Original Article

POLYMORPHIC VARIANTS OF THE INSULINE-LIKE GROWTH FACTOR I RECEPTOR GENE IN BULGARIAN POPULATION

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Summary

An attenuation of the insulin-like growth factor I (IGF-I) signaling has been associated with elongation of lifespan. In humans, IGF-I level has an age-related modulation with a lower concentration in the elderly, depending on hormonal and genetic factors affecting the IGF-I receptor gene (IGF-IR). The aim of our study was to survey the genotype and allele frequency of +3179G>A (rs2229765) SNP in Bulgarian population according to age and gender. For this purpose, we used a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. We genotyped 246 healthy donors distributed in two groups: elderly and youth (n=108, age range 30-81 years and n=138, age 18 years, respectively). Genotype distribution in the group of elderly was: AA (21%), AG (44%) and GG (35%), and in the group of youth was: AA (19%), AG (44%) and GG (36%). The observed frequency of AA genotypes was slightly higher in males than in females in both groups. Based on the experimental data, we could conclude that there is no statistically significant differences in the distribution of genotypes in the +3179G>A polymorphism of *IGF-IR* between both age subgroups from the Bulgarian population we studied.

Key words: single nucleotide polymorphism, insulin-

Introduction

Insulin-like growth factors (IGF-I and IGF-II), their binding proteins (IGFBPs) and the receptors mediating their signaling (types I and II IGF-R) play critical roles in normal development, growth, metabolism, and homeostasis [1, 2]. The IGF-I pathway exerts such diverse influence on mammalian biology that the scope of its function is only now beginning to be understood. It has been insinuated in fundamental processes such as determining life span and coping with oxidative stress in rodents [3]. IGF-IR bears both structural and functional resemblance to other closely related tyrosine kinase receptors, such as InR in *Drosophila melanogaster* [4, 5] and DAF-2 in *Caenorhabditis elegans* [6, 7]. It starts functioning during fetal development and retains its importance throughout life, although the consequences of its normal or abnormal activation change with aging. IGF-IR and

its related proteins have been implicated in many diseases, including growth abnormalities, metabolic disorders, and several forms of cancer [8-10]. The very early events that rescue cells from cell cycle arrest are mediated through signals transmitted by a group of peptides, collectively known as growth factors [11, 12]. These molecules can be classified into two subgroups, namely the "competence" factors, such as the platelet-derived growth factor that enable cells to enter into the G_1 phase, and the "progression" factors, such as the insulin-like growth factor (IGF) that are required for progression from G1 into the S phase and, ultimately, cell division [13]. Thus, this pathway continues to attract interest as a potentially useful target for therapeutic design.

The IGF system is comprised of ligands (IGF-I and IGF-II), receptors (IGF-IR and IGF-IIR), and a family of six binding proteins. The IGF-IR is activated by binding of either IGF-I or IGF-II. Ligand binding induces a conformational change and autophosphorylation of key residues in the β subunits of the receptor, and docking proteins then interact with the phosphorylated residues of the activated receptor. Activation of the receptor and transduction of the intracellular signaling kinase cascades culminates in cell proliferation and anti-apoptotic effects [8, 14]. Through the insulin-like I receptor (IGF-IR), the proliferative activity of IGF-I is mainly regulated by the mitogen-activated protein kinase (MAPK) signaling pathway, and its antiapoptotic activity - by the PI-3 kinase pathway. It would seem that local concentration of IGF-I and relative levels of IGF-IR determine the activity of this pathway.

As a polymorphic gene, IGF-IR SNPs are subjects of intensive studies, as perspective markers for determining genetic predispositions to growth abnormalities, metabolic disorders, cancer, autoimmune and infectious diseases. A synonymous transition +3179G>A [GenBank: NM_000875.3] in exon 16 of IGF-IR was found associated with lowest levels of free IGF-I (homozygous for A allele) and human longevity in Italian population [15]. Increased frequency of AA genotype has also been reported in multiple myeloma and ischemic stroke [16, 17].

In regard to the regulatory role of IGF-I/ IGF-IR pathway, the aim of our study was to survey the genotype and allele frequency of +3179G>A SNP (rs2229765) in Bulgarian population according to age and gender.

Materials and Methods

Study participants

A total group of 246 healthy volunteers from Stara Zagora district, Bulgaria was included in the study. The group was divided into two subgroups according to the age – a group of 108 healthy volunteers with a mean age of 45 years (range 30-81 years; 51% males and 49% females) and a group of 138 youth healthy volunteers with an age 18 (42% males and 58% females).

Informed consent was obtained from all participants and authorization was given by the Ethics Review Board of the Faculty of Medicine, Trakia University.

Blood sampling and rs 2229765 genotyping

Blood samples were collected by venipuncture and gDNA from blood samples was extracted using a column-based blood genomic DNA purification kit (Amersham Biosciences, Buckinghamshire, UK) and stored at -70°C until use. To assess the rs2229765 genotype, a gDNA aliquot (about 30-50ng/μL) was amplified by polymerase chain reaction (PCR) using the following primers: forward 5'-tcttctccagtgtacgttcc-3' and reverse 5'-ggaactttctctttaccacatg-3'. The cycling parameters for IGF-IR G/A SNP in +3179 were as follows: initial incubation step of 2 min at 95°C; 35 cycles: 30 sec at 94°C, 30 sec at 58°C, and 30 sec at 72°C and a final extension step of 7 min at 72°C completed the reaction. The resulting PCR product of 255bp containing the polymorphic site were digested using 2.5 units of restriction endonuclease MnlI (Fermentas, Latvia) per reaction for 3hr at 37°C. The rs2229765 genotype was examined after loading the corresponding enzymatic digestions on an agarose gel electrophoresis unit with standard 50 bp DNA marker ladder (Fermentas, Latvia) for instrumental lining-up. The +3179G allele yields four fragments: 132bp, 80bp, 23bp, and 20bp, respectively; the +3179A allele yields three fragments: 132bp, 100bp, and 20bp (Fig.1). PCR products and restriction fragments were visualized on a 4% agarose gel stained with ethidium bromide (0.5 mg/mL). In each PCR run, heterozygous control template was used to ensure accuracy. For quality control, 10% of randomly selected samples were analyzed for the second time without finding any discrepancies. PCR amplification was performed in a GeneAmp PCR

System 9700 (Applied Biosystems). Kits for PCR reactions were supplied by Fermentas, Latvia.

Statistical Analysis

Allele and genotype frequencies were calculated by direct counting. Using an interactive Online Software Package at http://statpages.org/index.html, the statistical significance of the difference was calculated by 2×2 table test and the goodness of fit for Hardy-Weinberg equilibrium, calculating the expected frequencies of each genotype. Comparison of expected frequencies with the values observed was performed using $\chi 2$-test goodness-of-fit test.

Results

The demographic data, genotype and allele frequency of IGF-IR +3179G>A are presented in Table 1. The genotype distribution for the polymorphism was in agreement with Hardy-Weinberg equilibrium for both investigated groups.

The distribution of +3179G>A genotypes among 108 healthy volunteers aged 45±15.5 was

Table 1. Genotype and allele distribution of IGF-IR dbSNP +3179G>A (rs2229765) in the group and subgroups of investigated Bulgarian population

	IGF-IR genotypes			Allele frequency		
	AA (%)	**AG (%)**	**GG (%)**	**A allele**	**G allele**	
Age 45 (15.5†) n=108	23 (21%)	48 (44%)	37 (35%)	94 (44%)	122 (56%)	**$\chi 2$ =0.16 P=0.923**
Males (48.3‡) n=55	13 (23.6%)	22 (40%)	20 (36.4%)	48 (43.6)	62 (56.4%)	
Females (42.1‡) n=53	10 (19%)	26 (49%)	17 (32%)	46 (43.4%)	60 (56.6%)	**$\chi 2$=0.47 P=0.791**
Age 18 n = 138	26 (19%)	61 (44%)	51 (36 %)	113 (41%)	163 (59%)	
Males n=59	14 (23.7%)	26 (44%)	19 (32.3%)	54 (45.7%)	64 (54.3%)	
Females n=79	12 (15.2%)	35 (44.3%)	32 (40.5%)	59 (37.3%)	99 (62.7%)	**$\chi 2$=1.41 P=0.496**
Total n = 246	49 (20%)	109 (44.3%)	88 (35.7%)	207 (42%)	285 (58%)	

†SD of age in subgroups; ‡ the age mean of males and females, respectively

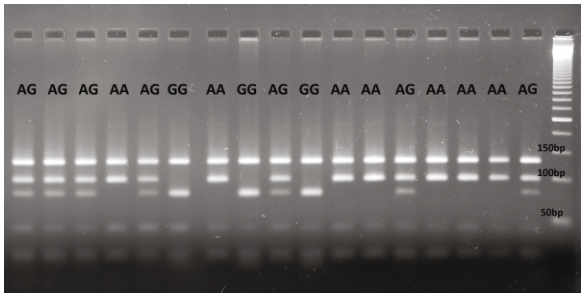

Figure 1. Genotyping of IGF-IR polymorphism rs2229765 (+3179G>A) on a 4% agarose gel electrophoresis. The +3179G allele yields four fragments: 132bp, 80bp, 23bp, and 20bp; respectively, the +3179A allele yields three fragments: 132bp, 100bp, and 20bp

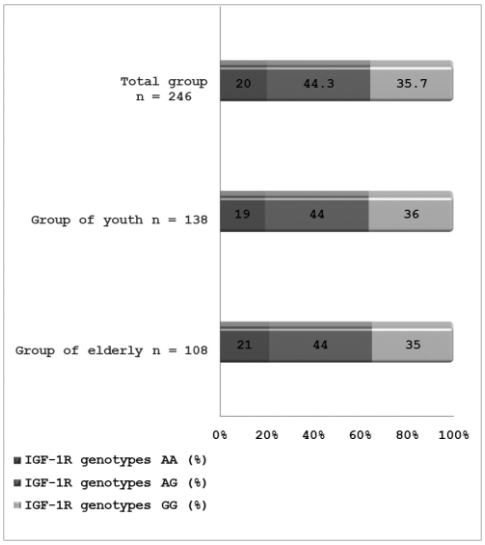

Figure 2. Genotype distribution of IGF-IR dbSNP +3179G>A (rs2229765) in the group and subgroups of investigated Bulgarian population

similar to that observed in the youth group of 138 healthy volunteers aged 18 years ($\chi 2 = 0.16$; df = 2; p=0.923). Genotype distribution in the group of elderly was 21%AA, 44%AG and 35%GG, and in the group of youth it was 19%AA, 44% AG and 36%GG. The observed allelic frequency of "A" – 0.44 in the group of elderly and 0.41 in the group of youth, was similar to frequencies reported for other Caucasian populations [15, 18]. Significant differences between allelic frequencies in the two subgroups were not found (p=0.566). We also investigated the association between the IGF-IR +3179G>A genotypes and the gender in the studied subgroups. Although a significant association between the +3179G>A genotypes distribution and the gender was not found in the elder group ($\chi 2$=0.47; df=2; p=0.791), the frequency of AA genotypes was slightly higher in males than in females (23.6% vs 19%). This tendency was also observed in the youth group, where we found 23.7% AA for males vs 15.2% AA for females though the differences did not reach statistical significance ($\chi 2$=1.41; df 2; p=0.496). However, no statistically significant differences in the allelic frequencies were detected in both investigated groups (p=0.164, p=0.159, respectively).

Discussion

This is the first study which investigates the distribution of +3179G>A (rs2229765) polymorphism in IGF-IR among Bulgarian population. Our findings for allele and genotype distribution in Bulgarian population are somewhat close to the results obtained by Bonafe et al. [18] who have demonstrated that the frequency of genotypes were as follows: 19.4%AA; 40.7%AG and 39.9%GG for young Italian citizens; 27.8%AA, 42.6AG and 29.6%GG for long-lived Italian citizens. The +3179G>A polymorphism of IGF-IR has been reported to modulate plasma IGF-I levels and therefore represents a likely candidate gene to study genetic predispositions to growth abnormalities, metabolic disorders, cancer, autoimmune and infectious diseases. The homozygous genotype for A allele has been associated with lowest levels of free IGF-I and human longevity in Italian population [15]. Human longevity is influenced by genetic and environmental factors. Genetic data about the rs2229765 did not indicate any major differences when the population was grouped in five-year age brackets or split in the two groups (70–85) and 85+, but there was an increase of the A-allele in the males over 85, suggesting a sex-specific effect on male longevity. The significance of this increase was not robust, (p = 0.04, $\chi 2$-test) probably because of the sample size of 85+ males. The (70-85) male group had a lower A-allele frequency than (70-85) females. This might be interpreted as a sex-specific effect but might also hide a chance or recruitment bias [15].

However, as the amino acid encoded by the polymorphic A variant of the IGF-IR +3179G>A is unchanged from wild-type, it would appear that this polymorphism would have no direct effect on IGF-IR receptor function. Based on present data we could suggest that +3179G>A most likely modulates IGF-IR function by influencing gene transcription or mRNA stability [18].

In our investigation there was no association between the IGF-IR +3179G>A genotypes and the two age-depended subgroups of the investigated Bulgarian population (P = 0.923, $\chi 2$ test). We did not find significant differences between observed allelic frequencies in the two subgroups either (p= 0.566). It is necessary to measure the plasma levels of IGF-I among the healthy volunteers in our study, to find the association of homozygous for A allele and IGF-I concentration. The small differences between genotypes of +3179G>A within males and females might be interpreted as a sex-specific effect, as have been pointed by Albani et al. [15].

Determination of the genotypes and allele distribution of IGF-IR +3179G>A polymorphism according to different age and gender is a prerequisite for further case-control studies for the diseases predisposition.

Conclusion

On the basis of experimental evidence, we could conclude that polymorphisms rs2229765 in +3175G>A of IGF-IR do not have statistically significant differences in genotypes and allelic distribution among the age and gender subgroups of the Bulgarian population investigated.

Acknowledgments

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