Exploring the phenotypes of individuals with midline brain defects

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Declaration

I confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. The ethical application was written and submitted by Dr E Webb. Patients were recruited by Dr E Webb. Anthropometric measurements were performed by a trained auxologist Victor Mead. Anthropometric data were collated and analysed by Dr E Webb. All hormonal assays were performed by the biochemistry laboratory at Great Ormond Street Hospital for Children (GOSH) under the supervision of Dr Helen Aitkenhead. Calculation of centiles for IGF-1 and IGFBP-3 SDS was carried out by Dr Emma Webb under the supervision of Professor Tim Cole. Bone age analysis was performed by Dr Emma Webb and Professor Mehul Dattani. Sleep data was acquired and analysed by Dr E Webb. Melatonin samples were taken by Dr E Webb and melatonin assays performed by Dr Benita Middleton. Endocrine investigations were performed on the inpatient endocrine ward at GOSH as part of routine clinical care. Baseline clinical data (age, sex, socioeconomic status etc) were collected and analysed by Dr E Webb. MRI studies were performed by the MRI department at GOSH. Analysis of the MRI scans was undertaken by Dr E Webb (TBSS, camino, VBM). FreeSurfer was run on the UCL cluster by Dr Jon Clayden. The output was analysed by Dr E Webb. Sleep data were acquired and analysed by Dr E Webb. Behavioural questionnaires were completed by the parents and analysed by Dr E Webb. Cognitive testing was performed by Dr Michelle O'Reilly. Statistical analysis of the cognitive data in relation to the MRI findings, auxological and GH status (IGF-1 and IGFBP-3 SDS) was performed by Dr Emma Webb. Screening for genetic changes in the patients with IGHD was performed by Dr E Webb.

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Background

The prevalence, aetiology and optimal management of the behavioural and cognitive difficulties, and circadian rhythm disturbances in children with midline brain abnormalities including isolated growth hormone deficiency (IGHD), isolated optic nerve hypoplasia (ONH) and septo-optic dysplasia (SOD) have to date not been adequately addressed.

Aims and Methods

This thesis aims to assess the prevalence of cognitive/behavioural problems and circadian rhythm abnormalities in children with midline brain abnormalities, and to further investigate any problems identified using high resolution MRI brain, actigraphy and melatonin profiling.

Results

Children with IGHD have significant impairments in motor skills and lower cognitive function scores, and children with ONH have significantly higher scores on the child behavioural checklist than controls. Children with SOD have significant sleep abnormalities.

In IGHD corticospinal tract and corpus callosum fractional anisotropy (FA) and specific neural volumes are significantly lower than in controls, with neural abnormalities correlating significantly with IQ and motor skills scores. In ONH ventral cingulum, corpus callosum and optic radiation FA are significantly reduced, with right ventral

cingulum FA correlating significantly with behavioural assessment scores. In SOD melatonin production was absent in one child in association with a fragmented sleep pattern. Three children had normal melatonin profiles, one with an arrhythmic and two with fragmented sleep patterns. The remaining child had fragmented sleep and a modest increase in daytime melatonin concentrations.

Conclusions

These studies suggest that the GH-IGF-1 axis plays a role in brain and cognitive development. They also show that children with ONH require behavioural assessment, not previously part of routine clinical care, and that the behavioural abnormalities identified in children with ONH may be related to underlying whiter matter abnormalities. We have also demonstrated that the aetiology of the sleep disturbances found in SOD is complex, and not solely due to abnormal nocturnal melatonin production.

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PUBLICATIONS AND ABSTRACTS

- Webb EA, O'Reilly MA, Clayden JD, Seunarine KK, Dale N, Salt A, Clark CA, Dattani MT. Reduced ventral cingulum integrity and increased behavioural problems in children with isolated optic nerve hypoplasia and mild to moderate or no visual impairment. Accepted for publication *PLOS ONE* Feb 2013.
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- Webb,EA, O'Reilly, MA, Orgill, J, Dale, N, Salt, A, Gringras P, Dattani, MT. Melatonin secretion in children with sleep disturbance and Septo-optic dysplasia

Oral presentation at 37th meeting of the British Society for Paediatric Endocrinology and Diabetes 10 - 12 November 2009, Reading

ABBREVIATIONS

- Adenosine triphosphate (ATP)
- Adrenocorticotropic hormone (ACTH)
- Adult-onset GHD (AO GHD)
- Agenesis of Corpus Callosum (ACC).
- α-Melanocyte-Stimulating Hormone (α-MSH)
- Analysis of Covariance (ANCOVA)
- Anterior Pituitary (Hypoplasia) (AP(H))
- Arginine vasopressin (AVP)
- Attention deficit hyperactivity disorder (ADHD)
- Autistic spectrum disorder (ASD)
- Behaviour Rating Inventory of Executive Function (BRIEF)
- Binding proteins (BPs)
- Body mass index (BMI)
- CANTAB (Cambridge Neuropsychological Test Automated Battery)
- Central nervous system (CNS)
- Cerebro-spinal fluid (CSF)
- Child Communication Checklist (CCC)
- Child behaviour checklist (CBCL)
- Childhood onset growth hormone deficiency (CO-GHD)
- Circadian rhythm sleep disorders (CRSD)
- Coefficients of variation (CVs)
- Diffusion Magnetic Resonance Imaging (dMRI)
- Diffusion tensor imaging (DTI)

- Digit Symbol Test (DST)
- Digit Span Subset (DSS)
- Ectopic Posterior Pituitary (EPP)
- Equivalent degrees of freedom (EDF)
- Evaluation of treatment questionnaire (ETQ)
- Evoked response potential (ERP)
- False discovery rate (FDR)
- Follicle-stimulating hormone (FSH)
- Fractional Anisotropy (FA)
- Family Wise Error (FWE)
- Full-Scale IQ (FSIQ)
- Functional Magnetic Resonance Imaging (fMRI)
- Functional Magnetic Resonance Imaging of the Brain Software Library (FSL)
- Gamma amino butyric acid (GABA)
- Great Ormond Street Children's Hospital (GOSH)
- GH receptor (GHR)
- Growth hormone (GH)
- Growth Hormone Binding Protein (GHBP)
- Growth hormone deficiency (GHD)
- Growth hormone releasing hormone (GHRH)
- Idiopathic short stature (ISS)
- Insulin-like growth factor I (IGF-I)
- Insulin-like growth factor binding protein-3 (IGFBP-3)
- Insulin-like growth factors (IGFs)
- Intelligence quotient (IQ)

- Intramuscular (IM)
- Isolated growth hormone deficiency (IGHD)
- Intrauterine growth retardation (IUGR)
- Locus control region (LCR)
- London Centre for Paediatric Endocrinology (LCPE)
- Long term memory (LTM)
- Luteinising hormone (LH)
- Magnetic Resonance Imaging (MRI)
- Mean diffusivity (MD)
- Melanocyte-stimulating hormone (MSH)
- Movement Assessment Battery for Children 2nd Edition (M-ABC2)
- Multiple pituitary hormone deficiency (MPHD)
- National Institute for Clinical Excellence (NICE)
- A Developmental NEuroPSYchological Assessment (NEPSY)
- Optic nerve hypoplasia (ONH)
- Perceptual Reasoning Index (PRI)
- Processing Speed indices (PSI)
- Prolactin (PRL)
- Pro-opiomelanocortin (POMC)
- Prophet of Pit1 (PROP1)
- QOL-assessment of GHD (QoL-AGHDA)
- Quality of life (QOL)
- Recombinant human growth hormone (rhGH)
- Reynell-Zinkin Scales (RZS)
- Septo-optic dysplasia (SOD)

- Short-term memory (STM)
- Small for gestational age (SGA)
- Social Communication Questionnaire (SCQ)
- Standard deviation scores (SDS)
- Standard Error (SE)
- Statistical parametric map (SPM)
- Suprachiasmatic nucleus (SCN)
- Tract Based Spatial Statistics (TBSS)
- Thyroid hormone (TH)
- Thyroid stimulating hormone (TSH)
- Total brain volume (TBV)
- Verbal Comprehension Index (VCI)
- Visual impairment (VI)
- Voxel Based Morphometry (VBM)
- Weschler Adult intelligence Scale (WAIS)
- Weschler Intelligence Scales for Children IV edition (WISC-IV)
- Weschler Preschool and Primary Scale of Intelligence-Third Edition (WPPSI-III)
- Working Memory Index (WMI)

1. INTRODUCTION

Congenital growth hormone deficiency (GHD) may occur in isolation, or in association with other hormone deficiencies, with or without other brain abnormalities such as underdevelopment of the nerves to the eye and midline forebrain defects (Figure 1). Septo-optic dysplasia (SOD) is a rare congenital anomaly in which children have one or more of the following; underdevelopment of the fore-brain, the nerves to the eyes and the pituitary gland. The pituitary gland produces several different hormones including growth hormone which is necessary for normal growth (Figure 2). We have observed that there is a high incidence of neuro-developmental delay in patients with septo-optic dysplasia with aberrant behaviors that lie within the autistic continuum. Anecdotally, we have also found that children with isolated GHD have developmental delay and behavioural problems. It is unclear whether these difficulties are related to the underlying lack of growth hormone or whether they are related to underlying subtle abnormalities of brain development. That the former may be the case is suggested by parental reports of marked improvement in; strength, behaviour, concentration, energy levels, appetite and school performance in children once they have started on GH treatment. Many of the parents are surprised by the apparent benefits of GH treatment in those areas of functioning that are not normally believed to dependent on GH.

Parents of children with hypopituitarism manage well with the day-to-day medical management and the regular hospital visits that their children require. However, they frequently complain during clinic visits of associated problems which present alongside the underlying medical condition. These include behavioural and cognitive difficulties and circadian rhythm disturbances. The prevalence of these problems, which impact

significantly on the well-being of the child and their family has not clearly been defined and their aetiology is not well understood.

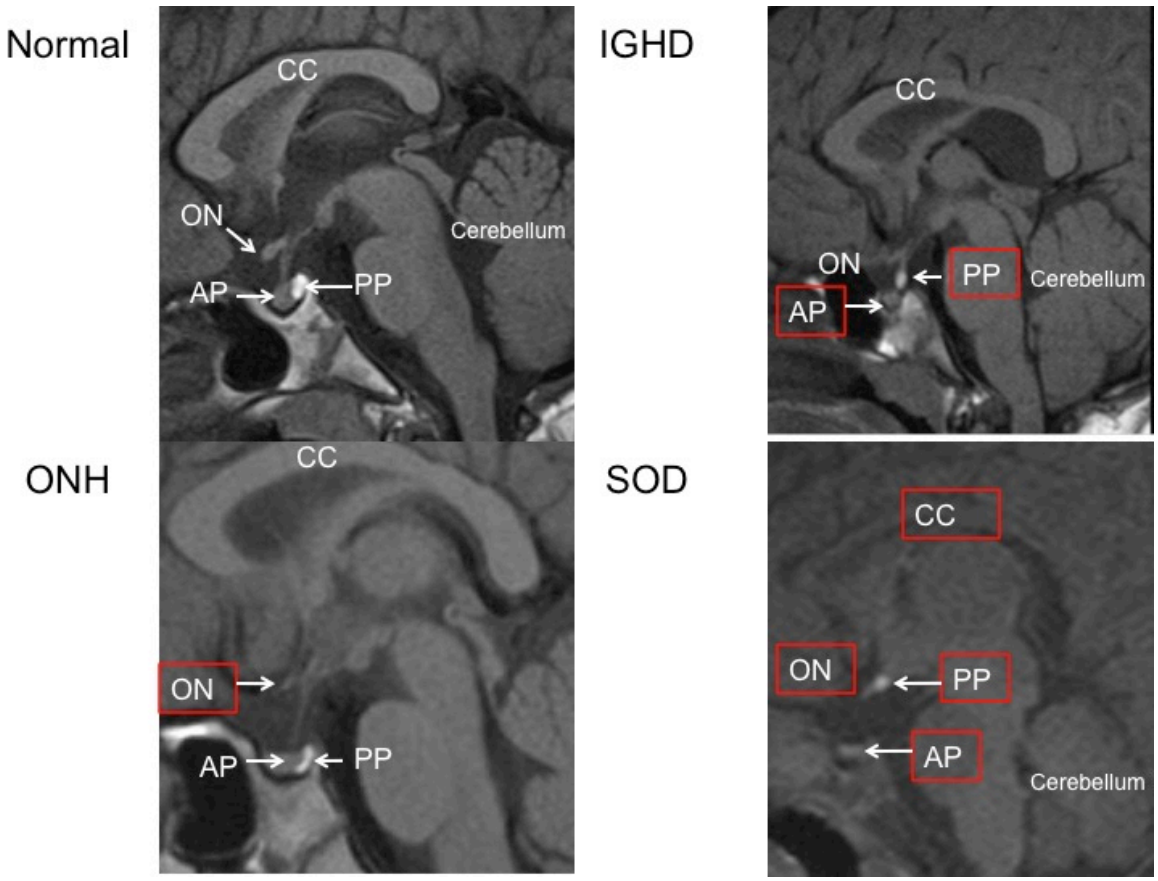
Aristotle first suggested that there may be an association between 'dwarfism' and impaired cognition. Subsequently, many studies, including in vitro and in vivo animal and human studies, have attempted to clarify the exact nature of the cognitive deficit found in individuals with an abnormal growth hormone (GH) axis. Whilst some studies have drawn similar conclusions, there remains no consensus as to whether the GH-Insulin like growth factor-1 (IGF-1) axis plays a significant role in neural development. The advent of high resolution magnetic resonance imaging (MRI) scanning, and the development of computer programmes which enable us to statistically analyse these images in detail, allows us an opportunity to revisit and potentially draw some conclusions in this much disputed area.

In children with septo-optic dysplasia (SOD) there is a high incidence of neuro-developmental delay and behavioural difficulties. The neural basis for these behavioural problems is unknown. Whilst conventional methods of neuroimaging have been unable to detect neural abnormalities, other than optic nerve hypoplasia (ONH) in individuals with isolated ONH, using diffusion tensor imaging (DTI) may offer us further insights into the pathophysiology of the behavioural problems found in children with isolated ONH.

A significant proportion of children with SOD have disordered sleep patterns. This circadian rhythm disturbance impacts significantly both the child's overall health and cognitive function and on the family's ability to manage their child's complex condition. Although melatonin is routinely administered to normalise sleep patterns in children with

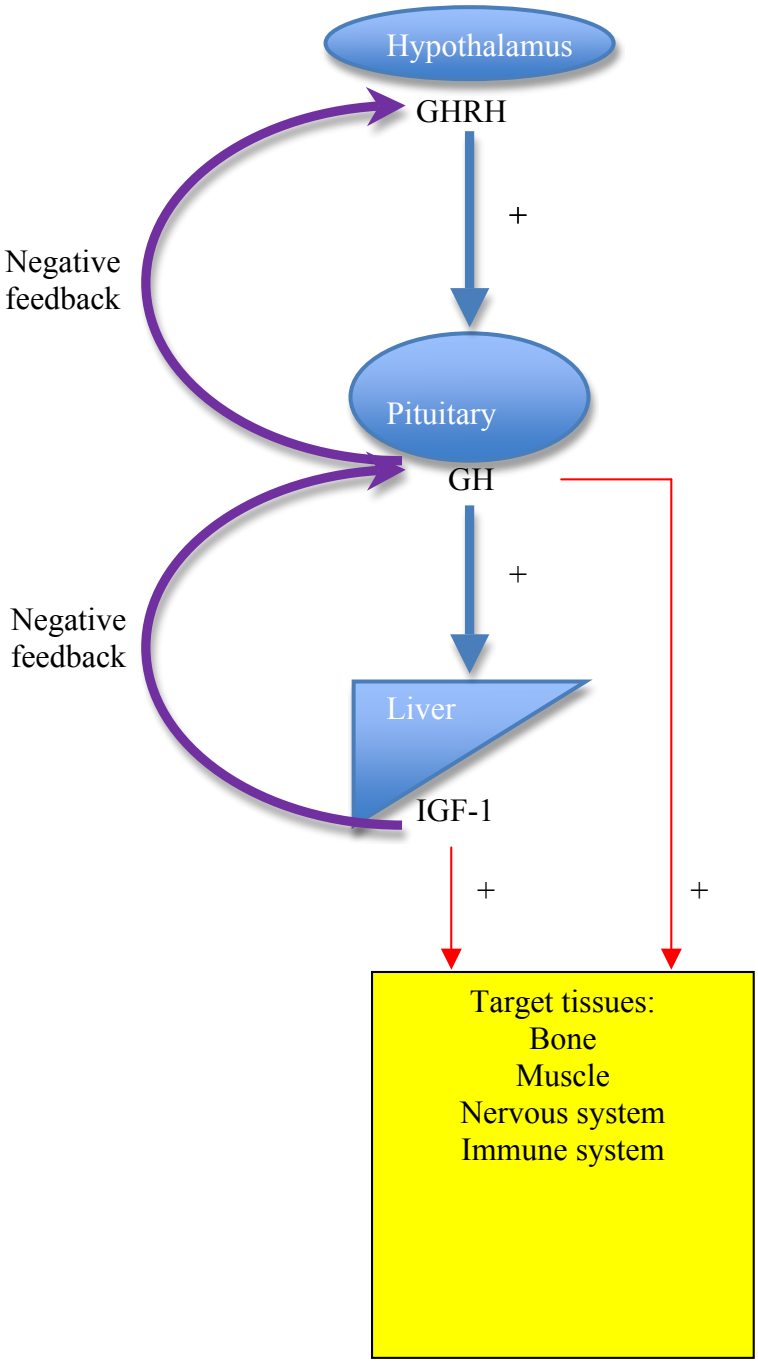
SOD, the pathophysiological basis for the underlying sleep disorder in these children, and consequently the optimum medical management of their problem, remains largely unknown. Using new equipment such as actigraphy, designed to assess circadian rhythms in infants and children, in conjunction with assaying melatonin concentrations, may lead to improved understanding of the mechanisms underlying the disordered sleep patterns in children with SOD.

Figure 1 Midline brain MR Images demonstrating the differing effects of isolated growth hormone deficiency (IGHD), optic nerve hypoplasia (ONH) and septo-optic dysplasia (SOD) on brain structure



Key Figure 1 CC: corpus callosum, ON: optic nerves, PP: posterior pituitary, AP: anterior pituitary, Brain regions highlighted in red structurally abnormal in condition specified in title.

Figure 2 Schematic representation of the growth hormone Insulin-like growth factor axis (Isolated growth hormone deficiency and septo-optic dysplasia disrupt GHRH and GH production, leading to GH and IGF-1 deficiency. Optic nerve hypoplasia has no effect on the GH-IGF-1 axis)



1.1 The role of the growth hormone and Insulin-like growth factor axis in neural development and cognitive function

A. The growth hormone and insulin-like growth factor axis

Growth hormone is an anabolic peptide secreted by the somatotroph cells of the anterior pituitary gland in a pulsatile fashion (1). The main isoform of human GH (75% circulating GH) is a 191 amino acid protein with two disulphide bridges and a molecular weight of 22 kiloDaltons (2). The other major isoform present in the circulation is 20kDa GH (10%), which lacks amino acids 32-46 and is generated by alternative splicing of exon 3 (3). GH has a relatively short half-life of approximately 5 minutes; however, the majority of GH circulates in the plasma bound to a specific high-affinity GH-binding protein (GHBP) (1;4). Bound GH has a slightly longer half-life of around 20 minutes (5). GH mediates its effects by binding to a specific cell surface receptor, the GH receptor (GHR), a 620 amino acid, 70kDa protein (6), forming a complex with two dimerized GHR components; this activates JAK2 tyrosine kinase and leads to the phosphorylation of intracellular signalling molecules (7).

Concentrations of GH are highest approximately 1 hour after sleep begins, with concentrations generally being undetectable during the day (8). In addition to being released during sleep, GH is secreted in response to stimuli including exercise, hypoglycaemia and physical stress, with levels being reduced in the context of psychosocial or emotional deprivation and obesity (9;10). Pulsatility is regulated by the interaction between two main hypothalamic hormones: growth hormone releasing hormone (GHRH), a 44 amino acid protein that stimulates GH secretion, and somatostatin, an inhibitory hormone containing 14 amino acids (4;11). The secretion of

these hypothalamic hormones is further influenced by neurotransmitters and neuropeptides such as dopamine, catecholamines, histamine, serotonin, gamma amino butyric acid (GABA), ghrelin and opiates. GH secretion is also under negative feedback control by itself and by IGF-1, which is generated in response to GH (12). Both GH and growth factors such as IGF-I and IGF-II also negatively feedback on the hypothalamic regulators of GH secretion, whereas sex steroids such as testosterone and oestrogen increase GH synthesis and secretion.

Whilst GH has both anabolic and metabolic actions of its own which include increasing linear growth, fat mobilisation and an increase in muscle mass, most of its effects are mediated through the generation of IGF-1. IGF-1 is a 70 amino acid peptide produced ubiquitously, with the majority of circulating IGF-1 being derived from the liver (4). IGF-1 circulates in the blood either as a free molecule, or bound to specific high affinity binding proteins (BPs) that prolong its half-life and regulate the availability of IGF-1 to specific tissues, modulating IGF-1 effects as well as having actions independent of IGF-1 (13;14). There is little circadian variation in the concentration of plasma IGF-1 (half-life of bound IGF-1: 12-15hrs) and it is therefore a convenient surrogate measure of the overall 24-hr secretion of GH (1;15). IGF-1 mediates many of the growth-promoting effects of GH acting throughout the body to promote somatic growth and to regulate metabolism (16).

B. Growth hormone deficiency

The reported incidence of congenital growth hormone deficiency (GHD) is 1 in 4,000 to 1 in 10,000 live births (17-20). GHD occurs more frequently in males with a 2:1 ratio and cases can be sporadic or familial. Familial cases account for 5-30% of cases (21),

suggesting a genetic aetiology for the condition. GHD can occur in isolation (isolated growth hormone deficiency [IGHD]), in association with other anterior and posterior pituitary hormone deficiencies (multiple pituitary hormone deficiency [MPHD]), and with or without extra-pituitary features such as optic nerve hypoplasia and midline forebrain defects (SOD).

C. Phenotypic variability in growth hormone deficiency

There is significant phenotypic variability both in the severity of GHD, the age at presentation, and in the association with other hormonal and brain abnormalities (22).

IGHD is the commonest pituitary endocrinopathy, with children classically presenting with a reduced height velocity and delayed bone age, frequently in association with a past medical history of neonatal hypoglycaemia, jaundice and micropenis (23). Well-established phenotypic features of IGHD include frontal bossing, midfacial hypoplasia, immature facies, truncal adiposity, fat dimpling, male hypogenitalism, a high-pitched voice and/or other malformations. There is not yet consensus regarding whether there is also a characteristic cognitive and behavioural phenotype in individuals with GHD, the evidence for this association will be discussed in detail later in the introduction.

When designing prospective studies to assess the impact of GH treatment in GHD the considerable degree of controversy regarding the diagnosis of GHD needs to be taken into account. The debate is partly secondary to the continuum which exists between deficiency and normal GH secretion, and also due to the significant difficulties surrounding the interpretation of GH stimulation tests (24). Prior to the introduction of the radioimmunoassay in the mid-1960s the diagnosis was relatively straightforward

being made on a clinical basis only in individuals with severe growth failure and other clinical features supporting a diagnosis of GHD. Subsequently, both clinical criteria (growth velocity, body composition, facial appearance), and biochemical serum GH responses to a variety of provocation tests have been used to define the condition. At present the "gold standard" for the diagnosis of GHD in the UK is defined by the National Institute for Clinical Excellence (NICE) guidelines as a subnormal response to two pharmacological stimulation tests. The majority of the debate pertains to the peak serum GH concentration that should be used to define GHD, as the peak changes according to the assay and stimulation test performed. Severe GHD remains easier to diagnose with the majority of studies concluding that individuals with severe GHD have a more consistent response to provocative testing (24). In addition, when reduced GH production in response to stimulation is associated with either other pituitary hormone abnormalities, clinical signs of GHD such as hypoglycaemia or microphallus, genetic mutations known to cause GHD, or MRI evidence of structural abnormalities of the hypothalamo-pituitary axis, the diagnosis can be made with a greater degree of certainty (24).

D. Short Stature

Children with GHD frequently present to the endocrine clinic with short stature, with the investigations outlined above being used to differentiate between individuals with and endocrine abnormality and those with non-GHD short stature. Children with short stature (secondary to GHD and non-GHD causes) have been found to have reduced IQ when compared to normal stature controls(25), with short stature in childhood being associated with emotional immaturity, behavioural problems, underachievement at school and poor learning skills (26;27). It has previously been postulated that adults and other children

relate to children according to their size rather that their chronological age and short stature can lead to a child being infantilized with consequent impacts on development (28;29). Studies including select groups of children with short stature, such as individuals with GHD therefore need to control for any possible effects of stature on development which may occur independent of the disease process being studied.

E. Evidence for actions of growth hormone and insulin-like growth factor-1 in the central nervous system

Factors including thyroid hormone, cortisol and nutrition (30) have been found to impact significantly on cognition during infancy and childhood, whilst the role of GH on neural development and cognition remains unclear with many incongruous reports.

A large body of evidence suggests that IGF and IGF receptors, and GH and GHR's, are expressed in the parts of the brain responsible for learning and memory. GH is produced both in the pituitary and to a lesser degree by other tissues in the central nervous system (CNS), for example the hippocampus (31;32). There is also evidence to support the transport of GH into the CNS across the blood brain barrier. There is a high density of GHRs in the choroid plexus (33) (34), and several studies report an increase in cerebro-spinal fluid (CSF) IGF-1 and GH after peripheral recombinant human GH (rhGH) administration (35) (36). Peripheral rhGH administration also increases levels of CSF neurotransmitters including vasoactive intestinal peptide, noradrenaline, homovanillic acid (dopamine metabolite) and aspartate (an excitatory amino acid which acts as a ligand for the N- methyl-D-aspartic acid receptor which in turn is involved in memory formation in the hippocampus) (37).

GH binding sites are found on various cell types in the brain including neurons, astrocytes, oligodendrocytes and microglia. In the rodent, GH has been identified in the amygdala, cortex, hippocampus, and thalamus (38), and the GHR in the choroid plexus, cortex, hippocampus, hypothalamus, striatum and spinal cord, with levels being higher in females than in males (39). The human brain has a similar pattern of GHR expression with concentrations being highest in the choroid plexus, thalamus, hypothalamus, pituitary, putamen and hippocampus (40;41). The putamen plays an important role in the processing of social perceptions, with the hippocampal and perihippocampal regions playing roles in learning and memory (42-44). Interestingly GHR expression is two to four-fold higher in the hippocampus than elsewhere in the brain suggesting that GH may play an important role in memory formation. GHR concentration is highest in the fetal and infant brain when they are located specifically in areas known to be actively involved in neurogenesis within the juvenile brain, including the hippocampal dentate gyrus, the olfactory bulb and in the sub-ventricular zone (34;45). Subsequently GHR concentrations decline with increasing age (34;46).

There is accumulating evidence that in addition to GH, IGF-1 also plays an important role in brain growth, development and myelination, and that it has an ongoing role in determining childhood and adult cognitive function (47;48). IGF-1 is actively transported across the blood brain barrier (49) acting to stimulate the viability and function of a variety of different neuronal cell-types through the IGF receptor family (50). It promotes neuronal DNA synthesis in addition to myelination, stabilises tubulin mRNA, enhances oligodendrocyte proliferation, increases neuron and glia survival and neuromuscular synaptogenesis (51;52). IGF-1 stimulates neuronal acetylcholine release (53) and activates the NMDA receptor. In addition to its neurotrophic effects it also has several

neuroprotective effects, limiting neuronal loss after ischaemic injury (54) and improving neurological functioning in rats when administered after spinal cord injury (55;56). IGF-1 gene expression has been found in humans in the hypothalamus, hippocampus, olfactory bulb, cerebellum, neocortex and striatum (51) with IGF-1 receptors being most dense in the hippocampus, amygdala, caudate nucleus, cortex, cerebellum, prefrontal and parahippocampal cortex (57). Together these regions make up the neural circuit known as the social brain (58).

The importance of GH and IGF-1 in neural development is highlighted by findings in GH and IGF-1 deficient animal and human disease models. Transgenic mice over-expressing IGF-1 have increased brain size with a marked increase in myelin content, whereas mice with a targeted IGF-1 gene deletion have reduced brain size (59). *GHR* knockout mice are of a normal size at birth, *IGF-1* knockout mice are 60% of their normal birth weight, whereas mice lacking both *IGF-I* and *GHR* are only 17% of their normal size at birth (60). These findings suggest that whilst IGF-I is an important fetal growth factor, GH also has some growth-promoting effects independent of IGF-I. Human disease models also exist where either the IGF-I gene is deleted or there is impairment in the functioning of the GHR leading to reduced production of IGF-1. Deletion of the insulin-like growth factor I gene is universally associated with significant intrauterine growth retardation, microcephaly, significant cognitive impairment and postnatal growth failure (48). On the other hand, individuals with *GHR* mutations have varying cognitive phenotypes. Guevara-Aguirre et al have reported on an Ecuadorian population with a single-point mutation (E180 splice mutation) in the GHR preventing dimerisation and normal functioning (56). In this population, who have elevated concentrations of GH but

undetectable IGF-1, intelligence has been found to be normal or slightly higher than in the general population (61;62). This contrasts with the initial reports of GH-insensitivity or Laron syndrome in an Israeli cohort with a different pathogenic mutation, in which affected individuals were found to have significantly reduced cognitive performance (63;64). The normal functioning of the GHR does not therefore appear to be a prerequisite for normal cognitive development. Scheepens et al (65) have suggested that this may indicate, 'some central role for high levels of GH acting via a receptor that does not require homodimerization.' The evidence that IGF-1 is not important in brain development is not however conclusive as studies of affected adults with GHR deficiency have found that they have significantly higher circulating concentrations of both IGF-1 and Insulin-like growth factor binding protein-3 (IGFBP-3) than affected children (66;67). It may therefore be that alternative pathways to the classical GH-GHR pathway are leading to IGF-1 production in these individuals.

Additional evidence to support the hypothesis that the concentration and activity of CNS IGF-1 impacts on cognition, in addition to promoting neural growth, comes from murine studies. These have shown that intraventricular infusion of IGF-1 improves cognitive performance, in particular in the domains of working and reference memory, and that conversely the inhibition of IGF-1 binding to its receptor leads to impairment in learning and reference memory (68).

In humans, fetal cord IGF-1 and IGFBP-3 concentrations have been reported to be related to head circumference at birth (69) and in childhood one study found that serum IGF-1 concentrations correlated positively with verbal intelligence (70). Amongst

elderly subjects, those with higher concentrations of IGF-I perform better on tests of cognitive function and have lower rates of cognitive decline (71).

Several neuropsychological studies have documented impairments in cognitive functioning in adults with childhood-onset GHD (CO GHD) (72;73) and adult-onset GHD (AO GHD) (65;72;74-76). They have also reported improvements in cognitive performance, especially in the domains of memory and attention, when GH is replaced (73;77-79) (Table 1). Concerns raised about previous studies in a recent meta-analysis of the literature included the use of healthy controls (i.e. non-short stature controls), the inclusion of heterogeneous patient groups with a variety of aetiologies (post-radiation, childhood onset, adult-onset) and duration of their GHD, the wide age-ranges of the patients included, and the lack of uniformity in the cognitive tests used to measure performance (80). In addition many studies have not adjusted their statistical analyses to account for type 1 error despite performing multiple comparisons. Despite these failings, Falletti *et al* concluded that when compared to matched controls individuals with GHD have consistently been found to have impairments in attention, memory and executive function and that there is some evidence for improvements in certain areas of cognitive functioning (memory and attention tasks) with GH treatment (80).

Table 1 Studies which assessed cognitive performance in Adults with GHD

Baseline studies investigating whether GHD impairs cognitive performance in individuals not receiving GH treatment				
Author(s) (age of recruits)	**Number participants (diagnosis)**	**Assessment Used**	**Conclusions**	**Problems**
Deijen 1996 (73) (19-37yrs)	48 (31 MPHD) (17 CO-GHD) 41 healthy controls	Groninger Intelligence Test long and short term memory tasks	Subnormal memory performance associated with GHD	Mixed patient groups, healthy controls
Lijffijt 2003 (75) (17-40yrs)	10 (CO-GHD) 10 controls	Selective attention task	Target detection was sig impaired in GHD group compared to controls i.e. selective attention affected	Healthy aged-matched controls
Meyer-Bahlburg 1978 (81)	29 patients with CO-GHD (MPHD, IGHD)	Intelligence quotient (IQ)	Normal IQ distribution MPHD lower IQ than IGHD (not significant)	No control group
Bulow 2002 (82) (39-77yrs)	33 women with AO-GHD and MPHD (lots had visual impairment (VI), radiotherapy or surgery to brain) with matched controls	Wechsler Adult intelligence Scale (WAIS) Swedish vocabulary test WAIS-R digit symbol test Block design test	Reduced performance on neuropsychological tests evaluating perceptual and fine-motor speed. Lower median scores than controls on vocab, digit symbol test, the	Healthy aged-matched controls Mixture of aetiologies Large number of individuals with VI

		Cronholm-Molander verbal memory test	Austin maze and attention process training inhibition tasks	
Peace 1998 (83)	69 (Post-treatment pituitary gland tumour) 23 controls	Digit span Auditory-verbal learning test Story recall test (immediate and delayed) Controlled oral word association test Block design	Performance in all patient groups was significantly below that in controls on story recall and auditory verbal learning tests	Patients all had pituitary tumours
Prospective studies in adults assessing the effect of rhGH on cognitive function				
Almqvist 1986 (84) (22-36yrs)	5 (IGHD)	Face recognition Simple addition (speed and accuracy) letter substitution	4wks of GH sig improved performance on face recognition tasks	Short follow up, small number of patients
Lasaite 2004 (85) (21-40yrs)	18 (MPHD) No controls	Digit Symbol Test (DST) and the Digit Span Subset (DSS) of the Wechsler. Quality of life (QOL) questionnaire	Improvement in mood & cognition after GH treatment. Statistically significant differences were observed on Profile of Mood State and on scores of DST.	Other hormonal deficits No control group
Sartorio 1995 (79)	8 (CO-GHD)	Bem Sex role test	Psychological difficulties improve	Small number of patients

(29.6yrs)		Non-verbal scales of WAIS State-trait anxiety inventory Experiential world inventory Draw a person test	with GH treatment After 6 months performance significantly better on the symbol-number associated compared with baseline.	
Baum 1998 (86) (24-64 yr)	40 (IGHD &MPHD)	WAIS-Revised Wechsler Memory Scale-Revised	No significant changes in cognitive function or quality of life after 18mths GH treatment	Differing aetiologies-brain tumour/IGHD
Pavel 2003 (87)	16 MPHD	Pupillographic sleepiness test and a choice reaction time test.	Improvement in sleep latency and quality.	Small group Short term follow up
Arwert 2005 (77) (mean 28yrs)	23 CO-GHD (IGHD&MPHD)	Associate learning task Delayed recognition task	Memory scores were significantly better at 2, 5, and 10 years than at baseline.	Mixed patient groups
Blinded placebo controlled trials assessing the effect of GH on cognitive function				
Degerblad 1990 (88) (20-38yrs)	6 (MPHD)	Verbal learning Non-verbal learning Reaction time Symbol digit substitution Finger tapping	No sig differences between placebo and GH treated groups	Small group
Deijen 1996 (73)	48 (MPHD-31,	Sensory register (iconic	Baseline: Impaired memory	Healthy age-matched

	IGHD-17) age-matched controls-41	memory) Short-term memory (STM) (including recognition and recall) Long term memory (LTM)	performance (STM, LTM and iconic memory) and lower intelligence scores After 2yrs GH (placebo control): normalization of memory performance, but not of perceptual motor skill or emotional well-being	controls
Oertel 2004 (89) (21-63yrs)	18 AO-IGHD & MPHD	Raven standard progressive matrices test Verbal STM and LTM Test reproduction tasks	After 6mths GH significant improvement in attention functioning compared to placebo. No change in verbal memory and non-verbal intelligence	Mixture of aetiologies, wide age-range
P300 auditory event related potentials (ERP) studies in adults with GHD				
Tanriverdi (90)	19 GHD, 18 acromegalic patients & 16 age, education and sex matched healthy controls	Evoked response potential (ERP)	Prolongation of P300 latencies in patients with severe GH deficiency and reduction of P300 amplitudes in patients with acromegaly	Healthy aged-matched controls
Golgeli (78) (mean age	14 patients with Sheehan's syndrome	ERP	Before GH therapy, P300 latencies were prolonged compared	Healthy age-matched controls

49.5yrs)	10 controls		to controls. Improved after 6mths GH.	

There are only a small number of studies in the literature pertaining to the cognitive impact of GHD in children (Table 2). In one study children with GHD were found to score lower in two subtests of the Leistungsprtif system intelligence test, measuring spatial orientation and speed of closure when compared to controls despite there being no difference in full scale IQ between the two groups (91;92). The largest study to date was carried out by Stabler *et al* who investigated the benefit of GH treatment in 72 individuals with IGHD and 59 with idiopathic short stature (ISS) (88). IQ, academic achievement, social competence and behaviour problems were assessed before and yearly after GH treatment for 3yrs. IQ and achievement scores did not change with GH therapy. However, after GH treatment, child behaviour checklist scores for total behaviours were improved. This effect was found to be more significant in those with GHD ($p<0.001$) than in those with ISS ($p<0.003$) (93). GH treatment in individuals with GHD also improved scores on internalizing subscales (withdrawn: $p<0.007$, somatic complications $p<0.001$, anxious/depressed $p<0.001$) and on attention, social problems and thought problems ($p=0.001$).

In the majority of studies assessing the impact of GH on neurodevelopment, children with idiopathic GHD have been reported to have a normal IQ. However, despite having IQ's within the normal range, a high percentage of patients with GHD have to repeat a class (94-96) and have difficulties with problem solving (97).

Conversely children with MPHD have consistently been found to have cognitive deficits (98). This may be due to the other hormonal abnormalities, including thyroid hormone and cortisol deficiency, present in children with MPHD. Both thyroid hormones and cortisol are known to be important determinants of cognitive function and brain development. Children with MPHD are also at greater risk than those with IGHD of having hypoglycaemic episodes in infancy, hypoglycaemia early in life being an independent risk factor for poor cognitive outcome (99).

In the paediatric literature, similar to the adult literature, many of the studies investigating the effect of GH on cognition have significant limitations. The studies have included small, heterogeneous, poorly defined patient groups and have frequently lacked appropriate control groups. Findings from previous studies have varied according to diagnosis, timing of diagnosis (infancy or onset later in childhood), presence of IGHD, MPHD or SOD, research methodologies, GH type, dose and duration of treatment. They have also tended to include individuals with visual impairment (VI), but not to control for the neurodevelopmental impact of the visual problems (93). This has made drawing conclusions regarding the cognitive benefits of GH treatment, if any, in children extremely difficult.

Motor skills have also been evaluated in children with IGHD and MPHD by assessing individuals ability to copy a number of designs. In these combined (IGHD & MPHD) cohorts abnormalities in visual-motor skills were identified (100;101). However again the existence of pituitary hormone abnormalities other than isolated GHD in these studies makes it hard to extrapolate these findings to children with IGHD. Specific assessment of motor skills have not previously been performed in individuals with IGHD (101).

Table 2 Studies which assessed the impact of growth hormone deficiency and growth hormone replacement on cognition in the paediatric population

Author(s) (age of recruits)	**Number participants (diagnosis)**	**Assessment Used**	**Conclusions**	**Problems**
Steinhausen, Stahnke 1976 (91) (9-19 years, mean 14.9 years)	16 GHD (6 IGHD/10 MPHD) 16 short stature controls Assessed before GH treatment	Thurstones test of primary mental abilities Wechsler scales Personality test (questionnaire assessing: extraversion, neuroticism, aggressiveness) Children's personality questionnaire of Cattell	Impaired social and coping behaviour in all short children Reduced spatial orientation and speed of closure No difference in IQ Less aggressive, less excitable, less dominant, more conscientious and less tense than the short stature controls	Mixed patient group
Stabler 1998 (93) (5-16 years)	72 IGHD 59 ISS Assessed before and after 3yrs GH	IQ Child behaviour checklist	IQ and achievement scores did not change with GH therapy. After GH treatment, child behaviour checklist scores for total behaviours were improved. (effect larger in those with GHD ($p<0.001$) than in	Did not include detailed assessment of attention measures

Stabler 1998 (93) (5-16 years)	72 IGHD 59 ISS Assessed before and after 3yrs GH	IQ Child behaviour checklist	IQ and achievement scores did not change with GH therapy. After GH treatment, child behaviour checklist scores for total behaviours were improved. (effect larger in those with GHD ($p<0.001$) than in those with isolated short stature ($p<0.003$)) GH improved scores on internalizing subscales (withdrawn: $p<0.007$, somatic complications $p<0.001$, anxious/depressed $p<0.001$) and on	Did not include detailed assessment of attention measures

F. Can magnetic resonance imaging assess the effect of growth hormone deficiency on neural development?

Since its introduction in the mid 1980s MRI has proved valuable in defining the gross structural abnormalities present in individuals with pathological conditions. For example, in individuals with IGHD the anterior pituitary gland is frequently small, the posterior pituitary ectopic and the infundibulum thin or absent (102). More recently advances in MRI have enabled more detailed examination of the structure of white matter in the brain, so that volumes of different regions within the brain can be determined (103;104) and visualization of brain activity during cognitive tasks can be performed (105). All of these techniques can be used to investigate the phenotype of individuals with pathological conditions in more depth. Newer MRI methods have also enabled the detailed study of brain maturation during childhood in the healthy population (106).

However, whilst significant relationships between IGF-1, IGFBP-3 and neural volumes (total brain volume, unmyelinated white matter and cerebellum) have been described in children born extremely preterm (<31weeks at birth) in whom there is a relative IGF-1 and IGFBP-3 deficiency postnatally (107), the relationship between neural volumes and IGF-1 and IGFBP-3 concentrations have not previously been studied in healthy children.

The only previous MRI studies using the advances outlined above in individuals with GHD have been performed in adults with CO-GHD using functional MRI (fMRI) (77). Arwert *et al* investigated the influence of growth hormone status in adults using fMRI, a non-invasive technique which enables visualization of brain activity during a cognitive task. The effects of 6 months of GH treatment were studied in 13 adults with CO-GHD using neuropsychological tests and fMRI, comparing the findings in individuals with CO-

GHD to those of 13 age, sex and education matched healthy controls (77). Using a working memory paradigm, they observed subnormal memory speed (with normal memory performance) in conjunction with increased activity in the dorsolateral/ventrolateral prefrontal cortex, anterior cingulate cortex, parietal and motor cortices as well as in the thalamus and precuneus area in patients with CO-GHD (77). Increasing task load was associated with an increase in brain activity in similar areas in patients compared to control subjects. They concluded that these findings suggest that the memory performance is not impaired due to the compensatory recruitment of dorsal prefrontal brain regions in those with CO-GHD.

Their follow-up study (108) used the same fMRI paradigm to examine the effects of GH treatment on cognitive function in CO-GHD patients. fMRI showed activation during the working memory task in prefrontal, parietal, motor, and occipital cortices, as well as in the right thalamus and anterior cingulate cortex. Decreased activation in the ventrolateral prefrontal cortex was observed after GH treatment as compared with placebo treatment, indicating decreased effort and more efficient recruitment of the neural system involved. These studies indicate how the GH/IGF-1 axis contributes to prefrontal functioning in GHD patients and how the benefits of GH in terms of cognitive functioning can be visualized using neuroimaging.

DTI is a non-invasive MRI technique which can provide quantitative indices of brain development, enabling the visualization of white matter microstructure and characterization of white matter anatomy including the degree of connectivity between different regions of the brain (103;109). The majority of white matter fasciculi can be identified from birth, with slow modifications occurring during the second year of life,

after which they remain relatively stable (110). White matter tracts are compiled of axons surrounded by myelin sheaths aligned in a parallel fashion. Within large white matter tracts this structure limits the Brownian motion of water molecules, leading to the preferential diffusion of water molecules along the tract as opposed to perpendicular to it (103;111). DTI describes the diffusion profile of water molecules in every voxel in a three-dimensional Gaussian function. This is classically represented as an ellipsoid, with the direction and lengths of the three principal axes of the ellipsoid being termed eigenvectors and eigenvalues respectively. The, 'major axis', or predominant direction of the white matter tract within a given voxel is represented by the eigenvector with the highest eigenvalue and is considered to be orientated along the dominant direction of the white matter tract. The diffusion of water molecules within the brain is affected by the underlying tissue microstructure; this enables the study of the orientation and integrity of neural fibres, a useful tool when assessing the impact of physiological and pathological processes on neurological development (112). The principal disadvantage of DTI is found in areas where white matter tracts come together or intersect, although so called high angular resolution diffusion imaging and appropriate reconstruction approaches can overcome this problem. Classically DTI identifies the one principal direction of fibre orientation within a voxel, this limits its ability to describe regions of more complex white matter structure.

Fractional anisotropy (FA), which describes the degree of diffusion directionality and is related to axonal density and degree of myelination, and ranges from 0 for isotropic diffusion to 1 for anisotropic diffusion, is calculated by relating the amount of diffusion along the major axis to that along the minor axes (109;112). FA can be affected by a range of microstructural differences in neural tissue including axonal membranes,

myelin sheaths, percentage brain water, compactness of fibre tracts and amount of axonal space (112). Studies conducted in the healthy paediatric population have found that FA correlates with neurocognitive function (113).

Since the basic principles of diffusion MRI were established in the mid-1980s many clinical studies have found that abnormalities in the brain's white matter tracts can be identified in a wide range of pathological conditions (e.g. multiple sclerosis, Alzheimer's disease and depression) (114-116). Studies in these conditions have, for example, improved the ability of MRI to identify clinically important white matter lesions in multiple sclerosis which were previously unseen on conventional MRI sequences (117). They have also enabled the identification of defects in conditions not classically associated with abnormalities on conventional neuroimaging, such as ASD (118;119).

Studies of white matter integrity require group contrast analysis where the patient group of interest is compared to an age-matched control group (120). Comparisons can either be made by looking at the white matter tracts across the whole brain (e.g. tract based spatial statistics (TBSS) or voxel based morphometry (VBM)) or by identifying tracts of interest, a technique which increases the statistical power of the study (tractography) (120;121). By averaging the measurements of FA over an entire white matter tract, a quantitative measure of tract organization can be generated (122).

In contrast with VBM analysis which examines FA throughout the white matter of the brain, TBSS looks only at the major white matter tracts in a computer generated, 'white matter skeleton'. This reduces the likelihood of registration errors and bias occurring

and removes the requirement for arbitrary smoothing of the data necessary for VBM analysis (121).

VBM is a technique which can be used to analyse 3D MRI datasets, such as T1-weighted MRI to detect subtle structural differences in neural architecture that may not be identified by visual inspection of MRI scans (123). FreeSurfer is a method developed by Fischl *et al* which generates neural volumes for total brain, cortical and sub-cortical grey and white matter structures (104). There is a large body of evidence from fMRI and voxel-based analyses detailing the neural regions most consistently associated with IQ, attention and memory (124). Although both IGF-1 and IGFBP-3 concentrations have been shown to relate to total brain, cerebellar and unmyelinated white matter volumes in children born extremely preterm, these relationships have not been studied in a healthy cohort. Neither VBM nor FreeSurfer have previously been used to look at MRI datasets in individuals with GHD.

These methods could potentially be used in this cohort both to assess whether there are changes in regions known to be important in memory, attention and executive function, all domains which appear to be affected consistently in adult studies (80), and to assess whether regions of the brain where there are very high levels of GH and IGF-1 receptors are different in individuals with GHD when compared to those without GHD (e.g. cerebellum, hippocampus, parahippocampal gyrus) (40;41;53;57).

G. Summary

Inter-child variability in IGF-I concentrations within the normal range relates to measures of cognition, but has not been related to anatomical studies of brain structure and

myelination. Previous studies in children with GHD have focussed on assessment of cognitive function but have not investigated whether differences found, for example in memory, correlate with neural structure and volume changes in brain regions known to be important in memory (e.g. hippocampus and parrahippocampal gyrus) (125;126). Clinicians prescribe GH therapy with the aim of optimising final height, maintaining normoglycaemia and minimising the metabolic effects (increased fat mass and reduced muscle bulk) of GHD. If GH has a significant impact on cognitive and psychosocial outcomes this may change the timing of treatment in individuals with GHD in whom currently the main aim is to optimize final height.

1.2 Behavioural problems in children with optic nerve hypoplasia and septo-optic dysplasia

A. Optic nerve hypoplasia and septo-optic dysplasia

ONH, a developmental abnormality of the optic nerves, is one of the leading causes of VI in the developed world, with a reported prevalence of 10.9 per 100,000 in England (2006) (127;128). All studies assessing the prevalence of ONH have only included individuals with a diagnosis of VI, therefore the true incidence of ONH is likely to have been underestimated because individuals with isolated ONH and mild VI have been excluded. The diagnosis of SOD is a clinical one and can be made when two or more features of the classical triad of (i) ONH, (ii) pituitary hormone abnormalities and (iii) midline brain defects, including agenesis of the septum pellucidum and/or corpus callosum, are present (129). The severity of the clinical presentation is highly variable with some children manifesting only mild VI in association with IGHD and others having the full spectrum of clinical abnormalities including panhypopituitarism, severe VI, hearing impairment, developmental delay, obesity, sleep disturbance and behavioural abnormalities (130).

B. The prevalence of behavioural problems in children with septo-optic dysplasia and optic nerve hypoplasia

Neuro-developmental delay is common in children with SOD with different studies citing incidences ranging from 46-73% (131). Behavioural problems are also frequent with aberrant behaviours within the Autistic spectrum being particularly common. Margalith *et al* reported that 21% of the cohort they studied (51 children) had behavioural difficulties

including attention deficit hyperactivity disorder (ADHD) and autistic spectrum disorder (ASD) (132). Subsequently Parr *et al* found that 46% of the children recruited into their study with ONH and severe/profound VI had developmental impairments related to ASD (133). Increased behavioural problems have also been associated with impaired cognition in previous studies (133). The presence of the associated learning difficulties present in many of the children previously studied makes it difficult to assess the true presence of isolated behavioural problems in children with ONH.

Children and adolescents with severe visual impairment (VI), secondary to varying aetiologies, have a significantly increased prevalence of behavioural and social communication problems (134-136). The neural basis for these behavioural problems is unknown. There are no studies reporting the prevalence of behavioural problems in children with mild/moderate VI in whom functionally normal or near normal vision is preserved. This is significant since previous studies have attributed the increased prevalence of behavioural problems in children with ONH to the absence of normal visual cues they experience.

C. The psychosocial burden of behavioural problems

Behavioural abnormalities are diagnosed using rating scales, which through application of cut-off scores identify children with common characteristics (137). Behavioural difficulties can be categorized as internalizing or externalizing. Externalizing behaviours include defiance, impulsivity, disruptiveness, aggression, antisocial features, and overactivity, whilst internalizing behaviours include withdrawal, dysphoria and anxiety

(138;139). Associations between academic underperformance and behavioural difficulties have long been recognized (140), with externalising behaviours diagnosed in childhood being associated with an increased risk of adult antisocial behaviour and substance abuse (141). Children who are underachieving academically are also at increased risk of other problems including deficits in self-esteem, difficulties with acquiring language skills and an increased prevalence of interpersonal difficulties (142).

D. Can magnetic resonance imaging help us to understand the increased prevalence of behavioural problems found in children with optic nerve hypoplasia?

To date conventional methods of neuroimaging have been unable to detect neural abnormalities, other than hypoplastic optic nerves, in individuals with isolated ONH. Only two studies have been published using DTI to better define the phenotype in ONH and SOD. Both have been in small numbers of subjects with severe VI (one and two individuals) and have focused solely on the optic tracts (143;144). These studies concluded that children with SOD have both pre- and post-chiasmatic diffusion tensor abnormalities in the visual pathway.

There is a wide literature debating the reasons as to why children with VI, secondary to a wide range of aetiologies, are at increased risk of behavioural and social communication difficulties. However, thus far, the underlying reason for this increased prevalence remains unknown (145). It has, however, previously been suggested that reductions in exposure to visual social cues and visually guided experiences in children with VI may predispose them to developing social development abnormalities (146).

Whilst the increased prevalence of behavioural deficits found in children with ONH and severe VI may be related to their underlying VI and reduced visual experience, we hypothesized that on the basis of the emerging literature regarding neuroimaging and social communication disorders such as ASD (119), the underdevelopment of other white matter tracts may also contribute to the behavioural abnormalities found in this cohort.

E. Summary

Previous studies investigating the prevalence of behavioural difficulties in children with ONH have all been in children with severe VI (133;134). Children with ONH and severe/profound VI frequently have co-morbidities which may potentially confound behavioural and DTI assessments, including significant learning difficulties, attention deficit disorder, seizures, and cerebral palsy (132;147-150).

We therefore firstly aimed to assess whether children with isolated ONH with vision ranging from normal range acuity to mild to moderate VI and no developmental delay have an increased prevalence of behavioural problems compared to a control group of typically developing children without ONH. Secondly we aimed to perform detailed DTI studies in this cohort to identify whether specific white matter abnormalities, not previously identified on standard structural brain MRI scans, are present that may provide neural correlates for any behavioural abnormalities identified.

1.3 Circadian rhythm abnormalities in children with septo-optic dysplasia

A. Circadian rhythm

The circadian clock controls daily cycles of physiology and behaviour, including the sleep-wake cycle, temperature and hormone production (151). These rhythms are regulated by environmental cues including food intake and light-dark cycles. In humans circadian rhythms are predominantly entrained by the transmission of light from the retina via the retinohypothalamic tract to the master clock or suprachiasmatic nucleus (SCN), located in the hypothalamus (152). Melatonin (N-acetyl-5-methoxytryptamine) is subsequently released from the pineal gland in response to the reduced light stimulus stimulating drowsiness and lowering body temperature. Melatonin is secreted in a circadian pattern. It is normally produced in the pineal gland in the evening with blood concentrations peaking at approximately 3am, after which levels fall, with secretion being extremely low during daylight hours (152). Sleep onset is associated with a peak in melatonin concentrations, however a subsequent effect of melatonin on maintaining sleep is less evident (153). Melatonin deficiency, regardless of its cause, is in the main factor associated with sleep abnormalities (154).

Circadian rhythm sleep disorders (CRSD) are diagnosed in individuals with a persistently disturbed sleep pattern (155). There are various aetiological factors underlying CRSD, which include structural abnormalities in the circadian timekeeping system such as absence of the pineal gland or SCN (156).

B. The prevalence and impact of disordered sleep patterns in children with septo-optic dysplasia

Thirty-two percent of children with SOD have disordered sleep patterns; these include free-running rest-activity cycles, fragmented and arrhythmic sleep (157). Free-running rest-activity patterns are most commonly found in individuals who have no light awareness and are therefore unable to register light cues. Abnormal sleep patterns impact significantly both on the child's well being and on the family's overall lifestyle and ability to cope with their child's complex condition, and can adversely impact on children's cognitive development (152;158).

C. The pathophysiology of circadian rhythm abnormalities in septo-optic dysplasia

Arendt *et* al showed that light perception is all that is required for synchronization with the SCN, with normally entrained circadian rhythms being found in the majority of individuals with light perception only, despite significant VI (159). It is therefore unlikely that the sleep abnormalities found in individuals with SOD are secondary to their VI as the majority have light perception. The pathophysiology of the sleep abnormalities found in SOD has therefore been hypothesized to be secondary to abnormalities at other stages in the pathway of melatonin production. For example, the neurons of the paired SCN have been shown to be absent in one patient with SOD (156). Abnormalities in melatonin production could therefore potentially arise either secondary to dysfunction of the SCN or to abnormalities of the pineal gland which is the main producer of melatonin (160). It is also possible that abnormalities in the pineal gland or SCN could lead to a failure in the feedback loop which plays a role in

regulating melatonin production, leading to a relative insensitivity to melatonin action (161).

No objective measurement of sleep/activity patterns with 24-hour melatonin profiles have been published in individuals with SOD. The pathophysiological basis underlying sleep disorders in SOD therefore remains largely unknown.

D. The management of sleep disorders in septo-optic dysplasia

A trial of melatonin treatment in children with SOD and sleep disruption is accepted clinical practice in many centres. However as stated above, no objective measurement of sleep/activity patterns with 24 hour melatonin profiles have been published for these individuals, and the pathophysiological basis underlying sleep disorders in SOD remains largely unknown.

A recent review on the use of melatonin in the treatment of sleep disorders stated that, 'the use of melatonin is frequently based on anecdotal evidence or small clinical trials' (162). There are possible side-effects associated with melatonin use with one study suggesting that melatonin may have pro-convulsant effects (163) and others suggesting that it impacts on the hypothalamo-pituitary axis, potentially affecting the patterns of oxytocin, adrenocorticotropic hormone (ACTH), vasopressin and GH release, although it remains difficult to predict whether these endocrine effects will have long-term clinical outcomes (152;164;165).

E. Summary

In view of the uncertainty surrounding the aetiology of the sleep abnormalities found in individuals with SOD, and consequently the optimum management of this problem, we aimed to establish whether children with SOD who experience sleep pattern disorders also have defective melatonin production.

1.4 The genetics of pituitary development

A. The pituitary gland

The pituitary gland acts as a central co-ordinator for growth, reproduction and homeostasis (129). It is situated in the sella turcica at the base of the brain and plays a key role in the transmission of information between the brain, hypothalamus and peripheral target organs; including the adrenals, gonads and thyroid (5). The hypothalamus regulates the pituitary gland via the secretion of trophic factors which act to modulate cell proliferation, hormone synthesis and secretion.

The pituitary is made up of two anatomically and functionally distinct components, the adenohypophysis, which consists of the anterior and intermediate lobes and the neurohypophysis or posterior lobe (5). The anterior lobe contains 5 different cell types each of which secretes a different hormone (Table 3). The posterior lobe of the pituitary is made up of axonal terminals of the magnocellular neurons, encircled by specialized astroglia known as pituicytes, it releases oxytocin (important during parturition and lactation) and ariginine vasopressin (AVP) (involved in regulation of osmotic balance). Oxytocin and AVP are produced in the hypothalamus and transported to the posterior lobe in hypothalamic axons.

Table 3 Anterior pituitary gland: Cell types and hormones released

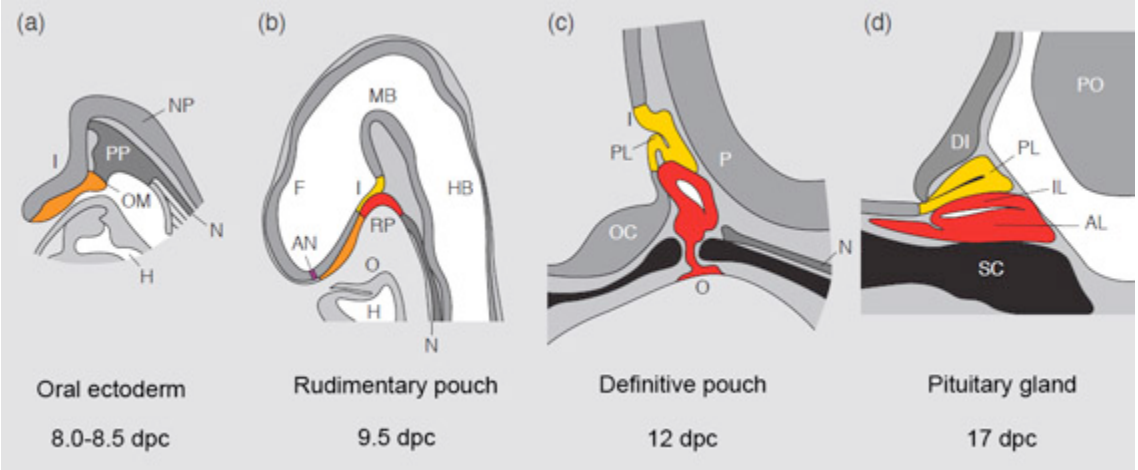
Cell type	Hormones produced	Action
Anterior Lobe		
Somatotropes	GH	Regulates linear growth and metabolism
Lactotropes	Prolactin	Regulates milk production in females
Corticotropes	ACTH	Regulates metabolic function through stimulation of glucocorticoid synthesis in the adrenal gland
Thyrotropes	Thyroid-stimulating hormone (TSH)	Promotes thyroid follicle development, thyroid hormone secretion and modulates skeletal remodelling
Gonadotropes	Lutenising hormone (LH) Follicle-stimulating hormone (FSH)	Act on the gonad to initiate sexual maturation and maintain reproductive function
Intermediate lobe		
Melanotropes	α-Melanocyte-stimulating hormone (α-MSH) Corticotrophin-like intermediary peptide B-endorphin γ-lipotropin	MSH controls production and distribution of melanin by melanocytes

B. The genetics of pituitary development

The mature pituitary gland is made up of two distinct lobes with different functions and developmental origins. The anterior and the intermediate lobes of the pituitary originate from the oral ectoderm, whilst the neurohypophysis (posterior pituitary) is derived from neural ectoderm (166).

The majority of our knowledge regarding pituitary development has been derived from extensive rodent studies (167;168); however the stages of pituitary development appear to be similar in all vertebrates (22). Development occurs in 4 distinct stages (Figure 3) culminating in the formation of a complex secretory organ consisting of 5 different cell types secreting 6 different hormones (Table 3).

Figure 3 Stages of pituitary development (Sheng and Westphal 1999)



Key Figure 3 Mouse pituitary development in sagittal section. Stages of development are indicated in days post-coitum; AL-anterior lobe, AN-anterior neural pore, DI-diencephalon, F-forebrain, H-heart, HB-hindbrain, I-infundibulum, MB-midbrain, N-notochord, NP-neural plate, O-oral cavity, OC-optic chiasm, OM-oral membrane, P-pontine flexure, PL-posterior lobe, PO-pons, RP-Rathke's pouch, SC-sphenoid cartilage.

It is now clear that normal anterior pituitary development is critically dependent upon a complex genetic cascade of signalling molecules and transcription factors that play a crucial role in organ commitment, cell proliferation, cell patterning and terminal differentiation (169). Complex genetic interactions direct normal pituitary development, with repression and activation of target genes enabling normal development to occur. In contrast with the large amount of information available on pituitary development in the rodent, there is minimal information pertaining specifically to human pituitary development (22). However, there appear to be significant similarities between transcription factors which govern both murine and human pituitary development, with insights into human pituitary disease having mainly been made by studying spontaneous

or experimentally introduced mutations affecting the mouse hypothalamo-pituitary axis (166;170;171).

There is significant phenotypic variability in individuals with pituitary hormone deficits, some of which may be secondary to the underlying aetiology of their condition. In those individuals in whom a genetic abnormality can be identified as the cause of their pituitary developmental abnormality the phenotype can be more clearly defined (Table 4). Consequently in individuals with a defined genetic aetiology we are better able to counsel the patient and their parents regarding likely associated clinical features and disease evolution.

Table 4 Human mutations causing abnormal hypothalamo–pituitary development and function.

Gene	Phenotype	MRI	Inheritance
Isolated Growth hormone deficiency			
GH-1	IGHD. No GH response to stimulation. Evolution of endocrinopathy with Type 2 AD GHD.	Hypoplastic or normal AP	Recessive, dominant.
GHRHR	IGHD	Hypoplastic AP	Recessive
Multiple pituitary hormone deficiency			
POU1F1	GH, TSH, prolactin deficiencies; usually severe.	Hypoplastic or normal AP	Recessive, dominant
PROP1	GH, TSH, LH, FSH, prolactin deficiencies; evolving ACTH deficiency.	Small, normal or enlarged AP	Recessive
Specific syndrome			
HESX1	IGHD, CPHD, SOD.	APH, EPP, absent infundibulum, ACC	Recessive, dominant
LHX3	MPHD (GH, TSH, LH, FSH, prolactin, ACTH deficiencies), short neck, limited rotation; short cervical spine, sensorineural hearing loss.	Small, normal or enlarged AP	Recessive
LHX4	MPHD (GH, TSH, ACTH deficiencies)	Small AP, EPP, cerebellar abnormalities	Dominant
SOX3	IGHD, mental retardation, panhypopituitarism.	APH, infundibular hypoplasia, EPP	X Linked

SOX2	Hypogonadotrophic hypogonadism; APH, bilateral anophthalmia/microphthalmia, abnormal CC, learning difficulties, oesophageal atresia, sensorineural hearing loss.	APH, hypoplasia of CC, abnormal hippocampi, hypothalamic hamartoma	*De novo*
GLI2	MPHD, Multiple midline defects.	APH, holoprosencephaly,	Dominant

AP(H), anterior pituitary (hypoplasia); EPP, ectopic posterior pituitary; (A)CC, (agenesis of) corpus callosum.

C. Summary

Currently we are able to identify genetic mutations in approximately 10% of those with IGHD and in <1% of those with SOD/ONH (130). Understanding the genetic basis for pituitary hormone deficiency may help us to further characterise any cognitive deficits, behavioural and neurological abnormalities we identify in this heterogeneous group of patients. I therefore aim to screen all patients recruited for mutations in genes previously implicated in pituitary development.

1.5 Study aims

A. To define the neurodevelopmental and behavioural phenotype of children with IGHD.

B. To identify whether MRI abnormalities can be identified using VBM and DTI in children with IGHD.

C. To investigate whether it is possible to identify neural correlates of any cognitive/motor skills deficits identified in children with IGHD.

D. To assess the prevalence of behavioural problems in children with isolated ONH and mild to moderate or no visual impairment.

E. To identify whether MRI abnormalities can be identified using DTI in children with isolated ONH and mild to moderate or no VI.

F. To investigate whether it is possible to identify neural correlates of any behavioural problems identified in children with isolated ONH and mild to moderate or no VI.

G. To evaluate whether any white matter abnormalities identified in children with isolated ONH are more widespread when children with SOD (restricted to those without brain MRI abnormalities [other than those in the hypothalamo-pituitary axis] visible on standard brain MRI) are also included in the DTI analysis.

H. Evaluate the pathophysiology of circadian rhythm abnormalities in SOD using sleep actigraphy and serum melatonin profiling.

I. To screen all individuals with IGHD, ONH and SOD recruited to the study for mutations in genes previously implicated in pituitary development.

2. STUDY SUBJECTS, PROTOCOLS, MATERIAL AND METHODS

A. Ethics

Ethical permission was granted for this study from the Joint Research Ethics Committee of Great Ormond Street Hospital/Institute of Child Health. Prior to participating in the study all parents/subjects gave written informed consent/assent as appropriate according to the Declaration of Helsinki (BMJ 1991; 302: 1194) (Appendices 8.A and 8.C).

B. Recruitment

Patients with IGHD, ISS, SOD or ONH (inclusion and exclusion criteria below) attending the paediatric endocrine clinic and/or the developmental vision clinic at Great Ormond Street Hospital for Children between 20th December 2007 and 20th December 2009 were recruited prospectively. Our control groups for the cognitive assessments performed in children with IGHD and ONH were individuals with ISS. As children with ISS have been shown to have reduced IQ when compared to normal stature controls, we chose children with ISS as our "controls", thereby controlling for the effect of stature and isolating the effect of GHD (25). As these children had normal development and no VI they were also used as controls for the study looking at behaviour, cognitive function and brain structure in children with ONH.

- Inclusion criteria IGHD
    - Aged 5-11years at entry into study
    - Prepubertal *i.e.* boys with testes volume >3 ml, girls with Tanner breast stage of ≥2, and patients with Tanner pubic hair stage ≥2 were excluded.

- Peak GH on stimulation <7ng/ml
- Height velocity <-0.8SDS

- Exclusion criteria IGHD
  - Subjects with chronic renal failure or liver failure
  - Previous/current brain tumour and/or cranio-spinal irradiation
  - Previously on GH treatment
  - Coeliac disease
  - Bone age >10years
  - Other pituitary hormone abnormalities
  - Seizures, ASD, Attention deficit hyperactivity disorder (ADHD)

- Inclusion criteria ISS
  - Aged 1-11years at entry into study
  - Prepubertal *i.e.* boys with testes volume >3 ml, girls with Tanner breast stage of ≥2, and patients with Tanner pubic hair stage ≥2 were excluded.
  - Peak GH on stimulation >10ng/ml
  - IGF-1 within the normal range (defined as -2 to +2 SDS)
  - Height <2SDS below mean for population
  - Normal growth velocity (>-0.8SDS)

- Exclusion criteria ISS
  - Intrauterine growth retardation: defined as birth weight <10^{th} centile for gestational age (independent risk factor for neurodevelopmental delay)
  - Chronic illness

- Bone age >10years
- Seizures, ASD, Attention deficit hyperactivity disorder (ADHD)

- Inclusion criteria SOD
  - Presence of two or more features of the classical triad of
    (i) ONH
    (ii) pituitary hormone abnormalities
    (iii) midline brain defects, including agenesis of the septum pellucidum and/or corpus callosum

- Exclusion criteria SOD
  - Seizures
  - Associated brain abnormalities including schizencephaly, cerebellar hypoplasia and aplasia of the fornix.

- Inclusion criteria ONH
  - Isolated ONH
  - Normal range visual acuity to mild/moderate reduction in visual acuity
  - Normal cognition

- Exclusion criteria ONH
  - Pituitary hormone abnormalities
  - Midline brain defects, including agenesis of the septum pellucidum and/or corpus callosum
  - Seizures

- Developmental delay
- Severe VI

C. Definition of clinical phenotype and determination of pituitary hormone function

i. Subject characteristics

Data including age at entry to the study, date of birth, ethnicity, antenatal and delivery history, birth weight, handedness, maternal highest educational level and paternal employment were collected on a questionnaire from all patients on entry to the study (Appendix 8.B). Socioeconomic status was calculated using the Standard Occupational Classification (172). Maternal highest educational level and socioeconomic status were used as a proxy for parental IQ.

ii. Vision assessment in children with septo-optic dysplasia and optic nerve hypoplasia

Visual acuity was assessed using the Sonksen LogMAR Test of Visual Acuity, Kay Picture cards (letter, symbol naming or matching), or the Keeler card (preferential looking) tests. These different acuity tests were selected depending on the child's developmental age, level of vision and ability to co-operate and communicate.

iii. Anthropometry

At -6 and 0 months the following measurements were taken from all study subjects. The serial measurements were performed by the same trained auxologist at each visit.

- Sitting and standing height to the nearest *mm* with a stadiometer (*Doherty).*
- Weight was measured by Seca scale.
- Head circumference (cm).
- The body mass index (BMI), defined as the ratio of the subject's weight in kilograms to the square of the subject's height in meters, was calculated from this data.

Height velocity was calculated at baseline, over a period of not less than 4 months, to determine annual growth rate in centimetres per year. Individuals with a height velocity ≤2SDS below the mean for age were further investigated as outlined below.

iv. Measurement of IGF-1 and IGFBP-3

IGF-1 and IGFBP-3 were measured in duplicate on all study subjects using the Immulite® 2500 solid-phase, enzyme-labeled chemiluminescent immunometric assay. The within-assay coefficients of variation (CVs) for IGF-1 were 3.9% and 3.0% at 77 and 689 mg/L respectively. The between-assay CVs for IGF-1 were 7.7% and 8.1% at 77 and 689 mg/L, and the detection limit of the assay was 25 mg/L. The within-assay CVs for IGFBP-3 were 4.4 % and 4.6 % at 0.91 and 4.82 mg/L respectively. The between-

assay CVs for IGFBP-3 were 6.6 % and 7.3 % at 0.91 and 4.82 mg/L, and the detection limit of the assay was 0.5 mg/L.

Normative data were required to interpret the IGF-1 and IGFBP-3 concentrations we obtained in our study subjects to enable us to convert them in to age and sex specific standard deviation scores. IGF-1 and IGFBP-3 reference values for the paediatric population were obtained from Immulite, and from 254 members of a twin study in whom serum samples had been taken at a mean age of 11.7 years (range 2.3 years), and IGF-1 and IGFBP-3 SDS were calculated using the LMS method developed by Tim Cole (173) (Figures 4-7). The twins were members of a study designed to assess how variability in birth weight between twin pairs (and therefore prenatal nutrition) impacts on cognitive and cardiovascular outcomes (174). 230 of the 254 individuals who underwent measurement of IGF-1 and IGFBP-3 were twin pairs and 24 were siblings. All of them had normal height and weight. Mean serum IGF-1 and IGFBP-3 concentrations were 257ng/ml (range: 66-644ng/ml) and 4.2ng/ml (range: 1.6-7.9ng/ml) respectively in the twin cohort.

The LMS method was developed to enable SDS to be calculated for scenarios where a measurement is significantly affected by a covariate e.g. age. In this scenario the reference range changes as age increases and a centile chart is required to generate SDS. The variation in a variable with age is represented by the reference centile curves. The LMS method uses 3 parameters to define the change in the distribution of a parameter with age.

These parameters are the median (M), coefficient of variation (S) and skewness (L), which is expressed as a Box-Cox power. The change in each of these parameters with age is represented by a graph, with the three curves being fitted as cubic splines with non-linear regression using penalised likelihood. When generating the graphs for each of these parameters, the equivalent degrees of freedom (EDF) for the L, M and S curves needs to be defined. The EDF measures the complexity of each fitted cubic spline curve. Deciding which value to use for EDF defines the smoothness of the curves for the individual parameters (L, M, S) and significantly affects the fit of the final SDS curves to the data. A high EDF leads to the curves being undersmoothed as the curves become relatively more complex.

The chosen EDF for the L, M and S curves were 2, 7, 3 for IGFBP-3 in females and, and 2, 8 and 3 for IGFBP-3 in males. The chosen EDF for the L, M and S curves were 3, 10 and 3 for IGF-1 in both sexes. There were inadequate control data to create SDS curves for IGFBP-3 in children aged <5 years.

Individuals with an IGF-1 ≤2SDS below the mean for age, a subnormal height velocity and a height ≤2SDS below the mean for age were further investigated with a glucagon stimulation test (protocol below).

Figure 4 IGF-1 in females standard deviation with age (years) (L,3 M,10 S,3)

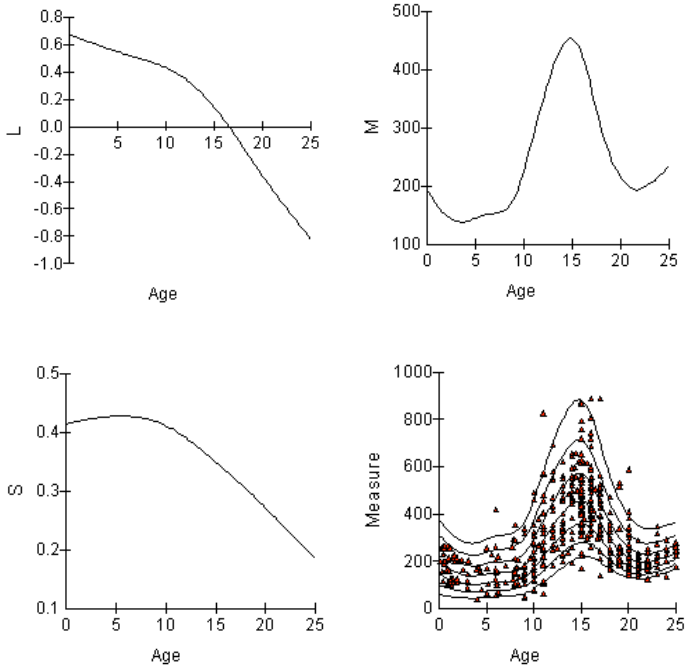


Figure 5 IGF-1 males standard deviation with age (years) (L,3 M,10 S,3)

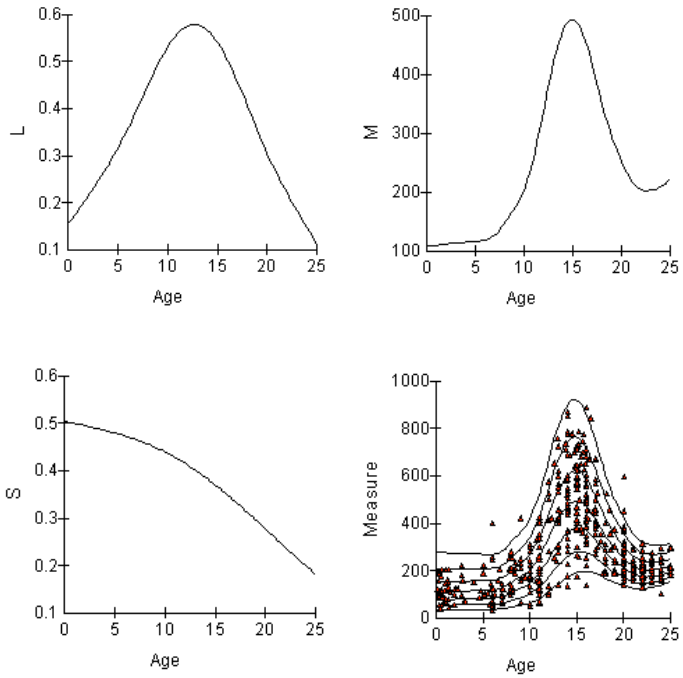


Figure 6 IGFBP-3 in females standard deviation with age (years) (L,2 M,8 S,3)

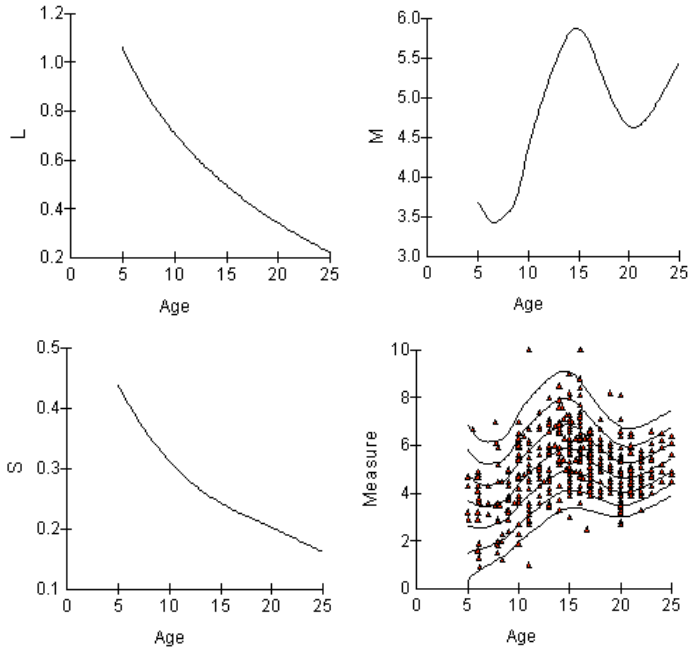
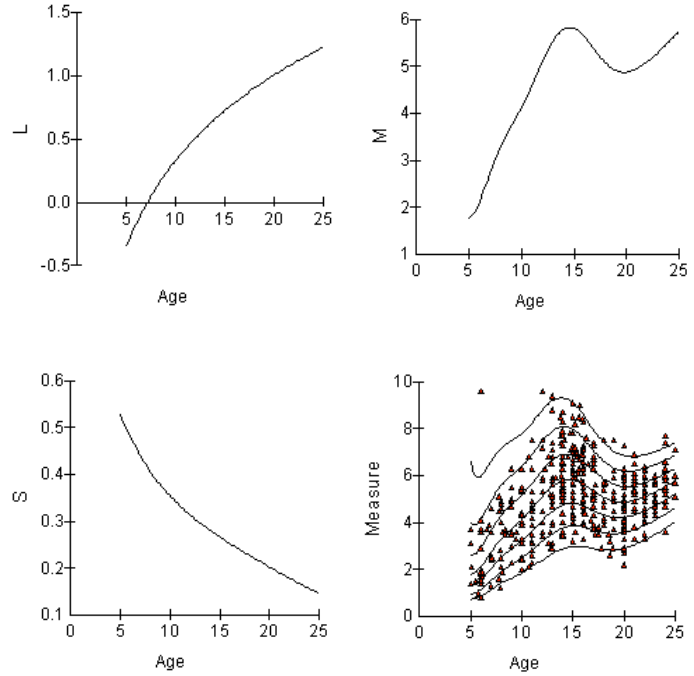


Figure 7 IGFBP-3 males standard deviation with age (years) (L,2 M,7 S,3)



Key Figures 4-7 LMS graph demonstrating the change in standard deviation scores for IGF-1 (Figures 4 and 5) and IGFBP-3 (Figures 6 and 7) with increasing age (over the range 0-25 years). The variation in IGF-1 and IGFBP-3 with age is represented by the reference centile curves. The LMS method uses 3 parameters to define the change in the distribution of a parameter with age. These parameters are the median (M), coefficient of variation (S) and skewness (L), which is expressed as a Box-Cox power. The change in each of these parameters with age is represented by the graphs in Figures 4-7.

v. Bone age

In all children presenting with short stature, (height <2SDS below mean for population) a plain radiograph was performed of the left hand and wrist. Bone age was determined using the Tanner-Whitehouse method (175). The same investigators (Dr Emma Webb and Professor Mehul Dattani) calculated bone age in all patients.

vi. Pubertal stage

One paediatric endocrinologist (Dr Emma Webb) performed pubertal staging (Tanner method) on all children (176).

vii. Endocrine assessment

Subjects were admitted to the endocrine ward at Great Ormond Street Hospital at 08.00 hours having fasted from midnight the night before. Sex-steroid priming was not performed as all children were prepubertal and had a bone age <10years. A heparin locked cannula was placed in a forearm vein 30 minutes prior to glucagon administration. Baseline bloods for basic

haematology and biochemistry, thyroid function, prolactin, erythrocyte sedimentation rate and tissue transglutaminase were taken at the time of cannula insertion.

Serum prolactin was measured in duplicate using the Immulite® 2500 solid-phase, two-site chemiluminescent immunometric assay. The within-assay CVs for prolactin were 2.8% and 2.5% at 187 and 1018 mU/L respectively. The between-assay CVs for prolactin were 8.2% and 6.9 % at 187 and 1018 mU/L respectively, and the detection limit of the assay was 20 mU/L.

A standardised glucagon stimulation test was then performed by the ward staff. Samples for measurement of blood glucose and serum GH concentrations were obtained at time -30 and 0, and 30, 60, 90, 120, 150 and 180 minutes after intramuscular (IM) injection of 100microgrammes/kg body weight glucagon (maximum 1 mg). Peak GH response to glucagon testing of ≤6.7ng/ml was diagnostic of GHD. Peak GH response to glucagon testing of ≥10ng/ml was defined as normal. Children with peak GH response to stimulation between 7 and 10ng/ml were excluded from participation in the study. Serum GH was measured in duplicate using the Immulite® 2500 solid-phase, two-site chemiluminescent immunometric assay. The within-assay CVs for GH were 3.5% and 2.9% at 2.6 ug/L and 7.9 ug/L respectively. The between-assay CVs for GH were 6.5% and 4.2% at 2.6 ug/L and 7.9 ug/L, and the detection limit of the assay was 0.1 ug/L.

In all children diagnosed with GHD, SOD and ONH a 24 hr cortisol profile (24-hour two-hourly blood samples to measure cortisol and glucose), was performed according to standardised protocols to exclude cortisol deficiency. Mean cortisol of >145nmol/L over a 24 hour period and/ or a morning peak cortisol of >175nmol/L were defined as normal (177). Serum cortisol was measured in duplicate using the Immulite® 2500 solid-phase, competitive chemiluminescent enzyme immunoassay. The within-assay CVs for cortisol were 6.0% and 6.2% at 91 and 717 nmol/L respectively. The between-assay CVs for cortisol were 8.1% and 7.3% at 91 and 717 nmol/L, and the detection limit of the assay was 28 nmol/L.

D. MRI acquisition

MR imaging was performed on an Avanto 1.5 Tesla MRI scanner (Siemens, Erlangen, Germany). A standard brain and pituitary scan (3mm thick image acquisition of the pituitary, in the sagittal and coronal planes using a combination of T_1 and T_2-weighted sequences), was performed on all subjects recruited to the study. All images were reviewed by a single experienced neuroradiologist, who was blinded to the clinical data (Dr Kling Chong). Posterior pituitary gland location was established. Hypoplastic anterior pituitary gland was a subjective diagnosis, defined by the visual appearance of the anterior gland on all slices. A diagnosis of ONH and laterality of disease were determined by one experienced pediatric neuroradiologist (Dr Kling Chong) blinded to the clinical data who reviewed all images. The diagnosis of ONH was confirmed by ocular fundus photography performed by a pediatric ophthalmologist.

3D FLASH (TR 11 ms, TE 4.94 ms, FOV 256 mm, voxel size 1 mm^3) (acquisition time 5mins) and 3D FLAIR (TR 6000 ms, TE 353 ms, TI 2200 ms, FOV 256 mm, voxel size 1 mm^3) (acquisition time 15mins) datasets were acquired. Diffusion tensor imaging was also performed. Twenty diffusion sensitised directions were acquired with a *b* value of 1000 s mm^{-2} along with 1 image at *b*=0 for normalisation. This protocol was repeated 3 times to improve signal to noise ratio. The image dimensions for the diffusion sequence are 96 × 96 × 45, consisting of 2.5mm isotropic voxels.

E. MRI analysis

i) FreeSurfer analysis: *IGHD AND ISS GROUPS*

Neural volumes were acquired using FreeSurfer, an MRI brain imaging software package which performs whole brain segmentation and automated labelling of the neuroanatomical structures based on probabilistic information estimated from a manually labelled atlas using the T1-weighted images (104;178).

To generate hypotheses as to which structures in the brain could be affected by IGF-1 and IGFBP-3 deficiency and, in order to reduce the number of multiple comparisons made, a literature search for articles pertaining to the distribution of brain GH and IGF-1 expression was performed (summarized in Introduction). Human brain GH receptor expression is highest in the choroid plexus, thalamus, hypothalamus, pituitary, putamen and hippocampus (40). IGF-1 gene expression has been found in humans in the hypothalamus, hippocampus, olfactory bulb, cerebellum, neocortex and striatum (179) with IGF-1 receptors most dense in the hippocampus,

amygdala, caudate nucleus, cortex, cerebellum and prefrontal cortex (53;179). On the basis of this literature we hypothesized that IGF-1 and IGFBP-3 SDS would correlate with total brain volume and the volumes of the thalamus, putamen, hippocampus, amygdala, caudate nucleus, parahippocampal gyrus, prefrontal cortex and cerebellum. As several of these structures form part of the basal ganglia, volumes for all components of the basal ganglia generated by FreeSurfer (104;178) were acquired. We also selected structures based on cognitive findings in children with IGHD using previous studies correlating cognitive performance with neural abnormalities (180-182). The automated parcellation of the neural volumes acquired was reviewed to assess that the correct structures had been extracted and that the structure margins defined by freesurfer were appropriate.

The volumetric data for the two groups were compared using t-tests. Where regions of significant difference ($p<0.05$) were identified neural volumes were compared using analysis of covariance, controlling for age at scan, sex and total brain volume (to ensure that differences found in specific structure volumes were not secondary to a generalized effect of variability in total brain volume). As multiple correlations were performed, the p-values for significance were adjusted to control for the false discovery rate (FDR) (183). Stepwise linear regression was also performed to assess the significance of study group in determining neural volumes with age, sex, study group and total brain volume being entered into the model.

ii) Voxel-based morphometry: *IGHD AND ISS GROUPS*

The 3D MRI data sets were also processed using statistical parametric mapping software version 8 (SPM8; Wellcome Trust Centre of Neuroimaging, Institute of Neurology, University College of London) running in Matlab R2009a (version 7.8.0) (184). The same investigator (EW) located the anterior commissure in each dataset and reoriented datasets to set the origin to 0,0,0 at the anterior commissure. Images were segmented using the segmentation tool in SPM8. The Diffeomorphic anatomic registration through exponentiated Lie algebra algorithm (DARTEL) toolbox (185) was then used to generate a template and to co-register the images. Registration achieved using DARTEL is superior to standard voxel based morphometry in which scans are spatially normalized to a standard template. DARTEL adjusts for the fact that different brains vary in shape and size and results in improved inter-subject alignment as compared with previous methods. This toolbox is especially useful in paediatric studies where collecting large amounts of control data to collate templates from can be challenging. By using DARTEL warping the images to an adult template and thus distorting findings is avoided. DARTEL was used to derive Jacobian scaled (modulated) warped tissue class images for white and grey matter. The modulated warped gray and white matter images were subsequently smoothed with an 8mm full width half-maximum isotropic Gaussian kernel, and then entered into statistical analyses. Smoothing reduces the effect of inter-subject anatomical variability, which may remain after image co-registration. The final smoothed modulated warped images had a normalized voxel size of 1.5 x 1.5 x 1.5mm.

Voxel based analysis was then performed using independent t-test on the smoothed, modulated, and segmented gray and white matter images with a voxel threshold of $p < 0.05$, family-wise error (FWE)-corrected to compare the maps of the 2 groups of subjects (IGHD and ISS) (all t-tests corrected for age and sex). Previous studies in children have shown that age has a significant effect on grey and white matter (186); age was therefore used as a covariate in all analyses. Where a significant difference in grey or white matter is present, voxels are displayed on an output map (statistical parametric map [SPM]).

iii) Diffusion tensor imaging: *IGHD, ISS AND ONH GROUPS*

Diffusion-weighted images were initially processed using the Functional Magnetic Resonance Imaging of the Brain Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl).

Data were inspected for movement artefacts and then correction for eddy current induced distortions, brain extraction, and calculation of diffusion tensor FA and mean diffusivity (MD) maps was carried out using FSL tools (http://www.fmrib.ox.ac.uk/fsl). Participants were excluded from image analysis if data quality was poor, for example secondary to excessive head movement or if the acquisition was incomplete.

iv) **Tract Based Spatial Statistics:** *IGHD, ISS AND ONH GROUPS*

The FA images were initially processed using TBSS (121;187), and automated, observer-independent, voxel-by-voxel whole-brain between-group analysis performed.

Initially, every FA image was aligned to every other one using the -n flag. This selects the most representative study image as a target image which is then affine-aligned into MNI152 standard space. Other study images were then transformed into 1x1x1mm MNI152 space by combining the nonlinear transform to the target FA image with the affine transform from that target to MNI152 space. Secondly the mean of all FA images was created using the -s option. The mean image was subsequently thinned and thresholded at an FA value of 0.2 to create a white matter tract skeleton representing the center of the tracts common to all subjects. FA data projected onto these skeletons was then used in voxel-wise statistical comparisons using the Threshold-Free Cluster Enhancement option (which is fully corrected for multiple comparisons across space).

v) **Tract Based Spatial Statistics:** *IGHD AND ISS GROUPS*

TBSS was used to calculate tract-based differences in FA values between the GHD group and the ISS control group. TBSS has the advantage over VBM that it does not require smoothing. However it only compares the skeletonised white matter tracts between subjects and regions of FA outside the white matter tracts i.e. in the hippocampus are not assessed. Values for corpus callosum and corticospinal tract FA were extracted from the TBSS

analysis by masking the mean skeleton with the appropriate structure label from the Johns Hopkins University white-matter tractography atlas (188).

FA and MD of the total white matter skeleton, corpus callosum and cortico-spinal tract were compared using Analysis of Covariance (ANCOVA). Age and sex are known to affect brain growth and myelination and were therefore used as a covariates in all analyses. Total brain volume (TBV) was compared using ANCOVA, controlling for age at scan, and sex. For all other neural volumes, TBV was used as an additional covariate.

vi) **Tract Based Spatial Statistics:** ONH AND ISS GROUPS

The FA images were analyzed using tract-based spatial statistics (121;187), an automated, observer-independent, voxel-by-voxel whole-brain between-group analysis technique, as described above. All analyses were corrected for age and sex. Values for the ventral cingulum, corpus callosum and optic radiation FA were extracted from the TBSS analysis by masking the mean skeleton with the appropriate structure label from the Johns Hopkins University white-matter tractography atlas (188).

F. Rest-activity pattern assessment

i. Actigraphy

All patients with SOD, IGHD and ISS wore the Actiwatch mini® (CamNtech Ltd), which detects and logs movement intensity and duration using an internal accelerometer, on the wrist of their non-dominant hand for a 2 week period. The actiwatch has been validated against polysomnography for the

assessment of sleep wake patterns and sleep efficiency in children (189). Actigraphy is more useful than laboratory based sleep studies when assessing circadian rhythm as it enables data collection over a longer time period, with a large body of evidence supporting its use in describing sleep-wake cycles and the identification of sleep abnormalities (190). The watches were calibrated by the manufacturer prior to use to ensure that data collection and sensitivity were comparable between the devices used.

ii. Sleep diary

Parents of children with SOD, IGHD and ISS were asked to keep a detailed sleep diary over the 2 week period recording the time of sleep onset and offset, and the number and length of night awakenings, daytime naps and actiwatch removal.

iii. Analysis of actiwatch data

Actiwatch data were analyzed in conjunction with the sleep diary. Sleep wake patterns were ascertained and actual time asleep and sleep efficiency calculated using Actiware software (Minimitter). Sleep efficiency was defined as the ratio of total sleep time to sleep period (191). Actogram analysis was used to define the sleep pattern as normal (tau 24hrs), free running (tau>24hrs), fragmented (sleep efficiency <85%), or arrhythmic (no discernable rhythmicity) (157). When these definitions are applied to healthy control populations very few individuals are identified with free running, fragmented or arrhythmic sleep patterns (157;190). Sleep efficiency in

children with SOD was compared to that of the healthy controls using the independent Student's t test.

iv. Melatonin profile

Children with SOD were admitted to hospital on the morning of the melatonin profile. Plasma samples were collected hourly over a 24hr period by Dr Emma Webb from an indwelling venous cannula, with overnight samples being taken under dim light (20:00-06:00). Samples were collected into lithium heparin tubes, mixed by inversion and spun immediately in a refrigerated centrifuge. Plasma was then removed and stored at -20^{o}C until assayed.

v. Melatonin analysis

Melatonin concentrations were measured by Stockgrand Ltd. Within assay CVs for melatonin were 11.8%, 9.6%, 8.1%, 7.8% and 5.5% at 18, 26, 50, 88 and 107 pg/ml. Between-assay CVs for melatonin were 13.1%, 11.3%, 7.7% and 8.1% at 14, 33, 90 and 118 pg/ml, and the detection limit of the assay was 3.3 pg/ml. The melatonin results were compared to historical controls collected by Waldhauser *et al* to assess normality (192).

G. Behavioural data acquisition

All parents were asked to complete the Child Behaviour Checklist (CBCL) (193), a standardized and validated rating scale that screens for emotional, social, and behavioural problems. The checklist includes 8 domains of behaviour: Social Withdrawal, Somatic

Complaints, Anxiety/Depression, Social Problems, Thought Problems, Attention Problems, Delinquent Behaviour, and Aggressive Behaviour. Each category is a compilation of observations about the child's behaviour with Likert scale values: 0 = no, 1 = sometimes, and 2 = very often; the sum of the values in each category signifies the severity of the behaviour. The scores for internalising and externalising problems and total scores were also calculated. Internalising problems consist of syndrome scales for emotionally reactive behaviour, anxious/depressed behaviour, somatic complaints and withdrawn behaviour. Externalising problems consist of syndrome scales for attention problems and aggressive behaviour. For these scores, cut-offs for subclinical and clinical problems were set at the 84th and 90th percentiles, respectively, following the CBCL manual.

H. Behavioural data analysis

Behavioural assessment scores were compared using ANCOVA controlling for socioeconomic status and maternal educational attainment (IGHD vs ISS and ONH vs ISS). Partial correlations were also used to assess the relationships of plasma IGF-1 and IGFBP-3 SDS to behavioural measures, adjusted for socioeconomic status and maternal educational attainment in children with IGHD. As multiple comparisons were performed, the p-values for significance were adjusted to control for the false discovery rate (FDR) (183).

I. Cognitive data acquisition

One assessor (Dr Michelle O Reilly) blinded to participant group performed all cognitive assessments.

i. IGHD AND ISS GROUPS

A detailed assessment of cognitive function was performed at baseline. Intellectual functioning was assessed using the Wechsler Intelligence Scales for Children IV edition (WISC-IV). Full-Scale IQ, Verbal Comprehension Index, Perceptual Reasoning Index, Working Memory and Processing Speed indices (FSIQ, VCI, PRI, WMI and PSI respectively) were calculated (population mean=100, SD=15) (194). Younger participants (one child in the IGHD group and two children in the SS control group) were assessed using the Wechsler Preschool and Primary Scale of Intelligence-Third Edition (WPPSI-III UK), with Full Scale IQ, Verbal and Performance IQ and Processing Speed scores generated. For the sake of brevity, these children's Verbal IQ was designated as VCI data and Performance IQ as PRI data. In our clinical experience we have found that parents of children with IGHD frequently report that they have delayed motor development when compared to their siblings. Therefore neuromotor function was assessed with the Movement Assessment Battery for Children 2nd Edition (M-ABC2) (195;196). Standard scores for the three motor skills components of manual dexterity, aiming & catching and balance and for the total test score are based on a distribution with a mean of 10 and a standard deviation of 3.

Attention, memory, executive function and language were assessed using the Developmental NEuroPSYchological Assessment (NEPSY) and CANTAB (Cambridge Neuropsychological Test Automated Battery) neuropsychological test batteries. Measures of memory included Memory for Faces, Memory for Names, Narrative Memory and List Memory from the NEPSY-II battery (197), and Pattern Recognition Memory, Spatial Working Memory and Paired Associate Learning tests from the CANTAB (198). Attention was assessed using the Big/Little Circle, Intra-dimensional

Extra-dimensional Set shift, Rapid Visual Information Processing tests from CANTAB. The Inhibition and Word Generation tests from the NEPSY-II battery and Intra-dimensional Extra-dimensional Set shift from CANTAB were used to assess Executive Function.

ii. ONH AND SOD GROUPS

Children aged over 6 years were assessed using WISC-IV; FSIQ, VCI, PRI, WMI and PSI were calculated (population mean=100, SD=15) (199). Younger participants were assessed using the WPPSI-III UK, with FSIQ, Verbal and Performance IQ scores generated (200). The SOD group and the two ONH participants who were too young for the WPPSI assessment were assessed using the semi-standardized Reynell-Zinkin Scales (RZS) (201). The RZS were performed as part of routine clinical assessment by clinical psychologists and paediatricians, who were experienced in assessing children with visual impairment. Raw scores were converted to age equivalent levels from the normative values in the RZS manual that are appropriate for the child's level of vision. Developmental Quotients were derived from the mid-points of the age equivalent level divided by the chronological age of the child at the time of assessment (200). Normal developmental status is typically defined as developmental quotient $\geq$ 80 (134), and FSIQ $\leq$ 80 is considered below average (200).

J. Cognitive data analysis

In the IGHD and ISS groups total scores for FSIQ, CBCL and Movement ABC were compared using ANCOVA controlling for socioeconomic status and maternal educational attainment. Scores were adjusted to control for FDR. Where total scores showed significant differences between groups sub-test analysis was then performed.

Partial correlations were used to assess the associations between plasma IGF-1 and IGFBP-3 SDS and cognitive measures, adjusted for socioeconomic status and maternal educational attainment in children with IGHD [in population based studies both maternal IQ and socioeconomic status have been found to be strong predictors of a child's IQ (202)]. As multiple comparisons were performed, the p-values for significance were adjusted to control for the FDR (183).

K. DNA collection and analysis

DNA samples were collected as 2 x 5-10 ml EDTA blood samples from all individuals with IGHD, ONH and SOD for genetic studies. The Institute of Child Health DNA laboratory processed the EDTA samples and generated DNA in 250 mg/ml aliquots of differing volumes. DNA was sequenced for mutations in the following genes (IGHD: *GH-1* and *GHRHR*; ONH and SOD: *HESX-1*, *SOX-2* and *SOX-3*), depending on the phenotype (Table 4) using Polymerase Chain Reaction, a method for amplifying a DNA base sequence, producing a rapid and highly specific amplification of the desired region. DNA was amplified using primers designed to include coding and splicing regions of individual exons for *GH1*, *GHRHR*, *HESX1*, SOX2, and *SOX3* as previously described (203-208). For any novel mutations identified, the sequence change was ascertained by screening 100 samples derived from an appropriate control population to establish whether the change was polymorphic.

3. THE EFFECT OF ISOLATED GROWTH HORMONE DEFICIENCY ON COGNITION, MOTOR FUNCTION AND BRAIN STRUCTURE

'Even among human beings, children when compared with adults, and dwarf adults, when compared with others, may have some characteristics in which they are superior, but in intelligence at any rate, they are inferior. And the reason is that in many of them the principle of the soul is sluggish and corporeal…'

(Aristotle, Parts of animals, IV.x.)

A. Introduction

Evidence is accumulating that both IGF-1 and IGFBP-3 play an important role in normal brain growth and development (209), with an ongoing role in determining childhood and adult cognitive function (13;48;210). There is also evidence to suggest that IGF-1 and IGFBP-3 deficiency such as that found in children and adults with GHD, and in children born extremely preterm (<31weeks at birth) in whom there is a relative IGF-1 and IGFBP-3 deficiency postnatally (107), is associated with cognitive deficits and changes in brain structure.

The impact of IGF-1 and IGFBP-3 deficiency on cognitive function has primarily been studied in adults and children with GHD. In adults (with childhood and adult onset GHD) cognitive function deficits, principally in the domains of memory and attention, have previously been documented. These deficits are ameliorated by GH replacement

(73;80;94). In the majority of studies examining children with GHD, IQ has been found to be within the normal range. Despite this, a significant proportion of children with GHD have difficulties with education particularly in the domains of reading, spelling and arithmetic (211). However, to date, the precise nature of the cognitive impairments associated with childhood IGHD remains unclear with the findings from previous studies being difficult to interpret due to: (1) the definition of GHD used (e.g. If GHD is defined as a peak GH response of <10μg/L, and normal as a peak GH response of >10μg/L, some individuals will be misclassified), (2) the inclusion of heterogeneous patient groups with varying aetiologies (e.g. post-radiation GHD, congenital GHD, multiple pituitary hormone deficiency), (3) variation in the duration of GHD, (4) the wide age-range of patients studied, and (5) the lack of uniformity in the cognitive tests used to measure performance (80).

Motor skills have previously been evaluated in children with IGHD and MPHD by assessing their ability to copy a number of designs. In this combined (IGHD & MPHD) cohort abnormalities in visual-motor skills were identified (100;101). However again the existence of pituitary hormone abnormalities other than isolated GHD in this study makes it hard to extrapolate these findings to children with solely IGHD. Specific assessment of motor skills have not previously been performed in individuals with IGHD (101).

Detailed neuroimaging studies using techniques such as volumetric MRI and DTI brain have not previously been performed in individuals with IGHD. MRI can be used to identify correlations between neural volumes, FA and parameters of interest such as IGF-1 and IGFBP-3 SDS to further assess the role of GH in neural development. The only similar population studied to date using some of these techniques is children born

extremely preterm, in whom there is a relative IGF-1 and IGFBP-3 deficiency post-natally. In these children Hansen-Pupp *et al* identified significant associations between IGF-1 and IGFBP-3, verbal IQ and total brain volume (107).

To further analyse the roles of IGF-1 and IGFBP-3 in brain development, we therefore investigated a cohort of children with IGHD using cognitive and motor assessment together with MRI, DTI and VBM and compared the findings with those of a cohort of children with ISS.

We hypothesized that in children with IGHD, IQ would correlate significantly with IGF-1 and IGFBP-3 SDS, and that FA, TBV and the volumes of the thalamus, putamen, hippocampus, amygdala, caudate nucleus, parahippocampal gyrus, prefrontal cortex and cerebellum (regions of the brain with the highest GHR, IGF-1 receptor and IGF-1 gene expression (40) (57;179)) would be significantly lower than in the ISS control group.

B. Methods

i. Study subjects

Two cohorts (IGHD and ISS, aged 5-11 years) were recruited prospectively from the paediatric endocrine clinic at Great Ormond Street Hospital between 2007 and 2009. IGHD was diagnosed in children with a height and growth velocity ≤2 SDS below the mean for age, a peak GH <6.7μg/L on two tests of GH release (glucagon and clonidine), or on one stimulation test in association with a pathologically low IGF-1 concentration for age and sex (below -2 SDS). Bone age delay was also used to support the diagnosis of IGHD. The presence of other hormonal abnormalities was excluded in children with

IGHD. ISS was diagnosed in children with a height ≤2 SDS below the mean for age, a normal height velocity, normal brain and pituitary MRI, normal IGF-1 concentration for age and sex (defined as between -2 and +2 SDS), and a normal peak GH in response to stimulation (>10 μg/L).

ii. Baseline characteristics

IGF-1, IGFBP-3 and growth velocity were measured and brain DTI and volumetric MRI acquired. All study subjects (IGHD and controls) completed 2 weeks actigraphy and a detailed sleep diary. Actiwatch data were analyzed in conjunction with the sleep diary.

iii. Cognitive (intellectual and neuromotor) and behavioural assessment

One assessor (MOR) blinded to participant group performed all cognitive assessments. Intellectual functioning was assessed using the full WISC-IV. FSIQ, VCI, PRI, WMI and PSI were calculated (population mean=100, SD=15) (200). Younger participants (aged 5-6 years, 1 IGHD, 2 ISS) were assessed using the WPPSI-III UK, with FSIQ, Verbal and Performance IQ and processing speed index scores generated. Neuromotor function was assessed with the M-ABC2 (195). Behaviour was assessed using the CBCL.

iv. MRI analysis

MRI data was processed using FreeSurfer 5.0 and volumes extracted for the basal ganglia, thalamus, hippocampus and corpus callosum, (51;113;212;213). Structures were selected based both on studies detailing the expression pattern of GH and IGF-1 receptors in the brain and on previous studies correlating cognitive and motor skills

performance with neural abnormalities (57;180-182). Total brain volume was also calculated. No other neural volumes were extracted from the FreeSurfer analysis to ensure all analyses performed were hypothesis driven. The difference in total brain volume between groups was compared using ANCOVA, controlling for age at scan, and gender. For all other neural volumes, total brain volume was used as an additional covariate. Freesurfer segmentation was checked for all neural volumes extracted. As multiple ANCOVA's were carried out to compare the difference in neural volumes between the two groups, the p-values for significance were adjusted to control for the FDR (183). Volumetric data were also processed using VBM with SPM8 software (Wellcome Department of Cognitive Neurology, Institute of Neurology), running in Matlab R2009a (version 7.8.0) (184). Voxel based analysis was then performed using independent t-test on the smoothed, modulated, and segmented gray and white matter images with a voxel threshold of $p < 0.05$, FWE corrected to compare the maps of the 2 groups of subjects (IGHD and ISS) (all t-tests corrected for age and sex). FA and MD images were processed using TBSS and automated, observer-independent, voxel-by-voxel whole-brain between-group analysis performed (corrected for age) (121). Values for corpus callosum and corticospinal tract FA were extracted from the TBSS analysis (detailed description in Methods). FA and MD of the total white matter skeleton, corpus callosum and cortico-spinal tract were compared using ANCOVA. Age and sex are known to affect brain growth and myelination and were therefore used as a covariate in all analyses.

v. Statistics

Baseline characteristics of the two groups, including age, sex, birth weight, gestational age, handedness, maternal highest educational level and socioeconomic status, peak GH (μg/L), IGF-1 SDS and IGFBP-3 SDS, height SDS, growth velocity SDS and BMI SDS were compared using independent Student's *t* test. Total scores for FSIQ, CBCL and Movement ABC were compared using ANCOVA controlling for socioeconomic status and maternal educational attainment. Scores were adjusted to control for FDR. Where total scores showed significant differences between groups sub-test analysis was then performed.

Partial correlations were used to assess the relationships between plasma IGF-1 and IGFBP-3 SDS and cognitive and motor skills assessment scores, (adjusted for socioeconomic status and maternal educational attainment) in children with IGHD and in the group as a whole (IGHD and ISS). Partial correlation was used to assess the relationship between neural volumes and the processing speed index, verbal comprehension index and IGF-1 and IGFBP-3 SDS (controlled for age at scan, sex and TBV).

Partial correlation was used to assess the relationship between neural volumes (where there was a significant difference in volumes of brain structures between the 2 groups) and FA of the corpus callosum and cortico-spinal tract to FSIQ, the Perceptual Reasoning Index, Verbal Comprehension Index and scores on the M-ABC2 and IGF-1 and IGFBP-3 SDS (controlled for age at scan, gender and total brain volume) in children with IGHD.

As children with ISS do not have an entirely normal GH-axis (as demonstrated by their mean IGF-1 SDS which falls below 0) partial correlation was also performed to assess the relationship between IGF-1 and IGFBP-3 SDS and MRI measures in the whole study group (IGHD and ISS) (controlled for age at scan, gender and total brain volume).

vi. Genetic analysis

Children with ectopic posterior pituitary gland and IGHD were screened for mutations in *SOX-3*. All children with IGHD were screened for mutations in *GH-1* and *HESX-1*.

C. Results

i. Subject characteristics

Fifteen children (mean 8.6yrs) with IGHD (peak GH <6.7µg/l [mean 3.9µg/l]), and 14 (mean 8.5yrs) with ISS (peak GH >10µg/l [mean 14µg/l] and normal growth rate) were recruited (3 children with ISS declined to participate). All children were right-handed, had no abnormal neurological findings and were in mainstream schooling. No subjects were hypoglycaemic (blood glucose <3.5mmol/L) after a 12hour fast. No children had abnormal cortisol profiles. Two children with IGHD and one with ISS did not tolerate the DTI scan but completed all other study components. Subject characteristics are summarised in Table 5. Table 6 outlines the baseline endocrinology of the two groups, and the anthropometric characteristics of the two groups are summarized in Table 7. No mutations in any of the genes screened (*HESX-1*, 15 children screened, *GH-1*, 15 children screened, and *SOX-3*, 7 individuals screened) were identified. Actigraphic studies were normal in all children with IGHD and all ISS controls.

Table 5 Subject characteristics

	IGHD	**ISS**	**p-value***
Subject characteristics			
Number	15	14	
Mean Age (SD, Standard Error (SE))	8.75 (1.8, 0.5)	8.36 (1.65, 0.4)	0.55
% Male (Number)	80 (12/15)	64 (9/14)	0.62
Gestational Age (SD)	39.4 (1.4)	39 (0.6)	0.97
Birth weight (g) (SD, SE)	3.56 (0.9, 0.2)	3.28 (0.3, 0.1)	0.3
% Right Handed	100	100	
Social Status (SD, SE)	2.6 (1.1, 0.3)	2.85 (0.8, 0.2)	0.48
Maternal Education Status (SD, SE)	2.5 (1.2, 0.3)	3.1 (1, 0.28)	0.15
% Hypoplastic anterior pituitary on MRI (number)	60 (9/15)	0 (0/14)	
% Ectopic posterior pituitary on MRI (number)	47 (7/15)	0 (0/14)	

- Baseline characteristics of the two groups (IGHD & ISS) were compared using independent Student's *t* test.

Table 6 Baseline endocrinology

(SD, SE)	**IGHD**	**ISS**	**p-value***
GH baseline μg/L	0.8 (1.4, 0.4)	3 (2.7, 1)	0.03
GH peak μg/L	3.5 (2.3, 0.6)	15 (5.7, 1.9)	**0.001**
IGF-1 SDS	-2 (0.7, 0.2)	-0.5 (0.8, 0.2)	**0.001**
IGFBP-3 SDS	-1 (0.8, 0.2)	0.1 (0.7, 0.2)	**0.001**
Fasting blood glucose (mmol/L)	3.9 (0.4, 0.1)	4.1 (0.21, 0.1)	0.3
Free thyroxine (pmol/L)	16 (3.3, 0.9)	16.2 (2.2, 0.7)	0.86
Thyroid stimulating hormone (mU/L)	3.1 (2.1, 0.55)	3 (1.9, 0.5)	0.88
Prolactin (mU/L)	212 (180, 46)	234 (256, 74)	0.79
Peak cortisol (nmol/L)	747 (208, 55)	713 (230, 87)	0.74

*p-values highlighted in bold remained significant after adjustment to control for FDR (183)

Table 7 Anthropometric characteristics

	IGHD	**ISS**	**p-value***
Baseline (SD, SE)			
Mean Age	8.75 (1.8, 0.5)	8.36 (1.65, 0.4)	0.55
Growth velocity (cm/yr)	3.8 (1.3, 0.3)	5.8 (1.4, 0.5)	**0.006**
Growth velocity SDS	-1.6 (1, 0.29)	-0.3 (1, 0.34)	**0.009**
Target height SDS	-0.54 (0.8, 0.3)	-0.74 (1.1, 0.3)	0.61
Height SDS	-2.9 (0.7, 0.2)	-2.5 (0.5, 0.2)	0.85
Weight SDS	-2.3 (1.5, 0.4)	-1.7 (0.56, 0.2)	0.4
BMI SDS	-0.2 (1.1, 0.3)	-0.1 (0.6, 0.2)	0.8
Occipitofrontal circumference SDS	-1.7 (1.2, 0.4)	-1.8 (0.9, 0.4)	0.8
Bone Age	6.2 (2.3, 0.6)	6.7 (1.8, 0.4)	0.62

*p-values highlighted in bold remained significant after adjustment to control for FDR (183)

ii. Cognitive (intellectual) and behavioural assessment

When compared to controls, children with IGHD had significantly lower FSIQ ($p<0.02$), verbal comprehension index ($p<0.006$) and processing speed index ($p<0.05$) scores (Table 9). Scores for Perceptual Reasoning Index and Working Memory Index were also lower in children with IGHD than in children with ISS, however these differences did not reach statistical significance ($p=0.09$, $p=0.2$). Examination of the IQ data showed that there were 2 outliers (defined as Z score > or < 2). The IQ data were reanalyzed without these 2 outliers, and significant group differences in FSIQ ($p<0.01$) and the Verbal Comprehension Index ($p<0.01$) remained. There was no significant difference in executive function, attention and memory measures (performance on the CANTAB and NEPSY) between children with IGHD and ISS controls (Table 8). There were no significant difference in scores on the CBCL between children with IGHD and controls (Table 8). One child with ISS had behavioural scores in the borderline range, no children with IGHD had CBCL scores in the borderline or clinical range.

iii. Neuromotor assessment

When compared to controls, children with IGHD had significantly lower scores on the manual dexterity ($p<0.03$), balance ($p<0.008$) and total scores ($p<0.008$) of the M-ABC2 test (Table 8).

Table 8 Difference between cognitive, memory, behavioural and motor skills assessment scores in children with isolated growth hormone deficiency and idiopathic short stature

Wechsler Intelligence Scale for Children IV *	**IGHD**	**ISS**	**p-value**
Full Scale IQ (SD, SE)	92.8 (16, 4.2)	102.6 (8.6, 2.4)	**0.01**
Perceptual Reasoning Index (SD, SE)	98.5 (18.6, 4.8)	107.7 (11, 3)	0.09
Verbal Comprehension Index (SD, SE)	91 (14, 3.7)	106 (10, 2.9)	**0.01**
Working Memory Index (SD, SE)	93.6 (22, 4.1)	95.9 (13, 2.1)	0.2
Processing Speed Index (SD, SE)	85.3 (16.6, 2.4)	95.7 (13, 1.8)	**0.05**
NEPSY*	**IGHD**	**ISS**	**p-value**
Inhibition total error (SD, SE)	10.2 (3.1, 0.8)	10.4 (3.6, 1)	0.9
Inhibition naming time (SD, SE)	9.2 (4, 1)	10.4 (3.9, 1.1)	0.50
Inhibition switching time (SD, SE)	9.7 (4, 1.1)	11.7 (3, 0.9)	0.19
List memory (SD, SE)	9 (4.2, 1.1)	9.1 (3.4, 1)	0.96
Memory faces (SD, SE)	9.3 (4.6, 1.2)	9.9 (2.6, 0.8)	0.68
Memory names (SD, SE)	9.4 (2.4, 0.6)	10.7 (3.7, 1.1)	0.28
Phonic processes (SD, SE)	10.9 (1.6, 0.6)	12.2 (1.9, 0.9)	0.21
Word generation (SD, SE)	10.8 (4.6, 1.2)	12.2 (3.5, 1)	0.4
Memory faces (SD, SE)	9.3 (4.6, 1.2)	9.9 (2.6, 0.8)	0.68
Memory names (SD, SE)	9.4 (2.4, 0.6)	10.7 (3.7, 1.1)	0.28

Phonic processes (SD, SE)	10.9 (1.6, 0.6)	12.2 (1.9, 0.9)	0.21
Word generation (SD, SE)	10.8 (4.6, 1.2)	12.2 (3.5, 1)	0.4
Behavioural Assessment*	**IGHD**	**ISS**	**p-value**
CBCL Total Score (SD, SE)	53 (4.7, 1.3)	53.6 (5.5, 1.6)	0.88
Movement-ABC2 (scaled scores)*	**IGHD**	**ISS**	**p-value**
Total score (SD, SE)	6.9 (2.5, 0.7)	9.8 (2.6, 0.7)	**0.008**
Manual Dexterity (SD, SE)	7.2 (2.3, 0.6)	9.3 (2.6, 0.7)	**0.03**
Aiming & Catching (SD, SE)	7.7 (2.9, 0.7)	8.8 (2.8, 0.7)	0.3
Balance (SD, SE)	7.9 (3, 0.8)	10.4 (2.4, 0.6)	**0.009**

*Results corrected for socioeconomic status and maternal educational attainment

* Total scores for FSIQ, CBCL and Movement ABC were adjusted to control for FDR. Where total scores showed significant differences between groups sub-test analysis was then performed. P-values highlighted in bold remain significant after adjustment to control for FDR.

iv. Associations between cognitive assessment scores, IGF-1 and IGFBP-3 SDS

Children with IGHD

In children with IGHD, verbal comprehension index scores correlated significantly with IGF-1 and IGFBP-3 SDS ($r=0.7$, $p<0.03$; $r=0.7$, $p<0.02$) (Figure 8) and FSIQ correlated significantly with IGFBP-3 SDS ($r=0.6$, $p<0.03$), but not IGF-1 SDS ($r=0.5$, $p=0.08$).

ISS Controls

There were no significant correlations between IGF-1 and IGFBP-3 and IQ in the control group.

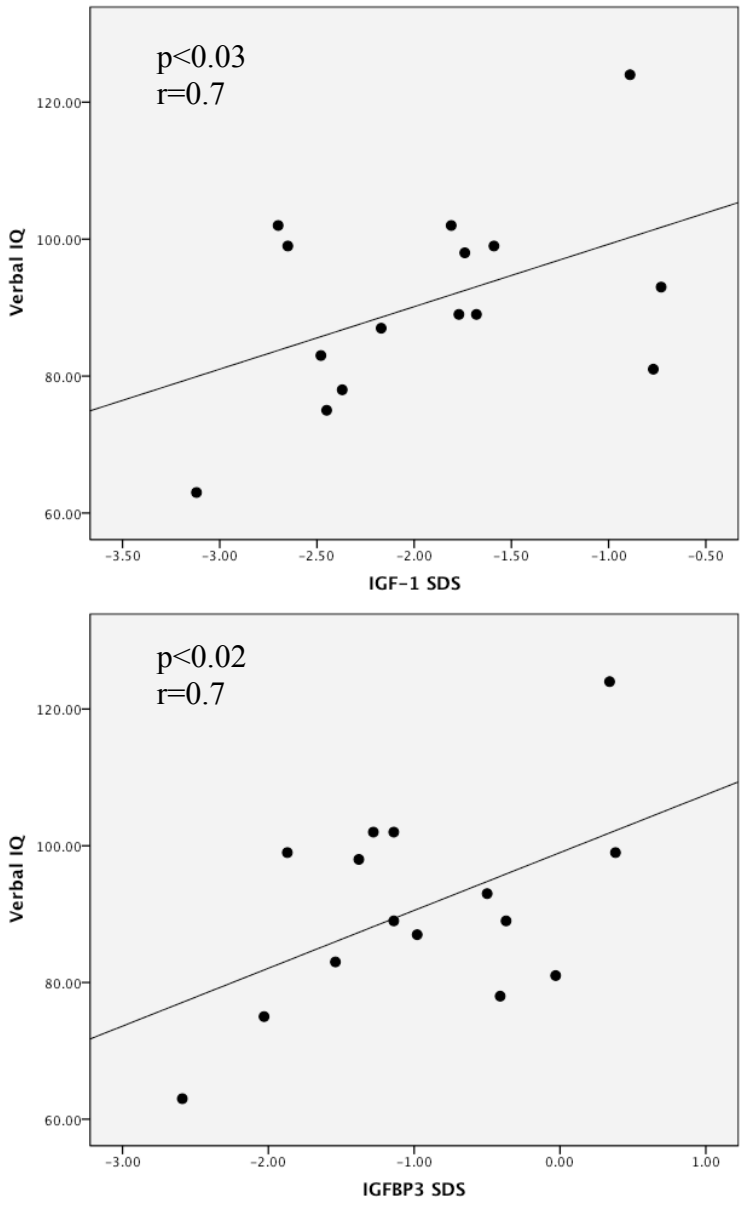
Whole Cohort (IGHD & ISS combined)

There was a significant positive correlation between whole cohort (IGHD and ISS) FSIQ and VIQ scores and plasma IGF1 SDS ($r=0.4$, $p<0.04$ and $r=0.5$, $p<0.02$, respectively) and IGFBP3 SDS ($r=0.4$, $p<0.04$, and $r=0.5$, $p<0.015$ respectively).

v. Associations between neuromotor assessment scores, IGF-1 and IGFBP-3 SDS

There were no significant correlations between neuromotor assessment scores, IGF-1 and IGFBP-3 SDS in children with IGHD, ISS controls or the whole cohort (IGHD and ISS combined).

Figure 8 Relationship between Verbal Comprehension Index (Verbal IQ), Insulin-like growth factor-1 (IGF-1) and Insulin-like growth factor binding protein-3 (IGFBP-3) standard deviation scores (SDS) in children with isolated growth hormone deficiency



Key Figure 8 Partial correlations were used to assess the relationships of plasma IGF-1 and IGFBP-3 SDS to the VIQ, adjusted for socioeconomic status and maternal educational attainment in children with IGHD. In children with IGHD, the VIQ scores correlated significantly with IGF-1 and IGFBP-3 SDS ($r=0.7$, $p<0.03$; $r=0.7$, $p<0.02$).

vi. MRI: Volumetric (freesurfer) results

The splenium of the corpus callosum, right pallidum, left thalamus and right hippocampus volumes were significantly smaller in children with IGHD ($p<0.01$, $p<0.007$, $p<0.01$ and $p<0.01$ respectively) than in ISS controls after correction for the FDR (Table 9).

vii. Correlations between volumetric results and IGF-1 and IGFBP-3 SDS

Children with IGHD

IGF-1 and IGFBP-3 SDS did not correlate significantly with neural volumes in children with IGHD.

Children with ISS

IGF-1 and IGFBP-3 SDS did not correlate significantly with neural volumes in children with ISS.

Whole Cohort (IGHD & ISS combined)

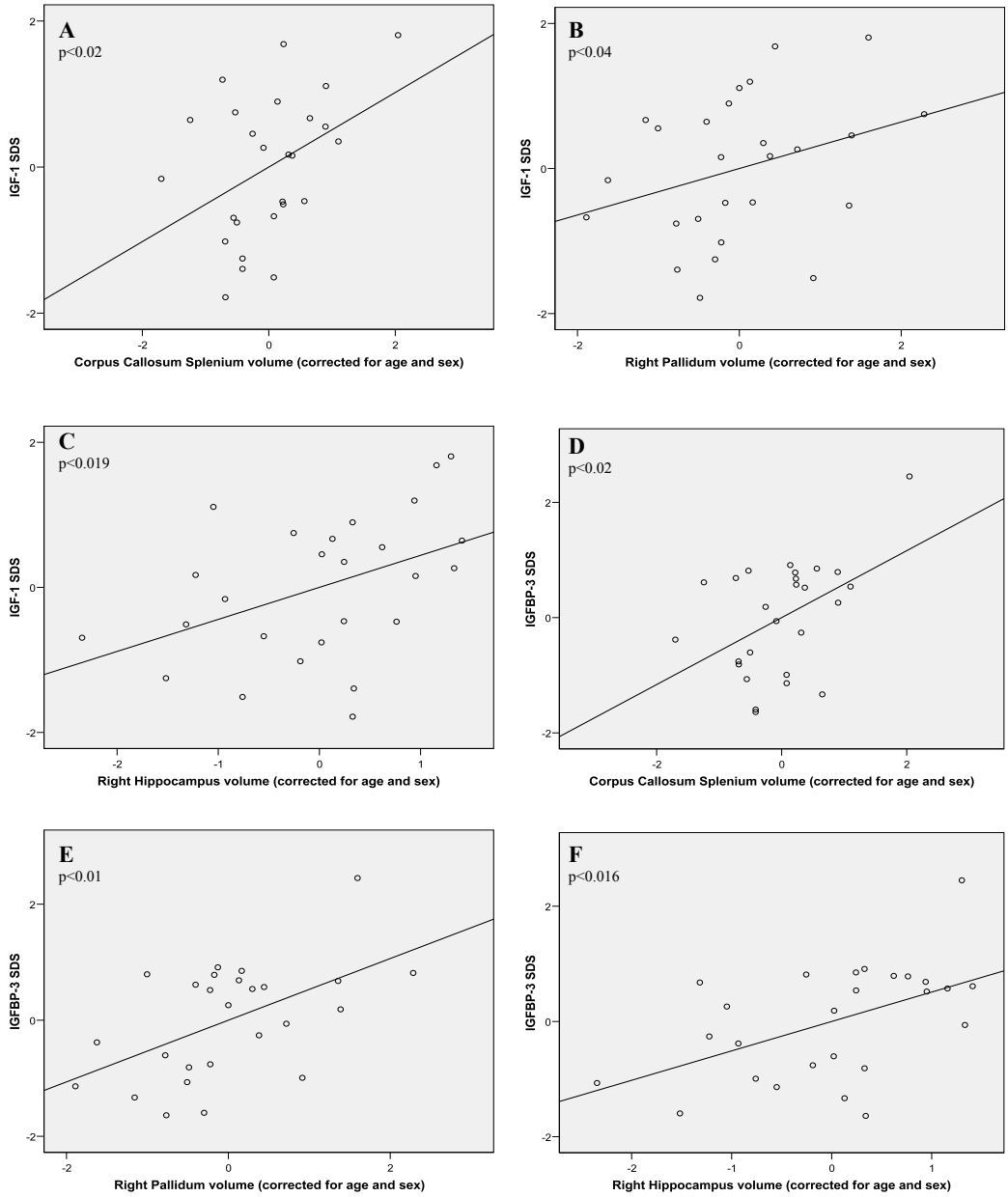
Corpus callosum splenium, right pallidum and right hippocampus volumes correlated significantly with IGF-1 ($P<0.02$, $p<0.04$ and $p<0.019$, respectively) and IGFBP-3 SDS ($p<0.02$, $p<0.01$ and $p<0.016$, respectively) (Figure 9). Whole group left pallidum and left and right thalami volumes correlated significantly with IGFBP-3 SDS ($p<0.04$, $p<0.05$ and $p<0.002$, respectively) (Figure 9).

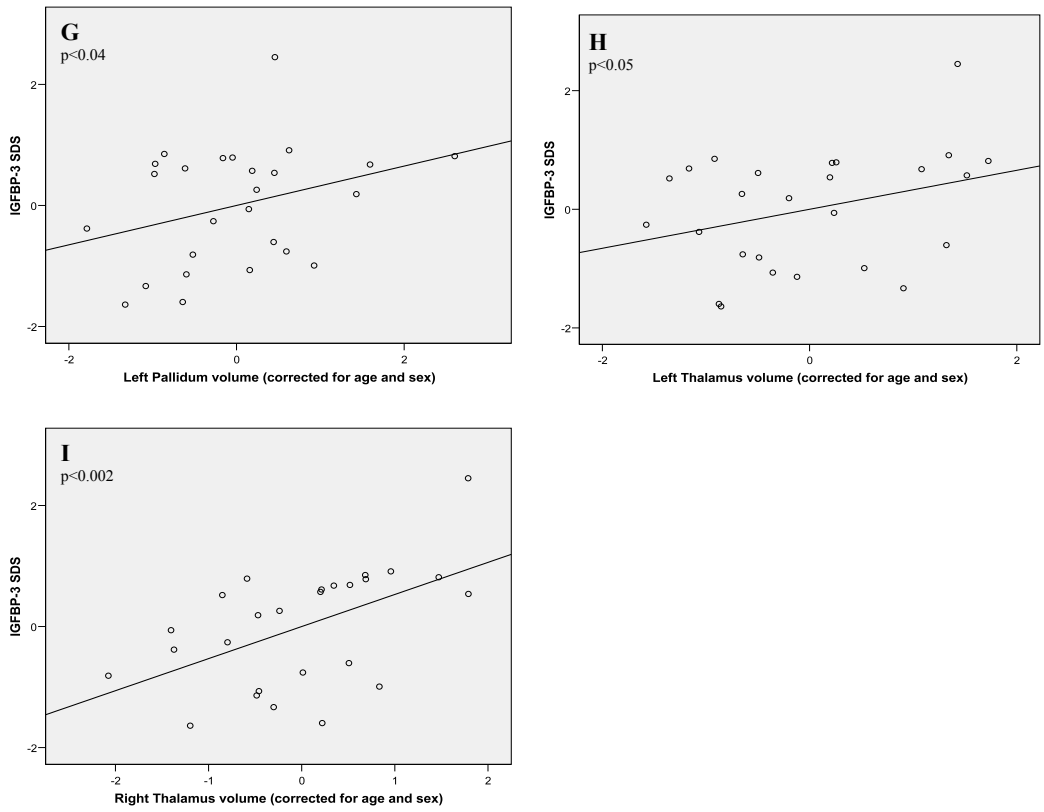
Table 9 The difference in Neural volumes between children with isolated growth hormone deficiency and idiopathic short stature

	GHD	**ISS**	**p-value**
Neural volumes (mm^3)** (SD, SE)			
Total brain volume	1.3 (2.1)	1.4 (1)	0.25
Splenium corpus callosum	1020 (220, 43)	1300 (210, 64)	**0.01**
Left caudate	3470 (780, 208)	3720 (606, 168)	0.7
Right caudate	3493 (747, 200)	3821 (581, 161)	0.3
Left pallidum	1600 (210, 50)	1780 (210, 56)	0.04
Right pallidum	1480 (190, 51)	1700 (220, 58)	**0.007**
Left putamen	5530 (772, 193)	5740 (575, 159)	0.54
Right putamen	5419 (660, 176)	5723 (570, 158)	0.2
Left amygdala	1455 (177, 30)	1519 (160, 33)	0.6
Right amygdala	1445 (164, 37)	1546 (170, 41)	0.63
Left hippocampus	4103 (430, 123)	4310 (260, 72)	0.12
Right hippocampus	4083 (460, 115)	4320 (280, 115)	**0.01**
Left thalamus	7040 (800, 218)	7600 (520, 167)	**0.01**
Right thalamus	7070 (782, 209)	7430 (530, 170)	0.056
Left cerebellum	71552 (10150, 1803)	72754 8856, 1952)	0.67
Right cerebellum	72538 (10879, 1845)	72586 (7779, 1998)	0.36

** Results corrected for age at scan, gender and total brain volume, p-values significant at $p<0.01$ corrected to control for the FDR

Figure 9 Significant associations between IGF-1 and IGFBP-3 standard deviation scores and neural volumes in children with isolated growth hormone deficiency and isolated short stature





Key Figure 9 Corpus callosum splenium, right pallidum and right hippocampus volumes correlated significantly with IGF-1 ($P<0.02$; $p<0.04$ and $p<0.019$, respectively) and IGFBP-3 SDS ($p<0.02$; $p<0.01$ and $p<0.016$, respectively) in children with IGHD. Whole group (IGHD and ISS) left pallidum and left and right thalami volumes correlated significantly with IGFBP-3 SDS ($p<0.04$, $p<0.05$ and $p<0.002$, respectively).

viii. MRI: Voxel Based Morphometry results

No significant differences in grey or white matter volumes (including in the regions of the parahippocampal gyrus and prefrontal cortex) were identified between children with IGHD and ISS controls using VBM.

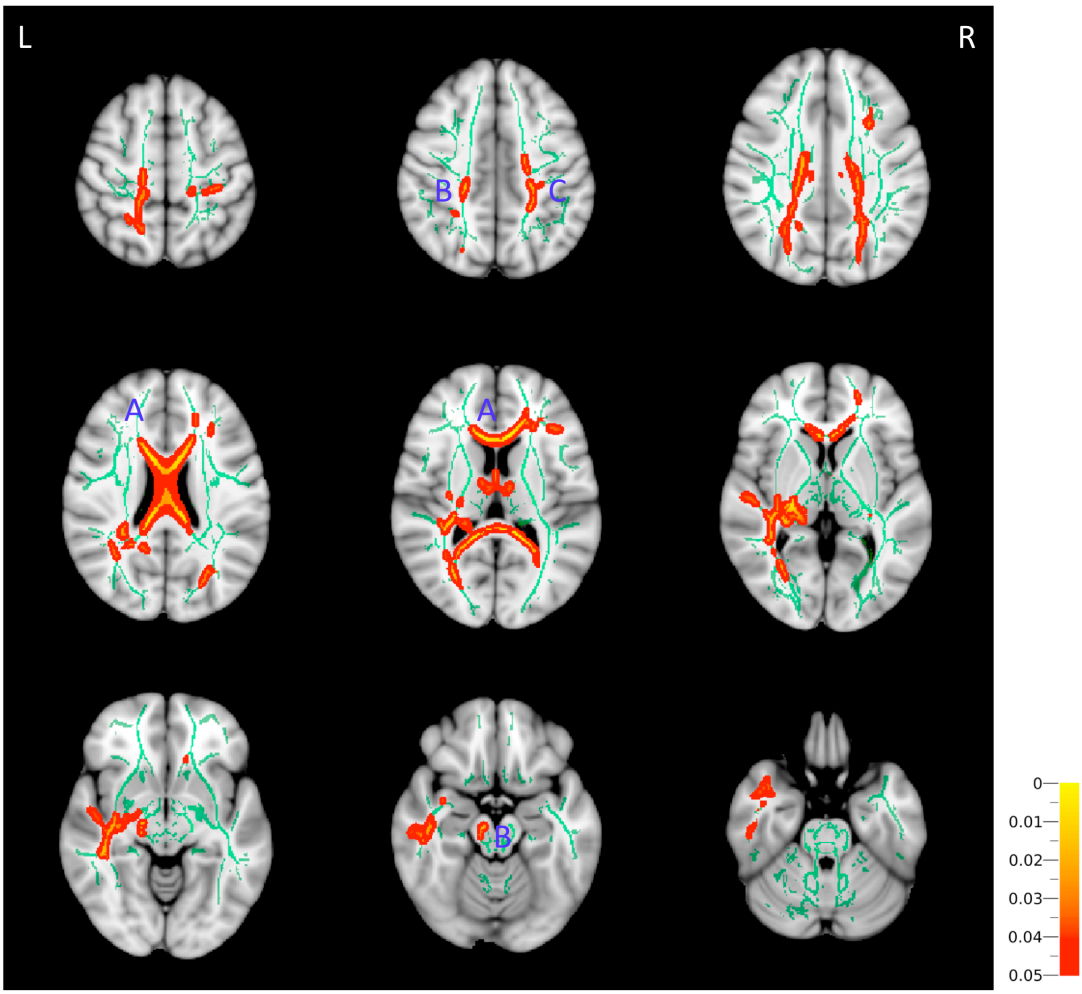
ix. MRI: TBSS Results

The TBSS analysis is summarized in Figure 8. Corpus callosum FA was significantly lower in children with IGHD ($p<0.05$) (Figure 10). Left cortico-spinal tract MD was significantly higher ($p<0.03$) and bilateral FA significantly lower ($p<0.045$, $p<0.05$) in children with IGHD (Figures 10 and 11).

x. Correlations between TBSS results and IGF-1 and IGFBP-3 SDS

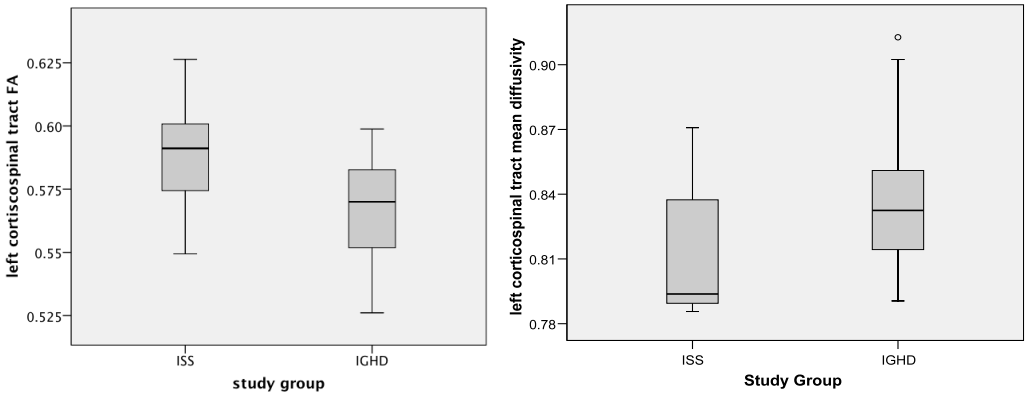
IGF-1 and IGFBP-3 SDS did not correlate significantly with FA or MD in children with IGHD or in the group as a whole (IGHD and ISS).

Figure 10 The association between Isolated Growth Hormone Deficiency and Fractional Anisotropy (Tract Based Spatial Statistics Analysis comparing isolated growth hormone deficiency to idiopathic short stature controls)



Key Figure 10 Mean FA skeleton overlaid on the mean FA map. Regions of the mean FA skeleton in green represent areas where there were no significant differences in FA values in the IGHD infants compared to idiopathic short stature (ISS) controls. Areas in red/yellow are regions where the FA was significantly lower in the IGHD group, and can be observed in the (a) corpus callosum, (b) right cortico-spinal tract, and (c) left cortico-spinal tract. Colour map indicates the degree of significance for red and yellow regions.

Figure 11 Difference in left corticospinal tract fractional anisotropy and mean diffusivity between children with isolated growth hormone deficiency and idiopathic short stature



P<0.045 **p<0.03**

ISS: Idiopathic short stature, IGHD: Isolated growth hormone deficiency, FA: fractional anisotropy, MD: mean diffusivity.

Key Figure 11 FA and MD ($x10^{-3}$ mm^2 s^{-1}) of the cortico-spinal tract were compared using Analysis of Covariance. Age and gender are known to affect brain growth and myelination and were therefore used as a covariate in all analyses. Left cortico-spinal tract FA was significantly lower ($p<0.045$) and MD significantly higher ($p<0.03$) in children with IGHD

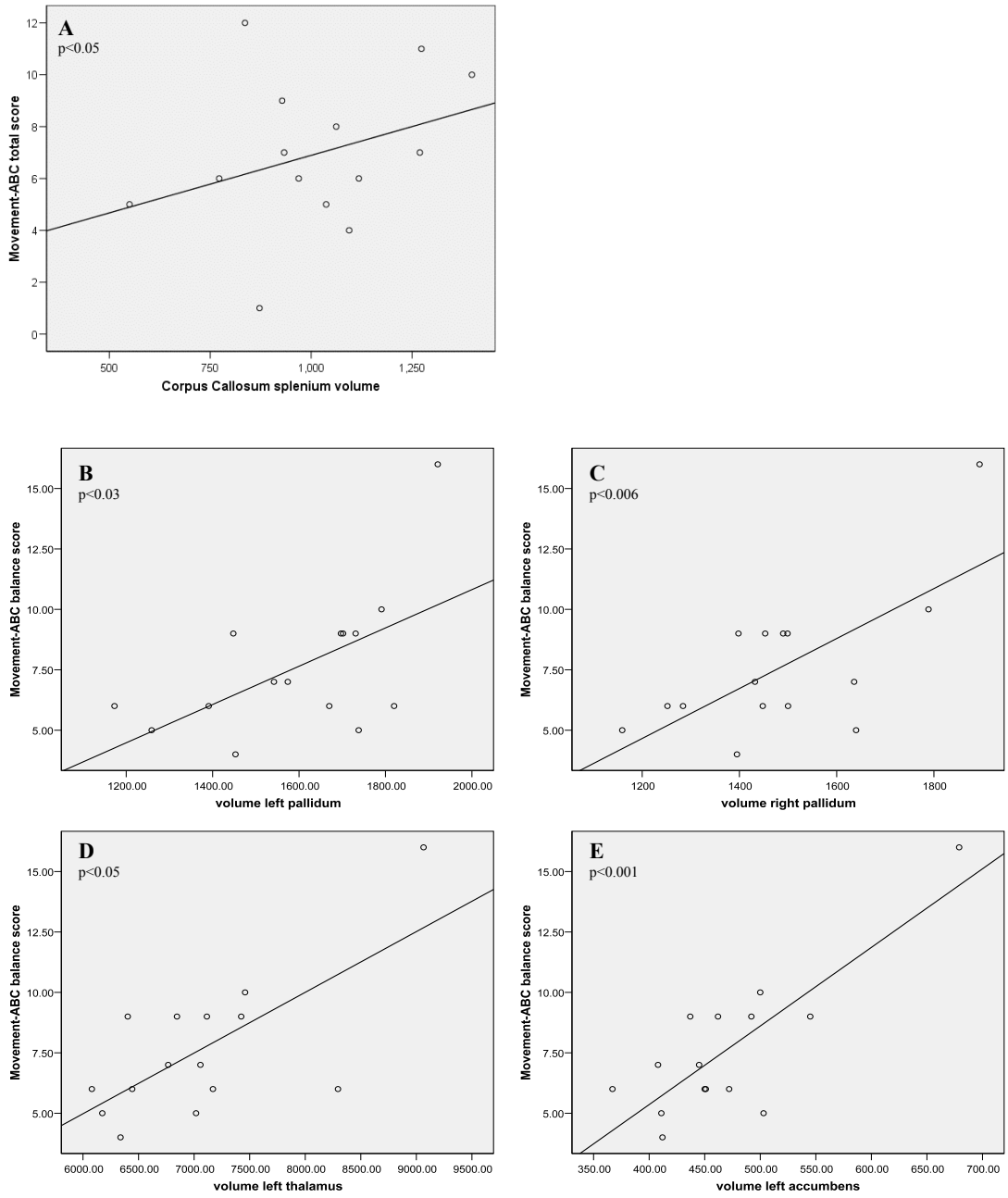
xi. Significant associations between volumetric MRI findings and cognitive assessment scores

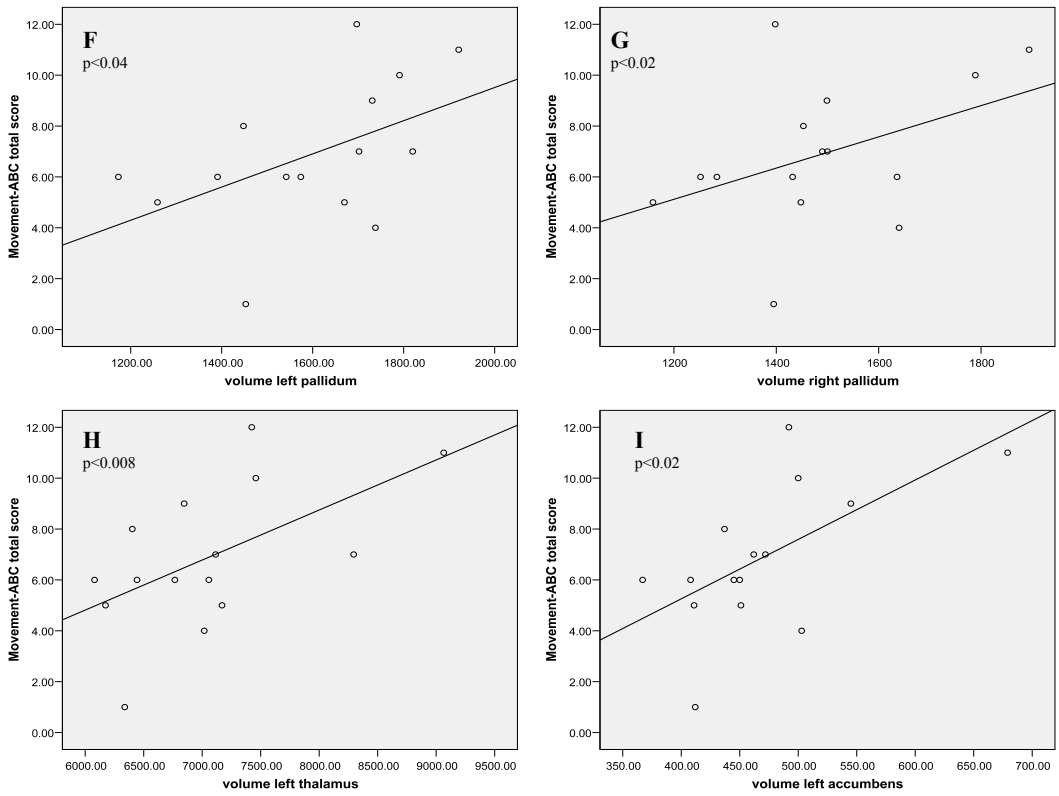
The significant associations between neural volumes and cognitive and motor skills assessment scores are summarized in Figure 12. The volume of the splenium of the corpus callosum correlated significantly with VIQ ($p<0.05$) and the total M-ABC2 score in children with IGHD ($p<0.05$). The volumes of the left and right pallidum, left thalamus and left accumbens correlated significantly with the performance on the balance ($p<0.03$, $p<0.006$, $p<0.05$ and $p<0.001$) and total ($p<0.04$, $p<0.02$, $p<0.008$ and $p<0.02$) scores of the M-ABC2 in children with IGHD.

xii. Significant associations between TBSS MRI findings and cognitive assessment scores

The significant associations between FA and cognitive and motor skills assessment scores are summarized in Figure 13. In children with IGHD, left cortico-spinal tract FA correlated significantly with performance on the M-ABC2 aiming and catching component ($r=0.6$, $p<0.04$) and with Perceptual Reasoning Index ($r=0.5$, $p<0.04$) and right cortico-spinal tract FA correlated significantly with the aiming and catching and balance components of the M-ABC2 ($r=0.4$, $p<0.02$; $r=0.5$ $p<0.04$) and Processing Speed Index ($r=0.4$, $p<0.02$). Corpus callosum FA correlated significantly with FSIQ ($r=0.5$, $p<0.05$) and Processing Speed Index ($r=0.8$, $p<0.009$).

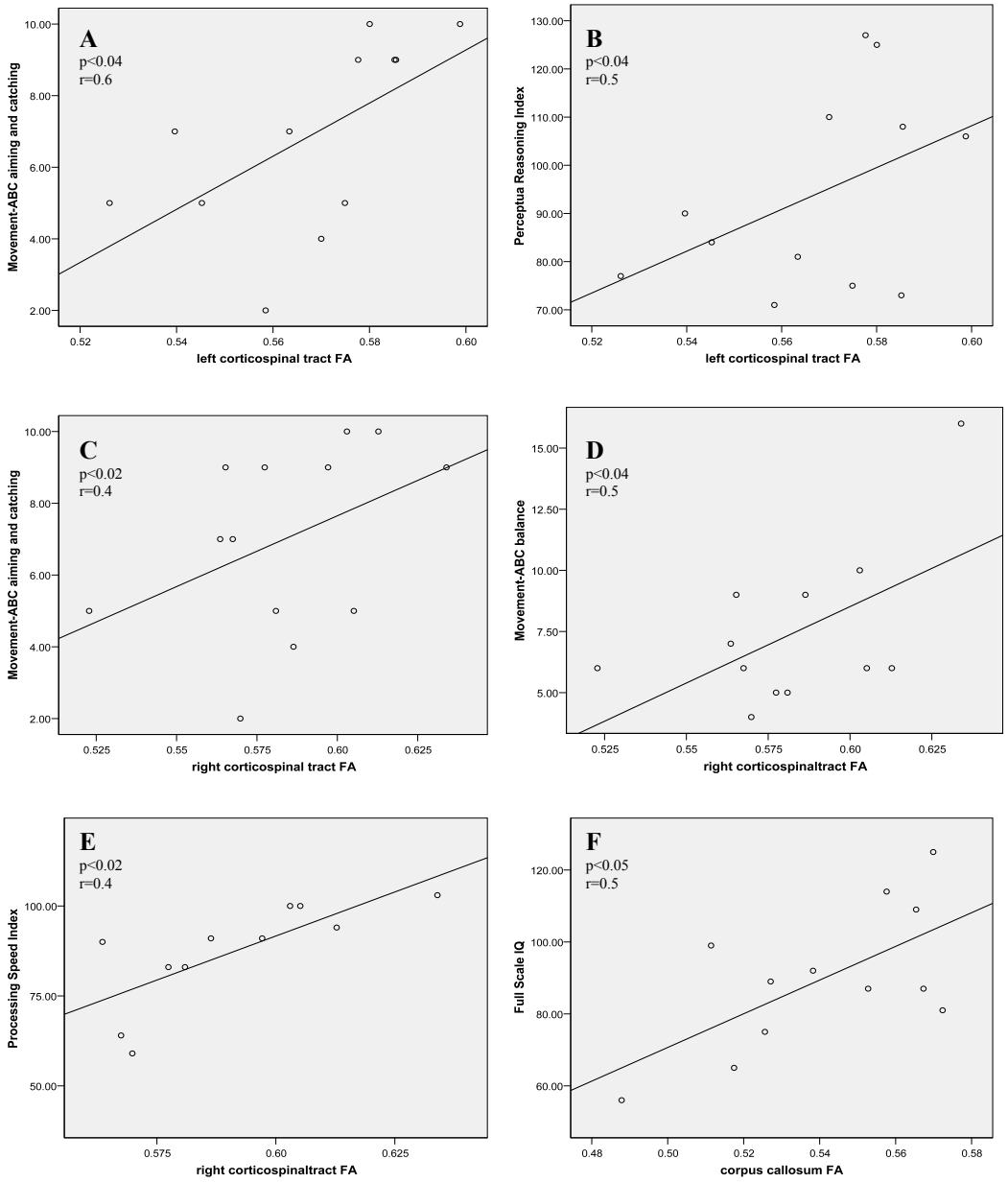
Figure 12 Significant associations between neural volumes, cognitive function and motor skills assessment scores in children with isolated growth hormone deficiency

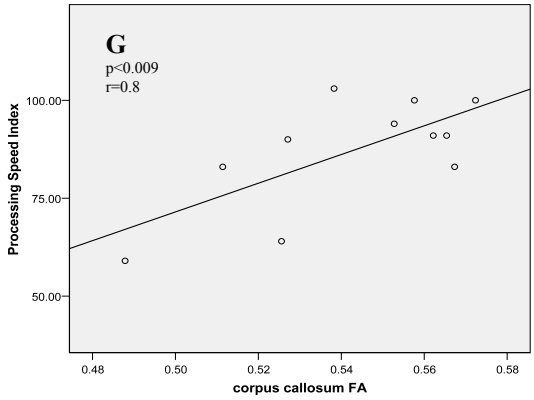




Key Figure 12 Partial correlations were used to assess the relationships between scaled scores on the M-ABC2 test (mean score 10, standard deviation 3), and neural volumes (controlled for age at scan and gender) in children with isolated growth hormone deficiency (IGHD). The volume of the corpus callosum splenium correlated significantly with total score of the M-ABC2 test in children with IGHD (A. $p<0.05$). The volumes of the left and right pallidum, left thalamus and left accumbens correlated significantly with the performance on the balance (B. $p<0.03$, C. $p<0.006$, D. $p<0.05$ and E. $p<0.001$) and total (F. $p<0.04$, G. $p<0.02$, H. $p<0.008$ and I. $p<0.02$) scores of the M-ABC2 in children with IGHD

Figure 13 The significant associations between cognitive function tests, corticospinal tract and corpus callosum fractional anisotropy in children with isolated growth hormone deficiency





Key Figure 13 Partial correlations were used to assess the relationships between scaled scores on the M-ABC2 test (mean score 10, standard deviation 3), cortico-spinal tract and corpus callosum FA (controlled for age at scan and gender) in IGHD. In children with IGHD, left cortico-spinal tract FA correlated significantly with performance on the aiming and catching component of the M-ABC2 (A) ($p<0.04$) and with the Perceptual Reasoning Index (B) ($p<0.03$) and right cortico-spinal tract FA correlated significantly with the Aiming and Catching (C) and Balance components of the M-ABC2 (D) ($p<0.02$, $p<0.04$) and the Processing Speed Index (E) ($p<0.02$). Corpus callosum FA correlated significantly with FSIQ (F) ($p<0.05$) and the Processing Speed Index (G) ($p<0.009$). Average MD is expressed in units of $mm^2\ s^{-1} \times 10^{-3}$, FA is a dimensionless index.

D. Discussion

We aimed to identify whether children with IGHD have abnormalities in cognitive function and brain structure. We found that children with IGHD have a significantly lower FSIQ, Verbal Comprehension Index and processing speed than controls. We also identified significant impairments in motor skills in children with IGHD. These have not previously been described and for the first time to our knowledge we have demonstrated structural brain differences in children with IGHD.

Children with IGHD had a significantly lower FSIQ and Verbal Comprehension Index than children with ISS although the mean FSIQ and Verbal Comprehension Index of both groups lay within the average WISC-IV range (as in previous studies) (94-96;211). FSIQ and Verbal Comprehension Index correlated significantly with IGF-1 SDS, with concentrations of IGF-1 and IGFBP-3 explaining 49% of the variance in Verbal Comprehension Index in the IGHD group. Verbal IQ has previously been shown to correlate positively with IGF-1 (70), and IGFBP-3 SDS has been shown to predict verbal IQ independent of age, social class, maternal educational level, IGF-1 SDS, height or TBV ($t=2.245$, $p=0.032$) (182) in healthy children but not in children with IGHD.

Although our cognitive findings parallel those found in normal children in whom serum IGF-1 concentrations correlate with verbal IQ (40;179), we did not identify the same pattern of cognitive deficits in children with IGHD as have previously been found in adults with GHD (namely deficits in attention and memory). It has previously been hypothesized that the cognitive abnormalities found in adults with GHD may be secondary to changes in the hippocampus which has a high density of IGF-1 receptors,

although there have been no previous imaging studies performed to further investigate this hypothesis.

Based on the results of the cognitive and motor skills assessment scores we focused our volumetric MRI analysis specifically on the basal ganglia (40;41;53;57) (which play an important role in motor function), and the corpus callosum (53) (in which volume changes have previously been shown to correlate with IQ). We also extracted hippocampal volume as this is the brain region with the highest density of IGF-1 receptors (214). In light of these previous studies, which have demonstrated associations between neural volumes and cognitive abilities, our observations of significantly smaller basal ganglia and corpus callosum volumes, and significantly lower corpus callosum and cortico-spinal tract FA in individuals with IGHD, are entirely consistent with the cognitive results.

Interestingly, the pattern of neural volume changes we identified was not limited to areas with a high density of GH and IGF-1 receptor expression (prefrontal cortex, cerebellum, thalamus, putamen, hippocampus, caudate) (40;41;53;179), but was restricted to certain structures (the right hippocampus, globus pallidum, left thalamus and corpus callosum), suggesting that these structures may be more vulnerable to variations in the GH-IGF-1 axis. As not all structures with a high concentration of GH and IGF-1 receptors were affected it may be that the effects of variations in the GH-IGF-1 axis are being mediated via the activation of other biochemical processes. For example, IGF-1 has also been found to stimulate acetylcholine release from hippocampal neurons (53), and to impact on N-methyl-D-aspartate-R2a and R2b receptor density. Normal central nervous system

functioning is dependent on glutamate signalling through the N-methyl-D-aspartate receptor (212).

Another possibility is that these findings are secondary to the selective neuronal vulnerability of these brain regions to the underlying disease process (215). In animal models of GHD a significant reduction in glucose metabolism throughout the brain has been identified, with levels of glucose utilization being significantly reduced in the thalamus and hippocampus (216). The thalamus and globus pallidum form an integral part of the motor system and therefore require high concentrations of energy in the form of adenosine triphosphate (ATP) to function normally. GH and IGF-1 act centrally to modulate neural ATP concentrations (217). ATP controls neurotransmitter synthesis, release and reuptake, and is also required to maintain the transmembrane ion gradients necessary for signal conduction (218). Regions of high energy demand such as the thalamus and globus pallidum are more susceptible to injury than other brain regions with lower energy requirements (selective neuronal vulnerability). The increased sensitivity of these neural regions to reduced ATP availability may lead to increased cell injury or cell-death, which may in turn be visualised on brain MRI as reduced neural volumes in these areas.

In children with IGHD, bilateral cortico-spinal tract FA correlated significantly with performance on the M-ABC2 and with processing speed index (which has many motor components) and corpus callosum FA correlated significantly with FSIQ. No previous studies have investigated neuromotor performance in individuals with IGHD or the relationship between the GH-IGF-1 axis and corticospinal tract structure in humans. However there is evidence to suggest that IGF-1 may play a specific role in

corticospinal tract development with murine in vivo and in vitro studies having previously found that IGF-1 acts specifically to significantly enhance corticospinal motor neuron outgrowth, development and maturation (219). This study provides evidence to suggest that abnormalities in the GH-IGF-1 axis also affect corticospinal tract development in humans, leading to reduced FA in individuals with GHD; and that these changes have functional consequences, namely impairment in motor skills performance.

In children with IGHD, corpus callosum volume and FA were also significantly reduced, with these structural changes correlating with reductions in cognitive and motor skills scores. Corpus callosum size also correlated significantly with IGF-1 and IGFBP-3 SDS suggesting that the differences in the size of the corpus callosum identified are not secondary to midline brain abnormalities which have not been identified by conventional MRI acquisition, but are related to the severity of the underlying GHD.

E. Study limitation

IGHD is a rare condition (1 in 4,000-1 in 10,000 live births (20)) and it is therefore difficult to recruit large numbers of carefully phenotyped children to studies such as this. The reduction in IQ that we have identified, despite our small cohort size, is likely to reflect the rigorous criteria we have used to define IGHD as compared with previous studies.

We are confident that our IQ findings represent reliable differences between IGHD and ISS groups even though the numbers studied were small. Even when the 2 outliers were removed, re-analysis of the IQ data revealed a very similar pattern of findings (namely

significant differences in FSIQ and Verbal Comprehension Index). Seven participants in the IGHD group had FSIQ scores below the 'average' range, compared to 8/12 having scores in the average range and 4/12 in the 'high' average range in the ISS group.

Children with IGHD are at risk of hypoglycaemia, which in itself can be associated with cognitive and neuroradiological abnormalities. It is however unlikely that the differences found are secondary to hypoglycaemia (no children had a history, were symptomatic or displayed evidence of hypoglycaemia), although premature infants with recurrent hypoglycaemia have a cognitive profile that is reminiscent of that of children with IGHD, with a reduced IQ and motor scores. In addition, studies in patients with diabetes mellitus with recurrent hypoglycaemia (and hyperglycemia) have identified different MRI findings to those that we have demonstrated in children with IGHD, with reduced FA present only in the posterior corona radiata and optic radiations. The later age at presentation in these children also makes it unlikely that they were at risk of neonatal hypoglycaemia.

F. Summary

Our findings provide evidence that the GH-IGF-1 axis plays a role in brain and cognitive development. Currently the main aims of GH treatment are to optimise final height, bone mass and body composition. Treatment is therefore often not started in infancy when GH is not the main driver of growth. Early intervention studies are now required to determine whether GH treatment can rectify some of these abnormalities in brain and cognitive functioning as this would have major implications for clinical practice.

4. ASSESSMENT OF THE PREVALENCE AND NEURAL CORRELATES OF BEHAVIOURAL PROBLEMS IN CHILDREN WITH ISOLATED OPTIC NERVE HYPOPLASIA

A. Introduction

Children and adolescents with severe VI, secondary to varying aetiologies, have a significantly increased prevalence of behavioural and social communication problems (134-136). The neural basis for these behavioural problems is unknown. Underdevelopment of the optic nerves early on in embryogenesis leads to ONH, one of the leading causes of VI in the developed world, with a prevalence of 10.9/100,000 (127;129). A recent study in children with severe/profound VI and ONH, reported that 26% had clinically significant behavioural difficulties (social, communicative or repetitive behaviours) (133). Interestingly children with severe profound VI and isolated ONH are diagnosed with behavioural and developmental abnormalities at a similar rate to children with SOD, who, in addition to ONH, have other midline brain and/or pituitary hormone abnormalities (133). However, the prevalence of behavioural difficulties in children with mild to moderate or no VI and ONH has not previously been recorded in the literature. Additionally, to date conventional methods of neuroimaging have been unable to detect neural abnormalities, other than ONH, in individuals with isolated ONH.

DTI is a non-invasive imaging technique, which can provide quantitative indices of brain microstructure and enables the visualization of white matter microstructure

(103;109). Since the basic principles of DTI were established in the mid-1990s many clinical investigations have found that abnormalities in the white matter tracts of the brain can be identified in a wide range of pathological conditions (e.g. multiple sclerosis, Alzheimers disease and depression) (114;116). They have also enabled the identification of defects in conditions not classically associated with abnormalities on conventional neuroimaging, such as ASD (118;119;148). Previous neuroimaging studies in children with ONH have been limited to those using qualitative radiological assessments of conventional MR images, and to two small studies (one and two individuals) using DTI to better define the phenotype in ONH and SOD. Both have been in small numbers of subjects with severe VI (one and two individuals) and have focused solely on the optic tracts (143;144). There is a wide literature debating the reasons as to why children with VI, secondary to a wide range of aetiologies, are at increased risk of behavioural and social communication difficulties. However, thus far, the underlying reason for this increased prevalence remains unknown (145). It has, however, previously been suggested that reductions in exposure to visual social cues and visually guided experiences in children with VI may predispose them to developing social development abnormalities (146). Whilst the increased prevalence of behavioural deficits found in children with ONH and severe VI may be related to their underlying visual impairment and reduced visual experience, we hypothesized that, on the basis of the emerging literature regarding neuroimaging and social communication disorders such as ASD (119), the underdevelopment of white matter tracts may also contribute to the behavioural abnormalities found in this cohort.

Previous studies investigating the prevalence of behavioural difficulties in children with ONH have all been in children with severe VI (133;134). Children with ONH and

severe/profound VI frequently have co-morbidities, which may potentially confound behavioural and DTI assessments including significant learning difficulties, attention deficit disorder, seizures, and cerebral palsy (132;147-150). We therefore firstly aimed to assess whether children with isolated ONH with vision ranging from normal range acuity to mild to moderate VI and no developmental delay have an increased prevalence of behavioural problems compared to a control group of typically developing children without ONH. Secondly we aimed to perform detailed DTI studies in this cohort to identify whether specific white matter abnormalities, not been previously identified using conventional MRI, are present that may provide neural correlates for any behavioural abnormalities identified. Volumetric datasets were also acquired and analysed to assess whether children with isolated ONH have subtle structural brain abnormalities not easily identifiable on conventional T1-weighted MRI brain datasets.

As children with SOD frequently have reduced white matter bulk and midline brain abnormalities including hypoplasia of the corpus callosum which may confound the DTI results, we initially performed our study in a cohort of children with isolated ONH. However, as a follow up to this initial study we also acquired DTI sequences in children with SOD to evaluate whether white matter abnormalities identified in children with isolated ONH were more widespread when children with SOD were also included in the analysis. The children with SOD included in this analysis were restricted to those without brain MRI abnormalities (other than those in the hypothalamo-pituitary axis) visible on standard MRI brain.

B. Methods

Children aged 1-11 years, diagnosed with isolated ONH or SOD by a paediatric ophthalmologist, paediatric neuroradiologist and paediatric endocrinologist, were recruited prospectively. A diagnosis of ONH and laterality of disease were determined by one experienced paediatric neuroradiologist (Dr Kling Chong) blinded to the clinical data who reviewed all images. The diagnosis of ONH was confirmed by ocular fundus photography performed by a paediatric opthalmologist. SOD was diagnosed when two or more features of the classical triad of (i) ONH, (ii) pituitary hormone abnormalities and (iii) midline brain defects were present.

We investigated children with isolated ONH, normal range visual acuity to mild/moderate reduction in visual acuity (mild/moderate VI) and normal cognition to remove the confounding effects of severe or profound VI and learning difficulties (isolated ONH study). We recruited children with SOD with only abnormalities of the hypothalamo-pitutiary axis and optic nerves visible on brain MRI (i.e. no midline brain abnormalities) and without TSH deficiency (known to affect brain myelination (220;221)) (SOD study). A control group of typically developing children without ONH aged 1-11years, matched for age and gender, were recruited concurrently. The controls were all recruited from the endocrine clinic where they presented with short stature. We chose children with short stature as our controls as although they have no clinically significant medical condition their parents had been concerned enough regarding their health to seek specialist opinion at a tertiary referral hospital. The CBCL is a parental questionnaire, which may be biased for parents of children with perceived (e.g. short stature) or actual (e.g. VI) medical conditions. We therefore used children presenting to the tertiary referral hospital rather than sibling controls to control for the effect of this.

Participants with isolated ONH and controls were all asked to complete the CBCL and to undergo brain MRI and cognitive assessment. All children in the SOD group underwent brain MRI and a detailed developmental and behavioural assessment, which was performed by a clinical psychologist and paediatrician with a special interest in children with VI. CBCL data were not collected in the SOD group. Standardised measures of social communication and autism are not available for children with VI; the diagnosis of ASD was therefore made on the basis of clinical assessment of behaviour by clinicians during standardised developmental assessments (133). Deficits in social and communication skills and the presence of repetitive or restricted behaviours were then assessed according to ICD-10 criteria for ASD (222). All children with SOD and ONH were screened for mutations in *HESX-1* and some with SOD were also screened for mutations in *SOX-2* and *SOX-3* depending on their phenotype (Table 4).

iii. Isolated optic nerve hypoplasia study

Volumetric analysis of the anatomical scans from the 3-D datasets was performed using the FreeSurfer technique of Fischl et al (223). This involves whole brain segmentation and automated labelling of the neuroanatomical structures based on probabilistic information estimated from a manually labelled atlas (224). Total brain, corpus callosum and cuneus (occipital lobe visual processing centre) volumes were extracted from this data. The difference in total brain volume between groups was compared using ANCOVA, controlling for age at scan, and gender. For the corpus callosum total brain volume was used as an additional covariate.

Diffusion-weighted images were initially processed using FSL software (http://www.fmrib.ox.ac.uk/fsl). The FA images were analyzed using TBSS (121;187). All analyses were corrected for age and gender. FA was found to be significantly reduced in the optic radiations, corpus callosum and ventral cingulum on the TBSS analysis (Figure 14). In order to assess whether there was any association between FA for these structures and behavioural scores, values for the ventral cingulum, corpus callosum and optic radiation FA were extracted from the TBSS analysis by masking the mean skeleton with the appropriate structure label from the Johns Hopkins University white-matter tractography atlas (188).

Baseline characteristics including age and cognitive level were compared using the independent Student's *t* test. Gender of the two groups (isolated ONH and control) was compared using the chi-squared test of equal proportions. Behavioural assessment scores were compared using the independent Student's *t* test. Partial correlations were used to assess the relationship between the ventral cingulum, corpus callosum and optic radiation FA and CBCL scores (controlled for age at scan and gender).

iv. Septo-optic dysplasia Study

Children with SOD but without visible white matter abnormalities on conventional MRI brain (7 individuals) were added to the ONH cohort, and the TBSS analysis described above was rerun. Diffusion-weighted images were initially processed using FSL software (http://www.fmrib.ox.ac.uk/fsl). The FA images were analyzed using TBSS (121;187). All analyses were corrected for age and gender.

C. Results

i. Subject characteristics

Isolated optic nerve hypoplasia study

All children (mean age 5.9 years, 81% males) with ONH and 24 controls (mean age 6.4 years, 68% males) were recruited. Of the eleven children with ONH, seven had bilateral ONH and four had unilateral ONH. Eleven children with ONH completed the developmental and behavioural assessment battery. Brain MRI was otherwise normal (including the hypothalamo-pituitary axis) in all subjects. Significant motion artefact was not noted in any study participants. Visual acuity in the better eye was between 6/6 and 6/24 Snellen (6/6 in 4 children, 6/9 in 2 children, 6/15 in 2 children, 6/19 in 2 children and 6/24 in 1 child), indicating that the vision of the ONH children was within the functionally normal to mild/moderate VI range. IGF-1 and IGFBP-3 concentrations, thyroid function tests and glucose and cortisol profiles were normal in all children. No mutations in any of the genes screened were identified.

Twenty-four controls were recruited, of whom 15 completed the developmental assessment and 11 completed the behavioural questionnaires. The time required to undertake the developmental assessment precluded some controls from consenting to undergo this component of the study. Subject characteristics are summarised in Table 10.

There were no significant differences between the age, cognitive level and gender of subjects and controls (Tables 10 and 11). All children were right-handed, had no abnormal neurological findings, were in the average range for cognition and were in

mainstream schooling. There were no significant differences in age and gender between those children who underwent behavioural assessment and those who did not consent to behavioural assessment (Table 10). No significant group differences in full scale IQ, verbal or non-verbal indices were observed (Table 11). All study recruits underwent brain MRI and data quality was deemed to be adequate in all subjects (visual inspection by an experienced observer (Dr Chris Clark)).

Septo-optic dysplasia study

Seven children with SOD (mean age 5.93 years, 2 males) were recruited. Of the seven children five had bilateral ONH and 2 had unilateral ONH. Brain MRI was otherwise normal (excluding the hypothalamo-pituitary axis) in all subjects; findings summarized in Table 12. Significant motion artefact was not noted in any study participants. Six of the children with SOD had severe or profound VI, with only one having mild VI. All children with SOD were GHD, two children with SOD also had a diagnosis of ACTH deficiency. Three children in the SOD group had a diagnosis of ASD. There was a wide range in cognitive abilities within this group; detailed subject characteristics are summarised in Table 12.

Table 10 Number of participants, age and gender for the children with isolated optic nerve hypoplasia and controls (whole group) and for the group with only behavioural and MRI data available. Significance levels from statistical tests comparing the two sub-groups are also presented

Variable	**Total group [1]**		**P values**	**Behavioural group [2]**		**P values**
Group	ONH	Control		ONH	Control	
Number	11	24		11	11	
Age (SD)	5.9 (3.3)	6.4 (3)	0.56	5.9 (3.3)	6.8 (3.1)	0.58
Male (%)	9 (81)	17 (68)	0.78	9 (81)	10 (91)	0.91

[1] MRI only [2] MRI, developmental and behavioural data available.

Table 11 Cognitive and Child Behaviour Checklist standard score means (SD) for the optic nerve hypoplasia and control participants (significant values p<0.05 in bold)

	ONH*	Control*	p value
Full Scale IQ	101.1 (22.5)	102.9 (7.1)	0.8
Verbal Comprehension/Verbal IQ	98.4 (22.6)	106.9 (7.4)	0.3
Perceptual Reasoning/Performance IQ	96.5 (19.5)	105.4 (9.9)	0.18
CBCL Anxious/Depressed	61.3 (11.6)	50.8 (2.4)	**0.014**
CBCL Withdrawn	63.5 (10.3)	52.5 (6.2)	**0.006**
CBCL Somatic Complaints	61 (8.2)	53.6 (10.7)	0.086
CBCL Social Problems	63.5 (10.7)	53.4 (10.1)	0.053
CBCL Thought Problems	65.8 (8.8)	51.5 (2.3)	**0.002**
CBCL Attention	68.3 (14.6)	53.1 (3.7)	**0.006**
CBCL Rule Breaking	58.6 (11.9)	52.7 (4.6)	0.151
CBCL Aggressive	61.8 (11.8)	51.3 (2.6)	**0.015**
CBCL Internalizing	63 (10.7)	51.7 (7.5)	**0.01**
CBCL Externalizing	60.6 (10.1)	51.3 (4.8)	**0.015**
CBCL Total Score	63.6 (11.4)	51.3 (6.4)	**0.006**

*11 children with ONH underwent behavioral and IQ assessment; *15 controls underwent IQ assessment, 11 of the controls who underwent IQ assessment also completed the behavioral questionnaires.

Table 12 Characteristics of study subjects with septo-optic dysplasia

	1	**2**	**3**	**4**	**5**	**6**	**7**
Age (years)	9.94	4.93	13.73	9.46	3.2	1.67	1.8
Gender	F	F	M	F	F	F	M
Degree Visual Impairment (size of object visualised at 30cm)	Severe (2.5mm)	Mild	Severe (5mm)	Profound (light awareness)	Severe (2.5mm)	Severe (2.5mm)	Severe (5mm)
Hormonal Abnormalities	GHD	GHD	GHD, ACTHD	GHD	GHD, ACTHD	GHD	GHD
MRI findings	Small AP Normal PP B/L ONH	Small AP Normal PP U/L ONH	Small AP Normal PP Absent SP B/L ONH	Small AP Normal PP B/L ONH Immature myelination	Small AP Normal PP B/L ONH	Small AP Normal PP B/L ONH	Small AP Normal PP U/L ONH
Cognitive Assessment (RZS, VI norms (225))	Severe delay ASD	Age Appropriate	Mixed profile: delayed non-verbal cognition ASD	Severe delay	Severe delay ASD	Age Appropriate	Mixed profile: delayed non-verbal cognition

AP: anterior pituitary gland; PP: posterior pituitary gland; SP: septum pellucidum; ONH: optic nerve hypoplasia; CC corpus callosum, GHD: growth hormone deficiency, TSHD: thyroid stimulating hormone deficiency; ACTHD: ACTH deficiency; DI: diabetes insipidus; B/L: bilateral; U/L: unilateral.

ii. Cognitive results

No significant group differences in FSIQ (p=0.8), verbal (p=0.3) or non-verbal (p=0.18) indices were observed (Table 11) (ONH versus controls).

iii. Behavioural assessment

Isolated optic nerve hypoplasia Group

Independent samples *t* tests revealed significantly more behaviour problems (indicated by higher CBCL scores) in the ONH compared to the control group (Total scores $p<0.006$, Table 11). This pattern was found across most of the CBCL's component subscales. Four out of the 11 children who underwent behavioural assessment (36%) in the ONH group had scores in the 'clinical' range (indicating more problems than were reported for 97% of the normative range), 1 of the 4 had unilateral ONH. One child, with bilateral ONH, had a score within the 'borderline' range, with the remainder reported as within the 'normal' range. One child in the control group had a score in the borderline range with the remainder of the control participants having scores within the 'normal' range.

Septo-optic dysplasia Group

Three children with SOD were diagnosed with ASD.

iv. Neuroimaging findings

Isolated optic nerve hypoplasia group

The volumetric analysis revealed no significant differences in total brain or corpus callosum volume between ONH subjects and controls (Table 13).

FA was significantly reduced in the optic radiations (bilaterally), corpus callosum and ventral cingulum (bilaterally) in children with ONH when compared to control subjects (Figure 14) (TBSS analysis). There were no regions in which FA was found to be lower in the control subjects than in the children with ONH.

Optic nerve hypoplasia and septo-optic dysplasia groups

In the combined ONH and SOD groups FA was significantly reduced in the optic radiations (bilaterally), corpus callosum, prefrontal cortex and ventral cingulum (bilaterally) (Figure 15). There were no regions in which FA was found to be lower in the control subjects than in the children with SOD and ONH.

v. Correlations between imaging measures and behavioural scores in children with isolated optic nerve hypoplasia and controls

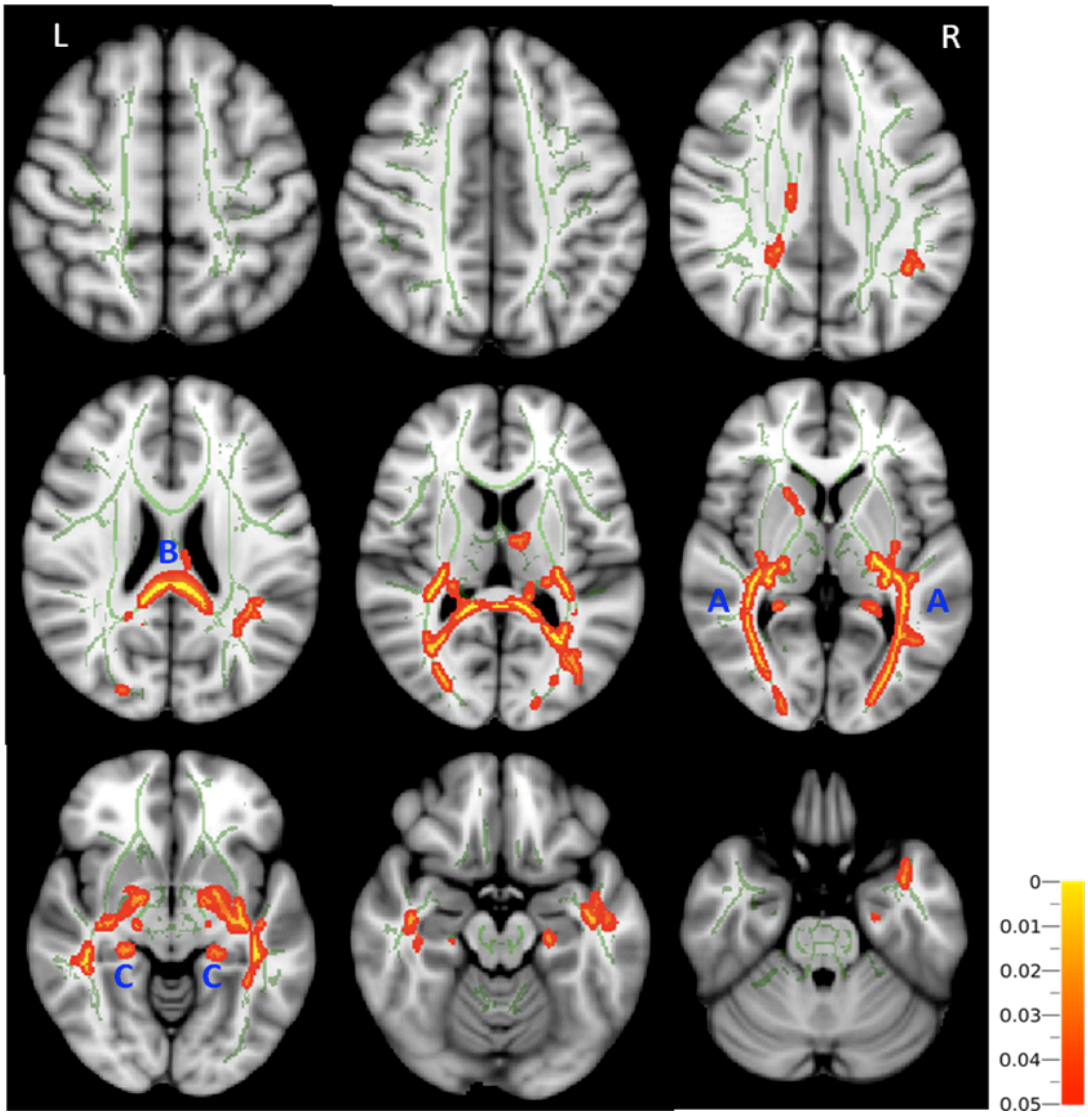
Right ventral cingulum FA correlated significantly with total CBCL score ($r=-0.52$, $p<0.02$) and the externalising score on the CBCL ($r=-0.46$, $p<0.049$) (Figure 16), but not the internalising score on the CBCL ($r=-0.45$, $p=0.056$). Correlations between left ventral cingulum FA, total CBCL score and externalising and internalising scores on the CBCL did not reach statistical significance. There were no significant correlations between corpus callosum or optic radiation FA and CBCL scores.

Table 13 The differences in neural volumes between children with isolated optic nerve hypoplasia and controls

Neural volumes (mm^3)**	**ONH**	**Controls**	**p-value**
Total brain volume	1506607	1560102	0.78
Splenium corpus callosum	647	786	0.12
Corpus callosum Total	2561	2905	0.45
Left Cuneus	3819	3696	0.93
Right Cuneus	4347	3916	0.2

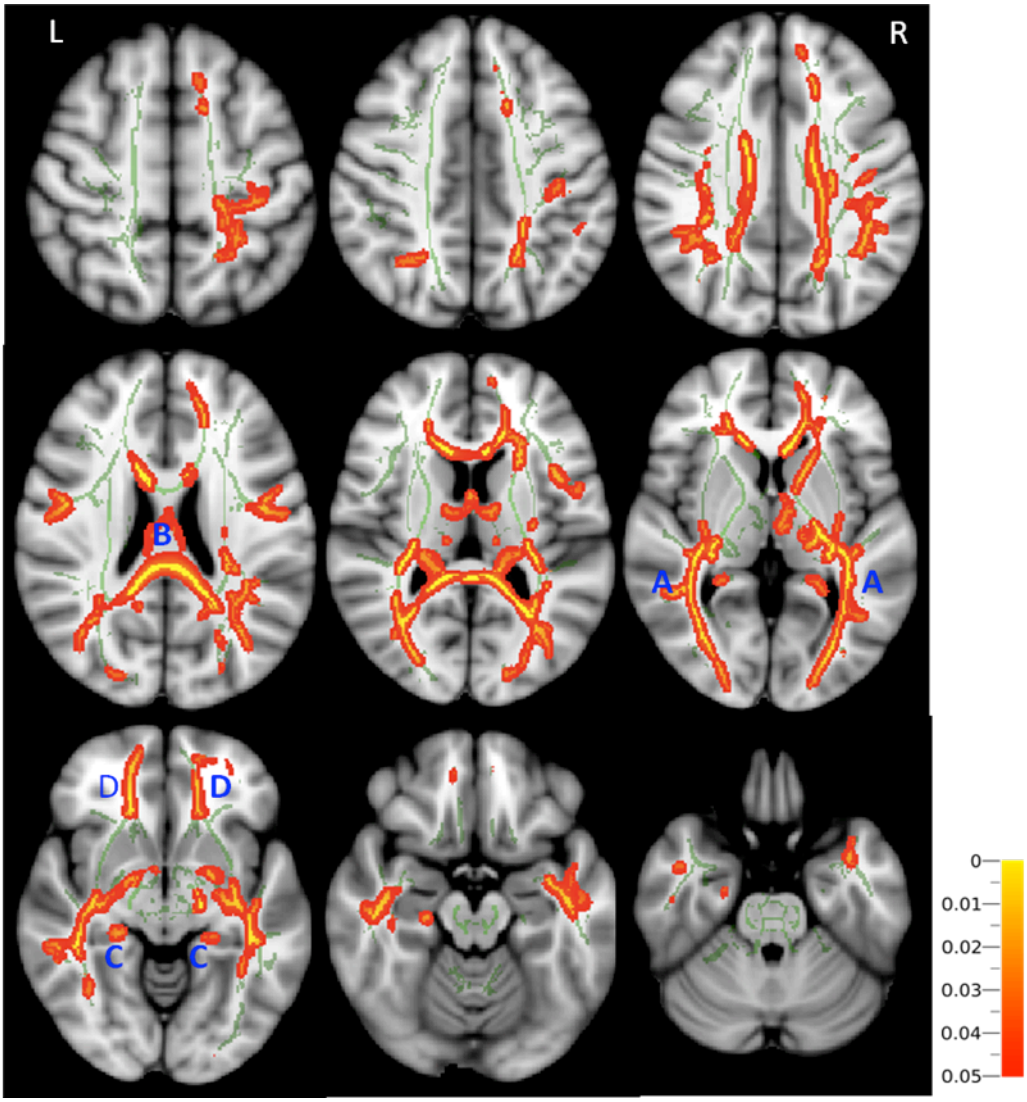
** Results corrected for age at scan, gender and total brain volume

Figure 14 Difference in fractional anisotropy between children with isolated optic nerve hypoplasia and normal controls (Tract Based Spatial Statistics analysis)



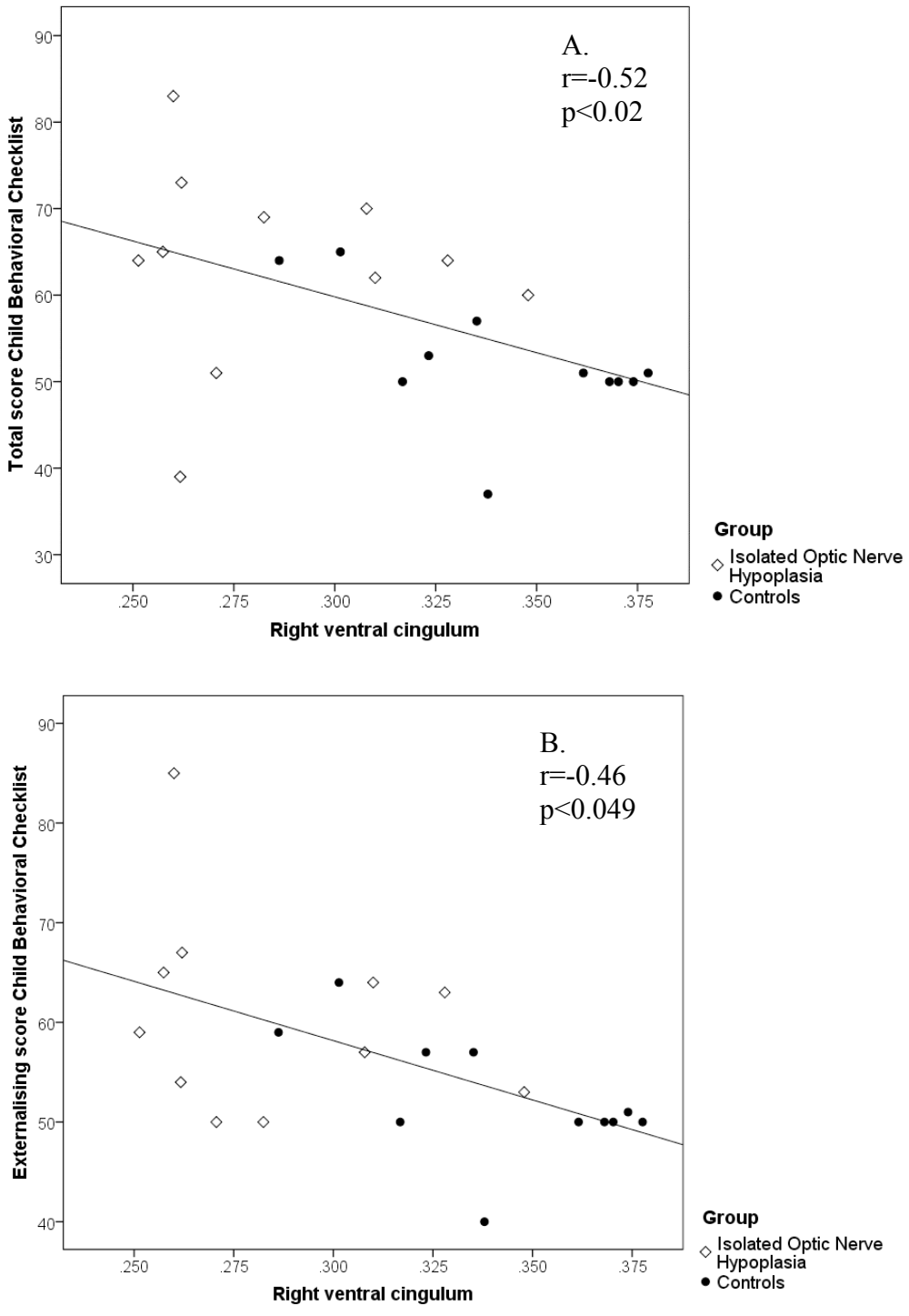
Key Figure 14 Mean FA skeleton overlaid on the mean FA map. Regions of the mean FA skeleton in green represent areas where there were no significant differences in FA values in the ONH children compared to controls. Areas in red/yellow are regions where the FA was significantly lower in the ONH group, and can be observed bilaterally in the (A) optic radiation, (B) corpus callosum, and (C) ventral cingulum. Colour map indicates the degree of significance for red and yellow regions.

Figure 15 Difference in fractional anisotropy between children with isolated optic nerve hypoplasia and septo-optic dysplasia and normal controls (Tract Based Spatial Statistics analysis)



Key Figure 14 Mean FA skeleton overlaid on the mean FA map. Regions of the mean FA skeleton in green represent areas where there were no significant differences in FA values in the ONH+SOD children compared to controls. Areas in red/yellow are regions where the FA was significantly lower in the ONH/SOD group, and can be observed bilaterally in the (A) optic radiation, (B) corpus callosum, (C) ventral cingulum and (D) prefrontal cortex. Colour map indicates the degree of significance for red and yellow regions.

Figure 16 Correlations between child behaviour checklist performance and right ventral cingulum fractional anisotropy



Key Figure 16 Ventral cingulum fractional anisotropy (FA) was extracted from the tract based spatial statistics analysis. FA was significantly lower in the ventral cingulum in children with ONH as compared to controls. Partial correlations were used to assess the relationships between scores on the child behavioural checklist (CBCL) and right ventral cingulum FA (controlled for age at scan and gender). Higher scores on the CBCL indicate more behavioural problems. FA correlated significantly with the total (A. $r=-0.52$, $p<0.02$) and externalising score on the CBCL (B. $r=-0.46$, $p<0.049$).

D. Discussion

Children with isolated ONH, normal development and mild to moderate or no VI have an increased prevalence of clinically significant behavioural problems. They also show evidence of reduced white matter integrity in the corpus callosum and ventral cingulum, the anterior optic pathway and optic radiations bilaterally, regions of the brain that are implicated in behavioural and emotional regulation and vision . The identified abnormalities in white matter fibre density in the ventral cingulum correlate significantly with behavioural scores. When children with ONH and SOD were grouped together they were found to have reduced FA in the corpus callosum, ventral cingulum and prefrontal cortex; together these structures form part of the social brain circuitry (148). The association between white matter abnormalities in the ventral cingulum and corpus callosum have previously been identified in adults with obsessive compulsive disorder and schizophrenia (226-228), which suggests that the behavioural difficulties experienced by children with ONH may not solely be due to reduced visual input, but may be related to other underlying neuroanatomical abnormalities which cannot be detected using conventional brain MRI.

iv. Cognition

Cognitive performance in children with isolated ONH was not significantly different to controls. This finding differs from previous studies that have documented developmental delay in children with ONH (147-149). This discrepancy is probably due to the associated pituitary hormone insufficiencies, midline brain abnormalities and more severe VI present in these cohorts. In support of this, the children with SOD included in the study had a high prevalence of moderate/severe developmental

delay.

v. Behaviour

We identified a similar prevalence of clinically significant behavioural difficulties (36%) in children with isolated ONH and mild to moderate or no VI to that reported in populations with severe VI (47 and 49%) (135;136;149). The CBCL profile showed increased scores on all subscales (except rule-breaking and somatic complaints) for the ONH group relative to the control group, suggesting that difficulties exist across most behavioural domains assessed by the CBCL. More specifically, the ONH group presented with a mix of internalizing and externalizing problems, showing significant elevations in attention problems, aggressive behaviour, and the anxious-depressed, withdrawn and thought problem scales of the CBCL (Table 11). Whilst we have not assessed the wider clinical significance of these behavioural difficulties, previous data demonstrate that children with a profile characterized by co-existing elevations in attention problems, aggressive behaviour, and anxious-depressed scales of the CBCL have increased rates of psychiatric disorders including paediatric bipolar disorder, suicide and a high rate of adult psychopathology (229). Our current findings highlight the importance of being alert to potential behaviour difficulties even in children with mild/moderate or no VI. Although it is not currently part of routine clinical care to perform behavioural assessment in children with ONH and mild to moderate or no VI, the incidence of clinically significant behavioural difficulties in our ONH sample (36%) is comparable to that reported previously in populations of children with severe VI (47 and 49%) (135;136) in whom behavioural assessment is advocated.

vi. Neuroimaging

Isolated optic nerve hypoplasia group

In addition to the well-established abnormalities of the optic nerve, we have demonstrated that children with ONH also show evidence of reduced white matter integrity in the ventral cingulum bilaterally, the corpus callosum and the optic radiations bilaterally. We were unable to demonstrate concurrent reductions in grey matter volumes in the corpus callosum in children with ONH.

The reduced white matter integrity (FA) identified in the ventral cingulum and corpus callosum is likely to reflect changes in underlying brain structure. Although FA changes can be attributed to a number of microstructural changes, including changes in axonal density, axon diameter distributions, myelin density, intra-voxel axon dispersion, unmasking due to selective tract degeneration, greater tract maturation in one tract compared to another in a crossing fibre situation in white matter, changes in cell membrane permeability, reduction in extra-cellular tortuosity as a result of increased water content in the extra-cellular space as might occur in a diffuse oedematous process and replacement gliosis, we expect that the axon density and myelin content explanation is the most plausible in ONH (112). Focal reductions in FA have also been described in other paediatric conditions in which there is an increased prevalence of behavioural problems. For example, DTI studies in children with autistism spectrum disorder report reduced FA in the corpus callosum, occipitotemporal tracts, and white matter structures adjacent to the ventromedial prefrontal cortex, anterior cingulate gyrus, fusiform gyrus, superior temporal gyrus, and amygdala (230;231), and studies in children born preterm have identified reduced FA in the corpus callosum, external capsule and the posterior

aspect of the posterior limb of the internal capsule (232). However, in these other disorders, the specific pattern of abnormalities we report has not been identified.

The DTI findings we describe are novel in the context of ONH but perhaps unsurprising when one examines the wider literature pertaining to brain development. Murine studies have shown that axons from the cingulate cortex cross the midline prior to those of the corpus callosum, acting as pioneering axons for the corpus callosum (233;234). Nakata *et al* performed brain DTI in 12 individuals with agenesis of the corpus callosum to explore the hypothesis that callosal dysgenesis may represent the most obvious anatomical manifestation of a more widespread white matter developmental disorder. They identified concomitant abnormalities in the volume and structure of the ventral cingulum bundle in individuals with agenesis of the corpus callosum, concluding that this provides further evidence for a relationship between the embryonic formation of the ventral cingulum and corpus callosum (148). Corpus callosum abnormalities are frequently found in association with ONH in SOD, an early developmental abnormality of forebrain development occurring at 4-6 weeks gestation (130). The presence of reduced FA in the corpus callosum in the current study suggests that ONH may represent a milder end of the SOD spectrum.

Reduced ventral cingulum FA was significantly associated with CBCL scores. The cingulum provides important white matter connections within the corticolimbic neural system which is involved in regulating emotion (235). Individuals with elevated CBCL scores in childhood are at increased risk of fulfilling criteria for Diagnostic and Statistical Manual of Mental Disorders fourth revised edition [DSM-

IV] diagnoses in adulthood. In adults with obsessive-compulsive disorder and schizophrenia (both DSM-IV diagnoses) white matter abnormalities in the cingulum bundle and corpus callosum have been identified (226-228). The finding of reduced cingulum FA in association with increased CBCL scores therefore supports our initial hypothesis that white matter abnormalities not identifiable using conventional neuroimaging methods may help to explain the increased prevalence of behavioural problems found in children with ONH.

In contrast with the two previous published studies using DTI to better understand SOD (143;144), both of which were performed in children classified as blind, we investigated a cohort of children with mild-moderate or no VI. Both previous studies have been in small numbers of subjects (one and two individuals respectively) and focused their analysis on the optic radiations. These studies demonstrated that children with SOD have both pre- and post-chiasmatic diffusion abnormalities in the visual pathway. Previous investigations concluded that the presence of reduced FA in the optic radiations demonstrated the need for an afferent input from the retina to the lateral geniculate nucleus to stimulate normal optic radiation development (143;144). It is therefore noteworthy that in our cohort of children with ONH and functionally normal vision to mild/moderate VI, we have also identified significant reductions in FA in the optic radiations. This suggests that either there is some other pathophysiological process underlying the reduced structural integrity of their optic radiations, or that even small reductions in visual stimulation can affect the development of the optic radiations. A genetic aetiology for SOD is currently only identified in <1% cases, and it has therefore been suggested that environmental factors including drugs, alcohol and anterior cerebral arterial supply may also be

impacting on normal forebrain development in SOD (<1%) (130). However, neither the genetic aetiologies nor the environmental factors hypothesized to impact on forebrain development would be expected to affect the development of the posterior optic radiations.

Combined optic nerve hypoplasia and septo-optic dysplasia groups

When children with SOD and isolated ONH were combined in the TBSS analysis FA was also significantly reduced in the prefrontal cortex, in addition to the previously identified structures (corpus callosum, optic radiations and ventral cingulum). This finding may reflect the increased power of the larger group studied. It is an interesting finding in view of the known association between the development of the prefrontal cortex and social and behavioural problems. The frontal lobe forms a key part of the neural network which makes up the 'social brain', together with the amygdala, superior temporal and cingulate gyri (58). Multiple previous neuro-imaging studies performed in individuals with autism, a condition defined by the social communication difficulties that individual's experience (118), have identified structural and functional abnormalities in the prefrontal cortex. Structural studies describe increases in white matter volume in the brains of individuals with ASD (236;237), with functional studies showing reduced activation in the prefrontal and anterior cingulate cortex (238). DTI studies report reduced FA in the fusiform gyrus, superior temporal gyrus, amygdala, prefrontal cortex, anterior cingulate gyrus and corpus callosum (230;231). Serotonin production has also been found to be abnormal in the frontal lobes of individuals with ASD (239;240), thought possibly to reflect abnormal underlying connectivity of the frontal lobe. Previous studies in children with SOD have also identified an

increased prevalence of behavioural difficulties including ASD (133). Although the numbers of children with SOD included in this study were small, three of the children we included in our cohort had a diagnosis of ASD. Our data suggests that children with SOD may have abnormalities in the development of their social brain, namely their prefrontal cortex, corpus callosum and ventral cingulum and this may to some extent explain the increased prevalence of behavioural and social communication difficulties found within this group of children (58). This work will need to be repeated in larger cohorts of children with SOD and ASD to confirm these preliminary findings.

E. Study limitations

Although the present study was limited by small sample size, the numbers of children included in the study are significantly larger than in previous studies in children with ONH and SOD and the results are statistically significant. Ideally, the age-range of patients studied would have been narrower, as we know myelination varies significantly during childhood (241); however the subject and control groups were well-matched in terms of age. We recruited a group of children with ONH and mild-moderate or no VI and therefore were unable to assess whether the neuro-anatomical, cognitive and behavioural abnormalities identified also relate to the degree of VI. The number of children with SOD and significant VI recruited were not large enough to further examine this question. To further investigate the relationship between developmental visual history and early and later levels of available functional vision and the development of the posterior optic radiations, this study should be repeated in children with a range of visual acuities, in the context of ONH (separating unilateral and bilateral ONH) and in VI secondary to

other pathologies (e.g. retinal dystrophy). To clarify whether the abnormal myelination/axon density in the posterior optic radiations is due to an antenatal insult or due to postnatal reductions in visual stimulation, the MRI component of this study would ideally be repeated in children at, or shortly after, birth.

Children with SOD and severe-profound VI frequently have co-morbidities which may potentially confound DTI assessments. Although we selected children with SOD and no other midline brain abnormalities, no cerebral palsy or history of seizures and with limited endocrine hormone abnormalities, it is not ideal to have combined the cohort of children with SOD with those with isolated ONH. Future studies would ideally be able to recruit larger numbers of children in both cohorts to assess whether the abnormalities of the prefrontal cortex identified in the larger group of combined children reflects the greater power of the larger group or something specific about the children with SOD.

Ideally we would have performed volumetric analysis of the ventral cingulum to assess whether DTI-based measures of white matter alone relate to behavioural problems in children with ONH or whether gray or white matter changes also impact on the behavioural pattern seen. Unfortunately, at present obtaining estimates of the volumes of white matter tracts remains a problem without a reliable solution, particularly in children. While a rough estimate of volume can in principle be obtained using diffusion tractography, partial volume effects in this narrow tract will be significant, and we therefore would not expect such a measure to be dependable enough to draw reliable conclusions from it.

F. Summary

To our knowledge, this is the first study to present converging information from neuroimaging (DTI) and behavioural measures in children with ONH and SOD. Although the sample size is relatively small and has a wide age range, the sample is more homogeneous than previous studies performed in children with ONH. We have demonstrated that children with ONH, normal intelligence and mild/moderate or no VI have an increased prevalence of clinically significant behavioural problems. Our findings suggest that children with ONH require behavioural assessment to exclude the presence of behavioural problems. The behavioural difficulties are found in association with reduced structural integrity of the ventral cingulum, suggesting that they are of neuro-behavioural origin. The finding of reduced FA in the corpus callosum suggests that ONH may be part of the spectrum of SOD, a condition frequently associated with corpus callosum hypoplasia (130). These children with mild/moderate or no VI and isolated ONH also have abnormalities in their optic radiations. This raises the question of whether abnormalities in the optic radiation in children with ONH are solely secondary to reduced visual stimulation. Further research within this population, controlling for visual levels, and presence of uni- or bilateral ONH, is required to explore the possible mechanisms affecting neural visual development.

5. EXPLORING THE SLEEP PHENOTYPE OF CHILDREN WITH SEPTO-OPTIC DYSPLASIA

A. Introduction

Thirty-two percent of children with SOD have disordered sleep patterns; these include free-running rest-activity cycles, and fragmented and arrhythmic sleep (157). Abnormal sleep patterns impact significantly both on the child's well being and on the families' overall lifestyle and ability to cope with their child's complex condition, and can adversely impact on children's cognitive development (152;158). A trial of melatonin treatment in children with SOD and sleep disruption is accepted clinical practice in many centres. However, no objective measurement of sleep/activity patterns with 24 hour melatonin profiles have been published for these individuals with the pathophysiological basis underlying sleep disorders in SOD remaining largely unknown.

A recent review on the use of melatonin in the treatment of sleep disorders stated that, 'the use of melatonin is frequently based on anecdotal evidence or small clinical trials' (162). There are possible side-effects associated with melatonin use with one study suggesting that melatonin may have pro-convulsant effects (163) and others suggesting that it impacts on the hypothalamo-pituitary axis, potentially affecting the patterns of oxytocin, ACTH, vasopressin and GH release, although it remains difficult to predict whether these endocrine effects will have long-term clinical outcomes (152;164;165).

We studied six children with rest-activity disturbances and SOD. All wore an Actiwatch-mini (a non-invasive method of detecting and recording movement intensity) for two weeks and were admitted to hospital for a 24-hour period during which hourly measurements of serum melatonin were taken. Sleep data were analyzed in conjunction with a detailed sleep diary. All children also underwent cognitive assessment, MRI brain scans and detailed endocrinological assessment.

In view of the uncertainty surrounding the etiology of the sleep abnormalities found in individuals with SOD, and consequently the optimum management of this problem, we aimed to establish whether children with SOD who experience sleep pattern disorders also have defective melatonin production.

B. Methods

Children aged 1-7 years with a diagnosis of SOD who reported significant rest-activity disturbances, but were naïve to melatonin treatment, were recruited prospectively. All were on adequate hormonal replacement at the time of recruitment to the study. All children were evaluated by an endocrinologist and an ophthalmologist, underwent brain MRI and completed a detailed sleep diary and 2 weeks actigraphy. A control group of children matched for age and gender; (4 aged 1-2 years (mean 1.4), and 6 aged 6-7 years (mean 6.8)); were recruited; all completed 2 weeks actigraphy and a detailed sleep diary. Participants were asked to complete the CBCL and to undergo brain MRI and cognitive assessment.

Actiwatch data were analyzed in conjunction with the sleep diary. Sleep wake patterns were ascertained and actual time asleep and sleep efficiency calculated using Actiware software (Minimitter). Sleep efficiency was defined as the ratio of total sleep time to sleep period (191). Actogram analysis was used to define the sleep pattern as normal (tau 24hrs), free running (tau>24hrs), fragmented (sleep efficiency <85%), or arrhythmic (no discernable rhythmicity) (157). When these definitions are applied to healthy control populations very few individuals are identified with free running, fragmented or arrhythmic sleep patterns (157;190). Sleep efficiency in children with SOD was compared to that of the healthy controls using the independent Student's t test.

Children with SOD were admitted to hospital on the morning of the melatonin profile. Plasma samples were collected hourly over a 24hr period from an indwelling venous cannula, with overnight samples being taken under dim light (20:00-06:00). The melatonin results were compared to historical controls collected by Waldhauser *et al* to assess normality (192).

C. Results

i. Subject characteristics

Subject characteristics are outlined in detail in table 14. Six children with SOD (4 male) were assessed, all of whom had some degree of light perception. All completed all components of the study.

Table 14 Characteristics of actigraphy study subjects with septo-optic dysplasia

	1	**2**	**3**	**4**	**5**	**6**
Age (years)	1.62	1.27	1.67	6.4	6.12	1.8
Gender	M	M	F	F	M	M
Degree Visual Impairment (size of object visualised at 30cm)	Profound (light awareness)	Profound (light awareness)	Severe (2.5mm)	Severe (5mm)	Severe (2.5mm)	Severe (5mm)
Hormonal Abnormalities	GHD, TSHD, ACTHD	GHD, TSHD, ACTHD, DI	GHD	GHD, TSHD, ACTHD	GHD, ACTHD	GHD
MRI findings	Small AP Normal PP B/L ONH Immature myelination Absent SP	Small AP Absent PP U/L ONH Immature myelination Thin CC	Small AP Normal PP B/L ONH	Small AP Small PP B/L ONH Immature myelination, b/l parietal clefts, perisylvian, frontal & parietal polymicrogyria	Small AP Normal PP B/L ONH Absent CC Absent SP	Small AP Normal PP U/L ONH
Cognitive Assessment (RZS, VI norms (225))	Age Appropriate	Mixed profile: delayed non-verbal cognition	Age Appropriate	Severe delay	Severe delay	Mixed profile: delayed non-verbal cognition
Mean actual sleep time hrs* **(normal reference range (242))**	10.2	6.29	8.2	7.4	5.4	7.6
Mean Sleep efficiency % (normal >85%)	74.7	61	71.4	66.4	56	78
Time of awakening h-min	05.19-08.54	03.03-09.35	04.20-08.56	05.26-12.10	04.02-10.14	05.24-08.06

Key Table 14 AP: anterior pituitary gland; PP: posterior pituitary gland; SP: septum pellucidum; ONH: optic nerve hypoplasia; CC corpus callosum, GHD: growth hormone deficiency, TSHD: thyroid stimulating hormone deficiency; ACTHD: ACTH deficiency; DI: diabetes insipidus; B/L: bilateral; U/L: unilateral.

Sleep data: mean over 14 days of actiwatch recording, time of awakening: range over 14 days of actiwatch recording.

ii. Sleep

Actigraphic studies showed reduced sleep efficiency in all children with SOD, mainly due to frequent and often prolonged night awakenings (Figure 17). Only one child (child 2) presented with an arrhythmic sleep pattern. Actigraphic studies were normal in all controls. Sleep efficiency was reduced in all subjects studied when compared with controls (sleep efficiency: SOD mean 69.5, SD 10.6; normal controls mean 89.5, SD 3.8; $p<0.001$) and total night-time sleep duration was >2 standard deviations below that of normal controls for age (242).

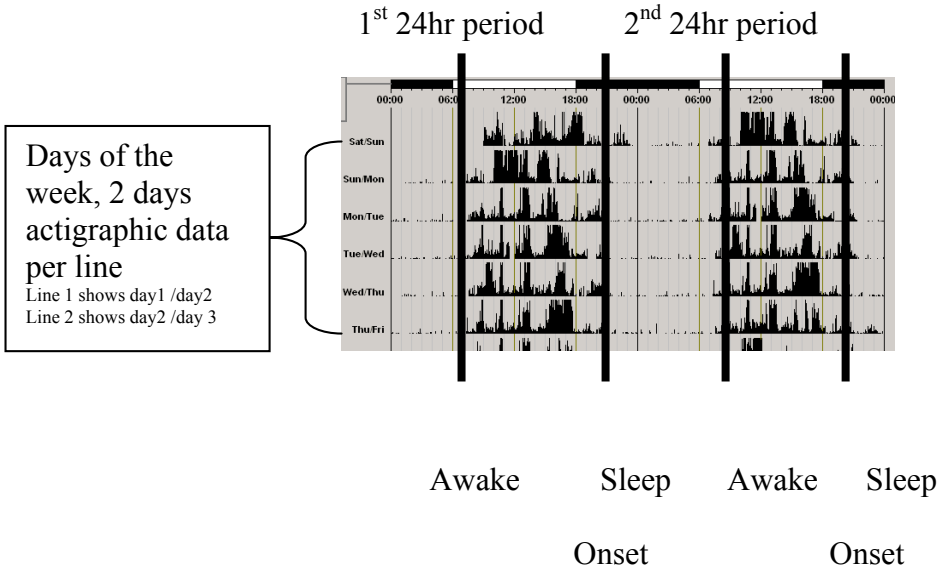
iii. Melatonin

Two children (child 4 and child 5) appeared to produce virtually no melatonin throughout the 24 hour period of measurement with a lack of clear circadian rhythmicity.

None of the remaining children had an 'inverted circadian rhythm', with all showing a normal circadian pattern with mean serum concentrations being lowest during the day (mean 56pg/ml) and peaking overnight (mean 380pg/ml) (Figure 18). Dim-light melatonin onset times (a commonly used marker to determine sleep phase) were normal (19.00 hours) in three of the children, but harder to discern in child 2 where melatonin concentrations appear to rise early in the day (advanced phase) but then peak normally at night.

Figure 17 Actigrams of rest-activity patterns

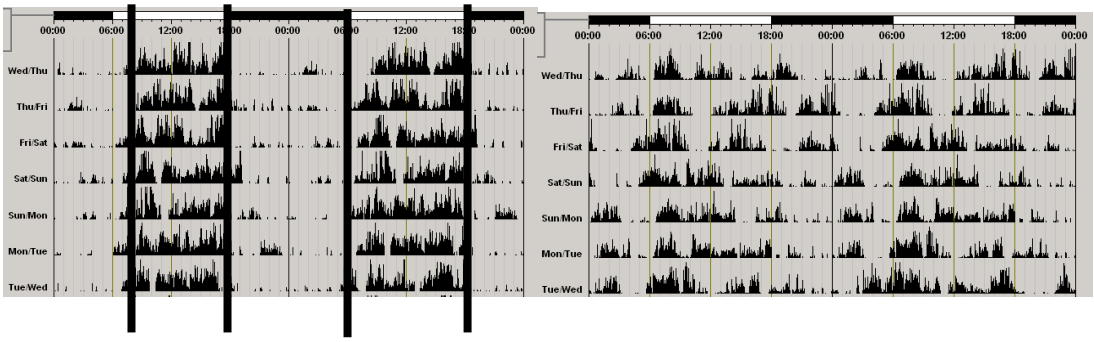
Example of a Normal sleep pattern Sleep efficiency (SE: 93%)



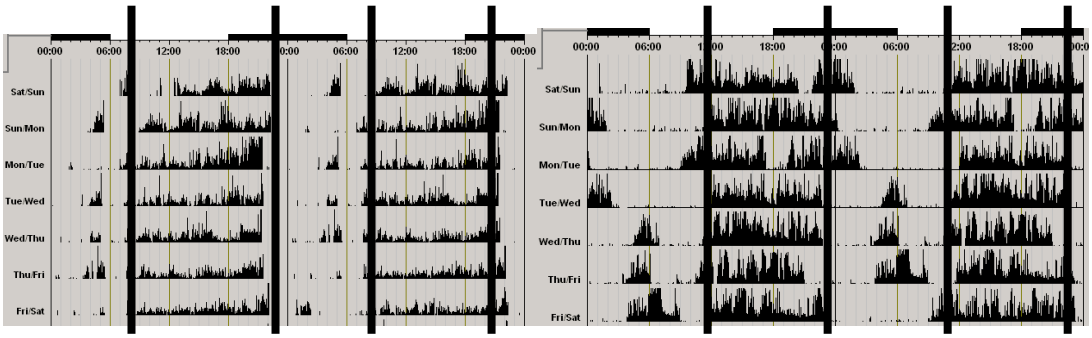
(data from normal individual [6.2year old boy wearing actiwatch mini© for a 12 day period])

Key Figure 17 Actigrams of rest-activity patterns in one normal individual and six children with septo-optic dysplasia. Actigraphic studies show reduced sleep efficiency (the ratio of total sleep time to sleep period) in all children with SOD, mainly due to frequent and often prolonged night awakenings (children 1, 3, 4, 5 & 6). Child 2 displays an arrhythmic sleep pattern.

Patient 1 (fragmented sleep [SE: 74.7%]) Patient 2 (arrhythmic sleep [SE: 61%])



Patient 3 (fragmented sleep [SE: 71.4%]) Patient 4 (fragmented sleep [SE: 66.4%])



Patient 5 (fragmented sleep [SE: 56%]) Patient 6 (fragmented sleep [SE: 78%])

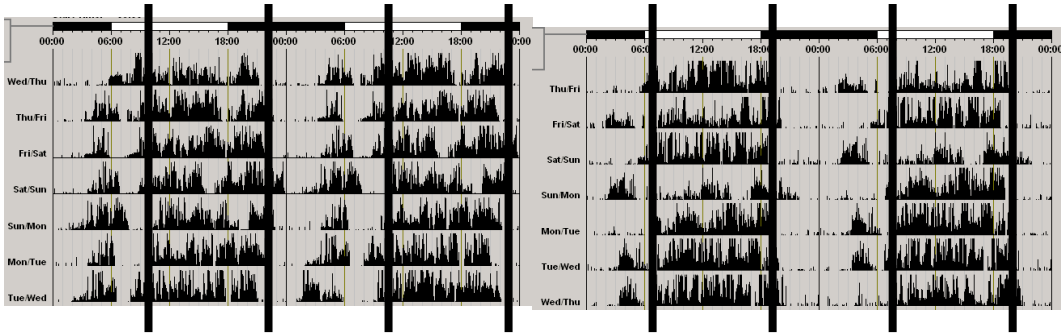
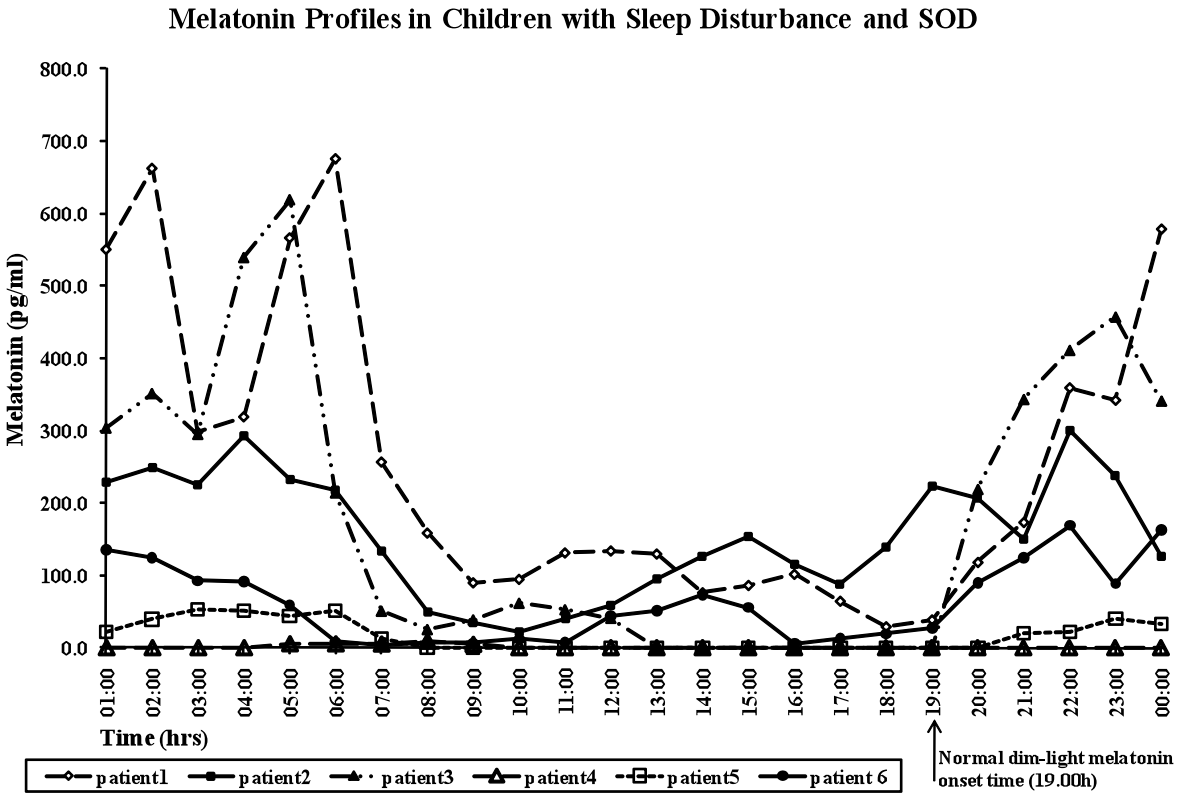


Figure 18 Melatonin profiles in children with sleep disturbance and septo-optic dysplasia



Key Figure 18 Plasma melatonin profiles in six children with septo-optic dysplasia (n=6). Plasma samples were collected hourly over a 24hr period from an indwelling venous cannula, with overnight samples being taken under dim light (20:00-06:00). Two children (child 4 and child 5) produce virtually no melatonin throughout the 24 hour period. The remaining children have a normal circadian pattern with mean serum melatonin concentrations being lowest during the day (mean 56pg/ml) and peaking overnight (mean 380pg/ml).

D. Discussion

The commonest problem in this cohort of SOD patients is sleep fragmentation, even in the three children with normal 24-hour melatonin profiles. We can only speculate about the abnormal melatonin profiles found in the other children. Very low, or absent, production of melatonin is an unusual finding, rarely reported in the literature, which has been associated with genetic defects in the enzyme that allows melatonin to be produced from serotonin (243). Due to the heterogeneous patterns of brain abnormality seen in patients with SOD, any point in the pathway from the retina, to the SCN, to the pineal gland could be severely disrupted and cause such a picture. Even with current neuroimaging resolution, we are still not able to directly image structures as small as the SCN. It is possible that anomalies in sleep and melatonin production may both arise from perturbations in the clock without necessarily being inter-dependent, which is to some extent supported by the poor relationships in this series between melatonin profiles and objective sleep patterns.

The SCN of the hypothalamus plays a principal role in the circadian regulation of sleep wake cycles and the diurnal release of endocrine factors including melatonin. This endogenous circadian rhythm can be modified by light exposure or exogenous melatonin administration. The pathophysiology of the disordered sleep patterns found in SOD could be caused by perturbations at any single, or indeed multiple points in the mechanisms responsible for normal, consolidated sleep-wake cycles. In individuals with light perception but significant VI, light will still entrain their circadian rhythms as this function is served by the non-image forming melanopsin ganglion cells (159). It is therefore unlikely that the sleep abnormalities found in individuals with SOD are secondary to their VI.

However, the circadian rhythm of melatonin, although entrained and suppressed by light, is endogenously driven by the SCN via a multisynaptic sympathetic noradrenergic pathway. Thus abnormalities in sleep and melatonin production could arise secondary to any dysfunction of the SCN itself (shown to be absent in one patient with SOD) (156), any disruption to the above complex pathways, or any abnormalities in the pineal gland.

Other possible influential factors include the fact that the circadian clock is entrained not only by light but also by behavioural and social cues (zeitgebers) (244). An inability to correctly interpret these zeitgebers in children with neurodevelopmental disorders can lead to abnormalities in circadian rhythms (245). Fifty-seven percent of children with bilateral ONH have significant developmental delay (246). This may be impacting on their ability to interpret environmental zeitgebers, leading to difficulty in establishing normal sleep-wake cycles. Significant improvement in time to sleep onset has been shown in children with neurodevelopmental delay treated with melatonin; however since children in our cohort did not have abnormalities in sleep latency, melatonin may not be the most appropriate treatment option (247).

With regard to treatment of sleep disorders in these children, we know that exogenous melatonin has a soporific effect, even in the presence of normal endogenous circadian melatonin production (e.g. sedation for MRI studies) (247). It is therefore unsurprising that some children with SOD benefit from melatonin treatment even in the presence of normal melatonin concentrations and circadian rhythm (248). However, the profiles we obtained do not suggest that delayed sleep phase syndrome, which has been suggested to be a good predictor of response to exogenous melatonin treatment, was present. This

differs from findings in conditions such as Rett and Angelman syndromes where behaviour and sleep disturbances have been shown to be associated with phase delay in melatonin secretion (165).

E. Study limitations

This study had several limitations including the small size of the cohort studied. This was in part due to the very intensive protocol used to ascertain melatonin peaks. The half life of melatonin is 30-53 minutes, and as it was previously unknown whether the normal circadian pattern of melatonin production would be maintained in children with SOD, hourly sampling over a 24 hour period was felt to be the most accurate way of ensuring peaks were not missed (249). Salivary melatonin measurement could offer an easier alternative means in the future of determining melatonin profiles in this group of patients. As a large amount of historical control data is available in the public domain; we did not therefore prospectively recruit age-matched controls because the number required to accurately delineate the significant variations found in melatonin concentrations with increasing age would have been extremely high (192).

F. Summary

Our findings indicate that abnormalities in timing and amount of melatonin secretion vary, but do not account for the majority of the sleep abnormalities observed in these children, and suggest that there are other as yet unexplored factors contributing to their disordered sleep patterns. In view of the significant socioeconomic and neurocognitive

burden associated with sleep deprivation, other methods for treating the chronic sleep abnormalities found in this cohort need to be explored.

5. DISCUSSION

A. Introduction

The studies summarized in the results chapters provide new data regarding the cognitive, behavioural and circadian rhythm difficulties experienced by children with midline brain abnormalities including IGHD, ONH and SOD. For parents of children with IGHD, their concerns regarding academic and motor skills performance often outweigh those regarding daily injections once the diagnosis of IGHD has been confirmed and treatment started. However, previously it has been hard for parents and physicians to know whether these children's difficulties with, for example, motor skills, are related to or independent of their diagnosis of IGHD. Similarly in children with isolated ONH and functionally normal vision, ongoing paediatric follow-up has not previously been advocated. However the increased prevalence of clinically significant behavioural difficulties in this cohort highlights the need for a shift in management practice. Children with SOD have complex clinical needs, which include MPHD and learning difficulties. However, the disturbances in circadian rhythm found in individuals with SOD and the disruption that they can inflict on the whole family is often the most difficult component of the syndrome for both the family and physician to manage. Previously a lack of understanding of the aetiology and therefore best management of this problem has hampered progress in this area. Whilst we have made conclusions in many of the areas outlined above there remain many unanswered questions, these are outlined in detail below under the headings of IGHD, ONH and SOD.

B. Isolated growth hormone deficiency

In summary, the study to investigate the effect of IGHD on cognition, motor function and brain structure found that the hippocampus, corpus callosum, thalamus and globus pallidum are smaller (in relation to brain size) in children with IGHD compared to those with ISS, and that children with IGHD also have reduced coherence of white matter tracts in association with cognitive and motor dysfunction. Our findings provide evidence to suggest that the GH-IGF-1 axis plays a role in brain and cognitive development. This study was however unable to ascertain whether the abnormalities identified occur as a result of GH-deficiency or are part of the underlying disorder. We have also not been able to assess whether cognitive and motor skills performance scores improve with GH therapy. Future studies are therefore require to address the hypothesis that GH/IGF1 affects both structural brain growth as well as brain function and that GH-treatment improves cognitive function in children with GHD.

Interestingly, the present study did not identify an increased prevalence of behavioural abnormalities in children with IGHD when compared to controls with ISS. This is surprising in the context of the one large previous study investigating the prevalence of behavioural problems in children with IGHD, which reported an increased prevalence of behavioural difficulties in children with IGHD (211;250). At baseline, Stabler *et al* found that children with both IGHD and ISS showed significant discrepancy ($p<0.01$) between IQ and achievement scores in reading (6%), spelling (10%), and arithmetic (13%) and a higher-than-expected rate of behavioral problems (GHD, 12%, $p<0.0001$; ISS, 10%, $p<0.0001$) (211). There were however no between group differences for IQ or behavioural measures. This differs from our findings of significantly lower FSIQ ($p<0.02$), verbal comprehension index ($p<0.006$) and processing speed index ($p<0.05$)

scores in children with IGHD as compared to ISS controls. As one of our primary aims was to assess whether we could identify neural correlates for any cognitive/motor skills or behavioural abnormalities identified we wanted to compare the subjects to the least different control group possible. We therefore chose children with short stature as our controls as, although they have no identifiable medical condition their parents have been concerned enough regarding their health to seek a specialist opinion at a tertiary referral hospital. Additionally, children with ISS have previously been found to have reduced IQ when compared to normal stature controls (25). In choosing children with ISS as our "controls", we thereby hoped to control for the effect of stature and isolate the effect of GHD. In addition the CBCL is a parental questionnaire, which may be biased for parents of children with perceived (e.g. short stature) or actual (e.g. VI) medical conditions. We therefore used children presenting to the tertiary referral hospital rather than sibling controls to control for the effect of this. However, in choosing short stature controls who are at increased of risk of having undiagnosed partial GH insensitivity or IGF-1 deficiency (251;252), we may have chosen a control population who are also at increased risk of neurological deficits due to subtle abnormalities of the GH axis. Looking at the concentrations of peak GH, baseline IGF-1 and IGFBP-3 (Table 7) of the controls with ISS, there is limited evidence to suggest the presence of either GH insensitivity or IGF-1 deficiency, with the mean GH peak of ISS controls not being excessively elevated at 15μg/L, and the IGF-1 SDS of ISS controls falling just below 0 (-0.5 SDS). However, the ISS group may have included individuals with both partial GH insensitivity and IGF-1 deficiency making interpretation of the means of GH peak and IGF-1 SDS unhelpful (251;252). Ideally we would therefore have also have recruited a control group of age and gender matched children of normal stature with a normal GH axis to assess whether there were any differences in behavioural measures

between children with IGHD and normal controls, and/or between children with ISS and normal controls.

Stabler *et al* also identified improvements in behavioural profiles following treatment with GH (93). This effect was found to be larger in those with GHD ($p<0.001$) than in those with ISS ($p<0.003$) (93). GH treatment in individuals with GHD also improved scores on internalizing subscales (withdrawn: $p<0.007$, somatic complications $p<0.001$, anxious/depressed $p<0.001$) and on attention, social problems and thought problems ($p=0.001$). We were not however able to follow our cohort up after GH treatment was started, and have not therefore been able to assess whether improvements in cognitive or behavioural measures were present after GH treatment was started. The cohort of children with IGHD we studied was relatively small and as two children with IGHD did not comply with GH treatment and two further children declined to participate in the follow-up component of the study the numbers of children remaining were not large enough to power follow-up, on treatment, studies. Future, larger, studies with a 12-24 month follow up post onset of GH treatment are therefore required.

Unlike adult studies in which individuals with GHD have consistently been found to have impairments in attention, memory and executive function we identified no abnormalities in attention, memory or executive function in children with IGHD (80). This may also have been due to the control group we selected. Assessing the difference in cognitive function, attention and memory skills performance and behaviour between children with IGHD and normal stature controls may have identified further cognitive abnormalities present in children with IGHD. It may also be that cognitive deficits in the domains of

attention, memory and executive function only become apparent in individuals with chronic untreated GHD (i.e. adults).

Although we identified reductions in specific neural volumes (the right hippocampus, globus pallidum, thalamus, corpus callosum and cerebellum) with reduced corticospinal tract and corpus callosum FA in children with IGHD, which we hypothesized may either be mediated by mechanisms such as the stimulation of acetylcholine release by IGF-1 from hippocampal neurons (53), or due to the selective neuronal vulnerability of these brain regions to the underlying disease process (215), we have been unable to further investigate the underlying pathogenesis of the abnormalities identified. One previous study performed in a cohort of adults with childhood onset GH deficiency (a combination of individuals with IGHD and MPHD) used nuclear magnetic resonance spectroscopy in conjunction with measurements of event-related potentials to further investigate this question (75;253). Magnetic resonance spectroscopy can be used to obtain in vivo measurements of brain metabolites such as N-acetylaspartate (a marker of neuronal density and integrity) and choline (a marker of membrane synthesis/breakdown). Future studies could use techniques such as magnetic resonance spectroscopy in addition to those used in the present study, to confirm the significant findings we have identified and to clarify the pathophysiology of the abnormalities present. It would also be extremely helpful to have follow-up MRI studies in children with IGHD and controls 12 months following the onset of GH treatment in patients.

C. Optic nerve hypoplasia

In the study to investigate the prevalence of behavioural problems in children with isolated ONH and to use DTI to identify neural abnormalities not detectable using conventional neuroimaging, we found that children with isolated ONH, normal development and mild to moderate or no VI have an increased prevalence of clinically significant behavioural problems. We also showed evidence of reduced white matter integrity in the corpus callosum and ventral cingulum, the anterior optic pathway and optic radiations bilaterally, regions of the brain that are implicated in behavioural and emotional regulation and vision. These identified abnormalities in white matter fibre density in the ventral cingulum correlated significantly with behavioural scores. When children with ONH and SOD were grouped together they were found to have reduced FA in the corpus callosum, ventral cingulum and prefrontal cortex, together these structures form part of the social brain (148).

Currently children with isolated ONH do not undergo behavioural assessment as part of their routine clinical care. However, the association between abnormal behaviours in childhood and later psychiatric morbidity in other childhood cohorts highlights the need for the identification of behavioural abnormalities in these children with isolated ONH. In children with behavioural difficulties strategies could then be put into place to prevent later psychiatric illnesses.

Although previous research in different clinical populations suggests that the behavioural abnormalities we have identified are likely to be clinically significant, we have not

assessed the wider clinical significance of these behavioural difficulties in children with ONH (229). Future long-term follow-up studies are therefore required to address this question. A more detailed assessment as to whether any of these behavioural problems are impacting on school performance would also be helpful.

In addition to the well-established abnormalities of the optic nerve, we demonstrated that children with ONH also show evidence of reduced white matter integrity in the ventral cingulum bilaterally, the corpus callosum and the optic radiations bilaterally. We were unable to demonstrate concurrent reductions in gray matter volumes in the corpus callosum and occipital visual cortex in children with ONH although there was a trend for children with ONH to have smaller corpus callosum splenium volumes. This is interesting as Garcia-Filon *et al* previously reported that, in children with optic nerve hypoplasia, for each $2cm^2$ reduction in corpus callosum size the risk for developmental delay increased twofold (131). However, the majority of children (54/60) studied in that paper had SOD as opposed to isolated ONH. It would be interesting to repeat the current study in a much larger cohort to assess whether in children with isolated ONH corpus callosum splenium volume also correlates with cognitive performance.

These children with mild/moderate or no VI and isolated ONH have abnormalities in their optic radiations. This raises the question of whether abnormalities in the optic radiation in children with ONH are, as previously hypothesized, solely secondary to reduced visual stimulation. Further research within this population, controlling for visual levels and uni- or bilateral ONH, is required to explore the possible mechanisms affecting neural visual development.

We recruited a group of children with ONH and mild-moderate or no VI and therefore were unable to assess whether the neuro-anatomical, cognitive and behavioural abnormalities identified also relate to the degree of VI. The number of children with SOD and significant VI recruited were not large enough to further examine this question. To further investigate the relationship between developmental visual history and early and later levels of available functional vision and the development of the posterior optic radiations this study should be repeated in children with a range of visual acuities, in the context of ONH (separating unilateral and bilateral ONH) and in VI secondary to other pathologies (e.g. retinal dystrophy). To clarify whether the abnormal myelination/axon density in the posterior optic radiations is due to an antenatal insult or due to postnatal reductions in visual stimulation, the MRI component of this study would ideally be repeated in children at, or shortly after, birth.

Our data suggests that children with SOD may have abnormalities in the development of their social brain, namely their prefrontal cortex, corpus callosum and ventral cingulum and this may to some extent explain the increased prevalence of behavioural and social communication difficulties found within this group of children (58). This work will need to be repeated in larger cohorts of children with SOD and ASD to confirm these preliminary findings.

D. Septo-optic dysplasia

Abnormal sleep patterns impact significantly both on the child's well being and on the families' overall lifestyle and ability to cope with their child's complex condition, and can adversely impact on children's cognitive development (152;158). Thirty-two

percent of children with SOD have disordered sleep patterns; these include free-running rest-activity cycles, fragmented and arrhythmic sleep (157). In view of the uncertainty surrounding the aetiology of the sleep abnormalities found in individuals with SOD, and consequently the optimum management of this problem, we aimed to establish whether children with SOD who experience sleep pattern disorders also have defective melatonin production. Out of the six children studied five children had sleep fragmentation and one had a completely arrhythmic sleep pattern. All children had a significant reduction in sleep efficiency. Two children produced virtually no melatonin throughout the 24-hour period of measurement with a lack of clear circadian rhythmicity, with the remaining four children having relatively normal melatonin production profiles. We have therefore shown that the aetiology of the sleep disturbances found in children with SOD is complex, and not solely due to abnormal nocturnal melatonin production. These may be due to a vriability in the aetiology of SOD in the children we studied. These findings are clinically important as they question current clinical practice. Previous papers have recommended that circadian rhythm disturbance in children with SOD can be resolved by administering low doses (0.1-0.5 mg) of melatonin in the evening, or soporific doses (3-5 mg) at bedtime. These recommendations form part of standard practice in many endocrine centres; however as stated in the above paper, and unsurprisingly in view of our findings, melatonin does not improve sleep dysregulation in all children with SOD (254).

Whilst we have now demonstrated why not all children with SOD respond to treatment with melatonin there remain many unanswered questions. Three possible future studies to address these issues are outlined below. Firstly, studying a larger cohort of children with SOD and sleep abnormalities using a less intensive study protocol (for example,

samples to measure melatonin concentration could be taken in the evening only to assess whether the normal nocturnal rise in melatonin was present rather than aiming to assess the entire 24 hour pattern of melatonin production) and following them up with a trial of melatonin treatment may help to clarify which children with SOD are most likely to benefit from melatonin treatment. Secondly, as at present the majority of medication prescribed to regulate sleep patterns in children are either unlicensed of off-label, it is difficult to know what the long-term safety profile of medications such as melatonin is. This reflects the limited number of therapeutic trials that have been performed in children with sleep disorders, not only in those with SOD (255). There are putative side-effects associated with melatonin use with one study suggesting that melatonin may have pro-convulsant effects (163) and others suggesting that it impacts on the hypothalamo-pituitary axis, potentially affecting the patterns of oxytocin, ACTH, vasopressin and GH release, although it remains difficult to predict whether these endocrine effects will have long-term clinical outcomes (152;164;165). Future long-term studies assessing the safety of melatonin use in children are therefore required. Thirdly, as significant sleep disturbances have a significant adverse impact on cognitive functioning and behaviour (256), as well as affecting the family as a whole, other solutions for the extremely challenging sleep disorders found in children with SOD need to be identified. This will require trials of novel medications targeting sleep disruption in children with SOD.

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8. APPENDICES

A. Examples of Information Sheets

The Wider Effects of Growth Hormone
Information to answer your questions
(Children with IGHD)

You are invited to take part in a research study. The study will look at some of the genes in your body that are important for making you grow and for allowing you to be active. Before you decide to say YES or NO, it is important for you to know why the research is being done and what it will involve. Please read, or have someone to read for you, this fact sheet. Do not worry if you do not understand it straight away. Your parents have also been told about this, and you can ask them to help you understand. Ask us if there is anything that is not clear or if you would like more information.

Introduction

A special part of the brain makes a number of chemicals called hormones that help you to grow. In some children who do not grow well, the brain cannot make these hormones. These children would need to be given the hormones that are not there. We know that the way your body changes and grows depends on the genetic material (called DNA, which is made up of thousands of genes) that you inherit from your parents. Changes within genes are the reason why we are all very different to each other. Normally, you have two copies of a gene, one that comes to you from your father and one

that comes to you from your mother. Sometimes a change within a gene that you get from your parents can lead to a medical condition.

Sometimes, when these hormones are missing, the way in which people behave and learn things are different.

We want to understand how the medicine changes the way children with these missing hormones learn

Why are you asking for my help?

We want to know why you have to take medicine, and why you have been ill in the past. We want to look at your genetic material (called DNA) for changes. We want to understand how giving you medicine changes the way in which you learn. These studies will help us understand why children have these particular conditions and how they can be helped best.

We ask all children who have a condition like yours to take part.

What will happen to me if I take part?

A few extra samples of blood will be taken from you after putting on a special cream to stop the blood test from hurting.

We will ask you some questions about how you sleep. We will ask you and your mum and dad these questions before you start

taking your medicine. We will also ask your teacher about your school work (if you don't mind). We will ask you to wear a small watch for 2 weeks to see how well you sleep.

We would like to see how you learn new things, use words and play. All the tests that we would like to use are like games and are made for children so we think that you will have fun taking part. But, if you get tired, we will take a break or stop.

Your doctors have decided that they want to take some special pictures of you. They are going to use a special machine called an MRI scanner. This machine can take pictures of the inside of you almost like an X-ray camera. When you are already in the MRI scanner, having all your pictures taken, we will take some extra pictures. Normally, all the pictures can be taken in about half an hour. If you let us take extra pictures, the whole scan will last about 10 minutes more.

The doctors or nurses will tell you what is going to happen. You will have to lie quite still when you are being scanned. The machine is also rather noisy, so you will have some headphones on. If you want to, you can watch a video whilst you are having your pictures taken.

Is it dangerous?

No, all of these tests are safe. MRI scanning is very safe. We take pictures of children every day with the MRI scanner.

What about my results?

The doctors looking after you will talk to you and your parents about the results of these tests. We hope that these tests will help us to look after both you and other children with similar conditions better now and in the future.

Do I have to take part?

No. It is up to you and your parents to decide. If you decide you don't want to, then that's fine. The doctors and nurses will look after you as best as they can anyway.

Who will get to see this information about me?

The doctors looking after you will be able to see your test results. We will look at all the results and may print the results in a magazine so that other people can learn what we have learned. Your name will be taken off, so that nobody knows whose results they are.

Who can I speak to if I have any questions?

You can speak to your parents who have also been given facts to read about this study. You can also speak to the doctors or nurses who look after you in the hospital.

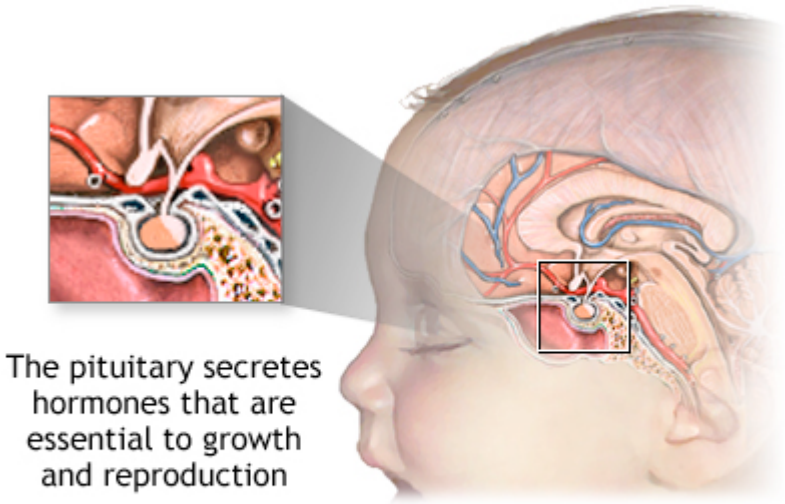
Emma Webb
Phone: 0787 2021356
Email: emmaalicewebb@yahoo.com
Dr Michelle O'Reilly
Phone: 07947 663371
Email: m.oreilly@ich.ucl.ac.uk

The Wider Effects of Growth Hormone

Information to answer your questions (IGHD parent Information Leaflet)

Your child is being invited to take part in a research study. This study is being carried out by Dr Emma Webb and Dr Michelle O'Reilly with the assistance of Prof. Mehul Dattani, Dr Naomi Dale and Dr Alison Salt at Great Ormond Street Hospital. Before you consider being involved, we want to make sure you know why we are doing this research so please read the following information carefully and discuss it with other people if it helps. You can call, email or write to us if anything is not clear or if you have more questions. Take time to decide whether or not you wish to take part. Thank you for reading this.

Introduction



The pituitary secretes hormones that are essential to growth and reproduction

The pituitary gland in the brain makes a number of chemicals called hormones that help you grow and develop normally. In some children who do not grow well, the pituitary gland may not make growth hormone (GH) and/or other hormones. The gland may be very small or even absent. These children would need to be treated with the hormones that are missing. Occasionally, the small pituitary gland may be associated with abnormalities affecting the eyes and the brain; this is called septo-optic dysplasia or SOD.

We now know that the way your body changes and grows depends on the genetic material (called DNA, which is made up of thousands of genes) that you inherit from your parents. Changes within genes are the reason why we are all very different to each other. Normally, you have two copies of a gene, one that comes to you from your father and one that comes to you from your mother. Sometimes a change within a gene that you get from your parents can lead to a medical condition. So far, we only know some of the genes that are important for pituitary development.

We have noticed that children who do not make enough GH are more likely to have abnormal behaviour and to be slow with their development. We know that treatment with GH makes you grow taller and it has been suggested that treatment may also improve behaviour.

What is the purpose of the study?

In this study, we aim to study the genes controlling the development of the brain and pituitary gland. We have recently found that changes in 3 genes are associated with a small pituitary gland and a lack of growth hormone (GH) in some of our patients. We now want to look at children with these conditions in more detail and to examine their DNA for abnormalities in these and other genes that are important for the normal development of the brain. We hope that this will help us to understand why children suffer from these particular conditions.

We're also interested in the effect of GH treatment on brain structure (using MRI). We hope that this research will help us gain better understanding of the strengths and weaknesses that children with GH deficiency may have and make us think of the ways that parents and professionals may help if specific difficulties arise.

How is the study being done? (Summary attached)

All of the tests outlined below will be arranged around your routine visits to the hospital to minimise any disruption the study may have on your child and your family.

1. Blood tests

The blood test/cannula insertion will be performed by a member of the Paediatric Endocrinology team at Great Ormond Street Children's Hospital during one of your routine visits to the hospital. We need to take a small blood sample (2 teaspoonfuls) from your child for the genetic) tests. To minimise discomfort, we will perform the blood test using local anaesthetic cream.

Only a single sample of blood is required for the genetic study. If a change (mutation) is found within one of the genes that are important for pituitary development, then further blood samples may be required from your child and the rest of your family. However, the requirement for these samples will be discussed in detail with you and your family.

2. Questionnaires

We will ask you some questions about your child (including date of birth, medical problems, birth history) the first time we see you. This will take about half an hour. We will ask you to keep a diary recording your child's sleep pattern for a 2week period whilst they wear a small comfortable watch which records how well they sleep before starting treatment with GH.

3. Developmental Assessment

We are interested to see how your child is learning about their world, uses language, interacts with other people, and responds in social situations like story-time. To understand this we would assess your child's language, cognitive and social development using a selection of tests. These will be carried out by a team with a special interest in the development of children with and without visual impairment. For this we will arrange for you to come with your child to the Developmental Clinic on the same day as your endocrine clinic visit or we can arrange a home, nursery or school visit if this is more convenient for you. All the tests that we would like to use in this research are game-like and designed especially for children so we anticipate that most children will have fun taking part. However, if your child gets tired we will take a break or stop altogether. We will ask your child's school teacher how they are doing in school (if you and your child are happy for us to contact them). This will provide a clear picture outlining the developmental progress and any developmental delay/behavioural problems present in your child.

4. MRI Scan

We also want to add 10mins extra pictures onto your child's routine magnetic resonance imaging (MRI) scan to get a better picture of the overall brain structure and the volumes of the different areas of the brain that may be important in GH deficiency. MRI does not involve ionising radiation, unlike computed tomography (CT) or x-rays, and is therefore a good and safe imaging investigation to use in children.

It is very important to keep very still throughout any MRI scan as otherwise, the pictures become blurred and of no use, so it is routine clinical practice to use sedation or general anaesthesia in young children during a scan. Sometimes the scans can be done in babies if they are asleep and wrapped so that they do not move. Usually however sedation or general anaesthesia is used as the

scanner is noisy, and the scan can last up to 40 minutes. Your doctor will talk to you about whether your child will be sedated or given general anaesthetic for his/her scan and will get separate consent from you for this. This is routine clinical practice and is not a consequence of our project. The choice between non-sedation, sedation, or general anaesthesia will not be altered whether or not your child participates in the project. MRI is a well established investigation at Great Ormond Street Hospital for Children and is performed under sedation or anaesthesia on several children every day.

We will also collect additional images during scanning to look at the white matter pathways connecting different parts of the brain – this is known as diffusion tensor imaging or DTI.

During the scan, we will need to inject a small volume of contrast fluid (dye) intravenously. This too is routine practice, independent of project participation, and is regarded as a very safe procedure. Our project involves taking extra pictures while your child has his/her MRI scan. This means that the scan will take 10 minutes longer than a routine scan.

What are the risks and discomfort?

No risk to the child can be foreseen. There is discomfort from a single needle prick for blood testing, but this will be minimised by using local anaesthetic cream. MRI uses electromagnetic pulses to produce images of organs and tissues. It is used routinely in all age groups, and there are no identified side effects. Compared to a normal scan, the scan time in the project will be longer, and we will apply more electromagnetic pulses. However, we will always operate within the limitations that are set for clinical use of MRI. If your child is sedated or under general anaesthetic, this will not have any effect on their level

of discomfort. If they are awake, then it will mean they may have to keep still for a little longer.

What are the potential benefits?

This study may help us to understand why your child has developed their problems. Additionally, we may be able to make the diagnosis earlier in future children who may have the condition, and where one child in the family already has the disorder, we may be able to make a diagnosis before birth in future pregnancies. An improved understanding of these conditions will help us to find the best ways of making the diagnosis i.e. using the best tests available so that the diagnosis can be made with minimal discomfort to the child. Some of these genes are associated with a changing (evolving) picture, with more hormonal abnormalities appearing with time. If that is the case, then we will be in a better position to anticipate these abnormalities once we have the genetic information and hence we will be able to diagnose the problem more rapidly. We hope that a better understanding of the role GH has on behaviour and brain structure will help us to guide the use of GH in children with these problems in the future.

Who will get to see this information about my child?

Only the researchers and a representative of the Research Ethics Committee will have access to the data collected during the study. We will tell people what we have learned in the study in reports and publications, but nobody will learn anything personal about your child, or any other child, by reading these reports or publications. The use of some types of personal information is safeguarded by the Data Protection Act 1998 (DPA). The DPA places an obligation on those who record or use personal information, but also gives rights to people about whom information is held. If you have any questions about data protection, contact the Data Protection officer via the switchboard on 020 7405 9200 extension 5217.

Who has approved the study and who is funding it?

The Child Growth Foundation is funding this study. The project has been approved by an independent research ethics committee who believe that it is of minimal risk to you. However, research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this study.

This research is covered by a compensation scheme, which may apply in the event of any significant harm resulting to your child from involvement in the study. Under this scheme, it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

If taking part in this study means that you have to make additional trips to hospital we will reimburse your travel costs.

Does my child have to take part in this study?

No. If you decide, now or at a later stage, that you do not wish to participate in this research project, that is entirely your right, and will not in any way prejudice any present or future treatment.

Who do I speak to if problems arise?

Please contact Dr Emma Webb or Dr Michelle O'Reilly directly with any problems relating to the study. If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researchers. If the problems are not resolved, or you wish to comment in any other way, please contact the Principal Investigator, Professor Mehul Dattani by post at the Institute of Child Health, 30 Guilford Street, London WC1N

1EH, or if urgent, by telephone on 4059200, and the switchboard will put you in contact with him.

Dr Emma Webb,
Research fellow in Paediatric Endocrinology
Clinical and Molecular Genetics Unit, Institute of Child Health,
30 Guilford Street, London, WC1N 1EH.
Telephone: 07872021356
Email: emmaalicewebb@yahoo.com

Dr Michelle O'Reilly,
Research Associate, Neurosciences Unit, Institute of Child Health, the Wolfson Centre, Mecklenburgh Square, London WC1N 2AP.
Telephone: 0794 7663371
Email: m.oreilly@ich.ucl.ac.uk

Thank you for your time and for considering taking part in this study!

GHD STUDY SCHEDULE

Developmental assessment (3 hrs):

- Age appropriate developmental assessment to assess verbal and cognitive development, attention and behaviour
- Parental questionnaire about behaviour

Questionnaire (30 min)

- Basic information

Imaging (additional 10 min):

- Volumetric MRI
- DTI

Genetics (1 min):
1 teaspoon blood sample

Sleep assessment:

- Actiwatch-worn for 2weeks
- 2week- sleep diary

The Wider Effects of Growth Hormone
Information to answer your questions
(Children with SOD)

You are invited to take part in a study. Before you decide to say YES or NO, it is important for you to know why the study is being done and what it will involve. Please read, or have someone to read for you, this fact sheet. Do not worry if you do not understand it straight away. Your parents have also been told about this, and you can ask them to help you understand. Ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

A special part of the brain makes a number of chemicals called hormones that help you to grow. In some children who do not grow well, the brain cannot make these hormones. These children would need to be given the hormones that are not there.

We know that the way your body changes and grows depends on the genetic material (called DNA, which is made up of thousands of genes) that you inherit from your parents. Changes within genes are the reason why we are all very different to each other.

Normally, you have two copies of a gene, one that comes to you from your father and one that comes to you from your

mother. Sometimes a change within a gene that you get from your parents can lead to a medical condition. Sometimes, when these hormones are missing, the way in which people behave and learn things are different.

We want to understand how in children that need medicine to help them to grow the medicine changes the way in which they learn.

Why are we asking for your help?

We want to know why you have to take medicine, and why you have been ill in the past. We want to look at your genetic material (called DNA) for changes. We want to understand how giving you medicine changes the way in which you learn, and changes your body shape. These studies will help us understand why children have these particular conditions and how they can be helped best.

What will happen to me if I take part?

A few extra samples of blood will be taken from you after special cream to stop the blood test from hurting has been put on.

We will ask you some questions about how you sleep. We will ask you and your mum and dad these questions We will also ask your teacher about your school work (if you don't mind). We will ask you to wear a small watch for 2 weeks to see how well you sleep.

We would like to see how you learn new things, use words and play. All the tests that

we would like to use to see how you learn are like games and are made for children so we think that you will have fun taking part. But, if you get tired, we will take a break or stop.

Your doctors have decided that they want to take some special pictures of you. They are going to use a special machine called an MRI scanner. This machine can take pictures of the inside of you almost like an X-ray camera. When you are already in the MRI scanner, having all your pictures taken, we will take some extra pictures. Normally, all the pictures can be taken in about half an hour. If you let us take extra pictures, the whole scan will last about 10minutes more.

Is it dangerous?

No, all of these tests are safe. MRI scanning is very safe. We take pictures of children every day with the MRI scanner.

What about my results?

The doctors looking after you will talk to you and your parents about the results of these tests. We hope that these tests will help us to look after both you and other children with similar conditions better now and in the future.

Who will get to see this information about me?

The doctors looking after you will be able to see your test results. We will look at all the results and may print the results in a magazine so that other people can learn what we have learned. Your name will be taken off, so that nobody knows whose results they are.

Do I have to take part?

No. It is up to you and your parents to decide. If you decide you don't want to, that's fine. The doctors and nurses will look after you as best as they can anyway.

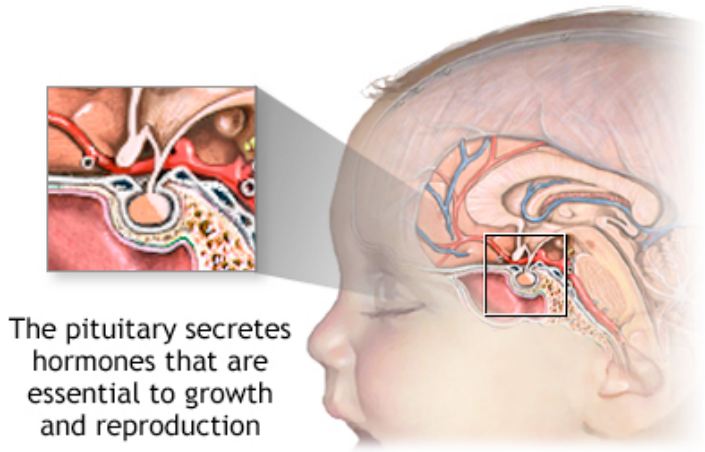
Who can I speak to if I have any questions?

You can speak to your parents who have also been given some facts to read about this study. You can also speak to the doctors or nurses who look after you in the hospital.

The Wider Effects of Growth Hormone
Information to answer your questions
(SOD parent Info Leaflet)

Your child is being invited to take part in a research study. This study is being carried out by Dr Emma Webb and Dr Michelle O'Reilly with the assistance of Prof. Mehul Dattani, Dr Naomi Dale and Dr Alison Salt at Great Ormond Street Hospital. Before you consider being involved, we want to make sure you know why we are doing this research so please read the following information carefully and discuss it with other people if it helps. You can call, email or write to us if anything is not clear or if you have more questions. Take time to decide whether or not you wish to take part. Thank you for reading this.

Introduction



The pituitary secretes hormones that are essential to growth and reproduction

The pituitary gland in the brain makes a number of chemicals called hormones that help you grow and develop normally. In some children who do not grow well, the pituitary gland may not make growth hormone (GH) and/or other hormones. The gland may be very small or even absent. These children would need to be treated with the hormones that are missing. Occasionally, the small pituitary gland may be associated with abnormalities affecting the eyes and the brain; this is called septo-optic dysplasia or SOD.

We now know that the way your body changes and grows depends on the genetic material (called DNA, which is made up of thousands of genes) that you inherit from your parents. Changes

within genes are the reason why we are all very different to each other. Normally, you have two copies of a gene, one that comes to you from your father and one that comes to you from your mother. Sometimes a change within a gene that you get from your parents can lead to a medical condition. So far, we only know some of the genes that are important for pituitary development.

We have noticed that children who do not make enough GH are more likely to have abnormal behaviour and to be slow with their development. We know that treatment with GH makes you grow taller and it has been suggested that treatment may also improve behaviour and sleep.

What is the purpose of the study?

In this study, we aim to study the genes controlling the development of the brain and pituitary gland. We have recently found that changes in 3 genes associated with a small pituitary gland and a lack of growth hormone (GH) in some of our patients. We now want to look at children with these conditions in more detail and to examine their DNA for abnormalities in these and other genes that are important for the normal development of the brain. We hope that this will help us to understand why children suffer from these particular conditions.

We hope that this research will help us gain better understanding of the strengths and weaknesses that children with GH deficiency may have and make us think of the ways that parents and professionals may help if specific difficulties arise.

How is the study being done? (Summary attached)

All of the tests outlined below will be arranged around your routine visits to the hospital to minimise any disruption the study may have on your child and your family.

1. Blood tests

The blood test/cannula insertion will be performed by a member of the Paediatric Endocrinology team at Great Ormond Street Children's Hospital during one of your routine visits to the hospital. We need to take a small blood sample (2 teaspoonfuls) from your child for the genetic tests. To minimise discomfort, we will perform the blood test using local anaesthetic cream.

Only a single sample of blood is required for the genetic study. If a change (mutation) is found within one of the genes that are important for pituitary development, then further blood samples may be required from your child and the rest of your family. However, the requirement for these samples will be discussed in detail with you and your family.

All children with growth hormone deficiency routinely have hourly measurements of a stress hormone called cortisol for a 24hr period. In addition to the routine measurements of cortisol, we will take one extra blood sample every hour (1.5ml-1 teaspoon) to measure the hormone melatonin if your child weighs more than 10kg. Melatonin is very important in determining when we fall asleep and if it is not produced correctly children may have problems sleeping.

2. Questionnaires

We will ask you some questions about your child (including date of birth, medical problems, birth history) the first time we see you. This will take about half an hour. This will help us to understand all of the benefits of GH treatment. We will ask you to keep a diary recording your child's sleep pattern for a 2week period whilst they wear a small comfortable watch which records how well they sleep.

3. Developmental Assessment

We are interested to see how your child is learning about their world, uses language, interacts with other people, and responds in social situations like story-time. To understand this we would assess your child's language, cognitive and social development using a selection of tests. These will be carried out by a team with a special interest in the development of children with and without visual impairment. For this we will arrange for you to come with your child to the Developmental Clinic on the same day as your endocrine clinic visit or we can arrange a home, nursery or school visit if this is more convenient for you. All the tests that we would like to use in this research are game-like and designed especially for children so we anticipate that most children will have fun taking part. However, if your child gets tired we will take a break or stop altogether. We will ask your child's school teacher how they are doing in school (if you and your child are happy for us to contact them).This will provide a clear picture outlining the developmental progress and any developmental delay/behavioural problems present in your child.

4. MRI Scan

We also want to add 10mins extra pictures onto your child's routine magnetic resonance imaging (MRI) scan to get a better picture of the overall brain structure and the volumes of the different areas of the brain that may be important in GH deficiency. MRI does not involve ionising radiation, unlike computed tomography (CT) or x-rays, and is therefore a good and safe imaging investigation to use in children. MRI is a well established investigation at Great Ormond Street Hospital for Children and is performed under sedation or anaesthesia on several children every day.

It is very important to keep very still throughout any MRI scan as otherwise, the pictures become blurred and of no use, so it is

routine clinical practice to use sedation or general anaesthesia in young children during a scan. Sometimes the scans can be done in babies if they are asleep and wrapped so that they do not move. Usually however sedation or general anaesthesia is used as the scanner is noisy, and the scan can last up to 40 minutes. Your doctor will talk to you about whether your child will be sedated or given general anaesthetic for his/her scan and will get separate consent from you for this. This is routine clinical practice and is not a consequence of our project. The choice between non-sedation, sedation, or general anaesthesia will not be altered whether or not your child participates in the project.

During the scan, we will need to inject a small volume of contrast fluid (dye) intravenously. This too is routine practice, independent of project participation, and is regarded as a very safe procedure. Our project involves taking extra pictures while your child has his/her MRI scan. This means that the scan will take 10minutes longer than a routine scan.

What are the risks and discomfort?

No risk to the child can be foreseen. There is discomfort from a single needle prick for blood testing, but this will be minimised by using local anaesthetic cream. MRI uses electromagnetic pulses to produce images of organs and tissues. It is used routinely in all age groups, and there are no identified side effects. Compared to a normal scan, the scan time in the project will be longer, and we will apply more electromagnetic pulses. However, we will always operate within the limitations that are set for clinical use of MRI. If your child is sedated or under general anaesthetic, this will not have any effect on their level of discomfort. If they are awake, then it will mean they may have to keep still for a little longer.

What are the potential benefits?

This study may help us to understand why your child has developed their problems. Additionally, we may be able to make the diagnosis earlier in future children who may have the condition, and where one child in the family already has the disorder, we may be able to make a diagnosis before birth in future pregnancies. An improved understanding of these conditions will help us to find the best ways of making the diagnosis i.e. using the best tests available so that the diagnosis can be made with minimal discomfort to the child. Some of these genes are associated with a changing (evolving) picture, with more hormonal abnormalities appearing with time. If that is the case, then we will be in a better position to anticipate these abnormalities once we have the genetic information and hence we will be able to diagnose the problem more rapidly. We hope that a better understanding of the role GH has on behaviour and brain structure will help us to guide the use of GH in children with these problems in the future. Additionally, these studies will help us to understand why some children with GH deficiency develop sleep problems.

Who will get to see this information about my child?

Only the researchers and a representative of the Research Ethics Committee will have access to the data collected during the study. We will tell people what we have learned in the study in reports and publications, but nobody will learn anything personal about your child, or any other child, by reading these reports or publications. The use of some types of personal information is safeguarded by the Data Protection Act 1998 (DPA). The DPA places an obligation on those who record or use personal information, but also gives rights to people about whom information is held. If you have any questions about data protection, contact the Data Protection officer via the switchboard on 020 7405 9200 extension 5217.

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This research is covered by a compensation scheme, which may apply in the event of any significant harm resulting to your child from involvement in the study. Under this scheme, it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

If taking part in this study means that you have to make additional trips to hospital we will reimburse your travel costs.

Do I have to take part in this study?

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Who do I speak to if problems arise?

Please contact Dr Emma Webb or Dr Michelle O'Reilly directly with any problems relating to the study. If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researchers. If the problems are not resolved, or you wish to comment in any other way, please contact the Principal Investigator, by post at the Institute of Child Health, 30 Guilford Street, London WC1N

1EH, or if urgent, by telephone on 0207405 9200, and the switchboard will put you in contact with him.

Thank you for your time and for considering taking part in this study!

STUDY SCHEDULE

Developmental assessment (3hrs):

- Age appropriate developmental assessment to assess verbal and cognitive development, attention and behaviour
- Parental questionnaire about behaviour and development

Sleep assessment:

- Actiwatch-worn for 2weeks
- 2week- sleep diary
- 24hr Cortisol, Melatonin, blood sugar + temperature (hourly)

Questionnaire (30min):

- Basic information

Imaging (extra 10min):

- Volumetric MRI

Genetics (1r
1teaspoon bl
sample

Key: Text in black: Routine investigation
Text in blue: Additional investigation

B. Confidential Data Sheet for GH/Development Study (page 1 to be held separately from main data form)

Study Number			

Patient DNA Sample Number	

	D	D	M	M	Y	Y
Date of Birth						

Forename	
Surname	
Hospital Consultant	
Hospital Number	
Postcode	
Home phone no	
Mobile phone no	
Right/left handed	

1. Study Number			

	M	F
2. Sex		

	Yrs	Mths
3. Age at baseline		
4. Age at 6mths into study		
5. Age at 12mths into study		

	D	D	M	M	Y	Y	Y	Y
6. Date of 1st study visit								

7. First language

8. Ethnicity(mark one box)

White British		Asian Pakastani		Black African		Chinese	
White other		Asian Indian		Black Caribbean		Any other ethnic background	
Asian Bangladeshi		Asian other		Black other		Specify:	

9. Study Group (mark one box)

VI		IGHD		SOD	
ONH		MPHD			

10. Antenatal History	Y	N
11. Maternal Smoking		
12. Maternal Alcohol		
13. Consanguinity		

14. Family History:

15. Mothers Highest Educational level:

16. Mums occupation _____

17. Dads Occupation: _____

	Yrs		Mths	
18. Maternal Age (years) at delivery				

19. Delivery _____

20. Gestation at birth			wks			Days

	Y	N
21. IUGR		
22. Breast fed		
23. Exclusive breast feeding		

24. Length Breast Fed	Exclusive:	Mixed:

25. Birth length | | | Cms

26. Birth weight | | | Cms

27. Birth head circumference | | | Cms

28. Neonatal complications _____

MEASUREMENTS AT PRESENTATION, 6 and 12mths

29. Weight (kg) Baseline ___ ___ ___ . ___ ___ Kg

6mth ___ ___ ___ ___ ___ Kg

12mth ___ ___ ___ ___ ___ kg

30. Height (cm) Baseline ___ ___ ___ Cm

6mth ___ ___ ___ Cm

12mth ___ ___ ___ cm

31. Head circumference (cm) Baseline ___ ___ ___ cm

6mth ___ ___ ___ cm

12mth ___ ___ ___ cm

32. Mothers height ___ ___ ___ Cm

33. Fathers height ___ ___ ___ Cm

34. Mid-parental height ___ ___ ___ Cm

35. Final height ___ ___ ___ Cm

36. Age at which diagnosed (years) ___ ___ Yrs

	Y	N	U
37. Spontaneuous puberty			

EYE FEATURES

38. Visual Acuity right eye	
39. Visual Acuity left eye	
40. Right Optic disc	
41. Left optic disc	
42. EEG	
43. VER	
44. Other opthalmological features:	

CHILD CARE	YES	NO
45. Nanny		
46. hrs/week	Hrs	
47. Nursery attendance		
48. hrs/week	Hrs	
49. Child minder		
50. hrs/week	Hrs	

ENDOCRINE FUNCTION TESTS

51. Free Thyroxine				
52. Basal TSH				
53. TRH test peak TSH				
54. Basal prolactin				
55. TRH test peak prolactin				
56. Basal LH				
57.Peak LH on LHRH				
58. Basal FSH				
59. Peak FSH on LHRH				
60. Basal Cortisol				
61. Peak Cortisol				
62. Peak GH				

63. OGTT-baseline

0 Insulin		0 Glucose	
30 Insulin		30 Glucose	
60 Insulin		60 Glucose	
90 Insulin		90 Glucose	
120 Insulin		120 Glucose	
150 Insulin		150 Glucose	

180 Insulin		180 Glucose	

64. OGTT after 6mths on GH

0 Insulin		0 Glucose	
30 Insulin		30 Glucose	
60 Insulin		60 Glucose	
90 Insulin		90 Glucose	
120 Insulin		120 Glucose	
150 Insulin		150 Glucose	
180 Insulin		180 Glucose	

65. 24hr	**Cortisol**	**Glucose**	**Melatonin**
0100			
0200			
0300			
0400			
0500			
0600			
0700			
0800			
0900			
1000			
1100			
1200			
1300			
1400			
1500			
1600			
	Cortisol	**Glucose**	**Melatonin**

1700			
1800			
1900			
2000			
2100			
2200			
2300			
2400			
66. 2nd profile	**Cortisol**	**Glucose**	**Melatonin**
0100			
0200			
0300			
0400			
0500			
0600			
0700			
0800			
0900			
1000			
1100			
1200			
1300			
1400			
1500			
1600			
1700			
1800			
	Cortisol	**Glucose**	**Melatonin**

1900			
2000			
2100			
2200			
2300			
2400			

67. Baseline IGF-1				
68. 6mth after onset Tx IGF-1				
69. Baseline IGFBP-3				
70. 6mth after onset Tx IGFBP-3				
71. Baseline Leptin				
72. 6mth after onset Tx Leptin				
73. Baseline Ghrelin				
74. 6mth after onset Tx Ghrelin				
75. Baseline Adiponectin				
76. 6mth after onset Tx Adiponectin				

	YES	NO
77. Diabetes Insipidus		
78. Any other endocrine results		

MEDICATION	YES	Date started
79. Hydrocortisone		
80. Thyroxine		
81. GH		
82. Any change in GH dose		
83. DDAVP		
84. Sex steroids		
85. Zoladex		

Medication Dose		**DOSE**	**UNITS**
86. Hydrocortisone	am Pm		
87. Thyroxine			
88. GH			
89. Any change in GH dose			
90. DDAVP	am Pm		
91. Sex steroids			
92. Zoladex			

93. Result of neuroimaging _____

_____

_____

94.Candidate Gene to be tested | |

95. Any genetic finding? | |

	M	M	D	D	Y	Y	Y	Y
96. 1st developmental assessment								
97. 2nd developmental assessment								
98. 3rd developmental assessment								

99. Result developmental assessment 1

A _____

B _____

C _____

D _____

E _____

100. Result developmental assessment 2

A

B

C

D

E

101. Result developmental assessment 3

A

B

C

D

E

C. Consent Form

Great Ormond Street Hospital for Children NHS Trust and Institute of Child Health Research Ethics Committee

Consent Form for PARENTS OR GUARDIANS of Children Participating in Research Studies

Title: A genetic analysis of children with forebrain, eye and / or pituitary defects

NOTES FOR PARENTS OR GUARDIANS

1. Your child has been asked to take part in a research study. The person organising that study is responsible for explaining the project to you before you give consent.

2. Please ask the researcher any questions you may have about this project, before you decide whether you wish to participate.

3. If you decide, now or at any other stage, that you do not wish your child to participate in the research project, that is entirely your right, and if your child is a patient it will not in any way prejudice any present or future treatment.

4. You will be given an information sheet which describes the research project. This information sheet is for you to keep and refer to. ***Please read it carefully.***

5. If you have any complaints about the way in which this research project has been or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee, by post via The Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH or if urgent, by telephone on 020 7905 2620 and the committee administration will put you in contact with him.

CONSENT

I/We _____, being the parent(s)/guardian(s) of _____ agree that the Research Project named above has been explained to me to my/our satisfaction, and I/We give permission for our child to take part in this study. I/We have read both the notes written above and the Information Sheet provided, and understand what the research study involves.

SIGNED (Parent (s)/Guardian (s))	**PRINTED**	**DATE**
SIGNED (Researcher)	**PRINTED**	**DATE**