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Original Article

Relationship between initial treatment effect of recombinant human growth hormone and exon 3 polymorphism of growth hormone receptor in Chinese children with growth hormone deficiency

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Abstract: The aim of this study is to investigate the frequency distribution of exon 3 deleted (d3-GHR) genetic polymorphism of growth hormone receptor (GHR) in growth hormone deficient (GHD) Chinese children and to explore the correlation between the growth promoting effects of recombinant human growth hormone (rhGH) and exon 3 genetic polymorphism of GHR in GHD children. In this study, 111 GHD (excluded small for gestational age) children were treated with rhGH (0.20 mg/kg/week) for six months. The body height (Ht), body weight, bone age (BA) and growth velocity (GV) were measured before and after six months of treatment. The d3-GHR and full length GHR (fl-GHR) were analyzed to detect the frequency distribution of two isoforms and their influence on growth promoting effect of rhGH. The results indicated that the frequencies of fl/fl, fl/d3 and d3/d3 GHR genotypes were 67.6%, 18.9% and 13.5%. After six months of GH therapy, there were significant differences of ΔGV (ΔGV: 10.77±3.40 cm/year vs 12.18±3.08 cm/year) ($P<0.05$) and ΔHt (ΔHt: 5.38±1.70 cm vs 6.09±1.54 cm) ($P<0.05$) were found among GHD children with different genotypes (fl/fl vs fl/d3 and d3/d3). In conclusion, the frequency distribution of three GHR genotypes in 111 Chinese GHD children was different from that reported in Caucasian, indicating the existence of ethnic difference of exon 3 GHR polymorphism. There was a closely relationship between GHR genotypes and growth-promoting effect of rhGH in Chinese GHD children.

Keywords: Growth hormone deficiency, growth hormone receptor, exon 3, polymorphism, recombinant human growth hormone

Introduction

Growth hormone deficiency (GHD) is an important cause of short stature. It was reported incidence of GHD was about 1/30,000 in children [1] and 1.2/100,000 in adult [2]. A recent nationwide survey in Denmark [3] revealed that among 1823 GHD patients, childhood onset of GHD was documented in 303 males and 191 females suggesting a significantly higher incidence in males. Recombinant human growth hormone (rhGH) was approved by U.S. Food and Drug Administration (FDA) in 1985 for treatment to GHD and replaced human pituitary derived growth hormone which was discontinued because of safety issues. In the following 20 years, it was confirmed by a series of clinical trials that rhGH could improve the growth and final height of short patients with non-GHD diseases such as Turner syndrome (TS), chronic renal failure (CRF), small for gestational age infant (SGA), Prader-Willi syndrome (PWS) and idiopathic short stature (ISS).

Long-term clinical observations have revealed that the growth promoting effect of GH varied significantly between individuals. The growth response to treatment depends on age, bone age delay, height standard deviation score at onset of treatment, mid-parent height, drug dosage and treatment duration, but also might be related to genetic background and epigenetic influences on the individual sensitivity to drugs [4].

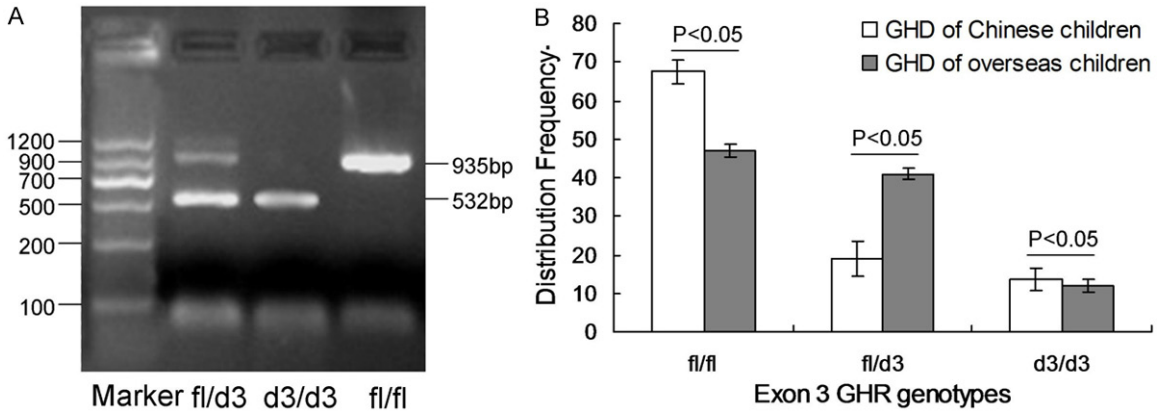

Figure 1. Multiplex PCR results of GHR gene exon 3. fl/fl indicates homozygous full-length genotype; d3/d3 indicates homozygous deletion genotype; fl/d3 indicates heterozygous exon 3 genotype.

Growth hormone receptor (GHR OMIM*600946) is one of candidate genes which is recognized to affect the response to rhGH treatment. GH, a decisive factor of postnatal growth, exerts its role through binding to GHR in a majority of conditions [5]. GHR gene is located on the short arm of chromosome 5 (p13.1-p12) and consists of nine exons. There are two GHR isoforms including full-length GHR (fl-GHR) and exon 3-deleted GHR (d3-GHR), and three genotypes including fl/fl, fl/d3 and d3/d3. A single allele of fl-GHR or d3-GHR has been shown to be sufficient for growth. It was reported that the frequency distribution of d3-GHR in human was approximately 50% and seemed to be less frequent in Japanese and West Africans [6, 7]. Study on the effect of d3-GHR and rhGH responsiveness was first carried out by Dos Santos et al, and a significant correlation between d3-GHR allele and rhGH therapeutic efficacy was found in ISS and SGA children [7]. However, subsequent studies did not always confirm the findings of the study. After 2 years of high-dose GH therapy (66 μg/kg.d), no significant difference in height between patients with d3-GHR genotype and fl-GHR genotype was found [8]. An Italian report found that patients with GHD carrying the d3-GHR allele was not related to better growth-promoting effect of rhGH as compared to those with fl-GHR [9]. Meanwhile, another report indicated that the presence of d3-GHR was related to a better growth-promoting effect of rhGH in the treatment severe GH deficient patients [4]. Although there are many related studies in recent years, the positive growth promoting effect of rhGH in patients carrying the d3-GHR genotype is still controversial.

Therefore, this study aims to investigate the frequency distribution of exon d3-GHR genetic polymorphism of GHR in GHD Chinese children and compare the GHR exon 3 polymorphism distribution of overseas children.

Material and methods

Inclusion criteria of subjects

We selected a total of 111 prepubertal (assessed by Tanner Staging method) isolated GHD children, with a mean age of 11.05±3.24 y, from Department of Pediatric Endocrinology and Inborn Metabolic Diseases, Children's Hospital, Fudan University, China. Inclusion criteria of subjects were as follows: (1) Ht is more than 2SD below the sex and age specific population mean in the same region (based on the Normal Body Height Standard of Urban and Rural Children published in China in 1995 [10]; (2) growth velocity (GV) <4 cm/year; (3) bone age (BA) delay; (4) peak GH value to provocation test <10 ng/ml (stimulated by insulin induced hypoglycaemic or intravenous arginine); (5) never receive any GH therapy. 206 normal Han children were recruited as control for genotype comparison.

This study was approved by the Local Research Ethics Committee. Informed consent for DNA sampling and for participation in this study was obtained from the subjects' parents.

Data collection and evaluation method

All GHD children were treated with rhGH (0.20 mg/kg/week in divided doses given daily) subcutaneously for six months. No other hormone was administrated during treatment. Body

Table 1. Baseline clinical data and the growth response to rhGH six months treatment in GHD children carrying the fl-GHR or d3-GHR genotype

Genotypes	fl/fl	fl/d3 and d3/d3	*P* value
Sex (male: female)	60:15	31:5	*NS*
Age (year)	10.70±2.95	10.54±3.57	*NS*
Ht (cm)	118.82±11.26	117.42±14.60	*NS*
Wt (kg)	23.65±6.98	23.56±8.31	*NS*
Ht of father (cm)	166.89±6.65	167.14±4.79	*NS*
Ht of mother (cm)	155.68±5.84	155.94±6.05	*NS*
SD of Ht (sd)	-3.70±1.42	-4.04±2.12	*NS*
BA (years)	6.58±2.51	6.33±2.89	*NS*
GV (cm/year)	2.75±0.79	2.55±0.95	*NS*
Peak GH value (ng/ml)	3.65±2.79	3.33±2.70	*NS*
ΔGV (cm/year)	10.77±3.40	12.18±3.08	0.038
ΔHt (cm)	5.38±1.70	6.09±1.54	0.038
ΔWt	3.13±1.82	3.36±1.81	*NS*

NS: not significant.

weight, Ht, BA and GV were measured before treatment and after six months of rhGH therapy.

All Ht of GHD children were measured by researchers using a wall-mounted stadiometer using a standardized technique and body weight measured with an electronic scale. BA was determined by the method of Greulich-Pyle [11]. The heights of the patients were expressed as standardized deviation scores based on Chinese growth standard [10].

DNA extraction and PCR proliferation

Peripheral blood genome DNA was extracted by phenol- protease method [12]. GHR exon 3 was amplified by multiplex PCR with primer G1 (5'-3') TGTGCTGGTCTGTTGGTCTG. fl-GHR (935 bp) was amplified with primer G2 (5'-3') AGTCGTTCTGGGACAGAGA, and d3-GHR (532-bp) was amplified with primer G1 and primer G3 (5'-3') CCTGGATTAACACTTTGCAGACTC.

Reaction conditions: In sequence, initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 20 sec, annealing at 60°C for 30 sec and extension at 68°C for 1 min by DNA polymerase (TOYOBO Co., Ltd., Japan), and a final extension at 68°C, preservation at 4°C.

PCR product electrophoresis: 10 μL PCR product was added into 2 μL 6× Loading Buffer and fully mixed, and then sample was directly loaded into 2% agarose gel. After electrophoresis in 1× TBE buffer solution on 100 v, electrophoresis strips were observed under UV lamp, and then a picture was taken and printed (**Figure 1A**).

Statistical analysis

All data were expressed as mean ± standard deviation. T-test was performed by using SPSS Version 17.0 statistical software. P<0.05 was considered statistically significant. (1) Frequency distribution of three exon 3 genotypes (fl/fl, fl/d3 and d3/d3) in GHD children and general Han population was calculated and compared with related data overseas. (2) According to measurement results of three genotypes in GHD children treated with rhGH, GHD children were divided into two groups (group fl/fl and group fl/d3 and d3/d). GV change (ΔGV = GV after treatment-GV before treatment), Wt change (ΔWt = Wt after treatment-Wt before treatment) and Ht change (ΔHt = Ht after treatment-Ht before treatment) were analyzed by t test with SPSS Version 17.0 statistical software. P<0.05 was considered statistically significant.

Results

GHR genotypes distribution and frequency in Chinese children

After agarose gel electrophoresis of multiplex PCR products, two electrophoresis bands were observed, and the former (935 bp) was fl-GHR isoform and the latter (532 bp) was d3-GHR isoform (**Figure 1A**). The frequency of fl/fl, fl/d3 and d3/d3 was 67.6%, 18.9% and 13.5% respectively in 111 GHD children. The frequency distribution of three exon 3 GHR genotypes in GHD children reported by overseas studies (fl/fl was 47%, fl/d3 was 41% and d3/d3 was 12%). For all of the 3 GHR genotypes, there were significant difference in GHD of Chinese children compared to the GHD of overseas children (**Figure 1B**, P<0.05). Also, the GHD distribution in overseas children was different from that observed in the present study (chi-square test *P* value = 0.0001) [4].

Table 2. Comparison of GHR exon 3 polymorphism distribution

	fl/fl	d3/fl	d3/d3
Caucasian GHD children*	100 (50%)	100 (50%)	
SGA children*	30 (44%)	32 (47%)	6 (9%)
TS children*	27 (51%)	15 (28%)	11 (21%)
ISS children*	23 (50%)	22 (50%)	
Our study data	75 (67%)	21 (19%)	15 (14%)

*Significant difference respectively compared with our study.

Analyses of baseline data and effect in GHD children

The clinical characteristics of prepuberted GHD children before treatment were shown in **Table 1**. No significant differences in age, GV, Ht of parents, peak GH value and BA were found among three GHR genotypes before treatment. Furthermore, no significant differences in ΔGV, ΔWt and ΔHt were found after GHD children were treated with rhGH for six months.

GHR exon 3 genotypes frequency was distinguish from overseas children

We also compared the GHR exon 3 genotypes frequency in different nationality. The frequency of each GHR exon 3 genotype in our study is different from that in Caucasian GHD children or that in SGA, ISS or TS children (**Table 2**, $P<0.05$).

Discussion

Effective growth promotion with high-dose GH in short children with GHD is influenced by the GH sensitivity, which depends on multiple gene loci involved in the functioning of the GH-IGF-1-signaling cascade as well as on the response of the epiphyseal growth plate [13, 14]. Pharmacogenetics of GH should enable us to relate the polymorphic sites of these gene loci to the individual growth response. Moreover, it should improve the prediction of the profit of therapy under ideal circumstances [15]. The pharmacogenetic data presented here point to the importance of the GHR genotype for GH responsiveness although the magnitude of this effect was group specific.

As early functional studies showed no difference in GHR exon 3 isoforms, the first report of a positive influence of the GHRd3 allele on the growth rate of children treated with hGH was received with skepticism and, soon after its publication, several authors investigated the reproducibility of this discovery in their own cohorts. Five studies investigated the influence of GHR exon 3 isoforms on the growth response of children with GHD, and contradictory results were observed [4, 9, 16-18].

Rare pharmacogenetic study of Chinese GHD children was carried out. In this study we analyzed the impact of GHR genotypes (GHR-d3/d3, GHR-d3/fl, and GHR-fl/fl) on growth response to rhGH replacement therapy in patients suffering from severe IGHD. A total 111 Chinese Han children were followed and prepubertal GV during the first 6 months on rhGH replacement (the dose was 0.2 mg/kg/wk on each patient) were assessed.

Firstly, the frequency of each GHR exon 3 genotype is different from that in Caucasian GHD children [18] or that in SGA [8], TS [19] or ISS [20] children (**Table 2**). It suggested that the GHR-fl was the predominant isoform in Chinese GHD children, which may contributed to the significant different of the GV between GHR3+ and GHR3-group. Secondly, in Chinese GHD children, there is a obvious relationship between d3-GHR and treatment effect of rhGH within initial treatment. It is clear that a remarkable initial treatment effect benefits a lot including better compliance and higher cost-effective. Alexander Jorge et al. [4] reported on the comparison of growth response to GH of three GHR genotypes (fl/fl, fl/d3 and d3/d3) among 58 severe GHD children [with a mean age of 8.9±3.8 y, peak GH value to provocation test of <1 ng/ml and extremely low serum IGF-1 and IGFBP3 (IGF1: 29±30 μg/l; IGFBP3: 0.9±0.5 mg/l)]. A significantly higher GV, difference in HtSDS and target HtSDS, and final adult height was found after one year of high dose of rhGH (31±5 μg/kg.d) treatment in GHD children with d3-GHR allele as compared to those children carrying the fl-GHR variant. Barbara Raz et al. [16] studied 181 severe GHD children treated with rhGH (31.5 μg/kg·d) for 4 years, the results indicated that in patients with IGHD, GHR genotype might play a role in GH responsiveness, at least at the beginning of treatment, there is no effect on final height.

GH ligand biding to GHR represents the first step in GHR activation. GH binding to preformed

dimers of GHRs located on the cell membrane surface [21] induces a relative rotation of intracytoplasmatic subunits of the dimerized receptors, resulting in activation of Janus kinase 2 and downstream signaling pathways [22]. The 22 residues codified by exon 3 are located in the extracellular domain of the GHR, but not in the GH binding interface [23]. Initial experimental studies demonstrated that GHRd3 and GHRfl present similar binding capacity to GH [24]. Dos Santos et al. reported that cells transiently transfected with GHRd3 induce higher transcription activity in luciferase reporter assays after treatment with 22-kDa hGH than cells transfected with GHRfl [7]. No difference in transcriptional activity between cells transfected with GHRd3 alone or together with GHRfl was observed [7], suggesting a dominant effect of GHRd3 allele. The mechanism by which the deletion of the region encoded by GHR exon 3 could increase receptor activity has not been elucidated.

In summary, we discovered a direct relation between genetic variability and rhGH treatment effect after initial treatment in Chinese GHD children for the first time. Patients with GHD who carry at least one GHRd3 allele had a small but statistically significant higher initial growth response and GV than patients who are homozygous for the GHR fl allele, treated under the same conditions. Further prospective studies are needed to confirm the importance of GHR exon 3 genotype in the response to hGH therapy contributed to the final adult height in Chinese GHD children.

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Disclosure of conflict of interest

None.

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