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The relationship of lmp2 and DR3 genes with susceptibility to type I diabetes mellitus in south China Han population

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Subject headings diabetes mellitus; Imp2 genes; DR3 genes ; polymerase chain reaction; restriction fragment length polymorphism; genetic susceptibility

Abstract

AIM To study the relationship of *lmp2* and DR3 genes with type I diabetes mellitus.

METHODS *lmp2* genotypes and DR3 were identified in 68 patien ts with type I diabetes mellitus (I-DM) and 71 healthy controls. Then, I-D M patients and controls were respectively allocated into DR3-positive and DR3- negative groups. The frequencies of *lmp2* and DR3 gene in random subjects, an d *lmp2* genotypes in DR3-matched subjects were compared between I-DM patie nts and controls. At the same time, I-DM patients were divided into 3 groups b ased on the onset age of diabetics: group A ⩽14 years, group B 15-30 years and group C⩾31 years.

RESULTS The frequency of DR3 in I-DM patients was significantly higher than that in controls (47% *vs* 21%, *P*<0.005), and it was significantly higher in group A than that in group B+C (70% *vs* 36%, $\chi^2 = 7.07$, *P*<0.01). There was a significant difference among groups with different onset age of diabetics ($\chi^2 = 8.19$, rp = 0.33, *P*<0.05). In random subjects, the frequency of *lmp2* R/R in I-DM patients was lower (43% *vs* 61%, *P*<0.05) and *lmp2*-R/H higher (53% *vs* 28%, *P*<0.05) than that in controls, and there was no significant difference among groups with different onset age of diabetics. In DR3-positive subjects, the frequency of *lmp2*-R/R in I-DM patients was lower (47% *vs* 87%, *P*<0.05) and *lmp2*- R/H higher (47% *vs* 13%, *P*<0.05) than that in controls. In DR3-negative subjects, the frequency of *lmp2*-R/H in I-DM patients was higher than that in controls (58% *vs* 32%, *P*<0.01), but the frequency of *lmp 2*-R/R and *lmp2*-H/H was not significantly different between these two groups.

CONCLUSION DR3 gene may be one of the susceptible genes of I-DM, and significantly related to the onset age of diabetics, and the persons with DR3 may have an younger onset age of diabetes. The *lmp2*-R/R may be the protective genotype of I-DM, and *lmp2*-R/H the susceptible genotype. These were not affected by DR3 gene. *lmp*^{2} genotypes were not related with the onset age of diabetics.

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INTRODUCTION

Type I diabetes mellitus (I-DM) is an autoimmune disease due to insufficient insulin secretion resulting from immunologically mediated destruction of pancreatic beta cells. Previous studies suggested that some genes (including DR3 gene) within Major Histocompatibility Complex (MHC) class II region determined the susceptibility to I-DM in other populations[1-4]. We investigated the relationship of DQA1 and DQB1 with I-DM[5,6], in south China Han population. However, the relationship between DR3 and I-DM has not yet been studied. *lmp2* is another gene locus within MHC class II region, its polymorphism site is at R/H-60. When the amino acid at position 60 is arginine (R) or histidine (H), the allele will be *lmp2*-R or *lmp2*-H. *lmp2*-has 3 genotypes, i.e., *lmp2*-R/R, *lmp2*-R/H and *lmp2*-H/H. The r elationship between *lmp2* and I-DM is still controversial. This study aims at investigating the relationship of *lmp2* and DR3 gene with I-DM in south China Han population.

MATERIALS AND METHODS

Subjects

Sixty-eight I-DM patients and 71 healthy persons (as controls) were included in this study. All the subjects were Han population without relatives from southern China. The controls were the healthy

persons having no family history of autoimmune or hereditary diseases. The diagnosis of I-DM was based on 1985 WHO criteria. Both IDM patients and controls were subdivided into D R3 positive and DR3-negative groups. The I-DM patients were divided based on the onset age of diabetics into 3 groups: group A≤14 years, group B 15-30 years and group C≥31 years.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes treated with protease-K, saturated phenol/ chloroform extraction and collected by cold ethanol preci pitation.

Identification of DR3 gene

DR3 gene was identified by the nested-PCR[7]. First, the exon2 of DRB1 was amplified from genomic DNA, and the PCR primers were exon2. 1, 5'-CCGGATC CTTCGTGTCCCCACAGCAC-3' and exon 2.2, 5'-TCGCCGCTGCACTGTGAAG-3'. Then, DR3 gene was amplified from exon2, the PCR primers were DR3.1, 5'-TACTTCCATAACCA GGAGGAGA-3', DR3.2, 5'-TGCAGTAGTTGTCCACCCG-3'.The primer amplifying DR3 was used to amplify all the alleles (except for DR10) within exon2 of DRB1 to justify its specificity.

Genotyping of lmp2

lmp2 was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)[2].

PCR amplication The PCR primers were *lmp2*-1, 5'-GCCAGCA AGAGCGGAAACAAG-3'and *lmp2*-2, 5'-GTGAACCGAGTGTTTGAGAAGC-3'. The PCR product was a DNA fragment of 252bp containing the *lmp2* polymorphism site R/H-60. PCR was performed in 50 μL of reactive volume containing 100 ng of genomic DNA, 0.8 μmol/L primer, 0.2 μmol/L dNTP, 5 μl 10×PCR buffer and 1.5 u Taq DNA polymerase. The samples were subjected to 35 thermal cycles of 50 s at 94 ℃ for denaturing, 60 s at 52 ℃ for annealing, and 60 s at 72 ℃ for extension, 7 min at 94 ℃ for denaturing before the first cycle, and 5 min at 72 ℃ for extension after the last cycle.

Hha-I digestion of PCR production The reactive volume 20 μL contained 10 μL of PCR product, and 10 u of Hha-I (Gibco). The samples were incubated in warm water bath at 37 ℃ overnight. The allele *lmp2*-R contained the *Hha*-I site, however, the *lmp2*-H did not. Therefore, the polymorphism of *lmp2* can be revealed by *Hha*-I. When Hha-I digestion was performed, the PCR products with known *lmp2* genotype were used as controls.

Statistical analysis

The χ^2 test in the 2×2 table was used to compare the frequenci es of *lmp2* genotypes and DR3 gene between I-DM patients and controls, and if the results were significant, the odds ratio (Ψ) would be calculated. The frequencies of *lmp2* genotypes and DR3 gene were compared among groups with different onset age of diabetics by the χ^2 test in the R×C table, and if the results were significant, the Pearson's *rp* would be calculated.

RESULTS

Genotyping of lmp2 and identification of DR3 gene Figure 1 shows the DR3 gene by nested-PCR. Figure 2 shows the various genotypes of *lmp2* and the product of PCR. The identification of DR3 was made twice in 90 samples, getting 99% (89/90) precision. Genotyping of *lmp2* was performed twice in 70 samples with 100% coincidence. The primer amplifying DR3 and Hha-I digestion had excellent specificity.

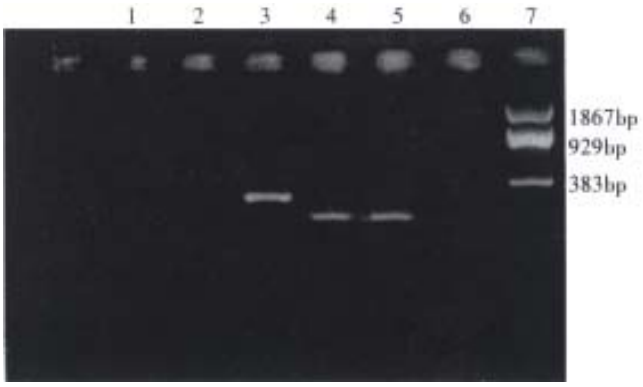

Figure 1 PCR products of DR3 and exon2 of DRB1.1. negative control of PCR; 2. exon2; 3. homozygous cell line of DR3; 4. DR3-positive sample; 5. DR3-negative sample; 6. pBR322DNA / -BstN-I Marker.

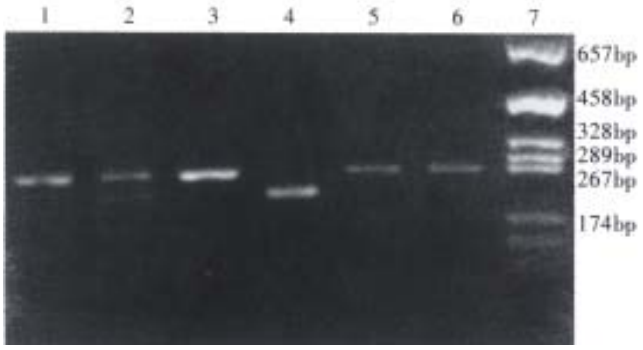

Figure 2 Genotyping of LMP2 gene by PCR-RFLP.1, 3, 5. products of PCR; 2. LMP2-R / H; 4. LMP2-R / R; 6. LMP2-H / H; 7. Pge m7Zf (+)Hae-III DNA marker.

Distribution of DR3 and lmp2 in random subjects

The frequency of *lmp2*-R/R in 1-DM patients was significantly lower, but the frequency of *lmp2*-R/H and DR3 was significantly higher than that in controls. And *lmp2*-H/H showed no significant difference between these two groups (Table 1).

Table 1 Comparison of frequencies of *lmp2* genotypes and DR3 gene between I-DM patients and controls in random subjects

	n	DR3	lmp2		
			R/R	R/H	H/H
1-DM	68	32(0.47)[a]	29(0.43)[b]	36(0.53)[c]	3(0.04)
Controls	71	15(0.21)	43(0.61)	20(0.28)	8(0.11)

Compared with controls: [a]x^2 = 10.44, ψ = 3.32, P<0.0 05; [b]x^2 = 4.47, ψ = 0.48, P<0.05; [c]x^2 = 8.86, ψ = 2.87, P<0.005.

Distribution of lmp2 in DR3-matched subjects

In DR3-positive subjects The frequency of *lmp2*-R/R and *lmp2*-R/H in I-DM patients was respectively lower and higher respectively than that in controls with significant difference, but *lmp2*-H/H had no significant difference between these 2 groups (Table 2).

In DR3-negative subjects The frequency of lmp2-R/H in I-DM patients was significantly higher than that in controls, while *lmp2*-R/R and lmp2-H/H had no significant differences between these two groups (Table 2).

Table 2 Frequencies of *lmp2* genotypes compared between I-DM patients and controls in DR3-matched subjects

	Positive DR3		Negative DR3	
	1-DM (n = 32)	Controls (n = 15)	1-DM (n = 36)	Controls (n = 56)
R/R	15(0.47)[a]	13(0.87)	14(0.39)	30(0.54)
R/H	15(0.47)[b]	2(0.13)	21(0.58)[c]	18(0.32)
H/H	2(0.06)	0(0.00)	1(0.03)	8(0.14)

Compared with controls: [a]x^2 = 7.50, ψ = 0.14, P <0.01; [b]x^2 = 4.98, ψ = 5.74, P<0.05; [c]x^2 = 6.15, ψ = 2.96, P<0.01.

Distribution of lmp2 and DR3 in groups with different diabetic onset age

There was a significant difference in DR3 frequency among different groups of diabetic onset age. The frequency of DR3 in group B was not significantly different from that in group C, thus, groups B and C were merged into group B+C, and its frequency of DR3 was 36% (16/45). When group A was compared with group B + C, the χ^2 was 7.07, and P<0.01, indicating that, the younger the age of diabetic onset, the higher the DR3 frequency (Table 3).

There were no significant differences in *lmp2* frequencies among different groups of diabetic onset age (Table 3).

Table 3 Frequencies of *lmp2* genotypes and DR3 gene compared amon g different age groups of diabetic onset

	n	DR3		lmp2		
		Positive	Negative	R/R	R/H	H/H
Group A	23	16(70%)	7(30%)	11(48%)	10(43%)	2(9%)
Group B	26	11(42%)	15(58%)	8(31%)	17(65%)	1(4%)
Group C	19	5(26%)	14(74%)	10(53%)	9(47%)	0

Difference of DR3 frequency among different age groups of diabetic onset was χ^2 = 8.19, rp = 0.33, P<0.05, *lmp2* was χ^2 = 4.53, P>0.05.

DISCUSSION

The relation between DR3 and I-DM Previous studies showed that DR3 is one of the susceptible genes of I-DM in some populations[1]. We studied the Han population in south China, and found that the frequency of DR3 in I-DM patients was significantly higher than that in controls. It su ggests that DR3 may be one of the susceptible genes of I-DM, and the persons with DR3 have a higher risk of suffering from I-DM.

Relation between *lmp2* and I-DM The *lmp2* encoded product is LMP protease, which is responsible for processing antigen, and may play a n important role in antigen presentation[8]. Therefore, *lmp2* may be an attractive candidate as a gene related with susceptibility to I-DM. Studies on the relation between *lmp2* and I-DM were still controversial. A recent study by Deng *et al*[9] suggested that *lmp2*-HR may be the protective genotype, and *lmp2*-R/H the susceptible genotype of I-DM, an d they may have no linkage disequilibrium to HLA-DR/DQ. Undlien *et al* [10] divided the subjects into many subgroups according to HLA-DRB1-DQA1-DQB1, and found that *lmp2* genotypes had no association with I-DM, but, the samplesize was too small after divided into subgroups. The studies by Van End ert *et al*[11], Kawaguchi *et al*[12] and Chauffert *et al*[13] yielded the similar results.

Our data indicated that, in random subjects, the frequencies of *lmp2*-R/R and *lmp2*-R/H in I-DM patients were significantly lower, and higher than those in controls respectively (Table 1). Therefore, *lmp2*-R/R may be the protective genotype, and *lmp2*-R/H the susceptible genotype of I-DM. In order to make sure that the effect of *lmp2* on I-DM will be affected by DR3, we investigated the frequency of *lmp2* genotypes in DR3-matched subjects. We divided the subjects into DR3 positive and DR3 negative groups and compared the frequencies of *lmp2* genotypes between I-DM and controls respectively in these groups, and obtained nearly the same results as

those in random subjects (Table 2). Therefore, our data suggested that the relationship between *lmp2* and I-DM may not be affected by DR3 gene. However, we have not study the relationship between *lmp2* and other protective and susceptible genes of I -DM within MHC class II region, so we can not confirm whether *lmp2* genotype s have independent effects on I-DM or not. Nevertheless, our data show that *lmp2* genotypes can predict the risk of I-DM occurrence. The persons with *lmp2*-R/R have a decreased risk, and those with *lmp2*-R/H a increase d risk of suffering from I-DM.

Relation among *lmp2* genotype, DR3 gene and the onset age of diabetics Recently, many studies have shown that I-DM is a heterogeneitic disease. The study by Caillat-Zucman *et al*[8] showed that DRB1, DQA1 and DQB1 were not only associated with the predisposition to I-DM, but also with the onset age of diabetics, and the younger the onset age of diabetics, the higher the frequencies of these genes. Some recent studies by my colleagues show ed the same results in DQA1 and DQB1[9,10]. This study also indicated that DR3 was associated with the age of onset in I-DM and the frequency of DR3 in patients with I-DM developed in childhood ($\leqslant$ 14 years) was significantly higher than that in adulthood ($\geqslant$15 years), suggesting that the persons with DR3 may have an earlier diabetic onset.

However, our data did not show any differences in frequencies of *lmp2* genotypes among various age groups of diabetic onset, suggesting that the *lmp2* genotypes may not have any association with the age of diabetic onset, and the distribution of *lmp2* genotypes may not be the same as that of DR3, D QA1 and DQB1 in I-DM patients.

REFERENCES

1 Caillat Zucman S, Garchon HJ, Timsit J, Assan R, Boitard C, Djilali-Saiah I, Bougneres P, Bach JF. Age-dependent HLA genetic heterogeneity of type-1 (insulin-dependent) diabetes mellitus.*J Clin Invest*,1992;90:2242-2250

2 Thorsby E, Ronningen KS. Particular HLA-DQ molecules play a dominant role determining susceptibility or resistance to type-1 (insulin-dependent) diabetes mellitus. *Diabetologia,* 1993;36:371-377

3 Ikegami H, Kawaguchi Y, Yamato E, Kuwata S, Tokunaga K, Noma Y, Shima K, Ogihara T. Analysis by the polymerase chain reaction of histocompatibility leukocyte antigen-DR9-linked susceptibility to insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*,1992;75:1381-1385

4 Undilien DE, Friede T, Rammensee HG, Joner G, Dahl-Jorgensen K, Sovik O, Akselsen HE, Knutsen I, Roningen KS, Thorsby E. HLA-encoded genetic predisposition in IDDM: DR4 subtypes may be associated with different degree of protection. *Diabetes*,1997; 46:143-149

5 Cheng H, Zhang Y, Fu ZZ, Yan T. Age-of-onset related HLA-DQA1 genetic heterogeneity of insulin-dependent diabetes mellitus. *Chin Med J*,1997;109:474-477

6 Zhang SL, Cheng H, Fu ZZ, Zhong GS, Yan T. Contribution of the absence of aspartic acid at position 57of the HLA-DQβ chain to predisposition to insulin-dependent diabetes mellitus in a Southern Chinese population. *Chin Med J*,1998;111:694-697

7 Bein G, Glaser R, Kirchner H. Rapid HLA-DRB1 genotyping by nested PCR amplication.*Tissue Antigen*,1992;39:68-73

8 Dick IR, Aldrich C, Jameson SC, Moomaw CR, Pramanik BC, Doyle CK, DeMartino GN. Proteolytic processing of ovalbumin and beta-galactosidase by the proteasome to yield antigen peptides. *J Immunol,* 1994;152:3884-3894

9 Deng GY, Muir A, Maclaren NK, She JX. Association of LMP2 and LMP7 genes with in the major histocompatibility complex with insulin-dependent diabetes mellitus: population and family studies.*Am J Hum Genet*,1995;56:528-534

10 Undlien DE, Akselsen HE, Joner G, Dahi-Jorgensen K, Sovik O, Ronningen KS, Thorsby E. No independent association of LMP2 and LMP7 polymorphisms with susceptibility to develop IDDM. *Diabetes*,1997;46:307-312

11 Van Endert PM, Liblau RS, Patel SD, Fugger L, Lopez T, Pociot F, Nerup J, Mcdevitt H. Major histocompatibility complex- encoded antigen processing gene polymorphism in IDDM.*Diabetes*,1994; 43:110-117

12 Kawaguchi Y, Ikegami H, Fukuda M, Takekawa K, Fujioka Y, Fujisawa Y, Ueda H, Ogihara T. Absence of association of TAP and LMP genes with type-1 (insulin-dependent) diabetes mellitus. *Life Sci*,1994;54:2049-2053

13 Chauffert M, Cisse A, Chevenne D, You JE, Michel S, Trivin E. Susceptibility to type- diabetes in the Senegalese population is linked to HLA-DQ and not TAP and LMP genes.Diabetes Care, 1997;20:1299-1303

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