TURUN YLIOPISTO
UNIVERSITY OF TURKU

IMMUNOLOGICAL MECHANISMS IN THE PATHOGENESIS OF TYPE 1 DIABETES

Milla Valta

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1715 | MEDICA – ODONTOLOGICA | TURKU 2023

TURUN YLIOPISTO
UNIVERSITY OF TURKU

IMMUNOLOGICAL MECHANISMS IN THE PATHOGENESIS OF TYPE 1 DIABETES

Milla Valta

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1715 | MEDICA – ODONTOLOGICA | TURKU 2023

University of Turku

Faculty of Medicine
Institute of Biomedicine
Medical Microbiology and Immunology
Turku Doctoral Programme of Molecular Medicine (TuDMM)

Supervised by

Docent Johanna Lempainen
Institute of Biomedicine
University of Turku, Finland

Professor Emeritus Jorma Ilonen
Institute of Biomedicine
University of Turku, Finland

Reviewed by

Docent Eliisa Kekäläinen
Department of Bacteriology and Immunology
University of Helsinki, Finland
Clinical Microbiology
HUS Diagnostic Center, Finland

Docent Niina Sandholm
Folkhälsan Research Center, Finland
University of Helsinki and Helsinki University Hospital, Finland

Opponent

Associate Professor Richard Oram
University of Exeter Medical School, United Kingdom

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-9275-1 (PRINT)
ISBN 978-951-29-9276-8 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2023

To my family

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Biomedicine
Medical Microbiology and Immunology
MILLA VALTA: Immunological mechanisms in the pathogenesis of type 1 diabetes
Doctoral Dissertation, 165 pp.
Turku Doctoral Programme of Molecular Medicine (TuDMM)
May 2023

ABSTRACT

Type 1 diabetes is a chronic autoimmune disease, in which insulin secreting beta cells in the pancreas are destroyed. The disease pathogenesis can be divided into an asymptomatic preclinical phase and symptomatic disease. The preclinical phase is characterised by the appearance of autoantibodies targeting pancreatic islet antigens and clinical onset of disease happens after the amount of functional beta cells becomes too low to sustain glucose homeostasis. While several genetic and environmental type 1 diabetes risk factors have been identified, the exact mechanism of the autoimmune process leading to the disease remains unknown. Additionally, strong heterogeneity in the disease pathogenesis has been observed and different possible pathways, or endotypes, to type 1 diabetes are suspected. The focus of this thesis was to investigate factors leading to heterogeneity in type 1 diabetes pathogenesis.

Several type 1 diabetes predisposing genetic polymorphisms, including the loci *NRP1*, *INS*, *UBASH3A* and *STAT4,* were found to associate with specific phases of disease pathogenesis. Moreover, other disease risk polymorphisms, like *PTPN22* and *INS*, associated significantly with suspected type 1 diabetes endotypes defined through the first appearing islet autoantibody. The autoimmune risk variant of *PTPN22* was also associated with elevated total and naïve regulatory T cell frequencies. No gene expression differences could be detected in individual genes between children positive for multiple type 1 diabetes associated autoantibodies and their healthy controls in monocytes and monocyte-depleted peripheral blood mononuclear cells. However, gene sets relating to viral responses and a type I interferon response were upregulated in monocytes of multiple autoantibody positive children, compared to healthy controls.

These data lend support to heterogeneity of type 1 diabetes with multiple possible pathways to disease onset.

KEYWORDS: Type 1 diabetes, genetics, endotypes, PTPN22, regulatory T cells, viral response, monocytes

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Biolääketieteen laitos
Lääketieteellinen mikrobiologia ja immunologia
MILLA VALTA: Immunologiset mekanismit tyypin 1 diabeteksen patogeneesissa
Väitöskirja, 165 s.
Molekyylilääketieteen tohtoriohjelma
Toukokuu 2023

TIIVISTELMÄ

Tyypin 1 diabetes on krooninen autoimmuunisairaus, jossa insuliinia tuottavat haiman betasolut tuhoutuvat. Taudinkulku voidaan jakaa oireettomaan prekliiniseen vaiheeseen ja oireiseen sairauteen. Prekliinisessä vaiheessa ilmaantuu autovasta-aineta haiman saarekesoluja vastaan ja sairauden kliininen puhkeaminen tapahtuu toiminnallisten betasolujen määrän laskiessa liian pieneksi sokeritasapainon ylläpitämiseen. Vaikka useita tyypin 1 diabetekselle altistavia perimä- ja ympäristötekijöitä tunnetaan, tautiin johtavan autoimmuniiprosessin tarkkaa mekanismia ei tunneta. Taudinkulussa on lisäksi havaittu suurta vaihtelevuutta ja useiden rinnakkaisten tyypin 1 diabetekseen johtavien tautimekanisminen tai taudin endotyyppien arvellaan olevan mahdollisia. Tässä väitöskirjassa keskitytttiin tyypin 1 diabeteksen patogeneesin heterogeniaan johtavien tekijöiden tutkimiseen.

Useiden tyypin 1 diabetekselle altistavien geneettisten polymorfismien, kuten *NRP1*, *INS*, *UBASH3A* ja *STAT4*, havaittiin assosioituvan tiettyihin taudin patogeneesin vaiheisiin. Lisäksi toiset taudin riskipolymorfismit, kuten *PTPN22* ja *INS*, assosioituivat merkittävästi ensimmäisen ilmestyneen autovasta-aineen mukaan määritettyihin, oletettuihin taudin endotyyppeihin. *PTPN22*-geenin autoimmuunitaudeille altistava riskivariantti assosioitui myös kohonneisiin regulatoristen T-solujen määriin kokonaispopulaatiossa ja naiiveissa soluissa. Useita tyypin 1 diabetekseen liitettyjä autovasta-aineita kehittäneiden lasten monosyyteissä ja monosyytittömissä muissa perifeerisen veren mononukleaarisissa soluissa ei havaittu yksittäisten geenien välisiä ekspressioeroja terveisiin kontrollilapsiin verrattuna. Virusvasteisiin ja tyypin I interferonivasteeseen liittyvät geeniryhmien ekspressiotasot olivat kuitenkin voimistuneet useita autovasta-aineita kehittäneiden lasten monosyyteissä terveisiin kontrolleihin verrattuna.

Nämä tulokset tukevat näkemystä tyypin 1 diabeteksen heterogeniasta ja useista mahdollisista poluista taudin puhkeamiseen.

AVAINSANAT: tyypin 1 diabetes, genetiikka, endotyypit, PTPN22, regulatoriset T-solut, virusvaste, monosyytit

Table of Contents

Abbreviations 9

List of Original Publications 11

1 Introduction 12

2 Review of the Literature 13
2.1 Immune system 13
2.1.1 Innate and adaptive immunity 13
2.1.1.1 Innate immunity 13
2.1.1.1.1 Monocytes 15
2.1.1.2 Adaptive immunity 16
2.1.2 Maturation of immunity 17
2.1.2.1 Exposure to microorganisms 17
2.1.2.2 Infections 18
2.1.2.3 Immunological memory during the first years of life 18
2.1.3 Immune tolerance 19
2.1.3.1 Central tolerance 19
2.1.3.1.1 T cells 19
2.1.3.1.2 B cells 21
2.1.3.2 Peripheral tolerance 21
2.2 Type 1 diabetes 23
2.2.1 Pathogenesis 24
2.2.1.1 Prediabetic period 24
2.2.1.1.1 Initiation of cell-mediated autoimmunity 25
2.2.1.1.2 Appearance of humoral beta cell autoimmunity 27
2.2.1.1.3 Decline of first-phase insulin response 30
2.2.1.2 Appearance of clinical disease 31
2.2.1.2.1 Insulitis and destruction of pancreatic beta cells 31
2.2.2 Heterogeneity and endotypes 32
2.2.2.1 Variability in the rate of disease pathogenesis 32
2.2.2.2 Heterogeneity in initial disease severity 33
2.2.2.3 IAA-first and GADA-first endotypes 34
2.3 Genetic and environmental risk factors of type 1 diabetes 35

2.3.1 Genetic risk factors of type 1 diabetes 35
2.3.1.1 Human leukocyte antigen 35
2.3.1.2 Single nucleotide polymorphisms................. 39
2.3.1.2.1 Insulin.. 40
2.3.1.2.2 Protein tyrosine phosphatase non-receptor type 22 41
2.3.1.2.3 Interleukin 2 receptor alpha subunit.. 42
2.3.1.2.4 Erb-B2 receptor tyrosine kinase 3 and zinc finger protein Eos 43
2.3.1.2.5 Protein tyrosine phosphatase non-receptor type 2 44
2.3.2 Environmental risk factors of type 1 diabetes............ 45
2.3.2.1 Microbiome and type 1 diabetes 45
2.3.2.2 Antibiotics, health and type 1 diabetes.......... 46
2.3.2.3 Nutrition and type 1 diabetes 46
2.3.2.4 Viral infections and type 1 diabetes............... 47

3 Aims 49

4 Materials and Methods.. 50
4.1 Subjects 50
4.1.1 Study I 51
4.1.2 Study II 51
4.1.3 Study III 52
4.2 Methods 52
4.2.1 Genotyping 52
4.2.1.1 HLA genotyping 52
4.2.1.2 SNP genotyping... 53
4.2.1.2.1 Sequenom.................................. 53
4.2.1.2.2 TaqMan Microarray 53
4.2.1.2.3 TaqMan genotyping assays.......... 53
4.2.2 Autoantibody analyses.. 54
4.2.3 Isolation of peripheral blood mononuclear cells 55
4.2.4 Detection of lymphocyte subpopulations using flow cytometry... 55
4.2.4.1 Study II 55
4.2.4.2 Study III 56
4.2.4.3 Data processing... 57
4.2.5 Fractionation of lymphocytes using magnetic separation... 57
4.2.6 RNA sequencing.. 57
4.2.6.1 RNA extraction.. 57
4.2.6.2 Library preparation....................................... 57
4.2.6.3 Sequencing.. 58
4.2.7 Statistical analyses 58
4.2.7.1 Survival analysis 58
4.2.7.2 Analysis of covariance 58
4.2.7.3 Analysis of sequencing data 59

5 Results 60

5.1 The effect of single nucleotide polymorphisms on the pathogenesis of type 1 diabetes ... 60
5.1.1 Non-HLA polymorphisms in different phases of autoimmunity in type 1 diabetes ... 60
5.1.2 Non-HLA polymorphisms in the endotypes of type 1 diabetes ... 65
5.2 The effect of *PTPN22* polymorphism on regulatory T cells ... 65
5.2.1 *PTPN22* rs2476601 risk allele A increases regulatory T cell frequencies in the context of type 1 diabetes ... 66
5.2.2 *PTPN22* rs2476601 risk allele A elevates regulatory T cell frequencies in healthy children ... 72
5.3 Evidence of viral infection-related gene upregulation in monocytes ... 75
5.3.1 Peripheral blood mononuclear cell gene expression in children positive for multiple type 1 diabetes associated autoantibodies ... 76
5.3.2 Analysis of monocyte and lymphocyte compartments in a larger cohort of children positive for multiple type 1 diabetes associated autoantibodies ... 76

6 Discussion ... 83
6.1 Non-HLA genetic factors in the pathogenesis of type 1 diabetes ... 83
6.2 *PTPN22* autoimmune risk variant rs2476601 in regulatory T cells ... 87
6.3 Monocyte function and viral infections in type 1 diabetes ... 89
6.4 Strengths and limitations of the study ... 92
6.5 Future perspectives ... 94

7 Conclusions ... 96

Acknowledgements ... 97

References ... 99

Original Publications ... 131

Abbreviations

AIRE	autoimmune regulator
APC	antigen presenting cell
APECED	autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
APS1	autoimmune polyglandular syndrome type 1
BCR	B cell receptor
CI	confidence interval
CTLA4	cytotoxic T-lymphocyte-associated protein 4
DIPP	the Finnish Diabetes Prediction and Prevention Study
DMSO	dimethyl sulfoxide
ERBB3	Erb-B2 receptor tyrosine kinase 3 gene
FDR	false discovery rate
FPIR	first phase insulin response
GADA	glutamic acid decarboxylase autoantibodies
GSEA	gene set enrichment analysis
GWAS	genome wide association study
HLA	human leukocyte antigen
HR	hazard ratio
IA-2A	islet antigen-2 autoantibodies
IAA	insulin autoantibodies
IFN	interferon
Ig	immunoglobulin
IKZF4	zinc finger protein Eos
IL	interleukin
IL2RA	interleukin 2 receptor alpha subunit gene
INS	insulin gene
IPEX	immunodysregulation, polyendocrinopathy, enteropathy X-linked syndrome
LADA	latent autoimmune diabetes in adults
MDA5	melanoma differentiation-associated protein 5
MHC	major histocompatibility complex
NOD	non-obese diabetic

nPOD	Network for Pancreatic Organ Donors with Diabetes
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PTPN22	protein tyrosine phosphatase non-receptor type 22 gene
RNA	ribonucleic acid
RT	room temperature
SD	standard deviation
SNP	single nucleotide polymorphism
STAT	signal transducers and activators of transcription
STAT4	signal transducer and activator of transcription 4
STRT	Single-cell Tagged Reverse Transcription
T1D	type 1 diabetes
TFG	transforming growth factor
TCR	T cell receptor
Tfh	follicular T helper cell
TNF	tumor necrosis factor
Treg	regulatory T cell
TRIGR	Trial to Reduce Insulin-Dependent Diabetes Mellitus in the Genetically at Risk
UBASH3A	Ubiquitin-associated and SH3 containing A
VNTR	variable number tandem repeat
ZnT8A	zinc transporter 8 autoantibodies

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Laine AP, Valta M, Toppari J, Knip M, Veijola R, Ilonen J and Lempainen J. Non-HLA Gene Polymorphisms in the Pathogenesis of Type 1 Diabetes: Phase and Endotype Specific Effects. *Frontiers in Immunology*, 2022; 13:909020. doi: 10.3389/fimmu.2022.909020.

II Valta M, Gazali AM, Viisanen T, Ihantola EL, Ekman I, Toppari J, Knip M, Veijola R, Ilonen J, Lempainen J and Kinnunen T. Type 1 diabetes linked PTPN22 gene polymorphism is associated with the frequency of circulating regulatory T cells. *European Journal of Immunology*, 2020; 50: 581-588.

III Valta M, Yoshihara M, Einarsdottir E, Pahkuri S, Ezer S, Katayama S, Knip M, Veijola R, Toppari J, Ilonen J, Kere J and Lempainen J. Viral infection-related gene upregulation in monocytes in children with signs of β-cell autoimmunity. *Pediatric Diabetes*, 2022; 23(6): 703- 713.

The original publications have been reproduced with the permission of the copyright holders.

1 Introduction

Type 1 diabetes is an endocrine disorder resulting from the destruction of pancreatic, insulin producing beta cells through an autoimmune process. The loss of beta cells leads to progressive insulin deficiency and ultimately to a life-long need of insulin replacement therapy. Compared to the first half of the 20th century, a significant increase in the disease incidence has been observed globally.

The autoimmune process leading to type 1 diabetes requires both a genetic predisposition and an environmental component and its initiation often takes place years before the onset of disease symptoms. While many genetic and environmental factors have been associated with the risk of type 1 diabetes over decades of intensive study, no clear disease pathway has emerged. Contrarily, disease heterogeneity has recently become apparent in type 1 diabetes pathogenesis, as for instance specific risk factors have been associated with disparate disease progression times. These results suggest several parallel disease mechanisms in type 1 diabetes.

The aim of this study was to increase the knowledge about the heterogenetic autoimmune process leading to type 1 diabetes. Disease associated genetic factors were investigated for associations with specific phases of type 1 diabetes pathogenesis and suspected disease endotypes. Genetic variants associated with type 1 diabetes were also studied for changes in regulatory T cell frequencies. Additionally, transcriptional changes in peripheral blood mononuclear cells were examined in children with beta cell autoimmunity compared to their healthy peers.

2 Review of the Literature

2.1 Immune system

2.1.1 Innate and adaptive immunity

The human immune system consists of two parts working parallel. These parts, innate and adaptive immunity, can be defined through the speed and specificity of their response to a pathogen (Parkin & Cohen, 2001). As its name suggest, we are born with our innate immunity, whose swift response remains largely the same throughout life. The adaptive immune response takes longer to form but is also more precise. Additionally, memory cells are formed and upon second exposure to the same pathogen, the adaptive immune response reacts quicker. Both innate and adaptive immunity are needed for a well-rounded immune system, and they interact and support each other against external and internal threats.

2.1.1.1 Innate immunity

Innate immunity encompasses the parts of the immune system that provide immediate defence to the host. The response to the detected threat is rapid and many of the elements are highly conserved. Some of the elements are not only shared with other animal species, but also with plants. The function of the innate immune system is identical with each encounter with the same pathogen and its lack of specificity sometimes results in damaged host tissues.

Innate immune cells are, for the most part, derived from the myeloid progenitors of leukocytes (Figure 1). Phagocytes of the innate immunity, neutrophils, monocytes, conventional dendritic cells, and macrophages, come from this cell line, as well as many inflammatory molecule secreting cell types like, mast cells, basophils, and eosinophils. Phagocytes engulf particles and digest them with proteases and reactive oxygen species. Inflammatory molecules secreted by other cell types include proteolytic enzymes, antimicrobial peptides, and cytokines, which are proteins mediating communications between different immune cells (Riera Romo et al., 2016). Some cytokine subgroups include interferons (IFN), interleukins (IL), tumour necrosis factors (TNF), and transforming growth factors (TGF).

The lymphoid cell line produces mostly adaptive immune cells, but plasmacytoid dendritic cells and natural killer cells are considered a part of the innate immune system. Macrophages and dendritic cells link innate immunity to the adaptive branch by acting as antigen presenting cells (APC) that introduce foreign antigens to T cells and activate them (Robertson, 1998). Natural killer cells destroy host cells that have become damaged. This includes cells infected with viruses and cancer cells.

Multiple conserved receptor types expressed on innate immune cells recognise and mediate the destruction of encountered pathogens. Some of the most important are the so-called pattern recognition receptors that detect pathogen associated and damage associated molecular patterns. Among their targets are microbial carbohydrates, like lipopolysaccharide, nucleic acids, and extracellular ATP. Cytoplasmic pattern recognition receptors include NOD-like and RIG-I-like receptors and membrane bound Toll-like and C-type lectin receptors (Takeuchi & Akira, 2010). The RIG-I-like receptor melanoma differentiation-associated protein 5 (MDA5) is encoded by the gene *IFIH1* and some polymorphisms in this gene have been linked with type 1 diabetes (Smyth et al., 2006). Some innate immune cells, like basophils and eosinophils, express immunoglobulin (Ig) receptors that recognise pathogens coated with antibodies (Parkin & Cohen, 2001). Natural killer cells have

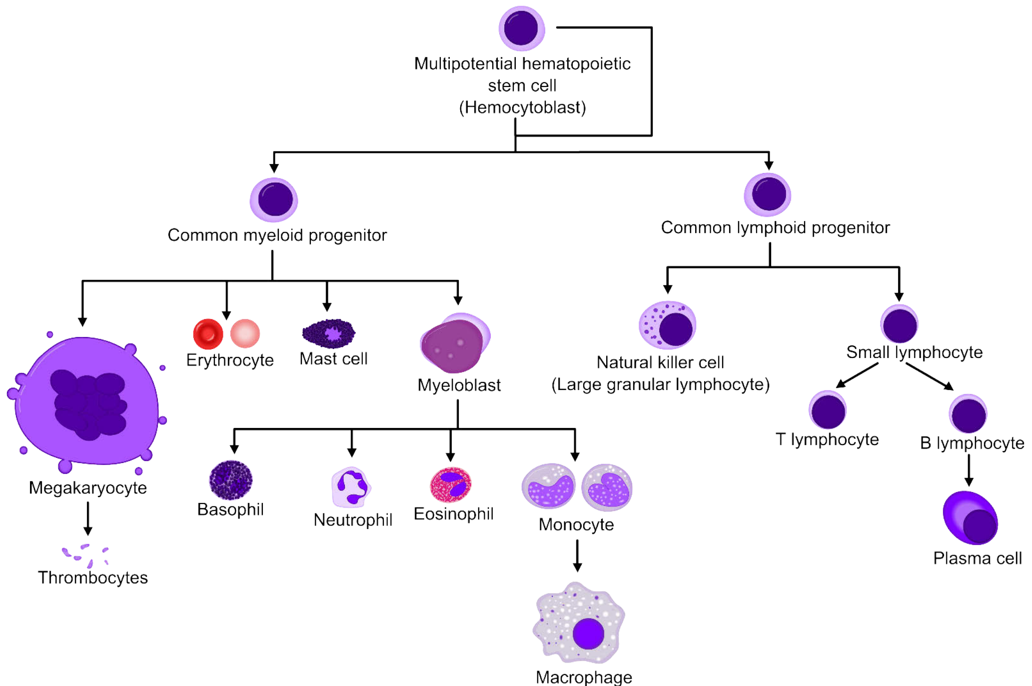

Figure 1. Myeloid and lymphoid cell lines. Reprinted with permission from Häggström under CC BY-SA 3.0 licence via Wikimedia Commons, adapted from work by A. Rad.

receptors for major histocompatibility complex (MHC) class I and the reduction of MHC class I molecules on the surface of cells, that can happen for example due to a viral infection, activates the natural killer cell (Kärre, 1993).

In addition to innate immune cells, several other factors and functions of the body can be considered parts of the innate immune system. Physical and chemical barriers, such as skin, mucous membranes, tears, and digestive enzymes, prevent microbes from accessing the body and causing infections. The complement system tags pathogens for destruction and activates the recruitment of immune cells to the site of infection, as well as damages the pathogen directly by forming holes on the pathogens' cell membranes (Parkin & Cohen, 2001).

2.1.1.1.1 Monocytes

Monocytes are a versatile group of innate immune cells with complex roles in innate immunity. They are produced in the bone marrow from the myeloid cell line and then move to the blood stream, from where they can reach most tissues and infection sites easily (Jakubzick et al., 2017; Robinson et al., 2021). Traditionally, they are known for being a precursor to macrophages, but the last few decades have expanded our understanding of their multiple functions and plasticity. According to our current understanding, monocytes are also involved with tissue repair, antigen presentation, as well as phagocytosis and are precursors to monocyte derived dendritic cells (Ożańska et al., 2020).

In humans, monocytes can be divided into three main populations based on their expression of CD14 and CD16 that function as a coreceptor for lipopolysaccharide and a low-affinity receptor for IgG, respectively (Jakubzick et al., 2017; Ożańska et al., 2020). The most abundant of these, constituting approximately 90 % of all monocytes, is the CD14+CD16- classical monocyte, which has the ability to migrate peripheral tissues from the circulation. While tissue macrophages generally stem from embryonic progenitors, classical monocytes participate in maintaining the tissue macrophage pool in some tissues, like the gut, lung, and heart (Robinson et al., 2021). Additionally, classical monocytes can migrate to sites of acute inflammation and take part in the proinflammatory reaction by producing cytokines, phagocytosis and differentiating into proinflammatory macrophages or dendritic cells (Jakubzick et al., 2017; Robinson et al., 2021). However, they also support wound healing and tissue repair (Ożańska et al., 2020).

The rest of the monocyte population is made up by CD14-CD16+ non-classical monocytes and CD14+CD16+ intermediate monocytes. Non-classical monocytes patrol vasculature for the presence of pathogens and are known to be closely involved in CD4+ T cell stimulation (Ożańska et al., 2020). There is also evidence that they prevent the formation of metastases in cancer (Robinson et al., 2021). Non-classical

monocytes seem to be derived from classical monocytes via the intermediate subpopulation (Robinson et al., 2021). Intermediate monocytes have diverse transcriptional profiles, reflecting features from both classical and non-classical monocytes (Robinson et al., 2021). They support T cell proliferation and participate in their stimulation, as well as act as antigen presenting cells (Ożańska et al., 2020).

2.1.1.2 Adaptive immunity

Compared to innate immunity, adaptive immune responses initiate slowly. However, while the response of the innate immune system is limited by its conserved receptors, the same is not true for the adaptive immune system. Instead, a unique response to each pathogen is created and fine-tuned for maximum specificity. Finally, memory cells against the pathogen are created for fast and precise reaction upon future encounters. This is possible, because of the random recombination of antigen sensing receptor genes.

Cells of the adaptive immune system come from the lymphoid cell line of the hematopoietic stem cell (Figure 1). The two main cell types are B and T cells, but both types can differentiate into numerous subtypes. B cells are adaptive immune cells that are responsible for producing antibodies, the humoral component of adaptive immunity. They develop and go through the random rearrangement of the antigen binding parts of the B cell receptor (BCR) in the bone marrow (Melchers, 2015). Antibodies are a soluble version of the BCR and also known as immunoglobulins. Naïve mature B cells locate to secondary lymphoid tissues, where, upon encountering an antigen they recognise, they differentiate into antibody secreting plasma cells or memory B cells (Nutt et al., 2015). After B cell activation, the BCR is subject to class switching and affinity maturation through somatic hypermutation (Melchers, 2015). In addition to producing humoral immunity, B cells are professional APCs (Hua & Hou, 2020).

T cells produce cell-mediated responses to specific antigens through direct cytotoxicity and recruitment of other cell populations. They are initially produced in the bone marrow as well, but they mature and go through recombination of the T cell receptor (TCR) in the thymus. Most T cells are αβ T cells that have TCRs that are heterodimers of alpha and beta chains, but in the gut, there are also γδ T cells, whose TCRs are heterodimers of gamma and delta chains. γδ T cells are functionally different from most other T cells and their TCRs are coded by separate genes. After TCR recombination, conventional T cells undergo positive and negative selection. Positive selection ensures that the developing T cell can only bind one class of MHC molecules, that is, it determines whether the cell will be a CD4+ or CD8+ T cell, as they are coreceptors for class II and class I MHC molecules, respectively (Wieczorek et al., 2017). In negative selection, self-antigens are introduced to T cells and those

cells that bind them either strongly go through apoptosis (Starr et al., 2003). CD4+ T cells, or the so-called T helper cells, have numerous subgroups, including type 1 and 2 T helper cells, regulatory T cells (Treg) and follicular T helper cells (Tfh). Their tasks are largely in support and control of immune responses, including assisting B cells' class switching (Caza & Landas, 2015). CD8+ T cells, or cytotoxic T effector cells, kill infected, damaged, or cancerous cells through the targeted release of cytotoxins, like perforin and granzymes (Mittrücker et al., 2014). T cells circulate between secondary lymphoid tissues and are mostly activated in lymph nodes by APCs. The differentiation of T cells is dependent on their surrounding cytokine environment and other signals. Especially CD4+ T cells can also retain some plasticity after initial differentiation and alter their phenotype according to their surroundings (Caza & Landas, 2015).

Most B and T cells do not survive after the pathogen that activated them has been cleared, but a fraction does. These memory cells are capable of reacting to the same pathogen quickly and can survive from months to decades.

2.1.2 Maturation of immunity

2.1.2.1 Exposure to microorganisms

An individual's microbial environment is the primary educator of the early immune system, starting already in utero (Rautava et al., 2012; Stinson et al., 2019). Especially commensal microbes interact with the early immune system, inducing tolerance in the gut and facilitating the maturation and differentiation of adaptive immune cells (Edelman & Kasper, 2008; Zhao & Elson, 2018). Limited contact with microbes in infancy and early childhood results in poorer gut homeostasis and susceptibility to infections (Chung et al., 2012). Additionally, several autoimmune diseases have been associated with dysbiosis and altered gut microbiome (Gomaa, 2020).

Cell type specific effects of early exposure to microorganisms have been studied using germ-free animal models. These studies have observed alterations in B and T cell compartments of germ-free mice, in comparison to mice with natural gut microbiomes. For instance, the proliferation and induction of mucosal IgA producing cells is vastly better after encountering commensal bacteria (Hapfelmeier et al., 2010). In addition to IgA levels, IgG levels are lower in germ-free mice too (Benveniste et al., 1971). Germ-free mice have fewer intestinal αβ T cells and Tregs compared to conventional animals (Bandeira et al., 1990; Geuking et al., 2011). Defective immune responses to viral infections have been observed in CD4+ and CD8+ T cells and B cells in a mouse model after depleting commensal bacteria with an antibiotic (Ichinohe et al., 2011).

2.1.2.2 Infections

Commensal microbes are not the only microbial contacts of a newborn child. In addition to them, neonates encounter various pathogenic agents, such as viruses. At the beginning of life, when previous pathogenic encounters are limited, infants have low immunological memory and most cells of the adaptive immunity are naïve (Hannet et al., 1992). In addition, neonatal adaptive responses are highly variable and, compared to adult responses, often lacking (Basha et al., 2014). For these reasons young children are particularly susceptible to infectious disease and to date infections are one of the leading causes of childhood mortality.

Each infection that we have induces the creation of memory cells that protect us from the same disease upon future encounters of the pathogen. Some pathogens shape the immune system more than others. For example, cytomegalovirus that establishes a latent infection induces a strong T cell response and over time so-called memory inflation, where a sizable proportion of an individual's memory cell pool targets only the one pathogen (Klenerman & Oxenius, 2016). Vaccinations have also very successfully been used to educate immune systems and protect people from more dangerous infections, without exposing them to the adverse effects of the pathogen. In Finland, for instance, the national immunisation programme starts at two months of age covering diseases such as polio, pertussis, mumps, and rubella within the first 18 months of life (Terveyden ja hyvinvoinnin laitos, 2022).

During the last three decades, the hygiene hypothesis has prompted numerous studies about the effect of infections on the maturation of immunity (Strachan, 1989). The hypothesis states that early life exposure to microorganisms, especially certain infections, helps the immune system develop and reach an appropriate level of tolerance against self and environmental antigens (Bach, 2021; Pfefferle et al., 2021). The decrease of infectious disease has been implicated in the increase of both allergic and autoimmune disease (Bach, 2021; Pfefferle et al., 2021).

2.1.2.3 Immunological memory during the first years of life

Immunological memory in humans is mostly mediated by dedicated memory cells of the adaptive immune system. Both B and T cells can differentiate to long-lived memory cells after encountering a new pathogen. During the first six months of life an infant is protected by antibodies from the mother, received through the placenta and later by breastfeeding, but as their protection fades, the child's developing immune system must deal with threats independently (Basha et al., 2014). Neonates have had only limited contact with microbes and thus have low amounts of memory cells in comparison to adults (Tosato et al., 2015).

The ratio of naïve/memory T and B cells declines throughout life and the rate of change is fastest in childhood (Hannet et al., 1992; Morbach et al., 2010; Saule et

al., 2006). For example, more than 90 % of CD4+ T cells in cord blood can be naïve, whereas in primary school aged children the amount is approximately 70 % and in young adults 50 % (Hannet et al., 1992; Saule et al., 2006). A similar trend can also be seen in naïve/memory B cells (Morbach et al., 2010). In addition, the phenotypes of adaptive immune cells are more immature in very young children. While neonates are capable of adult-like T and B cell responses, the efficacy and scale of the response are often limited (Adkins et al., 2004). Young children have fewer class-switched B cells. At the age of one year, children have approximately 70 % and 30 % of adult IgG and IgA levels, respectively, which may be due to incomplete maturation of germinal centres and the spleen (Ygberg & Nilsson, 2012). T cells from cord blood have also been observed to have considerably poorer INFγ and IL2 production compared to adults (Sautois et al., 1997).

2.1.3 Immune tolerance

The ability to differentiate between foreign and self-antigens is crucial for the adaptive immune system. As its response to pathogens is not static, but different for every individual and evolved to become as specific as possible, the possibility for mistakes is great. For this reason, the adaptive immune system must have built-in mechanisms to prevent responses to self-antigens and maintaining general immune homeostasis to minimise damage to the individual's own tissues. These mechanisms are collectively termed immune tolerance.

Immune tolerance can be divided into two main components: central tolerance and peripheral tolerance (Theofilopoulos et al., 2017). The former encompasses all processes that eliminate autoreactive T and B cells during T and B cell maturation. While mechanisms of central tolerance filter out most self-reactive cells, its effectiveness is not absolute. Autoreactive cells that escape central tolerance are regulated by peripheral tolerance. The presence of autoreactive cells in healthy people suggests that some autoimmune disorders are caused by broken peripheral tolerance (Theofilopoulos et al., 2017).

2.1.3.1 Central tolerance

2.1.3.1.1 T cells

T cell mediated tolerance begins with the maturation of T cells in the thymus or the foetal liver. T cells recognise their targets through the TCR and therefore T cell tolerance is built on the regulation of TCR interaction with its ligands. The most significant mechanisms ensuring the proper interaction between the TCR and an MHC molecule presenting a peptide antigen take place during positive and negative

selection of T cell maturation, which together maintain central T cell tolerance. Both processes depend on thymic epithelial cells that present self-antigens to the maturing T cells (Starr et al., 2003; Klein et al., 2014).

Positive selection ensures the ability of the TCR to bind to a complex formed by an MHC molecule and an antigen (Starr et al., 2003). Positive selection takes place in the thymic cortex (Klein et al., 2014). T cells receiving survival signals at positive selection are capable of binding either class I or class II MHC molecules and thus MHC restricted and destined to become either CD4+ or CD8+ cells. While this step of the selection process somewhat paradoxically involves the presentation of self-antigens, there is evidence that proteolytic pathways in cortical thymic epithelial cells differ from usual antigen processing and therefore the antigens presented may not be quite the same as in negative selection or in peripheral tissues (Klein et al., 2014). Accordingly, this step of T cell maturation tests that antigens are specifically recognised bound to an MHC molecule, which is important for the proper function of the immune system. This contributes to the high level of specificity in the TCR signalling system and therefore also tolerance, as antigens outside of these circumstances are not recognised. A murine model lacking class I and II MHC molecule coreceptors CD8 and CD4 has been shown to be capable of interacting with antigens independent of MHC molecules (Van Laethem et al., 2007). Additionally, specific genetic variants in the human leukocyte antigen (HLA) region, especially in the genes coding for the MHC molecules, have been associated with several types of autoimmunity (Matzaraki et al., 2017).

The main mechanism of central tolerance in T cell development is negative selection, during which T cells are tested for their ability to bind self-antigens. While it is important to ensure that TCRs are capable of binding to MHC molecules, making sure that they do not have a high affinity to self-antigens is even more important for self-tolerance. In negative selection, medullary thymic epithelial cells present self-peptides from peripheral tissues to T cells with newly rearranged TCRs (Klein et al., 2014). If a T cell binds one of these antigens with high enough affinity, it will be directed to apoptosis (Starr et al., 2003). The presentation of self-antigens in thymic epithelial cells is controlled by a transcription factor called autoimmune regulator (AIRE). In medullary thymic epithelial cells, AIRE enables the expression of proteins that are normally strictly tissue restricted through the activation of super enhancers (Besnard et al., 2021; Theofilopoulos et al., 2017). Mutations in the gene encoding AIRE have been known to have catastrophic consequences for the function of central immune tolerance. In autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, also known as APS1, autoimmune polyglandular syndrome type 1), defects in AIRE function in the thymus lead to a severe autoimmune disease characterised by the destruction of multiple endocrine organs, often including the insulin producing pancreas (Besnard et al., 2021). Decreased

AIRE expression has also been implicated in the development of diabetes in non-obese diabetic (NOD) mice (Fornari et al., 2010).

In addition to the classic negative selection by medullary thymic epithelial cells, some alternate mechanisms leading to the same outcome exist. Thymic dendritic cells take part in negative selection in the thymic medulla by presenting self-antigens captured from blood flow and transferred from thymic epithelial cells (Klein et al., 2014). Additionally, some negative selection happens in the thymic cortex, and it too is carried out by dendritic cells (Klein et al., 2014).

2.1.3.1.2 B cells

Just as T cell responses, B cell responses to pathogens are adaptive and the main mechanism of immune response adaptation is stochastic BCR recombination. Accordingly, B cells, like T cells, need to go through central tolerance checkpoints during their maturation. In fact, due to their capability of secreting antibodies that bind their antigens without costimulation, it can be argued that their potential for autoreactivity is even greater than that of T cells.

B cells mature in the foetal liver and later in the bone marrow. The mechanisms screening the cells for autoimmunity are integrated to BCR recombination and analogous to central tolerance in T cells. However, due to disparities in BCR and TCR signalling, there are also differences. For example, positive selection in B cells happens through so-called tonic signalling of the BRC. That is, the functional BCR in its unligated form emits a low-level signal to the B cells that is important for the survival of the cell during B cell maturation (Namazee, 2017).

Negative selection happens, similar to T cells, through clonal deletion. Self-antigens are presented to immature B cells several times during B cell development. The forming BCR is tested for autoreactivity after the recombination of both the heavy and light chains and then again once the immature B cell expresses a functioning BCR (Melchers, 2015). Cells carrying high-affinity autoreactive BCRs are often disposed of through apoptosis. Alternatively, the developing B cell can go through receptor editing, a process where the light chain of the BCR goes through additional recombination steps due to initial self-reactivity (Nemazee, 2017). If a BCR is edited successfully and loses its self-reactivity, the cell does not need to be deleted. Perhaps because of its location, comparatively little is known about antigen presentation to immature B cells in the bone marrow (Melchers, 2015).

2.1.3.2 Peripheral tolerance

Because the effectiveness of central tolerance is not absolute, there are mechanisms in place that maintain tolerance to self-antigens peripherally. In addition to

prevention of autoimmunity, some of the processes of peripheral tolerance inhibit reactions to harmless foreign antigens, like food antigens.

Several T cell populations take part in different aspects of directing and modulating immune responses and peripheral immune tolerance is one of the key areas of control for them. The subpopulation most directly associated with peripheral immune tolerance is Tregs that regulate immune reactions, not only against self-antigens, but also against cancer cells, commensal microbiota, allergens, and pathogens, to limit damage to host tissues (Grover et al., 2021). Most Tregs have been though to differentiate already in the thymus from a small section of autoreactive CD4+ T cells into so-called natural Tregs, however, they can also be induced in the periphery from conventional CD4+ T cells and naïve T cells (Lucca & Dominguez-Villar 2020; Grover et al., 2021). Therefore, it is not surprising that Tregs comprise a heterogenous group of cells and there is still ongoing discussion about the definition of Tregs (Zemmour et al., 2018; Gover et al., 2021). Several markers in different combinations are used to characterise Tregs and their subtypes. The classic Treg marker is the FoxP3 transcription factor, which is a master regulator for Treg differentiation and therefore found on most Tregs (Zemmour et al., 2018; Grover et al., 2021). Mutations in the gene encoding it lead to immunodysregulation polyendocrinopathy, enteropathy X-linked syndrome (IPEX) (Grover et al., 2021), that presents commonly with, but not limited to, autoimmune enteropathy, type 1 diabetes, and dermatitis. Another important marker is CD25 or the IL2 receptor alpha subunit (IL2RA), whose signalling is important for the homeostasis and differentiation of Tregs (Furtado et al., 2002) and alerts them of T cell activity, as IL2 is produced by activate T cells. Additionally, the lack of CD127 or IL7 receptor alpha subunit has also widely been associated with Tregs (Lucca & Dominguez-Villar 2020). In addition to these three markers, a handful of others are used to identify subpopulations of Tregs. For example, Helios and neuropilin-1 have both been used to distinguish natural Tregs from peripherally derived ones (Lucca & Dominguez-Villar 2020; Grover et al., 2021).

Tregs suppress several types of immune cells and operate though many different mechanisms. The Treg surface marker IL2RA forms a higher affinity complex with the beta and gamma subunits of the receptor, and it has been suggested that this form of the receptor competes with those on effector T cells, regulating their responses by cytokine deprivation and often resulting in apoptosis (Grover et al., 2021). Moreover, Tregs can produce granzyme and perforin that suppress effector T cell function by cytolysis (Grover et al., 2021). In addition to effector T cell suppression, Tregs can modulate the maturation and function of dendritic cells through cytotoxic T-lymphocyte associated protein 4 (CTLA4) and lymphocyte-activation gene 3 (Grover et al., 2021). Tregs are also capable of secreting immunosuppressive cytokines, like IL10 and transforming growth factor beta.

In addition to Treg suppression, T cell activation and differentiation themselves are tightly controlled processes. Several factors, like the presence of specific cofactors and cytokines, are needed for mounting a proper immune response to a pathogen. For example, suboptimal antigen stimulation conditions will lead to T cell anergy or apoptosis (ElTanbouly & Noelle, 2021). Regulatory epigenetic elements that are carried over in cell division and induce cell unresponsiveness in certain situations, have also been recognised (ElTanbouly & Noelle, 2021).

There are also several mechanisms of peripheral tolerance in B cells. Up to 20 % of mature naive B cells display autoreactivity (Wardemann et al., 2003). In addition to the autoreactive B cells that have escaped central tolerance, another source of self-reactive B cells is somatic hypermutation of activated B cells in germinal centres (Getahun, 2022). Since the affinity maturation of BCRs through somatic hypermutation is random, the probability of accidental autoreactivity is high. A major mechanism of peripheral tolerance in B cells is a state of functional unresponsiveness, B cell anergy (Getahun, 2022; Nemazee, 2017). Anergic B cells have a shorter lifespan compared to other B cells (Fulcher & Basten, 1994) and since the state of anergy is reversible (Gauld et al., 2005), they are a potential source of pathogenic cells in autoimmune disease. For example, B cells producing disease associated autoantibodies in type 1 diabetes have been observed to be exclusively anergic in healthy individuals but lose their anergy in new-onset disease (Smith et al., 2014).

2.2 Type 1 diabetes

Type 1 diabetes is an autoimmune disease that is defined by the immune response induced loss of insulin production of beta cells in the islets of Langerhans in the pancreas (Bach, 1994; Katsarou et al., 2017). The consensus is that the initiation of autoimmunity happens when genetic and environmental risk factors come together in an opportune manner. The autoimmune process, the length of which varies from months to decades (Ilonen et al., 2019; Pöllänen et al., 2019), culminates in the onset of clinical disease, which is commonly diagnosed according to the World Health Organization or American Diabetes Association criteria (Alberti & Zimmet, 1998; American Diabetes Association, 2020) (Table 1). Progression to clinical disease happens, when the amount of secreted insulin drops below sufficient and glucose homeostasis can no longer be maintained. Without insulin, glucose cannot be transferred to cells and blood glucose rises. The glucose that cannot be utilised as energy is secreted through urine and protein and fat of the body are used as an alternate source for sustenance. This leads to the build-up of ketone bodies and eventually ketoacidosis, which, left untreated, is a lethal metabolic state, where blood acidity rises. Therefore, an individual with type 1 diabetes requires lifelong insulin replacement therapy.

Table 1. The diagnostic criteria of diabetes. Modified from (Alberti & Zimmet, 1998; American Diabetes Association, 2020).

Fasting plasma glucose ≥7 mmol/l
OR
Plasma glucose ≥11 mmol/l at 2 hours during an oral glucose tolerance test
OR
Glycated haemoglobin ≥6.5 %
OR
Patient displaying classic symptoms of hyperglycaemia or hyperglycaemic crisis with a random plasma glucose of ≥11 mmol/l

Often development of type 1 diabetes takes place already in childhood and it is among the most common chronic conditions in children. The incidence of the disease in childhood varies drastically between different populations, which is largely explained by genetics, but the global trend indicates rising incidence in all parts of the world (Tuomilehto, 2013). In Finland, the incidence of childhood type 1 diabetes is the highest in the world (Tuomilehto, 2013). The rate has been increasing steadily in the last several decades: in 1953 the incidence in Finland was estimated only 12.5 per 100 000 children (Somersalo, 1954), but in 2006 it peaked at 64.9 per 100 000 children (Harjutsalo et al., 2013). In 2015-2018, the annual rate seems to have plateaued at 52.2 new cases per 100 000 children under the age of 15 years (Parviainen et al., 2020). Such a rapid rise in incidence is likely to be caused by changes in environment and lifestyle, which fits well into the timeframe of major societal developments in Finland.

2.2.1 Pathogenesis

2.2.1.1 Prediabetic period

The pathogenesis of type 1 diabetes can be divided into stages, based on measurable markers of autoimmunity and glucose homeostasis and lastly clinical symptoms (Figure 2). The time preceding clinical symptoms is called the prediabetic period and during it autoimmunity first initiates and then spreads from one detectable autoantibody to several and later the individual loses glycaemic control. In 1986, a three-stage model of the pathogenesis was suggested by George Eisenbarth: I detectable islet autoimmunity and no dysclycaemia; II autoimmunity and dysclycaemia but no clinical symptoms; III clinical type 1 diabetes (Eisenbarth,

1986). Since then, more details have been ascribed to the model. The phases can be divided even further by including additional steps to the preclinical period and metabolic changes in established type 1 diabetes (Couper et al., 2014).

The prediabetic period may take years, or even decades, but there are also rapid progressors who develop type 1 diabetes much faster (Ziegler et al., 2013). Therefore, the autoimmune process leading to type 1 diabetes is highly variable between individuals.

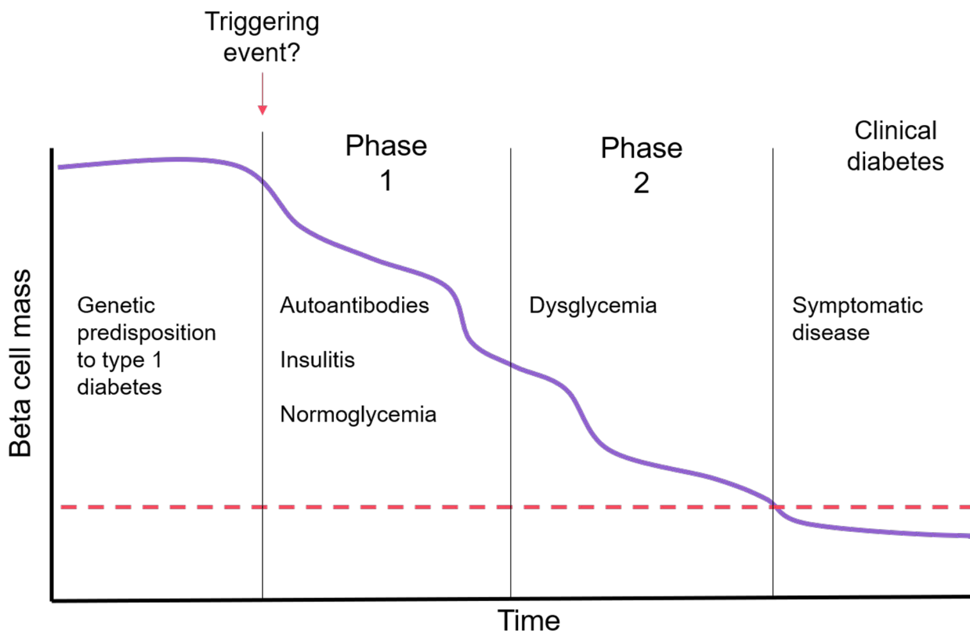

Figure 2. Stages of type 1 diabetes. Modified from (Eisenbarth 1986).

2.2.1.1.1 Initiation of cell-mediated autoimmunity

While we have a model of the general events of the pathogenesis of type 1 diabetes, the exact mechanism of the autoimmune process is inadequately understood. In fact, currently the general agreement is that there are likely several pathways to type 1 diabetes (Ilonen et al., 2019). Due to the autoimmune destruction taking place in the pancreas, most direct studies of the disease process have been conducted in animal models and thus may not be directly applicable to humans. The most important of these animal models is the NOD mouse, which develops spontaneous autoimmune diabetes due to strong genetic predisposition to the disease. While many of the disease features between NOD mice and humans are similar, like genetic risk factors and appearing autoantibodies, there are also differences, such as a milder

ketoacidosis upon disease onset and a female bias in diabetic cases (Pearson et al., 2016). Therefore, the cell-mediated autoimmunity, and especially its initiation, still require further studying. In situations, where human studies are not possible, more advanced, humanised animal models should be adopted.

Several pieces of evidence indicate that type 1 diabetes is a disease driven by T cells. In insulitis, the inflammatory immune infiltration of the pancreatic islets, the most abundant cell type in humans is the cytotoxic CD8+ T lymphocyte (Gianani et al., 2010; In't Veld et al., 2007; Willcox et al., 2009). The CD8+ T cells in insulitic lesions have also been observed to recognise islet antigens (Babon et al., 2016; Coppieters et al., 2012), with broader autoantigen repertoire in longer disease duration (Coppieters et al., 2012). There is also evidence of autoantigens like preproinsulin and glutamic acid decarboxylase being naturally processed and presented to cytotoxic CD8 T cells (Knight et al., 2015; Kronenberg et al., 2012; Skowera et al., 2008) in the pancreas. The predominance of CD8+ T cells in insulitis may therefore be due to the cells in the islets themselves expressing increased amounts of MHC class I molecules (Bottazzo et al., 1985; Foulis et al., 1987). While beta cell specific CD8+ cytotoxic T cells can also be found in healthy controls, in individuals with type 1 diabetes they display signs of chronic autoantigen exposure and expansion (Skowera et al., 2015). In a study by Yeo et al., the levels of circulating autoreactive CD8+ effector memory T cells were observed to mirror remaining beta cell function in recent onset type 1 diabetes cases (Yeo et al., 2018).

The strongest genetic risk to type 1 diabetes is conferred by genes coding MHC class II molecules (Erlich et al., 2008). It is therefore a reasonable conclusion that CD4+ helper T cells are also involved in the autoimmune process. They are also found in insulitic lesions in the pancreas, but in smaller numbers as CD8+ T cells (Gianani et al., 2010; In't Veld et al., 2007; Willcox et al., 2009). As CD8+ T cells, also CD4+ T cells have been observed to recognise self-antigens (Babon et al., 2016; Michels et al., 2017; Pathiraja et al., 2014). It is presumed that autoreactive CD4+ T cells initially avoid negative selection in the thymus and later encounter autoantigens presented by APCs and then activate (Sandor et al., 2019). Evidence of such a chain of events has also been found in NOD mice (Turley et al., 2003; Unanue et al., 2016) and in humans a type 1 diabetes risk associated variant of the insulin gene has been shown to correlate with lower insulin expression in the thymus (Pugliese et al., 1997). Compared to the cells of healthy controls, diabetogenic CD4+ cells take a proinflammatory phenotype and are capable of INF-γ and IL-17 secretion (Arif et al., 2004, 2014; Bellemore et al., 2015; Gomes et al., 2017). A study on naïve CD4+ cells from infants who would later develop type 1 diabetes related autoimmunity found that the CD4+ cells of these individuals had a disparate signature already before the initiation of autoimmunity (Heninger et al., 2017). In addition to cytokine secretion, there is evidence in mice that supporting CD8+ T cells through APC

recruitment and control is one of the main functions of CD4+ T helpers in type 1 diabetes (Espinosa-Carrasco et al., 2018). In other autoimmune diseases, strong CD4+ cell co-stimulation of CD8+ cells is also associated with worse outcomes (McKinney et al., 2015).

In addition to classical CD4+ T helper cells, other subtypes of this cell population have been associated to type 1 diabetes. Tfh cells bridge together cell-mediated and humoral immunity by directing B cell differentiation and affinity maturation in germinal centres. Some differences between individuals with beta cell autoimmunity and healthy controls have been observed in Tfh cells. Increased frequencies of circulating Tfh cells have been recorded in adults with established type 1 diabetes, children with new onset disease and children with prediabetes, but no clinical disease (Ferreira et al., 2015; Viisanen et al., 2016). Phenotypically similar CXCR5−PD-1^{hi} peripheral T helper cells, who are also capable of B cell help, were also increased in children with recent onset type 1 diabetes and children with advanced beta cell autoimmunity (Ekman, Ihantola, et al., 2019). In a study performed with samples from organ donors, the pancreatic lymph nodes and spleens of individuals with type 1 diabetes were found to have fewer follicular Tregs, the regulatory counterpart of Tfh cells, than their non-diabetic control subjects (Vecchione et al., 2021).

Another important subtype of the CD4+ T cell is the classical Treg, whose main purpose is to maintain peripheral tolerance. Since type 1 diabetes results from a lack of such tolerance, it is not surprising that alterations in Treg populations between healthy and subjects with beta cell autoimmunity have been described. Studies focusing on Treg frequencies in type 1 diabetes are not entirely unanimous: some have found decreased Treg levels (Kukreja et al., 2001; Ryba-Stanisławowska et al., 2014), but many others found no distinct differences (Brusko et al., 2007; Brusko et al., 2005; Lindley et al., 2005; Putnam et al., 2005). In children, even increased Treg levels have been observed (Viisanen et al., 2019). Despite this, many studies agree that in type 1 diabetes the function of Tregs is altered. For example, several studies have observed Tregs of diabetic individuals to have poorer suppressive function as those of their healthy controls (Brusko et al., 2005; Glisic-Milosavljevic et al., 2007; Lawson et al., 2008; Lindley et al., 2005). What is more, CD4+ effector T cells have been observed to be resistant to Treg-mediated suppression in type 1 diabetes patients and people with multiple disease associated AAB (Ihantola et al., 2018).

2.2.1.1.2 Appearance of humoral beta cell autoimmunity

Autoantibodies to pancreatic islet antigens can typically be detected at and already before onset of clinical type 1 diabetes and, in addition to genetic factors, are widely used for predicting the likelihood of developing diabetes. The appearance of disease associated autoantibodies is called seroconversion and gaining antibodies

recognising different autoantigens against several islet specific autoantigens as time passes is termed spreading of islet autoimmunity. How long the spreading takes is influenced by several factors, such as the specificity of the first appearing autoantibody and age at seroconversion (Bauer et al., 2019). In a large study with children genetically at risk for type 1 diabetes, the progression rate to clinical disease within 10 years was 69.7% after developing multiple type 1 diabetes associated autoantibodies (Ziegler et al., 2013). It is thought that these autoantibodies are a product of immune cells' continuous exposure to beta cell antigens and that they are rather symptomatic than pathogenic (Katsarou et al., 2017).

Islet cell autoantibodies

The first type 1 diabetes associated autoantibodies that were discovered, were the cytoplasmic islet cell autoantibodies (ICA) (Bottazzo et al., 1974). Unlike other disease associated autoantibodies in type 1 diabetes, ICA do not recognise a single self-antigen, but a collection of islet cell proteins, because ICA are measured from blood serum samples through an indirect immunofluorescence assay, where they stain pancreatic islets. This method also requires high quality samples of pancreatic tissue from organ donors or mice, which makes it difficult to standardise. ICA include antibodies to common disease associated antigens like insulin, glutamic acid decarboxylase and islet antigen-2, but their existence does not always correlate with these specific antigens (Andersson et al., 2014; Månsson et al., 2001). Because of the nature of the assay, ICA are not specific to beta cell antigens, but also include autoreactivity to other pancreatic cell types. Low levels of ICA can sometimes be found in otherwise healthy individuals and when not coupled with other known islet autoantibodies, they represent a relatively low risk of progressing to type 1 diabetes (Bonifacio et al., 1990).

Insulin autoantibodies

Autoantibodies targeting insulin (IAA) were the first of the so-called biochemical autoantibodies associated with type 1 diabetes to be discovered (Palmer et al., 1983). As other biochemical autoantibodies, they are traditionally detected with radio-binding assays. The appearance of IAA associates with the *HLA-DR4-DQ8* haplotype that is one of the strongest genetic risk factors of the type 1 diabetes (Ilonen et al., 2013; Triolo et al., 2021; Ziegler et al., 1991). IAA often appear already very early in life, and it is the most common first autoantibody in young children (Ilonen et al., 2013; Kupila et al., 2002; Parikka et al., 2012; Ziegler et al., 2012). Both the Finnish Diabetes Prediction and Prevention Study (DIPP) and the Environmental Determinants of Diabetes in the Young Study (TEDDY) observed a

peak in IAA appearance during the first two years of life (Krischer et al., 2015; Parikka et al., 2012). High IAA levels during follow-up are predictive of rapid progression to type 1 diabetes (Parikka et al., 2012; Pöllänen et al., 2017). This is especially true in high affinity IAA (Achenbach et al., 2004). Losing IAA during follow-up is associated with slower progression to type 1 diabetes (Endesfelder et al., 2016; Pöllänen et al., 2019). A variable number tandem repeat locus and the single nucleotide polymorphism rs689 in the insulin gene have been associated with both, the increased risk of IAA and type 1 diabetes (Graham et al., 2002; Hermann et al., 2005; Lempainen et al., 2013; Walter et al., 2003).

Glutamic acid decarboxylase autoantibodies

Glutamic acid decarboxylase is an enzyme taking part in the synthesis of γ-aminobutyric acid in the pancreas and the nervous system. The 65 kD isoform of the enzyme is expressed in pancreatic islets and glutamic acid decarboxylase autoantibodies (GADA) have been linked with type 1 diabetes (Baekkeskov et al., 1990; Rowley et al., 1992). GADA associate with the *HLA-DR3-DQ2* haplotype, which, along with *HLA-DR4-DQ8,* is one of the most important genetic predictors of type 1 diabetes (Ilonen et al., 2013; Vandewalle et al., 1997). In addition to IAA, GADA are also relatively common as the first appearing autoantibody, but generally appear later than IAA (Ilonen et al., 2013; Krischer et al., 2015). GADA as first autoantibodies have been linked to slower type 1 diabetes progression (Krischer et al., 2015; Pöllänen et al., 2019). As opposed to IAA titres, GADA titres have not been observed to influence progression to type 1 diabetes (Steck et al., 2015). In established type 1 diabetes, GADA are the most prevalent remaining autoantibodies (Long et al., 2021).

Islet antigen-2 autoantibodies

Type 1 diabetes associated autoantibodies against islet antigen-2 (IA-2A), also known as ICA-512, were first described in mid 1990s (Lan et al., 1996; Payton et al., 1995; Rabin et al., 1994). Islet antigen-2 is a transmembrane protein that is mainly localised in the secretory granules of pancreatic beta cells. Like IAA, IA-2A has been observed to occur more often in individuals with the *HLA-DRB1*0401-DQB1*0302* haplotype (M.-L. Mikk et al., 2020; Vandewalle et al., 1997). IA-2A is rarely the first appearing autoantibody and has been linked with rapid progression to type 1 diabetes both in children (Endesfelder et al., 2018; Ilonen et al., 2013) and in mixed or adult cohorts (De Grijse et al., 2010; Jacobsen et al., 2020). According to a hypothesis, this might be, because the main target of IA-2A is the intracellular part of the target protein and thus positivity for IA-2A might be a sign of advanced beta

cell autoimmunity (De Grijse et al., 2010). The affinity of IA-2A was not observed to correlate with progression to type 1 diabetes (Krause et al., 2012).

Zinc transporter 8 autoantibodies

Autoantibodies against zinc transporter 8 (ZnT8A) are the latest biochemical autoantibodies to be strongly associated with type 1 diabetes (Wenzlau et al., 2007). Like islet antigen-2, zinc transporter 8 is a transmembrane protein expressed in pancreatic islets and most abundant in beta cells. What is more, it has been shown to take part in insulin secretion (Chimienti et al., 2006). A polymorphism in the gene *SLC30A8* that codes zinc transporter 8 results in three different protein variants differing in the amino acid in position 325, and antibodies against the arginine and tryptophan variants are more common in type 1 diabetes compared to the antibodies against the glutamine variant (Vaziri-Sani et al., 2011; Wenzlau et al., 2008). ZnT8A usually appear later than IAA and GADA with titres increasing with disease progression (Achenbach et al., 2009; Wenzlau et al., 2007). This suggests an association between ZnT8A and rapid type 1 diabetes progression. However, according to some studies ZnT8A do not independently modify disease risk (Jacobsen et al., 2020; Vehik et al., 2020).

2.2.1.1.3 Decline of first-phase insulin response

After the appearance of humoral beta cell immunity, the next measurable marker of disease progression is the decline of first phase insulin response (FPIR). In healthy individuals, glucose intake triggers a biphasic insulin response (Rorsman & Renström, 2003). First, a rapid peak of insulin, FPIR, is released from readily available insulin granules in the beta cells. After this, the second phase insulin response starts and introduces a gradually rising insulin peak that is sustained through the glucose stimulus. FPIR can be measured with an intravenous glucose tolerance test (Park et al., 2021) and low FPIR values are strongly indicative of type 1 diabetes (Bingley, 1996; Bingley et al., 2006), especially when multiple autoantibodies are already present (Siljander et al., 2013). A study by Koskinen *et al*. detected significantly lower FPIR values in children who later developed type 1 diabetes already 4-6 years before the onset of the disease (Koskinen et al., 2016). Additionally, FPIR has showed an inverse correlation to the number of type 1 diabetes associated autoantibodies (Koskinen et al., 2017). A gradual loss of FPIR before clinical onset of type 1 diabetes has been observed (Colman et al., 1998; Srikanta et al., 1984) and an accelerated rate of decline 0.5-1.5 years before clinical disease has been observed (Sosenko et al., 2013).

2.2.1.2 Appearance of clinical disease

2.2.1.2.1 Insulitis and destruction of pancreatic beta cells

Immune infiltration in the islets of Langerhans and the autoimmune destruction of insulin producing beta cells are defining characteristics of type 1 diabetes. The immune cell infiltration of pancreatic islets is called insulitis. Its first description dates to over a hundred years ago (Schmidt, 1902) and a landmark study conducted in 1965 detailed its presence in recent onset patients and noted great variation in the degree of beta cell loss (Gepts, 1965). A later revision of the same dataset revealed that insulitis was mostly present in islets with insulin containing beta cells (Gepts & De Mey, 1978). Another landmark study by Foulis *et al.* confirmed these observations in a larger cohort, but also noted that insulitis could only be detected in 23% of the islets (Foulis et al., 1986). While the insulitis is commonly detected in recent onset cases type 1 diabetes, this is not the case for established disease (M. Campbell-Thompson et al., 2016; In't Veld, 2014). Additionally, according to pooled data from several studies, insulitis is significantly more common before adolescence (In't Veld, 2014).

The modern definition of insulitis is at least 15 CD45+ cells in at least three islets of Langerhans (Campbell-Thompson et al., 2013). It can be found on the periphery of the islet (peri-insulitis) or within the islet parenchyma (intra-insulitis) and out of these peri-insulitis is more common (Krogvold et al., 2016; Reddy et al., 2015). In insulitic lesions, CD8+ cytotoxic T lymphocytes are the most abundant cell type present, followed with CD4+ helper T cells and macrophages (Hänninen et al., 1992; Itoh et al., 1993; Willcox et al., 2009). Immunological heterogeneity regarding the presence of CD20+ B cells in insulitis has been detected (Arif et al., 2014) and their presence seems to associate uniformly with prepubertal age at diagnosis (Leete et al., 2016).

The initiation and exact mechanism of the immune process in the pancreatic islets are still poorly understood. Like other cell types, beta cells can encounter situations, like viral infections, where they undergo cellular stress and get damaged. The resulting secreted proteins, extracellular vesicles and apoptotic bodies may be a catalyst for autoimmunity (Sims et al., 2018). It has also been proposed that neoantigens forming in stressed beta cells through errors in protein production or defective cellular quality control could contribute to type 1 diabetes autoimmunity (Rodriguez-Calvo et al., 2021). According to a suggested disease model (Atkinson et al., 2011; Ilonen et al., 2019), autoreactive CD4+ T cells may become activated when they encounter these autoantigens in the pancreatic lymph node and subsequently activate B cells. CD8+ cytotoxic T cells may in turn be directly

activated by beta cell stress induced secretion of autoantigens and increased MHC class I expression.

To date, the studies by Gepts and Foulis *et al*. represent a significant portion of all the studied cases of insulitis (In't Veld, 2011). Most studies examining insulitis have been conducted with post-mortem samples, which are very rare. In 2007, the Network for Pancreatic Organ Donors with Diabetes (nPOD) was founded with the aim of collecting tissue samples from cadaveric organ donors with type 1 diabetes in an organised manner and it has since then collected a considerable amount of tissue samples. There have also been a few studies utilising biopsies (Imagawa et al., 2001; Krogvold et al., 2014, 2016), but because of the considerable risk of pancreatitis and other complications, studies like these are very rare. Insulitis has also been studied with rodent models, but there are fundamental differences between humans and mice, as is apparent from the vastly differing success rates of intervention studies between the species (In't Veld, 2014).

2.2.2 Heterogeneity and endotypes

There is increasing evidence that type 1 diabetes can be divided into subcategories based on distinct disease pathways or endotypes (Battaglia et al., 2020; Ilonen et al., 2019). Despite the long history of type 1 diabetes research, the exact molecular mechanisms behind its pathogenesis are insufficiently understood. Additionally, intervention studies in humans have had limited success (Primavera et al., 2020). One explanation for this is heterogeneity in the disease process and potential disease endotypes.

2.2.2.1 Variability in the rate of disease pathogenesis

The rate of pathogenesis progression in type 1 diabetes is highly variable. The duration of the prediabetic period can range from a few months to several decades (Knip et al., 2010). In children, some phenotypic differences have been detected between so called fast and slow progressors, like contrasting autoantibody patterns (Pöllänen et al., 2017, 2019). Different HLA haplotypes have been associated with rapid and slow progression (Ilonen et al., 2013; Krischer et al., 2015). Autoreactive CD8+ T lymphocytes with an activated memory phenotype have been linked with rapid progression to type 1 diabetes, whereas slow progressors seem to have an exhausted phenotype (Wiedeman et al., 2020). Seasonal variation in the time of diagnosis has also been seen and while generally the number of diagnoses goes up during cold seasons, in children 0-4 years, the peak is in the autumn (Turtinen et al., 2022). It has been observed that the disease incidence and the risk for developing multiple type 1 diabetes associated autoantibodies decline with age (Bonifacio et al.,

2021) and that slow progressors tend to lose autoantibodies over time (Hanna et al., 2020). The inherent reasons for these differences in disease progression remain obscure.

Autoimmune diabetes is widely regarded as a childhood disease, and most cases are thought to originate in childhood (Haller et al., 2005). However, it can develop at any age, although sometimes the characteristics differ from childhood type 1 diabetes. Despite the high incidence of type 1 diabetes in childhood, the cumulative number of cases in adulthood is higher and therefore adult-onset disease is, in fact, more common (Burahmah et al., 2022). Often autoimmune diabetes has a less aggressive form in adults. Latent autoimmune diabetes in adults (LADA) is considered its own subtype and is defined through diagnosis in adulthood (>30 years), presence of islet autoimmunity and a period of at least 6 months from diagnosis, during which insulin replacement therapy is not necessary (Buzzetti et al., 2017). Also, the polar opposite, fulminant type 1 diabetes is more common in adults than in children and mainly observed in Asian populations (Luo et al., 2020). In it, hypoglycaemia appears within a week, coupled with a rapid loss of beta cells and often infection like symptoms. Perhaps due to the sudden nature of the disease, humoral beta cell autoimmunity cannot be detected in most cases and there has been debate about how comparable to "classical" type 1 diabetes it is. True mechanistic differences between forms of autoimmune diabetes are not properly understood.

2.2.2.2 Heterogeneity in initial disease severity

As with rate of disease pathogenesis, also the initial severity of type 1 diabetes is subject to heterogeneity. Faster rate of disease progress seems to also go hand in hand with an aggressive disease phenotype (Leete et al., 2018). Young age at the onset of type 1 diabetes has been associated with more aggressive insulitis (Leete et al., 2016), lower remaining insulin secretion at diagnosis (Sosenko et al., 2018) and a higher chance of diabetic ketoacidosis at diagnosis (Hekkala et al., 2010; Rodacki et al., 2007). Interestingly, lean children have been observed to have lower residual beta cell function at diagnosis compared to overweight children (Redondo et al., 2012).

Two special cases of type 1 diabetes representing the opposite ends of the spectrum are LADA and fulminant type 1 diabetes. Compared to traditional type 1 diabetes, LADA has milder characteristics, and the initial attack of the beta cells seems to be less severe (Buzzetti et al., 2017; Howson et al., 2011). In fulminant type 1 diabetes the opposite may be true. There is evidence that it could be caused by viral infection in the beta cells and the subsequent immune infiltration of the islets, and it is associated with aggressive ketoacidosis (Imagawa et al., 2000; Luo et al., 2020).

2.2.2.3 IAA-first and GADA-first endotypes

The first autoantibodies to appear in type 1 diabetes are usually IAA or GADA (Ilonen et al., 2013). In recent years it has become increasingly evident that the first appearing autoantibody might have phenotypic implications in disease pathogenesis. This suggest different pathogenic pathways or disease endotypes, starting from the initiation of the disease. Cases with IAA or GADA as first autoantibodies have been compared and several phenotypic associations and characteristics have been discovered.

IAA are more likely to be the first autoantibody in cases, who seroconvert at a very young age and the peak for IAA seroconversion is before the age of two (Giannopoulou et al., 2015; Ilonen et al., 2013; Krischer et al., 2015; Nieto et al., 2021). Diabetic ketoacidosis is also associated to positivity for IAA (Nieto et al., 2021). Cases with IAA as first appearing autoantibodies are more likely to carry the type 1 diabetes predisposing *HLA-DR4-DQ8* haplotype, especially the *DRB1*04:01* positive one (Giannopoulou et al., 2015; Ilonen et al., 2013; Mikk et al., 2020; Triolo et al., 2021) and the risk associated A allele of the single nucleotide polymorphism (SNP) rs689 of the insulin gene (Ilonen et al., 2013). Another study by Ilonen *et al.* observed that the risk alleles A and C of *PTPN22*/rs2476601 and *PTPN2*/rs45450798, respectively, associated with IAA led autoimmunity (Ilonen et al., 2022). The same study observed an association between male sex and IAA as the first appearing autoantibody, as well (Ilonen et al., 2022). Additionally, type 1 diabetes initiated with IAA autoimmunity has been associated with Coxsackievirus B1 infections (Sioofy-Khojine et al., 2018).

GADA have been observed to generally appear later than IAA and the peak for GADA as first autoantibodies is between 3-5 years of age (Giannopoulou et al., 2015; Ilonen et al., 2013; Krischer et al., 2015). GADA as the initiating autoantibody has been linked with the *HLA-DR3-DQ2* haplotype that is strongly linked with increased risk of developing type 1 diabetes (Giannopoulou et al., 2015; Ilonen et al., 2013; Krischer et al., 2015; Triolo et al., 2021). From type 1 diabetes associated non-HLA polymorphisms, *IKZF4*/rs1701704 and *ERBB3*/rs2292239 the risk alleles C and A, respectively, associated with GADA-initiated autoimmunity (Lempainen et al., 2013). An interesting study by Johnson *et al.* found that certain maternal life events that induce psychological stress, coupled with genetic risk markers, were associated with beta cell autoimmunity, with GADA as the first autoantibodies (Johnson et al., 2021). Cases with GADA as the first autoantibodies are more likely to be female (Ilonen et al., 2022; Nieto et al., 2021).

2.3 Genetic and environmental risk factors of type 1 diabetes

2.3.1 Genetic risk factors of type 1 diabetes

The risk of type 1 diabetes is strongly affected by inherited factors, as is evident from the high concordance of diabetes among monozygotic twins. A study by Redondo *et al.* found that the cumulative incidence of type 1 diabetes in initially non-diabetic twins by 60 years of age was 65 % (Redondo et al., 2008). Even though genetic risk factors are necessary for disease onset, they alone are not sufficient and environmental factors modulate the realisation of their risk effect. The first genetic risk factor to be discovered was the HLA (Cudworth & Woodrow, 1975; Nerup et al., 1974; Singal & Blajchman, 1973), and it is to date considered the most important contributor to the risk of developing type 1 diabetes, conferring approximately half of the genetic risk attributed to the disease (Bakay et al., 2019). However, more than 70 other loci that affect the risk have since been discovered. Some of the best characterised are polymorphisms in the insulin gene (*INS*), the protein tyrosine phosphatase non-receptor type 22 gene (*PTPN22*) and the cytotoxic T-lymphocyte associated protein 4 gene (*CTLA4*) (Shapiro et al., 2021).

2.3.1.1 Human leukocyte antigen

The strongest known genetic risk to type 1 diabetes is conferred by the MHC molecules. In humans, the genes that code these molecules are located in the HLA region on chromosome 6p21. Most of the genes in this region are somehow involved in the function of the immune system and the region can be divided into HLA class I, class II, and class III (Shiina et al. 2009). Classes I and II code for MHC molecules. These genes are extremely polymorphic, and the molecules coded by them offer highly individual antigen presenting properties to each person. However, often certain genes appear together, forming conserved haplotypes, and some loci in the HLA are thus in strong linkage disequilibrium to each other.

MHC molecules are cell surface proteins that present peptide antigens to T cells. They can be divided into class I (HLA-A, B and C) and class II (HLA-DP, DQ and DR) molecules (Wieczorek et al., 2017). Both classes are heterodimers that form a peptide binding groove, through which antigen epitopes are presented to T cells through the TCR. The structure of these grooves determines the antigen presenting properties of an individual. Class I genes code the α-chain of the heterodimer and couple with an invariant β-2 microglobulin. The α-chain binds the peptides that the heterodimer presents and the β-2 microglobulin acts as a structural element. HLA class II contains genes for both α (*DPA1*, *DQA1* and *DRA*) and β chains (*DPB1*,

DQB1 and *DRB1*) (Wieczorek et al., 2017). MHC class I are expressed on the surface of most cells, and they present intracellular antigens to CD8+ T lymphocytes (Robertson, 1998). For example, in the event of viral infection or other cellular stress, they can present fragments of microbial proteins, but also mutated or otherwise altered self-antigens that CD8+ T cells recognise as foreign. The expression of class II MHC molecules is limited to APC, like macrophages, dendritic cells and B cells and they present antigens acquired by endocytosis to CD4+ T cells (Robertson, 1998). MHC class II also present self-antigens to T cells in thymic epithelial cells to ascertain self-tolerance. It has been suggested that some haplotypes present antigens less effectively, leading to less successful negative selection (Polychronakos & Li, 2011).

Due to the extremely polymorphic nature of the HLA, a naming convention has been established for all of the variants (Hurley, 2021). According to the current nomenclature convention, variant names take the following form: *HLA-XX*00:00:00:00*. The Xs denote the gene in question and the numbers from left to right the allele group, the specific amino acid sequence numbered in the order of discovery, synonymous exon variation and other non-coding variation. As a rule, shorter forms are presently used. For example, *HLA-DRB1*04:01* is specific variant of the *DRB1* gene belonging in the allele group 04, but no information of its exonic or non-coding variation has been shared.

HLA class II region contains the most important genetic factors for individual type 1 diabetes susceptibility that is *DRB1*, *DQA1* and *DQB1* (Bakay et al., 2019; Caillat-Zucman et al., 1992; Cucca et al., 1995; Erlich et al., 1993). Because of the close proximity of the genes and strong linkage disequilibrium between them, HLA class II associated type 1 diabetes risk predominantly evaluated by *DR-DQ* haplotype (Erlich et al., 2008) and some of the most common haplotypes associated with disease risk or protection are presented in Table 2. Two haplotypes *DRB1*03:01-DQA1*05:01-DQB1*02:01* and *DRB1*04-DQA1*03:01- DQB1*03:02* confer the highest risk for developing type 1 diabetes (Erlich et al., 2008; Hermann et al., 2003; Ilonen et al., 2016; Noble & Valdes, 2011). As a remnant of serological HLA nomenclature, they are often abbreviated *DR3-DQ2* and *DR4-DQ8,* respectively. In individuals carrying both risk haplotypes, their effect synergistically becomes even stronger (Ilonen et al., 2016; Thomson et al., 1988). The *DR4-DQ8* haplotype has subtypes that modulate the risk it confers. The highest type 1 diabetes risk is mediated by *DRB1*04:01* and *DRB1*04:05*, while the risk carried by *DRB1*04:02* and *DRB1*04:04* is lower and *DRB1*04:03* renders the haplotype protective (Erlich et al., 2008). The *DR4-DQ8* haplotype is also the most common risk haplotype in Finland, followed by *DR3-DQ2,* and their combination can be found in approximately one fourth in cases diagnosed in childhood (Hermann et al., 2003). Haplotypes can also offer protection to type 1 diabetes and protection is most

commonly provided by the *DRB1*15:01-DQB1*06:02* haplotype that is quite common in Caucasian populations and also neutralises the risk conferred by other variants (Erlich et al., 2008; Hermann et al., 2003; Noble & Valdes, 2011). HLA class II risk variants seem to associate to the initiation of autoimmunity in type 1 diabetes (Ilonen et al., 2016).

Although MHC class II are the most important genetic factors in the risk for type 1 diabetes, class I molecules do also have an effect. This is not surprising, considering the important role of MHC class I molecules in CD8+ T cell immunity. MHC class I molecules are hyperexpressed in insulitic lesions (Bottazzo et al., 1985; Foulis et al., 1987). The most notable type 1 diabetes associated HLA class I variants are *A*24:02* and *B*39:01* (Howson, Walker, Clayton, et al., 2009; Nejentsev et al., 2007; Noble et al., 2002). Both of these variants are not only associated with the disease but also a younger age of diabetes onset (Howson, Walker, Clayton, et al., 2009; Nejentsev et al., 2007). Unlike risk variants in class II, they have been associated with progression from established beta cell autoimmunity to disease onset (Mikk et al., 2017). This is in line with a theory, where naïve autoreactive CD4+ T cells are activated by APC as a result of beta cell stress and help B cells differentiate to plasma cells. Later, CD8+ T cells are recruited to islets with the start of insulitis (Ilonen et al., 2019).

In addition to type 1 diabetes risk, loci in the HLA region associate with several other autoimmune conditions, including, but not limited to, rheumatoid arthritis, celiac disease, systemic lupus erythematosus and multiple sclerosis (Matzaraki et al., 2017). Many HLA alleles are associated with several autoimmune diseases and sometimes alleles can predispose to one disease and protect from another. For instance, *HLA-DQA1*05-DQB1*02* predisposes for both type 1 diabetes and celiac disease, but *HLA-DQB1*06:02* protects from type 1 diabetes while being a risk allele for multiple sclerosis (Márquez & Martín, 2022; Tettey et al., 2015).

Table 2. Some commonly type 1 diabetes associated HLA DR/DQ haplotypes and genotypes. A) The risk effects to type 1 diabetes that the haplotypes confer are abbreviated in the table as follows: S = strong susceptibility; s = weak susceptibility; N = neutral; p = weak protection; P = strong protection. B) Different combinations of the haplotypes form risk groups. Modified from (Ilonen et al., 2016).

A

HAPLOTYPE	ODDS RATIO	RISK
DRB1*04:01-DQA1*03-DQB1*03:02	10.11	S
DRB1*04:05-DQA1*03-DQB1*03:02	3.01	S
DRB1*04:04-DQA1*03-DQB1*03:02	2.82	s
(DR3)-DQA1*05-DQB1*02	2.81	s
DRB1*04:02-DQA1*03-DQB1*03:02	1.75	S
(DR13)-DQB1*06:04	1.13	N
(DR9)-DQA1*03-DQB1*03:03	0.97	N
(DR8)-DQB1*04	0.95	N
(DR16)-DQB1*05:02	0.79	N
(DR7)-DQA1*0201-DQB1*02	0.63	N
(DR1/10)-DQB1*05:01	0.58	N
(DR4)-DQA1*03-DQB1*03:01	0.51	N
DRB1*0403-DQA1*03-DQB1*03:02	0.37	p
(DR13)-DQB1*06:09	0.36	N
(DR13)-DQB1*06:03	0.23	p
(DR11/12/13)-DQA1*05-DQB1*0:301	0.23	p
(DR7)-DQA1*02:01-DQB1*03:03	0.08	P
(DR15)-DQB1*06:01	0.07	P
(DR15)-DQB1*06:02	0.03	P
(DR14)-DQB1*05:03	0.03	P

B

GENOTYPE RISK GROUP	HAPLOTYPES
HIGH RISK	S/s*, s/s*
MODERATELY INCREASED RISK	S/s**, s/s, S/S**, S/N
SLIGHTLY INCREASED RISK	s/N, S/p
NEUTRAL	N/N, S/P, s/P, s/p
SLIGHTLY DECREASED RISK	p/N
STRONGLY DECREASED RISK	P/N, p/p, P/p, P/P

* if DR3-DQ2/DR4-DQ8
** if DR4-DQ8/ DR4-DQ8

2.3.1.2 Single nucleotide polymorphisms

To date, more than 70 non-HLA loci affecting the risk of type 1 diabetes have been identified (Robertson et al., 2021; Chiou et al., 2021). A few of these loci, like the *INS* and *PTPN22* genes, have been known to associate with type 1 diabetes for a longer time. However, it was not until some 15 years ago that genome wide association studies (GWAS) expanded the list of known risk genes exponentially. Typically, GWAS detect SNPs, who are gene variant, that differ only by one base from each other. In coding regions SNPs may change the amino acid sequence and in non-coding regions for example affect gene expression or messenger RNA structure.

In 2007 the Wellcome Trust Case Control Consortium published a GWAS (Burton et al., 2007), where they compared cases with seven common diseases, including type 1 diabetes, to a shared group of healthy controls and searched for genes associating to these diseases using the Affymetrix GeneChip 500K. Three completely new risk loci for type 1 diabetes were found in the study and most of the known ones were confirmed. Shortly after, most of the findings were validated in other cohorts and new associations added (Cooper et al., 2009; Todd et al., 2007). Meta-analyses combining different cohorts have since then solidified earlier findings and added new loci to the list of type 1 diabetes risk genes (Barrett et al., 2009; Bradfield et al., 2011; Cooper et al., 2008). In 2009, the Type 1 Diabetes Genetics Consortium combined and analysed the data from the Wellcome Trust cohort, several other studies and added some 16500 new samples of their own in a landmark study and observed 41 type 1 diabetes associated loci, of which 22 were novel (Barrett et al., 2009). A large study with a diverse population from 2021 identified 78 type 1 diabetes associated genomic regions, of which 36 were novel (Robertson et al., 2021). Another extensive GWAS from the same year investigating people with European ancestry found 81 statistically significant associations, of which 33 were novel (Chiou et al., 2021).

Most of the type 1 diabetes risk associated genes discovered in GWAS have functions in the immune system or pancreatic beta cells (Bakay et al., 2019). Many risk SNPs also colocalise with enhancer sequences that are active in immune cells (Onengut-Gumuscu et al., 2015). The effects on cell function can also be studied more precisely through quantitative trait loci (QTL) mapping, which associates loci with complex phenotype effects, and different chromatin assays. Studies focusing on the link between type 1 diabetes risk SNPs and altered cell functions may further our understanding of the mechanisms leading to type 1 diabetes. For example, alterations in genes involved in chemokine signalling have been found to affect T cell and especially Treg frequencies (Chu et al., 2022). Moreover, a study by Chiou and colleagues found that several of their disease associated GWAS loci held functional

associations with immune cells, such as T cells and monocytes, and cell types of the exocrine pancreas (Chiou et al., 2021).

Many of type 1 diabetes associated loci have been linked to other autoimmune disorders, like rheumatoid arthritis and multiple sclerosis, but interestingly, not all the associations are concordant in regards of association direction (Parkes et al., 2013). Sometimes a variant that confers risk to one autoimmune condition may protect from another. Autoimmune diseases that share characteristics and thus may share pathogenic pathways, often associate to the same risk variants. For example, type 1 diabetes and rheumatoid arthritis, that both present with autoantibodies, share several risk loci in common (Onengut-Gumuscu et al., 2015; Pociot & Lernmark, 2016).

Recently, non-HLA risk polymorphisms have been utilised in improving individual risk prediction. Several studies have set out to create genetic risk score models for more accurate population screening, disease recognition and prediction and have achieved improved prediction power (Ferrat et al., 2020; Frohnert et al., 2018; Oram et al., 2015; Sharp et al., 2019; Winkler et al., 2014).

2.3.1.2.1 Insulin

INS is located on chromosome 11p15 and encodes preproinsulin. According to several studies, *INS* confers the strongest risk to type 1 diabetes outside of the HLA region (Barrett et al., 2009; Laine et al., 2013; Smyth et al., 2008). It is therefore not surprising that it was the first non-HLA gene to be associated with the risk of type 1 diabetes. The discovery of the risk association took place in 1984 and the risk was initially attributed to a variable number tandem repeat (VNTR) approximately 600 bp upstream of *INS* (Bell et al., 1984). Different length variants were split to “classes” I, II and III and it was found that the shortest class I associated with type 1 diabetes, especially in Caucasian populations (Bell et al., 1984). Conversely, the long VNTR class III variants were generally associated with a protective effect (Stead et al., 2000). VNTR class III was also observed to have a considerably higher thymic expression level of insulin compared to class I (Vafiadis et al., 1997). It is widely accepted that insufficient insulin expression may affect the establishment of self-tolerance against insulin by letting insulin reactive T cells escape negative selection due to insufficient insulin expression.

With the advances in genetic studies, two type 1 diabetes associated SNPs, -23HphI and +1140A/C were soon discovered in the region (Barratt et al., 2004). The first of these, also known as rs689, is an intronic variant and the most widely studied *INS* variant to date. The rs689 polymorphism replaces the ancestral and protective T allele in position -23 of the gene promoter with the type 1 diabetes predisposing allele A. The risk allele A is in nearly complete linkage disequilibrium with VNTR

class I (Stead et al., 2003). The predisposing variant has been associated with IAA in type 1 diabetes (Hermann et al., 2003), especially as the first autoantibody (Lempainen et al., 2015). Interestingly, the risk variant was recently associated with the loss of B cell anergy in insulin binding B cells (Smith et al., 2018). The cells may be activated by insulin reactive T helper cells, and therefore the result supports the theory of the risk variant affecting negative selection. What is more, the risk allele has been observed to have higher levels of DNA methylation than the protective allele (Carry et al., 2020; Fradin et al., 2012). The *INS*/rs689 risk allele A has also been associated with a younger age at diagnosis (Howson, Walker, Smyth, et al., 2009).

Some other *INS* SNPs, like rs3842753 and rs3842729, have been associated with type 1 diabetes, but they have been studied far less and little else is known about them (Barratt et al., 2004; Laine et al., 2004).

2.3.1.2.2 Protein tyrosine phosphatase non-receptor type 22

Another important non-HLA risk associated locus, *PTPN22,* was discovered in 2004 (Bottini et al., 2004). The gene is located on chromosome 1p13 and codes for the protein tyrosine phosphatase non-receptor type 22. The protein is also known as lymphoid tyrosine phosphatase because it is expressed in all leukocyte lineages, but especially in thymocytes and mature T and B cells (Cohen et al., 1999). The murine homolog Pep has also been used especially in earlier PTPN22 studies. In T cells the protein functions as a negative regulator of TCR signalling and thus maintains immune homeostasis. PTPN22 acts by dephosphorylating the Src family kinase LCK and the ZAP-70 in the CD3 complex (Shapiro et al., 2021). Its suppression capacity improves considerably, when it forms a complex with the C-terminal Src kinase (Cloutier & Veillette, 1996, 1999), which is another negative regulator of immune response.

The discovered risk variant is caused by an exonic SNP rs2476601 that replaces the ancestral G allele with an A in the position 1858 of the gene. This in turn results in the replacement of the amino acid arginine to tryptophan in position 620. The risk variant of rs2476601 has been found to inhibit the interaction between PTPN22 and the C-terminal Src kinase (Bottini et al., 2004). The SNP therefore affects T cell activation. In a recent *in vitro* study, it was found that *PTPN22* rs2476601 decreases PTPN22 expression in T cells (Shaw et al., 2021). In addition to type 1 diabetes, the risk variant has also been linked with several other autoimmune diseases, like rheumatoid arthritis, juvenile idiopathic arthritis, and systemic lupus erythematosus (Begovich et al., 2004; Kyogoku et al., 2004; Viken et al., 2005).

While it is generally recognised that *PTPN22*/rs2476601 is a functional variant, a debate exists about whether it is a gain-of-function or a loss-of-function variant.

An intuitive loss-of-function mechanism for the *PTPN22*/rs2476601 risk variant is the diminished regulation of T cell responses and support for this theory has been found. It has been observed that the autoimmune variant leads to enhanced expansion and activation of T cells (Hasegawa et al., 2004; R. C. Sharp et al., 2018) and overexpression of PTPN22/Pep inhibits T cells (Cloutier & Veillette, 1996). Additionally, the variant promotes both positive and negative selection (Dai et al., 2013; Zhang et al., 2011). Conversely, other studies have observed very different functions that would suggest that *PTPN22*/rs2476601 is a gain-of-function variant. Some studies have found the variant to inhibit T and B cell responses better than the wild type (Aarnisalo, Treszl, et al., 2008; Arechiga et al., 2009; Rieck et al., 2007; Vang et al., 2005). The effects of *PTPN22*/rs2476601 therefore seem variable and possibly dependent on the target tissue. A so called "switch-of-function" model that includes both scenarios, has also been proposed (Vang et al., 2018).

In the context of type 1 diabetes, the autoimmune variant of rs2476601 associates to increased risk of disease in Caucasian populations (Tang et al., 2012). Additionally, the *PTPN22* rs2476601 risk allele A has been associated with IAA as the first appearing autoantibody (Krischer et al., 2019). In mice, the variant was observed to cause an increase in IAA, along with a higher frequency and an earlier onset of the disease (Lin et al., 2016). The risk variant has been associated with lower CD4+ T cell responsiveness in diabetic children (Aarnisalo, Treszl, et al., 2008).

2.3.1.2.3 Interleukin 2 receptor alpha subunit

The association between the gene encoding IL2RA and type 1 diabetes risk was also discovered outside of GWAS (Vella et al., 2005). The alpha subunit is a part of a high affinity receptor complex that binds IL2, an important cytokine of the immune system, with integral tasks in the regulation of T cell differentiation and function. The alpha subunit protein is capable of binding IL2 also on its own and it can initiate complex formation with the beta and gamma subunits. A lower affinity receptor complex with just the beta and gamma subunits also exists. The alpha subunit is expressed especially on Tregs, where IL2 receptor signalling is needed for Treg homeostasis and differentiation. Additionally, the signalling prompts Tregs to limit CD8+ responses. IL2RA associates to other autoimmune diseases too, such as juvenile idiopathic arthritis and multiple sclerosis (Hinks et al., 2009; The International Multiple Sclerosis Genetics Consortium, 2007).

Multiple *IL2RA* SNPs have been associated with the risk of type 1 diabetes. One of the best known is rs2104286, whose association with type 1 diabetes was discovered in the large study by the Wellcome Trust Case Control Consortium (Burton et al., 2007). Its minor allele G is neutral and its major allele A increases risk for type 1 diabetes. The risk allele was observed to associate with lower levels

of circulating IL2 receptor alpha subunit (Maier et al., 2009). The SNP rs12722495 switches an ancestral T into a C and associates with a lower risk of type 1 diabetes (Smyth et al., 2008). Another study in 2007 found risk associations to type 1 diabetes also with rs706778 and rs3118470 (Hui-Qi Qu et al., 2007).

2.3.1.2.4 Erb-B2 receptor tyrosine kinase 3 and zinc finger protein Eos

Two different genes located on chromosome 12q13 have been of special interest in the study of type 1 diabetes and strong linkage disequilibrium effects in the region have made the study of risk effects more complicated (Keene et al., 2012). The genes *ERBB3* and *IKZF4* code for the Erb-B2 receptor tyrosine kinase 3 and zinc finger protein Eos (also known as Ikaros family zinc finger 4) and both associate with type 1 diabetes (Burton et al., 2007; Hakonarson et al., 2008; Todd et al., 2007).

ERBB3, also known as HER3, is a cell surface protein from the epidermal growth factor family. Its ligands are NRG1 and NRG2 that both regulate development and cell differentiation. Binding a ligand allows ERBB3 to form a heterodimer with other epidermal growth factor receptors and transmit the growth factor signal. The ERBB2-ERBB3 heterodimer is considered the most active of all possible epidermal growth factor receptor heterodimers. The most widely studied risk variant of *ERBB3* is the SNP rs2292239 and in it an ancestral, risk inducing T allele is replaced with a protective G (Todd et al., 2007). Out of these, the ancestral risk variant has become the minor allele. The risk effect of the allele has been observed individually in several ethnic groups (D. Wang & Pan, 2019). The protective variant was observed to increase ERBB3 expression on APCs and modulate their ability to stimulate T cells (Wang et al., 2010). *ERBB3*/rs2292239 risk allele T hast been associated with both seroconversion (Törn et al., 2014) and progression from single to multiple autoantibodies (Vandewalle et al., 2021) in the pathogenesis of type 1 diabetes. Interestingly the latter association could only be seen in female cases. Additionally, the risk allele T associates with GADA led autoimmunity (Ilonen et al., 2018). Perhaps surprisingly, *ERBB3*/rs2292239 risk variant also associates with better residual beta cell function and metabolic control compared GT or GG individuals (Kaur et al., 2016).

Eos is a transcription factor belonging in the Ikaros family that is expressed in lymphocytes. Along with other transcription factors in the Ikaros family, it participates in the regulation of immune cell development and has been studied particularly in Tregs. In the *IKZF4* SNP rs1701704 the minor allele G replaces an ancestral T. The minor allele associates with an increased risk of developing type 1 diabetes, especially in individuals with GADA as their first autoantibody (Hakonarson et al., 2008; Lempainen et al., 2015). At the same time, the G allele seems to inversely associate with IAA (Lempainen et al., 2013). Children

homozygous for the risk variant have also been observed to have a faster deterioration of first phase insulin response (Koskinen et al., 2019). The protective T allele has been observed to associate with slower autoantibody spreading, but only in females (Vandewalle et al., 2021).

2.3.1.2.5 Protein tyrosine phosphatase non-receptor type 2

In addition to *PTPN22*, there is also a second protein tyrosine phosphatase encoding locus, protein tyrosine phosphatase non-receptor type 2 (*PTPN2*) that has been associated with type 1 diabetes (Burton et al., 2007). The gene is located in chromosome 18p11 and the protein it codes for is also known as the T cell protein tyrosine phosphatase, due to its original tissue of discovery (Cool et al., 1989). The protein has two primary isoforms that are expressed in the endoplasmic reticulum and the nucleus. The isoforms differ in their C-terminal ends, which determine their localisation. The epidermal growth factor receptor, Janus kinases JAK1 and JAK3 and transcription factors of the signal transducers and activators of transcription (STAT) family are some of the known substrates for PTPN2 and the downstream effects include modulation of immune cell differentiation and cytokine signalling (Cerosaletti & Buckner, 2012).

A few different SNPs in the *PTPN2* locus have been linked with type 1 diabetes. Perhaps the most widely studied one is an intronic variant rs1893217 that was originally found by Todd *et al.* and later confirmed in a large meta-analysis from Barrett *et al.* (Barrett et al., 2009; Todd et al., 2007). In it the ancestral A allele is replaced by a G. In addition to type 1 diabetes, it associates to several other autoimmune diseases like Crohn's disease and celiac disease (Cerosaletti & Buckner, 2012). In type 1 diabetes, the risk allele G associates particularly to IAA autoimmunity (Steck et al., 2012). The variant has been reported to reduce PTPN2 RNA levels and weaken IL2 receptor signalling in CD4+ T cells (Long et al., 2011). PTPN2 deficiency has been studied further in murine models and several effects have been observed. These include impairment of peripheral tolerance upkeep in CD8+ and B cells, modulation of pancreatic beta cell apoptosis and changes towards proinflammatory phenotypes in several immune cell populations, leading to faster disease progression (Santin et al., 2011; Smith et al., 2018; Wiede et al., 2014, 2017, 2019; Xi et al., 2015).

Another SNP, rs2542151, was discovered in the hallmark study by the Wellcome Trust Case Control Consortium, which also observed an association between the SNP and Crohn's disease (Burton et al., 2007). It was later associated with earlier progression to disease (Espino-Paisan et al., 2011). It is in strong linkage disequilibrium with rs1893217 in European populations (Todd et al., 2007).

A less known variant rs45450798 has been associated with a faster decline of the first-phase insulin response in children with multiple type 1 diabetes associated autoantibodies (Koskinen et al., 2019).

2.3.2 Environmental risk factors of type 1 diabetes

While genetic predisposition remains the most important predictor of type 1 diabetes, the importance of environmental risk factors has also become evident. In the past decades, the incidence of type 1 diabetes has risen considerably (Tuomilehto, 2013). Additionally, there is evidence that some migrant groups adapt to the risk levels of their new area of residence (Bodansky et al., 1992; Söderström et al., 2012). Therefore, the increase in incidence cannot be due to genetic factors, but changes in our environment and lifestyles. Consequently, considerable effort has been put to the study of these environmental factors and their effect on the immune system. Several associations between type 1 diabetes and environmental and lifestyle factors, such as mode of birth, viral infections, and dietary factors, have been discovered (Rewers & Ludvigsson, 2016). Additionally, the hygiene hypothesis has been implicated in the pathogenesis of type 1 diabetes, but the studies investigating the theory have so far been inconclusive (Bach & Chatenoud, 2012; Rewers & Ludvigsson, 2016).

2.3.2.1 Microbiome and type 1 diabetes

During the first years of life, several environmental aspects modify the maturing of the immune system. Things like infections and environmental microbes challenge a child's developing immune system and train it. An important process for normal maturation of the immune system is the establishment of the intestinal microbiome. The development of the gut microbiome takes place in phases and stabilises by three years of age (Stewart et al., 2018). This period coincides with the time, during which beta cell autoimmunity often initiates (Ilonen et al., 2013). Disturbances in the intestinal ecosystem have been linked with both autoimmune and allergic disease, as well as infection, inflammation, and obesity (Gomaa, 2020).

The development of the intestinal microbiome is affected by several factors, such as geographic location, mode of delivery, use of antibiotics, nutrition, and host genetics. In healthy individuals, the most abundant phyla are *Firmicutes* and *Bacteroidetes*, followed by *Actinobacteria* and *Proteobacteria* (Gomaa, 2020). The microbial diversity of the gut was observed to be lower in children with beta cells autoimmunity in several studies (Brown et al., 2011; de Goffau et al., 2013; Kostic et al., 2015; Murri et al., 2013; Russell et al., 2019). Additionally, changes in microbial composition have been reported (Brown et al., 2011; de Goffau et al., 2013; Murri et al., 2013; Stewart et al., 2018). Administration of probiotics within

the first month of life has been associated with reduced risk of beta cell autoimmunity in children with high HLA conferred risk of type 1 diabetes (Uusitalo et al., 2016).

2.3.2.2 Antibiotics, health and type 1 diabetes

One strong modulator of the gut microbiome is the use of antibiotics. While they are necessary in the combat against difficult bacterial infections, antibiotics are also harmful for our commensal bacteria. They are known to cause intestinal dysbiosis, marked by diminished microbial diversity, metabolic alterations, and lower colonisation resistance towards pathogenic species (Lange et al., 2016). There is evidence that antibiotics administered in early childhood may have long lasting effects on the gut microbiome (McDonnell et al., 2021), as well as metabolic effects and alterations in immune gene expression (Cox et al., 2014). Use of antibiotics has been associated, for example, with allergies, atopic disorders, and inflammatory bowel disease (Vangay et al., 2015).

Studies investigating the association between type 1 diabetes and antibiotics have had contradictory results. Some studies found no association between type 1 diabetes and antibiotics or the frequency of their use (Kemppainen et al., 2017; Tapia et al., 2018). Others have found evidence of increased disease risk after antibiotics use. One study reported an association between type 1 diabetes and frequent use of antibiotics (Mikkelsen et al., 2017). An association between antibiotics consumption and type 1 diabetes prevalence has also been observed (Ternák et al., 2022). Additionally, one study found a link between diabetes and the mother's use of antibiotics before pregnancy (Kilkkinen et al., 2006).

2.3.2.3 Nutrition and type 1 diabetes

Nutrition and dietary choices are simultaneously highly individual and tied to geographical location. Especially in Western cultures, our dietary habits have changed dramatically in the past several decades and the timing matches with the global increase in type 1 diabetes. As an example, the introduction of processed foods has made a large impact on many people's dietary choices. Several dietary factors, such as cow's milk, cereals, and duration of breastfeeding, have been associated with the risk of developing type 1 diabetes (Rewers & Ludvigsson, 2016).

Various studies have investigated the effect of breastfeeding on the risk of type 1 diabetes with varying results. A meta-analysis combining 43 studies found exclusive breastfeeding in early infancy to have a slight protective effect against type 1 diabetes (Cardwell et al., 2012). Some other studies, however, have failed to find such an effect (Couper et al., 1999; Virtanen et al., 2011; Ziegler et al., 2003).

Breastfeeding is also one of the strongest influencers of the early gut microbiome composition (Bäckhed et al., 2015).

Weaning and the introduction of foods in early childhood have been investigated in relation to type 1 diabetes risk. Baby formula containing cow's milk, for instance, has been investigated in several cohorts, because it is often the first non-breastmilk food introduced. Most studies have not found a link between cow's milk formula and type 1 diabetes (Couper et al., 1999; Norris et al., 2003; A.-G. Ziegler et al., 2003). However, others have found an association between the consumption of cow's milk in childhood and beta cell autoimmunity and type 1 diabetes, especially relating to certain cow's milk caseins (Elliott et al., 1999; Muntoni et al., 2000; Vaarala et al., 1999). The Trial to Reduce Insulin-Dependent Diabetes Mellitus in the Genetically at Risk study (TRIGR) investigated the effect of using hydrolysed casein baby formula instead of conventional cow's milk formula, but this did not decrease the incidence of type 1 diabetes or associated autoantibodies (Knip et al., 2014; Writing Group for the TRIGR Study Group, 2018).

The introduction of cereals and gluten (Norris et al., 2003; A.-G. Ziegler et al., 2003) have been associated with an increased risk of type 1 diabetes. The Swedish All Babies in Southeast Sweden (ABIS) study only observed an association between late introduction of gluten and GADA (Wahlberg et al., 2006), while the Finnish DIPP study did not find an association at all (Virtanen et al., 2011). Additionally, the early introduction of root vegetables has shown an association to disease (Virtanen et al., 2011).

Vitamin D has been studied as a potential protective factor in the context of type 1 diabetes and several studies have found lower vitamin D levels in diabetic patients (Infante et al., 2019). There is also evidence that higher serum vitamin D levels during pregnancy or childhood lower risk of islet autoimmunity (Norris et al., 2017; Sørensen et al., 2011) and some studies have observed vitamin D supplementation to have a positive effect to disease risk (Zipitis & Akobeng, 2008). However, several other studies have found no association between childhood vitamin D levels and type 1 diabetes (Cadario et al., 2015; Mäkinen et al., 2016; Miettinen et al., 2012).

Dietary risk associations are for the most part contradictory and no clear consensus regarding the effect of dietary factors has been found (Dedrick et al., 2020; Rewers & Ludvigsson, 2016). It is possible that the effects are specific to certain subpopulations of risk individuals.

2.3.2.4 Viral infections and type 1 diabetes

An association between type 1 diabetes and viruses has long been suspected. Viral infections are an important educator of the immune system in early childhood and young children go through many infections. The incidence of type 1 diabetes follows

a seasonal pattern (Turtinen et al., 2022). What is more, infections and other illnesses associate with higher insulin requirements, because of the stress they cause to the body and may therefore reveal the onset of diabetes in an early stage (Laffel, 2000).

The most compelling evidence for elevating the risk of type 1 diabetes is for enteroviruses. They are single stranded RNA viruses of the *Picornaviridae* family. A recent meta-analysis of 38 studies found an association between enterovirus infection and type 1 diabetes in European, African, Asian, Australian, and Latin American populations (Wang et al., 2021). Enteroviruses can infect the pancreas and particularly group B coxsackieviruses localise preferentially to pancreatic beta cells (Ylipaasto et al., 2004). Acute pancreatitis and infected islets have been observed in diabetic patients and cases with beta cell autoimmunity (Dotta et al., 2007; Oikarinen et al., 2008). Additionally, enteroviruses have been found to have damaged pancreases of children, who have died of enteroviral myocarditis (Foulis et al., 1990). It has been proposed that persistent low-grade enterovirus infection could cause beta cell damage leading to type 1 diabetes (Hyöty et al., 2018). Enteroviral RNA has been found in the blood and stools of cases with beta cell autoimmunity shortly before seroconversion (Honkanen et al., 2017; Oikarinen et al., 2010). Coxsackie B1 infections have been associated with IAA-initiated beta cell autoimmunity, but not with GADA led autoimmunity (Sioofy-Khojine et al., 2018). The risk effect was curiously strongest in children, for whom the coxsackie B1 infection was the first group B coxsackie virus infection. The risk effect of coxsackie B1 infection was also observed to be mitigated by maternal antibodies (Laitinen et al., 2014). According to the polio hypothesis, the diabetogenic risk effect of enteroviruses could be tied to the incidence of enterovirus infections in populations (Viskari et al., 2000). In countries, where enteroviruses have become rarer, the risk for an infection triggering type 1 diabetes would be higher.

Enteroviruses are, however, not the only viruses that have been investigated in the context of type 1 diabetes. The human cytomegalovirus has been detected in the islets of type 1 diabetes patients (Yoneda et al., 2017), but most studies have not been able to find an association between cytomegalovirus infection and type 1 diabetes (Aarnisalo, Veijola, et al., 2008; Hiltunen et al., 1995). Interestingly, a cytomegalovirus infection in early childhood may potentially decelerate type 1 diabetes progression (Ekman, Vuorinen, et al., 2019). Antibodies to the Ljungan virus have correlated with the *HLA-DR4-DQ8* haplotype and IAA (Nilsson et al., 2013, 2015). There is also some evidence that rotaviruses, mumps, and rubella, as well as various respiratory infections, may associate with an increased risk of type 1 diabetes (Rodriguez-Calvo et al., 2016).

3 Aims

The purpose of this thesis was to investigate factors influencing the heterogenic autoimmune process leading to type 1 diabetes.

The specific aims were:

1. To evaluate the effect of 21 known type 1 diabetes risk polymorphisms in the time periods from birth to seroconversion, seroconversion to onset of type 1 diabetes and birth to onset of type 1 diabetes in study I.
2. To assess the potentially differing effects of 21 known type 1 diabetes risk polymorphisms in the time periods from birth to seroconversion, seroconversion to onset of type 1 diabetes and birth to onset of type 1 diabetes to suspected disease endotypes in study I.
3. To determine the effect of seven type 1 diabetes risk polymorphisms to regulatory T cell frequencies in study II.
4. To examine transcriptional changes in peripheral blood mononuclear cells, in particular monocytes, between children positive for multiple type 1 diabetes associated autoantibodies and healthy controls in study III.

4 Materials and Methods

4.1 Subjects

Except for a small subset of children with type 1 diabetes in study II, all subjects studied in this thesis were participants in the DIPP study. DIPP is a prospective birth cohort study that recruits newborns with an increased genetic risk for T1D among the general population in the Turku university hospital, Oulu university hospital and Tampere university hospital since 1994, 1995 and 1997, respectively. The aim of the ongoing follow-up study is to investigate the pathogenic process leading to clinical type 1 diabetes and improve predictability of this process based on known risk factors such as genetic risk and appearance of humoral immunity. Additionally, the study seeks to eventually find effective preventative treatments for the disease.

When informed consent is obtained from the parents of the children born in the three participating hospitals, the children are genotyped for HLA alleles associated with risk for developing type 1 diabetes. After further consent, at-risk children are invited to take part in the follow-up study. For the first two years of life, the study participants visit a study clinic every three to six months. After this, the children have clinic visits every six to twelve months (Kupila et al., 2001). In these visits, the participants are monitored for the appearance of type 1 diabetes associated autoantibodies. Originally, children were first monitored only for ICA and after its appearance, also biochemical autoantibodies IAA, GADA and IA-2A were analysed from all available samples (Kupila et al., 2001). From 2003 all children participating in the study have been tested for all four autoantibodies as well as a subset of 1006 children born between November 1994 and July 1997 (Kukko et al., 2005). If autoantibodies are detected persistently that is, in two subsequent visits at three months intervals, frequency of visits is raised to every three months and glucose tolerance tests are regularly performed at following study clinic visits (Kupila et al., 2001). Progression to type 1 diabetes is diagnosed according to World Health Organization criteria (Alberti & Zimmet, 1998).

The study was approved by the local research ethics committees, and it is carried out according to the ethical principles outlined in the Declaration of Helsinki.

4.1.1 Study I

In study I, the effects of 21 type 1 diabetes associated SNPs on disease pathogenesis duration were investigated in the time periods from birth to seroconversion, seroconversion to onset of type 1 diabetes and birth to onset of disease. What is more, the same analyses were also conducted in suspected disease endotype subgroups that were defined through the first appearing autoantibody.

The study cohort in study I comprised 976 case subjects with beta cell autoimmunity and 1910 clinically healthy control subjects, who remained negative for type 1 diabetes associated autoantibodies throughout the follow-up. Beta cell autoimmunity was defined through having at least one persistently positive biochemical autoantibody (IAA, GADA or IA-2A). Most cases had two healthy controls, who were matched for sex, study centre and birth date with a range of two months before or after the birth of the connected case. During the follow-up, in 560 of the case subjects the islet autoimmunity spread to multiple autoantibodies and 426 case subjects received a diagnosis for type 1 diabetes. The median (interquartile range) ages for seroconversion and progression to clinical type 1 diabetes were 3.30 (1.49-5.11) years and 6.75 (3.68-9.82) years, respectively and the median age for all cases at the time of investigation was 10.47 (5.98-14.97) years. The median follow-up time for controls was 10.83 (6.65-15.01) years.

4.1.2 Study II

Study II of the thesis explored the effects of seven type 1 diabetes associated SNPs on Treg proportions in children. The children investigated formed two separate cohorts; the main cohort and a validation cohort that was used to verify the main finding of the study. The main cohort comprised 65 case subjects diagnosed with type 1 diabetes, 83 subjects with islet autoimmunity and 215 autoantibody negative and clinically healthy control subjects. The mean ages (SD) of these subgroups were 7.4 (±3.9), 8.6 (±4.7) and 8.8 (±3.9), respectively. Healthy control subjects and autoantibody positive case subjects were participants in the DIPP study and were carriers of HLA-conferred type 1 diabetes risk. Case subjects with type 1 diabetes had been recruited at the Department of Paediatrics, Turku University Hospital after hospitalisation with a diagnosis for type 1 diabetes. Persistent positivity for at least one biochemical type 1 diabetes associated autoantibody (IAA, GADA or IA-2A) was once more used as the definition of beta cell autoimmunity in subjects.

The validation cohort of the study consisted of ten trios of clinically healthy, autoantibody negative, unrelated children, also selected from the DIPP cohort. Each member of a trio carried a different variant of the three possible genotypes of *PTPN22* rs2476601 (AA, AG, GG). Each trio of children was matched for HLA class

II genotype, age, sex, and date of sampling. The mean age (SD) of the children in the validation cohort was 2.3 years (±1.5).

4.1.3 Study III

In study III of the thesis work, transcriptional changes in the peripheral blood mononuclear cell (PBMC) of children with beta cell autoimmunity were investigated compared to healthy controls. Two separate cohorts were studied. First, a preliminary analysis was carried out with a pilot cohort compiled from existing frozen PBMC samples. The pilot cohort comprised nine case-control pairs in which the cases were persistently positive for ICA and at least one biochemical autoantibody (IAA, GADA or IA-2A). In addition, the cases had developed type 1 diabetes later during their follow-up. The controls for these case subjects were matched for age at sampling, gender, HLA-DR/DQ genotype and length of freezing time of the samples used in the study.

Additionally, to validate the results from the pilot study, a confirmation cohort of 25 case-control pairs was gathered. The subjects of the confirmation cohort were participants in the DIPP follow-up at the time of study and the samples were used fresh. All case subjects in this cohort were persistently positive for at least two biochemical autoantibodies except for five cases, who had only one biochemical autoantibody and ICA. The controls for these children were matched for age at sampling, gender, HLA-DR/DQ genotype and date of sample collection.

4.2 Methods

4.2.1 Genotyping

4.2.1.1 HLA genotyping

All children invited to the DIPP study are routinely screened for and confirmed to carry type 1 diabetes associated HLA genotypes. The screening is carried out from a cord blood sample shortly after birth. The HLA DR-DQ haplotypes are determined after amplification by polymerase chain reaction (PCR), using sequence-specific, lanthanide-labelled oligonucleotide probes and time-resolved fluorometry detection as described earlier (Hermann et al., 2003; Kiviniemi et al., 2007).

In study I, the study subjects were additionally divided into four groups according to the risk conferred by their HLA genotype: strongly increased risk, moderately increased risk, slightly increased risk, and a combined group for neutral or protective genotypes (Table 2) (Ilonen et al., 2016). These groups were used in

statistical analyses. Screening procedure and eligibility criteria have been developed and modified during the long period of the study since year 1994 as described earlier (Ilonen et al., 2013). Follow-up cohorts from the early years of the study thus included small numbers of children whose genotypes later were classified as ineligible and not included in the newer cohorts.

4.2.1.2 SNP genotyping

4.2.1.2.1 Sequenom

The SNP genotyping of 521 cases and 989 controls in study I was conducted with the Sequenom (San Diego, California, USA) platform at the Genome Centre of Eastern Finland (Kuopio, University of Eastern Finland) as described earlier (Lempainen et al., 2015). The sequencing data was handled together with the TaqMan genotyping data and alleles were called for each SNP in the same software as the TaqMan results.

4.2.1.2.2 TaqMan Microarray

The SNP genotyping of the additional 455 cases and 921 controls in study I was done using TaqMan (Thermo Fisher Scientific, Waltham, Massachusetts, USA) platform. TaqMan OpenArray real-time PCR assays with QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific) were used to genotype 19 of 21 type 1 diabetes associated SNPs (Table 3) in study I. The genotyping was done according to the manufacturer's instructions at the Finnish Functional Genomics Centre core facility (Turku, Finland). Alleles were called in the ThermoFisher Connect cloud Genotyping application.

4.2.1.2.3 TaqMan genotyping assays

In study I, study subjects were genotyped for two of 21 type 1 diabetes associated SNPs (Table 3), *CD226* rs763361 and *NRP1* rs2666236, using TaqMan SNP Genotyping Assays (Thermo Fisher Scientific) according to the manufacturer's instructions with a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). The alleles of the SNPs were called using the QuantStudio Design and Analysis Desktop Software.

Seven type 1 diabetes associated SNPs *INS* (rs689), *PTPN22* (rs2476601), *IL2RA* (rs2104286 and rs12722495), *PTPN2* (rs45450798), *CTLA4* (rs3087243) and *ERBB3* (rs2292239), were determined in study II using the same method, apart from allele determination, which was done in the ThermoFisher Connect cloud Genotyping application.

Table 3. The 21 single nucleotide polymorphisms investigated in Study I. Risk allele frequencies are reported in the Finnish population and values are retrieved from the gnomAD database (Karczewski et al., 2020). Modified from (Laine et al., 2022).

SNP	CHROMOSOME	GENE REGION	MAJOR/MINOR ALLELE	RISK ALLELE	RISK ALLELE FREQUENCY
rs17388568	4q27	ADAD1	G/A	A	0.409
rs3757247	6q15	BACH2	G/A	A	0.365
rs763361	18q22.2	CD226	C/T	T	0.444
rs12708716	16p13.1	CLEC16A	A/G	A	0.643
rs3087243	2q33.2	CTLA4	A/G	G	0.667
rs3825932	15q25.1	CTSH	T/C	C	0.614
rs2292239	12q13.2	ERBB3	C/A	A	0.306
rs601338	19q13.4	FUT2	G/A	A	0.374
rs1990760	2q24.2	IFIH1	T/C	T	0.572
rs2104286	10p15.1	IL2RA	A/G	A	0.941
rs12722495	10p15.1	IL2RA	G/A	A	0.810
rs689	11p15.5	INS	A/T	A	0.797
rs630115	1p31.1	LOC646538	A/G	G	0.672
rs2666236	10p11.2	NRP1	C/T	T	0.376
rs45450798	18p11.2	PTPN2	G/C	C	0.147
rs2476601	1p13.2	PTPN22	G/A	A	0.149
rs2816316	1q31.2	RGS1	G/T	T	0.856
rs3184504	12q24.1	SH2B3	C/T	T	0.397
rs7574865	2q32.2	STAT4	G/T	T	0.217
rs6920220	6q23.3	TNFAIP3	G/A	A	0.187
rs9976767	21q22.3	UBASH3A	A/G	G	0.335

4.2.2 Autoantibody analyses

All study subjects taking part in the DIPP study are monitored for the appearance of autoantibodies in their clinic visits. ICA is determined with an indirect immunofluorescence assay (Bottazzo et al., 1974; Karjalainen, 1990) and IAA (Williams et al., 1997), GADA (Savola, Sabbah, et al., 1998) and IA-2A (Savola, Bonifacio, et al., 1998) with radiobinding assays. The cut-off values for autoantibody positivity were 2.5 Juvenile Diabetes Foundation Units (JDFU) for ICA and 3.48, 5.36 and 0.43 relative units (RU) for IAA, GADA and IA-2A, respectively (Siljander et al., 2009). The sensitivities and specificities of the assays were determined in the 2002 to 2015 Diabetes Antibody Standardization Program/Islet Autoantibody Standardization Program workshops and were 44% to 50% and 96% to 99% for IAA, 76% to 92% and 94% to 99% for GADA, and 64% to 76% and 97% to 100% for IA-2A, respectively (Bauer et al., 2019). Positivity for autoantibodies was considered persistent when the autoantibody could be detected on two consecutive clinic visits. Autoantibody analyses were carried out in the DIPP clinic in the University of Oulu.

4.2.3 Isolation of peripheral blood mononuclear cells

All cell separations were made from PBMC isolated from lithium heparin blood using the Ficoll-Paque Plus density gradient centrifugation method. After isolation, the PBMC were resuspended in RPMI 1640 medium.

In most cases, the cells were stored frozen at -150˚C after isolation, cryopreserved in RPMI 1640 with 10% human male AB serum and 10% dimethyl sulfoxide (DMSO). In study II, the flow cytometry samples of the primary cohort were resuspended in RPMI 1640 medium with 5% human AB serum. After this they were shipped at +4˚C, overnight from the DIPP centre in Turku to University of Eastern Finland in Kuopio. In the main cohort of study III, the cells were used for magnetic bead fractionation immediately.

4.2.4 Detection of lymphocyte subpopulations using flow cytometry

4.2.4.1 Study II

The samples in the primary cohort of study II were processed while fresh, after overnight shipping between study centres and the flow cytometry protocol has been previously described (Viisanen et al., 2019). Briefly, immunostaining was performed on 10^6 PBMC by incubating in room temperature (RT) with a panel of fluorochrome-labelled antibodies for 20-30 minutes. The panels are detailed in Table 4. Before staining intracellular cytokines and transcription factors, the cells were fixed and permeabilised using the Foxp3/Transcription Factor Staining Buffer set (eBioscience). A FACSCanto II flow cytometer (BD Biosciences) was used for acquisition of data.

Table 4. Fluorescent monoclonal antibody panels used in Study II.

PANEL	ANTIBODY	CLONE	MANUFACTURER
MAIN 1	CD3 APC-H7	SK-7	BD Biosciences
	CD4 BV510	RPA-T4	BioLegend
	CD45RO BV421	UCHL1	BioLegend
	CD25 PE-Cy7	BC96	BioLegend
	CD127 PerCp-Cy5.5	A019D5	BioLegend
	FOXP3 A488	259D	BioLegend
	HELIOS A647	22F6	BioLegend
	IFN-γ or IL-17A PE	4S.B3 or BL168	BioLegend
MAIN 2	CD4 APC-H7	RPA-T4	BD Biosciences
	CD25 PE-Cy7	BC96	BioLegend
	CCR6 PE	G034E3	BioLegend
	CXCR3 A647	G025H7	BioLegend
	CD127 PerCp-Cy5.5	A019D5	BioLegend
	CD45RA FITC	HI100	BioLegend
VALIDATION	CD3 APC-F750	SK-7	BioLegend
	CD4 PE-Cy7	RPA-T4	BioLegend
	CD25 PE	4E3	Miltenyi Biotec
	CD127 PerCP-Cy5.5	A019D5	BioLegend
	CD45RO BV421	UCHL1	BioLegend
	FOXP3 A488	259D	BioLegend
	HELIOS A647	22F6	BioLegend

4.2.4.2 Study III

In study III, the purity of separated cell fractions as well as the composition of the original PMBC sample were studied using flow cytometry. These cell fractions were immunostained for 30 min at +4˚C with anti-CD3 PE (SK7, BD Biosciences, San Jose, CA, USA) and anti-CD19 APC (SJ25C1, BD Biosciences) to attain initial and remaining frequencies of T and B cells in each sample. Additionally, the original PBMC sample and the lymphocyte fraction were stained with anti-CD14 FITC (M5E2, BD Biosciences) to investigate the initial and remaining monocyte frequencies, respectively. The cells were washed twice with phosphate buffered saline (PBS) for 5 min at 2500 rpm with Sorvall MC 12V (Thermo Fischer Scientific, USA) after immunostaining and then fixed with 0.1 % formaldehyde in PBS. Lastly, the samples were analysed with an Accuri C6 flow cytometer (BD Biosciences) and the BD Accuri C6 software.

4.2.4.3 Data processing

With the exception of purity controls in study III, all analyses of flow cytometry data were performed with the FlowJo software (BD Biosciences).

The gating strategy of Tregs in the primary cohort of study II has been reported previously (Viisanen et al., 2019). The analysis of all samples in this study was performed with coded samples, blinded for cohort subgrouping.

4.2.5 Fractionation of lymphocytes using magnetic separation

PBMC samples from study III were separated fresh into a monocyte fraction and the remaining fraction, from which the name lymphocyte fraction is used here. The cell fractionation was made using EasySep Human CD14 positive selection kit II (STEMCELL Technologies, Vancouver, BC, Canada) according to manufacturer's instructions. The separated cell populations were lysed with Buffer RLT Plus (Qiagen) with 1% β-mercaptoethanol (V/V) and then stored at -80˚C before RNA isolation.

4.2.6 RNA sequencing

4.2.6.1 RNA extraction

The RNA samples in study III were extracted according to the manufacturer's instructions using the RNeasy Plus Mini Kit (Qiagen) and RNeasy Plus Micro Kit (Qiagen) in the pilot and confirmation cohorts, respectively. In the pilot cohort the starting material was frozen PBMC. In the confirmation cohort monocytes and remaining lymphocyte fraction were freshly isolated from PBMC. Agilent RNA 6000 Nano Kit (Agilent, Santa Clara, CA, USA) and Agilent RNA 6000 Pico Kit (Agilent), respectively, were used to confirm the quality of the RNA in pilot and confirmation cohorts on a 2100 Bioanalyzer (Agilent). Samples with an RNA integrity number ≥ 8 were included in the study.

4.2.6.2 Library preparation

The RNA libraries for the pilot and confirmation cohorts in study III were made in a 48-plex Illumina-compatible manner using the Single-cell Tagged Reverse Transcription (STRT) method (Islam et al., 2012; Krjutškov et al., 2016). The libraries from the pilot cohort and the confirmation cohort used 10 and 20 ng of original RNA samples as starting material, respectively. Samples were placed on a

48-well plate with universal primers, template-switching oligos and 6 base pair barcodes for sample identification (Katayama et al., 2013; Krjutškov et al., 2016). After this, the resulting cDNA samples were pooled and amplified with a single primer PCR with a universal primer sequence.

4.2.6.3 Sequencing

The pilot cohort of study III was sequenced on three Illumina HiSeq2000 (Illumina, San Diego, CA, USA) lanes. The used protocol was the Illumina TruSeq v3 60-bp single-read protocol. Sequencing took place at the Bioinformatics and Expression Analysis (BEA) core facility at Karolinska Institutet (Huddinge, Sweden).

The confirmation cohort was sequenced using on an Illumina NextSeq 500, High Output (75 cycles) (Illumina) at Biomedicum Functional Genomics Unit (FuGU), University of Helsinki, Finland.

4.2.7 Statistical analyses

4.2.7.1 Survival analysis

The effect of 21 SNPs to the emergence of beta cell autoimmunity and development of type 1 diabetes in study I was investigated using the Cox proportional hazards model in IBM SPSS Statistics 25.0 (Armonk, NY, USA). Analyses were conducted with three different timelines: birth to seroconversion, seroconversion to type 1 diabetes and birth to type 1 diabetes. Both recessive and dominant models were run for all SNPs and the one reaching higher statistical significance was selected. All models were adjusted for HLA-conferred genetic risk by including HLA risk group as a covariant. Additionally, all main analyses with all cases were replicated with endotype groups defined through IAA or GADA as the first sole appearing autoantibody. The p values obtained were corrected for multiple testing using the number of studied SNPs in each survival timeline in the Benjamini and Hochberg false discovery rate (FDR) step-up procedure ($\alpha=0.05$) with the R package multtest (R package version 2.46.0) (Pollard et al., 2005). After correction, a p value of < 0.05 was considered statistically significant.

4.2.7.2 Analysis of covariance

In study II, Treg frequencies were studied in the context of the seven type 1 diabetes predisposing SNPs using analysis of covariance. Post-hoc analyses for determining statistical differences between the risk variants, heterozygotes and non-risk variants were done with the Bonferroni method. In the main analysis, the analyses were

conducted with pooled data from the entire primary cohort. The age of the children was used as a covariate to account for the different levels of immune system maturation. P-values of < 0.05 were considered significant. In the main analysis, the level of significance was adjusted with a factor of 8 (seven SNPs and HLA class II genotype) using the Bonferroni correction. After this p-values of $p < 0.006$ were considered significant. The analyses for the validation cohort were conducted with ten trios of the *PTPN22*/rs2476601 SNP and it was not necessary to correct the p-values. The analyses of covariance were carried out with IBM SPSS Statistics 24.0 (Armonk, NY).

4.2.7.3 Analysis of sequencing data

In study III, Casava 1.8.2 (Illumina) was used to convert original sequence data to FASTQ files, and the processing of the data was done with the STRTprep pipeline, also described by Krjutškov *et al.* (Krjutškov et al., 2016). In the confirmation cohort, the conversion of data into FASTQ files was done with Picard tools (v2.10.10; http://broadinstitute.github.io/picard/). The processing of the sequence was performed according to the STRT2 pipeline (Ezer et al., 2021). The monocyte and lymphocyte fractions were analysed separately.
Analysing differential expression between cases and controls was done using the R (v3.6.2) package DESeq2 (v1.24.0) (Love et al., 2014) in both the pilot and confirmation cohorts. Sex of study subjects was used as a covariate. Additionally, gene set enrichment analysis (GSEA) was performed software version 4.0.3 with the GSEAPreranked tool. The data was preranked using p-values and fold changes.

5 Results

5.1 The effect of single nucleotide polymorphisms on the pathogenesis of type 1 diabetes

Study I of this thesis investigated the association between type 1 diabetes predisposing SNPs and different phases and suggested endotypes of the disease. For this purpose, we studied 976 case subjects with beta cell autoimmunity and their 1910 clinically healthy control subjects participating in the DIPP follow-up study. All these children were genotyped for 21 SNPs (Table 3) associated with type 1 diabetes and then phase and endotype specific risk effects were examined through survival analysis, more specifically Cox proportional hazards model. The investigated SNPs were selected based on previous evidence of association in two earlier studies (Laine et al., 2013; Lempainen et al., 2015). The analyses were conducted between several timepoints: from birth to seroconversion, from seroconversion to development of type 1 diabetes and from birth to development of type 1 diabetes (Figure 3). The first of these time periods contains the events culminating in the initiation of autoimmunity, and the second time period includes autoimmunity spreading and onset of the disease. The last time period reflects the overall risk of developing type 1 diabetes. What is more, all analyses were also performed with suspected disease endotype groups, defined through the first appearing autoantibody.

5.1.1 Non-HLA polymorphisms in different phases of autoimmunity in type 1 diabetes

The effects of type 1 diabetes associated SNPs in the pathogenesis of the disease, and especially the timing of the realised risk effects, were investigated in study I. This was done by applying multiple Cox regression on three separate periods leading to clinical type 1 diabetes: birth to seroconversion, seroconversion to disease onset and birth to disease onset (Table 5).

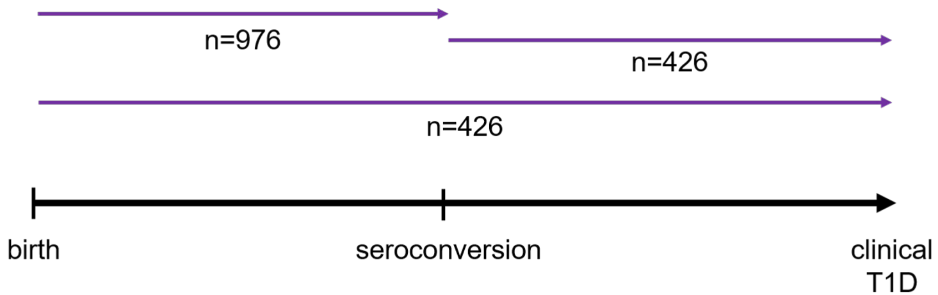

Figure 3. A schematic of the time periods analysed in Study I. The black arrow depicts type 1 diabetes pathogenesis, and the purple arrows are the analysed time periods. Author's own drawing.

In the broadest analysis, measuring the risk effects in the whole time period from birth to clinical disease in the entire cohort, four of the 21 studied SNPs, *PTPN22*, *INS*, *FUT2*, and *UBASH3A,* showed significant associations (p = 4.4×10^{-09}, 2.8×10^{-05}, 0.0088 and 0.0036, respectively). The effects of these four SNPs expectedly signify elevated risk of developing type 1 diabetes, out of which *PTPN22* reached the highest hazard ratio (HR) with 1.80. Suggestive associations that did not remain significant after correction for multiple testing, were seen in *NRP1*, *PTPN2* and *CD226* (p = 0.049, 0.045 and 0.048, respectively).

When the time period from birth to appearance of the first autoantibody was analysed, significant associations could be observed in *PTPN22, NRP1*, *INS, ERBB3* and *UBASH3A* (p = 2.2×10^{-07}, 0.0014, 7.9×10^{-05}, 0.0057 and 0.0072, respectively). Once again *PTPN22* displayed the strongest risk effect with a HR of 1.43. Additionally, *FUT2, BACH2, PTPN2, CLEC16A* and *IFIH1* displayed weak associations that did not survive the correction for multiple testing.

In the time period from seroconversion to clinical type 1 diabetes, SNPs in *PTPN22*, *STAT4*, *PTPN2* and *CD226* associated with progression to clinical disease. The strongest risk effect could be seen in *PTPN2*, whose HR was 2.41. In addition, weak, non-significant associations could be seen in *INS* and *FUT2*.

In conclusion, the strongest effect in the entire cohort was seen with *PTPN22*, which was significant in all studied time periods. In addition, *INS* and *UBASH3A* were significant in the time periods from birth to disease onset and birth to seroconversion.

Table 5. Hazard ratios (HR) and corresponding p-values with confidence intervals (CI) of Cox proportional hazards models calculated for time periods, birth and first appearing autoantibody, first appearing autoantibody and emergence of type 1 diabetes and between birth and emergence of type 1 diabetes. Results are shown for the complete cohort (A), cases with IAA as the first appearing autoantibody (B) and cases with GADA as the first appearing autoantibody (C). P-values that remained statistically significant after correction for multiple testing are bolded and underlined and p-values that are statistically non-significant, but show a suggestive association are bolded. A dominant heritance model with the non-risk homozygote as the reference genotype was used in the analyses unless otherwise indicated. Modified from (Laine et al., 2022).

A) COMPLETE COHORT		**BIRTH TO FIRST AAB**			**FIRST AAB TO T1D**			**BIRTH TO T1D**		
SNPS	**Gene region**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**
rs630115	LOC646538	0.48	1.07	0.88 - 1.31	0.48	0.9	0.68 - 1.20	0.58	1.09	0.81 - 1.45
rs2476601	PTPN22	**2.2E-07**	1.43	1.25 - 1.63	**0.00020**	1.45	1.19 - 1.77	**4.4E-09**	1.80	1.48 - 2.20
rs2816316	RGS1	0.57	0.88	0.57 - 1.36	0.3	1.45	0.72 - 2.93	0.58	1.22	0.60 - 2.45
rs1990760	IFIH1	**0.037**	1.22	1.01 - 1.46	0.55	1.09	0.82 - 1.45	0.05	1.32	1.00 - 1.75
rs7574865	STAT4	0.67	1.03	0.90 - 1.17	**0.0025**	1.35	1.11 - 1.63	0.16	1.15	0.95 - 1.39
rs3087243	CTLA4	0.32	1.12	0.90 - 1.39	0.37	0.86	0.62 - 1.19	0.68	0.93	0.68 - 1.29
rs17388568	ADAD1	0.79	1.02	0.89 - 1.16	0.46	1.08	0.88 - 1.33	0.28	1.12	0.91 - 1.38
rs3757247	BACH2	**0.036**	0.87	0.77 - 0.99	0.4	1.09	0.90 - 1.32	0.07	0.84	0.69 - 1.02
rs6920220	TNFAIP3	0.66	0.97	0.85 - 1.11	0.11	1.18	0.96 - 1.44	0.47	0.93	0.76 - 1.14
rs2104286	IL2RA	0.27	1.20	0.87 - 1.66	0.75	1.09	0.66 - 1.79	0.06	1.64	0.98 - 2.76
rs12722495	IL2RA	0.53	1.37	0.51 - 3.65	0.25	1.21	0.87 - 1.68	0.42	1.14	0.83 - 1.59
rs2666236	NRP1	**0.0014**	1.24	1.09 - 1.42	0.65	1.05	0.85 - 1.29	**0.049**	1.23	1.00 - 1.51
rs689	INS	**7.9E-05**	1.32*	1.15 - 1.51	**0.027**	1.28*	1.03 - 1.59	**2.8E-05**	1.59*	1.28 - 1.98
rs3184504	SH2B3	0.27	1.08	0.94 - 1.23	0.63	0.95	0.78 - 1.17	0.24	1.13	0.92 - 1.38
rs2292239	ERBB3	**0.0057**	1.20	1.05 - 1.36	0.41	0.92	0.76 - 1.12	0.22	1.13	0.93 - 1.37
rs3825932	CTSH	0.37	0.93	0.78 - 1.10	0.42	1.12	0.85 - 1.46	0.41	1.12	0.85 - 1.47
rs12708716	CLEC16A	**0.021**	1.16*	1.02 - 1.32	0.34	1.15	0.86 - 1.54	0.39	0.88	0.66 - 1.18
rs45450798	PTPN2	**0.048**	1.15	1.00 - 1.31	**0.00066**	2.41*	1.45 - 4.00	**0.045**	1.23	1.01 - 1.50
rs763361	CD226	0.26	1.09	0.94 - 1.25	**0.0021**	1.43	1.14 - 1.79	**0.048**	1.26	1.00 - 1.58
rs601338	FUT2	**0.019**	1.22*	1.03 - 1.44	**0.026**	1.26	1.03 - 1.54	**0.0088**	1.40*	1.09 - 1.80
rs9976767	UBASH3A	**0.0072**	1.20	1.05 - 1.37	0.37	1.10	0.90 - 1.34	**0.0036**	1.43*	1.12 - 1.82

B) IAA		BIRTH TO FIRST AAB			FIRST AAB TO T1D			BIRTH TO T1D		
SNPS	**Gene region**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**
rs630115	LOC646538	0.92	1.02	0.72 - 1.44	**0.012**	1.55*	1.10 - 2.18	0.73	1.11	0.62 - 1.96
rs2476601	PTPN22	**0.00077**	1.48	1.18 - 1.85	0.12	1.32	0.93 - 1.86	**0.0021**	2.47*	1.39 - 4.38
rs2816316	RGS1	0.60	1.24	0.55 - 2.78	0.32	2.03	0.50 - 8.21	0.24	2.30	0.57 - 9.32
rs1990760	IFIH1	0.07	1.34	0.98 - 1.83	0.68	0.9	0.55 - 1.48	0.30	1.31	0.79 - 2.15
rs7574865	STAT4	0.96	1.01	0.80 - 1.26	**0.0012**	1.76	1.25 - 2.48	0.15	1.29	0.91 - 1.82
rs3087243	CTLA4	0.53	1.13	0.77 - 1.64	0.79	0.92	0.51 - 1.67	0.86	0.95	0.52 - 1.72
rs17388568	ADAD1	0.67	0.95	0.76 - 1.20	0.30	1.22	0.84 - 1.79	**0.0077**	1.78*	1.17 - 2.72
rs3757247	BACH2	0.09	0.83	0.66 - 1.03	0.88	1.03	0.73 - 1.44	0.13	0.77	0.55 - 1.08
rs6920220	TNFAIP3	0.28	0.88	0.70 - 1.11	0.77	0.95	0.65 - 1.37	0.10	0.73	0.50 - 1.06
rs2104286	IL2RA	0.37	1.36	0.70 - 2.63	0.69	1.22	0.45 - 3.34	0.83	1.12	0.41 - 3.03
rs12722495	IL2RA	0.10	0.76*	0.55 - 1.05	0.43	1.24*	0.72 - 2.13	0.54	0.84*	0.49 - 1.45
rs2666236	NRP1	**0.010**	1.35	1.07 - 1.71	0.89	0.97	0.67 - 1.41	0.16	1.31	0.90 - 1.90
rs689	INS	**0.017**	1.34*	1.06 - 1.71	**0.020**	1.71*	1.09 - 2.69	**0.0013**	2.11*	1.34 - 3.31
rs3184504	SH2B3	0.32	1.12	0.89 - 1.41	0.98	1.00	0.70 - 1.42	0.19	1.27	0.89 - 1.81
rs2292239	ERBB3	0.06	1.24	0.99 - 1.54	**0.025**	0.67	0.47 - 0.95	0.88	1.03	0.73 - 1.45
rs3825932	CTSH	0.49	0.90	0.68 - 1.20	0.68	1.10	0.69 - 1.76	0.88	0.96	0.60 - 1.54
rs12708716	CLEC16A	0.78	1.05	0.75 - 1.46	0.91	0.97	0.61 - 1.55	0.17	0.72	0.46 - 1.15
rs45450798	PTPN2	**0.048**	1.27	1.00 - 1.61	0.63	0.91	0.63 - 1.32	0.13	1.33	0.92 - 1.91
rs763361	CD226	0.11	1.23	0.95 - 1.59	0.11	1.44	0.92 - 2.23	0.06	1.53	0.98 - 2.38
rs601338	FUT2	0.40	1.10	0.88 - 1.38	**0.046**	0.60*	0.37 - 0.99	0.62	1.09	0.77 - 1.55
rs9976767	UBASH3A	0.68	1.05	0.84 - 1.31	0.32	1.20	0.84 - 1.70	0.99	1.00	0.70 - 1.43

C) GADA		BIRTH TO FIRST AAB			FIRST AAB TO T1D			BIRTH TO T1D		
SNPS	**Gene region**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**
rs630115	LOC646538	0.36	1.18	0.83 - 1.66	0.37	0.75	0.39 - 1.42	0.50	1.28	0.63 - 2.59
rs2476601	PTPN22	0.31	1.13	0.89 - 1.45	0.76	0.92	0.54 - 1.57	0.69	1.12	0.65 - 1.91
rs2816316	RGS1	0.31	0.71	0.37 - 1.38	0.77	0.93	0.58 - 1.49	0.86	1.05	0.65 - 1.68
rs1990760	IFIH1	0.14	0.80	0.59 - 1.08	0.53	1.24	0.63 - 2.44	0.70	0.88	0.45 - 1.71
rs7574865	STAT4	0.33	1.12	0.89 - 1.39	0.52	1.16	0.74 - 1.84	0.93	0.98	0.62 - 1.55
rs3087243	CTLA4	0.60	1.11	0.75 - 1.66	0.26	0.65	0.31 - 1.366	0.49	0.77	0.36 - 1.62
rs17388568	ADAD1	0.55	1.07	0.86 - 1.35	0.71	0.92	0.58 - 1.45	0.73	1.09	0.68 - 1.74
rs3757247	BACH2	0.29	0.89	0.71 - 1.11	0.67	1.10	0.70 - 1.75	0.11	0.69	0.44 - 1.09
rs6920220	TNFAIP3	0.69	0.95	0.75 - 1.21	0.36	1.25	0.77 - 2.02	0.14	0.68	0.41 - 1.13
rs2104286	IL2RA	0.86	0.96	0.60 - 1.52	0.54	0.77	0.33 - 1.77	0.18	1.89	0.74 - 4.81
rs12722495	IL2RA	0.15	0.36	0.09 - 1.44	0.62	0.83*	0.41 - 1.69	0.91	1.04*	0.52 - 2.10
rs2666236	NRP1	0.10	1.21	0.96 - 1.53	0.86	0.96	0.59 - 1.54	0.62	1.13	0.70 - 1.80
rs689	INS	0.50	0.84	0.50 - 1.41	0.43	1.59	0.50 - 5.07	0.45	0.64	0.20 - 2.04
rs3184504	SH2B3	0.83	0.98	0.77 - 1.23	**0.0083**	0.53	0.34 - 0.85	0.18	0.72	0.45 - 1.16
rs2292239	ERBB3	0.08	1.22	0.97 - 1.52	**0.00068**	2.65*	1.51 - 4.66	**0.014**	2.01*	1.15 - 3.52
rs3825932	CTSH	0.68	0.94	0.70 - 1.26	0.71	1.12	0.61 - 2.04	0.42	1.31	0.68 - 2.54
rs12708716	CLEC16A	**0.048**	1.25*	1.00 - 1.55	0.94	1.03	0.50 - 2.11	0.18	0.62	0.31 - 1.25
rs45450798	PTPN2	0.60	1.07	0.84 - 1.35	0.30	1.28	0.80 - 2.06	0.65	1.12	0.69 - 1.81
rs763361	CD226	0.45	1.10	0.86 - 1.40	0.33	1.29	0.77 - 2.18	0.56	1.17	0.69 - 1.98
rs601338	FUT2	0.12	0.84	0.67 - 1.05	**0.030**	1.89*	1.06 - 3.37	0.54	0.86	0.54 - 1.38
rs9976767	UBASH3A	**0.036**	1.28	1.02 - 1.61	0.65	0.90	0.57 - 1.43	0.93	0.98	0.62 - 1.56

*combined heterozygote and non-risk homozygote were used as reference genotype

5.1.2 Non-HLA polymorphisms in the endotypes of type 1 diabetes

In addition to the survival analyses utilising the whole cohort, sub analyses were conducted with suspected endotypes of type 1 diabetes that were defined through the first appearing autoantibody. IAA and GADA were the most common first autoantibodies and thus used as the main subgroups in these analyses. Children, who had presented with some other initial autoantibody or whose first autoantibody could not be determined due to fast autoimmunity spreading and multiple autoantibodies at seroconversion, were left out from these analyses.

When the time period from birth to clinical type 1 diabetes was analysed, the SNPs in *PTPN22* and *INS* (p = 0.0021 and 0.0013, respectively) associated statistically significantly to the onset of disease in the IAA subgroup, with HRs greater than 2. In addition, a suggestive association could be observed in *ADAD1*, but it did not retain significance after correction for multiple testing. In the GADA subgroup, no SNP reached statistical significance, but *ERBB3* showed a suggestive association.

In the time period from birth to seroconversion, an association could be seen in *PTPN22* (p = 0.00077) in the IAA subgroup, with a HR of 1.48. Weak suggestive associations were observed in *NRP1*, *INS* and *PTPN2*. In the GADA subgroup, there were once again no statistically significant associations, but indicative observations in *CLEC16A* and *UBASH3A*.

The last time period from the first autoantibody to progression to type 1 diabetes displayed a significant association to *STAT4* (p = 0.0012) in the IAA subgroup. Four other associations that turned out to be insignificant after correction for multiple testing, could be seen in *INS, LOC646538, FUT2* and *ERBB3*. Curiously, the latter had a mildly protective effect with a HR of 0.67. The sole statistically significant association in the GADA subgroup was observed in *ERBB3* (p = 0.00068), with a HR of 2.65. Additionally, two suggestive associations were seen in *SH2B3* and *FUT2*.

To conclude, *PTPN22* and *INS* associated especially with the initiation of autoimmunity the IAA-first subgroup, while *ERBB3* associated with the GADA-first subgroup and was more important in the progression to disease.

5.2 The effect of *PTPN22* polymorphism on regulatory T cells

Study II explored the relationship between seven type 1 diabetes predisposing SNPs and Treg frequencies in children carrying HLA-conferred genetic risk for developing type 1 diabetes. Additionally, some of the children had already acquired autoantibodies or progressed into type 1 diabetes. Clinically healthy children and

autoantibody positive children were participants in the the DIPP study, and children with type 1 diabetes had been recruited after being admitted at the Turku University Hospital with the disease. The genotypes of *INS* (rs689), *PTPN22* (rs2476601), *IL2RA* (rs2104286 and rs12722495), *PTPN2* (rs45450798), *CTLA4* (rs3087243) and *ERBB3* (rs2292239) were determined and their effects, as well as those of HLA risk groups, on the frequencies of Tregs were studied using analysis of covariance. The studied loci were chosen among the ones with strongest associations to type 1 diabetes, with an emphasis on possible T cell functions like in *PTPN22* and *IL2RA* (Burton et al., 2007; Todd et al., 2007). If associations were found, post hoc analyses were used to assess differences between genotypes.

5.2.1 *PTPN22* rs2476601 risk allele A increases regulatory T cell frequencies in the context of type 1 diabetes

The main analyses were performed with two different definitions of Treg: CD4+CD25+CD127low and CD4+CD25+CD127lowFOXP3+. In both cases, total Tregs were also divided into naïve and memory Tregs based on CD45RA or CD45RO expression. Associations between the rs2476601 autoimmune risk allele A and elevated total Treg counts were observed in both CD4+CD25+CD127low and CD4+CD25+CD127lowFOXP3+ Treg definitions ($p = 0.002$ and 0.000006, respectively) in analyses performed in the pooled main cohort (Table 6). Post hoc analyses with the Bonferroni method revealed statistically significant elevations in Treg frequencies between children homozygous for the risk allele A compared to children homozygous for the protective allele G (Figure 4). Additionally, heterozygous AG children demonstrated a statistically significant difference to heterozygous GG children.

Table 6. Associations between investigated Treg populations and type 1 diabetes associated risk polymorphisms. Statistically significant p-values are bolded and underlined, and p-values that are under 0.05 but did not remain statistically significant after correction for multiple testing are bolded. A p-value of <0.006 was considered significant after correction for multiple testing using the Bonferroni method. Modified from (Valta et al., 2020).

	INS rs689	**PTPN22 rs2476601**	**IL2RA rs2104286**	**IL2RA rs12722495**	**PTPN2 rs45450798**	**CTLA4 rs3087243**	**ERBB3 rs2292239**	**HLA**
Total Treg (CD4+CD25+CD127- of CD4+)	0.537	**<u>0.002</u>**	0.804	0.271	0.583	0.11	0.35	0.166
Naive Treg (CD4+CD25+CD127-CD45RA+ of CD4+)	0.836	**<u>0.0002</u>**	0.426	0.431	0.71	0.068	0.477	0.14
Memory Treg (CD4+CD25+CD127-CD45RA- of CD4+)	0.231	0.19	0.898	0.314	0.635	0.811	0.572	0.531
FOXP3+ Treg (CD4+CD25+CD127-FOXP3+ of CD4+)	0.511	**<u>0.000006</u>**	0.68	**0.034**	0.566	**0.033**	0.925	0.111
FOXP3+ Naive Treg (CD4+CD25+CD127-CD45RO-FOXP3+ of CD4+)	0.596	**<u>0.00004</u>**	0.978	0.085	0.632	**0.042**	0.642	**0.049**
FOXP3+ Memory Treg (CD4+CD25+CD127-CD45RO+FOXP3+ of CD4+)	0.945	0.23	0.461	0.193	0.868	0.551	0.653	0.859

When analysing naïve and memory compartments within total Tregs, similar rs2476601 associations were once again observed in naïve Tregs in both CD4+CD25+CD127lowCD45RA+ and CD4+CD25+CD127lowCD45RO-FOXP3+ definitions (p = 0.0002 and 0.00004, respectively), but not in the corresponding memory compartments (Table 6). In addition, the risk genotype AA differed once again statistically from the protective GG genotype in post hoc analyses, as well as the AG genotype from the GG genotype in CD4+CD25+CD127lowCD45RO-FOXP3+ naïve Tregs (Figure 5).

In addition to the main analysis with the entire main cohort, analyses were also carried out separately with the clinically healthy children of the main cohort, as well as the autoantibody positive children and children with established type 1 diabetes. While similar tendencies were clearly to be seen in these analyses, likely due to the loss of statistical power, all previous observations could not be replicated in all subgroups (Table 7, Figure 5). In the healthy control subjects, total and naïve CD4+CD25+CD127lowFOXP3+ Tregs showed an elevated frequency (p = 0.004 and 0.003, respectively). A tendency for elevated naïve CD4+CD25+CD127lowCD45RA+ Tregs could also be observed in autoantibody

positive subjects (p = 0.023). In children with type 1 diabetes, rs2476601 associated tentatively to elevated total CD4+CD25+CD127lowFOXP3+ Tregs (p = 0.017).

To test the effect of type 1 diabetes to the frequency of Tregs, another analysis comparing children with GG genotypes in different subgroups was performed (Figure 6). Children with type 1 diabetes had a significantly elevated frequency of total CD4+CD25+CD127lowFOXP3+ Tregs compared to the healthy control children (p = 0.017).

No other SNPs or HLA risk groups associated significantly with alterations in Treg frequencies (Table 6). A few suggestive associations could, however, be seen. Total CD4+CD25+CD127lowFOXP3+ Tregs had a suggestive association with *IL2RA* rs2104286 and *CTLA4* rs3087243 (p = 0.034 and 0.033, respectively) and naïve CD4+CD25+CD127lowFOXP3+ Tregs with *CTLA4* rs3087243 and HLA risk groups (p = 0.042 and 0.049, respectively).

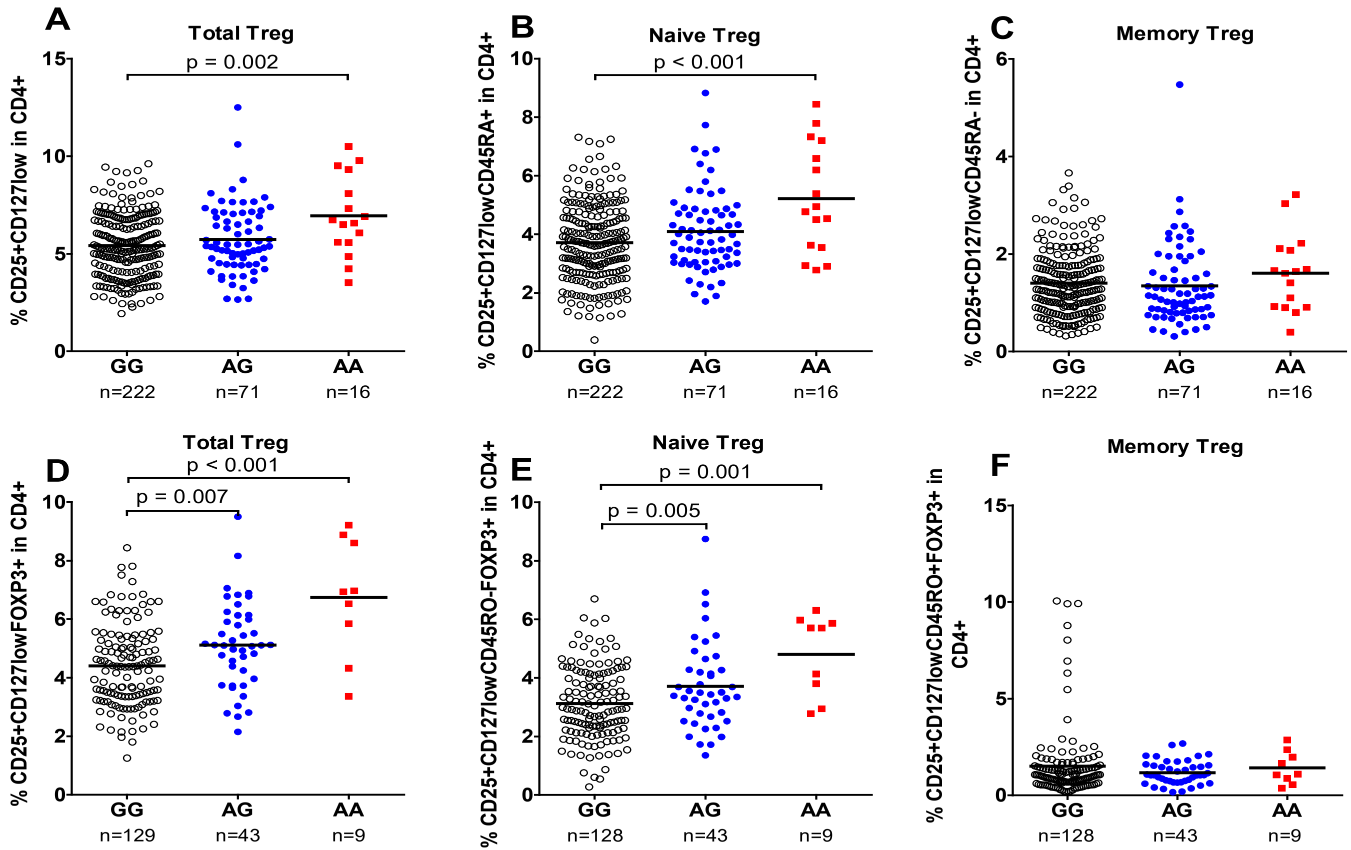

Figure 4. Frequencies of total, naïve and memory Treg cells by *PTPN22* rs2476601 genotype. The type 1 diabetes risk allele A associates with an increase in total (A, D) and naïve (B, E) regulatory T cells, but not memory (C, F) Tregs. Analysis of covariance was used to detect associations between cell populations and Bonferroni corrected post hoc analyses to investigate the variation between polymorphism genotypes. Reproduced from (Valta et al., 2019) with permission from *Eur. J. Immunol.*

Table 7. Associations between Treg populations and *PTPN22* rs2476601 in healthy control subjects (A), autoantibody positive children (B) and children who had progressed to type 1 diabetes (C). P-values under 0.05 were considered significant and are bolded. Reproduced from (Valta et al., 2019) with permission from *Eur. J. Immunol*.

A) CONTROLS	**PTPN22 rs2476601**
Total Treg (CD4+CD25+CD127- of CD4+)	0.806
Naive Treg (CD4+CD25+CD127-CD45RA+ of CD4+)	0.437
Memory Treg (CD4+CD25+CD127-CD45RA- of CD4+)	0.297
FOXP3+ Treg (CD4+CD25+CD127-FOXP3+ of CD4+)	**0.004**
FOXP3+ Naive Treg (CD4+CD25+CD127-CD45RO-FOXP3+ of CD4+)	**0.003**
FOXP3+ Memory Treg (CD4+CD25+CD127-CD45RO+FOXP3+ of CD4+)	0.205

B) AAB+	**PTPN22 rs2476601**
Total Treg (CD4+CD25+CD127- of CD4+)	0.68
Naive Treg (CD4+CD25+CD127-CD45RA+ of CD4+)	**0.023**
Memory Treg (CD4+CD25+CD127-CD45RA- of CD4+)	0.549
FOXP3+ Treg (CD4+CD25+CD127-FOXP3+ of CD4+)	0.084
FOXP3+ Naive Treg (CD4+CD25+CD127-CD45RO-FOXP3+ of CD4+)	0.083
FOXP3+ Memory Treg (CD4+CD25+CD127-CD45RO+FOXP3+ of CD4+)	0.366

C) T1D	**PTPN22 rs2476601**
Total Treg (CD4+CD25+CD127- of CD4+)	0.067
Naive Treg (CD4+CD25+CD127-CD45RA+ of CD4+)	0.126
Memory Treg (CD4+CD25+CD127-CD45RA- of CD4+)	0.421
FOXP3+ Treg (CD4+CD25+CD127-FOXP3+ of CD4+)	**0.017**
FOXP3+ Naive Treg (CD4+CD25+CD127-CD45RO-FOXP3+ of CD4+)	0.058
FOXP3+ Memory Treg (CD4+CD25+CD127-CD45RO+FOXP3+ of CD4+)	0.232

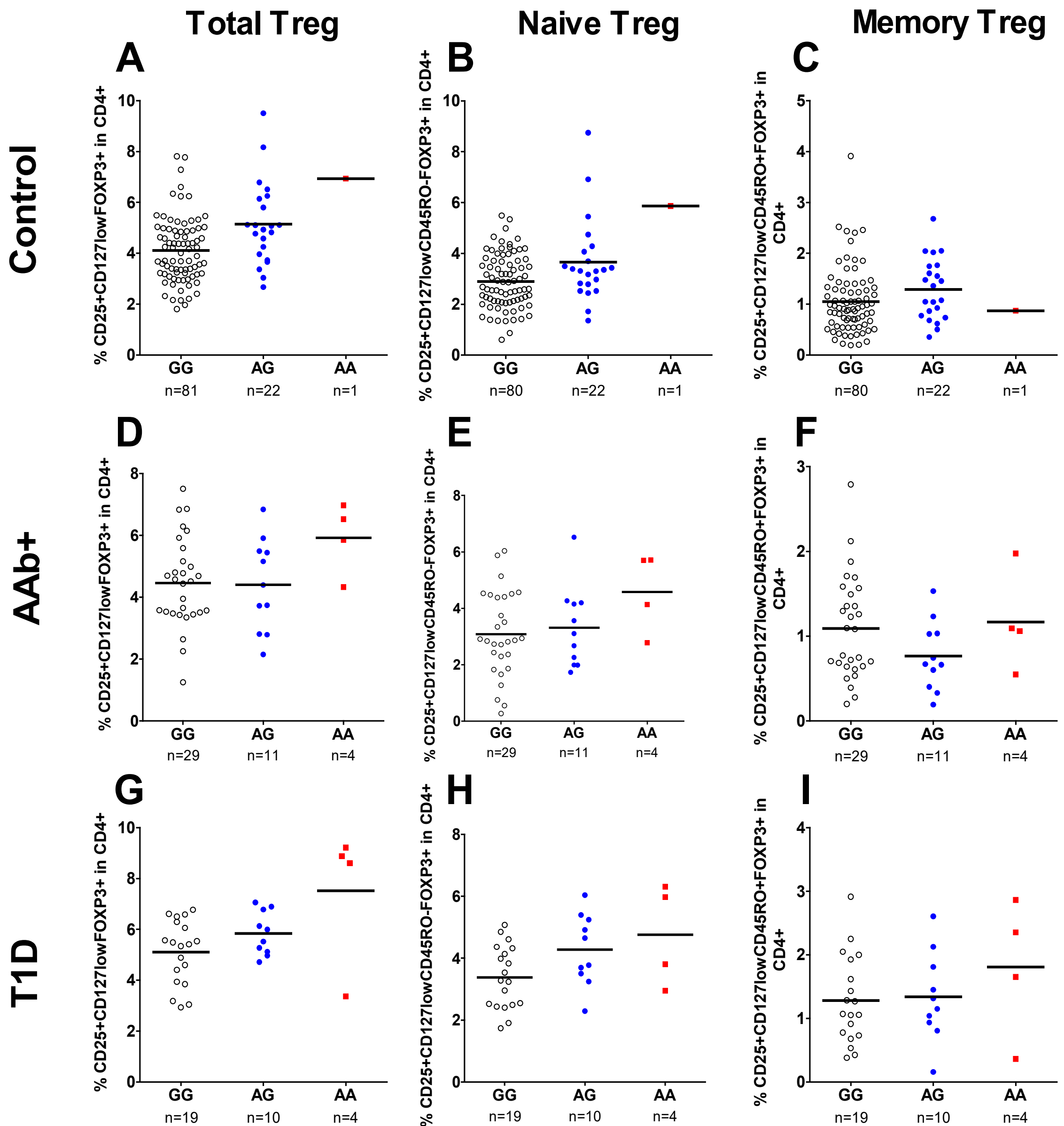

Figure 5. The relationships of Treg frequencies and *PTPN22* rs2476601 analysed separately in healthy control subjects (A-C) autoantibody positive children (D-F) and children who had progressed to type 1 diabetes (G-I). Reproduced from (Valta et al., 2019) with permission from *Eur. J. Immunol*.

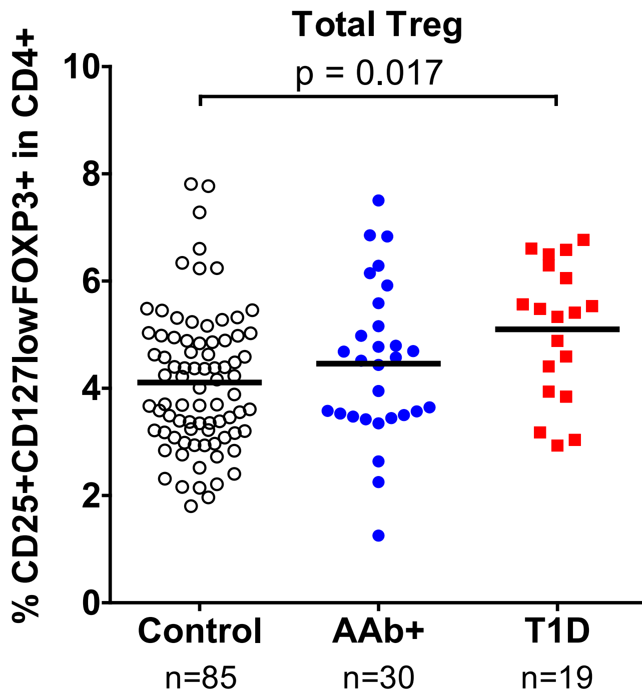

Figure 6. Frequencies of total Treg between healthy controls, children positive for type 1 diabetes associated autoantibodies and children, who had progressed to type 1 diabetes. Reproduced from (Valta et al., 2019) with permission from *Eur. J. Immunol*.

5.2.2 *PTPN22* rs2476601 risk allele A elevates regulatory T cell frequencies in healthy children

Due to the nature of the main cohort, we wanted to conduct further analyses confirming the associations in healthy individuals. A validation cohort of ten trios of healthy, unrelated children was collected. Each child in a single trio represented a different one of the three possible *PTPN22*/rs2476601 genotypes: AA, AG, or GG. All children in the validation cohort were participants in the DIPP study and carried HLA-conferred risk of developing type 1 diabetes. The analyses were once again performed with two Treg definitions: CD4+CD25+CD127lowFOXP3+ and CD4+CD25+CD127lowFOXP3+Helios+. The validation cohort confirmed the observation of *PTPN22* rs2476601 risk allele A associating with elevated CD4+CD25+CD127lowFOXP3+ Treg frequencies in total, naïve and memory cells (p = 0.005, 0.03 and 0.038 respectively) (Table 8). In addition, elevated frequencies could also be seen in total, naïve and memory CD4+CD25+CD127lowFOXP3+Helios+ Tregs (p = 0.008, 0.026 and 0.028,

respectively). As in the main cohort analyses, post hoc analyses examining the differences between genotypes were also conducted. In total and naïve CD4+CD25+CD127lowFOXP3+ Tregs, AA children had statistically significantly elevated cell frequencies compared to GG children ($p = 0.014$ and 0.041, respectively) (Figure 7). Additionally, heterozygous AG children had higher cell frequencies in total Tregs ($p = 0.012$). In CD4+CD25+CD127lowFOXP3+Helios+ total Tregs, AA vs. GG, as well as AG vs. GG, were statistically significant ($p = 0.028$ and 0.013, respectively) (Figure 8).

Table 8. Association between *PTPN22* rs2476601 and Treg populations in the validation cohort. P-values under 0.05 were considered significant and are bolded. Reproduced from (Valta et al., 2019) with permission from *Eur. J. Immunol*.

	PTPN22 rs2476601
Total FOXP3+ Treg (CD4+CD25+CD127lowFOXP3+ of CD4+)	**0.005**
Naive FOXP3+ Treg (CD4+CD25+CD127lowFOXP3+CD45RO- of CD4+)	**0.03**
Memory FOXP3+ Treg (CD4+CD25+CD127lowFOXP3+CD45RO+ of CD4+)	**0.038**
Total Helios+ Treg (CD4+CD25+CD127lowFOXP3+HELIOS+ of CD4+)	**0.008**
Naive Helios+ Treg (CD4+CD25+CD127lowFOXP3+HELIOS+CD45RO- of CD4+)	**0.026**
Memory Helios+ Treg (CD4+CD25+CD127lowFOXP3+HELIOS+CD45RO+ of CD4+)	**0.028**

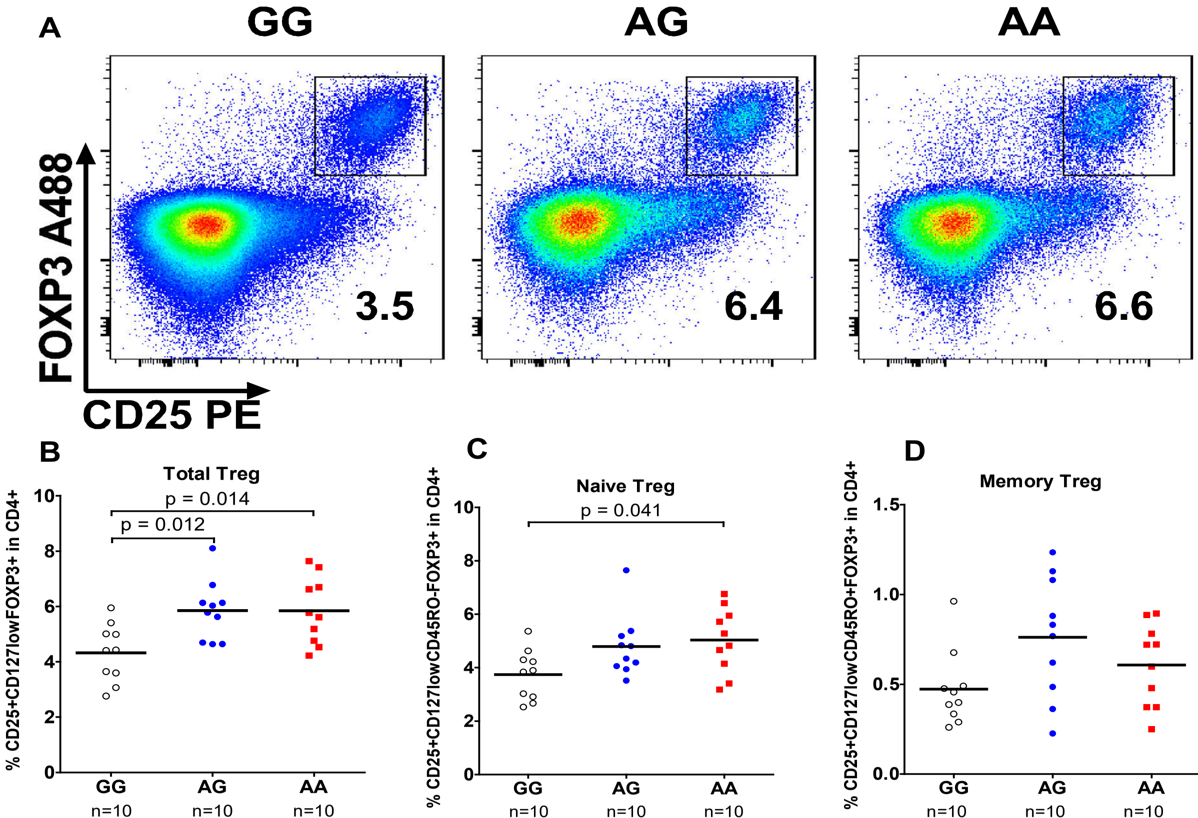

Figure 7. *PTPN22* rs2476601 risk allele A associates with elevated Treg cell frequencies in the validation cohort. Representative examples of CD3+CD4+CD25+CD127lowFOXP3+ Treg stainings in GG, AG and AA genotypes are displayed in panel (A). The polymorphism was associated with elevated total (B), naïve (C) and memory (D) Treg frequencies in analysis of covariance. Post hoc analyses corrected with the Bonferroni method revealed significant differences between GG and AA genotypes in total and naïve populations and also between GG and AG in total Tregs. Reproduced from (Valta et al., 2019) with permission from *Eur. J. Immunol.*

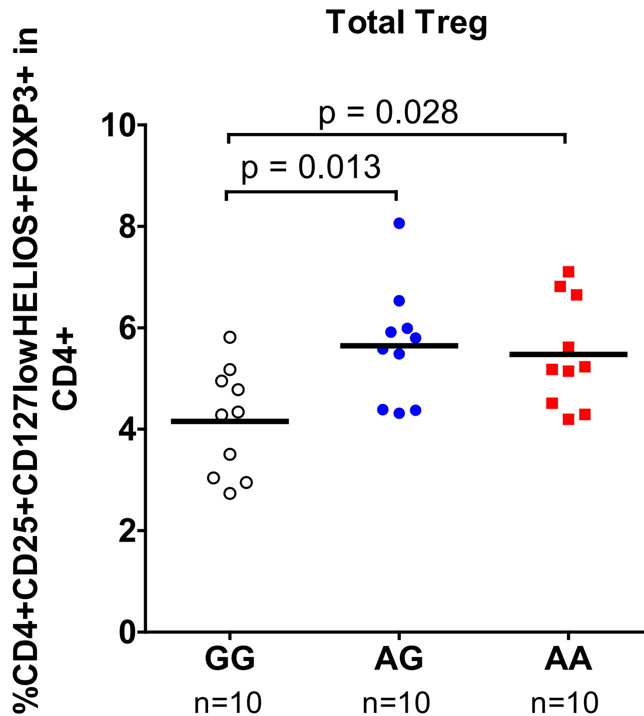

Figure 8. Frequencies of CD4+CD25+CD127lowHELIOS+FOXP3+ Tregs in the validation cohort representing all genotypes of *PTPN22* rs2476601. Post hoc analyses corrected with the Bonferroni method were used to analyse differences between each genotype and revealed significantly elevated cell frequencies both in AG and AA children compared to GG children. Reproduced from (Valta et al., 2019) with permission from *Eur. J. Immunol.*

5.3 Evidence of viral infection-related gene upregulation in monocytes

In study III, children with multiple type 1 diabetes associated autoantibodies and their healthy control subjects were compared for gene expression differences in peripheral immune cells. The study was conducted in two parts. First the PBMCs of nine case subjects, who had later progressed to type 1 diabetes, and their controls were compared in a pilot study. This part of the study was performed with frozen cells. The findings of the pilot were later confirmed in a larger main cohort with 25 children with advanced autoimmunity and their clinically healthy controls using freshly isolated monocytes and the remaining lymphocyte fraction from PBMC samples.

5.3.1 Peripheral blood mononuclear cell gene expression in children positive for multiple type 1 diabetes associated autoantibodies

Gene expression differences were initially studied in a pilot cohort of nine case-control pairs, where cases had advanced beta cell autoimmunity at the time of sampling and had later progressed to type 1 diabetes. Analysing differential gene expression between cases and healthy controls yielded no statistically differentially expressed genes. Nevertheless, gene-set enrichment analysis, GSEA was applied on the sequencing data that had been pre-ranked with fold change and significance of differential expression. The analyses were implemented on 200 of the most upregulated and downregulated genes. The GSEA performed with the upregulated data suggested associations to gene sets linked with virus associated immune activation. The gene sets “defence response to virus” (FDR < 0.001, ranking 2), “response to virus” (FDR < 0.001, ranking 3) and “response to type I interferon” (FDR = 0.002, ranking 12) were statistically significantly upregulated in the pilot cohort (Table 9).

5.3.2 Analysis of monocyte and lymphocyte compartments in a larger cohort of children positive for multiple type 1 diabetes associated autoantibodies

To confirm the observations from the pilot cohort, a larger confirmation cohort of 25 case-control pairs was compiled from children attending regular clinic visits at the time of study. PBMCs were isolated from the collected blood samples and after this, the samples were separated into monocytes and the remaining lymphocyte fraction while fresh to ensure good quality of data. Differential gene expression analysis between case and control subjects of this second dataset once again failed to reveal differentially expressed genes in either the monocyte or lymphocyte compartments. Still, a subsequent GSEA applied to the monocyte sequencing data confirmed an association between the upregulated genes and virus immunity. Statistically significant differences were observed in the upregulated genes of cases and controls in the gene sets “defence response to virus” (FDR = 0.02, ranking 2), “response to virus” (FDR = 0.04, ranking 3) and “response to type I interferon” (FDR = 0.02, ranking 1) (Table 9). Notably, these findings could not be seen in the lymphocyte fraction of the same cohort.

Table 9. Upregulated gene sets in cases with type 1 diabetes associated autoantibodies in pilot and main cohorts. Gene set enrichment analysis was used to investigate in 200 most upregulated genes, pre-ranked based on fold changes and significance of differential expression, in peripheral blood mononuclear cells in the pilot cohort and in the monocyte and lymphocyte fractions in the main cohort. The analyses uncovered monocyte specific upregulation of gene sets connected with viral response and response to type I interferon in autoantibody positive cases compared to healthy control children. The enrichment scores (ES), normalised enrichment scores (NES), nominal p-values (NOM p-val) and false discovery rate corrected q-values (FDR q-val) from the analyses are detailed in the table. Reproduced from (Valta et al., 2022) under the Creative Commons CC by 4.0 licence.

	PILOT						MONOCYTE						LYMPHOCYTE					
RANK	**NAME**	**SIZE**	**ES**	**NES**	**NOM p-val**	**FDR q-val**	**NAME**	**SIZE**	**ES**	**NES**	**NOM p-val**	**FDR q-val**	**NAME**	**SIZE**	**ES**	**NES**	**NOM p-val**	**FDR q-val**
1	Regulation of response to biotic stimulus	92	0.29	3.22	0.00E+00	0.00E+00	Response to type i interferon	71	0.28	2.88	0.00E+00	0.02	Electron transport chain	145	0.29	4.1	0.00E+00	0.00E+00
2	Defence response to virus	162	0.21	3.1	0.00E+00	0.00E+00	Defence response to virus	178	0.18	2.81	0.00E+00	0.01	Cellular respiration	162	0.28	4.04	0.00E+00	0.00E+00
3	Response to virus	215	0.19	3.1	0.00E+00	0.00E+00	Response to virus	240	0.15	2.6	0.00E+00	0.04	Small molecule catabolic process	293	0.21	4.01	0.00E+00	0.00E+00
4	Response to molecule of bacterial origin	197	0.19	3.09	0.00E+00	0.00E+00	Vesicle organization	250	0.14	2.57	0.00E+00	0.04	Cofactor metabolic process	351	0.18	3.98	0.00E+00	0.00E+00
5	Positive regulation of defence response to virus by host	20	0.57	3.04	0.00E+00	0.000244	Interferon gamma mediated signaling pathway	71	0.26	2.55	0.00E+00	0.04	Generation of precursor metabolites and energy	389	0.17	3.82	0.00E+00	0.00E+00
6	Defence response to other organism	273	0.16	2.99	0.00E+00	0.000204	Response to interferon gamma	141	0.18	2.5	0.00E+00	0.05	Mitochondrial translational termination	88	0.33	3.7	0.00E+00	0.00E+00
7	Response to bacterium	309	0.15	2.97	0.00E+00	0.00053	Ribonucleoprotein complex biogenesis	402	0.11	2.46	0.00E+00	0.06	Atp synthesis coupled electron transport	79	0.36	3.69	0.00E+00	0.00E+00
8	Lipopolysaccharide mediated signaling pathway	39	0.38	2.83	0.00E+00	0.00167	Regulation of gene silencing	111	0.19	2.38	0.00E+00	0.09	Mitochondrial translation	132	0.27	3.68	0.00E+00	0.00E+00

9	Positive regulation of cytokine production	282	0.15	2.81	0.00E+00	0.00176	Ribosome biogenesis	261	0.12	2.33	0.00E+00	0.11	Respiratory electron transport chain	95	0.32	3.65	0.00E+00	0.00E+00
10	Positive regulation of dna binding transcription factor activity	152	0.19	2.78	0.00E+00	0.00208	De novo protein folding	35	0.32	2.33	0.00E+00	0.1	Anaphase promoting complex dependent catabolic process	78	0.35	3.63	0.00E+00	0.00E+00
11	Negative regulation of viral genome replication	38	0.39	2.78	0.00E+00	0.002	Organic cyclic compound catabolic process	465	0.09	2.32	0.00E+00	0.1	Cellular amino acid metabolic process	218	0.21	3.59	0.00E+00	0.00E+00
12	Response to type I interferon	64	0.29	2.76	0.00E+00	0.00194	Ncrna metabolic process	403	0.1	2.31	0.00E+00	0.1	Mitochondrial respiratory chain complex assembly	89	0.33	3.59	0.00E+00	0.00E+00
13	Cytokine production	450	0.12	2.71	0.00E+00	0.00302	Multi organism localization	67	0.24	2.29	0.00E+00	0.1	Oxidative phosphorylation	114	0.28	3.5	0.00E+00	0.00E+00
14	Regulation of defence response to virus by host	29	0.42	2.68	0.00E+00	0.00412	Response to interferon alpha	17	0.45	2.26	0.00E+00	0.11	Aerobic respiration	75	0.34	3.48	0.00E+00	0.00E+00
15	Response to interferon beta	23	0.45	2.68	0.00E+00	0.00384	Organelle localization	477	0.09	2.24	0.00E+00	0.13	NADH dehydrogenase complex assembly	57	0.38	3.4	0.00E+00	0.00E+00
16	Leukocyte cell cell adhesion	218	0.16	2.68	0.00E+00	0.0036	Vesicle targeting	82	0.21	2.22	0.00E+00	0.13	Translational termination	100	0.29	3.4	0.00E+00	0.00E+00
17	Cellular response to biotic stimulus	148	0.18	2.66	0.00E+00	0.00404	Ncrna processing	343	0.11	2.21	0.00E+00	0.13	DNA dependent dna replication	134	0.25	3.28	0.00E+00	0.00E+00
18	Cytokine mediated signaling pathway	476	0.11	2.62	0.00E+00	0.00559	Trna transport	35	0.31	2.2	0.00E+00	0.14	Mitochondrial gene expression	156	0.23	3.24	0.00E+00	0.00E+00

19	Cell cell adhesion	344	0.12	2.58	0.00E+00	0.00755	Copii coated vesicle budding	64	0.24	2.2	0.00E+00	0.13	Energy derivation by oxidation of organic compounds	219	0.19	3.19	0.00E+00	0.00E+00
20	Regulation of defence response to virus	55	0.29	2.57	0.00E+00	0.00778	Nuclear transcribed mrna catabolic process nonsense mediated decay	114	0.18	2.19	0.00E+00	0.13	Nucleobase containing small molecule metabolic process	278	0.16	3.15	0.00E+00	0.00E+00
21	Response to interferon gamma	130	0.19	2.54	0.00E+00	0.00898	Ncrna export from nucleus	39	0.3	2.19	0.00E+00	0.12	Antigen processing and presentation of exogenous peptide antigen via MHC class I	75	0.3	3.11	0.00E+00	0.0000333
22	Inflammatory response	370	0.11	2.52	0.00E+00	0.01	Rrna metabolic process	193	0.13	2.14	0.01	0.16	Organic acid catabolic process	187	0.19	3.05	0.00E+00	0.0000943
23	Negative regulation of viral process	65	0.27	2.5	0.00E+00	0.01	Membrane fusion	111	0.17	2.13	0.00E+00	0.16	Regulation of cellular amino acid metabolic process	53	0.35	3.02	0.00E+00	0.00012
24	Regulation of multi organism process	253	0.13	2.5	0.00E+00	0.01	Regulation of nuclease activity	20	0.39	2.11	0.00E+00	0.18	Detoxification	90	0.27	2.99	0.00E+00	0.000144
25	Regulation of body fluid levels	229	0.14	2.47	0.00E+00	0.01	Endoplasmic reticulum to golgi vesicle mediated transport	165	0.14	2.11	0.00E+00	0.17	Mitochondrial electron transport nadh to ubiquinone	44	0.38	2.98	0.00E+00	0.000138
26	Regulation of cell cell adhesion	227	0.14	2.46	0.00E+00	0.01	Cotranslational protein targeting to membrane	94	0.18	2.09	0.00E+00	0.2	Nucleoside phosphate biosynthetic process	216	0.17	2.94	0.00E+00	0.000133

27	Negative regulation of multi organism process	109	0.2	2.45	0.00E+00	0.02	Dna recombination	208	0.12	2.09	0.00E+00	0.19	Regulation of cellular amine metabolic process	66	0.3	2.93	0.00E+00	0.000153
28	Adaptive immune response	261	0.13	2.43	0.00E+00	0.02	Apoptotic dna fragmentation	19	0.39	2.06	0.01	0.22	Drug metabolic process	219	0.17	2.91	0.00E+00	0.000148
29	Cellular response to interferon beta	15	0.51	2.4	0.00E+00	0.02	Glycosylation	164	0.14	2.06	0.00E+00	0.21	Cellular ketone metabolic process	142	0.21	2.88	0.00E+00	0.000167
30	Regulation of immune effector process	245	0.13	2.36	0.00E+00	0.03	Response to topologically incorrect protein	162	0.14	2.05	0.01	0.22	Regulation of cell cycle g2 m phase transition	197	0.17	2.87	0.00E+00	0.000208
31	Positive regulation of NF kappab transcription factor activity	102	0.2	2.31	0.00E+00	0.04	Positive regulation of defence response	360	0.09	2.04	0.00E+00	0.21	Antigen processing and presentation of peptide antigen via mhc class I	91	0.26	2.82	0.00E+00	0.00036
32	Negative regulation of immune system process	252	0.13	2.3	0.00E+00	0.04	Vesicle budding from membrane	90	0.18	2.03	0.00E+00	0.23	Cellular detoxification	84	0.26	2.81	0.00E+00	0.000436
33	Regulation of response to external stimulus	376	0.1	2.29	0.00E+00	0.04	Transport of virus	54	0.23	2.02	0.01	0.24	Amine metabolic process	102	0.23	2.75	0.00E+00	0.000824
34	Production of molecular mediator involved in inflammatory response	41	0.3	2.29	0.00E+00	0.04	Synapse organization	162	0.14	2.02	0.00E+00	0.23	Proteasomal ubiquitin independent protein catabolic process	21	0.49	2.73	0.00E+00	0.000944
35	Interleukin 6 production	86	0.21	2.28	0.00E+00	0.04	Vesicle localization	192	0.13	2.01	0.01	0.23	Purine containing compound biosynthetic process	154	0.19	2.72	0.00E+00	0.000977

36	Positive regulation of protein kinase b signaling	68	0.24	2.28	0.00E+00	0.04	Spliceosomal snrnp assembly	35	0.29	2	0.01	0.23	Nuclear dna replication	50	0.32	2.7	0.00E+00	0.00111
37	Interleukin 6 secretion	22	0.39	2.28	0.00E+00	0.04	Dna catabolic process endonucleolytic	23	0.35	2	0.00E+00	0.24	Meiotic cell cycle process	122	0.21	2.69	0.00E+00	0.00113
38	T cell mediated immunity	64	0.24	2.27	0.00E+00	0.04	Lymphocyte chemotaxis	32	0.29	1.99	0.01	0.24	Trna metabolic process	150	0.18	2.67	0.00E+00	0.00134
39	Immune response regulating signaling pathway	390	0.1	2.24	0.00E+00	0.05	Rna catabolic process	336	0.1	1.99	0.00E+00	0.24	Antigen processing and presentation of peptide antigen	170	0.18	2.66	0.00E+00	0.00143
40	Interferon gamma production	68	0.23	2.22	0.00E+00	0.05	Golgi vesicle transport	285	0.1	1.98	0.01	0.24	Cellular protein complex disassembly	182	0.17	2.61	0.00E+00	0.00215
41	Cytokine metabolic process	71	0.23	2.22	0.00E+00	0.05	Recombinational repair	95	0.17	1.96	0.00E+00	0.26	Negative regulation of cell cycle g2 m phase transition	94	0.23	2.6	0.00E+00	0.00229
42	Response to lipid	457	0.09	2.19	0.00E+00	0.06	Nuclear transport	279	0.1	1.96	0.00E+00	0.26	Branched chain amino acid catabolic process	19	0.48	2.59	0.00E+00	0.00245
43	Positive regulation of cytokine secretion	68	0.22	2.19	0.00E+00	0.06	Telomere maintenance via semi conservative replication	23	0.34	1.96	0.00E+00	0.25	Cofactor biosynthetic process	179	0.17	2.58	0.00E+00	0.00244
44	Cytolysis	17	0.44	2.18	00E+001	0.06	Golgi vesicle budding	72	0.19	1.96	0.00E+00	0.25	Nucleobase containing small molecule catabolic process	38	0.36	2.57	0.00E+00	0.00251

45	Cytokine production involved in inflammatory response	24	0.37	2.17	0.00E+00	0.07	Regulation of posttranscriptional gene silencing	88	0.18	1.96	0.01	0.25	Nucleoside monophosphate biosynthetic process	38	0.35	2.57	0.00E+00	0.00245
46	Regulation of lymphocyte migration	37	0.29	2.14	0.00E+00	0.08	Dna repair	440	0.08	1.94	0.01	0.27	Tricarboxylic acid cycle	32	0.38	2.57	0.00E+00	0.00245
47	Positive regulation of myeloid leukocyte mediated immunity	21	0.38	2.13	0.00E+00	0.09	Vesicle targeting to from or within golgi	67	0.2	1.93	0.01	0.28	Ribonucleoside catabolic process	17	0.52	2.56	0.00E+00	0.00254
48	Cytokine production involved in immune response	64	0.23	2.11	0.01	0.09	Rna export from nucleus	129	0.14	1.93	0.01	0.27	Dna conformation change	199	0.16	2.56	0.00E+00	0.00253
49	Positive regulation of ERK1 and ERK2 cascade	85	0.2	2.11	0.00E+00	0.09	Establishment of protein localization to endoplasmic reticulum	106	0.16	1.93	0.01	0.27	Monosaccharide catabolic process	32	0.38	2.56	0.00E+00	0.00254
50	Cytokine secretion	120	0.17	2.11	0.00E+00	0.09	Defence response to other organism	310	0.09	1.93	0.00E+00	0.26	Fatty acid beta oxidation	62	0.28	2.55	0.00E+00	0.00264

6 Discussion

The results of this work include findings on genetic and environmental factors in specific steps in the autoimmune process leading to type 1 diabetes and immune cell populations implicated in this process. These observations lend support to heterogeneity of type 1 diabetes and complex immunological mechanisms contributing to the onset of disease.

6.1 Non-HLA genetic factors in the pathogenesis of type 1 diabetes

Study I of this thesis explored the effect of 21 known type 1 diabetes risk polymorphisms on phases of disease pathogenesis and disease subtypes. While all studied SNPs are confirmed type 1 diabetes risk loci, little is known about the mechanisms by which they confer their risk effects. Since the disease process of type 1 diabetes can be divided into distinct phases, we wanted to see, if any of the selected risk polymorphisms associate with any in particular. In this study, the risk effects of the SNPs were studied from birth to type 1 diabetes diagnosis, from birth to seroconversion and from seroconversion to diagnosis. All analyses were also done in suspected type 1 diabetes endotypes, characterised by first appearing autoantibody. The effect of HLA genotype was included in the statistical models.

Study I builds on and adds to a previous study conducted with the DIPP cohort that aimed to find associations between SNPs and type 1 diabetes disease process (Lempainen et al., 2015). Our new study has almost twice as many autoantibody positive cases and controls than the earlier one. Instead of 521 cases and 989 controls in the previous study, we now had 976 cases and 1910 controls. Several findings of the previous study were now confirmed including the associations with *PTPN22* and *INS* the entire time period from birth to type 1 diabetes. Additionally, novel associations, compared to our previous study, were detected with *FUT2* and *UBASH3A*. The associations between type 1 diabetes and *PTPN22* is well documented and, in addition to genome wide association studies, has been observed in other follow up cohorts, like TEDDY and DAISY (Steck et al., 2014; Törn et al., 2014). The association between type 1 diabetes and *INS* has also previously been seen in DAISY (Steck et al., 2005, 2012).

In the time period from birth to the appearance of autoantibodies, previous associations with *PTPN22* and *INS* were confirmed, as well as a previously only suggestive association with *NRP1*. Novel associations from study I in this time period were *ERBB3* and *UBASH3A*. A previously statistically significant association between the appearance of autoantibodies and *IFIH1* did not reach significance in study I. Several of these have also been documented in other childhood follow-up cohorts. In TEDDY, associations between islet autoimmunity and *PTPN22* and *ERBB3* have been observed (Törn et al., 2014). Additionally, associations with *PTPN22*, *UBASH3A* and *INS* have been seen in DAISY, but in the case of *INS*, the effect was only seen in children with the high-risk HLA genotype *HLA-DR3/4* (Steck et al., 2005, 2014).

When the time from seroconversion to diabetes diagnosis was studied, we could confirm the earlier significant association with *PTPN2*, as well as previously suggestive associations with *PTPN22* and *CD226*. A novel association with *STAT4* was also seen. DAISY has previously reported a suggestive association between progression to type 1 diabetes and *PTPN22* (Steck et al., 2014).

In addition to conducting the phase specific analyses with the entire cohort, we also wanted to study the effect of the polymorphisms in suspected disease endotypes in study I. Heterogeneity within the autoimmune process leading to type 1 diabetes is presumed, but little is known about the mechanisms of possible alternate disease pathways. Birth cohort studies have shown that the peak incidences for IAA and GADA are at different ages (Krischer et al., 2015). As they are the also most common first autoantibodies in type 1 diabetes (Ilonen et al., 2013), there is reason to suspect that they may represent two different pathways to type 1 diabetes. Therefore, we separated two subsets from our study cohort, children with IAA or GADA as the first autoantibody, with the purpose of studying the effect of the selected SNPs in them separately. The same survival analyses conducted with the entire cohort were also ran with both subsets and several differences between these disease endotypes were observed.

In the time period from birth to type 1 diabetes, IAA-first disease type associated significantly with *PTPN22* and *INS* that could both also be seen in the entire cohort. Neither of these associations were present in the GADA-first group, but a suggestive association to *ERBB3* was detected. In our previous study, the GADA-first subset associated significantly with *ERBB3*.

In the time period from birth to seroconversion, the IAA-first group associated statistically significantly with *PTPN22*, which in line with results from the TEDDY cohort (Krischer et al., 2019; Krischer, Lynch, et al., 2017). Additionally, a borderline association with *INS* was detected that was statistically significant in our previous study and has also been seen in TEDDY (Krischer et al., 2022). In the GADA-first group we could not detect significant associations between SNPs and

islet autoimmunity, but we did see a suggestive association with *CLEC16A* that was recently reported in TEDDY (Krischer et al., 2022). There was also a suggestive association with *UBASH3A* in the GADA-first group, which we could also see in the whole cohort as a statistically significant association.

When the time period from seroconversion to type 1diabetes was studied, the IAA group had a significant association with *STAT4* and borderline associations with *INS* and *ERBB3*. The association with *INS* was also seen in our previous study, in which it was significant. In the GADA-first group, an association between *ERBB3* and progression to type 1 diabetes could be seen. Interestingly, the effects in the *ERBB3* associations in IAA- and GADA-led autoimmunity were opposite in our cohort. While in the GADA-first group the autoimmune risk-variant increased the risk of progression to disease, in the IAA-first group the same variant was mildly protective. We could not see any effect between *ERBB3* and progression while analysing the whole cohort, which may be due to these opposite effects in the subgroups.

Some previously reported associations from other cohorts could not be replicated in this study. These include an association between type 1 diabetes and *CTLA4* that was observed in DAISY (Steck et al., 2005). Additionally, TEDDY has detected an association between the appearance of islet autoimmunity and *SH2B3* that we could not duplicate (Törn et al., 2014). TEDDY also reported an association between *TNFAIP3* and progression to type 1 diabetes, which we could not see in this study (Krischer, Liu, et al., 2017). In DAISY, *IFIH1* was associated with progression to type 1 diabetes, but we could not see such an association in study I (Steck et al., 2014).

The SNP with the most associations in this cohort was rs2476601 in the gene encoding PTPN22. Genome wide association studies have shown that, apart from HLA genes, the locus has one of the strongest associations to type 1 diabetes and it is a risk factor in many other autoimmune diseases as well (Tizaoui et al., 2021; Todd, 2010). What is more, the autoimmune risk variant rs2476601 has been reported to have various and sometimes contradictory effects in the multiple immune cell populations, in which the protein is expressed (Tizaoui et al., 2021). Perhaps most importantly, it impairs the regulation of TCR signalling (Bottini et al., 2004), which is a key function of the protein in T cells. In our study, the SNP associated with all studied time periods and in endotype specific analyses, the risk effect was seen particularly in IAA led autoimmunity. These results support the narrative that the autoimmune variant of PTPN22 modulates several processes in the immune system. The association between *PTPN22* and IAA has also previously been reported in both humans and mice (Krischer et al., 2019; Lin et al., 2016).

ERBB3 encodes an epidermal growth factor receptor that takes part in pathways controlling cell differentiation and proliferation in various tissues. The minor variant

T in rs2292239 carries an increased risk for type 1 diabetes and it is in very strong linkage disequilibrium with *IKZF4* rs1701704 allele G, another known type 1 diabetes risk locus encoding the zinc finger protein Eos. The separate associations we and others have observed between the risk variant of *ERBB3* and initiation and progression of the autoimmune process leading to type 1 diabetes suggests that *ERBB3* may have several functions in disease pathogenesis. Moreover, we detected opposite effects in the IAA and GADA groups in the time period from seroconversion to type 1 diabetes, which supports the presence of functional differences between suspected disease endotypes. In a recent study by Vandewalle et al. the protective alleles of *ERBB3*/*IKZF4* were linked with slower spreading of autoimmunity, but the effect could only be detected in female cases (Vandewalle et al., 2021). This study did not observe an association with progression to type 1 diabetes, but the study analysed all autoantibody groups together, as in our main analysis. Another study found that the risk allele of *IKZF4* rs1701704 was associated with a faster decline in first phase insulin response in children with multiple autoantibodies (Koskinen et al., 2019). These results together suggest potentially complex differential mechanisms between patient groups.

UBASH3A codes for the Ubiquitin-associated and SH3 containing A (UBASH3A) protein, which has a role in the negative regulation of TCR signalling. The studied polymorphism rs9976767 was originally associated with type 1 diabetes in a genome wide association study (Grant et al., 2009), but little is known about its function. A NOD mouse study found that insufficient expression of UBASH3A in T cells leads to faster type 1 diabetes development (Chen et al., 2020). Additionally, *in vitro* studies in human T cells indicate that UBASH3A modulates TCR-CD3 interactions, CD28 signalling and IL2 expression in T cells (Ge et al., 2017, 2019). In our cohort the risk variant associated with both type 1 diabetes and appearance of autoantibodies and the latter finding seemed to associate particularly with the GADA subgroup. However, the association in the GADA group did not remain significant after correction for multiple testing. The specific association with appearance of type 1 diabetes related autoantibodies supports the theory that type 1 diabetes risk polymorphisms in *UBASH3A* result in poorer control of T cell activation.

FUT2 encodes an enzyme that is needed and therefore responsible for the secretion of ABO blood group antigens in an individual's bodily fluids. The studied SNP rs601338 allele A, when homozygous, results in a lack of ABO blood group antigens in bodily fluids. The same allele has also been associated with the risk of type 1 diabetes in a recessive risk model (Smyth et al., 2011). In our cohort, *FUT2* associated statistically significantly with developing type 1 diabetes and suggestively with both seroconversion and progression to type 1 diabetes. Additionally, *FUT2* associated with progression in both endotype subgroups, but as with *ERBB3*, the effects of the associations were opposite. In IAA led autoimmunity,

the allele had a mildly protective effect, but in the GADA group it associated with the risk of progression. Two earlier complementary studies characterising rapid and slow progressors in the DIPP cohort found that the secretory, non-autoimmune allele of *FUT2* rs601338 associated with rapid progression to the disease, while slow progressors had a suggestive association between delayed disease progression and the *FUT2* risk allele (Pöllänen et al., 2017, 2019). IAA and GADA led disease pathways could also be observed in these studies, as individuals with IAA as the first autoantibody were more likely to progress to disease compared individuals with GADA led autoimmunity. These results are in line with our differing observations in the IAA and GADA groups. In another study investigating the decline of the first phase insulin response in type 1 diabetes, children with the autoimmune risk allele experienced a faster decline of the response (Koskinen et al., 2019).

Signal transducer and activator of transcription 4 (STAT4) is a transcription factor involved in the differentiation of naïve CD4+ T cells into type 1 T helper cells. The ancestral T allele of the SNP rs7574865 has been associated with an increased risk of type 1 diabetes and other autoimmune diseases (Liang et al., 2012). In the present study, the risk allele associated with progression to type 1 diabetes, especially in the IAA subgroup. A study investigating *STAT4* SNPs in a Korean cohort found an association between the risk allele T of rs7574865 and earlier onset of disease (Lee et al., 2008). The effect seemed to be dosage dependent and individuals homozygous for the risk allele were the youngest to develop type 1 diabetes. Similarly, a study with Polish children found an association between the risk allele and earlier type 1 diabetes onset (Fichna et al., 2020). What is more, children with type 1 diabetes were found to express *STAT4* at higher levels in their PBMCs compared to healthy controls, especially in cases with multiple autoantibodies. Murine studies investigating Stat4 have found that Stat4 deficiency protects from type 1 diabetes (Holz et al., 1999; Yang et al., 2004).

6.2 *PTPN22* autoimmune risk variant rs2476601 in regulatory T cells

The purpose of Tregs in the immune system is to suppress excessive immune reactions and maintain tolerance to self-antigens. There is evidence that in diabetic patients these cells malfunction or are not as effective in their task compared to healthy individuals (Bettini & Bettini, 2021). However, the reasons behind these differences between patients and healthy individuals are not completely understood. Genetic factors shaping immune function are a strong possibility.

As in study I, the aim of study II of this thesis was to identify connections between disease predisposing genetic elements and the autoimmune process leading to type 1 diabetes. In study II, the effects of seven type 1 diabetes associated SNPs

in six loci to cell frequencies were investigated in Tregs. The study included a main cohort consisting of healthy children, children positive for multiple autoantibodies, and children who had progressed to type 1 diabetes and a later confirmation cohort with only healthy children. All healthy controls and autoantibody positive cases were participants in the DIPP study and thus carried type 1 diabetes associated HLA genotypes. Cases with type 1 diabetes had been recruited after hospitalisation for the disease. The flow cytometry analyses of the main cohort had been performed earlier (Viisanen et al., 2019). The children in the confirmation cohort were selected for equal representation of risk and protective SNP genotypes, as well as heterozygotes.

The main finding of study II was an association between the autoimmune risk variant of *PTPN22* rs2476601 and elevated Treg frequencies in peripheral blood. The finding was initially observed in the main cohort, but later confirmed also in the confirmation cohort. In the main cohort, an elevation in Tregs could be seen both in the total and naïve populations, but not in memory Tregs. Additionally, when the main cohort was divided into healthy, autoantibody positive and diabetic children, trends indicating similar increases in all subgroups could be seen. When the analysis was repeated in the confirmation cohort, elevated Treg frequencies were observed in total, naïve and memory Tregs.

A few other publications have also observed increases in Treg frequencies in relation to the risk allele A of *PTPN22* rs2476601. In a study aiming to characterise the Treg compartment in systemic lupus erythematosus, an association between the autoimmune risk variant of *PTPN22* and elevated Treg counts was observed (Ferreira et al., 2019). The association in this study was specific to thymically derived FOXP3+Helios+ Tregs and coupled with other markers of immune activation, like higher PD-1 expression. In our study the association was detected in CD4+CD25+CD127low Tregs, CD4+CD25+CD127lowFOXP3+ Tregs and CD4+CD25+CD127lowFOXP3+Helios+ Tregs. In a mouse model expressing a mutation analogous to the human *PTPN22* rs2476601 variant, the Treg compartment has been observed to expand with age (Dai et al., 2013). Following T cell depletion, mice carrying the risk variant showed higher T cell proliferation, especially in Tregs (Knipper et al., 2020). Additionally, increased Treg counts have been observed in Ptpn22 deficient mouse models (Brownlie et al., 2012; Maine et al., 2012; Zheng & Kissler, 2013). There are also studies, however, that have found no differences in Treg expression in relation to the autoimmune variant of *PTPN22* (Chemin et al., 2018; Wu et al., 2014).

The expression of PTPN22 is regulated negatively by the transcription factor FOXP3 (Marson et al., 2007) and simultaneously, PTPN22 has been implicated in the induction of FOXP3 expression (Fousteri et al., 2014). Therefore, the autoimmune variant of PTPN22 affecting Treg function and differentiation is a reasonable expectation. In our data, statistically significant differences could be

observed when GG children were compared to both AA and AG children. More human studies are needed to elucidate the effect of higher Treg frequencies in autoimmune processes.

In addition to the association between *PTPN22* rs2476601 and higher Treg counts, we also observed a separate association between type 1 diabetes and elevated Treg frequencies, when comparing healthy and diabetic children carrying the protective GG genotype. Higher Treg frequencies in type 1 diabetes patients have been discovered in other studies as well. For example, several studies have observed elevated CD25low Treg frequencies in patients compared to healthy controls (Parackova et al., 2016; Zhang et al., 2021; Zóka et al., 2019). Boldison and colleagues also noted an increase in activated memory Tregs in diabetics (Boldison et al., 2022).

Suggestive associations that did not remain statistically significant after correction for multiple testing could be seen between total FOXP3+ Tregs and *IL2RA* rs12722495 and *CTLA4* rs3087243, as well as naïve FOXP3+ Tregs and *CTLA4* rs3087243. In the case of in *CTLA4*, the type 1 diabetes associated risk allele associated with lower Treg frequencies. In *IL2RA* the risk allele associated with higher Treg frequencies. Both *CTLA4* and *IL2RA* are important for Treg function. The former codes for the cytotoxic T-lymphocyte-associated protein 4 (CTLA4) that competes with the co-stimulatory protein CD28 in TCR activation and in Tregs supports their inhibitory function. The risk variant rs3087243 has previously been associated with lower gene expression levels (de Jong et al., 2016; Zóka et al., 2019). IL2RA codes for a subunit of a high affinity IL2 receptor that is expressed particularly on Tregs. Little is known of the effects of rs12722495 on the function of the protein.

6.3 Monocyte function and viral infections in type 1 diabetes

The aim of the study III was to explore possible gene expression alterations in peripheral blood immune cells in prediabetic individuals with signs of advanced beta cell autoimmunity, but no clinical type 1 diabetes. While several studies have found gene expression differences between healthy individuals and diabetics (Kaizer et al., 2007; Rassi et al., 2006; M. Yang et al., 2015), less is known about the transcription profiles of prediabetic individuals. Further observations regarding the early stages of the autoimmune process leading to type 1 diabetes would potentially lead to better prediction of disease, new biomarkers, and even preventive treatments.

In the study, the transcription profiles of autoantibody positive children with genetic susceptibility to type 1 diabetes and their autoantibody negative controls were compared. The study was conducted in two parts: first a smaller pilot study of

nine case-control pairs was carried out with frozen PBMC and after this a larger main study with fresh cells consisting of 25 case-control pairs. In the pilot study the cases positive for multiple autoantibodies were selected among children that had later developed type 1 diabetes to specifically target individuals in the preclinical stage of the autoimmune process leading to diabetes. In the main cohort, a selection based on type 1 diabetes development could not be made, because of the use of fresh cells. However, it has been shown that the rate of progressing to type 1 diabetes after developing two or more biochemical, disease associated autoantibodies is nearly 70 % within 10 years (Ziegler et al., 2013).

Comparing gene expression profiles between healthy control children and children positive for multiple autoantibodies did not reveal any differentially expressed genes in the pilot cohort of study III. Additionally, routine quality assessment of the data from the pilot study revealed a significant batch effect in the samples based on time of storage. However, other quality control of the data did not uncover any problems, so it was decided to conduct GSEA analysis with the data to explore possible differences in up- or downregulated pathways between cases and controls. Among the upregulated pathways, several terms relating to responses to viral infections and function of the immune system were observed. Putting these findings together, it was decided to collect another larger data set with more statistical detection power.

The samples of the main cohort were handled while fresh to eliminate the effect of freezing on mRNA. Due to the involvement of monocytes in infection and antigen presentation (Jakubzick et al., 2017), it was also decided to divide the PBMC samples into a monocyte fraction and remaining PBMC cells that was termed the lymphocyte fraction. Monocytes are not only able to differentiate into macrophages and dendritic cells, but also act as independent immune cells and engage in phagocytosis, cytokine production and antigen presentation. These fractions were analysed for gene expression differences separately. Once more, analyses between transcriptional profiles of cases and controls yielded no statistically significantly differentially expressed genes. Because of this, the same further statistical analyses were conducted as in the pilot study. GSEA analyses in the lymphocyte fraction did not highlight any pathways outside of cell upkeep functions. However, in the monocyte fraction, terms relating to immune function could once again be seen among the upregulated pathways. Three of these, "defence response to virus", "response to virus" and "response to type I interferon", were statistically significant in both the pilot study and the monocyte fraction. In an earlier study by Irvine *et al*, pathways significantly associated with diabetes included tasks like cellular metabolism and survival, including endoplasmic reticulum and oxidative stress (Irvine et al., 2012). However, unlike study III, this study was conducted with new-onset diabetes.

The lack of an immune response from lymphocytes in the present study, combined with the observed upregulation of viral response pathways in monocytes, points to a possible infection in the monocytes themselves. The relationship between viral infections and increased disease risk has long been a point of investigation in the study of type 1 diabetes. There is also compelling evidence for a link between diabetes and enteroviruses from several populations (K. Wang et al., 2021; S. Yang et al., 2022). Peripheral monocytes and monocyte derived macrophages may, for example, be infected by Coxsackie virus B4 (Alidjinou, Sané, et al., 2015; Benkahla et al., 2018), which is a member of the *Enterovirus* genus. What is more, an in vitro study suggests that such an infection may turn persistent (Goffard et al., 2013). Interestingly, a Brazilian study investigating innate immune responses to enteroviruses in type 1 diabetes patients, non-diabetic siblings of patients and healthy, unrelated controls, found that innate immune responses were comparative in diabetic patients and their siblings and stronger than in healthy controls (Bergamin et al., 2020). This was also apparent in monocytes through increased toll-like receptor expression. In addition to monocytes, enteroviral infections have also been detected in the pancreatic cells of type 1 diabetes patients (Dotta et al., 2007; Foulis et al., 1990; Krogvold et al., 2022).

Study III of this thesis also highlighted the term “response to type I interferon”, particularly in monocytes. Type I interferons mediate immunoregulation and inflammatory signalling and include, for example, interferon alfa and beta. A transient interferon alfa signature, preceding the onset of type 1 diabetes, has been observed in multiple longitudinal studies (Ferreira et al., 2014; Kallionpää et al., 2014; Xhonneux et al., 2021). The phenomenon has previously been linked particularly with signs of infection in the TEDDY cohort (Xhonneux et al., 2021). What is more, at risk children with acute enteroviral infections in the DIPP study were accompanied by strong interferon responses (Lietzen et al., 2018). Similarly, low amounts of enterovirus RNA were detected in monocytes of patients with type 1 diabetes and the detected cases of infection were accompanied by interferon alpha expression (Alidjinou, Chehadeh, et al., 2015). A significant increase in type I interferons has also been noted in the insulitic pancreas (Huang et al., 1995). In a study by Dotta and colleagues the expression of type I interferons coincided with the presence of enterovirus infection (Dotta et al., 2007).

There are several accounts of transcriptional changes in the context of type 1 diabetes. For example, multiple studies investigating PBMCs of newly diagnosed patients have observed gene expression differences between cases and healthy controls (Kaizer et al., 2007; Rassi et al., 2006; Yang et al., 2015). However, gene expression level differences of different stages of the autoimmune process are not properly understood. In a study by Elo *et al*, the transcriptional profiles between autoantibody positive and cases with type 1 diabetes could not be separated (Elo et

al., 2010). In an analysis comparing cases with established type 1 diabetes, their first-degree relatives and unrelated healthy controls, the most significant gene expression difference was seen between autoantibody negative first-degree relatives and healthy controls (Stechova et al., 2012). On the other hand, Reynier and colleagues compared children with new-onset type 1 diabetes patients, autoantibody positive first-degree relatives and healthy controls and found the most differences between the diabetics and the two other groups (Reynier et al., 2010). Specific to monocytes, a study investigating identical twins discordant for type 1 diabetes found that 13 out of 16 genes expressed differentially in the diabetic twins compared to healthy controls were also abnormal in the non-diabetic twins (Beyan et al., 2010). Another study investigating gene expression differences in monocytes between newly diagnosed type 1 diabetes patients and healthy controls found that the transcriptional profiles of cases formed two subgroups with mild and strong deviation from controls (Irvine et al., 2012).

Put together, detecting transcriptional differences between different stages of the autoimmune process leading to type 1 diabetes may require the consideration of disease endotypes and genetic risk elements. Some of the differences in gene expression detected in earlier studies can perhaps be attributed to the ongoing metabolic stress caused by loss of glucose tolerance in the later stages of type 1 diabetes pathogenesis. Additionally, in several previous studies, separating prediabetic and/or genetically predisposed individuals from diabetic patients has been challenging (Bergamin et al., 2020; Beyan et al., 2010; Elo et al., 2010; Stechova et al., 2012). The relatively small differences observed in study III may therefore partly be explained by the close matching of cases and controls by genetic diabetes risk.

6.4 Strengths and limitations of the study

The greatest strength of this thesis work is being a part of the larger DIPP study. The study was initiated in 1994 and is a prospective follow-up study with a 15-year follow-up period. By August 2022, over 250 000 children had been screened for HLA-conferred genetic risk of type 1 diabetes, some 25 000 children had participated in the study, 1473 children had developed persistent, disease associated autoantibodies and 682 children had progressed to type 1 diabetes during the past 28 years. Children are recruited shortly after birth and monitored for signs of beta cell autoimmunity by measuring autoantibodies every three to twelve months, depending on the age of the child. Frequent study clinic visits and coupled with follow-up starting at birth allow the relatively accurate determination of age at seroconversion in all cases.

An aspect of the DIPP study that can be considered both a strength and a limitation is that it takes place in Finland, where the incidence of type 1 diabetes is the highest in the world. Accompanied with the fact that the recruitment is carried out in three major cities, the study provides a large-scale cohort with a high number of cases. On the other hand, due to the unique population, the results may not be easily extended to other populations. Similarly, because the study subjects are children, all results may not be generalisable to adults.

The antibodies measured in the DIPP protocol are ICA, IAA, GADA and IA-2A. A limitation of the sample collection acquired before 2003 is the early follow-up protocol that initially measured only non-specific ICA in primary screenings. Only after the appearance of ICA, were the children monitored also for the appearance of the biochemical autoantibodies. An exception to this is a subset of 1006 children born before 2003, from whom all autoantibodies were measured from the beginning of follow-up (Kukko et al., 2005). In this subset of children, only 25.9 % had ICA as the first appearing autoantibody and out of the 44 children positive for multiple autoantibodies, three did not have ICA at all. It is therefore possible that some autoantibody positive cases from before 2003 may have been missed.

Another limitation of the DIPP protocol is the sole inclusion of children carrying HLA-conferred genetic risk for type 1 diabetes. While most individuals, who develop diabetes also carry HLA-conferred risk to the disease, some do not. In a study characterising cases with and without diabetic family members, the 25.9-42.8 % of the cases had low or decreased genetic risk of type 1 diabetes, depending on familial type 1 diabetes cases (Veijola et al., 1996). Another study estimating the risk conferred by HLA genotypes similarly found that roughly 18 % of the studied diabetics had neutral or protective HLA genotypes (Ilonen et al., 2016). The DIPP study is conducted specifically in a high-risk cohort and the results may therefore not be generalisable to lower risk individuals.

In study I of this thesis, one confounding factor, in the investigation of SNPs, was the variable individual HLA-conferred genetic risk in the studied individuals. Type 1 diabetes associated HLA risk variants correlate with appearance of autoantibodies and progression to clinical disease (Anand et al., 2021). In this study, HLA genotypes were sorted into four groups according to risk level with groups high, moderate, slight risk and a combined group for low risk and protective genotypes. These groups were then used as a cofactor in statistical analyses. Ideally, future studies could adjust for individual HLA-conferred risk. However, here it was not possible without significant loss of statistical power. Similarly, despite the large study cohort, some of the analyses, especially in the IAA-first and GADA-first subgroups, suffer from a lack of power. Despite choosing well-established type 1 diabetes risk loci for the study, we did not observe associations to clinical disease in all SNPs. In the whole cohort, the number of cases in the time periods until

seroconversion and until type 1 diabetes onset were 976 and 426, respectively. Therefore, analyses in the time period from birth to seroconversion had higher statistical power compared to the other time periods in the study. In the IAA-first group, the corresponding case numbers were 330 and 135, respectively, and in the GADA-first group 329 and 77, respectively. The analyses in these subgroups had as a result less detection power as the analyses with the entire cohort. In addition, in the time periods from birth and seroconversion to onset of disease are somewhat more robust in the IAA-first group compared to the GADA-first group. In conclusion, the endotype subgroups and the time periods from birth and seroconversion to onset of type 1 diabetes could benefit from a new analysis in a larger cohort, especially SNPs with rarer minor alleles, such as *IL2RA* rs2104286.

In study II, the volume of the paediatric samples was a limiting factor in the study design. Further *in vitro* studies investigating functional differences between *PTPN22* rs2476601 genotypes could not be conducted due to the limited quantity of available cells. Additionally, some of the other SNP analyses in the study might have benefited from a larger cohort, because of the low minor allele frequencies of some SNPs. For instance, our cohort did not contain any homozygotes of the protective *IL2RA* rs12722495 allele C. Therefore, repeating the analysis in a larger cohort might lead to novel findings, for which we did not have the necessary power here. However, the inclusion of a validation cohort lends further credibility to the main result of increased Treg frequencies in the carriers of *PTPN22* rs2476601 risk allele A. In addition, the validation cohort revealed, that the effect exists also in healthy individuals.

In study III, one limitation was the lack of a control group without HLA-conferred risk of type 1 diabetes. Some studies in other cohorts in the context of type have similarly found it difficult to tell diabetic and at-risk individuals apart based on gene expression (Bergamin et al., 2020; Beyan et al., 2010; Elo et al., 2010; Stechova et al., 2012). Another confounding factor was the use of PBMC and a combined lymphocyte population in the pilot and main cohorts, respectively. While several immune cell subpopulations are implicated in the pathogenesis of type 1 diabetes, their roles in it are vastly different, which is also reflected in the cells' transcriptomic profiles. Additionally, it is unclear how well peripheral blood immune cells reflect the ongoing autoimmune process in the pancreas.

6.5 Future perspectives

This thesis explores the heterogenetic nature of type 1 diabetes and the observations made in it corroborate the existence of endotypes or different pathways to disease. Nevertheless, the study of type 1 diabetes endotypes is still in its infancy. Especially topics that have yielded contradictory results in past studies may benefit from

revisitation with special attention to the proposed disease endotypes. In the future, the endotype specific genetic findings in this thesis should be attempted to be replicated in other populations as well. Additionally, the inclusion of the newest discovered risk SNPs could facilitate the discovery of new disease stage and endotype specific associations. It is possible that taking mechanistic heterogeneity of type 1 diabetes into account in genetic analyses reveals stronger, situation specific risk effects in some of the lesser-known risk polymorphisms. Functional studies in cells could help link genetic risk factors and the mechanisms leading to type 1 diabetes.

Utilising longitudinal datasets to investigate transcriptomic and cell subset level findings may shed light to some of the remaining questions about the roles of different immune cells in the pathogenesis of type 1 diabetes. Moreover, additional controls without genetic type 1 diabetes risk may be beneficial in gene expression studies. In genetic studies new technologies may enable better adjustment for individual genetic risk.

7 Conclusions

The main conclusions of this thesis include:

1. Several type 1 diabetes risk polymorphisms associate expressly with a specific phase of the disease pathogenesis, including the loci *NRP1*, *INS*, *UBASH3A* and *STAT4*.
2. Many type 1 diabetes risk loci, like *PTPN22* and *INS*, seem to associate only with a specific disease endotype, defined by the first appearing autoantibody.
3. The type 1 diabetes risk polymorphism *PTPN22* rs2476601 associates with elevated total and naïve regulatory T cell frequencies.
4. Transcriptional profiles of children positive for multiple type 1 diabetes associated autoantibodies were not significantly altered in monocytes and monocyte-depleted peripheral blood mononuclear cells compared to genetically matched healthy control children.
5. Gene sets relating to responses to virus and a type I interferon response were significantly upregulated in the monocytes of children positive for multiple type 1 diabetes associated autoantibodies.

Acknowledgements

This work was carried out in the Immunogenetics laboratory, Institute of Biomedicine and Medicity Research Laboratory, University of Turku in the Turku Doctoral Programme of Molecular Medicine (TuDMM). TuDMM and past and present Directors of Medicity, Professor Sirpa Jalkanen and Professor Marko Salmi are warmly thanked for providing an encouraging working environment and excellent research facilities.

I wish to express my deepest gratitude to my supervisors, Professor Emeritus Jorma Ilonen and Docent Johanna Lempainen. Jorma, your exceptional understanding of science and our stimulating and wonderfully unpredictable discussions have provided great guidance and support. Johanna, your optimism, scientific ambition, and ability to always find a way forward have been equally important and inspiring during these years.

I thank the reviewers of this work, Docent Eliisa Kekäläinen and Docent Niina Sandholm for their excellent comments and constructive criticism. This thesis is much better for it.

I offer my gratitude to the members of my follow-up committee, Professor Juha Kere, Professor Tuure Kinnunen, and Professor Jorma Toppari, whose encouragement and advice have been a great help during all stages of this project.

I wish to thank the DIPP steering committee, Professors Riitta Veijola, Mikael Knip, Jorma Toppari, Heikki Hyöty, and Kalle Kurppa for the opportunity to participate in this one-of-a-kind study.

My sincere gratitude belongs to my co-authors: Professors Riitta Veijola, Mikael Knip, Jorma Toppari, Tuure Kinnunen, and Juha Kere, Doctors Antti-Pekka Laine, Ahmad Gazali, Tyyne Viisanen, Emmi-Leena Ihantola, Masahito Yoshihara, Elisabet Einarsdottir, Sini Ezer, and Shintaro Katayama, and Ilse Ekman and Sirpa Pahkuri.

I would like to thank my current and former colleagues in the Immunogenetics laboratory for their expertise and company. Antti-Pekka Laine has my gratitude for his invaluable assistance with genetic analyses and our insightful conversations about science, computers, and life in general. I thank Minna Kiviniemi, Mari-Liis Mikk, Zsófia Gombos, Sirpa Pahkuri, Meija Honkanen, and Lucas Nygård for their

friendship and company in the lab, as well as outside of it. The lab ladies, Terhi Laakso, Piia Nurmi, Anne Suominen, Mia Karlsson, and Ritva Suominen, I am grateful to you for making the lab such a welcoming and lively place. I would especially like to thank Terhi and Anne for their contributions to this thesis.

My university family, Janne, Marjaana, Salli, and Satu, you deserve my sincere gratitude for your endless peer support and all the laughs we have shared. After this decade, I am sure that we can soon rename the chat group Thesis 100 %. I want to thank my friend Roosa for all our shared pastries and Saturday afternoons.

I express my gratitude to my late grandmother Elli, who always wanted to know everything about my university studies. I would also like to thank my grandmother Terttu and my late grandfather Raimo for their kind support and encouragement for continuing my academic pursuits.

I wish to thank my parents Heli and Ari for their endless support and love. Thank you for providing me with every opportunity to get where I am today. I thank my brilliant brother Lari for challenging me and for being my oldest friend.

Finally, with all my heart, I wish to thank my Tobias. Your patience, love and positivity never fail to amaze me.

This work was funded by the Sigrid Juselius Foundation, the Päivikki and Sakari Sohlberg Foundation, the Emil Aaltonen Foundation, the Finnish Cultural Foundation, the Finnish Medical Foundation, and the Diabetes Research Foundation.

Turku, May 2032
Milla Valta

References

Aarnisalo, J., Treszl, A., Svec, P., Marttila, J., Öling, V., Simell, O., Knip, M., Körner, A., Madacsy, L., Vasarhelyi, B., Ilonen, J., & Hermann, R. (2008). Reduced CD4 +T cell activation in children with type 1 diabetes carrying the PTPN22/Lyp 620Trp variant. *Journal of Autoimmunity*, *31*(1), 13–21. <https://doi.org/10.1016/j.jaut.2008.01.001>

Aarnisalo, J., Veijola, R., Vainionpää, R., Simell, O., Knip, M., & Ilonen, J. (2008). Cytomegalovirus infection in early infancy: Risk of induction and progression of autoimmunity associated with type 1 diabetes. Diabetologia, 51(5), 769–772. <https://doi.org/10.1007/s00125-008-0945-8>

Achenbach, P., Koczwara, K., Knopff, A., Naserke, H., Ziegler, A.-G., & Bonifacio, E. (2004). Mature high-affinity immune responses to (pro)insulin anticipate the autoimmune cascade that leads to type 1 diabetes. Journal of Clinical Investigation, 114(4), 589–597. <https://doi.org/10.1172/JCI200421307>

Achenbach, P., Lampasona, V., Landherr, U., Koczwara, K., Krause, S., Grallert, H., Winkler, C., Pflüger, M., Illig, T., Bonifacio, E., & Ziegler, A. G. (2009). Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. Diabetologia, 52(9), 1881–1888. <https://doi.org/10.1007/s00125-009-1438-0>

Adkins, B., Leclerc, C., & Marshall-Clarke, S. (2004). Neonatal adaptive immunity comes of age. Nature Reviews Immunology, 4(7), 553-564. <https://doi.org/10.1038/nri1394>

Alberti, K. G. M. M., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. Diabetic Medicine, 15(7), 539–553. <https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S>

Alidjinou, E. K., Chehadeh, W., Weill, J., Vantyghem, M.-C., Stuckens, C., Decoster, A., Hober, C., & Hober, D. (2015). Monocytes of oatients with type 1 diabetes harbour enterovirus RNA. European Journal of Clinical Investigation, 45(9), 918–924. <https://doi.org/10.1111/eci.12485>

Alidjinou, E. K., Sané, F., Trauet, J., Copin, M.-C., & Hober, D. (2015). Coxsackievirus B4 can infect human peripheral blood-derived macrophages. Viruses, 7(11), 6067–6079. <https://doi.org/10.3390/v7112924>

American Diabetes Association. (2020). 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2021. Diabetes Care, 44(Supplement_1), S15–S33. <https://doi.org/10.2337/dc21-S002>

Anand, V., Li, Y., Liu, B., Ghalwash, M., Koski, E., Ng, K., Dunne, J. L., Jönsson, J., Winkler, C., Knip, M., Toppari, J., Ilonen, J., Killian, M. B., Frohnert, B. I., Lundgren, M., Ziegler, A.-G., Hagopian, W., Veijola, R., & Rewers, M. (2021). Islet autoimmunity and HLA markers of presymptomatic and clinical type 1 diabetes: Joint analyses of prospective cohort studies in Finland, Germany, Sweden, and the U.S. Diabetes Care, dc201836. <https://doi.org/10.2337/dc20-1836>

Andersson, C., Kolmodin, M., Ivarsson, S.-A., Carlsson, A., Forsander, G., Lindblad, B., Ludvigsson, J., Kockum, I., Marcus, C., Samuelsson, U., Örtqvist, E., Lernmark, Å., Elding Larsson, H., & Törn, C. (2014). Islet cell antibodies (ICA) identify autoimmunity in children with new onset diabetes mellitus negative for other islet cell antibodies. Pediatric Diabetes, 15(5), 336–344. <https://doi.org/10.1111/pedi.12093>

Arechiga, A. F., Habib, T., He, Y., Zhang, X., Zhang, Z.-Y., Funk, A., & Buckner, J. H. (2009). Cutting edge: The PTPN22 allelic variant associated with autoimmunity impairs B cell signaling. Journal of Immunology, 182(6), 3343–3347. <https://doi.org/10.4049/jimmunol.0713370>

Arif, S., Leete, P., Nguyen, V., Marks, K., Nor, N. M., Estorninho, M., Kronenberg-Versteeg, D., Bingley, P. J., Todd, J. A., Guy, C., Dunger, D. B., Powrie, J., Willcox, A., Foulis, A. K., Richardson, S. J., de Rinaldis, E., Morgan, N. G., Lorenc, A., & Peakman, M. (2014). Blood and islet phenotypes indicate immunological heterogeneity in type 1 diabetes. Diabetes, 63(11), 3835–3845. <https://doi.org/10.2337/db14-0365>

Arif, S., Tree, T. I., Astill, T. P., Tremble, J. M., Bishop, A. J., Dayan, C. M., Roep, B. O., & Peakman, M. (2004). Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. The Journal of Clinical Investigation, 113(3), 451–463. <https://doi.org/10.1172/JCI19585>

Atkinson, M. A., Bluestone, J. A., Eisenbarth, G. S., Hebrok, M., Herold, K. C., Accili, D., Pietropaolo, M., Arvan, P. R., Von Herrath, M., Markel, D. S., & Rhodes, C. J. (2011). How does type 1 diabetes develop?: The notion of homicide or β-cell suicide revisited. Diabetes, 60(5), 1370–1379. <https://doi.org/10.2337/db10-1797>

Babon, J. A. B., DeNicola, M. E., Blodgett, D. M., Crèvecoeur, I., Buttrick, T. S., Maehr, R., Bottino, R., Naji, A., Kaddis, J., Elyaman, W., James, E. A., Haliyur, R., Brissova, M., Overbergh, L., Mathieu, C., Delong, T., Haskins, K., Pugliese, A., Campbell-Thompson, M., … Kent, S. C. (2016). Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. Nature Medicine, 22(12), 1482–1487. https://doi.org/10.1038/nm.4203

Bach, J.-F. (1994). Insulin-dependent diabetes mellitus as an autoimmune disease. Endocrine Reviews, 15(4), 516–542. <https://doi.org/10.1210/edrv-15-4-516>

Bach, J.-F. (2021). Revisiting the hygiene hypothesis in the context of autoimmunity. Frontiers in Immunology, 11. <https://doi.org/10.3389/fimmu.2020.615192>

Bach, J.-F., & Chatenoud, L. (2012). The hygiene hypothesis: An explanation for the increased frequency of insulin-dependent diabetes. Cold Spring Harbor Perspectives in Medicine, 2(2), a007799. <https://doi.org/10.1101/cshperspect.a007799>

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M. T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y. S., Kotowska, D., Colding, C., … Wang, J. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host & Microbe, 17(5), 690–703. <https://doi.org/10.1016/j.chom.2015.04.004>

Baekkeskov, S., Aanstoot, H.-J., Christgai, S., Reetz, A., Solimena, M., Cascalho, M., Folli, F., Richter-Olesen, H., & Camilli, P.-D. (1990). Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature, 347, 151–156. <https://doi.org/10.1038/347151a0>

Bakay, M., Pandey, R., Grant, S. F. A., & Hakonarson, H. (2019). The genetic contribution to type 1 diabetes. Current Diabetes Reports, 19(11), 116. <https://doi.org/10.1007/s11892-019-1235-1>

Bandeira, A., Mota-Santos, T., Itohara, S., Degermann, S., Heusser, C., Tonegawa, S., & Coutinho, A. (1990). Localization of gamma/delta T cells to the intestinal epithelium is independent of normal microbial colonization. The Journal of Experimental Medicine, 172(1), 239–244. PubMed. <https://doi.org/10.1084/jem.172.1.239>

Barratt, B. J., Payne, F., Lowe, C. E., Hermann, R., Healy, B. C., Harold, D., Concannon, P., Gharani, N., McCarthy, M. I., Olavesen, M. G., McCormack, R., Guja, C., Ionescu-Tîrgovişte, C., Undlien, D. E., Rønningen, K. S., Gillespie, K. M., Tuomilehto-Wolf, E., Tuomilehto, J., Bennett, S. T., … Todd, J. A. (2004). Remapping the insulin gene/IDDM2 locus in type 1 diabetes. Diabetes, 53(7), 1884–1889. <https://doi.org/10.2337/diabetes.53.7.1884>

Barrett, J. C., Clayton, D. G., Concannon, P., Akolkar, B., Cooper, J. D., Erlich, H. A., Julier, C., Morahan, G., Nerup, J., Nierras, C., Plagnol, V., Pociot, F., Schuilenburg, H., Smyth, D. J., Stevens, H., Todd, J. A., Walker, N. M., & Rich, S. S. (2009). Genome-wide association study and

meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nature Genetics, 41(6), 703–707. <https://doi.org/10.1038/ng.381>

Basha, S., Surendran, N., & Pichichero, M. (2014). Immune responses in neonates. Expert Review of Clinical Immunology, 10(9), 1171–1184. <https://doi.org/10.1586/1744666X.2014.942288>

Battaglia, M., Ahmed, S., Anderson, M. S., Atkinson, M. A., Becker, D., Bingley, P. J., Bosi, E., Brusko, T. M., DiMeglio, L. A., Evans-Molina, C., Gitelman, S. E., Greenbaum, C. J., Gottlieb, P. A., Herold, K. C., Hessner, M. J., Knip, M., Jacobsen, L., Krischer, J. P., Long, S. A., … Peakman, M. (2020). Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. Diabetes Care, 43(1), 5–12. <https://doi.org/10.2337/dc19-0880>

Bauer, W., Veijola, R., Lempainen, J., Kiviniemi, M., Härkönen, T., Toppari, J., Knip, M., Gyenesei, A., & Ilonen, J. (2019). Age at seroconversion, HLA genotype, and specificity of autoantibodies in progression of islet autoimmunity in childhood. The Journal of Clinical Endocrinology & Metabolism, 104(10), 4521–4530. <https://doi.org/10.1210/jc.2019-00421>

Begovich, A. B., Carlton, V. E. H., Honigberg, L. A., Schrodi, S. J., Chokkalingam, A. P., Alexander, H. C., Ardlie, K. G., Huang, Q., Smith, A. M., Spoerke, J. M., Conn, M. T., Chang, M., Chang, S.-Y. P., Saiki, R. K., Catanese, J. J., Leong, D. U., Garcia, V. E., McAllister, L. B., Jeffery, D. A., … Gregersen, P. K. (2004). A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. The American Journal of Human Genetics, 75(2), 330–337. <https://doi.org/10.1086/422827>

Bell, G. I., Horita, S., & Karam, J. H. (1984). A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes melliitus. Diabetes, 33(2), 176–183. <https://doi.org/10.2337/diab.33.2.176>

Bellemore, S. M., Nikoopour, E., Schwartz, J. A., Krougly, O., Lee-Chan, E., & Singh, B. (2015). Preventative role of interleukin-17 producing regulatory T helper type 17 (Treg17) cells in type 1 diabetes in non-obese diabetic mice. Clinical and Experimental Immunology, 182(3), 261–269. <https://doi.org/10.1111/cei.12691>

Benkahla, M. A., Elmastour, F., Sane, F., Vreulx, A.-C., Engelmann, I., Desailloud, R., Jaidane, H., Alidjinou, E. K., & Hober, D. (2018). Coxsackievirus-B4E2 can infect monocytes and macrophages in vitro and in vivo. *Virology*, *522*, 271–280. <https://doi.org/10.1016/j.virol.2018.06.010>

Benveniste, J., Lespinats, G., Adam, C., & Salomon, J.-C. (1971). Immunoglobulins in intact, immunized, and contaminated axenic mice: Study of serum IgA. *The Journal of Immunology*, *107*(6), 1647–1655. <https://doi.org/10.4049/jimmunol.107.6.1647>

Bergamin, C. S., Pérez-Hurtado, E., Oliveira, L., Gabbay, M., Piveta, V., Bittencourt, C., Russo, D., Carmona, R. de C., Sato, M., & Dib, S. A. (2020). Enterovirus neutralizing antibodies, monocyte toll like receptors expression and interleukin profiles are similar between non-affected and affected siblings from long-term discordant type 1 diabetes multiplex-sib families: The importance of HLA background. *Frontiers in Endocrinology*, *11*. <https://doi.org/10.3389/fendo.2020.555685>

Besnard, M., Padonou, F., Provin, N., Giraud, M., & Guillonneau, C. (2021). AIRE deficiency, from preclinical models to human APECED disease. *Disease Models & Mechanisms*, *14*(2), dmm046359. <https://doi.org/10.1242/dmm.046359>

Bettini, M., & Bettini, M. L. (2021). Function, failure, and the future potential of Tregs in type 1 diabetes. *Diabetes*, *70*(6), 1211–1219. <https://doi.org/10.2337/dbi18-0058>

Beyan, H., Drexhage, R. C., van der Heul Nieuwenhuijsen, L., de Wit, H., Padmos, R. C., Schloot, N. C., Drexhage, H. A., & Leslie, R. D. (2010). Monocyte gene-expression profiles associated with childhood-onset type 1 diabetes and disease risk: A study of identical twins. *Diabetes*, *59*(7), 1751–1755. <https://doi.org/10.2337/db09-1433>

Bingley, P. J. (1996). Interactions of age, islet cell antibodies, insulin autoantibodies, and first-phase insulin response in predicting risk of progression to IDDM in ICA+ relatives: The ICARUS data set. *Diabetes*, *45*(12), 1720–1728. <https://doi.org/10.2337/diab.45.12.1720>

Bingley, P. J., Gale, E. A. M., & The European Nicotinamide Diabetes Intervention Trial (ENDIT) Group. (2006). Progression to type 1 diabetes in islet cell antibody-positive relatives in the

European Nicotinamide Diabetes Intervention Trial: The role of additional immune, genetic and metabolic markers of risk. *Diabetologia*, *49*(5), 881. <https://doi.org/10.1007/s00125-006-0160-4>

Bodansky, H. J., Staines, A., Stephenson, C., Haigh, D., & Cartwright, R. (1992). Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population. *British Medical Journal*, *304*(6833), 1020–1022. <https://doi.org/10.1136/bmj.304.6833.1020>

Boldison, J., Long, A. E., Aitken, R. J., Wilson, I. V., Megson, C., Hanna, S. J., Wong, F. S., & Gillespie, K. M. (2022). Activated but functionally impaired memory Tregs are expanded in slow progressors to type 1 diabetes. *Diabetologia*, *65*(2), 343–355. <https://doi.org/10.1007/s00125-021-05595-0>

Bonifacio, E., Shattock, M., Dean, B. M., Bottazzo, G. F., Bingley, P. M., Gale, E. A. M., & Dunger, D. (1990). Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *The Lancet*, *335*(8682), 147–149. <https://doi.org/10.1016/0140-6736(90)90013-U>

Bonifacio, E., Weiß, A., Winkler, C., Hippich, M., Rewers, M. J., Toppari, J., Lernmark, Å., She, J.-X., Hagopian, W. A., Krischer, J. P., Vehik, K., Schatz, D. A., Akolkar, B., Ziegler, A.-G., & TEDDY Study Group. (2021). An age-related exponential decline in the risk of multiple islet autoantibody seroconversion during childhood. *Diabetes Care*, *44*(10), 2260–2268. <https://doi.org/10.2337/dc20-2122>

Bottazzo, G. F., Dean, B. M., McNally, J. M., MacKay, E. H., Swift, P. G. F., & Gamble, D. R. (1985). In Situ Characterization of Autoimmune Phenomena and Expression of HLA Molecules in the Pancreas in Diabetic Insulitis. *New England Journal of Medicine*, *313*(6), 353–360. <https://doi.org/10.1056/NEJM198508083130604>

Bottazzo, G., Florin-Christensen, A., & Doniach, D. (1974). Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *The Lancet*, *304*(7892), 1279–1283. <https://doi.org/10.1016/S0140-6736(74)90140-8>

Bottini, N., Musumeci, L., Alonso, A., Rahmouni, S., Nika, K., Rostamkhani, M., MacMurray, J., Meloni, G. F., Lucarelli, P., Pellecchia, M., Eisenbarth, G. S., Comings, D., & Mustelin, T. (2004). A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nature Genetics*, *36*(4), 337-338. <https://doi.org/10.1038/ng1323>

Bradfield, J. P., Qu, H.-Q., Wang, K., Zhang, H., Sleiman, P. M., Kim, C. E., Mentch, F. D., Qiu, H., Glessner, J. T., Thomas, K. A., Frackelton, E. C., Chiavacci, R. M., Imielinski, M., Monos, D. S., Pandey, R., Bakay, M., Grant, S. F. A., Polychronakos, C., & Hakonarson, H. (2011). A genome-wide meta-analysis of six type 1 diabetes cohorts identifies multiple associated loci. *PLOS Genetics*, *7*(9), e1002293. <https://doi.org/10.1371/journal.pgen.1002293>

Brown, C. T., Davis-Richardson, A. G., Giongo, A., Gano, K. A., Crabb, D. B., Mukherjee, N., Casella, G., Drew, J. C., Ilonen, J., Knip, M., Hyöty, H., Veijola, R., Simell, T., Simell, O., Neu, J., Wasserfall, C. H., Schatz, D., Atkinson, M. A., & Triplett, E. W. (2011). Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS ONE*, *6*(10), e25792. <https://doi.org/10.1371/journal.pone.0025792>

Brownlie, R. J., Miosge, L. A., Vassilakos, D., Svensson, L. M., Cope, A., & Zamoyska, R. (2012). Lack of the phosphatase PTPN22 increases adhesion of murine regulatory T cells to improve their immunosuppressive function. *Science Signaling*, *5*(252), ra87. <https://doi.org/10.1126/scisignal.2003365>

Brusko, T. M., Wasserfall, C. H., Clare-Salzler, M., Schatz, D. A., & Atkinson, M. A. (2005). Functional defects and the influence of age on the frequency of CD4+CD25+ T-cells in type 1 diabetes. *Diabetes*, *54*(5), 1407–1414. <https://doi.org/10.2337/diabetes.54.5.1407>

Brusko, T., Wasserfall, C., McGrail, K., Schatz, R., Viener, H. L., Schatz, D., Haller, M., Rockell, J., Gottlieb, P., Clare-Salzler, M., & Atkinson, M. (2007). No alterations in the frequency of FOXP3+ regulatory T-cells in type 1 diabetes. *Diabetes*, *56*(3), 604–612. <https://doi.org/10.2337/db06-1248>

Burahmah, J., Zheng, D., & Leslie, R. (2022). Adult-onset type 1 diabetes: A changing perspective. *European Journal of Internal Medicine*, *104*, 7–12. <https://doi.org/10.1016/j.ejim.2022.06.003>

Burton, P. R., Clayton, D. G., Cardon, L. R., Craddock, N., Deloukas, P., Duncanson, A., Kwiatkowski, D. P., McCarthy, M. I., Ouwehand, W. H., Samani, N. J., Todd, J. A., Donnelly, P., & Barrett, J. C. (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*, *447*(7145), 661–678. <https://doi.org/10.1038/nature05911>

Buzzetti, R., Zampetti, S., & Maddaloni, E. (2017). Adult-onset autoimmune diabetes: Current knowledge and implications for management. *Nature Reviews Endocrinology*, *13*(11), 674–686. <https://doi.org/10.1038/nrendo.2017.99>

Cadario, F., Savastio, S., Pagliardini, V., Bagnati, M., Vidali, M., Cerutti, F., Rabbone, I., Fontana, F., Lera, R., De Donno, V., Valori, A., Gruden, G., Bona, G., & Bruno, G. (2015). Vitamin D levels at birth and risk of type 1 diabetes in childhood: A case–control study. *Acta Diabetologica*, *52*(6), 1077–1081. <https://doi.org/10.1007/s00592-015-0772-6>

Caillat-Zucman, S., Garchon, H. J., Timsit, J., Assan, R., Boitard, C., Djilali-Saiah, I., Bougnères, P., & Bach, J. F. (1992). Age-dependent HLA genetic heterogeneity of type 1 insulin-dependent diabetes mellitus. *Journal of Clinical Investigation*, *90*(6), 2242–2250.

Campbell-Thompson, M., Fu, A., Kaddis, J. S., Wasserfall, C., Schatz, D. A., Pugliese, A., & Atkinson, M. A. (2016). Insulitis and β-cell mass in the natural history of type 1 diabetes. *Diabetes*, *65*(3), 719–731. <https://doi.org/10.2337/db15-0779>

Campbell-Thompson, M. L., Atkinson, M. A., Butler, A. E., Chapman, N. M., Frisk, G., Gianani, R., Giepmans, B. N., von Herrath, M. G., Hyöty, H., Kay, T. W., Korsgren, O., Morgan, N. G., Powers, A. C., Pugliese, A., Richardson, S. J., Rowe, P. A., Tracy, S., & In't Veld, P. A. (2013). The diagnosis of insulitis in human type 1 diabetes. *Diabetologia*, *56*(11), 2541–2543. <https://doi.org/10.1007/s00125-013-3043-5>

Cardwell, C. R., Stene, L. C., Ludvigsson, J., Rosenbauer, J., Cinek, O., Svensson, J., Perez-Bravo, F., Memon, A., Gimeno, S. G., Wadsworth, E. J. K., Strotmeyer, E. S., Goldacre, M. J., Radon, K., Chuang, L.-M., Parslow, R. C., Chetwynd, A., Karavanaki, K., Brigis, G., Pozzilli, P., … Patterson, C. C. (2012). Breast-feeding and childhood-onset type 1 diabetes: A pooled analysis of individual participant data from 43 observational studies. *Diabetes Care*, *35*(11), 2215–2225. <https://doi.org/10.2337/dc12-0438>

Carry, P. M., Vanderlinden, L. A., Johnson, R. K., Dong, F., Steck, A. K., Frohnert, B. I., Rewers, M., Yang, I. V., Kechris, K., & Norris, J. M. (2020). DNA methylation near the INS gene is associated with INS genetic variation (rs689) and type 1 diabetes in the Diabetes Autoimmunity Study in the Young. *Pediatric Diabetes*, *21*(4), 597–605. <https://doi.org/10.1111/pedi.12995>

Caza, T., & Landas, S. (2015). Functional and phenotypic plasticity of CD4(+) T cell subsets. *BioMed Research International*, *2015*, 521957. <https://doi.org/10.1155/2015/521957>

Cerosaletti, K., & Buckner, J. H. (2012). Protein tyrosine phosphatases and type 1 diabetes: Genetic and functional implications of PTPN2 and PTPN22. *The Review of Diabetic Studies*, *9*(4), 188–200. <https://doi.org/10.1900/RDS.2012.9.188>

Chemin, K., Ramsköld, D., Diaz-Gallo, L., Herrath, J., Houtman, M., Tandre, K., Rönnblom, L., Catrina, A., & Malmström, V. (2018). EOMES-positive CD4+ T cells are increased in PTPN22 (1858T) risk allele carriers. *European Journal of Immunology*, *48*(4), 655–669. <https://doi.org/10.1002/eji.201747296>

Chen, Y.-G., Ciecko, A. E., Khaja, S., Grzybowski, M., Geurts, A. M., & Lieberman, S. M. (2020). UBASH3A deficiency accelerates type 1 diabetes development and enhances salivary gland inflammation in NOD mice. *Scientific Reports*, *10*(1), 12019. <https://doi.org/10.1038/s41598-020-68956-6>

Chimienti, F., Devergnas, S., Pattou, F., Schuit, F., Garcia-Cuenca, R., Vandewalle, B., Kerr-Conte, J., Van Lommel, L., Grunwald, D., Favier, A., & Seve, M. (2006). In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. *Journal of Cell Science*, *119*(20), 4199–4206. <https://doi.org/10.1242/jcs.03164>

Chiou, J., Geusz, R.J., Okino, M. L., Han, J. Y., Miller, M., Melton, R., Beebe, E., Benaglio, P., Huang, S., Korgaonkar, K., Heller, S., Kleger, A., Preissl, S., Gorkin, D. U., Sander, M. & Gaulton, K. J. (2021). Interpreting type 1 diabetes risk with genetics and single-cell epigenomics. *Nature, 594*, 398–402 <https://doi.org/10.1038/s41586-021-03552-w>

Chu, X., Janssen, A. W. M., Koenen, H., Chang, L., He, X., Joosten, I., Stienstra, R., Kuijpers, Y., Wijmenga, C., Xu, C.-J., Netea, M. G., Tack, C. J. & Li, Y. (2022). A genome-wide functional genomics approach uncovers genetic determinants of immune phenotypes in type 1 diabetes, *eLife, 11*, e73709. <https://doi.org/10.7554/eLife.73709>

Chung, H., Pamp, S. J., Hill, J. A., Surana, N. K., Edelman, S. M., Troy, E. B., Reading, N. C., Villablanca, E. J., Wang, S., Mora, J. R., Umesaki, Y., Mathis, D., Benoist, C., Relman, D. A., & Kasper, D. L. (2012). Gut immune maturation depends on colonization with a host-specific microbiota. *Cell*, *149*(7), 1578–1593. <https://doi.org/10.1016/j.cell.2012.04.037>

Cloutier, J.-F., & Veillette, A. (1996). Association of inhibitory tyrosine protein kinase p50csk with protein tyrosine phosphatase PEP in T cells and other hemopoietic cells. *The EMBO Journal*, *15*(18), 4909–4918.

Cloutier, J.-F., & Veillette, A. (1999). Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase. *Journal of Experimental Medicine*, *189*(1), 111–121. <https://doi.org/10.1084/jem.189.1.111>

Cohen, S., Dadi, H., Shaoul, E., Sharfe, N., & Roifman, C. M. (1999). Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase, Lyp. *Blood*, *93*(6), 2013.

Colman, P. G., McNair, P., Margetts, H., Schmidli, R. S., Werther, G. A., Alford, F. P., Ward, G. M., Tait, B. D., Honeyman, M. C., & Harrison, L. C. (1998). The Melbourne Pre-Diabetes Study: Prediction of type 1 diabetes mellitus using antibody and metabolic testing. *The Medical Journal of Australia*, *169*(2), 81–84. <https://doi.org/10.5694/j.1326-5377.1998.tb140188.x>

Cool, D. E., Tonks, N. K., Charbonneau, H., Walsh, K. A., Fischer, E. H., & Krebs, E. G. (1989). CDNA isolated from a human T-cell library encodes a member of the protein-tyrosine-phosphatase family. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(14), 5257–5261. <https://doi.org/10.1073/pnas.86.14.5257>

Cooper, J. D., Smyth, D. J., Smiles, A. M., Plagnol, V., Walker, N. M., Allen, J. E., Downes, K., Barrett, J. C., Healy, B. C., Mychaleckyj, J. C., Warram, J. H., & Todd, J. A. (2008). Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci. *Nature Genetics*, *40*(12), 1399–1401. <https://doi.org/10.1038/ng.249>

Cooper, J. D., Walker, N. M., Smyth, D. J., Downes, K., Healy, B. C., & Todd, J. A. (2009). Follow-up of 1715 SNPs from the Wellcome Trust Case Control Consortium genome-wide association study in type I diabetes families. *Genes and Immunity*, *10*(S1), S85–S94. <https://doi.org/10.1038/gene.2009.97>

Coppieters, K. T., Dotta, F., Amirian, N., Campbell, P. D., Kay, T. W., Atkinson, M. A., Roep, B. O., & von Herrath, M. G. (2012). Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *Journal of Experimental Medicine*, *209*(1), 51–60. <https://doi.org/10.1084/jem.20111187>

Couper, J. J., Haller, M. J., Ziegler, A., Knip, M., Ludvigsson, J., & Craig, M. E. (2014). Phases of type 1 diabetes in children and adolescents. *Pediatric Diabetes*, *15*(S20), 18–25. <https://doi.org/10.1111/pedi.12188>

Couper, J. J., Steele, C., Beresford, S., Powell, T., McCaul, K., Pollard, A., Gellert, S., Tait, B., Harrison, L. C., & Colman, P. G. (1999). Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. *Diabetes*, *48*(11), 2145–2149. <https://doi.org/10.2337/diabetes.48.11.2145>

Cox, L. M., Yamanishi, S., Sohn, J., Alekseyenko, A. V., Leung, J. M., Cho, I., Kim, S. G., Li, H., Gao, Z., Mahana, D., Zárate Rodriguez, J. G., Rogers, A. B., Robine, N., Loke, P., & Blaser, M. J. (2014). Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell*, *158*(4), 705–721. <https://doi.org/10.1016/j.cell.2014.05.052>

Cucca, F., Lampis, R., Frau, F., Macis, D., Angius, E., Masile, P., Chessa, M., Frongia, P., Silvetti, M., Cao, A., Virgiliis, S. D., & Congia, M. (1995). The distribution of DR4 haplotypes in Sardinia suggests a primary association of type I diabetes with DRB1 and DQB1 loci. *Human Immunology*, *43*(4), 301–308. https://doi.org/10.1016/0198-8859(95)00042-3

Cudworth, A. G., & Woodrow, J. C. (1975). Evidence for HL-A-linked genes in 'juvenile' diabetes mellitus. *The British Medical Journal*, *3*(5976), 133–135. <https://doi.org/10.1136/bmj.3.5976.133>

Dai, X., James, R. G., Habib, T., Singh, S., Jackson, S., Khim, S., Moon, R. T., Liggitt, D., Wolf-Yadlin, A., Buckner, J. H., & Rawlings, D. J. (2013). A disease-associated PTPN22 variant promotes systemic autoimmunity in murine models. *The Journal of Clinical Investigation*, *123*(5), 2024–2036. <https://doi.org/10.1172/JCI66963>

de Goffau, M. C., Luopajärvi, K., Knip, M., Ilonen, J., Ruohtula, T., Härkönen, T., Orivuori, L., Hakala, S., Welling, G. W., Harmsen, H. J., & Vaarala, O. (2013). Fecal microbiota composition differs between children with β-cell autoimmunity and those without. *Diabetes*, *62*(4), 1238–1244. <https://doi.org/10.2337/db12-0526>

De Grijse, J., Asanghanwa, M., Nouthe, B., Albrecher, N., Goubert, P., Vermeulen, I., Van Der Meeren, S., Decochez, K., Weets, I., Keymeulen, B., Lampasona, V., Wenzlau, J., Hutton, J. C., Pipeleers, D., Gorus, F. K., & the Belgian Diabetes Registry. (2010). Predictive power of screening for antibodies against insulinoma-associated protein 2 beta (IA-2β) and zinc transporter-8 to select first-degree relatives of type 1 diabetic patients with risk of rapid progression to clinical onset of the disease: Implications for prevention trials. *Diabetologia*, *53*(3), 517–524. <https://doi.org/10.1007/s00125-009-1618-y>

de Jong, V. M., Zaldumbide, A., van der Slik, A. R., Laban, S., Koeleman, B. P. C., & Roep, B. O. (2016). Variation in the CTLA4 3′UTR has phenotypic consequences for autoreactive T cells and associates with genetic risk for type 1 diabetes. *Genes & Immunity*, *17*(1), 75–78. <https://doi.org/10.1038/gene.2015.51>

Dedrick, S., Sundaresh, B., Huang, Q., Brady, C., Yoo, T., Cronin, C., Rudnicki, C., Flood, M., Momeni, B., Ludvigsson, J., & Altindis, E. (2020). The role of gut microbiota and environmental factors in type 1 diabetes pathogenesis. *Frontiers in Endocrinology*, *11*. <https://doi.org/10.3389/fendo.2020.00078>

Dotta, F., Censini, S., van Halteren, A. G. S., Marselli, L., Masini, M., Dionisi, S., Mosca, F., Boggi, U., Muda, A. O., Prato, S. D., Elliott, J. F., Covacci, A., Rappuoli, R., Roep, B. O., & Marchetti, P. (2007). Coxsackie B4 virus infection of β cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(12), 5115–5120. https://doi.org/10.1073/pnas.0700442104

Edelman, S. M., & Kasper, D. L. (2008). Symbiotic commensal bacteria direct maturation of the host immune system. *Current Opinion in Gastroenterology*, *24*(6), 720–724. <https://doi.org/10.1097/MOG.0b013e32830c4355>

Eisenbarth, G. S. (1986). Type I Diabetes Mellitus. *New England Journal of Medicine*, *314*(21), 1360–1368. <https://doi.org/10.1056/NEJM198605223142106>

Ekman, I., Ihantola, E.-L., Viisanen, T., Rao, D. A., Näntö-Salonen, K., Knip, M., Veijola, R., Toppari, J., Ilonen, J., & Kinnunen, T. (2019). Circulating CXCR5−PD-1hi peripheral T helper cells are associated with progression to type 1 diabetes. *Diabetologia*, *62*, 1681–1688. <https://doi.org/10.1007/s00125-019-4936-8>

Ekman, I., Vuorinen, T., Knip, M., Veijola, R., Toppari, J., Hyöty, H., Kinnunen, T., Ilonen, J., & Lempainen, J. (2019). Early childhood CMV infection may decelerate the progression to clinical type 1 diabetes. *Pediatric Diabetes*, *20*(1), 73–77. <https://doi.org/10.1111/pedi.12788>

Elliott, R. B., Harris, D. P., Hill, J. P., Bibby, N. J., & Wasmuth, H. E. (1999). Type I (insulin-dependent) diabetes mellitus and cow milk: Casein variant consumption. *Diabetologia*, *42*(3), 292–296.< https://doi.org/10.1007/s001250051153>

Elo, L. L., Mykkänen, J., Nikula, T., Järvenpää, H., Simell, S., Aittokallio, T., Hyöty, H., Ilonen, J., Veijola, R., Simell, T., Knip, M., Simell, O., & Lahesmaa, R. (2010). Early suppression of immune

response pathways characterizes children with prediabetes in genome-wide gene expression profiling. *Journal of Autoimmunity*, *35*(1), 70–76. <https://doi.org/10.1016/j.jaut.2010.03.001>

ElTanbouly, M. A., & Noelle, R. J. (2021). Rethinking peripheral T cell tolerance: Checkpoints across a T cell's journey. *Nature Reviews Immunology*, *21*(4), 257–267. <https://doi.org/10.1038/s41577-020-00454-2>

Endesfelder, D., Hagen, M., Winkler, C., Haupt, F., Zillmer, S., Knopff, A., Bonifacio, E., Ziegler, A.-G., zu Castell, W., & Achenbach, P. (2016). A novel approach for the analysis of longitudinal profiles reveals delayed progression to type 1 diabetes in a subgroup of multiple-islet-autoantibody-positive children. *Diabetologia*, *59*(10), 2172–2180. <https://doi.org/10.1007/s00125-016-4050-0>

Endesfelder, D., zu Castell, W., Bonifacio, E., Rewers, M., Hagopian, W. A., She, J., Lernmark, Å., Toppari, J., Vehik, K., Williams, A. J. K., Yu, L., Akolkar, B., Krischer, J. P., Ziegler, A.-G., Achenbach, P., & TEDDY Study Group. (2018). Time-resolved autoantibody profiling facilitates stratification of preclinical type 1 diabetes in children. *Diabetes*, *68*(1), 119–130. <https://doi.org/10.2337/db18-0594>

Erlich, H. A., Zeidler, A., Chang, J., Shaw, S., Raffel, L. J., Klitz, W., Beshkov, Y., Costin, G., Pressman, S., Bugawan, T., & Rotter, J. I. (1993). HLA class II alleles and susceptibility and resistance to insulin dependent diabetes mellitus in Mexican-American families. *Nature Genetics*, *3*(4), 358–364. <https://doi.org/10.1038/ng0493-358>

Erlich, H., Valdes, A. M., Noble, J., Carlson, J. A., Varney, M., Concannon, P., Mychaleckyj, J. C., Todd, J. A., Bonella, P., Fear, A. L., Lavant, E., Louey, A., & Moonsamy, P. (2008). HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: Analysis of the type 1 diabetes genetics consortium families. *Diabetes*, *57*(4), 1084–1092. <https://doi.org/10.2337/db07-1331>

Espino-Paisan, L., de la Calle, H., Fernández-Arquero, M., Figueredo, M. Á., de la Concha, E. G., Urcelay, E., & Santiago, J. L. (2011). A polymorphism in PTPN2 gene is associated with an earlier onset of type 1 diabetes. *Immunogenetics*, *63*(4), 255–258. <https://doi.org/10.1007/s00251-010-0500-x>

Espinosa-Carrasco, G., Le Saout, C., Fontanaud, P., Stratmann, T., Mollard, P., Schaeffer, M., & Hernandez, J. (2018). CD4(+) T helper cells play a key role in maintaining diabetogenic CD8(+) T cell function in the pancreas. *Frontiers in Immunology*, *8*, 2001. <https://doi.org/10.3389/fimmu.2017.02001>

Ezer, S., Yoshihara, M., Katayama, S., Daub, C., Lohi, H., Krjutskov, K., & Kere, J. (2021). Generation of RNA sequencing libraries for transcriptome analysis of globin-rich tissues of the domestic dog. *STAR Protocols*, *2*(4), 100995. <https://doi.org/10.1016/j.xpro.2021.100995>

Ferrat, L. A., Vehik, K., Sharp, S. A., Lernmark, Å., Rewers, M. J., She, J.-X., Ziegler, A.-G., Toppari, J., Akolkar, B., Krischer, J. P., Weedon, M. N., Oram, R. A., & Hagopian, W. A. (2020). A combined risk score enhances prediction of type 1 diabetes among susceptible children. *Nature Medicine*, *26*(8), 1247–1255. <https://doi.org/10.1038/s41591-020-0930-4>

Ferreira, R. C., Castro Dopico, X., Oliveira, J. J., Rainbow, D. B., Yang, J. H., Trzupek, D., Todd, S. A., McNeill, M., Steri, M., Orrù, V., Fiorillo, E., Crouch, D. J. M., Pekalski, M. L., Cucca, F., Tree, T. I., Vyse, T. J., Wicker, L. S., & Todd, J. A. (2019). Chronic immune activation in systemic lupus erythematosus and the autoimmune PTPN22 Trp620 risk allele drive the expansion of FOXP3+ regulatory T cells and PD-1 expression. *Frontiers in Immunology*, *10*, 2606. <https://doi.org/10.3389/fimmu.2019.02606>

Ferreira, R. C., Guo, H., Coulson, R. M. R., Smyth, D. J., Pekalski, M. L., Burren, O. S., Cutler, A. J., Doecke, J. D., Flint, S., McKinney, E. F., Lyons, P. A., Smith, K. G. C., Achenbach, P., Beyerlein, A., Dunger, D. B., Clayton, D. G., Wicker, L. S., Todd, J. A., Bonifacio, E., … Ziegler, A.-G. (2014). A type I interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes. *Diabetes*, *63*(7), 2538–2550. <https://doi.org/10.2337/db13-1777>

Ferreira, R. C., Simons, H. Z., Thompson, W. S., Cutler, A. J., Dopico, X. C., Smyth, D. J., Mashar, M., Schuilenburg, H., Walker, N. M., Dunger, D. B., Wallace, C., Todd, J. A., Wicker, L. S., & Pekalski, M. L. (2015). IL-21 production by CD4+ effector T cells and frequency of circulating

follicular helper T cells are increased in type 1 diabetes patients. *Diabetologia*, *58*(4), 781–790. <https://doi.org/10.1007/s00125-015-3509-8>

Fichna, M., Żurawek, M., Bogusz-Górna, K., Małecki, P. P., Niechciał, E., Sidoruk, A., Furman, K., & Fichna, P. (2020). STAT4 sequence variant and elevated gene expression are associated with type 1 diabetes in Polish children. *Central-European Journal of Immunology*, *45*(1), 22–28. PubMed. <https://doi.org/10.5114/ceji.2019.92492>

Fornari, T. A., Donate, P. B., Macedo, C., Marques, M. M. C., Magalhães, D. A., & Passos, G. A. S. (2010). Age-related deregulation of Aire and peripheral tissue antigen genes in the thymic stroma of non-obese diabetic (NOD) mice is associated with autoimmune type 1 diabetes mellitus (DM-1). *Molecular and Cellular Biochemistry*, *342*(1), 21–28. <https://doi.org/10.1007/s11010-010-0464-z>

Foulis, A. K., Farquharson, M. A., Cameron, S. O., McGill, M., Schönke, H., & Kandolf, R. (1990). A search for the presence of the enteroviral capsid protein VP1 in pancreases of patients with Type 1 (insulin-dependent) diabetes and pancreases and hearts of infants who died of coxsackieviral myocarditis. *Diabetologia*, *33*(5), 290–298. <https://doi.org/10.1007/BF00403323>

Foulis, A. K., Farquharson, M. A., & Hardman, R. (1987). Aberrant expression of Class II major histocompatibility complex molecules by B cells and hyperexpression of Class I major histocompatibility complex molecules by insulin containing islets in Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*, *30*(5), 333–343. <https://doi.org/10.1007/BF00299027>

Foulis, A. K., Liddle, C. N., Farquharson, M. A., Richmond, J. A., & Weir, R. S. (1986). The histopathology of the pancreas in Type I (insulin-dependent) diabetes mellitus: A 25-year review of deaths in patients under 20 years of age in the United Kingdom. *Diabetologia*, *29*(5), 267–274. <https://doi.org/10.1007/BF00452061>

Fousteri, G., Jofra, T., Debernardis, I., Stanford, S. M., Laurenzi, A., Bottini, N., & Battaglia, M. (2014). The protein tyrosine phosphatase PTPN22 controls forkhead box protein 3 T regulatory cell induction but is dispensable for T helper type 1 cell polarization. *Clinical and Experimental Immunology*, *178*(1), 178–189. <https://doi.org/10.1111/cei.12393>

Fradin, D., Fur, S. L., Mille, C., Naoui, N., Groves, C., Zelenika, D., McCarthy, M. I., Lathrop, M., & Bougnères, P. (2012). Association of the CpG methylation pattern of the proximal insulin gene promoter with type 1 diabetes. *PLOS ONE*, *7*(5), e36278. <https://doi.org/10.1371/journal.pone.0036278>

Frohnert, B. I., Laimighofer, M., Krumsiek, J., Theis, F. J., Winkler, C., Norris, J. M., Ziegler, A.-G., Rewers, M. J., & Steck, A. K. (2018). Prediction of type 1 diabetes using a genetic risk model in the Diabetes Autoimmunity Study in the Young. *Pediatric Diabetes*, *19*(2), 277–283. <https://doi.org/10.1111/pedi.12543>

Fulcher, D. A., & Basten, A. (1994). Reduced life span of anergic self-reactive B cells in a double-transgenic model. *Journal of Experimental Medicine*, *179*(1), 125–134. <https://doi.org/10.1084/jem.179.1.125>

Furtado, G. C., Curotto de Lafaille, M.A., Kutchukhidze, N. & Lafaille, J.J. (2002). Interleukin 2 signaling is required for CD4(+) regulatory T cell function. *Journal of Experimental Medicine, 196*(6), 851-857. <https://doi.org/10.1084/jem.20020190>

Gauld, S. B., Benschop, R. J., Merrell, K. T., & Cambier, J. C. (2005). Maintenance of B cell anergy requires constant antigen receptor occupancy and signaling. *Nature Immunology*, *6*(11), 1160–1167. <https://doi.org/10.1038/ni1256>

Ge, Y., Paisie, T. K., Chen, S., & Concannon, P. (2019). UBASH3A regulates the synthesis and dynamics of TCR–CD3 complexes. *The Journal of Immunology*, *203*(11), 2827–2836. <https://doi.org/10.4049/jimmunol.1801338>

Ge, Y., Paisie, T. K., Newman, J. R. B., McIntyre, L. M., & Concannon, P. (2017). UBASH3A mediates risk for type 1 diabetes through inhibition of T-cell receptor–induced NF-κB signaling. *Diabetes*, *66*(7), 2033–2043. <https://doi.org/10.2337/db16-1023>

Gepts, W. (1965). Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes*, *14*(10), 619–633. <https://doi.org/10.2337/diab.14.10.619>

Gepts, W., & De Mey, J. (1978). Islet cell survival determined by morphology an immunocytochemical study of the islets of langerhans in juvenile diabetes mellitus. *Diabetes*, *27*(Supplement_1), 251–261. <https://doi.org/10.2337/diab.27.1.S251>

Getahun, A. (2022). Role of inhibitory signaling in peripheral B cell tolerance. *Immunological Reviews*, *307*(1), 27–42. <https://doi.org/10.1111/imr.13070>

Geuking, M. B., Cahenzli, J., Lawson, M. A. E., Ng, D. C. K., Slack, E., Hapfelmeier, S., McCoy, K. D., & Macpherson, A. J. (2011). Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity*, *34*(5), 794–806. <https://doi.org/10.1016/j.immuni.2011.03.021>

Gianani, R., Campbell-Thompson, M., Sarkar, S. A., Wasserfall, C., Pugliese, A., Solis, J. M., Kent, S. C., Hering, B. J., West, E., Steck, A., Bonner-Weir, S., Atkinson, M. A., Coppieters, K., von Herrath, M., & Eisenbarth, G. S. (2010). Dimorphic histopathology of long-standing childhood-onset diabetes. *Diabetologia*, *53*(4), 690–698. <https://doi.org/10.1007/s00125-009-1642-y>

Giannopoulou, E. Z., Winkler, C., Chmiel, R., Matzke, C., Scholz, M., Beyerlein, A., Achenbach, P., Bonifacio, E., & Ziegler, A.-G. (2015). Islet autoantibody phenotypes and incidence in children at increased risk for type 1 diabetes. *Diabetologia*, *58*(10), 2317–2323. <https://doi.org/10.1007/s00125-015-3672-y>

Glisic-Milosavljevic, S., Wang, T., Koppen, M., Kramer, J., Ehlenbach, S., Waukau, J., Jailwala, P., Jana, S., Alemzadeh, R., & Ghosh, S. (2007). Dynamic changes in CD4+ CD25+ high T cell apoptosis after the diagnosis of type 1 diabetes. *Clinical and Experimental Immunology*, *150*(1), 75–82. <https://doi.org/10.1111/j.1365-2249.2007.03475.x>

Goffard, A., Alidjinou, E. K., Sané, F., Choteau, L., Bouquillon, C., Caloone, D., Lobert, P. E., & Hober, D. (2013). Antibodies enhance the infection of phorbol-ester-differentiated human monocyte-like cells with coxsackievirus B4. *Microbes and Infection*, *15*(1), 18–27. <https://doi.org/10.1016/j.micinf.2012.10.005>

Gomaa, E. Z. (2020). Human gut microbiota/microbiome in health and diseases: A review. *Antonie van Leeuwenhoek*, *113*(12), 2019–2040. <https://doi.org/10.1007/s10482-020-01474-7>

Gomes, K. F. B., Santos, A. S., Semzezem, C., Correia, M. R., Brito, L. A., Ruiz, M. O., Fukui, R. T., Matioli, S. R., Passos-bueno, M. R., & Da Silva, M. E. R. (2017). The influence of population stratification on genetic markers associated with type 1 diabetes. *Scientific Reports*, *7*, 43513. <https://doi.org/10.1038/srep43513>

Graham, J., Hagopian, W. A., Kockum, I., Li, L. S., Sanjeevi, C. B., Lowe, R. M., Schaefer, J. B., Zarghami, M., Day, H. L., Landin-Olsson, M., Palmer, J. P., Janer-Villanueva, M., Hood, L., Sundkvist, G., Lernmark, Å., Breslow, N., Dahlquist, G., for the Swedish Childhood Diabetes Study Group, & Blohmé, G. (2002). Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. *Diabetes*, *51*(5), 1346–1355. <https://doi.org/10.2337/diabetes.51.5.1346>

Grant, S. F. A., Qu, H.-Q., Bradfield, J. P., Marchand, L., Kim, C. E., Glessner, J. T., Grabs, R., Taback, S. P., Frackelton, E. C., Eckert, A. W., Annaiah, K., Lawson, M. L., Otieno, F. G., Santa, E., Shaner, J. L., Smith, R. M., Skraban, R., Imielinski, M., Chiavacci, R. M., … Hakonarson, H. (2009). Follow-up analysis of genome-wide association data identifies novel loci for type 1 diabetes. *Diabetes*, *58*(1), 290–295. <https://doi.org/10.2337/db08-1022>

Grover, P., Goel, P. N., & Greene, M. I. (2021). Regulatory T cells: Regulation of identity and function. *Frontiers in Immunology*, *12*. 750542. <https://doi.org/10.3389/fimmu.2021.750542>

Hakonarson, H., Qu, H.-Q., Bradfield, J. P., Marchand, L., Kim, C. E., Glessner, J. T., Grabs, R., Casalunovo, T., Taback, S. P., Frackelton, E. C., Eckert, A. W., Annaiah, K., Lawson, M. L., Otieno, F. G., Santa, E., Shaner, J. L., Smith, R. M., Onyiah, C. C., Skraban, R., … Polychronakos, C. (2008). A novel susceptibility locus for type 1 diabetes on Chr12q13 identified by a genome-wide association study. *Diabetes*, *57*(4), 1143–1146. <https://doi.org/10.2337/db07-1305>

Haller, M. J., Atkinson, M. A., & Schatz, D. (2005). Type 1 diabetes mellitus: Etiology, presentation, and management. *Pediatric Clinics of North America*, *52*(6), 1553–1578. <https://doi.org/10.1016/j.pcl.2005.07.006>

Hanna, S. J., Powell, W. E., Long, A. E., Robinson, E. J. S., Davies, J., Megson, C., Howell, A., Jones, T. J., Ladell, K., Price, D. A., Dayan, C. M., Williams, A. J. K., Gillespie, K. M., & Wong, F. S. (2020). Slow progressors to type 1 diabetes lose islet autoantibodies over time, have few islet antigen-specific CD8+ T cells and exhibit a distinct CD95hi B cell phenotype. *Diabetologia*, *63*(6), 1174–1185. <https://doi.org/10.1007/s00125-020-05114-7>

Hannet, I., Erkeller-Yuksel, F., Lydyard, P., Deneys, V., & DeBruyère, M. (1992). Developmental and maturational changes in human blood lymphocyte subpopulations. *Immunology Today*, *13*(6), 215–218. <https://doi.org/10.1016/0167-5699(92)90157-3>

Hänninen, A., Jalkanen, S., Salmi, M., Toikkanen, S., Nikolakaros, G., & Simell, O. (1992). Macrophages, T cell receptor usage, and endothelial cell activation in the pancreas at the onset of insulin-dependent diabetes mellitus. *The Journal of Clinical Investigation*, *90*(5), 1901–1910. <https://doi.org/10.1172/JCI116067>

Hapfelmeier, S., Lawson, M. A. E., Slack, E., Kirundi, J. K., Stoel, M., Heikenwalder, M., Cahenzli, J., Velykoredko, Y., Balmer, M. L., Endt, K., Geuking, M. B., Curtiss, R., McCoy, K. D., & Macpherson, A. J. (2010). Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science (New York, N.Y.)*, *328*(5986), 1705–1709. <https://doi.org/10.1126/science.1188454>

Harjutsalo, V., Sund, R., Knip, M., & Groop, P. (2013). Incidence of type 1 diabetes in Finland. *Journal of the American Medical Association*, *310*(4), 427–428. <https://doi.org/10.1001/jama.2013.8399>

Hasegawa, K., Martin, F., Huang, G., Tumas, D., Diehl, L., & Chan, A. C. (2004). PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science*, *303*(5658), 685–689. <https://doi.org/10.1126/science.1092138>

Hekkala, A., Reunanen, A., Koski, M., Knip, M., & Veijola, R. (2010). Age-related differences in the frequency of ketoacidosis at diagnosis of type 1 diabetes in children and adolescents. *Diabetes Care*, *33*(7), 1500–1502. <https://doi.org/10.2337/dc09-2344>

Heninger, A.-K., Eugster, A., Kuehn, D., Buettner, F., Kuhn, M., Lindner, A., Dietz, S., Jergens, S., Wilhelm, C., Beyerlein, A., Ziegler, A.-G., & Bonifacio, E. (2017). A divergent population of autoantigen-responsive CD4+ T cells in infants prior to β cell autoimmunity. *Science Translational Medicine*, *9*(378), eaaf8848. <https://doi.org/10.1126/scitranslmed.aaf8848>

Hermann, R., Laine, A. P., Veijola, R., Vahlberg, T., Simell, S., Lähde, J., Simell, O., Knip, M., & Ilonen, J. (2005). The effect of HLA class II, insulin and CTLA4 gene regions on the development of humoral beta cell autoimmunity. *Diabetologia*, *48*(9), 1766–1775. <https://doi.org/10.1007/s00125-005-1844-x>

Hermann, R., Turpeinen, H., Laine, A. P., Veijola, R., Knip, M., Simell, O., Sipilä, I., Åkerblom, H. K., & Ilonen, J. (2003). HLA DR-DQ-encoded genetic determinants of childhood-onset type 1 diabetes in Finland: An analysis of 622 nuclear families. *Tissue Antigens*, *62*(2), 162–169. <https://doi.org/10.1034/j.1399-0039.2003.00071.x>

Hiltunen, M., Hyöty, H., Karjalainen, J., Leinikki, P., Knip, M., Lounamaa, R., åkerblom, H. K., & The Childhood Diabetes in Finland Study Group. (1995). Serological evaluation of the role of cytomegalovirus in the pathogenesis of IDDM: A prospective study. *Diabetologia*, *38*(6), 705–710. <https://doi.org/10.1007/BF00401843>

Hinks, A., Ke, X., Barton, A., Eyre, S., Bowes, J., Worthington, J., Thompson, S. D., Langefeld, C. D., Glass, D. N., & Thomson, W. (2009). Association of the IL2RA/CD25 gene with juvenile idiopathic arthritis. *Arthritis and Rheumatism*, *60*(1), 251–257. <https://doi.org/10.1002/art.24187>

Holz, A., Bot, A., Coon, B., Wolfe, T., Grusby, M. J., & Herrath, M. G. von. (1999). Disruption of the STAT4 signaling pathway protects from autoimmune diabetes while retaining antiviral immune competence. *The Journal of Immunology*, *163*(10), 5374–5382.

Honkanen, H., Oikarinen, S., Nurminen, N., Laitinen, O. H., Huhtala, H., Lehtonen, J., Ruokoranta, T., Hankaniemi, M. M., Lecouturier, V., Almond, J. W., Tauriainen, S., Simell, O., Ilonen, J., Veijola, R., Viskari, H., Knip, M., & Hyöty, H. (2017). Detection of enteroviruses in stools precedes islet autoimmunity by several months: Possible evidence for slowly operating mechanisms in virus-

induced autoimmunity. *Diabetologia*, *60*(3), 424–431. <https://doi.org/10.1007/s00125-016-4177-z>

Howson, J. M. M., Rosinger, S., Smyth, D. J., Boehm, B. O., the ADBW-END Study Group, & Todd, J. A. (2011). Genetic analysis of adult-onset autoimmune diabetes. *Diabetes*, *60*(10), 2645–2653. <https://doi.org/10.2337/db11-0364>

Howson, J. M. M., Walker, N. M., Clayton, D., Todd, J. A., & Consortium, D. G. (2009). Confirmation of HLA class II independent type 1 diabetes associations in the major histocompatibility complex including HLA-B and HLA-A. *Diabetes, Obesity and Metabolism*, *11*(s1), 31–45. <https://doi.org/10.1111/j.1463-1326.2008.01001.x>

Howson, J. M. M., Walker, N. M., Smyth, D. J., & Todd, J. A. (2009). Analysis of 19 genes for association with type I diabetes in the Type I Diabetes Genetics Consortium families. *Genes and Immunity*, *10*(S1), S74–S84. <https://doi.org/10.1038/gene.2009.96>

Hua, Z., & Hou, B. (2020). The role of B cell antigen presentation in the initiation of CD4+ T cell response. *Immunological Reviews*, *296*(1), 24–35. <https://doi.org/10.1111/imr.12859>

Huang, X., Yuang, J., Goddard, A., Foulis, A., James, R. F. L., Lernmark, Å., Pujol-Borrell, R., Rabinovitch, A., Somoza, N., & Stewart, T. A. (1995). Interferon expression in the pancreases of patients with type I diabetes. *Diabetes*, *44*(6), 658–664. <https://doi.org/10.2337/diab.44.6.658>

Hui-Qi Qu, Alexander Montpetit, Bing Ge, Thomas J. Hudson, & Constantin Polychronakos. (2007). Toward further mapping of the association between the IL2RA locus and type 1 diabetes. *Diabetes*, *56*(4), 1174–1176. <https://doi.org/10.2337/db06-1555>

Hurley, C. K. (2021). Naming HLA diversity: A review of HLA nomenclature. *Human Immunology*, *82*(7), 457–465. <https://doi.org/10.1016/j.humimm.2020.03.005>

Hyöty, H., Leon, F., & Knip, M. (2018). Developing a vaccine for type 1 diabetes by targeting coxsackievirus B. *Expert Review of Vaccines*, *17*(12), 1071–1083. <https://doi.org/10.1080/14760584.2018.1548281>

Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., & Iwasaki, A. (2011). Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(13), 5354–5359. <https://doi.org/10.1073/pnas.1019378108>

Ihantola, E.-L., Viisanen, T., Gazali, A. M., Näntö-Salonen, K., Juutilainen, A., Moilanen, L., Rintamäki, R., Pihlajamäki, J., Veijola, R., Toppari, J., Knip, M., Ilonen, J., & Kinnunen, T. (2018). Effector t cell resistance to suppression and STAT3 signaling during the development of human type 1 diabetes. *The Journal of Immunology*, *Journal Article*. <https://doi.org/10.4049/jimmunol.1701199>

Ilonen, J., Hammais, A., Laine, A.-P., Lempainen, J., Vaarala, O., Veijola, R., Simell, O., & Knip, M. (2013). Patterns of β-cell autoantibody appearance and genetic associations during the first years of life. *Diabetes*, *62*(10), 3636–3640. <https://doi.org/10.2337/db13-0300>

Ilonen, J., Kiviniemi, M., Lempainen, J., Simell, O., Toppari, J., Veijola, R., & Knip, M. (2016). Genetic susceptibility to type 1 diabetes in childhood – estimation of HLA class II associated disease risk and class II effect in various phases of islet autoimmunity. *Pediatric Diabetes*, *17*, 8–16. <https://doi.org/10.1111/pedi.12327>

Ilonen, J., Laine, A.-P., Kiviniemi, M., Härkönen, T., Lempainen, J., Knip, M., & the Finnish Pediatric Diabetes Register. (2022). Associations between deduced first islet specific autoantibody with sex, age at diagnosis and genetic risk factors in young children with type 1 diabetes. *Pediatric Diabetes*, *23*(6), 693–702. <https://doi.org/10.1111/pedi.13340>

Ilonen, J., Lempainen, J., Hammais, A., Laine, A.-P., Härkönen, T., Toppari, J., Veijola, R., Knip, M., & the Finnish Pediatric Diabetes Register. (2018). Primary islet autoantibody at initial seroconversion and autoantibodies at diagnosis of type 1 diabetes as markers of disease heterogeneity. *Pediatric Diabetes*, *19*(2), 284–292. <https://doi.org/10.1111/pedi.12545>

Ilonen, J., Lempainen, J., & Veijola, R. (2019). The heterogeneous pathogenesis of type 1 diabetes mellitus. *Nature Reviews Endocrinology*, *15*(11), 635–650. <https://doi.org/10.1038/s41574-019-0254-y>

Imagawa, A., Hanafusa, T., Miyagawa, J., & Matsuzawa, Y. (2000). A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. *New England Journal of Medicine*, *342*(5), 301–307. <https://doi.org/10.1056/NEJM200002033420501>

Imagawa, A., Hanafusa, T., Tamura, S., Moriwaki, M., Itoh, N., Yamamoto, K., Iwahashi, H., Yamagata, K., Waguri, M., Nanmo, T., Uno, S., Nakajima, H., Namba, M., Kawata, S., Miyagawa, J., & Matsuzawa, Y. (2001). Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type 1 diabetes: Close correlation between serological markers and histological evidence of cellular autoimmunity. *Diabetes*, *50*(6), 1269–1273. <https://doi.org/10.2337/diabetes.50.6.1269>

Infante, M., Ricordi, C., Sanchez, J., Clare-Salzler, M. J., Padilla, N., Fuenmayor, V., Chavez, C., Alvarez, A., Baidal, D., Alejandro, R., Caprio, M., & Fabbri, A. (2019). Influence of vitamin D on islet autoimmunity and beta-cell function in type 1 diabetes. *Nutrients*, *11*(9), 2185. <https://doi.org/10.3390/nu11092185>

In't Veld, P. (2011). Insulitis in human type 1 diabetes. *Islets*, *3*(4), 131–138. <https://doi.org/10.4161/isl.3.4.15728>

In't Veld, P. (2014). Insulitis in human type 1 diabetes: A comparison between patients and animal models. *Seminars in Immunopathology*, *36*(5), 569–579. <https://doi.org/10.1007/s00281-014-0438-4>

In't Veld, P., Lievens, D., De Grijse, J., Ling, Z., Van der Auwera, B., Pipeleers-Marichal, M., Gorus, F., & Pipeleers, D. (2007). Screening for insulitis in adult autoantibody-positive organ donors. *Diabetes*, *56*(9), 2400–2404. <https://doi.org/10.2337/db07-0416>

Irvine, K. M., Gallego, P., An, X., Best, S. E., Thomas, G., Wells, C., Harris, M., Cotterill, A., & Thomas, R. (2012). Peripheral blood monocyte gene expression profile clinically stratifies patients with recent-onset type 1 diabetes. *Diabetes*, *61*(5), 1281–1290. <https://doi.org/10.2337/db11-1549>

Islam, S., Kjällquist, U., Moliner, A., Zajac, P., Fan, J.-B., Lönnerberg, P., & Linnarsson, S. (2012). Highly multiplexed and strand-specific single-cell RNA 5′ end sequencing. *Nature Protocols*, *7*(5), 813–828. <https://doi.org/10.1038/nprot.2012.022>

Itoh, N., Hanafusa, T., Miyazaki, A., Miyagawa, J., Yamagata, K., Yamamoto, K., Waguri, M., Imagawa, A., Tamura, S., & Inada, M. (1993). Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. *Journal of Clinical Investigation*, *92*(5), 2313–2322.

Jacobsen, L. M., Bocchino, L., Evans-Molina, C., DiMeglio, L., Goland, R., Wilson, D. M., Atkinson, M. A., Aye, T., Russell, W. E., Wentworth, J. M., Boulware, D., Geyer, S., & Sosenko, J. M. (2020). The risk of progression to type 1 diabetes is highly variable in individuals with multiple autoantibodies following screening. *Diabetologia*, *63*(3), 588–596. <https://doi.org/10.1007/s00125-019-05047-w>

Jakubzick, C. V., Randolph, G. J., & Henson, P. M. (2017). Monocyte differentiation and antigen-presenting functions. *Nature Reviews Immunology*, *17*(6), 349–362. <https://doi.org/10.1038/nri.2017.28>

Johnson, S. B., Lynch, K. F., Roth, R., Lundgren, M., Parikh, H. M., Akolkar, B., Hagopian, W., Krischer, J., Rewers, M., She, J.-X., Toppari, J., Ziegler, A. G., Lernmark, Å., & the TEDDY Study Group. (2021). First-appearing islet autoantibodies for type 1 diabetes in young children: Maternal life events during pregnancy and the child's genetic risk. *Diabetologia*, *64*(3), 591–602. <https://doi.org/10.1007/s00125-020-05344-9>

Kaizer, E. C., Glaser, C. L., Chaussabel, D., Banchereau, J., Pascual, V., & White, P. C. (2007). Gene expression in peripheral blood mononuclear cells from children with diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *92*(9), 3705–3711. <https://doi.org/10.1210/jc.2007-0979>

Kallionpää, H., Elo, L. L., Laajala, E., Mykkänen, J., Ricaño-Ponce, I., Vaarma, M., Laajala, T. D., Hyöty, H., Ilonen, J., Veijola, R., Simell, T., Wijmenga, C., Knip, M., Lähdesmäki, H., Simell, O., & Lahesmaa, R. (2014). Innate immune activity is detected prior to seroconversion in children

with HLA-conferred type 1 diabetes susceptibility. *Diabetes*, *63*(7), 2402–2414. <https://doi.org/10.2337/db13-1775>

Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., Kosmicki, J. A., Walters, R. K., Tashman, K., Farjoun, Y., Banks, E., Poterba, T., Wang, A., Seed, C., Whiffin, N., Chong, J. X., Samocha., K. E., Pierce-Hoffman, E., Zappala, Z., O'Donnell-Luria, A. H., Minikel, E. V., Weisburd, B., Lek, M., Ware, J. S., Vittal, C., Armean, I. M., Bergelson, L., Cibulskis, K., Connolly, K. M., Covarrubias, M., Donnelly, S., Ferriera., S., Gabriel, S., Gentry, J., Gupta, N., Jeandet, T., Kaplan, D., Llanwarne, D., Munshi, R., Novod, S., Petrillo, N., Roazen, D., Ruano-Rubio, V., Saltzman, A., Schleicher, M., Soto, J., Tibbetts, K., Tolonen, C., Wade, G., Talkowski, M. E., Neale, B. M., Daly, M. J. & MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature 581*, 434–443. <https://doi.org/10.1038/s41586-020-2308-7>

Karjalainen, J. K. (1990). Islet cell antibodies as predictive markers for IDDM in children with high background incidence of disease. *Diabetes*, *39*(9), 1144–1150. <https://doi.org/10.2337/diab.39.9.1144>

Kärre, K. (1993). Natural killer cells and the MHC class I pathway of peptide presentation. *Seminars in Immunology*, *5*(2), 127–145. <https://doi.org/10.1006/smim.1993.1016>

Katayama, S., Töhönen, V., Linnarsson, S., & Kere, J. (2013). SAMstrt: Statistical test for differential expression in single-cell transcriptome with spike-in normalization. *Bioinformatics*, *29*(22), 2943–2945. <https://doi.org/10.1093/bioinformatics/btt511>

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B. J., Jacobsen, L. M., Schatz, D. A., & Lernmark, Å. (2017). Type 1 diabetes mellitus. *Nature Reviews Disease Primers*, *3*, 17016. <https://doi.org/10.1038/nrdp.2017.16>

Kaur, S., Mirza, A. H., Brorsson, C. A., Fløyel, T., Størling, J., Mortensen, H. B., & Pociot, F. (2016). The genetic and regulatory architecture of ERBB3-type 1 diabetes susceptibility locus. *Molecular and Cellular Endocrinology*, *419*, 83–91. <https://doi.org/10.1016/j.mce.2015.10.002>

Keene, K. L., Quinlan, A. R., Hou, X., Hall, I. M., Mychaleckyj, J. C., Onengut-Gumuscu, S., & Concannon, P. (2012). Evidence for two independent associations with type 1 diabetes at the 12q13 locus. *Genes & Immunity*, *13*(1), 66–70. <https://doi.org/10.1038/gene.2011.56>

Kemppainen, K. M., Vehik, K., Lynch, K. F., Larsson, H. E., Canepa, R. J., Simell, V., Koletzko, S., Liu, E., Simell, O. G., Toppari, J., Ziegler, A. G., Rewers, M. J., Lernmark, Å., Hagopian, W. A., She, J.-X., Akolkar, B., Schatz, D. A., Atkinson, M. A., Blaser, M. J., … for The Environmental Determinants of Diabetes in the Young (TEDDY) Study Group. (2017). Association between early-life antibiotic use and the risk of islet or celiac disease autoimmunity. *JAMA Pediatrics*, *171*(12), 1217–1225. <https://doi.org/10.1001/jamapediatrics.2017.2905>

Kilkkinen, A., Virtanen, S. M., Klaukka, T., Kenward, M. G., Salkinoja-Salonen, M., Gissler, M., Kaila, M., & Reunanen, A. (2006). Use of antimicrobials and risk of type 1 diabetes in a population-based mother–child cohort. *Diabetologia*, *49*(1), 66–70. <https://doi.org/10.1007/s00125-005-0078-2>

Kiviniemi, M., Hermann, R., Nurmi, J., Ziegler, A. G., Knip, M., Simell, O., Veijola, R., Lövgren, T., & Ilonen, J. (2007). A high-throughput population screening system for the estimation of genetic risk for type 1 diabetes: An application for the TEDDY (The Environmental Determinants of Diabetes in the Young) study. *Diabetes Technology & Therapeutics*, *9*(5), 460–472. <https://doi.org/10.1089/dia.2007.0229>

Klein, L., Kyewski, B., Allen, P. & Hogquist, K. A. (2014). Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nature Reviews Immunology, 14*, 377–391. <https://doi.org/10.1038/nri3667>

Klenerman, P., & Oxenius, A. (2016). T cell responses to cytomegalovirus. *Nature Reviews Immunology*, *16*(6), 367–377. <https://doi.org/10.1038/nri.2016.38>

Knight, R. R., Dolton, G., Kronenberg-Versteeg, D., Eichmann, M., Zhao, M., Huang, G. C., Beck, K., Cole, D. K., Sewell, A. K., Skowera, A., & Peakman, M. (2015). A distinct immunogenic region

of glutamic acid decarboxylase 65 is naturally processed and presented by human islet cells to cytotoxic CD8 T cells. *Clinical and Experimental Immunology*, *179*(1), 100–107. <https://doi.org/10.1111/cei.12436>

Knip, M., Åkerblom, H. K., Becker, D., Dosch, H.-M., Dupre, J., Fraser, W., Howard, N., Ilonen, J., Krischer, J. P., Kordonouri, O., Lawson, M. L., Palmer, J. P., Savilahti, E., Vaarala, O., & Virtanen, S. M. (2014). Hydrolyzed infant formula and early β-cell autoimmunity: A randomized clinical trial. *Journal of the American Medical Association*, *311*(22), 2279–2287. <https://doi.org/10.1001/jama.2014.5610>

Knip, M., Korhonen, S., Kulmala, P., Veijola, R., Reunanen, A., Raitakari, O. T., Viikari, J., & Åkerblom, H. K. (2010). Prediction of type 1 diabetes in the general population. *Diabetes Care*, *33*(6), 1206–1212. <https://doi.org/10.2337/dc09-1040>

Knipper, J. A., Wright, D., Cope, A. P., Malissen, B., & Zamoyska, R. (2020). PTPN22 acts in a cell intrinsic manner to restrict the proliferation and differentiation of T cells following antibody lymphodepletion. *Frontiers in Immunology*, *11*. <https://doi.org/10.3389/fimmu.2020.00052>

Koskinen, M. K., Helminen, O., Matomäki, J., Aspholm, S., Mykkänen, J., Mäkinen, M., Simell, V., Vähä-Mäkilä, M., Simell, T., Ilonen, J., Knip, M., Veijola, R., Toppari, J., & Simell, O. (2016). Reduced β-cell function in early preclinical type 1 diabetes. *European Journal of Endocrinology*, *174*(3), 251–259. <https://doi.org/10.1530/EJE-15-0674>

Koskinen, M. K., Lempainen, J., Löyttyniemi, E., Helminen, O., Hekkala, A., Härkönen, T., Kiviniemi, M., Simell, O., Knip, M., Ilonen, J., Toppari, J., & Veijola, R. (2017). Class II HLA genotype association with first-phase insulin response is explained by islet autoantibodies. *The Journal of Clinical Endocrinology and Metabolism*, *103*(8), 2870–2878. <https://doi.org/10.1210/jc.2017-02040>

Koskinen, M. K., Mikk, M.-L., Laine, A.-P., Lempainen, J., Löyttyniemi, E., Vähäsalo, P., Hekkala, A., Härkönen, T., Kiviniemi, M., Simell, O., Knip, M., Veijola, R., Ilonen, J., & Toppari, J. (2019). Longitudinal pattern of first-phase insulin response is associated with genetic variants outside the class II HLA region in children with multiple autoantibodies. *Diabetes*, *69*(1), 12–19. <https://doi.org/10.2337/db19-0329>

Kostic, A. D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A.-M., Peet, A., Tillmann, V., Pöhö, P., Mattila, I., Lähdesmäki, H., Franzosa, E. A., Vaarala, O., de Goffau, M., Harmsen, H., Ilonen, J., Virtanen, S. M., Clish, C. B., Orešič, M., … Xavier, R. J. (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. <https://doi.org/10.1016/j.chom.2015.01.001>

Krause, S., Chmiel, R., Bonifacio, E., Scholz, M., Powell, M., Furmaniak, J., Rees Smith, B., Ziegler, A.-G., & Achenbach, P. (2012). IA-2 autoantibody affinity in children at risk for type 1 diabetes. *Clinical Immunology*, *145*(3), 224–229. <https://doi.org/10.1016/j.clim.2012.09.010>

Krischer, J. P., Liu, X., Lernmark, Å., Hagopian, W. A., Rewers, M. J., She, J.-X., Toppari, J., Ziegler, A.-G., Akolkar, B., & on behalf of the TEDDY Study Group. (2017). The influence of type 1 diabetes genetic susceptibility regions, age, sex, and family history on the progression from multiple autoantibodies to type 1 diabetes: A TEDDY study report. *Diabetes*, *66*(12), 3122–3129. <https://doi.org/10.2337/db17-0261>

Krischer, J. P., Liu, X., Lernmark, Å., Hagopian, W. A., Rewers, M. J., She, J.-X., Toppari, J., Ziegler, A.-G., Akolkar, B., TEDDY Study Group, & on behalf of the TEDDY Study Group. (2022). Predictors of the initiation of islet autoimmunity and progression to multiple autoantibodies and clinical diabetes: The TEDDY study. *Diabetes Care*, *45*(10), 2271–2281. <https://doi.org/10.2337/dc21-2612>

Krischer, J. P., Liu, X., Vehik, K., Akolkar, B., Hagopian, W. A., Rewers, M. J., She, J.-X., Toppari, J., Ziegler, A.-G., Lernmark, Å., & TEDDY Study Group. (2019). Predicting islet cell autoimmunity and type 1 diabetes: An 8-year TEDDY study progress report. *Diabetes Care*, *42*(6), 1051–1060. <https://doi.org/10.2337/dc18-2282>

Krischer, J. P., Lynch, K. F., Lernmark, Å., Hagopian, W. A., Rewers, M. J., She, J.-X., Toppari, J., Ziegler, A.-G., Akolkar, B., & the TEDDY Study Group. (2017). Genetic and environmental interactions modify the risk of diabetes-related autoimmunity by 6 years of age: The TEDDY study. *Diabetes Care*, *40*(9), 1194–1202. <https://doi.org/10.2337/dc17-0238>

Krischer, J. P., Lynch, K. F., Schatz, D. A., Ilonen, J., Lernmark, Å., Hagopian, W. A., Rewers, M. J., She, J.-X., Simell, O. G., Toppari, J., Ziegler, A.-G., Akolkar, B., & Bonifacio, E. (2015). The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: The TEDDY study. *Diabetologia*, *58*(5), 980–987. <https://doi.org/10.1007/s00125-015-3514-y>

Krjutškov, K., Katayama, S., Saare, M., Vera-Rodriguez, M., Lubenets, D., Samuel, K., Laisk-Podar, T., Teder, H., Einarsdottir, E., Salumets, A., & Kere, J. (2016). Single-cell transcriptome analysis of endometrial tissue. *Human Reproduction*, *31*(4), 844–853. <https://doi.org/10.1093/humrep/dew008>

Krogvold, L., Edwin, B., Buanes, T., Ludvigsson, J., Korsgren, O., Hyöty, H., Frisk, G., Hanssen, K. F., & Dahl-Jørgensen, K. (2014). Pancreatic biopsy by minimal tail resection in live adult patients at the onset of type 1 diabetes: Experiences from the DiViD study. *Diabetologia*, *57*(4), 841–843. <https://doi.org/10.1007/s00125-013-3155-y>

Krogvold, L., Genoni, A., Puggioni, A., Campani, D., Richardson, S. J., Flaxman, C. S., Edwin, B., Buanes, T., Dahl-Jørgensen, K., & Toniolo, A. (2022). Live enteroviruses, but not other viruses, detected in human pancreas at the onset of type 1 diabetes in the DiViD study. *Diabetologia*. <https://doi.org/10.1007/s00125-022-05779-2>

Krogvold, L., Wiberg, A., Edwin, B., Buanes, T., Jahnsen, F. L., Hanssen, K. F., Larsson, E., Korsgren, O., Skog, O., & Dahl-Jørgensen, K. (2016). Insulitis and characterisation of infiltrating T cells in surgical pancreatic tail resections from patients at onset of type 1 diabetes. *Diabetologia*, *59*(3), 492–501. <https://doi.org/10.1007/s00125-015-3820-4>

Kronenberg, D., Knight, R. R., Estorninho, M., Ellis, R. J., Kester, M. G., de Ru, A., Eichmann, M., Huang, G. C., Powrie, J., Dayan, C. M., Skowera, A., van Veelen, P. A., & Peakman, M. (2012). Circulating preproinsulin signal peptide–specific CD8 T cells restricted by the susceptibility molecule HLA-A24 are expanded at onset of type 1 diabetes and kill β-cells. *Diabetes*, *61*(7), 1752–1759. <https://doi.org/10.2337/db11-1520>

Kukko, M., Kimpimäki, T., Korhonen, S., Kupila, A., Simell, S., Veijola, R., Simell, T., Ilonen, J., Simell, O., & Knip, M. (2005). Dynamics of diabetes-associated autoantibodies in young children with human leukocyte antigen-conferred risk of type 1 diabetes recruited from the general population. *The Journal of Clinical Endocrinology & Metabolism*, *90*(5), 2712–2717. <https://doi.org/10.1210/jc.2004-1371>

Kukreja, A., Cost, G., Marker, J., Zhang, C., Sun, Z., Lin-Su, K., Ten, S., Sanz, M., Exley, M., Wilson, B., Porcelli, S., & Maclaren, N. (2001). Multiple immuno-regulatory defects in type-1 diabetes. *The Journal of Clinical Investigation*, *109*(1), 131–140. <https://doi.org/10.1172/JCI13605>

Kupila, A., Keskinen, P., Simell, T., Erkkilä, S., Arvilommi, P., Korhonen, S., Kimpimäki, T., Sjöroos, M., Ronkainen, M., Ilonen, J., Knip, M., & Simell, O. (2002). Genetic risk determines the emergence of diabetes-associated autoantibodies in young children. *Diabetes*, *51*(3), 646–651. <https://doi.org/10.2337/diabetes.51.3.646>

Kupila, A., Muona, P., Simell, T., Arvilommi, P., Savolainen, H., Hämäläinen, A.-M., Korhonen, S., Kimpimäki, T., Sjöroos, M., Ilonen, J., Knip, M., & Simell, O. (2001). Feasibility of genetic and immunological prediction of Type I diabetes in a population-based birth cohort. *Diabetologia*, *44*(3), 290–297. <https://doi.org/10.1007/s001250051616>

Kyogoku, C., Ortmann, W. A., Lee, A., Selby, S., Carlton, V. E. H., Chang, M., Ramos, P., Baechler, E. C., Batliwalla, F. M., Novitzke, J., Williams, A. H., Gillett, C., Rodine, P., Graham, R. R., Ardlie, K. G., Gaffney, P. M., Moser, K. L., Petri, M., Begovich, A. B., … Behrens, T. W. (2004). Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *The American Journal of Human Genetics*, *75*(3), 504–507. <https://doi.org/10.1086/423790>

Laffel, L. (2000). Sick-day management in type 1 diabetes. *Endocrinology and Metabolism Clinics of North America*, *29*(4), 707–723. <https://doi.org/10.1016/S0889-8529(05)70160-2>

Laine, A.-P., Hermann, R., Knip, M., Simell, O., Åkerblom, H. k., & Ilonen, J. (2004). The human leukocyte antigen genotype has a modest effect on the insulin gene polymorphism-associated susceptibility to type 1 diabetes in the Finnish population. *Tissue Antigens*, *63*(1), 72–74. <https://doi.org/10.1111/j.1399-0039.2004.00153.x>

Laine, A.-P., Knip, M., & Ilonen, J. (2013). Transmission disequilibrium analysis of 31 type 1 diabetes susceptibility loci in Finnish families. *Tissue Antigens*, *82*(1), 35–42. <https://doi.org/10.1111/tan.12143>

Laine, A.-P., Valta, M., Toppari, J., Knip, M., Veijola, R., Ilonen, J., & Lempainen, J. (2022). Non-HLA gene polymorphisms in the pathogenesis of type 1 diabetes: Phase and endotype specific effects. *Frontiers in Immunology*, *13*. <https://doi.org/10.3389/fimmu.2022.909020>

Laitinen, O. H., Honkanen, H., Pakkanen, O., Oikarinen, S., Hankaniemi, M. M., Huhtala, H., Ruokoranta, T., Lecouturier, V., André, P., Harju, R., Virtanen, S. M., Lehtonen, J., Almond, J. W., Simell, T., Simell, O., Ilonen, J., Veijola, R., Knip, M., & Hyöty, H. (2014). Coxsackievirus B1 is associated with induction of β-cell autoimmunity that portends type 1 diabetes. *Diabetes*, *63*(2), 446–455. <https://doi.org/10.2337/db13-0619>

Lan, M. S., Wasserfall, C., Maclaren, N. K., & Notkins, A. L. (1996). IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(13), 6367–6370. <https://doi.org/10.1073/pnas.93.13.6367>

Lange, K., Buerger, M., Stallmach, A., & Bruns, T. (2016). Effects of Antibiotics on Gut Microbiota. *Digestive Diseases*, *34*(3), 260–268. <https://doi.org/10.1159/000443360>

Lawson, J. M., Tremble, J., Dayan, C., Beyan, H., Leslie, R. D. G., Peakman, M., & Tree, T. I. M. (2008). Increased resistance to CD4+CD25hi regulatory T cell-mediated suppression in patients with type 1 diabetes. *Clinical and Experimental Immunology*, *154*(3), 353–359. <https://doi.org/10.1111/j.1365-2249.2008.03810.x>

Lee, H.-S., Park, H., Yang, S., Kim, D., & Park, Y. (2008). STAT4 polymorphism is associated with early-onset type 1 diabetes, but not with late-onset type 1 diabetes. *Annals of the New York Academy of Sciences*, *1150*(1), 93–98. <https://doi.org/10.1196/annals.1447.013>

Leete, P., Mallone, R., Richardson, S. J., Sosenko, J. M., Redondo, M. J., & Evans-Molina, C. (2018). The effect of age on the progression and severity of type 1 diabetes: Potential effects on disease mechanisms. *Current Diabetes Reports*, *18*(11), 115. <https://doi.org/10.1007/s11892-018-1083-4>

Leete, P., Willcox, A., Krogvold, L., Dahl-Jørgensen, K., Foulis, A. K., Richardson, S. J., & Morgan, N. G. (2016). Differential insulitic profiles determine the extent of β-cell destruction and the age at onset of type 1 diabetes. *Diabetes*, *65*(5), 1362–1369. <https://doi.org/10.2337/db15-1615>

Lempainen, J., Härkönen, T., Laine, A., Knip, M., & Ilonen, J. (2013). Associations of polymorphisms in non-HLA loci with autoantibodies at the diagnosis of type 1 diabetes: INS and IKZF4 associate with insulin autoantibodies. *Pediatric Diabetes*, *14*(7), 490–496. <https://doi.org/10.1111/pedi.12046>

Lempainen, J., Laine, A.-P., Hammais, A., Toppari, J., Simell, O., Veijola, R., Knip, M., & Ilonen, J. (2015). Non-HLA gene effects on the disease process of type 1 diabetes: From HLA susceptibility to overt disease. *Journal of Autoimmunity*, *61*, 45–53. <https://doi.org/10.1016/j.jaut.2015.05.005>

Liang, Y., Wu, H., Shen, X., Li, P., Yang, X., Liang, L., Tian, W., Zhang, L., & Xie, X. (2012). Association of STAT4 rs7574865 polymorphism with autoimmune diseases: A meta-analysis. *Molecular Biology Reports*, *39*(9), 8873–8882. <https://doi.org/10.1007/s11033-012-1754-1>

Lietzen, N., An, L. T. T., Jaakkola, M. K., Kallionpää, H., Oikarinen, S., Mykkänen, J., Knip, M., Veijola, R., Ilonen, J., Toppari, J., Hyöty, H., Lahesmaa, R., & Elo, L. L. (2018). Enterovirus-associated changes in blood transcriptomic profiles of children with genetic susceptibility to type 1 diabetes. *Diabetologia*, *61*(2), 381–388. <https://doi.org/10.1007/s00125-017-4460-7>

Lin, X., Pelletier, S., Gingras, S., Rigaud, S., Maine, C. J., Marquardt, K., Dai, Y. D., Sauer, K., Rodriguez, A. R., Martin, G., Kupriyanov, S., Jiang, L., Yu, L., Green, D. R., & Sherman, L. A. (2016). CRISPR-Cas9–mediated modification of the NOD mouse genome with Ptpn22R619W mutation increases autoimmune diabetes. *Diabetes*, *65*(8), 2134–2138. <https://doi.org/10.2337/db16-0061>

Lindley, S., Dayan, C. M., Bishop, A., Roep, B. O., Peakman, M., & Tree, T. I. M. (2005). Defective suppressor function in CD4+CD25+ T-cells from patients with type 1 diabetes. *Diabetes*, *54*(1), 92–99. <https://doi.org/10.2337/diabetes.54.1.92>

Long, A. E., George, G., & Williams, C. L. (2021). Persistence of islet autoantibodies after diagnosis in type 1 diabetes. *Diabetic Medicine*, *38*(12), e14712. <https://doi.org/10.1111/dme.14712>

Long, S. A., Cerosaletti, K., Wan, J. Y., Ho, J.-C., Tatum, M., Wei, S., Shilling, H. G., & Buckner, J. H. (2011). An autoimmune-associated variant in PTPN2 reveals an impairment of IL-2R signaling in CD4+ T cells. *Genes & Immunity*, *12*(2), 116–125. <https://doi.org/10.1038/gene.2010.54>

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. <https://doi.org/10.1186/s13059-014-0550-8>

Luo, S., Ma, X., Li, X., Xie, Z., & Zhou, Z. (2020). Fulminant type 1 diabetes: A comprehensive review of an autoimmune condition. *Diabetes/Metabolism Research and Reviews*, *36*(6), e3317. <https://doi.org/10.1002/dmrr.3317>

Maier, L. M., Lowe, C. E., Cooper, J., Downes, K., Anderson, D. E., Severson, C., Clark, P. M., Healy, B., Walker, N., Aubin, C., Oksenberg, J. R., Hauser, S. L., Compston, A., Sawcer, S., De Jager, P. L., Wicker, L. S., Todd, J. A., Hafler, D. A., & Multiple Sclerosis Genetics Consortium. (2009). IL2RA genetic heterogeneity in multiple sclerosis and type 1 diabetes susceptibility and soluble interleukin-2 receptor production. *PLoS Genetics*, *5*(1). <https://doi.org/10.1371/journal.pgen.1000322>

Maine, C. J., Hamilton-Williams, E., Cheung, J., Stanford, S. M., Bottini, N., Wicker, L. S., & Sherman, L. A. (2012). PTPN22 alters the development of T regulatory cells in the thymus. *Journal of Immunology*, *188*(11), 5267–5275. <https://doi.org/10.4049/jimmunol.1200150>

Mäkinen, M., Mykkänen, J., Koskinen, M., Simell, V., Veijola, R., Hyöty, H., Ilonen, J., Knip, M., Simell, O., & Toppari, J. (2016). Serum 25-hydroxyvitamin D concentrations in children progressing to autoimmunity and clinical type 1 diabetes. *The Journal of Clinical Endocrinology and Metabolism*, *101*(2), 723–729. <https://doi.org/10.1210/jc.2015-3504>

Månsson, L., Törn, C., & Landin-Olsson, M. (2001). Islet cell antibodies represent autoimmune response against several antigens. *International Journal of Experimental Diabetes Research*, *2*(2), 85–90. <https://doi.org/10.1155/EDR.2001.85>

Márquez, A., & Martín, J. (2022). Genetic overlap between type 1 diabetes and other autoimmune diseases. *Seminars in Immunopathology*, *44*(1), 81–97. <https://doi.org/10.1007/s00281-021-00885-6>

Marson, A., Kretschmer, K., Frampton, G. M., Jacobsen, E. S., Polansky, J. K., MacIsaac, K. D., Levine, S. S., Fraenkel, E., von Boehmer, H., & Young, R. A. (2007). Foxp3 occupancy and regulation of key target genes during T-cell stimulation. *Nature*, *445*(7130), 931–935. <https://doi.org/10.1038/nature05478>

Matzaraki, V., Kumar, V., Wijmenga, C., & Zhernakova, A. (2017). The MHC locus and genetic susceptibility to autoimmune and infectious diseases. *Genome Biology*, *18*(1), 76. <https://doi.org/10.1186/s13059-017-1207-1>

McDonnell, L., Gilkes, A., Ashworth, M., Rowland, V., Harries, T. H., Armstrong, D., & White, P. (2021). Association between antibiotics and gut microbiome dysbiosis in children: Systematic review and meta-analysis. *Gut Microbes*, *13*(1), 1870402. <https://doi.org/10.1080/19490976.2020.1870402>

McKinney, E. F., Lee, J. C., Jayne, D. R. W., Lyons, P. A., & Smith, K. G. C. (2015). T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature*, *523*(7562), 612-616. <https://doi.org/10.1038/nature14468>

Melchers, F. (2015). Checkpoints that control B cell development. *Journal of Clinical Investigation*, *125*(6), 2203.

Michels, A. W., Landry, L. G., McDaniel, K. A., Yu, L., Campbell-Thompson, M., Kwok, W. W., Jones, K. L., Gottlieb, P. A., Kappler, J. W., Tang, Q., Roep, B. O., Atkinson, M. A., Mathews, C. E., & Nakayama, M. (2017). Islet-derived CD4 t cells targeting proinsulin in human autoimmune diabetes. *Diabetes*, *66*(3), 722–734. <https://doi.org/10.2337/db16-1025>

Miettinen, M. E., Reinert, L., Kinnunen, L., Harjutsalo, V., Koskela, P., Surcel, H.-M., Lamberg-Allardt, C., & Tuomilehto, J. (2012). Serum 25-hydroxyvitamin D level during early pregnancy and type 1 diabetes risk in the offspring. *Diabetologia*, *55*(5), 1291–1294. <https://doi.org/10.1007/s00125-012-2458-8>

Mikk, M. -L, Heikkinen, T., El-Amir, M. I., Kiviniemi, M., Laine, A. -P., Härkönen, T., Veijola, R., Toppari, J., Knip, M., & Ilonen, J. (2017). The association of the HLA-A24:02, B39:01 and B39:06 alleles with type 1 diabetes is restricted to specific HLA-DR/DQ haplotypes in Finns. *HLA*, *89*(4), 215–224. <https://doi.org/10.1111/tan.12967>

Mikk, M.-L., Pfeiffer, S., Kiviniemi, M., Laine, A.-P., Lempainen, J., Härkönen, T., Toppari, J., Veijola, R., Knip, M., Ilonen, J., & The Finnish Pediatric Diabetes Register. (2020). HLA-DR-DQ haplotypes and specificity of the initial autoantibody in islet specific autoimmunity. *Pediatric Diabetes*, *21*(7), 1218–1226. <https://doi.org/10.1111/pedi.13073>

Mikkelsen, K. H., Knop, F. K., Vilsbøll, T., Frost, M., Hallas, J., & Pottegård, A. (2017). Use of antibiotics in childhood and risk of Type 1 diabetes: A population-based case–control study. *Diabetic Medicine*, *34*(2), 272–277. <https://doi.org/10.1111/dme.13262>

Mittrücker, H.-W., Visekruna, A., & Huber, M. (2014). Heterogeneity in the differentiation and function of CD8+ T cells. *Archivum Immunologiae et Therapiae Experimentalis*, *62*(6), 449–458. <https://doi.org/10.1007/s00005-014-0293-y>

Morbach, H., Eichhorn, E. M., Liese, J. G., & Girschick, H. J. (2010). Reference values for B cell subpopulations from infancy to adulthood. *Clinical & Experimental Immunology*, *162*(2), 271–279. <https://doi.org/10.1111/j.1365-2249.2010.04206.x>

Muntoni, S., Cocco, P., Aru, G., Cucca, F., & Muntoni, S. (2000). Nutritional factors and worldwide incidence of childhood type 1 diabetes. *The American Journal of Clinical Nutrition*, *71*(6), 1525–1529. <https://doi.org/10.1093/ajcn/71.6.1525>

Murri, M., Leiva, I., Gomez-Zumaquero, J. M., Tinahones, F. J., Cardona, F., Soriguer, F., & Queipo-Ortuño, M. I. (2013). Gut microbiota in children with type 1 diabetes differs from that in healthy children: A case-control study. *BMC Medicine*, *11*, 46. <https://doi.org/10.1186/1741-7015-11-46>

Nejentsev, S., Howson, J. M. M., Walker, N. M., Szeszko, J., Field, S. F., Stevens, H. E., Reynolds, P., Hardy, M., King, E., Masters, J., Hulme, J., Maier, L. M., Smyth, D., Bailey, R., Cooper, J. D., Ribas, G., Campbell, R. D., Clayton, D. G., & Todd, J. A. (2007). Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. *Nature*, *450*(7171), 887–892. <https://doi.org/10.1038/nature06406>

Nemazee, D. (2017). Mechanisms of central tolerance for B cells. *Nature Reviews Immunology*, *17*(5), 281–294. <https://doi.org/10.1038/nri.2017.19>

Nerup, J., Platz, P., Andersen, O. O., Christy, M., Lyngsøe, J., Poulsen, J. E., Ryder, L. P., Thomsen, M., Nielsen, L. S., & Svejgaard, A. (1974). HL-A antigens and diabetes mellitus. *The Lancet*, *304*(7885), 864–866. <https://doi.org/10.1016/S0140-6736(74)91201-X>

Nieto, J., Castillo, B., Astudillo, M., Tosur, M., Balasubramanyam, A., Pietropaolo, M., & Redondo, M. J. (2021). Islet autoantibody types mark differential clinical characteristics at diagnosis of pediatric type 1 diabetes. *Pediatric Diabetes*, *22*(6), 882–888. <https://doi.org/10.1111/pedi.13238>

Nilsson, A.-L., Vaziri-Sani, F., Andersson, C., Larsson, K., Carlsson, A., Cedervall, E., Jönsson, B., Neiderud, J., Elding Larsson, H., Ivarsson, S.-A., & Lernmark, Å. (2013). Relationship between Ljungan virus antibodies, HLA-DQ8, and insulin autoantibodies in newly diagnosed type 1 diabetes children. *Viral Immunology*, *26*(3), 207–215. <https://doi.org/10.1089/vim.2013.0008>

Nilsson, A.-L., Vaziri-Sani, F., Broberg, P., Elfaitouri, A., Pipkorn, R., Blomberg, J., Ivarsson, S.-A., Elding Larsson, H., & Lernmark, Å. (2015). Serological evaluation of possible exposure to Ljungan virus and related parechovirus in autoimmune (type 1) diabetes in children. *Journal of Medical Virology*, *87*(7), 1130–1140. <https://doi.org/10.1002/jmv.24127>

Noble, J. A., & Valdes, A. M. (2011). Genetics of the HLA region in the prediction of type 1 diabetes. *Current Diabetes Reports*, *11*(6), 533–542. <https://doi.org/10.1007/s11892-011-0223-x>

Noble, J. A., Valdes, A. M., Bugawan, T. L., Apple, R. J., Thomson, G., & Erlich, H. A. (2002). The HLA class I A locus affects susceptibility to type 1 diabetes. *Human Immunology*, *63*(8), 657–664. <https://doi.org/10.1016/S0198-8859(02)00421-4>

Norris, J. M., Barriga, K., Klingensmith, G., Hoffman, M., Eisenbarth, G. S., Erlich, H. A., & Rewers, M. (2003). Timing of initial cereal exposure in infancy and risk of islet autoimmunity. *Journal of the American Medical Association*, *290*(13), 1713–1720. <https://doi.org/10.1001/jama.290.13.1713>

Norris, J. M., Lee, H.-S., Frederiksen, B., Erlund, I., Uusitalo, U., Yang, J., Lernmark, Å., Simell, O., Toppari, J., Rewers, M., Ziegler, A.-G., She, J.-X., Onengut-Gumuscu, S., Chen, W.-M., Rich, S. S., Sundvall, J., Akolkar, B., Krischer, J., Virtanen, S. M., … The TEDDY Study Group. (2017). Plasma 25-hydroxyvitamin D concentration and risk of islet autoimmunity. *Diabetes*, *67*(1), 146–154. <https://doi.org/10.2337/db17-0802>

Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M., & Corcoran, L. M. (2015). The generation of antibody-secreting plasma cells. *Nature Reviews. Immunology*, *15*(3), 160–171. <https://doi.org/10.1038/nri3795>

Oikarinen, M., Tauriainen, S., Honkanen, T., Vuori, K., Karhunen, P., Vasama-Nolvi, C., Oikarinen, S., Verbeke, C., Blair, G. E., Rantala, I., Ilonen, J., Simell, O., Knip, M., & Hyöty, H. (2008). Analysis of pancreas tissue in a child positive for islet cell antibodies. *Diabetologia*, *51*(10), 1796. <https://doi.org/10.1007/s00125-008-1107-8>

Oikarinen, S., Martiskainen, M., Tauriainen, S., Huhtala, H., Ilonen, J., Veijola, R., Simell, O., Knip, M., & Hyöty, H. (2010). Enterovirus RNA in blood is linked to the development of type 1 diabetes. *Diabetes*, *60*(1), 276–279. <https://doi.org/10.2337/db10-0186>

Onengut-Gumuscu, S., Chen, W.-M., Burren, O., Cooper, N. J., Quinlan, A. R., Mychaleckyj, J. C., Farber, E., Bonnie, J. K., Szpak, M., Schofield, E., Achuthan, P., Guo, H., Fortune, M. D., Stevens, H., Walker, N. M., Ward, L. D., Kundaje, A., Kellis, M., Daly, M. J., … Rich, S. S. (2015). Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. *Nature Genetics*, *47*(4), 381–386. <https://doi.org/10.1038/ng.3245>

Oram, R. A., Patel, K., Hill, A., Shields, B., McDonald, T. J., Jones, A., Hattersley, A. T., & Weedon, M. N. (2015). A type 1 diabetes genetic risk score can aid discrimination between type 1 and type 2 diabetes in young adults. *Diabetes Care*, *39*(3), 337–344. <https://doi.org/10.2337/dc15-1111>

Ożańska, A, Szymczak, D & Rybka, J. (2020). Pattern of human monocyte subpopulations in health and disease. *Scandinavian Journal of Immunology, 92,* e12883. <https://doi.org/10.1111/sji.12883>

Palmer, J. P., Asplin, C. M., Clemons, P., Lyen, K., Tatpati, O., Raghu, P. K., & Paquette, T. L. (1983). Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science*, *222*(4630), 1337–1339. <https://doi.org/10.1126/science.6362005>

Parackova, Z., Kayserova, J., Danova, K., Sismova, K., Dudkova, E., Sumnik, Z., Kolouskova, S., Lebl, J., Stechova, K., & Sediva, A. (2016). T regulatory lymphocytes in type 1 diabetes: Impaired CD25 expression and IL-2 induced STAT5 phosphorylation in pediatric patients. *Autoimmunity*, *49*(8), 523–531. <https://doi.org/10.1080/08916934.2016.1217998>

Parikka, V., Näntö-Salonen, K., Saarinen, M., Simell, T., Ilonen, J., Hyöty, H., Veijola, R., Knip, M., & Simell, O. (2012). Early seroconversion and rapidly increasing autoantibody concentrations predict prepubertal manifestation of type 1 diabetes in children at genetic risk. *Diabetologia*, *55*(7), 1926–1936. <https://doi.org/10.1007/s00125-012-2523-3>

Park, S. Y., Gautier, J.-F., & Chon, S. (2021). Assessment of Insulin Secretion and Insulin Resistance in Human. *Diabetes & Metabolism Journal*, *45*(5), 641–654. <https://doi.org/10.4093/dmj.2021.0220>

Parkes, M., Cortes, A., van Heel, D. A., & Brown, M. A. (2013). Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nature Reviews Genetics*, *14*(9), 661–673. <https://doi.org/10.1038/nrg3502>

Parkin, J., & Cohen, B. (2001). An overview of the immune system. *The Lancet*, *357*(9270), 1777–1789. <https://doi.org/10.1016/S0140-6736(00)04904-7>

Parviainen, A., But, A., Siljander, H., & Knip, M. (2020). Decreased incidence of type 1 diabetes in young finnish children. *Diabetes Care*, *43*(12), 2953. <https://doi.org/10.2337/dc20-0604>

Pathiraja, V., Kuehlich, J. P., Campbell, P. D., Krishnamurthy, B., Loudovaris, T., Coates, P. T. H., Brodnicki, T. C., O'Connell, P. J., Kedzierska, K., Rodda, C., Bergman, P., Hill, E., Purcell, A. W., Dudek, N. L., Thomas, H. E., Kay, T. W. H., & Mannering, S. I. (2014). Proinsulin-specific, HLA-DQ8, and HLA-DQ8-transdimer–restricted CD4+ T cells infiltrate islets in type 1 diabetes. *Diabetes*, *64*(1), 172–182. <https://doi.org/10.2337/db14-0858>

Payton, M. A., Hawkes, C. J., & Christie, M. R. (1995). Relationship of the 37,000- and 40,000-M(r) tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). *Journal of Clinical Investigation*, *96*(3), 1506–1511.

Pearson, J. A., Wong, F. S. & Wen, L. (2016). The importance of the Non Obese Diabetic (NOD) mouse model in autoimmune diabetes. *Journal of Autoimmunity, 66*, 76-88. <https://doi.org 10.1016/j.jaut.2015.08.019>

Pfefferle, P. I., Keber, C. U., Cohen, R. M., & Garn, H. (2021). The hygiene hypothesis – learning from but not living in the past. *Frontiers in Immunology*, *12*. <https://doi.org/10.3389/fimmu.2021.635935>

Pociot, F., & Lernmark, Å. (2016). Genetic risk factors for type 1 diabetes. *Lancet*, *387*(10035), 2331–2339. <https://doi.org/10.1016/S0140-6736(16)30582-7>

Pöllänen, P. M., Lempainen, J., Laine, A.-P., Toppari, J., Veijola, R., Ilonen, J., Siljander, H., & Knip, M. (2019). Characteristics of slow progression to type 1 diabetes in children with increased HLA-conferred disease risk. *The Journal of Clinical Endocrinology & Metabolism*, *104*(11), 5585–5594. <https://doi.org/10.1210/jc.2019-01069>

Pöllänen, P. M., Lempainen, J., Laine, A.-P., Toppari, J., Veijola, R., Vähäsalo, P., Ilonen, J., Siljander, H., & Knip, M. (2017). Characterisation of rapid progressors to type 1 diabetes among children with HLA-conferred disease susceptibility. *Diabetologia*, *60*(7), 1284–1293. <https://doi.org/10.1007/s00125-017-4258-7>

Pollard, K. S., Dudoit, S., & van der Laan, M. J. (2005). Multiple testing procedures: The multtest package and applications to genomics. In R. Gentleman, V. J. Carey, W. Huber, R. A. Irizarry, & S. Dudoit (Eds.), *Bioinformatics and computational biology solutions using R and Bioconductor* (pp. 249–271). Springer. <https://doi.org/10.1007/0-387-29362-0_15>

Polychronakos, C., & Li, Q. (2011). Understanding type 1 diabetes through genetics: Advances and prospects. *Nature Reviews Genetics*, *12*(11), 781-792. <https://doi.org/10.1038/nrg3069>

Primavera, M., Giannini, C., & Chiarelli, F. (2020). Prediction and prevention of type 1 diabetes. *Frontiers in Endocrinology*, *11*. <https://doi.org/10.3389/fendo.2020.00248>

Pugliese, A., Zeller, M., Fernandez, A., Zalcberg, L. J., Bartlett, R. J., Ricordi, C., Pietropaolo, M., Eisenbarth, G. S., Bennett, S. T., & Patel, D. D. (1997). The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nature Genetics*, *15*(3), 293–297. <https://doi.org/10.1038/ng0397-293>

Putnam, A. L., Vendrame, F., Dotta, F., & Gottlieb, P. A. (2005). CD4+CD25high regulatory T cells in human autoimmune diabetes. *Journal of Autoimmunity*, *24*(1), 55–62. <https://doi.org/10.1016/j.jaut.2004.11.004>

Rabin, D. U., Pleasic, S. M., Shapiro, J. A., Yoo-Warren, H., Oles, J., Hicks, J. M., Goldstein, D. E., & Rae, P. M. (1994). Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. *The Journal of Immunology*, *152*(6), 3183–3188.

Rassi, D. M., Junta, C. M., Fachin, A. L., Sandrin-Garcia, P., Mello, S., Marques, M. M. C., Fernandes, A. P. M., Foss-Freitas, M., Foss, M. C., & Sakamoto-Hojo, E. (2006). Metabolism genes are among

the differentially expressed ones observed in lymphomononuclear cells of recently diagnosed type 1 diabetes mellitus patients. *Annals of the New York Academy of Sciences*, *1079*(1), 171–176. <https://doi.org/10.1196/annals.1375.026>

Rautava, S., Luoto, R., Salminen, S., & Isolauri, E. (2012). Microbial contact during pregnancy, intestinal colonization and human disease. *Nature Reviews Gastroenterology & Hepatology*, *9*(10), 565–576. <https://doi.org/10.1038/nrgastro.2012.144>

Reddy, S., Zeng, N., Al-Diery, H., Jung, D., Yeu, C., Joret, M. O., Merrilees, M. J., & Wu, F. (2015). Analysis of peri-islet CD45-positive leucocytic infiltrates in long-standing type 1 diabetic patients. *Diabetologia*, *58*(5), 1024–1035. <https://doi.org/10.1007/s00125-015-3519-6>

Redondo, M. J., Jeffrey, J., Fain, P. R., Eisenbarth, G. S., & Orban, T. (2008). Concordance for islet autoimmunity among monozygotic twins. *New England Journal of Medicine*, *359*(26), 2849–2850. <https://doi.org/10.1056/NEJMc0805398>

Redondo, M. J., Rodriguez, L. M., Escalante, M., O'Brian Smith, E., Balasubramanyam, A., & Haymond, M. W. (2012). Beta cell function and BMI in ethnically diverse children with newly diagnosed autoimmune type 1 diabetes. *Pediatric Diabetes*, *13*(7), 564–571. <https://doi.org/10.1111/j.1399-5448.2012.00875.x>

Rewers, M., & Ludvigsson, J. (2016). Environmental risk factors for type 1 diabetes. *The Lancet*, *387*(10035), 2340–2348. <https://doi.org/10.1016/S0140-6736(16)30507-4>

Reynier, F., Pachot, A., Paye, M., Xu, Q., Turrel-Davin, F., Petit, F., Hot, A., Auffray, C., Bendelac, N., Nicolino, M., Mougin, B., & Thivolet, C. (2010). Specific gene expression signature associated with development of autoimmune type-I diabetes using whole-blood microarray analysis. *Genes & Immunity*, *11*(3), 269–278. <https://doi.org/10.1038/gene.2009.112>

Rieck, M., Arechiga, A., Onengut-Gumuscu, S., Greenbaum, C., Concannon, P., & Buckner, J. H. (2007). Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. *The Journal of Immunology*, *179*(7), 4704–4710. <https://doi.org/10.4049/jimmunol.179.7.4704>

Riera Romo, M., Pérez-Martínez, D. & Castillo Ferrer, C. (2016). Innate immunity in vertebrates: an overview. *Immunology, 148*, 125-139. <https://doi.org/10.1111/imm.12597>

Robertson, C. C., Inshaw, J. R. J., Onengut-Gumuscu, S., Chen, W.-M., Santa Cruz, D. F., Yang, H., Cutler, A. J., Crouch, D. J. M., Farber, E., Bridges, S. L., Edberg, J. C., Kimberly, R. P., Buckner, J. H., Deloukas, P., Divers, J., Dabelea, D., Lawrence, J. M., Marcovina, S., Shah, A. S., … Rich, S. S. (2021). Fine-mapping, trans-ancestral and genomic analyses identify causal variants, cells, genes and drug targets for type 1 diabetes. *Nature Genetics*, *53*(7), 962–971. <https://doi.org/10.1038/s41588-021-00880-5>

Robertson, M. (1998). Antigen presentation. *Current Biology*, *8*(23), R829–R831. <https://doi.org/10.1016/S0960-9822(07)00523-4>

Robinson, A., Han, C. Z., Glass, C. K. & Pollard, J. W. (2021). Monocyte Regulation in Homeostasis and Malignancy. *Trends in Immunology, 42*(2), (104-119). <https://doi.org/10.1016/j.it.2020.12.001>

Rodacki, M., Pereira, J. R. D., de Oliveira, A. M. N., Barone, B., Dowell, R. M., Perricelli, P., Bravo, M. T., de Oliveira, M. M., Brum, J. D., Belem, L. C., de Ornellas, P. G., Berardo, R. S., Luescher, J., Campos, L., Vangelotti, A. de M., Kupfer, R., Zajdenverg, L., Milech, A., & de Oliveira, J. E. P. (2007). Ethnicity and young age influence the frequency of diabetic ketoacidosis at the onset of type 1 diabetes. *Diabetes Research and Clinical Practice*, *78*(2), 259–262. <https://doi.org/10.1016/j.diabres.2007.04.002>

Rodriguez-Calvo, T., Johnson, J. D., Overbergh, L., & Dunne, J. L. (2021). Neoepitopes in type 1 diabetes: Etiological insights, biomarkers and therapeutic targets. *Frontiers in Immunology*, *12*. <https://doi.org/10.3389/fimmu.2021.667989>

Rodriguez-Calvo, T., Sabouri, S., Anquetil, F., & von Herrath, M. G. (2016). The viral paradigm in type 1 diabetes: Who are the main suspects? *Autoimmunity Reviews*, *15*(10), 964–969. <https://doi.org/10.1016/j.autrev.2016.07.019>

Rorsman, P., & Renström, E. (2003). Insulin granule dynamics in pancreatic beta cells. *Diabetologia*, *46*(8), 1029–1045. <https://doi.org/10.1007/s00125-003-1153-1>

Rowley, M. J., Mackay, I. R., Chen, Q.-Y., Knowles, W. J., & Zimmet, P. Z. (1992). Antibodies to glutamic acid decarboxylase discriminate major types of diabetes mellitus. *Diabetes*, *41*(4), 548–551. <https://doi.org/10.2337/diab.41.4.548>

Russell, J. T., Roesch, L. F. W., Ördberg, M., Ilonen, J., Atkinson, M. A., Schatz, D. A., Triplett, E. W., & Ludvigsson, J. (2019). Genetic risk for autoimmunity is associated with distinct changes in the human gut microbiome. *Nature Communications*, *10*(1), 3621. <https://doi.org/10.1038/s41467-019-11460-x>

Ryba-Stanisławowska, M., Rybarczyk-Kapturska, K., Myśliwiec, M., & Myśliwska, J. (2014). Elevated levels of serum IL-12 and IL-18 are associated with lower frequencies of CD4+CD25highFOXP3+ regulatory T cells in young patients with type 1 diabetes. *Inflammation*, *37*(5), 1513–1520. <https://doi.org/10.1007/s10753-014-9878-1>

Sandor, A. M., Jacobelli, J., & Friedman, R. S. (2019). Immune cell trafficking to the islets during type 1 diabetes. *Clinical and Experimental Immunology*, *198*(3), 314–325. <https://doi.org/10.1111/cei.13353>

Santin, I., Moore, F., Colli, M. L., Gurzov, E. N., Marselli, L., Marchetti, P., & Eizirik, D. L. (2011). PTPN2, a candidate gene for type 1 diabetes, modulates pancreatic β-cell apoptosis via regulation of the BH3-only protein Bim. *Diabetes*, *60*(12), 3279–3288. <https://doi.org/10.2337/db11-0758>

Saule, P., Trauet, J., Dutriez, V., Lekeux, V., Dessaint, J.-P., & Labalette, M. (2006). Accumulation of memory T cells from childhood to old age: Central and effector memory cells in CD4+ versus effector memory and terminally differentiated memory cells in CD8+ compartment. *Mechanisms of Ageing and Development*, *127*(3), 274–281. <https://doi.org/10.1016/j.mad.2005.11.001>

Sautois, B., Fillet, G., & Beguin, Y. (1997). Comparative cytokine production by in vitro stimulated mononucleated cells from cord blood and adult blood. *Experimental Hematology*, *25*(2). <https://orbi.uliege.be/handle/2268/6983>

Savola, K., Bonifacio, E., Sabbah, E., Kulmala, P., Vähäsalo, P., Karjalainen, J., Tuomilehto-Wolf, E., Meriläinen, J., Åkerblom, H. K., Knip, M., & Childhood Diabetes Finland Study Group. (1998). IA-2 antibodies – a sensitive marker of IDDM with clinical onset in childhood and adolescence. *Diabetologia*, *41*(4), 424–429. <https://doi.org/10.1007/s001250050925>

Savola, K., Sabbah, E., Kulmala, P., Vähäsalo, P., Ilonen, J., & Knip, M. (1998). Autoantibodies associated with Type I diabetes mellitus persist after diagnosis in children. *Diabetologia*, *41*(11), 1293–1297. <https://doi.org/10.1007/s001250051067>

Schmidt, M. B. (1902). Über die Beziehung der Langerhans'schen Inseln des Pankreas zum Diabetes Mellitus. *Münchener Medizinische Wochenschrift*, *49*, 51–54.

Shapiro, M. R., Thirawatananond, P., Peters, L., Sharp, R. C., Ogundare, S., Posgai, A. L., Perry, D. J., & Brusko, T. M. (2021). De-coding genetic risk variants in type 1 diabetes. *Immunology & Cell Biology*, *99*(5), 496–508. <https://doi.org/10.1111/imcb.12438>

Sharp, R. C., Beg, S. A., & Naser, S. A. (2018). Polymorphisms in protein tyrosine phosphatase non-receptor type 2 and 22 (PTPN2/22) are linked to hyper-proliferative t-cells and susceptibility to mycobacteria in rheumatoid arthritis. *Frontiers in Cellular and Infection Microbiology*, *8*. <https://doi.org/10.3389/fcimb.2018.00011>

Sharp, S. A., Rich, S. S., Wood, A. R., Jones, S. E., Beaumont, R. N., Harrison, J. W., Schneider, D. A., Locke, J. M., Tyrrell, J., Weedon, M. N., Hagopian, W. A., & Oram, R. A. (2019). Development and standardization of an improved type 1 diabetes genetic risk score for use in newborn screening and incident diagnosis. *Diabetes Care*, *42*(2), 200–207. <https://doi.org/10.2337/dc18-1785>

Shaw, A. M., Qasem, A., & Naser, S. A. (2021). Modulation of PTPN2/22 function by spermidine in CRISPR-Cas9-edited t-cells associated with Crohn's disease and rheumatoid arthritis. *International Journal of Molecular Sciences*, *22*(16), 8883. <https://doi.org/10.3390/ijms22168883>

Shiina, T., Hosomichi, K., Inoko, H. & Kulski, J. (2009). The HLA genomic loci map: expression, interaction, diversity and disease. *Journal of Human Genetics, 54*, 15–39. <https://doi.org/10.1038/jhg.2008.5>

Siljander, H. T. A., Simell, S., Hekkala, A., Lähde, J., Simell, T., Vähäsalo, P., Veijola, R., Ilonen, J., Simell, O., & Knip, M. (2009). Predictive characteristics of diabetes-associated autoantibodies among children with HLA-conferred disease susceptibility in the general population. *Diabetes*, *58*(12), 2835–2842. <https://doi.org/10.2337/db08-1305>

Siljander, H. T., Hermann, R., Hekkala, A., Lähde, J., Tanner, L., Keskinen, P., Ilonen, J., Simell, O., Veijola, R., & Knip, M. (2013). Insulin secretion and sensitivity in the prediction of type 1 diabetes in children with advanced β-cell autoimmunity. *European Journal of Endocrinology*, *169*(4), 479–485. <https://doi.org/10.1530/EJE-13-0206>

Sims, E. K., Evans-Molina, C., Tersey, S. A., Eizirik, D. L., & Mirmira, R. G. (2018). Biomarkers of islet beta cell stress and death in type 1 diabetes. *Diabetologia*, *61*(11), 2259–2265. <https://doi.org/10.1007/s00125-018-4712-1>

Singal, D. P., & Blajchman, M. A. (1973). Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. *Diabetes*, *22*(6), 429–432. <https://doi.org/10.2337/diab.22.6.429>

Sioofy-Khojine, A.-B., Lehtonen, J., Nurminen, N., Laitinen, O. H., Oikarinen, S., Huhtala, H., Pakkanen, O., Ruokoranta, T., Hankaniemi, M. M., Toppari, J., Vähä-Mäkilä, M., Ilonen, J., Veijola, R., Knip, M., & Hyöty, H. (2018). Coxsackievirus B1 infections are associated with the initiation of insulin-driven autoimmunity that progresses to type 1 diabetes. *Diabetologia*, *61*(5), 1193–1202. <https://doi.org/10.1007/s00125-018-4561-y>

Skowera, A., Ellis, R. J., Varela-Calviño, R., Arif, S., Huang, G. C., Van-Krinks, C., Zaremba, A., Rackham, C., Allen, J. S., Tree, T. I. M., Zhao, M., Dayan, C. M., Sewell, A. K., Unger, W., Drijfhout, J. W., Ossendorp, F., Roep, B. O., & Peakman, M. (2008). CTLs are targeted to kill β cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. *The Journal of Clinical Investigation*, *118*(10), 3390–3402. <https://doi.org/10.1172/JCI35449>

Skowera, A., Ladell, K., McLaren, J. E., Dolton, G., Matthews, K. K., Gostick, E., Kronenberg-Versteeg, D., Eichmann, M., Knight, R. R., Heck, S., Powrie, J., Bingley, P. J., Dayan, C. M., Miles, J. J., Sewell, A. K., Price, D. A., & Peakman, M. (2015). β-cell–specific CD8 T cell phenotype in type 1 diabetes reflects chronic autoantigen exposure. *Diabetes*, *64*(3), 916–925. <https://doi.org/10.2337/db14-0332>

Smith, M. J., Packard, T. A., O'Neill, S. K., Henry Dunand, C. J., Huang, M., Fitzgerald-Miller, L., Stowell, D., Hinman, R. M., Wilson, P. C., Gottlieb, P. A., & Cambier, J. C. (2014). Loss of anergic B cells in prediabetic and new-onset type 1 diabetic patients. *Diabetes*, *64*(5), 1703–1712. <https://doi.org/10.2337/db13-1798>

Smith, M. J., Rihanek, M., Wasserfall, C., Mathews, C. E., Atkinson, M. A., Gottlieb, P. A., & Cambier, J. C. (2018). Loss of B-cell anergy in type 1 diabetes is associated with high-risk HLA and non-HLA disease susceptibility alleles. *Diabetes*, *67*(4), 697–703. <https://doi.org/10.2337/db17-0937>

Smyth, D. J., Cooper, J. D., Bailey, R., Field, S., Burren, O., Smink, L. J., Guja, C., Ionescu-Tirgoviste, C., Widmer, B., Dunger, D. B., Savage, D. A., Walker, N. M., Clayton, D. G., & Todd, J. A. (2006). A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. *Nature Genetics*, *38*(6), 617–619. <https://doi.org/10.1038/ng1800>

Smyth, D. J., Cooper, J. D., Howson, J. M. M., Clarke, P., Downes, K., Mistry, T., Stevens, H., Walker, N. M., & Todd, J. A. (2011). FUT2 nonsecretor status links type 1 diabetes susceptibility and resistance to infection. *Diabetes*, *60*(11), 3081–3084. <https://doi.org/10.2337/db11-0638>

Smyth, D. J., Plagnol, V., Walker, N. M., Cooper, J. D., Downes, K., Yang, J. H. M., Howson, J. M. M., Stevens, H., McManus, R., Wijmenga, C., Heap, G. A., Dubois, P. C., Clayton, D. G., Hunt, K. A., van Heel, D. A., & Todd, J. A. (2008). Shared and distinct genetic variants in type 1 diabetes and celiac disease. *The New England Journal of Medicine*, *359*(26), 2767–2777. <https://doi.org/10.1056/NEJMoa0807917>

Söderström, U., Åman, J., & Hjern, A. (2012). Being born in Sweden increases the risk for type 1 diabetes – a study of migration of children to Sweden as a natural experiment. *Acta Paediatrica*, *101*(1), 73–77. <https://doi.org/10.1111/j.1651-2227.2011.02410.x>

Somersalo, O. (1954). Studies of childhood diabetes. I. Incidence in Finland. *Annales Paediatriae Fenniae*, *1*(3), 239–249.

Sørensen, I. M., Joner, G., Jenum, P. A., Eskild, A., Torjesen, P. A., & Stene, L. C. (2011). Maternal serum levels of 25-hydroxy-vitamin D during pregnancy and risk of type 1 diabetes in the offspring. *Diabetes*, *61*(1), 175–178. <https://doi.org/10.2337/db11-0875>

Sosenko, J. M., Geyer, S., Skyler, J. S., Rafkin, L. E., Ismail, H. M., Libman, I. M., Liu, Y.-F., DiMeglio, L. A., Evans-Molina, C., & Palmer, J. P. (2018). The influence of body mass index and age on C-peptide at the diagnosis of type 1 diabetes in children who participated in the diabetes prevention trial-type 1. *Pediatric Diabetes*, *19*(3), 403–409. <https://doi.org/10.1111/pedi.12609>

Sosenko, J. M., Skyler, J. S., Beam, C. A., Krischer, J. P., Greenbaum, C. J., Mahon, J., Rafkin, L. E., Matheson, D., Herold, K. C., & Palmer, J. P. (2013). Acceleration of the loss of the first-phase insulin response during the progression to type 1 diabetes in Diabetes Prevention Trial – type 1 participants. *Diabetes*, *62*(12), 4179–4183. <https://doi.org/10.2337/db13-0656>

Srikanta, S., Ganda, O. P., Gleason, R. E., Jackson, R. A., Soeldner, J. S., & Eisenbarth, G. S. (1984). Pre-type I diabetes: Linear loss of beta cell response to intravenous glucose. *Diabetes*, *33*(8), 717–720. <https://doi.org/10.2337/diab.33.8.717>

Starr, T. K., Jameson, S. C., & Hogquist, K. A. (2003). Positive and negative selection of T cells. *Annual Review of Immunology*, *21*(1), 139–176. <https://doi.org/10.1146/annurev.immunol.21.120601.141107>

Stead, J. D. H., Buard, J., Todd, J. A., & Jeffreys, A. J. (2000). Influence of allele lineage on the role of the insulin minisatellite in susceptibility to type 1 diabetes. *Human Molecular Genetics*, *9*(20), 2929–2935. <https://doi.org/10.1093/hmg/9.20.2929>

Stead, J. D. H., Hurles, M. E., & Jeffreys, A. J. (2003). Global haplotype diversity in the human insulin gene region. *Genome Research*, *13*(9), 2101–2111. <https://doi.org/10.1101/gr.948003>

Stechova, K., Kolar, M., Blatny, R., Halbhuber, Z., Vcelakova, J., Hubackova, M., Petruzelkova, L., Sumnik, Z., Obermannova, B., Pithova, P., Stavikova, V., Krivjanska, M., Neuwirth, A., Kolouskova, S., & Filipp, D. (2012). Healthy first-degree relatives of patients with type 1 diabetes exhibit significant differences in basal gene expression pattern of immunocompetent cells compared to controls: Expression pattern as predeterminant of autoimmune diabetes. *Scandinavian Journal of Immunology*, *75*(2), 210–219. <https://doi.org/10.1111/j.1365-3083.2011.02637.x>

Steck, A. K., Bugawan, T. L., Valdes, A. M., Emery, L. M., Blair, A., Norris, J. M., Redondo, M. J., Babu, S. R., Erlich, H. A., Eisenbarth, G. S., & Rewers, M. J. (2005). Association of non-HLA genes with type 1 diabetes autoimmunity. *Diabetes*, *54*(8), 2482–2486. <https://doi.org/10.2337/diabetes.54.8.2482>

Steck, A. K., Dong, F., Wong, R., Fouts, A., Liu, E., Romanos, J., Wijmenga, C., Norris, J. M., & Rewers, M. J. (2014). Improving prediction of type 1 diabetes by testing non-HLA genetic variants in addition to HLA markers. *Pediatric Diabetes*, *15*(5), 355–362. <https://doi.org/10.1111/pedi.12092>

Steck, A. K., Vehik, K., Bonifacio, E., Lernmark, A., Ziegler, A.-G., Hagopian, W. A., She, J., Simell, O., Akolkar, B., Krischer, J., Schatz, D., Rewers, M. J., & the TEDDY Study Group. (2015). Predictors of progression from the appearance of islet autoantibodies to early childhood diabetes: The Environmental Determinants of Diabetes in The Young (TEDDY). *Diabetes Care*, *38*(5), 808–813. <https://doi.org/10.2337/dc14-2426>

Steck, A. K., Wong, R., Wagner, B., Johnson, K., Liu, E., Romanos, J., Wijmenga, C., Norris, J. M., Eisenbarth, G. S., & Rewers, M. J. (2012). Effects of non-HLA gene polymorphisms on development of islet autoimmunity and type 1 diabetes in a population with high-risk HLA-DR,DQ genotypes. *Diabetes*, *61*(3), 753–758. <https://doi.org/10.2337/db11-1228>

Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., Ross, M. C., Lloyd, R. E., Doddapaneni, H., Metcalf, G. A., Muzny, D., Gibbs, R. A., Vatanen, T., Huttenhower, C., Xavier, R. J., Rewers, M., Hagopian, W., Toppari, J., Ziegler, A.-G., …

Petrosino, J. F. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, *562*(7728), 583–588. <https://doi.org/10.1038/s41586-018-0617-x>

Stinson L. F., Boyce M. C., Payne M. S. and Keelan J. A. (2019). The Not-so-Sterile Womb: Evidence That the Human Fetus Is Exposed to Bacteria Prior to Birth. *Frontiers in Microbiology, 10*, 1124. <https://doi.org/10.3389/fmicb.2019.01124>

Strachan, D. P. (1989). Hay fever, hygiene, and household size. *British Medical Journal*, *299*(6710), 1259–1260. <https://doi.org/10.1136/bmj.299.6710.1259>

Takeuchi, O., & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell*, *140*(6), 805–820. <https://doi.org/10.1016/j.cell.2010.01.022>

Tang, S., Peng, W., Wang, C., Tang, H., & Zhang, Q. (2012). Association of the PTPN22 gene (+1858C/T, −1123G/C) polymorphisms with type 1 diabetes mellitus: A systematic review and meta-analysis. *Diabetes Research and Clinical Practice*, *97*(3), 446–452. <https://doi.org/10.1016/j.diabres.2012.04.011>

Tapia, G., Størdal, K., Mårild, K., Kahrs, C. R., Skrivarhaug, T., Njølstad, P. R., Joner, G., & Stene, L. C. (2018). Antibiotics, acetaminophen and infections during prenatal and early life in relation to type 1 diabetes. *International Journal of Epidemiology*, *47*(5), 1538–1548. <https://doi.org/10.1093/ije/dyy092>

Ternák, G., Németh, M., Rozanovic, M., & Bogár, L. (2022). Antibiotic consumption patterns in European countries might be associated with the prevalence of type 1 and 2 diabetes. *Frontiers in Endocrinology*, *13*. <https://doi.org/10.3389/fendo.2022.870465>

Terveyden ja hyvinvoinnin laitos. (2022, August 10). *Kansallinen rokotusohjelma*. Terveyden ja hyvinvoinnin laitos. <https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/tietoa-rokotuksista/kansallinen-rokotusohjelma>

Tettey, P., Simpson, S., Taylor, B. V., & van der Mei, I. A. F. (2015). The co-occurrence of multiple sclerosis and type 1 diabetes: Shared aetiologic features and clinical implication for MS aetiology. *Journal of the Neurological Sciences*, *348*(1), 126–131. <https://doi.org/10.1016/j.jns.2014.11.019>

The International Multiple Sclerosis Genetics Consortium. (2007). Risk alleles for multiple sclerosis identified by a genomewide study. *The New England Journal of Medicine*, *357*(9), 851–862. <https://doi.org/10.1056/NEJMoa073493>

Theofilopoulos, A. N., Kono, D. H., & Baccala, R. (2017). The multiple pathways to autoimmunity. *Nature Immunology*, *18*(7), 716–724. <https://doi.org/10.1038/ni.3731>

Thomson, G., Robinson, W. P., Kuhner, M. K., Joe, S., MacDonald, M. J., Gottschall, J. L., Barbosa, J., Rich, S. S., Bertrams, J., Baur, M. P., Partanen, J., Tait, B. D., Schober, E., Mayr, W. R., Ludvigsson, J., Lindblom, B., Farid, N. R., Thompson, C., & Deschamps, I. (1988). Genetic heterogeneity, modes of inheritance, and risk estimates for a joint study of caucasians with insulin-dependent diabetes mellitus. *American Journal of Human Genetics*, *43*(6), 799–816.

Tizaoui, K., Terrazzino, S., Cargnin, S., Lee, K. H., Gauckler, P., Li, H., Shin, J. I., & Kronbichler, A. (2021). The role of PTPN22 in the pathogenesis of autoimmune diseases: A comprehensive review. *Seminars in Arthritis and Rheumatism*, *51*(3), 513–522. <https://doi.org/10.1016/j.semarthrit.2021.03.004>

Todd, J. A. (2010). Etiology of type 1 diabetes. *Immunity*, *32*(4), 457–467. <https://doi.org/10.1016/j.immuni.2010.04.001>

Todd, J. A., Walker, N. M., Cooper, J. D., Smyth, D. J., Downes, K., Plagnol, V., Bailey, R., Nejentsev, S., Field, S. F., Payne, F., Lowe, C. E., Szeszko, J. S., Hafler, J. P., Zeitels, L., Yang, J. H. M., Vella, A., Nutland, S., Stevens, H. E., Schuilenburg, H., … Clayton, D. G. (2007). Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nature Genetics*, *39*(7), 857–864. <https://doi.org/10.1038/ng2068>

Törn, C., Hadley, D., Lee, H.-S., Hagopian, W., Lernmark, Å., Simell, O., Rewers, M., Ziegler, A., Schatz, D., Akolkar, B., Onengut-Gumuscu, S., Chen, W.-M., Toppari, J., Mykkänen, J., Ilonen, J., Rich, S. S., She, J.-X., Steck, A. K., Krischer, J., & the TEDDY Study Group. (2014). Role of type 1 diabetes–associated SNPs on risk of autoantibody positivity in the TEDDY Study. *Diabetes*, *64*(5), 1818–1829. <https://doi.org/10.2337/db14-1497>

Tosato, F., Bucciol, G., Pantano, G., Putti, M. C., Sanzari, M. c., Basso, G., & Plebani, M. (2015). Lymphocytes subsets reference values in childhood. *Cytometry Part A*, *87*(1), 81–85. <https://doi.org/10.1002/cyto.a.22520>

Triolo, T. M., Pyle, L., Broncucia, H., Armstrong, T., Yu, L., Gottlieb, P. A., & Steck, A. K. (2021). Association of high-affinity autoantibodies with type 1 diabetes high-risk HLA haplotypes. *The Journal of Clinical Endocrinology & Metabolism*, dgab853. <https://doi.org/10.1210/clinem/dgab853>

Tuomilehto, J. (2013). The emerging global epidemic of type 1 diabetes. *Current Diabetes Reports*, *13*(6), 795–804. <https://doi.org/10.1007/s11892-013-0433-5>

Turley, S., Poirot, L., Hattori, M., Benoist, C., & Mathis, D. (2003). Physiological β cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. *Journal of Experimental Medicine*, *198*(10), 1527–1537. <https://doi.org/10.1084/jem.20030966>

Turtinen, M., Härkönen, T., Ilonen, J., Parkkola, A., Knip, M., & the Finnish Pediatric Diabetes Register. (2022). Seasonality in the manifestation of type 1 diabetes varies according to age at diagnosis in Finnish children. *Acta Paediatrica*, *111*(5). <https://doi.org/10.1111/apa.16282>

Unanue, E. R., Ferris, S. T., & Carrero, J. A. (2016). The role of islet antigen presenting cells and the presentation of insulin in the initiation of autoimmune diabetes in the NOD mouse. *Immunological Reviews*, *272*(1), 183–201. <https://doi.org/10.1111/imr.12430>

Uusitalo, U., Liu, X., Yang, J., Aronsson, C. A., Hummel, S., Butterworth, M., Lernmark, Å., Rewers, M., Hagopian, W., She, J.-X., Simell, O., Toppari, J., Ziegler, A. G., Akolkar, B., Krischer, J., Norris, J. M., Virtanen, S. M., & for the TEDDY Study Group. (2016). Association of Early Exposure of Probiotics and Islet Autoimmunity in the TEDDY Study. *JAMA Pediatrics*, *170*(1), 20–28. <https://doi.org/10.1001/jamapediatrics.2015.2757>

Vaarala, O., Knip, M., Paronen, J., Hämäläinen, A. M., Muona, P., Väätäinen, M., Ilonen, J., Simell, O., & Akerblom, H. K. (1999). Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes*, *48*(7), 1389–1394. <https://doi.org/10.2337/diabetes.48.7.1389>

Vafiadis, P., Bennett, S. T., Todd, J. A., Nadeau, J., Grabs, R., Goodyer, C. G., Wickramasinghe, S., Colle, E., & Polychronakos, C. (1997). Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nature Genetics*, *15*(3), 289–292. <https://doi.org/10.1038/ng0397-289>

Valta, M., Gazali, A. M., Viisanen, T., Ihantola, E.-L., Ekman, I., Toppari, J., Knip, M., Veijola, R., Ilonen, J., Lempainen, J., & Kinnunen, T. (2020). Type 1 diabetes linked PTPN22 gene polymorphism is associated with the frequency of circulating regulatory T cells. *European Journal of Immunology*, *50*(4), 581–588. <https://doi.org/10.1002/eji.201948378>

Valta, M., Yoshihara, M., Einarsdottir, E., Pahkuri, S., Ezer, S., Katayama, S., Knip, M., Veijola, R., Toppari, J., Ilonen, J., Kere, J., & Lempainen, J. (2022). Viral infection-related gene upregulation in monocytes in children with signs of β-cell autoimmunity. *Pediatric Diabetes*, *23*(6), 703–713. <https://doi.org/10.1111/pedi.13346>

Van Laethem, F., Sarafova, S. D., Park, J.-H., Tai, X., Pobezinsky, L., Guinter, T. I., Adoro, S., Adams, A., Sharrow, S. O., Feigenbaum, L., & Singer, A. (2007). Deletion of CD4 and CD8 Coreceptors Permits Generation of αβT Cells that Recognize Antigens Independently of the MHC. *Immunity*, *27*(5), 735-750. <https://doi.org/10.1016/j.immuni.2007.10.007>

Vandewalle, C. L., Falorni, A., Lernmark, Å., Goubert, P., Dorchy, H., Coucke, W., Semakula, C., Van Der Auwera, B., Kaufman, L., Schuit, F. C., Pipeleers, D. G., & Gorus, F. K. (1997). Associations of GAD65- and IA-2-autoantibodies with genetic risk markers in new-onset IDDM patients and their siblings. The Belgian Diabetes Registry. *Diabetes Care*, *20*(10), 1547–1552. <https://doi.org/10.2337/diacare.20.10.1547>

Vandewalle, J., Van der Auwera, B. J., Amin, H., Quartier, E., Desouter, A. K., Tenoutasse, S., Gillard, P., De Block, C., Keymeulen, B., Gorus, F. K., Van de Casteele, M., & the Belgian Diabetes Registry. (2021). Genetic variation at ERBB3/IKZF4 and sexual dimorphism in epitope spreading

in single autoantibody-positive relatives. *Diabetologia*, *64*(11), 2511–2516. <https://doi.org/10.1007/s00125-021-05546-9>

Vang, T., Congia, M., Macis, M. D., Musumeci, L., Orrú, V., Zavattari, P., Nika, K., Tautz, L., Taskén, K., Cucca, F., Mustelin, T., & Bottini, N. (2005). Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nature Genetics*, *37*(12), 1317–1319. <https://doi.org/10.1038/ng1673>

Vang, T., Nielsen, J., & Burn, G. L. (2018). A switch-variant model integrates the functions of an autoimmune variant of the phosphatase PTPN22. *Science Signaling*, *11*(526), eaat0936. <https://doi.org/10.1126/scisignal.aat0936>

Vangay, P., Ward, T., Gerber, J. S., & Knights, D. (2015). Antibiotics, pediatric dysbiosis, and disease. *Cell Host & Microbe*, *17*(5), 553–564. <https://doi.org/10.1016/j.chom.2015.04.006>

Vaziri-Sani, F., Delli, A. J., Elding-Larsson, H., Lindblad, B., Carlsson, A., Forsander, G., Ivarsson, S. A., Ludvigsson, J., Marcus, C., & Lernmark, Å. (2011). A novel triple mix radiobinding assay for the three ZnT8 (ZnT8-RWQ) autoantibody variants in children with newly diagnosed diabetes. *Journal of Immunological Methods*, *371*(1), 25–37. <https://doi.org/10.1016/j.jim.2011.06.011>

Vecchione, A., Jofra, T., Gerosa, J., Shankwitz, K., Di Fonte, R., Galvani, G., Ippolito, E., Cicalese, M. P., Schultz, A. R., Seay, H. R., Favellato, M., Milardi, G., Stabilini, A., Ragogna, F., Grogan, P., Bianconi, E., Laurenzi, A., Caretto, A., Nano, R., … Fousteri, G. (2021). Reduced follicular regulatory T cells in spleen and pancreatic lymph nodes of patients with type 1 diabetes. *Diabetes*, *70*(12), 2892–2902. <https://doi.org/10.2337/db21-0091>

Vehik, K., Bonifacio, E., Lernmark, Å., Yu, L., Williams, A., Schatz, D., Rewers, M., She, J.-X., Toppari, J., Hagopian, W., Akolkar, B., Ziegler, A. G., Krischer, J. P., & The TEDDY Study Group. (2020). Hierarchical order of distinct autoantibody spreading and progression to type 1 diabetes in the TEDDY study. *Diabetes Care*, *43*(9), 2066–2073. <https://doi.org/10.2337/dc19-2547>

Veijola, R., Reijonen, H., Vähäsalo, P., Sabbah, E., Kulmala, P., Ilonen, J., Akerblom, H. K., & Knip, M. (1996). HLA-DQB1-defined genetic susceptibility, beta cell autoimmunity, and metabolic characteristics in familial and nonfamilial insulin-dependent diabetes mellitus. Childhood Diabetes in Finland (DiMe) Study Group. *The Journal of Clinical Investigation*, *98*(11), 2489–2495. <https://doi.org/10.1172/JCI119067>

Vella, A., Cooper, J. D., Lowe, C. E., Walker, N., Nutland, S., Widmer, B., Jones, R., Ring, S. M., McArdle, W., Pembrey, M. E., Strachan, D. P., Dunger, D. B., Rebecca Twells, C. J., Clayton, D. G., & Todd, J. A. (2005). Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. *The American Journal of Human Genetics*, *76*(5), 773–779. <https://doi.org/10.1086/429843>

Viisanen, T., Gazali, A. M., Ihantola, E.-L., Ekman, I., Näntö-Salonen, K., Veijola, R., Toppari, J., Knip, M., Ilonen, J., & Kinnunen, T. (2019). FOXP3+ regulatory t cell compartment is altered in children with newly diagnosed type 1 diabetes but not in autoantibody-positive at-risk children. *Frontiers in Immunology*, *10*, 19. <https://doi.org/10.3389/fimmu.2019.00019>

Viisanen, T., Ihantola, E.-L., Näntö-Salonen, K., Hyöty, H., Nurminen, N., Selvenius, J., Juutilainen, A., Moilanen, L., Pihlajamäki, J., Veijola, R., Toppari, J., Knip, M., Ilonen, J., & Kinnunen, T. (2016). Circulating CXCR5+PD-1+ICOS+ follicular T helper cells are increased close to the diagnosis of type 1 diabetes in children with multiple autoantibodies. *Diabetes*, *Journal Article*, db160714. <https://doi.org/10.2337/db16-0714>

Viken, M. K., Amundsen, S. S., Kvien, T. K., Boberg, K. M., Gilboe, I. M., Lilleby, V., Sollid, L. M., Foerre, O., Thorsby, E., Smerdel, A., & Lie, B. A. (2005). Association analysis of the 1858C> T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases. *Genes and Immunity*, *6*(3), 271–273. <https://doi.org/10.1038/sj.gene.6364178>

Virtanen, S. M., Takkinen, H.-M., Nevalainen, J., Kronberg-Kippilä, C., Salmenhaara, M., Uusitalo, L., Kenward, M. G., Erkkola, M., Veijola, R., Simell, O., Ilonen, J., & Knip, M. (2011). Early introduction of root vegetables in infancy associated with advanced ß-cell autoimmunity in young

children with human leukocyte antigen-conferred susceptibility to Type 1 diabetes. *Diabetic Medicine*, *28*(8), 965–971. <https://doi.org/10.1111/j.1464-5491.2011.03294.x>

Viskari, H. R., Koskela, P., Lönnrot, M., Luonuansuu, S., Reunanen, A., Baer, M., & Hyöty, H. (2000). Can enterovirus infections explain the increasing incidence of type 1 diabetes? *Diabetes Care*, *23*(3), 414–416. <https://doi.org/10.2337/diacare.23.3.414>

Wahlberg, J., Vaarala, O., Ludvigsson, J., & the ABIS-study Group. (2006). Dietary risk factors for the emergence of type 1 diabetes-related autoantibodies in 2½-year-old Swedish children. *British Journal of Nutrition*, *95*(3), 603–608. <https://doi.org/10.1079/BJN20051676>

Walter, M., Albert, E., Conrad, M., Keller, E., Hummel, M., Ferber, K., Barratt, B. J., Todd, J. A., Ziegler, A.-G., & Bonifacio, E. (2003). IDDM2/insulin VNTR modifies risk conferred by IDDM1/HLA for development of Type 1 diabetes and associated autoimmunity. *Diabetologia*, *46*(5), 712–720. <https://doi.org/10.1007/s00125-003-1082-z>

Wang, D., & Pan, G. (2019). The Association between rs2292239 Polymorphism in ERBB3 Gene and Type 1 Diabetes: A Meta-Analysis. *BioMed Research International*, *2019*, 7689642. <https://doi.org/10.1155/2019/7689642>

Wang, H., Jin, Y., Reddy, M. V. P. L., Podolsky, R., Liu, S., Yang, P., Bode, B., Reed, J. C., Steed, R. D., Anderson, S. W., Steed, L., Hopkins, D., Huang, Y., & She, J.-X. (2010). Genetically dependent ERBB3 expression modulates antigen presenting cell function and type 1 diabetes risk. *PLOS ONE*, *5*(7), e11789. <https://doi.org/10.1371/journal.pone.0011789>

Wang, K., Ye, F., Chen, Y., Xu, J., Zhao, Y., Wang, Y., & Lan, T. (2021). Association between enterovirus infection and type 1 diabetes risk: A meta-analysis of 38 case-control studies. *Frontiers in Endocrinology*, *12*, 706964–706964. PubMed. <https://doi.org/10.3389/fendo.2021.706964>

Wardemann, H., Yurasov, S., Schaefer, A., Young, J. W., Meffre, E., & Nussenzweig, M. C. (2003). Predominant autoantibody production by early human B cell precursors. *Science*, *301*(5638), 1374–1377. <https://doi.org/10.1126/science.1086907>

Wenzlau, J. M., Juhl, K., Yu, L., Moua, O., Sarkar, S. A., Gottlieb, P., Rewers, M., Eisenbarth, G. S., Jensen, J., Davidson, H. W., & Hutton, J. C. (2007). The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(43), 17040–17045. <https://doi.org/10.1073/pnas.0705894104>

Wenzlau, J. M., Liu, Y., Yu, L., Moua, O., Fowler, K. T., Rangasamy, S., Walters, J., Eisenbarth, G. S., Davidson, H. W., & Hutton, J. C. (2008). A common nonsynonymous single nucleotide polymorphism in the Slc30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. *Diabetes*, *57*(10), 2693–2697. <https://doi.org/10.2337/db08-0522>

Wieczorek, M., Abualrous, E. T., Sticht, J., Álvaro-Benito, M., Stolzenberg, S., Noé, F., & Freund, C. (2017). Major histocompatibility complex (MHC) class I and MHC class II proteins: Conformational plasticity in antigen presentation. *Frontiers in Immunology*, *8*. <https://doi.org/10.3389/fimmu.2017.00292>

Wiede, F., Brodnicki, T. C., Goh, P. K., Leong, Y. A., Jones, G. W., Yu, D., Baxter, A. G., Jones, S. A., Kay, T. W. H., & Tiganis, T. (2019). T-cell–specific PTPN2 deficiency in nod mice accelerates the development of type 1 diabetes and autoimmune comorbidities. *Diabetes*, *68*(6), 1251–1266. <https://doi.org/10.2337/db18-1362>

Wiede, F., Sacirbegovic, F., Leong, Y. A., Yu, D., & Tiganis, T. (2017). PTPN2-deficiency exacerbates T follicular helper cell and B cell responses and promotes the development of autoimmunity. *Journal of Autoimmunity*, *76*, 85–100. <https://doi.org/10.1016/j.jaut.2016.09.004>

Wiede, F., Ziegler, A., Zehn, D., & Tiganis, T. (2014). PTPN2 restrains CD8+ T cell responses after antigen cross-presentation for the maintenance of peripheral tolerance in mice. *Journal of Autoimmunity*, *53*, 105–114. <https://doi.org/10.1016/j.jaut.2014.05.008>

Wiedeman, A. E., Muir, V. S., Rosasco, M. G., DeBerg, H. A., Presnell, S., Haas, B., Dufort, M. J., Speake, C., Greenbaum, C. J., Serti, E., Nepom, G. T., Blahnik, G., Kus, A. M., James, E. A., Linsley, P. S., & Long, S. A. (2020). Autoreactive CD8+ T cell exhaustion distinguishes subjects

with slow type 1 diabetes progression. *The Journal of Clinical Investigation*, *130*(1), 480–490. <https://doi.org/10.1172/JCI126595>

Willcox, A., Richardson, S. J., Bone, A. J., Foulis, A. K., & Morgan, N. G. (2009). Analysis of islet inflammation in human type 1 diabetes. *Clinical & Experimental Immunology*, *155*(2), 173–181. <https://doi.org/10.1111/j.1365-2249.2008.03860.x>

Williams, A. J. K., Bingley, P. J., Bonifacio, E., Palmer, J. P., & Gale, E. A. M. (1997). A Novel Micro-assay for Insulin Autoantibodies. *Journal of Autoimmunity*, *10*(5), 473–478. <https://doi.org/10.1006/jaut.1997.0154>

Winkler, C., Krumsiek, J., Buettner, F., Angermüller, C., Giannopoulou, E. Z., Theis, F. J., Ziegler, A.-G., & Bonifacio, E. (2014). Feature ranking of type 1 diabetes susceptibility genes improves prediction of type 1 diabetes. *Diabetologia*, *57*(12), 2521–2529. <https://doi.org/10.1007/s00125-014-3362-1>

Writing Group for the TRIGR Study Group. (2018). Effect of hydrolyzed infant formula vs conventional formula on risk of type 1 diabetes: The TRIGR randomized clinical trial. *Journal of the American Medical Association*, *319*(1), 38–48. <https://doi.org/10.1001/jama.2017.19826>

Wu, D. J., Zhou, W., Enouz, S., Orrú, V., Stanford, S. M., Maine, C. J., Rapini, N., Sawatzke, K., Engel, I., Fiorillo, E., Sherman, L. A., Kronenberg, M., Zehn, D., Peterson, E., & Bottini, N. (2014). Autoimmunity-associated LYP-W620 does not impair thymic negative selection of autoreactive T cells. *PLoS ONE*, *9*(2), e86677. <https://doi.org/10.1371/journal.pone.0086677>

Xhonneux, L.-P., Knight, O., Lernmark, Å., Bonifacio, E., Hagopian, W. A., Rewers, M. J., She, J.-X., Toppari, J., Parikh, H., Smith, K. G. C., Ziegler, A.-G., Akolkar, B., Krischer, J. P., & McKinney, E. F. (2021). Transcriptional networks in at-risk individuals identify signatures of type 1 diabetes progression. *Science Translational Medicine*, *13*(587), eabd5666. PubMed. <https://doi.org/10.1126/scitranslmed.abd5666>

Xi, Y., Liu, S., Bettaieb, A., Matsuo, K., Matsuo, I., Hosein, E., Chahed, S., Wiede, F., Zhang, S., Zhang, Z.-Y., Kulkarni, R. N., Tiganis, T., & Haj, F. G. (2015). Pancreatic T cell protein–tyrosine phosphatase deficiency affects beta cell function in mice. *Diabetologia*, *58*(1), 122–131. <https://doi.org/10.1007/s00125-014-3413-7>

Yang, M., Ye, L., Wang, B., Gao, J., Liu, R., Hong, J., Wang, W., Gu, W., & Ning, G. (2015). Decreased miR-146 expression in peripheral blood mononuclear cells is correlated with ongoing islet autoimmunity in type 1 diabetes patients. *Journal of Diabetes*, *7*(2), 158–165. <https://doi.org/10.1111/1753-0407.12163>

Yang, S., Zhao, B., Zhang, Z., Dai, X., Zhang, Y., & Cui, L. (2022). Association between enterovirus infection and clinical type 1 diabetes mellitus: Systematic review and meta-analysis of observational studies. *Epidemiology & Infection*, *150*, e23. <https://doi.org/10.1017/S0950268821002442>

Yang, Z., Chen, M., Ellett, J. D., Fialkow, L. B., Carter, J. D., McDuffie, M., & Nadler, J. L. (2004). Autoimmune diabetes is blocked in Stat4-deficient mice. *Journal of Autoimmunity*, *22*(3), 191–200. <https://doi.org/10.1016/j.jaut.2003.08.006>

Yeo, L., Woodwyk, A., Sood, S., Lorenc, A., Eichmann, M., Pujol-Autonell, I., Melchiotti, R., Skowera, A., Fidanis, E., Dolton, G. M., Tungatt, K., Sewell, A. K., Heck, S., Saxena, A., Beam, C. A., & Peakman, M. (2018). Autoreactive T effector memory differentiation mirrors β-cell function in type 1 diabetes. *The Journal of Clinical Investigation*, *Journal Article*. <https://doi.org/10.1172/JCI120555>

Ygberg, S., & Nilsson, A. (2012). The developing immune system – from foetus to toddler. *Acta Paediatrica*, *101*(2), 120–127. <https://doi.org/10.1111/j.1651-2227.2011.02494.x>

Ylipaasto, P., Klingel, K., Lindberg, A. M., Otonkoski, T., Kandolf, R., Hovi, T., & Roivainen, M. (2004). Enterovirus infection in human pancreatic islet cells, islet tropism in vivo and receptor involvement in cultured islet beta cells. *Diabetologia*, *47*(2), 225–239. <https://doi.org/10.1007/s00125-003-1297-z>

Yoneda, S., Imagawa, A., Fukui, K., Uno, S., Kozawa, J., Sakai, M., Yumioka, T., Iwahashi, H., & Shimomura, I. (2017). A histological study of fulminant type 1 diabetes mellitus related to human

cytomegalovirus reactivation. *The Journal of Clinical Endocrinology & Metabolism*, *102*(7), 2394–2400. <https://doi.org/10.1210/jc.2016-4029>

Zemmour, D., Zilionis, R., Kiner, E., Klein, A. M., Mathis, D. & Benoist, C. (2018). Single-cell gene expression reveals a landscape of regulatory T cell phenotypes shaped by the TCR. *Nature Immunology, 19*, 291–301 <https://doi.org/10.1038/s41590-018-0051-0>

Zhang, J., Zahir, N., Jiang, Q., Miliotis, H., Heyraud, S., Meng, X., Dong, B., Xie, G., Qiu, F., Hao, Z., McCulloch, C. A., Keystone, E. C., Peterson, A. C., & Siminovitch, K. A. (2011). The autoimmune disease–associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness. *Nature Genetics*, *43*, 902–907. <https://doi.org/10.1038/ng.904>

Zhang, Y., Zhang, J., Shi, Y., Shen, M., Lv, H., Chen, S., Feng, Y., Chen, H., Xu, X., Yang, T., & Xu, K. (2021). Differences in maturation status and immune phenotypes of circulating Helios+ and Helios− Tregs and their disrupted correlations with monocyte subsets in autoantibody-positive T1D individuals. *Frontiers in Immunology*, *12*. <https://doi.org/10.3389/fimmu.2021.628504>

Zhao, Q., & Elson, C. O. (2018). Adaptive immune education by gut microbiota antigens. *Immunology*, *154*(1), 28–37. <https://doi.org/10.1111/imm.12896>

Zheng, P., & Kissler, S. (2013). Ptpn22 silencing in the NOD model indicates the type 1 diabetes–associated allele is not a loss-of-function variant. *Diabetes*, *62*(3), 896–904. <https://doi.org/10.2337/db12-0929>

Ziegler, A. G., Rewers, M., Simell, O., Simell, T., Lempainen, J., Steck, A., Winkler, C., Ilonen, J., Veijola, R., Knip, M., Bonifacio, E., & Eisenbarth, G. S. (2013). Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *Journal of the American Medical Association*, *309*(23), 2473–2479. <https://doi.org/10.1001/jama.2013.6285>

Ziegler, A. G., Standl, E., Albert, E., & Mehnert, H. (1991). HLA-associated insulin autoantibody formation in newly diagnosed type I diabetic patients. *Diabetes*, *40*(9), 1146–1149. <https://doi.org/10.2337/diab.40.9.1146>

Ziegler, A.-G., Bonifacio, E., & the BABYDIAB-BABYDIET Study Group. (2012). Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. *Diabetologia*, *55*(7), 1937–1943. <https://doi.org/10.1007/s00125-012-2472-x>

Ziegler, A.-G., Schmid, S., Huber, D., Hummel, M., & Bonifacio, E. (2003). Early Infant Feeding and Risk of Developing Type 1 Diabetes–Associated Autoantibodies. *JAMA*, *290*(13), 1721–1728. <https://doi.org/10.1001/jama.290.13.1721>

Zipitis, C. S., & Akobeng, A. K. (2008). Vitamin D supplementation in early childhood and risk of type 1 diabetes: A systematic review and meta-analysis. *Archives of Disease in Childhood*, *93*, 512–517. <http://dx.doi.org/10.1136/adc.2007.128579>

Zóka, A., Barna, G., Nyírő, G., Molnár, Á., Németh, L., Műzes, G., Somogyi, A., & Firneisz, G. (2019). Reduced GLP-1 response to a meal is associated with the CTLA4 rs3087243 G/G genotype. *Central-European Journal of Immunology*, *44*(3), 299–306. <https://doi.org/10.5114/ceji.2019.89604>

TURUN YLIOPISTO
UNIVERSITY OF TURKU

ISBN 978-951-29-9275-1 (PRINT)
ISBN 978-951-29-9276-8 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)

Painosalama, Turku, Finland 2023