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Genetic Signatures in Pemphigus Vulgaris: Decoding Immune Susceptibility through HLA Patterns and FCGR2B Variants

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Introduction & Objectives:

Pemphigus vulgaris (PV) is a rare autoimmune blistering disorder with a global annual incidence of 0.5–32 per million and 4.7 per million in Turkey. Though, being a relatively well understood disease, the initiation of the autoimmunity still warrants elucidation. It is generally accepted that polymorphic residues in the MHC class II β-chain play an important role in the selective binding of desmoglein epitopes, which are presented by antigen presenting cells to Dsg3-specific $CD4^+$ T cells, subsequently promoting class switching in Dsg3-specific B cells. Among Fcγ-receptors, the FcγRIIB was the only inhibitory one which is mainly expressed on B cells and primarily suppresses BCR signaling. In transgenic mice model, FcγRIIB was shown to inhibit pathogenic class switching of Dsg-3-specific B cells. Vice versa, attenuated FcγRIIB function may be a predisposing factor for PV, corresponding to the previous evidence for an association between *FCGRIIB*-I232T (rs1050501) polymorphism and systemic lupus erythematosus. Thus, this study aimed to investigate the effects of the rs1050501 variant in the *FCGR2B* gene and the *HLA-DRB1* gene allelic polymorphisms on the genetic etiology of PV.

Materials & Methods:

This single-center genetic epidemiology case-control study included adult PV patients diagnosed via clinical, histopathological, direct immunofluorescence microscopy and/or ELISA methods, and age, sex- and ethnicity-matched healthy controls without known autoimmunity, with power set at 80% (ES=1.5). The Hardy-Weinberg (H-W) equilibrium test was performed on controls using rs1050501 SNP alleles. Analysis of rs1050501 was performed through a two-step approach involving long-PCR followed by nested PCR, with the final PCR product analyzed via Sanger sequencing. *HLA-DRB1* genotyping was conducted using the PCR-SSOP-Luminex technique and analyzed by the LABScan3D platform. Demographic, clinical data and comorbidities were recorded. All statistical analysis performed by Chi-square and Fisher's exact test on open source RStudio (v.2024.04.2) platform using dplyr, tidyr, ggplot2, writexl and openxlsx packets. A Benjamini-Hochberg (FDR) corrected p-value ($p\ adj < 0.05$) was considered statistically significant.

Results:

A total of 91 PV and 200 healthy controls were analyzed. The H-W equilibrium test showed that rs1050501 variant alleles frequency was in equilibrium ($p > 0.05$). Thirty-three different HLA-DRB1 alleles were observed between groups. HLA-DRB1*04:02 and *14:01 alleles were significantly more frequently detected in PV patients compared

to the controls (**Table 1, Figure 1**). A significantly higher of the HLA-DRB1*16:01 allele was observed in controls as protective from PV. It was a very interesting observation for us that the HLA-DRB1*04:02/14:01 (compound heterozygous) genotype was seen only in PV patients (**Table 2**). Between the patients and controls, there was no statistically significant difference in terms of rs1050501 variants and genotypes (adj.p=0.12 and adj.p=0.35, respectively).

Conclusion:

HLA-DRB1*04:02 and 14:01 alleles and* HLA-DRB1*04:02/14:01* genotype were significantly more frequent in PV patients. To the best of our knowledge, this study is the first to demonstrate that the HLA-DRB1*04:02/14:01 genotype is significantly more frequent in PV patients compared to healthy individuals, based on a relatively large cohort. However, no association was found between rs1050501 and PV.

Table 1. HLA-DRB1 alleles and Chi-square test statistics

DRB1*	Controls (n)	PV (n)	p_value	p_adj	Odds_Ratio (OR)	95% CI
04:02	18	70	2.03×10^{-24}	**6.71×10^{-23}**	12.95	7.40-24.57
14:01	17	31	7.51×10^{-07}	**1.23×10^{-05}**	4.55	2.35-8.74
16:01	26	0	9.75×10^{-05}	**0.001**	0.03	0-0.31
11:01	39	6	0.00	**0.037**	0.33	0.10-0.75
14:04	0	4	0.00	0.06	20.19	1.46-Inf
15:01	33	6	0.02	0.10	0.40	0.12-0.91
13:03	12	0	0.02	0.10	0.08	0-0.77
07:01	37	7	0.03	0.13	0.41	0.17-0.97
09:01	8	0	0.06	0.22	0.12	0-1.27
04:01	16	1	0.07	0.23	0.19	0.029-1.14
11:04	39	9	0.07	0.23	0.50	0.22-1.09
08:01	0	1	0.09	0.25	6.61	0.40- Inf
11:11	0	1	0.09	0.25	6.61	0.40- Inf
03:01	26	5	0.12	0.28	0.43	0.16-1.23
04:05	6	0	0.18	0.37	0.16	0-1.86
04:08	5	0	0.18	0.37	0.19	0-1.86
15:02	12	1	0.24	0.44	0.25	0.03-1.62
10:01	11	2	0.24	0.44	0.46	0.03-1.64
04:04	4	4	0.26	0.46	2.22	0.40-12.06
04:03	9	7	0.30	0.49	1.76	0.59-5.10
04:07	3	0	0.31	0.49	0.31	0-3.34
13:02	12	7	0.46	0.69	1.3	0.51-3.98
13:01	19	6	0.51	0.74	0.72	0.21-1.72
01:01	23	7	0.55	0.76	0.68	0.27-1.67
08:04	2	1	0.59	0.78	1.31	0.15-30.33
01:02	3	2	1	1	1.57	0.1-7.80
04:06	1	0	1	1	0.72	0-11.78
13:05	1	0	1	1	0.72	0-11.78
16:02	1	0	1	1	0.72	0-11.78
08:02	2	0	1	1	0.43	0-11.70
08:03	7	3	1	1	1.02	0.24-4.15
11:03	6	1	1	1	0.50	0.07-4.01
12:01	2	0	1	1	0.43	0-11.71

Table 2. Six representative genotypes observed in study groups

DRB1* Genotype	Controls (n)	PV (n)	a*	b*	c*	d*	p	p_adj	OR	95% CI
04:02 / 14:01	0	20	20.5	71.5	0.5	200.5	1.2×10^{-11}	**2.62×10^{-9}**	115.0	13.1-inf*
01:01 / 04:02	0	5	5.5	86.5	0.5	200.5	0.0	0.003	25.5	2.7-inf
04:02 / 04:03	0	6	6.5	85.5	0.5	200.5	0.0	0.003	30.5	2.7-inf
04:02 / 13:02	0	6	6.5	85.5	0.5	200.5	0.0	0.003	30.5	2.7-inf
04:02 / 04:04	0	3	3.5	88.5	0.5	200.5	0.0	0.200	15.9	1.5-inf
04:02 / 13:01	0	4	4.5	87.5	0.5	200.5	0.0	0.200	20.6	1.5-inf

* Correction was made by adding 0.5 to the cell frequencies (Haldane-Anscombe correction). Therefore, the upper limit of CI is infinite.

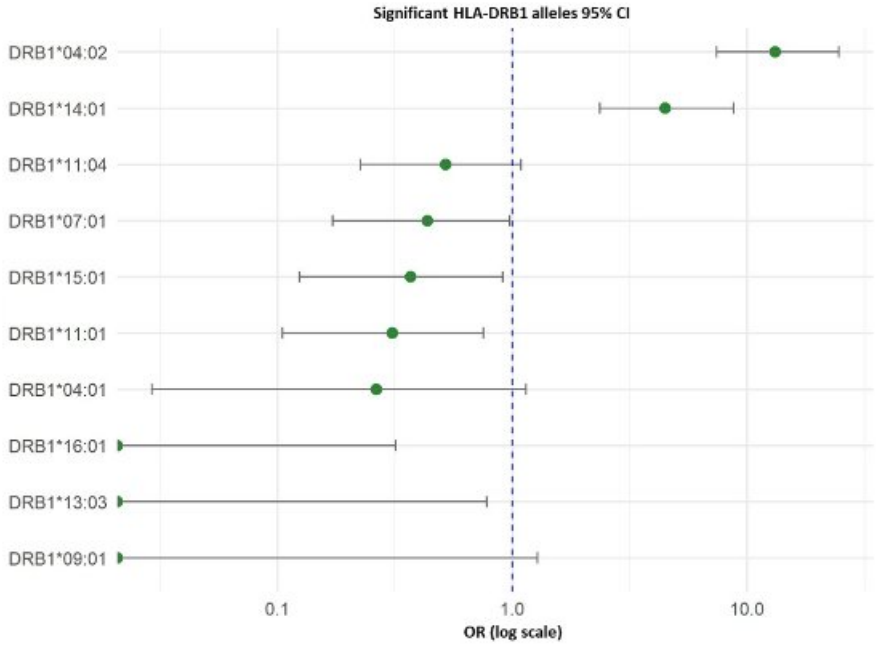


Figure 1. Ten most significant HLA-DRB1 alleles between groups