STAT5B mutations in heterozygous state have negative impact on height: another clue in human stature heritability

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Abstract

Context and objective: Growth hormone insensitivity with immune dysfunction caused by signal transducer and activator of transcription 5B *(STAT5B)* mutations is an autosomal recessive condition. Heterozygous mutations in other genes involved in growth regulation were previously associated with a mild height reduction. Our objective was to assess for the first time the phenotype of heterozygous *STAT5B* mutations.

Methods: We genotyped and performed clinical and laboratorial evaluations in 52 relatives of 2 previously described Brazilian brothers with homozygous *STAT5B* c.424_427del mutation (21 heterozygous). Additionally, we obtained height data and genotype from 1,104 adult control individuals from the same region in Brazil and identified 5 additional families harboring the same mutation (18 individuals, 11 heterozygous). Furthermore, we gathered the available height data from first-degree relatives of patients with homozygous *STAT5B* mutations (17 individuals from 7 families). Data from heterozygous individuals and non-carriers were compared.

Results: Individuals carrying heterozygous *STAT5B* c.424_427del mutation were 0.6 SDS shorter than their non-carrier relatives (p= 0.009). Heterozygous subjects also had significantly lower SDS for serum concentrations of IGF-1 (p=0.028) and IGFBP-3 (p=0.02) than their non-carrier relatives. The 17 heterozygous first-degree relatives of patients carrying homozygous STAT5B mutations had an average height SDS of -1.4 ± 0.8 when compared with population-matched controls ($p < 0.001$).

Conclusions: *STAT5B* mutations in heterozygous state have a significant negative impact on height (approximately 3.9 cm). This effect is milder than the effect seen in the homozygous state, with height usually within the normal range. Our results support the hypothesis that heterozygosity of rare pathogenic variants contributes to normal height heritability.

Introduction

Previous studies have demonstrated that while homozygous mutations in genes involved in growth regulation are causal of severe syndromic short stature, heterozygosity of the same variants can be associated with a milder height reduction [1-4]. For instance, in the growth hormone (GH) - insulin-like growth factor 1 (IGF-1) axis, heterozygous carriers of mutations in acid-labile subunit gene (*IGFALS*) [3] and *IGF1* gene [1] were shown to be significantly shorter than non-carriers, although generally still within the normal height range. These data support the concept that rare mono-allelic variants with moderate effects on phenotype can be associated with height variability [5] and, as such, can be an etiology for non-syndromic short stature [6, 7].

Signal transducer and activator of transcription 5B (STAT5B) is a key mediator of GH signaling, as well as of other signaling pathways, including those of prolactin and interleukin 2 (IL2) [8]. Since 2003, ten patients have been reported harboring seven different homozygous *STAT5B* mutations [9-16]. These rare homozygous mutations in *STAT5B* cause growth hormone insensitivity (GHI) and manifestations of immune dysregulation, such as increased susceptibility for opportunistic infections, lymphoid interstitial pneumonia and eczema. GHI syndrome, classically associated with homozygous mutations in the growth hormone receptor gene (*GHR*), is characterized by severe postnatal growth failure, normal to elevated GH levels and low serum concentrations of ALS, IGF1 and insulin-like growth factor binding protein 3 (IGFBP-3). Unlike most GHI patients carrying defects in *GHR*, however, serum concentrations of growth hormone binding protein (GHBP), the proteolytically-cleaved extracellular domain of GHR, were normal and prolactin levels were increased in patients carrying homozygous *STAT5B* mutations (reviewed in [8]).

To date, STAT5B deficiency is considered an autosomal recessive condition. The impact of heterozygous *STAT5B* mutations on growth and the GH-IGF axis, however, has not been carefully evaluated, due in part to the rarity of described cases and families. To address this issue, we evaluated a large community, in which multiple members carry a previously described *STAT5B* frameshift mutation [15]. By comparing their data with data from other families harboring other mutations in *STAT5B*, we provide evidence that heterozygous *STAT5B* mutations can influence stature.

Subjects and methods

Subjects

We evaluated 52 relatives of two Brazilian brothers with characterized GHI due to homozygous *STAT5B* c.424_427del mutation. Furthermore, an active search was done to investigate the prevalence of this mutation in the region where the index cases were born, identifying five unrelated heterozygous individuals among 1,104 evaluated adult control subjects. Relatives of these five individuals were subsequently evaluated, totaling 18 subjects. Height data gathered from the remaining 1,099 adult control individuals (non-carriers of *STAT5B* c.424_427del mutation) in the same region were used to assess the local population height.

Additionally, we gathered the available height data from first-degree heterozygous relatives of previously reported patients with homozygous *STAT5B* mutations. We also included in this group two recently diagnosed individuals heterozygous for *STAT5B* c.424_427del mutation, who lost two children with the same phenotype seen in patients with homozygous *STAT5B* mutations. In total, height data from 17 first-degree relatives from 7 families were analyzed.

These studies were approved by the local ethics committees, and the patients or guardians gave their written informed consent.

Genotyping in families with STAT5B c.424_427del mutation

Genomic DNA was isolated from peripheral blood leukocytes using standard techniques. Genotyping for *STAT5B* c.424_427del mutation was done by fragment analysis technique. The primers were designed to amplify the region around this mutation (primer sequences and amplification protocols are available upon request). Genotyping was performed after the clinical evaluation.

Clinical and laboratory assessment in families with STAT5B c.424_427del mutation

Individuals from families with *STAT5B* c.424-427del mutation were evaluated by an investigator blinded for *STAT5B* genotype. They were questioned about pneumopathies, eczema and other immune dysfunctions. Height and weight were assessed in all individuals. Total blood count, fasting glucose and

insulin, immunoglobulins G, A and E, basal GH, IGF-1, IGFBP-3 and prolactin were tested in 91% of the evaluated individuals. Serum GH, IGF-1, IGFBP-3, prolactin and immunoglobulin E were measured through chemiluminescence assays and immunoglobulins A and G through turbidimetry. IGF-1 and IGFBP-3 were transformed to SD scores (SDS) [17].

Whole-exome sequencing

Whole-exome sequencing of genomic DNA, obtained from the peripheral blood of one individual heterozygous for *STAT5B* c.424_427del mutation and with pneumopathy of unknown etiology, was performed with Illumina's Nextera Exome Enrichment kits (Illumina, San Diego, CA) for library preparation and exome capture, and the Illumina HiSeq sequencer. Alignments and variant annotation were made as previouly described [18].

Statistical methods

Because the patients came from many ethnic groups, height data were expressed as SDS for the appropriate country/ethnic group. The effect of one mutant allele *vs.* wild type was determined in the whole group.

Groups were compared by unpaired t-test or ANOVA followed by Tukey test for numerical variables with normal distribution. Numerical variables without parametric distribution were analyzed by Mann-Whitney Rank Sum Test or Kruskal-Wallis ANOVA on Ranks. Categorical data were compared between groups through chi-square test or Fisher's exact test as appropriate. Statistical significance was assumed for $p < 0.05$. Statistical analysis were made with SigmaStat 3.5 (Systat Software Inc. Chicago, USA) and MedCalc version 11.1.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

Families harboring STAT5B c.424_427del mutation

The largest Brazilian family consisted of two patients with homozygous *STAT5B* c.424_427del mutation, 21 heterozygous carriers (including their non-consanguineous parents) and 31 non-carrier relatives (Supplemental Figure 1). The other five families identified consisted of eleven heterozygous carriers and

seven non-carrier relatives. When polymorphic markers around this mutation were studied, the same haplotype was found in these six families, which was consistent with the presence of a founder effect (data not shown) [19]. Consequently, we analyzed all individuals from the six families together (Table 1).

In total, we analyzed data from 32 heterozygous carriers of *STAT5B* c.424_427del mutation (17 males) and 38 non-carrier family members (12 males). Unrelated spouses were not included. One heterozygous carrier was excluded from the height analysis because of severe short stature (height SDS -3.5) of unknown cause. Among the seventy evaluated individuals (aged 32.7 ± 18.5 years old), sixteen were children (7 heterozygous for *STAT5B* mutation).

Non-carrier subjects in these families had a similar height SDS to individuals from the local population (height SDS -0.2 ± 1.0 *vs.* -0.4 ± 0.8 respectively, p = 0.63). Heterozygous *STAT5B* c.424_427del individuals were significantly shorter (height SDS -0.8 ± 0.9) than their non-carrier relatives (height SDS difference of -0.6, p = 0.009, confidence interval 95% -1.1 to -0.2), although all were within the normal height range (Table 1). When the analysis was done excluding the children, the same results were obtained (height SDS -0.8 ± 0.9 *vs.* -0.2 ± 1.0 for heterozygous and non-carrier relatives respectively, p = 0.02, Figure 1). Furthermore, heterozygous carriers had significantly lower IGF-1 and IGFBP-3 SDS than their non-carrier relatives (Table 1). Other parameters, such as basal GH and prolactin concentrations, were not different between these groups.

Present or past history of dermopathies was reported in 9 out of 32 individuals heterozygous for *STAT5B* c.424_427del mutation and in 1 out of 38 individuals who were non-carriers (p = 0.004). We clinically diagnosed eczema in four carriers. No differences in total blood count and immunoglobulin levels between carriers and non-carriers were observed (Table 1).

One cousin of the probands, who was heterozygous for *STAT5B* c.424_427del mutation, presented with a moderate to severe restrictive pneumopathy of unknown etiology. Her disease was milder than the pneumopathy observed in patients homozygous for *STAT5B* mutations, since she was in her thirties and still not oxygen-dependent. Exome sequencing excluded other *STAT5B* mutations and mutations in genes normally associated with pneumopathies (data not shown). A lung biopsy of this patient showed areas of interstitial thickening near respiratory bronchioles, inflammatory interstitial infiltrate with lymphocytes, plasmocytes and histiocytes and mild interstitial fibrosis, which is compatible with lymphoid interstitial pneumonia. Her father, an obligatory heterozygous carrier for the same mutation, died of respiratory failure

secondary to an uninvestigated chronic pneumopathy, but he had confounding factors such as smoking and working as a miner.

First-degree relatives of index cases carrying STAT5B mutations

Height data were obtained in seventeen first-degree relatives of ten patients homozygous for *STAT5B* mutations (Table 2). Two Argentinian patients were adopted soon after birth and, consequently, data from their biological relatives were not available. Parents were consanguineous in four families and not consanguineous in three families. All these relatives were heterozygous for *STAT5B* mutations with an average height SDS of -1.4 ± 0.8 when compared with appropriate population-matched controls ($p < 0.001$).

Discussion

In adequate health and nutritional conditions, genetic variation is the main determinant of stature, accounting for approximately 80% of height variability [20]. Recent genome-wide association studies (GWAS) identified 697 variants in 423 loci that, together, accounted for only one-fifth of adult height heritability [21]. The individual effect of single nucleotide polymorphisms (SNPs) found in these studies, furthermore, is very small (less than 0.5 cm) [22]. The inability of GWAS to explain all height heritability, despite the increasing number of evaluated individuals, suggests that numerous rare variants with large to moderate effect have a role in height variability [23]. However, it is difficult to evaluate the importance of rare variants in height variability through the current available methods, since each private allele is restricted to a few families or small populations.

In the present study, the analysis of a large family with many heterozygous carriers of the *STAT5B* c.424_427del mutation showed that these individuals are significantly shorter than their non-carrier relatives and local population controls (mean height SDS difference of 0.6). Assuming that the mean SD for adult height distribution is 6.5cm, the mean height loss seen in these individuals can be estimated at 3.9 cm, which is a much larger individual effect than the 0.5 cm attributed to SNPs identified in GWAS. The significant reduction in IGF-1 SDS and IGFBP-3 SDS seen with *STAT5B* c.424_427del heterozygous carriers, furthermore, suggests that a decreased responsiveness to GH action could explain, at least in part, the observed height reduction.

Moreover, the analysis of the available height data from carriers of the different *STAT5B* mutations also displayed a significant reduction in height when compared to their population controls, and was equivalent to a height decrease of 9.1 cm, an even greater difference than that observed for carriers of *STAT5B* c.424_427del mutation. This difference could be due to the relatively smaller number of first-degree relatives available for study, and/or to variable effects on height dependent on the individual *STAT5B* mutation itself. For the *STAT5B* c.424_427del mutation, the lack of expression of the mutant protein in reconstitution experiments (Hwa V, unpublished data) suggests that partial haploinsufficiency could explain the modest height reduction seen in heterozygous carriers. No dominant-negative *STAT5B* mutations have been reported to date, although, interestingly, a heterozygous *STAT5B p.Gln177Pro* variant was recently described in two GHI patients with severe short stature but no immunological dysfunction [24].

Heterozygous mutations in other genes along the GH-IGF1 axis similarly show larger individual effects on height than SNPs, supporting our finding. For example, in a family carrying *IGF1* p.V44M mutation, individuals heterozygous for this mutation were 0.6 SDS shorter (equivalent to 3.9 cm) than their non-carrier relatives [1]. Moreover, heterozygous carriers of *IGFALS* mutations were 0.9 SDS shorter (equivalent to 5.8 cm) than their non-carrier relatives [3]. Heterozygous mutations in genes associated with bone growth regulation similarly impacted height: in a large family with many individuals heterozygous for a *NPR2* mutation, carriers were 1.4 SDS shorter than non-carriers (equivalent to 9.1 cm) [2]. In all of these studies, the clinical presentation of heterozygous individuals was much milder than the disorder seen in patients homozygous for the same mutations. Altogether, the presence of these rare pathogenic mutations in heterozygous state suggests that loss of one functional allele may result in low-normal height and borderline short stature.

Finally, we observed that individuals with heterozygous *STAT5B* c.424_427del mutation reported more dermopathies and skin allergies when compared to their non-carrier relatives ($p = 0.004$). There was no difference in pneumopathies or other allergies reported by both groups, although two heterozygous carriers (a cousin of the probands and her father) had severe pneumopathy of unknown etiology. Further investigations are necessary to better characterize the potential effects of heterozygous *STAT5B* mutations in immunologic alterations.

In conclusion, we demonstrated that *STAT5B* mutations in heterozygous state exert a significant negative impact on height. This effect is milder than the effect seen in homozygous state, with height usually

within the low normal range. Our results support the hypothesis that heterozygosity of rare pathogenic variants contributes to normal height heritability. Whether the cumulative effect of such variants could be responsible for a proportion of the missing height heritability posed by GWAS studies remains to be determined.

Declaration of interest: The authors have nothing to disclose.

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Figure legends:

Figure 1: Comparison of height SDS distribution among adult heterozygous carriers of *STAT5B* c.424_427del mutation (n = 25), their non-carrier relatives (n = 28) and a population sample from the same region in the south of Brazil (n=1,099).

Supplemental figure 1: The largest Brazilian family with STAT5B c.424_427del mutation. Subjects homozygous for *STAT5B* c.424_427del mutation are indicated by black symbols; heterozygous carriers by gray symbols and non-carriers by white symbols. The symbol * refers to two individuals with severe pneumopathy of unknown etiology.

Table 1: Clinical and biochemical characteristics of heterozygous carriers of *STAT5B c.424_427del* mutation *vs.* non-carriers

	WT/Mut	**WT/WT**	**p**
n	32	38	
Dermopathy	9:32	1:38	0.004
Severe pneumopathy	1:32	0:38	n.s.
Height SDS	-0.8 ± 0.9*	-0.2 ± 1.0	0.009
Basal GH (ng/mL)	1.4 ± 2.2	1.0 ± 1.6	n.s.
IGF-1 SDS	-0.4 ± 1.2	0.3 ± 1.2	0.028
IGFBP-3 SDS	-0.9 ± 1.4	0.0 ± 1.5	0.02
Prolactin (ng/mL)	12.5 ± 7.2	13.0 ± 10.6	n.s.
Glucose (mg/dL)	90 ± 14	83 ± 13	n.s.
Insulin (µUI/mL)	6.9 ± 7.3	6.4 ± 3.9	n.s.
Hemoglobin (g/dL)	13.3 ± 1.3	13.4 ± 1.5	n.s.
Leucocytes (cells/mm^3)	6893 ± 2008	6935 ± 2695	n.s.
Lymphocytes (cells/mm^3)	3998 ± 1292	3598 ± 1329	n.s.
IgG (mg/dL)	1022 ± 205	1054 ± 249	n.s.
IgA (mg/dL)	244 ± 141	225 ± 123	n.s.
IgE (UI/mL)	220 ± 325	143 ± 191	n.s.

SDS – standard deviation score; GH – growth hormone; IGF-1 – insulin-like growth factor 1; IGFBP-3 – insulin-like growth factor binding protein 3; IgG/IgA/IgE – immunoglobulins G, A and E; n.s. – non-significant.

* - excluding one heterozygous carrier with severe short stature (height SDS -3.5)

Values are expressed as mean ± SD

Table 2: Data of index patients homozygous for STAT5B mutations and their first-degree relatives.

Family n°	Ref	Consanguinity	cDNA mutation	Origin	Local height SDS			
					Patient	Fathers	Mothers	Siblings
1	9	Yes	c.1888G>C	Argentina	-7.5	-0.3	-1.2	
2	10	Yes	c.1191insG	Turkey	-7.8	-0.9	-0.6	
3	11	No	c.1102insC	Caribbean	-5.9	-2.8	-0.8	-2.3 / -0.8
4	12	No	c.454C>T	Argentina	-9.9	-2.2	-3.3	-2.0
5	13	Yes	c.1680delG	Kuwait	-5.6 / -5.8	-1.3	-0.6	
6	14	Adopted	c.454C>T	Argentina	-5.3	NA	NA	
7	15	No	c.424_427del	Brazil	-5.6 / -3.0	-1.5	-1.0	
8	16	Adopted	c.1937T>C	Argentina	-5.95	NA	NA	
9	19	Yes	c.424_427del	Brazil	NA*	-0.9	-1.9	
Mean ± SDS					-6.2 ± 1.8	-1.4 ± 0.8		
Median (range)					-5.9 (-9.9; -3.0)	-1.2 (-3.3; -0.3)		

NA: not available

* - patients died before anthropometric assessment.

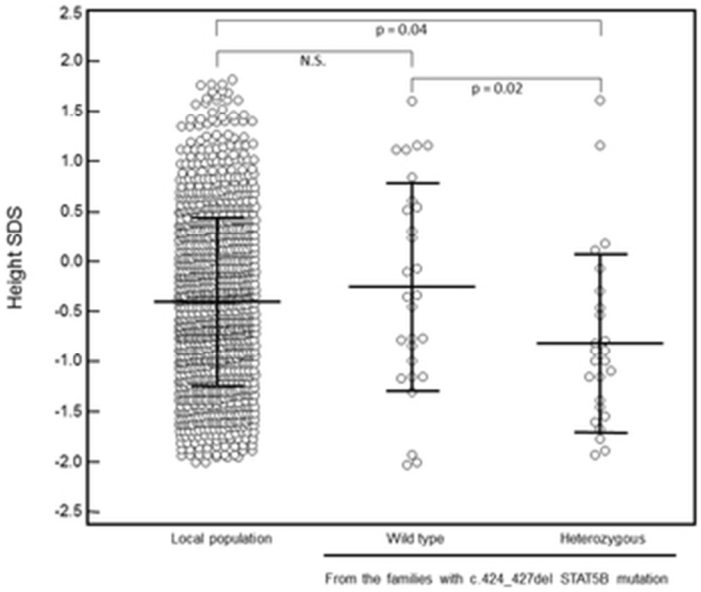

Figure 1: Comparison of height SDS distribution among adult heterozygous carriers of STAT5B c.424_427del mutation (n = 26), their non-carrier relatives (n = 28) and a population sample from the same region in the south of Brazil (n=1,099).
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