/54)	2.5% (1/40)
Ear Nose and Throat Doctor	3.7% (2/54)	5.0% (2/40)
Haematologist / Oncologist	3.7% (2/54)	5.0% (2/40)
Surgeons	3.7% (2/54)	2.5% (1/40)
Allergist	1.9% (1/54)	
Psychotherapist	1.9% (1/54)	
Hepatologist	1.9% (1/54)	
Stoma Nurse	1.9% (1/54)	
Paediatrician / Neonatologist		10.0% (4/40)
Respiratory Consultant		5.0% (2/40)

Table 30: Specialists involved in child's care

Footnote: The data in this table is taken from the 7-month and 12-month questionnaires. This question is not asked in the initial questionnaire. For many participants they had multiple specialists involved in their care

Table 31 shows the proportions of participants experiencing common childhood conditions. Half of the babies aged nine to 12 months had chest problems and this was a common issue at younger ages too. Gut problems including vomiting, diarrhoea and constipation were commonly experienced, with constipation reported by 35% to 48% of participants from age three to 12 months. Poor weight gain during the first month of life was reported by 23 participants; although this appeared to have improved for many by seven months, it became a more frequent issue again between the ages of 9-12 months. It is important to note that **all** study participants required hospital admissions prior to their first birthday.

Common condition by age					
	Age 0 – 1 month	Age 1- 3 months	Age 3 -7 Months	7 -9 Months	9-12 months
Chest problems / infection	8.2% (5/61)	9.8% (6/61)	24.1% (13/54)	20.0% (8/40)	50.0% (20/40)
Constipation	11.5% (7/61)	19.7% (12/61)	48.2% (26/54)	35.0% (14/40)	35.0% (14/40)
Vomiting	11.5% (7/61)	14.8% (9/61)	31.5% (17/54)	17.5% (7/40)	32.5% (13/40)
Diarrhoea	6.6% (4/61)	14.8% (9/61)	20.4% (11/54)	12.5% (5/40)	30.0% (12/40)
Ear problems / infection	1.6% (1/61)	1.6% (1/61)	7.4% (4/54)	5.0% (2/40)	12.5% (5/40)
Urinary tract infection	0	0	1.9% (1/54)	7.5% (3/40)	7.5% (3/40)
Colic	18.0% (11/61)	21.3% (13/61)	20.4% (11/54)	7.5% (3/40)	7.5% (3/40)
Thrush	0	3.3% (2/61)	7.4% (4/54)	2.5% (1/40)	7.5% (3/40)
Not gaining enough weight	37.7% (23/61)	14.8% (9/61)	13.0% (7/54)	5.0% (2/40)	22.5% (9/40)
Gaining too much weight	0	1.6% (1/61)	5.6% (3/54)	2.5% (1/40)	2.5% (1/40)
Something else	14.8% (9/61)	13.1 (8/61)	11.1% (6/54)	2.5% (1/40)	15.0% (6/40)

Table 31: Common conditions by age of participant.

5.2.4 Antibiotic use and infections

In this section, data on maternal use of antibiotics during pregnancy, labour, early feeding and infancy are explored. Data on the frequency of antibiotic use in infancy and the infections for which antibiotics were given are also presented. The antibiotics and the types of infections were coded from open questions.

Table 32 shows that 18% of mothers received antibiotics during pregnancy, 7% during labour and nearly 15% whilst breastfeeding (during the first three months of their baby's life). From open responses, urinary tract infections were the most common infection for which mothers received antibiotics during pregnancy. Urinary tract infection was one of the indications given by the mothers for antibiotics during the time that they were breastfeeding. Other infections included mastitis and cellulitis.

Maternal infections and antibiotic use		
Antibiotics given in first trimester	4.9%	(3/61)
Antibiotics given in second trimester	8.2%	(5/61)
Antibiotics given in third trimester	6.6%	(4/61)
Number of trimesters in which antibiotics were given		
None	82.0%	(50/61)
One trimester	16.4%	(10/61)
Two trimesters	1.64%	(1/61)
Antibiotics given during labour		
Yes	6.7%	(4/60)
No	88.3%	(53/60)
Not known	5.0	(3/60)
Antibiotics when breastfeeding		
Yes	14.5%	(8/55*)
No	76.3%	(42/55)
Not applicable as formula fed	12.7 %	(7/55)
Not known	1.8 %	(1/55)

Table 32: Maternal antibiotic use during pregnancy, labour and breastfeeding

Footnote: the details of the types of antibiotics given are in the Appendix 23 and 29. No mother said that they were taking antibiotics whilst breastfeeding their baby over the age of 3 months. *55 is the number of mother's who ever breastfed or gave expressed breastmilk to their baby (see Section 5.2.5).

The proportion of children receiving any antibiotics during the first year of life is shown in Table 33. Nearly 70% of children received antibiotics during the first 12 months with around half during the first few months of life. Infections for which antibiotics were required were given most often in the neonatal period and before the age of one month and then later in infancy after seven months. There were children who received multiple courses of antibiotics during the first year of life (Multiple courses of antibiotics were counted if parents said that antibiotics were given at more than one time point (further detail is given in Appendix 29). The list of which antibiotics were given is in Appendix 23.

Infant antibiotic use		
Ever received antibiotics	68.9%	(42/60)
Antibiotics age 0-3 months	45.9%	(28/61)
Antibiotics age 3-7 months*	32.8%	(20/61)
Antibiotics age 7-9 months	22.5%	(9/40)
Antibiotics age 9-12 months	47.5%	(19/40)

Table 33: Infant antibiotic use by age

*Footnote: For three participants their parents could not remember at which age they received antibiotics. * this number may be overrepresented - the question in the 7 month questionnaire was simply worded "My baby was aged 3 months plus when they were taking antibiotics". To see information on the number of antibiotic courses given between questionnaires see Appendix 29*

Suspected sepsis in the neonatal period was the most common indication for antibiotic treatment before the age of three months (18% (11/61)), followed by chest infections (6.6% (4/61)). Two babies were given antibiotics for umbilical infections and one for necrotizing enterocolitis. Chest infections including bronchiolitis was a frequent indication throughout infancy with 40% (16/40) of study participants being given antibiotics between the ages of nine and twelve months. Further details on the types of infections can be seen in Appendix 29 but other indications given for study participants to receive antibiotics during infancy included conjunctivitis, urinary tract infections, cellulitis, line infections, suspected meningitis and viral infections. Some babies were also given antibiotics as prophylaxis during surgery, chemotherapy and to prevent urinary tract infections.

5.2.5 Initial feeding after birth

This section summarises the initial feeds received after birth, maternal experiences of support received, and issues around feeding.

Table 34 shows that half of the mothers in the study had breastfed a previous child. Despite all attending antenatal check-ups for the study baby, they did not all attend sessions on feeding babies. Most of the mothers who had received a prenatal diagnosis of DS were told that babies with DS experience feeding problems but only a minority of these received any specific advice.

Prenatal Feeding Information		
Breastfed a previous child	73.2 %	(30/41*)
Attended antenatal check-ups	100 %	(61/61)
Attended a session which included talk on feeding babies	30 %	(18/60)
Told about specific problems related to feeding a baby with DS (if prenatal diagnosis)	86.7 %	(13/15**)
Received specific advice antenatally about help with breastfeeding a baby with DS	20.0 %	(3/15**)

Table 34: Prenatal feeding information

Footnote: *42 mothers had an older child (missing data on breastfeeding for one). **15 mothers had received a prenatal diagnosis for their baby.

The problems that babies with DS have with feeding that were described to mothers included baby's low tone, the size of tongue, difficulty of latching baby on to nipple, being tired or getting tired when feeding, taking longer to establish feeding, issues with baby coordinating suck and the potential complication of other medical diagnosis. One mother mentioned that she was told babies with DS could breast feed and a couple of the mothers said that they had been informed about the potential of using other feeding methods. Below are quotes from the questionnaire data to illustrate the information given to mothers in response to the question: 'If your baby was diagnosed with Down's syndrome before they were born, were

you told about any specific difficulties related to feeding a baby with Down's syndrome? What were you told?'

"The paediatrician explained it may be difficult to breastfeed and we may need to consider other forms of feeding due to a possible weak suck"

"Due to low muscle tone and size of tongue, breast feeding could be tricky. My midwife said that because I had breast fed before I would have a confidence that the baby would feed off and there was no reason breast feeding wouldn't work."

For specific information on who discussed feeding with the mothers antenatally and who gave specific advice regarding breastfeeding a baby with DS please see Appendix 29.

Table 35 shows the details of the babies first feeds after birth. The majority of mothers had skin to skin contact with their baby within 24 hours after birth. The majority of mothers initially breastfed their baby (for information on who helped putting the baby to the breast whilst in hospital and who helped with breastfeeding see Appendix 29). Feeding problems that were experienced in the hospital or birthing centre by the majority of mothers, included those commonly associated with babies with DS. Although low tone is one of the explanations frequently given as a cause for feeding difficulties in babies with DS only one parent described this.

First Feeds			
Ever breastfed or received expressed breast milk		90.2 %	(55/61)
Had skin to skin contact within 24hrs of birth – Yes		78.3 %	(47/60)
Maternal health problems after birth that affected feeding		6.6 %	(4/61)
Received help with breastfeeding within first few days – yes		66.7 %	(40/60)
How soon after birth was baby put to the breast	within a few minutes	25.5 %	(14/55)
	<30 min	30.9 %	(17/55)
	30 min to 1 hr	16.4 %	(9/55)
	>1 hr - 8 hrs	5.5 %	(3/55)
	>8 hrs – 24 hrs	5.5 %	(3/55)
	> 24 hrs later	16.4 %	(9/55)
Experienced problems when feeding their baby in the hospital, birth centre or unit		77.5 %	(45/58*)
Common problems with feeding**	Poor latch	44.4 %	(20/45)
	Medical issue	31.1 %	(14/45)
	Jaundice	31.1 %	(14/45)
	Tired / lethargic	28.9 %	(13/45)
	Maternal reason	8.9 %	(4/45)
	Tongue size/ position	6.7 %	(3/45)
	Low tone	2.2 %	(1/45)
Reported that they received enough help with feeding in hospital / birth centre		82.8 %	(48/58)

Table 35: First feeds

Footnote:* Three babies were home births ** These problems were coded from open responses.

The quotes below from the open question: 'Were there any problems feeding your baby while you were in the hospital, birth centre or unit? What problems were there?' illustrate some of these issues and the experiences of the mothers.

"X would not take anything from the breast and was very sleepy so had an NG tube put in."

"A feeding tube was inserted straight away, which prevented me from breast feeding. It also prevented her from becoming hungry enough to feed from me"

"baby continually pushing tongue out and could not latch on. Decided to try the bottle which she took immediately so did not try breast again and was not questioned by staff"

When describing problems many mentioned the need for expressing, NG tube feeding or the use of bottles.

At least half of the participants who completed the initial questionnaire required NG tube feeding as shown in Table 36, and the proportion may be higher due to the problems with the branching of the questionnaire. Of the babies who were admitted to SCBU, all were NG tube fed. Nearly 40% of the babies required NG tube feeds in SCBU due to issues with feeding the remainder due to medical co-morbidities (36.9% due to multiple medical indications). The milk that was given down the NG tube was mostly expressed breastmilk or a mixture of expressed breast milk and infant formula. Over half of the participants (61.3%) required NG tube feeds for less than two weeks. Nearly a third of the participants had feeds stopped at some point and were put onto IV fluids.

Feeding in SCBU and NG tube Feeds			
SCBU admission affected ability of mother to feed baby as she would have liked		67.7 %	(23/34)
Shown how to express milk in SCBU		85.3 %	(29/34)
Felt supported to express milk in SCBU		90.0 %	(27/30)
Had NG tube feeds		100 %	(31/31*)
Required NG tube feeds due to problems with feeding		38.7 %	(12/31)
Medical reason for NG tube feeds**	Prematurity	10.5 %	(2/19)
	Dehydration / hydration	10.5 %	(2/19)
	Ventilation	10.5 %	(2/19)
	Respiratory problems	42.1 %	(8/19)
	Jaundice / polycythaemia	21.1 %	(4/19)
	Vomiting or surgical abdomen	21.1 %	(4/19)
Number of medical reasons given for NG tube feed	1	63.2 %	(12/19)
	2	21.1 %	(4/19)
	3	10.5 %	(2/19)
	4	5.3%	(1/19)
Length of time NG tube fed	0 < 3 days	16.1 %	(5/31)
	3 < 7 days	19.4 %	(6/31)
	1 < 2 weeks	25.8 %	(8/31)
	2 < 4 weeks	19.4 %	(6/31)
	1 – 2 months	16.1 %	(5/31)
	>2months	3.2 %	(1/31)
Type of NG tube feeds given	Only expressed breast milk	32.3 %	(10/31)
	Only infant formula	6.5 %	(2/31)
	Expressed breast milk and infant formula	61.3 %	(19/31)
Feeds stopped and IV fluids given – yes		32.4 %	(11/34)
Length of time on IV fluids	0 – 3 days	22.2 %	(2/9)
	3 < 7 days	44.4 %	(4/9)
	7 days – 1 month	33.3 %	(3/9)

Table 36: Feeding in SCBU and NG tube feeds.

Footnote: Intra- venous fluids (IV fluids).

*Due to an issue with branching, only participants who had answered "yes" to "After the birth were you told that your baby needed to go to special care because of problems specifically with feeding?" or "yes" to "Was your baby put into special care for any other reason?" were able to answer the question regarding NG tube feeds. Furthermore, people who had answered yes to being admitted to SCBU but then answered not applicable to could not express or did not want to express were also unable to answer the question regarding NG tube feeds. This applied to three people. Where they were branched away, people have been counted as missing, so the denominator is 31.

**Coded from open responses.

Of 61 participants, 39 (63.9%) continued breastfeeding for over six weeks (Table 37) (this included babies who received infant formula as well as breastmilk). Over the age of six months, this had decreased to 54.2% (32/59) participants still breastfeeding.

The prevalence of exclusive breastfeeding in the study population at six weeks was 21.3% (13/61). By six months of age, less than 4% of participants were exclusively breast feeding (2/54). Although five participants said that they had only introduced formula over the age of six months, two of those had started solids between the ages of five to six months and one said that they had given a drink other than milk at the age of six months. Using data from the initial and 7-month questionnaires, the median age at which formula was introduced was five days (IQR 1, 21 days). Recommendations are that cow's milk should not be given as a drink until 12 months of age but can be mixed in food prior to this; the median age for starting cow's milk in the study population was 11 months so below the recommended age.

Whilst the seven-month questionnaire elicited a response from all mothers that going back to work had not influenced infant feeding (n=7), by 12 months three felt it did. However, by this stage more mothers (n=18) were back at work.

Types of feeds / Stopping breastfeeding		
Age stopped breastfeeding		
6 weeks or less	40.7%	(11/27*)
>6 weeks to 4 months	25.9%	(7/27)
>4 months to 6 months	11.1%	(3/27)
>6 months	22.2%	(6/27)
Age started formula feeds		
6 weeks or less	77.2%	(44/57**)
>6 weeks to 4 months	5.3%	(3/57)
>4 months to 6 months	8.8%	(5/57)
>6 months	8.8%	(5/57)
Age when given a non – milk drink (weeks)	Median 26 (IQR 20 - 28)	
Age at which first given cow's milk (months)	Median 11 (IQR 7-12)	

Table 37: Age at which participants stopped breastfeeding (including using expressed breastmilk and mixed feeding) and/or were introduced to other types of milk feeds or drinks. Non-milk drinks included any drink other than milk including water (which would be fine) and juices which would not be recommended.

*Footnote: *this denominator is the number of women who had stopped breastfeeding by the data cut off date. Some babies would still have been receiving breastmilk at the cut-off date for data analysis. Some would also have been still mixed feeding both breastmilk and formula. **this denominator is the number of participants who had started formula by September 2017.*

Just over half of the participants had problems with feeding after discharge from hospital as can be seen in Table 38. The most common problems experienced were with babies being lethargic or failing to thrive. Three of the mothers mentioned that their babies were readmitted to hospital due to issues relating to poor feeding. The majority did receive advice and help with their feeding issues.

General Feeding and feeding after discharge from hospital / birth centre		
Problems with feeding baby after discharge from hospital / birth centre	53.6%	(30/56)
Problems with feeding after discharge*		
Lethargy	21.3%	(13/61)
Poor weight gain / weight loss	16.4%	(10/61)
Low tone	1.6%	(1/61)
Latch	8.2%	(5/61)
Medical	4.9%	(3/61)
Jaundice	4.9%	(3/61)
Reflux	4.9%	(3/61)
Received help or information with feeding problems after discharge	84.4%	(27/32**)
Aware of the benefits of breastfeeding for their baby – yes	91.8%	(56/61)
Knowledge of specific health benefits of breastfeeding*		
Immune system / protection against infections	85.3%	(52/61)
Nutritional value / Natural / Vitamins	31.2%	(19/61)
For bonding and comfort	29.5%	(18/61)
Oro – motor development	23.0%	(14/61)
Good for digestion	19.7%	(12/61)
Healthy weight / preventing obesity	16.4%	(10/61)
Allergies	13.1%	(8/61)
General health	6.6%	(4/61)
Brain development	6.6%	(4/61)
Constipation	4.9%	(3/61)
Diabetes prevention	4.9%	(3/61)
Gut flora	4.9%	(3/61)
Sudden Infant Death Syndrome	3.3%	(2/61)
Other	4.9%	(3/61)

Table 38: General Feeding and feeding after discharge from hospital / birth centre

Footnote *coded from open responses. **This denominator comes from the 30 participants who experienced problems with feeding after discharge from hospital and two who experienced problems with feeding after their home birth.

The quotes below illustrate the information that the mothers provided in the questionnaire regarding the problems they experienced: 'Since you left the hospital, birth centre or unit have you had any problems with feeding your baby? What problems were there?"

"She slept a lot and had to be woken to feed. She was slow to gain weight at first. Once we got rid of the nipple shields which I introduced to help establish breastfeeding (and I believe helped initially) as I was advised that it helped babies with DS latch on, she gained more weight"

"About 2 days after getting her home, child's name stopped feeding and I was worried that she was becoming dehydrated. She was also very sleepy and had to be woken up for feeds. This was quite frightening as we had not been told to expect this. I used to need to strip her off to feed and make my hands cold so that she would wake up. I was scared she was going to die to be honest, it was awful. We were told to top up feed her with a bottle, we did this with expressed breast milk, and she gained weight well."

Over half of the mothers said that they would have liked to have breastfed for longer as shown in Table 39. The reasons for stopping breastfeeding included inadequate breastmilk supply, issues with expressing breast milk and their babies having lost weight.

Stopping breastfeeding and problems with feeding	Initial Q	7 month Q	12 month Q
Maternal opinion on how long they breastfed*			
Breastfed for as long as they intended	37.8% (14/37)	17.4% (4/23)	20.0% (4/20)
Breastfed for longer than they intended	8.1% (3/37)	13.0% (3/23)	15.0% (3/20)
Would have liked to breastfeed longer.	54.1% (20/37)	69.6% (16/23)	65.0% (13/20)
Reasons for stopping breastfeeding**			
Inadequate breastmilk supply	30% (6/20)	13.0% (7/54)	5.0% (2/40)
Problems expressing milk	25% (5/20)	5.6% (3/54)	10.0% (4/40)
Excessive time expressing milk	25% (5/20)	1.9% (1/54)	5.0% (2/40)
Baby losing weight	20% (4/20)	3.7% (2/54)	5.0% (2/40)
Maternal medical problem	15% (3/20)	3.7% (2/54)	2.5% (1/40)
Lethargy	5% (1/20)	1.9% (1/54)	
Weaning by choice	n/a	7.4% (4/54)	7.5% (3/40)
Other		5.6% (3/54)	10.0% (4/40)
Feeding problems since completing the previous questionnaire (including breast, bottle and / or giving solids)**		41.5% (22/53)	47.5% (19/40)
Swallowing difficulties / gagging / reflux		14.8% (8/54)	15.0% (6/40)
Difficulties managing bottles		9.3% (5/54)	
Feeding position		9.3% (5/54)	
Constipation		7.4% (4/54)	
Gastro – intestinal problems with solids (constipation / flatus / vomiting)		5.6% (3/54)	
Difficulty / slow weaning onto solids			15.0% (6/40)
Medical			12.5% (5/40)
Allergy			5.0% (2/40)
Other		7.4% (4/54)	7.5% (3/40)
Received help or found information to help with these feeding problems***		81.8% (18/22)	73.7% (14/19)

Table 39: Stopping breastfeeding and general problems with feeding including breastfeeding, bottle feeding and giving solids.

Footnote: *The denominator of 37 for the initial questionnaire is greater than the number who said they had stopped breastfeeding. It may have also been answered by some mothers who were giving expressed breastmilk rather than feeding directly from the breast. ** Coded from open responses. ***Further details on the sources of help and information can be found in Appendix 29

The quotes below from the questionnaire illustrate the reasons mothers gave for stopping breastfeeding 'Have you stopped breastfeeding (no longer give your baby any expressed milk or put your baby to your breast)? What were your reasons for stopping?' (the same question was asked in the initial, 7-month and 12-month questionnaires.

"Significant weight loss in early days, poor latch, inadequate milk being taken by baby, readmission to hospital for tube feeding, introduction of bottle feeding in hospital, milk supply subsequently drying up due to baby being put on bottle and use of pumps further made milk supply deteriorate."

"Baby fell asleep too much at the breast to feed properly. It was too time consuming and stressful to keep expressing."

The feeding problems experienced or reported changed as the babies got older as shown in Table 39. Both in the 7-month and 12-month questionnaire, problems with swallowing and reflux were found, but problems with positioning and managing bottles were more common at a younger age. These are illustrated in the quotes below from the 7-month questionnaire (Have you had any problems with feeding your baby (including breastfeeding, bottle feeding and / or giving your baby solids) since the time you filled in the previous questionnaire? Please specify)

"engaging interest in solid food, then getting her to swallow, 'chew' and eat solid food. Made more difficult because so hard to find practical comfortable feeding seating arrangement with 'floppy' nature of DS." (from 7-month questionnaire)

"Child's name has an unsafe swallow and is on a continual feed of 20ml hour via ng tube" (from 7-month questionnaire)

"was told when in intensive care unit that child's name likely had chronic aspiration, therefore she was nil by mouth until January and slowly starting purees while awaiting videofluroscopy in February. Child's name had been breastfed and I did not have enough supply for her needs, so she is currently on a mix of expressed breastmilk and formula via ng tube. Also, I add formula to the EBM to make it more calorific due to weight loss when sick. Child's name is still being treated as cow's milk protein allergy, I do not consume any dairy and the formula she receives is a mix of neocate and infatrini peptisorb" (from 12-month questionnaire)

Mothers were asked "What would have helped you breastfeed longer?" the answers included, not introducing bottles as early, not needing to express, being at home and having more specific support. Below are quotes from the initial, 7-month and 12-month questionnaires in response to this question. Some mothers also used this question to explain that they did breastfeed.

"Knowing as I do now that it's common to have feeding problems with Down syndrome. More specific breastfeeding support." (from initial questionnaire)

"My infections couldn't have been helped, however I feel I was forced to give Child's name a bottle when she was only a day old as the midwife was unwilling to entertain my concerns about the bottle meaning that she would never take the breast. I had wanted to give the milk by syringe or cup to give her a chance to keep trying the breast, but I was shot down and ridiculed." (from 7-month questionnaire)

"I just became too sick unwell but at the same time I fed longer than I thought I would. Thanks to the support I received." (from 12-month questionnaire)

"She lost 6% of her birth weight by day 9 and the NICU home midwife suggested we move onto top up. I think that was the pinch point. If I had said no, let's try harder to feed from the breast it might have established itself. But she had just been discharged from hospital and I was so reluctant to go back. You really have the threat of readmission hanging over you. I also had a three-year-old to look after so couldn't spend hours sat feeding her or encouraging her to feed. I would have loved to feed her myself. It is my biggest sadness but there were too many other variables at play. I am pleased I was able to exclusively breast feed her though for well over 4 months." (from 12-month questionnaire)

5.2.6 Weaning

In this section, results are given regarding the age at which participants were weaned onto solids, the reasons for starting solids at that age and issues that they encountered with weaning. Information is given on ingredients that parents excluded from their child's diets and the reason for this. Table 40 shows that just over half of the babies in the study who had been weaned onto solids by 1st September 2017 were weaned prior to or just up to the recommended age of 6 months. Of those who had started weaning by September 2017, 81.3% (43/53) had received advice on when to start weaning.

Weaning onto solid feeds		
Age when started weaning onto solid feeds		
4 months or less	5.7%	(3/53*)
5 months or less	20.7%	(11/53)
6 months or less	52.8%	(28/53)
7 months or less	94.3%	(50/53)
More than 9 months	100.0%	(53/53)

Table 40: Age at which weaned onto solids.

Footnote: Results are cumulative *53 had started solid feeds by September 2017

The reasons for weaning are given in Table 41, the most common reasons being; the advice of health professionals, previous experience with another baby and having read or seen information which advised starting solids. Advice on which types of solid feed should be given to their baby was received by 80 % (40/50). Further details on sources of advice that the mothers used and what types of feeds the babies had been given at different ages can be found in Appendix 29.

Reason for starting solid feeds	7-month Q	12-month Q
Advice of health professional	50.0% (27/54)	72.5% (29/40)
Previous experience (with another baby)	51.5% (28/54)	42.5% (17/40)
Read leaflets / seen information that advised me to	24.1% (13/54)	27.5% (11/40)
Baby was not satisfied with milk	13.0% (7/54)	5.0% (2/40)
Baby was able to sit up and hold food in hand	11.1% (6/54)	12.5% (5/40)
Start4Life	3.7% (2/54)	7.5% (3/40)
Baby was not gaining enough weight	3.7% (2/54)	7.5% (3/40)
Baby was waking up during the night	5.6% (3/54)	7.5% (3/40)
Advice of friend or relative	-	5% (2/40)
Other	20.4% (11/54)	10.0% (4/40)
Followed standard advice*	7.4% (4/54)	
Parental choice*	5.6% (3/54)	
Number of different reasons given for starting solids		
None	7.4% (4/54)	
1	31.5% (17/54)	40.0% (16/40)
2	40.7% (22/54)	32.5% (13/40)
3	14.8% (8/54)	22.5% (9/40)
4	3.7% (2/54)	2.5% (1/40)
6	1.9% (1/54)	2.5% (1/40)

Table 41: Reasons for starting solid feeds.

Footnote: *coded from free text where there was a commonality between the answers given by different participants

Table 42 shows that of those who were able to answer the question, less than half had issues with introducing solids and the most frequent issues were around the baby not taking certain foods or being disinterested. From the open question, issues around the mechanisms of coping with solid feeds became more apparent.

Issues with weaning onto solids	
Issues introducing solids – yes	41.2% (14/34*)
Issues with starting solid feeds	
Baby would not take certain solids	20.6% (7/34)
Baby was disinterested in food	11.8% (4/34)
Baby prefers to drink food	11.8% (4/34)
Baby would not take solids	8.8% (3/34)
Baby vomiting	8.8% (3/34)
Baby does not like to eat with a spoon	2.9% (1/34)
Other**	23.5% (8/34)
Swallow / gagging	11.8% (4/34)
Texture	11.8% (4/34)
Slow to wean / other	11.8% (4/34)
Number of difficulties with starting solids	
1	8.8% (3/34)
2	5.9% (2/34)
3	8.8% (3/34)
4	11.8% (4/34)
5	5.9% (2/34)

Table 42: Issues with weaning onto solids

Footnote: *There was a branching issue with this question so that people who answered "no" to "Did you get any information about when to start giving solids foods to your baby?" could not then go on to answer questions about issues with weaning onto solids. ** these were coded from open questions and have been included where there is a commonality.
Issues with the branching of the questionnaire meant that six of the mothers were branched away from the questions on issues experienced with weaning their babies

Table 43 shows that of the participants who were able to answer this question, half avoided including certain ingredients in their children's diet. The reasons given for excluding or restricting these foods was largely due to standard guidelines around healthy eating and also standard recommendations for young infants. Some parents gave specific reasons around DS as illustrated in the quotes below.

"Too much salt in any diet is bad leading to problems later in life. Too much added sugar is bad for teeth, and weight. Down's Syndrome has an increased risk of heart problems, weight gain and diabetes."

"I don't eat gluten myself and have read about some of the problems children with DS have with gluten. I do plan to trial her on it when she is older. Sugar-the negative effects of sugar on health Table salt-again don't think this is very good but do add a small amount of sea salt. Dairy- Has butter most days. I have read that can increase mucous production, butter is the most digestible and as she is quite congested have avoided so far but I will introduce more soon"

Restricting diet / omitting ingredients	
Avoids some solid foods with particular ingredients	50.0% (17/34*)
Foods which are avoided	
Refined sugar / sugar	26.5% (9/34)
Salt	14.7% (5/34)
Animal milk/ cow's milk	11.8% (4/34)
Honey	11.8% (4/34)
Soya	8.8% (3/34)
Gluten	8.8% (3/34)
Egg	5.9% (2/34)
Fish	5.9% (2/34)
Nuts	5.9% (2/34)
Other	11.8% (4/34)

Table 43: Ingredients omitted from participant's diets.

Footnote: *As with Table 40, six participants were branched away from this question.

5.2.7 Comparison of Study Cohort and General population

The results of the medical and feeding questionnaires in this chapter are descriptive as the numbers are too small to make any comments about significant differences when compared

to the general population in the UK. The tables below summarise some of the key findings and have been presented together with data from previously published literature on the general population. Comparisons will be explored further in the discussion section at the end of this chapter.

Table 44 shows a summary of the background characteristics for the mothers (refer to Table 23 for more detailed results). This table shows that mothers in the study were older, the majority of mothers were white British, and this was slightly greater than the proportion of mothers in the population for England. A larger proportion of mothers in the study were married, in a civil partnership or living together and a smaller proportion were in employment.

	General Population	FADES participants
Average age of mothers	30.4 years*[1]	Majority over the age of 35 (57.4%)
Ethnicity	83% White*[2]	87% White British
Marital Status of mother. (proportion of mothers who were married, in a civil partnership or living together)	84% *[3]	94%
Maternal Employment status (proportion employed or on paid maternity leave)	66% *[4]	50%*[5]

Table 44: Background characteristics of mothers in the study shown beside data from the general population

Footnote: ***[1]**(ONS 2016), ***[2]** Proportion of mothers classed as White in a study of 600,000 mothers in England (Heslehurst et al. 2010). *[3](ONS 2016). *[4] (ONS 2017) *[5] (ONS 2017).

The summary birth statistics for the general population are shown for comparison together with those of the participants in Table 45 (refer to Table 26 for more detailed results). The number of deliveries via C-section compared to normal vaginal delivery is similar to the

general population as were the proportion of home births (although numbers were very small). The mean birthweight for the babies in the study is also comparable to that of the general population of babies with DS (Morris et al. 2015).

	General Population	FADES Participants
Type of Delivery		
normal vaginal delivery	59%*	63%
C section	28%*	28%
Home Births	2%*[1]	5%
Pre-term births	8%*[2] (<37 weeks)	21% (<37 weeks) Median gestation 38 weeks
Birth weight	3.5 kg	Mean 3.0 kg (0.5 SD)
Admitted to SCBU	14%*[3]	56%

Table 45: Birth details and SCBU admissions for the study population shown beside data from the general population.

Footnote: * (NHS Digital 2017). *[1] (ONS 2015). *[2] (NHS Digital 2017). *[3] (RCPCH 2018)

A selection of the more common conditions found in children with DS are shown in Table 46. The prevalence of congenital heart disease, duodenal atresia/stenosis, Hirschsprung's disease and transient abnormal myelopoiesis within the study population are similar to those described for other children with DS.

	General population	General DS population	FADES participants
Congenital Heart Disease	0.5 – 0.9%*	40 – 60%*[1]	60%
AVSDs	0.2 – 0.3 per 1000 live births**	30 – 40%*[1]	22%*[2]
Duodenal atresia / stenosis	1 in 5000 to 10000 live births***	1-5%*[3]	3%
Hirschspungs Disease	1.6 per 10000 live births****	1-3%*[3]	3%
Transient abnormal myelopoesis	n/a	4-10%*[4]	5%

Table 46: Medical conditions for the study population shown beside data from the general population and the general population of children with DS

Footnote:*(BINOCAR 2013). **Of note this includes babies with DS (Craig 2006). *** This includes those with DS who make up approximately 30% of those with a diagnosis of duodenal stenosis atresia (Eovaldi and Cohen 2018). ****From a study in the North of England (Best et al. 2012).

*[1] (Freeman et al. 1998, Roizen and Patterson 2003). *[2] Of those with congenital heart disease. *[3] (Freeman et al. 2009) *[4] The frequency of transient abnormal myelopoiesis in the general DS population is unknown as it can be a silent condition and will not be picked up unless a full blood count and film are done. The estimated rate however is 4% to 10% of babies with DS (Yumura-Yagi et al. 1992).

Early feeding and breastfeeding statistics for the general population and for FADES participants are given in Table 47. This table shows that at all time points the proportion of babies within the study who were either breastfeeding (including those who received formula) or exclusively breastfeeding were higher (or in one case comparable) to those reported previously for the general population.

	General population[*1]	FADES participants
Skin to skin contact within 24 hours after birth	88%	78%
Initial breastfeeding rates	81%[*2]	90%
Prevalence of breastfeeding at 6 weeks[*3]	55%	64%
Prevalence of breastfeeding at 6 months	34%	54%
Exclusive breastfeeding rates at 6 weeks	23%	21%
Exclusive breastfeeding rates at 6 months	1%	4%

Table 47: Breastfeeding rates for FADES and the general population.

Footnote:[*1] IFS 2010 (McAndrew et al. 2012). [*2] UK rates for initial breastfeeding (babies who were breastfed even if just on one occasion or if received expressed breastmilk). [*3] prevalence of breastfeeding (which includes mixed feeding) in the general UK population at 6 weeks.

5.3 Discussion of Chapter 5: Medical and Feeding Questionnaires

The feasibility of the medical and feeding questionnaires were addressed in Chapter 4. This Chapter determines the secondary objectives of characterising the cohort and exploring the medical and feeding data. This was to establish whether the cohort is representative of the general population of children with DS. Also, whether the questionnaire data revealed information regarding the factors involved in the development of autoimmunity that they were designed to explore. These are discussed in detail in the following sections.

5.3.1 Cohort characteristics

Overall the results suggest that the FADES study population is representative of the overall UK population of children with DS.

Maternal Characteristics

Mothers were of a similar age and ethnicity to other mothers, although a higher proportion were married, in civil partnerships or living together. But fewer mothers were in employment. The majority were at least 35 years old when they completed the initial questionnaire. This is similar but slightly older than a previous research study in 2007/8 which found an average age for mothers of babies with DS of 34.4 years (Morris and Alberman 2009). This might simply reflect selection bias, with older mothers who have decided to go ahead with a pregnancy (a quarter of the babies in the study were diagnosed antenatally, higher than the 11% of live births of children with DS in England and Wales according to the NDSCR (Morris and Springett 2014b) then self-identifying and finding the study themselves. The average age of all mothers in England and Wales in 2016 was 30.4 years (ONS 2016). The majority of the mothers were white British similar to a recent national study which also showed over 80% of mothers in England are white British (Heslehurst, Rankin et al 2010). Interestingly only 60.6% of babies born in England and Wales in 2016 were classified White British by their mother. A larger proportion of these mothers were likely to have been White British but the father having differing ethnicity. A weakness of this study was that only parents who were English speaking were able to join the study inevitably leading to selection bias. The ONS report 2016, reported that 84% of babies in England and Wales were born to parents in marriage, civil partnership or living together (ONS 2016): the FADES population has even higher levels. Parents in stable relationships may feel better able to engage in research. Information on maternal employment status was collected as an indication of socio-economic and educational background. Compared to mothers in the general population in England, fewer mothers in the study were employed (ONS 2017) however due to their child's diagnosis, the

decisions regarding when or if to return to work may have been based on different factors to mothers in the general population. Very little can therefore be concluded from this question; the aim would have been better achieved by asking more detail regarding educational background instead (age left school, university education, qualifications).

Participant characteristics and birth details

Just over half of the participants were female (54%). The percentage of babies in the cohort delivered by spontaneous vaginal delivery or C section were similar to the 59.4% of spontaneous deliveries and 27.8% of C-section deliveries in England between 2016 and 2017 (NHS Digital 2017). The mode of delivery is of interest as the incidence of autoimmune conditions particularly T1D is higher in those born via C-section compared with the vaginal route (Norris et al. 2003). This difference may be due to the microbiota of babies born via C section being different from that of babies born vaginally (Stewart et al. 2018). As the proportion of babies born via C-section in the study is similar to the general population however, this is unlikely to contribute to any increased risk of autoimmune diabetes.

The proportion of preterm births (<37weeks) was relatively high in the study population compared to the 7.9 % of babies born at <37 weeks in England (NHS Digital 2017). None of the babies in the study were very or extremely preterm. The median gestation of 38 weeks within the study cohort fits with the modal gestation reported by Morris et al. of 38 weeks for babies with DS (Morris et al. 2015). Up to 38 weeks gestation the birth weight for babies with DS is similar to that of other babies but by 40 weeks new-borns with DS are smaller. The median birth weight from 37- 42 weeks gestation for boys with DS is 2.97 kg and for girls 2.93kg (Morris et al. 2015): FADES mean birthweight of 3kg is comparable.

The karyotype for the babies in the cohort was not requested which might be considered a weakness. FADES potentially might include children with mosaic Down's syndrome who can be phenotypically milder although this is rare: one to three children in every 100 children diagnosed as having DS have the Mosaic form (Devlin and Morrison 2004). This is unlikely to skew any conclusions. Two children with DS had a non-DS twin. Attempts were made to allow the twin siblings without DS to enter the study as controls. Although the parents were willing, ethics approval was not given.

The median length of hospital stay after birth was five days, often reflecting the amount of time it took to establish feeds but also the time to confirm the diagnosis of DS and check for other associated conditions. This is an important period for the families to adjust to the diagnosis. The range of time babies stayed in hospital was very wide with some babies going home 13 hours post-delivery and others not going home for several months due to medical problems.

Of concern is the observation that over a quarter of the mothers reported that they were not given the correct 'DS insert' in their Personal Child Health Record ('Red' book). This insert provides families and health professionals with important information about children with DS. It contains the correct growth charts for babies with DS (DSMIG 2019b, RCPCH 2019). Healthy babies with DS gain weight and grow more slowly than other children and it is important to understand this so that expectations are managed, and parents are given the correct advice about their baby's feeding. If a baby with DS is plotted on a standard growth chart they will appear to be failing to thrive and a mother who is successfully feeding her baby

may be told that she needs to supplement feeds, start formula feeds if she is breastfeeding or even that her baby needs admission to hospital.

5.3.2 Medical

SCBU admissions

Over half of the babies in the cohort were admitted to SCBU with approximately a third being due to problems with feeding and two thirds with medical complications. The most common reason for SCBU admission was respiratory problems other causes being jaundice, cardiac conditions/monitoring and infection. The degree to which the diagnosis of DS was a factor in the decision to admit the baby to SCBU is unknown. Having a period when their baby was in SCBU will have been helpful for some parents with additional support but for others it may have affected their autonomy particularly around decisions for infant feeding.

Medical conditions

The FADES cohort is similar to children with DS in general in terms of associated medical conditions. Within FADES, 61% of babies had congenital heart disease which aligns with around 40% to 60% of children with DS having a heart condition (Freeman et al. 1998, Roizen and Patterson 2003). AVSD is the most common defect seen (30%) in children with DS and accounted for 20% of the cardiac defects seen in the cohort ((Roizen and Patterson 2003, Freeman et al. 1998). Other medical conditions in the cohort included Hirchsprung's disease, duodenal atresia, transient abnormal myelopoesis, hypothyroidism and sleep apnoea. These are all conditions known to be associated with DS; duodenal atresia/ stenosis is found in one to five percent of children with DS, one to three percent have Hirschsprung disease (Freeman et al. 2009). Although the frequency of TAM in the general DS population is unknown because

it may be a silent condition (unless a full blood count is done), it is estimated that the rate is around four to 10% of babies with DS (Yumura-Yagi et al. 1992). Other conditions that were reported are relatively common amongst all newborns. Reflux was the most frequent condition with 13% of the cohort reporting that their child has reflux however as described by Hyman et al there are many common neonatal and childhood gastrointestinal disorders which might be mis-labelled as reflux (Hyman et al. 2006).

Having a representative sample of Trisomy 21 associated comorbidities is important as feeding is necessarily tied up with co-morbidity and this study focused significantly on the baby's ability to feed, the method by which they are fed and the types and quantities of feeds that were given.

This study was established to focus on the development of autoimmunity but no clinical diagnoses of coeliac disease or T1D were made in the study population during the period analysed. There were two children with hypothyroidism but neither child had antibodies suggesting the cause of hypothyroidism was not autoimmunity. Karlsson et al concluded that although autoimmune thyroid disease was common in people with DS over the age of eight, the causes of hypothyroidism before this age was not clear but maybe due to thyroid hypoplasia or dyshormonogenesis (Karlsson et al. 1998).

It was surprising that all participants required admission to hospital prior to their first birthday. Although acknowledging that children with DS have frequent infections and may have conditions which require surgical intervention, this universal re-admission highlights the severity of the condition. Hilton et al found that most hospital admissions for infants with DS were due to lower respiratory causes unlike admissions for children without DS (Bloemers et

al. 2007, Hilton et al. 1999). When parents were asked about common childhood conditions, chest and respiratory issues were frequent with half of the babies aged between nine and 12 months reporting a problem. Gut problems and weight gain were also common in all age groups up to 12 months. Moore et al previously reported that functional and structural abnormalities within the gastro-intestinal tract are common in children with DS (Moore 2008).

Delivery of care and input of professionals

Parents were asked about the professionals that were involved in their babys' care and the responses that were given show the wide variety of clinical specialties that may be involved. This review of clinical involvement has not been reported elsewhere but is important to inform plans for service provision for future families in the UK. It indicates that some families will be coping with multiple appointments. This should be and was considered when asking families to join a research study such as this one.

The professionals most commonly involved were community paediatricians, cardiologists, physiotherapists, speech and language therapists and dieticians. It would be interesting to compare the input of the allied health professionals between geographical regions, as from discussions with some of the charities, there is a suggestion that the availability of support is inequitable. The input or perceived input (as this is completed by parents not the professionals or from notes) of the community paediatricians was striking, 80% of families said that a community paediatrician was involved in their child's care up until the age of seven months but only a quarter thought that they were involved once their child was 12 months old. The RCPCH has produced a draft service specification for children and young people with

DS which suggests that children with DS are seen every three months in the first year of life by a lead paediatrician (with expertise in DS) and at least a minimum of once every year subsequently to that (RCPCH & DSMIG 2015). The lead paediatrician would in most areas be the community paediatrician and it is concerning that only quarter of parents reported a community paediatrician was involved in their child's care. Community paediatricians suggest that this confusion may arise where children are seen in multi-disciplinary settings where the face to face input may come mainly from the allied health professionals with the community paediatrician oversight (personal communication).

5.4 Antibiotic use and infections

Maternal use of antibiotics

Maternal infections during pregnancy, labour and breastfeeding and use of antibiotics were all asked about. This may lead to changes in the gut microbiota of the baby by altering the maternal microbiome which is vertically transmitted to the baby. Antibiotics given to the mother whilst breastfeeding may also alter the microbiome of the breastmilk which in turn alters the baby's microbiome. Only four mothers were given antibiotics in the last trimester but 18% received them at some point during their pregnancy and four received antibiotics whilst in labour. During pregnancy the type of infection itself may impact on the microbiome especially if they are gut or urogenital infections; some mothers reported having mastitis which again may alter the microbiome of the breastmilk. As the numbers are small it would be impossible to present any significant findings in relation to infant microbiome and maternal antibiotic use however, it is important that the study demonstrates this information can be successfully collected.

Infant antibiotic use

The majority of infants in the study had received antibiotics during the first 12 months of life (70%); but this is no higher than the rates of antibiotic use in a similarly aged general population. The rates for antibiotic use are poorly reported, but are around 70% for children in the pre-school age group and 65% in the first year of life in the general UK paediatric population (Rossignoli, Clavenna, and Bonati 2007, Schneider-Lindner et al. 2010). Given the known immune defects and propensity for respiratory infections it might be expected that antibiotic use might be higher in Downs syndrome. It might be of more relevance that at least a third of the children received multiple courses of antibiotics.

5.5 Feeding

Exclusive breastfeeding amongst the babies with DS at six months is comparable to that seen in the general population (4% vs 1%). Apart from exclusive breastfeeding at six weeks when prevalence was similar (21% of babies with DS versus 23% in the general population) breastfeeding (exclusive or combination feeding) amongst the study participants were higher at all time points than levels reported in the general population. This was surprising and does not support the hypothesis that low tone and oro-motor issues associated with DS preclude normal breastfeeding. This is an important and encouraging message for families, midwives advising them and other healthcare professionals. We need to change the current message which seems frequently heard (from the questionnaire results) that baby may be unable to breastfeed. This will be achieved by making this message a salient feature of a breast-feeding paper from this study. Another relevant finding in relation to the study hypothesis was that when formula was introduced, it was introduced early with a median of five days. Formula

feeds were introduced by 52% of mothers by a week of age in the IFS and 75% by six weeks. Similarly, in this cohort just over three-quarters had been started on formula feeds by the age of six weeks. As discussed in Chapter 1 (Section 1.5.3) the introduction of cow's milk protein in formula feeds leads to the production of anti–BSA antibodies. These antibodies have been associated with autoimmune conditions (Karjalainen et al. 1992, Atkinson et al. 1993). Linking the data from the feeding questionnaires and medical questionnaires with the results of the bloods samples as the cohort grows will enable this association to be investigated.

From the high proportion of mothers in the study who initially breastfed their baby, many of these mothers intended to breastfeed. Due to the wording of the questionnaire, it was not possible to distinguish between those who directly breastfed or those who gave expressed breastmilk, and this would be important to include in future questionnaires. Overall, over half of the mothers were still breastfeeding over the age of six months (including babies who combination fed with breastmilk and infant formula) which is a very positive message for families. This is higher than for the general population for whom prevalence of breastfeeding is 34% at six months.

The feeding questionnaire also demonstrated a high proportion of babies with DS are NG tube fed at some point. Prevalence on NG tube feeding rates in the general population and in the typical DS population are poorly reported. Lewis et al reported that 40% of DS babies in their study had been NG tube fed during the neonatal period (Lewis and Kritzinger 2004) which was less than in FADES. A third of those in the study whose baby was NG tube fed continued to give exclusively expressed breastmilk. Many of the babies were fed using expressed breastmilk either for NG tube feeds or for top up feeds via alternative feeding methods

including bottle. This allowed mothers to “exclusively” breastfeed, but some mothers found this to be “stressful” and “time consuming”. Over 60% of the babies who were NG tube fed received a combination of expressed breast milk and formula down the NG tube. This meant that these mothers were able to continue their supply of breastmilk and had the potential to breast feed in combination with receiving NG tube top ups or breastfeed once the NG tube was removed.

Any advice that is given to mothers on feeding a baby with DS based on the findings of this study would need to include the message that babies with DS can be exclusively breastfed or receive a combination of breast and formula feeds, but due to the challenges with feeding some may need to include expressed breastmilk and alternative feeding methods. Providing mothers with access to tailored support and advice specifically around expressing should be included, as should information on NG tube feeding. This would need to be sensitively presented so that the information is supportive and informative but not overwhelming.

The higher rates of breastfeeding in the study compared to the general population may be due to selection bias. Mothers who have chosen to take part in the study may already have a specific interest in infant feeding and therefore may be better informed regarding the advice to exclusively breastfeed until 6 months of age. In the UK the higher breastfeeding rates are seen in those mothers who are over 30 years of age (87%) (McAndrew et al. 2012) which may also explain the higher rate of initial breastfeeding in FADES.

Barriers to breastfeeding

There are two main breastfeeding barriers for mothers of babies with DS. The first being associated medical conditions which make the baby unwell in the neonatal period and may

require surgical intervention. The other is oro-motor difficulties described in previous publications and reported by some mothers in FADES (Desai 1997, Kumin and Bahr 1999). Mothers reported being advised about the difficulties babies with DS may have with feeding, but they were not told any specific advice on how to help with this. It was positive that one mother had been told that there was no reason why breastfeeding would not work. However, this study provides more accurate and optimistic information for future mothers. Of all the mothers (those with a prenatal diagnosis and those without) only a third had attended an antenatal session on feeding. As many of the issues experienced by the mothers in the study such as difficulties with latching and positioning are common to babies with and without DS, even generic feeding advice would have been helpful.

The majority of mothers in the study had skin to skin contact with their baby in the first 24 hours after birth as recommended as close to birth as possible to help with establishing breastfeeding. It is suggested to benefit both mother and baby although the evidence is weak (Moore et al. 2016) and is mostly for immediate skin to skin contact. The proportion of mothers with a baby with DS who had skin to skin contact within the first 24 hours was less than the proportion seen in the general population likely due to questions over the baby's diagnosis. When mothers of older children with DS talk about their experience of the immediate postnatal period, they wish that their baby had been treated the same way as any other baby and emphasise the importance of having time to bond, this includes time having skin to skin contact (McAndrew et al. 2012, Moore et al. 2016).

The majority did experience problems with feeding after birth. The problems were those typically experienced by any mother, but some also reported issues more commonly

associated with babies with DS. Of those who experienced problems, poor latch was given as the most common issue and this has previously been described (Desai 1997, Kumin and Bahr 1999). There was a high proportion with medical conditions as previously discussed, a third also commented that they had problems with feeding due to jaundice. This is more complex as it was difficult to discern whether the jaundice caused feeding difficulties, or the feeding difficulties contributed to the jaundice. Babies with jaundice tend to be more lethargic and if their jaundice is significant, they need interventions including spending periods of time under a UV light which may interrupt feeding. Lethargy was reported by almost 30% of the mothers for their baby and this continued to be a problem for mothers after discharge. Being more lethargic has been reported previously in babies with DS and unfortunately it is a vicious circle with a baby who feeds less often or for only a short period getting less nutrition and therefore becoming sleepier. Strategies for mothers to manage feeds for these babies is an area for future research. Although large or protruding tongues and low tone can be an issue for feeding babies with DS, the tongue size/position was only mentioned by three mothers and low tone by one mother during this immediate post-natal period.

The proportion of mothers with feeding problems after discharge reduced to just over half, but this is higher than in the general population (around 30% (McAndrew et al. 2012)). Lethargy was the most commonly reported problem with far fewer citing poor latch as an issue once they had gone home. It may be that many more babies had already started using alternative feeding methods including bottle feeds once they had been discharged. Importantly, 16% of the mothers said that poor weight gain or weight loss was an issue. Weight loss is a common problem for many babies at this age (with or without the diagnosis of DS) but as discussed earlier, it is important that babies with DS are plotted on the correct

syndrome specific growth chart. With over a quarter of the mothers saying that their red book did not contain this chart it is of concern that these babies may in fact have had normal weight gain for a DS baby but have been incorrectly plotted on the wrong chart. It was positive to see that most mothers (84%) did receive help or information regarding their feeding problems.

Facilitators to breastfeeding

Most mothers had breastfed a previous child which increases the likelihood of subsequent breastfeeding success (McAndrew et al. 2012). Over 90% of the mothers said they were aware of the benefits of breastfeeding to the immune system including protection against infection. Having the ability to proactively do something to help or benefit their child would have been a motivator for many of these mothers to breastfeed. Almost a quarter were aware of the benefits to oro-motor development but interestingly only 6% mentioned cognitive / IQ development which is the benefit with the most consistent evidence (Quigley et al. 2012, Heikkilä et al. 2011, Kramer et al. 2008). Children with DS have variable degrees of learning difficulties and the any potential improvement to cognitive function might well encourage mothers to breastfeed.

Amongst the mothers who breastfed, over half had put their baby to the breast within half an hour of the baby's birth. The support received by mothers during the postnatal period in the hospital, birth centre or unit was sufficient according to over 80% of the mothers. This will have had a positive impact on the number of mothers who then went on to successfully breastfeed and this is a credit to all the midwives and breastfeeding supporters who helped these families.

5.5.1 Special Care and NG Tube feeds

As previously discussed, over half of FADES babies were admitted to SCBU and all admitted were given NG tube feeds. In around 40% of babies issues with feeding were responsible for NG placement and for most of these participants this was also the reason why these babies were admitted to SCBU. Almost 70% of the mothers said that having their baby in SCBU had affected their ability to feed the way they would have liked. This is an area which requires further investigation to identify if some admissions might be prevented with more specialised/tailored support and input on the postnatal ward. Some hospitals will only have one or two babies born a year with DS, some of these babies may be automatically admitted to SCBU due to increased levels of anxiety around the other potential complications and associated conditions. In terms of oro-motor skill development it is important that babies have the chance to feed orally early on, this is postulated to be important for the development of oral skills, tolerance of tastes and textures and is also important for social development (Mason, Harris, and Blissett 2005). Most of the babies required NG tube feeds for less than two weeks but a third also required feeds to be stopped altogether at some point and were put onto IV fluids.

NG tube feeding was required for some babies due to dehydration, jaundice, polycythaemia and vomiting. Although classified as a 'medical' reason, inability to feed may have contributed or caused these issues. The other 'medical' reasons, prematurity, being on a ventilator, respiratory problems and abdominal issues requiring surgery are all standard reasons for any neonate to receive NG tube feeds. (World Health Organization 2009)

5.5.2 Stopping Breastfeeding

The findings in relation to stopping breast feeding varied between the questionnaires. At each consecutive time point, the data included babies who were older at the time breast feeding stopped. As found in the general population (McAndrew et al. 2012) over half of mothers would have liked to have breastfed for longer but over 40% breastfed for as long as they intended or even for longer than originally intended (initial questionnaire). The wording of the question may have affected the responses. Those who had low initial expectations of their ability or their baby's ability to breastfeed may have been exceeded the duration of breastfeeding that they initially anticipated but with opportunity would still have liked to breastfeed longer. By the 12-month questionnaire the proportion who 'would have liked to have breastfed for longer' increased to 65%. This may represent an increase in knowledge (through DS groups and social media) that some babies with DS can breastfeed and the development of feelings of guilt which are common for many mothers. The proportion who breastfed for longer than intended also increased slightly to 15%. This might be due to the group including babies who stopped feeding at an older age.

The reasons given for stopping breastfeeding were similar to the IFS (McAndrew et al. 2012) with inadequate breastmilk supply and weight loss being common. There were however a large proportion who mentioned issues surrounding expressing milk. This highlights again the need for mothers who are expressing to receive tailored support. Mothers were asked "What would have helped you breastfeed longer" the responses indicated that mothers understandably wanted 'normality', they wanted their babies to be well, to not require bottle or expressed milk and to be at home. Although it is not possible to change these factors for

many of the babies, every effort should be made to listen to the family's desires and assist where possible. Some mothers used this question to voice their frustration at alternative feeding methods being introduced as they perceived too early or against their wishes. The feeling from a few mothers was that NG tube feeds and/or bottle feeds reduced the chances of their baby subsequently breastfeeding.

5.5.3 Weaning - Introducing Solids

Timing of weaning onto solid feeds and the introduced to cow's milk is of interest due to the potential link with the development of autoimmunity. Except for specialised formulas, infant formula contains cow's milk protein. The timing of initiating cow's milk (as opposed to infant formula) was asked but this was in relation to nutritional guidelines which advise that cow's milk should not be introduced until 12 months of age (World Health Organization 2009). Cow's milk was introduced early (median 11 months).

The WHO guidelines are that babies should not be weaned onto solid food before six months of age (World Health Organization 2009). This advice is consistent with the advice to exclusively breast feed until six months of age. Amongst the study participants over 50% were weaned onto solids prior to 6 months of age. In the general UK population in 2010, three quarters of mother had introduced solids by five months of age (McAndrew et al. 2012). This is much higher than the 21% of the study population. The most common reasons given by mothers for starting solids was on the advice of a health professional or from previous experience. From the responses given, the advice to start solids was based on the baby being developmentally ready rather than due to concerns over weight gain. The early introduction of gluten into the diet as the trigger for coeliac disease is potentially also associated with T1D

as discussed in Chapter 1 (Section 1.4.6) (Norris et al. 2003). There is also thought to be a protective factor in mother's breastfeeding whilst introducing gluten (Sollid 2002, Akobeng et al. 2006)

Mothers were asked about feeding problems in the 7-month and 12-month questionnaires which include the period when babies are weaned onto solid feeds. They were also asked specifically about issues introducing solids. More mothers experienced problems with introducing solid feeds than in the general population, with 41.2% having problems compared to 11% in the IFS (McAndrew et al. 2012). In both questionnaires, mothers mentioned problems with swallowing, gagging and reflux, issues known to affect young children with DS (Kumin and Bahr 1999). Other problems associated with oro-motor issues were difficulty managing bottle, problems with feeding position and difficulty or slow weaning onto solids. The numbers were not high for any of these issues showing that although there is commonality with some of the problems experienced, they will not affect all or even most babies. Some mothers said in relation to weaning in the 12-month questionnaire that their babies fed well with no problems, one saying that their child was no different weaning than her other "typical" children. Support should be available where necessary to provide advice on positioning those babies with hypotonia, and suitable food textures for those with swallowing difficulties. As discussed earlier many of the babies had input from speech and language therapists and occupational therapists.

Half of the mothers did avoid giving certain foods to their infants but the reasons for doing so were largely based on standard advice for example around sugar and salt.

5.5.4 Overall ability to characterisation study participants

The data gained from the questionnaires was complete and informative. It revealed the desired information regarding ethnicity and maternal age. It was a weakness of the questionnaire design that the socio-economic background information was minimal, and this could be improved. The medical information was detailed with parents provided extra information in the free text boxes. Analysis of this data confirmed that FADES is representative of the general population of children with DS.

Parents provided details regarding the infections and antibiotic use, some of which was incomplete due to parent's inability to recall all of the information but did show that infections and antibiotic use was considerable in this population.

The feeding data was detailed and revealed new knowledge regarding this population. A weakness of the web-based questionnaire design study was that due to the branching of a few questions, some information was lost. From the questionnaires it was possible to deduce the ages and dates at which the babies were introduced to formula feeds, solids and the age at which breast feeding stopped. Similarly, ages at which the babies had infections and antibiotics could be determined. This was key to the aims of the overall study with the ability to link dates to the samples provided and explore whether feeding, infections and antibiotic use altered the development of autoimmunity.

CHAPTER 6

Initial analysis of clinical samples from the FADES cohort

Chapter 6 Initial analysis of Clinical Samples from the FADES Cohort

6.1 Overview of Chapter 6

In this chapter, results for the initial sample analysis are given for DNA, urine and blood samples. For the mouth swab samples DNA was extracted and HLA class II genotyping was completed. The FADES data is presented together with previously published populations of children with DS, children with DS and diabetes, healthy controls and children with T1D (from the Diabetes and metabolism team (Aitken et al. 2013)). The urine samples were tested for urine C-peptide/creatinine ratios (UCPCR) and the blood samples were tested for anti- bovine serum albumin (BSA) antibodies. For methods used in these analyses see Chapter 2 Methods Sections 2.16 and 2.17.

6.2 DNA samples: Analysis of HLA genotype

HLA genotyping was completed for 52 participants. The HLA genotypes were compared to previously published populations (Aitken et al. 2013) As shown in Table 48, six percent had the highest risk diplotype for autoimmune diabetes (*DR4-DQ8/DR3-DQ2*). As expected, this was less than the population with Down's Syndrome and Diabetes (17%) or Type 1 diabetes alone. Moderate risk haplotypes were present in 27% of the study participants (15% *DR4-DQ8* and 12% *DR3-DQ2*). The proportion of participants with no risk haplotypes was comparable to the DS control population and the healthy control population. Overall, the study population have a similar HLA class II risk profile to the DS control population.

HLA genotype*	FADES participants	Down's Syndrome	Down's Syndrome and Diabetes	Healthy Controls	Type 1 diabetes
N=	52	222	97	621	194
DR4-DQ8/DR3-DQ2 High risk (%)	6	2	17	3	38
DR4-DQ8/X (%)	15	12	24	13	40
DR3-DQ2/X (%)	12	22	32	27	17
X/X (%)	67	64	27	57	5

Table 48: HLA genotype. HLA risk genotypes of FADES participants, Down's syndrome control population, Down's syndrome and Diabetes, Healthy controls and population with Type 1 diabetes (control populations are described in (Aitken et al. 2013))

Footnote : **HLA DRB1*04-DQB1*0302/HLA DRB1*03-DQB1*0201, HLA DRB1*04-DQB1*0302/X* and *HLA DRB1*03-DQB1*0201/X* are described as *DR4-DQ8/DR3-DQ2*, *DR4-DQ8/X* and *DR3-DQ2/X* respectively where X is not *HLA DRB1*02-DQB1*0602* (*DR2-DQ6*)

6.3 Urine Samples: Analysis of Urine C-peptide

The urine, C-peptide creatinine ratio (UCPCR) was measured in 108 samples from 54 participants, 32 of whom had provided longitudinal samples. UCPCR measurements would not normally be done on children unless there was evidence of diabetes or an abnormal glucose tolerance test. UCPCR is a measurement of endogenous insulin secretion and is normally measured following a meal stimulus. As discussed in Chapter 2 Methods (Section 2.16.3.1) the timing of the urine sample in relation to feeds is important for interpretation.

Figure 30 shows the UCPCR threshold of 0.37nmol/mmol which was suggested by Besser et al as the threshold for significant endogenous insulin secretion in children (Besser et al. 2011). There were a proportion of children in FADES with low UCPCR levels (See Appendix 30): four samples with detectable but minimal UCPCR of ≥0.03nmol/mmol but less than 0.2 nmol/mmol (the cut off for intermediate insulin secretion ref Exeter) and 13 samples that had

undetectable UCPCR. These thresholds for minimal and detectable UCPCR are based on an adult population rather than a paediatric population. The minimal and undetectable samples were from 14 participants, none of whom had a diagnosis of diabetes and therefore were unlikely to have such low C-peptide levels although there is a dearth of data on C-peptide levels in infancy. This raises an issue regarding protocol compliance and their results were examined closely. Of the 14 participants with low C-peptide levels, there were six participants who had samples which were undetectable on the last available sample. The others (n=8) had at least one sample in which UCPCR was measured as at least intermediate insulin secretion. Interpretation of these results is explored further in the Discussion at the end of this chapter but there was non-compliance with the protocol in relation to the timing of sampling post feeds which will affect results. Of note, 38.5% (5/13) of those with an undetectable UCPCR were under the age of six months.

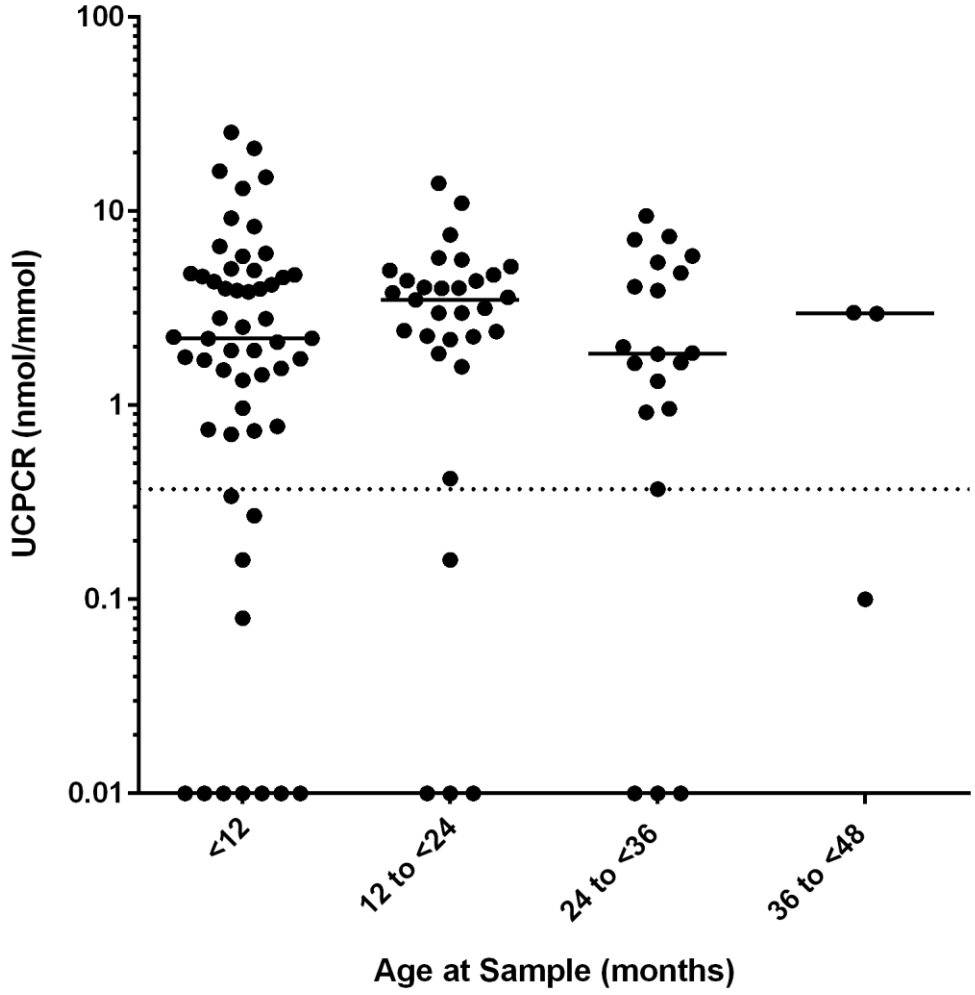


Figure 30: Urine C-peptide Creatinine Ratio (UCPCR nmol/mmol) levels in samples from participants N=54, followed longitudinally (total samples tested N=109). The dashed line represents the threshold for significant endogenous insulin secretion in children of 0.37nmol/mmol. The solid line represents the median UCPCR value at each timepoint.

6.4 Blood Samples: Analysis of Anti BSA Antibodies.

Analysis of anti-BSA antibodies was completed on serum samples from 53 participants, 30 of whom had longitudinal samples. A control group of 18 samples from children aged under two years from the BOX study were also analysed (males n=9, median age 1.6 years (IQR 1.3, 1.8years)). Anti-BSA antibodies were found to be positive in 58.5% (31/53) of the FADES participants tested. As seen in Figure 31, there was a distinct group of participants who had anti BSA antibodies and a group who were negative for anti BSA antibodies (below the threshold).

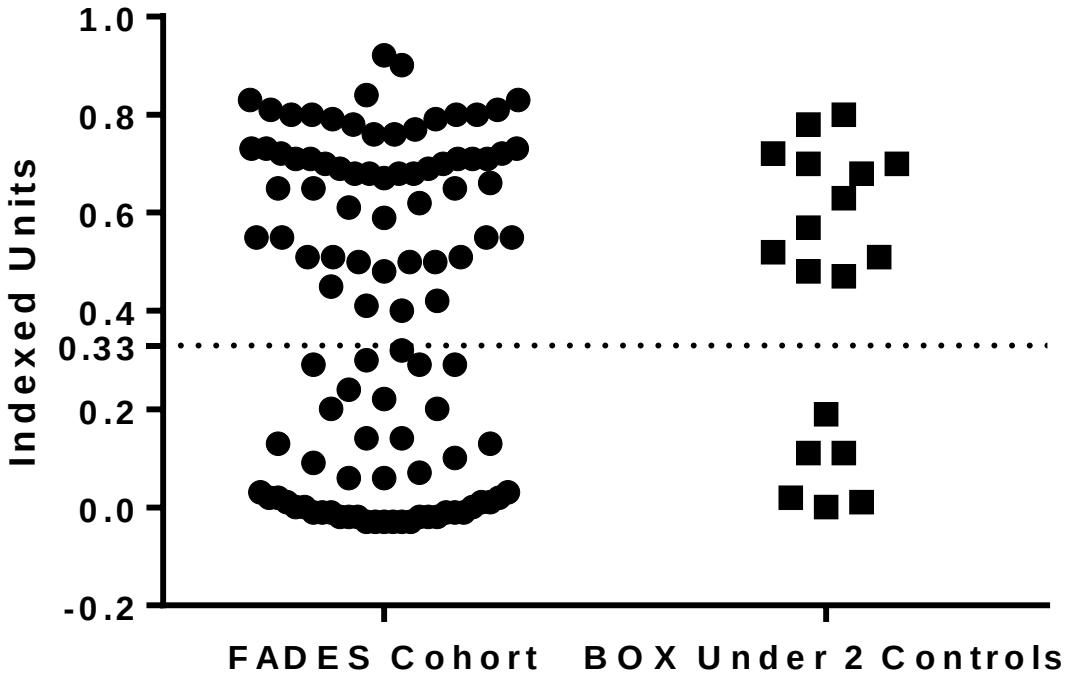


Figure 31: Anti BSA antibodies in the FADES cohort and BOX (Barts Oxford study) under 2 control population. The threshold for positivity 0.33 Indexed Units was determined using 244 healthy schoolchildren (90th centile) (see Chapter 2 methods).

Figure 32 shows that anti- BSA antibodies developed with age as would be expected with the introduction of formula feeds and solids containing cow's milk protein.

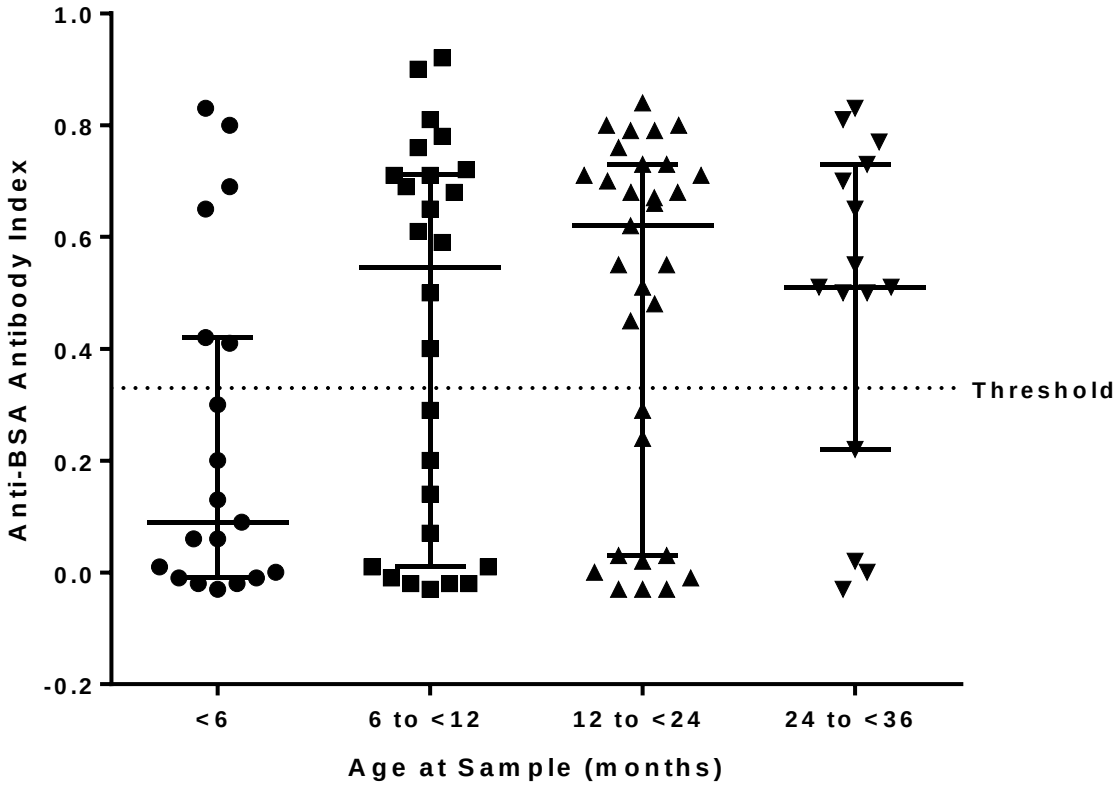


Figure 32: Anti-BSA antibodies in FADES participants from Baseline (<6 months) to 3 years. The threshold for positivity: 0.33 Indexed Units was determined using 244 healthy schoolchildren (90th centile).

The development of anti BSA antibodies with the introduction of feeds is shown in Figure 33. As the numbers of samples within some of the groups for age at which formula was introduced were small no clear trends can be ascertained. There were two samples provided by the same participant prior to any formula being introduced and these show that this participant was still negative for anti BSA antibodies at a year. Conversely there were also participants who had formula introduced before six weeks of age but who remained anti-BSA antibody negative at a year.

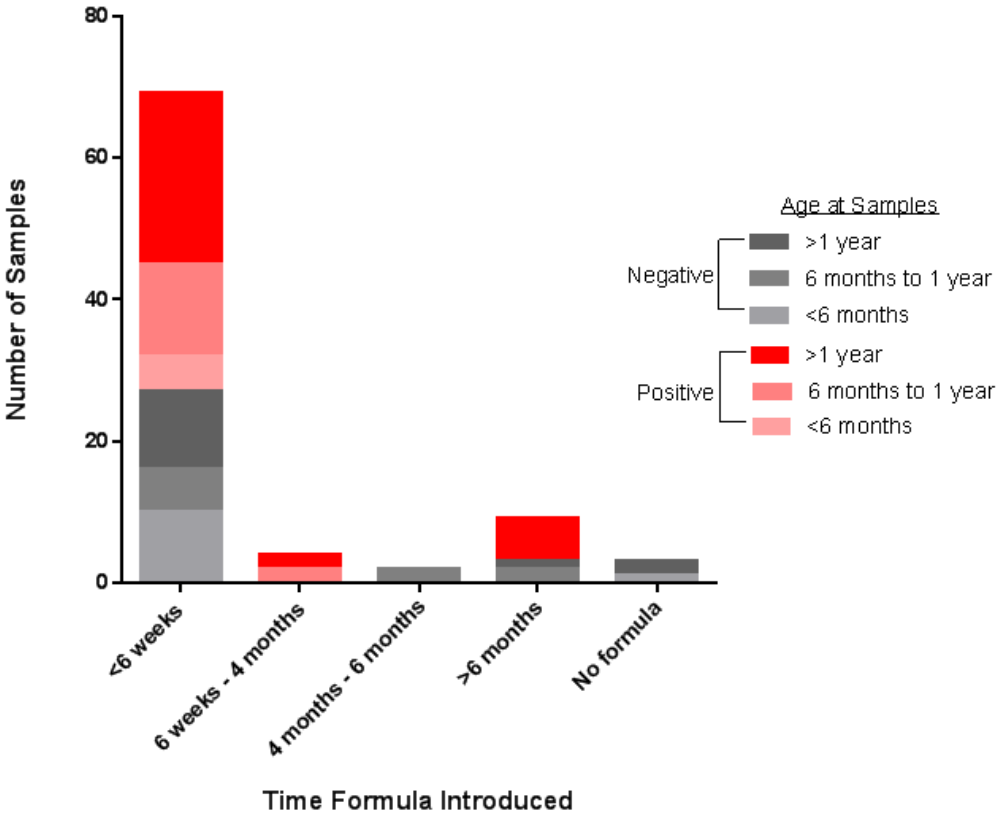


Figure 33: Anti BSA antibody positivity in relation to age and age at which formula was introduced.

Footnote: For two samples there was no feeding data (two different participants) available one was negative 0.29 Indexed Units for BSA the other was positive 0.42 Indexed Units.

6.5 Discussion of Chapter 6: Sample analysis

Overall the study showed the ability for parents to collect their own child's samples (DNA, urine and stool) using the instructions and kits provided and for them to return them in the

standard mail. The pilot studies for the collection of urine and stool samples provided validity for some of the collection methods used.

The sample analysis showed that the DNA samples collected were adequate for analysis of HLA genotype, urine samples were adequate for measurement of urine C-peptide and blood samples were suitable for analysis of Anti-BSA antibodies.

As the number of samples included was small, the findings were descriptive and not statistically analysed but showed that the FADES population had similar HLA class II risk profile to a previously published DS control population (Aitken et al. 2013). Aitken also shows that DS with diabetes have less HLA risk than those with T1DM without DS (Aitken et al. 2013).

Interpreting the results of the urine C-peptide measurement is challenging. Urine C-peptide is usually measured post prandially, with urine collected two hours after a standard mixed meal (Greenbaum et al. 2008). As previously mentioned, it is not possible to time exactly when a baby will pass urine following a feed and although it may be possible to provide a fixed feed volume consumption is not guaranteed. There are also no reference ranges for very young babies. The results showed 14 participants who had a sample with minimal or undetectable urine C-peptide which would be expected in someone with diabetes who has inadequate insulin production due to beta cell destruction. None of these babies had been diagnosed with diabetes and many went on to have further samples which did show evidence of detectable urine C-peptide. It is more likely that these results were due to samples being collected when the babies were fasted and had had a long period post feed. Another explanation might be that the samples were collected onto cotton wool (It is a weakness of the study that information on which collection method was used was only collected for a

small number of participants). Repeat 'clean catch' samples could be collected to confirm the results. The initial instructions had advised parents to collect the urine samples after a feed but had not asked for this time to be recorded on the sample sheet. An amendment was put in place, but the timing of the urine sample was still not consistently recorded and where it was recorded sometimes revealed the long period between feed and sample. The addition of information regarding the timing of the urine sample post feeds will help to improve this but was a weakness of the study. Nearly 40% of those who had an undetectable urine C-peptide were under the age of 6 months and levels at this age may be very low or drop considerably faster after a meal. Renal immaturity at this age may also account for reduced clearance of C-peptide via the kidney. Comparison with data from non-DS infants will be carried out in future to help explain the C-peptide results.

The methods used for the collection and transport of the blood samples was shown to be adequate and the analysis shows that most samples were suitable for testing. Importantly the results could be linked to longitudinal feeding data. One of the questions that the cohort was designed to answer in the future is whether the early introduction of cow's milk protein in the form of formula feeds correlates with increased risk of autoimmunity. The development of anti BSA antibodies is a marker of the immune system having been exposed to cow's milk protein and has been associated with the development of autoimmunity (Karjalainen et al. 1992, Atkinson et al. 1993). The analysis showed that there was a group who were positive for anti-BSA antibodies and a group who were not. Numbers were small and therefore it is not possible to draw any significant conclusions but it shows the feasibility of the collection, transport, storage and lab methods. The proportion of participants who were positive increased after the age of six months as would be expected after weaning. The percentage

of babies who are exclusively breastfed beyond this stage is around 1% in the general population and 4% in this cohort. However, when the feeding data were looked at in more detail, it showed some participants who had formula introduced early remained anti BSA antibody negative even at a year of age. These differences are most likely to be due to inherent differences in the participant's immune responses. They may also be related to level of exposure; some babies may have received only a few formula feeds whilst breastfeeding was established. However, Juvonen et al. suggested that development of anti-BSA antibodies was related to timing of exposure to formula rather than duration of exposure (Juvonen et al. 1999). Mothers who breastfeed and also consume dairy products will still pass some cow's milk protein to their baby and therefore the feeding data regarding exposure to cow's milk protein is not complete as mothers were not asked as to their own diet and exclusion of dairy.

Participants were able to collect stool samples at home and send them in the standard post. The stool samples have not yet been tested (currently stored frozen at -80^{0}C) so it is not possible to comment on how usable the samples are, but the methods have been tested in the feasibility study (Chapter 2 section 2.15).

The study continues to collect a bank of data on early feeding, medical conditions and infections for these children which can be linked to longitudinal samples. As the cohort progresses it is expected that Type 1 diabetes and other autoimmune conditions will be diagnosed in participants or that antibodies associated with such conditions will develop and be detectable. It will then be interesting to determine whether these participants are those with the higher risk HLA genotype although earlier data suggests this association is not strong in diabetes in children with DS. Having urine samples as part of the bank of biological samples

that have been collected from these participants adds to the completeness of this cohort. Urine samples maybe used in the future not only for urine C-peptide measurement but also potentially for looking at the metabolome of these participants. Future testing of the blood samples for auto antibodies and linking this data with the results of the feeding and medical questionnaires and other sample analysis will allow exploration of the factors involved in the development of autoimmunity in this population.

CHAPTER 7

Final Discussion and Future Direction

Chapter 7 Final Discussion and Future Direction

7.1 Overview of Chapter 7

This chapter summarises the principal findings in relation to the feasibility and secondary objectives. Strengths and weaknesses of FADES are discussed, as well as recommendations made for future national birth cohort studies of a relatively rare conditions. The final sections describe future directions and conclusions.

7.2 Principal Findings

The establishment of the only UK wide birth cohort of babies with DS to prospectively study early feeding and the development of autoimmunity in children with DS has been achieved. FADES forged successful collaborations with all major DS charities in the UK including the DSA, DSS and DHG, as well as with clinicians in the DSMIG. The cohort continues recruitment until 2020, and follow up until 2022, with over ninety participants to date. I have detailed information on early feeding, medical conditions, infections and antibiotic use during the first few years of life in children with DS. The input of professionals from a range of specialties in the care of these babies has been described. A bank of longitudinal samples were collected including DNA, urine for C-peptide and future metabolomics, stool for gut microbiome and blood samples for autoantibodies and other biomarkers.

The study was presented at the DSMIG meetings, the Royal College of Paediatrics and Child Health Conference and at the World Down's Syndrome Congress 2018. The Study team held a dedicated symposium at the World Down's Syndrome Congress where delegates included families, world experts, current and future collaborators.

A confidentiality agreement has been signed between members of the FADES team and a pharmaceutical company based in Norway (Pharmasum) who have developed a DRYK1A Kinase inhibitor, (DRYK1A also known as mini-brain in Drosophila flies). DRYK1A is found in the DS critical region on chromosome 21, is over-expressed and may be important in dementia and diabetes (Dirice et al. 2016, Nguyen et al. 2018).

My initial PPI work supporting the establishment of a birth cohort of children with DS was confirmed as important by families, clinicians and researchers. My paper describing challenges in recruiting babies with DS concluded that a flexible approach to recruitment is required whilst fully supporting alternative modes of engagement, including social media, parent groups, charities and websites. Over half of the FADES participants were recruited using these alternate routes. I confirmed that using the internet for recruitment is both a practical and successful solution for low risk studies looking at a relatively rare conditions over a large geographical area. In addition, providing web-based questionnaires was popular with participants with the majority of families completing the questionnaires online. This method proved convenient and practical for parents and the study team alike.

Analysis of questionnaires demonstrated that the desired key questions in relation to factors which have been hypothesised to be involved in autoimmunity were addressed. These included; mode of birth delivery, feeding and the introduction of formula feeds, introduction of solid feeds likely to contain gluten, antibiotic use and infections. Although no formal statistical comparison could be made, there were no clear differences seen in any of these areas when frequencies were compared to the non-DS, general population. A key finding from the study was that some babies with DS can breastfeed exclusively for six months as per WHO

guidelines. This may require significant perseverance from the mother and supportive professionals, as establishing breastfeeding was challenging for some. This was often due to oro-motor difficulties and significant medical needs, but the take home message from this study is that mothers who wish to breastfeed should be encouraged and supported to do so as success is possible.

The feasibility studies for the collection of urine and stool samples (see Chapter 2 Section 2.15) provided validity for the sampling methods used. The paper describing the optimal collection methodology for long distance gut microbiome sampling (Williams et al. 2019) should prove invaluable for future studies covering a wide geographical area. Overall, FADES demonstrated the ability for participants to collect their own child's samples (DNA, urine and stool) and for these to provide adequate material and quality for analysis after return to the laboratory by standard mail. HLA genotyping shows that the study population are representative with a risk profile similar to that of a previously published DS control population (Chapter 6 Section 6.2). Analysis of anti BSA antibodies demonstrated the development of antibodies with the introduction of formula feeds, although this was not entirely consistent for all participants. The differences seen are likely to relate to a variation in individual's immune system. Continued analysis of additional samples is ongoing. Describing these findings in relation to the development of autoimmunity in these participants will be possible once autoantibody testing has been completed.

Thabane et al (Thabane et al. 2010) described the four potential outcomes of a pilot/ feasibility study as: 1) Stop - not feasible and therefore not to continue with the main study. 2) Continue – the study is feasible but requires modifications to the protocol. 3) Continue –

without adjusting the protocol but would need to continue to monitor closely. 4) Continue – no modifications required, and the study can continue with the current protocol. This study falls under 'feasible with modifications' but these modifications are minor and easily achieved, although recruitment rates are still under those originally envisaged. Many aspects of the study design were successful and will remain including the use of web recruitment and online questionnaires. Sample collection methods have been shown to work but could be optimised further. The lessons learnt during this study which might inform a future cohort study are discussed below (Section 7.4). Improvements to the protocol and future directions for the study are also discussed in more detail (Section 7.5).

This study provides the springboard for future research. It has generated a bank of data and samples from which the associations between early life factors and the development of autoimmunity can be explored. Antibody testing needs to be completed in order to determine the number of autoantibody positive participants. The number of participants required to calculate statistical power for findings in relation to; feeding practices, differences in microbiome or antibiotic use, and infections could then be calculated and used to inform a major grant application.

7.3 Strengths and Weaknesses

The retention of participants was the main strength of this longitudinal cohort study. Over half of the participants provided all of the requested samples and questionnaires over two years. Attrition is a major issue for longitudinal cohort studies particularly those involving paediatric patients (Zook et al. 2010, Golding and Birmingham 2009). There are many publications describing tactics for retaining participants and many of these were adopted in

this study including, reminders, newsletters and regular email contact (Golding and Birmingham 2009, Marmor et al. 1991, Morton et al. 2014).

The main weakness of this study was the ability to recruit enough participants within the time period. However, compared to other cohorts involving young babies with DS who were followed prospectively, this is now a notably large cohort and continues to grow. A sample size calculation was not made for this study as the use of sample size calculations for feasibility studies is inappropriate (Lancaster, Dodd, and Williamson 2004, Arain et al. 2010, Thabane et al. 2010). Thus descriptive results using proportions and confidence intervals were acceptable. A true feasibility study should come before a 'main study' however, as this is an important cohort and recruitment will always be the main stumbling block, the current participants will remain whilst the study continues recruiting further participants. It would also be unethical to remove families from a low risk, longitudinal study such as this in which they have invested much time and effort.

The methods for assessing the feasibility of the study were robust with clear feasibility objectives (Eldridge, Chan, et al. 2016, Lancaster, Dodd, and Williamson 2004). This included a qualitative study providing important and deeper understanding of the issues that were being faced by families in relation to recruitment. A previously published study also explored the issues around recruiting children with DS into research but did not specifically look at recruiting very young babies (Fortnum et al. 2014). FADES did not include a dedicated longitudinal PPI advisory group which was a weakness but throughout the study there was close collaboration with the DSA, DSS and DHG which were all established as parent-led

charities. For initial PPI, a local parent support group (BADSS) was also consulted on the protocol, the participant-facing information and questionnaires.

The families recruited were representative of the general population of children with DS with similar numbers of children having associated conditions including cardiac abnormalities as reported in other series. The eligibility criteria were broad and did not exclude any medical conditions. A further strength is that the study was UK wide rather than specific to a regional population reducing selection bias. Those who could not speak English were excluded and this may have had altered feeding results with cultural differences in early feeding (Griffiths, Tate, and Dezateux 2005). Translating all study documentation however was not possible for the multiple languages represented in UK. The majority of mothers in the study were white, this is similar to the proportion seen in other national studies. It is acknowledged that feeding practices amongst different ethnicities varies with mothers who are white having lower breastfeeding rates than other ethnic groups according to the Millennium Cohort Study (Griffiths, Tate, and Dezateux 2005). The Millennium cohort also highlighted that partner's ethnicity influences breastfeeding rates but partners ethnicity was not obtained and this can be amended for the study going forward. Further information on the socio-economic status of the parents would also have provided valuable information particularly educational background as breastfeeding rates are lower amongst those without academic qualifications again this will be amended.

Ideally as a 'birth cohort', recruitment should occur prenatally allowing questionnaires to be completed without recall bias and samples completed at the time of birth for both mother and baby. This is particularly pertinent for the collection of microbiome samples from the

mother as it is the vertical transmission of microbiota at the time of birth which is of interest. However as previously described for many parents the diagnosis of DS is unexpected (Hedov, Wikblad, and Annerén 2002, Van Riper 2007). Samples analysed during a longitudinal cohort study should ideally be collected from a control group which can be tested at the same time. However, it is unlikely that ethical or parental approval would have been obtained for collecting multiple biological samples from 'healthy babies'.

It was not possible to collect preserved whole blood samples for detailed immune cell analysis from the babies as had been proposed in the original protocol. As described, obtaining adequate samples for antibody analysis already provided challenges. It was clearly a weakness of the study as alterations in the T cell population are likely to play a significant role in the development of autoimmunity in this population. It might be possible to obtain a greater volume of blood once the babies are older. Ethical approval was obtained, and families were consented to use the day five blood spot that is collected from every baby as part of the new born blood spot screening program (England 2018). This would mean that even if babies are recruited at an older age, a very early sample would be available for testing. Dried blood spots can be used to tested for auto-antibodies. Unfortunately, although FADES had ethics approval, the newborn screening laboratories were not prepared to provide such sample. Although in the past the blood spots had been used for research, this has recently been under consultation.

Other cohorts of children with DS have focused on the child's development or on very disease specific conditions (Bloemers et al. 2007, Carr 1988, CDSS 2018). FADES in comparison, is more holistic and includes the experiences of parents, clinicians/ charities and professionals.

It has achieved this through the qualitative work and through the free text boxes that were available to parents throughout their questionnaire sessions contributing to its value.

7.4 Researcher Impact

Throughout the study has been influenced by my own position as a paediatric doctor specialising in paediatric diabetes and endocrinology. I have worked with families and children with DS prior to my PhD studies both acutely in hospital and in the community. My medical position helped with understanding some of the complications and medical needs of the children after birth but from a very clinical perspective. This was important for the planning and set-up of the study and enabled me to answer questions from sites, collaborators and when talking to parents. However, my understanding of the personal journey that parents go through has developed and grown through this study and has been a valuable lesson for both my research and clinical career.

Initial hypothesis in relation to feeding were influenced by my prior experience particularly around the difficulties that some DS babies have with feeding, but it was important to recognise that as a clinician I was seeing those babies with difficulties more than those without (reflexivity was discussed separately in Chapter 3 with respect to the qualitative study).

It would be of value to the study to develop a dedicated PPI group which includes families already enrolled in the study as this would reduce some of the inherent biases that exist as a result of the study being designed and run by clinicians and scientists. It would be of particular

importance for such a group to review the results of the feeding data and when producing information leaflets for feeding recommendations.

7.5 Lessons learnt for future studies

From this study the following recommendation can be made when planning the development of future national, rare disease cohorts. Early involvement and engagement of all stakeholders is key. For a condition such as DS, diagnosed around the time of birth this should include the neonatal networks, parent organisations, community paediatricians, national charities and medical interest groups. A focus group including stakeholders and, in this case, new parents of children with DS can provide important insights when designing a study protocol, the participation information leaflets, consent form and overall support for the study. This is crucial to successful approvals processes and may potentially engage local collaborators earlier. Applying qualitative research methodologies to understand potential barriers and motivations for families to engage should occur prior to or early after study onset. Qualitative research can focus on various aspects which would benefit a study, providing a deeper understanding of parental perspectives which can aid recruitment and retention. Such an approach can highlight aspects of the study which might be unacceptable and logistical challenges that might not have been previously considered by the research team.

The time taken to gain R and D approvals across the UK prior to the start of the study was considerable but did reduce delays for the participants. For future studies, it may be advantageous to identify potential local collaborators and focus the gaining of permissions to

those sites. For those self–identifying through web recruitment in sites with no local collaborator this may however cause a delay. The process for gaining approvals across multiple sites has now changed to HRA which has been designed to be quicker (HRA. 2019). Therefore, aiming to obtain approvals at all sites may still be optimal.

Adopting the emerging technologies becoming available to research studies helps provide ease of access, accuracy and security. Participants will increasingly expect to be able to undertake research online at their convenience, as illustrated by most participants opting to complete the web-based questionnaire rather than the paper version. Therefore, I would recommend that study information 'sheets' be provided on websites. Consequently, potential participants should be able to identify themselves via an online form and questionnaires should become web-based. Availability of email access to the research team means that issues can be dealt with promptly and participants and researchers can develop a relationship during an otherwise non-participant facing study.

This study proves that parents can collect DNA, urine and stool samples from their babies independently at home and return them in the standard post. For large cohort studies this is convenient and reduces costs. The feasibility study for the collection of stool samples showed that the OMNIgene•GUT kit was the optimal method for preserving the samples whilst being posted in the standard post. For the collection of urine samples for urine C-peptide, a clean catch method is the most reliable system. Collection onto cotton wool could be used where parents are struggling, but if the UCPCR is very low, a repeat clean catch sample should be obtained. It is important that information is collected on the timing of feeds prior to the sample collection.

The recommendations from families were that more information should be provided regarding the sample collection. The use of online videos to demonstrate how samples are collected are being used in other studies and have received positive feedback (personal communication from the BOX study). Timing of blood samples and methods for collecting them continues to prove problematic. The use of research nurses who can meet participants at their appointments and collect the blood sample is the ideal; but may not be possible at all sites. Involving local collaborators and the clinical research networks as much as possible facilitates blood sample collection and discussions should be held with them early during the study set-up.

7.6 Future Directions and Unanswered questions

Why people with DS are at increased risk of autoimmunity still needs to be established. Autoimmune conditions are more common with a three-fold increased risk of diabetes (Bergholdt et al. 2006), ten-fold increased risk of coeliac disease and four-fold increased risk of thyroid disease compared to the general population (Karlsson et al. 1998, Bonamico et al. 2001). It is acknowledged that people with DS have variations in their immune system which leads to an increased number of infections, autoimmune conditions and haematological malignancies (Øster and Nielsen 1975, Kusters et al. 2009).They have an altered T cell population and T cell function is impaired, (Kusters et al. 2009). In addition, the histological appearance of the thymus which essentially acts as an immune system regulator in early life, in people with DS is different. The thymus is important in the development of the immune system: T regulatory cells (Tregs) expressing FOXP3 come from the thymus and suppress the immune response to self-antigens. In individuals with DS, there are an increased number of

Tregs but their function is impaired. These alterations in the immune system are the most likely cause of the increased risk of autoimmunity. However, there are some confounding factors including the increased number of infections seen in people with DS and antibiotic use which may alter microbiota which in turn, may affect the developing immune system. A complex web therefore needs to be explored and untangled.

There may also be a genetic explanation. A recent paper by Johnson et al looking at neonatal diabetes and DS has supported previous publications which show that autoimmune TID can occur early (before six months of age) in babies with DS and may not be HLA mediated (Johnson et al. 2019). Those with TID and DS do not have the prevalence of the high risk HLA diplotype *DR3/DR4* as seen in people with TID alone. The authors conclude that T1D in people with DS includes instances where T1D is linked to an HLA risk genotype and those which are not. Other genes described in Chapter 1 (Section 1.4.3) which are located on chromosome 21 are candidates for a causal link. These include Ubiquitin associated and SH3 domain containing A (*UBASH3A*) gene, autoimmune regulator (*AIRE*) and the cluster of genes which includes four interferon receptors (*IFNAR1, IFNAR2, IFNGR2* and *IL10RB*). These have all been implicated in T1D and other autoimmune conditions independently of DS and the mechanisms by which they affect the development of autoimmunity have been explored. Studies specifically examining the expression of *AIRE* in people with DS have revealed compelling arguments for its role. Mutations in *AIRE* lead to APECED which as previously described has overlaps with conditions seen in DS. The expression of *AIRE* within the thymus of people with DS has been shown to be reduced (Lima et al. 2011). It plays an important role by regulating the transcription of genes encoding antigens allowing the development of self-tolerance. The structural changes seen in the thymus of people with DS are also consistent

with a reduced expression of AIRE (Giménez-Barcons et al. 2014). Testing the FADES cohort for mutations within these candidate genes and analysing these together with auto-antibody data will provide further evidence for any potential links.

The role of the gut microbiome in autoimmune disease is becoming established with publications suggesting those with autoimmune conditions have a less diverse gut microbiome (Giongo et al. 2011, Kostic et al. 2015). A recent study of the longitudinal gut microbiome in children participating in TEDDY Study, showed that the developing gut microbiome undergoes three distinct phases of microbiome progression: a developmental phase (months 3 to 14), a transitional phase (months 15 to 30), and a stable phase (months 31 to 46) (Stewart et al. 2018). Receipt of breast milk, either exclusive or partial, was the most significant factor associated with the microbiome structure. It will therefore be important to link any microbiome data from this study with feeding data. Longitudinal stool samples have been collected but are yet to be tested, there are no other studies which have looked at the microbiota of those with DS nor related them to outcomes of relevance.

In order to recruit a cohort of sufficient size to answer some of these questions, FADES will probably need to be established internationally. In some countries, unlike the situation in the UK, there are national registers of children with DS making identification of potential participants much easier (Bergholdt et al. 2006). Professor E. Molloy (Trinity College Dublin) is establishing such a register in the Republic of Ireland and we are collaborating with her to establish FADES there. The amendment for this expansion has been approved through the HRA we are now awaiting local approval in Ireland. Up until January 2019, the number of women who could legally have an abortion in Ireland was very restricted, this changed after

the referendum in May 2018 to repeal the Eighth Amendment. However, due to cultural and religious beliefs in Ireland many women may still not consider screening for DS and termination of pregnancy would not be an acceptable option for them. The number of babies born in Ireland with DS is therefore still likely to be higher than in the UK despite the smaller overall population. Within the UK, the study will continue to involve new local collaborators with a particular focus on the neonatal networks.

This feasibility study has raised some important questions and highlighted potential opportunities for interventions in relation to early tube feeding in children with DS. A high proportion of babies required NG tube feeding but it is important to determine why, whether this practice is the same in all regions and if any recommendations or changes could be made in relation to this. In order to be able to develop guidelines for feeding babies with DS, some of the more detailed questions from the FADES questionnaires, including those around who provided feeding support in the hospital, birth centre and after discharge home can be investigated in more detail.

Another area for future development and potential improvement is in the screening guidelines for autoimmune conditions in the DS population. FADES will likely provide useful information on the age at which organ specific antibodies first develop. This is particularly pertinent for coeliac disease which can cause significant problems with abdominal symptomatology and thriving but is often diagnosed late (Csizmadia et al. 2000, Bonamico et al. 2001). There are new guidelines being developed for coeliac screening in DS which are likely to include HLA genotyping to help stratify risk of coeliac disease and guide screening. Although HLA genotype is known to confer risk in the general population (< 1% of patients

with coeliac disease lack DQ2 and DQ8), it is important that the role of the HLA genotype for coeliac disease is also confirmed for those with DS as screening and diagnostic testing may be different in this population as it is with T1DM (Wouters et al. 2009). FADES is perfectly positioned to answer this type of clinical question.

The cohort has collected questionnaire data and samples that could also be used for previously unrecognised research avenues in DS. A potential area of interest is to look at the developing longitudinal metabolome of the children in the cohort (Caracausi et al. 2018). Caracausi et al have suggested differences in the metabolomic profiles of children with DS compared to a control population identifying differences in metabolites related to mitochondrial metabolism. It has been postulated that there is a metabolic explanation for the intellectual impairment seen in people with DS. If this was confirmed it might lead to potential therapeutic targets.

The points below summarise the next steps this and future studies would need to take to test the hypotheses discussed in this PhD:

- Ongoing recruitment and establishment of the study in Ireland.
- Testing of T1D auto-antibodies, thyroid antibodies and TTG antibodies to determine the natural history and development of autoimmunity (specifically Type 1 diabetes, coeliac disease and thyroid disease) amongst the cohort.
- Relate the development of antibodies to HLA genotype, feeding and medical data, infections and antibiotic usage.
- Test for mutations within candidate genes on Chromosome 21 and relate these mutations to the development of antibodies.

- Analyse gut microbiome samples from the cohort to characterise the gut microbiome of infants with DS and to determine if the gut microbiome is altered in those who go on to develop autoimmunity.

7.7 Conclusion

The ongoing study of the development of autoimmunity in children with DS may inform us as to the factors that influence the maturation of the immune system. Whether alterations in the gut microbiome, differences in feeding practices, infections or use of antibiotics play a significant role in the increased risk of autoimmunity in these children remains to be determined. Due to the complex and variable medical, social and educational challenges for many people with DS, understanding the natural history and pathophysiological evolution of these issues is essential to develop future therapeutic options and support for these people and their families. FADES is a unique UK wide cohort which continues to expand our knowledge of children with DS, their families and the associated medical conditions which they may experience.

References

Abshire, Martha, Victor D Dinglas, Maan Isabella A Cajita, Michelle N Eakin, Dale M Needham, and Cheryl Dennison Himmelfarb. 2017. "Participant retention practices in longitudinal clinical research studies with high retention rates." *BMC medical research methodology* 17 (1):30.

Ahmad, T, D Vickers, S Campbell, MG Coulthard, and S Pedler. 1991. "Urine collection from disposable nappies." *The Lancet* 338 (8768):674-676.

Aitken, Rachel J, Kay L Mehers, Alistair J Williams, Jamie Brown, Polly J Bingley, Reinhard W Holl, Tilman R Rohrer, Edith Schober, Majedah M Abdul-Rasoul, and Julian PH Shield. 2013. "Early-onset, coexisting autoimmunity and decreased HLA-mediated susceptibility are the characteristics of diabetes in Down syndrome." *Diabetes care* 36 (5):1181-1185.

Akobeng, Anthony K, Athimalaipet V Ramanan, Iain Buchan, and Richard F Heller. 2006. "Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies." *Archives of disease in childhood* 91 (1):39-43.

Anderson, Ericka L, Weizhong Li, Niels Klitgord, Sarah K Highlander, Mark Dayrit, Victor Seguritan, Shibu Yooseph, William Biggs, J Craig Venter, and Karen E Nelson. 2016. "A robust ambient temperature collection and stabilization strategy: Enabling worldwide functional studies of the human microbiome." *Scientific Reports* 6.

Arain, Mubashir, Michael J Campbell, Cindy L Cooper, and Gillian A Lancaster. 2010. "What is a pilot or feasibility study? A review of current practice and editorial policy." *BMC medical research methodology* 10 (1):67.

Atkinson, Mark A, Mark A Bowman, Kuo-Jang Kao, Lalita Campbell, Paula J Dush, Shirish C Shah, Olli Simell, and Noel K Maclaren. 1993. "Lack of immune responsiveness to bovine serum albumin in insulin-dependent diabetes." *New England Journal of Medicine* 329 (25):1853-1858.

Atkinson, Mark A, George S Eisenbarth, and Aaron W Michels. 2014. "Type 1 diabetes." *The Lancet* 383 (9911):69-82.

Balkany, Thomas J, Marion P Downs, Bruce W Jafek, and Marilyn J Krajicek. 1979. "Hearing Loss in Down's Syndrome A Treatable Handicap More Common Than Generally Recognized." *Clinical Pediatrics* 18 (2):116-118.

Barnett, AH, C Eff, R DG Leslie, and DA Pyke. 1981. "Diabetes in identical twins." *Diabetologia* 20 (2):87-93.

Bate, Jessica, Neil Ranasinghe, Rebecca Ling, Jennifer Preston, Ruth Nightingale, Simon %J Archives of Disease in Childhood-Education Denegri, and Practice. 2016. "Public and patient involvement in paediatric research."edpract-2015-309500.

Berger, Roni. 2015. "Now I see it, now I don't: Researcher's position and reflexivity in qualitative research." *Qualitative research* 15 (2):219-234.

Bergholdt, R., S. Eising, J. Nerup, and F. %J Diabetologia Pociot. 2006. "Increased prevalence of Down's syndrome in individuals with type 1 diabetes in Denmark: a nationwide population-based study." 49 (6):1179. doi: 10.1007/s00125-006-0231-6.

Bernhardt, Jay M, and Elizabeth M %J Journal of medical Internet research Felter. 2004. "Online pediatric information seeking among mothers of young children: results from a qualitative study using focus groups." 6 (1).

Besser, Rachel EJ, Johnny Ludvigsson, Angus G Jones, Timothy J McDonald, Beverley M Shields, Bridget A Knight, and Andrew T Hattersley. 2011. "Urine C-peptide creatinine ratio is a noninvasive alternative to the mixed-meal tolerance test in children and adults with type 1 diabetes." *Diabetes care* 34 (3):607-609.

Best, Kate E, Svetlana V Glinianaia, Mary Bythell, and Judith Rankin. 2012. "Hirschsprung's disease in the North of England: prevalence, associated anomalies, and survival." *Birth Defects Research Part A: Clinical and Molecular Teratology* 94 (6):477-480.

Bingley, PJ, and EA Gale. 1989. "Incidence of insulin dependent diabetes in England: a study in the Oxford region, 1985-6." *BMJ: British Medical Journal* 298 (6673):558.

Bingley, Polly J, Ezio Bonifacio, Marion Shattock, Hilary A Gillmor, Pamela A Sawtell, David B Dunger, Robin DM Scott, Gian Franco Bottazzo, and Edwin AM Gale. 1993. "Can islet cell antibodies predict IDDM in the general population?" *Diabetes Care* 16 (1):45-50.

BINOCAR. 2013. "Congenital Anomaly Statistics 2011, England and Wales, British Isles Network of Congenital Anomaly Registers." accessed 04.07.2019. http://www.binocar.org/content/Annual%20report%202011_FINAL_040913.pdf.

Bland, J Martin, and DouglasG Altman. 1986. "Statistical methods for assessing agreement between two methods of clinical measurement." *The lancet* 327 (8476):307-310.

Bloemers, Beatrijs LP, A Marceline van Furth, Michel E Weijerman, Reinoud JBJ Gemke, Chantal JM Broers, Kimberly van den Ende, Jan LL Kimpen, Jan LM Strengers, and Louis J Bont. 2007. "Down syndrome: a novel risk factor for respiratory syncytial virus bronchiolitis—a prospective birth-cohort study." *Pediatrics* 120 (4):e1076-e1081.

Bodansky, HJ, A Staines, C Stephenson, D Haigh, and R Cartwright. 1992. "Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population." *Bmj* 304 (6833):1020-1022.

Bonamico, Margherita, Paolo Mariani, Helene Maria Danesi, Massimo Crisogianni, Pinella Failla, Gerolamo Gemme, Alberto Rasore Quartino, Aldo Giannotti, Massimo Castro, and Fiorella Balli. 2001. "Prevalence and clinical picture of celiac disease in Italian Down syndrome patients: a multicenter study." *Journal of pediatric gastroenterology and nutrition* 33 (2):139-143.

Bonifacio, Ezio, Michael Hummel, Markus Walter, Sandra Schmid, and Anette-G Ziegler. 2004. "IDDM1 and multiple family history of type 1 diabetes combine to identify neonates at high risk for type 1 diabetes." *Diabetes Care* 27 (11):2695-2700.

Book, Linda, Allison Hart, Jeff Black, Mike Feolo, John J Zone, and Susan L Neuhausen. 2001. "Prevalence and clinical characteristics of celiac disease in Downs syndrome in a US study." *American journal of medical genetics* 98 (1):70-74.

Bowen, Deborah J, Matthew Kreuter, Bonnie Spring, Ludmila Cofta-Woerpel, Laura Linnan, Diane Weiner, Suzanne Bakken, Cecilia Patrick Kaplan, Linda Squiers, and Cecilia Fabrizio. 2009. "How we design feasibility studies." *American journal of preventive medicine* 36 (5):452-457.

Bowling, Ann. 2005. "Mode of questionnaire administration can have serious effects on data quality." *Journal of public health* 27 (3):281-291.

Braun, Virginia, Victoria Clarke, and Gareth Terry. 2014. "Thematic analysis." *Qual Res Clin Health Psychol*:95-114.

Burgio, GR, AG Ugazio, L Nespoli, AF Marcioni, AM Bottelli, and F Pasquali. 1975. "Derangements of immunoglobulin levels, phytohemagglutinin responsiveness and T and B cell markers in Down's syndrome at different ages." *European journal of immunology* 5 (9):600-603.

Caldwell, Patrina HY, Phyllis N Butow, and Jonathan C Craig. 2003. "Parents' attitudes to children's participation in randomized controlled trials." *The Journal of pediatrics* 142 (5):554-559.

Caporaso, J Gregory, Christian L Lauber, William A Walters, Donna Berg-Lyons, James Huntley, Noah Fierer, Sarah M Owens, Jason Betley, Louise Fraser, and Markus Bauer. 2012. "Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms." *The ISME journal* 6 (8):1621-1624.

Caporaso, J Gregory, Christian L Lauber, William A Walters, Donna Berg-Lyons, Catherine A Lozupone, Peter J Turnbaugh, Noah Fierer, and Rob Knight. 2011. "Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample." *Proceedings of the National Academy of Sciences* 108 (Supplement 1):4516-4522.

Caracausi, Maria, Veronica Ghini, Chiara Locatelli, Martina Mericio, Allison Piovesan, Francesca Antonaros, Maria Chiara Pelleri, Lorenza Vitale, Rosa Anna Vacca, and Federica Bedetti. 2018. "Plasma and urinary metabolomic profiles of Down syndrome correlate with alteration of mitochondrial metabolism." *Scientific reports* 8 (1):2977.

Carnicer, Jesús, Carme Farré, Vicente Varea, Pere Vilar, Julio Moreno, and Josep Artigas. 2001. "Prevalence of coeliac disease in Down's syndrome." *European journal of gastroenterology & hepatology* 13 (3):263-267.

Carr, Janet. 1988. "Six weeks to twenty-one years old: A longitudinal study of children with Down's syndrome and their families." *Journal of child Psychology and Psychiatry* 29 (4):407-431.

CDSS. 2018. "The Children with Downs Syndrome Study." accessed 12.11.2018. https://www.cdss.org.uk.

Choo, Jocelyn M, Lex EX Leong, and Geraint B Rogers. 2015. "Sample storage conditions significantly influence faecal microbiome profiles." *Scientific reports* 5:16350.

Chowta, MN, PM Adhikari, NK Chowta, AK Shenoy, and S D'Souza. 2010. "Serum C peptide level and renal function in diabetes mellitus." *Indian journal of nephrology* 20 (1):25.

Colobran, Roger, Mireia Giménez-Barcons, Ana Marín-Sánchez, Eduard Porta-Pardo, and Ricardo Pujol-Borrell. 2016. "AIRE genetic variants and predisposition to polygenic autoimmune disease: The case of Graves' disease and a systematic literature review." *Human immunology* 77 (8):643-651.

Consortium, Human Microbiome Project. 2012a. "A framework for human microbiome research." *Nature* 486 (7402):215-221.

Consortium, Human Microbiome Project. 2012b. "Structure, function and diversity of the healthy human microbiome." *Nature* 486 (7402):207-214.

Cooper, Glinda S, and Berrit C Stroehla. 2003. "The epidemiology of autoimmune diseases." *Autoimmunity reviews* 2 (3):119-125.

Costello, Christine, and Anne Webber. 1976. "White cell function in Down's syndrome." *Clinical genetics* 9 (6):603-605.
Craig, Brian. 2006. "Atrioventricular septal defect: from fetus to adult." *Heart* 92 (12):1879-1885.
Csizmadia, Cassandra GDS, M Luisa Mearin, Anath Oren, Adrie Kromhout, J Bart A Crusius, B Mary E von Blomberg, A Salvador Peña, Marije NL Wiggers, and Jan P Vandenbroucke. 2000. "Accuracy and cost-effectiveness of a new strategy to screen for celiac disease in children with Down syndrome." *The Journal of pediatrics* 137 (6):756-761.
Cunningham, Cliff. 1996. "Families of children with Down syndrome." *Down Syndrome Research and Practice* 4 (3):87-95.
Da Cunha, Rosana Pires, and Jose Belmiro de Castro Moreira. 1996. "Ocular findings in Down's syndrome." *American journal of ophthalmology* 122 (2):236-244.
de Goffau, Marcus C, Kristiina Luopajärvi, Mikael Knip, Jorma Ilonen, Terhi Ruohtula, Taina Härkönen, Laura Orivuori, Saara Hakala, Gjalt W Welling, and Hermie J Harmsen. 2013. "Fecal microbiota composition differs between children with β-cell autoimmunity and those without." *Diabetes* 62 (4):1238-1244.
Desai, Sindoor S. 1997. "Down syndrome: a review of the literature." *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 84 (3):279-285.
Devlin, L, and PJ Morrison. 2004. "Mosaic Down's syndrome prevalence in a complete population study." *Archives of disease in childhood* 89 (12):1177-1178.
Di Sabatino, Antonio, and Gino Roberto Corazza. 2009. "Coeliac disease." *The Lancet* 373 (9673):1480-1493.
Dirice, Ercument, Deepika Walpita, Amedeo Vetere, Bennett C Meier, Sevim Kahraman, Jiang Hu, Vlado Dančík, Sean M Burns, Tamara J Gilbert, and David E Olson. 2016. "Inhibition of DYRK1A stimulates human β-cell proliferation." *Diabetes* 65 (6):1660-1671.
Dominianni, Christine, Jing Wu, Richard B Hayes, and Jiyoung Ahn. 2014. "Comparison of methods for fecal microbiome biospecimen collection." *BMC microbiology* 14 (1):103.
Down, J Langdon H. 1867. "Observations on an ethnic classification of idiots." *Journal of mental science* 13:121-123.
Downey, Laura, Rachel Houten, Simon Murch, and Damien Longson. 2015. "Recognition, assessment, and management of coeliac disease: summary of updated NICE guidance." *Bmj* 351:h4513.
DSMIG. 2019a. "Guidance for Essential Medical Surveillance , Down Syndrome Medical Interest Group." accessed 04.07.2019. https://www.dsmig.org.uk/information-resources/guidance-for-essential-medical-surveillance/.
DSMIG. 2019b. "Personal Child Health Record, Down Syndrome Medical Interest Group." accessed 04.07.2019. https://www.dsmig.org.uk/information-resources/personal-child-health-record-pchr/.
Durruty, P, F Ruiz, and M Garcia de los Rios. 1979. "Age at diagnosis and seasonal variation in the onset of insulin-dependent diabetes in Chile (Southern Hemisphere)." *Diabetologia* 17 (6):357-360.
E. Naserke, Heike, Ezio Bonifacio, and Anette-G Ziegler. 2001. "Prevalence, characteristics and diabetes risk associated with transient maternally acquired islet antibodies and persistent islet antibodies in offspring of parents with type 1 diabetes." *The Journal of Clinical Endocrinology & Metabolism* 86 (10):4826-4833.

Edgar, Robert C. 2013. "UPARSE: highly accurate OTU sequences from microbial amplicon reads." *Nature methods* 10 (10):996-998.

Eldridge, Sandra M, Claire L Chan, Michael J Campbell, Christine M Bond, Sally Hopewell, Lehana Thabane, and Gillian A Lancaster. 2016. "CONSORT 2010 statement: extension to randomised pilot and feasibility trials." *Pilot and Feasibility Studies* 2 (1):64.

Eldridge, Sandra M, Gillian A Lancaster, Michael J Campbell, Lehana Thabane, Sally Hopewell, Claire L Coleman, and Christine M Bond. 2016. "Defining feasibility and pilot studies in preparation for randomised controlled trials: development of a conceptual framework." *PLoS One* 11 (3):e0150205.

Elwyn, Glyn, Anne Seagrove, Kym Thorne, and Wai Yee Cheung. 2005. "Ethics and research governance in a multicentre study: add 150 days to your study protocol."

England, Public Health. 2018 "Newborn blood spot screening: programme overview."

Eovaldi, Benjamin J, and Harris L Cohen. 2018. "Duodenal Atresia And Stenosis." In *StatPearls [Internet]*. StatPearls Publishing.

Eriksson, Daniel, Matteo Bianchi, Nils Landegren, Frida Dalin, Jakob Skov, Lina Hultin-Rosenberg, Argyri Mathioudaki, Jessika Nordin, Åsa Hallgren, and Göran Andersson. 2018. "Common genetic variation in the autoimmune regulator (AIRE) locus is associated with autoimmune Addison's disease in Sweden." *Scientific reports* 8 (1):8395.

Fasano, Alessio, and Carlo Catassi. 2001. "Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum." *Gastroenterology* 120 (3):636-651.

Fell, JME, H Thakkar, DJ Newman, and CP Price. 1997. "Measurement of albumin and low molecular weight proteins in the urine of newborn infants using a cotton wool ball collection method." *Acta paediatrica* 86 (5):518-522.

Feng, Zhong-Jun, Shu-Lan Zhang, Hai-Feng Wen, and Yun Liang. 2015. "Association of rs2075876 polymorphism of AIRE gene with rheumatoid arthritis risk." *Human immunology* 76 (4):281-285.

Fergeson, Mark A, John J Mulvihill, G Bradley Schaefer, Kristi A Dehaai, Jamie Piatt, Kathleen Combs, Brianna C Bright, and Barbara R Neas. 2009. "Low adherence to national guidelines for thyroid screening in Down syndrome." *Genetics in Medicine* 11 (7):548.

Fernando, Michelle MA, Christine R Stevens, Emily C Walsh, Philip L De Jager, Philippe Goyette, Robert M Plenge, Timothy J Vyse, and John D Rioux. 2008. "Defining the role of the MHC in autoimmunity: a review and pooled analysis." *PLoS genetics* 4 (4):e1000024.

Ferreira, Ricardo C, Hui Guo, Richard MR Coulson, Deborah J Smyth, Marcin L Pekalski, Oliver S Burren, Antony J Cutler, James D Doecke, Shaun Flint, and Eoin F McKinney. 2014. "A type I interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes." *Diabetes* 63 (7):2538-2550.

Ferretti, Pamela, Edoardo Pasolli, Adrian Tett, Francesco Asnicar, Valentina Gorfer, Sabina Fedi, Federica Armanini, Duy Tin Truong, Serena Manara, and Moreno Zolfo. 2018. "Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome." *Cell host & microbe* 24 (1):133-145. e5.

Flores, Roberto, Jianxin Shi, Guoqin Yu, Bing Ma, Jacques Ravel, James J Goedert, and Rashmi Sinha. 2015. "Collection media and delayed freezing effects on microbial composition of human stool." *Microbiome* 3 (1):33.

Fortnum, Heather, Paul Leighton, Murray D Smith, Lisa Brown, Matthew Jones, Claire Benton, Elizabeth Marder, Andrew Marshall, and Kate Sutton. 2014. "Assessment of the feasibility and clinical value of further research to evaluate the management options for children with Down syndrome and otitis media with effusion: a feasibility study." *Health technology assessment (Winchester, England)* 18 (60):1.

Freeman, Sallie B, Lisa F Taft, Kenneth J Dooley, Katherine Allran, Stephanie L Sherman, Terry J Hassold, Muin J Khoury, and Denise M Saker. 1998. "Population-based study of congenital heart defects in Down syndrome." *American journal of medical genetics* 80 (3):213-217.

Freeman, SB, CP Torfs, PA Romitti, MH Royle, C Druschel, CA Hobbs, and SL %J Clinical genetics Sherman. 2009. "Congenital gastrointestinal defects in Down syndrome: a report from the Atlanta and National Down Syndrome Projects." 75 (2):180-184.

Gill, Denis %J European journal of pediatrics. 2004. "Ethical principles and operational guidelines for good clinical practice in paediatric research. Recommendations of the Ethics Working Group of the Confederation of European Specialists in Paediatrics (CESP)." 163 (2):53-57.

Gillespie, Kathleen M, Steven C Bain, Anthony H Barnett, Polly J Bingley, Michael R Christie, Geoffrey V Gill, and Edwin AM Gale. 2004. "The rising incidence of childhood type 1 diabetes and reduced contribution of high-risk HLA haplotypes." *The Lancet* 364 (9446):1699-1700.

Gillespie, Kathleen M, Rachel J Dix, Alistair JK Williams, Richard Newton, Zoey F Robinson, Polly J Bingley, Edwin AM Gale, and Julian PH Shield. 2006. "Islet autoimmunity in children with Down's syndrome." *Diabetes* 55 (11):3185-3188.

Gillespie, KM, SJ Valovin, J Saunby, KM Hunter, DA Savage, D Middleton, JA Todd, PJ Bingley, and EAM Gale. 2000. "HLA class II typing of whole genome amplified mouth swab DNA." *Tissue antigens* 56 (6):530-538.

Giménez-Barcons, Mireia, Anna Casteràs, Maria del Pilar Armengol, Eduard Porta, Paula A Correa, Ana Marín, Ricardo Pujol-Borrell, and Roger Colobran. 2014. "Autoimmune predisposition in Down syndrome may result from a partial central tolerance failure due to insufficient intrathymic expression of AIRE and peripheral antigens." *The Journal of Immunology* 193 (8):3872-3879.

Giongo, Adriana, Kelsey A Gano, David B Crabb, Nabanita Mukherjee, Luis L Novelo, George Casella, Jennifer C Drew, Jorma Ilonen, Mikael Knip, and Heikki Hyöty. 2011. "Toward defining the autoimmune microbiome for type 1 diabetes." *The ISME journal* 5 (1):82-91.

Glaser, Barney G. 1992. *Basics of grounded theory analysis: Emergence vs forcing*: Sociology press.

Golding, Jean, and Karen Birmingham. 2009. "Enrolment and response rates in a longitudinal birth cohort." *Paediatric and Perinatal Epidemiology* 23:73-85.

Green, Anders, Chris C Patterson, and Eurodiab Tiger Study Group. 2001. "Trends in the incidence of childhood-onset diabetes in Europe 1989–1998." *Diabetologia* 44 (3):B3-B8.

Green, Peter HR, and Bana Jabri. 2003. "Coeliac disease." *The Lancet* 362 (9381):383-391.

Greenbaum, CJ, T Mandrup-Poulsen, PF McGee, T Battelino, B Haastert, J Ludvigsson, P Pozzilli, JM Lachin, and H Kolb. 2008. "European C-Peptide Trial Study Group: Mixed-

meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes." *Diabetes Care* 31:1966-1971.

Griffiths, Lucy J, A Rosemary Tate, and Carol Dezateux. 2005. "The contribution of parental and community ethnicity to breastfeeding practices: evidence from the Millennium Cohort Study." *International Journal of Epidemiology* 34 (6):1378-1386.

Group, TEDDY Study. 2008. "The environmental determinants of diabetes in the young (TEDDY) study." *Annals of the New York Academy of Sciences* 1150 (1):1-13.

Hagen, Neil A, Patricia D Biondo, Penny MA Brasher, and Carla R Stiles. 2011. "Formal feasibility studies in palliative care: why they are important and how to conduct them." *Journal of pain and symptom management* 42 (2):278-289.

Hahn, THALIA, S Levin, and ZT Handzel. 1976. "Leucocyte migration inhibition factor (LIF) production by lymphocytes of normal children, newborns, and children with immune deficiency." *Clinical and experimental immunology* 24 (3):448.

Harris, Paul A, Robert Taylor, Robert Thielke, Jonathon Payne, Nathaniel Gonzalez, and Jose G %J Journal of biomedical informatics Conde. 2009. "Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support." 42 (2):377-381.

Hasle, Henrik, Inge Haunstrup Clemmensen, and Margareta Mikkelsen. 2000. "Risks of leukaemia and solid tumours in individuals with Down's syndrome." *The Lancet* 355 (9199):165-169.

Hedov, Gerth, Karin Wikblad, and Göran Annerén. 2002. "First information and support provided to parents of children with Down syndrome in Sweden: clinical goals and parental experiences." *Acta paediatrica* 91 (12):1344-1349.

Hegney, Desley, Tony Fallon, and Maxine L O'Brien. 2008. "Against all odds: a retrospective case-controlled study of women who experienced extraordinary breastfeeding problems." *Journal of clinical nursing* 17 (9):1182-1192.

Heikkilä, Katriina, Amanda Sacker, Yvonne Kelly, Mary J Renfrew, and Maria A Quigley. 2011. "Breast feeding and child behaviour in the Millennium Cohort Study." *Archives of Disease in Childhood* 96 (7):635-642.

Heslehurst, Nicola, Judith Rankin, JR Wilkinson, and CD %J International journal of obesity Summerbell. 2010. "A nationally representative study of maternal obesity in England, UK: trends in incidence and demographic inequalities in 619 323 births, 1989–2007." 34 (3):420.

Hill, Cian J, Jillian RM Brown, Denise B Lynch, Ian B Jeffery, C Anthony Ryan, R Paul Ross, Catherine Stanton, and Paul W O'Toole. 2016. "Effect of room temperature transport vials on DNA quality and phylogenetic composition of faecal microbiota of elderly adults and infants." *Microbiome* 4 (1):19.

Hilton, JM, DA Fitzgerald, DM %J Journal of paediatrics Cooper, and child health. 1999. "Respiratory morbidity of hospitalized children with Trisomy 21." 35 (4):383-386.

HRA. 2019. "Health Research Authority ". https://www.hra.nhs.uk/.

Hunter, Ian, Stephen A Greene, Thomas M MacDonald, and Andrew D %J Archives of Disease in Childhood Morris. 2000. "Prevalence and aetiology of hypothyroidism in the young." 83 (3):207-210.

Husby, S, S Koletzko, IR Korponay-Szabo, ML Mearin, A Phillips, R Shamir, R Troncone, K Giersiepen, D Branski, and C Catassi. 2012. "European Society for Pediatric

Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease." *Journal of pediatric gastroenterology and nutrition* 54 (1):136-160.

Hyman, Paul E, Peter J Milla, Marc A Benninga, Geoff P Davidson, David F Fleisher, and Jan Taminiau. 2006. "Childhood functional gastrointestinal disorders: neonate/toddler." *Gastroenterology* 130 (5):1519-1526.

Iliyasu, Z, WH Gilmour, and DH Stone. 2002. "Prevalence of Down syndrome in Glasgow, 1980-96--the growing impact of prenatal diagnosis on younger mothers." *Health bulletin* 60 (1):20-26.

IRAS. 2014. "The Integrated Research Application System (IRAS)." www.myresearchproject.org.uk.

Ivarsson, Anneli, Olle Hernell, Hans Stenlund, and Lars Åke %J The American journal of clinical nutrition Persson. 2002. "Breast-feeding protects against celiac disease." 75 (5):914-921.

James, Rebecca. 2011.

Johnson, Kelly, Randall Wong, Katherine J Barriga, Georgeanna Klingensmith, Anette-G Ziegler, Marian J Rewers, and Andrea K Steck. 2012. "rs11203203 is associated with type 1 diabetes risk in population pre-screened for high-risk HLA-DR, DQ genotypes." *Pediatric diabetes* 13 (8):611-615.

Johnson, Matthew B, Elisa De Franco, Siri Atma W Greeley, Lisa R Letourneau, Kathleen Gillespie, Matthew N Wakeling, Sian Ellard, Sarah E Flanagan, Kashyap A Patel, and Andrew T Hattersley. 2019. "Trisomy 21 is a Cause of Permanent Neonatal Diabetes that is Autoimmune but not HLA Associated." *Diabetes*:db190045.

Juvonen, Pekka, Majvi Månsson, N-I Max Kjellman, Bengt Björkstén, and Iréne Jakobsson. 1999. "Development of immunoglobulin G and immunoglobulin E antibodies to cow's milk proteins and ovalbumin after a temporary neonatal exposure to hydrolyzed and whole cow's milk proteins." *Pediatric allergy and immunology* 10 (3):191-198.

Kaprio, J, J Tuomilehto, M Koskenvuo, K Romanov, A Reunanen, J Eriksson, J Stengård, and YA Kesäniemi. 1992. "Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland." *Diabetologia* 35 (11):1060-1067.

Karjalainen, Jukka, Julio M Martin, Mikael Knip, Jorma Ilonen, Brian H Robinson, Erkki Savilahti, Hans K Akerblom, and Hans-Michael Dosch. 1992. "A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus." *New England journal of medicine* 327 (5):302-307.

Karlsson, Bengt, Jan Gustafsson, Gerth Hedov, SA Ivarsson, and Göran Annerén. 1998. "Thyroid dysfunction in Down's syndrome: relation to age and thyroid autoimmunity." *Archives of disease in childhood* 79 (3):242-245.

Karvonen, Marjatta, Maarit Viik-Kajander, Elena Moltchanova, Ingrid Libman, RONALD LaPorte, and JAAKKO Tuomilehto. 2000. "Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group." *Diabetes care* 23 (10):1516-1526.

Kaukinen, Katri, Jukka Partanen, Markku Mäki, and Pekka Collin. 2002. "HLA-DQ typing in the diagnosis of celiac disease." *The American journal of gastroenterology* 97 (3):695-699.

Kennedy, RL, TH Jones, and HS Cuckle. 1992. "Down's syndrome and the thyroid." *Clinical endocrinology* 37 (6):471-476.

Kimpimaki, T, Antti Kupila, A-M Hamalainen, Marika Kukko, Petri Kulmala, Kaisa Savola, Tuula Simell, P Keskinen, Jorma Ilonen, and Olli Simell. 2001. "The first signs of β-cell autoimmunity appear in infancy in genetically susceptible children from the general population: the Finnish Type 1 Diabetes Prediction and Prevention Study." *The Journal of Clinical Endocrinology & Metabolism* 86 (10):4782-4788.

Knip, Mikael, and Olli Simell. 2012. "Environmental triggers of type 1 diabetes." *Cold Spring Harbor perspectives in medicine* 2 (7):a007690.

Kostic, Aleksandar D, Dirk Gevers, Heli Siljander, Tommi Vatanen, Tuulia Hyötyläinen, Anu-Maaria Hämäläinen, Aleksandr Peet, Vallo Tillmann, Päivi Pöhö, and Ismo Mattila. 2015. "The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes." *Cell host & microbe* 17 (2):260-273.

Kozhakhmetova, A, RC Wyatt, C Caygill, C Williams, AE Long, K Chandler, RJ Aitken, JM Wenzlau, HW Davidson, and KM Gillespie. 2018. "A quarter of patients with type 1 diabetes have co-existing non-islet autoimmunity: the findings of a UK population-based family study." *Clinical & Experimental Immunology* 192 (3):251-258.

Kramer, Michael S, Frances Aboud, Elena Mironova, Irina Vanilovich, Robert W Platt, Lidia Matush, Sergei Igumnov, Eric Fombonne, Natalia Bogdanovich, and Thierry Ducruet. 2008. "Breastfeeding and child cognitive development: new evidence from a large randomized trial." *Archives of general psychiatry* 65 (5):578-584.

Krischer, Jeffrey P, Kristian F Lynch, Desmond A Schatz, Jorma Ilonen, Åke Lernmark, William A Hagopian, Marian J Rewers, Jin-Xiong She, Olli G Simell, and Jorma Toppari. 2015. "The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study." *Diabetologia*:1-8.

Kumin, Libby, and DC Bahr. 1999. "Patterns of feeding, eating, and drinking in young children with Down syndrome with oral motor concerns." *Down Syndrome Quarterly* 4 (2):1-8.

Kumin, Libby, Kimberly Chapman Von Hagel, and DC Bahr. 2001. "An effective oral motor intervention protocol for infants and toddlers with low muscle tone." *Infant Toddler Intervention* 11 (3/4):181-200.

Kusters, MAA, RHJ Verstegen, EFA Gemen, and E De Vries. 2009. "Intrinsic defect of the immune system in children with Down syndrome: a review." *Clinical & Experimental Immunology* 156 (2):189-193.

Lancaster, Gillian A, Susanna Dodd, and Paula R Williamson. 2004. "Design and analysis of pilot studies: recommendations for good practice." *Journal of evaluation in clinical practice* 10 (2):307-312.

Lejeune, Jerome, Martha Gautier, and Raymond Turpin. 1959. "Study of somatic chromosomes from 9 mongoloid children." *Comptes rendus hebdomadaires des seances de l'Academie des sciences* 248 (11):1721.

Lettre, Guillaume, and John D Rioux. 2008. "Autoimmune diseases: insights from genome-wide association studies." *Human molecular genetics* 17 (R2):R116-R121.

Levin, Stanley, Menahem Schlesinger, Zaev Handzel, Thalia Hahn, Yehudit Altman, Bernard Czernobilsky, and Jochanan Boss. 1979. "Thymic deficiency in Down's syndrome." *Pediatrics* 63 (1):80-87.

Lewis, Eilene, and Alta Kritzinger. 2004. "Parental experiences of feeding problems in their infants with Down syndrome." *Down Syndrome Research and Practice* 9 (2):45-52.

Ley, Ruth E, Fredrik Bäckhed, Peter Turnbaugh, Catherine A Lozupone, Robin D Knight, and Jeffrey I Gordon. 2005. "Obesity alters gut microbial ecology." *Proceedings of the National Academy of Sciences of the United States of America* 102 (31):11070-11075.

Li, Chi-Ming, Meirong Guo, Martha Salas, Nicole Schupf, Wayne Silverman, Warren B Zigman, Sameera Husain, Dorothy Warburton, Harshwardhan Thaker, and Benjamin Tycko. 2006. "Cell type-specific over-expression of chromosome 21 genes in fibroblasts and fetal hearts with trisomy 21." *BMC medical genetics* 7 (1):24.

Lima, Flavia A, Carlos A Moreira-Filho, Patrícia L Ramos, Helena Brentani, Leandro de A Lima, Magaly Arrais, Luiz C Bento-de-Souza, Luciana Bento-de-Souza, Maria I Duarte, and Antonio Coutinho. 2011. "Decreased AIRE expression and global thymic hypofunction in Down syndrome." *The Journal of Immunology* 187 (6):3422-3430.

Lombardi, Angela, Effie Tsomos, Sara S Hammerstad, and Yaron Tomer. 2018. "Interferon alpha: The key trigger of type 1 diabetes." *Journal of autoimmunity*.

LonDownS Consortium. www.ucl.ac.uk/london-down-syndrome-consortium.

Lönnrot, Maria, Karita Korpela, Mikael Knip, Jorma Ilonen, Olli Simell, Sari Korhonen, Kaisa Savola, Päivi Muona, Tuula Simell, and Pentti Koskela. 2000. "Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study." *Diabetes* 49 (8):1314-1318.

Lopez, Vicente, Hans D Ochs, Horace C Thuline, Starkey D Davis, and Ralph J Wedgwood. 1975. "Defective antibody response to bacteriophage ØX 174 in Down syndrome." *The Journal of pediatrics* 86 (2):207-211.

Lozupone, Catherine A, Jesse I Stombaugh, Jeffrey I Gordon, Janet K Jansson, and Rob Knight. 2012. "Diversity, stability and resilience of the human gut microbiota." *Nature* 489 (7415):220.

Lozupone, Catherine, and Rob Knight. 2005. "UniFrac: a new phylogenetic method for comparing microbial communities." *Applied and environmental microbiology* 71 (12):8228-8235.

Madan, V, J Williams, and JT Lear. 2006. "Dermatological manifestations of Down's syndrome." *Clinical and Experimental Dermatology: Clinical dermatology* 31 (5):623-629.

Malcova, Hana, Zdenek Sumnik, Pavel Drevinek, Jitrenka Venhacova, Jan Lebl, and Ondrej Cinek. 2006. "Absence of breast-feeding is associated with the risk of type 1 diabetes: a case–control study in a population with rapidly increasing incidence." *European journal of pediatrics* 165 (2):114-119.

Manji, N, JD Carr-Smith, K Boelaert, A Allahabadia, M Armitage, VK Chatterjee, JH Lazarus, SHS Pearce, B Vaidya, SC %J The Journal of Clinical Endocrinology Gough, and Metabolism. 2006. "Influences of age, gender, smoking, and family history on autoimmune thyroid disease phenotype." 91 (12):4873-4880.

Marmor, Jaci K, Susan A Oliveria, Richard P Donahue, Ellen J Garrahie, Mary Jo White, Lynn L Moore, and R Curtis Ellison. 1991. "Factors encouraging cohort maintenance in a longitudinal study." *Journal of Clinical Epidemiology* 44 (6):531-535.

Mason, Sarah J, Gillian Harris, and Jacqueline Blissett. 2005. "Tube Feeding in Infancy: Implications for the Developmentof Normal Eating and Drinking Skills." *Dysphagia* 20 (1):46-61.

McAndrew, Fiona, Jane Thompson, Lydia Fellows, Alice Large, Mark Speed, Mary J %J Leeds: Health Renfrew, and Social Care Information Centre. 2012. "Infant feeding survey 2010."

McDonald, Tim J., Bridget A. Knight, Beverley M. Shields, Pamela Bowman, Maurice B. Salzmann, and Andrew T. Hattersley. 2009. "Stability and Reproducibility of a Single-Sample Urinary C-Peptide/Creatinine Ratio and Its Correlation with 24-h Urinary C-Peptide." *Clinical Chemistry* 55 (11):2035-2039. doi: 10.1373/clinchem.2009.129312.

McLeod, Donald SA, and David S %J Endocrine Cooper. 2012. "The incidence and prevalence of thyroid autoimmunity." 42 (2):252-265.

Moore, Elizabeth R, Nils Bergman, Gene C Anderson, and Nancy Medley. 2016. "Early skin-to-skin contact for mothers and their healthy newborn infants." *Cochrane database of systematic Reviews* (11).

Moore, SW %J Pediatric surgery international. 2008. "Down syndrome and the enteric nervous system." 24 (8):873-883.

Morgan, Xochitl C, Timothy L Tickle, Harry Sokol, Dirk Gevers, Kathryn L Devaney, Doyle V Ward, Joshua A Reyes, Samir A Shah, Neal LeLeiko, and Scott B Snapper. 2012. "Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment." *Genome biology* 13 (9):R79.

Morris, JK, and A. Springett. 2014a. "The National Down Syndrome Cytogenetic Register for England and Wales 2013 Annual Report." *Queen Mary University of London, Barts and The London School of Medicine and Dentistry*.

Morris, Joan K, and Eva Alberman. 2009. "Trends in Down's syndrome live births and antenatal diagnoses in England and Wales from 1989 to 2008: analysis of data from the National Down Syndrome Cytogenetic Register." *BMJ: British Medical Journal* 339.

Morris, Joan K, Tim J Cole, Anna L Springett, and Jennifer %J American Journal of Medical Genetics Part A Dennis. 2015. "Down syndrome birth weight in England and Wales: implications for clinical practice." 167 (12):3070-3075.

Morris, Joan K, and Anna Springett. 2014b. "The National Down Syndrome Cytogenetic Register for England and Wales: 2013 Annual Report, ." accessed 04.07.2019. http://www.binocar.org/content/annrep2013_FINAL_nologo.pdf.

Morton, Susan MB, Cameron C Grant, Polly E Atatoa Carr, Elizabeth M Robinson, Jennifer M Kinloch, Courtney J Fleming, Te Kani R Kingi, Lana M Perese, and Renee Liang. 2014. "How do you recruit and retain a prebirth cohort? Lessons learnt from Growing Up in New Zealand." *Evaluation & the Health Professions* 37 (4):411-433.

Murch, Simon, Huw Jenkins, Marcus Auth, Ronald Bremner, Assad Butt, Stephanie France, Mark Furman, Peter Gillett, Fevronia Kiparissi, and Maureen %J Archives of disease in childhood Lawson. 2013. "Joint BSPGHAN and Coeliac UK guidelines for the diagnosis and management of coeliac disease in children."archdischild-2013-303996.

Murphy, Marianne, Marcia J Lempert, and Lois B Epstein. 1990. "Decreased level of T cell receptor expression by Down syndrome (trisomy 21) thymocytes." *American Journal of Medical Genetics* 37 (S7):234-237.

Nabulsi, Mona, Yvette Khalil, and Jihad Makhoul. 2011. "Parental attitudes towards and perceptions of their children's participation in clinical research: a developing-country perspective." *Journal of medical ethics* 37 (7):420-423.

Nakanishi, Koji, Tetsuro Kobayashi, Toshio Murase, Takako Nakatsuji, Hidetoshi Inoko, Kimiyoshi Tsuji, and Kinori Kosaka. 1993. "Association of HLA-A24 with complete β-cell destruction in IDDM." *Diabetes* 42 (7):1086-1093.

Nejentsev, Sergey, Joanna MM Howson, Neil M Walker, Jeffrey Szeszko, Sarah F Field, Helen E Stevens, Pamela Reynolds, Matthew Hardy, Erna King, and Jennifer Masters. 2007. "Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A." *Nature* 450 (7171):887-892.

Nguyen, Thu Lan, Arnaud Duchon, Antigoni Manousopoulou, Nadège Loaëc, Benoît Villiers, Guillaume Pani, Meltem Karatas, Anna E Mechling, Laura-Adela Harsan, and Emmanuelle Limanton. 2018. "Correction of cognitive deficits in mouse models of Down syndrome by a pharmacological inhibitor of DYRK1A." *Disease models & mechanisms* 11 (9):dmm035634.

NHS Digital. 2017. "NHS Maternity Statistics, England 2016-17,."

NIHR. 2019. "NIHR Evaluation, Trials and Studies Coordinating Centre Glossary." accessed 04.07.2019. https://www.nihr.ac.uk/glossary?letter=F&postcategory=-1.

NIHR Bristol BRU. 2016. "FADES Study ". http://www.uhbristol.nhs.uk/research-innovation/our-research/bristol-nutrition-bru/.

NIHR CRN Portfolio. 2014. "NIHR CRN Portfolio." www.nihr.ac.uk/funding-and-support/study-support-service/eligibility-for-nihr-support/.

Norris, Jill M, Katherine Barriga, Edward J Hoffenberg, Iman Taki, Dongmei Miao, Joel E Haas, Lisa M Emery, Ronald J Sokol, Henry A Erlich, and George S %J Jama Eisenbarth. 2005. "Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease." 293 (19):2343-2351.

Norris, Jill M, Katherine Barriga, Georgeanna Klingensmith, Michelle Hoffman, George S Eisenbarth, Henry A Erlich, and Marian Rewers. 2003. "Timing of initial cereal exposure in infancy and risk of islet autoimmunity." *Jama* 290 (13):1713-1720.

O'Cathain, Alicia, Pat Hoddinott, Simon Lewin, Kate J Thomas, Bridget Young, Joy Adamson, Yvonne JFM Jansen, Nicola Mills, Graham Moore, and Jenny L Donovan. 2015. "Maximising the impact of qualitative research in feasibility studies for randomised controlled trials: guidance for researchers." *Pilot and Feasibility Studies* 1 (1):32.

Office for National Statistics (ONS). 2015. "Birth characteristics in England and Wales: 2015." https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthcharacteristicsinenglandandwales/2015.

ONS. 2016. "Births in England and Wales: 2016." https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthsummarytablesenglandandwales/2016.

ONS. 2017. "Families and the labour market, England: 2017, Office for National Statistics." accessed 04.07.2019. Families and the labour market, England: 2017.

Oram, RichardA, AngusG Jones, RachelE J. Besser, BridgetA Knight, BeverleyM Shields, RichardJ Brown, AndrewT Hattersley, and TimothyJ McDonald. 2014. "The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells." *Diabetologia* 57 (1):187-191. doi: 10.1007/s00125-013-3067-x.

Orsmond, Gael I, and Ellen S Cohn. 2015. "The distinctive features of a feasibility study: Objectives and guiding questions." *OTJR: occupation, participation and health* 35 (3):169-177.

Øster, Jakob, and Arne Nielsen. 1975. "MORTALITY AND LIFE-TABLE IN DOWN'S SYNDROME." *Acta Paediatrica* 64 (2):322-326.

Patterson, Christopher C, Gisela G Dahlquist, Eva Gyürüs, Anders Green, Gyula Soltész, and EURODIAB Study Group. 2009. "Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study." *The Lancet* 373 (9680):2027-2033.

Patton, Michael Quinn. 1990. *Qualitative evaluation and research methods*: SAGE Publications, inc.

Paul, James, Rachael Seib, and Todd Prescott. 2005. "The Internet and clinical trials: background, online resources, examples and issues." *Journal of medical Internet research* 7 (1).

Pellegrini, FP, M Marinoni, V Frangione, A Tedeschi, V Gandini, F Ciglia, L Mortara, RS Accolla, and L Nespoli. 2012. "Down syndrome, autoimmunity and T regulatory cells." *Clinical & Experimental Immunology* 169 (3):238-243.

Plantin, Lars, and Kristian %J BMC family practice Daneback. 2009. "Parenthood, information and support on the internet. A literature review of research on parents and professionals online." 10 (1):34.

Quast, Christian, Elmar Pruesse, Pelin Yilmaz, Jan Gerken, Timmy Schweer, Pablo Yarza, Jörg Peplies, and Frank Oliver Glöckner. 2013. "The SILVA ribosomal RNA gene database project: improved data processing and web-based tools." *Nucleic acids research* 41 (D1):D590-D596.

Quigley, Maria A, Christine Hockley, Claire Carson, Yvonne Kelly, Mary J Renfrew, and Amanda Sacker. 2012. "Breastfeeding is associated with improved child cognitive development: a population-based cohort study." *The Journal of pediatrics* 160 (1):25-32.

RCPCH. 2018. "National Neonatal Audit Programme Annual Report 2018 - on 2017 data." accessed 04.07.2019. https://www.rcpch.ac.uk/resources/national-neonatal-audit-programme-annual-report-2018-2017-data.

RCPCH. 2019. "Personal Child Health Record , Royal College of Paediatric and Child Health ". https://www.rcpch.ac.uk/resources/personal-child-health-record-pchr.

RCPCH & DSMIG. 2015. "Paediatric Service Specification, Services for Children and Young People with

Down Syndrome." https://www.dsmig.org.uk/wp-content/uploads/2015/09/Draft-servcei-spec.pdf.

Redondo, Maria J, Joy Jeffrey, Pamela R Fain, George S Eisenbarth, and Tihamer Orban. 2008. "Concordance for islet autoimmunity among monozygotic twins." *New England Journal of Medicine* 359 (26):2849-2850.

Rewers, Marian. 2005. "Epidemiology of celiac disease: what are the prevalence, incidence, and progression of celiac disease?" *Gastroenterology* 128 (4):S47-S51.

Riessman, Catherine Kohler. 1993. *Narrative analysis*. Vol. 30: Sage.

Robinson, A %J The Journal of Laryngology, and Otology. 2014. "Patient and public involvement: in theory and in practice." 128 (4):318-325.

Rohrer, TR, P Hennes, A Thon, A Dost, M Grabert, B Rami, S Wiegand, RW Holl, and DPV Initiative. 2010. "Down's syndrome in diabetic patients aged< 20 years: an analysis of metabolic status, glycaemic control and autoimmunity in comparison with type 1 diabetes." *Diabetologia* 53 (6):1070-1075.

Roizen, Nancy J, and David Patterson. 2003. "Down's syndrome." *The Lancet* 361 (9365):1281-1289.

Rönningen, Kjersti S, Niels Keiding, and Anders Green. 2001. "Correlations between the incidence of childhood-onset type I diabetes in Europe and HLA genotypes." *Diabetologia* 44 (3):B51-B59.

Rook, Graham AW. 2009. "Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis." *Immunology* 126 (1):3-11.

Rosati, G. 2001. "The prevalence of multiple sclerosis in the world: an update." *Neurological sciences* 22 (2):117-139.

Rosner, F, Ph J Kozinn, and GA Jervis. 1973. "Leukocyte function and serum immunoglobulins in Down's syndrome." *New York state journal of medicine* 73 (5):672-675.

Rossignoli, Alessandra, Antonio Clavenna, and Maurizio %J European journal of clinical pharmacology Bonati. 2007. "Antibiotic prescription and prevalence rate in the outpatient paediatric population: analysis of surveys published during 2000–2005." 63 (12):1099-1106.

Rubio-Cabezas, Oscar, Jayne AL Minton, Richard Caswell, Julian P Shield, Dorothee Deiss, Zdenek Sumnik, Amely Cayssials, Mathias Herr, Anja Loew, and Vaughan Lewis. 2009. "Clinical heterogeneity in patients with FOXP3 mutations presenting with permanent neonatal diabetes." *Diabetes Care* 32 (1):111-116.

Sakaguchi, Shimon. 2005. "Naturally arising Foxp3-expressing CD25+ CD4+ regulatory T cells in immunological tolerance to self and non-self." *Nature immunology* 6 (4):345-352.

Salman, Rustam Al-Shahi, Elaine Beller, Jonathan Kagan, Elina Hemminki, Robert S Phillips, Julian Savulescu, Malcolm Macleod, Janet Wisely, and Iain Chalmers. 2014. "Increasing value and reducing waste in biomedical research regulation and management." *The Lancet* 383 (9912):176-185.

Sánchez-Albisua, Iciar, Wolfgang Storm, Iris Wäscher, and Martin Stern. 2002. "How frequent is coeliac disease in Down syndrome?" *European journal of pediatrics* 161 (12):683-684.

Sasaki, Motomichi, and Yoshitaka Obara. 1969. "Hypersensitivity of lymphocytes in Down's syndrome shown by mixed leucocyte culture experiments."

Schmidt, Hollie, Dhelia Williamson, and Allison Ashley-Koch. 2007. "HLA-DR15 haplotype and multiple sclerosis: a HuGE review." *American journal of epidemiology* 165 (10):1097-1109.

Schneider-Lindner, Verena, Caroline Quach, James A Hanley, and Samy %J Journal of Antimicrobial Chemotherapy Suissa. 2010. "Secular trends of antibacterial prescribing in UK paediatric primary care." 66 (2):424-433.

Shield, Julian PH, Emma JK Wadsworth, Terry J Hassold, Lu Ann Judis, and Patricia A Jacobs. 1999. "Is disomic homozygosity at the APECED locus the cause of increased autoimmunity in Down's syndrome?" *Archives of disease in childhood* 81 (2):147-150.

Simmonds, MJ, and SCL Gough. 2004. "Unravelling the genetic complexity of autoimmune thyroid disease: HLA, CTLA-4 and beyond." *Clinical & Experimental Immunology* 136 (1):1-10.

Smith, GC, and CM Taylor. 1992. "Recovery of protein from urine specimens collected in cotton wool." *Archives of disease in childhood* 67 (12):1486-1487.

Smith, Jonathan A, and Mike Osborn. 2004. "Interpretative phenomenological analysis." *Doing social psychology research*:229-254.

Smith, Trevor, Edward JH Moore, and Hugh Tunstall-Pedoe. 1997. "Review by a local medical research ethics committee of the conduct of approved research projects, by examination of patients' case notes, consent forms, and research records and by interview." *Bmj* 314 (7094):1588.

Snell-Bergeon, Janet K, Jennifer Smith, Fran Dong, Anna E Barón, Kathy Barriga, Jill M Norris, and Marian Rewers. 2012. "Early Childhood Infections and the Risk of Islet Autoimmunity The Diabetes Autoimmunity Study in the Young (DAISY)." *Diabetes care* 35 (12):2553-2558.

Snooks, Helen, Hayley Hutchings, Anne Seagrove, Sarah Stewart-Brown, John Williams, and Ian Russell. 2012. "Bureaucracy stifles medical research in Britain: a tale of three trials." *BMC medical research methodology* 12 (1):122.

Sollid, LM. 2002. "Breast milk against coeliac disease." *Gut* 51 (6):767-768.

Song, Se Jin, Amnon Amir, Jessica L Metcalf, Katherine R Amato, Zhenjiang Zech Xu, Greg Humphrey, and Rob Knight. 2016. "Preservation methods differ in fecal microbiome stability, affecting suitability for field studies." *mSystems* 1 (3):e00021-16.

Stewart, Christopher J, Nadim J Ajami, Jacqueline L O'Brien, Diane S Hutchinson, Daniel P Smith, Matthew C Wong, Matthew C Ross, Richard E Lloyd, HarshaVardhan Doddapaneni, and Ginger A Metcalf. 2018. "Temporal development of the gut microbiome in early childhood from the TEDDY study." *Nature* 562 (7728):583.

Stiehm, E Richard, and H Hugh Fudenberg. 1966. "Serum levels of immune globulins in health and disease: a survey." *Pediatrics* 37 (5):715-727.

Stock, Nicola Marie, Kerry Humphries, Beate St Pourcain, Maggie Bailey, Martin Persson, Karen M Ho, Susan Ring, Cathy Marsh, Liz Albery, and Nichola Rumsey. 2016. "Opportunities and challenges in establishing a cohort study: an example from cleft lip/palate research in the United Kingdom." *The Cleft Palate-Craniofacial Journal* 53 (3):317-325.

Sturges, Judith E, and Kathleen J Hanrahan. 2004. "Comparing telephone and face-to-face qualitative interviewing: a research note." *Qualitative research* 4 (1):107-118.

Sullivan, Kelly D, Hannah C Lewis, Amanda A Hill, Ahwan Pandey, Leisa P Jackson, Joseph M Cabral, Keith P Smith, L Alexander Liggett, Eliana B Gomez, and Matthew D Galbraith. 2016. "Trisomy 21 consistently activates the interferon response." *Elife* 5:e16220.

Sutnick, Alton I, W Thomas London, and Baruch S Blumberg. 1969. "Effects of host and environment on immunoglobulins in Down's syndrome." *Archives of internal medicine* 124 (6):722-725.

Sword, Wendy. 1999. "Accounting for presence of self: Reflections on doing qualitative research." *Qualitative Health Research* 9 (2):270-278.

TEDDY Study Group. 2007. "The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design." *J Pediatric diabetes* 8 (5):286-298.

Thabane, Lehana, Jinhui Ma, Rong Chu, Ji Cheng, Afisi Ismaila, Lorena P Rios, Reid Robson, Marroon Thabane, Lora Giangregorio, and Charles H Goldsmith. 2010. "A tutorial on pilot studies: the what, why and how." *BMC medical research methodology* 10 (1):1.

The Human Tissue Act, London, UK: Office of Public Sector Information. 2004. "Human Tissue Act 2004 (Chapter 30)."

Thompson, Andrew GH, and Emma F France. 2010. "One stop or full stop? The continuing challenges for researchers despite the new streamlined NHS research governance process." *BMC health services research* 10 (1):124.

Thomson, Gill, Katherine Ebisch-Burton, and Renee Flacking. 2015. "Shame if you do–shame if you don't: women's experiences of infant feeding." *Maternal & child nutrition* 11 (1):33-46.

Thorsby, E, and KS Rønningen. 1993. "Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type 1 (insulin-dependent) diabetes mellitus." *Diabetologia* 36 (5):371-377.

Todd, John A. 2010. "Etiology of type 1 diabetes." *Immunity* 32 (4):457-467.

Topolovec-Vranic, Jane, and Karthik Natarajan. 2016. "The use of social media in recruitment for medical research studies: A scoping review." *Journal of Medical Internet Research* 18 (11).

Turnbaugh, Peter J, Ruth E Ley, Micah Hamady, Claire Fraser-Liggett, Rob Knight, and Jeffrey I Gordon. 2007. "The human microbiome project: exploring the microbial part of ourselves in a changing world." *Nature* 449 (7164):804.

Ugazio, A Gi, SD Jayakar, AF Marcioni, M Duse, V Monafo, F Pasquali, and GR Burgio. 1977. "Immunodeficiency in Down's syndrome: relationship between presence of human thyroglobulin antibodies and HBsAg carrier status." *European journal of pediatrics* 126 (3):139-146.

University of Bristol. 2019. "Information Security Policies." accessed 09.07.2019. http://www.bristol.ac.uk/infosec/policies/.

University of Bristol REDCap. 2019. "Bristol REDCap." accessed 04.07.2019. https://brtcclinical.bris.ac.uk/redcap/.

Vaarala, Outi. 2013. "Human intestinal microbiota and type 1 diabetes." *Current diabetes reports* 13 (5):601-607.

Van Goor, JC, G Massa, and R Hirasing. 1997. "Increased incidence and prevalence of diabetes mellitus in Down's syndrome." *Archives of disease in childhood* 77 (2):183.

Van Riper, M. 2007. "Families of children with Down syndrome: responding to "a change in plans" with resilience." *J Pediatr Nurs* 22 (2):116-28. doi: 10.1016/j.pedn.2006.07.004.

Vatanen, Tommi, Aleksandar D Kostic, Eva d'Hennezel, Heli Siljander, Eric A Franzosa, Moran Yassour, Raivo Kolde, Hera Vlamakis, Timothy D Arthur, and Anu-Maaria Hämäläinen. 2016. "Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans." *Cell* 165 (4):842-853.

Virtanen, Suvi M, Leena Räsänen, Katrina Ylönen, Antti Aro, David Clayton, Bryan Langholz, Janne Pitkäniemi, Erkki Savilahti, Raisa Lounamaa, and Jaakko Tuomilehto. 1993. "Early introduction of dairy products associated with increased risk of IDDM in Finnish children." *Diabetes* 42 (12):1786-1790.

Watanabe, Norihiko, Yi-Hong Wang, Heung Kyu Lee, Tomoki Ito, Yui-Hsi Wang, Wei Cao, and Yong-Jun Liu. 2005. "Hassall's corpuscles instruct dendritic cells to induce CD4+ CD25+ regulatory T cells in human thymus." *Nature* 436 (7054):1181-1185.

Weetman, Anthony P. 2004. "Autoimmune thyroid disease." *Autoimmunity* 37 (4):337-340.

Williams, Georgina M, Sam D Leary, Nadim J Ajami, Saranna Chipper Keating, Joseph F Petrosin, Julian P Hamilton-Shield, and Kathleen M Gillespie. 2019. "Gut microbiome analysis by post: Evaluation of the optimal method to collect stool samples from infants within a national cohort study." *PloS one* 14 (6):e0216557.

Williams, Georgina M, Patricia Neville, Kathleen M Gillespie, Sam D Leary, Julian P Hamilton-Shield, and Aidan J Searle. 2018. "What factors influence recruitment to a birth cohort of infants with Down's syndrome?" *Archives of disease in childhood* 103 (8):763-766.

World Health Organization. 2009. "The optimal duration of exclusive breastfeeding: report of an expert consultation. 2002." *World Health Organization: Geneva*.

Wouters, Jeroen, Michel E Weijerman, A Marceline van Furth, Marco WJ Schreurs, J Bart A Crusius, B Mary E von Blomberg, Laura R de Baaij, Chantal JM Broers, and Reinoud JBJ Gemke. 2009. "Prospective human leukocyte antigen, endomysium immunoglobulin A antibodies, and transglutaminase antibodies testing for celiac disease in children with Down syndrome." *The Journal of pediatrics* 154 (2):239-242.

Yang, Quanhe, Sonja A Rasmussen, and JM Friedman. 2002. "Mortality associated with Down's syndrome in the USA from 1983 to 1997: a population-based study." *The Lancet* 359 (9311):1019-1025.

Yatsunenko, Tanya, Federico E Rey, Mark J Manary, Indi Trehan, Maria Gloria Dominguez-Bello, Monica Contreras, Magda Magris, Glida Hidalgo, Robert N Baldassano, and Andrey P Anokhin. 2012. "Human gut microbiome viewed across age and geography." *Nature* 486 (7402):222-227.

Yumura-Yagi, Keiko, Junichi Hara, Hiroki Kurahashi, Tetsuo Nishiura, Yoshio Kaneyama, Yuko Osugi, Naoki Sakata, Masami Inoue, Akio Tawa, and Shintaro %J British journal of haematology Okada. 1992. "Mixed phenotype of blasts in acute megakaryocytic leukaemia and transient abnormal myelopoiesis in Down's syndrome." 81 (4):520-525.

Zhernakova, Alexandra, Eli A Stahl, Gosia Trynka, Soumya Raychaudhuri, Eleanora A Festen, Lude Franke, Harm-Jan Westra, Rudolf SN Fehrmann, Fina AS Kurreeman, and Brian Thomson. 2011. "Meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis identifies fourteen non-HLA shared loci." *PLoS genetics* 7 (2):e1002004.

Ziegler, Anette-G, Michael Hummel, Michael Schenker, and Ezio Bonifacio. 1999. "Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study." *Diabetes* 48 (3):460-468.

Ziegler, Anette G, Marian Rewers, Olli Simell, Tuula Simell, Johanna Lempainen, Andrea Steck, Christiane Winkler, Jorma Ilonen, Riitta Veijola, and Mikael Knip. 2013. "Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children." *Jama* 309 (23):2473-2479.

Zook, Patricia M, Carolina Jordan, Bernadette Adams, Cynthia M Visness, Michelle Walter, Kathryn Pollenz, Jennette Logan, Elizabeth Tesson, Ernestine Smartt, and Amy Chen.

2010. "Retention strategies and predictors of attrition in an urban pediatric asthma study." *Clinical trials* 7 (4):400-410.

APPENDIX

APPENDIX

Appendix 1: Common autoimmune conditions and associated HLA genotypes and autoantibodies

Condition	HLA risk genotype	Antibodies
T1D	DRB1*03 - DQA1*0501 – DQB1 0201 (DR3-DQ2) and DRB1*04 – DQA1*0301 – DQB1 0302 (DR4 – DQ8)	GADA IAA IA2A ZnT8A
CD	DQ2 and DQ8	tTG Anti-gliadin antibody EMA
Thyroid	DR3	Antibodies to tyroxine peroxidase (TPO) Antithyroglobulin

Appendix 1 Common autoimmune conditions and associated HLA genotypes and autoantibodies

Appendix 2: FADES Protocol

University Hospitals Bristol NHS
NHS Foundation Trust

NIHR Bristol Biomedical Research Centre
Nutrition Theme

Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)

PROTOCOL

Researchers:

Biomedical Research Unit

Chief Investigator: Georgina Williams (Clinical PhD Student)

Principal Investigator: Julian Hamilton-Shield (Clinical)

Laura Birch (Dietetics)

Andy Ness (Epidemiology/ cohort studies)

Sam Leary (Statistician)

Jenny Ingram (Breast feeding specialist)

Katie Berryman (Trials Coordinator)

Vanessa Marshall (BRU Manager)

Stu Toms (Database Manager)

Shirley Jenkins (Management Assistant BRU)

Sofia Leadbetter (Administrative Support)

Patricia Neville (Lecturer in social sciences / qualitative research)

Diabetes & Metabolism Unit

Kathleen Gillespie (Immunology)

Alistair Williams (Immunology)

Rachel Aitken (Research Technician)

Saranna Chipper-Keating (Research Technician)

Georgina Mortimer (Research Technician)

Down's Syndrome Association

Sheila Heslem (Policy manager)

Downs's Syndrome Scotland

Sarah Van Putten (Family Support Service Manager)

University of Oxford, Department of Paediatrics

Irene Roberts (B cell analysis)

Sponsor

University of Bristol (Contact Dr Birgit Whitman),

University Hospitals Bristol NHS
NHS Foundation Trust

NIHR BRISTOL BIOMEDICAL RESEARCH CENTRE NUTRITION THEME

Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)

PROTOCOL

Aim: To develop a family acceptable study protocol and establish the feasibility of creating a national cohort of infants with Down's syndrome (DS) to study the associations between early infant feeding, infections and the development of autoimmunity in Down's syndrome.

Setting: This study has beenset up as a partnership between the NIHR, Bristol Biomedical Research Unit in Nutrition, The University of Bristol, School of Clinical Sciences, Diabetes Research Group, Imperial College London, Department of Medicine, the Down's Syndrome Medical Interest Group, the Down's Syndrome Association and Down's Syndrome Scotland. The study is being extended to the Republic of Ireland with the support of Down's Syndrome Ireland Fiona McGrane (research nurse) and Professor E Molloy Trinity College Dublin.

Summary: Children with Down's Syndrome (DS) have increased risk of autoimmune conditions where the body's immune system attacks its own cells, such as thyroid problems, diabetes and

coeliac disease (which causes malabsorption). In DS, autoimmunity is likely to be related to lifelong inherent defects in the immune system. The increased risk of diabetes-related autoimmunity is despite a reduced prevalence of the usual HLA haplotypes commonly associated with type 1 diabetes. Infant feeding practice has been linked to diabetes and coeliac risk with some evidence that prolonged breastfeeding is protective. We hypothesise that in infants with DS, already at increased risk, early feeding practices may be related to the development of autoimmunity. Children with DS are more floppy and therefore have difficulties with breastfeeding leading to the rapid introduction of formula feeds which contain modified cow's milk protein.

We aim to create a cohort of infants with DS recruited through the Down's Syndrome Association (DSA), Down's Syndrome Scotland (DSS), Down's Syndrome Ireland (DSI), community paediatricians and neonatologists to study the association between early infant feeding, infections and the development of autoimmunity. We initially anticipated that we would recruit 100 patients per year.

Parents are asked to complete questionnaires at baseline detailing family history, birth history, weight, medical problems and early feeding. They have further feeding questionnaires to complete at 7 months and 12 months, and medical questionnaires annually. Samples arecollected at baseline including faeces to store from mother and baby a brushing from the infants cheek for genotyping (looking at their DNA), a blood sample from the baby to look at development of auto-antibody production (antibodies which act against their own cells), and a urine specimen to detect development of diabetes. Further stool, urine and blood samples are collected at 6 and 12 months and once a year thereafter. The study is currently funded to run until January 2022 and participants will continue to complete annual questionnaires and samples around the time of their birthday until this date.

Phase 1 and Phase 2

There has also been a small pilot study (**Phase 1**) to assess the stool collection kit that we are now using in the main study (**Phase 2**). The kit that we are using in the main study is a new kit designed to preserve stool samples. We wanted to compare the new kit with the standard stool collection method to see if it is better at keeping samples fresh for analysis in the laboratory. The protocol for the pilot study can be found in appendix 1.

Phase 2 Recruitment: Is across the UK and the Republic of Ireland recruiting families with newborns with Trisomy 21 from as near to birth as possible. The Down's Syndrome Association (DSA) and Down's Syndrome Scotland (DSS) with whom we are collaborating on this project, assess they are able to contact around 500 new mothers with a child born with Down's syndrome a year (Information supplied by Sheila Heslam: Policy Manager DSA and Sarah Van Putten: Family Support Service Manager DSS). With a relatively conservative estimate of 20% interest in this study we therefore thought that we would be able to recruit 100 patients per year. Interested community paediatricians, neonatologists and research nurses also help with recruitment and display flyers for the study in community clinics and neonatal units. The DSA and Down's Syndrome Ireland provide links to the FADES study via their website and social media links. We are recruiting participants until January 2020.

Background: Children with Down's Syndrome (DS) have increased risk of thyroid, pancreatic[2] and coeliac[3] autoimmunity likely related to lifelong defects in intrinsic immunity[4]. Furthermore, the increased risk of pancreatic autoimmunity is associated with an earlier age of diabetes presentation suggesting an accelerated or exaggerated process in the autoimmune destruction of islet β cells[5 6]. We have documented this increased risk of diabetes-related autoimmunity despite a significantly reduced prevalence of the classical DR3/4 genotype in those with DS getting type 1 diabetes compared to children developing type 1 diabetes with normal chromosome number[7].

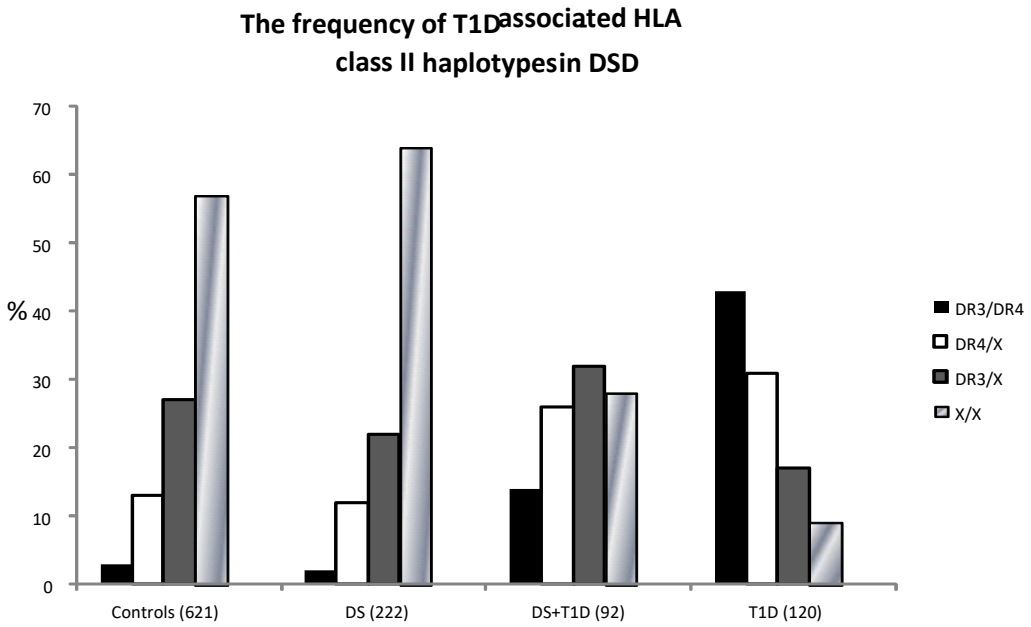


Figure 1

HLA high risk genotypes in healthy controls, Down's syndrome (DS), Down's syndrome and diabetes (DS+T1D) and type 1 diabetes(T1D)

A recent study in DS may be pertinent documenting a liability to elevated circulating T regulatory cells expressing FOXP3 but associated with a reduced T-cell inhibitory ability[8]. Uibo et al identified a similar picture with regards to FOXP3 expression in a cohort of patients positive for classical GADA or IA-2 antibodies regardless of clinical status[9]. In another study from the same group undertaken in mucosal biopsies of patients with coeliac or coeliac disease with diabetes, a picture of increased CD4 positive cells at the site of inflammation was associated with greatly increased FOXP3 positive Tregs[10].

Infant feeding practice has been linked to diabetes and coeliac disease risk with some evidence that prolonged breast feeding is protective[11 12]. The link between infant feeding and diabetes risk may

be through an abnormal immune response to Bovine Serum Albumin (BSA) present in cow's milk derived formula feeds. In animal studies and children developing diabetes, there is evidence of abnormally high titres to BSA not present in control groups[13]. A study by Atkinson and colleagues confirmed increased levels of anti-BSA antibodies in those with and at risk of autoimmune diabetes as well as those with other autoimmune conditions. In this study however it was shown that T cells in autoimmune diabetes are not activated against BSA peptides. They concluded that "Anti-BSA antibodies may reflect a general defect in the process of immunologic tolerance associated with a predisposition to autoimmunity rather than immunity specific to beta cells"[14].

We have identified that children with DS and especially DS and diabetes have a greatly increased prevalence of low affinity antibodies to BSA[15]. In theory this may be related to an increased gut permeability and immature immune system associated with trisomy 21. **We hypothesise that in infants with DS, already at increased risk of autoimmunity, early feeding practices such as a rapid introduction of modified cow's milk protein possibly related to a reduced ability to breast feed due to hypotonia[16] that classically accompanies DS, is related to the development of autoimmunity.** A recent study in Scandinavia suggested that dietary manipulation to prevent early exposure to complex cow's milk protein in non-DS, but at risk infants might impact upon the development of autoimmunity.[17] Whilst infant milk feeding practice may associate with an increased risk of diabetes, it is also possible that weaning practice with regards to gluten containing cereals may also be of importance. There is evidence that the development of islet autoimmunity in non-DS children may be related to an early introduction of gluten-containing cereal feeds possibly influenced by early gastro-intestinal infections[18-20]. One further avenue by which early feeding practices may influence autoimmunity of gut and pancreas is through the microbial population present in the intestine, the so-called 'gut microbiome'. There have been well documented differences in this microbiome recorded both in those developing diabetes[21 22] and coeliac disease[23] when compared to non-affected controls. Furthermore, the

development of the gut microbiome has been linked to early infant feeding practices such breast versus cow's milk feeding and time of weaning[24]. Resident gut microflora are known to contribute to immune function and homeostasis. At the epidemiological level, children born by Caesarian section are at greater risk of T1D[25] indicating that early exposure to maternal bacteria may be protective. Furthermore NOD mice, kept in non-germ free conditions are relatively protected from diabetes. Increased gastrointestinal permeability or a "leaky gut" has been associated with the risk of developing T1D and other autoimmune conditions. For example individuals with, or at increased risk, of T1D[26] have been shown to have abnormal intestinal permeability to the sugars mannitol and lactulose[27], upregulation of the gut permeability modulator zonulin[28] and mucosal alterations on electron microscopy (Secondulfo et al. 2004). In the Biobreeding rat model of T1D there are distinct bacterial populations resident in diabetes prone and resistant strains and oral transfer of Lactobacillus johnsonii strain 6.2 from the resistant to susceptible strains transfers resistance[29 30]. In the NOD mouse, it has been shown that interaction of intestinal microbes with the innate immune system is a critical factor in T1D predisposition[31]. Most recently in humans, differences in diversity of the gut microbiome have been identified in those who develop type 1 diabetes, compared with non-autoimmune prone individuals[21].

Overall the rationale of this study is to study factors known to be important in autoimmunity in the general population, in a population known to be at increased risk of organ specific autoimmunity, namely children with Down's syndrome. The research design allows longitudinal follow-up of the natural history of feeding regimes, the appearance of antibodies to BSA and markers of autoimmunity as well as analysis of the gut microbiome.

Methodology:

Phase 1: Was a pilot study to evaluate the performance of the DNA Genotek Stool Collection Kit compared to a standard stool self collection kit. The methodology for Phase 1 can be found in Appendix 1.

Phase 2:

Recruitment:

When a parent is told either antenatally or after birth that their baby has a diagnosis of Down's syndrome they are provided with the details of The Down's Syndrome Association (DSA) and/or Down's Syndrome Scotland (DSS) and/or Down's Syndrome Ireland (DSI) as a source of information and support. DSA and DSI send out new parent information packs to all families who request them and the DSS make contact with families who request their input. The DSA and DSI have agreed to send out a flyer with their 'New Parent Packs' with simple details about the study. DSS have also agreed to mail out our flyers to all families with a baby under 8 months of age with whom they have contact, and will also take the flyer with them on the home visits they make to new parents. They will also have information about the study posted on the DSA, DSI and DSS websites on their research pages and through Blogs. The DSA has also created a link via their Facebook page and Twitter feed to our webpage. The study flyer and the information on the website will include a web address by which parents can register an interest in taking part and download a more detailed information sheet. In areas where there are interested community paediatricians and neonatologists who will act as local collaborators potentially eligible families will also be identified by their community paediatrician or neonatologist or research nurse who will provide them with details of the study, how to register. Community paediatricians, neonatologists and research nurses who are local collaborators will be able to support them through the study. When registering an interest, parents can indicate how they would like to be contacted by the

research team, either email or telephone. We then send them 2 copies of the consent form in the post, with a copy of the information sheet we then contact them after at least 48 hours in order for them to have a chance to ask any questions and discuss any concerns. If happy to proceed we ask them to sign both consent forms, one to post back to us in the stamped addressed envelope provided and one for them to keep for their own records. Where there is a community paediatrician, neonatologist or research nurse acting as a local collaborator the information sheet and consent forms may be provided by them and they may consent the participant.

Once a participant has been consented we write to their GP and community paediatrician to inform them that they are in the study. We also write to inform the mother's GP.

Inclusion Criteria:

- Babies recruited antenatally or in the first 8 months of life born with Down's syndrome (3 copies of chromosome 21) as confirmed by karyotype after birth will be eligable for the study.

Exclusion Criteria

- Babies with Down's Syndrome who have a child protection plan or who are no longer with birth mother.
- Babies with Down's syndrome over 8 months of age
- Babies with Down's syndrome in whom the parents do not speak English. The study is recruiting families who are trying to cope with a difficult diagnosis and may wish to have conversations with the research staff to establish what the implications of recruitment would involve for them. Participants are also be required to fill out 7 questionnaires over 5 years which are in English.

Period of Enrolment

Infants are ideally enrolled in the antenatal or early postnatal period (maximum age 8months) and are being followed up until January 2022. We will be recruiting participants until January 2020.. For those that were initially recrutited in 2014 they will now be followed up until they are 7 years

old, those recruited in 2015 until they are 6years old and then for each additional year they will be followed for one year less (see table below)

Year of birth / recruitment	Follow up period
2019	2 years
2018	3 years
2017	4 years
2016	5 years
2015	6 years
2014	7 years

Data collection:

Overview: We are collecting a variety of data from each participant and their family. There are questionnaires that the family can either complete online or in a paper version. In addition are seeking consent from the baby's parents to access stored blood spots taken on day 5 of life (as part of the Newborn Screening Programme) to look at baseline levels of autoimmunity. There are a series of samples that we ask parents to help us take during the study. These include samples of DNA from a brushing of the inside of the baby's cheek, blood, urine and stool specimens to be collected annually around the time of their birthday until January 2022..

Months of age	0* Baseline	6	7	12	**Every year around the time of the participant's birthday until January 2022**
Day 5 blood spot for autoantibodies	**X**				
Combined feeding and medical questionnaire	**X**		**X**	**X**	
Medical questinnaire					**X**
Mouth swab for DNA extraction	**X**				
Stool for microbiome	**X**	**X**		**X**	**X**
Blood for autoantibodies and BSA antibodies.	**X**	**X**		**X**	**X**
Urine for urinary C	**X**			**X**	**X**

peptide analysis					

Fig 1. Timeline for sample collection. (*at recruitment)

1. **Baseline:** Once parents have consented for their baby to take part in the study we send them out a pack which will include the instructions of how to log on to the online questionnaire (if they requested a paper version of the questionnaire this will be sent out in this pack). Parents are asked to fill in some details about their own medical history, family history, details of birth history, weight, related medical problems such as congenital cardiac disease and any history of infections. The initial questionnaire also include feeding questions exploring parental expectations and barriers to breast feeding, introduction of modified cow's milk feeding if applicable and any other problems with early feeding.
   Having gained consent from the parent we will also access the baby's day 5 blood spot (Guthrie card) from which we can look for any autoantibodies that were present in the first week of life.

Baseline Samples:

(a) A stool specimen from infant to the laboratory for storage for later evalaution of stool microbiome. This will be a sample collected from the baby's nappy by the parents.
A stool specimen from the mother to the laboratory for storage for later evaluation of stool microbiome. The mother is asked to take a swab from a piece of soiled toilet paper.

(b) A swab from infant's buccal mucosa for DNA extraction for HLA genotyping. - The parents are provided with a swab for taking a painless brushing from the inside of the baby's cheek.

(c) Urine specimen for urinary 'C' peptide analysis. Parents are provided with sterile cotton wool which can be placed in the nappy to collect urine on after a feed. Or if the parents prefer they can catch the urine in the pot. We advise that either the nappy is taken off prior to the feed or the cotton wool is placed in the nappy just prior to a feed in order that the first urine after the feed can be collected

(d) A heel prick blood test for analysis of auto-antibody production (approx 0.5ml blood) and immune cell analysis at Imperial College (0.5ml) collected by a health professional at a routine health check as detailed above.

2. **Month 6 - 7:** At 7 months parents are asked to fill in a further feeding questionnaire regarding length of time of breast feeding alone, timing of cow's milk introduction, type, quantity consumed per 24 hours, and time of weaning with which 'solid food' products. The questionnaire also explores the medical history of the infant including, primary and secondary care consultations/admissions, indication for consultation and use of antibiotics in first 6 months and for what indications.

   Samples: At 6 months parents are asked to send:

   (a) A stool specimen from infant to the laboratory for for gut microbiome analysis.

   (b) A heel prick blood test for analysis of auto-antibody production. Having observed the initial blood test parents should be able to do this blood test at home if happy to do so.

3. **Month 12:** A further feeding and medical history questionnaire is requested for life events between 7-12 months.

   Samples: Parents are asked to send:

   (a) A stool specimen from infant to the laboratory for gut microbiome analysis.

   (b) A fingerprick blood test for analysis of auto-antibody production. At this age the blood test can be taken from a finger prick by the parents or alternatively at the time of routine thyroid disease screening.

   (c) Urine for urinary 'C' peptide analysis

4. **Once a year around the time of the child's birthday up until January 2022**

   Parent complete medical questionaire

Samples: Every year parents are asked to send:

(a) A stool specimen from infant to the laboratory for gut microbiome analysis.

(b) A finger prick blood test for analysis of auto-antibody production.

(c) Urine for urinary 'C' peptide analysis

Samples:

Apart from the initial blood test, samples can be collected by the parents at home. We send them out a pack which contains instructions for taking all the samples (brushing from the baby's cheek, blood, stool and urine) as well as the equipment for taking it. The parents can take the initial brushing from the baby's cheek and collect the urine and stool samples at home

After birth, all babies have a number of routine health checks carried out by health professionals including their midwife, GP, GP practice nurse and for babies with Down syndrome, they will also have an appointment with a paediatrician. With consent from the families, we contact one of the key health professionals with which they are having their routine appointments to ask if they can assist with the first blood test. We also provide the parents with a factsheet that can be shown to the health professional, outlining the study, and basic requirements for blood sampling. Where there is a local collaborator for the FADES study, the initial blood test may be organised by them to coincide with one of their appointments. They may also help with coordinating the future blood tests.Once this initial blood test has been taken with the parents observing, the heel prick blood test at 6months can be done by the parents at home if they are happy to do so. The finger prick blood tests at a year and annually after that can also be done by the parents at home although we provide advice on the alternative of having these samples taken at the same time as their routine clinical yearly samples taken for thyroid disease screening. Once the samples have been taken they aregiven to the parents to send back in the packaging that they have been provided with.

We also provide the necessary pre-paid packaging so that parents can post the samples back to us at the University of Bristol's Learning and Research Centre at Southmead. The packaging ensures that the samples do not deteriorate during transportation and all samples are labelled with the participant's ID number so that traceability is maintained. Any samples that need to be sent out to other laboratories for analysis are sent from Southmead using university approved couriers. This will include blood for immune cell analysis by Prof Irene Roberts at Imperial College, urine will be sent to Exeter for analysis of urinary C peptide and the stool will be sent for microbiome analysis. All samples will be used, stored and disposed of in accordance with The Human Tissue Act 2004.

If the questionnaires are not completed or samples are not sent back we initially email the family if they have previously expressed that they are happy to be contacted by email, or send a reminder letter out to the family 2 weeks after the date that we sent out the pack. If we still have no response from the family after a further 2 weeks we email or phone them to discuss whether they need a new pack, or if they would prefer to send us only some of the samples or whether they wish to withdraw from the study.

Laboratory methods:

A dedicated password protected Access database has generated to hold patient data. A separate database has been generated for laboratory data. The databases are linked by an ID number only to permit data analysis.

1. **HLA class II analysis**
   DNA samples will be genotyped for all HLA class II HLA DRB1 and DQB1 haplotypes by polymerase chain reaction using a well established PCR-SSP method (Gillespie et al. Tissue Antigens 2001). This analysis will take place at the Learning and Resource centre at Southmead**.**

2. **Urinary C Peptide analysis**
   Urine samples will be sent from the Learning and Research Centre at Southmead to Dr Timothy Macdonald for analysis of urinary c peptide using university approved couriers. The samples will be labelled with the participant's ID to ensure traceability is maintained.

3. **Autoantibody analysis**
   Serum from all individuals recruited will be tested for islet autoantibodies to insulin, GAD, IA-2 and ZnT8R/W using established standardized radioimmunoassays with ^{125}I or ^{35}S labeled antigens (Long et al. Diabetes 2012). Anti-BSA antibody analysis will also be determined by radioimmunoassay. Thyroid and gut autoimmunity will be analysed by testing for antibodies to tissue transglutaminase (Tg) as previously published (Williams AJ, Norcross AJ, Lock RJ, Unsworth DJ, Gale EA, Bingley PJ. Diabetes Care. 2001 Mar;24(3):504-9). Antibodies to thyroid peroxidase (TPO) will be measured using a radioimmunoassay kit purchased from RSR Limited, Cardiff UK. I125 labelled TPO will be incubated with patient sera and calibrators. Antigen-antibody complexes will then precipitated using solid phase protein A and centrifugation. Unbound labelled TPO will be removed by aspiration and the remaining pellet measured in a gamma counter. Percentage binding is calculated as the pellet count divided by total count multiplied by 100. A calibration curve is produced using the calibrator data allowing patient TPO autoantibody levels to be determined. Antibodies to gastric H+/K+ ATPase 4A subunit antibodies will be measured using a method recently described (Development of a novel autoantibody assay for autoimmune gastritis in type 1 diabetic individuals. (Wenzlau JM, Gardner TJ, Frisch LM, Davidson HW, Hutton JC. Diabetes Metab Res Rev. 2011 Nov;27(8):887-90. doi: 10.1002/dmrr.1267. This analysis will be done at the Learning and Research Centre at Southmead.

4. **Immune cell analysis (Prof Irene Roberts)**

Blood samples will be sent from the Learning and Research Centre at Southmead to Irene Roberts using university approved couriers. The samples will be labelled with the participants ID to ensure traceability is maintained. EDTA blood samples would be red cell- and granulocyte-depleted by density gradient centrifugation and mononuclear cells isolated for: (i.) immunophenotyping by multiparameter flow cytometry (BD Fortessa) using a panel of B-cell (CD19, CD10, CD27, IgM, IgD) and T-cell (CD4, CD8, CD45RO, CD62L/CCR7, CD25/cFoxP3, Vα24/vβ11) antigens; and (ii) RNA extraction to assess expression of lineage-associated genes by flow-sorted B cells.

5. **Gut microbiome**

We have run a pilot study **(Phase1)** to evaluate the performance of the DNA Genotek Stool Collection Kit compared to a standard stool self collection kit. See Appendix 1 for the pilot study protocol..

Parents collect small samples of stool from their babies nappies using a swab and will transfer it into containers which will be provided. Maternal stool samples will be collected by swabing a piece of soiled toilet paper and transferring into the provided container. The gut bacterial DNA will be extracted and sent for analysis in a laboratory with expereince in microbiome analysis. All samples sent out by the research team will be anonymous and linked only to the patient by a four digit number.

Feasibility study objectives

As this is a feasibility study there are a number of objectives that we will use to assess the acceptability and feasibility of the study. These fall under the following headings:

Recruitment

The feasibility of our recruitment methods will be determined by the number of participants that we are able to consent to the study, our target is 100 participants a year recruiting over a 2 year period (20% of the DS families who are in contact with the DSA)

The acceptability and feasibility of web recruitment will be explored. Potential participants are asked to complete an online registration/ expression of interest form, from this the participant information sheets and consent forms are sent out to them in the post (the exception are those recruited and consented by local collaborators). We will keep a record of any problems that occur which prevent or delay participants being recruited as a result of the online system.

Qualitative telephone interviews with community paediatricians, neonatologists, research nurses, other relevant healthcare professionals and family support workers from charities such as the DSA and DSS will be used to explore the barriers and motivations to families with new babies with DS in taking part in research. We will also be asking them about the feasibility and acceptability of the FADES protocol (appendix 3)

Questionnaires

Online and paper questionnaires are completed at initial recruitment, 7 months, a year and annually until January 2022. The feasibility and acceptability of the questionnaires will be determined by the percentage of participants that complete the questionnaires as well as by the quality of the data.

We are aiming for 75 % of recruited participants to complete initial questionnaires, the questionnaires at 7 months and to be completing questionnaires at a year. We aim for 50% to complete the and annual questionnaires up until the age of 5 years.

The feasibility and accepatbility of using online questionnaires will be determined by the number of participants who choose to use the online versions. We hope that over 60% of participants will opt to use the online questionnaire. We will also be assessing whether the data produced is in a

form which is ready for analysis. A record of any problems that occur which prevent or delay participants completion of the online questionnaire and any issues that are raised by participants in relation to the online or paper questionnaires will be recorded.

Sample collection

Families are asked to collect a number of samples at home which they post back to us. The feasibility of the sample collection is being assesed in several ways:

The method for collection of urinary c peptide using cotton wool was established in a small pilot study prior to the start of the FADES study.

Phase 1 (appendix 1) is being used to establish whether gut microbiome samples are preserved whilst being sent in the post.

The feasibility and acceptability of collecting all the samples within the desired timeline will be determined by the percentage of participants who return the requested samples. We hope that 75% of participants will provide the requested initial samples before 8 months of age. Of the samples provided 90% of samples should be adequate for analysis. For many participants they will not need to collect samples at 7 months because of the age at recruitment therefore acceptability and feasibility will be determined by the percentage of participants who return the requested samples at 12 months. Our target would be for 75% of participants to provide the requested 12 month samples before 14 months of age. The aim will be for 50% of participants to provide all samples up to the age of 5 years.

In order to determine the acceptability and feasibility of the study we will also be recording any issues or problems that occur with sample collection at home. Blood samples can be collected by health professionals during participants routine health appointments we will record any issues that arise in organising and collecting these samples.

Retention of participants

The acceptability and feasibility of the study will be determined by the number of participants who reamain active in the study up until the age of 5 years old with a traget of retaining 50% of participants.

Patient and Family Involvement (PPI

The DSA, DSI andDSS are parent/member led charities with whom we are collaborating with on this study. They have had input into the study design as well as advising us on the wording of the information sheets, consent forms and questionnaires.

Conflict Of Interest: None of the researchers have any affiliations or involvement in any organisation or entity with any financial interest or non-financial interest which would impact on this study.

Monitoring and Audit: The University of Bristol has a Service Level Agreement in place with a local NHS Trust (UH Bristol). As part of this, UH Bristol will undertake maonitoring of research projects where University of Bristol is fulfilling the responsibilities of a research sponsor. A minimum of 10% of UoB projects will be monitored.

All study related documents will be made available on request for monitioring and audit by UH Bristol and the relevant Research Ethics Committee.

Safety Reporting: For safety reporting procedures we will follow the UH Bristol Research Adverse Event Policy

Indemnity: This is a University of Bristol sponsored research study. The University of Bristol has arranged Public Liability insurance to cover the legal liability of the Univeersity as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University. The university of Bristol holds Professional Negligence insurance to

cover the legal liability of the University, for harm to participants arising from the design of the research, where the research protocol was designed by the University. The University of Bristol's Public Liability insurance policy provides an indemnity to our employees for their potential liability for harm to participants during the conduct of the research.

Ethics and R & D Approvals: The study will be carried out subject to Research Ethics Committee (REC) approval and Research and Development (R&D) approval from the relevant NHS Trusts.

Research Governance Statement: This study will be sponsored by the University of Bristol and conducted in accordance with the Research Governance Framework for Health and Social Care and Good Clinical Practice.

Data Protection: Data will be collected and retained in accordance with the data protection act 1998.

Recruitment

Month	0	6	7	12	
Annually until Jan 2022					
Combined Feeding and Medical Questionnaire	X		X	X	
Medical Questionnaire					X
Samples					
DNA extraction	X				
Stool for microbiome	X	X		X	X
Blood for auto-antibodies	X	X		X	X
Bovine serum albumin					
antibody analysis	X	X		X	
Urine Protein C	X			X	X

References

1. Vanhaelst L, Hayez F, Bonnyns M, Basteinie PA. Thyroid auto-immune disease and thyroid function in families of subjects with Down's syndrome. J Clin Endocrinol Metab 1970;30(6):792-7.

2. Van Goor JC, Massa GG, Hirasing R. Increased incidence and prevalence of diabetes mellitus in Down's syndrome. Arch Dis Child 1997;77(2):186.

3. Gale L, Wimalaratna H, Brotodiharjo A, Duggan JM. Down's syndrome is strongly associated with coeliac disease. Gut 1997;40(4):492-6.

4. Kusters MA, Verstegen RH, Gemen EF, de Vries E. Intrinsic defect of the immune system in children with Down syndrome: a review. Clinical and experimental immunology 2009;156(2):189-93.

5. Shield JP, Wadsworth EJ, Hassold TJ, Judis LA, Jacobs PA. Is disomic homozygosity at the APECED locus the cause of increased autoimmunity in Down's syndrome? Arch Dis Child 1999;81(2):147-50.

6. Rohrer TR, Hennes P, Thon A, Dost A, Grabert M, Rami B, et al. Down's syndrome in diabetic patients aged <20 years: an analysis of metabolic status, glycaemic control and autoimmunity in comparison with type 1 diabetes. Diabetologia 2010;53(6):1070-5.

7. Aitken RJ, Mehers KL, Williams AJ, Brown J, Bingley PJ, Holl RW, et al. Early-Onset, Coexisting Autoimmunity and Decreased HLA-Mediated Susceptibility Are the Characteristics of Diabetes in Down Syndrome. Diabetes Care 2012.

8. Pellegrini FP, Marinoni M, Frangione V, Tedeschi A, Gandini V, Ciglia F, et al. Down syndrome, autoimmunity and T regulatory cells. Clinical and experimental immunology 2012;169(3):238-43.

9. Link M, Salur L, Kisand K, Rajasalu T, Tillmann V, Uibo R. Higher FoxP3 mRNA expression in peripheral blood mononuclear cells of GAD65 or IA-2 autoantibody-positive compared with autoantibody-negative persons. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica 2008;116(10):896-902.

10. Vorobjova T, Uibo O, Heilman K, Rago T, Honkanen J, Vaarala O, et al. Increased FOXP3 expression in small-bowel mucosa of children with coeliac disease and type I diabetes mellitus. Scandinavian journal of gastroenterology 2009;44(4):422-30.

11. Malcova H, Sumnik Z, Drevinek P, Venhacova J, Lebl J, Cinek O. Absence of breast-feeding is associated with the risk of type 1 diabetes: a case-control study in a population with rapidly increasing incidence. Eur J Pediatr 2006;165(2):114-9.

12. Sollid LM. Breast milk against coeliac disease. Gut 2002;51(6):767-8.

13. Karjalainen J, Martin JM, Knip M, Ilonen J, Robinson BH, Savilahti E, et al. A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. N Engl J Med 1992;327(5):302-7.

14. Atkinson MA, Bowman MA, Kao KJ, Campbell L, Dush PJ, Shah SC, et al. Lack of immune responsiveness to bovine serum albumin in insulin-dependent diabetes. N Engl J Med 1993;329(25):1853-8.

15. Brown JA, R. Newton, R. Uibo,R. Bingley,P. Shield, JP. Williams,A. Gillespie, K. Increased levels of low affinity anti-BSA antibodies in individuals with Down's syndrome at genetic risk for autoimmunity. European Congress of Immunology Glasgow, 2012.

16. McBride MC, Danner SC. Sucking disorders in neurologically impaired infants: assessment and facilitation of breastfeeding. Clinics in perinatology 1987;14(1):109-30.

17. Knip M, Virtanen SM, Seppa K, Ilonen J, Savilahti E, Vaarala O, et al. Dietary intervention in infancy and later signs of beta-cell autoimmunity. N Engl J Med 2010;363(20):1900-8.

18. Snell-Bergeon JK, Smith J, Dong F, Baron AE, Barriga K, Norris JM, et al. Early Childhood Infections and the Risk of Islet Autoimmunity: The Diabetes Autoimmunity Study in the Young (DAISY). Diabetes Care 2012.

19. Norris JM, Barriga K, Klingensmith G, Hoffman M, Eisenbarth GS, Erlich HA, et al. Timing of initial cereal exposure in infancy and risk of islet autoimmunity. JAMA 2003;290(13):1713-20.

20. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. JAMA 2003;290(13):1721-8.

21. Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, et al. Toward defining the autoimmune microbiome for type 1 diabetes. The ISME journal 2011;5(1):82-91.

22. Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PloS one 2011;6(10):e25792.

23. Di Cagno R, De Angelis M, De Pasquale I, Ndagijimana M, Vernocchi P, Ricciuti P, et al. Duodenal and faecal microbiota of celiac children: molecular, phenotype and metabolome characterization. BMC microbiology 2011;11:219.

24. Thompson AL. Developmental origins of obesity: early feeding environments, infant growth, and the intestinal microbiome. American journal of human biology : the official journal of the Human Biology Council 2012;24(3):350-60.

25. Cardwell CR, Stene LC, Joner G, Cinek O, Svensson J, Goldacre MJ, et al. Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. Diabetologia 2008;51(5):726-35.

26. Bosi E, Molteni L, Radaelli MG, Folini L, Fermo I, Bazzigaluppi E, et al. Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia 2006;49(12):2824-7.

27. Kuitunen M, Saukkonen T, Ilonen J, Akerblom HK, Savilahti E. Intestinal permeability to mannitol and lactulose in children with type 1 diabetes with the HLA-DQB1*02 allele. Autoimmunity 2002;35(5):365-8.

28. Sapone A, de Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F, et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes 2006;55(5):1443-9.

29. Valladares R, Sankar D, Li N, Williams E, Lai KK, Abdelgeliel AS, et al. Lactobacillus johnsonii N6.2 mitigates the development of type 1 diabetes in BB-DP rats. PloS one 2010;5(5):e10507.

30. Lau K, Benitez P, Ardissone A, Wilson TD, Collins EL, Lorca G, et al. Inhibition of type 1 diabetes correlated to a Lactobacillus johnsonii N6.2-mediated Th17 bias. J Immunol 2011;186(6):3538-46.

31. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 2008;455(7216):1109-13.

Appendix 3: Protocol for Feasibility study for the collection of gut microbiome samples

NIHR Bristol Nutrition Biomedical Research Unit

Pilot Study Design – University Hospitals Bristol

OBJECTIVE

This pilot study will evaluate the performance of the DNA Genotek Stool Collection Kit (OMR 200) for use in the FADES study. The DNA Genotek Stool Collection Kits to be evaluated in this pilot study will be prototypes still under development and thus will require participants to strictly follow the collection instructions provided. The DNA Genotek Stool Collection Kit will be compared to a standard stool self-collection kit (without stabilization liquid).

STUDY DESIGN

Recruitment

10 Participants will be recruited from patients admitted to Bristol Royal Hospital for Children for non-infectious causes (likely infants being admitted for elective surgery). We will provide flyers to parents of babies under 1 year of age who are admitted to the Bristol Royal Hospital for Children. If they are interested, we will give them a study information sheet. If they are happy to proceed, we will meet them on the ward to answer any questions, complete the consent form and provide them with the stool collection kit.

Sample Collection

Samples will be collected by parents on the ward from their baby's nappy. The samples need to be 'fresh' (passed within an hour) and they will need to take 3 samples from the same "dirty

nappy" (i.e. from the same stool). In order to acquire a 'fresh' stool we will ask parents to check their baby's nappy regularly within one hour after a feed as babies tend to have a pronounced gastro-colic reflex. We will arrange to be on the ward the day that the sample is collected so that we can freeze one of the samples immediately and package the other samples for posting.

The first sample from the nappy will be collected using the DNA Genotek stool collection kit (OMR 200) ("Tube 1") which will be sent in the post. The second and third samples will be collected using the standard stool self-collection kit without stabilization liquid; "Tube 2" which we will send in the post with "Tube 1" in order to compare to the DNA Genotek Stool Collection Kit. "Tube 3" we will freeze immediately (the current accepted method for collecting stool for gut microbiome).

Prior to collection the tubes will be labelled as Tube 1, Tube 2 and Tube 3 appropriately. A unique identifier will be assigned to each tube and each tube will be weighed (to be used as baseline to determine amount of sample collected post-collection).

The parents of the ten participants will each be provided with the required materials for sample collection as described in Section 3 Study Materials. The parents of the 10 participants, will each collect stool samples from one "dirty" nappy. A small amount of the stool will be put into each of the three provided tubes following the appropriate provided Collection Instructions. Tube 1 will be the DNA Genotek Stool Collection kit (OMR 200 tube contains mixing bead and stabilization liquid) Tube 2 and Tube 3 will be the standard stool collection kit without stabilization liquid.

The parents will then be asked to complete the questionnaire in Appendix 2 which asks how easy they found the collection kit to use.

Once the sample has been collected

Tube 1(Genotek sample kit): - we will package with Tube 2 and send to the Diabetes and Metabolism Unit at Southmead Hospital. These samples will be in standard packaging and labelled so as to maintain traceability. It is anticipated that shipping will comprise ambient temperature conditions for 4 -5 days.

Tube 2(Standard collection kit): - we will package with tube 1 and send to the Diabetes and Metabolism Unit at Southmead hospital.

Tube 3 (Standard sample kit): - we will put on dry ice (-78.5°C) and transport to the Diabetes and Metabolism Unit at Southmead Hospital. Tube 1 will then be placed in the Freezer at -80^{o}C

Sample Testing

On arrival at the Diabetes and Metabolism Unit the tubes will be weighed and sample weights determined based on matched baseline values measured pre-collection, the samples will then all be placed in the freezer at -80^{o}C until DNA extraction.

Three aliquots (0.25mL each) of each sample will be removed and DNA extracted using the MoBio PowerFecal DNA Isolation Kit or MoBio PowerSoil DNA Isolation Kit, following manufacturer's instructions, specifically:

- Vortex the DNA Genotek tubes containing sample and stabilization liquid to ensure homogenization
- Remove 0.25mL of stool sample mixed with stabilization liquid from Tube 1 ("DNA Genotek kit") or 0.25g of stool sample from Tube 2 (no stabilization liquid) and add to the Dry Bead Tube as described in Step 1 of the Experienced User Protocol for the PowerFecal kit, or add to the PowerBead Tubes as described in Step 1 of the Experienced User Protocol for the PowerSoil kit.

Continue with the remainder of the protocol.

Each purified DNA sample will be tested for the following endpoints:

- DNA concentration (using fluorescence)
- High molecular weight DNA using agarose gel electrophoresis
- Microbiome stability assessed using Denaturing Gradient Gel Electrophoresis (DGGE) and/or 16S rRNA microbiome sequencing and/or whole metagenomic sequencing

STUDY MATERIALS

Each participant will be provided with the following materials for collection:

- Tube 1: One DNA Genotek Stool Collection Kit (tube containing the DNA Genotek stabilization liquid and a collection tool) (provided by DNA Genotek)
- Collection Instructions for Tube 1 (in Appendix 1)
- Tube 2 and 3: Two empty tubes and collectors as the standard stool collection kit.
- Collection Instruction for Tube 2 and 3
- A rack to hold tubes during collection
- Disposal bag

STUDY SAMPLES

Ten (10) donors will each provide three tubes each containing a small amount of stool sample for a total of 30 samples. Each sample will be extracted in triplicate for a total of 90 extracted DNA samples.

DATA ANALYSIS

Study Endpoints

Questionnaire results from DNA Genotek Collection Kit and standard stool collection kit

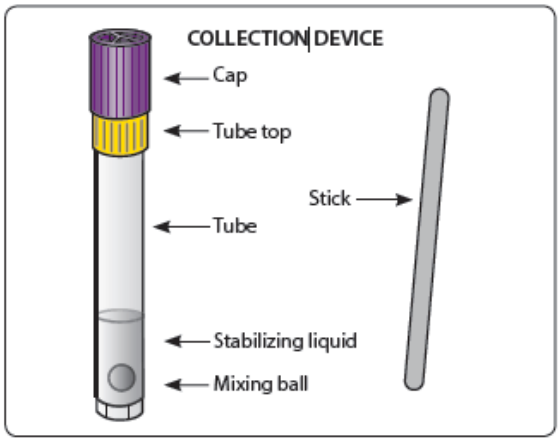
Sample weight

DNA concentration (using fluorescence)

High molecular weight DNA measured using agarose gel electrophoresis

Microbiome stability assessed using Denaturing Gradient Gel Electrophoresis (DGGE) and/or 16SrRNA microbiome sequencing and/or whole metagenomics sequencing

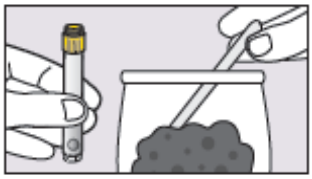
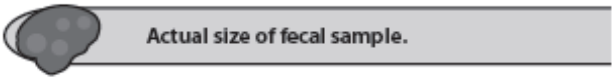
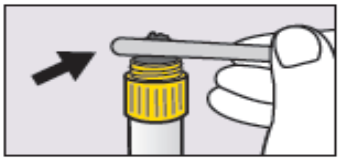
APPENDIX 1: Collection Instructions for the DNA Genotek Stool Collection Kit - Omnigene GUT (OMR 200)

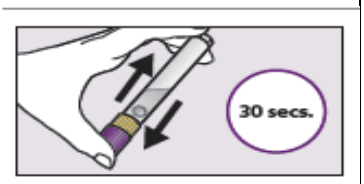
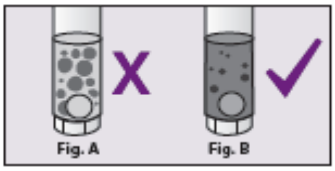


Warnings and precautions:

- FOR EXTERNAL USE ONLY.
- Do NOT remove the yellow tube top from the tube.
- Do NOT spill the stabilizing liquid in the tube.
- Wash with water if liquid comes in contact with eyes or skin. Do NOT ingest.
- If fecal sample is liquid or donor has diarrhea wait until the next bowel movement to collect the sample.
- Small items may pose a choking hazard.

Step	Procedure	
1	As soon as you are aware that your baby has passed a stool (done a poo) in their nappy remove the nappy. We want the stool to be as fresh as possible when the sample is collected.	
2		Unscrew ONLY the purple cap from the collection device and set it aside for later use.

		IMPORTANT: Do not remove the yellow cap  Do not spill the stabilization liquid in the tube
3		For the first sample Tube 1 use the stick to collect a small amount of faecal matter (poo) from the nappy 
4		Transfer the stool (poo) sample into the yellow tube top. Repeat until the sample reaches the top and fills it completely  IMPORTANT: Try not to push the sample into the tube.
5		Scrape horizontally across the tube top to level the sample and remove any excess. Discard the stick. Wipe the exterior of the tube and top with the toilet paper or tissue as needed.

6		Screw the purple cap back onto the yellow top tube until tightly closed 
7		Shake the sealed tube as hard and fast as possible in a back and forth motion for a minimum of 30 seconds.
8		The stool (poo) sample will be mixed with the stabilizing liquid in the tube. Not all particles will dissolve. IMPORTANT: Keep shaking if large particles remain as shown in Fig. A
9	Proceed with the collection of the sample into Tube 2. Open the tube and use the scoop to pick up some stool from the nappy put the cap back on including the scoop.	
10	Repeat step 9 with Tube 3	
11	Give all 3 tubes to the member of the research team who will be present on the ward together with a completed questionnaire.	

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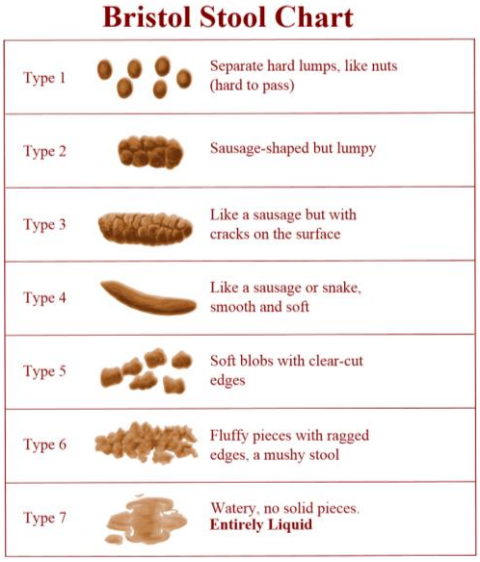
APPENDIX 2: Questionnaire for the DNA Genotek Stool Collection Kit – Tube 1

1. How old was your baby (in weeks) at the time of stool collection?
2. Using this Bristol Stool Chart, please **circle** the Type (1-7) which most closely resembles the consistency/hardness of stool sampled for this collection.



3. When collecting the sample into Tube 1 how did you find transfer of the sample into the yellow top : (please circle all that apply)
   a. How many collections (step 3) or transfers were required to fill the top of the yellow cap with the stick?
      i. **1**
      ii. **2**
      iii. **3**
      iv. **More than 3**

4. Regarding the overall collection experience:
   b. Were the components provided in the kit for tube 1 sufficient to collect and transport samples in a comfortable manner? **Yes / No**

      i. I would add to the kit: _____

ii. I would remove from the kit: _____

iii. I would change the kit by: _____

c. Were the collection instructions easy to follow? **Yes / No**

d. Was enough detail provided in the instructions for a first-time donor? **Yes / No**

e. Were you overwhelmed by the number of collection steps? **Yes / No**

f. Would you consider your collection successful? **Yes / No**

5. Do you have any comments/feedback that would help us improve the sample collection process?

_____
_____
_____
_____
_____
_____

Appendix 4: Protocol for qualitative study

NIHR Bristol Nutrition Biomedical Research Unit

Qualitative Research exploring the barriers and motivations to research in young babies with Down's Syndrome

STUDY PROTOCOL

Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES)

Final Version: 1
Date: 2nd September 2015

Short title: FADES Quali
Study Sponsor: University of Bristol
Funding source: NIHR Bristol Nutritional Biomedical Research Unit, University of Bristol

STUDY PERSONNEL AND CONTACT DETAILS

Sponsor:	University of Bristol
Contact name:	Dr Birgit Whitman Head of Research Governance Senate House, Tyndall Avenue Clifton BS8 1TH Phone: 0117 331 7130 Email: birgit.whitman@bristol.ac.uk
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Co-investigators:	Dr Patricia Neville, Professor Julian Hamilton Shield Dr Sam Leary Dr Kathleen Gillespie
Study Coordinating Centre:	NIHR Biomedical Research Unit in Nutrition, Diet and Lifestyle Level 3, University Hospitals Bristol Education Centre Upper Maudlin Street, Bristol BS2 8AE

SYNOPSIS

Title	Feeding and Autoimmunity in Down's Syndrome Evaluation Study
Short title	FADES
Chief Investigator	Georgina Williams

Objectives	The overall aim of the research is to establish the feasibility of creating a national cohort of infants with Down's syndrome (DS) to study the associations between early infant feeding, infections and the development of autoimmunity in DS. The objectives are: To explore the views and opinions of healthcare professionals and support workers about their experience of working with parents of new babies with DS (babies under the age of 8 months). To explore the views and opinions of healthcare professionals and support workers about research in this population. To explore the views and opinions of healthcare professionals and support workers about potential barriers to families taking part in research and how these may be overcome. To explore the views and opinions of healthcare professionals and support workers about potential motivations to families taking part in research. To explore healthcare professionals and support workers experience of the FADES study and its acceptability for families that they work with. To explore the views and opinions of healthcare professionals and family support workers on how recruitment in the FADES study might be improved. To establish the feasibility of creating a national cohort of infants with DS to study the associations between early infant feeding, infections and the development of autoimmunity in DS
Study Configuration	The study will consist of approximately 15-20 interviews with community paediatricians, neonatologists, research nurses, other relevant healthcare professionals and family support workers from charities such as the Down's Syndrome Association and Down's Syndrome Scotland. Each participant will undergo one telephone interview lasting for approximately an hour. Interviews will take place over a 6 week period, at a time convenient to the participant.
Sample size estimate	It is anticipated that approximately 15-20 healthcare professionals and support workers who work with parents of new babies with DS will be interviewed

Number of participants	It is anticipated that a total of 15-20 interviews will take place.
Eligibility criteria	• Community paediatricians, neonatologists, research nurses and other relevant healthcare professionals with experience in working with parents of new babies with Down's syndrome. And/or • Family support workers from charities such as the DSA and DSS with experience in working with parents of new babies with DS.
Description of interventions	Community paediatricians, neonatologists, research nurses, other relevant healthcare professionals and family support workers from charities such as the DSA and DSS with experience in working with parents of new babies with DS will be invited to take part in a telephone interview with the researcher at a time that would be convenient to them. The telephone interviews will last about an hour and will be digitally audio recorded. Interviews will be conducted individually, with one researcher and one participant. The interviews will focus on a range of aspects relating to their experience of working with parents with a new baby with DS. We want to explore their overall experience and explore the pressures that families are under at this time and the concerns that parents have. We want to explore the opinion and views of the health care professionals and support workers on the acceptability to families of taking part in research during the first few months with their baby. We want to find out if they are aware of potential barriers to families taking part in research and how these may be overcome and also what might motivate parents to want to engage in research. We also want to explore their opinions on the FADES study, specifically its acceptability for families and how recruitment might be improved. We will provide participants with copies of the FADES flyer, protocol and participant information sheet prior to the telephone interviews.
Duration of study	Intended Start date: September 2015 It is anticipated that the interviews will take place over a 6 week period, thus recruitment and data collection will run until November 2015.

	Following recruitment, ie. asking to participate and providing consent, participants will only be involved for the duration of their interview. This is expected to last about one hour. Some participants may already be local collaborators for the FADES study and would therefore continue in this role after the interviews have taken place.
Randomisation and blinding	Randomisation will not take place. Healthcare professionals and support workers will be asked to participate in the research following a purposive sampling strategy.
Outcome measures	As qualitative research, there will not be quantifiable outcomes. The key outcomes of the research are to understand the barriers and possible motivations for families with a young baby with DS taking part in research.
Statistical methods	Qualitative data will be collected during this research, and statistical analysis will therefore not be required. Interview data will be digitally audio recorded, transcribed and analysed thematically using a framework approach.

STUDY BACKGROUND INFORMATION AND RATIONALE

We aim to create a cohort of infants with DS recruited through the Down's Syndrome Association (DSA), Down's Syndrome Scotland (DSS), community paediatricians and neonatologists to study the association between early infant feeding, infections and the development of autoimmunity. We initially anticipated that we would recruit 100 participants per year, recruiting over 2 years but initial recruitment has been slow.

Children with Down's Syndrome (DS) have increased risk of autoimmune conditions where the body's immune system attacks its own cells, such as thyroid problems, diabetes and coeliac disease (which causes malabsorption). In DS, autoimmunity is likely to be related to lifelong inherent defects in the immune system. The increased risk of diabetes-related autoimmunity is despite a reduced prevalence of the usual HLA haplotypes commonly associated with type 1 diabetes. Infant feeding practice has been linked to diabetes and coeliac risk with some evidence that prolonged breastfeeding is protective. We hypothesise that in infants with DS, already at increased risk, early feeding practices may be related to the development of autoimmunity. Children with DS are more floppy and therefore have difficulties with breastfeeding leading to the rapid introduction of formula feeds which contain modified cow's milk protein.

Parents are asked to complete questionnaires at baseline detailing family history, birth history, weight, medical problems and early feeding. They have further feeding questionnaires to complete at 7 months and 12 months, and medical questionnaires annually until the age of 5 years. Samples are collected at baseline including faeces to store from mother and baby, a brushing from the infants cheek for genotyping (looking at their DNA), a blood sample from the baby to look at development of auto-antibody production (antibodies which act against their own cells), and a urine specimen to detect development of diabetes. Further stool, urine and blood samples are collected at 6 and 12 months and once a year thereafter until 5years of age.

This study is a feasibility study and therefore we need to understand whether recruiting families and asking them to participate in such a study is acceptable and feasible. The proposed research will use the experiences of those that work with these families; community paediatricians, neonatologists, and family support workers from the DSA and DSS to understand the potential barriers and motivations for families to take part in research shortly after their baby has been born[1, 2]. We want to explore the pressures that families are under at this time and the concerns that parents have[3, 4]. We want to explore the opinion and views of the health care professionals and support workers on the acceptability to families of taking part in research during the first few months with their baby. We want to find out if they are aware of potential barriers to families taking part in research and how these may be overcome and also what might motivate parents to want to engage in research.

We would also like their opinion on the FADES study its acceptability for families and how recruitment might be improved. We will provide participants with copies of the FADES flyer, protocol and participant information sheet prior to the telephone interviews

The primary aim is to establish the feasibility of creating a national cohort of infants with DS to study the associations between early infant feeding, infections and the development of autoimmunity in DS.

STUDY OBJECTIVES AND PURPOSE

PURPOSE

PRIMARY OBJECTIVES

The primary objectives are:

To explore the views and opinions of healthcare professionals and family support workers about their experience of working with parents of new babies with DS (babies under the age of 8 months).

- To explore the views and opinions of healthcare professionals and family support workers about research in this population.
- To explore the views and opinions of healthcare professionals and family support workers about potential barriers to families taking part in research and how these may be overcome.
- To explore the views and opinions of healthcare professionals and family support workers about potential motivations to families taking part in research.
- To explore healthcare professionals and family support workers experience of the FADES study and its acceptability for families that they work with.
- To explore the views and opinions of healthcare professionals and family support workers on how recruitment in the FADES study might be improved.
- To establish the feasibility of creating a national cohort of infants with DS to study the associations between early infant feeding, infections and the development of autoimmunity in DS

STUDY DESIGN

STUDY CONFIGURATION

The study will consist of approximately 15-20 telephone interviews with community paediatricians, neonatologists, research nurses, other relevant healthcare professionals and family support workers from charities such as the Down's Syndrome Association and Downs Syndrome Scotland who have experience in working with these families. Each participant will undergo one interview lasting for approximately an hour. Interviews will take place over a 6 week period at a time that will be convenient to the participant.

Primary endpoint

As qualitative research, there will not be quantifiable outcomes. The key endpoint of the research is to have gained an understanding of the barriers and motivations for families with new babies with Down's syndrome to taking part in research. The key outcomes of the research are to use this knowledge to establish the acceptability and feasibility of FADES.

RANDOMIZATION AND BLINDING

Randomisation will not take place. Healthcare professionals and support workers will be asked to participate in the research following a purposive sampling strategy.

As this is not an intervention study, blinding is not necessary.

STUDY MANAGEMENT

The Chief Investigator has overall responsibility for the study and shall oversee all study management. There is not a Trial Steering Committee for this research, as this is a small qualitative study.

The data custodian will be the Chief Investigator.

DURATION OF THE STUDY AND PARTICIPANT INVOLVEMENT

It is anticipated that the interviews will take place over a 6 week period, thus recruitment and data collection will run until November 2015.

Following recruitment, ie asking to participate and providing consent, participants will only be involved for the duration of their interview. This is expected to last about an hour.

Some participants may already be local collaborators for the FADES study and would therefore continue in this role after the interviews have taken place.

End of the study

The end of the study for each participant will be following their interview. Following recruitment, the participants will only speak with the researcher on one occasion; for the interview. Those who are already local collaborators for the FADES study will continue in this role after the interviews have taken place.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

The target population will be community paediatricians, neonatologists, other relevant healthcare professionals and family support workers from charities such as the Down's Syndrome Association and Down's Syndrome Scotland who have experience in working with families of babies with Down's syndrome in the first few months of life. Participants will be recruited through the local collaborators that we already have helping with the study, through the Down's Syndrome Medical Interest Group and through contacts with the charities including the DSA and DSS. We will email participant information sheets to our local collaborators and to members of the DSMIG and to family support workers through the charities to find out who would be interested in taking part. We will then send out consent forms to those that have expressed an interest.

Participants will be recruited using a purposive sampling method.

Consent forms and information sheets will not be available printed in other languages. Participants must have a sufficient understanding of the English language to participate.

It will be explained to the potential participant that participating in the research is entirely voluntary and that all data will be anonymised and treated as confidential. They can withdraw at any time but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

Removal of participants from the research

Participants would be removed from the study if they request to withdraw their participation. However if a participant indicates a wish to withdraw attempts will be made to persuade the participant to at least permit primary outcome data to be collected, ensuring that enough data are recorded to support the planned analysis. Participants will not be accepted as lost to follow-up unless we receive no response from phone calls, emails and letters. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Informed consent

After interested potential participants have been identified information about the research will be provided by the researcher, in the form of the Information Sheet and any questions the potential participants have about the research will be answered.

Potential participants will be asked whether they are happy to provide a contact telephone number for the researcher. Their name and telephone number will be stored within the Bristol Nutrition BRU in a locked file and will not leave the Bristol Nutrition BRU. The researcher will ask whether the potential participant is interested in participating in the research, and if they are, a date for the interview will be set. When the researcher calls the participant on the date of the interview, the information sheet and consent form will be discussed in detail and signed. Again, participants will be informed that participation is voluntary, they can withdraw at any time and that all data will be anonymised and treated as confidential.

If the potential participant states that he does not want to participate in the research when they are called by the researcher, then their name and telephone number will be securely destroyed.

Consent will be taken over the phone, the researcher will discuss the information sheet (which will have been emailed or posted to the potential participant) with the participant over the phone and ask if they have any queries. Once they have had an opportunity to ask any questions if they are happy to take part the researcher will complete the consent form and consent will be recorded. (Consent over the phone has been used in previous qualitative studies NRES reference 13/NW/0228)

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the research, continuing consent will be obtained using an amended consent form which will be signed by the participant.

STUDY TREATMENT AND REGIMEN

Once a participant has orally agreed to participate, an interview date will be arranged between the individual and the researcher. We will email and/or post copies of the FADES protocol, FADES flyer and FADES participant information sheet to the participant for them to view prior to the telephone interview as these will be discussed.

The researcher will conduct the interview in a quiet, private location, where they will not be interrupted.

The interview will last for approximately an hour, and will be digitally audio recorded. Recordings will be transcribed using a University of Bristol approved transcription service who have signed a data protection agreement. All names and identifying information will be removed from the transcripts upon transcription. An ID will be allocated in order to identify whether the participant is a community paediatrician, neonatologist, another healthcare professional or a family support worker. Interviews will be conducted individually, with one researcher and one participant.

Interviews will be semi-structured, and will cover the following topics. The interviews will focus on a range of aspects relating to their experience of working with new parents of children with Down's syndrome. We want to find out their opinions on the barriers that these parents might have to taking part in research and also the possible motivations they may have. We also want to find out their opinion on the acceptability of the FADES study having viewed the flyer, protocol and participant information sheet. We want to know if they have any ideas or suggestions for improving recruitment.

Criteria for terminating research

There are no anticipated criteria for terminating the study.

ANAYLSIS AND STATISTICS

Qualitative data will be collected during this research, and statistical analysis will therefore not be required. Interviews will be digitally audio-taped and transcribed verbatim. Thematic analysis of the interview data will be conducted using a qualitative method, such as the Framework Approach (Ritchie and Spencer, 2002). Individuals will not be identified by name in the written transcript. A qualitative analysis computer program, such as NVivo (NVivo10, QSR International, 2012) will be used to assist with the analysis.

ADVERSE EVENTS

The research consists of participant interviews, following a standardised interview procedure. It is not anticipated that any adverse events will occur as a result of this research. However in the occurrence of adverse events, the event will be reported immediately to the Chief Investigator and necessary advisory boards. The event will be investigated, resolved where appropriate and a report written.

Participant removal from the study due to adverse events

Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator.

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The research will not be initiated before the protocol, informed consent forms and participant information sheets have received approval from the Research Ethics Committee (REC). Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant information sheets have been reviewed and received approval from the REC. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The research will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical

Practice, and the Department of Health Research Governance Framework for Health and Social care, 2005.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. A copy of the participant information sheet and consent form will be emailed or sent to the potential participant. Consent will be taken over the phone, the researcher will discuss the information sheet with the participant over the phone and ask if they have any queries. Once they have had an opportunity to ask any questions if they are happy to take part the researcher will complete the consent form and record consent.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty. No research-specific data collection will be carried out before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they

wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

RECORDS

Case Report Forms

Each participant will be assigned a unique identity code number, for use on Case Report Forms (CRFs), other research documents and the electronic database. The unique identity code number will consist of a CP for a community paediatrician, NT for a Neonatologist, RN for research nurse or an SW for a family support worker and for other health care professionals similar code numbers will be created. A unique letter ie. A is the first participant, B is the second, C is the third, within the community paediatrician, neonatologist or support worker's interviews and a number eg. 001 for the first participant, 002 for the second, 003 for the third. Examples of the unique identity code include CP_A_001, NT_E_005, SW_G_007. Each participant will additionally be assigned a pseudo-name, for the purpose of transcription and analysis. This will correspond to the unique letter in their identity code, it will also correspond to their gender. For example, in the three codes above, potential pseudo-names could

be Andrew, a community paediatrician (CP_A_001), Emma, a neonatologist (PA_E_005), Gemma, a support worker (ST_G_007). This system will ensure anonymity for the participants throughout analysis and dissemination of research findings.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, contact details, and unique identity code number, to permit identification of all participants enrolled in the study, in accordance with regulatory requirements.

CRFs shall be restricted to those personnel approved by the Chief Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms and audio tapes. A CRF may also completely serve as its own source data. Only research staff shall have access to research documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF, and all source documents, shall be made available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities.

DATA PROTECTION

All study staff and investigators will endeavour to protect the rights of the research participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998. The CRF will only collect the minimum required information for the purposes of the research. CRFs will be held securely, in a locked room, and locked cabinet. Access to the information will be limited to the study staff and investigators and relevant regulatory authorities (see above).

Computer held data including the study database and audio tapes will be held securely and password protected. Access will be restricted by user identifiers and

passwords (encrypted using a one way encryption method) Electronic data will be backed up regularly to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

The University of Bristol has arranged Public Liability insurance to cover the legal liability of the University as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University.

The University of Bristol holds Professional Negligence insurance to cover the legal liability of the University, for harm to participants arising from the design of the research, where the research protocol was designed by the University.

The University of Bristol's Public Liability insurance policy provides an indemnity to our employees for their potential liability for harm to participants during the conduct of the research.

RESEARCH CONDUCT

Research conduct will be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the research; Trial Delegation Log; CVs of research staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; accountability of research materials and equipment calibration logs.

RESEARCH DATA

Monitoring of research data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Research Coordinator, or where required, a nominated designee, shall carry out monitoring of research data as an ongoing activity.

Research data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Bristol Research Code of Conduct, the Chief Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The research documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Bristol. This archive shall include all research databases and associated meta-data encryption codes.

DISCONTINUATION OF THE RESEARCH BY THE SPONSOR

The Sponsor reserves the right to discontinue this research at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons.

STATEMENT OF CONFIDENTIALITY

Participant confidentiality will be further ensured by utilising identification code numbers to correspond to data in the computer files.

Data generated as a result of this study will be available for inspection on request by the University of Bristol representatives, the REC, and regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

Data collected during this research may contribute to peer reviewed publications in journals and presentations at conferences. All data will be anonymised, and participants will not be identified in any publications, reports or future grant applications.

USER AND PUBLIC INVOLVEMENT

User and public involvement has not been included in the design of the research to date, as there has yet to be an opportunity for this to occur.

STUDY FINANCES

Funding source

NIHR Bristol Nutritional Biomedical Research Unit, University of Bristol. Participants will not be paid to participate in the research.

SIGNATURES

Signatories to Protocol:

Chief Investigator: (name)_____

Signature:_____

Date: _____

References

1. Hoehn, K.S., et al., *What factors are important to parents making decisions about neonatal research?* Archives of Disease in Childhood-Fetal and Neonatal Edition, 2005. **90**(3): p. F267-FF269.
2. Zupancic, J.A., et al., *Determinants of parental authorization for involvement of newborn infants in clinical trials.* Pediatrics, 1997. **99**(1): p. e6-e6.
3. Van Riper, M., *Families of children with Down syndrome: responding to "a change in plans" with resilience.* J Pediatr Nurs, 2007. **22**(2): p. 116-28.

4. Brasington, C.K., *What I wish I knew then... reflections from personal experiences in counseling about Down syndrome.* Journal of Genetic Counseling, 2007. **16**(6): p. 731-734.

Appendix 5: Qualitative Study Topic Guide

NIHR BRISTOL NUTRITION BIOMEDICAL RESEARCH UNIT

Questions / Topic Guide for Professionals Telephone Interviews

Intro statement

Hi I'm Georgina, I'm a paediatric registrar currently doing a clinical PhD looking at how early life influences may affect the development of Autoimmune conditions in children with Down's syndrome.

Thank you for talking to me I hope that from these telephone interviews I will get a better understanding of the motivations and barriers for new parents of babies with Down's syndrome to taking part in research and particularly in the FADES study. Children with Down's syndrome are a minority group in whom little research has been conducted, understanding why recruitment in this group is challenging will be important for anyone undertaking research in Down's syndrome in the future. I am conducting these interviews with community paediatricians, support workers, neonatologists, research nurses and midwives.

This will be a general conversation about families with children with Down's syndrome and research as well as some more specific questions in relation to the FADES study (protocol, flyer and participant information sheet).

The interview should take about an hour - is now a good time for you? If you are pressed for time please let me know and I can adjust some of the questions accordingly. (grey questions are omitted in shortened version)

Have you had the opportunity to read the participant information sheet?

Do you have any questions or anything you would like me to clarify?

I am now going to take consent, I will read through the consent form please let me know if you are happy for me to sign the consent form on your behalf? – Read through consent form and take consent.

Do you have any questions before we start?

We will be putting together a report based on what is said in the telephone interviews and to make this possible we would like to record what is said. We will of course treat this discussion as confidential, and will not use your real names anywhere in the transcripts or report. So I will just turn on the recorder on if that's OK and then I will ask you again and record your answer….

(TURN ON RECORDER)

Are you happy for the phone call to be recorded? (pause) do you consent to taking part in the study?

Thank you.

Opening

If you don't mind I will start with some questions about your background

1. Please could you tell me a bit about your job?

2. What are your particular areas of interest/ specialism?

3. Tell me about your experience of working with families with Down's syndrome?
   - Have you had any particular training in working with families with Down's syndrome?
   - How long have you been working with children with Down's syndrome and their families?

4. How many families with a child with Down's syndrome do you see each year?

5. What are your first impressions when you meet a parent of a new baby with Down's syndrome? – do you find for example that they are in usually in shock or coping well are they information seeking or in denial?

6. What do your interactions with these families involve?

7. How often would you see / follow up these families?

Research specific questions

8. Do you have any experience of participating in research with children with Down's syndrome?
    - Please tell me about your experience of recruiting families.

9. What do you think are the barriers for these families to participating in research in general?

10. What motivations might families with Down's syndrome have to taking part in research in general?

11. How do you think parent's experiences of when they first receive the diagnosis of Down's syndrome might affect their engagement in research or interaction with the medical profession or researchers?
    - Does this change over time?

We are trying to recruit families as close to birth as possible our maximum age for recruitment is 8 months

12. Some parents may need time to adjust to the diagnosis that they have received, sometimes referred to as a period of "adaptation" – what is your experience of this?
    - when do you think parents might be the most amenable / accepting of the idea of joining a study?

13. What would be the best ways do you think of letting new parents of a child with DS know about research and how to get involved?
    - Who should do the recruiting?

- What methods should be used for example face to face / invitation letters ?

Thinking about barriers to research….

14. What pressures are families under at this time in these first few months?
    - Do they have multiple appointments to attend? – how do you think this might effect their participation?
    - Do they talk to you about other family pressures that may exist – siblings, marital relationships? – how do you think this might effect their participation? - (feels a bit leading?)
    - Do they have many "hoops" to go through for example claiming disability benefits/ support? – how do you think this might effect their participation?
    - Do you feel these parents feel under a "burden of care"? – how do you think this might effect their participation?

15. What sort of support do you find families are looking for? How might this relate to being part of a study?

16. Are parents particularly protective of their baby with DS, more so than other parents during the newborn period?

    - Do you think this effects their participation in research?

Thinking about motivations to research….

17. Have you found that many parents become "advocates" for Down's syndrome?

- Do you think they feel a responsibility towards other families and children with Down's syndrome?
- Does this change over time?
- Are these families often looking for a "voice"?

FADES specific questions

18. How have you heard about the FADES study and how aware of it have you been?
    - Have any families spoken to you about the FADES study without you mentioning it to them? (if yes) please can you tell me what they said or why they were talking about it.
    - Have any colleagues spoken to you about the FADES study without you mentioning it to them? (if yes) please can you tell me what they said or why they were talking about it.

19. *Have you had an opportunity to look at the FADES protocol, flyer and participant information sheet?*

20. If you were a parent why might you join this study?

21. What might stop you from joining this study?

This study is looking at early life influences particularly feeding and how this might affect the development of autoimmunity

22. What do you think are parent's early experiences of feeding their newborn baby with DS? Do they have many concerns about feeding their baby?

23. Some parents may feel that they would like, or would have liked increased support with feeding, is this something you hear from the parents that you meet?

24. How do you think parents will view this area of research?

25. In your opinion, would this motivate parents to take part in the FADES study or be a barrier?

26. What did you think about the FADES flyer? Do you think new families receiving information about Down's syndrome would be engaged by seeing this flyer?

27. Was there anything in the participant information sheet for parents that might concern families considering taking part in the study?

The study involves participants completing questionnaires as close to birth as possible, at 6 months, 12months and yearly thereafter.

28. How do you think these families would feel about completing these questionnaires – what might encourage / motivate them? What might put them off?

We also ask parents to collect a mouth swab sample, urine sample and stool sample close to birth. We as them for further stool sample at 6 months and a urine and stool sample at 12 months and yearly thereafter until the age of 5 years. These samples are collected at home by the families and posted back to us in prepaid packaging.

29. How do you think these families would feel about collecting these samples – what might encourage / motivate them? What might put them off?

We also ask for a blood sample close to birth at 6 months, 12months and yearly thereafter until the age of 5 years. We explain that they should not need any additional appointments in order to have these bloods collected that they can be done at the same time as their routine appointments

30. How do you think these families would feel about collecting these samples – what might encourage / motivate them? What might put them off?

31. Do you have any specific comments about anything else that you saw in the protocol that might affect recruitment to the study

32. Do you have any comments about your experience of the FADES study?

Closing

Summarise back to them

Thank you so much for the time you have taken for this interview and I would just like to reiterate that the recording is confidential and will be anonymised.

Is there anything else you think would be helpful for me to know so that I can try and increase recruitment for the FADES study and establish the feasibility of conducting research in babies with Down's syndrome.

Appendix 6: REC Queries

Query and comments from ethics committee	**Changes made in response**
How would inclusion criteria be assessed?	• Within the GP letter a paragraph was added to say "Children are excluded from the study if they are no longer with their birth mother or are subject to a child protection plan. We would be grateful if you could inform us if you become aware that this child is no longer eligible for this study" • The question "Are you the birth mother of this child?" was added to the consent form
The PIS should be amended to emphasise that samples could be taken by health professionals at routine appointments, but that if parents wished to they could be shown how to take the samples themselves at home.	• The wording was changed to make it clear that samples could be taken at routine appointments but that there is an option for collecting samples at home as well.
The PIS should be amended to reflect the burdensome nature of the study - An estimate of	• The estimated time to complete each of the questionnaires and a table of study procedures was added to the PIS.

the time to complete each questionnaire should be included in the PIS	• It was highlighted that under the heading "what are the possible disadvantages and risks of taking part" on the PIS that there was a comment "We do understand that this is a considerable commitment in terms of being part of an on-going study as you will have additional papers and samples to be collected from your child that families with a child with Down's syndrome do not normally have to do"
Clarification on whether the storage and use of data and samples in future research is optional	• The PIS was changed to explain that the storage and use of data and samples in future research is optional and [Yes] and [No] boxes were added to the relevant items on the Consent Forms for participants to indicate their agreement as appropriate.
It was requested that a section be added to the PIS to explain how the samples would be analysed and what would happen to them at the end of the study	• A section was added "what will happen to any samples provided?" it did not contain detailed explanation of the biochemical analytical methods, but it was explained that the study team would be happy to answer any questions parents had on the analysis prior to consent. • "What will happen to samples at the end of the study?" was answered in other sections on the PIS.

The PIS for the stool collection feasibility (Phase 1 study) should be amended to explain why three samples are collected and the committee suggested an online instruction video be produced that could be viewed by participants.	• The PIS was amended to explain the need for three samples. Genotek the company who produced the stool collection kit were emailed to see if they had an instruction video, but they did not. However a researcher would be present and part of the study was to test it's ease of use. • For the main study (Phase 2) the instructions for collecting stool samples were simplified following discussion with the lab conducting the sample collection for the TEDDY study.
It was requested that the PIS be amended to include a section on insurance arrangements for the Study	• It was explained that the study was a low risk study and that it was not anticipated that any harm would come to participants. • A new section "What if something goes wrong?" was added to the PISs
It was requested that the questionnaires should be proof read to ensure the branching and order of the questions was correct.	• The questionnaires were renumbered and simplified. • Dr Sam Leary statistician proof read the questionnaires and checked them again once they were online.

Appendix 7: Confirmation of ethical approval

NRES Committee South West - Central Bristol

Bristol Research Ethics Committee Centre
Whitefriars
Level 3, Block B
Lewin's Mead
Bristol BS1 2NT
Email: nrescommittee.southwest-bristol@nhs.net

Telephone:
0117 342 1335 Facsimile: 0117 342 0445 23 April 2014

Dr Georgina Williams

PhD Studentship / Clinical Research Fellow

Bristol Nutrition BRU

Level 3, University Hospitals Bristol Education & Research Centre

Upper Maudlin Street

Bristol BS2 8AE

Dear Dr Williams

Study title:	**Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)**
REC reference:	**14/SW/0030**
Protocol number:	**2103**

IRAS project ID: **130663**

Thank you for your letter of 24 March 2014, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager Mrs Naazneen Nathoo, nrescommittee.southwest-bristol@nhs.net.

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Advertisement	FADES Flyer v.1	18 November 2013
Advertisement	Pilot Study Flyer v.1	18 November 2013
Covering Letter		30 January 2014
Evidence of insurance or indemnity	CT1704 insureconfirm.doc	03 December 2013
GP/Consultant Information Sheets	Factsheet for Health Professional v.1	22 January 2014
GP/Consultant Information Sheets	2	28 March 2014
Investigator CV	(Academic Supervisor - Kathleen Gillespie)	01 January 2014
Investigator CV	(Academic Supervisor - Sam Leary)	06 February 2014
Investigator CV	(Chief Investigator - Dr Georgina Williams)	28 January 2014
Investigator CV	(Academic Supervisor - Julian Shield)	02 December 2013
Other: Organisational Approval from DSS	1	10 January 2014
Other: Organisational Approval from DSA	1	10 January 2014
Other: Parent Contact	1	06 March 2014

Other: Generic instructions	2	25 March 2014
Participant Consent Form	2. Pilot	28 March 2014
Participant Consent Form	2	28 March 2014
Participant Information Sheet	2	24 March 2014
Participant Information Sheet	2 Pilot study (stool collection)	28 March 2014
Protocol	FADES v.1	20 January 2014
Questionnaire: Initial	2	25 March 2014
Questionnaire: Annual medical qn.	2	25 March 2014
Questionnaire: 12mo feeding qn.	2	25 March 2014
Questionnaire: 7 mo feeding Qn	2	25 March 2014
REC application		24 January 2014
Response to Request for Further Information	covering ltr	24 March 2014
Summary/Synopsis	Instructions for collecting Blood Samples	

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

14/SW/0030	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Pamela Cairns Chair

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Dr Birgit Whitman*

Diana Benton, University Hospitals Bristol NHS Foundation Trust

Appendix 8: Scientific Review

NIHR Biomedical Research Unit in Nutrition, Diet and Lifestyle
Level 3, University Hospitals Bristol Education Centre
Upper Maudlin Street
Bristol
BS2 8AE

e: georgina.williams@bristol.ac.uk

t: 0117 342 1754

Dear FADES Research Team,

Study Title: **Feeding and Autoimmunity in Down's Syndrome Evaluation Study (FADES)**

The FADES study was scientifically reviewed by the Professor Andy Ness and Professor Richard Martin on the 1st May 2013. As part of the scientific review of the study the following aspects were considered:

Originality and importance of the work
Scientific Reliability
Is the research question is clearly defined and answered?
Overall design of the study
Are the participants adequately described and conditions defined?
Are the methods adequately defined?

The study has been approved.

Yours sincerely

Professor Andy Ness Professor Richard Martin

Appendix 9: DSA Organisational approval

Prof JP Hamilton Shield

Professor of Diabetes and Metabolic Endocrinology

University Hospital

Bristol 21st March 2013

Dear Prof Shield

Feeding and Autoimmunity in Down's syndrome – Evaluation Study (FADES)

I am writing to confirm that the Down's Syndrome Association is delighted to be able to collaborate as part of the research study named above.

We agree to send out the initial information about the study to families as they contact the DSA with our ‘new parent packs’, and we also agree to approve content of information sent out to those families.

This is a really important study and we will do whatever we can to help.

Yours sincerely

Carol Boys

Chief Executive

Appendix 10: DSS Organisational Approval

Down's Syndrome Scotland
helping people realise their potential

Dr Georgina Williams
Clinical Research Fellow
NIHR Biomedical Research Unit in Nutrition, Diet and Lifestyle
Level 3, University Hospitals Bristol Education Centre
Upper Maudlin Street
Bristol
BS2 8AE

10th January 2014

RE: FADES Study

Dear Georgina

Thank you for the information regarding your proposed research project involving children with Down's syndrome. Having read this information on the expected outcomes of the research project, we would be happy to support the research.

Our support would involve the advertising of the research project to families through our media such as our website, ebulletin, through our membership mailing lists and through the direct contact with families our Family Support Service. Afterwards we would be happy to include an article written by you summarising the outcomes of the project for our bi annual newsletter, should this be desirable.

I hope this is sufficient and good luck with your ethics approval. If in the meantime I can of any further assistance please do not hesitate to get back in touch.

Kind Regards

[redacted]

Sarah Van Putten
Family Support Service Manager/Depute CEO

Appendix 11: FADES Flyer

Would you like to take part in our study?

Feeding and Autoimmunity in Down's Syndrome Evaluation Study

George Armour

What is the study about? The study will investigate early feeding in babies and children with Down's Syndrome (DS).

Who can take part? We are looking for new parents who have a baby under the age of 8 months.

How will this benefit children with Down's syndrome? We hope the study will help us understand why children with Down's syndrome are more likely to experience problems with their hormones and their gut, help reduce this risk and lead to the development of new treatments to help with feeding.

What will it involve? We will be asking parents to complete a questionnaire about their child's feeding and health as a young baby and at 6 and 12 months. We will also ask about the child's health yearly after this until the age of 5 years old.

We would need to collect some samples from your baby soon after birth, at six and twelve months and yearly thereafter if possible until the age of 5 years. All questionnaires can be completed online (or paper versions if preferred) at home, and apart from the initial blood sample all samples can be taken at home, or during your baby's routine health checks. You would not need any additional hospital attendances. We will provide pre-paid packaging so that all samples and questionnaires can be sent back to Bristol.

The Study Team: Professor J Hamilton-Shield, Dr Kathleen Gillespie, and Dr Georgina Williams at University of Bristol and University Hospitals Bristol NHS Foundation Trust. We are collaborating with the Down's Syndrome Association, Down's Syndrome Scotland, Down's Syndrome Ireland and Professor E Molloy Trinity College Dublin.

What next? If you would like to take part in this study, or just want to know more please contact us:

EMAIL: fades-study@bristol.ac.uk

There is also our website: **www.bristolnutritionbru.org.uk. Click on the 'FADES Study' tab.**

Here you can register your interest and also download an information sheet.

NIHR BRISTOL BIOMEDICAL RESEARCH CENTRE
NUTRITION THEME

Study Information Sheet

Study title: Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)

Dear Parent(s)

Congratulations on the birth of your baby!

Thank you for showing interest in our research study, and for asking for more information.

We would like to follow up your baby's early life looking at any feeding problems you might encounter, any infections your child has and whether these are associated with the development of conditions more common in children with Down's syndrome.

What is the purpose of the study?

This study will look at early feeding in babies and children with Down's Syndrome and see how feeding and early infections may be related to the increased risk children with Down's

syndrome have of developing thyroid, coeliac disease (which causes gut problems) and diabetes. These are all known as autoimmune conditions.

Do we have to take part in this study?

The simple answer is no. If you do not wish to take part, it will in no way affect the care you and your child receives from any of your doctors.

What will I be asked to do if I take part?

You will be asked to fill in a questionnaire online (or a paper version depending on your preference) which mainly asks questions about your baby's birth history, medical history and early feeding. It also contains some questions about your family history and briefly about your medical history. This initial questionnaire will take around 30 minutes to complete. There are further questionnaires about your baby's medical history and feeding at six and twelve months these are shorter and will take around 20 minutes to complete. We will then send out an annual questionnaire each year (until January 2022) this is a short questionnaire about your child's health, which takes about 10 minutes to complete.

We would also like to collect a sample of stool (poo) from you (the mother) and from your baby, as well as urine from your baby at six and twelve months and yearly thereafter if possible. We will also ask you to use a special tiny brush, to brush the inside of your baby's cheek. All of these samples can all be collected at home.

Every baby born in the UK has a small sample of blood taken from a heel prick as part of their new born screening and with your permission we will also analyse this spot of blood.

Finally we would like to collect small samples of blood from your child using a special heel/finger prick sample collector which causes minimal discomfort. The first sample we will arrange to be done during one of your baby's routine health checks. We would also like to collect bloods samples at six and twelve months and yearly thereafter we will advise you on how these can be taken at your child's routine appointments with health care professionals, when your child would normally be having routine screening bloods. If however you would prefer to take these samples yourself at home once you have seen the first sample being taken we will advise you on how you could do this. All the samples can then be sent with any paper questionnaires back to Bristol in the supplied pre-paid packages. ***Your child should not need additional hospital attendances.***

We understand that this study involves considerable time and input from participants and if for any reason, you miss a sample/questionnaire we would still like your child to continue in the study as your contribution will still be important.

Below is a chart showing the timeline for questionnaires and sample collection for your baby.

Months of age	**0** **Baseline**	**7**	**12**	**Every year around the time of your child's birthday until January 2022**

Combined feeding and medical questionnaire	**X**	**X**	**X**	
Medical questinnaire				**X**
Mouth swab	**X**			
Stool (poo) sample	**X**	**X**	**X**	**X**
Urine sample	**X**		**X**	**X**
Heel or finger prick blood sample.	**X**	**X**	**X**	**X**

The study is currently running until 2022 we will therefore be asking you to complete questionnaires and provide samples around the time of your child's birthday until 1st January 2022.

What will happen to the samples that are provided?

We will be analysing your child's samples to see if we can find any common findings which might explain why babies with Down's Syndrome might develop autoimmune conditions. The mouth swab will be used to look at your baby's genes (little packets of information within your cells) particularly those that we know may be associated with autoimmune conditions. We will look at your stool and your baby's stool (poo) to see the natural bacteria that live within the gut. The urine sample will help us to know which babies may be developing diabetes although we expect that very few babies will develop diabetes during the period that

they are in the study. The blood samples will be used to look for antibodies which are associated with autoimmune conditions.

What are the possible disadvantages and risks of taking part?

We do not believe there to be any risks. Your child might find the heel / finger prick tests a little uncomfortable but it only lasts a minute or so and there will only be seven of these over five years. We do understand that this is a considerable commitment in terms of being part of an on-going study as you will have additional papers to fill in and samples to be collected from your child that families with a child with Down's syndrome do not normally have to do.

What are the possible benefits in taking part?

This study is designed to try and find out what difficulties babies and children with Down's Syndrome have with feeding and infections. We want to reduce the risk of these problems and develop new treatments to help with feeding. We also hope we will increase our knowledge of why children with Down's syndrome are at increased risk of developing problems with their hormones and gut known as "autoimmune conditions". These include thyroid or coeliac disease and diabetes.

Whilst our findings may not directly help your baby they will hopefully benefit children born with Down's Syndrome in the future. From the findings of this study we hope that we will be able to develop an intervention to help with feeding babies born with Down's Syndrome. We

also hope the study will enable us to provide parents and carers of children with Down's syndrome with more information regarding feeding and autoimmune conditions.

Will details on my child in this study be kept confidential?

All data collected in this study will be maintained and stored in strict accordance with the data protection regulations. All information that is collected about you and your child during the course of the research will be kept strictly confidential. All members of the study team will have a duty of confidentiality to you and your child as research participants.

We keep the information we collect about your child and the results from any samples collected separate from your child's personal details and we can only link this information together with a secure code. Only authorised people working on the study will have access to your child's information.

With your consent we will inform your child's GP and community paediatrician that your child is participating in the study and will send them a factsheet explaining what will be involved, we will also inform your GP. We will also contact key members of your child's health care team with your consent to arrange blood sampling. They will not have access to any of the information that is collected about your child during the course of the research; we will only contact them if any of the results are significant for your child's current or future health.

What happens to the data and samples collected when this research study is finished?

We will keep the data and samples for 15years and then destroy them securely unless you have chosen to consent for the data and / or samples to be stored for use in future ethically approved studies in this area of research. If this is the case we will give you the option to be re-contacted in order that you can provide your consent again if you so wish to do so.

Who is organizing and funding the research?

The study is sponsored by the University of Bristol and is funded by the National Institute of Health Research, Bristol Biomedical Research Unit in Nutrition. The study is being conducted as part of a Clinical PhD in Child Health.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people called a research ethics committee to protect your safety, rights, well–being and dignity. This study has been reviewed and given a favourable opinion by the NRES committee (South West Central Bristol Research Ethics Committee). This study has also been reviewed by the executive board of the Bristol Biomedical Research Unit, the University of Bristol Research and Enterprise development team and the University Hospitals Bristol Research and Development team.

What if something goes wrong?

The researchers do not anticipate that taking part in this study could cause any harm to your child. In the eventuality that its negligence does cause injury to your child, the University holds

Public Liability insurance. If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (contact details below). If you remain unhappy and wish to complain formally, you can do this by contacting the hospitals Patient Support and Complaints Team (contact details below).

Will I be informed of the results of the study?

If you would like to be updated regarding the progress of the study we can arrange to send you regular updates via email. At the end of the study we will provide you with a summary of the findings and copies of any publications that you would like to receive.

What do I do now?

Thank you for considering taking part in this research. We will contact you either by email or telephone depending on your preference and will answer any questions you may have. Once you have had an opportunity to ask any questions, if you are interested in your child taking part, please fill in the consent forms which we will send to you in the post with a copy of this information sheet. Keep one copy for your own records and send the other back to us in the stamped addressed envelope provided.

If you have any questions regarding the study please call the FADES research team on +44 (0)117 342 1756 or email fades-study@bristol.ac.uk.

If you have concerns about any aspect of the way you have been approached or treated during the course of this study you may wish to contact the hospital's Patient Support and Complaints Team on 0117 342 3604, email pals@uhbristol.nhs.uk or write to Patient Support & Complaints Team, Trust Headquarters, University Hospitals Bristol, Marlborough Street, Bristol, BS1 3NU

Professor J Hamilton-Shield, Dr Kathleen Gillespie and Dr Georgina Williams

Bristol Biomedical Research CentreNutrition Theme

University Hospitals Bristol NHS Foundation Trust

Appendix 13: FADES Initial Questionnaire

NIHR BRISTOL BIOMEDICAL RESEARCH CENTRE
NUTRITION THEME

Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)

Initial Questionnaire

What is this questionnaire about?

Thank you for taking part in this study. This questionnaire asks about the birth of your baby, your baby's health, a little about the health of you and your family and your baby's feeding. (This is the longest of the questionnaires that you will fill in, the questionnaire at 6 months and 12 months will be much shorter).

How to fill in the paper questionnaire

1. Please fill in the questionnaire in black biro.

2. Most questions on the following pages can be answered simply by putting a cross in the box next to the answer that applies to you.

   Example:

   Yes ☒
   No ☐

   Sometimes you are asked to write in a number or the answer in your own words. Please enter numbers as figures rather than words.

3. Occasionally you may have more than one answer to a question. Please cross all the boxes next to the answers that apply to you if the instruction ***"Please cross one or more boxes"*** is printed.

4. Usually after answering each question you go on to the next one unless a box you have crossed has an arrow next to it with an instruction to go to another question.

   Example:

   Yes □ → Go to Q8
   No □

   By following the instructions carefully you will miss out questions which do not apply, so ***the questionnaire will be shorter than it looks***.

5. If you cannot remember, do not know, or are unable to answer a particular question please put a cross in the box marked "do not know" or when there is an option to insert text please write in that you don't know.

6. If you would like to give any further information on any of your answers you can write this in at the end of the survey.

7. If at any point you have any problems or difficulties with the questionnaire please do contact us and so that we can help you. (Contact details below)

8. When you have finished, please post the questionnaire to us as soon as possible in the pre-paid envelope provided, even if you were not able to complete all of it.

We are very grateful for your help.

Contact:

EMAIL: Georgina.Williams@bristol.ac.uk

Tel: +44 (0)117 342 1755

What is your identification number for this study? (Please do not put your name or your baby's name anywhere on this questionnaire)

If you no longer wish to take part in this study please cross the box below and return the questionnaire to us so we do not trouble you further.

☐

SECTION 1: The birth of your baby and your baby's health

First of all we would like to ask some general questions before finding out how you feed your baby.

Q1.1. How old is your baby?
Please write numbers in both boxes
Write in how many whole weeks plus any additional days:

□ and □
Weeks days

Q1.2. Thinking about the birth of your baby, what kind of delivery did you have?

Normal (vaginal) birth □
A caesarean (through a cut in the abdomen) □
Delivery using forceps □
Delivery using vacuum cap on the baby's head (ventouse) □

Q1.3. How many weeks pregnant were you when your baby was born?
Please write numbers in both boxes if you are able to
Write in how many whole weeks plus any additional days:
□ and □
Weeks days

Q1.4. How much did your baby weigh when he/she was born?

Either in pounds and ounces:
□ lb □ oz

Or in kilograms:
□ Kg

Q1.5 Where was your baby born?

In hospital – in a midwife-led unit □ → go to q1.6
In hospital – in a consultant-led unit □ → go to q1.6
In a midwife-led unit or birth centre separate from hospital □ → go to q1.6

At home □ → go to q1.7
Somewhere else ***(Please cross and write in where)*** □

Q1.6 How long after the baby was born did you stay in the hospital, birth centre or unit?
*Please enter number in **one box only**. Please give an estimate if you are not sure.*

Either:
How many **hours** did you spend in the hospital, birth centre or unit?
□hours
OR
How many **days** did you spend in the hospital, birth centre or unit?
□Days

Q1.7a Is this your first baby?

Yes □ → go to Q1.8
No □ → go to Q1.7b

Q1.7b Did you breastfeed any of your other children/child?

Yes □
No □

Q1.8 Is your baby one of twins, triplets or other multiple births?

No □
Yes, twin □
Yes, triplets or other multiple birth □

Q1.9 Did you know prior to your baby's birth that they had Down's Syndrome?

Yes □
No □

Q1.10a Does anyone else in your *immediate* family have Down's syndrome?

Yes □ → go to Q1.10b
No □ → go to Q1.11a

Q1.10b Who in your family has Down's syndrome? (you do not need to give names) please cross the box which corresponds with how are they related to your baby? Please cross one or more boxes.

Mother □
Father □
Sibling □
Grandparent □
Cousin □

Q1.11a While you were pregnant did you have any antenatal check-ups?

Yes □ → Go to Q1.11b
No □ → Go to Q1.12

Q1.11b At the check-ups did anyone discuss feeding your baby with you?

Yes □ → Go to Q1.11c
No □→ Go to Q1.12

Q1.11c. At the check-ups, who discussed feeding your baby with you?
Please cross one or more boxes

Doctor □
Health visitor □
Midwife □
Nurse □
Peer supporter (a mum who has breastfed their own baby and been trained to give support to other mums) / Volunteer □
Someone else (***Please cross and write in)*** □

Q1.12 During this pregnancy did you attend any sessions that included talks or discussions about feeding babies?

Yes □
No □

Q1.13a If your baby was diagnosed with Down's Syndrome before they were born, were you told about any specific difficulties related to feeding a baby with Down syndrome?

Yes □ → go to Q1.13b
No □ → go to Q1.14a

Q1.13b What were you told?

Yes □ → go to question 1.14b
No □ → go to question 1.15

Q1.14b Where did you get that advice from?

Midwife □
Down's Syndrome Association □
Down's syndrome Scotland □
GP □
Somewhere else, please specify (***Please cross and write in)*** □

We are now going to ask you about infections that either you had during your pregnancy, or your baby has had since birth. We also ask about whether these infections required antibiotics, this gives us an indication of the level, number and type of infections. We know that babies with Down's syndrome are more prone to getting certain infections.

Q1.15a During your pregnancy did you have any infections requiring antibiotics?

Yes □ → go to question 1.15b
No □ → go to question 1.16

Q1.15b During which stage of your pregnancy did you take antibiotics? Please cross one or more boxes if you required antibiotics during more than one stage of your pregnancy.

In the first trimester (first 3 months of pregnancy) □

If you remember which antibiotic did you take? Please enter name of antiobiotic or write "not

If you were told what infection you had please specify (eg. Chest infection, ear infection), or

In the second trimester (between your 3rdth and 6th month of pregnancy)

□

If you remember which antibiotic did you take? Please enter name of antiobiotic or write "not known" if you don't know

If you were told what infection you had please specify (eg. Chest infection, ear infection), or

In the last trimester of your pregnancy (in the last 3 months of your pregnancy) □

If you remember which antibiotic did you take? Please enter name of antiobiotic or write "not

If you were told what infection you had please specify (eg. Chest infection, ear infection), or write "not known" if you don't know

Q1.16 Were you given antibiotics <u>during labour</u> because of a known risk of infection such as having a high temperature during labour or your waters breaking early?

Yes □
No □
I don't know □

Q1.17a If you have breastfed your baby, have <u>you</u> been on any antibiotics during the time you were breastfeeding?

Yes □ → go to question 1.17b
No □ → go to question 1.18
I don't know / I can't remember □ → go to question 1.18
Not applicable as I have only fed my baby with formula milk □→ go to question 1.18

Q1.17b How old was your baby when <u>you</u> were on antibiotics and breastfeeding?

I don't know □
Age 0-1 months □

If you remember which antibiotic did you take? Please enter name of antiobiotic or write "not known" if you don't know

If you were told what infection you had please specify (eg. Chest infection, ear infection), or write "not known" if you don't know.

Age 1-3months □

If you remember which antibiotic did you take? Please enter name of antiobiotic or write "not known" if you don't know.

If you were told what infection you had please specify (eg. Chest infection, ear infection), or write "not known" if you don't know.

Age 3months plus □

If you remember which antibiotic did you take? Please enter name of antiobiotic or write "not known" if you don't know.

If you were told what infection you had please specify (eg. Chest infection, ear infection), or write "not known" if you don't know.

Q1.18 Has your baby ever suffered from any of the following problems?

Please cross one or more boxes. Please indicate at roughly what age (eg 0-1month, 1-3months or more than 3 months old (3months plus)

Sickness or vomiting	age 0-1 month□	age 1-3months □	age 3months plus □
Diarrhoea	age 0-1 month□	age 1-3months □	age 3months plus □
Constipation	age 0-1 month□	age 1-3months □	age 3months plus □
Chest problems / infection	age 0-1 month□	age 1-3months □	age 3months plus □
Ear problems / infection	age 0-1 month□	age 1-3months □	age 3months plus □
Urinary tract infection	age 0-1 month□	age 1-3months □	age 3months plus □
Colic / painful wind	age 0-1 month□	age 1-3months □	age 3months plus□

Thrush	age 0-1 month□	age 1-3months □	age 3months plus□
Not gaining enough weight	age 0-1 month□	age 1-3months □	age 3months plus□
Gaining too much weight	age 0-1 month□	age 1-3months □	age 3months plus □
Something else (please cross the age and write in)	age 0-1 month□	age 1-3months □	age 3months plus □

□

None of these

Q1.19a. Since your baby was born has he/she been given any antibiotics?

Yes □ → go to question 1.19b
No □ → go to question 1.20
I don't know □ → go to question 1.20

Q1.19b How many separate courses of antibiotics has your baby had since they were born?

Please enter a number in the box below.

□courses of antibiotics

Q1.19c When was your child given antibiotics? *Please cross one or more boxes*

I don't know □

Age 0-1 months □

If you remember which antibiotic did they take? Please enter name of antiobiotic or write "not known" if you don't know

If you were told what infection you had please specify (eg. Chest infection, ear infection).

Age 1-3months □

If you remember which antibiotic did they take? Please enter name of antiobiotic or write

If you were told what infection you had please specify (eg. Chest infection, ear infection), or write "not known" if you don't know

Age 3 months plus □

If you remember which antibiotic did they take?? Please enter name of antiobiotic or write

If you were told what infection you had please specify (eg. Chest infection, ear infection), or

Q1.20 Does your baby have any of the following medical conditions:

a) A heart condition?

Yes □ **Please specify if you know the name of the heart condition**

No □

b) A problem with the gut known as duodenal atresia ?

Yes □

No □

c) A condition known as Hirschsprungs (causing very bad constipation from birth)?

Yes □
No □

d) Any other medical conditions?

Yes □ **Please specify below**

No □

There are now some questions about your own health and other members of your child's family

Q1.21 Have you ever been diagnosed with any of the following conditions? Please cross one or more boxes

Type 1 diabetes? (this does not refer to gestational diabetes which only develops during pregnancy or Type 2 diabetes) □
An underactive thyroid gland known as hypothyroidism? □
An overactive thyroid gland known as hyperthyroidism? □
Coeliac disease? □

Q1.22 Has the father of your baby ever been diagnosed with any of the following conditions? Please cross one or more boxes

Type 1 diabetes? (this does not refer to Type 2 diabetes) □
An underactive thyroid gland known as hypothyroidism? □
An overactive thyroid gland known as hyperthyroidism? □
Coeliac disease? □

Q1.23 If your baby has any siblings have any of them been diagnosed with any of the following conditions? Please cross one or more boxes

Type 1 diabetes? (this does not refer to Type 2 diabetes)	□
An underactive thyroid gland known as hypothyroidism?	□
An overactive thyroid gland known as hyperthyroidism?	□
Coeliac disease?	□
Not applicable as no siblings?	□

SECTION 2: About your baby's first feeds

We are now going to ask some questions about your baby's first feeds. We know that often babies with Down's Syndrome have difficulties with both breast and bottle feeding and we are interested to hear about your experience.

Please note that when we ask about 'breastfeeding' we also mean 'giving your baby expressed breast milk'.

Q2.1 Did you have skin-to-skin contact with your baby within the first 24 hours after he/she was born? (By skin-to-skin contact we mean you were holding the baby so that his/her bare skin was next to your bare skin.)

Yes ☐
No ☐

Q2.2 After the birth did you have any health problems that affected your ability to feed your baby the way you wanted to?

Yes ☐
No ☐

Q2.3a During the first few days, did anyone help you put your baby to the breast?

Yes ☐ → go to Q2.3b
No ☐ → go to Q2.4

Q2.3b Who was this?
Please cross one or more boxes

Midwife ☐
Midwifery Support Worker ☐
Nurse ☐
Nursery Nurse ☐
Healthcare assistant ☐
Health visitor ☐
Doctor / GP ☐

Voluntary or charitable organisation ☐
Peer supporter (a mum who has breastfed themselves and been trained to give support to other mums) ☐
Breastfeeding support group ☐
Partner, friend or relative ☐
Someone else ***(Please cross and write in who)*** ☐

Q2.4 **Have you ever breastfed your baby? (put them to your breast even if it was just once or ever given them expressed breast milk)**

Yes ☐ → go to Q2.5
No ☐ → go to Q2.6a

Q2.5. How soon after your baby was born did you first put him/her to the breast?
Please cross <u>one</u> box only. If you can't remember exactly please estimate which is the closest answer

Immediately / within a few minutes ☐
Within half an hour ☐
More than ½ hour, up to 1 hour later ☐
More than 1 hour, up to 4 hours later ☐
More than 4 hours, up to 8 hours later ☐
More than 8 hours, up to 12 hours later ☐
More than 12 hours, up to 24 hours later ☐
More than 24 hours later ☐

Q2.6a Were there any problems feeding your baby while you were in the hospital, birth centre or unit?

Yes ☐ → go to Q2.6b
No ☐ → go to Q2.7
Not applicable as I had a home birth ☐ → go to Q2.7

Q2.6b What problems were there?
Please write in

Q2.6c Did anyone give you any help or support with this/these problems?

Yes □ → go to Q2.6d
No □ → go to Q2.7

Q2.6d Who helped or supported you?
Please cross one or more boxes

Midwife □
Midwifery Support Worker □
Nurse □
Nursery Nurse □
Healthcare assistant □
Health visitor □
Doctor / GP □
Voluntary or charitable organisation □
Peer supporter (a mum who has breastfed themselves and been trained to give support to other mums) □
Breastfeeding support group □
Partner, friend or relative □
Someone else ***(Please cross and write in)*** □

Q2.7 While you were in the hospital, birth centre or unit did you get enough help and support with feeding your baby?

Yes – received enough help □
No – would have liked more help □
Not applicable as I had a home birth □

Q2.8a After the birth were you told that your baby needed to go to special care because of problems specifically with feeding?

Yes □ → go to Q2.9a
No □ → go to Q2.8b

Q2.8b Was your baby put into special care for any other reason?

Yes □ Please specify in the box below and then go to Q2.9a

No □ → go to Q2.13

Q2.9a For how long was your baby in special care?

One day or less □ → go to Q2.10
Two or three days □ → go to Q2.10
Four days to one week □ → go to Q2.10
More than one week up to one month □ → go to Q2.9b
More than one month □ → go to Q2.10b

Q2.9b How many weeks in total was your baby in special care?

If you are unsure please estimate
□weeks

Q2.10 Did having your baby in special care affect your ability to feed your baby the way you wanted to?

Yes □
No □

Q2.11a Whilst in Special Care were you shown how to express breast milk?

Yes □ → go to Q2.11b
No □ → go to Q2.11b
Not applicable to me (could not express or did not wish to express) □ → go to Q2.13a

Q2.11b Did you feel supported to express breast milk whilst in special care?

Yes □
No □

Q2.12a Did your baby need to be fed with a naso-gastric (NG) tube (a feeding tube which goes from the baby's nose to the baby's stomach)?

Yes □ → go to Q2.12b
No □ → go to Q2.13a

Q2.12b Was this solely because of problems with feeding or other medical conditions?

Problems with Feeding □
Other Medical condition **(*please cross and write below*)** □

Q2.12c For how long did your baby need to be fed with an NG tube (please estimate if you are unsure)

Either in days: □
OR
In whole weeks plus any additional days:
□ and □
Weeks days

Q2.12d Which milk was your baby given down the NG tube?

Only expressed breast milk □
Only infant formula □
Expressed Breast milk and infant formula □
I don't know □

Q2.13a Were all feeds stopped at any point and your baby put onto fluids given into a vein ie intravenous (iv) fluids?

Yes □ → go to Q2.13b
No □ → go to Q2.15
I don't know □ → go to Q2.14

Q2.13b How long was your baby on iv fluids?

□ and □
Weeks days

I don't know □

Q2.14 Were you given, or did your red book contain the specific Down's Syndrome insert with growth charts for babies with Down's Syndrome?

Yes □
No □

We *are now going to ask some questions about your experience of having your baby at home - either from the start because your baby was born at home or since leaving the hospital / birthing centre*

Q2.15 Was your baby born at home?

Yes □ → go to Q2.16
No □ → go to Q2.17

Q2.16 Have you had any problems feeding your baby?

Yes □ → go to Q2.18
No □ → go to Section 3 question 3.1.

Q2.17 Since you left the hospital, birth centre or unit have you had any problems with feeding your baby?

Yes □ → go to Q2.18
No □ → go to Section 3 question 3.1

Q2.18 What problems were there?
Please write in

Q2.19a Did you get any help or information about this/these problems?

Yes □ → go to Q2.19b
No □ → go to Section 3 question 3.1

Q2.19b Where did you get this help or information?
Please cross one or more boxes

SureStart or Children's Centre / Children's Health Clinic □
Voluntary or charitable organisation □
Peer supporter (a mum who has breastfed themselves and been trained to give support to other mums) □
Down's syndrome Association □
Down's syndrome Scotland □
Breastfeeding support group □
Partner, friend or relative □
Start4Life □
Books / leaflets / magazines □
Television / radio □
The internet / web based resources □
Breastfeeding clinic □
National Breastfeeding Helpline □
Doctor / GP □
Health visitor □

Midwife □
Nurse □
Somewhere else ***(Please cross and write in)*** □

SECTION 3: About the milk that you give your baby

We are now going to ask some questions about the milk that you give your baby. We know that often babies with Down's Syndrome have difficulties with both with breast and bottle feeding and we are interested to hear about your experience. The earlier sections were about your baby's first feeds, we now want to ask some more questions about feeding in general. We apologise that some of the questions in this section may feel repetitive, this is so we can collect as much detailed information as possible.

Please note that when we ask about 'breastfeeding' we also mean 'giving your baby expressed breast milk'.

Q3.1 Thinking about the milk that your baby has received over the last 7 days, has he/she had…
Please cross one box only

Only breast milk □ → go to Q3.2
Only infant formula □ → go to Q3.3
Breast milk and infant formula □ → go to Q3.5

Q3.2. Has your baby EVER been given infant formula, even if this was only once?

Yes (even if only once) □ → go to Q3.5
No □ → go to Q3.12

Q3.3 Has your baby EVER fed from your breast, even if this was only once?

Yes (even if only once) □ → go to Q3.5
No □ → go to Q3.4

Q3.4 Has your baby EVER been given expressed breast milk (via syringe, bottle or cup etc.)?

Yes (even if only once) □ → go to Q3.5
No □ → go to Q3.10

Q3.5 Have you stopped breastfeeding (no longer give your baby *any* expressed milk or put your baby to your breast)?

Yes □ → go to Q3.6
No □ → go to Q3.8

Q3.6 How old was your baby when you stopped breast feeding?
Please write the age in the appropriate box – Please estimate if you are not sure
Either in days: □
OR
In whole weeks plus any additional days:
□ and □
Weeks days

Q3.7 What were your reasons for stopping?
Please write in the reasons

Q3.8 Which of the following best describes how long you breastfed for?
Please cross one box only

I would have liked to breastfeed for longer □ → go to Q3.9
I breastfed for as long as I intended □ → go to Q3.10
I breastfed for longer than I intended □ → go to Q3.10

Q3.9 What would have helped you breastfeed for longer?
Please write in the box

Q3.10 How old was your baby when he/she FIRST received infant formula?
Please write the age in the appropriate box. Please estimate if you are not sure.
Either in days: □
OR
In whole weeks plus any additional days:

□ and □
Weeks days

Q3.11 Since your baby was born, how often has he/she been fed infant formula?
If your pattern of using infant formula has varied please select the answer you feel comes closest to describing your situation.
Please cross one box only

All or almost all feeds □
About half of all feeds □
One or two feeds a day □
A few feeds a week, but not every day □
A few feeds since they were born, but not every week □
Only once or twice since they were born □

Q3.12 Has your baby ever needed to have special milk which has been prescribed by a doctor or dietician?

Yes □ please specify the type of milk if known

No □

Q3.13a Are you aware of any health benefits in breastfeeding, for your baby?

Yes □ → go to Q3.13b
No □ → go to Q3.14

Q3.13b What health benefits, if any, are you aware of for the BABY?
Please write in

Q3.14 Is there anything else you would like to say about feeding your baby?

Yes □ **Please write in below**

Q4.1 How did you hear about the Down's Syndrome Association or Down's Syndrome Scotland?

Q4.2 What age are you now?
Please cross one box only

Under 20 □
20, up to 24 □
25, up to 29 □
30, up to 34 □
35, up to 39 □
40 or over □

Q4.3 Are you…

Married or in a civil partnership □
Living together □
Single □
Widowed, divorced or separated □

Q4.4 What is your ethnic group?
Please cross one box only

White
British □
Irish □
Any other white background ***(Please cross and write in)*** □

Mixed
White and Black Caribbean □
White and Black African □
White and Asian □
Any other mixed background ***(Please cross and write in)*** □

Indian □
Pakistani □
Bangladeshi □
Any other Asian background ***(Please cross and write in)*** □

Black or Black British
Caribbean □
African □
Any other black background ***(Please cross and write in)*** □

Chinese or Other ethnic group
Chinese □
Any other ***(Please cross and write in)*** □

Q4.5 Please give the date when you filled in this questionnaire

DD/MM/YYYY

/ /

Q4.6 Is there any further information that you would like to add?

Yes □ **Please write in below**

No □

Was there anything you intended to go back and complete?

Please check.

Appendix 14: FADES 7 Month Questionnaire

7 month questionnaire

What is the questionnaire about?

Thank you for filling in the first questionnaire we sent you. This questionnaire asks some more questions about your baby's health and feeding.

If you no longer wish to take part in this study please cross the box below and return the questionnaire to us so we do not trouble you further.

□

Section 1: About your baby and their health

Q1.1 How old is your baby?
Please write numbers in both boxes
Write in how many whole weeks plus any additional days:

□ and □
Weeks days

Q1.2 Some babies with Down's syndrome have medical conditions which mean that they need to spend some of the first few weeks or months of their early life in hospital. Please indicate how long your baby has spent in hospital?

Less than a week	□
1week – less than 4 weeks	□
1month – less than 2months	□
2 months – less than 3 months	□
3 months – less than 4 months	□
4 months - less than 5 months	□
5 months - less than 6 months	□
More than 6 months	□

Q1.3 Which specialists are involved in your baby's care? Please cross one or more boxes

Paediatric cardiologist/ heart doctor □
Paediatric gastroenterologist / gut doctor □
Paediatric Endocrinologist / hormone doctor □
Paediatric dietician □
Community Paediatrician □
Speech and language therapist □
Other (**please cross and write below**) □

Q1.4 Since completing the last questionnaire has your baby been diagnosed with any of the following:

a. A heart condition?

Yes □ **Please specify if you know the name of the heart condition**

No □

b. A condition known as Hirschsprungs?

Yes □
No □

c. Hypothyroidism requiring treatment?

Yes, diagnosed age less than 3 months □ 3 months plus □

No □

d. Type 1 diabetes requiring insulin?

Yes, diagnosed age less than 3 months □ 3 months plus □

No □

e. Coeliac disease, for which your child has been put on a gluten free diet?

Yes, diagnosed at age less than 3 months □ 3 months plus □

No □

f. Any other medical conditions?

Yes, diagnosed age less than 3 months □ 3 months plus □

(Please indicate and write in medical condition below)

No □

Q1.5 Has your baby ever suffered from any of the following problems?

Please cross one or more boxes. Please indicate at roughly what age (eg. 0-3months, 3months plus)

a. Sickness or vomiting age less than 3months □ age 3months plus □

b. Diarrhoea age less than 3months □ age 3months plus □

c. Constipation age less than 3months □ age 3months plus □

d. Chest problems / infection age less than 3months □ age 3months plus □

e. Ear problems / infection age less than 3months □ age 3months plus □

f. **Urinary tract infection** age less than 3months □ age 3months plus □

g. **Colic / painful wind** age less than 3months □ age 3months plus □

h. **Thrush** age less than 3months □ age 3months plus □

i. **Not gaining enough weight** age less than 3months □ age 3months plus □

j. **Gaining too much weight** age less than 3months □ age 3months plus □

k. **Something else (please** age less than 3months □ age 3months plus □

l. **None of these** □

Q1.6a Since completing the last questionnaire has your baby been given any antibiotics?

Yes □ → go to Q1.6b
No □ → go to section 2

Q1.6b How many separate courses of antibiotics has your baby had since they were born?

□courses

Q1.6c When was your child given antibiotics?

Please cross one or more boxes

I don't know □

Age less than 3months □

If known which antibiotic? Please enter name of antiobiotic or write "not known" if you don't

If you were told what infection you had please specify (eg. Chest infection, ear infection), or

Age 3months plus □

If known which antibiotic? Please enter name of antiobiotic or write "not known" if you don't

If you were told what infection you had please specify (eg. Chest infection, ear infection), or

Section 2: About the milk that you give your baby

We are now going to ask some questions about the milk that you give your baby. We know that often babies with Down's Syndrome have difficulties with both with breast and bottle feeding and we are interested to hear about your experience.

Some of these questions are repeated from the initial questionnaire that you filled in but please fill them in again as this is so we can collect as much detailed information as possible.

Please note that when we ask about 'breastfeeding' we also mean 'giving your baby expressed breast milk'.

Q2.1 Thinking about the milk that your baby has received over the last 7 days, has he/she had…

Please cross one box only

Only breast milk	□	→ go to Q2.2
Only infant formula / other milk	□	→ go to Q2.3
Breast milk and infant formula / other milk	□	→ go to Q2.5

Q2.2 Has your baby EVER been given any kind of milk other than breast milk, such as infant formula or cow's milk (even if this was only once)?

Yes (even if only once)	□	→ go to Q2.10
No	□	→ go to Section 3

Q2.3 Has you baby EVER fed from your breast, even if this was only once?

Yes (even if only once)	□	→ go to Q2.5
No	□	→ go to Q2.4

Q2.4 Has your baby EVER been given expressed breast milk (via syringe, bottle or cup etc)?

Yes (even if only once)	□	→ go to Q2.5
No	□	→ go to Q2.11

Q2.5 Have you stopped breast feeding (no longer give your baby any expressed milk or put your baby to your breast)?

Yes	□	→ go to Q2.6
No	□	→ go to Q2.10

Q2.6 How old was your baby when you stopped breastfeeding?
If you cannot remember exactly, please put in the approximate age.
Write in how many whole weeks plus any additional days
Please write numbers in both boxes

□	and	□
Weeks		days

Q2.7 What were your reasons for stopping breastfeeding?
Please write in the reasons

Q2.8 Which of the following best describes how long you breastfed for?
Please cross one box only

I would have liked to breastfeed for longer	□	→ go to Q2.9
I breastfed for as long as I intended	□	→ go to Q2.10
I breastfed for longer than I intended	□	→ go to Q2.10

Q2.9 What would have helped you breastfeed for longer?
Please write in the reasons

Q2.10 How often has your baby been given breast milk over the last 7 days on average?

Not at all	□
Once a day	□
Twice a day	□
3-4 times a day	□
5-6 times a day	□
7-8 times a day	□
More than 8 times a day	□

Q2.11 Which of the following kinds of milk has your baby EVER been given, even if this was only once?

Please cross one or more boxes

Infant formula (or "first" milk)	□
Follow-on formula	□
Cow‟s milk	□
Another kind of milk ***(Please cross and write in)***	□

Q2.12 How old was your baby when he/she was FIRST given any kind of milk other than breast milk such as infant formula or cow's milk?

If you cannot remember exactly, please put in the approximate age.
Please write in the age to the nearest whole week

□ weeks old

Q2.13 Excluding breast milk, which one of the following kinds of milk has your baby been given MOST OFTEN over the last 7 days?
Please cross one box only

Infant formula (or "first" milk) □
Follow-on formula □
Cow"s milk □
Another kind of milk ***(Please cross and write in)*** □

None of these □ → go to **Section 3**

Section 3: About other drinks and food that you may give to your baby

Q3.1a Has your baby EVER had anything else to drink apart from milk such as water, fruit juice, squash or herbal drink?

Yes (even if only occasionally) □ → go to Q3.1b
No □ → go to Q3.2a

Q3.1b How old was your baby when he or she was FIRST given something apart from milk, such as water, fruit juice, squash or herbal drink?
If you cannot remember exactly, please put in the approximate age.
Please write number in the box to nearest whole week
□ Weeks

Q3.2a Has your baby ever had any solid foods such as cereal, rusks, baby rice, fruit, vegetables or any other kind of solid food?

Yes □ → go to Q3.2b
No □ → go to section 4

Q3.2b How old was your baby when he/she first had any food apart from milk?
If you cannot remember exactly, please put in the approximate age.
Please write number in the box to nearest whole week
□ Weeks

Q3.3 Why did you start giving your baby solid foods?
Please cross one or more boxes

Doctor / health visitor / other health professional advised me to □
Friend or relative advised me to □

Read leaflets / seen information that advised me to	□
Start4Life	□
Previous experience (with another baby)	□
Baby was not satisfied with milk	□
Baby was not gaining enough weight	□
Baby was waking up during the night	□
Baby able to sit up and hold food in hand	□
Some other reason ***(Please cross and write in)***	□

Q3.4 What was the FIRST solid food given to your baby?
Please cross one box only

Ready made baby food	□
Rusk	□
Baby rice	□
Fruit (home prepared)	□
Vegetables (home prepared)	□
Homemade food	□
Any other food (for example, yoghurt, fromage frais or breakfast cereal)	□

Q3.5 What sort of solid foods has your baby EVER had?
Please cross one or more boxes

Ready made baby food	□
Rusk	□
Baby rice	□
Fruit (home prepared)	□
Vegetables (home prepared)	□
Homemade foods	□
Any other food (for example, yoghurt, fromage frais or breakfast cereal)	□

Q3.6 What sort of solid foods did your baby eat yesterday?
Please cross one or more boxes

Ready made baby food	□
Homemade foods	□
Rusk	□
Baby rice	□
Fruit (home prepared)	□

Vegetables (home prepared)	□
Homemade foods	□
Any other food (for example, yoghurt, fromage frais or breakfast cereal)	□
Didn't have solids yesterday	□

Q3.7 Did you get any information about when to start giving solid foods to your baby?

Yes □
No □

Q3.8 Did you get any information about the types of solid foods to give your baby?

Yes □
No □

If you answered Yes at Q3.7 or Q3.8 please go to Q3.9
Otherwise go to Section 4

Q3.9 Where did you get this information?
Please cross one or more boxes

SureStart or Children's Centre / Children's Health Clinic	□
Partner, friend or relative	□
Voluntary or charitable organisation	□
Peer supporter (a mum who has breastfed themselves and been trained to give support to other mums)	□
Breastfeeding support group	□
Start 4 Life	□
Books / leaflets / magazines	□
Television / radio	□
The internet / web based resources	□
Breastfeeding Clinic	□
National Breastfeeding Helpline	□
Doctor / GP	□
Health visitor	□
Midwife (including at antenatal sessions)	□
Nurse	□
Somewhere else ***(Please cross and write in)***	□

Section 4: Help and information for you about feeding your baby

Q4.1a Have you had any problems with feeding your baby (including breastfeeding, bottle feeding and/or giving you baby solids) since the time you filled in the previous questionnaire?

Yes □ → go to Q4.1b
No □ → go to section 5

Q4.1b What problems have you had?
Please write in

Q4.1c Did you get any help or information about this/these problems?

Yes □ → go to Q4.1d
No □ → go to section 5

Q4.1d Where did you get this help or information?
Please cross one or more boxes

SureStart or Children‟s Centre / Children‟s Health Clinic □
Partner, friend or relative □
Voluntary or charitable organisation □
Down's Syndrome Association □
Down's Syndrome Scotland □
Peer supporter (a mum who has breastfed themselves and been trained to give support to other mums) □
Breastfeeding support group □
Start 4 Life □
Books / leaflets / magazines □
Television / radio □
The internet / web based resources □
Breastfeeding Clinic □
National Breastfeeding Helpline □
Doctor / GP □
Health visitor □
Midwife (including at antenatal sessions) □
Nurse □
Somewhere else ***(Please cross and write in)*** □

Section

Q5.1 Are you doing any paid work at the moment?

Yes	□	→ go to Q5.2
On paid maternity leave	□	→ go to Section 6
On unpaid maternity leave	□	→ go to Section 6
No	□	→ go to Section 6

Q5.2 What age was your baby when you returned to work?

1 month, less than 2 months	□		
2 months, less than 3 months		□	
3 months, less than 4 months		□	
4 months, less than 5 months		□	
5 months, less than 6 months		□	
6 months, or older	□		

Q5.3a Has your return to work affected the way in which you are feeding your baby at all?

Yes	□	→ go to Q5.3b
No	□	→ go to Section 6

Q5.3b How has this affected the way in which you feed your baby?
Please write in

Section 6:

Q6.1 Is there anything else you would like to say about feeding your baby?

Yes □ **Please write in below**

No □

Q6.2 Is there anything else you would like to say about your baby's health?

Yes □ **Please write in below**

No □

Q6.3 Please give the date when you filled in this questionnaire

DD/MM/YYYY

/ /

Q6.4 Is there any further information that you would like to add?

Yes □ **Please write in below**

No □

Was there anything you intended to go back and complete? Please check.

NIHR BRISTOL BIOMEDICAL RESEARCH CENTRE NUTRITION THEME

Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)

12 month questionnaire

What is the questionnaire about?

Thank you for filling in the two previous questionnaires. This questionnaire asks some more questions about your baby's health and feeding.

Section 1: About your baby and their health

Q1.1 How old is your baby?
Please write numbers in both boxes
Write in how many whole weeks plus any additional days:

□ and □
Weeks days

Q1.2 Some babies with Down's syndrome have medical conditions which mean that they spend some weeks or months of their life in hospital.

Please indicate since you last filled in the questionnaire when your baby was 7 months old, how long your baby has spent in hospital?

Since my baby was 7 months old they have spent….

Less than a week □
1week – less than 4 weeks □
1month – less than 2months □
2months – less than 3 months □
3months – less than 4 months □
4months – less than 5 months □
5months – less than 6 months □
More than 6 months □

……**in hospital**

Q1.3a Has your baby been seen by any specialists since you last completed the questionnaire when your baby was 7 months old? (for example paediatric cardiologist / heart doctor, paediatric gastroenterologist/ gut specialist, community paediatrician etc)

Yes □ → go to Q1.3b
No □ → go to Q1.4

Q1.3b Which specialists has your baby been under the care of since they were 7months old?

Please cross one or more boxes

Paediatric cardiologist/ heart doctor □
Paediatric gastroenterologist / gut doctor □
Paediatric dietician □
Community Paediatrician □
Speech and language therapist □
Other ***please cross box and write in*** □

Q1.4 Since completing the last questionnaire has your baby been diagnosed with any of the following:

a. A heart condition?

Yes □ **Please specify if you know the name of the heart condition**

No □

b. Hypothyroidism requiring treatment?

Yes, diagnosed age 7months - less than 9 months □ 9-12 months plus □

No □

c. Type 1 diabetes requiring insulin?

Yes, diagnosed age 7months - less than 9 months □ 9-12 months plus □

No □

d. Coeliac disease, for which your child has been put on a gluten free diet?

Yes, diagnosed at age 7 months - less than 9 months □ 9-12 months plus □

No □

e. Any other medical conditions?

Yes, diagnosed age 7 months - less than 9 months □ 9-12 months plus □

(Please indicate and write in medical condition below)

No □

Q1.5 Has your baby ever suffered from any of the following problems since you last completed the questionnaire at around 7months?

Please cross one or more boxes. Please indicate at roughly what age (eg 7months – less than 9 months, 9-12 months)

a. Sickness or vomiting 7months, less than 9months □ 9-12months □

b. Diarrhoea 7months, less than 9months □ 9-12months □

c. Constipation 7months, less than 9months □ 9-12months □

d. Chest problems / infection 7 months, less than 9months □ 9-12months □

e. Ear problems / infection 7 months, less than 9months □ 9-12months □

f.	**Urinary tract infection**	7 months, less than 9months □	9-12months □
g.	**Colic / painful wind**	7 months, less than 9months □	9-12months □
h.	**Thrush**	7 months, less than 9months □	9-12months □
i.	**Not gaining enough weight**	7 months, less than 9months □	9-12months □
j.	**Gaining too much weight**	7months, less than 9months □	9-12months □
k.	**Something else (please cross age and write in)**	7 months, less than 9months □	9-12months □

l. None of these

□

Q1.6a Since completing the last questionnaire has your baby been given any antibiotics?

Yes □ → go to Q1.6b
No □ → go to Q1.7

Q1.6b How many separate courses of antibiotics has your baby had since they were 7 months old?

□

Q1.6c When was your child given antibiotics? *Please cross one or more boxes*

I don't know □

Age 7months – less than 9months □

If known which antibiotic? Please enter name of antiobiotic or write "not known" if you don't

If you were told what infection you had please specify (eg. Chest infection, ear infection), or

Age 9 months – less than 12months □

If known which antibiotic? Please enter name of antiobiotic or write "not known" if you don't

If you were told what infection you had please specify (eg. Chest infection, ear infection), or write "not known" if you don't know

Section 2: About the drinks and food that you give your baby

We are now going to ask some questions about the milk that you give your baby.

Please note that when we ask about 'breastfeeding' we also mean 'giving your baby expressed breast milk'.

Some of these questions are repeated from the last questionnaire that you filled in but please fill them in again as this will help us to collect as much detailed information as possible.

Q2.1a Since completing the last questionnaire at around 7months have you had any problems with feeding your baby?

Yes □ → go to Q2.1b
No □ → go to Q2.2

Q2.1b What problems have you had?
Please write in

Q2.1c Did you get any help or information about this/these feeding problem(s)?

Yes □ → go to Q2.1d

No □ → go to Q2.2

Q2.1d Where did you get this help or information?
Please cross one or more boxes

SureStart or Children's Centre / Children's Health Clinic	□
Partner, friend or relative	□
Voluntary or charitable organisation	□
Peer Supporter (a mum who has breastfed themselves and been trained to give support to other mums)	□
Breastfeeding support group	□
Start 4 Life	□
Books / leaflets / magazines	□
Television / radio	□
The internet / web based resources	□
Breastfeeding clinic	□
National Breastfeeding Helpline	□
Doctor / GP	□
Health visitor	□
Midwife (including at antenatal sessions)	□
Nurse	□
Somewhere else ***(Please cross and write in)***	□

Q2.2 Thinking about the milk that your baby has received over the last 7 days, has he/she had…
Please cross one box only

Only breast milk	□ → go to Q2.3
Only infant formula / other milk	□ → go to Q2.4
Breast milk and infant formula / other milk	□ → go to Q2.5

Q2.3 Has your baby EVER been given any kind of milk other than breast milk, such as infant formula or cow's milk (even if this was only once)?

Yes	□ → go to Q2.5
No	□ → go to Q2.15

Q2.4 Has your baby EVER fed from your breast or EVER been given expressed breast milk (via syringe, bottle or cup etc)?

Yes (even if only once)	□	→ go to Q2.5
No	□	→ go to Q2.11

Q2.5 Have you stopped breast feeding (no longer give your baby any expressed milk or put your baby to your breast)?

Yes	□	→ go to Q2.6
No	□	→ go to Q2.10

Q2.6 How old was your baby when he/she was LAST given breast milk or you put them to your breast?

10 weeks or less	□
More than 10 weeks, up to 4 months	□
More than 4 months, up to 5 months	□
More than 5 months, up to 6 months	□
More than 6 months, up to 7 months	□
More than 7 months, up to 8 months	□
More than 8 months, up to 9 months	□
More than 9 months	□

Q2.7 What were your reasons for stopping breastfeeding?
Please write in the reasons

Q2.8 Which of the following best describes how long you breastfed for?
Please cross one box only

I would have liked to breastfeed for longer □ → go to Q2.9
I breastfed for as long as I intended □ → go to Q2.10
I breastfed for longer than I intended □ → go to Q2.10

Q2.9 What would have helped you breastfeed for longer?
Please write in the reasons

Q2.10 On average how often has your baby been given breast milk over the last 7 days?

Not at all □
Once a day □
Twice a day □
3-4 times a day □
5-6 times a day □
7-8 times a day □
More than 8 times a day □

Q2.11 Which of the following kinds of milk has your baby EVER been given to drink, even if this was only once?
Please cross one or more boxes

Infant formula (or "first" milk) □
Follow-on formula (sometimes known as stage 2/3) □
Cow's milk □
Another kind of milk ***(Please cross and write in)*** □

Q2.12 How old was your baby when he/she was first given ANY KIND OF MILK OTHER THAN BREAST MILK, such as infant formula or cow's milk?

10 weeks or less □
More than 10 weeks, up to 4 months □
More than 4 months, up to 5 months □
More than 5 months, up to 6 months □

More than 6 months, up to 7 months □
More than 7 months, up to 8 months □
More than 8 months, up to 9 months □
More than 9 months □

Q2.13 Excluding breast milk, which one of the following kinds of milk has your baby been given MOST OFTEN over the last 7 days?
Please cross one box only

Infant formula (or "first" milk) □
Follow-on formula □
Cow"s milk □
Another kind of milk ***(Please cross and write in)*** □

None of these □

Q2.14a Has your baby ever been given any COW'S MILK?

Yes □ → go to Q2.14b
No □ → go to Q2.15

Q2.14b how old was he/she when COW'S MILK was first given?
If you cannot remember exactly, please put in the approximate age.
Please write number in the box to nearest whole month
□ Months

Q2.15 Has your baby ever had any solid foods such as cereal, rusks, baby rice, fruit, vegetables or any other kind of solid food?

Yes □ → go to Q2.16
No □ → go to Section 3

Q2.16 How old was your baby when he/she first had any food apart from milk?
If you cannot remember exactly, please put in the approximate age.

Please write number in the box to nearest whole week

□ Weeks

Q2.17 Why did you start giving your baby solid foods?

Please cross one or more boxes

Doctor / Health visitor / Other Health	□
Professional advised me to	□
Friend or relative advised me to	□
Read leaflets / seen information that advised me to	□
Start4Life	□
Previous experience (with another baby)	□
Baby was not satisfied with milk	□
Baby was not gaining enough weight	□
Baby was waking up during the night	□
Baby able to sit up and hold food in hand	□
Some other reason ***(Please cross and write in)***	□

Q2.18 What was the FIRST solid food given to your baby?

Please cross one box only

Ready made baby food	□
Homemade foods	□
Rusk	□
Baby rice	□
Fruit (prepared at home)	□
Vegetables (prepared at home)	□
Any other food (for example, yoghurt, Fromage frais or breakfast cereal)	□

Q2.19 What sort of solid foods has your baby EVER had?

Please cross one or more boxes

Ready made baby food	□
Homemade foods	□
Rusk	□
Baby rice	□
Fruit (prepared at home)	□
Vegetables (prepared at home)	□

Any other food (for example, yoghurt, fromage frais or breakfast cereal) □

Q2.20 What sort of solid foods did your baby eat yesterday?

Please cross one or more boxes

Ready made baby food □
Homemade foods □
Rusk □
Baby rice □
Fruit (prepared at home) □
Vegetables (prepared at home) □
Any other food (for example, yoghurt, fromage frais or breakfast cereal) □
Didn't have solids yesterday □

Q2.21 Please estimate how often do you usually give your baby these particular TYPES of solid food?

Type of food	More than	Once a day	3 or more times a	Once or twice a	Less than once a	Never
Breakfast cereals	□	□	□	□	□	□
Rice or pasta	□	□	□	□	□	□
Bread	□	□	□	□	□	□
Potatoes	□	□	□	□	□	□
Potato products (inc. chips,	□	□	□	□	□	□
Butter/Margarine and other	□	□	□	□	□	□
Beef	□	□	□	□	□	□
Lamb	□	□	□	□	□	□
Pork (including ham)	□	□	□	□	□	□
Chicken / other poultry	□	□	□	□	□	□

Fish (including tuna)	□	□	□	□	□	□
Eggs	□	□	□	□	□	□
Beans, lentils, chickpeas	□	□	□	□	□	□
Tofu, Quorn, textured vegetable	□	□	□	□	□	□
Nuts (including ground nuts)	□	□	□	□	□	□
Fruit	□	□	□	□	□	□
Vegetables	□	□	□	□	□	□
Cheese, yoghurt,	□	□	□	□	□	□
Puddings or desserts	□	□	□	□	□	□
Biscuits, sweets, chocolate or	□	□	□	□	□	□
Crisps and corn snacks	□	□	□	□	□	□

Q2.22 Did you get any information about when to start giving solid foods to your baby?

Yes □
No □

Q2.23 Did you get any information about the types of solid foods to give your baby?

Yes □
No □

If you answered Yes at Q2.22 or Q2.23 please go to Q2.24, otherwise go to Section 3

Q2.24 Where did you get this information?
Please cross one or more boxes

SureStart or Children's Centre / Children's Health Clinic □
Partner, friend or relative □
Voluntary or charitable organisation □
Peer Supporter (a mum who has breastfed themselves and been trained to give support to other mums) □
Breastfeeding support group □

Start 4 Life	□
Books / leaflets / magazines	□
Television / radio	□
The internet / web based resources	□
Breastfeeding clinic	□
National Breastfeeding Helpline	□
Doctor / GP	□
Health visitor	□
Midwife (including at antenatal sessions)	□
Nurse	□
Somewhere else ***(Please cross and write in)***	□

Q2.25a Do you avoid giving your baby solid foods with particular ingredients?

Yes □ → go to Q2.25b
No □ → go to Q2.26

Q2.25b Which ingredient(s) do you avoid giving your baby? Please write in below, if there is more than one ingredient that you avoid please number them

Ingredient (s)

Q2.25c Why do you avoid giving your baby this / these ingredient(s)? Please write in below indicating which ingredient you are referring to.

Reason for avoiding

Q2.26a Has it been difficult to introduce your baby to solid foods?

Yes □ → go to Q2.26b
No □ → go to Section 3

Q2.26b In what way has it been difficult?
Please cross one or more boxes

Baby would not take solids	□
Baby would only take certain solids	□
Baby was disinterested in food	□
Baby prefers drinks to food	□
Baby vomiting	□
Baby doesn't like eating from a spoon	□
Other reason ***(Please cross and write in)***	□

Section 3: About your plans for work

Q3.1 Are you doing any paid work at the moment?

Yes	□ → go to Q3.2
On paid maternity leave	□ → go to Section 4
On unpaid maternity leave	□ → go to Section 4
No	□ → go to Section 4

Q3.2 What age was your baby when you returned to work?

less than 3 months	□
3 months, less than 4 months	□
4 months, less than 5 months	□
5 months, less than 6 months	□
6 months, less than 9 months	□
9 months or older	□

Q3.3a Has your return to work affected the way in which you are feeding your baby at all?

Yes	□ → go to Q3.3b
No	□ → go to Section 4

Q3.3b How has this affected the way in which you feed your baby?

Please write in

Section 4:

Q4.1 Is there anything else you would like to say about feeding your baby?

Yes □ **Please write in below**

No □

Q4.2 Is there anything else you would like to say about your baby's health?

Yes □ **Please write in below**

No □

Q4.3 Please give the date when you filled in this questionnaire

DD/MM/YYYY

/ /

Q4.4 Is there any further information that you would like to add?

Yes □ **Please write in below**

No □

Was there anything you intended to go back and complete? Please check.

NIHR BRISTOL BIOMEDICAL RESEARCH CENTRE NUTRITION THEME

Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)

Annual Medical Questionnaire

Thank you for filling in the previous questionnaires. This questionnaire is only about your baby's health and you will be asked to fill in a similar questionnaire every year around the time of your baby's birthday, until they are 5 years old.

We are very grateful for your help.

Contact:

EMAIL: Georgina.Williams@bristol.ac.uk

Tel: +44 (0)117 342 1755

What is your identification number for this study? (Please do not put your name or your baby's name anywhere on this questionnaire)

If you no longer wish to take part in this study please cross the box below and return the questionnaire to us so we do not trouble you further.

☐

Q1 How old is your child?

Please write numbers in both boxes

Write in how many months plus any additional weeks:

□ and □

Months weeks

Q2 Some children with Down's syndrome have medical conditions which mean that they spend some weeks or months of their early life in hospital.

Please estimate since you last filled in the questionnaire (which would have been around the time of their last birthday) how long your child has spent in hospital

Less than a week	□
1 week – less than 4 weeks	□
1 month – less than 2months	□
2 months – less than 3 months	□
3 months – less than 4 months	□
4 months – less than 5 months	□
5 months – less than 6 months	□
More than 6 months	□

Q3a Has your child been seen by any specialists since you last completed the questionnaire? (for example paediatric cardiologist / heart doctor, paediatric gastroenterologist/ gut specialist, community paediatrician etc)

Yes □ → go to Q3b

No □ → go to Q4

Q3b Which specialists has your child been under the care of in the last 6months? Please cross one or more boxes

Paediatric cardiologist/ heart doctor	□
Paediatric gastroenterologist / gut doctor	□
Paediatric dietician	□
Community Paediatrician	□
Speech and language therapist	□
Other (please cross and write in)	□

Q4 Since completing the last questionnaire has your baby been diagnosed with any of the following:

a. A heart condition?

Yes □ **Please specify if you know the name of the heart condition**

No □

b. Hypothyroidism requiring treatment?

Yes □
No □

c. Type 1 diabetes requiring insulin?

Yes □
No □

d. Coeliac disease, for which your child has been put on a gluten free diet?

Yes □
No □

e. Any other medical conditions?

Yes □ **Please specify**

Q5 Has ed the questic
Please

Sickness or vomiting □
Diarrhoea □
Constipation □
Chest problems / infection □
Ear problems / infection □
Urinary tract infection □
Colic / painful wind □
Thrush □
Not gaining enough weight □
Gaining too much weight □
Something else ***(please cross and write in)*** □

None of these □

Q6a. Since completing the last questionnaire has your baby been given any antibiotics?

Yes □ → go to Q6b
No □ → go to Q7a

Q6b. How many separate courses of antibiotics has your baby had in the last year, since you completed the last questionnaire? Please estimate if you are unsure.

□

Q7 Is there anything else you would like to say about your baby's health?
Please write in box

DD/MM/YYYY
/ /

Q9 Is there any further information that you would like to add?

Was there anything you intended to go back and complete?
Please check

NIHR BRISTOL BIOMEDICAL RESEARCH CENTRE NUTRITION THEME

Feeding and Autoimmunity in Down's syndrome

Evaluation Study (FADES)

Instructions for sample Collection

Thank you for participating in this study. We appreciate that completing the questionnaires and collecting the samples will take time and we want to support you with this in any way we can.

If you have any questions regarding either the questionnaires or collecting the samples please do not hesitate to contact us either by email fades-study@bristol.ac.uk or telephone 0117 342 1756. You may also find the answer you are looking for on our website (www.bristolnutritionbru.org.uk click on the FADES tab).

This pack contains:

Kit for mouth swab collection

Kit for urine collection

2 x Kit for stool collection

And a sheet for recording the date and time that your child's samples were taken.

The instructions for collecting the mouth swab, urine and stool samples are all within this booklet. The instructions and kit for collecting blood samples are in a separate booklet.

We would be grateful if you could collect the samples as soon as you can and post these back to us in the packaging provided. These need

to be posted within 24hours of collection to prevent the samples degrading.

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9-10 Stool Collection Instructions for baby's sample

11-13 Stool Collection Instructions for maternal sample

Instructions for taking Mouth swabs

Kit contents: Mouth swab, inner tube, outer mailing tube, outer Biohazard bag. You will also need a sharp pair of scissors.

1. Stand inner tube up in the lid of the outer tube. The inner tube contains some mouth swab collection buffer, please do not discard or spill.

2. Rub the swab very gently on the inside of your baby's cheeks and gums for 20-30 seconds this should not cause your baby any pain or discomfort.

3. Put used swab into the inner tube (so that the foam end is submerged in the collection buffer) and cut off some of the stick, enough so that the swab will fit into the tube with the lid closed. Close the lid tightly and remove from the stand.

4. Put the inner tube into the outer mailing tube (lid end up) and close the outer tube lid.

5. Place in the zip lock biohazard bag and then into the prepaid package and send back to us with the other samples.

INSTRUCTIONS FOR COLLCTING A URINE SAMPLE

Kit contents: Cotton wool bud packs x2, urine collection cup, gloves, 1 green top urine collection tube, disposable pipette, zip lock biohazard bag.

1. You can either "catch" your child's urine in the cup provided or use the sterile cotton wool buds in the nappy to catch the urine. When your child is older and if they are out of nappies urine can be collected in the cup provided.

2. If you have decided to catch your child's urine please take off your baby's nappy just prior to a feed and be ready to try and catch the first urine that your child does after their feed. If your child is out of nappies please catch the first urine after they have eaten. **Please note the time of the feed / meal.**

3. Once you have caught the urine in the cup transfer it into the urine tube pictured below using the plastic pipette provided. The tube has some white powder in it which is meant to be there, so please **do not** tip it out. Push the lid on securely.

4. If you have decided to use the cotton wool to collect the urine in the nappy - immediately prior to your baby's feed place 4 cotton wool balls in the nappy and fasten the nappy in the usual way. **Please note the time of the feed**

5. Half an hour after your child's feed check whether your baby has passed urine if they have, the cotton wool will be wet (if there is also poo on the cotton wool this will need to be discarded and try again at the next feed).Take the cotton wool out of the nappy and squeeze the urine from the cotton wool balls into the cup (you may wish to wear the gloves provided to do this), try to squeeze out as much as you can. Carefully transfer the urine from cup into the urine tube using the plastic pipette and push the lid on securely. The container has some white powder in it which is meant to be there, so please **do not** tip it out.

6. Write the **date** and the **time** the sample was taken on the sheet provided. **Please also note how long after the feed or meal the sample was collected.**

7. Put the green-topped container (with your child's urine in) inside the sealable biohazard plastic bag that contains some absorbent paper material.

8. Please post this either the **same** day with the other samples or, if the sample was taken in the evening you may post your sample the next morning if you keep it in the fridge or cool place overnight. Please send in the freepost packaging provided.

INSTRUCTIONS FOR COLLECTING A STOOL SAMPLE (BABY)

Kit contents: *BD BBL™ CultureSwab™ EZ II Swab, gloves, zip lock biohazard bag. OMNIgene GUT collection tube and spatula.*

Please collect 2 samples from a single nappy (from the same poo) one into the tube with the purple lid according to the instructions and one using the swab as per the instructions.

Things to consider:

• If the nappy is very wet from urine, do not collect a faecal (poo) sample from this nappy but wait until the next time your baby defecates.

• Avoid collecting the faecal specimen from the edges.

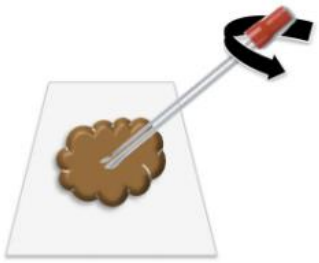
• Try to avoid scraping the nappy while collecting the sample

Procedure:

1. Remove the nappy as soon as you are aware that your baby has defecated (done a poo).

2. Pull/twist the BD™ CultureSwab™ from the covering tube by holding the swab by the purple cap. Set the tube aside at a reachable distance as you will need to re-use this.

3. While wearing gloves collect the faecal (poo) specimen by gently rubbing the foam ends of the double swab in the faeces that is on the nappy in a circular motion until both swab heads are covered with it. (See image below)

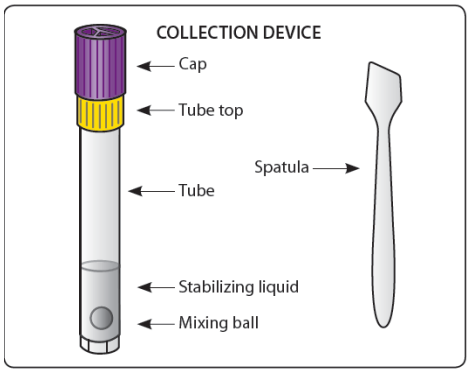


Avoid collecting too much sample so it is difficult to reintroduce the swab back into the tube.

4. Return the used double head swab into the covering tube and push firmly closed. Please write your child's name on the covering tube.

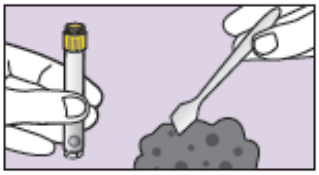
5. Place swab in the plastic biohazard zip lock bag, this can then be put in the mailing envelope with the other samples that you have collected.

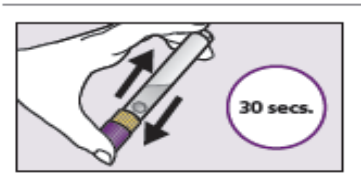
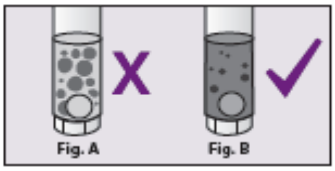
6. **DO NOT** throw the nappy away as you need to collect another sample from the same poo as per the instructions below.



Warnings and precautions:
• FOR EXTERNAL USE ONLY.
• Do NOT remove the yellow tube top from the tube.
• Do NOT spill the stabilizing liquid in the tube.
• Wash with water if liquid comes in contact with eyes or skin. Do NOT ingest.
• If stool sample is liquid or your baby has diarrhoea wait until the next bowel movement to

Step	Procedure	

1	Having collected one sample from the nappy using the swab you now need to collect another sample from the **SAME** nappy (same poo) using the instructions below.	
2		While holding the yellow tube top, unscrew ONLY the purple cap from the kit and set it aside for later use. IMPORTANT: Do not remove the yellow cap Do not spill the stabilization liquid in the tube
3		Use the spatula to collect a small amount of poo from  the nappy
4		Transfer the stool (poo) sample into the yellow tube top. If there is enough poo repeat until the sample reaches the top and fills it completely, if not fill it as much as you can.  IMPORTANT: Try not to push the sample into the tube.
5		Scrape horizontally across the tube top to level the sample and remove any excess. Discard the spatula.

		Wipe the exterior of the tube and top with toilet paper or tissue as needed.
6		Screw the purple cap back onto the yellow top tube until tightly closed 
7		Shake the sealed tube as hard and fast as possible in a back and forth motion for a minimum of 30 seconds.
8		The stool (poo) sample will be mixed with the stabilizing liquid in the tube. Not all particles will dissolve. IMPORTANT: Keep shaking if large particles remain as shown in Fig. A

Return postage:

1. Place both the swab and the purple top tube (OMNIgene GUT tube) in the plastic biohazard zip lock bag. This can then be put

in the mailing envelope with the other samples that you have collected.

2. Mail the envelope as soon as you can or store at refrigerated temperature until you are able to post it – this should be as soon as possible to preserve the sample.

INSTRUCTIONS FOR COLLECTING A STOOL SAMPLE (ADULT)

Kit contents: *BD BBL™ CultureSwab™ EZ II Swab, gloves, zip lock biohazard bag.* Fe-col collection device, OMNIgene- GUT collection kit.

Please collect **2** samples from the **same** poo. One into the tube with the purple lid according to the instructions and one using the swab as per the instructions.

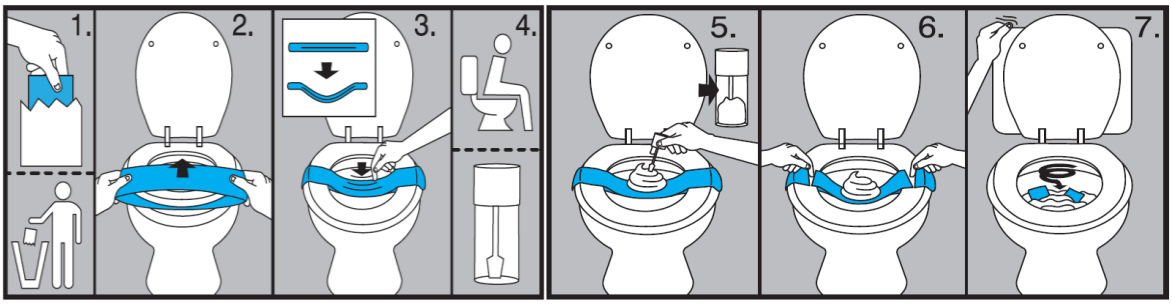
Please read the instructions before collecting the samples.

Things to consider:

- Avoid passing urine at the time of defecation.
- Avoid collecting the fecal specimen from the edges.

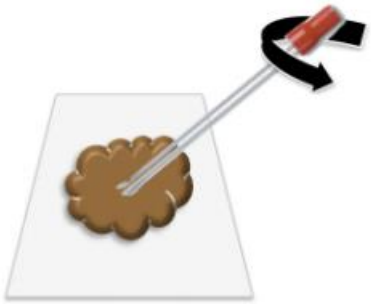
Procedure:

1. Pull the cap from the double swab, by grabbing the red cap and pulling. Set aside at a reachable distance
2. Follow the instructions on the Fe-Col collection device to capture the sample.



3. While wearing gloves collect the faecal (poo) specimen by gently rubbing the cotton end of the swab in the faeces

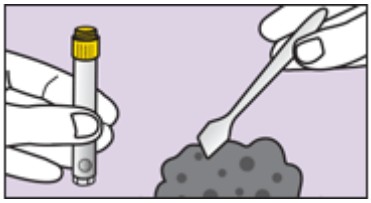
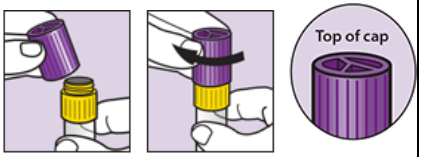
   that is on faeces collection device in a circular motion until both swab heads are covered with it. (See image below)

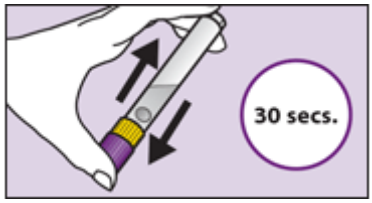
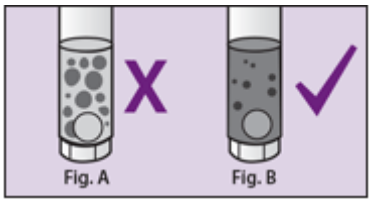


4. Return the used double head swab into the capsule and seal.

5. **DO NOT** flush the faeces (poo) away but collect a second sample following the instructions below.
6. Place swab in plastic zip lock this can then be put in the mailing envelope once you have collected the other sample.

Step	Procedure	
	Having collected one sample using the swab you now need to collect another sample from the **SAME** poo using the instructions below.	
1.		While holding the yellow tube top, unscrew ONLY the purple cap from the kit and set it aside for later use.

		IMPORTANT: Do not remove the yellow cap Do not spill the stabilization liquid in the tube
2.		Use the spatula to collect a small amount of faecal sample. 
3.		Transfer the faecal sample into the yellow tube top. Repeat until the sample reaches the top and fills it completely. IMPORTANT: Do NOT push sample into the tube. 
4.		Scrape horizontally across the tube top to level the sample and remove any excess. Discard the stick. Wipe exterior of tube and top with toilet paper or tissue as needed.
5.		Screw the purple cap back onto the yellow tube top until tightly closed.

6.		Shake the sealed tube as hard and fast as possible in a back and forth motion for a minimum of 30 - seconds.
7.		The faecal sample will be mixed with the stabilising liquid in the tube; not all particles will dissolve. IMPORTANT: Continue shaking if large particles remain as shown in Figure A.

<u>Return postage</u>:

- Place both the swab and the purple top tube (OMNIgene GUT tube) in the plastic biohazard zip lock bag. This can then be put in the mailing envelope with the other samples that you have collected.
- Mail the envelope as soon as you can or store at refrigerated temperature until you are able to post it – this should be as soon as possible to preserve the sample.

Thank you very much for your help with this study. If you have any queries please contact Dr Georgina Williams on email: fades-study@bristol.ac.uk or Tel:0117 3421756.

NIHR BRISTOL BIOMEDICAL RESEARCH CENTRE NUTRITION THEME

Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)

Instructions for sample Collection

Thank you for participating in this study. We appreciate that completing the questionnaires and collecting the samples will take time and we want to support you with this in any way we can.

If you have any questions regarding either the questionnaires or collecting the samples please do not hesitate to contact us either by email fades-study@bristol.ac.uk or telephone 0117 342 1756. You may also find the answer you are looking for on the website http://www.uhbristol.nhs.uk/research-innovation/our-research/bristol-nutrition-bru/fades-study/.

We will be in touch with you to help you to arrange having the blood test taken at the same time as one of your child's routine health care appointments.

Please take everything included within this pack including this instruction leaflet and your copy of the consent form to the appointment.

This pack contains:

Kit for blood collection

And a sheet for recording the date and time that your child's samples were taken. Please also note on this sheet whether the sample was from a heel prick / finger prick or from a vein (if taken at the same time as other routine blood tests)

The instructions for collecting the blood samples are within this booklet. There is also a factsheet for health care professionals at the back of the booklet.

We understand that you may not be able to collect the blood samples at the same time as the other samples.

Once the blood sample has been taken it can be sent back separately in the packaging which is marked blood sample.

Heel Prick Instructions For Parents and/or Health Professional

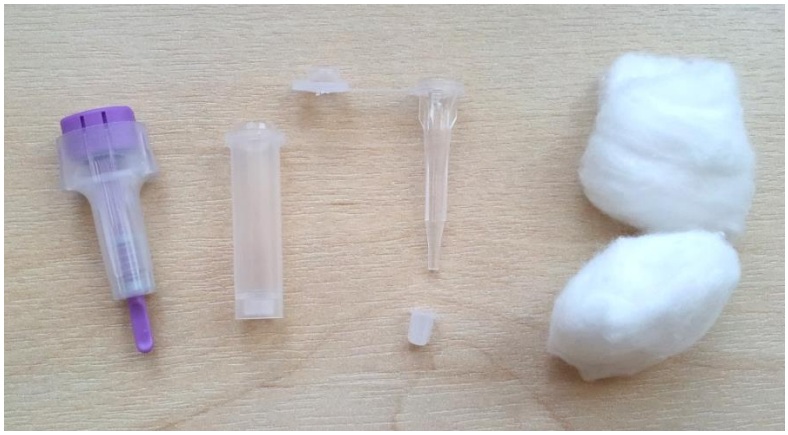
The initial blood test should be taken by a health professional and we will contact you in order to help you with coordinating this with one of your baby's routine health checks.

We understand that it is not easy for a parent to watch blood being taken from their child but the discomfort only lasts a few minutes and your baby will be quickly comforted after by a cuddle with you.

If having observed the initial sample you feel you would prefer to take the other samples (at 6months, a year and then annually) at home yourself please follow the instructions below:

It is much easier to take the blood with two people, one to cuddle your baby and one to take the blood.

1. Lay out the equipment so that you can reach it all easily - dry cotton wool, warm water (never place hot water near your baby), lancet (very small needle, we have provided you with a spare lancet) and blood collecting tubes x2.



To prepare the blood collection tube you need to **remove the small cap from the bottom of the tube** but keep it close as you will need to put it back on at the end.

2. There is evidence that the discomfort for your baby is reduced if you are able to have your baby next to your skin (skin to skin) whilst the blood is being taken.

3. The person taking the blood should wash their hands with soap and water or alcohol gel.

4. Wash your baby's heel with warm water and dry. The person taking the blood should encircle your baby's heel in their hand, they should not need to squeeze the foot or hold the foot tightly.

5. The blood is taken from the side of the heel (shaded areas on picture below) do not use the bottom of the foot or the back of the heel.

6. Prepare the safety lancet by twisting off the green tab at the end. Whilst holding the heel with one hand, place the lancet against the area where you want to take the blood and depress the top of the lancet. This may make your baby startle and cry but it is important to collect the blood before it stops bleeding.

7. Wipe the first drop away with some dry cotton wool and then, holding the sample collection tube horizontally (with both ends open), place the thin end of the tube next to the next blood drop and start collecting. The blood should be sucked into the tube via capillary action. Once the tube starts to get full you may need to place the lid on the bigger end to prevent the blood from dripping out. You may need to gently squeeze and release the heel in order to keep the blood flowing but this can be done very gently and does not need to be firm.

8. Please collect as much as you are able to, if possible into **one** tube if the heel keeps bleeding please then also use the spare tube (Sometimes it helps to wipe the heel with the cotton wool part way through collection, particularly if the blood spreads rather than going in the bottle). If the blood is no longer going into the tube from the bottom but the heel is still bleeding place the small cap on the bottom and allow the drops to go in from the top.

9. Once you have finished collecting the blood first place the cap on the bottom

…and then place the lid on the top of the bottle. Make sure it is <u>on securely</u>.

This tube can now be placed back in the outer tube.

10. You may find that your baby's heel stops bleeding after only a few drops, or they may continue bleeding even after you have filled the bottles. If their heel is still bleeding just gently press some dry cotton wool against their heel for a few seconds and it should stop.

11. The used lancet can be placed in the clear plastic bag marked 'biohazard' with the blood bottle these can both go into the prepaid package to be sent back with the other samples.

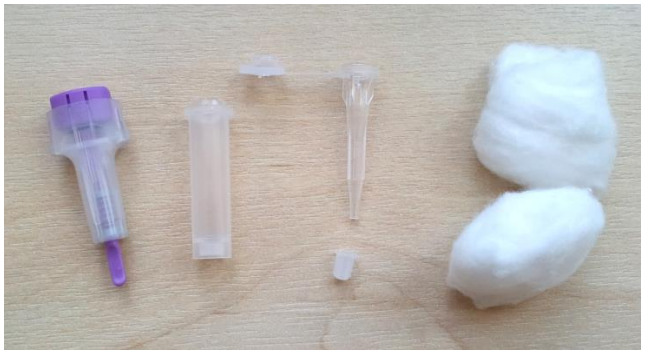
INSTRUCTIONS FOR COLLECTING A FINGER PRICK BLOOD SAMPLE

(For babies over the age of 12 months only, babies younger than 12 months should have a heel prick blood test)

You may be taking this sample yourself at home or this maybe done during one of your child's routine health checks. We understand that

it is not easy for a parent to take blood from their own child but the discomfort only lasts a few minutes and your child will be quickly comforted after by a cuddle with you. This will be much easier with two people, one to cuddle your child and one to take the blood.

1. Lay out the equipment so that you can reach it all easily - dry cotton wool, warm water, lancet (very small needle, we have provided you with a spare lancet) and blood collecting tubes x2.

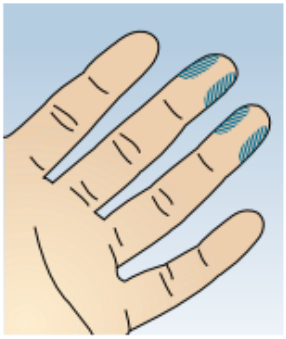


To prepare the blood collection tube you need to **remove the small cap from the bottom of the tube** but keep it close as you will need to put it back on at the end.

2. The person taking the blood should wash their hands with soap and water.

3. Wash your child's hand with warm water and dry.

4. Select one of the following (shaded)sites to puncture:



5. Prepare the safety lancet and puncture the skin as shown:

Twist the tab at the bottom until it separates from the lancet.

Press the lancet **very firmly** against the finger to ensure a good puncture site and blood flow. You may wish to rest the finger on a flat surface, and press the

6. Discard the first drop of blood by blotting finger on the gauze swab.

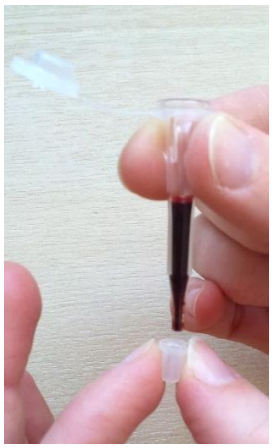
7. Collect the blood as shown below

To maintain good blood flow, hold arm downwards and you can gently massage your child's hand.

Avoid repeated strong pressure around the puncture site (milking) as this will be uncomfortable for your child and may spoil the sample.

Please collect as much as you are able to, if possible into **one** tube if the finger keeps bleeding please then also use the spare tube. (Sometimes it helps to wipe the area with the cotton wool part way through collection if the blood spreads rather than going in the bottle). If the blood is no longer going into the tube from the bottom but the finger is still bleeding place the small cap on the bottom and allow the drops to go in from the top.

10. Once you have finished collecting the blood first place the cap on the bottom



and then place the lid on the top of the bottle. Make sure it is <u>on securely</u>.

This tube can now be placed back in the outer tube.

11. You may find that your child's finger stops bleeding after only a few drops, or they may continue bleeding even after you have filled the bottles. If their finger is still bleeding just gently press some dry cotton wool against their finger for a few seconds and it should stop.

The used lancet can be placed in the clear plastic bag marked 'biohazard' with the blood bottle these can both go into the prepaid package to be sent back with the other samples.

Study Fact Sheet for Health Professionals

Study title: Feeding and Autoimmunity in Down's syndrome Evaluation Study (FADES)

What is the aim of the FADES study?

To develop a family acceptable study protocol and establish the feasibility of creating a national cohort of infants with Down's syndrome (DS) to study the associations between early infant feeding, infections and the development of autoimmunity in Down's syndrome.

Who is responsible for this study?

This study has been set up as a partnership between the NIHR, Bristol Biomedical Research Unit in Nutrition, The University of Bristol, School of Clinical Sciences, Diabetes Research Group, Imperial College

London, Department of Medicine, the Down's Syndrome Association and Down's Syndrome Scotland.

What is the background to the study?

Children with Down's Syndrome (DS) have increased risk of autoimmune conditions including thyroid problems, diabetes and coeliac disease. In DS, autoimmunity is likely to be related to lifelong inherent defects in the immune system. The risk of diabetes-related autoimmunity is increased despite a reduced prevalence of the usual HLA haplotypes commonly associated with type 1 diabetes. Infant feeding practice has been linked to diabetes and coeliac risk with some evidence that prolonged breastfeeding is protective. We hypothesise that in infants with DS, already at increased risk, early feeding practices may be related to the development of autoimmunity. Children with DS have hypotonia and therefore have difficulties with breastfeeding leading to the rapid introduction of formula feeds which contain modified cow's milk protein.

How were the participants recruited?

Infants are recruited through the Down's Syndrome Association (DSA) and Down's Syndrome Scotland (DSS).

What would be my involvement?

During this study we will be collecting a variety of data from each participant and their family as shown in the table below, the majority of the samples will be collected by the parents at home and they have been given all the necessary instructions and equpiment. They will also complete the questionnaires at home. **We are asking if you could kindly assist with taking the blood samples when you see the participant for their routine health checks**. The blood samples at 12months and yearly thereafter can be taken at the same time as their recommended annual thyroid check.

Months of age	**0* Baseline**	**6**	**12**	**Every year around the time of the participant's birthday until January 2022**
Day 5 blood spot for autoantibodies	**X**			
Combined feeding and medical questionnaire	**X**	**X**	**X**	
Medical questinnaire				**X**
Mouth swab for DNA extraction	**X**			
Stool for microbiome	**X**	**X**	**X**	**X**
Heel or finger prick blood	**X**	**X**	**X**	**X**

sample for autoantibodies and BSA antibodies.**				
Urine for protein C analysis	**X**		**X**	**X**

Fig 1. Timeline for sample collection. (*at recruitment, **Bovine serum Albumin)

All the necessary equipment for collecting the sample will be provided by us and the parents will bring this to their appointment with you.

How do I return the samples?

The blood bottles have already been labelled with the participants unique identifier code. The parents have been provided with special pre-paid packaging in which they can return the samples too us.

Will I be given the results of the tests?

We will only contact the participant's GP with the results of the tests if they are of clinical significance to the participant

Will I be informed of the outcomes of the Study?

If you are interested in the study and would like to be updated regarding its progress we can arrange to send you regular updates via email. At the end of the study we can provide you with a summary of the findings and copies of any publications that you would like to receive.

Thank you for your help and support with this study!

We hope that the outcomes of the study may benefit children born with DS in the future. From this study we hope to go on and do a further study to develop an intervention to help with feeding in babies born with DS. We also hope the study will increase our knowledge of autoimmune conditions and will provide increased information in this area for the parents of children with DS as well as those that care for them.

NIHR BRISTOL NUTRITION BIOMEDICAL RESEARCH UNIT

Feeding and Autoimmunity in Down's Syndrome Evaluation Study

(FADES)

Dear Parents,

Thank you so much for your ongoing participation in the FADES study. We are trying to improve this study and also make recommendations for future research for babies with Down's Syndrome.

Why have I been contacted?

We are contacting families who are currently participating in the FADES study as well as those who have shown an interest in the study but have not gone on to take part. We want to find out what initially interested them and either their experience of being part of the study or why they did not go on to enrol in the study.

This will help us to improve the study and will help to inform future research with families who have a child with Down's Syndrome.

What am I being asked to do?

If you would like to help us please complete this short anonymous questionnaire online it should take less than 10 minutes to complete. If you would prefer a paper version of the questionnaire please let us know and we will send this to you together with a stamped addressed envelope.

Thank you

Dr Georgina Williams / FADES Research Team

Email: fades-study@Bristol.ac.uk

1. How did you hear about the FADES Study? Please tick any that apply

Through the Down's Syndrome Association website	☐
From the flyer in the Down's Syndrome Association New Parent pack	☐
From a support worker from Down's Syndrome Scotland	☐
Through the flyer in the Down's Syndrome Scotland new parent pack	☐
From a community paediatric doctor	☐
From a neonatal doctor	☐
Through a friend	☐
Through social media: please provide detail if you can	☐
Other: please provide detail if you can	☐

2. What initially interested you in the FADES Study?

3. Why did you decide to take part in the FADES study?

4. What did you think about the study website? (please circle on the scale below)

Attractive	1	2	3	4	5	Unattractive
Clear to understand	1	2	3	4	5	Difficult to understand

5. How would you recommend we improve the information that is provided about the study?

6. What improvements in the study do you think we could we make to encourage families to take part?

7. If you have any further comments to make about the FADES study or any general comments about recruiting families who have recently had a baby with Down's Syndrome, to research studies please make them here.

Appendix 20: Non-Engagement / Non- Participant Questionnaire

NIHR BRISTOL NUTRITION BIOMEDICAL RESEARCH UNIT

Feeding and Autoimmunity in Down's Syndrome Evaluation Study

(FADES)

Dear Parents,

Thank you so much for having contacted and shown an interest in the FADES study. We are trying to improve this study and also make recommendations for future research for babies with Down's Syndrome.

Why have I been contacted?

We are contacting families who have either shown an interest in the FADES study but have not gone on to take part, or those that have started in the study but are no longer actively participating. We want to find out what initially interested them in FADES and why they did not go on to enrol or complete the study.

This will help us to improve the study and will help to inform future research with families who have a child with Down's Syndrome.

What am I being asked to do?

If you would like to help us please complete this short anonymous questionnaire online it should take less than 10 minutes to complete. If you would prefer a paper version of the questionnaire please let us know and we will send this to you together with a stamped addressed envelope.

Thank you

Dr Georgina Williams / FADES Research Team

Email: fades-study@Bristol.ac.uk

1. How did you hear about the FADES Study? Please tick any that apply

Through the Down's Syndrome Association website	□
From the flyer in the Down's Syndrome Association New Parent pack	□
From a support worker from Down's Syndrome Scotland	□
Through the flyer in the Down's Syndrome Scotland new parent pack	□
From a community paediatric doctor	□
From a neonatal doctor	□
Through a friend	□

Through social media: please provide detail if you can □
Other: please provide detail if you can □

2. What initially interested you in the FADES Study?

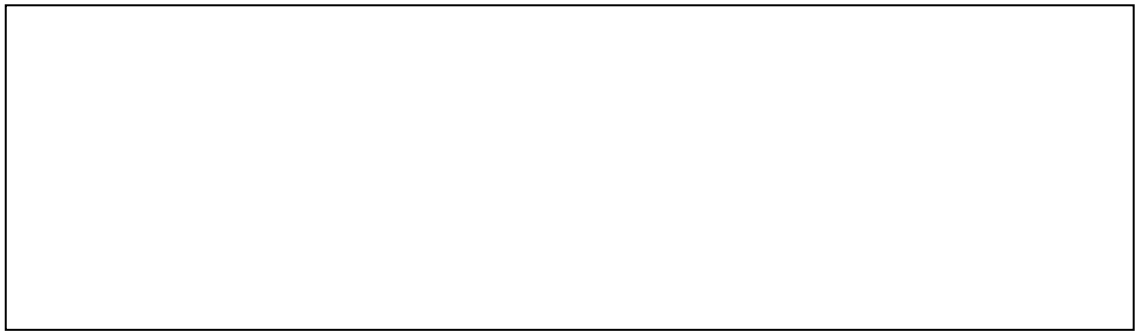
3. Why did you feel unable to participate or continue in the study? Please tick all that apply and provide any further detail or comments below

Our baby was too unwell □
We meant to take part but did not get round to it □
We decided not to take part because of the time that the study would involve □
We did not want to take the stool, urine and mouth brush samples □
We did not want to take the blood samples □
We were worried about completing the questionnaires □
Other: Please describe in box below

4. What did you think about the study website? (please circle on the scale below)

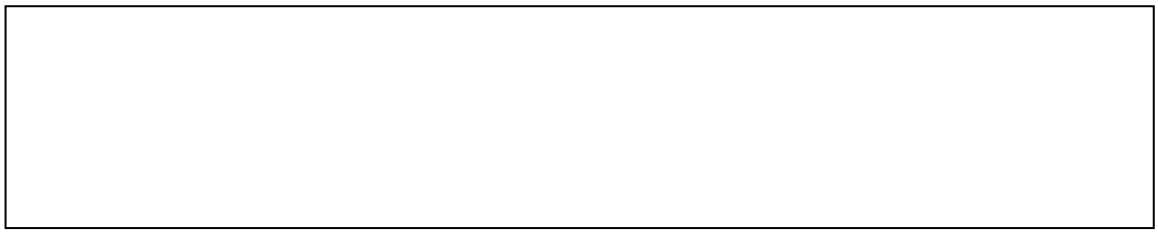
Attractive	1	2	3	4	5	Unattractive
Clear to understand	1	2	3	4	5	Difficult to understand

5. How would you recommend we improve the information that is provided about the



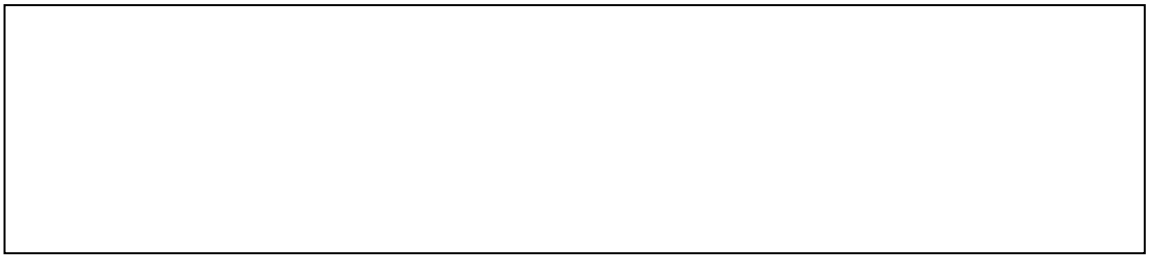
study?

6. What improvements in the study do you think we could we make to encourage



families to take part?

If you have any further comments to make about the FADES study or any general comments



about recruiting families who have recently had a baby with Down's Syndrome, to research studies please

Appendix 21: Examples of errors corrected during study:

- 9th September 2015 it was found that prior to this date the question Q2.14 “Were you given, or did your red book contain the specific Down’s Syndrome insert with growth charts for babies with Down’s Syndrome?” had not been available to all participants due to branching. It had not therefore been answered by everyone. If they had answered “no” to 2.8b it had skipped to 2.15 (paper version was also wrong). The online / REDCap questionnaire and paper questionnaire were both corrected appropriately to prevent participants being branched away from this question. Participants who had incorrectly missed this question were emailed to ask Q2.14 and they responded to the email.
- 14th November 2016 – found that the age limit on the 12 month questionnaire was not enough. Some parents who were completing it late could not enter their child’s age, as up until this point the limits within that field only allowed up to 62 weeks to be entered onto the online/ REDCap questionnaire. The limits on this question was changed

Appendix 22: Example of Variables Master Sheet

Excerpt from 7 month master sheet

Example from the 7 month questionnaire variable master sheet. The first column shows which variables were newly created. For example, the first two variables are variables which were too long straight from RedCAP and therefor had to be shortened. The other variables which say "NEW" were created from free text responses.

	Renamed and new variables	Old variable names from RedCAP	Question from Questionnaire
	m7_spec_inv_bab_care_1_3_6	m7_speclsts_invol_babys_care_1_3___6	Which specialists are involved in your baby's care? (choice=Other)
	m7_spec_inv_bab_care_oth_1_3a	m7_speclsts_invol_babys_care_other_1_3a	If someone else please specify
NEW	m7_num_spc_inv		Number of specialists invoved in baby's care
NEW	m7_spec_inv_care_allergy_1_3acd		Allergy specialist
NEW	m7_spec_inv_care_audio_1_3acd		Audiologist
NEW	m7_spec_inv_care_Comnurse_1_3acd		Community nurse/ health visitor /Paediatric nurse
NEW	m7_spec_inv_care_cranio_1_3acd		Cranio-facial /head specialist
NEW	m7_spec_inv_care_ENT_1_3acd		Ear nose and throat speciallist (ENT)
NEW	m7_spec_inv_care_haemonc_1_3acd		Haematology/ oncology
NEW	m7_spec_inv_care_hepat_1_3acd		Hepatologist
NEW	m7_spec_inv_care_surg_1_3acd		Surgeons
NEW	m7_spec_inv_care_OT_1_3acd		Occupational Therapist
NEW	m7_spec_inv_care_opthal_1_3acd		Opthalmology
NEW	m7_spec_inv_care_port_1_3acd		Portage / early years teacher

NEW	m7_spec_inv_care_Psych_1_3acd		Psychotherapist
NEW	m7_spec_inv_care_Physio_1_3acd		Physiotherapist
NEW	m7_spec_inv_care_stoma_1_3acd		Stoma nurse
		m7_bby_cnd_heart_1_4a	Since completing the last questionnaire has your baby been diagnosed with any of the following medical conditions: A heart condition?
		m7_bby_cnd_heart_spec_1_4a1	Please specify if you know the name of the heart condition

Appendix 23: Antibiotic codes and answers

Examples from questions:

Antibiotics used in first trimester (1_15b1a) abx mother 1st trim
Not known
Amoxicillin

Infections in first trimester (init_frst_tri_wht_infc_1_15b1b) mother conditions first trim
Pleurisy
UTI
Tooth infection

Antibiotics given to baby age 0-1 month (init_bby_age_antb_1_19c2a) bby abx age 0-1 month
Not known
Ciprofloxacin
IV benzylpenicillin
Penicillin
Amoxicillin
Benzylpenicillin and gentamicin
Trimethoprim (prophylaxis)

Infections for which baby received antibiotics age 0-1 month (init_bby_age_antb_1_19c2b) what infection age 0-1 month
Not known
Suspected sepsis
Help with chemo
Prophylactic
Bronchiolitis / cough / chest infection
NEC
Umbilical infection

Antibiotics

For Antibiotics Mother during preg

Not known
Amoxicillin
nitrofurantoin

anti viral
iv meropenem and teicoplanin
cephalexin

For antibiotics mother BFdg

Not known
co amoxiclav
metronidazole
clarithromycin
Flucloxacillin
Augmentin

Antibiotics for baby

Not known
Ciprofloxacin
IV benzylpenicillin
Penicillin
Amoxicillin
Benzylpenicillin and gentamicin
Trimethoprim (+prophylaxis)
Flucloxacillin
Chloramphenicol
Co-amoxiclav
Azithromycin

Baby infections

Not known
Suspected sepsis
Help with chemo
Prophylactic
Bronchiolitis / cough / chest infection
NEC
Umbilical infection
cellulitis
conjunctivitis
prophylaxis (surgery)
UTI
Prophylaxis for chest
Post op infection
Tonsillitis
Suspected meningitis

Antibiotics Code

1. Amoxicillin
2. Azithromycin
3. Cephalexin
4. Chloramphenicol
5. Ciprofloxacin
6. Clarithromycin
7. Co-amoxiclav
8. Flucloxacillin
9. Penicillin
10. Trimethoprim
11. Cephalexin and Nitrofurantoin
12. Co-amoxiclav and Metronidazole
13. IV Benzylpenicillin
14. IV Benzylpenicillin and Gentamicin
15. IV Meropenem and Teicoplanin
16. IV Meropenem and Teicoplanin and Cephalexin
17. Anti-viral
18. Not Known
19. IV Benzylpenicillin, gentamicin and prophylactic trimethoprim
20. IV Benzylpenicillin and Gentamicin and chloramphenicol
21. IV Benzylpenicillin, gentamicin, metronidazole and prophylactic trimethoprim
22. Amoxicillin and erythromycin
23. IV co-amoxiclav and flucloxacillin
24. Fucidin cream
25. Erythromycin and penicillin
26. Amoxicillin, azithromycin and vancomycin
27. Co- amoxiclav, clindamycin and Tamiflu

Stata coding :

0 "None" 1 "Amoxicillin" 2 "Azithromycin" 3 "Cephalexin" 4 "Chloramphenicol" 5 "Ciprofloxacin" 6 "Clarithromycin" 7 "Co-amoxiclav" 8 "Flucloxacillin" 9 "Penicillin" 10 "Trimethoprim" 11 "Cephalexin and Nitrofurantoin" 12 "Co-amoxiclav and Metronidazole" ///

13 "IV Benzylpenicillin" 14 "IV Benzylpenicillin and Gentamicin" 15 "IV Meropenem and Teicoplanin" 16 "IV Meropenem and Teicoplanin and Cephalexin" 17 "Anti-viral" 18 "Not Known" 19 "IV Benzylpenicillin, gentamicin and prophylactic trimethoprim" ///

20 "IV Benzylpenicillin and Gentamicin and chloramphenicol" 21 "IV Benzylpenicillin, gentamicin, metronidazole and prophylactic trimethoprim" 22 “Amoxicillin and erythromycin” 23 “IV co-amoxiclav and flucloxacillin” 24 “Fusidin cream” 25 “Erythromycin and penicillin” 26 “Amoxicillin, azithromycin and vancomycin” 27 “Co- amoxiclav, clindamycin and Tamiflu”

Codes for infections in baby/child

1. Cellulitis
2. Chest infection/ Bronchiolitis / cough
3. Tonsillitis
4. Umbilical infection
5. Urinary Tract Infection

6. Suspected sepsis in neonatal period / precautionary
7. Suspected Meningitis
8. Necrotising Enterocolitis
9. Post Op infection
10. Not Known
11. Prophylaxis for chest
12. Prophylaxis for surgery
13. Prophylaxis UTI
14. Prophylaxis other/ chemo
15. Conjunctivitis
16. Suspected sepsis and conjunctivitis
17. Chest infection and cellulitis
18. Chest infection and UTI prophylaxis
19. Viral infection (unspecified)
20. Central line infection – Streptococcal
21. Viral infection (specified)
22. Suspected meningitis, conjunctivitis, chest and ear infection
23. UTI and C.Diff

Stata coding:

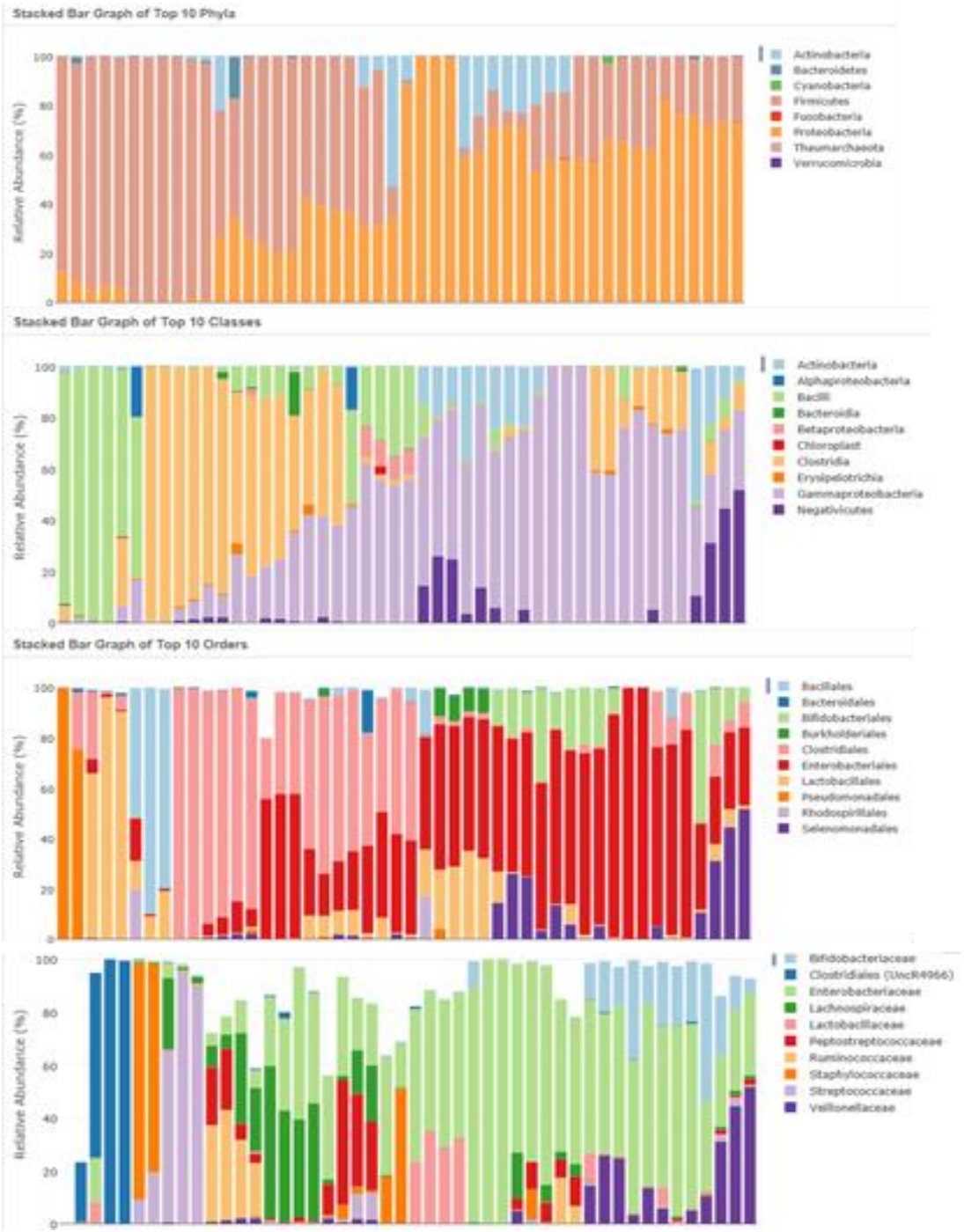
0 "No infection" 1 "Cellulitis" 2 "Chest infection/ Bronchiolitis/ Cough" 3 "Tonsilitis" 4 "Umbilical Infection" 5 “Urinary Tract Infection(UTI)" 6 "Suspected Sepsis in the neonatal period / precautionary" 7 "Suspected meningitis" 8 "Necrotising Enterocolitis (NEC)" 9 " Post Op Infection" 10 "Not Known" 11 "Prophylaxis for chest" 12 "Prophylaxis for surgery" 13 "Prophylaxis for UTI" 14 "Prophylaxis other/chemo" 15 “Conjunctivitis” 16 "Suspected Sepsis and Conjunctivitis" 17 "Chest infection and cellulitis" 18 "Chest infection and UTI prophylaxis" 19 "Viral infection" 20 "Central line infection - Streptococcal" 21 “Viral infection (specified)” 22 “Suspected meningitis, conjunctivitis, chest and ear infection” 23 “UTI and C.Diff”

label define init_bby01_inf_1_19c2bcd 0 "No infection" 2 "Chest infection/ Bronchiolitis/

Cough" 4 "Umbilical Infection" 6 "Suspected Sepsis in the neonatal period / precautionary" 8

"Necrotising Enterocolitis

Appendix 24: Relative Abundance of Phyla, Classes, Order, Families and Genera observed in the 48 samples analysed

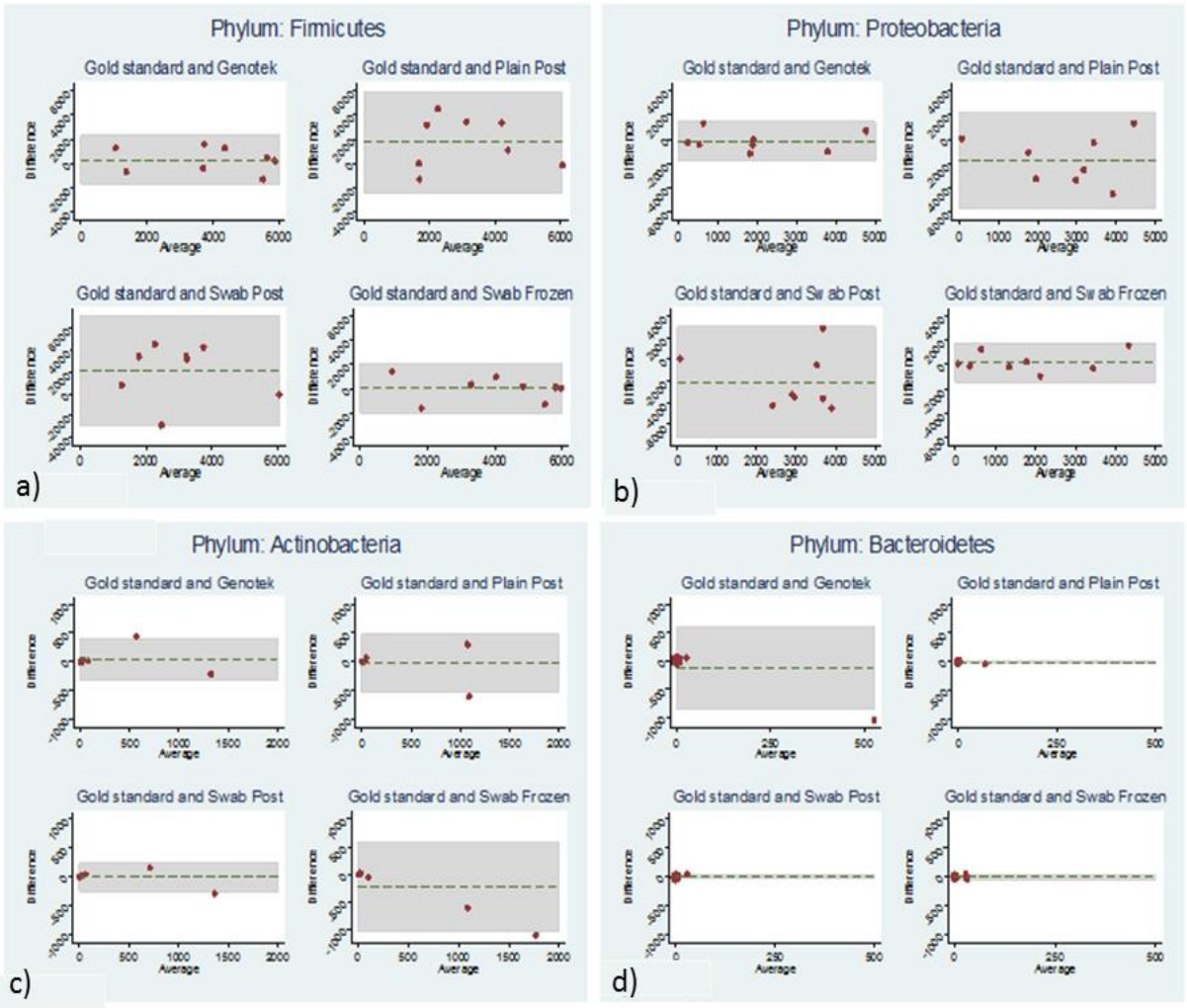


Stacked bar chart showing Relative Abundance of Phyla, Classes, Order, Families and Genera observed in the 48 samples analysed.

Appendix 25: The Bland–Altman analysis for abundance of a)*Firmicutes*, b)*Proteobacteria*, c)*Actinobacteria* and d)*Bactriodetes* for the four different methods of sample collection compared to the frozen standard of immediate freezing.

	Methodology compared to frozen standard (Average difference, (95% Limit of Agreement)			
Phylum	**OMNIgene•GUT**	**Plain post**	**Swab post**	**Swab frozen**
Actinobacteria	35.6 (-320.5, 391.7)	-24.6 (-522.2, 473)	-6.3 (-249.2, 236.7)	-213.4 (-1019.8, 593)
Bacteroidetes	-124 (-851.8, 603.8)	-3.3 (-30.7, 24.2)	6.8 (-24.6, 38.1)	-0.2 (-47.4, 46.9)
Firmicutes	288 (-1729.2, 2305.2)	1755.5 (-2379.7, 5890.7)	2081 (-2960.2, 7122.7)	34.8 (-1966.1, 2035.6)
Proteobacteria	-198.4 (-1822.3, 1425.6)	-1727.5 (-5683.6, 2228.6)	-2081.4 (7182.5, 3019.8)	180.8 (-1452.2, 1813.7

BA analysis of the relative abundance of the four most abundant phyla for the four different methods of sample collection compared to the frozen standard.

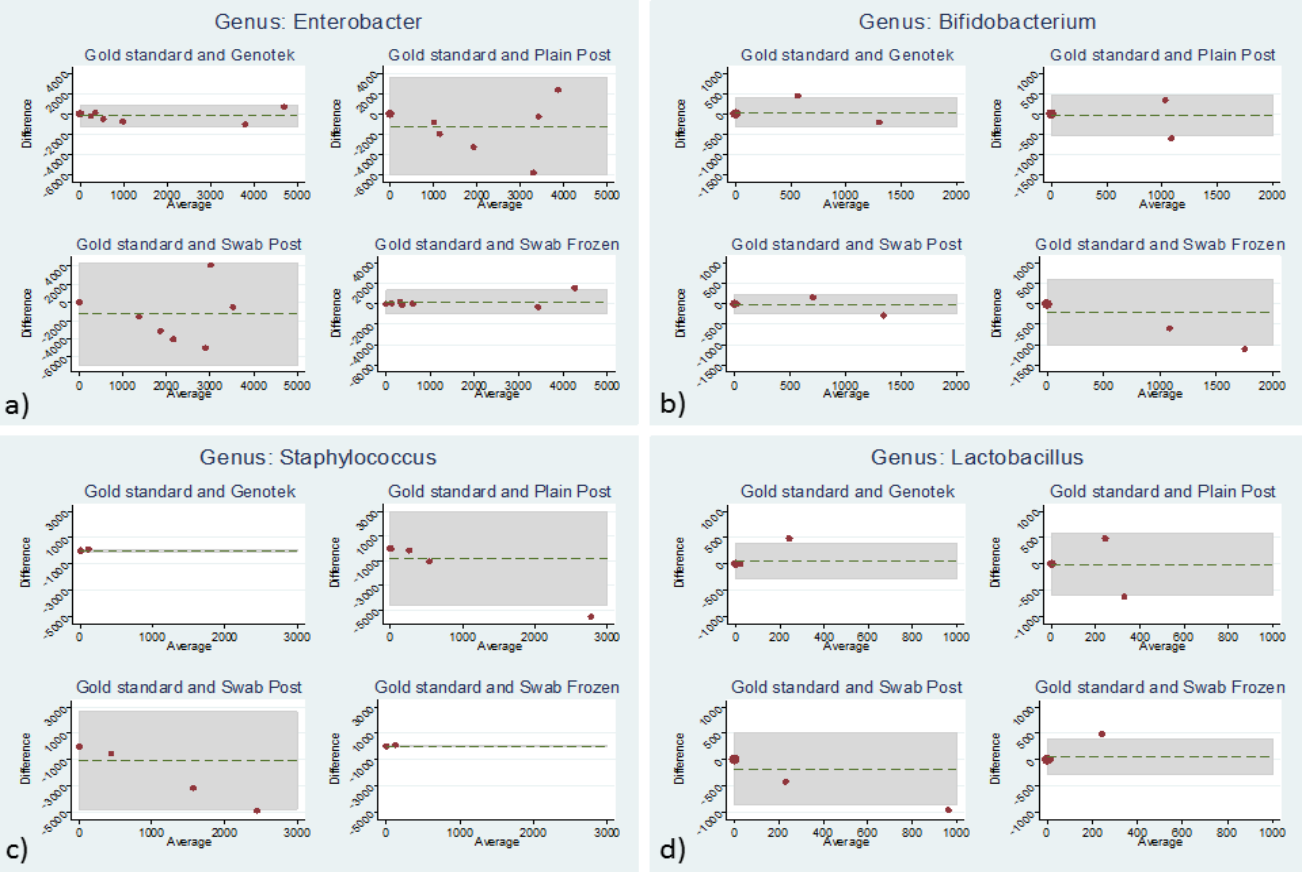


The Bland–Altman plots for abundance of a)*Firmicutes*, b)*Proteobacteria*, c)*Actinobacteria* and d)*Bactriodetes* for the four different methods of sample collection compared to the frozen standard of immediate freezing.

Appendix 26: The BA analysis for abundance of a)Enterobacter, b)Bifidobacterium, c)Staphylococcusand d)Lactobacillus f or the four different methods of sample collection compared to the frozen standard of immediate freezing.

	Methodology compared to Frozen standard (Average difference, (95% Limit of Agreement)			
Genus	**OMNIgene•GUT**	**Plain post**	**Swab post**	**Swab frozen**
Enterobacter	-206.6 (-1271.6, 858.4)	-1217 (-6025.2, 3590.7)	-1266.9 (-6866.4, 4332.6)	162.4 (-982.2, 1307.0)
Staphylococcus	19.4 (-65.1, 103.9)	-848.3 (-4631.4, 2934.9)	-1066.4 (-4761.4, 2628.6)	17.3 (-54.4, 88.9)
Lactobacillus	60.88 (-275.8, 397.6)	-16.6 (-600.9, 567.7)	-170.5 (-853.1, 512.1)	61.8 (-274.3, 397.8)
Bifidobacterium	31.0 (-325.7, 387.7)	-32.8 (-538.1, 472.6)	-15.9 (-252.3, 220.5)	-211.6 (-1029.0, 605.7)

Bland Altman analysis of the relative abundance of *Enterobacter, Staphylococcus, Lactobacillus* and *Bifidobacterium* for the four different methods of sample collection compared to the gold standard of immediate freezing.



The Bland–Altman plots for abundance of a)*Enterobacter*, *b)Bifidobacterium*, *c)Staphylococcus*and *d)Lactobacillus* for the four different methods of sample collection compared to the frozen standard of immediate freezing.

Appendix 27: Table Summarising the Study in relation to the features of a feasibility study

	Supporting	Against	Amendments or improvements to be made for main study
Ability to complete measures			
Comprehension	Participants were able to follow the instructions for data collection. No major issues with either the questionnaires or sample collection were identified	As data was collected at a distance via post and internet rather than face to face. Potentially the research team were unaware of those who did have issues with comprehension	No changes made
Capacity	The research team were able to manage the data collection, storage of samples and sample analysis.	None	No changes made

	There were no concerns raised about capacity		
Appropriateness of the amount of data collected	Participants completed the detailed questionnaires and included extra detail in the free text boxes. A reasonable proportion of participants collected all the requested samples.	The ethics committee and the DSA queried whether the questionnaires were too long. Due to the vast amount of data collected from the questionnaires, detailed analysis of all the data was not completed. Some participants completed the questionnaires but did not go on to collect the biological samples.	The questionnaire was shortened during the planning stages but could be shortened further. No changes made to the samples requested from participants

Whether data was complete and usable	The use of web-based questionnaires reduced the amount of missing data so this was minimal. The data was usable. As discussed in more detail in the sample collection section the proportion of samples that were collected and were usable was within the feasibility target. The collected samples were usable for analysis.	Due to the incorrect branching of some questions, participants were unable to complete some questions. This is discussed in more detail for the relevant questions	Correct the errors in the branching. Develop videos for demonstrating sample collection methods
Acceptability and suitability of study procedures			

Retention	Retention of participants was good.		
Adherence to study procedures	Participants followed the procedures well with no concerns. Flexibility with regards to the timings of data collection were built in to the protocol.	When local collaborators recruited participants the EOI form was not always completed and this prevented participants being fully enrolled into the study.	Inform local collaborators about the completion of the EOI form.
Engagement	Participants were very engaged as demonstrated by the high questionnaire completion rate.		

Time	As participants complete the questionnaires and most samples are collected at home this reduces the amount of researcher time.	Participants do spend considerable time completing questionnaires and collecting samples Organising and coordinating blood sample collection takes up a lot of researcher time with often multiple phone calls and emails. The approval process for multiple sites took up a large amount of time.	Shorten questionnaires Now approvals are largely in place the time involved in liaising with sites has reduced.
Burden	There is flexibility built in to enable families to complete questionnaires and samples in their own time.	The study requires a high input from the families	

	The timing of blood sample collection is designed so that the participants do not need to go to a separate appointment.		
Acceptability and suitability of the procedures	Participants collected samples without significant issues. Pilot studies for the collection of the urine and stool samples showed the suitability of the collection methods used for these.		

Appendix 28: FADES Newsletter (Example from March 2017)

FADES Newsletter

(Feeding and Autoimmunity in Down Sydrome Evaluation Study)

March 2017

The FADES study team are celebrating World Down Syndrome Day!

The FADES study team have put on their best socks and baked cakes ready to celebrate World Down Syndrome Day. We will be raising money for the Down's Syndrome Association and Down's Syndrome Scotland, as well as raising awareness of research for children with Down's syndrome.

The FADES Study Team and their socks!!

Dr Sam Leary, Professor Julian Hamilton-Shield, Georgina Mortimer, Stu Toms, Dr Aidan Searle, Dr Georgina Williams and Sofia Leadbetter.

FADES Update

We are thrilled to say that we now have 55 families enrolled in the FADES study.

The information you have provided from the initial questionnaires will be presented as an oral presentation at the Royal College of Paediatrics and Child Health Conference in May.

RCPCH Conference 2017
24-26 May 2017, Birmingham

Professor Julian Hamilton-Shield met with Professor Eleanor Molloy (Trinity College University, Dublin) and Down's Syndrome nurse Fiona McGrane, (Trinity Centre, Tallaght Hospital Dublin) in March to discuss an extension of FADES recruitment to Ireland in the near future

as a collaboration between the two groups to improve our understanding of Down's syndrome

FADES Families

Amos our newest FADES participant

We love to hear your stories and about your babies progress. Please do send us photos and updates that you would like to be included in the newsletter. Below Cheryl tells us about her experience of being in the FADES study.

"We found out about the FADES study not long after Alexander was born, through our local Down syndrome support group. After I found out more about it, we were very keen to take part. The research being undertaken could be crucial to understanding the nutritional needs of children with Down syndrome.

It is very easy to take part in the study. Every 6 months, we fill in a questionnaire and they have a blood, stool and urine sample test.

The extra support we have had with questions and queries around other areas of Alexander's health have been wonderful, and we feel privileged to be part of improving the lives of children with Down syndrome. We would encourage anyone thinking about joining the study to take the plunge!"

Children with Down's Syndrome and Research

We are very grateful to all of you who completed the survey asking for your views on FADES. These will help us to understand how to make it easier for families to get involved in research and will inform future studies.

If you have not completed this survey but would still like to, please send us an email and we would be happy to forward you the link.

The Poo (Stool) Collection Kit

Why are we collecting stool samples?

Our gut contains trillions of bacterial cells called the microbiome. Research has shown that the microbiome plays a role in health and disease. We are interested to find out whether the gut microbiome in children with Down's syndrome is different, and if this might have an effect on their risk of getting autoimmune conditions.

Why is there a new tube for collecting the samples?

The samples you collect at home are sent in the post back to us. During the time the samples are in the post we want to preserve them and stop the bacteria from growing. The new kit contains a liquid, which has been shown to preserve the stool microbiome at room temperature for several days.

If you have any queries about collecting any of the samples or would like more details about any part of the study to feature in the next newsletter please let us know

A BIG THANK YOU FROM FADES

We want to take this opportunity to thank all our participants and their families, the Down's Syndrome Association, Down's Syndrome Scotland, the Down's Syndrome Medical Interest Group and all of our collaborators: we couldn't do it without you!

Don't forget to let your friends know about the FADES study, we are still looking for new families.

If you would like any further information or if you would like to feature in the next newsletter please contact us:

fades-study@bristol.ac.uk

Appendix 29: Supplementary Tables for Feeding and Medical Questionnaires.

Length of hospital stay **between** questionnaires time points		
	From 7 month questionnaire (between initial and 7month questionnaire)	From 12 month questionnaire (between 7 month and 12 month questionnaire)
Less than a week	37.7 % (20/53)	67.6 % (25/37)
1 to <4 weeks	43.4 % (23/53)	18.9 % (7/37)
1 to <2 months	7.6 % (4/53)	8.1 % (3/37)
2 to <3 months	5.7 % (3/53)	0
3 to <4 months	3.8 % (2/53)	0
5 to <6 months	0	2.7 % (1/37)
More than 6 months	2.9 % (1/53)	2.7 % (1/37)

Table: Length of hospital stay between questionnaire time points

Maternal antibiotic use						
	First trimester	Second trimester	Third trimester	During labour	Whilst breastfeeding baby age 0 – 1 month	Whilst breastfeeding baby age 1 – 3 months

Yes antibiotics given	4.9 % (3/61)	8.2 % (5/61)	6.6 % (4/61)	6.7 % (4/60)	75.0 % (6/8)	57.1 % (4/7)
Amoxicillin	1.6 % (1/61)	1.6 % (1/61)	3.3 % (2/61)	n/a		
Clarithromycin				n/a	1.6 % (1/61)	1.6 % (1/61)
Co – amoxiclav				n/a	1.6 % (1/61)	1.6 % (1/61)
Flucloxacillin				n/a		1.6 % (1/61)
Co- amoxiclav and metronidazole				n/a	1.6 % (1/61)	
Cephalexin and nitrofurantoin		1.6 % (1/61)		n/a		
Anti-viral		1.6 % (1/61)		n/a		
IV Meropenem, teicoplanin and cephalexin			1.6 % (1/61)	n/a		
Type not known	3.3 % (2/61)	3.3 % (2/61)	1.6 % (1/61)	n/a	4.9% (3/61)	1.6 % (1/61)
None given	95.1 % (58/61)	92.9 % (56/61)	93.4 % (57/61)	n/a	90.2% (55/61)	93.4 % (57/61)

Table of Maternal Antibiotic use by type of antibiotic.

Infants antibiotic use		
Received any antibiotics since birth		
Yes	51.7 %	(31/60)
No	46.7 %	(28/60)

Don’t Know	1.7 %	(1/60)
Number of antibiotic courses between birth and completing initial questionnaire		
1	50.0 %	(15/30)
2	33.33 %	(10/30)
3	10.0 %	(3/30)
4	6.7 %	(2/30)
Received antibiotics since completing initial questionnaire (initial to 7 month)	28.0 %	(15/53)
Number of antibiotic courses between birth and 7 months mnth		
1	28.6 %	(4/14)
2	21.4 %	(3/14)
3	28.6 %	(4/14)
4	14.3 %	(2/14)
5	7.1 %	(1/14)
Received antibiotics since completing 7 month questionnaire (7 month to 12month)	62.5 %	(25/40)
Number of antibiotic courses that their child has had since completing the 7m questionnaire		
1	52.0 %	(13/25)

2	24.0 %	(6/25)
3	12.0 %	(3/25)
4	8.0 %	(2/25)
10	4.0 %	(1/25)

Table: Infants antibiotic use by number of courses of antibiotics.

	Age 0-1 month	Age 1-3 months	Age 7- 9 months	Age 9 -12 months

Amoxicillin	1.6 % (1/61)		5.0 % (2/40)	12.5 % (5/40)
Azithromycin			2.5 % (1/40)	2.5 % (1/40)
Chloramphenicol		3.3 % (2/61)		
Ciprofloxacin	1.6 % (1/61)	1.6 % (1/61)		
Co – amoxiclav		1.6 % (1/61)	2.5 % (1/40)	5.0 % (2/40)
Flucloxacillin		1.6 % (1/61)		
Trimethoprim	1.6 % (1/61)	1.6 % (1/61)		
IV benzylpenicillin	1.6 % (1/61)			
Amoxicillin, azithromycin and vancomycin				2.5 % (1/40)
Co-amoxiclav, clindamycin and Tamiflu				2.5 % (1/40)
IV Benzylpenicillin and gentamicin	4.9 % (3/61)			

IV Benzylpenicillin and gentamicin and trimethoprim	1.6 % (1/61)			
Type not known	21.3 (13/61)	8.2 % (5/61)	7.5 % (3/40)	15.0 % (6/40)
None given	65.6 % (40/61)	82.0 % (50/61)	82.5 % (33/40)	

Table: Infant Antibiotic use by antibiotic type

Infections or condition requiring antibiotics by age

	Age 0-1 month	Age 1-3 months	Age 3 months plus	Age 7-9 months	Age 9-12 months
Suspected sepsis in neonatal period	18.0 % (11/61)		n/a	n/a	n/a
Umbilical infection	3.3 % (2/61)		n/a	n/a	n/a
Necrotising enterocolitis	1.6 % (1/61)		n/a	n/a	n/a
Chest infection / bronchiolitis / cough	6.6 % (4/61)	4.9 % (3/61)	16.7% (9/54)	15.0 % (6/40)	40.0 % (16/40)
Conjunctivitis		3.3 % (2/61)		2.5 % (1/40)	
Urinary tract infection (UTI)		1.6 % (1/61)	1.9% (1/54)		2.5 % (1/40)
Cellulitis		1.6 % (1/61)			
Central line infection				2.5 % (1/40)	
Suspected meningitis			1.9% (1/54)		2.5 % (1/40)
Viral infection (specified)			1.9% (1/54)		2.5 % (1/40)

Prophylaxis other / chemo	3.3 % (2/61)	1.6 % (1/61)	1.9% (1/54)		
Prophylaxis for surgery		1.6 % (1/61)	1.9% (1/54)		
Prophylaxis for UTI	1.6 % (1/61)		1.9% (1/54)		
Not known	3.3 % (2/61)	3.3 % (2/61)			

Table: Type of Infection for which infant was given antibiotics

People who discussed feeding antenatally		

Doctor	11.5%	(7/61)
Health Visitor	18.0 %	(11/61)
Midwife	65.6 %	(40/61)
Nurse	3.3 %	(2/61)
Other	3.3 %	(2/61)
Where did you get specific advice regarding breastfeeding a baby with DS from		
DSA	3.3%	(2/61)
Other	1.6 %	(1/61)
Who helped put the baby to the breast (whilst in hospital)		
Midwife	51%	(31/61)
Midwifery support worker	13.1 %	(8/61)
Nurse	23.0 %	(14/61)
Nursery nurse	6.6 %	(4/61)
Healthcare assistant	6.6 %	(4/61)
Breastfeeding support group	4.9 %	(3/61)
Partner, friend or relative	8.2 %	(5/61)
Someone else	4.9 %	(3/61)
Who helped with feeding problems whilst in hospital		
Midwife	37.7 %	(23/61)
Midwifery support worker	13.1 %	(8/61)
Nurse	34.4 %	(21/61)
Nursery nurse	13.1 %	(8/61)
Healthcare assistant	6.6 %	(4/61)
Doctor / GP	3.1 %	(2/61)
Voluntary or charitable organisation	1.6 %	(1/61)

Peer supporter	1.6 %	(1/61)
Breastfeeding support group	4.9 %	(3/61)
Partner, friend or relative	6.6 %	(4/61)
Someone else	13.1 %	(8/61)
Source of help or information regarding feeding problems (after leaving hospital)		
Sure start / Children's centre / children's health clinic	8.2 %	(5/61)
Peer supporter	6.6 %	(4/61)
DSA	8.2 %	(5/61)
Breastfeeding support group	8.2 %	(5/61)
Partner, friend or relative	4.9 %	(3/61)
Start4Life	1.6 %	(1/61)
Books/leaflets/magazines	4.9 %	(3/61)
The internet / web based resources	13.1 %	(8/61)
Breastfeeding clinic	11.5 %	(7/61)
Doctor / GP	8.2 %	(5/61)
Health visitor	19.7 %	(12/61)
Midwife	18.0 %	(11/61)
Nurse	8.2 %	(5/61)
Somewhere else	6.6 %	(4/61)

Table: Feeding information and sources at different stages.

Solid feeds and weaning		
The first type of solid food given to baby		
Fruit (home prepared)	10.2 %	(5/61)

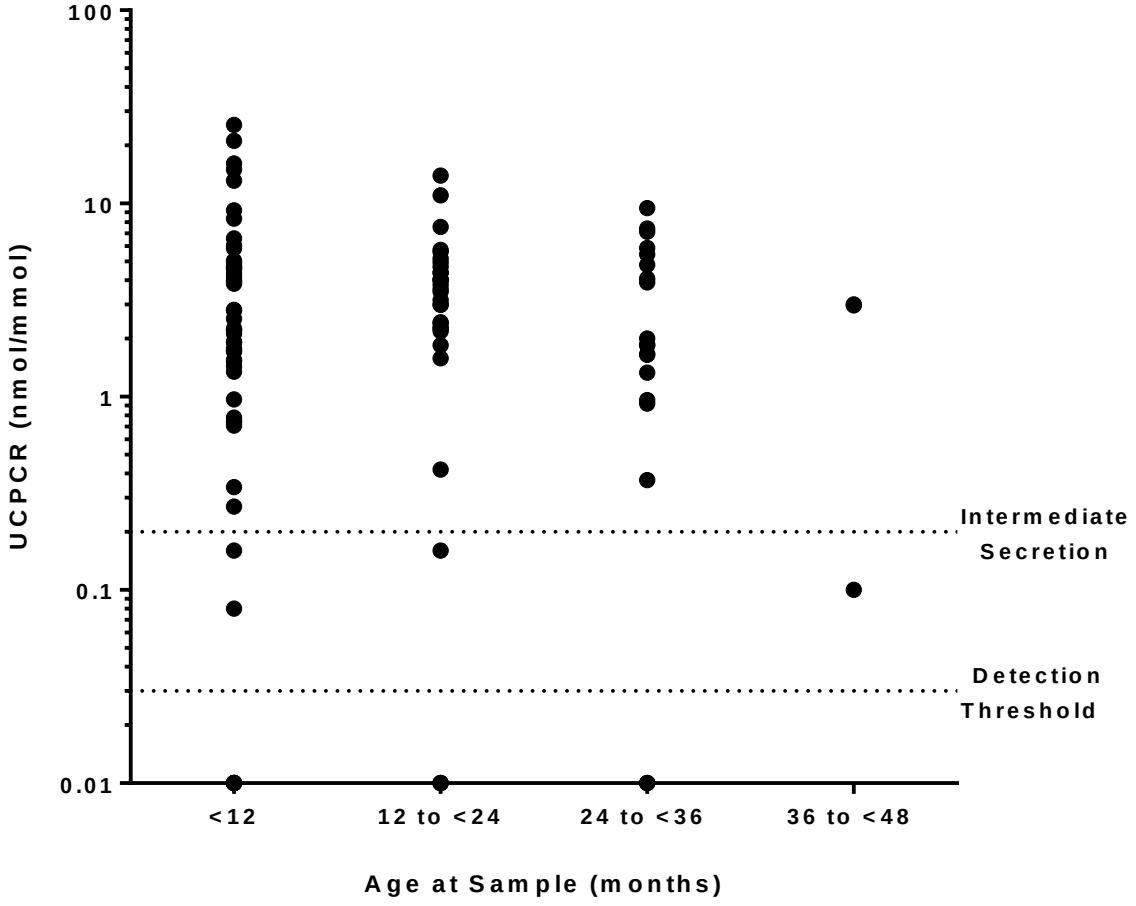
Vegetables (home prepared)	28.6 %	(14/49)
Homemade food	6.1 %	(3/49)
Baby rice	36.7 %	(18/49)
Ready made baby food	2.0 %	(1/49)
Any other food	16.3 %	(8/49)
Source (s) of information on which types of solid feeds to start on used by the parents		
SureStart or Children's Centre / Children's Health Clinic	24.1 %	(13/54)
Partner / Friend or relative	7.4 %	(4/54)
Voluntary or charitable organisation	1.9 %	(1/54)
Peer supporter	5.6 %	(3/54)
Breastfeeding support group	3.7 %	(2/54)
Start4Life	13.0 %	(7/54)
Books / Leaflets / magazines	35.2 %	(19/54)
Internet / web based resources	20.4 %	(11/54)
Health Visitor	46.3 %	(25/54)
Midwife (including at antenatal sessions)	1.9 %	(1/54)
Nurse	1.9 %	(1/54)
Somewhere else	16.7 %	(9/54)
Speech and language therapist	5.6 %	(3/54)
Dietician	5.6 %	(3/54)

Table: Weaning onto solids. Initial solid feeds and sources of information on solid feeds.

Types of solid feeds ever given		
	At 7 months	At 12 months
Ready made baby food	61.1 % (33/54)	80.0 % (32/40)
Rusk	18.5 % (10/54)	40.0 % (16/40)
Baby rice	66.7 % (36/54)	72.5 % (29/40)
Fruit	83.3 % (45/54)	90.0 % (36/40)
Vegetables	81.5 % (44/54)	90.0 % (36/40)
Homemade food	64.8 % (35/54)	90.0 % (36/40)
Other food	66.7 % (36/54)	97.5 % (39/40)

Table : Types of solid feed given

Appendix 30: Urine C-peptide Creatinine Ratio (UCPCR nmol/mmol) levels in samples from participants N=54, followed longitudinally (total samples tested N=109).



Urine C-peptide Creatinine Ratio (UCPCR nmol/mmol) levels in samples from participants N=54, followed longitudinally (total samples tested N=109). The threshold for detection is ≥0.03 nmol/mmol and the threshold for intermediate insulin excretion > 0.2 nmol/mmol.