Association of HLA Class I and II Antigen with Vitilig in Egyptian Population

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Abstract

The association of HLA antigens with vitiligo remains controversial issue. In order to determine which, if any, HLA markers were associated with an increased risk of vitiligo, we investigated HLA antigens in 50 patients with vitiligo and in 50 controls. Vitiligo patients had significant increase in HLA-A1, A19, B16, CW4, CW 6, DR 4, DR7 and DQ3 compared to controls. Familial vitiligo is marked by increased frequency of HLA-A28, B16, B27, B48, CW2 and DQ3, while non-familial vitiligo is marked by increased frequency of HLA-A1, CW6 and DR13. These findings suggest that immunogenetic mechanism may be responsible for vitiligo. Also there may be heterogeneity in the pathogenic process between familial and non-familial vitiligo.

Keywords HLA Class I and II antigens; Vitiligo; Egypt

Introduction

Vitiligo is a pigmentary disorder that affects 0.38-1.13% of the population in different regions of the world. The pathogenesis of vitiligo is still unknown, but genetic and immunological factors have an important role in the origin of vitiligo [1]. Human leukocyte antigen (HLA) molecules perform a crucial function in the regulation of the immune response [2].

The human leucocyte antigen (HLA) is now recognized as a major contributing factor for susceptibility to a variety of autoimmune diseases, and numerous associations with vitiligo have focused on the HLA system. Most of them reported multiple HLA class II alleles to be associated with vitiligo in different populations [3,4]; however, a few studies had consistent association evidence, which might be explained by weak genetic effects or complex gene-gene or gene-environmental interactions, population stratification or genetic differences between populations. Interestingly, abnormal expression of HLA-DR in perilesional epidermis has also been reported and was suggested to contribute to HLA class II restricted melanocyte killing [5]. Recently, several studies have further suggested that HLA class II alleles/haplotypes have been inclined towards patients who have earlier-onset age, with autoimmune diseases or with family history [6-8].

This study was conducted to determine which, if any, HLA markers were associated with Vitiligo in Egyptian Population. Also, in this study we tried to compare HLA phenotypes in patients with familial and non-familial vitiligo.

Patient and Methods

One hundred subjects were included in this study, 50 patients with vitiligo and 50 normal subjects as a control group. Patients and controls were selected from the attendants of the dermatology and venereology clinic, Benha University Hospital.

I-Patient

Fifty patients of vitiligo were included in our study. There were 27 males and 23 females, with age ranging from 11 to 69 years (mean=39.2), with a duration from 1 to 3 years (mean=1.5). In order to analyse the association of HLA phenotypes based on family history we grouped the patients into familial cases (had at least one first-degree relative affected with vitiligo), (n=13) and non-familial cases (n=37). Physical examination was performed on each patient to determine the clinical type and extent of vitiligo. Our patients were free from any other autoimmune disorder e.g. diabetes mellitus, thyroid disorder or alopecia areata.

II-Control group

The control group was composed of 50 healthy blood donors coming to Benha University Hospital. There were 32 males and 18 females with age ranging from 10 to 50 years (mean=31.3).

HLA Class I and II polymorphism

HLA-A, B, C, DR and DQ typing was carried out in all patients and controls for class I antigens and on B-enriched suspensions, obtained by resetting with sheep's red blood cells for class II antigens.

Statistic Analysis

The antigenic frequencies of class I and class II from patients and controls were compared. Z-test was used to calculate the P. values. The strength of the association was estimated by relative risk according to Woolf's formula.

Methods

HLA-DNA Typing

Briefly, DNA extraction was carried out by classical phenol-chloroform method from fresh whole blood samples which anticoagulated by EDTA. Polymerase chain reaction/sequence specific primer (PCR/SSP) method was used to determine HLADNA typing. A dried primer stock solution consisting of an HLA specific primer mix, i.e., allele and group specific primers and internal positive control primer pairs, was aliquoted in 0.2 ml PCR tubes. To check PCR conditions, the internal positive control primer pairs that amplify the human growth hormone gene segments were used in each specimen. The PCR master mix contains: 0.4 U/ll Taq Polymerase, 200 lM dNTPs, 50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl, pH 8.3, 0.001% w/v gelatin, 5% glycerol, 100 lg/ml cresol red at final concentration. Amplification reaction contains: 2 ll template DNA (20-100 ng/ml), 3 ll PCR master mix, 5 ll nuclease freed H_2O for one SSP reaction. PCR cycling parameters: denaturation at 94°C for 2 min; one cycle, denaturation at 94°C for 10 s, annealing and extension at 65°C for 60 s; 10 cycles, denaturation at 94°C for 10 s, annealing at 61°C for 50 s, extension at 72°Cfor 30 s; 20 cycles, as the manufacturer described (HLA DR&DQ Combi SSP, Olerup SSP AB, Sweden). PCR products were electrophoresed on 2% agarose gel. Presence and relative lengths of the specific PCR products were interpreted with the Helmberg-Score interpretation Software.

Results

When all the vitiligo patients are compared to controls a significantly increased frequency of HLA-A1(50% versus 20% RR=4), A19 (34% versus 8%, RR=5.926), B16 (16% versus 4%, RR=4.571), CW4 (34% versus 6%, RR=3.5),CW6 (50% versus 30%, RR=2.041), DR4 (42% versus16%, RR=3.812), DR7 (50% versus 10%, RR=8.2) DQ3 (50% versus 30%, RR=2.333) and a significantly decreased frequency of CW3 (26% versus 46%, RR=0.412) and DQ1 (34% versus 56%, RR=0.400) are found Frequencies of CW3 (RR=0.412) and DQ1 (RR=0.400) were significantly decreased (Table 1).

Table 1: Frequency of HLA antigens in Vitiligo patients and controls.

HLA antigens	Patient (N=50)		Controls (N=50)		Relative risk (PR)	Z	P
	No.	%	No.	%			
A1	25	50	10	20	4	3.145	<0.005
A2	17	34	15	30	1.202	0.429	>0.05
A19	17	34	4	8	5.926	3.192	<0.05
A28	4	8	4	8	1	-	-
B5	17	34	19	38	0.841	0.417	>0.05
B8	4	8	3	6	1.362	0.392	>0.05
B12	17	34	19	38	0.841	0.417	>0.05
B14	4	8	4	8	1	-	-
B16	8	16	2	4	4.571	2	<0.05
B17	5	10	2	4	2.089	0.848	>0.05
B18	5	10	3	6	1.366	0.397	>0.05
B21	4	8	3	6	1.362	0.392	>0.05
B27	4	8	2	4	2.087	0.842	>0.05
B35	4	8	3	6	1.362	0.392	>0.05
B48	4	8	2	4	2.087	0.842	>0.05
CW2	4	8	4	8	1	-	-
CW3	13	26	23	46	0.412	2.003	<0.05
CW4	17	34	3	6	8.07	3.5	<0.05
CW6	25	50	15	30	2.333	2.041	>0.05
CW7	8	16	10	20	0.672	0.521	>0.05
DR3	8	16	7	14	1.17	0.28	>0.05

DR4	21	42	8	16	3.812	2.865	<0.05
DR7	25	50	5	10	9	4.364	<0.05
DR8	4	8	2	4	2.087	0.842	>0.05
DR11	4	8	2	4	2.087	0.842	>0.05
DR13	13	26	6	12	2.577	1.784	>0.05
DR14	3	6	5	10	0.782	0.347	>0.05
DQ1	17	34	28	56	0.4	2.211	<0.05
DQ2	21	42	21	42	1	-	-
DQ3	25	50	15	30	2.333	2.041	<0.05

The familial form of vitiligo was associated with a significant increase in HLA-A19 (RR=18.39), A28 (RR=5.1), B16 (RR=10.66), B27 (RR=10.66), B48 (RR=10.66), CW2 (RR=5.1) and DQ3 (RR=5.24) (Table 2).

Table 2: Frequency of HLA antigens in familial Vitiligo compared to controls.

HLA antigens	Patient (N=13)		Controls (N=50)		Relative risk (PR)	Z	P
	No.	%	No.	%			
A1	4	30.76	10	20	1.77	0.832	>0.05
A2	4	30.76	15	30	1.03	0.854	>0.05
A19	8	61.53	4	8	18.39	4.379	<0.05
A28	4	30.76	4	8	5.1	2.197	<0.05
B5	8	61.53	19	38	2.6	1.528	>0.05
B12	8	61.53	19	38	2.6	1.528	>0.05
B16	4	30.76	2	4	10.66	2.929	<0.05
B27	4	30.76	2	4	10.66	2.929	<0.05
B48	4	30.76	2	4	10.66	2.929	<0.05
CW2	4	30.76	4	8	5.1	2.197	<0.05

CW4	4	30.76	3	6	6.95	2.532	<0.05
CW7	5	38.46	10	20	2.49	1.392	>0.05
DR4	8	61.53	8	16	8.39	3.36	<0.05
DR7	5	38.46	5	10	5.62	2.502	<0.05
DQ2	4	30.76	21	42	0.5	0.737	>0.05
DQ3	9	69.23	12	30	5.24	2.595	<0.05

The non-familial form of vitiligo was associated with significant increase in HLA-A1 (RR=5.22) and CW6 (RR=5.24), DR13 (RR=3.97) compared to controls (Table 3). Both familial and non-familial forms of vitiligo was associated with significant increase in HLA-A19, HLA-CW4, DR4 and DR7 compared to controls (Tables 2 and 3).

Table 3: Frequency of HLA antigens in non-familial Vitiligo compared to controls.

Antigens	Patients (N=37)		Controls (N=50)		Relative risk (PR)	Z	P
	No.	%	No.	%			
A1	21	56.75	10	20	5.24	3.539	<0.05
A2	13	35.13	15	30	1.26	0.507	>0.05
A19	9	24.32	4	8	3.69	2.112	<0.05
B5	9	24.32	19	38	0.52	1.35	>0.05
B8	4	10.81	3	6	1.89	0.816	>0.05
B12	9	24.32	19	38	0.52	1.35	>0.05
B14	4	10.81	4	8	1.39	0.449	>0.05
B16	4	10.81	2	4	2.9	1.239	>0.05
B17	5	13.51	2	4	2.95	1.23	>0.05
B18	5	13.51	3	6	1.92	0.44	>0.05
B21	4	10.81	3	6	1.89	0.449	>0.05
B35	4	10.81	3	6	1.89	0.449	>0.05
CW3	13	35.13	23	46	0.63	1.017	>0.05
CW4	13	35.13	3	6	8.48	3.468	<0.05
CW6	9	69.23	12	30	5.24	2.595	<0.05
CW7	3	8.1	10	20	0.35	1.538	>0.05

Discussion

The association of multiple HLA class I antigens and II alleles have been suggested for vitiligo. It is thought that individuals with certain HLA subtypes may be more susceptible to vitiligo than others. In distinct ethnic/racial population, disease and

associated HLA antigens distribution is variable. For example, HLA associations with vitiligo include HLA-B21, Cw6 and DR53 in the Kuwaiti population [9], A30, B27 Cw6, DR07 and DQw3 in Italians [10], DR4 and DQw3 in African-Americans [11], B13 in Jewish Moroccans and Bw35 in Jewish Yemenites [12], A2, Bw60 and DRw12 in Northern Germans [13], Cw7 DQB1 DR06 and DRB4*010 in the Dutch population [14,15], and Bw6 and DR07 in native Omanis [16]. Negative associations of A19, DR52 and DR01, DR03 Antigens were observed in the Kuwaiti population [9] and Italians [10], respectively. Genotyping of HLA complex showed a positive association of DRB1*0701, DQB1*0201, HLA-A2 and HLA-Dw7 DPB1*1601 in the Slovak population [17,18].

Our results show significantly increased frequency of HLA-DR4. These results thus confirm reports of vitiligo association with DR4 in black American [11], Dutch [15] and Turkish patients [19]. Dunston et al. identified DR4 to be associated with positive family history of autoimmune disease and early onset of disease (younger than 20 years) [11]. Also, both Fain et al. and Hu, et al. reported that HLA class II haplotype DRB1*04-DQB1*0301 and DRB1*07, respectively, contributes to risk of familial generalized vitiligo [7,20]. Orozco-Topete et al. reported that DRB1*04 is associated with the genetic susceptibility to develop vitiligo with autoimmune thyroid disorders [6].

Our study demonstrated positive association of vitiligo with DQw3 and DR7 which confirmed results of Finco et al. [10] Patients with adult onset of vitiligo are marked by increased frequency of HLA-DR7 antigen, which could represent a possible marker for this form [10].

Several small scale studies suggested that HLA class II alleles/haplotypes may determine type, stability, and severity of vitiligo [6,7,20].

HLA-DR is a MHC class II, cell surface receptor encoded by the HLA complex on chromosome 6 region 6p21.31. HLA-DR molecules regulate immune response and are one of the chief markers for dendritic cells. It has been implicated in susceptibility to a variety of autoimmune diseases as HLA-D region plays an important role in the presentation of auto antigens to T cells and the induction of subsequent immune response that results in the production of autoantibodies and autoreactive T cells responsible for the pathogenesis of disease [21]. A contribution of immune activation to progressive depigmentation is also supported by abnormal expression of HLA-DR in perilesional epidermis, which is likely relevant to explain melanocytes destruction, as melanocytes can present antigens in the context of MHC class II [22,23].

Our study shows a significant increase in HLA-A1, A19, and B16. Our findings are in agreement with Abanmi et al. [24] who demonstrated a significant association of HLA-19 in Saudi patients.

Other studies demonstrated the positive associations of vitiligo with A31, B46, and B13 in Jewish patients [12], A2 in Slovak patients [18] and A30 in Italian patients [25].

However, the frequencies of HLA-A*02, A30,A*31,B*46, were not significantly increased in patients in our study, which suggested that HLA genetic markers associated with vitiligo made some differences in the race and ethnic distribution.

Our study showed a significant increase in HLA-CW4 which confirmed results of Ando et al. [26] in Japanese patients. Previous studies demonstrated positive association of HLA-CW6 in Italian patients [25], Kuwaiti patients [9], Saudi patients [23] and Chinese patients [4].

Also, Egyptian patients included in our study showed significant increase in HLA-CW6 which confirmed previous studies. Orecchia et al. described that HLA-A30 and -Cw6 antigens increased in generalized vitiligo in Italian patients [25]. Also, Zhang et al. demonstrated that the allelic frequencies of HLA-A*2501, -A*30, -B*13, -B*27 and -Cw*0602 were significantly increased in generalized vitiligo compared with the controls [4].

In a genome-wide association study (GWAS) of generalized vitiligo, carried out in European-derived whites (EUR), Jin et al. identified 16 loci that contribute to risk of generalized vitiligo [27]. Within the major histocompatibility complex (MHC), a major generalized vitiligo association signal localized to HLA-A, in the class I gene region. At HLA-A, the most highly associated single-nucleotide polymorphism (SNP)

was rs12206499, which tags HLA-A*02 in the EUR and Japanese patients [27,28]. HLA-A*02:01 presents peptide antigens on the surfaces of all cells. In melanocytes, HLA-A*02:01 presents the major vitiligo autoimmune antigen, tyrosinase (TYR). TYR in turn activates and recruits antimelanocyte autoreactive cytotoxic T lymphocytes to the skin, which then target and destroy melanocytes [29]. These studies suggested that these alleles might be positively associated with generalized vitiligo patients. In our study we did not compare the distribution of HLA allelic frequencies between the cases and the controls according to the clinical type of vitiligo.

Our study showed that frequencies of CW3 and DQ1 were significantly decreased indicating that this allele might be negatively associated with the development of vitiligo in Egyptians.

Class I major histocompatibility complex (MHC) which involves HLA-A, HLA-B and HLA-C plays an important role in the presentation of intracellular antigens e.g. Virus to cytotoxic T cell (T8) that destroys viral-infected cell [30]. So, vitiligo may be due to external agents such as viruses, selectively interacting with class I MHC with subsequent activation of cytotoxic T cells that destroy viral-infected cells which may be melanocytes. This theory was supported by the findings of Grimes et al. who demonstrated cytomegalovirus (CMV) DNA in the depigmented and uninvolved skin of some patients with vitiligo and its absence in control subjects suggesting that vitiligo may indeed be triggered by a viral infection [31]. In addition, Fujinami et al. have demonstrated homology between a viral intermediate early protein and the HLA-DR antigen [32]. The expression of these autoreactive antigens in the plasma membrane of cells suggests that they could serve

as binding sites for cytolytic IgM antibodies identified on CMV-Infected cells, thereby mediating cell destruction.

CMV-infected cells also produce a glycoprotein similar to class I major histocompatibility complex (MHC) antigens [33]. Hence immune-mediated injury by CMV could be directed at cells that express the appropriate HLA-DR or class I MHC antigen.

Relationship between the regressing melanoma and HLA-A2 has been detected, So, both psoriasis and vitiligo show melanocyte specific cytotoxic T lymphocytes seem to be effective in the destruction of pigment cells [34].

When vitiligo patients are divided based on the family history of vitiligo, our study showed that HLA-A19, HLA-A28, B16, B27, B48, CW2 and DQ3 are significantly increased in the familial vitiligo patients compared to controls, HLA-A1,CW6 and DR13 are significantly increased in the non-familial vitiligo patients, while HLA-A19,HLA-CW4,DR4 and DR7 were associated with either familial or non-familial vitiligo.

Also, previous studies detected differences in HLA frequencies between familial and non-familial vitiligo patients [8,25,26]. Ando et al. reported an association of HLA-B46 with familial vitiligo and an association of HLA-A31 and HLA-Cw4 with non-familial vitiligo in the Asian Japanese population [26], while Misri et al. found significant increase in HLA A2, A28, A31, and B44 allele in Indian familial vitiligo [8]. Orecchia et al. demonstrated that non-familial vitiligo is marked by increases in HLA-A30 and DQw3 [25].

Fain et al. stated that variation within the MHC contributes to the unique characteristics of familial vitiligo, including earlier onset of the vitiligo phenotype and a greater risk and broader spectrum of other autoimmune/autoinflammatory phenotypes compared with non-familial vitiligo.The differences in the frequencies between familial and non-familial vitiligo from the same ethnic population suggest heterogeneity in the pathogenic process between familial and non-familial vitiligo patients [7].

Association has been reported between generalized vitiligo and HLA-DRB4*0101 and HLA-DQB1*0303 in Dutch patients [14], with HLA-DRB1*03, DRB1*04 and HLA-DRB1*07 alleles in Turkish patients [19], and with alleles of microsatellites located in the MHC in Columbian patients [35].

It have been shown that, in multiplex generalized vitiligo families, the MHC class II haplotype HLA DRB1A*04-(DQA1*0302)-DQB1*0301 is associated with both increased risk of vitiligo and with relatively early disease onset [7]. The association between MHC and the type of vitiligo has not been detected in our patients.

Inconsistencies between populations and even between studies of the same population are common in case-control disease association studies, and may be explained by weak genetic effects or complex gene-gene or gene-environmental interactions, population stratification, or genetic differences between populations [36].

Conclusion

The significance of HLA phenotype in the pathogenesis of vitiligo remains to be elucidated. Overall, our results indicate that specific MHC-linked genetic variation contributes to risk of vitiligo. HLA associations with vitiligo contribute evidence that the disease has an autoimmune component to its pathogenesis. The apparent difference in HLA phenotype suggests a different genetic background of familial and non-familial vitiligo. Further investigations for other vitiligo-risk factors are necessary.It is known that genetic variation may affect response of patients to medications (pharmacogenetics). So, more studies are needed to assess the effects of HLA phenotypes on response of patients to the different treatment modalities of vitiligo.

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