Original Article

The Effect of Vitamin D SNP FokI (rs2228570) and ApaI (rs7975232) on Axial Spondyloarthritis in Patients on Biological Treatment

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ABSTRACT

Objective: Examine associations between FokI and ApaI genotypes and axSpA disease activity, extra-articular manifestations (EAMs), and infliximab (INF) response in Iraqi patients.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Baghdad Tertiary Centers from 1st August 2024 to 3th December 2024.

Methods: This cross-sectional analysis of 150 axSpA patients on INF (≥3 months) at Baghdad Tertiary Centers. Disease activity was staged by ASDAS; genotypes were determined by PCR-RFLP. Logistic models adjusted for age, sex, smoking, and disease duration assessed genotype-phenotype links; serum VDR activity and an INF "effect-length" index were evaluated.

Results: The mean age 38.8±8.9 years; 72% male; smokers 30%. FokI showed CC 60% and CT 40% (TT absent). CC was confined to inactive ASDAS and associated with remission (OR 19.2; p=0.04), whereas CT increased odds of high activity (OR 3.27; p=0.001). CT carriers had lower frequencies of uveitis, dactylitis, and UTI. ApaI genotypes did not associate with ASDAS or EAMs. Neither SNP related to serum VDR activity or INF effect-length.

Conclusions: FokI acts as a phenotypic modifier—CC protective for axial activity; CT associated with higher activity yet fewer select EAMs-while ApaI appears neutral. Pharmacodynamic measures were unaffected.

Key Words: Axial spondyloarthritis; Vitamin D receptor; FokI; ApaI; infliximab

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INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disorder with sacroiliac and spinal involvement and substantial impacts on function and quality of life.[1] Despite global advances in axSpA management[2,3], regional data from the Middle East remain scarce and diagnostic delay persists, partly due to the lack of specific biomarkers for early detection (unlike in other diseases.[4,5]

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TNF-α is pivotal in axSpA pathogenesis, underpinning the effectiveness of inhibitors such as infliximab[6,7]; however, a considerable subset shows primary or secondary non-response.[8]

Well-established genetic factors such as HLA-B27 influence axSpA susceptibility and phenotype[9], and variants in other genes including the vitamin D receptor (VDR) may further shape disease activity and extra-articular manifestation profiles.[10] The FokI start-codon polymorphism alters VDR transactivation potential[11], whereas ApaI is an intronic variant with inconsistent clinical associations.[12] We investigated whether FokI and ApaI genotypes relate to ASDAS-defined disease activity, EAMs, and infliximab response in Iraqi axSpA patients.

METHODS

This cross-sectional study conducted at the Rheumatology Clinics of Baghdad Hospital/Medical City and the National Center for Educational Laboratories from 1st August 2024 to 30th December 2024. A total of 150 consecutive adults (≥18 years) with axSpA verified by MRI or radiography, all receiving INF for ≥3 months. Data collection:

Structured interview and examination captured demographics, smoking, and EAMs; laboratory indices included ESR. Disease activity was staged using ASDAS. Genotyping: VDR FokI (rs2228570) and ApaI (rs7975232) were genotyped by PCR-RFLP. Serum VDR activity was quantified by ligand-binding assay. Using SPSS-24, data was analyzed. Between-group comparisons used t-tests/ANOVA where appropriate. Associations with ASDAS stages used Chi-square and logistic regression adjusted for age, sex, smoking, and disease duration. $p \leq 0.05$ was considered significant.

RESULTS

The mean age was 38.8±8.9 years and BMI was 28.9±6.5 kg/m²) (Table 1). The males were predominated (72%). Only 16% had a first-degree family history suggesting mostly sporadic disease and 30% were current smokers (Table 2).

ASDAS stage correlated with gender (p=0.02; all inactive were male), inflammatory back pain (IBP) (p=0.003; 74% overall, peaking 87% in low activity), colitis (p=0.02; clustered in low activity), and smoking (p<0.001; 46.7% of smokers in the high-activity group). Peripheral arthritis (p=0.41), uveitis (p=0.10), dactylitis (p=0.13), and psoriasis (p=0.19) showed no significant stage trends; enthesitis was borderline (p=0.055; highest 12.5% in very high activity). The males were predominance in the inactive stage; IBP and smoking are strong markers of higher activity; colitis clustering at low activity may indicate a distinct phenotype (Table 3).

ASDAS staging: inactive 12 (8%), low 69 (46%), high 45 (30%), very high 24 (16%). Mean axSpA duration was 4.77±4.22 years; infliximab exposure was 3.07±2.23 years. The INF effect index averaged 1.53±0.2, indicating maintained efficacy in most patients (Table 4).

FokI (rs2228570): CC 60%, CT 40%, TT absent; allele frequencies C 80%, T 20% consistent with HWE and no genotyping bias. ApaI (rs7975232): CC 42%, CA 40%, AA 18%; allele frequencies C 62%, A 38% also HWE-consistent, indicating study genetic stability (Table 5).

FokI (rs2228570): Strong stage association (p<0.001). All inactive cases were CC (12/12). CT frequency rose with activity (low 30.4%, high 60%, very high 50%). Regression: CC → remission (OR 19.2; 95% CI 1.11-331.9; p=0.04); CT → high activity (OR 3.27; 95% CI 1.58–6.77; p=0.001). ApaI (rs7975232). No significant (p>0.05) association with ASDAS stage (Table 6). Genotype–EAMs (Tables 1-6 and 1-7): For FokI, CT carriers had lower uveitis and dactylitis (each p=0.04). Enthesitis trended lower in CT (ns). ApaI showed no significant EAM associations. Serum VDR activity and INF effect-length did not vary by genotype (Tables 7-8).

Table No. 1: Descriptive statistics of the patients

Variable	Mean±SD
Age (years)	38.82±8.88
BMI (mg/m^2)	28.92±6.45

Table No. 2: Demographic information of the patients

Variable	No.	%
Gender		
Male	108	72.0
Female	42	28.0
Family of SPA		
Yes	24	16.0
No	126	84.0
Smoking		
Yes	45	30
No	105	70.0

Table No. 3: Association between disease stages with gender, extra-articular manifestations and smoking

Variable		Disease Activity				Total	P value
		Inactive disease	Low disease activity	High disease activity	Very high activity		
Gender	Female	-	24 (34.8%)	12 (26.7%)	6 (25%)	42 (28%)	0.02*
	Male	12 (100%)	45 (65.2%)	33 (73.3%)	18 (75%)	108 (72%)	
Inflammatory back pain	No	6 (50%)	9 (13%)	18 (40%)	6 (25%)	39 26%)	0.003*
	Yes	6 (50%)	60 (87%)	27 (60%)	18 (75%)	111 (74%)	
Arthritis	No	6 (50%)	24 (34.8%)	15 (33.3%)	12 (50%)	57 (38%)	0.41
	Yes	6 (50%)	45 (65.2%)	30 (66.7%)	12 (50%)	93 (62%)	
Enthesitis	No	12 (100%)	66 (95.7%)	45 (100%)	21 (87.5%)	144 (96%)	0.055
	Yes	-	3 (4.3%)	-	3 (12.3%)	6 (4%)	
Uveitis	No	12 (100%)	57 (82.5%)	42 (93.3%)	21 (97.5%)	132 (88%)	0.10
	Yes	-	12 (17.5%)	3 (6.7%)	3 (2.5%)	18 (12%)	
Dactylitis	No	12 (100%)	57 (82.6%)	42 (93.3%)	21 (87.5%)	132 (88%)	0.13

	Yes	-	12 (17.4%)	3 (6.7%)	3 (12.5%)	18 (12%)	
Psoriasis	No	12 (100%)	66 (85.7%)	45 (100%)	24 (100%)	147 (98%)	0.19
	Yes	-	3 (14.3%)	-	-	3 (2%)	
Colitis	No	12 (100%)	63 (91.3%)	45 (100%)	24 (100%)	144 (96%)	0.02*
	Yes	-	6 (8.7%)	-	-	6 (4%)	
Smoking	No	12 (100%)	48 (69.6%)	24 (53.3%)	21 (87.5%)	105 (70%)	<0.001*
	Yes	-	21 (30.4%)	21 (45.7%)	3 (12.5%)	45 (30%)	

Table No.4: Mean duration of axiel spondylarthritis, infliximab usage and infliximab effect

Variable	**Mean±SD**	**SE**	**Range**
Duration of AxSpA	4.77±4.22	0.34	3.00-26.00
Duration of INF.	3.07±2.23	0.18	1.00-9.00
Effect of INF.	1.53±0.21	0.01	1.0-2.0

Table No.5: Vitamin D receptor gene FokI and ApaI polymorphism variant distribution

Variable		**N**	**Mean ± SD**	**SE**	**p-value**
FokI	CT	69	4.75 ± 2.13	0.25	0.92
	CC	90	5.21 ± 3.70	0.39	
ApaI	CC	60	5.16 ± 1.53	0.19	0.93
	AA	27	5.24±1.85	0.35	
	AC	60	5.08±2.98	0.36	

Table No.6: Association between VDR polymorphism variants and disease stage

Variable		**Disease Activity**				**Total**	**P value**
		Inactive disease	**Low disease activity**	**High disease activity**	**Very high activity**		
Fok I	CC	12 (100%)	48 (69.6%)	18 (40%)	12 (50%)	90 (60%)	<0.001*
	Odd	19.2 (1.11-331.9)	1.71 (0.88-3.31)	0.30 (0.14-0.63)	0.61 (0.25-1.47)		
	p	0.04	0.10	0.003	0.27		
	CT	-	21 (30.4%)	27 (60%)	12 (50%)	60 (40%)	
	Odd		0.58 (0.3-1.12)	3.27 (1.58-6.77)	1.62 (0.67-3.9)		
	p		0.10	0.001	0.27		
ApaI	AA	4 (33.3%)	11 (15.9%)	9 (20%)	3 (12.5%)	27 (18%)	0.50
	Odd	2.5 (0.64-9.0040)	0.77(0.33-1.7)	1.20(0.49-2.94)	0.57 (0.15-2.07)		
	P	0.16	0.54	0.60	0.39		
	AC	6 (50%)	29 (42%)	15 (33.3%)	10 (41.7%)	60 (40%)	
	Odd	1.55(0.44-5.07)	1.2 (0.63-2.33)	0.66 (0.31-1.3)	1.08(0.44-2.6)		
	P	0.46	0.58	0.23	0.88		
	CC	2 (16.7%)	29 (42%)	21 (46.7%)	11 (45.8%)	63 (42%)	
	Odd	0.45(0.09-2.12)	1.001(0.52-1.9)	1.13(00.69-2.48)	1.20(0.50-2.89)		
	p	0.31	0.99	0.40	0.60		

Table No.7: Association between FokI genotypes and gender/extra-articular manifestations

Variable		**Fok**		**Total**	**P value**
		CC	**CT**		
Gender	Female	24 (26.7%)	18 (30%)	42 (28%)	0.71
	Male	66 (73.3%)	42 (70%)	108 (72%)	
Inflammatory back pain	No	21 (23.3%)	18 (30%)	39 (26%)	0.41
	Yes	69 (76.7%)	42 (70%)	111 (74%)	
Arthritis	No	33 (36.7%)	24 (40%)	57 (38%)	0.73
	Yes	57 (63.3%)	36 (60%)	93 (62%)	
Enthesitis	No	84 (93.3%)	60 (100%)	144 (96%)	0.08
	Yes	6 (6.7%)	-	6 (4%)	
Uveitis	No	75 (83.3%)	57 (95%)	132 (88%)	0.04
	Yes	15 (16.7%)	3 (5%)	18 (12%)	

Dactylitis	No	75 (83.3%)	57 (95%)	132 (88%)	0.04
	Yes	15 (16.7%)	3 (5%)	18 (12%)	
Psoriasis	No	87 (96.7%)	60 (100%)	147 (98%)	0.27
	Yes	3 (3.3%)	-	3 (2%)	
Colitis	No	84 93.3%)	60 (100%)	144 (96%)	0.08
	Yes	6 (6.7%)	-	6 (4%)	

Table No.8: Association between ApaI genotypes and gender/extra-articular manifestations

Variable		Apa			Total	P value
		AA	AC	CC		
Gender	Female	5 (18.5%)	18 (30%)	19 (30.2%)	42 (28%)	0.45
	Male	22 (81.5%)	42 (70%)	44 (69.9%)	108 (72%)	
Inflammatory back pain	No	10 (37%)	21 (35%)	26 (41.3%)	57 (38%)	0.76
	Yes	17 (63%)	39 (65%)	37 (58.7%)	93 62%)(	
Arthritis	No	10 (37%)	21 (35%)	26 (41.3%)	57 (38%)	0.76
	Yes	17 (63%)	39 (65%)	37 (58.7%)	93 (62%)	
Enthesitis	No	26 (96.3%)	57 (95%)	61 (96.8%)	144 (96%)	0.87
	Yes	1 (3.7%)	3 (5%)	2 (3.2%)	6 (4%)	
Uveitis	No	24 (88.9%)	50 (83.3%)	58 (92.1%)	132 (88%)	0.32
	Yes	3 (11.1%)	10 (16.7%)	5 (7.9%)	18 (12%)	
Dactylitis	No	25 (92.6%)	49 (81.7%)	58 (92.1%)	132 (88%)	0.15
	Yes	2 (7.4%)	11 (18.3%)	5 (7.9%)	18 (12%)	
Psoriasis	No	27 (100%)	58 (96.7%)	62 (98.4%)	147 (98%)	0.44
	Yes	-	2 (3.3%)	1 (1.6%)	3 (2%)	
Colitis	No	26 (96.3%)	58 (96.7%)	60 (95.2%)	144 (96%)	0.91
	Yes	1 (3.7%)	2 (3.3%)	3 (4.8%)	6 (4%)	

DISCUSSION

In this Iraqi study, FokI - but not ApaI was clinically informative for axSpA. The absence of TT and predominance of CC/CT align with regional allele frequencies. Mechanistically, the CC isoform's higher transactivation capacity may augment anti-inflammatory signaling[11], consistent with its restriction to inactive ASDAS and strong remission odds. Conversely, CT tracked with higher axial activity yet coincided with reduced uveitis and dactylitis, suggesting tissue specific or pathway selective immunomodulation. ApaI neutrality mirrors reports with limited clinical translation for this intronic variant.[10,12]

Importantly, serum VDR activity and infliximab effect - length were genotype - independent, implying that while FokI shapes phenotype, pharmacodynamic readouts under TNF blockade remain largely driven by treatment and other non-genetic factors.[13-15] Smoking was concentrated in the high-activity ASDAS group, reinforcing its adverse role in the inflammatory tone.[2] Collectively, these results support using FokI as a prognostic phenotypic marker rather than a predictor of infliximab pharmacodynamics.

CONCLUSION

FokI (rs2228570) is a bidirectional modifier: CC associates with inactive ASDAS (remission odds ↑), while CT associates with high activity but fewer select EAMs. ApaI (rs7975232) shows no meaningful association with activity, EAMs, VDR activity, or INF effect-length. Pharmacodynamic measures under infliximab were genotype-independent.

Recommendations

- Validate findings in larger, longitudinal, multi-ethnic cohorts.
- Undertake functional studies to dissect genotype-specific immune pathways.
- Consider FokI in phenotype profiling and risk stratification; prioritize smoking-cessation strategies in axSpA care.

Author's Contribution:

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