

ORIGINAL INVESTIGATION

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Proteasome modulator 9 and macrovascular pathology of T2D

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Abstract

Aims: Coronary artery disease (CAD) and stroke share a major linkage at the chromosome 12q24 locus. The same chromosome region entails at least a major risk gene for type 2 diabetes (T2D) within NIDDM2, the non-insulin-dependent-diabetes 2 locus. The gene of Proteasome Modulator 9 (*PSMD9*) lies in the NIDDM2 region and is implicated in diabetes in mice. *PSMD9* mutations rarely cause T2D and common variants are linked to both late-onset T2D and maturity-onset-diabetes of the young (MODY3). In this study, we aimed at determining whether *PSMD9* is linked to macrovascular pathology of T2D.

Methods and Results: In our 200 T2D families from Italy, we characterized the clinical phenotype of macrovascular pathology by defining the subjects for presence or absence of CAD, stroke and/or transitory ischemic attacks (TIA), plaques of the large arterial vessels (macro-vasculopathy) and arterial angioplasty performance. We then screened 200 T2D siblings/families for *PSMD9* +nt460A/G, +nt437C/T and exon E197G A/G single nucleotide polymorphisms (SNPs) and performed a non-parametric linkage study to test for linkage for coronary artery disease, stroke/TIA, macro-vasculopathy and macrovascular pathology of T2D. We performed 1,000 replicates to test the power of our significant results. Our results show a consistent significant LOD score in linkage with all the above-mentioned phenotypes. Our 1000 simulation analyses, performed for each single test, confirm that the results are not due to random chance.

Conclusions: In summary, the *PSMD9* IVS3+nt460A/G, +nt437C/T and exon E197G A/G SNPs are linked to CAD, stroke/TIA and macrovascular pathology of T2D in Italians.

Introduction

Type 2 diabetes (T2D) has long-term complications, both microvascular and macrovascular. The microvascular complications entail the diabetic retinopathy, diabetic neuropathy and diabetic nephropathy. The macrovascular complications are represented by coronary artery disease (CAD), stroke, transitory ischemic attacks (TIA), and atherosclerotic plaques in major arterial vessels. The history of arterial angioplasty in a patient also implicates the presence of macro-vasculopathy. Given the high impact of macrovascular pathology on life quality and life expectancy, it is quite important to identify risk genes contributing to such polygenic and complex phenotype.

Recently, a study reported a significant linkage to coronary artery disease (CAD) [1], myocardial infarction [1,2], stroke [3] to the chromosomal locus 12q24, within the NIDDM2 locus in which *PSMD9* is located.

PSMD9 unique mutations rarely cause T2D [4]. We identified a significant linkage of *PSMD9* common single nucleotide polymorphism (SNPs) to late-onset T2D, via recessive/additive model [5], as well as to early-onset T2D/MODY3 via additive model in Italians families [6]. The *PSMD9* role in beta cell dysfunction and T2D in mice is known [7].

The aim of the present study was to determine whether the IVS3+nt460A/G, +nt437C/T and exon E197G A/G T2D risk SNPs of *PSMD9* cause evidence for linkage with other T2D-associated phenotypes such as CAD, stroke/TIA, vasculopathy and macrovascular pathology of T2D in Italians.

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Methods

Ethical Statement

The subjects were all recruited from Rome, Italy following the Helsinki declaration guidelines. Subjects gave written informed consent and the Penn State College of Medicine Ethical Committee approved the study.

Families

We previously recruited 200 Italian T2D affected siblings and families with both T2D affected and unaffected members [8]. We excluded all families with identical twins, both parents affected and genetic admixture with no Italians. We confirmed that the families were for at least three generations Italians. In addition, the Italians are suitable for genetic studies as they are a homogeneous population from a geographically restricted peninsula.

Clinical Phenotyping

We characterized the family subjects with T2D and without T2D for presence/absence of coronary arterial disease (CAD: angina or myocardial infarction/coronary angioplasty/presence of pathological Q waves on the electrocardiogram), cerebrovascular events (stroke/transitory ischemic attacks), vasculopathy (plaque(s) on carotid arteries/lower extremities arteries, and/or arterial angioplasty), and macrovascular pathology (vasculopathy and/or CAD and/or cerebrovascular events). Phenotypes of macrovascular pathology are described as unknown if diagnosis is unclear or data are lacking; subjects are described as affected if they carry the phenotype under study and as unaffected if they do not have the phenotype. In our 200 Italian families, for each phenotype, data are less than 100%.

Sequencing

We sequenced the *PSMD9* *IVS3+nt460/+nt437/E197G* SNPs in all family members. We directly sequenced the PCR products, status post-purification via EXOSAP-IT on an automated ABI 3730 Sequencer.

Statistical Analysis

We tested in the 200 Italian families for linkage of the *PSMD9* SNPs with all described phenotypes. The three SNPs studied are in linkage disequilibrium as previously shown [4]. The map distance between each SNP pair given for the linkage analysis was 0.1 cM. We performed our statistical analysis using the latest version of Merlin software [9] and the allelic information of a total of 200 genotyped families. Merlin analyzed all the informative families within this dataset. Non-parametric linkage analysis was performed for the *IVS3+nt460A/G*, *+nt437C/T* and exon *E197G A/G* SNPs. Each phenotype was tested independently using for the above-mentioned three

SNPs the same map distance (cm 139.1, 139.2, 139.3 on the same chromosome, respectively). Merlin calculated the linkage score for each of these SNPs in linkage disequilibrium as a single locus, as it reported only one LOD score per statistical test, which implies that Merlin considered the SNPs located on a single locus.

All results are reported as LOD scores calculated by Merlin. For each test performed for each specific phenotype, to exclude false positive results, we calculated the empirical P-value in 1,000 replicates of simulations by using the gene dropping method: this analysis replaces real data with simulated data, while maintaining the pedigree structure, allele frequencies and recombination fraction. These datasets are generated under the null hypothesis of no linkage. The empirical P-value for each phenotype-specific test was calculated based on the P-value corresponding to the single LOD score given by Merlin for the three SNPs analysis at one locus and using the same map distance of the real analysis. Further, the outcome of the simulations was calculated taking in consideration how many times in 1,000 replicates there was a P-value equal to or smaller than the P-value of the real analysis.

Results

The LOD scores of the non-parametric linkage analysis, performed for the various phenotypes, are reported, jointly for the three tested SNPs, with their corresponding P-value as well as with the results of the 1000 simulations (Table 1). Linkage is shown significantly for the CAD phenotype, the stroke/TIA phenotype and the macrovascular pathology phenotype and with a less significant empirical P-value with the vasculopathy phenotype.

Discussion

Our study shows that the *PSMD9* *IVS3+nt460A/G*, *+nt437C/T* and exon *E197G A/G* SNPs studied are in linkage with CAD, cerebrovascular events, vasculopathy, and macrovascular pathology. The simulation analyses rule out false results for the linkage to CAD, stroke and macrovascular pathology at the significance level of at least $\alpha = 0.005$, however the significance level of the vasculopathy linkage is only at $\alpha = 0.053$. Thus, *PSMD9* or any of its variants contribute to linkage of the reported phenotypes.

Of note, the potential contribution of intronic variants to complex disorders such as atherosclerosis and/or major cardiovascular events is widely accepted.

However, we think it is important to take in consideration that the linkage signal detected for the macrovascular pathology phenotypes in our study may indeed reflect the underlying linkage for T2D [5]. In fact, we recognize that it is difficult to disentangle associated

Table 1 Non-parametric Linkage Analysis of Various Phenotypes of the 200 Italian Families by Merlin software

Phenotype	Prevalence	Families	Lod Score	P-value	Empirical P-value
CAD	28.20%	15	1.39	0.006	0.005
Stroke	14.80%	5	1.04	0.014	0.002
Vasculopathy	53.80%	48	0.64	0.040	0.053
Macrovascular Pathology	70.10%	78	2.21	0.0007	0.002

Prevalence = phenotype prevalence among the family subjects studied; Families = families number analyzed; Lod score = derived from the non-parametric linkage analysis by Merlin; Empirical P-value = derived from 1000 replicates by using the gene dropping method; CAD = coronary artery disease; Vasculopathy = based on the presence of plaques and/or angioplasty of carotid arteries, coronary arteries, lower extremities arteries; Macrovascular Pathology = based on the presence of CAD and/or Stroke/TIA and/or Vasculopathy.

complex phenotypes in a family-based linkage study targeted at one phenotype only at a time. Despite the necessity to raise concerns about the potential implications of the statistical data, we think that it is possible that a gene responsible for T2D is also contributing to macrovascular disease. This interpretation is based on the fact that the two complex phenotypes are strongly associated as well as that the same locus is linked in multiple studies to T2D [10,11] and macrovascular disease [3,1].

PSMD9 gene has the potential of having pleiotropic effects. Recently, we published the contribution of the same gene to T2D-nephropathy [12], which is a microvascular complication of T2D and is linked to the 12q24 locus [13].

Our results should be replicated in different T2D ethnic groups and are of relevance to the T2D families as well as to the families with macrovascular pathology without T2D carrying linkage in the chromosome 12q24 locus, which may be explained by the *PSMD9* variants, at least partially. This locus in fact was reported as linked to macrovascular pathology in other populations [1-3]. In addition, we hereby prove the strength and validity of the SNP-based linkage approach in identifying genes in complex disorders. Microsatellite used mostly in the past as markers for linkage studies in polygenic diseases should be revisited, as they may have disempowered the linkage strategy. Also, family-based linkage studies are stronger in their statistical power compared to association studies. Our Italian family dataset is ethnically homogenous, thus it allows significant results to be revealed. The mechanism by which the *PSMD9* protein may contribute to the macrovascular pathology is unknown at the present time.

Lifetime expectancy is highly affected by the presence of atherosclerosis and macrovascular pathology, thus the impact of the identification of a risk gene to macrovascular pathology is inestimable.

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Authors' contributions

CG conceived and designed the study, collected the clinical information, performed the statistical analysis and drafted the manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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