Research Square

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Whole Exome Sequencing in Idiopathic Short Stature: rare mutations affecting growth

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Abstract

Introduction: one of the most common causes of referrals to paediatricians is short stature (ISS), some pathogenic mutations may present exactly similar to non-pathogenic causes, our goal is to identify and treat these patients labelled ISS with these mutations and hopefully treat them correctly.

Materials and Methods: We assessed All children under the age of fifteen years labelled as ISS. Fourteen of them were confirmed to be ISS and thus were allowed in our study. Afterwards, we pooled their blood specimens and ordered a whole-exome sequencing (WES) test.

Results: five patient had normal WES results. Four patients had rare motions that were not studied in the previous literature but due to the functions of the genes, and our patients' phenotypes it is highly possible that these mutations caused our patients' short stature. Four patients had known genetic mutations causing short stature. One patient had a mutation with no effect on height. With the help of WES, some rare mutations were found, with the patients' phenotype and evaluation we identified their function, we diagnosed some other patients' rare genetic disorders and assessed the possible effect of their mutation on their height and phenotype we aimed to determine how many children labelled as ISS are correctly diagnosed. By WES most of our patient achieved the correct diagnosis which would be impossible to diagnose without WES; thus the reason for their short stature was identified, with the correct diagnosis now we can aim for the proper treatment.

Introduction

One of the most common causes of referrals to paediatricians is short stature, a child with short stature is defined by a height that is minus two standard deviations (SD) or less than the corresponding average height for children of that age, sex, and population which may be pathologic or non-pathologic.

Due to not finding any cause and pathologies in the majority of the patients, most patients are considered to be non-pathologic, yet our goal is to identify, assess and treat children with pathologic causes, especially those who were wrongly labelled as non-pathologic. [1–3]. Non pathologic causes could be 1.familial that is the most seen cause of short stature and can be distinguished from pathologic causes of short stature by its normal growth velocity [4] 2.Constitutional delays of growth and puberty that have a low to normal growth velocity and their growth curvature is below but parallel to the 3rd percentile but they tend to catch up when entering puberty [4] 3. Idiopathic short stature (ISS) 4. Small for gestational age infants with catch-up growth, although they may have a short stature their growth usually catches up after two years of age [5]. As for the pathologic causes, 1.undernutrition[6] 2.systematic disease such as renal, pulmonary or cardiac impairments, metabolic disorders, endocrine conditions, etc. Come to mind. [3,7,8]

Other important reasons are genetic diseases with effects on growth, like Turner syndrome, Noonan syndrome, silver Russell syndrome, Short Stature homeobox (Shox) gene mutations, etc. [9–11]. The Differentiation of pathologic reasons from non-pathologic causes is essential, yet some pathologic causes may present exactly similar to non-pathologic causes hence their distinction is difficult. There are hundreds of different genetic variations with small effects on the height of a person [4]. Yet some mutations tend to greatly influence our adult height, One important example of these are shox mutations, interestingly these patients are diagnosed with ISS that nearly comprise 4% of all patients diagnosed with ISS [1] Shox mutations are just one example, many monogenic disorders affect our growth with no other sign or symptom present at the time of evaluation and thus are diagnosed as ISS [12]. ISS is defined as a height of minus 2 SD (the FDA criterion is −2.25 SD) or more below the average for that sex and age with the absence of any diseases and underlying causes that explain the short stature [1,2,13]. By this definition about 80% of children

with short stature are diagnosed with ISS due to not finding any other underlying cause [1,14] yet a proper medical evaluation may result in identifying an underlying cause in up to 40% of the patients[4], when a single gene mutation is suspected in a patient with short stature single gene based tests are indicated [12] and these tests lead to a proper diagnosis. But what if a patient with short stature and concomitant genetic defects has no indicating phenotype, no sign and symptom of any kind? With most of them being monogenic defects as seen in many cases, how should we approach and treat these patients? Hormonal therapy Is FDA approved for treating ISS patients although treatment for all patients labelled as ISS may result in a large number of expenses for the patient and the society, not to forget some patients marked as ISS have some underlying pathologies that hormonal therapy is not advised if not contraindicated.

In patients 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) in ISS patients is very useful and efficient to determine the cause of short stature [15] and helps us to find monogenic mutations that greatly affected the patient's height, especially in patients that were incorrectly diagnosed as ISS due to their significant similarity that makes it almost impossible to distinguish them from one another with clinical examinations and without a proper genetic study, thus WES helps us to aim for the correct treatment and reducing all expenses.

In this study, we try to determine the efficacy of WES in the correct diagnosis of ISS patients and the cost benefits that it may have for the patient and the society and most importantly the best method for approaching a patient with ISS.

Materials And Methods

patient selection and recruitment

We assessed All children under the age of fifteen years labelled as ISS which were referred to any of our clinics (clinics under supervision of Alborz University of medical sciences) and extracted their sex, age, weight, parental heights, and past medical histories from their medical records, then we ordered routine blood tests and electrolytes and evaluated their thyroid function, growth hormone (GH) levels and Insulin-like growth factor 1 (IGF1) levels. After completion of the examinations of the initial patients and rolling out all systemic and syndromic causes of short stature, 14 of them where confirmed to be ISS and thus were allowed in our study, patients with other medical comorbidities and dimorphisms were included in the study as long as these other conditions had no relation to their short stature we excluded all children with intrauterine growth retardation from the study. After the approval of this study in the university's research committee and ethics in research committee of medical sciences, we explained the study to the participants and their legal guardians then a written consent was obtained from all of them.

Afterwards, we pooled blood specimens from the patients and ordered whole-exome sequencing (WES).

WES

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

Data analysis

we used SPSS version 19 for our data analysis, x2 And Fisher's exact test was used for comparing and categorising of means, and a p-value of less than 0.05 was considered statistically significant.

Results

From the patients tested, only five of them had utterly normal WES results and thus were correctly diagnosed as ISS. Eight of them had mutations that indeed could be the cause of their short stature. And one patient had a mutation unrelated to his short height, thus diagnosed correctly as ISS. The mutations are shown in table 1

mutations of unknown significance.

The first patient, an eight-year-old female with a height of 112cm and –2.8 SD for her age with normal height parents, was heterozygous for GHSR gene (NM_198407 exon2: c.847>T) leading to amino acid change p.R283. This gene encodes a member of the G-protein receptor family. [16] although this mutation is of uncertain significance, and its inheritance could be both autosomal recessive (AR) or autosomal dominant (AD) [16] pathologic mutations of this gene results in isolated partial growth hormone deficiency (GHDP) [17], which result in growth delay and short stature and sometimes episodes of abdominal pain, vomiting and ketosis and hyperglycemia may accrue [18]. In our case this mutation of uncertain significance with its concomitance with the patient's phenotype and her low levels of GH, we could conclude that that the mutation is likely pathologic and her short stature may be associated with this mutation.

Due to her GH levels we began hormonal therapy for her and the response was well.

The second patient a 10 year old female with a height of 112 cm and –4.2 for her age and a short mother with the height of 144 cm was heterozygous for CLCN5 gene (NM_001127898 exon14: c.2333T>G) leading to amino acid change p.L778R. This gene provides instructions for making a protein called CLC–5 [19] which transports chloride ions across cell membranes and plays an important role in proximal tubule cells of the kidney. It is inherited through an X-linked recessive pattern[19]. Pathologic mutations of this gene are related to dent disease which is a chronic kidney disorder and is almost exclusively seen in males and results in kidney failure[19]. This mutation also affects serum calcium and vitamin D levels.[20], it has been observed that some female carriers could manifest a few of these conditions signs and symptoms due to random X-chromosome inactivation [21,22]. Short stature is seen in many patients with Dent disease [22]. If carriers present some of these signs and symptoms of this condition, maybe our patient's short stature is the result and one of the signs of this mutation. With her mother being positive for the same mutation and its concomitance with short stature in her mother as well, it's highly likely that this mutation is the cause of her short stature.

Despite her normal GH levels we began hormonal therapy for her and the results were poor as if she was resistant to hormonal treatment Thus it could be concluded that hormonal therapy may not be indicated in this condition

The third patient a two-year-old female with short stature, microcephaly and hearing loss that is homozygous for c.395T>G in exon4 of the CLPP gene (NM_006012) that are inherited through AR-pattern and is associated with Perrault syndrome type 3 [23]. There were no studies on functional effects of this specific mutation, yet with the patient's phenotype and symptoms that are mainly seen in pathologic mutations of Perrault syndrome, likely, this mutation is also pathologic. Perrault syndrome type 3 is a rare condition that may present with different signs and symptoms and affects both male and females, a key feature of the disease is hearing loss as in our patient[23]. Females with this condition may have ovarian dysgenesis with normal external genitalia. Patients may also suffer from neurological conditions such as ataxia, peripheral neuropathy, and intellectual disability,[23].short stature is

another sign of this condition[23].not to forget sometimes low levels of GH is seen in these patients [24] It is highly likely that our patient's short stature with hearing loss and microcephaly resulted from Perrault syndrome. Due to our patient's low levels of GH, we initiated hormonal therapy, but sadly, we observed no significant difference after treatment. Thus it could be concluded that hormonal therapy may not be indicated in this condition

The other patient a ten and a half-year-old male with a height of 126 cm and –2.2SD for his age and hearing loss had three different mutations, the first one is a heterozygous variant in TMPRSS3 (NM_032404.2 exon5: c.266G>A) which leads to amino acid change p.R89H, the second one is a heterozygous variant in HOMER2 gene (NM_199330 exon3: c.188C>T) that results in amino acid change p.P63L. The third one is 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 may cause autosomal recessive deafness (DFNB8)[25,26]. Since it has an autosomal recessive pattern and our patient is heterozygous for this gene, it is highly unlikely as a reason for his hearing loss. The HOMER2 gene encodes a dendritic protein from the homer family and mutations in this gene may cause autosomal dominant deafness (DFNA68) a postlingual onset sensory hearing loss resulted from neural receptors damage in the inner ear [26]. Although the mutation in our patient is of uncertain significance due to its AD inheritance and our patient's condition, this mutation is the likely cause of his hearing loss. The third gene, FGFR3 provides instructions for making a protein called fibroblast growth factor receptor 3, mutations in this gene may cause hypochondroplasia which is a form of short-limbed dwarfism [27] as seen in our patient with very mild short limbs and the predicted height for these patients range between 138 to 165 cm in males [28] although this mutation is of uncertain significance and no studies where done on the effects of this particular mutation, due to our patients phenotype and its AD pattern of inheritance it is highly probable that this mutation is the cause of his short, stature.

Although the patients GH levels were normal we began Hormonal therapy but the results were not as expected thus continuance of hormonal therapy for this patient is questionable.

Pathologic mutations

the first one a 5.3-year-old boy with a height of 99 cm and –2.2SD for his age and a short father with the height of 150 cm with no other medical condition was heterozygous for UROD gene (NM_000347 exon9: c. 912C>A) with an AD or AR inheritance that leads to amino acid change p.N304K.this gene provides instruction for making uroporphyrinogen decarboxylase enzyme [29] and thus pathologic mutations in this gene disrupts chemical steps that leads to heme production resulting in porphyria[30]. There are several types of porphyria distinguished by their genetics and signs and symptoms, in our case the patients genetics was known to cause a type of porphyria similar to porphyria cutanea tarda called porphyria hepatoerythropoietic (HEP) (that primarily effects the skin, when exposed to sun light the skin becomes fragile and blistered thus increasing the risk of infection scaring and pigmentation)[30,31], some signs and symptoms of HEP are osteolysis and shortening of distal phalanges and sclerodactyly and joint deformities that progress over time[32] although at first it may seem like that this condition is not the cause of our patients short stature. Short stature can be seen in other types of porphyria (congenital erythropoietic porphyria)[33] and in one case report a patient with HEP had a noticeable short stature [34] although no clear path between short stature and HEP has been found due to the rarity of this disease, only very few patients are described with this condition, it seems that it's not by chance that short stature has been seen in two patients with such a rare disease, mainly when his father tested positive for this mutation and he had short stature as well, yet indeed further studies are required.

Despite the patient's normal GH levels, we began hormonal therapy, but the response was feeble thus it could be concluded that GH therapy in this condition is not indicated

The second patient with a pathologic mutation was 2.6-year-old male with the height of 84cm and SD of −2.2 for his age and normal height parents, was heterozygous for RYR1 gene (NM_000540 exon15: c. 1589G>A) that leads to amino acid change p.R530H and is inherited through AD pattern[35,36]. RYR1 provides instructions for making ryanodine receptor 1 protein that transports calcium ions and plays a critical role in muscles and movement[35,37], mutations in this gene makes patients prone to malignant hyperthermia, a condition called malignant hyperthermia susceptibility 1 [38] especially when underwent an invasive surgery or general anesthetics [39]. At first it may seem like that this mutation is not responsible for our patients short stature, but on the contrary it has been observed that RYR1 gene mutation can result in short stature (King–Denborough syndrome)[40,41] to answer if this mutation is the reason of our patients short stature further studies are indicated yet we can't rule it out as a probable cause. Other coexisting conditions with our patient's condition were seizures, as previously reported in another paper we presume his seizures could be due to stress induced hyper pyrexia [42]

despite the patient's normal GH levels we began hormonal therapy and we observed a well response

the third patient an 11 month year old girl with short stature and a SD of −3 for her age and normal parents was heterozygous for SMAD4 gene (NM_005359 exon 12: c. 1498 A>G) that leads to amino acid change p.1500V. And is of AD pattern. [43] SMAD4 provides instructions for making SMAD4 protein which is part of transforming growth factor betas (TGF-β) signaling pathway and acts as both a transcription factor and tumor suppressor thus controlling the activity of some particular genes and preventing uncontrolled cell division and cancer.[44,45] the mutation in our patient is associated with Myhre syndrome that is a condition that may result in short stature and it has a characteristic facial feature [43,46] other signs and symptoms include: hearing loss, joint stiffness, limited joint mobility fibrosis, cardiovascular problems, respiratory complications, and muscular and skeletal problems. And short stature [43,47]. The patients' phenotype and development of the conditions mentioned above varies and depends on mutation types and domains [43]. In our patient beside some mild facial features and short stature abnormal TSH changes and lower normal limits of insulin-like growth factor 1 (IGF−1) was 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 other patient a two-year-old female with short stature and an SD of −2.2 and normal parents was heterozygous, for COL9A3 gene (NM_001853: exon 18:c. 920G>A) that leads to amino acid change p.G307D and is associated with multiple epiphyseal dysplasia. (OMIM 600969) [48,49]. And is inherited through an autosomal dominant pattern [50].

Multiple epiphyseal dysplasia (MED) is a mild and variable condition in which irregular ossification of the epiphyseal cartilage occurs [50]. Common signs and symptoms may include early-onset arthritis, knee and hip cartilage anomalies and short stature [49,50]. The patient's phenotype correlated with her genotype and thus this mutation is the cause of her short stature.

Although the patient had an average level of GH, we began GH therapy, but despite our best efforts, we observed a week response; thus, we might conclude that hormonal therapy in this patient is not indicated.

The last patient, a 6.9-year-old boy with a height of 99 cm and an SD of −4.2 for his age with normal height parents, was heterozygous for CFTR gene (NM_000492 exon11: c.1397C>G) resulting in amino acid change p.S466,and is inherited through an AR pattern, mutations in this gene may cause cystic fibrosis [51] although this condition is

inherited through an AR pattern and our patient was heterozygous for the mutation, he did not present any sign and symptom of the disease. It is highly unlikely that his short stature is related to this mutation

despite the patient's normal GH levels We began hormonal therapy and we observed a very good response to GH therapy.

The treatment results are shown in summery in table 2

Discussion

we aimed to determine how many of children labeled as ISS are correctly diagnosed and as a result of wess performed only five had absolutely no pathologic genetic mutations. By WES most of our patient achieved the correct diagnosis which would be impossible to diagnose without WES due to the rarity of their diseases and symptom presentations, thus the reason for their short stature was identified, with the correct diagnosis now we can aim for the correct treatment, when we should and shouldn't initiate GH therapy, and with the correct diagnosis we may predict possible outcomes of GH therapy and its effectiveness. Thus as seen in our study we think WES is essential and needed for the correct diagnosis of all patients labeled as ISS, not only we can find the underlying cause of their short stature we may find other diseases that their signs and symptoms hasn't started yet and we may have a chance to improve the patient's quality of live and reduce the diseases morbidity, as for the patients with short stature correct diagnosis leads to proper treatment.

List Of Abbreviations

*s*tandard deviations (SD), Idiopathic short stature (ISS)Short Stature, homeobox (Shox), whole-exome sequencing (WES), Insulin-like growth factor 1 (IGF1), 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-β), Multiple epiphyseal dysplasia (MED), Porphyria hepatoerythropoietic (HEP), growth hormone (GH), Variant of unknown significance (VUS), X linked recessive (XLR), Heterozygous (het), Homozygous (hom)

Declarations

Ethics approval and consent to participate

After the approval of this study in the university's research committee and ethics in research committee of medical sciences, the study was explained to the participants and their legal guardians then a written consent was obtained from all of them to use the data for 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 analysed 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. N GH and M GH helped in data management, revision and preparing the final manuscript

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 for publication purposes.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author in response to reasonable requests and with the permission of the patient's legal guardians.

Competing interests

The authors declare that they have no competing interests

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Tables

Table1. Detected mutations, pathogenicity in this study and related conditions								
Height SD	Related condition	Variant location	Pathogenicity in literature	Patient zygosity	Variant	Pathogenicity in this study	Inheritance	Mutation
2.8-	Isolated partial GH deficiency	exone2	VUS[4]	het[3]	C.847>T T.R283	pathogenic	AD[1],AR[2]	GHSR NM_198407
4.2-	Dent disease	exon14	VUS	het	c.2333T>G p.L778R	pathogenic	XLR[5]	CLCN5 NM_001127898
4-	Perrault syndrome	exon4	VUS	hom[6]	c.395T>G	pathogenic	AR	CLPP NM_006012
2.25-	DFNB8[7]	exon5	pathogenic	het	c.266G>A p.r89h	pathogenic	AR	TMPRSS3 NM_032404.2
	DFNA68[8]	exon3	VUS	het	c.188C>T p.p63l	pathogenic	AD	homer2 NM_199330
	hypochondroplasia	exon8	VUS	het	c.992G>A	pathogenic	AD	FGFR3 NM_001163213
2.25-	HEP[9]	exon9	pathogenic	het	912C>A p.n304k	pathogenic	AD,AR	UROD NM_000347
2.25-	malignant hyperthermia susceptibility 1	exon15	pathogenic	het	.c 1589G>A p.R530H	Likely Pathogenic	AD	RYR1 NM_000540
3-	Myhre syndrome	exon 12	pathogenic	het	c. 1498 A>G p.1500V	pathogenic	AD	SMAD4 NM_005359
2.25-	MED[10]	exone 18	pathogenic	het	c. 920G>A p.G307D	pathogenic	AD	COL9A3 NM_001853
4.2-	Cystic fibrosis	exon11	pathogenic	het	c.1397C>G p.s466	pathogenic	AR	CFTR NM_000492

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. Porphyria hepatoerythropoietic, 10. Multiple epiphyseal dysplasia

Table 2		
Hormone therapy response	Related condition	mutation
excellent	Isolated partial GH deficiency	GHSR
poor	Dent disease	CLCN5
poor	Perrault syndrome	CLPP
weak	hypochondroplasia	FGFR3
poor	HEP[1]	UROD
good	malignant hyperthermia susceptibility 1	RYR1
weak	Myhre syndrome	SMAD4
weak	MED[2]	COL9A3
Excellent	Cystic fibrosis	CFTR

1. Porphyria hepatoerythropoietic, 2. Multiple epiphyseal dysplasia