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HLA-DM Polymorphism and Risk of Trichloroethylene Induced Medicamentosa-like Dermatitis[1]

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Objective To establish the association between genetic polymorphisms of HLA-DMA and HLA-DMB and risk of developing trichloroethylene-induced medicamentosa-like dermatitis (TIMLD). **Methods** Sixty-one cases were medically confirmed to have been affected with TIMLD and 60 controls were selected from exposed workers who were free from TIMLD. The TIMLD cases and controls were similar in terms of age, sex, and duration of exposure. DNA was extracted both from the TIMLD cases and controls, HLA-DMA and HLA-DMB loci were amplified by using Touchdown PCR, and the alleles and genotypes were confirmed by restriction fragment length polymorphism (RFLP) and direct sequencing. Finally, the frequencies of HLA-DMA and HLA-DMB variants were compared between the two groups. **Results** The results showed that the frequency of HLA-DMA*0101 and HLA-DMB*0103 alleles was significantly increased in TIMLD patients than in controls (71.3% *vs*. 55.0% for HLA-DMA*0101; $P<0.05$) (11.5% *vs*. 3.3% for HLA-DMB*0103; $P<0.05$). In addition, the frequency of HLA-DMA*0102-*0102 homozygous genotype was also significantly higher in the controls than in the patients (25.0% *vs*. 8.2%, $P<0.05$), whereas the frequency of heterozygous HLA-DMB *0101-*0102 genotype was lower in the patients in comparison with the controls. **Conclusion** The polymorphisms of HLA-DM may be associated with the susceptibility to TIMLD.

Key words: Trichloroethylene, Dermatitis, Genetic polymorphism

INTRODUCTION

Trichloroethylene (TCE) can induce erythema and dermatitis bullosa in some occupationally exposed workers, and such an induced syndrome has been called trichloroethylene-induced medicamentosa-like dermatitis (TIMLD) (GBZ18-2002)[1]. TIMLD has occurred frequently in China in recent years, which leads to death in some cases. Symptoms develop within 3 months of exposure and are considered as the outcomes of delayed hypersensitivity. TIMLD is characterized by a latent period after the first contact, no obvious dose dependence (symptoms occur in some cases after exposure to a very low dose), low incidence rate among exposed workers (<5%), and lesions on both contacted and un-contacted skin. Therapeutically, glucocorticoids, such as methylprednisolone, display a good curative effect on these cases[2]. That is why the condition is considered as the outcome of hypersensitivity.

In 1947 Schwartz first reported that TCE could induce TIMLD, and to date only 18 cases have been reported from other countries excluding China. However, since 1988, more than 200 cases have been identified with 20 deaths among occupationally exposed workers in Guangdong Province of South China[2-4]. Since several thousands of TCE-exposed workers did not develop TIMLD, it may be hypothesized that hereditary predisposition in immune response to TCE exposure may play a role in

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Abbreviation: Trichloroethylene, TCE; Trichloroethylene Induced Medicamentosa-like Dermatitis, TIMLD; polymerase chain reaction, PCR; human leukocyte antigen DM, HLA-DM; restriction fragment length polymorphism, RFLP.

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the development of TIMLD.

Human leukocyte antigen DM (HLA-DM) plays an important role in immunological responses to xenobiotics[5], and it has two regions of HLA-DMA and HLA-DMB. Their respective gene products, α chain and β chain, form a HLA-DM molecule. HLA-DM molecule is a HLA class II-like glycoprotein that is required for class II restriction processing and presentation of protein antigens[6]. HLA-DM catalyzes dissociation of class II-associated invariant chain peptide (CLIP), a fragment of the invariant chain, from the peptide groove of the class II molecules and stabilizes empty HLA-DR molecules[7]. Our previous study showed that variants of the N-acetyltransferase 2 (NAT2) gene might have an influence on the susceptibility to TIMLD[8]. It will be of great interest to us if HLA-DM polymorphism has different reactions on different NAT2-induced metabolite. Based on the fatal effect of HLA-DMA on other HLA genes, we have in the present study investigated the association between HLA-DMA and HLA-DMB polymorphisms and risk of developing TIMLD.

MATERIALS AND METHODS

Study Population

The study population comprised 61 TIMLD patients and 60 genetically unrelated TCE exposed workers who were free from TIMLD. The former were diagnosed by the National Committee for Occupational Disease Diagnosis based on the Chinese National Diagnostic Criteria of Occupational Skin Diseases (General Guideline) (GBZ18-2002)[1], while the latter served as controls. The sex, age, smoking, and other information about the subjects recruited in the study are shown in Table 1. The controls were exposed to TCE for a period of 3 months to 4 years, while the TIMLD cases were occupationally exposed to TCE with similar length of time. The TIMLD cases and controls had similar distribution of sex and age. Blood samples of 5 mL was obtained with informed consents. This study was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science & Technology.

TABLE 1

Information About the Subjects Recruited in the Study

Group	Age (y)	Sex		Smoking		Period of TCE Contact
		Male	Female	Yes	No	
Control	22.9±3.2	35	25	32	28	3 Months to 4 Years
TIMLD Cases	23.2±2.9	37	24	35	26	8 Days to 5 Months

HLA-DMA and HLA-DMB Genotyping

Genomic DNA was isolated from 1 mL peripheral blood using DNAzol BD Reagent (Invitrogen, CA, USA), according to the manufacturer's instructions. DNA was dissolved in ddH_2O and stored at -20℃ for genotyping. HLA-DM genotyping was performed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP), and samples with undecided genotyping were directly sequenced to confirm the genotype. Briefly, polymorphisms of the DMA gene and DMB gene were determined by PCR amplification of the third exon using primers as defined by previously published sequences[9-10]. Each reaction tube contained 1.25 μL primer (10 mmol/L, Bioasia, China), 1.5 μL dNTP (each 2.5 mmol/L, Takara, China), 6 μL 10×buffer (containing Mg^{2+}2.5 mmol/L, Takara, China), 1.5 μL template DNA (150 ng), 0.8 μL Taq polymerase (5 units/μL, Takara, China), 47.7 μL ddH_2O, and the total volume of reaction was 60 μL. Touch-Down PCR was performed on a Mastercycler Gradient 5331 (Eppendorf, Hamburg, Germany) with the following profile: denaturation at 94℃ for 3 min for 3 cycles, at 94℃ for 30 s, anneal at 58℃ for 30 s (per cycle descended 0.5℃) and at 72℃ for 1 min for 10 cycles, then at 94℃ for 30 s, at 53℃ for 30 s, at 72℃ for 1 min for 20 cycles, a final extension at 72℃ for 5 min. The length of DMA and DMB was 370 bp and 348 bp respectively (Fig. 1). DMA and DMB were determined using restriction fragment length polymorphism (RFLP). Three restriction enzymes (BseGI, HinfI, and SsiI; MBI Fermentas, Lithuania) were used to digest DMA, and 3 restriction enzymes (HhaI, BseNI, and Alw44I; MBI fermentas, lithuania) were used to digest DMB. The digested fragments were separated by 8% polyacrylamide gel electrophoresis (10 V/cm, 10℃) for 3 h and silver stain was used to determine the RFLP patterns. Based on these patterns, DMA*0101 to DMA*0104 and DMB*0101 to DMB*0106 were identified. The standard of DMB*0105 and *0106 digestion pattern was used for the first time (Table 2, Fig. 2). Sequence-base typing using the Applied Biosystem 3730 (ABI, CA, USA) was also used to determine the

genotypes when the RFLP patterns were not easy to identify (Fig. 3).

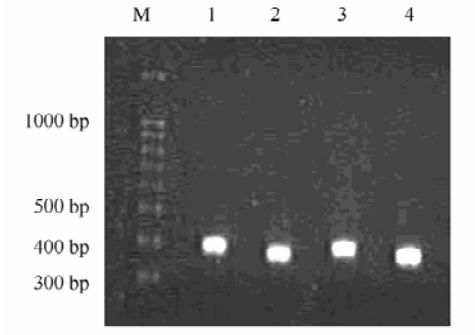

FIG. 1. Length of PCR products of DMA and DMB. M, DNA marker; lane1 and lane3: PCR products of DMA (370 bp); lane2 and lane 4: PCR products of DMB (348 bp).

Statistical Analysis

Chi-square test and Fisher's exact test were used to compare the distribution of HLA-DMA and DMB alleles and genotypes between TIMLD patients and controls. The crude and age- and sex-adjusted odd ratios (OR) and the 95% confidence intervals (CI) were calculated from the logistic regression models and used to estimate the strength of the association between genetic variants and risk of TIMLD. According to Bonferroni's method, *P* values were corrected (*Pc*) by the number of comparisons (2 for DMA alleles and 4 for DMB alleles, 3 for DMA genotypes and 7 for DMB genotypes). All the significant levels were 0.05 and two-sided, and all calculations were performed with SPSS 10.0 software.

TABLE 2

Determination of HLA-DMA and DMB Alleles and RFLP Patterns

	Allele	Codon144	Codon155	Code180	*BseG*I	*Hif*I	*Ssi*I
DMA	0101	Val	Ile	Arg	370 bp	207+93+70 bp	292+78 bp
	0102	Ile	Ile	Arg	224+146 bp	207+93+70 bp	292+78 bp
	0103	Val	Thr	His	370 bp	277+93 bp	370 bp
	0104	Ile	Thr	Cys	224+146 bp	207+93+70 bp	370 bp
	Allele	Codon144	Codon177		*Alw44*I	*Hha*I	*BseN*I
DMB	0101	Ala	Ile	-	189+159 bp	220+105+23 bp	348 bp
	0102	Glu	Ile	-	348 bp	220+105+23 bp	348 bp
	0103	Ala	Thr	-	189+159 bp	220+77+28+23 bp	348 bp
	0104	Val	Thr	-	348 bp	220+77+28+23 bp	187+161 bp
	0105	Val	Ile	-	348 bp	220+105+23 bp	187+161 bp
	0106	Glu	Thr	-	348 bp	220+77+28+23 bp	348 bp

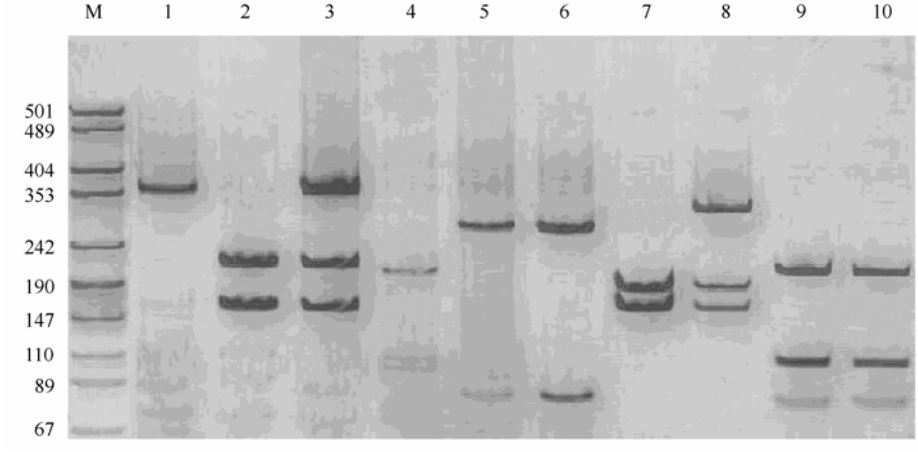

FIG. 2. DMA and DMB digest pattern. M: DNA Marker; lane 1: *DMA**0101-*0101(370 bp); lane 2: DMA*0102-*0102 BseG I digest pattern (224+146 bp); lane 3: DMA*0101-*0102 BseG I (370+224+146 bp); lane 4: DMA*0101-*0101 *HinfI* digest pattern (207+93+70 bp); lane 5, 6: DMA*0101-*0101 and DMA*0102-*0102 SsiI digest pattern (292+78 bp); lane 7: DMB*0101-*0101 *HhaI* digest pattern (189+159 bp); lane 8: DMB*0101-*0102 *HhaI* digest pattern (348+189+159 bp); lane 9, 10: DMA*0101-*0101 *HinfI* digest pattern (207+93+70 bp).

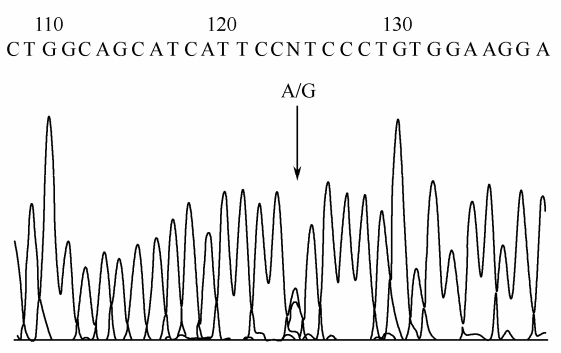

FIG. 3. Two apexes in sequence base typing of heterozygote.

RESULTS

The frequencies were successfully established for all HLA-DMA and DMB alleles in TIMLD patients and controls. The frequencies of DMA and DMB alleles are given in Table 3. Two DMA alleles and four DMB alleles were detected in both TIMLD patients and controls, and no other alleles were found in this study. The DMA*0101 allele was more frequent in TIMLD patients (71.3%) than in controls (55.0%) (Pc=0.022, OR=2.03, CI=1.19-3.46). The frequency of DMB*0103 allele was also higher in TIMLD patients, but the difference was not significant after the correction (P>0.05). No other significant differences in alleles were observed between them.

The frequencies of DMA and DMB genotypes are shown in Table 4. We detected 3 DMA genotypes and 7 DMB genotypes in both TIMLD cases and controls, and no other genotypes were found in this study. The frequency of DMA*0102-0102 genotype was statistically higher in the controls (25.0%) than in the cases (8.2%, Pc=0.045, OR=3.05, CI=2.78-4.94), and the frequency of DMB*0101-*0102 heterozygote was also statistically higher in the controls (38.3%) than in the cases (14.8%, Pc=0.028, OR=3.59, CI=2.71-4.47). The frequencies of other DMA and DMB genotypes were not different between the two groups.

TABLE 3

Comparison of Frequencies of HLA-DMA and DMB Alleles Between TIMLD Patients and Controls

Gene	Allele	Controls		TIMLD Patients		χ^2	P	Pc
		No.	%	No.	%			
DMA	*0101	66	55.0	87	71.3	6.922	0.011	0.022△
	*0102	54	45.0	35	28.7	-	-	-
DMB	*0101	89	74.2	90	73.8	0.005	0.944	NS
	*0102	27	22.5	17	13.9	2.984	0.084	NS
	*0103	4	3.3	14	11.5	5.825	0.016	NS
	*0106	0	0	1	0.8	0.988	0.320	NS

Note. P = uncorrected P values, Pc = corrected P values, △ = statistically significant, NS = not significant.

TABLE 4

Comparison of Frequencies of HLA-DMA and DMB Genotypes Between Controls and TIMLD Patients

	Genotype	Controls	%	TIMLD Patients	%	χ^2	P	Pc
	*0101-*0101	21	35.0	31	50.8	3.089	0.079	NS
DMA	*0102-*0102	15	25.0	5	8.2	6.190	0.013	0.039△
	*0101-*0102	24	40.0	25	41.0	0.012	0.912	NS
	*0101-*0101	32	53.3	37	60.7	0.662	0.416	NS
	*0102-*0102	2	3.3	3	4.9	0.192	0.661	NS
	*0103-*0103	1	1.7	3	4.9	1.000	0.317	NS
DMB	*0101-*0102	23	38.3	9	14.8	8.645	0.003	0.021△
	*0101-*0103	2	3.3	7	11.4	2.913	0.088	NS
	*0102-*0106	0	0	1	1.6	0.992	0.319	NS
	*0102-*0103	0	0	1	1.6	0.992	0.319	NS

Note. P = uncorrected P values, Pc = corrected P values, △ = statistically significant, NS = not significant.

DISCUSSION

TCE is mainly used as degreasing agent, solvent and extractive solvent. In industry, TCE can cause severe medicamentosa-like dermatitis as reported by Schwartz in 1947[11]. To date only 18 cases have been reported from other countries excluding China[12-16]. But since 1998, over 200 cases presented with skin damage (severe medicamentosa-like dermatitis), fever, abnormal liver function, and superficial lymph node enlargement have been recorded among TCE exposed workers in China. About 30 new cases occur annually. Symptoms in all these cases developed within 3 months of exposure. However, the exposed TCE concentrations ranged between 0.3 and 4085.0 mg/m^3 (Permissible concentration-time weighted average, PC-TWA, is 30 mg/m^3 based on the National Standard of the People's Republic of China GBZ2-2002), and no dose-depend relationship was found between their severity and exposure concentration. Neither findings of occupational health surveys nor clinical manifestations supported the assumption that such dermatitis was a kind of acute TCE poisoning. Because its incidence rate among exposed workers is <5%, difference in individual susceptibility may play a role in the etiology. Based on the above reasons, the condition is considered as a delayed-type hypersensitivity and its main clinical symptom is severe medicamentosa-like dermatitis, with liver dysfunction and superficial lymph node enlargement observed in some cases.

It is well known that there is a considerable variation in susceptibility to environment-induced diseases. Therefore, identification of genetic factors is critical in identifying subpopulation at risk. HLA-DM gene is a nonclassical HLA-II gene first detected in the 1990s, which has two subregions (DMA, DMB). The two subregions code αchain and βchain of DM molecule separately. DM molecule has a fatal effect on antigen presentation function of the classical HLA-II (such as HLA–DR, DQ, DP)[17].

The susceptibility to allergy reaction largely depends on genetic predisposition. Our previous researches have found that the frequency of slow metabolic type NAT2 (N-acetylase2) gene is higher in TIMLD patients than in controls, and this NAT2 gene may be one of the gene factors influencing the variation of susceptibility to TIMLD in TCE-exposed workers[8]. In the present study, we have focused on the difference in DM between the controls and TIMLD patients, by establishing two specific polymorphisms in the DM genes.

When compared to the controls, the frequencies of DMA*0101 allele and DMB*0103 allele were higher in TIMLD patients, suggesting that these two alleles might contribute to the genetic susceptibility to TIMLD, although the underlying molecular mechanisms remain unknown. On the other hand, we have also found that the frequency of DMA*0101 allele and DMB*0101-*0102 heterozygote is lower in TIMLD patients than in the controls, suggesting that the effect of HLA-DMA*0101 and HLA-DMB *0103 could be modulated by other unknown factors.

How do these alleles affect the TIMLD susceptibility? According to recent development and theoretical exploration[17-18], DM is directly involved in the removal of CLIP (class II-associated invariant chain peptide) from class II molecules, and DM enhances the release of CLIP from DR[7,19]: CLIP forms complexes in a dose-dependent manner, thereby accelerating loading of DR with antigenic peptides. DM can also stabilize empty HLA-DR molecules and modify the MHC II-peptide complex in terms of its presenting power[6]. The chaperone-like function of HLA-DM seems to be allele dependent[20]. Different DM molecules have their unique spatial structures, and therefore the ability to present antigen is different. However, there are many other steps in the processing of DM molecule that could affect its association with and processing of MHC class-II molecules that are subject to alteration, leading to a functional change. If a different HLA-DM has a different antigen presenting power to TCE and its metabolite or concomitant or HLA-DM affects the antigen presence by regulating other MHC II, the relationship between HLA-DM and TIMLD susceptibility needs further investigations. There may be a genetic difference in the metabolism of TCE in exposed population, which needs to be further addressed in future studies

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