Original Article

Association between the SUMO4 M55V Polymorphism and Susceptibility to Type 2 Diabetes Mellitus: A Meta-analysis*

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Abstract

Objective The aim of this study is to determine whether the SUMO4 M55V polymorphism is associated with susceptibility to type 2 diabetes mellitus (T2DM).

Methods A meta-analysis was performed to detect the potential association of the SUMO4 M55V polymorphism and susceptibility to T2DM under dominant, recessive, co-dominant (homogeneous and heterogeneous), and additive models.

Results A total of eight articles including 10 case-control studies, with a total of 2932 cases and 2679 controls, were included in this meta-analysis. The significant association between the SUMO4 M55V polymorphism and susceptibility to T2DM was observed in the dominant model (GG + GA versus AA: OR = 1.21, 95% CI = 1.05-1.40, P = 0.009), recessive model (GG versus GA + AA: OR = 1.29, 95% CI = 1.07-1.356, P = 0.010), homozygous model (GG versus AA: OR = 1.41, 95% CI = 1.06-1.56, P = 0.001), and additive model (G versus A: OR = 1.18, 95% CI = 1.08-1.29, P = 0.080). In subgroup analyses, significant associations were observed in the Chinese population under four genetic models excluding the heterozygous model, whereas no statistically significant associations were observed in the Japanese population under each of the five genetic models.

Conclusion The meta-analysis demonstrated that the G allele of the SUMO4 M55V polymorphism could be a susceptible risk locus to T2DM, mainly in the Chinese population, while the association in other ethnic population needs to be further validated in studies with relatively large samples.

Key words: SUMO4; Type 2 diabetes mellitus (T2DM); Polymorphisms; Meta-analysis

INTRODUCTION

Diabetes mellitus, a complex and chronic disease, has become a major public health problem and imposes new challenges on health systems[1]. The International Diabetes Federation’s latest assessment shows that 387 million people (8.3% of adults) suffer from diabetes, and the number of patients is expected to rise above 592 million by 2035[2]. Diabetes has had a

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Biographical note of the first author: ZHANG Qun, male, born in 1973, PhD, majoring in medical informatics.
massive impact on China in recent decades. Growing evidence shows that China has a very high incidence of diabetes, and approximately 92.4 million adults currently have diabetes in China[3]. Type 2 diabetes mellitus (T2DM), the most common form of diabetes, is characterized by insulin resistance[4]. Several studies have indicated that susceptibility genes and environmental factors play significant roles in the development of T2DM[5-6]. Multiple genes are thought to be involved, each producing a small effect on T2DM risk[7].

Despite the differing pathogenesis of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), epidemiological data have indicated that these two types of diabetes display familial clustering, which suggests a common genetic basis between them[8]. Several susceptibility genes have been reported to be associated with both T1DM and T2DM[9]. The small ubiquitin-like modifier (SUMO) is a protein that attaches to target proteins and controls the target proteins’ stability or activity[10]. SUMO4 is the fourth member of the SUMO family and is mainly expressed in the immune system[11]. The SUMO4 gene is located at a T1DM susceptibility locus (IDDM5) on chromosome 6q25, and a single nucleotide polymorphism (SNP) is associated with susceptibility to T1DM[11-13]. In vitro studies have shown that SUMO4 negatively regulates nuclear factor κB (NF-κB) transcriptional activity, which is a key transcriptional pathway involved in immune response and inflammation[14]. Inflammation induces inhibition of the insulin signaling pathway, which leads to insulin resistance and contributes to the development of T2DM[15].

Some epidemiology studies reported that the SUMO4 M55V polymorphism is associated with susceptibility to T2DM[15-19], while other studies did not favor the association[20-23]. For example, Nosu et al. (2009) reported that the SUMO4 M55V polymorphism is associated with an increased risk of T2DM in a population recruited from the western region of Japan[15], whereas other studies conducted in Wakayama and Toyoto did not confirm this association, neither in an allelic model, nor in additive, dominant, and recessive models[20]. Therefore, the association between the SUMO4 M55V polymorphism and T2DM remained controversial. Two separate meta-analyses were conducted to explore the association between the SUMO4 M55V polymorphism and T2DM, and revealed an association[24-25]. However, these studies only conducted meta-analyses based on alleles or combined genotypes, without taking genetic models into account.

The patterns of inheritance of the SUMO4 M55V polymorphism are not clear, and more than half of the studies did not support the association between the SUMO4 M55V polymorphism and T2DM. Therefore, we performed this updated meta-analysis to validate the association between the SUMO4 M55V polymorphism and T2DM under dominant, recessive, co-dominant (homozygous and heterozygous), and additive models.

METHODS

Search Strategy and Inclusion Criteria

A comprehensive literature search was conducted of the online databases MEDLINE (Medical Literature Analysis and Retrieval System Online), China National Knowledge Infrastructure (CNKI), Sinomed, and WanFang up to December 21, 2016. The following medical subject headings and keywords were used in the search strategy: ‘Small ubiquitin-like modifier 4’ or ‘SUMO4,’ ‘type 2 diabetes mellitus’ or ‘type 2 diabetes’ or ‘T2DM,’ and ‘rs237025’ or ‘Met55Val’ or ‘M55V,’ or ‘A163G.’ References lists of the retrieved articles and reviews were also screened for additional articles not captured by electronic search.

Eligible studies included in the meta-analysis met all the following criteria: (1) the associations of polymorphisms in the SUMO4 gene with T2DM; (2) case-control or cohort design; (3) provided odds ratio (OR) with 95% confidence interval (CI) or genotype frequency among case and control group; (4) study samples being unrelated individuals drawn from clearly defined populations; (5) written in English or Chinese; (6) for duplicate publications from the same population, only the paper that had the largest population, contained more useful information, or the latest one was selected. The exclusion criteria were defined as studies on animals, case reports, reviews, abstracts, editorial comments, and reports with incomplete data.

Data Extraction and Quality Appraisal

The following information was extracted: first author, year of publication, ethnicity, country of origin, sample size, age, gender, allele/genotypic frequencies and minor allele frequency in cases and controls and P-value for the allele frequency. The quality of studies was evaluated independently by
two investigators (ZHANG Qun and LIU Di) according to the Newcastle-Ottawa Scale (NOS). Uncertainties were resolved by discussions or by consensus with a third reviewer (ZHAO Zhong Yao). NOS evaluated studies with a star-rating system ranging from 0 (lowest) to 8 (highest) stars, which was based on three study components including selection, comparability, and outcome assessment. Studies with more than 5 points were evaluated as qualified.

**Statistical Analysis**

The associations of polymorphisms in the SUMO4 gene with T2DM were estimated by calculating pooled OR and 95% CI by using RevMan 5.3. Hardy-Weinberg equilibrium (HWE) among controls was evaluated by chi-square test and \( P < 0.05 \) was considered as significant disequilibrium. The chi-square test and the inconsistency index \( (I^2) \) were applied to assess heterogeneity among studies. The Z-test was used to calculate the \( P \)-value of the overall effect for the meta-analysis. Because the true effect size might differ from study to study, random effects models were used for combined data, regardless of whether or not heterogeneity was detected in the meta-analysis. Pooled OR and 95% CI were computed by the random-effects method of Mantel-Haenszel for combined data. Publication bias was evaluated by Begg’s funnel plot and Egger’s test. All the tests were two-sided, and results were considered statistically significant at \( P \leq 0.05 \) unless otherwise stated.

**RESULTS**

**Characteristics of Eligible Studies**

A total of 52 articles were identified after an initial search, 20 of which were duplicate articles (Figure 1). Twenty-three articles were further excluded because of duplicate sample use (5), animal or function studies (4), studies on other diseases (8), and review or comments (6) (Figure 1). From the above, we identified nine articles, which were included in the SUMO4 M55V analysis (Table 1). One study was further excluded because of significant disequilibrium in the Hardy-Weinberg equilibrium test. Finally, eight articles including 10 case-control studies, with a total of 2932 cases and 2679 controls, were included in this meta-analysis. The study quality is summarized in Table 2. The quality of the studies included in this meta-analysis was acceptable (at least 5 points).

![Figure 1. Flowchart of study selection.](image-url)
### Table 1. Characteristics of Case-control Studies Included in Meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Ethnicity/Region</th>
<th>Population</th>
<th>Group</th>
<th>Subject Size</th>
<th>Diagnosis</th>
<th>Genotyping Method</th>
<th>P-value for HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nosó S[15]</td>
<td>2007</td>
<td>Western area</td>
<td>Japanese</td>
<td>T2DM</td>
<td>355</td>
<td>ADA</td>
<td>Taqman SNP genotyping assay</td>
<td>0.690</td>
</tr>
<tr>
<td>Shimada T[20]</td>
<td>2009</td>
<td>Tokyo</td>
<td>Japanese</td>
<td>T2DM</td>
<td>451</td>
<td>WHO</td>
<td>Taqman SNP genotyping assay</td>
<td>0.408</td>
</tr>
<tr>
<td>Ji [17]</td>
<td>2010</td>
<td>Hunan</td>
<td>Chinese</td>
<td>T2DM</td>
<td>427</td>
<td>WHO</td>
<td>PCR-RFLP</td>
<td>0.830</td>
</tr>
<tr>
<td>Li B[21]</td>
<td>2011</td>
<td>Yunan</td>
<td>Chinese</td>
<td>T2DM</td>
<td>232</td>
<td>WHO</td>
<td>PCR-RFLP</td>
<td>0.260</td>
</tr>
<tr>
<td>Lin HY[16]</td>
<td>2007</td>
<td>Taiwan</td>
<td>Chinese</td>
<td>T2DM</td>
<td>574</td>
<td>NA</td>
<td>PCR-RFLP</td>
<td>0.193</td>
</tr>
<tr>
<td>Fallah S[12]</td>
<td>2010</td>
<td>Tehran</td>
<td>Irani</td>
<td>T2DM</td>
<td>50</td>
<td>NA</td>
<td>PCR-RFLP</td>
<td>0.493</td>
</tr>
<tr>
<td>Hu RT[23]</td>
<td>2009</td>
<td>Va</td>
<td>Chinese</td>
<td>T2DM</td>
<td>96</td>
<td>WHO</td>
<td>PCR-RFLP</td>
<td>0.487</td>
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<tr>
<td>Hu RT[23]</td>
<td>2009</td>
<td>Lalu</td>
<td>Chinese</td>
<td>T2DM</td>
<td>54</td>
<td>WHO</td>
<td>PCR-RFLP</td>
<td>0.088</td>
</tr>
<tr>
<td>Pu LM[18]</td>
<td>2012</td>
<td>Beijing</td>
<td>Chinese</td>
<td>T2DM</td>
<td>270</td>
<td>WHO</td>
<td>PCR-HRM</td>
<td>1.92 × 10⁻⁶</td>
</tr>
</tbody>
</table>


### Table 2. Assessment of Case-control Studies Using Newcastle-Ottawa Scale for Evaluating Methodological Quality

<table>
<thead>
<tr>
<th>Study</th>
<th>Case Definition</th>
<th>Representativeness of the Case</th>
<th>Selection of Controls</th>
<th>Definition of Controls</th>
<th>Comparability</th>
<th>Ascertainment of Exposure</th>
<th>Same Method of Ascertainment for Cases and Controls</th>
<th>Non-Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nosó S 2007[15]</td>
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<td>Shimada T 2009[20]</td>
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<td>Lin HY 2007[16]</td>
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<td>Li B 2011[21]</td>
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<td>Hu RT 2009[24]</td>
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</table>
Meta-analysis

Figure 2 presents the forest plot of the association between the SUMO4 M55V polymorphism (also named G163A in SUMO4, or rs237025) and T2DM in each study and the meta analysis. The significant association between the SUMO4 M55V polymorphism and susceptibility to T2DM was observed in the dominant model (GG + GA versus AA: \(OR = 1.21, 95\% CI = 1.05-1.40, P = 0.009\)), recessive model (GG versus GA + AA: \(OR = 1.29, 95\% CI = 1.06-1.56, P = 0.010\)), homozygous model (GG versus AA: \(OR = 1.41, 95\% CI = 1.15-1.71, P = 0.001\)), and additive model (G versus A: \(OR = 1.18, 95\% CI = 1.08-1.29, P = 0.001\)), and marginally significant in the heterozygous model (GA versus AA: \(OR = 1.16, 95\% CI = 0.98-1.36, P = 0.080\)).

No significant subgroup differences were observed (\(P\) ranged from 0.52 to 0.56 in all five genetic models). Subgroup analysis showed significant associations between the SUMO4 M55V polymorphism and susceptibility to T2DM in the Chinese population under all but the heterozygous model (GA versus AA: \(OR = 1.12, 95\% CI = 0.86-1.44, P = 0.090\)) and marginally significant associations in the Japanese population (\(P\) ranged from 0.13 to 0.40 in all five genetic models).

Evaluation of Publication Bias and Sensitivity

Funnel plot asymmetry was evaluated by Egger’s regression test. If the line passed through the origin, it would indicate the absence of publication bias. The funnel plots and Egger’s linear regression test are shown in Figure 3. No publication biases were detected under the five genetic models (all \(P > 0.05\)).

A sensitivity analysis was conducted to explore the sources of heterogeneity, and the results showed that no single study affected the pooled \(OR\) and \(CIs\) in a qualitative manner.

**DISCUSSION**

Our study found a statistically significant association between the SUMO4 M55V polymorphism and T2DM under dominant, recessive, co-dominant (homozygous and heterozygous), and additive models, especially in the Chinese population. In addition, publication bias tests and sensitivity analysis showed that the overall results were robust. The implication of this finding is that subjects with the G allele in the SUMO4 M55V polymorphism are at a high risk of developing T2DM late in life.

The meta-analysis demonstrated that the SUMO4 M55V polymorphism was associated with T2DM, consistent with several domestic and foreign reports\(^{15-19}\). In subjects living in the western region of Japan, Noso et al. (2007)\(^{15}\) found that the frequency of the G allele was higher in T2DM patients (\(P < 0.05\)), and the frequency of the GG and GA genotypes in the case group was also higher than in controls, whereas these findings were not validated in subjects recruited from Wakayama and Toyoto of Japan\(^{20}\). A study in the Taiwan, China population showed that SUMO4 M55V polymorphism G allele carriers have a higher risk of suffering from T2DM\(^{16}\), as did two studies conducted...
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in Beijing and Hubei\(^{[17-18]}\). However, studies conducted in Han Chinese and two ethnicities (Va, Lahu) recruited from the Yunnan province of China did not demonstrate the same association\(^{[21-23]}\). In addition, Fallah et al. (2010) found that the SUMO4 M55V polymorphism was not associated with T2DM in the Iranian population\(^{[22]}\), while Sozen et al. (2014) reported that the SUMO4 M55V polymorphism was associated with T2DM in the Turkish population\(^{[19]}\). The study conducted in Turkey was excluded from this meta-analysis because of the significant Hardy-Weinberg disequilibrium, while the study conducted in Iran may have lacked statistical power (50 in each of disease and control groups). This disparity in the association between the SUMO4 M55V polymorphism and T2DM between studies might be caused by heterogeneity in ethnicity and genetic background\(^{[26]}\). In addition, the relatively small sample size in several studies also contributes to this discrepancy.

Genome-wide scans for T2DM revealed linkage of T2DM at chromosome 6q, where a susceptibility gene for T2DM (1DDM5) was mapped in African-American\(^{[27]}\), Chinese\(^{[28]}\) and Finnish populations\(^{[29]}\). In addition, strong associations between the SUMO4 M55V polymorphism and T2DM were validated in several meta-analyses\(^{[24,30]}\). The consistency in the association between the SUMO4 M55V polymorphism and T1DM or T2DM suggested that SUMO4 may explain the common genetic predisposition to T1DM and T2DM.

SUMO4 regulates the activation of \(\text{I} \kappa \text{B}\alpha\) by sumoylation, and thus negatively regulates the activity of \(\text{NF}-\kappa\text{B}\)\(^{[31]}\). \(\text{I} \kappa \text{B}\alpha\) inhibits \(\text{NF}-\kappa\text{B}\) by masking the nuclear localization signals (NLS) of \(\text{NF}-\kappa\text{B}\) proteins and keeping them sequestered in an inactive state in the cytoplasm; it also blocks the ability of \(\text{NF}-\kappa\text{B}\) transcription factors to bind to DNA, which is required for proper functioning of \(\text{NF}-\kappa\text{B}\)\(^{[32]}\). \(\text{NF}-\kappa\text{B}\), a transcriptional factor for inflammation, is relevant to the pathogenesis of diabetes mellitus\(^{[33]}\). In addition, the interaction between \(\text{NF}-\kappa\text{B}\) and \(\text{I} \kappa \text{B}\alpha\) result in the insulin resistance in \emph{vivo} and \emph{in vitro} studies\(^{[34-37]}\). Furthermore, the association of SUMO4 Met55Val variation with increased insulin resistance was reported in newly diagnosed T2DM in a Chinese population\(^{[38]}\). Therefore, the Met55Val variation in SUMO4 may affect its capacity to modify \(\text{I} \kappa \text{B}\alpha\), leading to increased activity of \(\text{NF}-\kappa\text{B}\) and increased insulin resistance and thus increase the risk of diabetes mellitus.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Publication biases indicated by the funnel plots. Figure A, dominant model, \(P = 0.655\) and 0.550 in Begg’s and Egger’s test, respectively. Figure B, recessive model, \(P = 0.245\) and 0.063 in Begg’s and Egger’s test, respectively. Figure C, homozygous model, \(P = 0.074\) and 0.131 in Begg’s and Egger’s test, respectively. Figure D, heterozygous model, \(P = 0.655\) and 0.237 in Begg’s and Egger’s test, respectively. Figure E, additive model, \(P = 0.788\) and 0.866 in Begg’s and Egger’s test, respectively.}
\end{figure}
However, there are two limitations to our meta-analysis. First, confounding factors were not accounted for because most of the studies did not consider these. Unmeasured confounding factors might affect the observed association. Secondly, heterogeneity might lead to publication bias, because studies with negative results might not be published. Although the funnel plots and Egger’s linear regression test indicated no biases, it is possible that the relatively small number of studies included in this meta-analysis led to less statistical power to detect publication biases.

To summarize, our meta-analysis demonstrated that the G allele in the SUMO4 M55V polymorphism may be a susceptible risk locus to T2DM, especially in the Chinese population. The disparity among studies suggests that further investigations are required on the associations between the SUMO4 M55V polymorphism and T2DM susceptibility in different ethnic groups. The SUMO4 M55V polymorphism might be a causal factor in T2DM via its regulation of NFκB signaling.

AUTHORS CONTRIBUTIONS

ZHANG Qun designed the study, collected the data, and wrote the manuscript; LIU Di, ZHAO Zhong Yao, and SUN Qi collected and analyzed the data; DING Lixiang designed the study. WANG You Xin designed the study, revised the manuscript.

CONFLICT OF INTEREST

All authors have no conflicts of interest.

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REFERENCES

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