Genetic Polymorphisms as Predictive Markers of Response to Growth Hormone Therapy in Paediatric Growth Hormone Deficiency

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Introduction

Linear growth is regulated through the interaction of different hormones. One of the most important regulators is growth hormone (GH), which is produced by the pituitary gland. GH deficiency (GHD) is the most common endocrine cause of impaired growth and is commonly treated with recombinant human GH (rhGH). Response to treatment is highly variable and a considerable proportion of patients do not reach an adult height within the target range for their family even after long-term rhGH therapy (1).

The influence of clinical factors such as age and height at treatment initiation, treatment duration and genetic target size on the success of rhGH therapy are estimated at 40–61% (2). By contrast, the influence of genetic factors on growth remains poorly understood. There is, nonetheless, a growing body of evidence that variation in growth-related genes, e.g. deletion of exon 3 in the growth hormone receptor (GHR) gene, may impact response to GH treatment (3).

The objective of our present study was to identify genetic factors that might serve as predictive markers of response to rhGH treatment. To this end, we analysed thirteen known single nucleotide polymorphisms (SNPs) and investigated whether the complete deletion of exon 3 in the growth hormone receptor (GHR) and the growth response were associated.

	SNPs (all patients)	Exon 3 deletion (prepubertal subgroup)
Patients, n	101	81
Male sex, n (%)	62 (61.4)	47 (58.0)
Female sex, n (%)	39 (38.6)	34 (42.0)
Prepubertal patients, n (%)	81 (80.2)	81 (100.0)
Patients at onset of puberty (max. Tanner stage 2), n (%)	20 (19.8)	0 (0.0)
Mean (SD) age at treatment initiation, years	9.0 (3.6)	8.0 (3.2)
Median (range) age at treatment initiation, years	9.5 (0.2–15.2)	7.9 (0.2–13.3)
Mean (SD) peak serum GH level (stimulation tests), µg/l	5.8 (2.4)	5.7 (2.6)
Median (range) peak serum GH level (stimulation tests), µg/l	6.2 (0.1–9.9)	5.9 (0.1–9.9)
Mean (SD) daily rhGH dose, µg/kg BW	22.5 (4.5)	23.0 (4.8)
Median (range) daily rhGH dose, µg/kg BW	20.0 (20.0–40.0)	20.0 (20.0–40.0)
Mean Ht-SDS (SD) at treatment initiation	-3.0 (1.0)	-3.2 (1.1)
Mean Ht-SDS (SD) after treatment of one year	-2.5 (1.1)	-2.5 (1.1)
Mean HV-SDS (SD) at treatment initiation	-0.7 (2.1)	-0.7 (2.2)
Mean HV-SDS (SD) after treatment of one year	2.0 (2.2)	2.1 (2.3)

SD, standard deviation; GH, growth hormone; rhGH, recombinant human GH; BW, body weight.

Table 1: Study population characteristics by type of analysis

Results

Two of the SNPs we analysed, rs2888586 in the SOS1 gene and rs2069502 in the CDK4 gene, were associated with statistically significant differences in IoR (see table 2).

With rs2888586 in SOS1, the TT genotype was associated with higher IoR values than the CT or the CC genotype (P=0,014).

With rs2069502 in CDK4, the GG allele was associated with increased IoR values than the GG or the AA genotype (P=0,011).

Furthermore, we analysed the association between the exon 3-deleted GHR and the IoR. Our results show that patients with the exon 3 deletion had higher IoR values than those with the full-length variant of the receptor whether homozygous or heterozygous deletion variant (P=0,022). Comparison by unpaired t-test of the median IoR values for the specific genotypes revealed a higher median IoR for the homozygous deletion than for the homozygous full length GHR variant (P = 0.016). Interestingly, patients who were heterozygous for the deletion had lower IoR values than those who carried two copies of the full length variant (P = 0.006).

Conclusions

In conclusion, we found two of the 13 SNPs investigated and the exon 3 deletion in the GHR gene to be associated with the IoR in paediatric GHD patients. As regards the rs2888586 SNP in the SOS1 gene, patients with the TT genotype showed better response to rhGH therapy than did those with the TC or CC genotype. Furthermore, the GG and AG genotypes of the rs2069502 SNP in the CDK4 gene were associated with better response to rhGH treatment than was the AA genotype. Complete deletion of exon 3 in the GHR gene was also associated with better response to rhGH therapy.

Thus, the genetic variations we studied may serve as predictive markers of response to rhGH treatment in paediatric patients with GHD. The results of the present study indicate that genetic analyses are a valid starting point for the individualized treatment of GHD. Further prospective studies are needed to overcome the limitations of our study.

Materials & Methods

In total, 101 children with GHD receiving rhGH therapy at our paediatric endocrinology outpatient clinic underwent genetic analysis for 13 known SNPs in genes of the GH axis (SOS1, IGFR1, GAB1, LHX4, IGFBP3, GRB10, GHRHR, GHSR), growth plate (VDR, ESR1) and cell cycle (CDK4) by high resolution melting analysis (HMR) and sequencing (see figure 1).

The stage of pubertal development was not to exceed Tanner 2, i.e. before the beginning of the pubertal growth spurt . SNP analysis was performed in all patients and prepubertal participants (81 children) were additionally examined for complete deletion of exon 3 in the GHR gene by PCR (4). Patients with GHD after treatment for malignant disease were excluded from participation (see table 1).

An objective measure of response to rhGH therapy is provided by the index of responsiveness (IoR), which is calculated from the observed height velocity and the predicted height velocity based on factors such as age at treatment initiation, dosage, maximum GH concentration after stimulation, height and weight at treatment initiation, birth weight and mid-parental height (5). Individual index of responsiveness (IoR) values were calculated and analysed by genotype by one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

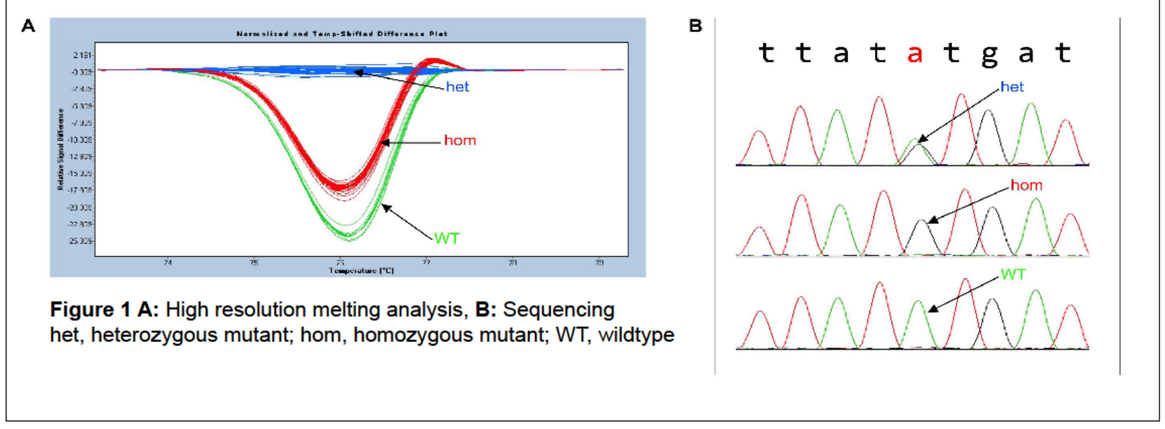

Figure 1 A: High resolution melting analysis, **B:** Sequencing
het, heterozygous mutant; hom, homozygous mutant; WT, wildtype

SNP / Gene	Alleles / Mean IoR			*P*
rs2888586 ***SOS1***	TT 0.7483	CT 0.0959	CC −0.4324	**0.014**
rs2871865 *IGF1R*	CC –/–	CG 0.2304	GG –/–	–/–
rs300919 *GAB1*	TT 0.1607	CT 0.1574	CC −0.3870	0.271
rs3845395 *LHX4*	GG 0.0023	CG 0.0423	CC 0.5400	0.493
rs3110697 *IGFBP3*	TT 0.4479	CT −0.0196	CC −0.0463	0.503
rs933360 *Grb10*	GG −0.0100	AG 0.3679	AA −0.1145	0.320
rs2854744 *IGFBP3*	CC −0.1588	AC 0.2766	AA −0.1929	0.278
rs2267723 *GHRHR*	AA 0.0500	AG −0.0138	GG 0.1400	0.917
rs572169 *GHSR*	GG 0.0595	AG 0.0595	AA −0.5750	0.383
rs2228570 *VDR*	TT −0.2308	TC −0.2100	CC 0.3098	0.178
rs2347867 *ESR1*	GG 0.3357	AG 0.0065	AA −0.0373	0.690
rs2069502 ***CDK4***	GG 0.2539	AG 0.1065	AA −1.1455	**0.011**
rs2270777 *CDK4*	GG 0.0025	AG 0.1243	AA −0.0762	0.859
Exon 3 Deletion GHR	fl/fl -0.2638	fl/d3 -0.4792	d3/d3 0.8000	**0.022**

P-values in bold-face indicate statistical significance.

Table 2: Mean IoR values in relation to genotypes for the SNPs/Exon 3 Deletion analysed in the present study and respective P-values of the analysis of variance (ANOVA)

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