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ORIGINAL ARTICLE

Genetic *IGF1R* defects: new cases expand the spectrum of clinical features

E. N. Gonc[1] · Z. A. Ozon[1] · S. Oguz[2] · S. Kabacam[3] · E. Z. Taskiran[2] · P. O. S. Kiper[3] · G. E. Utine[3] · A. Alikasifoglu[1] · N. Kandemir[1] · O. K. Boduroglu[3] · M. Alikasifoglu[2]

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Abstract

Purpose We aimed to identify the phenotypic variability of *IGF1R* defects in a cohort of short children with normal GH secretion gathered through the last decade.

Patients and methods Fifty children (25 girls) with short stature and a basal/stimulated growth hormone (GH) over 10 ng/ml having either a low birth weight or microcephaly were enrolled. MLPA and then Sanger sequence analysis were performed to detect *IGF1R* defects. The auxological and metabolic evaluation were carried out in index cases and their first degree family members whenever available.

Results A total of seven (14%) *IGF1R* defects were detected. Two *IGF1R* deletions and five heterozygous variants (one frameshift, four missense) were identified. Three (likely) pathogenic, one VUS and one likely benign were classified by using ACMG. All children with *IGF1R* defects had a height < − 2.5SDS, birth weight < − 1.4SDS, and head circumference < − 1.36SDS. IGF-1 ranged from − 2.44 to 2.13 SDS. One child with a 15q terminal deletion had a normal phenotype and intelligence, whereas low IQ is a finding in a case with missense variant. Two parents who carried *IGF1R* mutations had diabetes mellitus, hypertension and hyperlipidemia, one of whom also had hypergonadotropic hypogonadism.

Conclusion We found a deletion or variant in *IGF1R* in 14% of short children. Birth weight, head circumference, intelligence, dysmorphic features, IGF-1 levels and even height are not consistent among patients. Additionally, metabolic and gonadal complications may appear during adulthood, suggesting that patients should be followed into adulthood to monitor for these late complications.

Keywords Short stature · Intrauterine growth retardation · Insulin-like growth factor 1 receptor · Insulin-like growth factor 1 · Microcephaly · Small for gestational age

Introduction

Persistent short stature affects 10–15% of children born small for gestational age (SGA) [1]. Genetic, chromosomal and acquired disorders affecting the fetus may lead to both poor fetal growth and postnatal growth failure [2]. Genetic factors usually cause persistent short stature. Two major proteins associated with pre- and postnatal growth are IGF-1, and its receptor IGF-1 receptor (IGF-1R). IGF-1 promotes growth primarily by binding to IGF-1R and is expressed in fetal tissues as early as the formation of the zygote. Thus, defects in either IGF-1 or its receptor can result in poor pre- and postnatal growth [3, 4].

Animal studies have shown that the average birth weight of *IGF1R*-null mice is 45% of that in wild-type alleles, and the mutants die of respiratory failure just after birth [5]. Heterozygous mutant mice are phenotypically normal [5]. Heterozygous mutations in *IGF1R* in the human cause a syndrome of resistance to IGF-1 (MIM # 270450) with intrauterine and postnatal growth failure. Since the first report of patients with *IGF1R* defects in 2003, accumulating evidence has revealed a human growth phenotype characterized by

✉ E. N. Gonc
ngonc@hacettepe.edu.tr

[1] Department of Pediatric Endocrinology, Hacettepe University Faculty of Medicine, 06100 Ankara, Turkey

[2] Department of Medical Genetics, Hacettepe University Faculty of Medicine, Ankara, Turkey

[3] Department of Pediatric Genetics, Hacettepe University Faculty of Medicine, Ankara, Turkey

low birth weight, failure of catch up growth, microcephaly and relatively high serum IGF-1 [6, 7].

The gene encoding IGF-1R (*IGF1R*) is located on the distal long arm of chromosome 15. Heterozygous terminal 15q deletions or ring chromosome 15 encompassing *IGF1R* may lead to clinical findings similar to *IGF1R* gene mutations, particularly intrauterine growth retardation and postnatal short stature. However, these patients usually have additional findings involving different organ systems, attributed to concomitant monosomy of the flanking genes in that region. Intellectual impairment, cardiac defects, genitourinary anomalies, skeletal malformation and facial dysmorphic features have been frequently reported in patients with *IGF1R* deletions [8–10].

Case selection for the analysis of *IGF1R* defects is still a challenge for pediatric endocrinologists. Patients with short stature and a history of low birth weight as well as a normal GH response to stimulation tests may be candidates for the investigation of *IGF1R* defects. Previous studies to determine the prevalence of *IGF1R* defects have yielded variable results. This may be related to patient selection as well as the choice of genetic methods. Patient populations are usually confined to short children with unexplained intrauterine growth retardation. Some studies have also used other phenotypic features. Moreover, the genetic analyses differ [6, 9, 11–13]. Studies solely screening mutations in the *IGF1R* gene can miss *IGF1R* deletions. In contrast, sole analysis of copy number changes can detect *IGF1R* deletions, but would omit *IGF1R* mutations.

IGF1R defect is not only uncommon, but its clinical and laboratory findings have also not been thoroughly defined. Restriction of inclusion criteria in study populations have led to the inclusion of patients with predefined characteristics and carry a risk of confining the clinical variability of the condition. Thus, in the current study, we aimed to identify the phenotype variability in a cohort of children presumed to be *IGF1R* defect gathered over the last decade.

Patients and methods

Study population

The cohort consisting of 50 children (25 girls, 25 boys) was recruited from a database of cases with short stature (height ≤ 3rd percentile) and normal GH secretion who had additionally at least one of the two major phenotypic features, either intrauterine growth retardation or microcephaly. No chronic or inflammatory disorder causing growth retardation was identified in any of the children. All children had basal/stimulated growth hormone (GH) over 10 ng/ml. Serum IGF-1 level was measured however; this was not used as an inclusion/exclusion criterion. The auxological and laboratory parameters of the cohort are shown in Table 1.

The clinical, auxological parameters as well as blood samples for IGF-1 levels and metabolic measurements were collected from the parents and siblings who were available; the pedigrees were recorded. Genetic analysis was carried out in whole cohort and the family members of the affected children.

The study protocol was approved by the local institutional review board (Decision No. GO 17/231-15), and all participants and their parents provided written informed consent.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes by salt precipitation. In the first step, MLPA analysis was performed in each of the patients to detect any copy number change in exons of *IGF1R*. MLPA analysis was performed with the SALSA MLPA® Probemix P217-B2 kit, following the manufacturer's instructions (MRC-Holland, Amsterdam, Netherlands). In the case of any copy number change detected by MLPA, a microarray analysis was carried out to determine the exact breakpoints on DNA. Microarray analyses were performed using the Affymetrix platform (CytoScan Optima and CytoScan HD) according to the manufacturer's instructions. In the second step, Sanger sequence analysis was performed to scan exonic or splice site mutations in the *IGF1R* gene in all patients with normal MLPA results (the sequences of primer pairs and reaction conditions are available upon request). Variants were classified using mainly the consensus recommendations of ACMG [14]. However, there were some shortcomings in the classification of variants according to the 2015 ACMG criteria. Thus, new Sherloc criteria developed in 2017 were also used in the classification [15]. In the new criteria, the frequency of the variant is prioritized and the positive clinic is considered to be more significant than negative functional test. In addition, 3B score (which means three points of

Table 1 Clinical and laboratory characteristics of the cohort (50 patients)

	Mean (min–max)
Clinical characteristics	
Age (years)	10.3 (2.5 to 16.1)
Birth weight SDS	−2.7 (−6.7 to −1.2)
Height SDS	−3.14 (−5.96 to −1.8)
BMI SDS	−1.23 (−4.69 to 1.62)
Head circumference SDS	−2.64 (−7.80 to −0.15)
Midparental height SDS	−1.39 (−5.82 to 1.55)
Laboratory findings	
IGF-1 SDS	−0.34 (−4.8 to 4.3)

benign characteristics) was considered sufficient for likely benign, and 4P (which means four points of pathogenic characteristics) score was required for likely pathogenic. This asymmetric condition prevents a variant to be considered as pathogenic just because it is rare. However, in such a scoring system difficulty in determining pathogenicity of the variants is inevitable when majority of the disease causing variants are missense changes in a gene, which is also true for IGF1R. Thus, missense variants are more likely to be classified as benign or VUS at best according to ACMG and Sherloc [14, 15]. What is more, short stature is not only a common trait, but it is a multifactorial (multigenic) one as well. Since IGF1R defects are a rare cause of short stature, and stature is affected by more than one gene, lack of segregation should not be considered as a strong evidence to make the case for a benign variant. Same caveat was stated in the 2015 ACMG criteria [14]. It has also been supported by the study of Giabicani et al. [16] in which two heterozygous *IGF1R* missense variants, classified as VUS due to non-segregation, were found to be likely pathogenic after functional studies. It is suggested that development of more focused guidance regarding the classification of variants in specific genes in specific disease groups might be necessary given that the applicability and weight assigned to certain criteria may vary by gene and disease [14]. Thus, gene-specific variant classification approach will probably be more accurate, but unfortunately unavailable for *IGF1R* defects or growth failure of prenatal onset yet.

In the current study, two cases had deletion and were classified as pathogenic. Classification of the identified missense variants according to ACMG and Sherloc are shown in Table 2.

Growth hormone stimulation tests

GH stimulation tests were carried out early in the morning after a 12-h fast. Blood was drawn before and 60 and 90 min after the administration of levodopa (10 mg/kg, max 500 mg) per oral. A clonidine stimulation test was performed if the peak GH response was < 10 ng/ml during the L-dopa stimulation test. Blood was drawn at 0, 30, 60, 90, 120 min after clonidine hydrochloride (150 μg/m^2; max: 200 μg) administration orally. A peak GH level ≥ 10 ng/ml was considered a normal GH response to pharmacologic stimulation.

Auxological parameters and calculations

Standing height was measured in patients older than 2 years using a wall-mounted stadiometer that measured to the nearest 0.1 cm. Body mass index (BMI) was calculated by dividing the body weight in kilograms by the square meters of height. Height-SDS and BMI-SDS were calculated [17, 18]. Bone age was assessed using the Greulich–Pyle method, and puberty was assessed using Tanner staging [19, 20].

Hormone assays

GH was measured using an immunochemiluminometric assay (ICMA), which was performed on an IMMULITE 2000 System (Siemens, England). The intra- and interassay CVs were 3.7 and 5.7%, respectively, and the analytic sensitivity of the test was 0.01 ng/ml. The serum IGF-1 level was measured using Beckman Coulter trademark assay with the immunoradiometric (IRMA) method. The intra- and interassay CVs of the IGF-1 level were 2.6 and 4.5%, respectively, with an analytical sensitivity of 2 ng/ml. The serum IGF-1 and SDS were calculated using the reference tables for age and gender [21].

Results

A total of 7 (14%) cases with *IGF1R* defects were detected in a cohort of 50 children. The clinical, biochemical and molecular details of the patients and the affected parents are summarized in Tables 3 and 4, respectively. *IGF1R* deletions were identified in 2 (4%) out of 50 cases by MLPA and microarray analyses. These were de novo mutations since the parents did not carry the deletions. The two cases with a deletion in the distal part of chromosome 15q (cases 1 and 2) also had concomitant duplications in chromosomes 9p and 1p, respectively. These duplications were not considered to contribute to the clinical presentations. Sanger sequencing revealed heterozygous variants in 5 (cases 3–7) out of 48 cases with normal MLPA. A schematic presentation of these variants on *IGF1R* is shown in Fig. 1; four were missense, and one was a frameshift variant.

The patient in case 1 first drew clinical attention for short stature (height 62 cm, − 3.4 SDS) at 11 months of age. No intellectual disability or dysmorphic finding was noted. The echocardiographic examination and renal scan were normal. The patient's parents were third degree cousins. GH treatment was administered at the age of 4.2 years, and her growth velocity increased from 4 cm per year to 8 cm per year.

The patient in case 2 had a 15q terminal deletion as well as minor dysmorphic features, such as bilateral epicanthal folds, a high-arched palate and sandal gap toes. He had normal hearing and his IQ level was 45. Cardiac examination was normal.

Case 3 had a heterozygous missense variant (c.236C > T) that was classified as likely pathogenic in ACMG. The mutation was previously reported in a patient with short stature [22]. The patient had a twin brother with a birth weight of 1600 g. The twin brother who did not carry the variant had a

Table 2 Interpretation of sequence variants in five cases according to ACMG and Sherloc [14, 15]

Patient	Mutation	References	Variant ınformation	Parental origin	ACMG classification	Used criterias
Case 3	c.236C > T p.Thr79Met	[20]	ExAC: (-) GnomAD: 5 allele count (allele frequency: % 0,00,177) allele number: 282814) MT: 'disease causing' PolyPhen-2: probably damaging SIFT: damaging Evolutionary conserved	Mother ht and short (144 cm; − 2.9 SDS) Father wt (167 cm; − 1.4 SDS) Sibling wt (164.5 cm; − 1.5 SDS) No consanguinity	Likely pathogenic	PM2, PP1, PP3, PP4, PP5
Case 4	c.54_57delTCTC p.Leu19Profs*27	This study	Frameshift R89stop: pathogenic	Mother wt (154 cm; − 1.4 SDS) Father ht and short (151 cm; − 3.6SDS) Consanguinity (+)	Pathogenic	PVS1, PM2, PP1, PP3, PP4
Case 5[a]	c.1382G > A p.Arg461His	This study	p.Arg461Leu pathogenic ExAC: 4 ht, hom (-) GnomAD: 5 allele count (allele frequency: % 0,00,199) allele number: 251160) MT: 'disease causing' PolyPhen-2: benign SIFT: tolerated Evolutionary conserved	Mother wt and short (149 cm; − 2.2 SDS) Father ht and short (165 cm; -1.6 SDS) Sibling wt (125 cm; − 1.9 SDS) No consanguinity	Likely pathogenic	PM2, PM5, PP1, PP4
Case 6	c.2111A > G p.Lys704Arg	This study	ExAC: (-) GnomAD: 1 allele count (allele frequency: % 0,0031 allele number: 31386) MT: 'disease causing' PolyPhen-2: possibly damaging SIFT: tolerated Evolutionary conserved	Mother ht and short (152 cm; − 1.9 SDS) Father ht (171 cm; − 0.8 SDS) Consanguinity (+)	VUS	PM2, PP1, PP3, PP4
Case 7[b]	c.2032A > G p.Ile678Val	This study	ExAC: (-) GnomAD: 1 allele count (allele frequency: % 0,000,398) allele number: 251476) MT: 'disease causing' PolyPhen-2: benign SIFT: tolerated Evolutionary conserved?	Mother ht (161 cm; − 0.2 SDS) Father wt (178 cm; 0.2 SDS) Sibling wt and short (− 1.9 SDS) but taller than the index case, dysmorphic features are very similar Consanguinity (+)	Likely benign	PM2, PP4 BP5

Variants that are absent (PS4) or present at extremely low frequencies in control subjects from the ExAC (PM2) are considered as strong or moderately strong evidence for pathogenicity, respectively. Sherloc classifies a variant seen in ≤ 8 total alleles in healthy subjects (ExAC or GnomAd) as rare enough to be considered in the pathogenic range for autosomal dominant disorders, as long as high quality and abundant (> 15,000 alleles) data are present. Also when a missense variant is previously reported to be pathogenic, another variant in the same locus resulting in a different amino acid change is considered to be moderate evidence for pathogenicity (PM5). Familial segregation (PP1), multiple pieces of computational evidence supporting a deleterious effect on the gene (PP3), presence of at least two specific clinical characteristics out of three (PP4), and report of pathogenicity for same variation previously without provision of the evidence (PP5) were all considered as supportive evidence for pathogenicity of a missense variation. Lack of segregation (BS4) was considered as supportive evidence for benign impact rather than strong, since short stature is a common trait and a multigenic one. Also variant in a case with an alternate molecular basis for disease (BP5) was supportive evidence for benign impact

ExAC Exome Aggregation Consortium, *GnomAD* Genome Aggregation Database, *MT* Mutation Taster, *ht* heterozygote, *hom* homozygote, *wt* wild-type, *ACMG* American College of Medical Genetics, *VUS* variant of uncertain significance

[a]*In silico* analysis suggested benign impact in two out of three algorhitms for case 5; however, when the number of prediction algorithms were increased to ten (using VARSUN), computational evidence suggested pathogenic variant, and *in silico* results did not change the resultant classification and thus was not included in the overall analysis

[b]In case 7, since a sibling with milder but positive phenotype with wild-type *IGF1R* as well as possible AR transmission was present, BP5 was considered. However, caveat was short stature is a common trait in a multigenic background, and *IGF1R* defect might be an additional abnormality in this case. Still, we categorized the patient as likely benign using BP5

Table 3 Clinical and laboratory features of seven cases involving *IGF1R* defects

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Variant	(15q26.2q26.3 × 1), (9p24.3p13.1 × 3)	(15q26.3 × 1), (1p36.33p36.31 × 3)	c.236C > T p.Thr79Met	c.54-57delTCTC p.Leu19Profs*27	c.1382G > A p.Arg461His	c.2111A > G p.Lys704Arg	c.2032 A > G p.Ile678Val
Allele frequency			5/282,814	novel	5/251,160	1/31,386	1/251,476
Age (years)/gender	3.1/F	12.8/M	13.8/F	16.3/M	13.5/M	15/M	13/M
Birth weight SDS	− 3.1	− 1.4	− 4.7	− 2.4	Low	− 6.8	− 2.9
Ht SDS	− 2.8	− 3.6	− 2.9	− 2.9	− 3.2	− 4.2	− 2.6
BMI SDS	0.98	0.24	0.2	− 1.6	− 1.8	− 3.2	− 1.6
HC SDS	− 2.2	− 2.4	− 2.0	− 1.4	− 1.5	− 3.3	− 5.5
Bone age (years)	3	10	15	14.5	11	10	11.5
Puberty stage (Tanner)	1	1	5	4	1	3	2
Maternal Ht SDS	− 0.3	− 2.4	− 2.9[a]	− 1.5	− 2.2	− 1.9[a]	− 0.2[a]
Paternal Ht SDS	− 1.1	− 1.6	− 1.3	− 3.6[a]	− 1.5[a]	− 0.5[a]	0.5
Adult Ht SDS	NA	NA	-3.0	NA	− 1.5	-3.2	NA
Additional findings	No	Dysmorphic findings	No	Past history of chronic diarrhea	No	No	Dysmorphic findings Hypergonadotropic hypogonadism
Intelligence capacity	Normal	Low	Normal	Normal	Normal	Low	Low
Parental Consanguinity	Yes	No	No	Yes	No	Yes	Yes
Basal GH (ng/ml)	2.39	0.151	25.3	0.744	0.458	0.633	3.67
Peak GH (ng/ml)	14.3	11.2	NA	11.7	10	20.3	12.9
IGF-1 (ng/ml)	268	291	671	537	100	112	350
IGF-1 SDS	2.1	1.53	2.13	0.85	− 1.11	− 2.44	0.6
Fasting glucose (mg/dl)	90	95	78	79	86	81	113 (postprandial)
HbA1c (%)	NA	NA	NA	NA	NA	5.5	NA
Hypertension	No	No	No	No	No	No	No

NA not available, *N/A* not applicable, *HC SDS* head circumference standard deviation score, *Ht SDS* height standard deviation score, *BMI SDS* body mass index standard deviation score, *GH* growth hormone

[a]Represents the parent who carries the mutation

normal growth rate and reached an adult height of 164.5 cm (− 1.5 SDS), whereas the patient could never catch up in growth. She had had regular menstrual bleeding since the age of 11. At the age of 19, her physical examination was normal except for short stature. Her mother, who carried the same mutation had also severe short stature and has exhibited hypertension, hyperlipidemia and diabetes mellitus since her late 30s. The mother had also experienced premature menopause at the age of 38 years with FSH 78.2 mIU/ml (normal 3.03–8.08), LH 27.1 mIU/ml (normal 1.8–11.7) and estradiol 13 pg/ml (normal 21–251). The maternal grandmother's height was 145 cm (− 2.9 SDS); she also had diabetes mellitus and hypertension and had died of a hemorrhagic stroke at the age of 48 years. The maternal uncle also had short stature; his height was 160 cm (− 2.3 SDS). Moreover, the maternal grand-grandmother had exhibited short stature and had died of cancer at a young age.

Table 4 Clinical and laboratory features of parents who carried the variants

	Mother of case 3	Father of case 4	Father of case 5	Mother of case 6	Father of case 6	Mother of case 7
Mutation	c.236C > T p.Thr79Met	c.54-57delTCTC p.Leu19Profs*27	c.1382G > A p.Arg461His	c.2111A > G p.Lys704Arg	c.2111A > G p.Lys704Arg	c.2032 A > G p.Ile678Val
Allele frequency	5/282,814	Novel	5/251,160	1/31,386	1/31,386	1/251,476
Age (years)/gender	44/F	47/M	38/M	45/F	45/M	41/F
Birth weight SDS	Low	Low	NA	NA	NA	NA
Ht SDS	− 2.9	− 3.6	− 1.6	− 1.9	− 0.5	− 0.2
BMI (kg/m2)	31.8	21.24	NA	34.9	24.8	NA
HC SDS	− 2.7	− 2.8	NA	NA	NA	NA
Maternal Ht SDS	− 2.85	NA	NA	NA	NA	NA
Additional findings	Hypertension, DM, Hyperlipidemia, premature menopause	Hypertension, DM, hyperlipidemia	No	No	No	No
Intelligence capacity	Normal	Normal	Normal	Normal	Normal	Normal
Parental Consanguinity	Yes	No	NA	No	No	Yes
Basal GH (ng/ml)	< 0.05	< 0.05	NA	0.253	0.849	NA
IGF-1 (ng/ml)	94	140	NA	66	92	NA
IGF-1 SDS	− 2.0	− 0.1	NA	− 2.5	− 2.0	NA
Fasting glucose (mg/dl)	97	325	NA	82	NA	NA
HbA1c (%)	6.2	7.4	NA	NA	NA	NA
Hypertension	Yes	Yes	NA	No	No	NA

NA not available, *N/A* not applicable, *HC SDS* head circumference standard deviation score, *Ht SDS* height standard deviation score, *BMI SDS* body mass index standard deviation score, *GH* growth hormone, *DM* diabetes mellitus

Case 4 had a frameshift variant causing an early stop codon that was previously unreported in the GnomAd data; thus, it was classified as pathogenic. The patient was a 9.8-year-old boy with a height of 121.5 cm (− 2.5 SDS) at admission. He had a history of chronic diarrhea lasting over 2 years with an unknown etiology. Examination of the upper and lower gastrointestinal system with endoscopy and biopsies was normal. His height reached 153.5 cm (− 2.9 SDS) by the age of 16.3 years. His parents were third-degree cousins. His 47-year-old father carried the same variant, had severe short stature and had been diagnosed with hypertension and diabetes mellitus in his early 40s. He had normal gonadotropin and testosterone levels (FSH: 3.7 mIU/ml (normal 0.95–11.95), LH: 5.8 mIU/ml (normal 0.37–12) and testosterone: 235 ng/dl (normal 150–980). The patient's paternal aunt also had short stature, with a height of 145 cm.

The patient in case 5 with a likely pathogenic variant had a height of − 3.2 SDS at the age of 13.5 years; however, he reached an adult height of − 1.5 SDS. His adult height was similar to that of his father, who had the same molecular defect.

The patient in case 6 with a heterozygous missense *VUS* variant had an IQ score of 80 and no significant dysmorphic features. His parents were first cousins. The patient, his mother and father all carried the same heterozygous *IGF1R* variant; however, only the patient and the mother had short

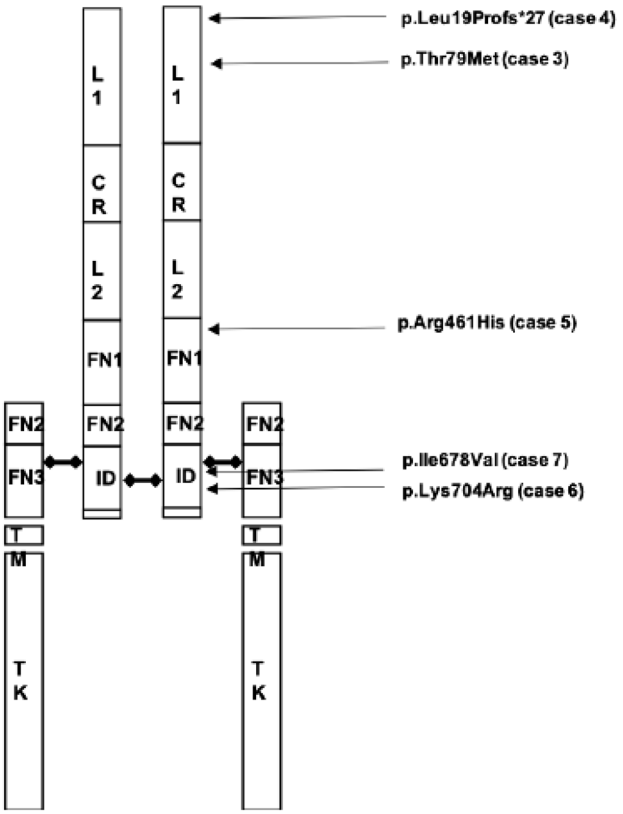

Fig. 1 Heterozygous point mutations in the *IGF1R* gene. L1 and L2, large domains 1 and 2 (leucine-rich repeats); *CR* Cys-rich domain, *FN* fibronectin type III domains, *TM* transmembrane domain, *ID* insert domain, *TK* tyrosine-kinase domain

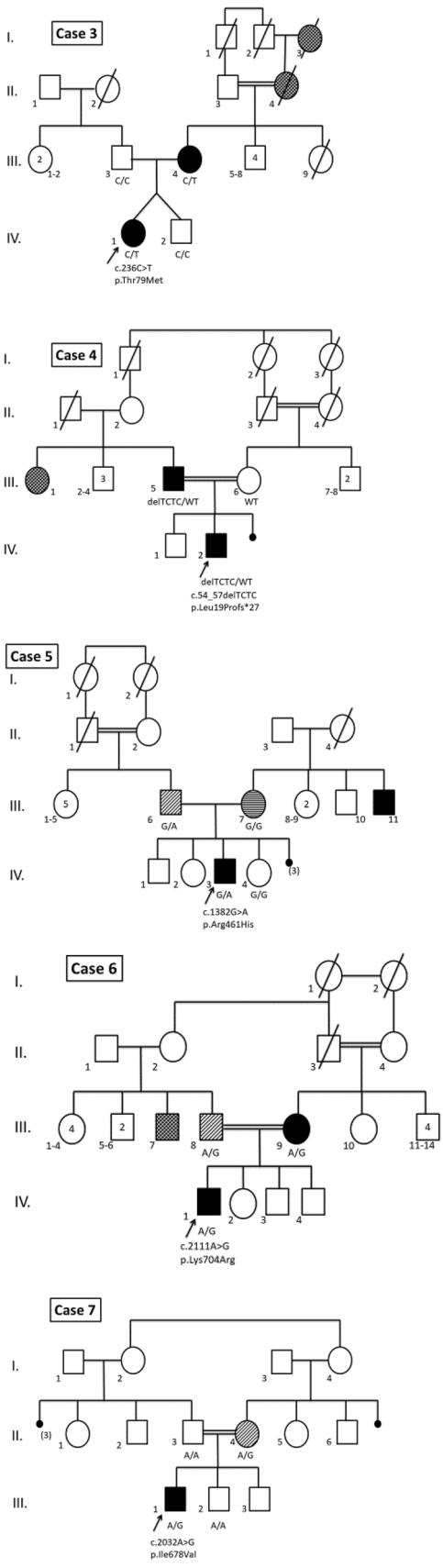

Fig. 2 Pedigree of the five cases involving *IGF1R* variations. Black ▶ circles and squares represent patients with short stature + *IGF1R* mutation. Dotted circles and squares represent family members with short stature + no genetic analysis. Diagonal striped circles and squares represent family members with normal height + *IGF1R* mutation. Horizontal striped circles and squares represent family members with short stature + wild type *IGF1R*. The results of *IGF1R* gene analysis are shown below the cases

stature. Family history revealed that one of the paternal uncles also had severe short stature (155 cm).

Case 7, who had a heterozygous missense variant classified as likely benign, had an IQ score of 46. Bilateral orchiopexy was performed at the age of 7 years. He had wide palpebral fissures and a pointed chin, bilateral clinodactyly at the 5th finger and syndactyly between the second and third toes. At the age of 13 years, serum gonadotropins were elevated [FSH: 56 mIU/ml (normal: 0.95–11.95 mIU/ml), LH: 8.57 mIU/ml (normal: 0.57–12.07 mIU/ml), T: 72.9 ng/dl (normal: 36–633). His 3.5-year-old younger brother had similar findings with low birth weight, severe microcephaly, dysmorphic features and intellectual disability. His height was at 3rd percentile. He had a wild-type *IGF1R*. So, the two brothers having first degree-related parents shared similar pathologic characteristics that cannot be attributed to *IGF1R* defect alone. Another autosomal recessive disorder may be considered in this family. However, *IGF1R* defect might contribute to the severity of short stature in case 7.

The pedigrees of the five children with *IGF1R* variants are shown in Fig. 2.

Discussion

Studies screening for *IGF1R* defects reported so far have shown heterogeneity in population characteristics and genetic analysis tools [6, 9, 11–13]. The sequential analysis of copy number using MLPA and microarrays, followed by Sanger sequencing in the current study, enabled a more effective delineation of the frequency of *IGF1R* defects. A total of seven (14%) molecular defects in *IGF1R* included pathogenic and likely pathogenic variants in 6% (3/50), deletions in 4% (2/50), VUS in 2% (1/50), and likely benign in 2% (1/50) of the patients.

Patients with *IGF1R* defects may have distinct clinical features regarding whether the defect is due to a deletion or a mutation. Terminal deletions of the long arm or the ring form of chromosome 15, which lead to deletion in *IGF1R,* are generally associated with intellectual disability, dysmorphic features and the involvement of several organs in addition to intrauterine growth retardation, short stature and microcephaly. Clinical presentation depends on the extent of the deleted genes along with the *IGF1R* [8–10]. In contrast, *IGF1R* mutations are generally reported to lead

to auxological findings without involvement of intellectual development and other organs [23]. However, it should be kept in mind that some patients with 15q terminal deletions may have normal appearance and intelligence without organ involvement (as in our first case), most likely due to very small deletions. Consistently, additional involvement of genomic regions poor in functional genes would not cause any further clinical features, as in our first two cases with duplications at chromosome 9p, and 1p. Interestingly, some patients with *IGF1R* missense variants show additional features such as low intellectual capacity (case 6), gastrointestinal (case 4) and gonadal (the mother in case 3) involvement. Similar discordance has also been described in three large series of *IGF1R* defects [12, 16, 22].

Intrauterine growth retardation, microcephaly and short stature are hallmarks of *IGF1R* defects. However, in the present cohort, case 2 had a birth weight of − 1.4 SDS and cases 4 and 5 had head circumferences in the lower half of the normal range. This is in line with findings from three large cohorts [16, 22, 24] as well as other individual cases in the literature [11, 12, 25, 26]. Similarly, postnatal growth and adult height of *IGF1R* carriers also vary in a wide range. One of our three index cases, who attained adult height, reached a low-normal final height, similar to his father with the same molecular defect. In addition, case 6 attained an adult height of 153.5 cm (− 3.2 SDS), in contrast to his parents (with the same heterozygous variants) who were not so short. Therefore, a short-normal adult height can be a feature of *IGF1R* defects. There is also a wide variation in height potential and adult height among patients with *IGF1R* defects, ranging between − 5.5 and 1 SDS [16, 22]. The heights of the carrier parents and family members also had significant variability in adult height, ranging from very short stature to a normal height [12, 22, 27]. Thus, the scoring system proposed by Walenkamp et al. [22] for *IGF1R* defect should be revised to account for these clinical variabilities.

A reliable laboratory marker indicating IGF-1R deficiency is yet unavailable. IGF-1, as well as basal/peak GH levels, is far from being a marker for diagnosis, since there is a great variation in their levels as in our cases [22, 25, 28–30]. Patients with undernutrition tend to have low IGF-1 levels, which may be restored with weight gain [30, 31].

In the present cohort, two families with *IGF1R* mutations had a significant burden of early-onset cardiovascular risk factors. The mother (with a heterozygous missense mutation), grandmother and maternal uncle of case 3 all had severe short stature and relatively early onset diabetes mellitus, dyslipidemia and hypertension (in their late 30s or early 40s). Moreover, the grandmother died of stroke at the age of 48 years. Case 4 also had a father (with a heterozygous frameshift mutation) and a paternal aunt who had very short stature and were diagnosed with diabetes mellitus and hypertension in their early 40s. Animal studies suggest a possible link between *IGF1R* defects and glucose metabolism. *Igf1r* ± male mice had higher glucose levels during fasting and after a glucose load [32]. Knockout models of *Igf1r* on β cells show impaired glucose-dependent insulin release without a decrease in β cell mass, as well as age-dependent glucose intolerance in mice [33, 34]. A few case presentations in the literature show a propensity for impairment in glucose metabolism during adulthood [16, 27, 31]. Interestingly, diabetes mellitus at 14.5 years of age with relative insulin deficiency, as well as steroid-induced diabetes at 5 years of age, was reported in two siblings with a compound heterozygous *IGF1R* mutation [35]. A comparison between HbA1c and prevalence of type 2 diabetes between a limited number of adult *IGF1R* carriers versus adults with a history of small for gestational age using questionnaire revealed no difference [24]. Further studies in the adult carriers are required to elucidate whether there is a relationship between *IGF1R* defect and glucose metabolism.

The impact of *IGF1R* defect on gonadal function is unclear. *Igf1r* ± female mice have normal fertility, mating behavior and estrous cycle length compared to the corresponding features in wild-type mice [32]. Specific knockdown of IGF-1R in granulosa cells results in apoptosis, blocks folliculogenesis and ultimately leads to loss of fertility [36], suggesting IGF1R is essential for ovarian granulosa cell survival, estrogen production and formation of preovulatory follicles in female mice. Similarly, a lack of *INSR* and *IGF1R* in Sertoli cells of mouse testes gives rise to a decrease in testes size and sperm production [37]. In the current cohort, a carrier parent (case 3) experienced premature ovarian failure and early menopause. Also one patient (case 7) had hypergonadotropic hypogonadism; however, his molecular defect was classified as likely benign, and thus its association with gonadal defect is questionable. This issue requires further evaluation in adult carriers of *IGF1R* defect.

In conclusion, microcephaly, significant intrauterine growth retardation and severe short stature are not consistently present in *IGF1R* defects due to the multifactorial nature of these traits. What is more, IGF-1 and basal GH levels are not definitive diagnostic markers in most instances Adult cases with *IGF1R* defects should be investigated more thoroughly with respect to diabetes mellitus, cardiovascular risk factors and gonadal functions. We believe there is still a lot to learn about the characteristics and natural history of *IGF1R* defects.

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Data availability The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the local institutional review board (Decision No. GO 17/231–15).

Informed consent All participants and their parents provided written informed consent for participation in the study and publication.

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