

# Effect of growth hormone deficiency on brain structure, motor function and cognition

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The growth hormone-insulin-like growth factor-1 axis plays a role in normal brain growth but little is known of the effect of growth hormone deficiency on brain structure. Children with isolated growth hormone deficiency (peak growth hormone  $<6.7 \mu\text{g/l}$ ) and idiopathic short stature (peak growth hormone  $>10 \mu\text{g/l}$ ) underwent cognitive assessment, diffusion tensor imaging and volumetric magnetic resonance imaging prior to commencing growth hormone treatment. Total brain, corpus callosal, hippocampal, thalamic and basal ganglia volumes were determined using Freesurfer. Fractional anisotropy (a marker of white matter structural integrity) images were aligned and tract-based spatial statistics performed. Fifteen children (mean 8.8 years of age) with isolated growth hormone deficiency [peak growth hormone  $<6.7 \mu\text{g/l}$  (mean  $3.5 \mu\text{g/l}$ )] and 14 controls (mean 8.4 years of age) with idiopathic short stature [peak growth hormone  $>10 \mu\text{g/l}$  (mean  $15 \mu\text{g/l}$ ) and normal growth rate] were recruited. Compared with controls, children with isolated growth hormone deficiency had lower Full-Scale IQ ( $P < 0.01$ ), Verbal Comprehension Index ( $P < 0.01$ ), Processing Speed Index ( $P < 0.05$ ) and Movement-Assessment Battery for Children ( $P < 0.008$ ) scores. Verbal Comprehension Index scores correlated significantly with insulin-like growth factor-1 ( $P < 0.03$ ) and insulin-like growth factor binding protein-3 ( $P < 0.02$ ) standard deviation scores in isolated growth hormone deficiency. The splenium of the corpus callosum, left globus pallidum, thalamus and hippocampus ( $P < 0.01$ ) were significantly smaller; and corticospinal tract (bilaterally;  $P < 0.045$ ,  $P < 0.05$ ) and corpus callosum ( $P < 0.05$ ) fractional anisotropy were significantly lower in the isolated growth hormone deficiency group. Basal ganglia volumes and bilateral corticospinal tract fractional anisotropy correlated significantly with Movement-Assessment Battery for Children scores, and corpus callosum fractional anisotropy with Full-Scale IQ and Processing Speed Index. In patients with isolated growth hormone deficiency, white matter abnormalities in the corpus callosum and corticospinal tract, and reduced thalamic and globus pallidum volumes relate to deficits in cognitive function and motor performance. Follow-up studies that investigate the course of the structural and cognitive deficits on growth hormone treatment are now required to confirm that growth hormone deficiency impacts significantly on brain structure, cognitive function and motor performance.

**Keywords:** hormones and cognition; diffusion tensor imaging; corticospinal pathways

**Abbreviation:** IGF = insulin-like growth factor; IGFBP-3 = IGF-binding protein-3; IGHD = isolated growth hormone deficiency

## Introduction

There is a large amount of literature regarding the role of the growth hormone insulin-like growth factor-1 (IGF-1) signalling pathway in neurogenesis and brain development. There are minimal data; however, addressing the effect of a reduction in growth hormone and IGF-1 on brain structure (Annenkov, 2009).

Growth hormone is produced in the pituitary gland and, to a lesser degree, by other tissues in the CNS (Donahue *et al.*, 2006), and is also transported across the blood–brain barrier (Burman *et al.*, 1996). Growth hormone binding sites are present on neurons, astrocytes, oligodendrocytes and microglia, with IGF-1 promoting neuron growth, dendritic arborization and synaptogenesis (Bondy and Lee, 1993; Lai *et al.*, 1993). Concentrations of growth hormone receptors are highest in the choroid plexus, thalamus, hypothalamus, pituitary, putamen and hippocampus (Lai *et al.*, 1993), and IGF-1 receptors are most dense in the hippocampus, amygdala, caudate nucleus, prefrontal and parahippocampal cortex (Araujo *et al.* 1989; Bondy and Lee, 1993). Growth hormone receptor expression is 2- to 4-fold higher in the hippocampus than elsewhere in the brain (Lai *et al.*, 1993).

Several of the structures with a high density of growth hormone and IGF-1 receptors are known to play a role in aspects of cognitive functioning including learning and memory (Nyberg and Burman, 1996). Studies in mice 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 (Markowska *et al.*, 1998). In humans, *IGF-1* gene deletion is associated with intrauterine growth retardation, microcephaly, significant cognitive impairment (global developmental delay) and post-natal growth failure (Woods *et al.*, 1996). Individuals with growth hormone receptor mutations, which lead to reduced production of IGF-1, have varying cognitive phenotypes (Laron and Klinger 1994; Kranzler *et al.*, 1998). Foetal cord IGF-1 and IGF-binding protein-3 (IGFBP-3) concentrations are related to head circumference at birth (a correlate of total brain volume; Geary *et al.*, 2003) and serum IGF-1 concentrations correlate positively with verbal intelligence in childhood (Gunnell *et al.*, 2005). Amongst elderly subjects, those with higher concentrations of IGF-1 perform better on tests of cognitive function and have lower rates of cognitive decline, suggesting that the growth hormone-IGF-1 axis affects cognitive performance throughout life (Aleman *et al.*, 1999).

In view of the above, it is unsurprising that several neuropsychological studies have documented impairments in cognitive functioning (memory and attention) in adults with childhood- or adult-onset growth hormone deficiencies (Falleti *et al.*, 2006), which improve when growth hormone is replaced (Deijen *et al.*, 1998). In the majority of studies assessing the impact of growth hormone on neurodevelopment, children with growth hormone deficiency have been reported to have a normal IQ. However,

despite having IQs within the normal range, a high percentage of children with growth hormone deficiency have difficulties with education (Frisch *et al.*, 1990) particularly in the domains of reading, spelling and arithmetic (Stabler *et al.*, 1994). Motor skills performance improves in children with Prader–Willi syndrome following treatment with growth hormone; however, motor skills have not previously been assessed in children with growth hormone deficiency (Carrel *et al.*, 2010).

The exact nature of the cognitive deficits associated with growth hormone deficiency remains unclear as the findings from previous studies are difficult to interpret due to: (i) the definition of growth hormone deficiency used (e.g. some individuals will be misclassified if growth hormone deficiency is defined as a peak response of  $<10\mu\text{g/l}$ , and normal as a peak response of  $>10\mu\text{g/l}$ ); (ii) the inclusion of heterogeneous patient groups with varying aetiologies (e.g. post-radiation or congenital growth hormone deficiency and multiple pituitary hormone deficiency); (iii) variation in the duration of the deficiency; (iv) the wide age-range of patients studied; and (v) the lack of uniformity in the cognitive tests used to measure performance (Falleti *et al.*, 2006).

MRI has proven valuable in defining gross structural changes in the brain in growth hormone deficiency (Tillmann *et al.*, 2000). More recently, advances in MRI, including the development of diffusion tensor imaging, have allowed examination of white matter structure in the brain. Diffusion tensor imaging provides quantitative indices of white matter microstructure such as mean diffusivity (a measure of the overall degree of mobility of water in brain tissue) and fractional anisotropy (a measure of the degree of directionality of water diffusion, which is affected by axonal calibre, fibre density and degree of myelination). A low fractional anisotropy (high mean diffusivity) reflects lower structural integrity of the white matter (Beaulieu, 2002). Conventional  $T_1$ -weighted brain MRI can also be segmented to provide volumetric analysis of cortical and sub-cortical structures (e.g. hippocampus, globus pallidum and thalamus) using techniques such as Freesurfer (Fischl *et al.*, 2002). These volume measurements can then be entered into statistical analyses to compare structure volumes between groups or to correlate a neural volume with a parameter of interest such as IGF-1 standard deviation score.

To examine the effect of growth hormone deficiency on brain structure, we investigated a cohort of children with isolated growth hormone deficiency (IGHD) using cognitive and motor assessment in conjunction with volumetric analysis and diffusion tensor imaging and compared the findings with those of a cohort of children with idiopathic short stature. As children with short stature have been shown to have an increased prevalence of behavioural problems when compared with control subjects with normal stature, we chose children with idiopathic short stature as our 'controls', thereby controlling for the effect of stature and isolating the effect of growth hormone (Voss *et al.*, 1991).

A comprehensive battery of cognitive tests, including assessments of IQ, memory and attention was used, as the studies outlined above have previously identified these domains as being vulnerable in growth hormone deficiency. Behavioural and motor skills assessments were also performed. We performed a voxel-based analysis using tract-based spatial statistics (Smith *et al.*, 2006) to examine whether there were differences in white matter structure between the two groups and used the results of our cognitive assessment to focus our volumetric analyses on relevant brain structures.

## Patients and methods

### Patients

All patients with IGHD or idiopathic short stature (aged 5–11 years) attending the paediatric endocrine clinic at Great Ormond Street Hospital for Children were recruited prospectively between 2007 and 2009. All assessments were performed prior to the study subjects starting treatment with growth hormone. We restricted the study to children over 5 years of age as myelination changes rapidly during early childhood (Schmithorst *et al.*, 2005). As children with short stature have been shown to have an increased prevalence of behavioural problems when compared to normal stature controls, we chose children with idiopathic short stature as our ‘controls’, thereby controlling for the effect of stature and isolating the effect of growth hormone (Voss *et al.*, 1991). Children with intrauterine growth restriction (birth weight <10th centile for gestational age), chronic illness, bone age >10 years, or in whom puberty had commenced (boys testes volume >3 ml, girls breast stage of  $\geq 2$  and patients with pubic hair stage  $\geq 2$ ) were excluded from entry into the study. The study was approved by the Joint Research Ethics committee of Great Ormond Street Hospital/Institute of Child Health and all parents and children gave written informed consent/assent as appropriate.

At the first presentation, ethnicity, antenatal and delivery history, birth weight, handedness, maternal highest educational level and paternal employment were recorded. Socioeconomic status was calculated using the Standard Occupational Classification (OPCS, 1991). Maternal highest educational level and socioeconomic status were used as a proxy for parental IQ. Standing height was measured to the nearest millimetre with a stadiometer and height velocity calculated at baseline (over  $\geq 6$  months) to determine annual growth rate (serial measurements performed by one trained auxologist).

### Definition of clinical phenotype and determination of growth hormone status

IGHD was diagnosed in children with a height and growth velocity  $\leq 2$  standard deviation scores below the mean for age, a peak growth hormone <6.7  $\mu\text{g/l}$  on two tests of growth hormone release (glucagon and clonidine), or on one stimulation test in association with a pathologically low IGF-1 concentration for age and sex (below  $-2$  standard deviation scores). A cut-off of <6.7  $\mu\text{g/l}$  was chosen to differentiate clearly between the study groups (IGHD and idiopathic short stature). Locally, in conjunction with our chemical pathology laboratory a ‘normal cut-off’ (<6.7  $\mu\text{g/l}$ ) has been established for the diagnosis of growth hormone deficiency. Bone age delay was also used to support the diagnosis of growth hormone deficiency. Idiopathic short stature

was diagnosed in children with a height  $\leq 2$  standard deviation scores 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  $+2$  standard deviation scores; methods outlined below), and a normal peak growth hormone in response to glucagon stimulation (>10  $\mu\text{g/l}$ ). A single paediatric endocrinologist (M.T.D.) performed pubertal staging (Tanner method) on all children.

All children diagnosed with IGHD underwent a 24 h glucose and cortisol profile (two-hourly blood samples) to exclude adrenocorticotrophic hormone deficiency and hypoglycaemia (Mehta *et al.*, 2005). Mean cortisol of >145 nmol/l over a 24 h period and/or a morning peak cortisol of >175 nmol/l was defined as normal (Mehta *et al.*, 2005). Thyroid function was performed on all children on at least two occasions.

### IGF-1 and IGFBP-3 assays

IGF-1 and IGFBP-3 were measured in duplicate for all study subjects using the Immulite® 2500 solid-phase, enzyme-labelled chemiluminescent immunometric assay. The within-assay coefficients of variation for IGF-1 were 3.9 and 3.0% at 77 and 689 mg/l, respectively. The between-assay coefficients of variation 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 coefficients of variation for IGFBP-3 were 4.4 and 4.6% at 0.91 and 4.82 mg/l, respectively. The between-assay coefficients of variation 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 obtained from Immulite and IGF-1 and IGFBP-3 standard deviation scores and were calculated using the least mean squares method (Cole, 1990).

### Growth hormone assay

Serum growth hormone was measured in duplicate using the Immulite® 2500 solid-phase, two-site chemiluminescent immunometric assay. The within-assay coefficients of variation for growth hormone were 3.5 and 2.9% at 2.6 and 7.9  $\mu\text{g/l}$ , respectively. The between-assay coefficients of variation for growth hormone were 6.5 and 4.2% at 2.6 and 7.9  $\mu\text{g/l}$ , and the detection limit of the assay was 0.1  $\mu\text{g/l}$ .

### DNA collection and analysis

DNA samples were collected as two 10 ml Ethylenediaminetetraacetic acid (EDTA) blood samples from all individuals with IGHD for genetic studies. DNA was sequenced for mutations known to cause IGHD (*GH1*, *HESX1*, *SOX3*) (Kelberman *et al.*, 2009).

### Behavioural assessment

Behavioural assessment was assessed by parental report using the Achenbach child behaviour checklist (Achenbach and Rescorla, 2001).

### Cognitive assessment

One assessor blinded to participant group performed all cognitive (intellectual and neuromotor) assessments. Neuromotor function was assessed with the Movement-Assessment Battery for Children 2nd edition (Henderson and Sugden, 1992). Intellectual functioning was assessed using the full Wechsler Intelligence Scales for Children 4th edition (WISC-IV). Full-Scale IQ, Verbal Comprehension Index, Perceptual Reasoning Index, Working Memory and Processing Speed Indices were calculated (population mean = 100, SD = 15) (Wechsler,

2003). Younger participants (one child in the IGHD group and two children in the short stature control group) were assessed using the Wechsler Preschool and Primary Scale of Intelligence 3rd 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 defined as Verbal Comprehension Index and Performance IQ as Perceptual Reasoning Index. A comprehensive battery of tests was also used to assess memory, attention and executive function. Measures of memory consisted of Memory for faces, Memory for names, Narrative memory and List memory from the NEPSY®-II (A Developmental NEuroPSYchological Assessment) battery (Korkman *et al.*, 2001), and Pattern recognition memory, Spatial working memory and Paired associate learning tests from the Cambridge neuropsychological test automated battery (CANTAB). Tests used to assess attention and executive function included the Big/little circle, Intra-dimensional extra-dimensional set shift, Rapid visual information processing tests from CANTAB (Sahakian *et al.*, 1988) and the Inhibition and word generation tests from the NEPSY®-II battery.

## MRI acquisition

MRI was performed on a Siemens Avanto 1.5 Tesla scanner (Siemens). A standard MRI of the brain and pituitary (3-mm thick image acquisition of the pituitary, in the sagittal and coronal planes using a combination of T<sub>1</sub>- and T<sub>2</sub>-weighted sequences) was acquired.

Echo-planar diffusion-weighted images were acquired for an isotropic set of 20 non-collinear directions, using a weighting factor of  $b = 1000 \text{ s mm}^{-2}$ , along with a T<sub>2</sub>-weighted ( $b = 0$ ) volume. This protocol was repeated three times in a single scan session, and the data merged together without averaging. Forty-five contiguous axial slices of width 2.5 mm were imaged, using a field of view of  $240 \times 240 \text{ mm}$  and  $96 \times 96$  voxel acquisition matrix, for a final image resolution of  $2.5 \times 2.5 \times 2.5 \text{ mm}$ . Echo time was 89 ms and repetition time was 6300 ms. In addition, a T<sub>1</sub>-weighted 3D FLASH (fast low angle shot) structural image was acquired using 176 contiguous sagittal slices, a  $256 \times 224 \text{ mm}$  field of view, a flip angle of  $15^\circ$  and  $1 \times 1 \times 1 \text{ mm}$  image resolution. Echo time in this case was 4.9 ms and repetition time was 11 ms.

One experienced neuroradiologist (W.K.C.) blinded to the clinical data reviewed all images. Posterior pituitary gland location was established. Hypoplastic anterior gland was a subjective diagnosis, defined by the visual appearance of the anterior gland on all slices (Tillmann *et al.*, 2000).

## Image analysis

Diffusion-weighted images were initially processed using the functional MRI of the brain software library (FMRIB software library; <http://www.fmriv.ox.ac.uk/fsl>). Data were inspected for movement artefacts and then corrected for eddy current induced distortions. Brain extraction and calculation of diffusion tensor fractional anisotropy and mean diffusivity maps were carried out using FMRIB software library tools. Scans were excluded from further image analysis if data quality was poor or if the acquisition was incomplete.

Basal ganglia, thalamic, hippocampus, corpus callosum and total brain volume were determined from the T<sub>1</sub>-weighted MRI using Freesurfer (Fischl *et al.*, 2002). These structures were selected based on previous studies correlating cognitive and motor skills performance with neural abnormalities (Schmithorst *et al.*, 2005; Kontis *et al.* 2009; Draganski and Bhatia 2010; Baudrexel *et al.*, 2011). No other neural volumes were extracted from the Freesurfer analysis to ensure all analyses performed were hypothesis driven. Fractional anisotropy and

mean diffusivity images were processed using tract-based spatial statistics and automated, observer-independent, voxel-by-voxel whole-brain between-group analysis performed (corrected for age) (Smith *et al.*, 2006).

Initially, every fractional anisotropy image was aligned to every other image using the  $-n$  flag. This selects the most representative study image as a target image which is then affine-aligned into MNI (Montreal Neurological Institute) 152 standard space. Other study images were then transformed into  $1 \times 1 \times 1 \text{ mm}$  MNI152 space by combining the non-linear transform to the target fractional anisotropy image with the affine transform from that target to MNI152 space. Secondly the mean of all fractional anisotropy images was created using the  $-s$  option. The mean image was subsequently thinned and thresholded at a fractional anisotropy value of 0.2 to create a white matter tract skeleton representing the centre of the tracts common to all subjects. Fractional anisotropy data projected onto these skeletons were then used in voxel-wise statistical comparisons using the threshold-free cluster enhancement option (which is fully corrected for multiple comparisons across space).

Values for corpus callosum and corticospinal tract fractional anisotropy were extracted from the tract-based spatial statistics analysis by masking the mean skeleton with the appropriate structure label from the Johns Hopkins University white matter tractography atlas (Hua *et al.*, 2008).

## Statistical analysis

Baseline characteristics of the two groups, including age, sex, socio-economic status, peak growth hormone ( $\mu\text{g/l}$ ) to provocative testing, baseline IGF-1, IGFBP-3, height, growth velocity and body mass index standard deviation scores were compared using an unpaired Student's *t*-test. Children with IGHD were further subdivided into those with and without a normally sited posterior pituitary gland to assess whether those with an ectopically sited posterior pituitary gland had more significant cognitive, motor skills and neural abnormalities. Behavioural and cognitive assessment scores were compared using analysis of covariance (ANCOVA) controlling for socio-economic status and maternal educational attainment. Partial correlations were used to assess the relationships of plasma IGF-1 and IGFBP-3 standard deviation scores to cognitive and motor skills scores, adjusted for socio-economic status and maternal educational attainment in children with IGHD.

Fractional anisotropy and mean diffusivity of the total white matter skeleton, corpus callosum and corticospinal 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. Total brain volume was compared using ANCOVA, controlling for age at scan, and sex. For all other neural volumes, total brain volume was used as an additional covariate. As several ANCOVAs were performed to assess the difference in neural volumes between the two groups the *P*-values for significance were adjusted to control for the false discovery rate (Benjamini *et al.*, 2001). Partial correlation was used to assess the relationship between neural volumes (where there was a significant difference in structure volume between the two groups) and fractional anisotropy of the corpus callosum and corticospinal tract to Full-Scale IQ, Perceptual Reasoning Index, Verbal Comprehension Index and scores on the Movement-Assessment Battery for Children and IGF-1 and IGFBP-3 standard deviation scores (controlled for age at scan, sex and total brain volume) in children with IGHD.

As children with idiopathic short stature do not have an entirely normal Growth hormone-axis (as demonstrated by their mean IGF-1 standard deviation scores, which falls below zero) partial correlation was

also performed to assess the relationship between IGF-1 and IGFBP-3 standard deviation scores and neural volumes (basal ganglia and corpus callosum) in the whole study group (IGHD and idiopathic short stature controlled for age at scan, sex and total brain volume). As multiple comparisons were performed the *P*-values for significance were adjusted to control for the false discovery rate (Benjamini *et al.*, 2001).

## Results

### Group characteristics

Fifteen children (mean age 8.8 years) with IGHD [peak growth hormone  $<6.7 \mu\text{g/l}$  (mean  $3.5 \mu\text{g/l}$ )] and 14 controls (mean age 8.4 years) with idiopathic short stature [peak growth hormone  $>10 \mu\text{g/l}$  (mean  $15 \mu\text{g/l}$ ) and normal growth rate] were recruited (three children with idiopathic short stature declined to participate). All children were right-handed, had no abnormal neurological findings and were in mainstream schooling. No genetic abnormalities were identified. Nine children with IGHD had a hypoplastic anterior pituitary gland and seven with IGHD had an ectopic posterior pituitary gland; brain MRI was otherwise normal in all subjects. Children with IGHD and an ectopically sited posterior pituitary gland had significantly lower peak growth hormone and IGF-1 concentrations ( $P < 0.05$ ,  $P < 0.05$ ). There were no significant differences in measures of cognitive function, motor skills and neural volumes between children with IGHD and a normally sited posterior pituitary gland and children with IGHD and an ectopically sited posterior pituitary gland. No subjects were hypoglycaemic (blood glucose  $<3.5 \text{ mmol/l}$ ) after a 12 h fast or had prior history or documented evidence of episodes of hypoglycaemia. No children had abnormal thyroid function tests or cortisol profiles. Two children with IGHD and one with idiopathic short stature did not tolerate the diffusion tensor imaging scan but completed all other study components. Data quality was deemed to be adequate in all subjects (visual inspection by an experienced observer, C.A.C.). Subject characteristics are summarized in Table 1.

### Behavioural assessment results

There were no significant differences on scores for the child behavioural checklist between children with IGHD and children with idiopathic short stature (IGHD mean 55.4, standard deviation (SD) 9, standard error of the mean (SEM) 2.4; idiopathic short stature mean 53.4, SD 5.4, SEM 1.4). One child with IGHD and one with idiopathic short stature had scores in the clinical range (total *t*-score  $\geq 70$ ).

### Neuromotor skills assessment results

When compared with controls, children with IGHD had significantly lower scores on the manual dexterity ( $P < 0.03$ ), balance ( $P < 0.009$ ) and total scores ( $P < 0.008$ ) of the Movement-Assessment Battery for Children test (Table 1).

## Cognitive assessment results

When compared with controls, children with IGHD had significantly lower Full-Scale IQ ( $P < 0.01$ ), Verbal Comprehension Index ( $P < 0.01$ ) and Processing Speed Index ( $P < 0.05$ ) scores (Table 1). Scores for the Perceptual Reasoning Index and Working memory index were also lower in children with IGHD than in children with idiopathic short stature; these differences, however, did not reach significance ( $P = 0.09$ ,  $P = 0.2$ ). There was no significant difference in performance on tests of attention and memory (NEPSY<sup>®</sup>-II and CANTAB) between children with IGHD and idiopathic short stature controls. Examination of the IQ data showed that there were two outliers (defined as *z*-score  $> +2$  or  $< -2$ ). The IQ data were reanalysed without these two outliers, and significant group differences in Full-Scale IQ ( $P < 0.01$ ) and the Verbal Comprehension Index ( $P < 0.01$ ) remained. In children with IGHD, the Verbal Comprehension Index scores correlated significantly with IGF-1 and IGFBP-3 standard deviation scores ( $r = 0.7$ ,  $P < 0.03$ ;  $r = 0.7$ ,  $P < 0.02$ ; Fig. 1) and Full-Scale IQ correlated significantly with IGFBP-3 standard deviation scores ( $r = 0.6$ ,  $P < 0.03$ ), but not IGF-1 standard deviation scores ( $r = 0.5$ ,  $P = 0.08$ ).

## Diffusion tensor imaging

The tract-based spatial statistics analysis is summarized in Fig. 2. Corpus callosum ( $P < 0.05$ ) and bilateral corticospinal tract fractional anisotropy (right  $P < 0.05$ , left  $P < 0.045$ ) were significantly lower in children with IGHD. Left corticospinal tract mean diffusivity was significantly higher ( $P < 0.03$ ) in children with IGHD (Fig. 3). IGF-1 and IGFBP-3 standard deviation scores did not correlate significantly with fractional anisotropy or mean diffusivity in children with IGHD. Results were corrected for age at scan and sex.

## Volumetric (Freesurfer) results

The splenium of the corpus callosum, right pallidum, right hippocampus and left thalamus volumes were significantly smaller in children with IGHD ( $P < 0.02$ ,  $P < 0.007$ ,  $P < 0.01$  and  $P < 0.01$ , respectively) (Table 1). IGFBP-3 standard deviation scores correlated significantly with right hippocampus volume ( $P < 0.04$ ,  $r = 0.63$ ) (Supplementary Fig. 1A). IGF-1 and IGFBP-3 standard deviation scores did not correlate significantly with other neural volumes in children with IGHD. Results were corrected for age at scan, sex and total brain volume.

## Correlations between MRI findings and motor skills scores in children with IGHD

In children with IGHD, left corticospinal tract fractional anisotropy correlated significantly with performance on the Movement-Assessment Battery for Children aiming and catching component ( $P < 0.04$ ) and with the Perceptual Reasoning Index (which has some motor components) ( $P < 0.04$ ) (Fig. 4A and B). Right corticospinal tract fractional anisotropy correlated significantly with both the aiming and catching and balance components of the

**Table 1** Subject characteristics and the difference between cognitive assessment scores and neural volumes in children with IGHD and idiopathic short stature

	Growth hormone deficiency	Idiopathic short stature	P-value
Subject characteristics			
Number	15	14	
Mean age (SD, SEM)	8.75 (1.8, 0.5)	8.36 (1.65, 0.4)	0.55
Male (%)	80 (12/15)	64 (9/14)	0.62
Birth weight (g)	3.56 (0.9, 0.2)	3.28 (0.3, 0.1)	0.3
Growth velocity (cm/year) (SD, SEM)	3.8 (1.3)	5.8 (1.4)	<b>0.006</b>
Growth velocity SDS (SD)	−2.7 (0.6)	−0.5 (0.6)	<b>0.001</b>
Height SDS (SD)	−2.9 (0.7)	−2.5 (0.5)	0.85
Body mass index SDS (SD)	−0.2 (1.1)	−0.1 (0.6)	0.8
Fasting blood glucose (mmol/l) (SD, SEM)	3.9 (0.4, 0.1)	4.1 (0.21, 0.1)	0.3
IGF-1 SDS (SD, SEM)	−2 (0.7, 0.2)	−0.5 (0.8, 0.2)	<b>0.001</b>
IGFBP-3 SDS (SD, SEM)	−1 (0.8, 0.2)	0.1 (0.7, 0.2)	<b>0.001</b>
Peak growth hormone µg/l (SD)	3.5 (2.3, 0.6)	15 (5.7, 1.9)	<b>0.001</b>
Hypoplastic anterior pituitary on MRI (%)	67 (10/15)	0 (0/14)	
Ectopic posterior pituitary on MRI (%)	53 (8/15)	0 (0/14)	
Movement-Assessment Battery for Children II (scaled scores)*			
Total score (SD, SEM)	6.9 (2.5, 0.7)	9.8 (2.6, 0.7)	<b>0.008</b>
Manual dexterity (SD, SEM)	7.2 (2.3, 0.6)	9.3 (2.6, 0.7)	<b>0.03</b>
Aiming and catching (SD, SEM)	7.7 (2.9, 0.7)	8.8 (2.8, 0.7)	0.3
Balance (SD, SEM)	7.9 (3, 0.8)	10.4 (2.4, 0.6)	<b>0.009</b>
Wechsler Intelligence Scale for Children IV*			
Full-Scale IQ (SD, SEM)	92.8 (16, 4.2)	102.6 (8.6, 2.4)	<b>0.01</b>
Perceptual Reasoning Index (SD, SEM)	98.5 (18.6, 4.8)	107.7 (11, 3)	0.09
Verbal Comprehension Index (SD, SEM)	91 (14, 3.7)	106 (10, 2.9)	<b>0.01</b>
Working Memory Index	93.6 (22, 4.1)	95.9 (13, 2.1)	0.2
Processing Speed Index (SD, SEM)	85.3 (16.6, 2.4)	95.7 (13, 1.8)	<b>0.05</b>
Neural volumes (mm <sup>3</sup> )**			
Total brain volume	1.3 (2.1)	1.4 (1)	0.25
Splenium corpus callosum (SD, SEM)	1020 (220, 43)	1300 (210, 64)	<b>0.01</b>
Left pallidum (SD, SEM)	1600 (210, 50)	1780 (210, 56)	0.04
Right pallidum (SD, SEM)	1480 (190, 51)	1700 (220, 58)	<b>0.007</b>
Left hippocampus (SD, SEM)	4103 (430, 123)	4310 (260, 72)	0.12
Right hippocampus (SD, SEM)	4083 (460, 115)	4320 (280, 115)	<b>0.01</b>
Left thalamus (SD, SEM)	7040 (800, 218)	7600 (520, 167)	<b>0.01</b>
Right thalamus (SD, SEM)	7070 (782, 209)	7430 (530, 170)	0.056
Left caudate (SD, SEM)	3470 (780, c 208)	3720 (606, 168)	0.7
Right caudate (SD, SEM)	3493 (747, 200)	3821 (581, 161)	0.3
Left putamen (SD, SEM)	5530 (772, 193)	5740 (575, 159)	0.54
Right putamen (SD, SEM)	5419 (660, 176)	5723 (570, 158)	0.2

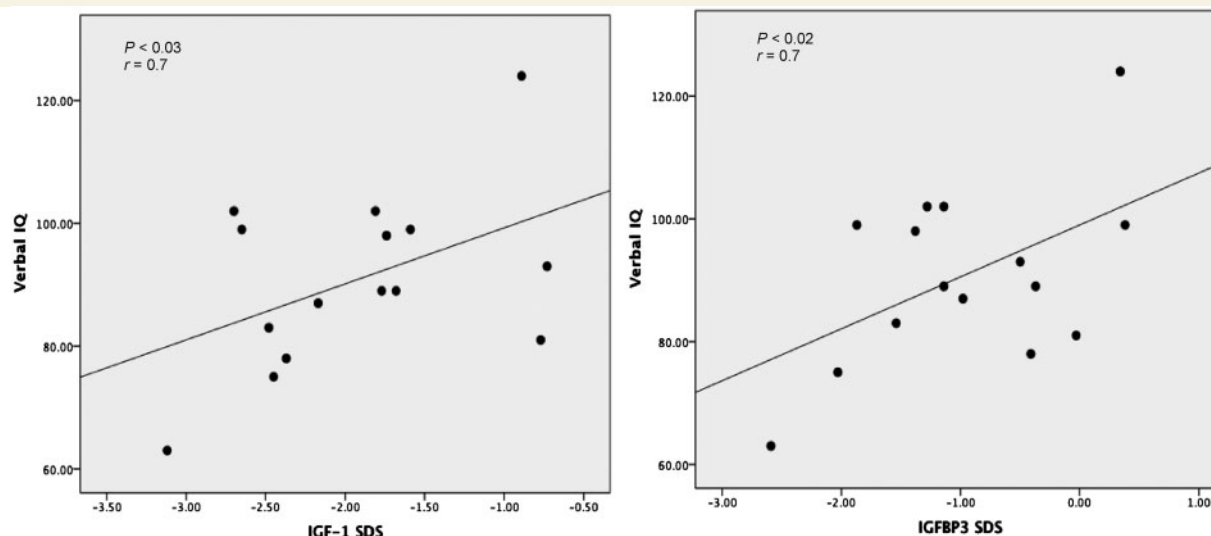
\*Results corrected for socio-economic status and maternal educational attainment.

\*\*Results corrected for age at scan, sex and total brain volume, P-values significant at  $P < 0.01$  corrected to control for the false discovery rate SDS = standard deviation score.

Movement-Assessment Battery for Children ( $P < 0.02$ ,  $P < 0.04$ ) (Fig. 4C and D). The volume of the splenium of the corpus callosum correlated significantly with the total Movement-Assessment Battery for Children score in children with IGHD ( $P < 0.05$ ) (Supplementary Fig. 1B). The volumes of the left and right pallidum and left thalamus correlated significantly with the performance on the Balance ( $P < 0.03$ ,  $P < 0.006$  and  $P < 0.05$ , respectively) (Supplementary Fig. 1C–E) and total ( $P < 0.04$ ,  $P < 0.02$  and  $P < 0.008$ , respectively) (Supplementary Fig. 1F–H) scores of the Movement-Assessment Battery for Children in children with IGHD.

## Correlations between MRI findings and cognitive assessment scores in children with IGHD

Right corticospinal tract fractional anisotropy correlated significantly with the Processing Speed Index ( $P < 0.02$ ) (Fig. 4E). Corpus callosum fractional anisotropy correlated significantly with Full-Scale IQ ( $P < 0.05$ ) and the Processing Speed Index ( $P < 0.009$ ) (Fig. 4F and G). The volume of the splenium of the corpus callosum correlated significantly with Verbal Comprehension Index ( $P < 0.05$ ).



**Figure 1** The relationship between the Verbal Comprehension Index, IGF-1 and IGFBP-3 standard deviation scores in children with IGHD. Partial correlations were used to assess the relationships of plasma IGF-1 and IGFBP-3 standard deviation scores (SDS) to the Verbal Comprehension Index, adjusted for socioeconomic status and maternal educational attainment in children with IGHD. In children with IGHD, the Verbal Comprehension Index scores correlated significantly with IGF-1 and IGFBP-3 standard deviation scores ( $r = 0.7$ ,  $P < 0.03$ ;  $r = 0.7$ ,  $P < 0.02$ ).

## Correlations between the IGF-1 axis and MRI findings in the whole study group (IGHD and idiopathic short stature)

The splenium of the corpus callosum, right pallidum and right hippocampus volumes correlated significantly with IGF-1 ( $P < 0.02$ ;  $P < 0.04$  and  $P < 0.019$ , respectively, Supplementary Fig. 2A–C) and IGFBP-3 standard deviation scores ( $P < 0.02$ ,  $P < 0.01$  and  $P < 0.016$ , respectively, Supplementary Fig. 2D–F). Whole group left pallidum and left and right thalami volumes correlated significantly with IGFBP-3 standard deviation scores ( $P < 0.04$ ,  $P < 0.05$ ,  $P < 0.002$ , respectively, Supplementary Fig. 2G–I). However, IGF-1 and IGFBP-3 standard deviation scores did not correlate significantly with fractional anisotropy or mean diffusivity.

## Discussion

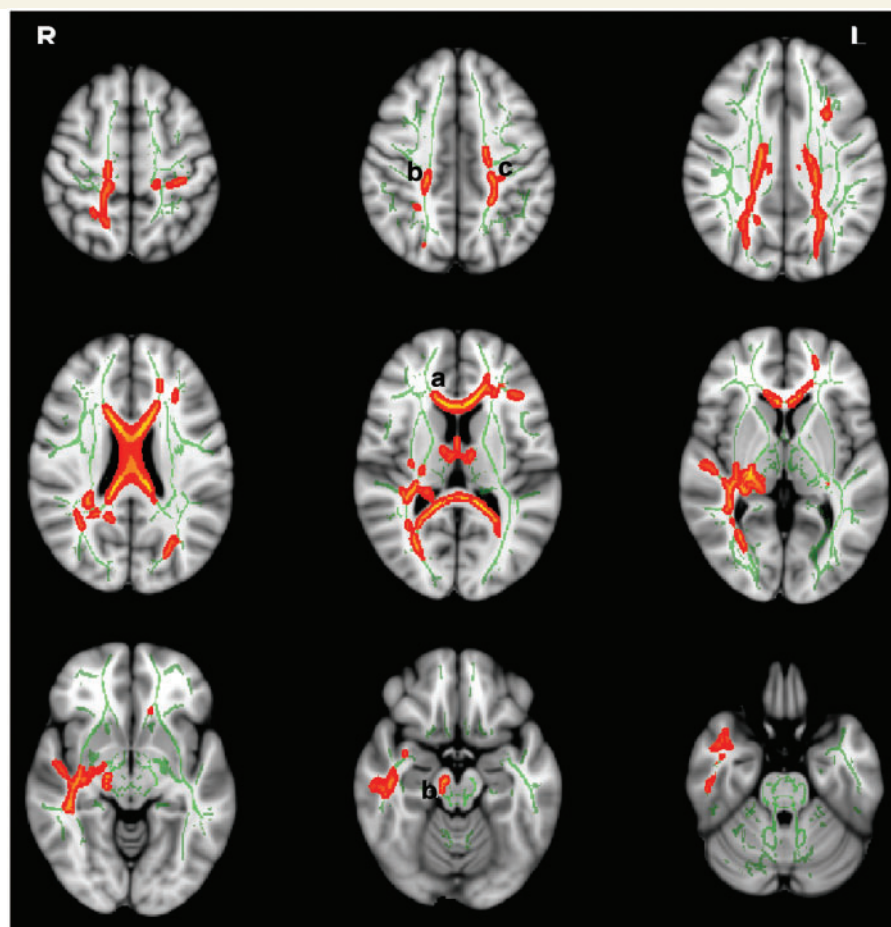
We aimed to determine the effect of growth hormone deficiency on developing brain structure and function. Reductions in white matter integrity in the corticospinal tract and corpus callosum and selective reductions in neural volumes were identified in individuals with growth hormone deficiency. The abnormalities in white matter fibre density and neural volumes correlated significantly with cognitive function and motor skills abilities, which were also significantly impaired in the IGHD cohort.

No previous studies have investigated neuromotor performance in individuals with IGHD or the relationship between the growth hormone-IGF-1 axis and corticospinal tract structure in humans. 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 reported that IGF-1 acts specifically to significantly enhance corticospinal motor neuron outgrowth, development and maturation, with interruption of IGF-1 signalling leading to defasciculation and discontinuation of corticospinal axonal growth (Ozdinler and Macklis, 2006). Disease processes that impact on the structural integrity of the corticospinal tract have also previously been shown to affect motor performance (Lindenberg *et al.*, 2010). This study provides evidence to suggest that abnormalities in the growth hormone-IGF-1 axis also affect corticospinal tract development in humans, leading to reduced fractional anisotropy in individuals with growth hormone deficiency; and that these white matter tract changes are associated with impairments in motor skills performance.

Corpus callosum fractional anisotropy and volume were also significantly reduced in children with IGHD, with the structural white matter and volume abnormalities correlating with decreases in cognitive and motor skills scores. Abnormalities in oligodendrocyte turnover have previously been found in the corpus callosum of mice with growth hormone and IGF-1 deficiency, with no previous studies having examined corpus callosum fractional anisotropy in humans with IGHD (Hua *et al.*, 2009). Corpus callosum size correlated significantly with IGF-1 and IGFBP-3 standard deviation scores, thereby suggesting that the differences in the size of the corpus callosum found in our study cohorts 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 growth hormone deficiency. Importantly children with an ectopically sited posterior pituitary gland who may be suspected to have an increased likelihood of having other brain abnormalities did not have significantly lower neural volumes than children with IGHD and normally sited posterior pituitary glands.

IGHD is a rare condition (1:4000–10 000 live births; Lindsay *et al.*, 1994) and it is therefore difficult to recruit large numbers

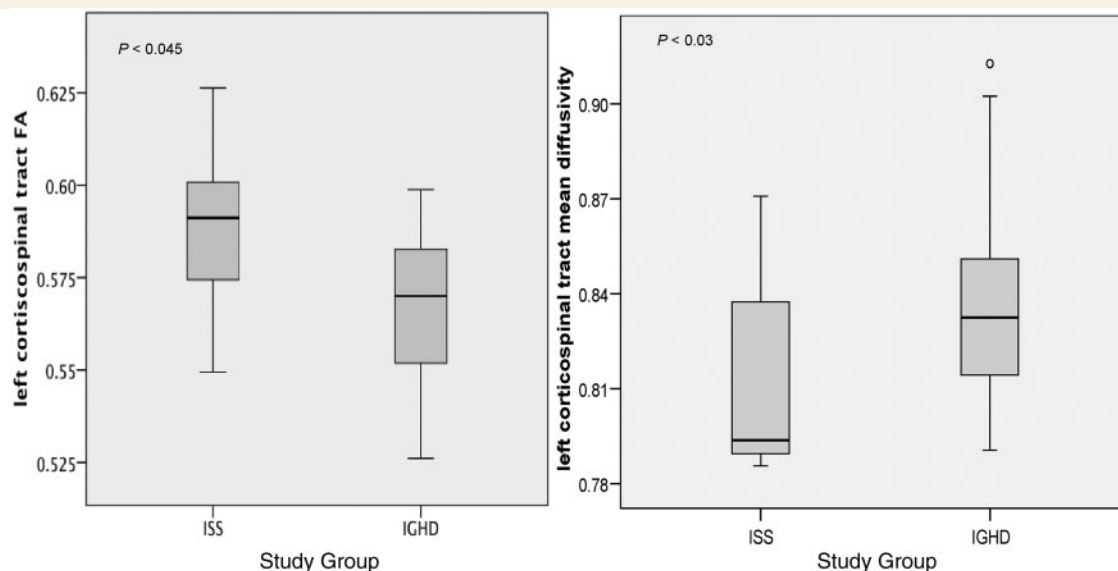


**Figure 2** The association between IGHD and fractional anisotropy (tract-based spatial statistics analysis comparing IGHD to idiopathic short stature controls). Mean fractional anisotropy skeleton is overlaid on the mean fractional anisotropy map. Regions of the mean fractional anisotropy skeleton in green represent areas where there were no significant differences in fractional anisotropy values in the infants with IGHD compared to idiopathic short stature controls. Areas in red/yellow are regions where the fractional anisotropy was significantly lower in the IGHD group and can be observed in the (a) corpus callosum, (b) right corticospinal tract and (c) left corticospinal tract. L = left; R = right.

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. Although our cognitive findings parallel those found in normal children in whom serum IGF-1 concentrations correlate with Verbal IQ (Gunnell *et al.*, 2005), we did not identify the same pattern of cognitive deficits in children with IGHD that have been previously found in adults with growth hormone deficiency (deficits in attention and memory) (Falleti *et al.*, 2006). This suggests that the effect of growth hormone deficiency on the developing brain may be different to the impact of growth hormone deficiency on the adult brain.

Based on the results of the cognitive and motor skills assessment scores we focused our volumetric MRI analysis specifically on the basal ganglia and the thalamus (Draganski and Bhatia, 2010; Baudrexel *et al.*, 2011), which play an important role in motor function, and the corpus callosum (Schmithorst *et al.*, 2005; Kontis *et al.*, 2009), 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 (Bondy and Lee 1993; Lai *et al.*, 1993). Children with growth hormone deficiency did not have global reductions in brain volumes; instead specific structures (hippocampus, globus pallidum and left thalamus) were affected, suggesting that these structures may be more vulnerable to variations in the growth hormone–IGF-1 axis. The asymmetry of the results found are likely to reflect the size of the groups studied rather than an asymmetry of growth hormone/IGF-1 effects on the brain hemispheres. Interestingly, the pattern of neural volume changes we identified was not limited to areas with a high density of growth hormone and IGF-1 receptor expression (thalamus, putamen, hippocampus, caudate) (Araujo *et al.*, 1989; Bondy and Lee 1993; Lai *et al.*, 1993). As not all structures with a high concentration of growth hormone and IGF-1 receptors were affected it may be that the effects of variations in the growth hormone–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 (Araujo *et al.*, 1989), and to impact on



**Figure 3** Difference in left corticospinal tract fractional anisotropy and mean diffusivity between children with IGHD and idiopathic short stature (ISS). Fractional anisotropy (FA) and mean diffusivity (MD;  $\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ ) of the corticospinal 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. Left corticospinal tract fractional anisotropy was significantly lower ( $P < 0.045$ ) and mean diffusivity significantly higher ( $P < 0.03$ ) in children with IGHD.

N-methyl-D-aspartate (NMDA)-R2a and R2b receptor density (Sonntag *et al.*, 2000). Normal CNS functioning is dependent on glutamate signalling through the NMDA receptor, with studies in which normal NMDA receptor function is disrupted by pharmacologic or genetic means highlighting its critical role in motor co-ordination (Kadotani *et al.*, 1996). Another possibility is that these findings are secondary to the selective neuronal vulnerability of these brain regions to the underlying disease process (variations in IGF-1 and IGFBP-3 concentrations) (Wang and Michaelis, 2010). In animal models of growth hormone deficiency a significant reduction in glucose metabolism (not availability) throughout the brain has been identified, with levels of glucose utilization being significantly reduced in the thalamus and hippocampus (Lynch *et al.* 2001; Sonntag *et al.*, 2006). Selective vulnerability of neurons within these regions to local reductions in glucose metabolism may be a further mechanism by which they are affected.

It is unlikely that the differences found are secondary to neonatal hypoglycaemia (no children had a history, were symptomatic or displayed evidence of hypoglycaemia), although children with IGHD are at risk of hypoglycaemia, which in itself can be associated with cognitive and neuroradiological abnormalities (Lucas *et al.* 1988a; Kodl *et al.* 2008a). The later age at presentation in our cohort also reduces the likelihood of neonatal hypoglycaemia. Premature infants with recurrent neonatal hypoglycaemia have a cognitive profile that is reminiscent of that of children with IGHD, with reduced IQ and motor scores (Lucas *et al.* 1988b). However, studies in adults with diabetes mellitus with recurrent hypoglycaemia (and hyperglycaemia) have identified different regions of the brain (posterior corona radiata and optic radiation) as having reduced fractional anisotropy compared with those described in the current study (Kodl *et al.*, 2008).

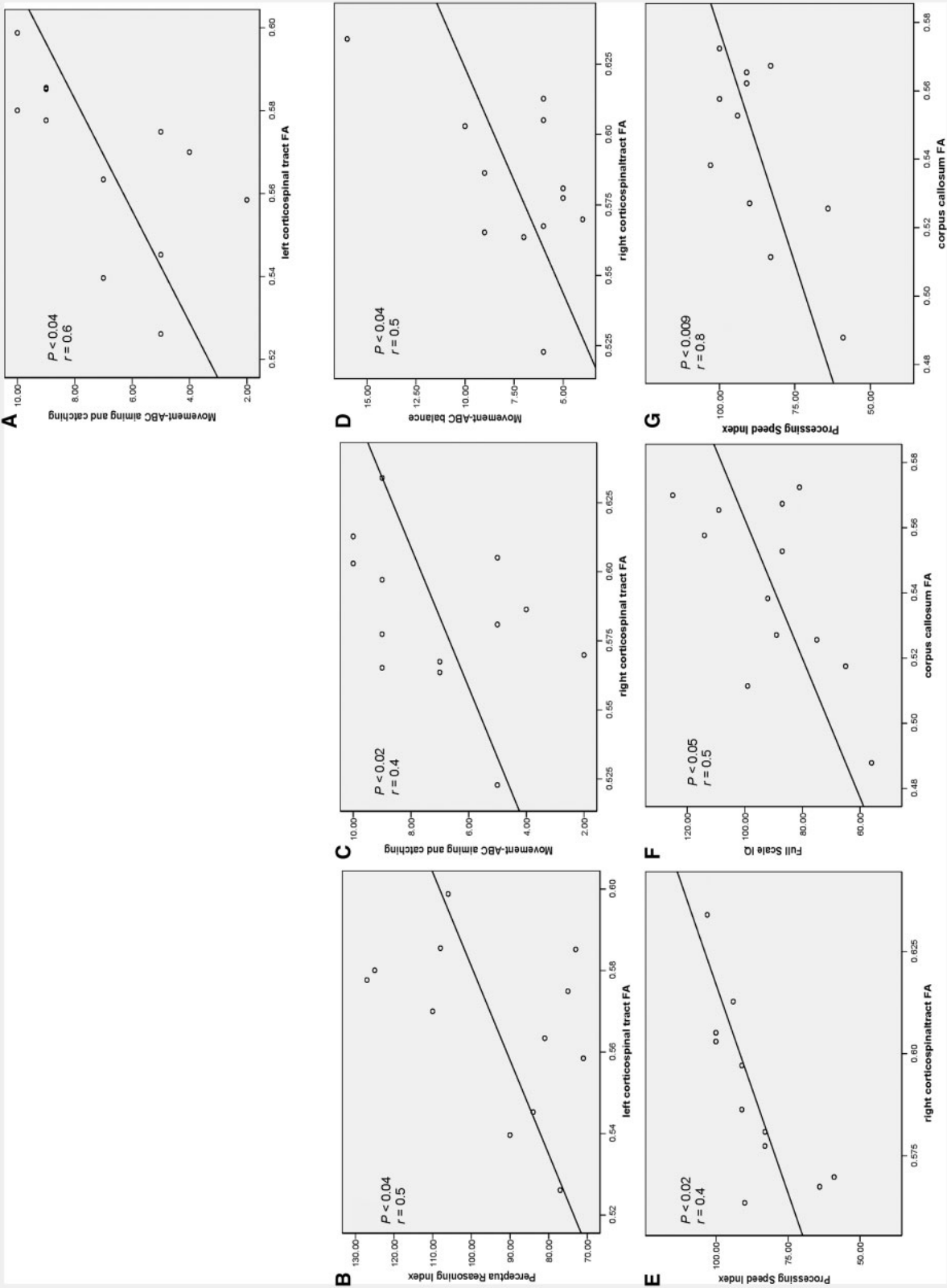
In common with all imaging studies, spatial resolution was limited by current medical imaging technology; currently we are unable to examine the cellular-level mechanisms underlying the differences we observed. Nevertheless, changes to the ensemble properties of white matter microstructure, and to the volumes of neural structures, are proven characteristics of the normal development process, and disruptions or alterations of these changes due to differences in the IGF-1 axis are therefore very substantive findings.

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

This study provides new quantitative evidence to suggest that IGF-1 and IGFBP-3 are important contributors to structural brain development affecting the volume of brain sub-structures and mediating the magnitude and coherence of water diffusion, likely reflecting underlying myelin and/or axonal density. These structural brain differences alter the connectivity of the brain leading to specific deficits in both cognition and motor function.

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**Figure 4** Correlations between cognitive function tests, corticospinal tract and corpus callosum fractional anisotropy in children with IGHD. Partial correlations were used to assess the relationships between scaled scores on the Movement-Assessment Battery for Children (ABC) test (mean score 10, SD 3), corticospinal tract fractional anisotropy (FA) and corpus callosum fractional anisotropy (controlled for age at scan and sex) in children with IGHD. In children with this condition, left corticospinal tract fractional anisotropy correlated significantly with performance on the aiming and catching component of the Movement-Assessment Battery for Children (A) ( $P < 0.04$ ) and with the Perceptual Reasoning Index (B) ( $P < 0.03$ ) and right corticospinal tract fractional anisotropy correlated significantly with the Aiming and Catching (C) and Balance components of the Movement-Assessment Battery for Children (D) ( $P < 0.02$ ,  $P < 0.04$ ) and the Processing Speed Index (E) ( $P < 0.02$ ). Corpus callosum fractional anisotropy correlated significantly with Full-Scale IQ (F) ( $P < 0.05$ ) and the Processing Speed Index (G) ( $P < 0.009$ ). Average mean diffusivity is expressed in units of  $\text{mm}^2 \text{ s}^{-1} \times 10^{-3}$ ; fractional anisotropy is a dimensionless index.

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## Supplementary material

Supplementary material is available at *Brain* online.

## References

- Achenbach TM, Rescorla LA. Manual for the ASEBA school-age forms and profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth, and Families; 2001.
- Aleman A, Verhaar HJ, de Haan EH, De Vries WR, Samson MM, Drent ML, et al. Insulin-like growth factor-I and cognitive function in healthy older men. *J Clin Endocrinol Metab* 1999; 84: 471–5.
- Annenkov A. The insulin-like growth factor (IGF) receptor type 1 (IGF1R) as an essential component of the signalling network regulating neurogenesis. *Mol Neurobiol* 2009; 40: 195–215.
- Araujo DM, Lapchak PA, Collier B, Chabot JG, Quirion R. Insulin-like growth factor-1 (somatomedin-C) receptors in the rat brain: distribution and interaction with the hippocampal cholinergic system. *Brain Res* 1989; 484: 130–8.
- Baudrexel S, Witte T, Seifried C, et al. Resting state fMRI reveals increased subthalamic nucleus-motor cortex connectivity in Parkinson's disease. *Neuroimage* 2011; 55: 1728–38.
- Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed* 2002; 15: 435–55.
- Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behaviour genetics research. *Behav Brain Res* 2001; 125: 279–84.
- Bondy CA, Lee WH. Patterns of insulin-like growth factor and IGF receptor gene expression in the brain. Functional implications. *Ann NY Acad Sci* 1993; 692: 33–43.
- Burman P, Hetta J, Wide L, Mansson JE, Ekman R, Karlsson FA. Growth hormone treatment affects brain neurotransmitters and thyroxine [see comment]. *Clin Endocrinol* 1996; 44: 319–24.
- Carrel AL, Myers SE, Whitman BY, Eickhoff J, Allen DB. Long-term growth hormone therapy changes the natural history of body composition and motor function in children with prader-will syndrome. *J Clin Endocrinol Metab* 2010; 95: 1131–6.
- Cole TJ. The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 1990; 44: 45–60.
- Deijen JB, de Boer H, van der Veen EA. Cognitive changes during growth hormone replacement in adult men. *Psychoneuroendocrinology* 1998; 23: 45–55.
- Donahue CP, Kosik KS, Shors TJ. Growth hormone is produced within the hippocampus where it responds to age, sex, and stress. *Proc Natl Acad Sci USA* 2006; 103: 6031–6.
- Draganski B, Bhatia KP. Brain structure in movement disorders: a neuroimaging perspective. *Curr Opin Neurol* 2010; 23: 413–9.
- Falletti MG, Maruff P, Burman P, Harris A. The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: a meta-analysis of the current literature. *Psychoneuroendocrinology* 2006; 31: 681–91.
- Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002; 33: 341–55.
- Frisch H, Hausler G, Lindenbauer S, Singer S. Psychological aspects in children and adolescents with hypopituitarism. *Acta Paediatr Scand* 1990; 79: 644–51.
- Geary MP, Pringle PJ, Rodeck CH, Kingdom JC, Hindmarsh PC. Sexual dimorphism in the growth hormone and insulin-like growth factor axis at birth. *J Clin Endocrinol Metab* 2003; 88: 3708–14.
- Gunnell D, Miller LL, Rogers I, Holly JM. Association of insulin-like growth factor I and insulin-like growth factor-binding protein-3 with intelligence quotient among 8- to 9-year-old children in the Avon Longitudinal Study of Parents and Children. *Pediatrics* 2005; 116: e681–6.
- Henderson SE, Sugden DA. Movement assessment battery for children: manual. London: The Psychological Corporation; 1992.
- Hua K, Forbes ME, Lichtenwalner RJ, Sonntag WE, Riddle DR. Adult-onset deficiency in growth hormone and insulin-like growth factor-I alters oligodendrocyte turnover in the corpus callosum. *Glia* 2009; 57: 1062–71.
- Hua K, Zhang J, Wakana S, Jiang H, Li X, Reich DS, et al. Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. *Neuroimage* 2008; 39: 336–47.
- Kadotani H, Hirano T, Masugi M, Nakamura K, Nakao K, Katsuki M, et al. Motor discoordination results from combined gene disruption of the NMDA receptor NR2A and NR2C subunits, but not from single disruption of the NR2A or NR2C subunit. *J Neurosci* 1996; 16: 7859–67.
- Kelberman D, Rizzoti K, Lovell-Badge R, Robinson IC, Dattani MT. Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev* 2009; 30: 790–829.
- Kodl CT, Franc DT, Rao JP, Anderson FS, Thomas W, Mueller BA, et al. Diffusion tensor imaging identifies deficits in white matter microstructure in subjects with type 1 diabetes that correlate with reduced neurocognitive function. *Diabetes* 2008a; 57: 3083–9.
- Kontis D, Catani M, Cuddy M, Walshe M, Nosarti C, Jones D, et al. Diffusion tensor MRI of the corpus callosum and cognitive function in adults born preterm. *Neuroreport* 2009; 20: 424–8.
- Korkman M, Kemp SL, Kirk U. Effects of age on neurocognitive measures of children ages 5 to 12: a cross-sectional study on 800 children from the United States. *Dev Neuropsychol* 2001; 20: 331–54.
- Kranzler JH, Rosenbloom AL, Martinez V, Guevara-Aguirre J. Normal intelligence with severe insulin-like growth factor I deficiency due to growth hormone receptor deficiency: a controlled study in a genetically homogeneous population. *J Clin Endocrinol Metab* 1998; 83: 1953–8.
- Lai Z, Roos P, Zhai O, et al. Age-related reduction of human growth hormone-binding sites in the human brain. *Brain Res* 1993; 621: 260–6.
- Laron Z, Klinger B. Laron syndrome: clinical features, molecular pathology and treatment. *Horm Res* 1994; 42: 198–202.
- Lindenberg R, Renga V, Zhu LL, Betzler F, Alsop D, Schlaug G. Structural integrity of corticospinal motor fibers predicts motor impairment in chronic stroke. *Neurology* 2010; 74: 280–7.
- Lindsay R, Feldkamp M, Harris D, Robertson J, Rallison M. Utah Growth Study: growth standards and the prevalence of growth hormone deficiency. *J Pediatr* 1994; 125: 29–35.
- Lucas A, Morley R, Cole TJ. Adverse neurodevelopmental outcome of moderate neonatal hypoglycaemia. *BMJ* 1988a; 297: 1304–8.
- Lucas A, Morley R, Cole TJ. Adverse neurodevelopmental outcome of moderate neonatal hypoglycaemia. *BMJ* 1988b; 297: 1304–8.
- Lynch CD, Lyons D, Khan A, Bennett SA, Sonntag WE. Insulin-like growth factor-1 selectively increases glucose utilization in brains of aged animals. *Endocrinology* 2001; 142: 506–9.
- Markowska AL, Mooney M, Sonntag WE. Insulin-like growth factor-1 ameliorates age-related behavioral deficits. *Neuroscience* 1998; 87: 559–69.
- Mehta A, Hindmarsh PC, Dattani MT. An update on the biochemical diagnosis of congenital ACTH insufficiency. *Clinical Endocrinology* 2005; 62: 307–14.
- Nyberg F, Burman P. Growth hormone and its receptors in the central nervous system—location and functional significance. *Horm Res* 1996; 45: 18–22.

- OPCS. Standard Occupational Classification. Vol. Vol. 3. London: HMSO; 1991.
- Ozdinler PH, Macklis JD. IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. *Nat Neurosci* 2006; 9: 1371–81.
- Sahakian BJ, Morris RG, Evenden JL, Heald A, Levy R, Philpot M, et al. A comparative study of visuospatial memory and learning in Alzheimer-type dementia and Parkinson's disease. *Brain* 1988; 111 (Pt 3): 695–718.
- Schmithorst VJ, Wilke M, Dardzinski BJ, Holland SK. Cognitive functions correlate with white matter architecture in a normal pediatric population: a diffusion tensor MRI study. *Hum Brain Mapp* 2005; 26: 139–47.
- Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage* 2006; 31: 1487–505.
- Sonntag WE, Bennett C, Ingram R, Donahue A, Ingraham J, Chen H, et al. Growth hormone and IGF-I modulate local cerebral glucose utilization and ATP levels in a model of adult-onset growth hormone deficiency. *Am J Physiol Endocrinol Metab* 2006; 291: E604–10.
- Sonntag WE, Bennett SA, Khan AS, Thornton PL, Xu X, Ingram RL, Brunso-Bechtold JK. Age and insulin-like growth factor-1 modulate N-methyl-D-aspartate receptor subtype expression in rats. *Brain Res Bull* 2000; 51: 331–8.
- Stabler B, Clopper RR, Siegel PT, Stoppani C, Compton PG, Underwood LE. Academic achievement and psychological adjustment in short children. The National Cooperative Growth Study. *J Dev Behav Pediatr* 1994; 15: 1–6.
- Tillmann V, Tang VW, Price DA, Hughes DG, Wright NB, Clayton PE. Magnetic resonance imaging of the hypothalamic-pituitary axis in the diagnosis of growth hormone deficiency. *J Pediatr Endocrinol Metab* 2000; 13: 1577–83.
- Voss LD, Bailey BJ, Mulligan J, Wilkin TJ, Betts PR. Short stature and school performance—the Wessex Growth Study. *Acta Paediatr Scand Suppl* 1991; 377: 29–31.
- Wang X, Michaelis EK. Selective neuronal vulnerability to oxidative stress in the brain. *Front Aging Neurosci* 2010; 2: 12.
- Wechsler D. Wechsler Preschool and Primary Scale of Intelligence-Third UK Edition. London: Psychological Corporation; 2003.
- Woods KA, Camacho-Hubner C, Savage MO, Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med* 1996; 335: 1363–7.