Molecular scanning of candidate mitochondrial tRNA genes in type 2 (non-insulin dependent) diabetes mellitus

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Abstract

Mitochondrial DNA (mtDNA) gene defects may play a role in the development of non-insulin dependent diabetes mellitus (NIDDM). In order to search for potentially diabetogenic mtDNA defects we have applied the technique of single stranded conformational polymorphism (SSCP) analysis to 124 patients with a history of NIDDM and 40 non-diabetic controls. No new heteroplasmic mutations were detected. However, a variety of homoplasmic variants were found in patients with NIDDM; some of these merit further investigation.

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Key words: diabetes; mitochondrial DNA; MELAS.

An excess of maternal transmission in NIDDM suggests that mtDNA defects might play a role in the disease[1]; several groups have reported the cosegregation of an A to G point mutation at position 3243 bp in the mitochondrial $tRNA^{leu(UUR)}$ gene and maternally inherited diabetes/deafness.[2] This mutation had previously been reported in the syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). A single pedigree with diabetes and deafness has been found to carry a mtDNA duplication.[3] In the mitochondrial myopathies, a variety of different mtDNA mutations may result in a similar phenotype and it is our hypothesis that NIDDM may be associated with several mtDNA mutations. Therefore we have applied the molecular scanning technique of SSCP analysis to candidate mtDNA regions in order to search for potentially diabetogenic mutations. A total of 124 patients with a history of NIDDM were studied. Group A (n=33) consisted of patients with NIDDM and a history of an affected mother. Group B (n=56) represented NIDDM patients with at least one affected sib irrespective of parental diabetes status. Group C consisted of 35 women with previous gestational diabetes. The race matched control group D (n=40) had no

mtDNA mutations detected in subjects with diabetes and controls

mtDNA region	*Mutation detected*	*Position*	*Gene*	*Occurrence in subject groups*			
				A(n=33)	*B(n=56)*	*C(n=35)*	*D(n=40)*
3130–3558	A–G[h]	3243	$tRNA^{leu}$	0	1	0	0
	A–G	3348	ND1	1	0	0	0
	T–C	3394	ND1	0	1	0	0
	T–C	3396	ND1	0	1	0	0
	A–G	3434	ND1	0	1	0	0
	G–A	3438	ND1	0	1	0	0
	A–G	3447	ND1	0	2	2	0
	A–G	3480	ND1	0	1	0	1
	G–A	3483	ND1	0	0	0	1
5454–5920	A–G	5656	nc[a]	0	1	0	0
	G–A	5780	$tRNA^{cys}$	0	0	0	1
7377–7650	C–T	7476	$tRNA^{ser}$	0	1	0	2*
8196–8445	A–G	8245	CoII	0	1	0	0
	G–A	8251	CoII	0	1	0	1
	C–T	8252	CoII	0	1	0	0
	G–A	8269	nc[b]	1	1	0	3
	9bp deletion	8277	nc[b]	0	0	0	1
	9bp repeat	8277	nc[b]	0	1	0	0
	G–A	8290	nc[b]	0	1	0	0
15788–16150	A–G	15924	$tRNA^{thr}$	3	nd	nd	2
	G–A	15927	$tRNA^{thr}$	1	1	1	0*
	G–A	15928	$tRNA^{thr}$	2	7	6	7*
	C–T	16069	D loop	5	nd	nd	4
	T–C	16093	D loop	19	nd	nd	18
	C–T	16126	D loop	1	nd	nd	0

nc[a] = non-coding 1 bp junction between $tRNA^{asn}$ and $tRNA^{aln}$ genes.
nc[b] = non-coding intergenic region between CoII and $tRNA^{lys}$ genes.
* = control group (n=97) screened by PCR/RFLP.
nd = not determined.
h = heteroplasmic.
Group A = NIDDM with maternal history.
Group B = NIDDM with affected sibs.
Group C = gestational diabetes.
Group D = non-diabetic controls.

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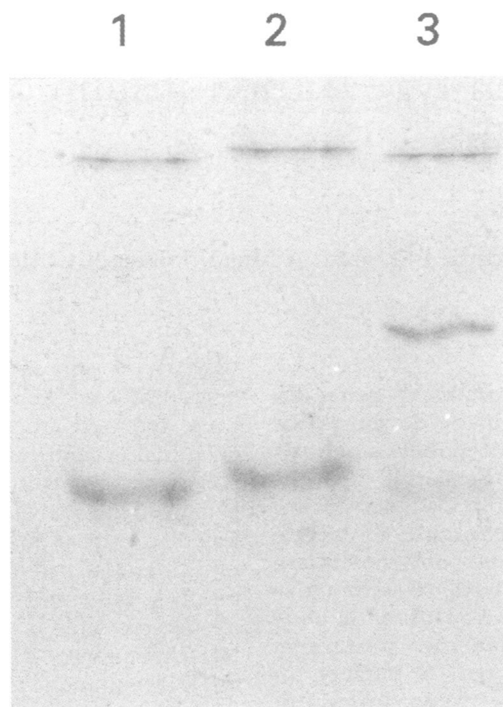

Figure 1 Single stranded conformation polymorphism (SSCP) analysis of the mitochondrial tRNA^{lys} gene using the Pharmacia Phast System. Lane 1, wild type. Lane 2, 8251 bp homoplasmic mutation (NIDDM patient). Lane 3, 8344 bp heteroplasmic mutation (kind gift from the late Professor A E Harding).

known personal or family history of diabetes. For PCR/RFLP studies, the control group was increased to 97 subjects. No control subject or patient with NIDDM had clinically obvious features of mitochondrial disease, although detailed screening such as audiometry and EMG were not carried out. One pedigree with diabetes and deafness, previously reported to harbour the 3243 bp mtDNA mutation, and one subject without diabetes but with a heteroplasmic mtDNA mutation in the tRNA^{lys} gene were used as positive controls for heteroplasmic mutations.

Primers were designed to amplify candidate regions of the mitochondrial genome including those where potentially pathogenic mutations have been reported previously (table). SSCP analysis was performed using precast polyacrylamide gels in the PhastSystem (Pharmacia, Uppsala, Sweden), as described elsewhere.[4] PCR products harbouring SSCPs were purified and then sequenced on an Applied Biosystems Automated DNA Sequencing System (Model 373A). Where a mtDNA variant was found to alter a restriction site (that is, *Alu*I site loss at 7476 bp and *Msp*I site loss at 15927 bp and 15928 bp), PCR products from all diabetic subjects and a total of 97 controls were genotyped using the appropriate restriction enzyme followed by electrophoresis on ethidium bromide stained gels.

Only two heteroplasmic mutations were detected; these were in the positive control samples. A further 25 homoplasmic mtDNA variants (22 in diabetic subjects) were detected (fig 1, table). Although most of the homoplasmic variants found in NIDDM patients probably represent neutral polymorphisms, a number may merit further attention. These include a T to C substitution at 3394 bp which has previously been implicated in Leber's hereditary optic neuropathy (LHON),[5] and a 9 bp direct triplicate repeat in the intergenic region between CoII and tRNAlys which has been reported in a family with a mitochondrial encephalomyopathy as well as in two other people with symptoms indicative of mitochondrial disease.[6] The mutation at 5656 bp alters a single nucleotide at the site of mitochondrial transcript scission and further studies will be required to determine whether this might interfere with tRNA processing. The G to A mutation at 15927 bp in the tNRA^{thr} was found in 3/124 NIDDM patients but 0/97 controls. This mutation has also been reported in a single

*Figure 2 (A) Single stranded conformation (SSCP) analysis of the mitochondrial tRNA^{thr} gene. Lane 1 control (wild type), lane 2 15924 bp mutation, lane 3 15927 bp mutation, lane 4 15928 bp mutation. (B) Comparison of normal nucleotide sequence of the anticodon stem and loop regions of the mitochondrial tRNA^{thr} gene to mutated sequences. * = mutated mucleotide in each case. (C) Diagram of the mitochondrial tRNA^{thr} showing molecular position of mutations in the anticodon stem and loop region.*

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patient with hypertrophic cardiomyopathy and a second patient with chronic external ophthalmoplegia.[7] The mutation occurs in the anticodon stem of the tRNA molecule and may adversely effect the tertiary structure required for mRNA binding. Evidence for this structural alteration arises from the large SSCP mobility shifts between the three distinct mtDNA base pair substitutions identified in the $tRNA^{thr}$ gene (fig 2). We detected a homoplasmic mutation at 7476 bp in one diabetic patient and two controls. This mutation lies close to others reported in patients with maternally inherited deafness.[8,9] The significance of this finding is unclear since we did not screen our subjects for deafness.

Review of the clinical notes of patients with NIDDM failed to show any unusual phenotypic characteristics in those subjects with the homoplasmic mtDNA variants discussed above. However, detailed studies (for example, audiometry, echocardiography) will have to be performed in these patients and their relatives in order to define the importance of the mtDNA sequences observed. The results of this study suggest that in patients with NIDDM, heteroplasmic pathogenic mutations are rare within the regions of the mitochondrial genome we have studied. It is still possible that pathogenic mutations are present in other parts of the mitochondrial genome. Further studies are also required to determine whether acquired, somatic mtDNA defects in pancreatic beta cells play a role in the development of the disease.

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