The genetic association between LMP2 and LMP7 polymorphisms and susceptibility of insulin dependent diabetes mellitus

A meta-analysis

Yang Xu, MM, Guotao Liu, BA, Yv Zhou, MM, Zengzhen Lu, MM, Zhaorui Shi, BA, Jun Wang, MD

Abstract

Background: Insulin dependent diabetes mellitus (IDDM) is a kind of heterogeneous disease caused by the interaction of polygene inheritance and environmental factors. The LMP2 and LMP7 are 2 loci in LMP gene, and although genetic association between LMP2 and LMP7 polymorphisms were reported, the results are inconclusive. The aim of this study was to investigate the association between LMP2 and LMP7 polymorphisms and IDDM risk.

Methods: An exhaustive search was performed out through the electronic databases including PubMed, Embase, and Chinese National Knowledge Infrastructure (CNKI). The pooled odds ratio (OR) and 95% confidence interval (CI) were used to assess the strength association between LMP2 CfoI and LMP7 G37360T polymorphisms and IDDM risk.

Results: A total of 7 studies with 707 cases and 821 controls were included in the present study. The results indicated that the dominant model of LMP2 CfoI was significantly associated with IDDM in Asian population (OR=1.96, 95% CI: 1.24–3.10, P = .004). In addition, the allelic and dominant models of LMP7 G37360T were associated with IDDM in Caucasian population (allelic model: OR=0.69, 95% CI: 0.56–0.85, P = .0005; dominant model: OR=0.67, 95% CI: 0.50–0.89, P = .007).

Conclusions: The dominant model of LMP2 CfoI might be a risk factor for IDDM in Asian population. Whereas, the allelic and dominant models of LMP7 G37360T might be protective factors for IDDM in Caucasian population.

Abbreviations: CI = confidence intervals, CNKI = Chinese National Knowledge Infrastructure, HLA = human lymphocyte antigen, HWE = Hardy–Weinberg equilibrium, IDDM = insulin dependent diabetes mellitus, LMP = large multifunctional protease, MHC = major histocompatibility complex, NOS = Newcastle-Ottawa Scale, ORs = odds ratios, SNP = single nucleotide polymorphisms, TAP = tissue antigen presentation.

Keywords: insulin dependent diabetes mellitus, large multifunctional protease, meta-analysis, single nucleotide polymorphism

1. Introduction

Insulin dependent diabetes mellitus (IDDM), also known as type 1 diabetes, is a heterogeneous disease caused by polygene inheritance and associated with autoimmune disorders.[1,2] In recent years, it has been found that some genes of MHC II region (DR3, DQA1, and DQB1) are susceptible factors for IDDM.[3,4] However, these could not explain all of the genetic features of IDDM. Many researchers are still looking for new genes associated with the development of IDDM. The majority protease (large multifunctional protease, LMP) gene located in the region of (MHC) II and was a major histocompatibility complex in human beings. LMP genes including LMP2 and LMP7, are polymorphic and their products constitute 2 subunits of the proteasome complex involved in the degradation of cytosolic proteins and generation of antigenic peptides.[5–7]

There were no consistent results in the publications of the genetic association between LMP gene polymorphisms and IDDM. Deng et al.[8] found that LMP2 is a susceptibility gene for type 1 diabetes, but only in DR4-DQB1*0302 haplotypes. And the association between the LMP gene and type 1 diabetes is shown to be secondary to the linkage imbalance between the gene and HLA-DR/DQ.[9] Ding et al.[10] has indicated that LMP2-R/H genotype may be an independent protective factor for type 1 diabetes mellitus in South China. However, no association between LMP2 and IDDM has been observed in other ethnic groups.[11,12] These results suggested that the association between the polymorphism of the LMP2 gene and IDDM needs to be further strengthened. In addition, significantly increased risk was detected between LMP7 polymorphism and IDDM independent of the HLA-DRB1-DQ haplotypes.[9] Furthermore,
a study in Norwegian population has indicated that stratification of data according to HLA-DRB1*04 subtypes was important when considered the role of LMP7.\[11\]

Given the controversy role of LMP2 and LMP7 in genetic susceptibility to IDDM, the aim of this study was to determine whether polymorphisms of LMP2 and LMP7 were associated with IDDM by using a meta-analysis.

2. Materials and methods

2.1. Patient and public involvement

No patient and public involvement and ethical approval is necessary for the present meta-analysis.

2.2. Search strategy

A comprehensive search examining the association between the LMP2 and LMP7 polymorphisms with IDDM was conducted through the electronic databases including PubMed, Embase, and Chinese National Knowledge Infrastructure (CNKI) using the following terms: “Large multifunctional protease 2” or “LMP2” and “Large multifunctional protease 7” or “LMP7” and “polymorphism” or “variation” or “single nucleotide polymorphisms” or “SNP” and “insulin dependent diabetes mellitus” or “IDDM” or “Type I diabetes.” No language and publication year were restricted. Meanwhile, other potentially relevant literature was identified by searching cross-references within available studies.

2.3. Inclusion and exclusion criteria

Inclusion criteria: studies were case-control designed; studies that documented to the association between LMP2 or LMP7 polymorphism and IDDM risk; studies contained available genotypes for the calculation of odds ratios (ORs) and 95% confidence intervals (CIs); the distributions of genotypes in controls were in Hardy-Weinberg equilibrium (HWE).

Exclusion criteria: duplicated studies, letters, reviews, case reports, or abstracts; raw data of LMP2 and LMP7 genotypes were not available; the distributions of genotypes in controls were not in HWE.

2.4. Data extraction and quality assessment

Two independent authors (GL and YZ) selected the relevant articles according to the inclusion and exclusion criteria and performed the data extraction process. The following information from each study were extracted: the first author, publication year, ethnicity, age, sex, genotype-methods, the number of cases and controls, evidence of HWE in controls. All the data were summarized in Table 1. All discrepancies were resolved by discussion. The Newcastle-Ottawa Scale (NOS) was used to evaluate the study quality.\[13\] Total score ranged from 0 (lowest quality) to 8 (highest quality). A study with a score of ≥6 was classified as high quality and recruited in the further analysis.

2.5. Statistical analysis

The association between the allelic, dominant, and recessive models of LMP2 and LMP7 polymorphisms and IDDM risk was evaluated by pooled OR and 95% CI. The significance of the pooled OR was assessed by the Z test. Heterogeneity was evaluated by the Q-statistics (significant at $P<0.05$) and $I^2$ statistics (where $>50\%$ indicates significant heterogeneity). The pooled OR estimate of each study was calculated by the fixed-effect model when there was lack of heterogeneity. Otherwise, the random-effect model was used. Subgroup analysis stratified by ethnicity was also performed in this meta-analysis. The stability of the results was assessed using sensitivity analysis by omitting single study each time to evaluate the influence of each study on the pooled OR. The funnel plot was used to assess potential publication bias. Egger test and Begg tests were performed to evaluate potential publication bias of the literatures. A $P<0.05$ was considered significant. Statistical analyses were performed with the STATA 12.0 (StataCorp, College Station, TX) and Revman 5 (Cochrane Collaboration, London, UK) software.

3. Results

3.1. Selection of eligible study

The study selection process is shown in Fig. 1. A total of 939 publications (612 for LMP2 and 327 for LMP7) were initially retrieved from electronic databases including PubMed, Embase, and CNKI. After reviewing the titles, abstracts, and full text, 716 were excluded for duplicated studies (478 for LMP2 and 238 for LMP7). One hundred eighty nine were excluded for irrelevant studies (118 for LMP2 and 71 for LMP7). Twenty four were excluded for not related to the association between LMP2 or LMP7 polymorphisms and IDDM risk (9 for LMP2 and 18 for LMP7). Finally, 7 articles published with 707 cases and 821 controls were included in the current meta-analysis.\[8-12,14,15\]

The main characteristics of all eligible studies are shown in

---

Table 1  
The characters of included studies.

| First author | Year | Ethnicity | Age (mean ± SD) | Gender (M/F: case/control) | Case | Control | Polymorphisms |
|--------------|------|-----------|----------------|---------------------------|------|---------|--------------|
| Kawaguchi    | 1994 | Japanese | NA             | NA                        | 45   | 53      | LMP2 CfoI    |
| Deng         | 1995 | Caucasian| NA             | NA                        | 188  | 192     | LMP2 CfoI LMP7 G37360T |
| CHAUFFERF    | 1997 | African  | 19±15.3/29.2±7.7| 47/45:59/58              | 92   | 117     | LMP2 CfoI    |
| Ding-1       | 1999 | Chinese  | NA             | 39/29:36/35              | 68   | 73      | LMP2 CfoI LMP7 G37360T |
| Undlien      | 1997 | Norway   | NA             | NA                        | 191  | 217     | LMP2 CfoI LMP7 G37360T |
| VAN ENDERT   | 1993 | Danish   | NA             | NA                        | 50   | 83      | LMP2 CfoI    |
| Ding-2       | 1999 | Chinese  | NA             | NA                        | 73   | 86      | LMP2 CfoI    |

F = female, LMP = large multifunctional protease, M = male, NA = not available, SD = standard deviation.
Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart of studies inclusion and exclusion.
**Table 2**
The association between LMP2 CfoI and LMP7 G37360T polymorphisms and insulin dependent diabetes mellitus.

| Polymorphism | Genotype | Subgroups | Number of studies | OR  | 95% CI | P-value | Model | P-value | I² (%) |
|--------------|----------|-----------|------------------|-----|--------|---------|-------|---------|--------|
| LMP2         | Allele   | Total     | 7                | 1.07| [0.92, 1.24] | .41     | F     | .14     | 38     |
|              |          | Caucasian | 4                | 1.02| [0.86, 1.21] | .83     | R     | .09     | 50     |
|              |          | Asian     | 2                | 1.31| [0.92, 1.87] | .14     | F     | .83     | 0      |
|              |          | African   | 1                | 0.79| [0.53, 1.16] | .25     | -     | -       | -      |
| LMP2         | Dominant | Total     | 7                | 1.21| [0.86, 1.72] | .28     | R     | .02     | 60     |
|              |          | Caucasian | 4                | 1.02| [0.69, 1.52] | .91     | R     | .04     | 59     |
|              |          | Asian     | 2                | 1.96| [1.24, 3.10] | .004    | F     | .69     | 0      |
|              |          | African   | 1                | 0.73| [0.42, 1.27] | .27     | -     | -       | -      |
| LMP2         | Recessive| Total     | 7                | 0.72| [0.48, 1.10] | .13     | F     | .66     | 0      |
|              |          | Caucasian | 4                | 0.84| [0.52, 1.35] | .48     | F     | .66     | 0      |
|              |          | Asian     | 2                | 0.44| [0.18, 1.00] | .08     | F     | .77     | 0      |
| LMP2         |          | African   | 1                | 0.75| [0.33, 1.67] | .48     | -     | -       | -      |
| LMP7         | Allele   | Total     | 3                | 0.69| [0.57, 0.83] | .0001   | F     | .39     | 0      |
|              |          | Caucasian | 2                | 0.69| [0.56, 0.85] | .0005   | F     | .17     | 46     |
|              |          | Asian     | 1                | 0.69| [0.43, 1.13] | .14     | -     | -       | -      |
| LMP7         | Dominant | Total     | 3                | 0.70| [0.53, 0.94] | .02     | F     | .35     | 4      |
|              |          | Caucasian | 2                | 0.67| [0.50, 0.89] | .007    | F     | .72     | 0      |
|              |          | Asian     | 1                | 1.54| [0.49, 4.83] | .46     | -     | -       | -      |
| LMP7         | Recessive| Total     | 3                | 0.60| [0.26, 1.41] | .24     | R     | .007    | 80     |
|              |          | Caucasian | 2                | 0.72| [0.15, 3.51] | .69     | R     | .002    | 90     |
|              |          | Asian     | 1                | 0.47| [0.25, 0.89] | .02     | -     | -       | -      |

CI = confidence interval, F = fixed model, LMP = large multifunctional protease, OR = odd ratio, R = random model.

Table 1. Among the studies, 4 were conducted in Caucasian population, 2 were in Chinese Han population, and 1 was in African population. Furthermore, 7 studies with 707 cases and 821 controls documented to the association between LMP2 polymorphism and IDDM risk. And 3 studies with 450 cases and 495 controls documented to the association between LMP7 polymorphism and IDDM risk.

3.2. Association between LMP2 CfoI and IDDM

The results of this meta-analysis are shown in Table 2. The pooled risk estimates indicated that all the genetic models (allelic, dominant, and recessive models) of LMP2 CfoI were not significantly associated with the susceptibility of IDDM ($P > .05$). Subgroup analysis stratified by ethnicity showed that the significant association between the dominant model of LMP2 CfoI and IDDM was detected in Asian population (OR=1.96, 95% CI: 1.24–3.10, P=.004) (Fig. 2).

3.3. Association between LMP7 G37360T and IDDM

For LMP7 G37360T, subjects with T allele and dominant model had a significantly decreased model: (OR=0.69, 95% CI: 0.57–0.83, P=.001); dominant model: OR=0.70, 95% CI: 0.53–0.94, P=.02). No association was found between the recessive model of LMP7 G37360T and IDDM risk ($P > .05$). In subgroup analyses based on ethnicity, we found that the allelic and dominant models of LMP7 G37360T were associated with a decreased risk of IDDM in Caucasian population (allelic model: OR=0.69, 95% CI: 0.56–0.85, P=.0005; dominant model: OR=0.67, 95% CI: 0.50–0.89, P=.007), but not in Asian population ($P > .05$) (Fig. 3).

3.4. Heterogeneity

Heterogeneity was found for the dominant model of LMP2 CfoI and IDDM ($P=.02$, $I^2=60$). Therefore, subgroup analysis was carried out based on ethnicity. Significant heterogeneity remained in the dominant model of LMP2 CfoI and IDDM in Caucasian population, but not in Asian population (Table 2). The significant heterogeneity in these genetic models were contributed mainly by Van Endert et al.\cite{14} Removal of this study from meta-analysis gave 20% heterogeneity ($P > .05$). In addition, significant heterogeneity was also found for the recessive model of LMP7 G37360T and IDDM. The heterogeneity remained in Caucasian subgroup analysis (Table 2). The significant heterogeneity in this genetic model was contributed mainly by Undlien et al.\cite{15} Removal of this study from meta-analysis gave 0% heterogeneity ($P > .05$).

3.5. Sensitive analysis and publication bias

The sensitivity analysis showed that no single study altered the pooled ORs qualitatively, which provided the evidence of the stability of the meta-analysis (Fig. 4). Publication bias was assessed by Begg test and Egger test. As shown in Fig. 5, no evidence of publication bias was found.

4. Discussion

The products encoded by LMP2 gene are mostly functional protease, which are responsible for processing antigens and participating in the initiation of immune responses (including autoimmune reactions)\cite{16,17}. Therefore, we speculate that the LMP2 gene may be associated with autoimmune diseases. In recent years, the relationship between LMP2 gene and IDDM has
been studied abroad, however, no consistent conclusion has been reached. A recent study by Deng et al[8] suggested that LMP2-HR may be the independent protective genotype, and LMP2-R/H may be the independent susceptible genotype of IDDM. In addition, Ding et al[10] has detected that the LMP2-R/H and LMP2-R/R were susceptible to IDDM. However, Undlien et al[11] found that the LMP2 gene was not a IDDM-independent susceptible gene in Norwegian population. Van Endert et al[12] also analyzed the LMP2 polymorphism at position 60 in DR3-DQ2/DR4-DQ8 Danish IDDM patients compared with HLA-DR and -DQ matched Danish control subjects. Even though the numbers studied were small, those authors found no evidence for an independent association. Kawaguchi et al[15] have studied the same LMP2 polymorphism in IDDM patients and controls in Japanese, and again found no evidence for an independent association. In the present study, we firstly detected a significant association between the dominant model of LMP2 CfoI polymorphism and IDDM risk in Asian population by using a meta-analysis. To confirm this result, further researches with larger number of cases and controls is necessary.

The products encoded by LMP7 gene are mostly functional protease, which are involved in the processing of antigens to form small molecular peptides[18,19]. Together with the products encoded by LMP2 and the transporter associated with antigen processing, it is involved in the initiation of
immune response. Fehling et al\cite{20} showed that the expression level of MHC I region on the cell surface was significantly decreased, and endogenous antigens could not be presented to lymphocytes effectively, but did not affect the expression of tissue antigen presentation (TAP) gene. The addition of exogenous antigenic peptide could restore the expression of MHC I molecule, which proved that the expression product of \textit{LMP7} gene played an important role in the process of antigen presentation.

The relationship between \textit{LMP7} gene and IDDM has been reported in recent years, but the same conclusion has not been reached. Deng et al\cite{8} found that the frequency of \textit{LMP7-A/A} in insulin dependent diabetes mellitus group was significantly higher than that in control group, and the frequency of \textit{LMP7-B/B} in insulin dependent diabetes mellitus group was significantly lower than that in control group, and there was no linkage imbalance between \textit{HLA-DQ/DR} and insulin dependent diabetes mellitus. It is considered that \textit{LMP7-A/A} is an independent susceptible genotype and \textit{LMP7-B/B} is an independent protective genotype. Undlien et al\cite{11} found that the frequency of \textit{LMP7-B/A} was significantly lower than that of the control group. Further \textit{DRB1-DQA1-DQB1} pairing study showed that there was no significant difference in the frequencies of \textit{LMP7} genotypes between IDDM group and control group. In our meta-analysis, we observed that the allelic and dominant models of \textit{LMP7 G37360T} were significantly associated with IDDM. Subgroup analysis stratified by ethnicity indicated that these positive associations can only be found in Caucasian population. Furthermore, we also found a significant association between the recessive model of \textit{LMP7 G37360T} in Asian population. For there was only 1 study included in Asian subgroup analysis, the result needs to be confirmed by much more studies with larger number of subjects in the future. Notable, the G37360T polymorphism locates at intron 6 of the \textit{LMP7} gene, which does not reveal any amino acid substitutions. The significant association between this polymorphism and IDDM indicate that the G37360T polymorphism might link to polymorphisms affecting the expression of the \textit{LMP7} gene.

Limitations should be taken into account. First, the number of included studies and subjects were relatively small, especially in Asian population. To confirm these results, much more studies with larger number of cases and controls are necessary. Second, ethnic specific effect is an important consideration in meta-analysis. However, there were only 2 different ethnicities in the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{forest_plot.png}
\caption{Forest plots of odds ratios for the association between \textit{LMP7 G37360T} polymorphism and Insulin dependent diabetes mellitus. A = Allelic model; B = Dominant model; C = Recessive model.}
\end{figure}
present study. Larger number of studies with more subjects in multiple ethnicities is needed in the future. Third, the IDDM is caused by multiple factors including genetics and environment, as well as the interaction between the 2 factors. While, we could not assess the influence of environmental factor in the development of IDDM for lack of data.

5. Conclusion
Our combined results suggested an increased risk of the dominant model of LMP2 CfoI and a decreased risk of the allelic and dominant models of LMP7 G37360T in IDDM. To confirm these results, further study with larger sample size and multiple ethnicity is necessary.
Figure 5. Publication bias of literatures for allelic model of LMP2 CfoI and LMP7 G37360T was tested by Begg funnel plot and Egger test. A = LMP2 CfoI; B = LMP7 G37360T.
Author contributions
W.J. and X.Y. designed the study and wrote the manuscript, L.G. T. and Z.Y. retrieved the data base and extracted the whole data, L.Z.Z. and S.Z.R. evaluated the included studies, X.Y. revised the article. All the authors reviewed the article.

References
[1] Sutton DL, Lyle DM, Pierce JP. Incidence and prevalence of insulin-dependent diabetes mellitus in the zero- to 19-years’ age-group in Sydney. Med J Aust 2018;151:140–1.
[2] Pérezbravo F, Carrasco E, Gutierrezlópez MD, et al. Genetic predisposition and environmental factors leading to the development of insulin-dependent diabetes mellitus in Chilean children. J Mol Med (Berl) 1996;74:105–9.
[3] Yasunaga S, Kimura A, Hamaguchi K, et al. Different contribution of HLA-DR and -DQ genes in susceptibility and resistance to Insulin-dependent diabetes mellitus (IDDM). Tissue Antigens 2010; 47:37–48.
[4] Valdes AM, Mcweeney S, Thomson G. HLA class II DR-DQ amino acids and insulin-dependent diabetes mellitus: application of the haplotype method. Am J Hum Genet 1997;60:717–28.
[5] Fumio K, Yoshinori S, Monaco JJ. Genomic organization of the mouse LMP-2 gene and characteristic structure of its promoter. Gene 1993;133:243–8.
[6] Ozbas-Gerceker F, Micioglu D. LMP2 and LMP7 gene polymorphisms in the Southeastern Anatolia population of Turkey. Int J Hum Genet 2013;13:165–70.
[7] Frenzel S, Kuhn-Hartmann I, Gernold M, et al. The major-histocompatibility-complex-encoded beta-type proteasome subunits LMP2 and LMP7. Evidence that LMP2 and LMP7 are synthesized as proproteins and that cellular levels of both mRNA and LMP-containing 20S proteasomes are differentially regulated. Eur J Biochem 2010;216:119–26.
[8] Deng GY, Muir A, Maclaren NK, et al. Association of LMP2 and LMP7 genes within the major histocompatibility complex with insulin-dependent diabetes mellitus: population and family studies. Am J Hum Genet 1995;56:328–34.
[9] Chauffert M, Cissé A, Chevenne D, et al. Susceptibility to type 1 diabetes in the Senegalese population is linked to HLA-DQ and not TAP and LMP genes. Diabetes Care 1997;20:1299–303.
[10] Ding H, Cheng H, Fu Z. Relationship of polymorphism of LMP2 gene to insulin-dependent diabetes mellitus and DR3 gene. Nat Med J China 1999;79:28–30.
[11] Undlien DE, Akselsen HE, Joner G, et al. No independent associations of Lmp2 and Lmp7 polymorphisms with susceptibility to develop IDDM. Diabetes 1997;46:307–12.
[12] Van Endert PM, Liblau RS, Patel SD, et al. Major histocompatibility complex-encoded antigen processing gene polymorphism in IDDM. Diabetes 1994;43:110–7.
[13] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603–5.