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Relationship Between HLA-DMA, DMB Alleles and Type 1 Diabetes in Chinese

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Abstract The Human Leucocyte Antigen (HLA)-DMA and DMB genes are located in the HLA-D region between DQ and DP. HLA-DM molecules play an important role in the process of peptide loading to HLA class II antigens, both in regulating the dissociation of class II-associated invariant chain peptides (CLIP) and the subsequent binding of exogenous peptides to HLA class II molecules. In order to investigate the immunogenetic heterogeneity within the HLA-D region, we designed this study to explore the relationship between HLA-DMA, DMB, and genetic susceptibility to type 1 diabetes in Chinese. Our results showed that HLA-DMA*0103 and HLA-DMB*0103 alleles contributed to the predisposition, while HLA-DMA*0102 and HLA-DMB*0101 alleles conferred protection to type 1 diabetes in Chinese. HLA-DMA*0101/0102 and DMB*0101/0101 genotypes were significantly increased in the controls, HLA-DMB*0103/0103 and DMA*0101/0103 genotypes were significantly increased in the patients. In Chinese, HLA-DMA*0102/DMB*0101 heterodimer confers protection to type 1 diabetes, while HLA-DMA*0103/DMB*0102, DMA*0103/DMB*0103 and DMA*0103/DMB*0101 heterodimers confer susceptibility to type 1 diabetes. In general, our study suggests that HLA-DMA and DMB genes may be associated with the susceptibility to type 1 diabetes in Chinese.

Key words Human leucocyte antigen; Non-classical gene; Type 1 diabetes

Introduction

Type 1 diabetes is a polygenic autoimmune disease which results from an autoimmune destruction of the pancreatic islet β-cells. The strongest susceptibility is conferred by the human leucocyte antigen (HLA) region on chromosome 6P21.[1] Studies have identified a cluster of genes within the class II region.[2] Recently, two novel genes, HLA DMA and DMB were found in the major histocomptibility complex (MHC) region between HLA-DQ and HLA-DP. HLA-DMA and DMB products play an essential role in the mechanism of class II-restricted antigen presentation by promoting the exchange of class II-associated invariant-chain peptides normally bound to newly synthesised class II molecules and peptides derived from antigen processing.[3,4] Limited genetic variation has been found within human HLA-DMA and DMB loci,[5] and recent studies have shown that HLA-DMA and DMB alleles could be associated with the genetic susceptibility to type 1 diabetes.[6] Our present research was designed to study the relationship between HLA-DMA, DMB, DQA1 and genetic susceptibility to type 1 diabetes in Chinese.

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Methods

Subjects

A total of 80 children (36 males, 44 females) with type 1 diabetes were selected as research subjects. Diagnosis of

type 1 diabetes was made according to WHO criteria. The age at onset of type 1 diabetes ranged from 2.5 to 14 years of age.

Ninety-one healthy adult blood donors were selected as normal controls. They had neither type 1 nor type 2 diabetes, nor a family history of these disorders, and their fasting blood glucose levels were normal. Informed consent for research was obtained from all the subjects.

DMA, DMB and DQA1 Typing

Genomic DNA was prepared from peripheral blood leucocytes obtained from type 1 diabetes patients and healthy controls. Samples of DNA were separately amplified for the third exon of DMA and DMB genes and the second exon of DQA1 genes by polymerase chain reaction (PCR) techniques.

The sequences of DMA and DMB primers were listed as following:
DMAAMP-A: 5'-GGGTTTCCTATCGCTGAAGTG-3';
DMAAMP-B: 5'-CCAATAGGCAATTGCTGTGTA-3';
DMBAMP-A: 5'-CGGCCACCATCTGTGCAAGT-3';
DMBAMP-B: 5'-CCAGTCCCGAAGGATGGGCT-3';[7]

The sequences of DQA1 primers:
DQAAMP-A: 5'-ATGGTGTAAACTTGTACCAGT-3';
DQAAMP-B: 5'-TTGGTAGCAGCGGTAGAGTTG-3'.

PCR-amplified DNA was denatured and dotted onto nylon membrane, DNA was ultraviolet bound to the membrane after which DMA, DMB and DQA1 oligotyping was performed to classify four HLA-DMA, five HLA-DMB and one DQA1 allele (DQA1*0501). Sequences of the specific oligonucleotide probes were listed in Table 1.[8]

Statistical Analysis

Statistical analysis of the distribution of HLA-DMA, DMB and DQA1 alleles in patients and healthy subjects was done by the chi-square test or Fisher's exact test when any value was less than five. Values was corrected (Pc) by multiplying the number of alleles compared, i.e, × 4 for DMA alleles and × 5 for DMB alleles.

Results

Our study showed that HLA-DMA*0103 and DMB*0103 alleles were significantly increased in the patients with type 1 diabetes mellitus, which confer predisposition to type 1 diabetes. HLA-DMA*0102 and DMB*0101 alleles were significant decreased in patients which confer protection to type 1 diabetes (Tables 2 & 3). DMA*0101/0102 and DMB*0101/0101 phenotypes were significantly increased in the controls (42% vs 10.8%; 46% vs 7.1%, respectively, Pc<0.01), these two phenotypes are protective ones in Chinese. DMB*0103/0103 and DMA*0101/0103 phenotypes were susceptive phenotypes in Chinese (Tables 4 & 5).

The patients were classified into two groups according to whether carry the DQA1*0501 allele or not, The frequency distribution of DMA and DMB alleles in the two groups were studied (Tables 6 & 7). Results showed that frequencies of susceptible DM alleles in two groups having no significant difference. We also studied the distribution of DMA, DMB alleles in patients and controls carrying DQA1*0501 allele (Tables 8 & 9). DMA*0103 allele is significantly increased in controls with DQA1*0501 allele. DMA*0102 and DMB*0101

Table 1 Sequences of probes used in the study

Probe	Sequence	Specificity	Position
DMA*01:	5'-AAATTGACCGCTACACAG-3'	0101+0102	247-265
DMA*02:	5'-ATCATTCCGTCCCTGTGG-3'	0101+0103	116-133
DMA*03:	5'-ATTCCATCCCTGTGGAAG-3'	0102+0104	119-136
DMA*04:	5'-GAAATTGACCACTACACAG-3'	0103	247-265
DMA*05:	5'-GAAATTGACTGCTACACAG-3'	0104	247-265
DMB*01:	5'-AGCAGTGCGCACAAGACT-3'	0101+0103	142-159
DMB*02:	5'-AGCAGTGAGCACAAGACT-3'	0102	142-159
DMB*03:	5'-AGCAGTGTGCACAAGACT-3'	0104+0105	142-159
DMB*04:	5'-GTAGAGCACATTGGGGCTC-3'	0101+0102+0105	244-262
DMB*05:	5'-GTAGAGCACACTGGGGCTC-3'	0103+0104	244-262
DQA1*7504:	5'-CTT GAA CAG TCT GAT TAA-3'	0501	

Table 2 Frequencies of HLA-DMA alleles in patients with type 1 diabetes and controls

Alleles	Patients (N=74)		Controls (N=88)		RR	Pc
	n	%	n	%		
DMA*0101	96	64.9	115	65.3		NS
DMA*0102 (PR)	8	5.4	37	21.0	0.17	<0.01
DMA*0103 (SU)	37	25	7	4.0	11.57	<0.01
DMA*0104	7	4.7	17	9.7	0.44	NS

PR: protective; SU: susceptive

Table 3 Frequencies of DMB alleles in patients with type 1 diabetes and controls

Alleles	Patients (N=70)		Controls (N=87)		RR	Pc
	n	%	n	%		
DMB*0101(PR)	40	28.6	104	59.8	0.34	<0.01
DMB*0102	32	22.9	28	16.1	1.30	NS
DMB*0103(SU)	37	26.4	19	10.9	2.68	<0.05
DMB*0104	27	19.3	18	21.8	1.05	NS
DMB*0105	4	2.9	5	3.6	0.73	NS

PR: protective; SU: susceptive

Table 4 Frequencies of HLA-DMA phenotype in patients with type 1 diabetes and controls

DMA phenotype	Patients (N=74)		Controls (N=88)		RR	Pc
	n	%	n	%		
0101/0101	23	31.1	27	30.7	1.02	NS
0101/0102 (PR)	8	10.8	37	42.0	0.17	<0.01
0101/0103 (SU)	37	50.0	7	8.0	11.57	<0.01
0101/0104	5	6.8	17	19.3	0.30	NS
0104/0104	1	1.4	0			NS

PR: protective; SU: susceptive

Table 5 Frequencies of HLA-DMB phenotype in patients with type 1 diabetes and controls

DMB phenotype	Patients (N=70)		Controls (N=87)		RR	Pc
	n	%	n	%		
0101/0101 (PR)	11	15.7	40	46.0	0.22	<0.01
0101/0102	10	14.3	8	9.2	1.65	NS
0101/0103	11	15.7	10	11.5	1.44	NS
0101/0104	1	1.4	2	2.3	0.62	NS
0101/0105	1	1.4	4	4.6	0.30	NS
0102/0102	2	2.9	4	4.6	0.61	NS
0102/0103	8	11.4	2	2.3	5.48	NS
0102/0104	10	14.3	9	10.3	1.44	NS
0102/0105	0	0	1	1.1		NS
0103/0103 (SU)	7	10.0	0	0		<0.05
0103/0104	4	5.7	7	6.9	0.69	NS
0104/0104	3	4.3	0	0		NS
0104/0105	1	1.4	0	0		NS
0105/0105	1	1.4	0	0		NS

PR: protective; SU: susceptive

Table 6 Frequencies of DMA alleles in patients with or without DQA1*0501 alleles

Alleles	DQA1*0501 (+) Patients N=44	%	DQA1*0501 (-) Patients N=30	%	Pc
DMA*0101	43	97.7	30	100	NS
DMA*0102	5	11.4	3	10	NS
DMA*0103	23	52.3	14	46.7	NS
DMA*0104	6	13.6	3	10	NS

Table 7 Frequencies of DMB alleles in patients with or without DQA1*0501 alleles

Alleles	DQA1*0501 (+) Patients N=41	%	DQA1*0501 (-) Patients N=29	%	Pc
DMB*0101	19	46.3	15	51.7	NS
DMB*0102	14	34.1	12	41.4	NS
DMB*0103	20	48.8	10	34.5	NS
DMB*0104	8	19.5	7	24.1	NS
DMB*0105	1	2.4	2	6.9	NS

Table 8 Distribution of DMA alleles in subjects with DQA1*0501 alleles

Alleles	DQA1*0501+Patient (N=44)		DQA1*0501+Control (N=30)		RR	Pc
	n	%	n	%		
DMA*0101	43	97.7	30	100		NS
DMA*0102 (PR)	5	11.3	12	40	0.20	<0.05
DMA*0103 (SU)	23	52.3	2	6.7	15.88	<0.01
DMA*0104	6	13.6	5	16.7	0.82	NS

PR: protective; SU: susceptive

Table 9 Distribution of DMB alleles in subjects with DQA1*0501 alleles

Alleles	DQA1*0501+Patient (N=41)		DQA1*0501+Control (N=29)		RR	Pc
	n	%	n	%		
DMB*0101	19	46.3	23	79.3	0.23	<0.05
DMB*0102	14	34.1	9	31.0	1.15	NS
DMB*0103	18	43.9	6	20.7	3	NS
DMB*0104	8	19.5	5	17.2	1.16	NS
DMB*0105	1	2.4	2	6.8	0.34	NS

allele is significantly higher in controls with DQA1*0501 allele indicating that these two alleles conferring strong protection to type 1 diabetes. In general, the above results indicated that relationship between DMA, DMB genes and type 1 diabetes maybe not from linkage disequilibrium with class II classical allele-DQA1*0501.

Discussion

Genetic studies have shown that type 1 diabetes is a polygenic disease of which HLA play the most important role in susceptibility of type 1 diabetes. Within the class II region of HLA, DQB1, DQA1 and DRB1 genes provide the major contribution to type 1 genetic susceptibility and protection.[9-12] Other genes in close proximity to the DQ and DR loci also encode molecules which participate in metabolic pathways leading to antigen presentation, such as DMA and DMB.[13]

Till now, several studies have examined the association between DMA and DMB alleles and susceptibility of type 1 diabetes with varying conclusions. Cucchi-Mouillot studied the relationship between HLA-DMA alleles and type 1 diabetes in Corsician[6] and revealed that DMA*0101 allele conferred predisposition to type 1 diabetes, while DMA*0102 confers protection to type 1 diabetes. In a similar study on German patients with type 1 diabetes mellitus,[7] HLA-DMA*0102 and DMB*0101 conferred protection, but DMB*0103 contributed to the susceptibility to type 1 diabetes. A study from Italy reported that HLA-DMB alleles have no significant association with type 1 diabetes.[14] The type 1 diabetes susceptible MHC DQA1-DQB1 haplotypes were not associated with specific HLA-DMB alleles. We report here for the first time the distribution of DMA and DMB variants in type 1 diabetes and normal controls in Chinese. A significant increase of DMA*0103 and DMB*0103 alleles in type 1 diabetes was observed, these alleles confer susceptibility while DMA*0102 and DMB*0101 alleles confer protection to type 1 diabetes in Chinese. The above results indicated that HLA-DMA and DMB non-classical genes may also have significant effect on genetic susceptibility of type 1 diabetes, and the relationship between DM and type 1 diabetes have racial differences.

Being worthy to be mentioned, DMA*0103 gene confers susceptibility to type 1 diabetes both in German and Chinese. In several studies on rheumatoid arthritis and multiple sclerosis,[15,16] this gene also present strong predisposition to these diseases, demonstrating that DMA*0103 could represent an additional genetic risk marker for susceptibility of autoimmune disease such as type 1 diabetes. However, these results need to be confirmed by further HLA-polymorphism studies on different type 1 diabetes populations.

In our study, a higher frequency of DMA*0101/0103 and DMB*0103/0103 phenotypes and decrease of DMA*0101/0102 and DMB*0101/0101 phenotypes in patients were observed. In addition, relationship between heterodimers and type 1 diabetes showed that DMA*0102/DMB*0101 heterodimer confer protection, while DMA*0103/DMB*0102, DMA*0103/DMB*0103 and DMA*0103/DMB*0101 heterodimers confer susceptibility to type 1 diabetes in Chinese.

Combined study of relationship between DQA1*0501, DMA, DMB and susceptibility of type 1 diabetes indicated that relationship between DMA, DMB and type 1 diabetes maybe not from the linkage disequilibrium with class II classical allele DQA1*0501.

DM molecules play an important role in antigen loading process. DM is an essential cofactor in peptide loading, and it also serves as a editor of antigen loading and presentation, by which to determine the property of antigen to be presented by class II molecules.[17-19] Large amount studies have shown that type 1 diabetes is a Th1-dominant disease.[20-23] It can be deduced that in the presentation of antigen, individuals with DM susceptive gene or heterodimer will enable the "susceptive" peptide-class II molecule complex to be survived and presented to the surface of the APC. The peptide may trigger the immune reaction which will cause up-regulation of TH1/Th2 ratio, and thus leading to the onset of type 1 diabetes. On the contrary, the individual with DM protective genes or heterodimers will enable the "protective" peptide-class II molecule complex to be survived and presented to the surface of the APC. This antigen may down-regulate the ratio of Th1/Th2, and thus avoid the onset of type 1 diabetes.

In general, our results showed that HLA- class II non-classical alleles-DMA and DMB are involved in pathogenesis of type 1 diabetes. More studies will be needed to confirm the role of DM in the pathogenesis of type 1 diabetes, since the distribution of DM alleles and HLA-class II alleles differs among different ethnic groups.

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