Genetic characterization of short stature patients with overlapping features of growth hormone insensitivity syndromes

Authors' full names and institutions: Afiya Andrews[1], Avinaash Maharaj[1], Emily Cottrell[1], Sumana Chatterjee[1], Pratik Shah[2], Louise Denvir[3], Katja Dumic[4], Artur Bossowski[5], Talat Mushtaq[6], Rade Vukovic[7], Mohamed Didi[8], Nick Shaw[9], Louise A. Metherell[1], Martin O. Savage[1], Helen L. Storr[1]

[1]Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK, [2]Barts Health NHS Trust, London, UK, [3]Nottingham University Hospital, Nottinghamshire, UK, [4]University Hospital Centre, Zagreb, Croatia, [5]Department of Peadiatrics, Endocrinology and Diabetes with a Cardiology Unit, Medical University of Białystok, Poland, [6]Leeds Teaching Hospital, Leeds, UK, [7]Mother and Child Health Care Institute of Serbia, "Dr Vukan Cupic", [8]Alder Hey Children's Hospital, Liverpool, UK [9]Birmingham Children's Hospital, Birmingham, UK

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Corresponding author's contact information: Professor Helen L. Storr, Professor and Honorary Consultant in Paediatric Endocrinology, Centre for Endocrinology, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, UK. Tel: +44 (0)20 7882 6198. E-mail: h.l.storr@qmul.ac.uk

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ABSTRACT

Context and objective: Growth hormone insensitivity (GHI) in children is characterized by short stature, functional IGF-I deficiency and normal or elevated serum GH concentrations. The clinical and genetic etiology of GHI is expanding. We undertook genetic characterization of short stature patients referred with suspected GHI and features which overlapped with known GH-IGF-I axis defects.

Design and methods: Between 2008 and 2020, our center received 149 GHI referrals for genetic testing. Genetic analysis utilized a combination of candidate gene sequencing (CGS), whole exome sequencing (WES), array comparative genomic hybridization (aCGH) and a targeted whole genome short stature gene panel**.**

Results*:* Genetic diagnoses were identified in 80/149 subjects (54%) with 45/80 (56%) having known GH-IGF-I axis defects (*GHR* n=40, *IGFALS* n=4, *IGFIR* n=1). The remaining 35/80 (44%) had diagnoses of 3M syndrome (n=10) (*OBSL1* n=7, *CUL7* n=2 and *CCDC8* n=1), Noonan syndrome (n=4) (*PTPN11* n=2, *SOS1* n=1 and *SOS2* n=1), Silver-Russell syndrome (n=2) (Loss of methylation on chromosome 11p15 and uniparental disomy for chromosome 7), Class 3-5 copy number variations (n=10) and disorders not previously associated with GHI (n=9) (Barth syndrome, Autoimmune lymphoproliferative syndrome, Microcephalic osteodysplastic primordial dwarfism Type II, Achondroplasia, Glycogen storage disease Type IXb, Lysinuric protein intolerance, Multiminicore Disease, MACS syndrome and Bloom syndrome).

Conclusion: We report the wide range of diagnoses in 149 patients referred with suspected GHI, which emphasizes the need to recognize GHI as a spectrum of clinical entities in undiagnosed short stature patients. Detailed clinical and genetic assessment may identify a diagnosis and inform clinical management.

INTRODUCTION

The evaluation of children presenting with short stature comprises detailed clinical, phenotypic, auxological and biochemical assessments alongside genetic analyses in selected cases(1-3). Advances in molecular technology and bioinformatic pipelines have broadened the genetic investigative modalities available to clinicians and unveiled numerous genetic causes for growth failure. This work has advanced the understanding of the physiology of normal human linear growth, identified new genetic causes of short stature and enhanced patient diagnosis.

Growth hormone insensitivity (GHI) encompasses a range of defects of GH action presenting clinically as extreme, dysmorphic short stature or milder short stature associated with normal physical appearance(4). 'Laron syndrome' (OMIM: 262500) or 'classical' GHI due to defects of the GH receptor gene (*GHR)* presents at the extreme end of the spectrum with marked postnatal growth failure and IGF-I deficiency secondary to severe GH resistance(5). Laron syndrome is clinically recognizable and associated with severe deficiencies of serum IGF-I, IGFBP-3 and ALS(6). To date, more than 90 homozygous, compound heterozygous, missense, nonsense, and splice site *GHR* mutations have been identified with significant phenotypic and biochemical variability(7).

Most cases of GHI associated with *GHR* mutations exhibit autosomal recessive inheritance. The majority have defects in the extracellular domain of the *GHR* and present with severe phenotypes(8). 'Non-classical' GHI disorders have mild to moderate phenotypic and biochemical presentations. These milder forms tend to be caused either by heterozygous *GHR*

mutations in the intracellular and transmembrane domains (dominant negative (DN) effect)(9-11) or by the homozygous intronic *GHR* pseudoexon (6Ψ) mutation(12,13).

The known GHI spectrum evolved further with genetic defects discovered in key downstream GH-IGF-I axis genes such as *STAT5B(14)*, *IGFI(15)*, *IGF2(16)*, *IGFALS(17)* and *PAPPA2(18).* We can now conceptualize a continuum of phenotypic GHI presentations from very mild to very severe(4,19). Each known defect in the GH pathway often has a distinct clinical, biochemical, metabolic and/or genetic signature(4,20). Other molecular defects impacting GH signaling and causing GHI phenotypes include *STAT3*, *IKBKB*, *IL2RG*, *PIL3R1* and *FGF21* mutations(21-23).

The cardinal features of GHI defects are short stature, normal GH secretion and IGF-I deficiency. Investigation of a child with short stature should follow a standard protocol(24) leading to logical determination of GH status. If GH secretion is normal, the finding of a low serum IGF-I concentration, particularly when there is severe short stature, requires formal genetic sequencing of known GH-IGF-I axis genes.

Our group and others have reported congenital growth disorders 3M (OMIM: 273750), Silver Russell (OMIM: 180860) and Noonan (OMIM: 163950) syndromes presenting with features of GHI(4,25,26) and phenotypic overlap with known GH-IGF-I axis defects. It is estimated that currently approximately 80% of children referred with short stature do not obtain identifiable primary diagnosis(27). Many of these children have normal GH secretion and receive a presumed designation of GHI, but no specific diagnosis is reached. The identification of an

underlying genetic defect will enable access to effective treatment, specific genetic counselling, early detection of likely co-morbidities and will inform prognosis(3).

Our center is an international referral center for patients with undiagnosed short stature, many with mild to moderate GHI features. The present study reports the clinical, endocrine, and genetic characterization of a series of patients, with suspected GHI, referred for genetic sequencing.

MATERIALS AND METHODS

Ethical Approval

Informed consent for genetic research was obtained from patients and/or their parents or carers. Ethical approval was gained from the Health Research Authority, East of England Cambridge East Research Ethics Committee (REC reference: 17/EE/0178).

Subjects

We performed genetic analyses on 149 subjects referred with short stature (height SDS ≤-2.0) and suspected GHI (functional IGF-I deficiency) between 2008 and 2020. They were assessed by the referring clinicians at their home institution. No precise criteria for the presumptive diagnosis of GHI were set for referring clinicians. However, the combination of short stature, normal GH secretion and IGF-I deficiency as a basis for genetic investigation have been reported in previous publications(20,28). A consanguineous marriage was defined as a union between a couple related as second cousins or closer(29,30).

Phenotypic and endocrine characterization

Referring clinicians excluded GH deficiency (peak GH level of ≥6.7 μg/L) during standard provocation testing according to the British Society for Paediatric Endocrinology and Diabetes (BSPED) clinical standards or baseline GH of ≥10 μg/ and causes of secondary GHI e.g. malnutrition and chronic inflammation. The clinicians completed a referral proforma which consisted of detailed clinical, biochemical and auxological data prior to sending a blood or DNA sample for genetic analysis. Birth weight, height and BMI values were expressed as standard deviation scores (SDS) according to the appropriate UK-WHO growth national standards. IGF-I generation tests (IGFGT) were performed at the referring centers according to established protocols (rhGH 0.033 mg/kg/day for 4 days with IGF-I measurements before the first and 12 hours after the fourth GH injections) in 61/149 (41%) subjects and an increase in IGF-I level of <15 ng/ml between the basal and peak values, consistent with severe GH resistance, was noted in 39/61 (64%) subjects(31). IGF-I levels were expressed as SDS based on age and sex appropriate ranges provided by the referral centers. Where serum IGF-I levels were undetectable (less than the lower limit of the assay) the lowest detectable SDS was calculated. Patients were categorized as having 'biochemical' GHI if they met the criteria above associated with severe IGF-I deficiency (IGF-I SDS ≤-2)(19).

Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes (Qiagen DNeasy kit). Candidate gene sequencing (CGS) was performed in 88 patients with GHI according to their clinical and biochemical phenotype as previously described(28). Briefly, all patients had *GHR* and *IGFALS* sequencing, patients with evidence of immunodeficiency and/or atopy or eczema also had *STAT5B* sequencing. GHI patients who did not have a molecular diagnosis following this initial approach and were born SGA underwent *IGFI, OBSL1, CUL7*, *CCDC8* and *IGFIR* gene analysis.

Patients undiagnosed following CGS underwent WES **(Figure 1)**. WES methodology was described in our previous publication(28).

Genomic sequencing using a custom designed NGS short stature gene panel analyses incorporated whole genomic sequences (including coding, promotor and intronic regions) of 60 genes of interest, 3 non-protein coding regions and one intergenic region. The targeted gene panel was created in 2017 to enable detailed exploration of key genes of interest in GHI and overlapping syndromes. Genes were selected for the panel based on their relevance to GHI phenotypes. Recognized genetic causes of overlapping syndromes (SRS, 3M, and NS) were included, in addition to other short stature genes of interest that may present with similar phenotypes. Several novel genes which were good candidates, such as genes with key roles in known growth pathways but without currently recognized human mutations causing growth failure, were also included. Otogenetics (Otogenetics Corporation, 4553 Winters Chapel Road, Ste 100 Atlanta, GA CLIA CERTIFIED 11D2066426, GA St Clinical laboratory License 067-071) designed the probes to cover genetic regions of interest in as much detail as possible, within the limitations of highly repetitive regions. The total number of probes was 89527, and the average coverage of the panel for the regions of interest was 97%.

Bioinformatic analysis

Ingenuity Variant Analysis (IVA), a bioinformatic tool, was used to filter genetic variants(32). Variant Call Files (VCFs) generated from the NGS methodologies were uploaded to the software and changes observed in the patient cohort were compared to the reference genome. VCFs contain thousands of genetic variants per patient, many of which are synonymous, and IVA allowed filtering based on several parameters e.g. type of variant or

inheritance pattern as previously described(32). Novel missense variants were investigated *in silico* by SIFT (score range 0, predicted deleterious to 1, predicted benign), PolyPhen-2 (score range 0, predicted benign to 1, predicted deleterious) and CADD Scores for coding regions and intronic variants. A CADD score ≥20 was the threshold for inclusion. A CADD score of 20 indicates the top 1% most deleterious missense variants and one of 30 indicates the variant is in the top 0.1%. Mutation Taster predicted whether a variant was disease causing or benign and Human Splicing Finder predicted whether exon skipping was more likely in the variant compared to the reference allele by calculating the consensus values of potential splice sites, splice enhancer and splice silencer sites(33).

Copy Number Variation (CNV) analysis

DNA samples were analyzed by array comparative genomic hybridization (aCGH), using a 60K oligonucleotide array (Agilent design 028469 or 085030) as previously outlined(34). In summary, 1µg DNA was labelled using CGH Labelling Kit for Oligo Arrays (Enzo Life Sciences, USA). Labelled DNA was then purified using QIAquick PCR purification Kit (Qiagen, USA). DNA samples were applied to a 60K oligonucleotide array (Agilent, USA) and hybridization, washing and scanning was performed following the manufacturers' protocols. Copy Number variations (CNVs) were classified into 5 categories (class 1, benign; class 2, likely benign; class 3; variant of uncertain significance (VUS); class 4, likely pathogenic and class 5, pathogenic) based on evidence including population, computational, functional and segregation data in line with accepted best practice guidelines(35). Class 1 and 2 CNVs were excluded from further analysis.

Statistical analysis

Statistical analyses of differences in height SDS, IGF-I SDS, birth weight SDS, peak GH, age, gender and consanguinity between those with genetic defects identified in the GH-IGF-I axis and overlapping disorders identified external to the GH-IGF-I axis as well as the diagnosed and undiagnosed groups were completed using an unpaired t test and Fisher's exact t test (GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA). P values of <0.05 were considered significant.

RESULTS

Subjects referred for genetic testing

Demographics and Biochemical Features

149 subjects (58% male; mean age 6.9 years, range 0.1 to 20.0 years) were referred with suspected GHI (mean height SDS -4.2, range -9.4 to -2.0; mean peak GH levels 41.9 µg/L, range 6.9 to 1195.0 µg/L and mean IGF-I SDS -2.3, range: -8.2 to 3.6) between 2008 and 2020. The mean birth weight SDS of the cohort was-1.3 (range: -6.0 to 2.6). The majority were from UK centers (n=76) but there were international patients from Kuwait (n=19), Poland (n=10), Mexico (n=8), India (n=4), Germany (n=4), Jordan (n=4), Serbia (n=3), Thailand (n=3), Sri Lanka (n=2), Italy (n=2), Egypt (n=2), Argentina (n=2) and the United Arab Emirates (n=2) as well as single patient referrals from Greece, Sweden, Turkey, Croatia, Slovakia, Belgium, Portugal and Qatar.

Consanguinity

Parental consanguinity was documented in 51 (34%) patients, 77 (52%) did not have a consanguineous background and in 21 (14%), consanguinity was not known.

Genetic Diagnoses

The genetic analyses and the diagnostic outcomes of the GHI subjects are shown in **Figure 1**. In 80/149 (54%) subjects a genetic diagnosis was made **(Group 1, Table 1)**. Genetic diagnoses were identified by: CGS in 37/149 (25%), WES in 16/149 (11%), the genomic short stature gene panel in 12/149 (8%), aCGH in 10/149 (7%) and by another modality in 5/149 (3%) (**Figure 2A**). All the 37/88 (42%) patients diagnosed by CGS and 10 of the patients diagnosed by WES were previously reported(28). No genetic diagnosis was found in 69/149 (46%) subjects **(Group 2, Table 1)**. The diagnosed cohort comprised 56% (45/80) with known GH-IGF-I axis defects (**Table 1, Group 3**) and 44% (35/80) with an overlapping disorder external to the GH-IGF-I axis (**Table 1, Group 4**).

Subjects identified with genetic variants in known GH-IGF-I axis genes

Of the 80 subjects with identified genetic diagnoses, 45/80 (56%) had variants in known GH-IGF-I axis genes (*GHR,* n=40; *IGFALS,* n=4; *IGFIR,* n=1) (**Figure 2B**). Within this group, there was a high rate of consanguinity, 29/45 (64%). In 11/45 (25%) of these subjects, there was no consanguinity and in 5/45 (11%) consanguinity was not known. The majority of *GHR* subjects had features of classical GHI including frontal bossing and midfacial hypoplasia (34/45, 76%). The majority of *GHR* variants were homozygous (n=35), 3 were compound heterozygous and 2 were heterozygous dominant negative *GHR* variants. Three homozygous and 1 compound heterozygous *IGFALS* variants and a heterozygous *IGFIR* variant were also identified. *GHR* variants not previously reported in the literature were predicted deleterious by at least one *in silico* functional prediction method (Mutation Taster, SIFT, PolyPhen-2 and/or CADD scores). These included 3 patients with homozygous *GHR* variants (c.689A>G p.Ile167Val in 2 siblings and c.730T>C, p.Leu229Pro).

Overlapping short stature disorders

Of the 80 patients with a genetic diagnosis, 35 (44%) had defects associated with genes outside the GH-IGF-I axis (**Table 1, Group 4**). The clinical and biochemical features of the patients are detailed in **Tables 2 and 3**. The range of diagnoses are shown in **Figure 2B**.

3M syndrome

3M syndrome was diagnosed in 10/35 (29%) subjects. Their mean age was 2.9 years (range 0.1-10.0 years), mean height SDS -5.4 (range -7.4 to -2.0) and mean IGF-I SDS -2.1 (range -3.3 to -0.2). Seven subjects had homozygous mutations in *OBSL1* and 6 had consanguineous parents(28). Two subjects had *CUL7* variants; patient 8 was diagnosed with novel compound heterozygous c.3490C>T, p.Arg1164Trp and c.3349C>T, p>Arg1117Trp *CUL7* variants both predicted disease causing by Mutation taster by altering the amino acid sequence and affecting protein features. The other *CUL7* variant (patient 9) is previously published(36,37). Patient 10 had the previously described *CCDC8* variant(38). 8/10 (80%) 3M subjects had overlapping facial features with the established GHI phenotype including frontal bossing (**Table 2**). SGA birth weights were present in 6/10 (60%) (mean SDS -3.8, range -5.8 to -2.1) as previously described in 3M syndrome(36).

Noonan and Silver Russell Syndromes

Four subjects had heterozygous variants in genes associated with Noonan syndrome (*PTPN11* n=2, *SOS1* n=1, *SOS2* n=1). Three of these patients were described in detail in our previous publication(28). Patient 14 presented with features of Noonan syndrome and was diagnosed with a rare missense heterozygous *SOS2* c.572C>G, p.Pro191Arg variant predicted damaging

by SIFT with a CADD score of 23.4. Patients 15 and 16 were diagnosed with SRS (11p15LOM and mUPD7) and were previously published(28). They were both SGA; patient 15 had a birth weight SDS -2.0 and patient 16 a birth weight SDS -2.3 (**Table 2**).

Copy Number Variations (CNVs)

Class 3-5 CNVs were identified in 10/35 (29%) subjects with mean height SDS -3.7 (range -5.7 to -2.0), mean IGF-I SDS -1.6 (range -2.7 to 1.3) and mean peak GH 38.6 µg/L (range 8.8 to 120.0 µg/L). There were 2 patients with Class 4, 1q21 deletions. This deletion was also identified in a sibling who shared the same clinical phenotype. One patient was diagnosed with a Class 5, 12q14 deletion. The other subjects had a 5q12 deletion (Class 3), Xq26 deletion (Class 4), duplication of chromosome 10, a combination of 7q21 (Class 3) and 7q31 (Class 4) deletions, combined (Class 3) 7q21 and Xp22 duplication, 7q36 duplication (Class 3) and combined 3p22 deletion (Class 3) and combined 15q13 (Class 4) duplication and 3p22 (Class 3) deletion. Nine of these CNVs are described in our recent publication(39).

Other overlapping short stature disorders

Novel overlaps with other disorders were diagnosed in 9/35 (26%) patients with mean height SDS -4.4 (range -9.4 to -2.0) and mean IGF-I SDS -2.2 (range -4.1 to -0.3). The clinical, biochemical, and genetic features are described in **Table 3**.

Barth syndrome

A novel hemizygous c.182delC, p.Thr61fs*22 *TAZ* variant predicted damaging by SIFT was identified by WES in patient 17 consistent with Barth syndrome (OMIM: 302060). This patient presented with failure to thrive, hypoglycemic episodes (associated with both Barth and Laron

syndrome) and typical features of severe GHI including frontal bossing, midfacial hypoplasia and small hands. Echocardiography showed left ventricular trabeculation and mild left ventricular dysfunction which are known associations of Barth syndrome.

Glycogen Storage disease Type IXb

A novel homozygous variant in *PHKB* (c.56-1G>A, p?) was identified in patient 18 with a history of parental consanguinity, severe short stature (height SDS -4.5) and features of congenital chloride diarrhea. This variant alters a canonical splice site base and is predicted to cause exon skipping and to be damaging to protein structure. *PHKB* variants are associated with Glycogen Storage disease Type IXb (OMIM: 306000)(40). A novel homozygous c.2007+1G>C *SLC26A3* variant (associated with congenital chloride diarrhea), predicted disease causing by Mutation taster, was also identified.

Multiminicore Disease

WES analysis identified a homozygous *SEPN1* mutation (c.1396C>T, p.Arg466Trp) predicted deleterious/damaging by SIFT and PolyPhen-2 in patient 19 who presented with short stature (height SDS -2.0), severe progressive thoracic scoliosis and solitary maxillary central incisor. The diagnosis of Multiminicore Disease (OMIM: 255320) was subsequently confirmed and the patient unfortunately died following scoliosis surgery soon after referral. Susceptibility to serious complications and sudden death are recognized in this disorder following general anesthesia.

MACS syndrome

Compound heterozygous mutations in *RIN2* including a missense mutation and novel splice site mutation (c.2648A>T and c.205-4A>G) were identified in patient 20 diagnosed with Macrocephaly, Alopecia, Cutis Laxa and Scoliosis syndrome (MACS syndrome; OMIM: 613075). The novel splice site c.205-4A>G variant was predicted to lead to loss of acceptor site and aberrant splicing and the missense c.2648A>T, p.Tyr883Phe variant was predicted damaging by SIFT and had a CADD score of 25. This patient presented with isolated proportionate short stature (height SDS -2.4) and detailed phenotyping is ongoing.

Bloom syndrome

Patient 21 was born small for gestational age (SGA) (BW SDS -4.7) with a history of recurrent upper and lower respiratory tract infections requiring repeated courses of antibiotics. She had severe short stature (height SDS -5.3), micrognathia, long, narrow face, brachydactyly and multiple café au lait spots(41). She was reviewed by a geneticist and described as 'SRS-like' but 11p15LOM testing was negative. A homozygous c.1933C>T, p.Gln645* mutation in the *BLM* gene was identified by WES and is recognized to cause Bloom syndrome(42). Both parents were heterozygous for this mutation.

Achondroplasia

Patient 22 was referred with severe short stature (height SDS -6.2) and WES confirmed a known deleterious missense *FGFR3* variant (c.1138G>A, p.Gly380Arg)(43) which was consistent with a diagnosis of achondroplasia (OMIM: 100800). The mother had the same genetic variant with severe short stature (height SDS -5.8), and both had clinical features of achondroplasia.

ALPS, MOPD Type II and Lysinuric protein intolerance

Detailed clinical, biochemical, and genetic interrogation at the referring centers confirmed the diagnosis in 3 additional subjects. These patients underwent CGS at our center. Patient 23 was from a consanguineous family and had a family history of splenomegaly and immune thrombocytopenia. The referring team suspected *STAT5B* deficiency but a diagnosis of Autoimmune lymphoproliferative syndrome (ALPS; OMIM: 601859) was made based on clinical features including recurrent childhood infections, lymphadenopathy, bronchiectasis, Type 1 diabetes mellitus, hypothyroidism, splenomegaly, pancytopenia and hypogammaglobulinemia. Genotyping by the local team identified a heterozygous c.794A>G, p.Asp265Gly missense *FAS* mutation consistent with a diagnosis of ALPS.

Patient 24 had a history of intrauterine growth restriction (BW SDS -5.7), severe short stature (height SDS -9.4) and microcephaly. He was investigated from 11 months, and following our initial genetic testing, his features evolved with the development of progressive bone dysplasia with hip contractures, pronounced rhizomelia and dysmorphic features such as a large nose with hypoplastic alae nasi and micrognathia. A diagnosis of Microcephalic osteodysplastic primordial dwarfism Type II (MOPD Type II; OMIM: 210720) was subsequently assigned at the age of 3 years by the referring team. Genotyping confirmed the known *PCNT* heterozygous c.1345-1G>A splice site mutation(44).

Lysinuric protein intolerance (OMIM: 222700) was diagnosed in patient 25 with a history of parental consanguinity, short stature (height SDS -3.8), poor weight gain, low energy levels and a history of fractures. Biochemical investigations revealed a picture in keeping with Lysinuric protein intolerance with reduced plasma lysine, arginine, and ornithine levels. This

was confirmed genetically with the identification of a homozygous c.625+1G>A *SLC7A7* mutation(45) which is predicted to disrupt the canonical splice donor site of intron 4 of the *SLC7A7* gene and is considered a pathogenic mutation.

Diagnoses in the subset of subjects with 'biochemical' GHI (IGF-I deficiency)

IGF-I deficiency (IGF-I SDS ≤-2) was present in 69/80 (86%) patients with a genetic diagnosis. The 11 patients who did not have IGF-I deficiency included 3 patients with mutations in the GH-IGF-I axis. The first patient had a heterozygous dominant negative *GHR* mutation (height SDS -3.2, IGF-I SDS 2.2), the second had a homozygous *GHR* mutation(28) (height SDS -5.0, IGF-I SDS 2.2) and the third had a heterozygous *IGFIR* mutation(28) resulting in IGF-I resistance (height SDS -3.1, IGF-I SDS 2.0). Three patients had CNVs, the first was diagnosed with Class 3 7q21 and Xp22 duplication (Height SDS -2.7, IGF-I SDS -0.6), the second had a Class 3 7q36 duplication (Height SDS -2, IGF-I SDS -0.8) and the third combined 15q13 (Class 4) duplication and 3p22 (Class 3) deletion (Height SDS -3.6, IGF-I SDS 1.3). An additional 2 patients were diagnosed with 3M syndrome (patients 4 and 9) and one patient was diagnosed with NS (patient 14) (**Table 2**). Patients 21 and 24 were diagnosed with Bloom syndrome and MOPD Type II, respectively (**Table 3**).

Analysis of phenotypic and biochemical associations

Comparison between patients with and without genetic diagnoses

Patients with genetic diagnoses were significantly shorter (mean height SDS -4.9 vs -3.4, $p<0.0001$), had a lower IGF-I SDS (mean -2.5 vs -1.9, $p<0.05$) and a higher consanguinity rate (53% vs 13%, $p<0.0001$) than the undiagnosed group (**Figure 3**). There was no significant

difference in the age of presentation, gender, birth weight SDS and peak GH levels between the diagnosed and undiagnosed subjects (**Table 1; Groups 2 & 3**).

Comparison between patients with genetic diagnoses external to and those involving the GH-IGF-I axis

Patients with diagnoses external to the GH-IGFI axis were more likely to be SGA (mean BW SDS -2.2 vs -0.8, $p<0.01$). Height SDS was significantly lower in patients with known GH-IGF-I axis defects (mean height SDS-5.3 vs -4.4, $p<0.05$) and they had a higher consanguinity rate (64% vs 37%, $p<0.05$). (**Figure 4**). There was no significant difference in peak GH levels, IGF-I SDS, age of presentation and gender between these two groups (**Table 1; Groups 4 & 5**).

DISCUSSION

Growth hormone insensitivity (GHI) encompasses a spectrum of defects of GH action and evidence of GHI is found in approximately 30% of children referred for investigation of short stature(46). This study confirmed our previous findings in a smaller series of 107 patients that a genetic diagnosis is more likely to be identified in patients from consanguineous families, and in patients presenting with a lower height SDS and IGF-I SDS values(28). The incidence of consanguinity is high in our patient cohort (34%), which significantly increases the likelihood of detecting recessive disorders.

Genetic defects of the GH-IGF-I axis are recognized to cause GHI; however, their exact prevalence is not well established. GH-IGF-I axis genetic variants comprised the most common cause of GHI, accounting for 56% (45/80) of patients in whom a diagnosis was made. This was not unexpected, given that the patients were referred with suspected GHI and there was a

high incidence of consanguinity in the cohort. The majority (40/45, 89%) had *GHR* variants and 95% (38/40) of these were located in the extracellular domain. These *GHR* mutations are recognized to present at the more severe end of the GHI continuum(4) and consistent with this, 76% had clinical features of GHI.

Our study highlights the wide range of additional genetic diagnoses that may exist in patients presenting to the clinician with short stature and apparent GHI. We observed a high diagnostic rate of 'overlapping' short stature disorders (35/80; 44%) which may also reflect the high rates of consanguinity in our cohort. 3M, Noonan and Silver Russell syndromes were present in 16/80 (20%) and a further 11% (9/80) had diagnoses not previously associated with the GHI spectrum. The patients diagnosed with 3M, NS and SRS had some corresponding characteristics of their underlying syndromes. Most of the patients (80%) diagnosed with 3M had the classical phenotype of frontal bossing, disproportionately large head, triangular face, anteverted nares and full fleshy lips(36,47,48). Some of these features were identified following genetic diagnosis, stressing the importance of detailed phenotypic documentation as part of the initial clinical assessment to aid diagnosis.

The clinical diagnosis of SRS can be made using the Netchine-Harbison clinical scoring system (NH-CSS)(49). However, many of the NH-CSS are non-specific and overlap with other conditions presenting with GHI. Nevertheless, it is indicated in patients presenting with pre- and post-natal growth restriction associated with relative macrocephaly to ensure the diagnosis of SRS is not overlooked. Two patients had SRS diagnoses due to 11p15LOM and mUPD7 as previously reported(28).

Our results demonstrate significant clinical and biochemical overlap between patients diagnosed with known GH-IGF-I axis genetic variants and those with short stature disorders external to the GH-IGF-I axis. Specifically, there were no significant differences in peak GH levels, IGF-I SDS, age at presentation and gender between these two groups emphasizing the substantial diagnostic challenges for clinicians. However, the patients with GH-IGF-I axis gene defects did have lower height SDS and higher consanguinity rates compared to those with overlapping short stature disorders.

Birth weight SDS was also significantly lower in the overlapping group which is consistent with the finding that most patients with *GHR* variants have normal prenatal growth. Patients with *IGF-I* and *IGFIR* variants typically have prenatal growth restriction however these defects are less common, and we only identified one individual with a *IGFIR* variant. Additionally, 12/35 (34%) patients in the overlapping disorders group had 3M and SRS which are characterized by pre- and post-natal growth restriction. Accordingly, both the SRS and 60% of the 3M patients were born SGA. Bloom syndrome is also frequently associated with prenatal growth restriction and was diagnosed in one subject(50). Different short stature disorders have variances in head circumference e.g. macrocephaly observed in MACS(51), relative macrocephaly and frontal bossing in SRS(49) and 3M(47) and microcephaly associated with *IGFIR* variants(52) and syndromes such as MOPD II(53). Hence accurate head circumference may guide clinical diagnosis and genetic testing.

The clinical and biochemical presentations of patients with known GH-IGF-I axis gene defects can be useful diagnostic tools to aid genetic differentiation. Diagnostic pointers include birth weight and length, head circumference, facial dysmorphisms, the degree of post-natal growth

failure, presence of immune deficiency and GH and IGF-I levels. GH-IGF-1 assessment should be considered in all undiagnosed short children. However, in those with clinical features consistent with a specific phenotype e.g. achondroplasia, GH-IGF assessment would not routinely be indicated. Comprehensive algorithms for targeting genetic investigations have previously been published(20). However there has been no emphasis on differentiating patients with known genetic variants in the GH-IGF-I axis from those with overlapping disorders external to the GH-IGF-I axis. This is likely due to the rarity of many of these disorders and the previous lack of association with GHI. Furthermore, the absence of a genetic diagnosis within the GH-IGF-I axis does not rule out the possibility of an undefined molecular abnormality in this axis. Our data demonstrate the overlap between these groups proving that clinical differentiation is challenging. However, genetic, biochemical and clinical evaluation for other overlapping disorders may prove beneficial to improving the diagnostic yield in undiagnosed short stature with GHI features.

Many rare short stature disorders pose diagnostic challenges due to the wide spectrum of phenotypic features that exist under each diagnostic umbrella. The clinical diagnosis of known genetic syndromes traditionally relies on identifying 'classical' features. We demonstrate that the predominant consistent feature of many of these conditions is short stature. The associated dysmorphic features can be subtle, overlap with other disorders and are frequently non-specific. Diagnostic confusion is even more likely if these coexist with biochemical features of known GHI disorders.

This was evident in our patients diagnosed with Microcephalic osteodysplastic primordial dwarfism type II (MOPD II) and Glycogen storage disease Type IXb (GSD IXb). MOPD II has a

heterogeneous phenotype(53) and our patient was referred for genetic sequencing at 11 months due to severe growth failure (Height SDS -9.4). Diagnosis of MOPD II was subsequently confirmed by genotyping as the dysmorphic features became more evident. GSD IX is a metabolic disorder with significant clinical variability even amongst individuals with the same genetic mutation. Our patient had no distinguishable clinical features except for growth delay which is present in ~88% patients (54). Hepatomegaly is usually observed but in ~6% GSD IXb patients it is not reported (54). Interestingly, GSD IX secondary to *PHKB* gene defects (as identified in our patient) may be associated with milder phenotypes than the other known underlying genetic causes (55). An accurate molecular diagnosis eliminates the need for invasive investigations such as liver biopsies and allows for genetic counselling of the patient and family.

Some of the other rare overlapping syndromes identified are associated with more serious co-morbidities such as predisposition to neoplasia. Bloom syndrome is characterized by pre- and post-natal growth restriction in association with photosensitivity, telangiectasia, immune deficiency and chromosomal instability causing enhanced cancer risk(50). Clinical features of autoimmune lymphoproliferative syndrome (ALPS) include lymphadenopathy, hepatosplenomegaly, autoimmunity and an increased malignancy risk. Short stature is not typically associated with ALPS and there are no reported cases presenting with GHI. A number of targeted therapies and avoidance of environmental mutagens can improve clinical outcomes in APLS and Bloom syndrome, respectively (50,56), hGH therapy should also be avoided. This highlights the importance of genetic diagnoses for ongoing management of these conditions.

Many of the patients with overlapping syndromes had presumptive diagnoses of GHI / primary IGF-1 deficiency and as such, rh-IGF-I therapy was considered. The patient diagnosed with Barth syndrome presented with failure to thrive (BMI SDS -4.1), hypoglycemia and clinical features of GHI (frontal bossing, deep set eyes and small hands). The phenotype of Barth syndrome is variable but it typically presents with growth failure in association with dilated cardiomyopathy, neutropenia, proximal myopathy and organic aciduria(57). Severe cases are associated with fetal cardiomyopathy, still birth and early neonatal death, thus timely diagnosis and genetic counselling is vital(57). Mild left ventricular dysfunction was noted on echocardiography in our patient and there was a good growth response to rhIGF-I therapy. Barth syndrome is associated with lower anabolic IGF levels and higher catabolic cytokine IL-6 levels when compared to healthy controls(58). This may account for the growth delay and the patient's responsiveness to rhIGF-I therapy.

Lysinuric protein intolerance (LPI) is a rare condition associated with vomiting, diarrhea, failure to thrive, hepatomegaly, osteopenia, osteoporosis, hyperammonemia and low blood urea. The symptoms are highly variable and about a third of apparently asymptomatic individuals are identified in the context of familial screening(59). Our patient had back pain and vertebral fractures were confirmed on radiological investigations. The diagnosis was made biochemically with reduced plasma lysine, arginine, and ornithine with increased urine levels. The association of this condition with GHI is not established.

Achondroplasia is an autosomal dominant condition with numerous associated comorbidities including delayed motor milestones, communicating hydrocephalus and spinal stenosis. This is usually an uncomplicated diagnosis given its characteristic phenotype and established

genetic defect. Our patient was referred for genetic analysis given the unusual biochemical picture of GHI, not usually associated with achondroplasia. The patient diagnosed with Multiminicore disease had short stature with no obvious syndromic features except for scoliosis; one of the recognized features of this disorder. Multiminicore disease is a congenital myopathy disorder with a clinically heterogenous phenotype(60). The classic clinical form accounts for ~75% of cases and is characterized by neonatal hypotonia, delayed motor development, weakness and muscle atrophy(60). Failure to thrive, short stature and low body weight are described. This patient died unexpectedly shortly after spinal surgery and there was also a history of sudden unexplained death in a sibling, highlighting the importance of genetic counselling for this family.

The characteristic clinical features of macrocephaly, alopecia, cutis laxa and scoliosis (MACS) syndrome includes downward slanting palpebral fissures, puffy eyelids, gingival hyperplasia and short stature(51). Isolated short stature was the main presenting feature in our patient and WES aided the diagnosis by identifying predicted deleterious compound heterozygous variants in *RIN2*.

In summary, we have identified a wide spectrum of growth disorders, including several not previously considered part of the GHI spectrum, presenting analogously with short stature and normal GH production. Although the underlying disease mechanisms are diverse, we suggest these overlapping disorders be considered part of an extended GHI spectrum. We also highlight the benefits of integrating NGS technology such as WES into the diagnostic framework. Our current pipeline uses aCGH and the whole genome short stature gene panel as first-line to assess for CNV and a range of genes known to cause GH-IGF-1 axis

defects/overlapping syndromes, respectively. Subsequently WES is utilised in undiagnosed subjects to seek novel causalities/aetiologies. We anticipate this strategy will evolve to whole genome sequencing in all patients, once costs and bioinformatic tools are equivalent.

Many overlapping disorders have significant co-morbidities and a definitive genetic diagnosis allowed screening tests to be initiated. A diagnosis also informs prognosis, clinical management and countenances genetic counselling. Advancing molecular knowledge of the GHI continuum has added likely benefits of facilitating targeted clinical therapies and preventing inappropriate use of rhGH in pre-malignant conditions.

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DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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FIGURE LEGENDS

Figure 1: Flowchart showing the genetic analyses undertaken and the diagnostic outcomes of the GHI subjects (n=149)

Genes for Candidate gene sequencing (CGS) were chosen depending on the clinical and biochemical features of the patients. Next generation sequencing included: Whole exome sequencing (WES), short stature genomic panel and array comparative genomic hybridization (aCGH). Diagnoses were made in a total of 80/149 (54%) subjects, leaving 69/149 (46%) undiagnosed. Our center identified a genetic defect in 75 (50%) subjects (94% of those diagnosed) and a further 5 diagnoses were made at the local referring institution ('other modality'). These included 2 patients with molecular defects consistent with Silver Russell syndrome (SRS; 11p15LOM and mUPD7, respectively) and 3 patients with Autoimmune lymphoproliferative syndrome (ALPS), Lysinuric protein intolerance and Microcephalic osteodysplastic primordial dwarfism Type II (MOPD Type II), respectively. These diagnoses were suspected by the referring clinician or clinical geneticist and confirmed by genotyping.

Figure 2: The range of genetic diagnoses and the diagnostic modality in the patients with suspected growth hormone insensitivity

A) The range of diagnostic modalities that secured the genetic diagnoses in 80/149 (54%) diagnosed subjects. CGS, Candidate gene sequencing; WES, Whole exome sequencing; Panel, short stature genomic panel; aCGH, array comparative genomic hybridization; OM, other modality.

B) Range of genetic diagnoses. Group 1; Known GH-IGF-I axis genetic variants (n=45; *GHR* n=40, *IGFALS* n=4 and *IGFIR* n=1), group 2; overlapping disorders comprising 3M syndrome

genetic variants (n=10; *OBSL1* n=7, *CUL7* n=2 and *CCDC8* n=1), Noonan syndrome (NS) genetic variants (n=4; *PTPN11* n=2, *SOS1* n=1 and *SOS2* n=1), Silver-Russell syndrome (SRS) (n=2; Loss of methylation on chromosome 11p15, uniparental disomy for chromosome 7), CNV, Class 3-5 copy number variations (n=10, Class 4 1q21 deletion n=2, Class 5 12q14 deletion n=1, Class 3 5q12 deletion n=1, Class4 Xq26 duplication n=1, duplication of Chromosome 10 n=1, Class 3 7q21 and Class 4 7q31 deletion n=1), Class 3 7q21 duplication and Xp22 duplication n=1, Class 3 7q36 duplication n=1, Class 3 3p22 deletion and 15q13 duplication n=1) and additional overlapping disorders (n=9; Barth syndrome, Autoimmune lymphoproliferative syndrome, Microcephalic osteodysplastic primordial dwarfism Type II, Achondroplasia, Glycogen storage disease Type IXb, Lysinuric protein intolerance, Multiminicore disease, MACS syndrome and Bloom syndrome). GH-IGF-I, growth hormone-insulin-like growth factor-I; NS, Noonan syndrome; SRS, Silver Russell syndrome; CNV, Copy Number Variants.

Figure 3: Comparison of Height SDS, IGF-I SDS and consanguinity between patient groups with and without a genetic diagnosis

A) Height SDS was significantly lower in the diagnosed group (n=78) compared with the undiagnosed group (n=68) (mean height SDS -4.9 vs -3.4, respectively), $p < 0.0001$. B) IGF-I SDS was significantly lower in the diagnosed group (n=71) compared with the undiagnosed group (n=58) (mean IGF-I SDS -2.5 vs -1.9, respectively), $p=0.0384$. C) Consanguinity rates were significantly higher in the diagnosed group (n=80) compared with the undiagnosed group (n=69) (53% vs 13%, $p < 0.0001$). *$p \leq 0.05$, ****$p \leq 0.0001$.

Figure 4: Comparison of Birth Weight SDS, Height SDS and consanguinity between patients with known genetic diagnoses in the GH-IGF-I axis and overlapping disorders

A) Birthweight SDS was significantly lower in the overlapping disorders group (n=31) compared to the known GH-IGF-I axis defect group (n=40) (mean BW SDS -2.2 vs -0.8, respectively), p=0.0027. B) Height SDS was significantly lower in the known GH-IGF-I axis defect group (n=44) compared to the overlapping short stature disorders group (n=34) (mean height SDS-5.3 vs -4.4, respectively), p=0.0174. C) Consanguinity rates were significantly higher in the GH-IGF-I axis group (n=45) compared with the overlapping disorders group (n=35) (64% vs 37%, p =0.0236). *p ≤0.05, **p ≤0.01.

Table 1: Comparison of clinical and biochemical features among the different patient groups

	Group 1 Subjects with an identified genetic diagnosis (n= 80)	**Group 2 Subjects without an identified genetic diagnosis (n=69)**	**Group 3 Patients with known variants in the GH-IGF-1 axis (n=45)**	**Group 4 Overlapping disorders (3M, NS, SRS, CNV and other syndromes) (n=35)**	**P Value (95% CI) Group 1 vs Group 2**	**P value (95% CI) Group 3 vs Group 4**
Age (years)	6.5 (0.1 to 17.0) n=77	7.4 (0.8 to 20.0) n=67	6.9 (1.1 to 16.5) n=44	6.0 (0.1 to 17.0) n=33	0.3276 (NS)	0.5245 (NS)
Sex M: F (%)	48:32 (60:40)	39:30 (57:43)	27:18 (60:40)	21:14 (60:40)	0.7396 (NS)	0.9999 (NS)
Consanguinity	42 (53%)	9 (13%)	29 (64%)	13 (37%)	**<0.0001(****)**	**0.0236 (*)**
Birth Weight SDS	-1.4 (-6.0 to 2.6) n=71	-1.0 (-4.6 to 1.6) n=59	-0.8 (-6.0 to 2.6) n=40	-2.2 (-5.8 to 0.3) n=31	0.2103 (NS)	**0.0027 (**)**
Height SDS	-4.9 (-9.4 to -2.0) n=78	-3.4 (-6.3 to -2.1) n=68	-5.3 (-8.9 to -2.0) n=44	-4.4 (-9.4 to -2.0) n=34	**<0.0001(****)**	**0.0174 (*)**
IGF-1 SDS	-2.5 (-8.2 to 2.2) n=71	-1.9 (-4.1 to 3.6) n=58	-3.0 (-8.2 to 2.2) n=39	-2.2 (-4.1 to 4.4) n=32	**0.0384 (*)**	0.0623 (NS)
Peak GH (µg/L)	57.8 (7.0 to 1195.0) n=73	20.4 (6.9 to 66.9) n=54	81.9 (9.6 to 1195.0) n=39	27.4 (7.0 to 104.3) n=34	0.0533 (NS)	0.1224 (NS)

GH levels were defined as normal or raised if baseline GH ≥10µg/L and/or peak GH on provocation testing ≥6.7µg/L. NS; Noonan syndrome, SRS; Silver-Russell syndrome, CNV; Copy Number Variation. *P value ≤ 0.05; **P value ≤ 0.01; ***P value ≤ 0.001; ****P value ≤ 0.0001. NS, P value not significant (> 0.05).

Table 2: Endocrine, phenotypic and genetic characteristics of patients diagnosed with 3M, Noonan, and Silver Russell syndromes

Pt no.	Diagnosis	Age at referral (years)	Sex	BW SDS	HSDS	BMI SDS	IGF-I SDS	Basal GH (µg/L)	Peak GH (µg/L)	Clinical Features	Genetic Variant	Diagnostic Modality	Predicted outcome (unpublished variants)
1	3M syndrome	1.1	F	-3.8	-4.9	-0.4	**ND**	4.2	37.2	Frontal bossing, hypermobile joints, protuberant abdomen. Elongated face *Consanguineous parents*	Homozygous *OBSL1* mutation(61) *c.1359insA, p.Glu454Argfs *11* (CI093476)*	CGS	-
2	3M syndrome	0.1	F	-1.5	-4.5	0.5	-2.6	41.0	33.0	Frontal bossing, depressed nasal	Homozygous *OBSL1*	CGS	-

										bridge, hypermobility of joints, prominent heels, short fingers, trident hands, short rib cage, bilateral hip dysplasia. *Consanguineous parents*	mutation(61) *c.1359insA, p.Glu454Argfs *11* (CI093476)*		
3	3M syndrome	3.0	F	-5.2	-5.7	-4.7	-3.3	9.1	15.0	frontal bossing, depressed nasal bridge, bitemporal hair	*OBSL1* Homozygous mutation(61) *c.1359insA,*	CGS	-

										thinning with sparse hair, short neck, short trunk, joint hypermobility, prominent heels. *Consanguineous parents*	*p.Glu454Argfs *11* (CI093476)*		
4	3M syndrome	0.1	F	-2.6	-5.1	0.7	-0.2	5.4	10.8	Frontal bossing, prominent heels, hypermobile joints	Homozygous *OBSL1* mutation(61) *c.1359insA, p.Glu454Argfs *11*	CGS	-

										Consanguineous parents	(CI093476)*		
5	3M syndrome	1.0	M	-1.6	-6.4	-2.3	-2.5	2.1	18.2	Prominent forehead, depressed nasal bridge, hypotonia, short neck, hypermobility, prominent heels, short chest *Consanguineous parents*	Homozygous *OBSL1* mutation(61) *c.1463C>T p.Arg489** (rs121918216)*	CGS	-

6	3M syndrome	4.6	M	-3.2	-7.4	1.5	-2.5	6.0	>32.0	Frontal bossing, depressed nasal bridge, bitemporal hair thinning, high pitched voice *Consanguineous parents*	Homozygous *OBSL1* mutation(61) *c.1463C>T, p.Arg489** (rs121918216)*	CGS	-
7	3M syndrome	10.0	F	-0.8	-4.5	0.7	ND	ND	ND	Frontal bossing, flat nasal bridge, relatively large head with increased antero-posterior diameter, mid	Homozygous *OBSL1* splice site mutation(62) *c.2134+1G>A* (CS148259)*	Panel	-

										facial hypoplasia, dolichocephaly, bushy eyebrows, mild hirsutism, lumber lordosis, and protuberant abdomen. *Consanguinity ND*			
8	3M syndrome	7	M	ND	-2.0	-0.1	-2.6	ND	19	Pectus carinatum and high-pitched voice	Compound heterozygous *CUL7* mutation	Panel	Both variants SIFT: Damaging, PolyPhen-2:

										Consanguinity ND	**c.3490C>T, p.Arg1164Trp** (rs201135654)* **and c.3349C>T, p.Arg1117Trp** (rs375832364)*		Possibly damaging CADD score 23.1 for c.3490C>T and 28.1 for c.3349C>T
9	3M syndrome	0.3	F	-5.8	-5.5	-0.6	-1.1	22.5	26.7	Frontal bossing, depressed nasal bridge, epicanthic folds, bilateral hip dysplasia *Consanguineous parents*	Homozygous *CUL7* mutation(36) *c.2988G>A, p.Trp996X* (CM121245)*	WES	-

10	3M syndrome	1.6	M	-3.7	-7.4	-2.6	-2.4	5.1	9.9	Triangular face, prominent sternum *Non-consanguineous parents*	Homozygous *CCDC8* mutation(63), *c.612dupG, p.Lys205fs*59* (rs752254407)*	Panel	-
11	Noonan syndrome	6.9	M	0.3	-2.1	-2.7	-2.4	1.1	>32	Bilateral orchidopexy, right undescended testis, prominent eyes, low set ears, single palmer crease	Heterozygous *PTPN11* mutation(64) *c.417G>C, p.Glu139Asp* (rs397507520)*	WES	-

										Consanguineous parents			
12	Noonan syndrome	8.9	F	-2.1	-3.2	-1.6	-2.4	21.7	10.5	Low set ears, downward slanting eyes, hypertelorism, mild ptosis, low posterior hairline. *Non-consanguineous parents*	Heterozygous *PTPN11* mutation(64) *c.853T>C, p.Phe285Leu* (rs397507531)*	WES	-
13	Noonan syndrome	13.1	M	-3.0	-3.8	-1.5	-2.6	0.4	26.6	Nasal speech, frontal bossing but not typical	Heterozygous *SOS1* mutation(28)	WES	-

										Laron, Failure to thrive since birth, feeding difficulties, right undescended testes *Non-consanguineous parents*	*c.3418T>A, p.Leu1140Ile* (rs375550588)*		
14	Noonan syndrome	9.4	M	1.2	-2.0	0.1	-1.2	1.0	10.3	Low set ears, hypertelorism, joint hypermobility	Heterozygous *SOS2* mutation **c.572C>G, p.Pro191Arg** (rs72681869)*	Panel	SIFT: Damaging CADD score 23.4

										Non-consanguineous parents			
15	Silver Russell syndrome	1.1	M	-2.0	-3.7	**ND**	-2.8	12.6	38.7	Midfacial hypoplasia, frontal bossing *Non-consanguineous parents*	11p15LOM	SRS testing	-
16	Silver Russell syndrome	4	F	-2.3	-4.3	-4.9	-3.4	4.6	12.5	Frontal bossing, blue sclera, high pitched voice, normal cranial circumference, small face	MatUPD7	SRS testing	-

										Non-consanguineous parents			

BW, birth weight; HSDS, height SDS; ND, not documented; WES, Whole exome sequencing; CGS, candidate gene sequencing; SRS testing for loss of methylation on chromosome 11p15 (11p15LOM) and uniparental disomy for chromosome 7 (MatUPD7) was requested concomitantly by clinical geneticists in referring centers. Genetic variants in bold are not published. Patient variants in italics were previously reported in Storr et al 2015(26)and Shapiro et al 2017(28) * Reference SNP ID number or "rs" ID, the identification tag assigned by NCBI to a group (or cluster) of single nucleotide polymorphisms (SNPs) that map to an identical location or reference as listed on The Human Gene Mutation Database.

866 **Table 3: Endocrine, phenotypic and genetic characteristics of patients diagnosed with additional overlapping short stature disorders**

Pt no.	**Diagnosis**	**Age at referral (years)**	**Sex**	**BW SDS**	**HSDS**	**BMI SDS**	**IGF-I SDS**	**Basal GH (µg/L)**	**Peak GH (µg/L)**	**Clinical Features**	**Genetic Variant**	**Diagnostic Modality**	**Predicted outcome (unpublished variants)**
17	Barth syndrome	1.9	M	-2.9	-4.4	-4.1	-2.9	32.0	**ND**	Hypoglycemic episodes, frontal bossing, deep set eyes, small hands. *Non-consanguineous parents*	Hemizygous *TAZ* variant **c.182delC, p.Thr61fs*22**	WES	SIFT: Damaging Mutation Taster: Disease causing

18	Glycogen storage disease Type IXb	8.0	M	0.2	-4.5	0.6	-4.1	6.7	24.7	Congenital chloride diarrhea *Consanguineous parents*	Homozygous variant in *PHKB* **c.56-1G>A** and *SLC26A3* **c.2007+1G>C**	WES	Mutation Taster: Disease causing
19	Multiminicore Disease	13.8	F	-1.5	-2.0	-4.6	-2.2	1.7	104.3	Solitary median maxillary central incisor, severe thoracic scoliosis. Subsequent sudden death during sleep.	Homozygous SELENON (*SEPN1)* variant **c.1396C>T, p.Arg466Trp**	WES	SIFT: deleterious PolyPhen-2: probably damaging CADD score: 33

										Consanguinity ND			
20	MACS syndrome	3.3	M	-0.5	-2.4	-1.3	-3.1	9.3	16.9	Referred with isolated short stature Specific features of MACS syndrome not sought. *Consanguineous parents*	Compound heterozygous *RIN2* variants **c.205-4A>G** and **c.2648A>T, p.Tyr883Phe** (rs183141566)*	WES	**c.205-4A>G** splice site variant leads to loss of acceptor splice site and aberrant splicing **c.2648A>T** SIFT: deleterious

													PolyPhen-2: probably damaging CADD score: 25
21	Bloom syndrome	5.9	F	-4.7	-5.3	-1.8	-0.3	**ND**	8.9	'SRS-like' long, narrow face, brachydactyly, micrognathia, cafe-au-lait spots on abdomen and right popliteal fossa	Homozygous variant in *BLM* gene(42) c.1933C>T, p.Gln645X (rs373525781)*	WES	-

										Non-consanguineous parents			
22	Achondroplasia	3.2	F		-6.2	1.7	-2.1	1.9	11.7	Mother has Achondroplasia *Consanguinity ND*	Heterozygous *FGFR3* variant(43) c.1138G>A, p.Gly380Arg (rs28931614)*	WES	-
23	ALPS	15.7	M	**ND**	-3.8	-0.7	-3.7	0.1	19.0	T1DM, splenomegaly, pancytopenia, lymphadenopathy	Diagnosis made by referring clinical team Heterozygous *Fas* variant	Clinical confirmed by genotyping	Mutation Taster: Disease causing

										Consanguineous parents	**c.794A>G, p.Asp265Gly**		
24	MOPD Type II	0.9	M	-5.7	-9.4	-4.3	-1.8	66	**ND**	IUGR, microcephaly, progressive bone dysplasia with hip contractures, micrognathia *Non-consanguineous parents*	Diagnosis made by referring clinical team. Heterozygous *PCNT* splice site mutation c.1345-1G>A[42](44) (CS080729)*	Clinical confirmed by genotyping	-
25	Lysinuric protein intolerance	7.0	F	**ND**	-3.8	**ND**	-3.1	6.5	25.5	Chubby cheeks. Not typical mid-	Diagnosis made by	Bio-chemical	-

										facial hypoplasia, *Consanguineous parents*	referring clinical team. Homozygous c.625+1G>A *SLC7A7(45)* splice site mutation (rs386833822)*	(urine analysis) confirmed by genotyping	

867 BW, birth weight; HSDS, height SDS; ND, Not documented; WES, Whole exome sequencing; MOPD type II, Microcephalic osteodysplastic primordial dwarfism type II; ALPS,
868 Autoimmune lymphoproliferative syndrome; MACS syndrome, macrocephaly, alopecia, cutis laxa and scoliosis; T1DM, Type 1 diabetes mellitus; IUGR, intrauterine growth
869 restriction. Genetic variants in bold are not published. *Reference SNP ID number or "rs" ID, the identification tag assigned by NCBI to a group (or cluster) of single nucleotide
870 polymorphisms (SNPs) that map to an identical location or reference as listed on The Human Gene Mutation Database.

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Figure 1: Flowchart showing the genetic analyses undertaken and the diagnostic outcomes of the GHI subjects (n=149)

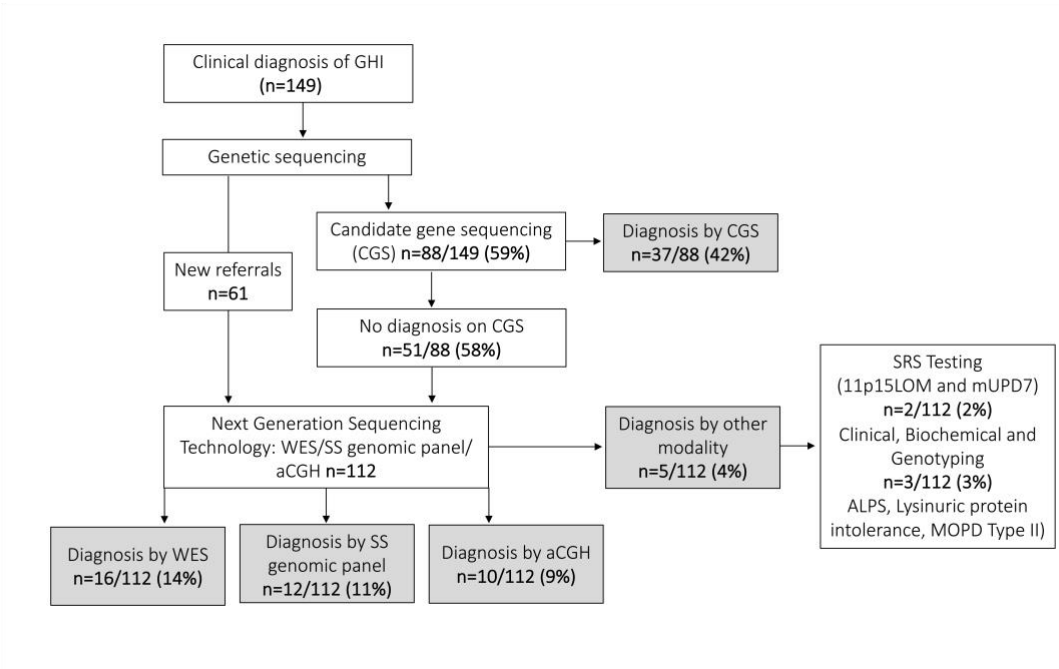
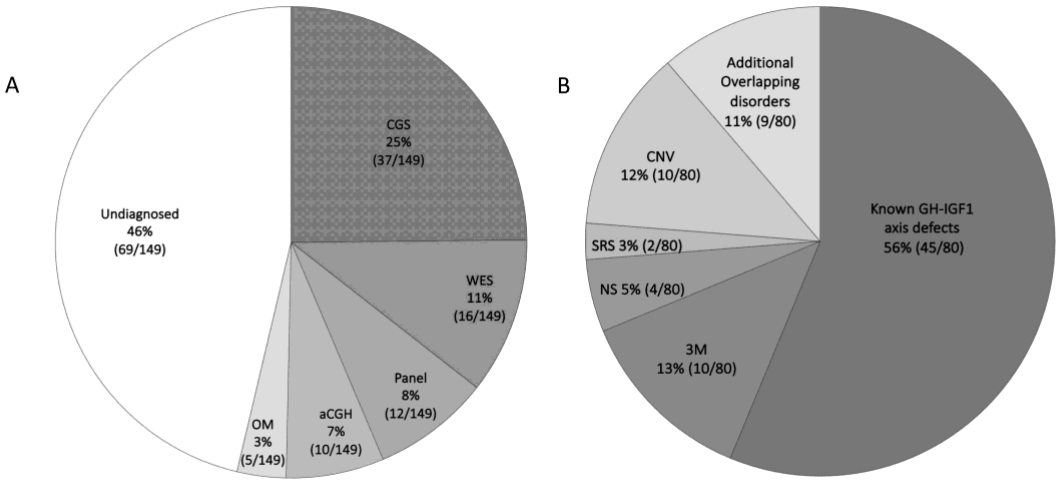


Figure 2: The range of genetic diagnoses and the diagnostic modality in the patients with suspected growth hormone insensitivity



907 **Figure 3: Comparison of Height SDS, IGF**

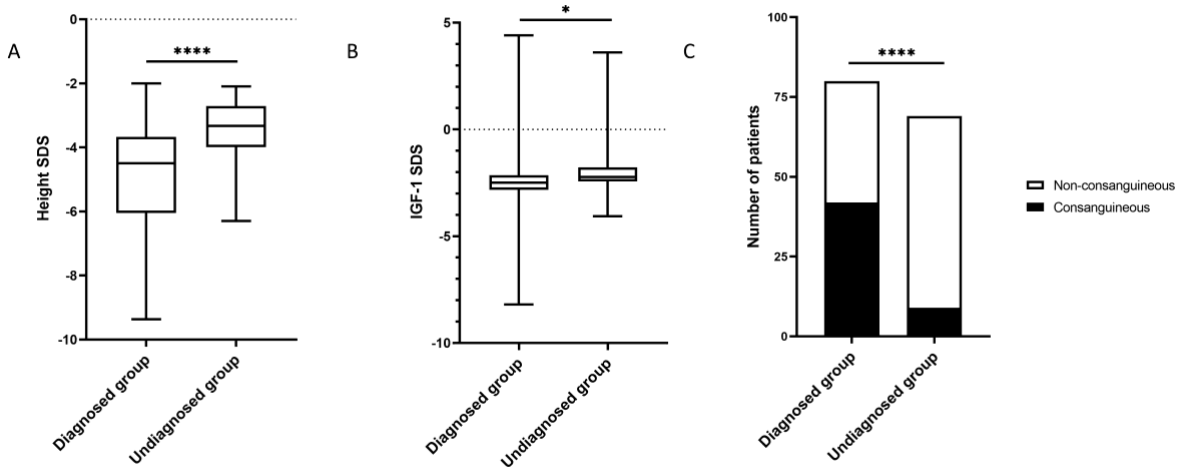


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Figure 4: Comparison of Birth Weight SDS, Height SDS and consanguinity between patients with known genetic diagnoses in the GH-IGF-I axis and overlapping disorders