The acetyl-coenzyme A carboxylase beta (ACACB) gene is associated with nephropathy in Chinese patients with type 2 diabetes

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Abstract

Background. A single-nucleotide polymorphism (SNP), rs2268388, in the acetyl-coenzyme A carboxylase beta (ACACB) gene is associated with susceptibility to type 2 diabetic nephropathy (T2DN) in Japanese and European-American populations. Whether this association also exists in Chinese patients is unclear. Attempts at replication in small Singaporean and Korean samples were not significant.

Methods. Eight ACACB SNPs were genotyped in 595 subjects with type 2 diabetes mellitus born in Hong Kong or southern China, 295 with advanced T2DN and 300 with long-standing diabetes lacking nephropathy. Association analyses were focused primarily on SNP rs2268388 and secondarily on flanking SNPs and haplotypes.

Results. Adjusting for age, gender and diabetes duration, ACACB SNP rs2268388 was significantly associated with advanced T2DN (odds ratio = 2.39; recessive model; P = 0.0129).

Conclusion. These results in the Chinese replicate the association between T2DN and rs2268388, as seen in Japanese and European Americans. The ACACB gene and attendant alterations in fatty acid oxidation may play important roles in susceptibility to T2DN. Targeting this pathway may provide novel treatment options for the prevention of diabetic nephropathy.

Keywords: ACACB; Chinese; diabetic nephropathy; kidney; type 2 diabetes mellitus

Introduction

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD) in developed countries where type 2 diabetes mellitus (T2DM) has reached epidemic proportions. Although the exact pathogenesis of type 2 diabetic nephropathy (T2DN) is not fully understood and is likely diverse in nature, there are convincing data that genetic susceptibility plays an important role. Maeda et al. [1–3] performed a genome-wide association study (GWAS) using gene-based single-nucleotide polymorphisms (SNPs) in Japanese T2DN subjects and identified genes encoding solute carrier family 12 (sodium/chloride) member 3 (SLC12A3), engulfment and cell motility 1 (ELMO1) and neurocalcin δ (NCALD) as associated with T2DN. The ELMO1 association has since been replicated
in African Americans with T2DN [4] and European Americans with type 1 DN [5].

More recently, a single SNP in intron 18 of the acetyl-coenzyme A carboxylase beta (ACACB) gene was found to be associated with proteinuria in Japanese patients [6]. The frequency of the T allele for SNP rs2268388 was consistently higher in Japanese patients with T2DN and overt proteinuria, an observation that was replicated in European Americans with T2DN-associated ESRD and in an Asian meta-analysis for proteinuria [6]. This SNP was not in linkage disequilibrium with any other known HapMap SNPs in ACACB across different populations.

The present study tested for the association between the ACACB gene and DN in Chinese patients with T2DM to determine whether the association between the ACACB polymorphism observed in Japanese and European Americans with DN also exists in the Chinese.

Research design and methods

Participants

Self-described Chinese subjects born in Hong Kong or southern China formed the study sample. Peripheral blood samples for DNA extraction were collected from unrelated, prevalent ESRD patients on peritoneal dialysis, haemodialysis, after renal transplantation, with renal biopsy-proven DN plus urine albumin excretion >3 g/24 h or deemed unfit for renal replacement therapy with a serum creatinine concentration >4.5 mg/dL, who fulfilled the inclusion criteria after medical record review by a single investigator (S.C.W.T.). T2DM was clinically diagnosed in those treated with oral hypoglycaemic agents and/or insulin, in the absence of insulin-only treatment for more than the first year after diagnosis. DN was confirmed by either renal biopsy or the clinical criteria of T2DM duration ≥5 years prior to ESRD with diabetic retinopathy and/or historic overt proteinuria ≥2 g/24 h or urine protein-to-creatinine ratio ≥3.0 g/g, or T2DM duration <5 years prior to ESRD with diabetic retinopathy and historic overt proteinuria in the absence of other causes of ESRD. Subjects with T2DM lacking nephropathy who had diabetes for at least 10 years with a spot urine albumin-to-creatinine ratio <30 mg/g or negative dipstick for microalbumin, and serum creatinine concentration <1.5 mg/dL in men or <1.3 mg/dL in women. The study was approved by the Institutional Review Boards at The University of Hong Kong/Hospital Authority Hong Kong West, Kowloon Central and East Clusters of Hospitals, and met the criteria outlined in the Declaration of Helsinki. All participants provided written informed consent.

Genetic analyses

Eight SNPs were genotyped in the 595 Chinese subjects: SNPs were chosen based on prior gene-based GWAS evaluating 80 000 SNPs by Maeda et al. [6] who identified SNP rs2268388 in Japanese, Singaporean, Korean, European and European-American samples, alongside seven additional SNPs without association in ACACB flanking this marker. We listed the results of all these eight SNPs to allow cross-comparison. All SNPs had minor allele frequencies >0.05. SNPs were genotyped using the MassARRAY genotyping system (Sequenom Inc., San Diego, CA). Polymerase chain reaction primers were designed using the MassARRAY Assay Design 3.4 Software (Sequenom Inc., San Diego, CA) and will be provided upon request. SNPs that had genotyping success rates lower than 99% were excluded from further analysis.

Statistical analyses

Each SNP was tested for departures from Hardy–Weinberg equilibrium (HWE) expectations via a chi-square goodness-of-fit test [7]. All tests for association between T2DN and a SNP were computed using a logistic regression model that adjusted for age, gender and duration of T2DM. For each SNP, the two degrees of freedom and the three a priori genetic models (dominant, additive and recessive) were computed. If the overall two degrees of freedom test of genotypic association was significant, then three a priori genetic models were examined for association. This is consistent with the Fisher’s protected least significant difference multiple comparisons method, and the additional seven polymorphisms apart from the SNP of major interest were compared to a Bonferroni-adjusted P-value of 0.0007 [8]. The power to detect an association at the flanking SNPs varies by minor allele frequency (MAF). For SNPs with MAF=0.10 and assuming a type 1 error rate of 0.05, the power for an additive genetic model was 0.23, 0.64 and 0.80 for odds ratios of 1.25, 1.50 and 1.64, respectively. Similar calculations for MAF=0.25 yielded power estimates of 0.40 and 0.80 for odds ratios of 1.25 and 1.44, respectively. All statistics were computed using the programme SNPGWA (http://www.phs.wfubmc.edu). In addition, the two- and three-marker haplotype test was computed using the expectation-maximization algorithm as implemented in SNPGWA.

Results

DNA from 295 Chinese cases with T2DN and 300 T2DM controls lacking nephropathy were genotyped for eight ACACB SNPs. Of the cases with T2DN, 60% were male and the demographic data for all subjects are shown in Table 1. Cases with DN consisted of 174 subjects on dialysis, 26 after kidney transplantation, 79 who met the study criteria with serum creatinine concentrations >4.5 mg/dL but who declined dialysis or were deemed unsuitable and 16 had kidney biopsy evidence of DN with >3 g/day proteinuria (8 of these 16 subjects had serum creatinine values above 2 mg/dL at the time of biopsy). Genotyped SNPs spanned 63.2 kb of ACACB. Genotyping success rates in all SNPs exceeded 99%. No SNPs were out of HWE in controls. The genotype frequencies for the individual SNPs are shown in Table 2.

Table 3 summarizes the allele frequencies and results of the association analyses in T2DN cases versus T2DM non-nephropathy controls. The previously implicated SNP rs2268388 showed significant evidence of association with T2DN in the overall genotypic test of association (P-value=0.0227), with the recessive genetic model showing the evidence of association (odds ratio=2.39, P-value=0.0129 after adjustment for age, gender and diabetes duration). None of the other seven SNPs genotyped provided evidence of association and the haplotype analysis did not provide evidence of haplotypic association (data not shown).

Discussion

In this study, the ACACB association with T2DN observed in Japanese subjects is replicated for the first time in

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Table 1. Demographic characteristics of diabetic cases and controls

<table>
<thead>
<tr>
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<th>T2DN cases, N=295</th>
<th>T2DM non-nephropathy controls, N=300</th>
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<tbody>
<tr>
<td>Sex, % female</td>
<td>40%</td>
<td>58%</td>
</tr>
<tr>
<td>Age at blood draw (years)</td>
<td>65.0 (66) ± 11.8</td>
<td>68.3 (69) ± 9.4</td>
</tr>
<tr>
<td>Age at ESRD diagnosis (years)</td>
<td>62.0 (63) ± 13.8</td>
<td>NA</td>
</tr>
<tr>
<td>BMI at recruitment (kg/m²)</td>
<td>25.3 (25) ± 4.6</td>
<td>23.9 (23) ± 3.6</td>
</tr>
<tr>
<td>Duration of ESRD (years)</td>
<td>2.8 (1.4) ± 4.4</td>
<td>NA</td>
</tr>
<tr>
<td>Duration of T2DM (years)</td>
<td>15.5 (14) ± 8.3</td>
<td>15.4 (13) ± 6.2</td>
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*aMean (median) ± SD.
Chinese subjects. Our findings suggest that a SNP within ACACB (rs2268388, intron 18 + 4139 C > T) increases susceptibility to development of severe nephropathy in Chinese patients with T2DM by more than 2-fold per copy of the risk variant. These results are particularly important since 94.6% (279/295) of these Chinese cases had ESRD, replicating the association results in dialysis patients. However, the association was also observed in cases with proteinuria and diabetes (without reduced GFR or ESRD) among Japanese, Singaporean, and Koreans with T2DM. In summary, SNP rs2268388 in ACACB confers susceptibility to DN through altered fatty acid oxidation and lipid accumulation in renal tissue.

There are limitations to this study. Although similar to other published reports, the relatively modest sample size limits our statistical power to convincingly distinguish among the different genetic models. Here, there was evidence for a recessive genetic model, whereas the previous study reported allelic and additive genetic models [6]. Our sample contained 42 subjects homozygous for the T risk allele in rs2268388; hence, the statistical analysis of the recessive model is robust. Our calculation of the recessive model for the data provided in the previous study [6] shows a weaker but still present association under a recessive model (P=0.0088). Finally, survival bias could affect the association results in dialysis patients. However, the fact that association was also observed in cases with proteinuria and diabetes (without reduced GFR or ESRD) from Maeda et al. [6] supports true association.

**Conclusion**

In summary, SNP rs2268388 in ACACB confers susceptibility to DN in Chinese patients with T2DM, extending the original ACACB association with proteinuria in the Japanese and with ESRD in European Americans with T2DN. The ACACB genetic association appears to be with severe and progressive nephropathy, as evidenced by this and the prior European-American report. Fatty acid oxidation pathways have been implicated in the development of
obesity and metabolic syndrome; however, this replicated genetic association suggests that it makes important contributions to nephropathy susceptibility in Asian- and European-derived individuals with T2DM. Novel treatments to prevent diabetic kidney disease could result from this observation.

Acknowledgments. This study was supported by the Seed Funding Programme for Basic Research of the University of Hong Kong (S.C.W.T.) and NIH grants R01 DK053591 (D.W.B.) and R01 DK070941 (B.I.F.). The authors are grateful to Wendy W.S. Tsui, Desmond Y.H. Yap, Maggie K.M. Ma, all Sai Ying Pun GOPC doctors and nurses for helping with patient recruitment, to Anita Tsang for specimen sorting, to Sandra Luen for coordination and to all the study participants.

Conflict of interest statement. None declared.

References

Race differences in prevalence of chronic kidney disease among young adults using creatinine-based glomerular filtration rate-estimating equations

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Abstract

**Background.** Despite a higher incidence of end-stage renal disease (stage 5), blacks have been shown to have the same or lower prevalence of chronic kidney disease (CKD stages 3 and 4). Current creatinine-based glomerular filtration rate (GFR)-estimating equations may misclassify young, healthy blacks.

**Methods.** Among 3501 young adults (mean age 45), we compared the prevalence of CKD in blacks and whites using the Modification of Diet in Renal Disease (MDRD) and the CKD Epidemiology Collaboration (CKD-EPI) equations. In addition, we used measured creatinine excretion rates to determine the actual excretion ratio for CARDIA (race coefficient 12%) and applied this to the CKD-EPI equation.