

Short Report: Genetics

Genetic risk factors affecting mitochondrial function are associated with kidney disease in people with Type 1 diabetes

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Abstract

Aim To evaluate the association with diabetic kidney disease of single nucleotide polymorphisms (SNPs) that may contribute to mitochondrial dysfunction.

Methods The mitochondrial genome and 1039 nuclear genes that are integral to mitochondrial function were investigated using a case (n = 823 individuals with diabetic kidney disease) vs. control (n = 903 individuals with diabetes and no renal disease) approach. All people included in the analysis were of white European origin and were diagnosed with Type 1 diabetes before the age of 31 years. Replication was conducted in 5093 people with similar phenotypes to those of the discovery collection. Association analyses were performed using the PLINK genetic analysis toolset, with adjustment for relevant covariates.

Results A total of 25 SNPs were evaluated in the mitochondrial genome, but none were significantly associated with diabetic kidney disease or end-stage renal disease. A total of 38 SNPs in nuclear genes influencing mitochondrial function were nominally associated with diabetic kidney disease and 16 SNPs were associated with end-stage renal disease, secondary to diabetic kidney disease, with meta-analyses confirming the same direction of effect. Three independent signals (seven SNPs) were common to the replication data for both phenotypes with Type 1 diabetes and persistent proteinuria or end-stage renal disease.

Conclusions Our results suggest that SNPs in nuclear genes that influence mitochondrial function are significantly associated with diabetic kidney disease in a white European population.

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Introduction

Diabetic kidney disease is a complex renal disorder representing a major global economic burden [1,2]. There is an inherited genetic predisposition for diabetic kidney disease, but the majority of studies to date have focused on genetic variation in the nuclear genome [3]. Mitochondria are specialized organelles that generate energy by oxidative phosphorylation, but they are susceptible to oxidative

damage from cellular stress, including hyperglycaemia and exposure to a uraemic environment. Chronic inflammation is a common contributor to progressive renal failure and leads to premature mitochondrial DNA (mtDNA) damage. Pathogenic mutations in mtDNA represent an increasingly recognized cause of chronic morbidity, and mitochondrial mutations have been implicated in a range of complex disorders, including kidney disease [4–6]. There is a synergistic relationship between the mitochondrial and nuclear genomes, with the expression of nuclear genes being essential for critical mitochondrial functions.

The aim of the present study was to investigate genetic variation that may contribute to mitochondrial dysfunction, both across the mitochondrial genome and in nuclear genes

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What's new?

- Mitochondrial dysfunction has been identified in diabetic kidney disease, but relatively large-scale genetic and epigenetic studies focused on mitochondria have not yet been described.
- We report a novel case–control analysis, with independent replication, of genetic variation focused on the mitochondrial genome and 1039 nuclear genes that are important for mitochondrial function.
- Single nucleotide polymorphisms (SNPs) in nuclear genes affecting mitochondrial function were found to be associated with diabetic kidney disease. The highlighted SNPs were within the genes implicated in regulation of epigenetic processes. Further research to explore the interactions between hyperglycaemia, uraemia and epigenetic modifications of the genome could shed new light on how these nuclear genome SNPs are associated with kidney disease.

that affect mitochondrial function, using a case–control approach, followed by independent replication.

Methods**Participants**

Written informed consent was obtained from all individuals in the discovery cohort who were recruited to the All-Ireland-Warren 3-Genetics of Kidneys in Diabetes (GoKinD) UK collections. Cases ($n = 823$) were white European individuals with Type 1 diabetes of at least 10 years duration, who had persistent proteinuria (> 500 mg/24 h), hypertension ($\geq 135/85$ mmHg) and retinopathy. End-stage renal disease ($n = 246$), secondary to diabetic kidney disease, was defined as requiring renal replacement therapy (chronic dialysis or kidney transplantation). Control subjects ($n = 903$) were people with at least 15 years' duration of Type 1 diabetes, with no evidence of renal disease on repeated testing. Control subjects were not prescribed anti-hypertensive medication to avoid misclassification of phenotype, as blood pressure-lowering treatment may reduce proteinuria. The discovery and replication groups have been previously described and include the FinnDiane (Finland), GoKinD (USA), and Steno Diabetes Centre (Denmark) cohorts (Table S1) [7]. In all, 3245 people with diabetic kidney disease, 1422 people with end-stage renal disease and 3574 control subjects were analysed in the present study.

Gene, single-nucleotide polymorphism selection and genotyping

Twenty-five single nucleotide polymorphisms (SNPs) in the mitochondrial genome were investigated using existing

genotype data from individuals genotyped using the Omni1-quad array using a case vs control group approach (Table S2) [7]. Relevant nuclear genes ($n = 1096$) were extracted from MITOCARTA [8] ($n = 1012$ genes) and MITOMAP [9] ($n = 84$ genes). Downloaded gene lists were revised to approved unique HUGO Gene Nomenclature Committee [10] symbols, resulting in the removal of 57 duplicate gene names; a total of 1039 unique nuclear genes were analysed.

The SNPs were downloaded from the Ensembl genome browser [11] using Ensembl genes 74 database on the Homo sapiens (GRCh37.p13) dataset. The SNPs ($n = 9\,243\,501$) associated with 1039 genes in the nuclear genome that influence mitochondrial function were investigated for their association with diabetic kidney disease. Genotype data for nuclear genes were extracted for relevant genes from imputed, publicly accessible directly typed genome-wide data for the discovery cohort (dbGaP Study Accession: phs000389.v1.p1; [7]). *In silico* replication was conducted for all top-ranked SNPs, where $P \leq 0.05$ in the discovery study when considering either diabetic kidney disease or end-stage renal disease as the phenotype; data were retrieved from the GENIE genome-wide association study meta-analysis [7]. *De novo* genotyping was conducted for replication of the top seven independent signals using commercially available TaqMan 5' nuclease assays (Life Technologies, Warrington, UK) according to the manufacturer's instructions.

Statistical analysis

Allelic association was adjusted for age, duration of diabetes, gender and multiple testing using PLINK software (version 1.07 [12]) for *de novo* genotyping. PLINK (<http://pngu.mgh.harvard.edu/~purcell/plink>) is a free, open-source whole-genome association analysis toolset, which efficiently performs association studies for single SNPs or computationally intensive genome-wide association studies. Replication was conducted *in silico* for the FinnDiane ($n = 2910$) and USA ($n = 1595$) populations, followed by *de novo* genotyping in a Danish ($n = 588$) cohort. Genotype data were evaluated for their association with diabetic kidney disease and end-stage renal disease.

Results

Concordance between genotype duplicates ($n = 12$) was 100%, with genotype completion rates of $> 89\%$ for SNPs in the mitochondrial genome and $> 97.5\%$ for SNPs in the nuclear genome.

One mtDNA SNP was monomorphic in the UK collection; MitoT9951C. A further 19 mtDNA SNPs were present with a minor allele frequency $\leq 5\%$. Five SNPs were observed with a minor allele frequency of $> 5\%$, but no SNPs in the mitochondrial genome were associated with diabetic kidney disease (Table S2).

A total of 9 243 501 SNPs in mitochondrial-related nuclear genes were downloaded from Ensembl [11]. A comparison of 823 cases with Type 1 diabetes and nephropathy with 903 control subjects with Type 1 diabetes and no evidence of nephropathy identified 421 SNPs in 86 unique genes with a nominal association ($P \leq 0.05$) with diabetic kidney disease, without correction for multiple testing. *In silico* replication in the FinnDiane ($n = 2910$), and USA ($n = 1595$) cohorts supported the association of 38 SNPs in 17 unique genes with consistent direction of effect across all collections (Table S3). Consideration of the more extreme phenotype of end-stage renal disease (246 cases vs. 903 controls) identified 139 SNPs in 50 unique genes where $P \leq 0.05$. *In silico* replication in the FinnDiane and GoKinD USA cohorts supported the association of 16 SNPs in eight genes (seven independent signals, $P < 0.05$), with a consistent direction of effect observed across all three case-control collections for end-stage renal disease (Table 1). SNPs were investigated for regulatory features using HAPLOREG and Blood eQTL browsers (Tables S4 and S5, respectively).

Seven SNPs (representing three independent signals) were common to both diabetic kidney disease and end-stage renal disease for the *in silico* replication analyses; SNPs representing each of these three independent signals were taken forward for additional *de novo* genotyping using independent samples. As the recent GENIE genome-wide association study [7] identified more significant hits with end-stage renal disease, all independent signals observed for end-stage renal disease ($n = 7$; Table 1) were also *de novo* genotyped in a Danish cohort of 588 individuals. Four SNPs (rs2147653, rs7387720, rs1167726, rs7213412) in the Danish cohort showed non-significant trends in the same direction as the earlier results, with rs17745445 (*TXNRD2* gene on 22q11.21) showing the most evidence for association.

Discussion

The mitochondrial genome is circular, is 16 569 DNA bases in length, encodes 37 genes, and can be transcribed and translated independently of nuclear DNA. There are several differences between mitochondrial and nuclear genomes; mtDNA is primarily coding (lacking introns), and has overlapping genes, different codons, a higher mutation rate and a greater number of copies of mtDNA present in cells that have high energy consumption.

Previous publications have reported that SNPs in the mitochondrial and nuclear genomes affect mitochondrial function and have been associated with renal dysfunction [5,6,13–16]. In the present study, none of the genotyped SNPs in the mitochondrial genome were significantly associated with diabetic kidney disease, but the majority were present at very low frequency meaning that the discovery collection was only adequately powered to identify a statistically significant association for five of the 25 SNPs investigated. It should be noted that only 25 SNPs,

from the mitochondrial genome, were present on the Omni1-quad array so only a limited number of mitochondrial SNPs were considered for their association with diabetic kidney disease using this array-based method. It is conceivable that more comprehensive examination of the entire mitochondrial genome by next-generation sequencing methods would identify hundreds of inherited and somatic mutations and some of these mitochondrial variants may be associated with diabetic kidney disease. Seven SNPs, representing three independent signals across five nuclear genes (*COQ5*, *COX6A1*, *GATC*, *TOP1MT* and *PARCRG*) were associated with both diabetic kidney disease and end-stage renal disease in the discovery and *in silico* replication analyses.

Six SNPs identified as associated with end-stage renal disease and diabetic kidney disease after *in silico* replication were co-located, representing one independent signal at 12q24.31. There are multiple genes within this linkage disequilibrium block, making it challenging to identify potential biological roles for these intronic or intergenic mutations (Fig. S1). Coenzyme Q is an antioxidant essential for electron-transport in the respiratory chain of the mitochondria; coenzyme Q5 homologue (*COQ5*) encodes methyltransferase. Cytochrome *c* oxidase (*COX*) is an enzyme, catalysing the last step in the respiratory cycle, transferring electrons from reduced cytochrome *c* to molecular oxygen. This process is then coupled to the translocation of protons in the inner mitochondrial membrane. A total of 13 subunits comprise *COX*, of which three larger subunits are encoded by mtDNA and the remaining 10 are encoded by nuclear DNA [17]. *COX* deficiencies have been previously associated with clinical phenotypes including neurological disorders, Leigh syndrome, cardiomyopathy and hepatopathy [17]. The effect of the intronic SNP located in the *COX6A1* may lead to an increase in oxidative stress within the kidney. Glutamyl-tRNA(Gln) amidotransferase, subunit C (*GATC*) is a protein-coding gene, and clusters with long non-coding RNA.

Two SNPs comprised a second independent signal around the *TOP1MT* gene (mitochondrial topoisomerase I), which encodes an enzyme, topoisomerase, that exclusively targets mitochondria and plays a crucial role in the modification of DNA [18]. *TOP1MT* relieves the tension of mtDNA supercoiling generated from mtDNA transcription and replication, within the mitochondrial genome. *TOP1MT* is a type 1B topoisomerase. It catalyses the temporary breaking of the DNA and allows the DNA to turn in a controlled rotation, before covalently linking the DNA to relax the super coiling [18].

The third independent signal showed a consistent direction of effect across all four collections (UK-ROI, Finnish, American and Danish) for two intronic SNPs in the *TXNRD2* gene. This gene plays a key role in regulating cellular redox environment, particularly scavenging of reactive oxygen species in mitochondria. *TXNRD2* is a stress

Table 1 Discovery and replication results for SNPs with $P \leq 0.05$ for end-stage renal disease in the UK collection

SNP	Gene	Location of SNP	Chromosome	Independent signal	Allele 1	Allele 2	Allele frequency, %	End-stage renal disease P value for UK collection	End-stage renal disease P value (UK, FD, GoKInD USA)	Direction of effect (UK, FD, GoKInD USA)	Diabetic kidney disease P value for UK collection	Diabetic kidney disease P value (UK, FD, GoKInD USA)	End-stage renal disease P value Steno	Diabetic kidney disease P value Steno
rs1167726*	COQ5	intronic	12q24.31	1	A	C	84.29	0.0019	1.00E-06	+++	0.006	2.00E-05	0.32 [†]	0.39
rs614226*	COQ5	intronic	12q24.31	1	T	C	84.29	0.0018	1.00E-06	---	0.006	2.00E-05		
rs1167725	COQ5	intergenic	12q24.31	1	A	T	17.76	0.003	2.00E-06	---	0.028	4.00E-05		
rs12310837*	COX6A1	intergenic	12q24	1	A	G	84.22	0.002	2.00E-06	+++	0.007	3.00E-05		
rs2235222*	GATC	intronic	12q24.31	1	C	G	15.66	0.001	3.00E-06	---	0.004	4.00E-05		
rs7137953	GATC	intronic	12q24.31	1	T	C	84.34	0.001	3.00E-06	---	0.004	4.00E-05		
rs7387720	TOP1MT	intergenic	8q24.3	2	A	G	53.97	0.003	7.00E-06	---	0.01	9.00E-05	0.70 [†]	0.94
rs724037*	TOP1MT	intronic	8q24.3	2	T	G	54.03	0.004	2.00E-05	---	0.008	2.00E-04		
rs17745445	TXNRD2	intronic	22q11.21	3	A	G	13.69	0.003	7.00E-05	+++	0.08	3.00E-04	0.06 [†]	0.07
rs17745433	TXNRD2	intronic	22q11.21	3	T	C	14.28	0.004	7.00E-05	---	0.08	4.00E-04		
rs5992495	TXNRD2	missense	22q11.21	4	T	G	15.85	0.004	5.00E-04	---	0.06	0.004	0.14 [†]	0.14
rs5992493	TXNRD2	intronic	22q11.21	4	A	G	84.16	0.004	5.00E-04	---	0.06	0.004		
rs176903	SPTLC2	intronic	14q24.3	5	A	G	34.62	0.003	0.002	---	0.0003	0.002	0.14 [†]	0.09
rs7213412	COX10	intronic	17p12	6	C	G	65.99	0.002	0.002	+++	0.08	0.048	0.33 [†]	0.29
rs2147653*	PACRG	intronic	6q26	7	C	G	92.64	0.002	0.003	---	0.0001	0.004	0.63 [†]	0.81
rs1408705	PACRG	intronic	6q26	7	A	G	92.70	0.001	0.004	---	0.0001	0.004		

SNP, single-nucleotide polymorphism; FD, FinnDiane; GoKInD, All-Ireland-Warren 3-Genetics of Kidneys in Diabetes cohort; COQ5, coenzyme Q5 homologue, methyltransferase (*S. cerevisiae*); COX6A1, cytochrome c oxidase subunit VIa polypeptide 1; GATC, glutamyl-tRNA(Gln) amidotransferase, subunit C; TOP1MT, topoisomerase (DNA) I, mitochondrial; TXNRD2, thioredoxin reductase 2; SPTLC2, serine palmitoyltransferase, long-chain base subunit 2; COX10, COX10 heme A:farnesyltransferase cytochrome c oxidase assembly factor; PACRG, PARK2 co-regulated.

*SNPs that were common to diabetic kidney disease and end-stage renal disease *in silico* replication.

[†]Combined meta-analysis for rs1167726 = 0.0000004; rs7387720 = 0.000004; rs17745445 = 0.000002; rs5992495 = 0.0003; rs176903 = 0.0008; rs7213412 = 0.001; rs2147653 = 0.001.

response gene involved in many signalling pathways, including WNT which is associated with renal fibrosis in diabetic kidney disease. Gene expression of *TNFRD2* is differentially regulated by epigenetic mechanisms in response to high glucose and is a probable target of microRNAs miR335 and miR34a [19,20].

Complementary to the single-SNP analysis described above, interaction analyses have produced novel findings for common phenotypes such as cancer [21], heart disease [22] and BMI [23], particularly when considered in association with environmental variables whose effects are under genetic and epigenetic control such as smoking. It is possible that a cumulative effect of common and/or rare genetic variants influence diabetic kidney disease, particularly where combined SNPs are located in the same biological pathway. More comprehensive analysis of the mitochondrial genome and genotyping in a larger population, such as the recently funded Juvenile Diabetes Research Foundation Genetics of Diabetic Nephropathy Collaborative Research Initiative, are required to robustly consider this approach for diabetic kidney disease. SNP–SNP interaction analyses for SNPs in the mitochondrial genome, did not reveal further associations in the present study. Clusters of correlated SNPs were identified at top-ranked genetic loci, as shown in the supplementary regional association plots (Figures S1–7).

Our data support the hypothesis that variants in genes affecting mitochondrial function are associated with renal disease including diabetic kidney disease. The identified SNPs are found within nuclear genes implicated in the regulation of epigenetic processes. Further research to explore the interactions between hyperglycaemia, uraemia and epigenetic modifications of the genome could shed new light on how these nuclear genome SNPs are associated with the phenotypes of diabetic kidney disease and end-stage renal disease.

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Competing interests

P.-H.G. has received research grants from Eli Lilly and Roche, is an advisory board member for Boehringer-Ingelheim, Novartis, Cebix and Abbott. He has also received lecture fees from Boehringer-Ingelheim, Eli Lilly, Genzyme, Novartis, Novo Nordisk, Sanofi and MSD.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of populations participating in the discovery and replication analyses.

Table S2. Association results for diabetic kidney disease and SNPs in the mitochondrial genome sorted by minor allele frequency.

Table S3. Discovery and meta-analysis results combined, where $P \leq 0.05$ for diabetic kidney disease.

Table S4. HAPLOREG annotations for variants described in Table 1.

Table S5. eQTL data retrieved from Westra *et al.*, *Nature Genetics* 2013, **45**: 1238–1243.

Fig. S1. LocusZoom plot showing the genetic region surrounding rs1167726 (signal 1).

Fig. S2. LocusZoom plot showing the genetic region surrounding signal 2.

Fig. S3. LocusZoom plot showing the genetic region surrounding signal 3.

Fig. S4. LocusZoom plot showing the genetic region surrounding signal 4

Fig. S5. LocusZoom plot showing the genetic region surrounding signal 5.

Fig. S6. LocusZoom plot showing the genetic region surrounding signal 6.

Fig. S7. LocusZoom plot showing the genetic region surrounding signal 7.