

ONLINE MUTATION REPORT

Mitochondrial DNA haplogroups and type 2 diabetes: a study of 897 cases and 1010 controls

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J Med Genet 2007;44:e80 (<http://www.jmedgenet.com/cgi/content/full/44/6/e80>). doi: 10.1136/jmg.2007.048876

Mitochondria play a central role in the secretion of insulin by pancreatic β -cells, and pathogenic mutations of mitochondrial DNA (mtDNA) can cause diabetes. The aetiology of type 2 diabetes has a strong genetic component, raising the possibility that genetic variants of mtDNA alter the risk of developing the disorder. Recent studies have produced conflicting results. By studying 897 UK cases of type 2 diabetes and 1010 population-matched controls, it is shown that European mtDNA haplogroups are unlikely to play a major role in the risk of developing the disorder.

Insulin secretion by pancreatic β -cells is critically dependent on ATP synthesis by mitochondrial oxidative phosphorylation (OXPHOS). Thirteen essential OXPHOS proteins are synthesised within mitochondria from the maternally inherited mitochondrial DNA (mtDNA). Specific point mutations of mtDNA, such as m.3243A→G, cause maternally inherited diabetes, often in association with deafness, cardiomyopathy or a multisystem neurological disorder, and account for <1% of patients with type 2 diabetes. This raises the possibility that a more subtle genetic variation of the mtDNA might contribute to the risk of developing the disorder by interacting with other genetic and environmental factors.

mtDNA is maternally inherited and highly polymorphic. Mutations have divided the population into a number of discrete haplogroups, with members of the same haplogroups sharing a common maternal ancestor. Emerging evidence suggests that different mtDNA haplogroups are associated with subtle differences in OXPHOS capacity and the generation of reactive oxygen species.¹ Given the central role played by mitochondria in insulin secretion and signal transduction, coupled with the greater risk of type 2 diabetes developing in the offspring of affected women when compared with the offspring of affected men, mtDNA is an obvious candidate for genetic susceptibility studies on type 2 diabetes.

Small case-control studies reported an association between type 2 diabetes and the non-coding region of mtDNA (the 16184–16193 polyC tract),² which was not confirmed in a larger study with meta-analysis.³ More recently, a large multicentre genetic association study designed to investigate the effect of common mtDNA variants on the risk of developing the metabolic syndrome concluded that there was no evidence of a link between mtDNA variation and type 2 diabetes.⁴ However, this study used a new approach, with haplotype tags derived from a mixed dataset of healthy controls and controls with a number of different disorders, not directly based on pre-existing knowledge of the structure of mtDNA within the European population. Close scrutiny of the published data revealed a strong association between haplogroup J markers and type 2 diabetes in one population, which did not emerge from the whole dataset after a correction for multiple

significance testing.⁴ Haplogroup J is of particular interest because of a recently reported association with insulin resistance and type 2 diabetes in a Brazilian study of 347 patients and 350 controls.⁵ Given the potential importance of these controversial findings, we carried out an independent study of 897 cases of type 2 diabetes and 1010 controls based on a conventional haplogroup-association design, aimed at identifying deep-rooted ancient genetic variants of mtDNA that predispose to type 2 diabetes, or clades containing one or more recent functional variants that contribute to the risk of the disorder.

METHODS

The 897 cases of type 2 diabetes were part of the Diabetes UK Warren 2 cohort (mean (SD) age 62.9 (8.2) years; 55.6% male; mean (SD) body mass index 30.3 (6.4) kg/m²).³ The clinical details of the type 2 diabetes cases have been published previously.⁶ All were anti-glutamic acid decarboxylase antibody negative. The 1010 controls were part of the UK Medical Research Council 1958 birth cohort. mtDNA haplogroups H, J, K, U, T, W, X, I and V were determined by PCR-restriction fragment length polymorphism analysis of polymorphic variants that unambiguously define the mtDNA haplogroup when part of a well-established sequential algorithm.^{7,8} The exact probability of observing the different overall haplogroup distributions in cases and controls was compared using Monte Carlo simulation as described (1000 iterations); 95% CIs were determined by the Clopper-Pearson method. Individual haplogroups were compared using Fisher's exact test.

RESULTS AND DISCUSSION

The haplogroup distribution in the controls was no different from other large published series from different regions of the UK, confirming that the control group was representative of the general UK population. There was no difference in the frequency of non-European haplogroups (included in the group "other", which refers to samples of European origin that could not be classified into one of the ten major European haplogroups; table 1) between cases and controls, supporting demographic data showing that the two study groups were ethnically well matched. There was no difference in the overall haplogroup distribution between cases of type 2 diabetes and controls ($p = 0.182$), and direct comparison of the individual haplogroups showed a striking correspondence between both groups, with no significant difference in frequency for any haplogroup (table 1).

The parental phenotype was known in 88% of the type 2 diabetes cases. A significantly greater number of cases had an affected mother (27.9%) than an affected father (14.9%),

Abbreviations: OXPHOS, oxidative phosphorylation; mtDNA, mitochondrial DNA

Table 1 Haplogroup distribution for 897 cases of type 2 diabetes and 1010 controls

mtDNA haplogroup	Controls		Type 2 diabetes cases		p Value
	Number in each haplogroup	Proportion, % (95% CI, %)	Number in each haplogroup	Proportion, % (95% CI, %)	
H	448	44.4 (41.3 to 47.5)	409	45.6 (42.3 to 48.9)	0.61
V	32	3.2 (2.2 to 4.4)	19	2.1 (1.3 to 3.3)	0.20
T	99	9.8 (8.0 to 11.8)	78	8.7 (6.9 to 10.7)	0.43
J	123	12.2 (10.2 to 14.4)	108	12.0 (10.0 to 14.4)	0.94
K	91	9.0 (7.3 to 11.0)	77	8.6 (6.8 to 10.6)	0.81
U	119	11.8 (9.9 to 13.9)	125	13.9 (11.7 to 16.4)	0.17
I	33	3.3 (2.3 to 4.6)	21	2.3 (1.5 to 3.6)	0.27
W	11	1.1 (0.5 to 1.9)	20	2.2 (1.4 to 3.4)	0.07
X	15	1.5 (0.8 to 2.4)	18	2.0 (1.2 to 3.2)	0.48
M	6	0.6 (0.2 to 1.3)	1	0.1 (0.0 to 0.6)	0.13
Other	33	3.3 (2.3 to 4.6)	21	2.3 (1.5 to 3.6)	0.27
Total	1010		897		

95% CIs calculated according to the Clopper–Pearson method. p Value is uncorrected Fisher’s exact p. For historical reasons, haplogroups K and U are shown separately, but K may actually be a sub-haplogroup of U. “Other” refers to European subjects who could not be classified into one of the 10 major European haplogroups.

Table 2 Haplogroup distribution for cases of type 2 diabetes with either a maternal or a paternal family history of diabetes

mtDNA haplogroup	Type 2 diabetes cases with affected fathers		Type 2 diabetes cases with affected mothers		p Value
	Number in each haplogroup	Proportion, % (95% CI, %)	Number in each haplogroup	Proportion, % (95% CI, %)	
H	47	39.8 (30.9 to 49.3)	91	40.8 (34.3 to 47.6)	0.91
V	3	2.5 (0.5 to 7.3)	4	1.8 (0.5 to 4.5)	0.70
T	9	8.3 (3.9 to 15.1)	22	9.9 (6.3 to 14.6)	0.56
J	16	13.6 (8.0 to 21.1)	29	13.0 (8.9 to 18.1)	0.87
K	12	10.2 (5.4 to 17.1)	18	8.1 (4.9 to 12.5)	0.55
U	15	12.7 (7.3 to 20.1)	34	15.3 (10.8 to 20.7)	0.63
I	4	3.4 (0.9 to 8.5)	6	2.7 (1.0 to 5.8)	0.74
W	3	2.5 (0.5 to 7.3)	6	2.7 (1.0 to 5.8)	1.00
X	4	3.4 (0.9 to 8.5)	5	2.2 (0.7 to 5.2)	0.50
M	1	0.9 (0.0 to 4.6)	0	0.0 (0.0 to 1.6)	0.35
Other	4	3.4 (0.9 to 8.5)	6	2.7 (1.0 to 5.8)	0.74
Total	118		221		

95% CIs calculated according to the Clopper–Pearson method. p Value is uncorrected Fisher’s exact p comparing each haplogroup in cases with an affected father versus cases with an affected mother. For historical reasons, haplogroups K and U are shown separately, but K may actually be a sub-haplogroup of U. “Other” refers to European subjects who could not be classified into one of the 10 major European haplogroups.

Fisher’s exact $p < 0.001$), although this could partly be explained by maternal longevity. However, there was no relationship between maternal family history and the mtDNA haplogroup distribution (table 2; type 2 diabetes cases with an affected mother compared with cases with an affected father, $p = 0.979$; type 2 diabetes cases with an affected mother compared with population controls, $p = 0.538$).

Therefore, two large studies (our study and the study by Saxena *et al*⁴) found no evidence of an association between type 2 diabetes and polymorphic variation of mtDNA. However, there is good evidence that geographical differences in mtDNA genetic background can alter the association of specific haplogroups with disease. For example, the well-established strong association between mtDNA haplogroup J and the m.11778G→A and m.14484T→C mutations that cause Leber hereditary optic neuropathy is not apparent in Iran,⁸ probably because the association is actually due to specific variants in *MTCYB* that are not found in the Iranian population.⁸ Secondly, simulation has shown that even larger studies (>10 000 cases and controls) are required for adequate power to detect moderate associations (odds ratios of 1.1–1.2) for haplogroups, which are present in <10% of controls.⁹ Finally, there are a number of different ways in which mtDNA variation could be associated with disease. The haplogroup approach can be used to test an association with ancient or recent functional variants,

or polymorphisms that are strongly associated with a specific haplogroup. By contrast, an equally plausible hypothesis is that one or more recently acquired genetic variants add together to

Key points

- Pathogenic mutations of mitochondrial DNA (mtDNA) can cause diabetes, raising the possibility that natural genetic variation of mtDNA might contribute to the risk of developing type 2 diabetes.
- Some studies have reported an association between mtDNA haplogroups and type 2 diabetes, but others have not. To resolve this issue, we studied 897 cases of type 2 diabetes and 1010 population-matched controls from the UK.
- We found no evidence of an association between type 2 diabetes and the major European mtDNA haplogroups, even when the cases were stratified on the basis of maternal family history. Our observations confirm that a major mtDNA haplogroup effect is unlikely to be important in the aetiology of the disorder.

compromise OXPHOS. Given that ~35% of European mtDNA genetic variants are homoplasies, occurring more than once on the phylogenetic tree, and that a large proportion of controls harbour unique polymorphisms, a more complex and massive-scale approach will be required to provide an absolute conclusion regarding the role of mtDNA in type 2 diabetes.

ACKNOWLEDGEMENTS

PFC is a Wellcome Trust Senior Fellow in Clinical Science, and receives additional grant support from The Wellcome Trust, The United Mitochondrial Diseases Foundation and the EU FP6 programme MITOCIRCLE. MW and SKP are supported by Diabetes UK. We acknowledge the use of DNA from the British 1958 Birth Cohort collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02, and Diabetes UK for funding the collection of the Warren 2 resource.

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Competing interests: None declared.

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Received 2 January 2007

Revised 26 January 2007

Accepted 5 February 2007

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