Research in Pharmaceutical Sciences, December 2014; 9(6): 489-494
Received: Dec 2013
Accepted: Jan 2014

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Short Communication

Exon 3-deleted and full-length growth hormone receptor polymorphism frequencies in an Iranian population

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Abstract

The functional role of the exon 3 growth hormone receptor (*d3*GHR) polymorphism in human and its distributions in different populations is not clearly understood. The presence of full length growth hormone (*fl*GHR) is the most important in metabolic risk factors. The aim of this study was to define the frequency distribution of *d3*GHR/full-length GHR in an Iranian population. The presence of the *d3*GHR polymorphism in healthy volunteers blood DNA (n=80, male=30 and female=50) was assessed by PCR using specific primers. The 935-bp and 592-bp fragments indicate the presence of the *fl*GHR and the exon3 deletion of GHR, respectively. The distribution of the GHR genotypes in this study were 31.4% (n=24) for *fl/fl*GHR, 49.7 % (n=41) for *fl/d3*GHR, and 19.0 % (n=15) for *d3/d3*GHR. Frequencies of *fl* allele and *d3* allele were 55.4% and 44.4% within whole population, respectively. There was no difference in allels frequencies of GHR in male (*fl*=0.583, *d3*=0.417) and female (*fl*=0.540, *d3*=0.460) when compared with whole population. The results showed that the frequency of *d3/d3*GHR isoform was significantly lower than that of the *fl*/*fl*GHR and *d3*/*fl*GHR. The frequencies of GHR polymorphisms were likely consistent with previous reports. Our finding is also consistant with Mexican population. The advantage of existence of the *d3/d3* rather than *fl/fl*GHR polymorphisms in individuals and in correlation with diseases opens new insights for GH and insilin-like-growth factor-1 (IGF-I) axis.

Keywords: *d3*GHR; *d3*GHR/*fl*GHR; Polymorphism; Population; GH/IGF-I ***axis***

INTRODUCTION

Growth hormone (GH) exerts somatotropic and metabolic effects through its receptor to which has a high affinity for binding (1). GH receptor is composed of three parts including integrated memberane part and extracellular and intracellular domains. In addition to the membrane-bound growth hormone receptor (GHR), GH binding proteins (GHBPs), which essentially corresponds to the extracellular domain of GHR (1,2), has been identified in human serum (3,4).

Little is known about the physiological role of this soluble receptor (5,6). The integral segment of GHR is a part of the type 1 cytokine receptor super family which consist of a single membrane-spanning domain. The intracellular part of GHR conveys the signal transduction, after dimerization, via activation of JAK-STAT and MAPK pathways (7). The function of hormones receptor and cell survival are likely depend on the presence of trace elements (8). The zinc (Zn^{2+}) is an important element in GH function which causes the dimerization of GHR on the cell surface membrane (9).

This trimer complex at the extracellular domain of GHR might be triggered by *d3*GHR and consequently affects signal transductions, which reflects sensitivity to GH. The study of gene variability in different populations is very important for prediction and prognostigation of some diseases such as diabetes (10).

Based on the presence or absence of exon3, there are two variants of GHR namely *fl*GHR and *d3*GHR. Genomic deletion of exon 3 (*d3*), results in polypeptide that lacks 22 amino acids at the N-terminal part. The function of the N-terminal are still unknown (11,12).

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Experimental results strongly suggest that both the *d3*GHR and *fl*GHR are efficiently translated as functional receptors (11-13). The isoform of *d3*GHR has more receptor activity than *fl*GHR due to an increase in the signal transmission process.

The *d3*GHR signal transduction shows more activity than that of *fl*GHR. Inclusion or exclusion of exon 3 in GHR, facilitates critical alterations in hormone binding and physiological function (14). One study reported that the polymorphism of GHR is associated with increased responsiveness to exogenous GH.

The observation suggested that the presence of exon 3 in GHR length has an important role in GH pharmacogenetics (15). The presence of at least one receptor with exon 3 deleted allele could be associated with increased responsiveness to growth hormone. This phenomenon is probably due to the higher sensitivity to GH and subsequent increase in insuline-like growth factor-1 (IGF-I) secretion (16).

Recent studies have shown that *fl*GHR versus *d3*GHR has a stronger association with metabolic risk factors (12). The *d3*GHR in comparison with *fl*GHR has higher effects on biochemical and clinical parameters in patients with acromegaly (17-20).

Following that, a study on girls with Turner syndrome showed that homozygous for *d3*GHR is associated with more influence of growth hormone action with reduction of body mass index (21). The studies on children born small for gestational age (SGA) show that the children with homozygote *d3/d3* and *heterozygote d3/fl* genotype has greater growth in comparison to with homozygote *fl/fl*GHR genotype (22).

In a population of adults with type 2 diabetes the frequency of genotype homozygous *d3*/*d3*GHR is lower than that of normal.

It is suggested that the *d3/d3* GHR genotype may have a protective role against the development of insulin resistance (16). In order to gain more insight into the regulation of exon 3-deleted and full-length growth hormone receptor polymorphism frequencies, this study was conducted to investigate the individual distribution of the *d3*/*d3*GHR and the *d3/fl*GHR in human geneome. The data that will be obtained in these subjects help to estimate the role of GHR polymorphism in disease.

MATERIALS AND METHODS

This cross-sectional study was conducted on a population of healthy volunteers from city of Isfahan (30 to 60 years old, mean ± SD: 36.0 ± 11.4). The subjects did not have any underlying disease that could affect lipid, glucose and hemoglobin. Written informed consent was obtained from each individual taking part in the study.

Blood sampling

Blood samples were collected into EDTA evacuated tubes from each individual following overnight fasting. Aliquot of the extracted DNA was stored at -20 °C until analysis. Experiments on the stored samples should be performed up to two months after

Biochemical assay

Clinical and biochemical characteristics of the participants in this study were measured. Plasma glucose levels were determined using the glucose oxidize method.

DNA extraction

DNA was extracted from peripheral blood mononuclear cells by using DNA purification kit. Then, the extracted DNA was maintained at -20 °C for further analysis.

Growth hormone receptor genotyping

Extracted DNA used for multiplex polymerase chain reaction (PCR) analysis. The 935bp and 592bp products indicate respectively the presence of the flGHR and d3/d3 allele.

As shown in Table 1, the primer sequences used for investigating the GHR gene polymorphism were taken from Genebank Accession *No. AF155912*. The specific PCR protocols used for GHR (*fl/d3*) polymorphism analyses are summarized in Table 2 (23,24). Fig. 1 shows the PCR for growth hormone receptor polymorphism.

Table 1. The primer sequences used for growth hormone receptor gene polymorphism analyses.

Primer name	Sequence (5'-3')	Length (base pair)	Amplified fragment (base pair)
G1-G2	*TGTGCTGGTCTGTTGGTCTG*	20bp	532bp
	AGTCGTTCCTGGGACAGAGA	20bp	
G1-G3	*TGTGCTGGTCTGTTGGTCTG*	20bp	935bp
	CCTGGATTAACACTTTGCAGACTC	24bp	

Table 2. PCR protocols used for growth hormone receptor (*fl/d3*) polymorphism analyses.

Polymorphism	PCR protocol
GHR *fl/d3*	Pre-denaturation: 94 °C for 5 min 30 cycles at: Denaturation: 94 °C for 30 s Annealing: 54.7 °C for 30 s Extension: 72 °C for 1 min Final extension: 72 °Cfor 5 min

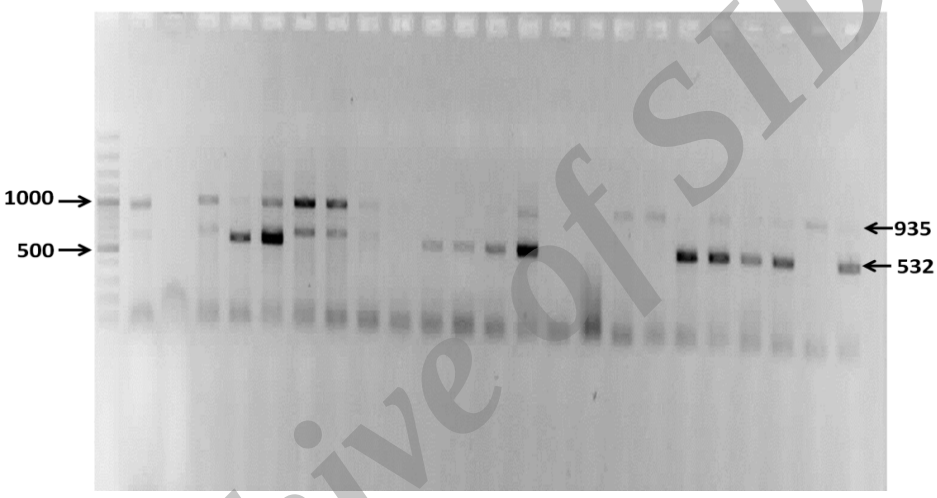

Fig 1. PCR for growth hormone receptor (*fl/d3*). 1% agarose gel electrophoresis stained with ethidiumbromide. The 500 and 1000 bp DNA of the ladder are shown at the left side of the gel. It is a pre-mixed, ready-to-load molecular weight marker containing bromophenol blue as a tracking dye. The amplified fragments of growth hormone receptor polymorphisms are shown at the right side of the gel.

Statistical analysis

SPSS Software Version 16.0 (SPSS Inc) was used for all statistical analysis. The Hardy–Weinberg equilibrium (HWE) was applied to calculate the genotype frequencies. Frequencies of distribution of alleles within the lines were compared using χ^2-test.

RESULTS

The distribution of the GHR genotypes in the population (n=80) was 31.4% (n=24) for *fl/fl*GHR, 49.7 % (n=41) for *fl/d3*GHR, and 19.0 % (n=15) for *d3/d3*GHR. The proportion of *fl/fl*GHR to *d3/d3*GHR is 1.65. Frequencies of *fl* allele and *d3* allele within the whole population were 55.4% and 44.4%, respectively. There was no difference between genders in GHR exon 3 deleted genotypes ($p<0.05$). The allel frequencies of GHR in male (*fl*=0.583, *d3*=0.417) and female (*fl*=0.540, *d3*=0.460) were compared with whole population (Table 3).

The frequencies of polymorphisms were likely consistent with previous reports in other populations (Table 3). The critical chi-square value for 1 degree of freedom was calculated to assess the allele frequencies and Hardy-Weinberg equilibrium for GHR genotype (Table 4).

Table 3. The differences in genotype and allele frequency distributions of growth hormone receptor polymorphism among different ethnic groups.

Study, reference	Population	n	Genotype			Allel	
			d3/d3 (%)	*d3/fl* (%)	*fl/fl* (%)	*fl* (%)	*d3* (%)
Palizban *et al*. (The present study)	Iranian	78	19.0 (n=15)	49.7 (n=41)	31.4 (n=24)	55.6	44.4
Montefusco *et al*. (12)	Italian	79	9.2	31.6	59.2	75.0	25.0
Mercado *et al*. (17)	Mexican	148	32.4	21.6	45.9	56.7	43.2
Bianchi *et al*. (18)	Italian	84	17.9	29.8	52.4	67.3	32.8
Kamenicky *et al*. (19)	French	105	19.0	29.5	51.4	66.2	33.8
Turgut *et al*. (20)	Turkish	35	14.3	31.4	54.3	70.0	30.0
McKay *et al*. (25)	Swedish	92	7.0	39.0	54.0	73.3	26.6

Table 4. The critical chi-square value for 1 degree of freedom. Allele frequencies and Hardy-Weinberg equilibrium for growth hormone receptor .

Genotype	Normal subjects					
	Male	Female	Total%	Observed	Expected	χ^2
d3/d3	N = 6(20.0%)	N = 9(18.0%)	19.0%	24	24.8	
fl/d3	N = 13(43.3%)	N = 28(56.0%)	49.7%	41	39.4	0.123
fl/fl	N = 11(36.7%)	N = 13(26.0%)	31.4%	15	15.8	
Total	30	50	100%	80	80	

DISCUSSION

The real impact of the presence of d3GHR in individuals and population are still unknown. Therefore, the main objective of this study was to investigate the distribution of GHR gene polymorphism in healthy subjects in Iranian population. Several studies revealed that the GHR polymorphisms are associated with many diseases (16,20, 25-27). The importance of the d3 allele and its impact on growth parameters before and during GH treatment in individuals with Prader–Willi syndrome (PWS) were also considered (25,26). The results revealed that the frequency of GHR gene variants were likely similar to that of previous reports (6,12,17-26).

The recent studies showed that the *allele* distribution was significantly different among members of different ethnic groups. There are no differences in genotype and allele frequency distributions of GHR polymorphism among Iranian and Mexican populations (17). The frequency of d3 allele (44.4%), in our studied population is slightly higher than that of other populations (12,18-20,25-27). Recent studies suggest that the individuals with the d3/d3GHR isoform are more sensitive to respond to human GH action, which subsequently presents a protection function against diabetes (16). In other word, the screening of GHR polymorphism could be an effective strategy for disease prevention. Since the protective role of this homozygous form (d3/d3GHR) against the development of diseases has not been studied so far, further studies can be specificaly performed in diabetes patients to find if the d3GHR is an important element for genetic predisposition to type 2 diabetes.

CONCLUSION

The distribution of the GHR genotypes in this study were 31.4% for *fl/fl*GHR, 49.7 % for *fl/d3*GHR, and 19.0 % for *d3/d3*GHR. Frequencies of *fl*allele and *d3* allele were 55.4% and 44.4% within whole population, respectively. There was no difference in allels frequencies of GHR in male and female. The frequency of *d3/d3*GHR isoform was significantly lower than that of the *fl*/*fl*GHR and *d3*/*fl*GHR. The frequencies of GHR polymorphisms were likely consistent with previous reports. The advantage of existence of the *d3/d3* rather than *fl/fl*GHR polymorphisms in individuals and in correlation with diseases opens new insights for GH and IGF-I *axis*.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Isfahan University of Medical Sciences for financial support (grant number 391178).

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