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Impact of Heterozygosity for Acid-Labile Subunit (*IGFALS*) Gene Mutations on Stature: Results from the International Acid-Labile Subunit Consortium

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Context: To date, 16 *IGFALS* mutations in 21 patients with acid-labile subunit (ALS) deficiency have been reported. The impact of heterozygosity for *IGFALS* mutations on growth is unknown.

Objective: The study evaluates the impact of heterozygous expression of *IGFALS* mutations on phenotype based on data collected by the International ALS Consortium.

Subjects/Methods: Patient information was derived from the IGFALS Registry, which includes patients with *IGFALS* mutations and family members who were either heterozygous carriers or homozygous wild-type. Within each family, the effect of *IGFALS* mutations on stature was analyzed as follows: 1) effect of two mutant alleles (2ALS) *vs*. wild-type (WT); 2) effect of two mutant alleles *vs.* one mutant allele (1ALS); and 3) effect of one mutant allele *vs.* wild-type. The differences in height SD score (HtSDS) were then pooled and evaluated.

Results: Mean HtSDS in 2ALS was -2.31 ± 0.87 (less than -2 SDS in 62%); in 1ALS, -0.83 ± 1.34 (less than -2 SDS in 26%); and in WT, -1.02 ± 1.04 (less than -2 SDS in 12.5%). When analyses were performed within individual families and pooled, the difference in mean HtSDS between 2ALS and WT was -1.93 ± 0.79; between 1ALS and WT, -0.90 ± 1.53; and between 2ALS and 1ALS, -1.48 ± 0.83.

Conclusions: Heterozygosity for *IGFALS* mutations results in approximately 1.0 SD height loss in comparison with wild type, whereas homozygosity or compound heterozygosity gives a further loss of 1.0 to 1.5 SD, suggestive of a gene-dose effect. Further studies involving a larger cohort are needed to evaluate the impact of heterozygous *IGFALS* mutations not only on auxology, but also on other aspects of the GH/IGF system. **(*J Clin Endocrinol Metab* 95: 0000–0000, 2010)**

Since the first report on acid-labile subunit (ALS) deficiency and its molecular basis in humans in 2004 (1), 20 additional cases have been reported worldwide (2–12). A total of 16 novel mutations in the *IGFALS* gene have been discovered in children and adolescents with congenital ALS deficiency, characterized by total ALS and severe circulating IGF-I/IGF binding protein-3 (IGFBP-3) deficiencies. These 21 patients come from 16 families and represent high divergency in ethnicity and countries of residence. The spectrum of *IGFALS* mutations, both homozygous and compound heterozygous, covers the majority of the 20 leucine-rich repeats (LRR), as well as both the amino and carboxy termini, all of which are encoded by exon 2 of the *IGFALS* gene.

Clinical and hormonal implications of homozygous and compound heterozygous *IGFALS* mutations in pa-

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Abbreviations: ALS, Acid-labile subunit; 1ALS, family members, heterozygous carriers for *IGFALS* mutations; 2ALS, patients with homozygous or compound heterozygous *IGFALS* mutations; HtSDS, height SDS; IGFBP-3, IGF binding protein-3; ISS, idiopathic short stature; LRR, leucine-rich repeats; SDS, SD score; WT, family members, homozygous wild-type.

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tients are well characterized (8). In general, all patients meet the criteria for primary IGF deficiency, including postnatal short stature, normal GH response in GH stimulation tests, and low serum concentrations of IGF-I and IGFBP-3. The main phenotypic features of congenital ALS deficiency include: 1) mild or moderate short stature; 2) undetectable or extremely low serum concentrations of ALS; 3) extremely low serum levels of IGF-I and IGFBP-3, with predominant deficit of IGFBP-3 over IGF-I; 4) delayed puberty in boys; 5) poor response to GH treatment; and 6) some degree of insulin insensitivity. All of these observations, however, are influenced by some degree of ascertainment bias because evaluation of the GH-IGF axis is not commonly performed in normal individuals.

The impact of heterozygosity for *IGFALS* mutations on growth and the GH-IGF axis is not clearly understood and is still a matter of debate. When studied, serum concentrations of ALS, IGF-I, and IGFBP-3 in heterozygotes have tended to be within the broad normal range, but a subtle impact of ALS haploinsufficiency on the GH-IGF axis and statural growth cannot be ruled out without a thorough evaluation.

To evaluate the issue of heterozygous expression of *IGFALS* gene mutations, an International ALS Consortium was established in an effort to collate data from as many affected patients and families with mutations of *IGFALS* as possible. One of the goals of the International ALS Consortium is to evaluate the effect of heterozygosity for *IGFALS* mutations on auxological and metabolic characteristics. To pursue this objective, genotype:phenotype analysis of families with patients harboring defects of the *IGFALS* gene has been initiated.

This report summarizes current data from the ALS Consortium on the impact of heterozygous expression of *IGFALS* mutations on growth.

Patients and Methods

Patient information was derived from the IGFALS Registry, which consists of patients with homozygous or compound heterozygous *IGFALS* mutations (2ALS), as well as family members who were either heterozygous carriers (1ALS), or homozygous wild-type (WT). The IGFALS Registry includes all cases reported in a total of 12 publications (eight articles, four abstracts) covering the years 2004–2009.

To estimate the effect of *IGFALS* heterozygosity, height (measured in centimeters) and height SD score (HtSDS) were used as target auxological parameters. Because the patients come from a wide variety of ethnic groups, anthropometric data within each family were expressed as both centimeters of height and SDS for the appropriate country/ethnic group. HtSDS was also calculated using 2000 U.S. height standards of the National Center for Health Statistics, Centers for Disease Control and Prevention (CDC). To minimize any ascertainment bias and to limit interethnic height variations, a family analysis approach was employed, *i.e.* comparison within each individual family of affected patients (2ALS), family members who are heterozygous for *IGFALS* defects (1ALS), and family members who are homozygous wild-type (WT). According to this approach, the following effects were determined within each family: 1) the effect of two mutant alleles *vs.* one (HtSDS of each homozygous or compound heterozygous case minus mean HtSDS of all heterozygous relatives); 2) the effect of one mutant allele *vs.* wild-type (HtSDS of each heterozygous carrier minus mean HtSDS of all WT relatives); 3) the effect of two mutant alleles *vs.* wild-type (HtSDS of each homozygous or compound heterozygous case minus mean HtSDS of all WT relatives). The differences in HtSDS obtained in each individual family were then pooled for all of the families, for further analysis of the entire cohort.

At the end of December 2009, the IGFALS Registry consisted of a total of 65 subjects, of whom 21 were patients, harboring homozygous or compound heterozygous *IGFALS* defects (2ALS); 36 were heterozygous carriers (1ALS), *i.e.* parents, siblings, cousins, and uncles; and eight were homozygous WT relatives (Fig. 1).

The cohort of heterozygous (1ALS) carriers (n = 36) included mothers (n = 14), fathers (n = 14), siblings (n = 6), and other relatives (cousin and uncle, n = 2). Statistical analysis was performed in 30 of the 36 heterozygotes, in whom both auxological and genetic analysis data were available.

The cohort of homozygous WT relatives, or noncarriers (n = 8), included siblings (n = 3) and other relatives (cousins, aunts, and uncles; n = 5).

The majority (66%) of patients live in countries belonging to the European Union (Austria, Spain, The Netherlands, United Kingdom, Sweden), 24% of patients live in the United States, 5% live in South America (Argentina), and 5% live in Canada.

Data are presented as mean ± SD. U.S. height standards were used for the analysis of HtSDS.

Results

A total of 16 *IGFALS* mutations were reported between 2004 and 2009 in 21 children, adolescents, and young adults with total ALS and severe circulating IGF-I/IG-

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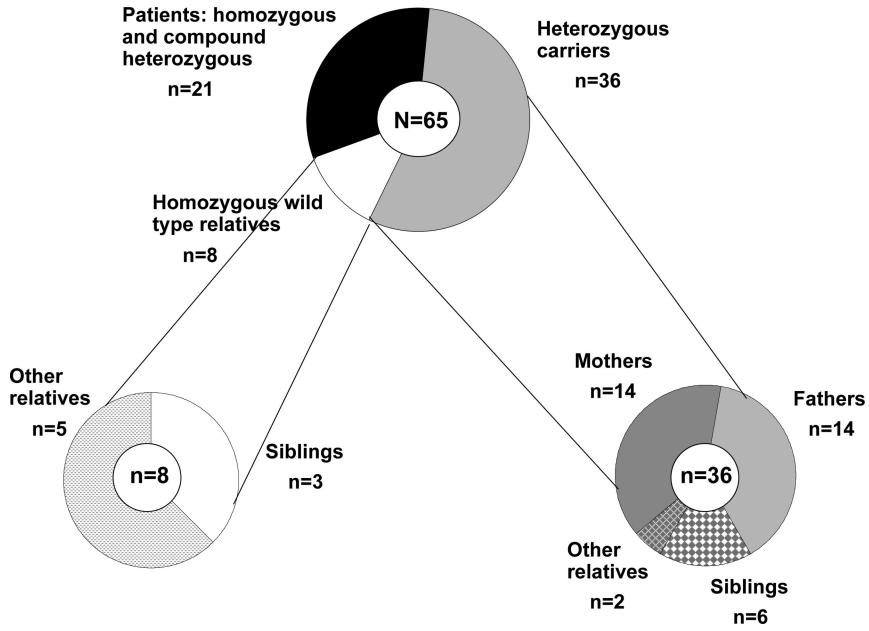

FIG. 1. Distribution of homozygous/compound heterozygous *IGFALS* patients, heterozygous *IGFALS* carriers, and WT relatives in the IGFALS Registry.

FBP-3 deficiency (Table 1). Among the 16 families analyzed, parents were consanguineous in three (19%) families (2, 6, 9); in 11 (69%) families, parents were nonconsanguineous. There is no record on consanguinity in two (12%) families because children were adopted at an early age (1, 7).

Approximately one third (38%; 8 of 21) of patients were familial cases: five patients came from two nonconsanguineous families (3, 11), and three patients came from one consanguineous family (6).

The majority (56%; 9 of 16) of the *IGFALS* mutations were missense, four (25%) were frameshifts with premature stop codons, two (13%) were in-frame insertions, and one (6%) was nonsense. Of particular interest is the finding of two different 9-bp duplications that resulted in in-frame insertion of three extra amino acids, Ser195_Arg197dup in LRR 7 (3) and Leu437_Leu439dup in LRR 17 (7) in four patients from two unrelated families.

All mutations were located in exon 2 of the *IGFALS* gene. Involvement of the LRR was as follows: amino-terminal flank and LRRs 1, 6, and 17 had two mutations each; carboxyl-terminal flank and LRRs 4, 7, 9, 10, 12, and 19 had one mutation each. One mutation was located between LRR 8 and LRR 9.

Among all *IGFALS* mutations (n = 16), homozygous and compound heterozygous mutations were equally

TABLE 1. Mutations in the *IGFALS* gene included in the IGFALS Registry

Family no.	Case no.	Mutation no.	Mutation	Type of mutation	Ref.
1	1[b]	1	*1338delG* (Glu35LysfsX85)	Frameshift, premature stop codon (NH2-flank)	1
2[c]	2	2	Asp440Asn	Missense (LRR 17)	2
3	3[a], 4[a], 5[a]	3–4	Cys540Arg/Ser195_Arg197dup	Missense (COOH-flank)/in-frame insertion (LRR 7)	3
4	6	5	Asn276Ser	Missense (LRR 10)	5
5	7				5
6	8	6	Gln320X	Nonsense (LRR 12)	5
7[c]	9[a], 10[a], 11[a]	7	*1490dupT* (Leu497PhefsX40)	Frameshift, premature stop codon (LRR 19)	6
8	12[b]	8	*1308_1316 dup9* (Leu437_Leu439dup)	In-frame insertion (LRR 17)	7
9	13	9–10	Cys60Ser/Leu244Phe	Missense (LRR 1)/missense (LRR 9)	7
10	14	11	Leu134Gln	Missense (LRR 4)	7
11	15	12–13	Pro73Leu/Leu241Pro	Missense (LRR 1)/missense (LRR 8–9)	7
12	16	14	Leu172Phe	Missense (LRR 6)	10
13	17		Leu172Phe/Ser195_Arg197dup	Missense/in-frame insertion	
14	18	15	Leu134Gln/Ala183SerfsX149	Missense/frameshift, premature stop codon (LRR 6)	9
15[c]	19		Pro73Leu	Missense	9
16	20[a], 21[a]	16	*103_104insG* (Glu35GlyfsX17)/Asn276Ser	Frameshift, premature stop codon (NH2-flank)/missense	11

[a] Siblings.

[b] Adopted children.

[c] Consanguineous marriage.

TABLE 2. Homozygosity and compound heterozygosity for *IGFALS* mutations in the IGFALS Registry

	IGFALS mutations		Patients	
	n	% of total	n	% of total
Homozygous	8	50	12	57
Compound heterozygous	8	50	9	43
Total	16	100	21	100

distributed (Table 2). Among all patients (n = 21), 12 (57%) harbored homozygous *IGFALS* mutations, and nine (43%) harbored compound heterozygous *IGFALS* mutations.

Patients harboring *IGFALS* mutations showed highly diverse ethnicity (Table 3).

The clinical data of 2ALS patients are presented in Table 4. Only three girls (14%) were reported (3, 7, 10), most likely representing the common male-oriented ascertainment bias commonly observed in evaluation of short stature. Mean chronological age at diagnosis was 11.1 ± 4.8 yr (range, 4.1–19.6 yr).

In the cohort of 2ALS patients (n = 21), mean HtSDS was −2.31 ± 0.87 (range, −3.61 to −0.39). HtSDS fell below −2 SDS in 62% (13 of 21) of patients (Fig. 2 and Table 4). The mild to moderate degree of growth retardation in *IGFALS* defects was confirmed by the distribution of HtSDS in patients. The majority (43%; 9 of 21) fell within the −3 to −2 HtSDS range, with only 19% (4 of 21) below −3 HtSDS; 38% (8 of 21) were within the 0 to −2 HtSDS range. Patients harboring homozygous *IGFALS* mutations were shorter than patients with compound heterozygous mutations, with mean HtSDS of −2.76 ± 0.58 (range, −3.61 to −1.77) and −1.71 ± 0.85 (range, −2.87 to −0.39), respectively ($P = 0.003$).

The anthropometry data of 1ALS and WT relatives are presented in Tables 5 and 6, respectively. In the 1ALS cohort (n = 30), mean HtSDS was −0.83 ± 1.34 (range, −3.32 to +1.57), with 26% (8 of 30) below −2 SDS (Fig. 2 and Table 5). Eleven of 30 (37%) 1ALS were between 0 and −2 HtSDS, and 37% were above average (0 to +2) HtSDS. In the latter group, most cases were between 0 and +1 HtSDS, with only one case above +1 HtSDS.

In the WT cohort (n = 8), mean HtSDS was −1.02 ± 1.04 (range, −2.81 to +0.31). Surprisingly, one of eight WT (12.5%) fell below −2 SDS (Fig. 2 and Table 6). Only

TABLE 3. Ethnic origin and countries of residence of patients in the IGFALS Registry

Family no.	Case no.	Mutation	Sex	Ethnic origin	Country of residence
1	1[b]	*1338delG* (Glu35LysfsX85)	M	Argentinean	Argentina
2[c]	2	Asp440Asn	M	Turkish	Austria
3	3[a]	Cys540Arg/Ser195_Arg197dup	M	Norwegian/German	United States
3	4[a]	Cys540Arg/Ser195_Arg197dup	M	Norwegian/German	United States
3	5[a]	Cys540Arg/Ser195_Arg197dup	F	Norwegian/German	United States
4	6	Asn276Ser	M	Spanish	Spain
5	7	Asn276Ser	M	Spanish	Spain
6	8	Gln320X	M	Spanish	Spain
7[c]	9[a]	*1490dupT* (Leu497PhefsX40)	M	Kurdish	Netherlands
7[c]	10[a]	*1490dupT* (Leu497PhefsX40)	M	Kurdish	Netherlands
7[c]	11[a]	*1490dupT* (Leu497PhefsX40)	M	Kurdish	Netherlands
8	12[b]	*1308_1316 dup9* (Leu437_Leu439dup)	M	Mayan	Canada
9	13	Cys60Ser/Leu244Phe	F	Jewish/Eastern European (Polish, Russian, Austrian-Hungarian)/Icelandic/European (French, English)	United States
10	14	Leu134Gln	M	Indian	United Kingdom
11	15	Pro73Leu/Leu241Pro	M	Ashkenazi Jewish	United States
12	16	Leu172Phe	F	Swedish	Sweden
13	17	Leu172Phe/Ser195_Arg197dup	M	Swedish	Sweden
14	18	Leu134Gln/Ala183SerfsX149	M	NA	United Kingdom
15[c]	19	Pro73Leu	M	NA	United Kingdom
16	20[a]	*103_104insG* (Glu35GlyfsX17)/Asn276Ser	M	Spanish	Spain
16	21[a]	*103_104insG* (Glu35GlyfsX17)/Asn276Ser	M	Spanish	Spain

M, Male; F, female; NA, data not available.

[a] Siblings.

[b] Adopted children.

[c] Consanguineous marriage.

TABLE 4. Anthropometry data at diagnosis in patients included in the IGFALS Registry

Family no.	Case no.	Mutation	Sex	CA (yr)	Height (cm)	HtSDS national	HtSDS USA standards
1	1[b]	*1338delG* (Glu35LysfsX85)	M	14.6	145.2	−2.05	−2.66
2[c]	2	Asp440Asn	M	12.1	130.3	−2.90	−2.71
3	3[a]	Cys540Arg/Ser195_Arg197dup	M	15.3	156.2	−2.00	−1.84
3	4[a]	Cys540Arg/Ser195_Arg197dup	M	19.6	174.0	−0.50	−0.39
3	5[a]	Cys540Arg/Ser195_Arg197dup	F	15.4	156.2	−1.00	−0.92
4	6	Asn276Ser	M	4.5	98.0	−2.37	−1.77
5	7	Asn276Ser	M	4.7	93.2	−3.90	−3.02
6	8	Gln320X	M	15.0	144.9	−2.55	−2.95
7[c]	9[a]	*1490dupT* (Leu497PhefsX40)	M	14.6	136.4	−4.20	−3.58
7[c]	10[a]	*1490dupT* (Leu497PhefsX40)	M	6.8	108.0	−3.00	−2.36
7[c]	11[a]	*1490dupT* (Leu497PhefsX40)	M	4.2	92.5	−3.20	−2.60
8	12[b]	*1308_1316 dup9* (Leu437_Leu439dup)	M	6.7	104.6	−2.80	−2.91
9	13	Cys60Ser/Leu244Phe	F	4.1	92.5	−2.14	−2.14
10	14	Leu134Gln	M	15.2	143.7	−3.00	−3.17
11	15	Pro73Leu/Leu241Pro	M	12.7	132.3	−2.70	−2.87
12	16	Leu172Phe	F	11.4	133.0	−2.5	−1.84
13	17	Leu172Phe/Ser195_Arg197dup	M	12.5	138.6	−2.4	−1.87
14	18	Leu134Gln/Ala183SerfsX149	M	10.6	123.0	−2.80	−2.81
15[c]	19	Pro73Leu	M	13.4	130.0	−3.20	−3.61
16	20[a]	*103_104insG* (Glu35GlyfsX17)/Asn276Ser	M	4.1	96.0	−2.14	−1.66
16	21[a]	*103_104insG* (Glu35GlyfsX17)/Asn276Ser	M	16	166.8	−1.22	−0.89

CA, Chronological age; HtSDS National, HtSDS data provided by authors; HtSDS USA standards, HtSDS data based on CDC (2000), the National Center for Health Statistics, Centers for Disease Control and Prevention; M, Male; F, female.

[a] Siblings.

[b] Adopted children.

[c] Consanguineous marriage.

one WT (12.5%) relative had a height above 0 HtSDS, and, in contrast to the 1ALS cohort, none was above +1 HtSDS. The majority of WT fell in the 0 to −2 HtSDS range, three (37%) were between −2 and −1, and three were between −1 and 0 HtSDS.

When the analyses within each family are pooled, distinct mean height differences among 2ALS, 1ALS, and WT emerged. The difference in mean HtSDS between 2ALS and WT was -1.93 ± 0.79 (n = 6; four families). The difference in mean HtSDS between 2ALS and 1ALS was -1.48 ± 0.83 (n = 15; 11 families). The difference in mean HtSDS between 1ALS and WT was -0.90 ± 1.53 (n = 12; four families). Based on these data, heterozygosity for *IGFALS* mutations results in approximately 1.0 SD height loss in comparison with wild-type, whereas homozygosity or compound heterozygosity results in a further loss of 1.5 SD, for a cumulative height deficit of approximately 2 SD.

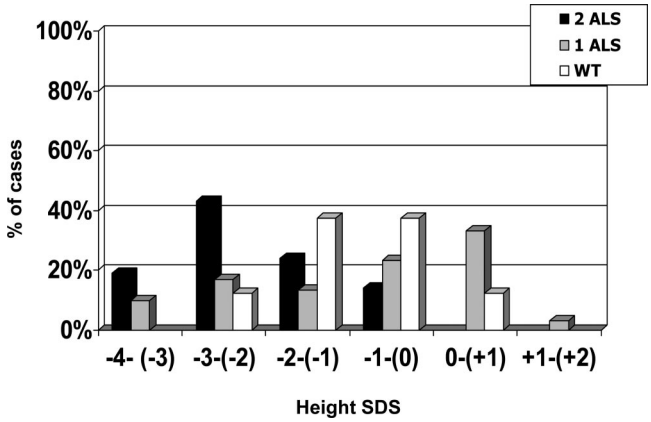

FIG. 2. Distribution of HtSDS in patients, heterozygous carriers, and WT relatives in the IGFALS Registry.

Discussion

In the 6 yr since the first report of a homozygous *IGFALS* mutation in an Argentinean boy (1), a total of 16 novel *IGFALS* mutations in 21 patients have been published. Other than the *GHR* gene, the *IGFALS* gene now appears to be the most frequently affected gene among gene candidates (*GHR*, *STAT5b*, *IGF1*, *IGFALS*) responsible for primary IGF deficiency. Indeed, because *IGFALS* gene defects cause more modest growth failure compared with the other genes and molecular studies are subject to ascertainment bias, their prevalence may well be currently underestimated. Because all of the cases reported to date have involved homozygous or compound heterozygous gene defects, the potential impact of heterozygosity for *IGFALS* mutations on growth has not yet been systematically evaluated.

The preliminary findings of the International ALS Consortium presented here, based on the families evaluated to date, appear to show a clinically significant impact of het-

TABLE 5. Anthropometry data of heterozygous carriers in families of patients with *IGFALS* mutations

	Mother			Father			Sibling 1/sibling 2/ cousin/uncle		
Family no.	Height (cm)	HtSDS national	HtSDS USA	Height (cm)	HtSDS national	HtSDS USA	Height (cm)	HtSDS national	HtSDS USA
2[a]	143.5	−2.80	−3.04	161.0	−2.0	−2.20			
3	157.5	−0.93	−0.90	188.0	1.50	1.57	168.9	0.50	0.60
4	165.0	0.65	0.26	175.0	−0.10	−0.26	180.0	0.73	0.48
							168.0	−1.20	−1.09
5	158.3	−0.90	−0.78	162.0	−2.27	−2.06	154.5	−1.20	−1.36
6	159.5	−0.60	−0.59	173.0	−0.46	−0.54	163.0	0.86	0.33
7[a]	141.7	−3.40	−3.32	155.5	−3.20	−2.96	149.8	−2.0	−2.08
							126.3	0.30	0.40
							154.8	−3.30	−3.06
9	149.5	−2.13	−2.13	179.0	0.30	0.30			
10	157.5	NA	−0.90	167.2	NA	−1.35			
11	152.4	−1.68	−1.68	182.9	0.85	0.85			
12	166.0	−0.10	0.41	182.0	0.30	0.72			
13	157.6	NA	−0.88	180.2	NA	0.47			

HtSDS National, HtSDS data provided by authors; HtSDS USA, HtSDS data based on CDC (2000), the National Center for Health Statistics, Centers for Disease Control and Prevention.

[a] Consanguineous family.

erozygous *IGFALS* mutations on growth. Heterozygosity for *IGFALS* mutations results in approximately 1.0 SD height loss in comparison with wild-type. Homozygosity for *IGFALS* mutations gives a further loss of 1.0 to 1.5 SD. These findings are consistent with a gene-dose effect.

Evaluation of the impact of heterozygosity on growth is challenging. It is recognized that patients typically come to the attention of endocrinologists because of their short stature, so an important element of ascertainment bias exists. Additionally, it is recognized that short individuals often tend to mate with short individuals, so that the presence of a documented genetic abnormality does not, in itself, prove that this abnormality is the major etiological factor in growth failure. Finally, ethnic differences in growth patterns throughout the world make analysis of international growth data complicated.

It should be noted that the mean height of the WT relatives was −1.02 ± 1.04 SDS (range, −2.81 to +0.31) because one WT case had a height below −2 SDS. We can speculate that factors other than *IGFALS* may have influenced the growth potential in this individual. The mean HtSDS in the WT group was not significantly different from that of 1ALS subjects (mean, −0.83 ± 1.34; range, +1.57 to −3.32), with 26% having heights of less than −2 SDS. When analyses were performed within individual families and then pooled, however, the real impact of heterozygosity upon height became more apparent.

The possibility that heterozygosity for *IGFALS* mutations may contribute to idiopathic short stature (ISS) and constitutional delay of growth and puberty has had some preliminary consideration, although with conflicting results. One study of 90 children with constitutional delay of growth and puberty found no *IGFALS* mutations (13). In another study of 52 ISS children, four heterozygous defects in the *IGFALS* gene were identified in five patients (14). The same defects were found in three of seven parents

TABLE 6. Anthropometry data of WT relatives in families of patients with *IGFALS* mutations

Family no.	Relative	Sex	Height (cm)	HtSDS national	HtSDS USA
2[a]	Sibling	F	160.0	0.00	−0.50
5	Sibling	F	162.7	0.25	−0.10
7[a]	Cousin 1	M	114.5	−0.10	0.31
	Cousin 2	M	107.0	−1.80	−1.21
	Aunt 1	F	151.3	−1.70	−1.85
	Aunt 2	F	160.6	0.00	−0.42
	Uncle	M	156.6	−3.00	−2.81
11	Sibling	M	129.4	−1.61	−1.61

HtSDS National, HtSDS data provided by authors; HtSDS USA, HtSDS data based on CDC (2000), the National Center for Health Statistics, Centers for Disease Control and Prevention.

[a] Consanguineous marriage.

and four of six siblings with short stature. Unfortunately, no detailed evaluation within the individual families was performed, so that the impact of heterozygosity within each family remained unclear. Nevertheless, ISS individuals with heterozygous *IGFALS* defects were reported to be significantly shorter than those without *IGFALS* defects. However, no differences in serum concentrations of IGF-I, IGFBP-3, and ALS in these two groups were found. A recent study of 89 ISS children found five so-called "severe" ("probably or possibly damaging") nonsynonymous heterozygous single nucleotide polymorphisms in six cases, as well as in three of 46 normal controls (15). In families of ISS children, HtSDS, as well as serum IGF-I, IGFBP-3, and ALS levels were reportedly lower in heterozygous carriers for these single nucleotide polymorphisms compared with wild-type relatives. The authors speculated that heterozygous *IGFALS* mutations can be considered the molecular mechanism of short stature in a subset of ISS children presenting with low serum levels of IGF-I, IGFBP-3, and ALS.

The questions concerning the impact of *IGFALS* homozygosity, compound heterozygosity, and simple heterozygosity on growth continue to be confounded by ascertainment bias issues. To avoid such bias issues, multiple approaches are required: the IGFALS Registry must be expanded to make it as large as possible and to include all new reported cases. Secondly, a family analysis approach should continue to be undertaken, allowing genotype: phenotype analysis of first-degree relatives. Thirdly, the frequency of *IGFALS* gene defects must be ascertained, not only in the short stature population, but also in a suitable control group. Finally, functional studies should be performed, where possible, to determine whether any specific defect of the *IGFALS* gene results in a dysfunctional (or absent) protein.

The last decade has seen the identification of significant homozygous and compound heterozygous defects of important genes in the GH-IGF axis, such as *STAT5b*, *IGF-I*, and *IGFALS*, in addition to the earlier identified mutations of the *GHR*. It should not surprise us if some of these genes, such as *IGFALS*, prove to have a subtle impact upon growth, even with heterozygous defects, either by themselves or in combination with other seemingly minor perturbations of the many genes involved in skeletal growth (16, 17).

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