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貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書

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2．研究テーマ

中国人低身長患者における成長ホルモン受容体の変異解析

3．成果の概要（100字程度）

Growth hormone receptor （GHR）において、Exon3を有するか（GHRfl）あるいは欠失するか（GHRd3）という多型が知られている。漢民族49名とウイグル民族23名および日本人52名の低身長者を対象にGHR多型を解析した。GHRfl/GHRfl、GHRfl/GHRd3、GHRd3/d3の割合はそれぞれ、漢民族では56%、40%、4%、ウイグル民族では72%、18.2%、9%、日本人では83.7%、14.3%、2%であった。以上から、漢民族はウイグル民族、日本人よりもGHRd3頻度が高いが、Caucasianよりは低いことが分かった。

4．研究業績

(1) 学会における発表 ☑無 ・ □有（学会名・演題）

(2) 発表した論文 ☑無 ・ □有（雑誌名・題名）

－日中医学協会助成事業－

中国人低身長患者における成長ホルモン受容体の変異解析

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Abstract

Exon 3 insertion or deletion is one common polymorphism in the growth hormone receptor (*GHR*) of humans. Deletion of exon 3 has been associated with the degree of height increase in response to GH therapy. The objective of the study was to evaluate the proportion of *d3/f1*-GHR polymorphism genotypes in Chinese 72 Short stature Han and Uihgur patients compare with Japanese 52 GHR genotypes. The Chinese Han group was classified into GHRf1/GHRf1 (56%) GHRf1/GHRd3 (40%) and GHRd3/GHRd3 (4%) based on genotypes of GHR-exon3. Genotypes of the Uihgur group and Japanese Group consisted of 72%, 83.7%with GHRf1/GHRf1, 18.2%, 14.3% with GHRf1/GHRd3 and 9%, 2% with GHRd3/GHRd3 respectively. The proportion with GHRf1/GHRd3 exon3 genotype in Chinese Han nation was found to be higher than Chinese Uighur and Japanese genotype but lower than that in Europeon people was with significant statistically.

Keywords: Growth hormone receptor, polymorphism, short stature, Chinese, different races.

Introduction

The human *GHR* gene is a single copy gene that spans 300 kb of chromosome 5. It contains nine coding exons that encode the receptor and several additional exons in the 5-prime untranslated region. (1) A polymorphism the human GH receptor gene (*d3/f1*-GHR) resulting in genomic deletion of exon 3 (2, 3). Recently associated with the degree of height increase in response to GH therapy in short French children born small for gestational age (SGA) or with idiopathic short stature (4), German Turner syndrome patients (5), and Brazilian GH-deficient children (6), although other studies found no such association (7, 8).However, a significant association between GHRd3 genotype and hypertension have been demonstrated, lacking a copy of the GHRd3 alleles to be a risk factor for hypertension has been reported(9). At the present the common polymorphism of GHR is as yet not documented statistically XinJiang of China. In an attempt to understand the polymorphism change of exon3 in GHR-gene in my area we examined 72 Chinese-Han and Uighur ethnic (totally deferent with main Han nation) compared to 52 Japanese sample genotype.

Materials and methods

Subjects

A total of 72 Chinese Short stature patient live in Xinjiang(biggest minority area in

China)aged 2-15(Uighur boy 11, Han boy 31,Uighur girl 12,Han girl 18); were recruited from June 2006 to October 2006. Participate in this study. Twenty-four were living rural area in XinJiang , twenty hospitals participated,28 from out patient . Inclusion criteria were: height lower than 3% of normal, never having been treated with GH or other anabolic agents; normal gastrointestinal, pulmonary, and liver function;. Exclusion criteria were: neonatal brain injury, chronic diseases, and steroid therapy. None were in an acute care situation。Height and weight were recorded by our-self at inclusion and the corresponding heights SDS were calculated. Serum IGF-I were measured at entry into the study in 41boys and 31girls and expressed as SDS according to age- and sex-matched controls and the ratio IGF-I calculated (Table 1).

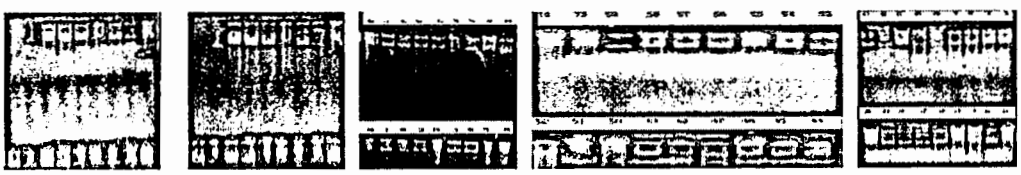
Table1.Height, IGF-1 date in the Chinese short Children

	Uighur Patient	Han Patient
Age	11.3±2.7	8.9±3.1
Height-SDS	-3.17±0.43	-2.93±0.93
IGF-1-SDS	-1.07±1.01	-0.89±1.34

Genotyping

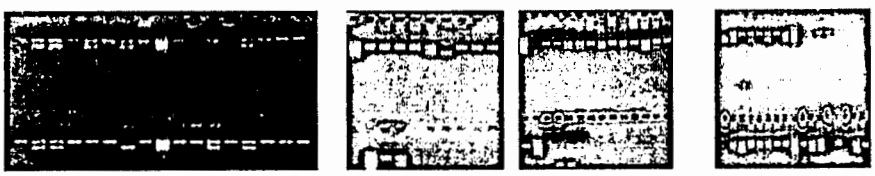
Genomic DNA was extracted from white blood cells of patient and Patient' s mother using a PUREGENE DNA Purification Kit (Gentra) and used as template DNA (Figure1).

Figure1: 72 template DNA in short stature from XinJiang



To determine genotype of *GHR* exon 3, we applied one revised polymerase chain reaction (PCR)-based assay method.9 The pair of primers G1 (5′ -GTTGGTCTGCTGGTCT GCTT-3′) and G3 5′ -GTGCTCTGCTAAGGATAGC TG-3′) allowed amplification of the exon 3 allele only, whereas primers G1 and G2 (ACTTTAGCCAGTCGT TCCTG) amplified *GHRd3* allele under specific conditions thereby discriminating the three possible alleles in this locus (i.e. homozygous of *GHRf1/f1*, *GHRd3/d3* and combination heterozygous *GHRf1/d3*) (Fig. 1a). Parameters of PCR were the initial step of 5 min at 94° C, followed by 35 cycles consisting of 30 s at 94° C, 30 s at 55° C,1 min 30 s at 72° C, and the last extension of 5 min at72° C. The expected length of 934 bp indicates the *GHRf1* allele and 532 bp indicates the *GHRd3* allele (Figure2)

Figure 2



Statistical analysis

Hardy-Weinberg equilibrium (HWE) was calculated according to standard procedures using x2 analysis. Differences for *d3/fl*-GHR genotype frequencies between three groups and height-SDS groups were analyzed by the by the x2 test.

Results

Similar proportion for the GHRfl/GHRfl and GHRfl/GHRd3 genotypes was found in both Japanese group and Chinese Uihgur group. In the Uighur group *d3/d3* genotype was higher and did reach statistical significance. Mean values of Uighur group height- SDS were lower than Han groups (although not statistically significant). The proportion of *d3/fl*-GHR genotype in Chinese Han group was higher than Japanese group and Chinese Uighur group but lower than European people did reach statistical signification. (Table 2)

Table2. d3-GHR genotypes (%) in Chinese-Japanese short stature patient

	Japanese	Chinese Han nation	Chinese Uihgur nation
d3/d3	2	4	9
d3/fl	14.7	40	18.2
fl/fl	83.7	56	72.7

IGF-I-SDS lower in Uighur group than Han group although not statistically significant, was observed.

Discussion

Growth hormone is used to increase height in short children who are not deficient in growth hormone, but its efficacy varies widely across individuals. In which genetic disposition, nutrition, homeostasis, hormones, and growth factors interact. Among these factors, GHR plays an important role, and loss-of-function mutations in the GHR gene lead to growth delay during infancy, childhood, and adolescence and short stature in adulthood (10-12).

There are 2 isoforms of GHR in humans, generated by retention or exclusion of exon 3 during splicing: a full-length isoform and an isoform that lacks exon 3 (d3-GHR). The generation of 2 transcripts that differ by the skipping of a coding exon results from homologous recombination, which mimics alternative splicing between the 2 retroviral sequences that flank the skipped exon (13). The allele encoding d3-GHR is specific to humans. Results of the studies (14) supported the hypothesis that the GHRd3 isoform is transcribed from a GHR allele carrying a genomic deletion of exon 3 rather than by alternative splicing. An isoform of the growth hormone receptor gene that lacks exon 3 (d3-GHR) was associated with 1.7 to 2 times more growth acceleration induced by growth hormone than the full-length isoform(15) (P less than 0.0001). In transfection experiments, the transduction of growth hormone signaling through d3-GHR homo- or heterodimers was approximately 30% higher than through full-length GHR homodimers (P less than 0.0001). Thus, the polymorphism in exon 3 of GHR is important in growth hormone pharmacogenetics. Dos Santos et al. (2004) stated that one-half

of Europeans are heterozygous or homozygous with respect to the allele encoding the d3-GHR isoform, which is dominant over the full-length isoform. In our study the proportion of GHRf1/GHRf1, GHRf1/GHRd3, GHRd3/GHRd3 genotype in Short stature Chinese Uighur group differ statistically and significantly from those Short stature Chinese Han group. This date suggest that in addition to the multiple factors that may contribute to short stature in Uighur group. In a summary, our date showed significant differences in the proportion of the d3-GHR polymorphism genotypes between Japanese groups, Chinese Uighur group with Chinese Han group. In addition Further investigate will be need to investigate polymorphism of GHR-gene in normal height control populations in my area and compare with this date.

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