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Evidence of association of interleukin-23 receptor gene polymorphisms with Egyptian rheumatoid arthritis patients

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ABSTRACT

Background: The identification of additional genetic risk factor is an on-going process that will aid in the understanding of rheumatoid arthritis (RA) aetiology. A genome-wide association scan in Crohn's (CD) disease highlighted the interleukin-23 receptor (*IL23R*) gene as a susceptibility factor. Since the IL-23/IL-17 pathway is known to associate with other autoimmune disease, including rheumatoid arthritis and systemic sclerosis, we hypothesised that *IL23R* could be a shared susceptibility gene. The rare allele of *IL23R* single nucleotide polymorphism (SNP) rs11209026 (Arg381Gln) confers strong protection against CD. Our aim was to analyse *IL23R* SNP (rs11209026, rs2201841, and rs10889677) and to detect its association with RA in Egyptian patients.

Methods: A group of Egyptian patients with RA ($n = 120$) and apparently healthy persons as controls ($n = 120$) was genotyped for rs11209026, rs2201841 and rs10889677 by real time/polymerase chain reaction (real-time/PCR) for the first SNP and restriction fragment length polymorphism/PCR (RFLP/PCR) in the last two SNPs.

Results: Our data emphasise that the AA genotype of rs11209026 (Arg381Gln) was significantly associated with RA patients compared to the controls (P value = 0.001).We did not find any significant association between either rs2201841 or rs10889677 and the development of rheumatoid arthritis (P value = 1.000 & 0.562 respectively).

Conclusion: Our results suggest that IL23 receptor AA genotype variant of rs11209026 would contribute to RA aetiology; consequently, it might be a genetic marker for RA. We need to address the subgroup of patients who will benefit from the selective suppression of the IL23 signalling which would represent new perspectives toward a personalized therapy of RA patients by further studies.

1. Introduction

Rheumatoid arthritis (RA) is a chronic progressive systemic inflammatory autoimmune disease of the joints, which can lead to long-term joint damage, resulting in chronic pain, loss of function, and disability [1]. The pathogenesis of RA is multifarious, with suspected interrelated contributions from genetic, infectious, environmental, and hormonal factors [2].

Advances in genotyping technology and the use of single-nucleotide polymorphism (SNP) assays have facilitated the application of whole genome association approaches to link genetic variants with disease susceptibility [3].

Until recently, there were only two reproducible genetic associations. The strongest of these associations is of genes within the HLA region on chromosome 6p, particularly the HLA-DRB1 gene. A second, more modest, association identified has been of the protein tyrosine phosphatase non-receptor 22 (PTPN22) genes [4].

Soon researches were directed toward the interleukin-23 receptor (*IL23R*) gene polymorphism which maps to chromosome 1 (1p32.1–p31.2).The encoded protein forms a receptor for IL23, together with the β1 subunit of IL12 (IL12Rβ1) [4].

IL23R is a type I cytokine transmembrane protein, it consists of 629 amino acids. The extracellular domain contains a signal

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sequence, an N terminal Ig-like domain, and two cytokine receptor domains. [5]. 11 exons encode for the standard form of *IL23R*. Through alternative splicing, can generate at least six spliced isoforms (*IL23R1–6*) [6]. The splicing strategies most often result in the deletion of exon 7 and/or exon 10. These variants result in either premature termination to generate a diverse form of receptor ectodomain, or a frame shift to produce various lengths of *IL23R* intracellular domain. Differential expressions of *IL23R* spliced variants as well as immune regulatory cells were discovered in tumour cells [6].

In human immune cells, expression of IL23 receptors occurs on activated or memory T cells, on natural killer cells, and, to a smaller extent, on macrophages and dendritic cells. The corresponding ligand IL23, a heterodimeric cytokine that is a key component of the immunoregulatory pathway, plays an important role in the development, differentiation, and effectors functions of immune cells [7].

In chronic inflammation, the antigen-stimulated macrophages and dendritic cells produce IL23. Binding of IL23 to its receptor leads to the activation of Janus Kinases (Jak2 and Tyk2), which can phosphorylate *IL23R* at discrete locations and thus form docking sites for signal transducers and activators of transcription (STATs). The STATs are then phosphorylate by the Jaks, and are thus capable of dimerising and translocating to the nucleus where they influence the transcription of key pro-inflammatory genes [8]. IL23 promotes the development of Th17 cells characterized by the production of cytokine IL17, which enhances T cell priming and triggers potent inflammatory responses by inducing the production of several inflammatory mediators. IL23 also stimulates dendritic cells and macrophages in an autocrine/paracrine manner to generate other pro-inflammatory cytokines, like IL1, IL6, and TNF-α. The Th1 cells stimulated by IL12 produce interferon gamma (INF-γ) and suppress the differentiation of Th17 cells [9,10]. The pro-inflammatory cytokines IL23 & IL17 are present in RA synovial fluid in an increased level [11,12]. IL17 stimulates osteoclast differentiation by inducing the expression of receptor activator of NF-κB legend (RANKL) via a mechanism involving the synthesis of prostaglandin E2 in osteoblasts in vitro [13]. In addition, IL17 directly stimulates human osteoclastogenesis from human monocytes alone, via the TNF-α or RANK–RANKL pathway [14]. Authors have reported that IL17 is also important in joint destruction in animal models and in patients with RA [15,16].

Polymorphisms of *IL23R gene* are associated with numerous different autoimmune diseases like inflammatory bowel disease, ankylosing spondylitis, Graves' ophthalmopathy, and psoriasis [17–20]. Yet conflicting results from different researches as regard the role of allelic variants or haplo-groups of *IL23R* in the development of RA were illustrated.

Therefore, we aimed in the current study to assess the possible association of the *IL23R* Arg381Gln (rs11209026) substitution and rs2201841 and rs10889677 variant with the susceptibility for developing RA.

2. Subject and methods

We conducted this study on 120 patients with rheumatoid arthritis diagnosed according to the (2010) ACR criteria for the classification of rheumatoid arthritis [21]. We selected our cases from the Rheumatology and Rehabilitation and Internal Medicine departments and their outpatient clinics of Cairo, Al Fayoum and Zagazig, University hospitals; they were collected in the period from March 2012 till April 2014. A hundred and twenty apparently healthy volunteers matched for age and sex were taken as a control group. All patients were subjected to full history taking and thorough general & musculoskeletal examination (age, sex, disease duration, age of onset, duration of morning stiffness, presence of extra-articular manifestations and their current treatment). The disease activity in RA patients was assessed by the 28 joint count Disease Activity Score (DAS 28) using the number of swollen and tender joints, erythrocyte sedimentation rate (ESR) and patient's global status and pain evaluated by the visual analogue scale (VAS) range from 0 to 100 mm [22].

Blood samples were drawn from the cubital vein in patients and controls at the day of clinical examination after overnight fasting. Blood samples were collected on EDTA tubes and were stored at −20 °C till the time of assay which was performed for all samples at the same time. Erythrocyte sedimentation rate (ESR), Complete blood count (CBC), Rheumatoid factor (RF), Liver function test, Renal function test and Urine analysis were done. The molecular analysis was performed using DNA extracted from peripheral blood leukocytes of the patients and control. We obtained oral consent after proper orientation of the patients and volunteers about the objectives of the study.

The rs11209026 (*IL23R* Arg381Gln) was genotyped using real-time PCR method using Light Mix® Kit human *IL23R* Arg381GlnKit produced under license from Roche Diagnostics GmbH (TIB MOLBIOL GmbH, Berlin, Germany).

The rs2201841 and rs10889677 SNPs were genotyped separately using PCR-RFLP method from Huber et al. [19]. Instead of using fluorescently labelled forward primers, DNA was amplified using the ordinary primer pairs from Operon Biotechnologies (GmbH/Bio campus, Germany):

Forward primer 5′-GGCCTATGATTATGCTTTTTCCTG-3′, reverse primer 5′-GAACATAACCCTATTGACACCCTG-3′ for rs2201841 and forward primer 5′-AGGGGATTGCTGGGCCATAT-3′ and reverse primer 5′–TGTGCCTGTATGTGTGACCA-3′ for rs10889677. We added 2 U to digest amplification products separately.

2.1. Statistical analysis

All patients' data were tabulated, and processed using Statistical package for sciences and society (SPSS 12.0) (SPSS Inc., Chicago, USA).Quantitative variables were expressed by mean and standard deviation (SD) and then compared using Mann–Whitney *U* test for comparing two independent variables and Kruskal–Wallis analysis for more than two independent variables. We expressed Qualitative variables by frequency and percentage and compared it using chi-square test or Fischer's exact test. *P* value was considered significant if less than 0.05. Odd ratio (OR) and their 95% confidence interval (CI) were calculated for the interleukin 23 receptors genotype using multivariate logistic regression analysis.

3. Results

One hundred twenty cases with rheumatoid arthritis were included in the present study. These included 98 female and 22 male RA cases, with ages ranging from 18 to 69 years with a mean of 42.5 ± 13.42 years. Disease duration in these cases ranged from one year to 15 years with a mean of 5.24 ± 3.45 years. One hundred twenty healthy controls were also enrolled in the study. These controls included 95 females and 25 males, with ages ranging from 20 to 65 years with a mean 44.26 ± 12.36 years. There was no statisticaly significant difference between patients and controls as regards age and sex. RF was positive in 94 (78.3%) cases and 35 (29.16%) cases had deformity. Sixty-six (55%) patients had swollen joints ranged from 1 to 6 joints. At the time of the study, all patients were treated with NSAIDs, 51 patients (42.5%) were maintained on combination of methotrexate and hydroxychloroquine, 62 (51.6%) patients were treated with combination of methotrexate and leflunamide, and 7 (5.8%) patients were maintained on

Table 1
The main characteristics of RA patients included in the study.

RA patients (total No. = 120)	
Age of RA patients (y)	42.5 ± 13.42
Gender (male/female)	22/98
Disease duration (y)	5.24 ± 3.45
Joints deformity, number (%)	35/120 (29.16%)
Swollen joint, number (%)	No swollen joint 54 (45%) 1 swollen joint 18(15%) 2 swollen joint 15 (12.5%) 3 swollen joint 8 (6.67%) 4 swollen joint 14 (11.66%) 5 swollen joint 6 (5%) 6 swollen joint 5 (4.17%)
TLC ($\times 10^3/\mu l$)	7.54 ± 2.8
ESR (mm/h)	59.1 ± 37.5
Rheumatoid factor positive N (%)	94/120 (78.3%)
DAS (range, mean ± SD)	1.52–4.68 (3.23 ± 1.2)

combination of leflunamide and steroids. Clinical and demographic data of the RA patients are presented in Table 1.

We tested three *IL 23R gene* SNPs (rs11209026, rs2201841, and rs10889677) for association with RA. Our results revealed that the prevalence of the AA genotypes of rs11209026 (Arg381Gln) was 95% in the cases group compared with 61.7% in control group (*P* value = 0.001), OR (00.374) with CI (0.264–0.571), a significant difference between cases and control, while the allele and genotype frequencies of rs2201841 and rs10889677 showed no association with RA (*P* value = (1.000, 0.562) – OR (1.000, 1.353)) respectively. The comparison of allele and genotype frequencies between the control and RA cases has been illustrated in Table 2. The AA genotype of the rs11209026 was detected in 95.7% of RF positive patients and in 88.6% of those having deformity. The frequency of the interleukin 23 genotypes and alleles in cases with positive rheumatoid factor and deformity have been illustrated in Table 3.

4. Discussion

The present study was performed to investigate the association of three SNP of the *IL23R* gene in Egyptian rheumatoid arthritis patients. The results showed that the AA genotype of the rs11209026 (Arg381Gln) SNP was associated with an increased susceptibility to RA. However, there was no difference with regard to the genotypes and of rs7517847 and rs17375018 between patients with RA and normal controls.

Rheumatoid arthritis (RA) represents the most common form of chronic inflammatory joint disease. The range of presentations of RA is broad; the clinical course can range from mild, self-limiting arthritis to progressive multisystem inflammation. The first and most common manifestations are pain, stiffness and swelling of peripheral joints .Though not directly life threatening, RA severely affects the quality of life of a patient and has major economic consequences for society [2].

Table 3
The frequency of the interleukin-23 genotypes & alleles in cases with positive rheumatoid factor & deformity.

Rheumatoid arthritis patients No. = 120					
SNP	Genotype allele	RF positive No. = 94		Deformity positive No. = 35	
		NO	%	NO	%
rs11209026	AA	90	95.7	31	88.6
	AG	4	4.3	4	11.4
rs2201841	AA	78	83	23	65.7
	AG&GG	16	17	12	34.3
rs10889677	AA	55	58.5%	25	71.4%
	CC&AC	39	41.5%	10	28.6%

The strategies that guided us to select the studied gene were based on choosing a susceptibility gene already identified in other related diseases considering its relevant functions that might be functional in the pathogenesis of RA. In this study, we selected the IL23 receptor (*IL23R*) as a candidate gene principally based on the fact that the interaction of *IL23R* with its ligand IL23 results in an increase of signal transducers and activators of transcription signalling which can consequently promote the production of IL17. IL17 is a potent pro-inflammatory cytokine already identified in various chronic inflammatory diseases [8]. Up-regulated production of *IL23R* that is associated with certain SNP alleles in its gene could confer risk for the disease [23].

Our results showed that the AA genotype of rs11209026 (p.Arg381Gln) was significantly associated with RA patients compared to the controls (*P* value = 0.001). The association demonstrated here is consistent with earlier reports showing the association between the same genotypic variant and other autoimmune diseases including Behçet's disease, psoriatic arthritis, inflammatory bowel disease, and ankylosing spondylitis [24,25,17,18]. However, others [4,26–28] have reported contradictory to our result.

The biological impact of the investigated polymorphisms on the expression and functionality of *IL23R* is currently unknown but it is obvious that these SNPs can represent an important link in the development of autoimmune and inflammatory diseases [7]. Several mechanisms can be suggested by which polymorphisms can change the function of the receptor. The rs11209026 Arg381Gln SNP is located in the cytoplasmic domain and encodes the fifth amino acid internal to the transmembrane domain [29] .The arginine allele has a side chain with positive charge while the rare glutamine has an uncharged polar one and thus the substitution can have considerable effect on the protein structure [7].

To investigate association of other *IL23R* variants with RA, we genotyped SNPs rs2201841 and rs10889677 in our RA patients and control group. It has been reported that the rs10889677 located in the 3′-UTR can possibly cause over expression of the *IL23R* (e.g., by increasing mRNA stability), driving differentiation of T-cells towards a Th17 sub-population resulting in an increased release

Table 2
The comparison of allele and genotype frequencies between the control and RA cases.

SNP	Genotype allele	Cases (No. = 120)	Controls (No. = 120)	*P* value	Odds ratio	95% confidence interval
		Frequency (%)	Frequency (%)			
rs11209026	AA	114/120 (95)	74/120 (61.7)	0.001	0.374	0.264–0.571
	AG	6 /120 (5)	46/120 (38.3)			
rs2201841	AA	104/120 (86.7)	104/120 (86.7)	1.000	1.000	0.226–4.431
	AG&GG	16/120 (13.3)	16/120 (13.3)			
rs10889677	AA	82/120 (68.3)	89/120 (74.2)	0.562	1.353	0.426–3.834
	CC&AC	38/120 (31.7)	33/120 (27.5)			

of other cytokines causing inflammation. The rs2201841 is an intronic variant, is in significant linkage disequilibrium with rs10889677, and therefore is unlikely to confer independent risk [7].

In the current study, we did not find any significant association between either rs2201841 or rs10889677 and the development of rheumatoid arthritis. Despite a case–control study of Hungarian Caucasian RA-patients and healthy controls showed that both the rs10889677GG variant and rs2201841GG variant are risk alleles in RA [30]. The study on a Spanish Caucasian population supports our findings [26].

Like other candidate gene studies, there are several limitations in our study. As the power to detect disease susceptibility genes is influenced by the number of the patient's samples, the size of patient samples in our study seemed to be relatively small. The results observed in this study need to be confirmed using large sample size involving RA patients from different areas in upper and lower Egypt.

In conclusion, our results suggest that IL23 receptor AA genotype variant of rs11209026 would contribute to RA aetiology; consequently, it might be a genetic marker for RA. Further studies are needed to address the subgroup of patients who will benefit from the selective suppression of the IL23 signalling which would represent new perspectives toward a personalized therapy of RA patients.

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