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ORIGINAL ARTICLE

Comparisons of +3179G/A insulin-like growth factor 1 receptor gene distribution between two inflammatory arthritides and healthy adults

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ABSTRACT

Objectives: This study aims to investigate whether single nucleotide polymorphisms (SNPs) at the +3179G/A insulin-like growth factor 1 receptor (IGF-1R) locus were associated with rheumatoid arthritis (RA) and ankylosing spondylitis (AS) genetic susceptibility and also explore age and sex distribution of the rs2229765 in healthy adults.

Patients and methods: This cross-sectional study was conducted between September 2012 and October 2014. Seventy patients with RA (7 males, 63 females; mean age: 54±1 years; range, 32 to 78 years) and 56 with AS (44 males, 12 females; mean age: 38±9 years; range, 22 to 57 years) were genotyped using polymerase chain reaction-restriction fragment length polymorphism method. The genotype and allele frequencies of the rs2229765 polymorphism in both patient groups were compared to those in 308 healthy donors (141 males, 167 females; mean age: 35±19 years; range, 18 to 75 years) who were further subjected to analysis of sex- and age-related genetic variation.

Results: We identified the homozygous genotype AA (22.9% *vs.* 14.1%; odds ratio [OR]=2.33, p=0.034) and A-allele (47.9% *vs.* 37.5%; OR=1.53, p=0.032) associated with increased risk for RA, but not AS. The same genotype AA was non-significantly more common in healthy males than females, and the frequency of the A-allele was markedly higher in younger males (46% *vs.* 40%; p=0.039). The overall percentage of healthy carriers of the AA gene variant was 18%.

Conclusion: We primarily present an inverse effect of the +3179G/A IGF-1R polymorphism on disease susceptibility to RA and AS, confirming the distinctly different immune pathways involved in the pathogenesis of both inflammatory arthritides. In addition, we could also show trends regarding age- and sex-specific patterns of the rs2229765 genotype distribution in the general population.

Keywords: Ankylosing spondylitis, insulin-like growth factor 1 receptor, rheumatoid arthritis, rs2229765, single nucleotide polymorphism.

The axis consisting of insulin-like growth factors (IGF-1 and IGF-2), their binding proteins, and transmembrane receptors mediating their intracellular signaling (types I and II IGF-R) is critical for many physiological and pathological processes. IGF-2 acts primarily during embryogenesis and fetal development, whereas IGF-1 has been shown to function principally during the postnatal life. The latter plays key roles in body growth through promotion of cell reproduction and inhibition of cell death (apoptosis), in metabolic processes, and homeostasis. The proliferative activity of IGF-1 is mainly regulated by the mitogen-activated protein kinase signaling pathway, while its anti-apoptotic activity is predominantly controlled

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by the phosphoinositide 3-kinase pathway.[1,2] In humans, serum IGF-1 levels have an age-related modulation with lower concentrations in the elderly,[3,4] depending on hormonal and genetic factors, liver function.

The IGF-1 receptor (IGF-1R) is the major receptor of IGF-1 which binds its ligand with high affinity. The coding structural gene for this receptor is located on chromosome 15q26. IGF-1R has obviously been considered crucial for tumor transformation and survival of malignant cell. It is highly overexpressed in most malignant tissues where it functions as an anti-apoptotic agent by enhancing cell proliferation and viability.[5] The IGF/IGF-1R system plays a prominent role also in the regulation of immunity and inflammation. Inappropriate balance of the IGF-1 signaling has been reported in autoimmune disorders.[6] There are a few studies describing the contribution of the IGF-1 system to rheumatic diseases. The involvement of this signaling pathway has been recently revealed in disease pathogenesis of rheumatoid arthritis (RA),[7-9] systemic lupus erythematosus (SLE),[10,11] systemic sclerosis,[12,13] and some other rheumatic diseases. These published studies have investigated the modulation of the IGF chain activity at different levels of the signaling process, but the findings are conflicting or uncertain.

All these data support the notion that abnormalities in the IGF-1R pathway might play some part in the pathogenesis of diseases where immunity is altered. The scope of its function is only now beginning to be understood. Thus, there is a reason to explore the potential for this receptor as a regulator of chronic inflammation in various autoimmune disorders. Little is currently known about the impact of IGF-1R and the signaling pathways shared with IGF-1R on the development of ankylosing spondylitis (AS) and RA. On the other hand, involvement of IGF-IR in the pathogenesis of autoimmune diseases attracts interest in the context of therapeutic targeting of receptors.

Single nucleotide polymorphisms (SNPs) of the genes influencing the activity of the components of this pathway determine specific immunological reactivity, as well as susceptibility to diseases. Recently, SNP located in exon 16 (+3179G/A; rs2229765; GenBank: NM_000875.3) of the IGF-1R gene was found associated with serum levels of IGF-1 and human longevity in an Italian population.[14] Enhancing our knowledge about genetic control and mechanisms of signaling pathways modulation allows us to establish the role of the genes in pathological responses and is relevant to the search for new therapeutic targets. Besides, it might help us to gain important insights into the disease predisposition.

It is difficult to identify SNPs, which are most likely to have functional effects, and which could be useful to reveal the structural basis of disease mutations from a large number of neutral SNPs. rs2229765 was previously predicted to affect splicing regulation and associated with several diseases.[15-17] That is why this SNP should be considered an important candidate in causing diseases related to IGF-1R malfunction and we speculated that it is of likely functional importance also in rheumatic diseases. We have previously studied its role in a cohort of patients with SLE.[11] Up to now, the role of IGF-1R rs2229765 in AS and RA patients is unknown. In this study, we aimed to investigate whether SNPs at the +3179G/A IGF-1R locus were associated with RA and AS genetic susceptibility and also explore age and sex distribution of the rs2229765 in healthy adults.

PATIENTS AND METHODS

This cross-sectional study was conducted at Rheumatology clinic of University Hospital "St. Ivan Rilski", Sofia and Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria between September 2012 and October 2014. +3179G/A IGF-1R genotype was determined in 70 elderly subjects (7 males, 63 females; mean age: 54±12 years; range, 32 to 78 years) who met the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 RA classification criteria[18] and 56 AS patients (44 males, 12 females; mean age: 38±9 years; range, 22 to 57 years) fulfilling the modified New York criteria.[19] The mean disease duration was 11±7 (range, 1 to 30) years for AS patients and 9±6 (range, 1 to 24) years for RA patients. Also, 308 healthy individuals (141 males, 167 females; mean age: 35±19 years; range, 18 to 75 years)

were genotyped for the +3179G/A polymorphism of IGF-1R. To assess age-related differences in variants of this receptor gene in the absence of a detectable autoimmune pathology, the healthy population was divided into two age groups as young adults (age range, 18 to 39 years) and older adults (age range, 40 to 81 years). The current study used age 40 years as a cut-off based on the median value of this variable. The study protocol was approved by the University Hospital "St. Ivan Rilski" and Medical Faculty, Trakia University Ethics Committee. A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Genomic deoxyribonucleic acid (DNA) was extracted using a genomic blood DNA purification kit (NucleoSpin® Blood L, Macherey-Nagel, Düren, Germany) and stored at -80°C until use. The concentration of resulting DNA was measured spectrophotometrically at 260 nm using NanoVue™ Spectrophotometer (9GE Healthcare, Buckinghamshire, UK). The ratio of absorptions at 260 *vs.* 280 nm was used to assess the purity of DNA samples.

Genotyping for the +3179G/A polymorphism (rs2229765) was performed by polymerase chain reaction-restriction fragment length polymorphism method as described in details elsewhere.[17]

Statistical analysis

Statistical analysis was performed using the SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Inter-group comparisons were performed using the Chi-square (χ^2) analyses for categorical variables. The odds ratios (ORs) with 95% confidence intervals and p value were calculated using an interactive online software package at http://statpages.org/index.html, by 2×2 contingency table test. A 2-tailed *p* value of <0.05 was considered statistically significant.

RESULTS

The significance of differences in genotype and allele frequency across both RA and AS groups and healthy subjects was tested. Genotype distribution of polymorphisms was in conformity with Hardy-Weinberg equilibrium in all study groups. In the setting of these two types of arthritis, we observed an opposite effect of investigated IGF-1R polymorphism (+3179G/A; rs2229765) on disease susceptibility. Significantly different genotype and allele frequencies of the rs2229765 polymorphism with a higher incidence of homozygous genotype AA (22.9% *vs.* 14.1%; OR=2.33, p=0.034) and allele A (47.9% *vs.* 37.5%; OR=1.53, p=0.032) were apparent in patients with RA compared to healthy controls (Figure 1). The prevalence of AA genotype in the present sample of AS patients was approximately twice lower than in the healthy population (10.9% *vs.* 20.9%; OR=0.47; p=0.142), without reaching statistical significance (Figure 2).

In order to present information on the variants in the gene among the general population,

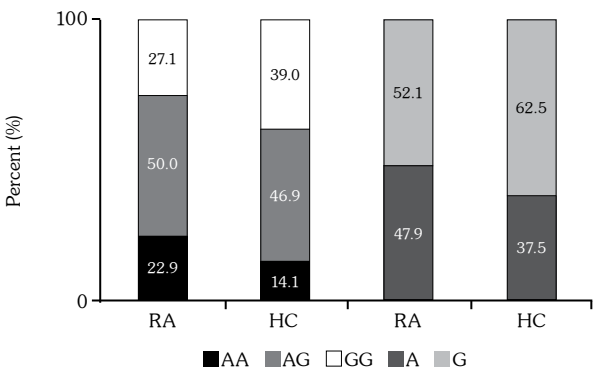

Figure 1. Genotype and allele distribution among rheumatoid arthritis patients and healthy controls.
HC: Healthy controls; RA: Rheumatoid arthritis.

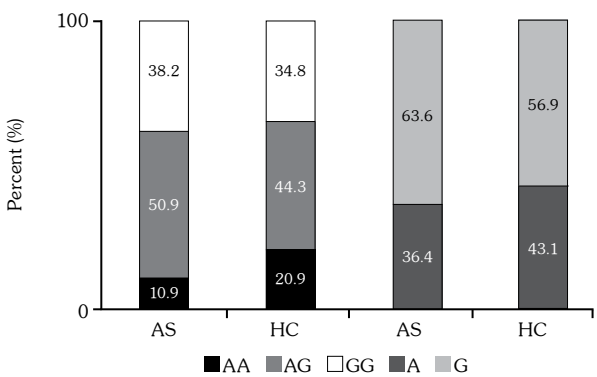

Figure 2. Genotype and allele distribution among ankylosing spondylitis patients and healthy controls.
AS: Ankylosing spondylitis; HC: Healthy controls.

Table 1. Age and sex distribution of +3179G/A insulin-like growth factor 1 receptor genotypes and alleles in healthy Bulgarian adults

		Age (year)	Genotype frequency						Allele frequency				
	n	Mean±SD	AA	%	AG	%	GG	%	A	%	G	%	*p*
Males													
Young	81	21.3±6.8	20	25	35	43	26	32	75	46	87	54	0.704
Older	60	58.7±10.5	12	20	26	43	22	37	50	42	70	58	
	141	37±20	32	23	61	43	48	34	125	44	157	56	
Females													
Young	98	20.9±6.8	17	17	44	45	37	38	78	40	118	60	0.159
Older	69	53.2±9.1	8	12	35	51	26	37	51)	37	87	63	
	167	34±18	25	15	79	47	63	38	129	39	205	61	
Total													
Young	179	21±6	37	21	79	44	63	35	153	43	205	57	0.214
Older	129	56±10	19	15	61	47	49	38	99	38	159	61	
	308	35±19	56	18	140	46	112	36	252	41	364	59	

SD: Standard deviation.

we investigated age and sex distribution of the +3179G/A genotypes in healthy adults. In total, 308 blood samples were collected from healthy donors. No significant association between +3179G/A genotypes distribution and sex was found in healthy older subset (χ^2=3.71; df=2; p=0.156). Genotype AA occurred in 18% of the general population and was more common in males than females (20% *vs.* 12%; p=0.287), but the difference did not reach statistical significance (Table 1). The same pattern was observed among younger cohort between 18 to 39 years of age (χ^2=0.9; df=2; p=0.634); AA genotype was also numerically but not significantly more prevalent in male sex (25% *vs.* 17%; p=0.216). However, there was a significant age and sex difference in the frequency of the A-allele at this locus which was higher in younger males than in females (46% *vs.* 40%; p=0.039) (Table 1).

DISCUSSION

The present study is an initial exploration of the possible genetic impact of the synonymous rs2229765 polymorphism as a disease predisposing factor for two rheumatic diseases, AS and RA. These two chronic arthritides reflect different disease spectrums in terms of the specific processes involved in the predominantly enthesopathic or predominantly synovial inflammation. SNPs determine genetic differences in disease susceptibility between individuals and that of the IGF-1R gene has not been sufficiently studied in rheumatic conditions. We have previously reported a non-significant higher frequency of the AA genotype and A-allele in association with SLE development in Bulgarian population. Moreover, the higher systemic IGF-1 concentrations have been linked to disease severity in SLE.[11]

In keeping with this, now we present estimates of rs2229765 genotype distributions in other rheumatic diseases. Our results revealed a significantly higher incidence of the homozygous genotype AA and A-allele in patients with RA compared to those from controls. The findings allow us to assume that this allelic variant is associated with leastwise twofold increase in the risk of RA in adults, which implies that it might have a potential contributory role in disease pathogenesis. It appears that SNPs of the IGF-1R might influence activation of the signaling cascades downstream from IGF-IR, and probably their regulatory function on different

immune cells. It is known that IGF-1/IGF-1R signaling exerts an influence on the development and function of various immune cell lineages.[6] Erlandsson et al.[20] observed that IGF-1R signaling contributes to rheumatoid inflammation, as IGF-1R expression in leukocytes of RA patients is related to higher serum interleukin (IL)-6 levels, and values of erythrocyte sedimentation rate. The role of the up-regulated IGF-1R signaling and an increase in the IGF-IR density on inflammatory cells (cluster of differentiation 4+ T-lymphocytes) in RA have been confirmed by Laurberg et al.[21] Studies on plasma and synovial fluid IGF-1 concentrations in RA found contradictory results with higher, similar or reduced levels compared to controls.[7-9,22,23] In a cohort consisting of 68 seropositive RA patients, Dhaunsi et al.[24] found that 94% of them were carriers of the 192-bp allele of the IGF-1 gene, which is associated with reduced serum levels of IGF-1. No previous data in the literature concerning +3179G/A IGF-1R polymorphisms in association with RA are available.

In contrast, we found that allele A and genotype AA of +3179G/A IGF-1R polymorphism were distributed in a non-significant lower proportion in AS cases than control subjects, supporting the notion that it might be primarily considered as linked to a lower risk of disease incidence. The knowledge about the role of the IGF-1/IGF-1R system in immune responses underlying the AS pathogenesis is so far even more limited.

Briefly, it turns out that the aforementioned genotype is a risk gene variant specifically for RA, but not AS. This might result from a disease-specific function of the IGF-1 pathway. Definitely, there exists a complex interplay between growth factors and pro-inflammatory cytokines, via sharing common signaling components and functional interactions. Receptor activation of IGF-1R leads to abnormal responses in various effector immune cells.[6] The origin of the incommensurability in the risk probabilities may be deeply rooted in the distinctive immunological features in both arthritides. The RA pathogenesis involves more adaptive immune features such as autoreactive B cells and production of autoantibodies (rheumatoid factors and antibodies to citrullinated peptides), whereas AS seems a pathology triggered to a greater extent by innate immunity. It is more driven by lymphocyte subsets T helper 17 cells producing IL-17A that stimulate signals typical of early inflammatory processes and thus serving as a bridge between adaptive and innate immunity.[25]

As part of our work, we examined this SNP at the IGF-1R locus in the healthy subjects from the viewpoint of age and sex differences. In our analysis, we observed a slightly higher prevalence of the AA genotype among males in Bulgarian population. In total, the proportion of healthy carriers of the identified risk gene variant AA for RA/AS was small, only 18%. This illustrates the overall picture in the context of the two rheumatic diseases.

The limitation of the present study is the small number of patients with AS and RA. As this is a small-scale study, our assumption needs to be confirmed in further studies. Furthermore, it is difficult to establish fully exactly the pathogenic role of the rs2229765 by evaluating the importance of single gene effects. However, the strength is that there is a lack of data in the literature on the issue we have examined.

In conclusion, the homozygous genotype AA of the +3179G/A IGF-1R was found to constitute a putative genetic risk factor for RA susceptibility and might be of importance for disease development. The impact of this risk gene variant was confined only to RA population, but not to that with AS. We have also shown that there exist at least partly sex differences in the genotype distribution of the rs2229765 with a predominance of AA among males in Bulgarian population.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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