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Genetic copy number variation of *TLR7*, *T-bet*, *IL12B*, and *FcγRIIIB* in Egyptian ankylosing spondylitis patients

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Background: Ankylosing spondylitis (AS) is a polygenic disorder. Copy number variations (CNVs) alter the expression of *TLR7, T-bet, IL-12B*, and *FcγRIIIB* genes, contributing to AS development via their immune system roles.

Objective: The current study aimed to assess the correlation between AS susceptibility and CNVs of *TLR7, T-bet, IL12B*, and *FcγRIIIB*, as well as their influence on disease activity.

Methods: The study involved 42 healthy controls and 72 patients with AS, recruited from the Rheumatology and Rehabilitation clinic of Zagazig University Hospitals, Zagazig, Egypt, from 01 November 2023 to 30 October 2024. Sociodemographic, clinical data and blood samples were collected from all participants. Copy number estimations for *TLR7, T-bet, IL12B*, and *FcγRIIIB* genes were performed using SYBR Green real-time polymerase chain reaction.

Results: Of 72 cases aged 19 to 52 years, 47 (65.2%) were men and 25 (34.8%) were women. Controls included 42 participants aged 27 to 55 years, 22 (52.4%) men and 20 (47.6%) women. Higher *IL12b* and *FcγRIIIB* gene copy numbers were significantly associated with a higher risk of AS ($p = 0.001$, odds ratio [OR]: 3.8, 95% confidence interval [CI]: 1.7–8.07, and $p < 0.001$, OR: 5.5, 95% CI: 2.31–13.08, respectively). While *T-bet* and *TLR7* gene CNVs showed no significant association with AS risk. No significant association was observed between the studied CNVs and AS activity.

Conclusion: High copy numbers of *IL12B* and *FcγRIIIB* genes may be associated with increased AS risk. However, no significant correlation was found between AS risk and *TLR7* or *T-bet* CNVs.

What this study adds: The findings of this study revealed that genetic copy number variations may contribute to the risk of AS.

Keywords: ankylosing spondylitis; copy number variations; *TLR7*; *T-bet*; *IL12B*; *FcγRIIIB*.

Introduction

Ankylosing spondylitis (AS) is a common seronegative autoimmune arthropathy, primarily characterised by axial skeleton and sacroiliac joint inflammation. In severe cases, inflammation may progress to fibrosis and calcification, reducing spinal flexibility and resulting in fusion and restricted mobility.[1] The primary clinical symptoms include back pain, progressive stiffness of the spine, and joint inflammation. Furthermore, extra-articular manifestations, particularly acute anterior uveitis and inflammatory bowel disease, may further complicate the clinical course.[2]

The aetiology of AS is multifactorial, involving numerous factors, such as genetic factors, environmental influences, prior infections, and socio-economic status.[3] The most substantial genetic correlation is found with *HLA-B27*, a key genetic factor accounting for 16% – 50% of disease genetic susceptibility.[4,5] Previous genetic studies on AS have primarily focused on disease susceptibility. Despite its significance, investigating genetic factors associated with radiographic damage and disease activity has been largely overlooked.[4,6]

Recent findings have highlighted the substantial role of copy number variations (CNVs) in the progression of AS.[3] Copy number variations are structural genomic alterations involving genome-

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wide deletions or duplications that affect one kilobase to several megabases.[7] Copy number variations can significantly alter gene expression by altering the copy number (CN) of dosage-sensitive loci, thereby perturbing gene expression.[8] Moreover, CNVs may reposition genes and regulatory regions, affecting gene expression.[9]

Research increasingly emphasises the significance of CNVs in various complex immune and nonimmune disorders.[10,11] Gene CNVs for Fc Gamma Receptor IIIB (*FcγRIIIB*), T-box expressed in T-cells (*T-bet*), Interleukin-12B (*IL12B*), and Toll-like receptor 7 (*TLR7*) have been identified as risk factors for the onset of several autoimmune disorders, such as rheumatoid arthritis, AS, and systemic lupus erythematosus.[12,13,14,15]

Lower CNs of *FcγRIIIB* genes have been linked to an increased risk of developing AS.[16] A high CN of the *T-bet* gene has been associated with increased susceptibility to AS, along with acute anterior uveitis, indicating the potential impact of the *T-bet* gene CNV on the clinical course of the disease.[15] A previous study demonstrated that extra gene copies are associated with higher messenger RNA levels of *T-bet* and *IL-12B*,[17] increasing *IL-12B* expression, which has been demonstrated to exacerbate the severity of AS.[18] A Chinese study indicated that CNVs in the *TLR7* gene are linked to AS, with a reduced CN posing a risk for men and a protective effect for women.[19]

Furthermore, a number of studies have connected several gene polymorphisms to the progression of AS and the activity of the disease. The Ankylosing Spondylitis Disease Activity Score based on C-reactive protein is the standard method for evaluating AS disease activity. This validated composite index combines C-reactive protein levels with patient-reported measures (back pain, morning stiffness length, and global disease activity), and categorises disease activity as inactive (< 1.3), low (1.3–2.1), high (2.1–3.5), or extremely high (≥ 3.5), based on the 2018 criteria.[20]

The link between CNVs and AS risk has been the subject of several studies in developed countries, but far less is known in North African countries with limited resources, especially Egypt. Moreover, to our knowledge, the influence of these CNVs on disease activity has not been previously examined. Thus, the purpose of this study was to examine the relationship between the risk of developing AS in Egyptian patients and CNVs in the *FcγRIIIB*, *T-bet*, *IL12B*, and *TLR7* genes. We also aimed to assess the influence of these CNVs on AS activity.

Methods

Ethical considerations

This study was approved by the Zagazig University Institutional Review Board (approval number: 11207) on 25 October 2023. All participants provided written informed consent prior to participating. Procedures were followed in accordance with the Helsinki Declaration. The patient's information had been concealed from the lab staff as the data records and sample tubes were coded.

Subjects

This study was a case-control study conducted at Zagazig University Hospital, Zagazig, Egypt, from 01 November 2023 to 30 October 2024. It comprised 72 consecutive patients with AS, recruited from the Rheumatology and Rehabilitation clinic of the Zagazig University Hospitals, Zagazig, Egypt. The control group comprised 42 healthy volunteers of the same ethnicity, matched for age and gender, devoid of any autoimmune or malignant disorders. Sample size of this study was calculated using Epi Info version 3.01 (Centers for Disease Control and Prevention [CDC], Atlanta, Georgia, United States), at 95% confidence interval (CI), based on the previously published data.[12,15,19]

Eligibility criteria of participating patients included fulfilling the Modified New York Criteria for AS diagnosis.[21] Patients with other coexisting immunological or malignant conditions were excluded from the study. The diagnosis of AS and the evaluation of disease activity have been conducted by two expert rheumatologists based on clinical examination, radiographic evidence, and laboratory investigations. A verbal questionnaire was given to the participants in order to gather sociodemographic data, including age, ethnicity, disease duration and family history. The following were obtained from patients' records: *HLA-B27* status, C-reactive protein, and erythrocyte sedimentation rate levels. Disease activity was assessed using ASDAS.[20]

Extraction of genomic DNA

Venous blood samples were collected in tubes containing ethylenediaminetetraacetic acid. Total DNA was extracted according to the manufacturer's instructions using DNA isolation kits (QIAamp DNA Minikit; QIAGEN GmbH, Hilden, Germany) and stored at −20 °C. Agarose gel electrophoresis was used to roughly assess the extracted DNA concentration before storage.[22]

Copy number estimation

Real-time quantitative polymerase chain reaction (PCR) (RTqPCR, SYBR Green; Enzynomics, Life Technologies, Delhi, India) was utilised to assess the CNVs of *FcγRIIIB*, *T-bet*, *IL12B*, and *TLR7*, following the previously described procedure.[23] The concentration of the extracted DNA was accurately measured using a Qubit Fluorometer immediately prior to the real-time PCR run for mixture preparation.[24] The PCR reactions were conducted using an Applied Biosystems 7500 Real-Time PCR machine, following the manufacturer's instructions for the PCR mixture and cycling conditions. Forkhead box protein P2 (*FOXP2*) gene (a non-CNV gene) served as the reference for normalisation. Table 1 lists the primer sequences used to estimate CNVs for the genes under study and the reference gene *FOXP2*.

Copy numbers (CNs) were calculated using the comparative cycle threshold (2-^^Ct) method.[23] Copy numbers for *FcγRIIIB, T-bet, IL12B,* and *TLR7* were categorised as low CN (< 2), normal CN (= 2), and high CN (> 2).

Data analysis

Data were tabulated and analysed using SPSS-22 (IBM Corporation, Armonk, New York, United States). Student's *t*-test and one-way analysis of variation with least significant difference post-hoc test were used to compare means, and the Kruskal-Wallis test was used to compare medians. Chi-square and Fisher's exact tests were used to compare frequencies. The level of statistical significance was set at a *p*-value of < 0.05.

Results

Demographic and clinical characteristics

The study included 114 participants: 72 AS patients and 42 healthy controls. Among the 72 AS patients, aged 19 to 52 years, 47 (65.2%) were men and 25 (34.8%) were women. The control group consisted of 42 individuals aged 27 to 55 years, with 22 (52.4%) men and 20 (47.6%) women. Among patients with AS, 11.1% exhibited low disease activity, 58.3% demonstrated high activity, and 30.6% presented with very high activity. Table 2 and Table 3 summarise the demographics and clinical characteristics of the study participants.

Frequency distribution of *FcγRIIIB, T-bet, IL12B,* and *TLR7* genes copy number variations

Patients and healthy controls were compared regarding the frequency of CNV in the genes under investigation (Table 4).

Elevated CNs of the *IL12b* and *FcγRIIIB* genes (> 2) were associated with an increased risk of AS (> 2) (*IL12b: p* = 0.001, odds ratio [OR]: 3.8, 95% CI: 1.7–8.07; *FcγRIIIB: p < 0.001,* OR: 5.5, 95% CI: 2.31–13.08). While low CNs of *IL12b* and *FcγRIIIB* genes (< 2) were found to be associated with reduced risk of AS (*IL12b: p* = 0.02, OR: 0.38, 95% CI: 0.16–0.87; *FcγRIIIB*: *p* = 0.001, OR: 0.22, 95% CI: 0.09–0.54). However, neither *T-bet* nor *TLR7* gene CNVs were significantly associated with the risk of AS.

Association between ankylosing spondylitis activity and copy number variations in *FcγRIIIB, T-bet, IL12B,* and *TLR7* genes

To determine the association between CNVs of the examined genes and AS activity, we analysed the differences in cases concerning CNVs and the erythrocyte sedimentation rate, C-reactive protein, *HLA-B27*, and Ankylosing Spondylitis Disease Activity Score – C-reactive protein Grade (Table 5). However, we could not identify any correlation between the clinical manifestations of AS and *FcγRIIIB, T-bet, IL12B,* and *TLR7* CNVs.

TABLE 1: Primer sequences for estimating copy number variations for the reference gene *FOXP2* and the genes under study, Zagazig University Hospitals, Zagazig, Egypt, November 2023 – October 2024.

Gene	Primer	Sequence
TLR7[25]	Forward	CAGTATTGTGCTGTCTTTGAAATGTAAA
	Reverse	TGGGCCCAATAGCATCAACT
IL-12B[17]	Forward	CCTGGGAAACATAACAA
	Reverse	CACAATCACGGCTCAC
T-bet[17]	Forward	CCTGGTGCTGCGTCTT
	Reverse	GCGGGCTGATGGTTAT
FcγRIIIB[26]	Forward	CAA GCA TCC TGG GAAAGC T
	Reverse	TACTCTCCCAAAGGTCTG C
FOXP2[27]	Forward	TGACATGCCAGCTTATCTGTTT
	Reverse	GAGAAAAGCAAT TTTCACAGTCC

Note: Please see the full reference list of this article for details on the articles cited: Said NM, Mekkawy EM, Said D, et al. Genetic copy number variation of *TLR7, T-bet, IL12B,* and *FCGR3B* in Egyptian ankylosing spondylitis patients. Afr J Lab Med. 2025;14(1), a2916. https://doi.org/10.4102/ajlm.v14i1.2916

TLR7, Toll-like receptor *7; IL-12B,* Interleukin-12B*; T-bet,* T-box expressed in T-cells*; FcγRIIIB,* Fc Gamma Receptor IIIB; *FOXP2,* Forkhead box protein P2.

TABLE 2: Demographic characteristics of the study participants, Zagazig University Hospitals, Zagazig, Egypt, November 2023 – October 2024.

Variable	Cases (*n* = 72)			Controls (*n* = 42)		
	Mean ± s.d.	*n*	%	Mean ± s.d.	*n*	%
Age (years)	35.2 ± 10.0	-	-	37.71 ± 7.7	-	-
Sex						
Male	-	47	65.2	-	22	52.4
Female	-	25	34.8	-	20	47.6

s.d., standard deviation.

TABLE 3: Clinical characteristics of the study participants, Zagazig University Hospitals, Zagazig, Egypt, November 2023 – October 2024.

Variable	Low activity (*n* = 8, 11.1%)†				High activity (*n* = 42, 58.3%)‡				Very high activity (*n* = 22, 30.6%)§			
	Median	Range	*n*	%	Median	Range	*n*	%	Median	Range	*n*	%
Duration (years)	13	4–22	-	-	7	1.5–36	-	-	7	4–20	-	-
MS (min)	0	0–5	-	-	20	5–60	-	-	30	10–120	-	-
Axial	3.5	1–6	-	-	7	0–10	-	-	8	5–10	-	-
PGA	1.5	0–5	-	-	5.5	0–7	-	-	6.5	0–9	-	-
CRP (mg/L)	10.75	6.1–24	-	-	15	1.64–107	-	-	13	2.8–50	-	-
ESR (mm/h)	33	11–42	-	-	24	2–107	-	-	15	5–95	-	-
HLA-B27												
Positive	-	-	6	75	-	-	18	42.9	-	-	10	45.5
Negative	-	-	2	25	-	-	24	57.1	-	-	12	54.5
Medication												
Biologic treatment	-	-	6	75	-	-	42	100	-	-	22	100
Sulfasalazine	-	-	2	25	-	-	0	-	-	-	0	-

MS, morning stiffness; min, Minutes; PGA, patient global assessment; ASDAS, Ankylosing Spondylitis Disease Activity Score; *HLA-B27*, Human leukocyte antigen-B27; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; s.d., standard deviation.

†, ASDAS-CRP Score mean ± s.d. = 1.8 ± 0.14; ‡, ASDAS-CRP Score mean ± s.d. = 2.9±0.45; §, ASDAS-CRP Score mean ± s.d. = 3.9 ± 0.67.

TABLE 4: *FcγRIIIB, T-bet, IL12B,* and *TLR7* gene copy number variation distribution in ankylosing spondylitis patients and healthy controls, Zagazig University Hospitals, Zagazig, Egypt, November 2023 – October 2024.

Variable	Case group (*n* = 72)		Control group (*n* = 42)		*p*†	Odds ratio	95% CI
	n	%	*n*	%			
TLR7							
< 2	43	59.7	18	42.9	0.13	1.8	0.8–3.9
= 2	4	5.5	6	14.2	0.11	0.3	0.09–1.3
> 2	25	34.6	18	42.9	0.38	0.7	0.3–1.5
T-bet							
< 2	30	41.7	24	57.1	0.11	0.54	0.25–1.16
= 2	5	6.9	0	0	-	-	-
> 2	37	51.4	18	42.9	0.38	1.41	0.65–3.0
IL-12B							
< 2	16	22.2	18	42.9	0.02**	0.38	0.16–0.87
= 2	0	0	4	9.5	-	-	-
> 2	56	77.8	20	27.8	0.001**	3.8	1.7–8.07
FcγRIIIB							
< 2	10	13.9	18	42.9	0.001**	0.22	0.09-0.54
= 2	2	2.8	4	9.5	0.14	0.27	0.05–1.56
> 2	60	83.3	20	27.8	< 0.001**	5.5	2.31–13.08

TLR7, Toll-like receptor 7; *IL-12B,* Interleukin-12B; *T-bet,* T-box expressed in T-cells; *FcγRIIIB*, Fc Gamma Receptor IIIB; CI, confidence interval.

**, *p*-value ≤ 0.05: Significant.

†, Chi-square test, *p*-value > 0.05: Non-significant.

TABLE 5: Ankylosing Spondylitis Disease Activity Score – C-reactive protein Grade and copy number variation frequencies of *FcγRIIIB, T-bet, IL12B,* and *TLR7*, Zagazig University Hospitals, Zagazig, Egypt, November 2023 – October 2024.

Variable	ASDAS-CRP grade			*p*†
	Low activity (1.3–2.1) (*n* = 8)	High activity (2.1–3.5) (*n* = 42)	Very High (≥ 3.5) (*n* = 22)	
TLR7				
< 2	8	24	11	0.15
= 2	0	2	2	-
> 2	0	16	9	-
T-bet				
< 2	4	18	8	0.15
= 2	2	1	2	-
> 2	2	23	12	-
IL-12b				
< 2	2	6	8	0.13
> 2	6	36	14	-
FcγRIIIB				
< 2	0	6	4	0.53
= 2	0	2	0	-
> 2	8	34	18	-

ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score – C-reactive protein; *TLR7,* Toll-like receptor 7; *IL-12B,* Interleukin-12B; *T-bet,* T-box expressed in T-cells; *FcγRIIIB*, Fc Gamma Receptor IIIB.

†, Chi-square test, *p*-value > 0.05: non-significant.

Discussion

The study examined the association between the risk of AS and CNVs in the *FcγRIIIB, T-bet, IL12B,* and *TLR7* genes among Egyptian patients, as well as their impact on disease activity.

The results of the current investigation showed a strong correlation between elevated *FcγRIIIB* and *IL12B* copy numbers (> 2 copies) and a higher risk of AS, with 3.8-fold and 5.5-fold increases in risk associated with each. As for the *T-bet* gene CNV, there was no significant distinction between AS patients and healthy controls, even though AS patients had a higher frequency (51.4%) of increased CNs (> 2 copies). Furthermore, AS patients had a higher frequency of low *TLR7* CN (59.7%). Nevertheless, no significant link between AS risk and *TLR7* CNVs was found. Meanwhile, we found no association between AS activity and CNVs of *TLR7, IL12B, T-bet,* or *FcγRIIIB*.

In contrast to our findings, the analysis of *FcγRIIIB* CNV frequency distribution in a study of Algerian AS patients in 2019 showed no statistically significant difference between patients and controls.[28] A Chinese study in 2016 indicated that a low CN (≤ 3) of the *FcγRIIIB* gene may be a risk factor for AS.[12] The observed discrepancy in results may be attributed to variations in the distribution of CN groups, as our study defined three copies as a high CN.

To the best of our knowledge, this is the first study to investigate the association of *IL12B* CNV and the risk of AS. Previous research, however, including a Chinese study in 2024 and a Bulgarian study in 2019, found a strong correlation between *IL12B* SNPs and an increased genetic risk for AS.[29,30] Additionally, *IL12B* has been identified as a possible therapeutic target for AS in two recent Chinese studies conducted in 2024.[30,31]

In contrast to our findings, a study conducted in China in 2016 identified a higher prevalence of a high *T-bet* CN in AS patients, which was significantly correlated with disease risk.[15]

A Chinese study in 2018 identified a significant correlation between low *TLR7* CN and the risk of AS. Nevertheless, the CNs found in this investigation were restricted to one or two copies, in contrast to our analysis, which found that more than two copies of the gene were found in 51.4% of AS patients and 42.9% of healthy controls.[19] However, our findings aligned with those of the Chinese 2018 study by Wang et al., which indicated no association between clinical manifestations and *TLR7* CNVs.[19] No prior research has examined the relationship between AS activity and *FcγRIIIB, T-bet*, and *IL12B* CNVs.

Limitations

It is important to take into account the limitations of this study when evaluating the results. First, the study was conducted at a single centre and had a relatively small sample size. Therefore, subsequent multicentre studies with larger cohorts may be necessary to corroborate the results, as they might be lacking sufficient power. Second, despite the established impact of genetic CNVs on the level of gene expression, which may influence the outcomes of therapy, the study did not address the effect of the genes under investigation on the response to therapy.

Conclusion

The study's findings indicate that possessing more than two copies of both *IL12B* and *FcγRIIIB* may increase the risk of developing AS. However, AS risk was not significantly correlated with *TLR7* or *T-bet* CNVs.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

N.M.S. and A.A.A. helped to conceptualise the study. N.M.S., E.M.M., D.S., and D.G.A. all made contributions to the study's investigation. Contributions to data curation came from E.M.M., D.S., D.G.A. and A.A.A. D.G.A., A.A.A., A.A.E.S., E.A.A., and F.Z.K. all made contributions to the methodology. The project administration and original draft preparation were handled by N.M.S. The article was reviewed and edited with assistance from E.M.M., D.S., F.Z.K., E.A.A., A.A.E.S. and A.A.A. F.Z.K., E.A.A., and A.A.E.S. assisted with the study supervision. All of the authors read and approved the article.

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Data availability

The data sets generated and analysed during the current study are available from the corresponding author, N.M.S., on reasonable request.

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