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# Association of *IL-23R* gene single nucleotide polymorphism; rs 11209026 with incidence and severity of ankylosing spondylitis in a cohort of Egyptian patients



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## ABSTRACT

The aim of the present study was to investigate the possible association between incidence and severity of ankylosing spondylitis (AS) in a cohort of Egyptians and Interleukin-23 Receptor (*IL23R*) gene single nucleotide polymorphism (rs11209026). **Methods:** The study included thirty-two AS patients and forty volunteers who serves as a control group. The studied polymorphism was genotyped using 5' Nuclease assay. **Results:** A statistically significant difference was detected between both studied groups as regards different *IL23R* gene single nucleotide polymorphism (rs11209026) genotypes. Heterozygous genotype was the most prevailing among both cases and controls. At a cutoff level of 110 pg/mL, a statically significant difference was observed between cases and controls as regards serum IL23 level. **Conclusions:** In Egyptians, *IL-23R* single nucleotide polymorphism (rs11209026) appears to be associated with ankylosing spondylitis occurrence not severity, while higher levels of IL-23 might be associated with disease severity.

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## 1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disorder that affects the axial skeleton.<sup>1</sup>

Even though AS is genetically associated with the major histocompatibility complex class 1 antigen (HLA-B27),<sup>2</sup> only 1–5% of B27- positive individuals develop AS.<sup>3</sup>

Several non-MHC (major histocompatibility complex) genes, that may be linked to AS, were identified including Interleukin-23R gene (*IL23R*). In addition, some studies reported an association of AS with many nonsynonymous single-nucleotide polymorphisms (SNPs) of *IL23R* gene.<sup>4</sup>

**Abbreviations:** ALT, alanine aminotransferase; AS, ankylosing spondylitis; ASDAS, ankylosing spondylitis disease activity score; AST, serum aspartate aminotransferase; BASDAI, Bath ankylosing Spondylitis Disease Activity Index; BASFI, Bath ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-23, Interleukin-23; *IL23R*, Interleukin-23 Receptor gene; SNP, single-nucleotide polymorphism; SpA, Spondyloarthritis; SPSS, Statistical Package for Social Sciences. Peer review under responsibility of Alexandria University Faculty of Medicine.

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The *IL23R* gene located on chromosome 1p31 encodes one of the subunits of the receptor for interleukin-23 (IL-23) and contains 11 exons spanning approximately 92 kb.<sup>5</sup>

IL-23 is a cytokine that is involved in the expansion of Th17 cells which secretes pro-inflammatory cytokines, such as IL-17A, IL-17F and IL-2, thus dysregulation of the IL-23/IL-17 axis plays a major role in the progression of chronic autoimmune inflammatory diseases.<sup>6</sup>

Studies strongly relate a group of autoimmune diseases sharing some clinical features, including AS, psoriasis and psoriatic arthritis, to some common autoimmune overlapping susceptibility loci such as *IL23R*.<sup>7</sup>

A loss of function, single-nucleotide polymorphism (SNP) (Arg381Gln; R381Q; 1142G > A polymorphism; rs11209026) is located within exon 9 of the human *IL-23R* gene. This polymorphism was reported to be associated with numerous autoimmune diseases as Crohn's disease, AS and psoriasis.<sup>7,8</sup> In addition, they reported that rs11209026 A allele might confer protection against some autoimmune inflammatory diseases.

The present study aimed at investigation of the possible association between incidence and severity of AS in a cohort of Egyptian patients and *IL23R* gene single nucleotide polymorphism (rs11209026).

## 2. Materials and methods

The study was approved by the ethics committee of the Faculty of Medicine, Alexandria University. All patients provided a written, signed consent to participate in this study.

The study included thirty-two AS patients fulfilling the modified New York criteria.<sup>9</sup> Forty healthy age- and sex-matched volunteers were enrolled as a control group.

Full disease history was inquired about including; time of disease onset, duration of disease, location of pain, and history of any musculo-skeletal affection as neck pain, neck stiffness, back pain, back stiffness and morning stiffness. The inquiry included medications used, family history of AS, inflammatory bowel disease, uveitis and psoriasis. Thorough clinical examination was performed with emphasis on musculoskeletal system examination. Assessment of disease activity was done using BASDAI (Bath ankylosing Spondylitis Disease Activity Index) and ankylosing spondylitis disease activity score (ASDAS), while assessment of the degree of disease functional limitation was done using BASFI (Bath ankylosing Spondylitis Functional Index). BASMI (Bath Ankylosing Spondylitis Metrology Index) was used for assessment of the spinal mobility.

For all those participating in the study, a complete blood count, renal and liver function tests were assayed.

Markers of inflammation assayed included erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). HLA-B27 positive cases were detected using flow-cytometry. Serum IL23 was assayed using Quantikine Human IL-23 Immunoassay kit (R&D, USA).

High frequency ultrasound and Doppler examination on peripheral joints were carried out to assess synovitis and effusion. In addition, sites of enthesitis were examined to assess vascularity, thickness, hypo-echogenicity and cortical irregularity.

### 2.1. Genotyping

Genomic DNA was extracted from whole blood by standard methods using the PureLink<sup>®</sup> DNA kit (life technologies, CA, USA). Checking DNA quality and quantity was performed using Nano-Drop (Thermo Scientific, USA). All samples were stored at  $-20^{\circ}\text{C}$  till further analysis.

Samples were genotyped for interleukin-23 receptor polymorphisms using a TaqMan 5'-allele discrimination Assay-By-Design method (Applied Biosystems, Foster City, CA). The genotyping was performed on Rotor-Gene Q (Qiagen, Hilden, Germany).

TaqMan allelic discrimination was performed in 25  $\mu\text{L}$  reaction volume containing the Universal 2x TaqMan<sup>®</sup> Master Mix (Applied Biosystems, Foster City, CA, USA), and the 20X Assay ready-made stock for *IL23R* gene (rs 11209026) (Cat No.C\_1272298\_10). DNA was added in a final concentration of 1–10 ng per reaction.

Thermal cycling profile included an initial step of polymerase activation at  $95^{\circ}\text{C}$  for 10 min followed by 40 cycles of  $95^{\circ}\text{C}$  for 15 s (denaturation step) and  $60^{\circ}\text{C}$  for 1 min (annealing/extension step). No template control (NTC) was included in each run.

### 2.2. Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS/version 20) software. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test, Fisher's Exact test or Monte Carlo correction. For normally distributed data, independent *t*-test and paired *t*-test were used. For abnormally distributed data, Mann Whitney test and Wilcoxon signed ranks test were used. Significance of the obtained results was judged at the 5% level.

## 3. Results

No statistically significant difference was observed between patients and controls as regards gender ( $p = 0.072$ ), or age ( $p = 0.21$ ).

The AS patients' group was composed of 68.75% males ( $n = 22$ ), and 31.25% ( $n = 10$ ) females with male predominance, while the control group was formed of 27 males (67.5%) and 13 females (32.5%).

Age at disease onset ranged between 12.0 and 45.0 years with disease duration ranging between 1.0 and 37.0 years. Eleven cases (34.4%) complained of inflammatory low back pain and peripheral arthritis.

A statically significant difference of CRP between cases and controls was detected ( $p = 0.007$ ), while no statically significant difference detected as regards ESR ( $p = 0.613$ ) between both studied groups.

Twenty-two cases (68.8%) showed HLA-B27 positivity, while only 3 of the control participants (7.5%) were HLA-B27 positive, thus a statistically significant difference between cases and controls as regards HLA-B27 was observed ( $P < 0.001$ ).

Table 1 shows genotypes of *IL23R* gene polymorphism (rs11209026) detected using 5' Nuclease assay.

The most prevailing genotype among both studied groups was the heterozygous AG genotype.

Using ROC curve analysis, a cutoff level of 110 pg/mL was set for IL-23 level giving the best sensitivity and specificity (46.9% and 85.0%, respectively), positive predictive value 71.4%, negative predictive value 66.7% and area under the curve representing diagnostic performance of 0.577.

At this cutoff, a statically significant difference was observed between cases and controls as regards serum IL-23 level ( $p = 0.003$ ) Table 2.

No statistically significant difference was observed between different *IL23R* SNP (rs11209026) genotypes and serum IL 23 level ( $p = 0.431$ ).

In addition, HLA B27 positivity was not related to different *IL23R* polymorphism (rs11209026) genotypes ( $p = 1$ ), or to serum IL-23 level ( $p = 0.153$ ). No correlation was detected between serum IL23 and different Metrologic parameters, functional indices and Measures of the disease activity.

Different *IL23R* SNP (rs11209026) genotypes were not related to BASDAI ( $p = 0.34$ ), BASFI ( $p = 0.188$ ), BASMI ( $p = 0.513$ ), ASDAS ESR ( $p = 0.078$ ) or ASDAS CRP ( $p = 0.110$ ).

As regards AS group, Serum IL23 level was related to inflammatory low back pain ( $p = 0.027$ ), but not to peripheral arthritis ( $p = 0.653$ ), while different *IL23R* gene polymorphism (rs11209026) genotypes were not related neither to inflammatory back pain ( $p = 0.513$ ) or peripheral arthritis ( $p = 1.0$ ).

Higher IL-23 levels were associated with BASFI score ( $p = 0.017$ ), but not BASDAI ( $p = 0.79$ ), BASMI ( $p = 0.412$ ), or BASGI ( $p = 0.160$ ) scores.

## 4. Discussion

Spondyloarthritis (SpA) is a frequent form of chronic inflammatory articular group of disorders, including AS, that share the same genetic background. This has led many studies to search for definite factors behind this disabling disease aiming at early diagnosis and severity assessment.<sup>10</sup>

The present study aimed at studying the association between the incidence and severity of AS in a cohort of Egyptian patients and *IL23R* gene SNP; rs11209026 in relation to HLAB27 status. Serum IL23 was assayed to assess its association with this *IL23R* gene single nucleotide polymorphism.

**Table 1**  
Different *IL23R* gene single nucleotide polymorphism (rs11209026) genotypes.

Genotypes	Cases (n = 32)		Control (n = 40)		<sup>MC</sup> p
	No.	%	No.	%	
AA	6	18.8	1	2.5	0.015 <sup>*</sup>
AG	20	62.5	36	90.0	
GG	6	18.8	3	7.5	

$\chi^2$ : Chi square test.

MC: Monte Carlo test.

<sup>\*</sup> Statistically significant at  $p \leq 0.05$ .

**Table 2**  
Comparison between the two studied groups as regards serum IL-23 level.

IL-23 (pg/mL)	Cases (n = 32)	Control (n = 40)	
$\leq 110$	17 (53.1%)	34 (85.0%)	0.003 <sup>*</sup>
$> 110$	15 (46.9%)	6 (15.0%)	

<sup>\*</sup> Statistically significant at  $p \leq 0.05$ .

In the current study, AS cases showed higher values for CRP mainly due to the inflammatory nature of the disease.

Benhamou et al.<sup>11</sup> evaluated the clinical value of CRP in two randomized controlled trials in which 851 patients with painful axial AS participated. The study concluded that elevated CRP was observed in patients with painful axial AS. In addition, the study reported that CRP is correlated to both disease activity and functional severity.

SpA is a complex heritable disease that involves multiple genetic factors, among which the HLA-B27 allele plays a prominent role.<sup>12</sup>

Even though AS, as one of the Spondyloarthropathies, is strongly associated with HLA-B27 positivity as proved by many studies,<sup>13,14</sup> only up to 5% of HLA-B27 positive individuals develop AS implying the presence of other non-HLA genes playing a major role in the development of AS.<sup>15</sup> In addition, susceptibility to AS cannot be fully explained by associations with the MHC genes.

It was reported that HLA-B27 positivity carry some racial differences being higher in those of North European ancestry.<sup>16</sup>

Studies concerning the association of AS with B27 positivity in the middle-east have shown a relatively weak strength of the association when compared with western countries ranging from (25 to 75%).<sup>17,18</sup>

In accordance with previous studies,<sup>17,18</sup> twenty-two cases (68.8%) of AS cases in the present work showed HLA-B27 positivity with a statistically significant difference detected between cases and controls.

Numerous SNPs have been associated with AS in many populations.<sup>19,20</sup> In the present work, the most prevailing genotype was the heterozygous AG in Egyptian AS cases (62.5%) and controls (90%). In addition, a strong association was detected between AS and *IL23R* SNP (rs11209026) with no relation between any of this SNP genotypes and HLA-B27 positivity. The rs11209026 SNP was not related to any of disease activity, spinal mobility or functional disability scores studied.

A study by Rahman et al.<sup>19</sup> demonstrated a strong association between AS and rs11209026 in multiple Canadian populations.

Kadi et al. genotyped the SNP rs11209026 in a French cohort of 415 SpA patients and 372 controls. The study identified a significant association between rs11209026 and AS and suggested that this SNP mostly affects disease severity rather than susceptibility.<sup>20</sup>

A meta-analysis that included European and Asian populations<sup>19</sup> revealed a significant association between rs11209026 and the risk of developing AS in Europeans only.

The minor A Allele of rs11209026 is underrepresented in patients of many diseases including Crohn's disease, ulcerative col-

itis<sup>21</sup> and many other autoimmune inflammatory disorders including psoriasis,<sup>8</sup> Rheumatoid arthritis,<sup>22</sup> and ankylosing spondylitis,<sup>23</sup> thus studies concluded that the loss of function mutant A allele mediates a protective effect through the occurrence of a condition of IL-23 reduced responsiveness leading to decreased IL-23-dependent IL17 and IL-22 production.<sup>19</sup>

In the current work, a statically significant difference was observed between cases and controls as regards serum IL23 level which was not related to HLA B27 positivity or to different *IL23R* genotypes. Higher levels of serum IL-23 correlated to Bath ankylosing spondylitis functional Index (BASFI) which is a measure of functional disability for patients suffering from AS.<sup>24</sup>

In the present study, no correlation was detected between serum IL-23 levels and other studied measures of disease activity (ASDAS) or spinal mobility (BASMI), while it related well to inflammatory low back pain.

In cases of AS, increased levels of IL-23 leads to secretion of IL-17 which is a pro-inflammatory cytokine that has been reported to be increased in the sera, synovial fluid and inflamed bone marrow of patients with AS.<sup>25</sup>

Although IL-17 provides mucosal immunity against bacteria and fungi, chronic exposure to IL-17 promotes inflammation that ends in bone and cartilage destruction as in cases of AS,<sup>26</sup> thus IL-23/IL-17 axis is implicated in pathogenesis of many autoimmune diseases including AS.<sup>27,28</sup>

Ugur et al.<sup>29</sup> reported a statistically significant elevation of serum level of IL-23 in AS patients that correlated to disease activity scores as BASDAI, BASFI, thus concluding that elevated serum IL-23 levels are related to AS activity.

Another study by Chen et al.<sup>30</sup> evaluated serum IL-23 and IL-17 in 49 Chinese AS patients and 25 healthy control subjects. The study concluded that elevated level of both studied cytokines correlated well to AS disease activity markers emphasizing on the role of IL-23 and IL-17 in the pathogenesis of AS.

It is recommended to investigate other single nucleotide polymorphisms in correlation to different proven candidate genes like *ERAP-1* as well as *HLA-B27*.

## 5. Conclusions

In the studied cohort of Egyptian ankylosing spondylitis patients, it is suggested that *IL-23R* single nucleotide polymorphism (rs11209026) might be associated with ankylosing spondylitis occurrence not severity, while higher levels of IL-23 might be associated with disease severity.

## Conflict of interest

We have no conflict of interest to declare.

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