Novel HLA alleles associated with pemphigus vulgaris in Indian population detected by DNA microarray analysis

Sir,

Numerous studies in different populations have confirmed the role of genetic factors in the pathogenesis of pemphigus vulgaris. The association of human leukocyte antigen (HLA) class II genes with susceptibility to pemphigus has been studied most extensively.[1] Identifying other contributing genes is also paramount for elucidation of the mechanisms that lead to pemphigus vulgaris which may help in the development of novel targeted therapeutic modalities. There has been a dearth of studies in Indian population to find the association of HLA and non-HLA genes, with susceptibility and severity, of pemphigus vulgaris. Genomic microarray analysis has emerged as a systematic, convenient and innovative technique to explore the genome and has contributed vastly in the understanding of various genetic diseases. In the present case–control study, we carried out genomic microarray analysis in a small number of pemphigus vulgaris patients to evaluate their genetic basis using global screening array.

The study was conducted at the immunobullous clinic of Postgraduate Institute of Medical Education and Research, Chandigarh, India, from April 2018 through September 2018. After obtaining written informed consent, eight patients with established diagnosis of pemphigus vulgaris (based on clinical, histopathological and direct immunofluorescence findings) were recruited and their clinicodemographic details were noted. Eight healthy volunteers without any history and examination findings suggestive of pemphigus vulgaris were recruited as controls. Venesection was performed and five milliliters of peripheral blood were withdrawn from both cases and controls for carrying out genomic microarray analysis using the Infinium Global Screening Array-24 v1.0.

The mean age of the patients was 42.6 ± 9.3 years compared to 33.4 ± 9.4 years among controls. There were two males and six females in the patient cohort, and four males and four females in the control group. The mean disease duration was 12.0 ± 15.1 months. Out of eight patients, two had severe disease and six had mild-to-moderate pemphigus. The severity was calculated based on the Pemphigus Disease Area Index score.[2]

Table 1 illustrates the single-nucleotide polymorphisms with significant association ($P < 0.05$). However, those polymorphisms with 0% frequency in the control population were excluded for further analysis. HLA-DQA1 (OR = 9), HLA-DPB2 (OR = 9), HLA-DMB (OR = 7) and HLA-DQB1 (OR = 5.571) genes were found to be statistically significantly associated with pemphigus (with $P < 0.05$ and odds ratio of >1.5). In addition to this, we tried to find the single nucleotide polymorphisms associated with severe pemphigus [Table 2]. HLA-DOB was found to be significantly associated with severe pemphigus (OR =15).

Among HLA genes, HLA DRB1*0401, DRB1*1402, DQB1*0503 and DQB1*0302 were found to be most commonly associated with pemphigus vulgaris in the previous studies.[1] In a meta-analysis including 18 studies, it was concluded that DRB1*04, DRB1*08 and DRB1*14 genes are significantly associated with susceptibility to pemphigus vulgaris.[3] Conversely, DRB1*03, DRB1*07 and DRB1*15 may be negatively associated with pemphigus vulgaris. Thus, the findings of meta-analysis suggested that specific HLA-DRB1 types may influence the susceptibility or resistance to pemphigus vulgaris.[3] However, studies which have conducted genomic microarray analysis in pemphigus vulgaris patients are lacking till date.

We found HLA-DQA1, HLA-DPB2, HLA-DMB and HLA-DQB1 to be significantly associated with pemphigus. Among these genes, association of both HLA-DQA1 and HLA-DQB1 has been previously reported in literature, especially among patients of Indian origin in the study by Delgado *et al*.[4] Although observations in the present study reaffirm the significance of these alleles in disease pathogenesis, interestingly, the single nuceleotide polymorphisms found herein – rs12722039 and rs12722042 in HLA-DQA1 and rs6689 in HLA-DQB1 – have not been previously reported to be associated with pemphigus. Two missense polymorphisms, rs12722042 and rs12722039, have previously been implicated

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Table 1: Single-nucleotide polymorphisms with significant association (*P* < 0.05 and OR >1.5) with pemphigus patients in comparison to healthy controls

Single-nucleotide polymorphisms	Gene	Frequency in cases (eight cases – 16 alleles) (%)	Frequency in controls (eight controls – 16 alleles) (%)	*P*-value
rs9277626	HLA-DPB2	8/16 (50)	0	0.001
rs733208	HLA-DPB2	5/16 (31.3)	0	0.015
rs733210	HLA-DPB2	5/16 (31.3)	0	0.015
rs9277628	HLA-DPB2	5/16 (31.3)	0	0.015
rs9277636	HLA-DPB2	5/16 (31.3)	0	0.015
rs9277761	HLA-DPB2	5/16 (31.3)	0	0.015
rs9277765	HLA-DPB2	5/16 (31.3)	0	0.015
rs9277766	HLA-DPB2	5/16 (31.3)	0	0.015
GSA-rs3129832	HCG17,HLA-L	4/16 (25)	0	0.033
GSA-rs3819285	HLA-B	4/16 (25)	0	0.033
rs3094070	HCG17,HLA-L	4/16 (25)	0	0.033
rs3094071	HCG17,HLA-L	4/16 (25)	0	0.033
rs3094072	HCG17,HLA-L	4/16 (25)	0	0.033
rs3094074	HCG17,HLA-L	4/16 (25)	0	0.033
rs3094630	HCG17,HLA-L	4/16 (25)	0	0.033
rs3129701	HCG17,HLA-L	4/16 (25)	0	0.033
rs3129703	HCG17,HLA-L	4/16 (25)	0	0.033
rs3129705	HCG17,HLA-L	4/16 (25)	0	0.033
rs3129831	HCG17,HLA-L	4/16 (25)	0	0.033
rs3129831	HCG17,HLA-L	4/16 (25)	0	0.033
rs3130398	HCG17,HLA-L	4/16 (25)	0	0.033
rs3130401	HCG17,HLA-L	4/16 (25)	0	0.033
rs3130402	HCG17,HLA-L	4/16 (25)	0	0.033
rs3130403	HCG17,HLA-L	4/16 (25)	0	0.033
rs3130405	HCG17,HLA-L	4/16 (25)	0	0.033
rs3132658	HCG17,HLA-L	4/16 (25)	0	0.033
rs3132659	HCG17,HLA-L	4/16 (25)	0	0.033
GSA-rs12722042	HLA-DQA1	8/16 (50)	1/16 (6.3)	0.006
rs12722039	HLA-DQA1	8/16 (50)	1/16 (6.3)	0.006
rs2395349	HLA-DPB2	6/16 (37.5)	1/16 (6.3)	0.032
rs9277642	HLA-DPB2	6/16 (37.5)	1/16 (6.3)	0.032
rs9277643	HLA-DPB2	6/16 (37.5)	1/16 (6.3)	0.032
rs9277657	HLA-DPB2	6/16 (37.5)	1/16 (6.3)	0.032
rs194675	HLA-DMB	8/16 (50)	2/16 (12.5)	0.022
GSA-rs6689	HLA-DQB1	9/16 (56.3)	3/16 (18.8)	0.028

Table 2: Single-nucleotide polymorphisms with significant association (*P*<0.05 and odds ratio >1.5) with severe pemphigus

Single-nucleotide polymorphisms	Gene	Frequency in severe pemphigus (cases – two, alleles – four) (%)	Frequency in mild-to-moderate pemphigus (cases – six, alleles – 12) (%)	*P*-value
rs2114226	ST18	3/4 (75)	0	0.0008
rs62500975	ST18	3/4 (75)	0	0.0008
GSA-rs7010548	ST18	2/4 (50)	0	0.008
rs17875379	HLA-F	2/4 (50)	0	0.008
GSA-rs11244	HLA-DOB	3/4 (75)	2/12 (16.67)	0.029

in childhood acute lymphoblastic leukemia through genome-wide association studies.[5]

Another novel observation in our study was the association of pseudogenes HLA-DPB2 – rs2395349, rs9277642, rs9277643 and rs9277657 and HLA-DMB – rs194675 with pemphigus. Pseudogenes are segments of DNA that have lost at least some functionality, relative to the complete gene, in cellular gene expression or protein-coding ability. The role of pseudogenes – the non-coding part of the human genome has

long been debated in the pathogenesis of autoimmune disease and carcinogenesis.

The major limitation of our study was the small sample size. Though there are few studies carried out in pemphigus patients in the Indian population, ours was the first pilot study in which whole-genome single nucleotide polymorphisms profiling was done. Many of the HLA loci associations found in our study were novel and have not been described in the previous studies which evaluated the genetic association of pemphigus. The results of this study need to be evaluated in a much larger population along with the functional expression of these alleles.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

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Impact on quality of life of family members of vitiligo patients in North India: A cross-sectional study using family dermatology life quality index

Sir,

Vitiligo is a common acquired depigmenting disorder caused due to progressive loss of melanocytes. The disease affects approximately one–two percentage of the global population, and the highest incidence has been recorded in the Indian subcontinent followed by Mexico and Japan.[1,2] Visible disfigurement, especially in pigmented races, can lead to a detrimental impact on patient's psychosocial status. Such patients are in a constant need of emotional support and motivation from family members/partners to address these issues. But dermatologic disorders like vitiligo have been shown to adversely affect the quality of life of a patient's family members as well.[3]

We conducted a cross-sectional study on 158 vitiligo patients and their family members attending dermatology outpatients' department of Era's Lucknow Medical College and Hospital in Lucknow from February 2019 to February 2020 with an aim to explore the level and specific domains in which quality of life of of family members of vitiligo patients are affected and any correlation of its impairment with the disease parameters. Our inclusion criteria were as follows: (i) all patients with clinically diagnosed vitiligo attending dermatology OPD without any other significant illness or disability, who give a written informed consent for the study and (ii) family members of age greater than 18 years, having a close relationship with the patient (parents/children/spouse/siblings) and who are living in same household. Exclusion criteria were as follows: (i) an individual with dermatological disorders other than vitiligo and (ii) partners and relatives who report having any skin diseases or other significant illness (including psychiatric illness) that may impair their

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