Genetic Predictors of Long-Term Response to Growth Hormone (GH) Therapy in Children With GH Deficiency and Turner Syndrome: The Influence of a SOCS2 Polymorphism

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Background: There is great interindividual variability in the response to GH therapy. Ascertaining genetic factors can improve the accuracy of growth response predictions. Suppressor of cytokine signaling (SOCS)-2 is an intracellular negative regulator of GH receptor (GHR) signaling.

Objective: The objective of the study was to assess the influence of a *SOCS2* polymorphism (rs3782415) and its interactive effect with *GHR* exon 3 and −202 A/C *IGFBP3* (rs2854744) polymorphisms on adult height of patients treated with recombinant human GH (rhGH).

Design and Patients: Genotypes were correlated with adult height data of 65 Turner syndrome (TS) and 47 GH deficiency (GHD) patients treated with rhGH, by multiple linear regressions. Generalized multifactor dimensionality reduction was used to evaluate gene-gene interactions.

Results: Baseline clinical data were indistinguishable among patients with different genotypes. Adult height SD scores of patients with at least one *SOCS2* single-nucleotide polymorphism rs3782415-C were 0.7 higher than those homozygous for the T allele ($P < .001$). *SOCS2* ($P = .003$), *GHR*-exon 3 ($P = .016$) and −202 A/C *IGFBP3* ($P = .013$) polymorphisms, together with clinical factors accounted for 58% of the variability in adult height and 82% of the total height SD score gain. Patients harboring any two negative genotypes in these three different loci (homozygosity for *SOCS2* T allele; the *GHR* exon 3 full-length allele and/or the −202C-*IGFBP3* allele) were more likely to achieve an adult height at the lower quartile (odds ratio of 13.3; 95% confidence interval of 3.2–54.2, $P = .0001$).

Conclusion: The *SOCS2* polymorphism (rs3782415) has an influence on the adult height of children with TS and GHD after long-term rhGH therapy. Polymorphisms located in *GHR*, *IGFBP3*, and *SOCS2* loci have an influence on the growth outcomes of TS and GHD patients treated with rhGH. The use of these genetic markers could identify among rhGH-treated patients those who are genetically predisposed to have less favorable outcomes. **(*J Clin Endocrinol Metab* 99: E1808–E1813, 2014)**

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Abbreviations: CI, confidence interval; GHD, GH deficiency; GHR, GH receptor; IGFBP, IGF-binding protein; rhGH, recombinant human GH; SDS, SD score; SNP, single-nucleotide polymorphism; SOCS, suppressor of cytokine signaling; TS, Turner syndrome.

Recombinant human GH (rhGH) is commonly used in the long-term treatment of children with growth disorders, with or without GH deficiency (GHD). This standard growth-promoting treatment is carried out with empirical and fixed doses of rhGH adjusted for body weight or surface, resulting in a variable growth response. The addition of genetic data may improve mathematical models to predict the response to rhGH therapy and consequently allow a more personalized rhGH treatment strategy (1).

GH binding to the GH receptor (GHR) activates the tyrosine kinase Janus kinase 2, initiating a multitude of signaling cascades that result in a variety of biological responses. GH promotes growth mainly by IGF-I. IGF-I transport in serum is mediated mostly by IGF-binding protein-3 (IGFBP-3) and acid labile subunit. The suppressor of cytokine signaling (SOCS)-2 is a member of a family of intracellular proteins involved in the negative regulation of cytokine signaling through the inhibition of the Janus kinase signal transducer (2). Several lines of evidence support the role of SOCS-2 as a negative regulator of GHR signaling (2, 3).

Several studies demonstrated that common polymorphisms in the GH/IGF-I system can modulate the effectiveness of rhGH treatment. Two meta-analyses confirmed a small, but significant, influence of *GHR* polymorphism on the growth response of children treated with rhGH (4, 5). Furthermore, a single-nucleotide polymorphism (SNP) located at position −202 of the *IGFBP3* promoter region (rs2854744) has also been involved in rhGH pharmacogenetics (6, 7). Noteworthy, a two-locus interactions analysis demonstrated the interactive effect of these two common polymorphisms on treatment outcomes, especially on adult height, of patients with Turner syndrome (TS) treated with rhGH (7).

Polymorphisms in *SOCS2* have been associated with adult height variation in healthy individuals (8–10). There are no studies analyzing the effect of these polymorphisms on GH therapy. Thus, the aim of this study was to assess the influence of *SOCS2* polymorphisms and their interactive effect with *GHR* exon 3 and −202 A/C *IGFBP3* polymorphisms on adult height of patients with TS and GHD treated with rhGH. Furthermore, we propose that these three loci, *GHR*, *IGFBP3* and *SOCS2*, could be used to identify poor responders to rhGH therapy.

Materials and Methods

Subjects

This study was approved by the local ethics committee. Patients, parents, or guardians gave their written informed consent. Sixty-five patients with TS and 47 patients with severe GHD were selected. The detailed inclusion and exclusion criteria are provided in the Supplemental Methods.

Genotyping

Four *SOCS2* tagSNPs (rs3782415, rs3816997, rs3825199, and rs11107116) were genotyped by allelic discrimination real-time PCR, two of them associated with variation in adult height in genome-wide association studies (rs3782415 and rs11107116) (8–10). The −202 A/C IGFBP3 and GHR exon 3 polymorphisms were genotyped as previously described (7).

Statistical analysis

A complete description of all statistical analyses is available in Supplemental Methods. In summary, to assess whether genetic variants had independent prognostic significance for adult height, regression analyses adjusting for the established influential factors were performed. The significant associations observed in TS patients were confirmed in patients with GHD. The effect of combined multiple-loci genotype was also assessed by pooling patients with GHD and TS. Patients with GHD and TS were classified as the worst responders when their adult height SD score (SDS) was at the lower quartile in each short stature etiology group. Generalized multifactor dimensionality reduction was applied to analyze gene-gene interactions (11).

Results

Clinical correlations

We did not detect deviations from the Hardy-Weinberg equilibrium for genotype distributions of all polymorphisms. The genotypes had a similar distribution in TS and GHD patients. Genotypic groups were similar concerning chronological and bone ages at the start of treatment, basal height, parental height, the frequency of spontaneous or induced puberty, age at puberty onset, mean rhGH dose, duration of therapy, karyotype distribution (for TS), and gender distribution (for GHD) (Table 1 and Supplemental Tables 1–10).

Associations with adult height SDS after rhGH therapy in patients with TS (with or without target height adjustment) were performed under an additive model or dominant model based on genotype frequencies, adjusted for height SDS at the start of rhGH therapy. Only SNPs rs3782415 and rs11107116 reached statistical significance level under an additive model ($P < .001$) at the linear regression after the correction for multiple tests. Therefore, the subsequent analyses were only performed with these SNPs under an additive model.

Multiple linear regressions have shown that *SOCS2* SNP rs3782415 is a predictive variable of adult height in patients with TS treated with rhGH. Together with height SDS at the start of rhGH therapy ($P < .001$), *GHR* exon 3 genotype ($P = .040$) and *−202 A/C IGFBP3* ($P < .001$), this *SOCS2* polymorphism ($P = .032$) accounted for 63% of adult height variation. *SOCS2* SNP rs11107116 was

Table 1. Clinical Characteristics of 65 Patients With TS and 47 Patients With GHD Grouped According to *SOCS2* rs3782415 Genotype

	SOCS2 rs3782415 Genotype				*P* Value	
	TT	TC	CC	*/C	TT vs. TC vs CC [a]	TT vs */C [b]
Patients with Turner syndrome						
n	35	26	4	30		
Target height SDS	−1.0 ± 0.8	−0.9 ± 1.0	−0.7 ± 0.8	−0.9 ± 0.9	NS	NS
Chronological age at the start of therapy, y	11.4 ± 3.7	11.4 ± 2.7	12.2 ± 3.0	11.8 ± 2.9	NS	NS
Bone age at the start of therapy, y	10.3 ± 3.0	9.5 ± 2.6	10.5 ± 0.7	10.0 ± 2.5	NS	NS
Height SDS at the start of therapy	−3.2 ± 1.4	−3.0 ± 1.1	−2.8 ± 0.4	−3.0 ± 1.0	NS	NS
Mean rhGH dose, μg/kg·d	48	48	48	48	NS	NS
Duration of rhGH therapy, y	5.1 ± 2.5	5.0 ± 2.6	4.0 ± 0.9	4.8 ± 2.4	NS	NS
First-year growth velocity, cm/y	6.8 ± 2.5	7.1 ± 1.9	7.2 ± 0.9	7.1 ± 1.8	NS	NS
Adult height SDS	−2.3 ± 1.1	−1.8 ± 0.8	−0.5 ± 0.4	−1.6 ± 0.9	.002	.007
Adult height-target height SDS	−1.4 ± 1.0	−0.8 ± 1.0	0.2 ± 0.4	−0.7 ± 1.2	.003	.013
Patients with GHD						
n	25	18	4	24		
Target height SDS	−1.2 ± 0.7	−0.8 ± 0.9	−1.0 ± 0.7	−0.9 ± 0.9	NS	NS
Chronological age at the start of therapy, y	10.1 ± 4.0	8.4 ± 3.8	10.5 ± 2.5	9.4 ± 3.4	NS	NS
Bone age at the start of therapy, y	4.7 ± 2.3	4.4 ± 2.8	4.0 ± 1.1	4.3 ± 2.6	NS	NS
Height SDS at the start of therapy	−4.9 ± 1.7	−4.0 ± 1.3	−4.6 ± 1.3	−4.0 ± 1.3	NS	NS
Mean rhGH dose, μg/kg·d	33	33	33	33	NS	NS
Duration of rhGH therapy, y	8.4 ± 3.5	8.8 ± 2.9	7.7 ± 0.6	8.6 ± 2.7	NS	NS
First-year growth velocity, cm/y	11.0 ± 2.3	11.3 ± 2.6	12.4 ± 3.0	11.5 ± 2.7	NS	NS
Adult height SDS	−1.3 ± 0.8	−0.6 ± 1.1	0.5 ± 0.6	−0.5 ± 1.1	<.001	.005
Adult height-target height SDS	−0.2 ± 0.9	0.2 ± 1.5	1.5 ± 1.0	0.4 ± 1.5	.031	NS

Abbreviations: C/*, CC and TC genotypes; NS, not significant.

[a] One-way ANOVA.

[b] Student's *t* test.

not an independent variable after combining multiple genetic variants and clinical data ($P = .151$). Therefore, only *SOCS2* SNP rs3782415 was included in further analysis. Females with TS carrying at least one *SOCS2* SNP rs3782415-C allele reached on average a 0.7 SDS higher adult height than those homozygous for T allele [confidence interval (CI) 95% 0.2–1.2, $P = .007$] (Figure 1A and Table 1). To confirm the role of *SOCS2* SNP rs3782415 polymorphism on rhGH pharmacogenetics, these results were replicated in patients with GHD. As observed in patients with TS, patients with GHD carrying at least one *SOCS2* SNP rs3782415-C allele reached on average 0.8 SDS higher adult height than those homozygous for the T allele (95% CI 0.3–1.3, $P = .005$) (Figure 1B and Table 1).

The global effect of the combined multiple-loci genotype on adult height after rhGH therapy was assessed by pooling the patients with GHD and TS. Adjusted for the clinical variables [short stature etiology (GHD or TS, $P < .001$) and the height SDS at the start of rhGH therapy ($P < .001$)], the *SOCS2* SNP rs3782415 ($P = .001$), *GHR* exon 3 ($P = .008$) and *−202 A/C IGFBP3* ($P = .006$) polymorphisms explained 58.3% of the variability in adult height after rhGH treatment. When the total height SDS gain was evaluated as the dependent variable, *SOCS2* SNP rs3782415 ($P = .001$), *GHR* exon 3 ($P = .008$) and *−202 A/C IGFBP3* ($P = .006$) polymorphisms combined with the clinical factors [short stature etiology ($P < .001$) and height SDS at the start of rhGH therapy ($P < .001$)] accounted for 82% of its variability.

The cohorts were also classified by quartiles of growth response. Patients with TS had a median adult height SDS of −1.9 and those with adult height SDS < −2.5 were in the worst response quartile, whereas patients with GHD had a median adult height SDS of −1.0, with the worst response quartile represented by patients with an adult height SDS of −1.5 or less. The genotype distribution of *SOCS2* rs3782415 ($P = .002$); *GHR* exon 3 ($P < .001$) and *−202 IGFBP3* ($P < .001$) polymorphisms was significantly different between the group of patients in the worst response quartile and the remaining quartiles (Figure 1C and Supplemental Table 3).

We applied the generalized multifactor dimensionality reduction method to our data to assess gene-gene interactions associated with increased risk to be in the worst response quartile to rhGH therapy (11). The models of GHR exon 3/−202 *IGFBP3* (two loci) and GHR exon 3/−202 *IGFBP3/SOCS2* SNP rs3782415 (three loci) suggested the presence of a gene-gene interaction. The two-loci model has the higher testing accuracy, whereas the three-loci model has the best cross-validation consistency

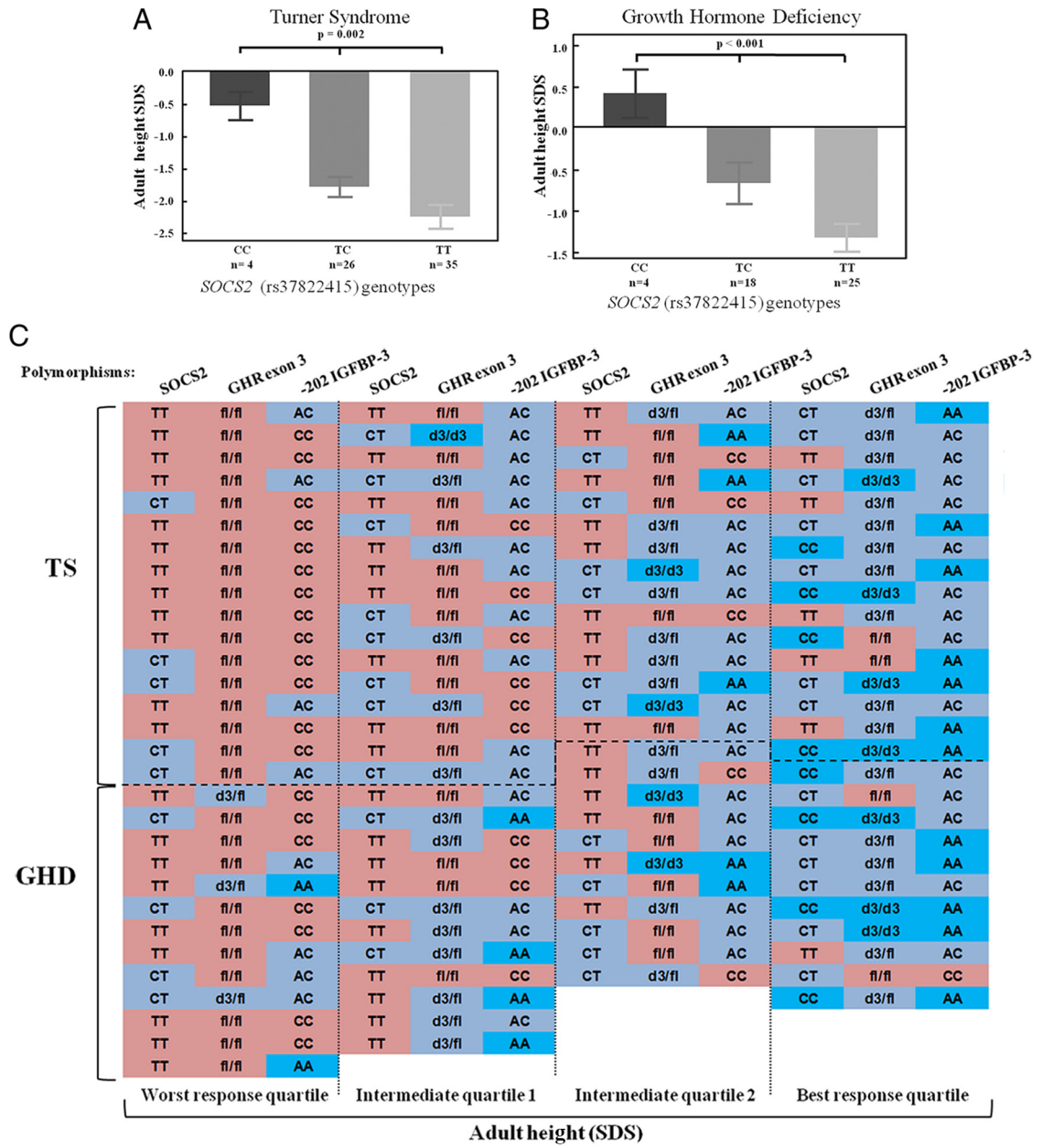

Figure 1. Influence of *SOCS2* rs3782415 genotypes on adult height in patients with TS (A) and in patients with GHD (B) treated with rhGH. *SOCS2* rs3782415, *GHR* exon 3 and −202 A/C *IGFBP3* individual genotypes (lines) according to quartiles of growth response (columns) in patients with Turner syndrome and GHD treated with rhGH (C). Genotypes associated with unfavorable outcome are shown in red, whereas genotypes associated with favorable adult height after rhGH therapy are shown in blue.

(Supplemental Table 4). Patients with TS and GHD harboring any two unfavorable genotypes in these three different loci [homozygosity for *SOCS2* SNP rs3782415-T allele; for *GHR* exon 3 full-length allele and/or for *−202 C-IGFBP3* allele] were more likely to achieve an adult height at the lower quartile (odds ratio 13.3; 95% CI 3.2–54.2, $P = .0001$].

Discussion

Considering that growth response to rhGH treatment is a complex trait determined by a network of clinical and genetic factors that can involve the entire GHR-dependent signaling cascade, it is important to expand the knowledge about gene-gene interactions at this network. The results of the present study demonstrate that *SOCS2* is an additional gene involved in rhGH pharmacogenetics. *SOCS2* SNP rs3782415 and rs11107116 T alleles were negatively associated with adult height after rhGH therapy, which is in accordance with genome-wide association studies results for adult height variation (8, 12, 13). The number of patients with the CC genotype was small, but this genotype was combined with CT as the better response genotypes compared with TT. It is noteworthy that the mag-

nitude of influence of these SNPs on rhGH pharmacogenetics is greater than that observed in adult height variation of healthy individuals (−2.8 cm vs −0.4 cm per each T allele in *SOCS2* rs3782415 in the adult height, respectively). It is reasonable to suppose that, in healthy individuals, slight differences in GH sensitivity could be compensated by a corresponding increase in GH secretion. On the other hand, differences in GH sensitivity would be more clearly observed in patients with an impairment of this adaptive response, such as those receiving rhGH therapy.

In addition, our results suggest an interaction among *SOCS2* rs3782415 SNP, *GHR* exon3 and *−202A/CIGFBP3* polymorphisms to influence the adult height of these patients. The similar results observed in patients with two distinct causes of short stature (GHD and TS) are not completely unexpected. It is acceptable to assume that some genetic polymorphisms that modulate GH action could have a role in rhGH pharmacogenetics independently of short stature etiology, similarly to what happens in growth prediction models in which different short stature causes share the same clinical predictors (14–16). In two recent studies from the same research group, the influence of several SNPs in 103 candidate genes on the first-year growth response with rhGH (17) and IGF-I generation test (18) was evaluated in a multiethnic group of girls with TS and in children with variable degrees of GHD. In these studies, the GHR exon 3 polymorphism was not genotyped. Interestingly, although there was some degree of overlap in the genes associated with the variability of response regarding these two end points, most the associations were end point specific (17, 18). Polymorphisms in *SOCS2* were associated with 1-month IGF-I generation in girls with TS (18), whereas polymorphisms in *IGFBP3* were associated with first-year growth response with rhGH (17).

We showed that homozygosity for *T-SOCS2* SNP rs3782415, *GHR* exon 3 full-length and *−202 C-IGFBP3* alleles was associated with less favorable long-term growth outcomes after rhGH treatment in patients with TS and severe GHD (Figure 1C). GHD and TS patients harboring a combination of unfavorable alleles in these three loci have a high probability to have a poor outcome regarding adult height after standard rhGH therapy. Predictive powers like those reached in the present study are sufficient to justify the realization of additional studies in different cohorts to verify if these associations are translatable to clinical practice. Due to the difficulty in obtaining larger and homogeneous cohorts of patients with TS and GHD, multicenter studies and/or future meta-analysis will be necessary to confirm the role of this polymorphism in rhGH pharmacogenetics.

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