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Genetic analysis of coronary artery disease single-nucleotide polymorphisms in diabetic nephropathy

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Abstract

Background. Diabetic nephropathy is a leading cause of end-stage renal disease. Premature mortality is common in patients with nephropathy, largely due to cardiovascular disease. Genetic variants implicated in macrovascular disease are therefore excellent candidates to assess for association with diabetic nephropathy. Recent genome-wide association studies have identified a total of 15 single-nucleotide polymorphisms (SNPs) that are reproducibly associated with cardiovascular disease.

Methods. We initially assessed these SNPs for association in UK type 1 diabetic patients with (cases; $n = 597$) and without (controls; $n = 502$) nephropathy using iPLEX™ and TaqMan® assays. Replication studies were performed with DNA genotyped in a total of 2668 individuals from the British Isles.

Results. One SNP (rs4420638) on chromosome 19q13 was found to be significantly associated with diabetic nephropathy before ($P = 0.0002$) and after correction for multiple testing ($P_{corrected} = 0.002$). We replicated this finding in a phenotypically similar case–control collection comprising 709 individuals with type 1 diabetes ($P = 0.002$; combined $P < 0.00001$; OR = 1.54, 95% CI: 1.29–1.84).

Conclusions. Our case–control data suggest that rs4420638, or a functional SNP in linkage disequilibrium with this SNP, may be associated with diabetic nephropathy.

Keywords: apolipoprotein; association; diabetic nephropathy; genetics; single-nucleotide polymorphisms

Introduction

Diabetic nephropathy is now the leading cause of end-stage renal disease in developed countries [1], and is associated with a 10-fold increased risk of macrovascular events [2]. Family studies have also shown that parents of individuals with diabetic nephropathy are more susceptible to hypertension, cardiovascular and cerebrovascular disease with associated premature mortality [3,4]. Aggressive management of hyperglycaemia, hypertension and cardiovascular risk factors in individuals with diabetic nephropathy has been recommended by the UK National Service Frameworks for diabetes and renal services [5].

Diabetic nephropathy exhibits a multifactorial aetiology, but determining definitive genetic risk factors has proved challenging [6,7]. Several recent large-scale association studies [8–10] have identified 15 single-nucleotide polymorphisms (SNPs) that demonstrated strong prior evidence of association or were robustly replicated loci associated with an increased risk of cardiovascular disease. We have assessed these SNPs for association with diabetic nephropathy in white individuals recruited as part of multicentre collections in the British Isles.

Subjects and methods

Approval to conduct this study was obtained from the appropriate Research Ethics Committees, and all those recruited provided written informed consent. We obtained a total of 1099 genomic DNA samples from white, unrelated individuals with type 1 diabetes diagnosed before 31 years of age and who were insulin dependent from diagnosis. This population includes individuals recruited as part of the UK Warren 3/Genetics of Kidneys in Diabetes (GoKinD) collection [11]. Cases ($n = 597$) had onset of persistent proteinuria (>0.5 g/24 h) at least 10 years after diagnosis of diabetes with evidence of hypertension (>135/85 mmHg or treatment with antihypertensive medication) and retinopathy. Controls ($n = 502$) had diabetes for more than 15 years, with no evidence of renal disease and were not prescribed antihypertensive medication.

Genomic DNA was also available from 709 white individuals of Irish descent (parents and grandparents born in the island of Ireland) [12]. Cases ($n = 267$) and controls ($n = 442$) were recruited with the same inclusion/exclusion criteria as the UK population except that these individuals had type 1 diabetes diagnosed before 35 years of age. Similar clinical characteristics were observed for both groups (Table 1). Genomic DNA was also available on 122 parent-affected offspring trios derived from the UK Warren 3 collection; affected offspring were recruited according to the same criteria as described above for UK cases (http://www.diabetes.org.uk/en/About_us/News_Landing_Page/2865). To minimize population heterogeneity, all recruited individuals were at least second generation born in the British Isles, with similar phenotypic inclusion criteria. Individuals with microalbuminuria were excluded from all collections.

Genotyping and data analysis

Genotyping of genomic DNA samples was performed using the MassARRAY iPLEX™ assay (Sequenom, San Diego, CA, USA) for 13 SNPs, and a further two SNPs were genotyped using commercially available

Table 1. Clinical characteristics of individuals with type 1 diabetes recruited to the case–control groups

	UK collection		All Ireland collection	
	Case	Control	Case	Control
Number of individuals	597	502	267	442
Age at diagnosis (years)	14.5 (± 7.6)	15.6 (± 8.0)	16.7 (± 11.5)	15.0 (± 8.1)
BMI (kg/m^2)	26.4 (± 4.8)	26.1 (± 4.2)	25.7 (± 4.8)	26.3 (± 4.2)
Duration of diabetes (years)	33.9 (± 9.3)	29.2 (± 9.1)	32.0 (± 9.3)	27.2 (± 9.3)
HbA_{1c} (%)	8.6 (± 1.7)	8.2 (± 1.4)	10.6 (± 1.5)	9.5 (± 1.2)
Systolic BP (mmHg)	136.0 (± 30.4)	127.0 (± 15.5)	146.6 (± 20.1)	123.6 (± 13.6)
Diastolic BP (mmHg)	80.9 (± 11.6)	75.3 (± 8.5)	84.7 (± 10.5)	76.0 (± 6.9)

Table 2. Location, frequency and significance values of genotyped SNPs in the UK case–control dataset

Unique SNP identifier	Alleles	Chromosome location	Affiliated gene	Minor allele frequency (%)	*P* value (genotype)	*P* value (allele)
rs4420638 [8]	G/A	19q13	APOC1	19	0.0003546	0.0001526[b]
rs17465637 [9]	A/C	1q41	MIA3	28	0.02182	0.009071[c]
rs2383206[a] [10]	A/G	9p21	None	49	0.4589	0.2898
rs10757274 [10]	G/A	9p21	None	49	0.6197	0.3379
rs2943634 [9]	A/C	2q36	None	34	0.5925	0.3094
rs6922269 [8,9]	A/G	6q25	MTHFD1L	29	0.4176	0.3462
rs383830 [8]	T/A	5q21	None	21	0.04421	0.3529
rs501120 [9]	G/A	10q11	None	14	0.4317	0.5454
rs688034 [8]	T/C	22q12	SEZ6L	34	0.4492	0.4682
rs17672135 [8]	C/T	1q43	FMN2	12	0.7447	0.6367
rs17228212 [9]	C/T	15q22	SMAD3	30	0.6222	0.662
rs8055236 [8]	T/G	16q24	CDH13	20	0.9462	0.7458
rs599839[a] [9]	G/A	1p13	PSRC1	22	0.9055	0.7927
rs1333049 [8,9]	C/G	9p21	None	47	0.5752	0.7858
rs7250581 [8]	A/G	19q12	None	20	0.7754	0.9058

[a]SNPs genotyped by TaqMan.
[b]$P_{corrected} = 0.002$.
[c]$P_{corrected} = 0.1$.
Initially, the genotypic test (2df, –model –cell 5) was used to provide a general test of association for the single phenotype (case/control) of diabetic nephropathy. The basic allelic chi-square test (1df, –assoc) was used to provide asymptotic *P*-values. Adjustment for multiple testing was performed using Bonferroni's method.

TaqMan® kits (Applied Biosystems, Warrington, UK), as previously described [11]. Quality checks of genotyping data were performed, including individual and SNP-based call rates, departures from the Hardy–Weinberg equilibrium, duplicate error rates and Mendelian inconsistencies.

Statistical analysis

Analyses were performed using the standard tests implemented in PLINK [13]. Initially, the genotypic test (2df, –model –cell 5) was used to provide a general test of association for the single phenotype (case/control) of diabetic nephropathy. The basic allelic chi-square test (1df, –assoc) was used to provide asymptotic *P*-values and odds ratios (95% confidence interval) for minor alleles. The level of statistical significance was set at 5%, and the adjustment for multiple testing was performed using Bonferroni's method. Allele counts from both case–control collections were combined using a stratified analysis for Mantel–Haenszel odds ratio to minimize potential confounding between populations. Haplotype analysis was conducted where SNPs formed a haplotype block using 95% confidence bounds on D′. The initial UK case–control collection had >80% power to identify an allele with an odds ratio of at least 1.5, assuming a minor allele frequency of 10% in controls.

Results

Prior to statistical analysis, nine individuals were removed from the UK case–control dataset where no genotypes were obtained for more than one SNP; remaining genotype completion rates were >99%. Minor allele frequencies exceeded 10% for all variants (Table 2) and the distributions of genotypes in cases and controls were consistent with the Hardy–Weinberg equilibrium. Experimental controls were as expected. Two SNPs demonstrated evidence for association with diabetic nephropathy in the UK case–control collection, but only one remained significant after Bonferroni correction for multiple testing; rs17465637 $P_{genotype} = 0.02$; $P_{allele} = 0.009$; $P_{corrected} = 0.2$ and rs4420638 $P_{genotype} = 0.0004$; $P_{allele} = 0.0002$; $P_{corrected} = 0.002$, OR = 1.5 (95% CI: 1.2–1.9).

Statistically significant evidence for association was also observed in a separate All Ireland population, providing convincing support for the involvement of this novel SNP in diabetic nephropathy ($P = 0.002$; Table 3). Combining these two collections by stratified analysis revealed an odds ratio of 1.54 (95% CI: 1.29–1.84; $P < 0.00001$). No significant distortion in transmission frequency was observed for rs4420638 in families ($P = 0.3$). Consistent with previous findings, strong LD ($D' > 0.95$) was observed for three variants localized to chromosome 9p21 (rs10757274, rs2383206, rs1333049); frequencies of estimated haplotypes for these markers were similar in case and control groups (data not shown). The two most commonly observed haplotypes were AAG (47% versus 50%, $P = 0.2$) and GGC (46% versus 46%, $P = 0.9$) in cases and controls, respectively.

Table 3. Genotype and allele data for rs4420638 in UK and All Ireland case–control collections

	UK collection		All Ireland collection	
	Case	Control	Case	Control
Genotypes	355/209/26	346/141/7	171/81/15	322/111/8
Alleles	261/919	155/833	111/423	127/755
Minor allele frequency (%)	22.1	15.7	20.8	14.4
Unadjusted $P_{genotype}$ value	0.0004		0.004	
Unadjusted P_{allele} value	0.0002		0.002	
$P_{corrected}$	0.002			

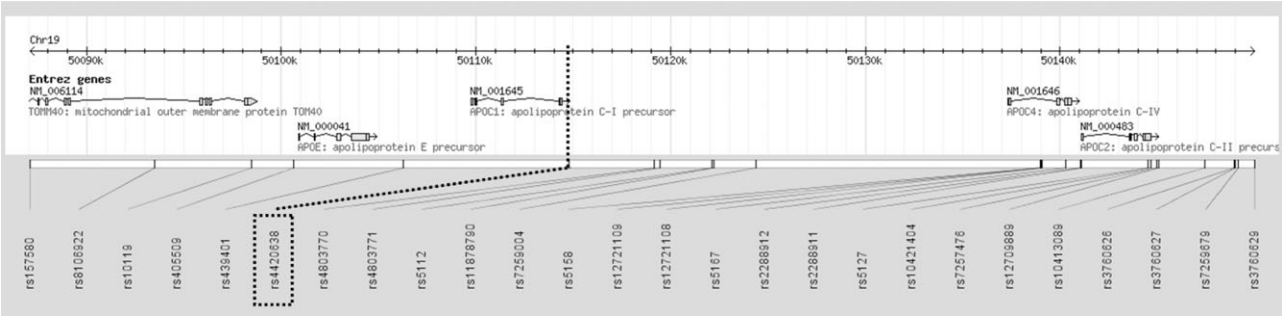

Fig. 1. Genomic context of associated SNP with the position of rs4420638 highlighted by dashed line. SNPs depicted are those genotyped in the CEU population in this region as part of HapMap project release 21.

Discussion

Utilizing a relatively large ($n = 1099$) collection of individuals with type 1 diabetes with or without nephropathy, we observed positive associations for two loci at the 5% level. One marker (rs4420638) remained statistically significant following stringent adjustment for multiple testing, and was replicated in a second case–control collection. The lack of statistically significant support in the family-based association study may be due to the small sample size, or explained by rs4420638 in linkage disequilibrium with the disease-causing allele. Of note, we also found a non-significant increase of the minor allele in diabetic nephropathy patients, compared to non-nephropathic controls, with type 2 diabetes from Northern Ireland ($n = 494$; 17.9% versus 14.6%). It is possible that the significant association identified in this study is due to susceptibility to cardiovascular disease in patients with nephropathy rather than a shared mechanism contributing to both end points. A large, long-term prospective study, such as the FinnDiane cohort, may ultimately be able to test the interaction between CVD, diabetic nephropathy and genotypes.

SNP rs17465637 (allele-based $P_{uncorrected} = 0.009$) was also genotyped in the All Ireland population to further investigate the association of this marker with diabetic nephropathy. The resultant allele-based $P = 0.7056$ supports the theory that this SNP is not strongly associated with diabetic nephropathy in individuals with type 1 diabetes from the British Isles.

Genome-wide association studies [9] suggest strong LD within this region of 19q13; however, the genomic region is poorly mapped for genomic variation based on information in online resources. Indeed, *APOC1/APOE* loci are poorly tagged by the Affymetrix 500K chip [8]. The SNP statistically associated with diabetic nephropathy (rs4420638) is linked to *APOC1*, but the adjacent region contains three other APO genes (*APOC2*, *APOC4, APOE*) in close proximity (Figure 1). *APOC1* has been suggested to exacerbate dyslipidaemia [14]. Increased apoCIII has been reported in patients with chronic renal failure, and an increase in apoCI has been specifically observed with diabetic nephropathy [15]. Several studies have examined a limited number of genetic variants in *APOC2*, *APOC4* and *APOE* genes for association with diabetic nephropathy [7]. It has been suggested that rs4420638 co-inherits with *APOE* genotypes [16], which are ~14 kb away. Both statistically significant association and a lack of association with nephropathy have been observed for genetic profiles at the *APOE* locus in individuals with diabetes [7]. Due to the lack of publicly available SNP data in this region, extensive resequencing is required to identify appropriate SNPs for genotyping. Fine mapping of the adjacent genomic region and replication by other groups will help to confirm this SNP as a genuine susceptibility allele or part of an important haplotype.

In conclusion, we have identified a previously unreported association of rs4420638 at 19q13 with diabetic nephropathy in two type 1 diabetic populations from the British Isles.

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Conflict of interest statement. None declared.

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Gluten sensitivity in patients with IgA nephropathy

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Abstract

Background. Coeliac disease is more frequent in IgA nephropathy (IgAN) patients compared to the healthy population. Several hypotheses postulate that food antigens like gluten may be involved in the onset of IgAN.

Methods. In this study, we used a recently developed mucosal patch technique to evaluate the rectal mucosal inflammatory reaction to gluten in patients with IgAN ($n = 27$) compared to healthy subjects ($n = 18$). The rectal mucosal production of nitric oxide (NO) and release of myeloperoxidase (MPO) and eosinophil cationic protein (ECP) were measured. Serum samples were analysed for IgA and IgG antigliadin antibodies (AGA), IgA antibodies against tissue transglutaminase and IgA endomysium antibodies.

Results. Gluten reactivity, defined as increase in MPO and/or NO after gluten exposure, was observed in 8 of 27 IgAN patients. The prevalence of HLA-DQ2 and DQ8 was not increased among gluten-sensitive patients, and the total prevalence among IgAN patients was the same as for the normal population. An elevated serum IgA AGA response was seen in 9 of 27 IgAN patients. The increase in IgA AGA