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CONCISE COMMUNICATION

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Association of NFKB1 -94ATTG ins/del polymorphism (rs28362491) with pemphigus vulgaris

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Abstract

Pemphigus vulgaris is a rare chronic blistering skin disease resulting from IgG autoantibodies directed against transmembrane desmosomal glycoprotein desmoglein 3 and is the most common form of pemphigus. Since interleukin-1 receptor-associated kinase (IRAK-1)/nuclear factor-kappa B (NF-kappa B) pathway plays an essential role in the pathogenesis of autoimmune diseases, the aim of the present study was to explore the role of polymorphisms in three genes, named IRAK1 (rs3027898), NFKBIA (rs696) and NFKB1 (-94ATTG insertion/deletion variant, - rs28362491), in PV susceptibility. Forty-four unrelated patients with PV (23 males) were enrolled in the study. Additionally, 77 ethnic matching healthy volunteers (45 males) with no personal or family history of chronic autoimmune or infectious diseases were studied. Strong statistical significant difference was observed between PV patients and controls for polymorphism -94 insertion/deletion ATTG in the promoter region of NFKB1 gene (P = 0.00005). Additional dedicated studies in larger groups of patients of various ethnicities are needed to replicate and confirm the preliminary findings.

KEYWORDS

IRAK1, NFKB1, NFKBIA, pemphigus vulgaris, polymorphism

1 | INTRODUCTION

Pemphigus is a group of autoimmune diseases that affect the skin and the mucous membranes. Several subtypes of pemphigus have been identified, based on clinical and histologic features as well as on the specific antigens targeted by circulating autoantibodies. Pemphigus vulgaris (PV) is the most prevalent type of pemphigus, comprising up to 70% of all cases of pemphigus.[1] PV is characterized by acantholysis and intraepidermal blister formation, resulting from IgG autoantibodies directed against transmembrane desmosomal glycoprotein desmoglein 3 and in some cases desmoglein 1.[2,3] However, many immunological steps are required prior to autoantibody production that is the key to the development of blisters in pemphigus.

Interleukin-1 receptor-activated kinases (IRAKs) are key mediators in the IRAK1/NF-κB signalling pathway. IRAK1 plays a significant role in NF-κB activation, which subsequently increases the expression of many genes related to immunological reactions.[4,5] NF-kB is inactivated by cytoplasmic trapping through IkB proteins (eg NFKBIA). Phosphorylation of serine residues on the I kappa B proteins, by kinases, marks them for destruction via the ubiquitination pathway, thereby allowing activation of the NF-kB.

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Subsequently, NF-kB, as a key regulator of a variety of genes, is implicated in diverse biological events including cell survival, apoptosis, inflammation, differentiation and autophagy.[6,7] To date, polymorphisms in IRAK1 (interleukin-1 receptor-activated kinase 1), NFKBIA (NFKB inhibitor alpha) and NF-kB (nuclear factor-kappa B1) have been implicated in the pathogenesis of many immune diseases such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, systemic lupus erythematosus, ulcer colitis, atherosclerosis and Crohn's disease.[8–14]

2 | QUESTION ASKED

PV is an immune blistering disease, but little is known about the role of these genes and related polymorphism in PV susceptibility.[15] Up to date, only the attenuation of pro-inflammatory cytokines by blocking NF-κB–mediated pathways was documented, which inhibited the pathogenesis of PV by suppressing oxidative stress and apoptosis in human keratinocytes.[16,17] The aim of the present study was to explore the role of polymorphisms in IRAK1/NF-κB signalling pathway in PV predisposition. Specifically, the following three variants were genotyped in PV patients and control subjects: a) rs3027898 (in the 3' UTR of IRAK1 gene); b) rs28362491 (-94ATTG insertion/deletion in the promoter region of NFKB1); and c) rs696 (in the 3' UTR of NFKBIA gene).

3 | EXPERIMENTAL DESIGN

Forty-four unrelated patients with PV (23 males and 21 females; mean age 56 ± 16 years; range 31–86 years) were enrolled in the study. Patients were diagnosed as described previously.[18] Additionally, 77 ethnic matching healthy volunteers (45 males and 32 females; mean age 50 ± 19 years; range 17–85 years) with no personal or family history of chronic autoimmune or infectious diseases were studied. The study protocol was approved by the Papageorgiou University Hospital Ethics Committee. A written informed consent was obtained from each patient.

Genomic DNA was extracted from peripheral blood lymphocytes according to PureLink Genomic DNA Kit (Invitrogen). Polymorphisms rs3027898 (IRAK1 gene), rs28362491 (-94ATTG insertion/deletion, NFKB1 gene) and rs696 (NFKBIA gene) were studied with restriction fragment length polymorphism (RFLP) assay. The primer pair 5'-AAA/ACC/TGA/CAC/GGG/AAG/TG-3' and 5'-TTT/GTG/TTC/AGC/CGT/GAG/TC-3' was used for the genetic variant rs3027898, the 5'-TGG/GCA/CAA/GTC/GTT/TAT/GA-3' and 5'-CTG/GAG/CCG/GTA/GGG/AAG-3' for the rs28362491, and the 5'-GCC/TGA/AAG/AAC/ATG/GAC/TTG-3' and 5'-GTA/CAC/CAT/TTA/CAG/GAG/GG-3' for the rs696. The restriction assay was performed with the following enzymes NlaIII, PflMI and HaeIII, respectively. All samples were run in duplicate, and random samples were sequenced to assure quality control of the genotyping.

TABLE 1 Statistical analysis of the three studied polymorphisms between pemphigus vulgaris patients and controls (* reference allele; OR, odds ratio; 95% CI, 95% confidence interval)

	rs3027898				rs28362491 (-94ATTG ins/del variant)			rs696		
	Genotypes				Genotypes			Genotypes		
	CC*	AC	AA		Ins*	Ins/Del	Del	GG*	GA	AA
PV patients (n = 44)	6	11	27		23	17	4	12	21	11
Controls (n = 77)	19	15	43		11	53	13	20	46	11
***P*-value**	0.33353				**0.00005**			0.2844		
	Alleles				Alleles			Alleles		
	C		A		Ins		Del	G		A
Male PV patients (n = 23)	5		18	**PV patients (n = 88)**	63		25	45		43
Male Controls (n = 45)	17		28	**Controls (n = 154)**	75		79	86		68
***P*- value**	0.186			***P*-value**	**0.0006**			0.480		
OR, 95% CI	0.476 (0.144–1.459)			**OR, 95% CI**	**2.65 (1.515–4.651)**			0.828 (0.489–1.399)		
	Genotypes									
	CC	AC	AA							
Female PV patients (n = 21)	1	11	9							
Female Controls (n = 32)	2	15	15							
***P*-value (Yates' correction)**	0.932									

SPSS statistical package (SPSS Inc.) was used to test differences in polymorphism distribution between PV patients and controls (Pearson's chi-square, Yates' chi-square if any expected frequency was below 1 or if the expected frequency was <5 in more than 20% of cells). Furthermore, the odds ratio (OR) with a confidence interval (CI) of 95% was calculated (reference allele vs variant allele). A difference at $P \leq 0.05$ was considered as statistically significant.

4 | RESULTS

Polymorphism rs696 located in the 3' UTR of NFKBIA gene was in Hardy-Weinberg (HW) equilibrium in the control group ($P = 0.0637$). The variant rs28362491 (-94ATTG insertion/deletion) located in the promoter region of NFKB1 gene was not in HW equilibrium ($P = 0.0009$), but this is in accordance with this reported for other European control groups.[19] As far as concerned, the polymorphism rs3027898 located in the 3' UTR of IRAK1 gene, which is mapped in X chromosome, was found in HW equilibrium in female control group where the three genotypes exist ($P = 0.4872$). Specifically, 18 male patients carried the A allele and 5 the C allele, and 9 female patients carried the AA genotype, 11 the AC genotype, and 1 the CC genotype. In the control group, 28 males carried the A allele and 17 the C allele, and 15 females carried the AA genotype, 15 the AC genotype, and 2 the CC genotype.

Strong statistical significant difference was observed between PV patients and controls for polymorphism -94 insertion/deletion ATTG (rs28362491) in the promoter region of NFKB1 gene (Table 1). On the other hand, the studied polymorphism rs3027898 and rs696 in IRAK1 and NFKBIA, respectively, did not differ significantly between PV patients and controls (Table 1). Taking into account the hemizygous state of rs3027898 polymorphism (X-linked) in males and the X inactivation in females which leads females to have randomly one active X chromosome in their cells, we replicated the analysis of rs3027898 genotypes' distribution separately in male patients vs male controls and in female patients vs female controls. Concerning female groups (in males, the hemizygous state of X genotypes coincides with alleles), the analysis of rs3027898 was not expanded to alleles since non-random X inactivation patterns were also described for several X-linked genes,[20] which could lead to biased categorization of alleles in case of heterozygous females.

5 | CONCLUSIONS

The altered function of IRAK1/NF-kB pathway, which could be the result of gene polymorphisms, may predispose to the susceptibility of pemphigus if we take into account the reported increase of several inflammatory cytokines and chemokines in skin inflammation conditions.[21,22] On the other hand, the blocking of NF-κB–mediated pathways by the appropriate pharmacological treatment was reported as a way of inhibiting the PV pathogenesis.[16,17] The present study focused on the association of three genetic variants that concern the IRAK1/NF-kB pathway and specifically the genes IRAK1, NFKB1 and NFKBIA.

Among the studied polymorphism, positive association was observed between the -94 insertion/deletion ATTG in the promoter region of NFKB1 gene. This association between NFKB1 gene and PV, where the insertion variant is the risk allele, is described for the first time in the literature. Previously, this polymorphism has been mainly associated with several cancer forms and heart diseases.[19,23–25] In the present study, over-presentation of the NFKB1 insertion allele was observed in PV patients as it was observed in the majority of studies referred to cancer.[19]

It is known that the deletion allele reduces or prevents the binding to nuclear proteins and leads to lower transcript levels of the NFKB1 gene.[26,27] Alternatively, the insertion allele of NFKB1 leads to increased expression of p50, the active form of NFKB1. This seems to be in accordance with the increased expression of NFKB1 at sites of inflammation in diverse diseases, which subsequently can induce transcription of pro-inflammatory cytokines, chemokines, adhesion molecules, cell cycle regulators, anti-apoptotic factors, matrix metalloproteinases, Cox-2 and nitric oxide species.[28] Additionally, increased expression of various chemokines and cytokines was reported to be associated with immune profiles in the pathogenesis of pemphigus,[29–31] while the naringenin was reported to protect keratinocytes from oxidative stress injury via inhibition of the NOD2-mediated NF-κB pathway in PV patients.[16]

To conclude, this is the first study to report association of -94ATTG insertion/deletion polymorphism in the promoter region of NFKB1 gene with PV. Since this study is an exploratory one, we do emphasize the need for additional dedicated studies in larger groups of patients of various ethnicities to replicate and confirm the preliminary findings.[32] In addition, in the future it would be important to conduct functional analyses in order to evaluate NFKB1 mRNA/protein levels in PV patients compared to controls and to elucidate its potential use as a target in disease treatment.

CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTION

AC had the concept and designed the study, performed the research, the data analysis and the literature review, and wrote the manuscript. AK performed sample collection and reviewed the manuscript. PM performed sample collection and reviewed the manuscript. AL edited and reviewed the manuscript. AP performed sample collection and reviewed the manuscript.

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