Whole Exome Sequencing in Idiopathic Short Stature: Rare Mutations Affecting Growth

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Abstract

Introduction Evaluation of short stature is a challenge for pediatricians and in the process, idiopathic short stature (ISS) is an often diagnosis of exclusion. Non-pathogenetic mutations affecting height may present with phenotypes similar to the pathogenetic mutations. In this study, we aim to identify the underlying genetic cause of short stature in patients diagnosed with ISS and investigate potential treatments for them. Materials and Methods We identified 14 children in our practice who were under the age of 15 and were initially labelled as ISS. Then, we evaluated their plasma whole-exome sequencing (WES). Results Out of the 14 patients assessed with WES, five had normal results and correctly diagnosed with ISS. However, four of them had rare mutations that have not been extensively studied in the past. Due to the functions of these mutated genes and our patients' phenotypes, we suspect that these mutations played a role in the short stature. Out of the remaining five patients, four had genetic mutations known to cause short stature and one had a mutation that was known not to affect height. Conclusion In patients who are initially diagnosed with ISS, WES can help to identify rare mutations that may play a role in short stature. Directing attention to these genes, may help with the correct diagnosis and choosing proper treatment for the patients.

Introduction

Management of short stature is a dilemma in the field of pediatrics. Short stature in children as a height that is more than 2 standard deviations (SD) below the mean of the same age, sex, and population[1,2]. Short stature may have an underlying pathogenetic cause[1,2]. However**,** in many affected patients is often labelled as non-pathogenetic or idiopathic due to lack of identifying mutations that thwart linear growth[1-3]**.** Common non-pathogenetic causes of short stature include familial cases which are distinguishable from pathogenetic short stature by their normal growth velocity, constitutional growth delays due to impeded puberty which have a low to normal growth velocity falling below but parallel to the 3rd percentile growth curve, small for gestational age infants whose displaying catch-up growth after two years of age, and idiopathic short stature (ISS)[4,5]. The pathologic causes of short stature include undernourishment as well as renal diseases, cardiopulmonary malady, metabolic ailment, endocrine conditions, and other systemic causes that are not the focus of this study[3,6,7,8]. Furthermore, pathogenetic short stature is observed in Turner syndrome, Noonan syndrome, Silver-Russell syndrome, and short stature homeobox (SHOX) gene mutations among other congenital conditions[9-11]. Differentiating pathogenetic and non-pathogenetic causes of short stature is essential in choosing treatment modalities but challenging as they often present with similar phenotypes. There are various mutations that are thought to affect a person's linear growth with some having a greater impact on the individual[4]. One example of these mutations in SHOX mutations. Interestingly, nearly 4% of patients diagnosed with ISS, have evidence of SHOX mutations[1]. Similarly, many monogenic disorders that may affect children's growth tend to no present with significant signs or symptoms and thus are misdiagnosed as ISS[12]. ISS is defined as a height that is more than 2 SD below the average for that sex and age with

the absence of any diseases and underlying causes of short stature[1,2,13]. By this definition, about 80% of children with short stature fall into the category of ISS due to lack of obvious evidence of the underlying cause [1,14]. Yet, proper medical evaluation results in identifying an underlying cause in up to 40% of the patients labelled as ISS[4]. Hormonal therapy is approved by the United States Federal and Drug Administration for treating ISS patients although the treatment for all patients who are labelled as ISS is not only cost-effective but may not be indicated for all the patients given their underlying pathologies. When a single gene mutation is suspected in a patient with short stature, single gene-based tests are indicated and these tests often lead to a proper diagnosis by the clinicians[12]. However, it becomes a real challenge when the patient presents with no indicating phenotype, and no obvious signs and symptoms. In patients who are suspected of having a genetic defect that causes short stature, exome sequencing is used to determine the underlying cause[12]. As reported in previous studies, whole-exome sequencing (WES) is a useful tool in determining the cause of short stature in patients labelled as ISS[15]. Also, WES allows clinicians to detect monogenic mutations that significantly influence the patients' height[15]. Ultimately, WES assists physicians in making a proper diagnosis and a suitable treatment modality for the patients with short stature. In this study, we investigated the efficacy of WES as an optimal diagnosis tool in a clinical setting for patients who are labelled as ISS. We also evaluated the role of proper diagnosis in choosing treatment modalities and reducing financial burden for the patients and the society.

Materials And Methods

Study population and criteria for participant inclusion

We assessed children under the age of 15 labelled as ISS who were referred to four medicals clinics that are under the supervision of Alborz University of Medical Sciences. The clinics were located in the city of Karaj, situated 20 kilometres west of Tehran, capital city of Iran. We obtained patients' sex, age, weight, height, medical history and their parental heights, and their medical histories from their medical records. Then, we ordered a comprehensive blood workup (complete blood count, erythrocyte sedimentation rate, fasting glucose, blood urea nitrogen, creatinine, serum iron, electrolytes) and evaluated their thyroid function, growth hormone (GH) levels and Insulin-like growth factor 1 (IGF-1) levels. After completion of the initial examination and rolling out all systemic causes of short stature, 14 of the children were confirmed to have ISS and thus were included in the study. Children with other medical comorbidities and dimorphisms were included in the study as long as these conditions had no obvious relation to their short stature. Children with intrauterine growth retardation were excluded from the study. After the approval by Alborz University of Medical Sciences research ethics committee, we explained the purpose of the study to the patients' parents/legal guardians in-depth and obtained written consent from them. Afterwards, we pooled blood specimens from the patients and submitted it to whole-exome sequencing.

Whole-exome sequencing

WES was performed to enrich exons of protein-coding genes and along other important genomic regions. Next-generation sequencing was performed to a sequence near to 100 million reads on Illumina sequencer. The test platform was Illumina HiSeq 4000 conducted by Macrogen Sequencing, South Korea and it examined more than 95% of all targeted regions with a sensitivity of above 99%. This test was capable of simultaneously detect point mutations, micro insertion and deletions and duplications less than 20bp.

Data analysis

Data analysis was conducted with SPSS version 19. And Fisher's exact test was used for comparing and categorizing of means, and a *p*-value of less than 0.05 was considered statistically significant.

Results

From the children tested, only five of them had utterly normal WES results and thus were correctly labelled as ISS. Eight of the patients had mutations that could potentially be the underlying cause of their short stature. One patient had a mutation unrelated to his short height, thus correctly diagnosed with ISS. The summary of the mutations is shown in table 1.

Mutations of unknown significance

The first patient, an eight-year-old girl with a height of 112 cm and an SD of -2.8 for her age with normal height parents. She was heterozygous for GHSR gene (NM_198407 exon2: c.847>T) that led to amino acid changes p.R283. This gene encodes a member of the G-protein receptors family[16]. The mutation was of uncertain significance, and its inheritance was either autosomal recessive (AR) or autosomal dominant (AD) pattern[16]. However, pathologic mutations of this gene could have developed isolated partial growth hormone deficiency (GHDP) in the individual[17]. The mutation in GHSR gene results in growth delay, short stature, and in some reported cases, episodes of abdominal pain, vomiting, ketosis, and hyperglycemia[18]. In our case report, with respect to the mutation of uncertain significance, the child's phenotype, and her low levels of GH, we concluded that the mutation is likely pathologic in nature and the cause of her short stature. Given her low GH levels, hormonal therapy was initiated, and she showed a great response to the intervention.

The second patient was a 10-year-old girl with a height of 112 cm and an SD of -4.2 for her age, with a relatively short mother who had a height of 144 cm. The patient was heterozygous for CLCN5 gene (NM_001127898 exon14: c.2333T>G) which led to amino acid change p.L778R. This gene provides instructions for the synthesis CLC-5 protein which transports chloride ions across cell membranes and has an integral role in proximal tubule cells of the kidney[19]. CLCN5 gene mutations are inherited through an X-linked recessive pattern[19]. Pathologic mutations of this gene are a known cause of Dent disease which is a chronic renal disorder and is almost exclusively observed in males[19]. This mutation affects serum calcium and vitamin D levels as well[20]. Also, it has been observed that some female carriers could

manifest the symptoms due to random X-chromosome inactivation[21,22]. Short stature is commonly seen among patients with Dent disease[22]. In our case report, the child and her mother both had evidence of CLCN5 gene mutation. With respect to the mother's short height, we concluded that the mutation in CLCN5 gene is the cause of short stature in both the child and her mother. Despite the normal GH levels of the child, we opted to initiate hormonal therapy. However, her response to the intervention was poor. Thus, we concluded that hormonal treatment may not be indicated for this condition.

The third patient was a two-year-old girl with short stature, microcephaly and hearing loss that was homozygous for c.395T>G in exon 4 of the CLPP gene (NM_006012). The mutation in CLPP gene is inherited through AR-pattern and is associated with Perrault syndrome type 3[23]. There are no formal studies conducted on the functional effects of this mutation, yet with respect to the patient's phenotype and symptoms that are mainly observed in pathologic mutations, we concluded that this mutation is in fact pathologic in nature. Perrault syndrome type 3 is a rare condition that may represent with different signs and symptoms and affects both male and females[23]. A key feature of the disease is hearing loss and females with this condition may have ovarian dysgenesis with normal external genitalia[23]. Patients may also suffer from neurological conditions such as ataxia, peripheral neuropathy, and intellectual disability[23]. Lastly, patients with Perrault syndrome type 3 have low levels of GH and evidence of short stature[23,24]. It is highly likely that this individual's short stature with hearing loss and microcephaly resulted from Perrault syndrome type 3. Due to our patient's low levels of GH, we initiated hormonal interventions, but no significant difference was observed after the treatment. Thus, we concluded that hormonal therapy may not be indicated in this condition.

The fourth case is a 10 and a half-year-old boy with a height of 126 cm and an SD of -2.2 for his age with normal height parents. He had hearing loss and three different mutations. The first mutation was a heterozygous variant in TMPRSS3 (NM_032404.2 exon5: c.266G>A) which led to amino acid change p.R89H. The second one was a heterozygous variant in HOMER2 gene (NM_199330 exon3: c.188C>T) that resulted in amino acid change p.P63L. The third one was a heterozygous variant in the FGFR3 gene (NM_001163213 exon8: c.992G>A). TMPRSS3 gene encodes a protein of serine protease family that is required for ear's saccular hair cell survival, and pathologic mutations of this gene could result in autosomal recessive deafness (DFNB8)[25,26]. However, since the mutation has an AR pattern of inheritance and our patient was heterozygous for this gene, we concluded that the hearing loss is not attributed to the first mutation. The HOMER2 gene encodes a dendritic protein from the Homer family and mutations in this gene may lead to autosomal dominant deafness (DFNA68)[26]. Although the mutation in this child was of uncertain significance due to its AD pattern inheritance, it was likely the cause of his hearing loss. The third gene, FGFR3 provides instructions for synthesizing fibroblast growth factor receptor 3[27]. Mutations in this gene may result in hypochondroplasia, which is a form of short-limbed dwarfism[27].

In our case report, the patient presented with mildly short limbs. Although this mutation was of uncertain significance and no formal studies were conducted on the effects of this mutation, with respect to our

patient's phenotype and its AD pattern of inheritance, we concluded that the mutation in FGFR3 gene is probably the cause of his short stature. The patient's GH levels were normal, however we opted for a trial of hormonal therapy, but the results were not promising, and the treatment was discontinued. It is questionable whether continuance of hormonal therapy for this patient is effective and indicated.

Pathologic mutations

The first case of pathologic mutations was a five-year-old boy with a height of 99 cm and a SD of -2.2 for his age with a short father who had a height of 150 cm. He was heterozygous for UROD gene (NM_000347 exon9: c. 912C>A) with an AD or AR pattern of inheritance that led to amino acid change p.N304K. This gene provides instruction for the synthesis of uroporphyrinogen decarboxylase enzyme[29]. Pathologic mutations in this gene disrupt chemical steps that lead to heme production and results in porphyria[30]. There are several types of porphyria distinguished by the genome sequences and the symptoms. In our case, the patient's genome sequence showed evidence of hepatoerythropoietic porphyria (HEP). HEP results in fragile and blistered skin in exposure to the direct sunlight and makes the individual more vulnerable to infection scaring and pigmentation[30,31]. HEP is characterized by osteolysis, i.e. shortening of distal phalanges, sclerodactyly, and progressive joint deformities[32]. Furthermore, short stature has been noted in some types of porphyria, mainly, congenital erythropoietic porphyria[33]. There is one case report of a patient with HEP who had a noticeable short stature[34]. Due to the rare nature of this disease, the correlation between HEP and short stature remains questionable. However, in our case, the child's father had evidence of the mutation and short stature. Given that the child and the father both have evidence of short stature and the mutation, we concluded that there is, in fact, a correlation between HEP and short stature. Yet, given the rarity of the disease, further studies and case reports are required to confirm our hypothesis. Despite the patient's normal GH levels, we decided to initiate hormonal therapy. The response was feeble thus we discontinued the treatment and concluded that GH therapy may not be indicated for this medical condition.

The second patient with a pathologic mutation was a two and a half-year-old boy with a height of 84 cm and an SD of -2.2 for his age with normal height parents. He was heterozygous for RYR1 gene (NM_000540 exon15: c. 1589G>A) that led to amino acid change p.R530H and it was inherited through AD pattern[35,36]. RYR1 provides instructions for synthesizing ryanodine receptor 1 protein that transports calcium ions and is critical in muscle contraction and the movement[35,37]. Mutations in this gene make patients prone to malignant hyperthermia susceptibility 1 especially in an event of invasive procedure or general anesthetics[38,39]. It has been observed that RYR1 gene mutation results in King-Denborough syndrome and short stature [40,41]. To conclude that this mutation is the reason for our patient's short stature, further studies are indicated but it cannot be ruled out as a probable cause. Other coexisting conditions with this patient were seizures. As reported in previous studies, we presume his seizures could be due to stress-induced hyperpyrexia[42]. Despite the patient's normal GH levels, we initiated hormonal therapy and observed a good response.

The third patient was an eleven-months-old girl with short stature and an SD of -3 for her age. Her parents were of normal height. She was heterozygous for SMAD4 gene (NM_005359 exon 12: c. 1498 A>G) that led to amino acid change p.1500V and was inherited through AD pattern[43]. SMAD4 provides instructions for the synthesis SMAD4 protein which is part of transforming growth factor betas (TGF-β) signalling pathway and acts as a transcription factor and a tumour suppressor[44,45]. The mutation in our patient is associated with Myhre syndrome. Myhre syndrome has a characteristic facial feature and may result in short stature[43,46]. Affected patients may also present with hearing loss, joint stiffness, limited joint mobility fibrosis, cardiovascular problems, respiratory complications, and muscular and skeletal problems[43,47]. The patient's phenotype and development of the aforementioned conditions vary and depend on the mutation types and the domains [43]. In our case, besides mild facial features and short stature, abnormal TSH changes and lower normal limits of IGF-1 were noticeable.

After GH therapy initiation at a proper age, the expected difference in her height and velocity of growth was not observed, although due to her age the response observed to treatment can not determine if hormonal therapy is indicated or not.

The third patient was a two-year-old girl with short stature and an SD of -2.2 with normal height parents. She was heterozygous, for COL9A3 gene (NM_001853: exon 18:c. 920G>A) that led to amino acid change p.G307D and was associated with multiple epiphyseal dysplasia (OMIM 600969)[48,49]. The mode of inheritance was of AD pattern[50]. Multiple epiphyseal dysplasia is a mild and variable condition in which irregular ossification of the epiphyseal cartilage occurs[50]. Common signs and symptoms include early-onset arthritis, knee and hip cartilage anomalies and short stature [49,50]. In our case, the patient's phenotype correlated with the known characteristics of the disease, thus we concluded that the mutation in COL9A3 is the cause of her short stature. Although the patient had an average levels of GH, we decided to initiate GH therapy, but despite our best efforts, we observed a weak response. Thus, we concluded that hormonal therapy is not indicated in this patient.

The last patient, was a seven-year-old boy with a height of 99 cm and a SD of -4.2 for his age with normal height parents. He was heterozygous for CFTR gene (NM_000492 exon11: c.1397C>G) that led to amino acid change p.S466. The mode of inheritance was of an AR pattern. Mutations in this gene may cause cystic fibrosis[51]. In our case, the patient had no evidence of cystic fibrosis and was heterozygous, hence it is unlikely that his short stature is attributed to the mutation in CFTR gene. Despite the patient's normal GH levels, we initiated hormonal therapy and observed an excellent response. The summary of treatment results is shown in Table 2.

Discussion

After conducting and analyzing WES, we noted that out of 14 children who were initially labelled at ISS, only five had absolutely no pathologic genetic mutations and thus correctly diagnosed. Four of the children had evidence of rare mutations that probably played a role in their short stature however, further

studies and case reports are needed to confirm the hypothesis. The other four children had mutations that are known to cause short stature and one child had a mutation that was known to not affect the height. The proper diagnosis would have not been possible without analyzing children's whole-exome sequences given the rarity of their diseases. We also noted that GH therapy is not the gold standard for all children with short stature and other treatment modalities need to be considered for some of these individuals.

Conclusion

Management of patients with short stature is challenging as it is often difficult to differentiate pathogenetic and non-pathogenetic causes given their similar phenotypes. Whole-exome sequencing is an essential procedure in determining the underlying cause of short stature in patients who have labelled as idiopathic short stature based on clinical evaluations. The correct diagnosis helps physicians in choosing proper management for the affected individuals, ultimately, improving their health and quality of life.

List Of Abbreviations

Standard Deviations (SD), Idiopathic Short Stature (ISS), Short Stature Homeobox (SHOX), Whole-Exome Sequencing (WES), Insulin-Like Growth Factor 1 (IGF-1), Isolated Partial Growth Hormone Deficiency (GHDP), Autosomal Recessive Deafness (DFNB8), Autosomal Dominant Deafness (DFNA68), Autosomal Recessive (AR), Autosomal Dominant (AD), Transforming Growth Factor Betas (TGF-B), Multiple Epiphyseal Dysplasia (MED), Porphyria Hepatoerythropoietic Porphyria (HEP), Growth Hormone (GH), Variant Of Unknown Significance (VUS), X Linked Recessive (XLR), Heterozygous (Het), Homozygous (Hom)

Declarations

Ethics approval and consent to participate

This research was approved by the Alborz University of Medical Sciences research ethics committee. The purpose of study was explained to the participant's parents/legal guardians in-depth and written consent was obtained from all of them to use the data for the research purposes.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. With the permission of the patients' legal guardians.

Authors contributions

SH N and F R designed the study, visited and carried out the treatment of the patients. SH S and H ZK performed genetic testing and analyzed genetic findings. K K designed the data collection instruments and carried out data analysis. N MKH aided in the genetic study, drafted the initial manuscript, reviewed and revised the manuscript, enrolled the patients in the study, collected the data and drafted the final manuscript. B HZ prepared, revised and reviewed the final manuscript N GH and M GH helped in data management.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work**.**

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Consent to publish

A separate informed written consent was obtained from all the patients' parents/ legal guardians for publication purposes.

Availability of data and materials

The datasets used and analyzed for this study are available from the corresponding author in response to reasonable requests and with the permission of the patients' parents/ legal guardians.

Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Tables

Table1. Detected mutations, pathogenicity in this study and their related conditions

Mutation	Inheritance	Pathogenicity in this study	Variant	zygosity Patient	Pathogenicity in literature	Variant location	Related condition	Height SD
GHSR NM_198407	AD[1],AR[2]	pathogenic	C.847>T T.R283	het[3]	VUS[4]	exon 2	Isolated partial GH deficiency	-2.8
CLCN5 NM_001127898	XLR[5]	pathogenic	c.2333T>G p.L778R	het	VUS	exon 14	Dent disease	-4.2
CLPP NM_006012	AR	pathogenic	c.395T>G	hom[6]	VUS	exon 4	Perrault syndrome	-4
TMPRSS3 NM_032404.2	AR	pathogenic	c.266G>A p.r89h	het	pathogenic	exon 5	DFNB8[7]	-2.25
homer2 NM_199330	AD	pathogenic	c.188C>T p.p63l	het	VUS	exon 3	DFNA68[8]	
FGFR3 NM_001163213	AD	pathogenic	c.992G>A	het	VUS	exon 8	hypochondroplasia	
UROD NM_000347	AD,AR	pathogenic	912C>A p.n304k	het	pathogenic	exon 9	HEP[9]	-2.25
RYR1 NM_000540	AD	Likely Pathogenic	c. p.R530H 1589G>A	het	pathogenic	exon 15	malignant hyperthermia susceptibility 1	-2.25
SMAD4 NM_005359	AD	pathogenic	c. 1498 p.1500V A>G	het	pathogenic	exon 12	Myhre syndrome	-3
COL9A3 NM_001853	AD	pathogenic	c. p.G307D 920G>A	het	pathogenic	exon 18	MED[10]	-2.25
CFTR NM_000492	AR	pathogenic	p.s466 c.1397C>G	het	pathogenic	exon 11	Cystic fibrosis	-4.2

1. Autosomal dominant, 2. Autosomal recessive, 3.Heterozygous, 4. A variant of unknown significance, 5.X linked recessive, 6. Homozygous, 7. Autosomal recessive deafness, 8. Autosomal dominant deafness, 9. Hepatoerythropoietic porphyria, 10. Multiple epiphyseal dysplasia

Table 2		
Mutation	Related condition	Response to hormone therapy
GHSR	Isolated partial GH deficiency	Excellent
CLCN5	Dent disease	Poor
CLPP	Perrault syndrome type 3	Poor
FGFR3	Hypochondroplasia	Weak
UROD	HEP[1]	Poor
RYR1	Malignant hyperthermia susceptibility 1	Good
SMAD4	Myhre syndrome	Weak
COL9A3	MED[2]	Weak
CFTR	? Cystic fibrosis	Excellent

1. Hepatoerythropoietic porphyria, 2. Multiple epiphyseal dysplasia