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Mutations in mitochondrial tRNA genes: non-linkage with syndromes of Wolfram and chronic progressive external ophthalmoplegia

Jody M.W.van den Ouweland, G.Jan Bruining[1], Dick Lindhout[2], Jan-Maarten Wit[3], Bernard F.E.Veldhuyzen[4] and J.Antonie Maassen*

Department of Medical Biochemistry, Sylvius Laboratories, University of Leiden, Departments of [1]Paediatrics and [2]Cell Biology and Genetics, Sophia Children Hospital/Academic Hospital Rotterdam, Erasmus University, Rotterdam, [3]Department of Paediatrics, University Hospital for Children and Youth 'Het Wilhelmina Kinderziekenhuis', University of Utrecht and [4]Department of Internal Diseases, Ignatius Hospital Breda, The Netherlands

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ABSTRACT

We have recently identified a point mutation in the mitochondrially encoded $tRNA^{Leu(UUR)}$ gene which associates with a combination of type II diabetes mellitus and sensorineural hearing loss in a large pedigree. To extend this finding to other syndromes which exhibit a combination of diabetes mellitus and hearing loss we have sequenced all mitochondrial tRNA genes from two patients with the Wolfram syndrome, a rare congenital disease characterized by diabetes mellitus, deafness, diabetes insipidus and optic atrophy. In each patient, a single different mutation was identified. One is an A to G transition mutation at np 12,308 in $tRNA^{Leu(CUN)}$ gene in a region which is highly conserved between species during evolution. This mutation has been described by Lauber *et al.* (1) as associating with chronic progressive external ophthalmoplegia (CPEO). The other is a C to T transition mutation at np 15,904 in $tRNA^{Thr}$ gene. Both mutations are also present in the general population (frequency $tRNA^{Leu(CUN)}$ mutation 0.16, $tRNA^{Thr}$ mutation 0.015). These findings suggest that evolutionarily conserved regions in mitochondrial tRNA genes can exhibit a significant polymorphism in humans, and that the mutation at np 12,308 in the $tRNA^{Leu(CUN)}$ gene is unlikely to be associated with CPEO and Wolfram syndrome.

INTRODUCTION

During the past three years, changes in the mitochondrial genome have been described in a number of human diseases. Large deletions of mitochondrial DNA (mtDNA) have been reported in patients with Kearn-Sayre Syndrome (KSS) and progressive external ophthalmoplegia (PEO) (2–4), Pearson's syndrome (5,6), Parkinson's disease (7), and even in healthy individuals (8). Duplications of mtDNA have been described in two patients with mitochondrial myopathy (9). Point mutations in mtDNA genes encoding subunits of respiratory complexes have been linked to Leber's hereditary optic neuropathy (10–12) and to a syndrome characterized by ataxia, retinitis pigmentosa, and peripheral neuropathy (13). Mutations in mitochondrial tRNA genes were first identified in patients with myoclonic epilepsy and ragged red fibers (MERRF) (14), and in patients with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes (MELAS) (15). Several more tRNA mutations, which are all linked to specific neuromuscular diseases, have recently been reported (1,16–20).

We have identified a mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene in a kindred of maternally transmitted diabetes mellitus and sensorineural hearing loss (21). To discover whether similar mutations occur in other patients having a combination of diabetes mellitus with deafness we examined patients with Wolfram (or DIDMOAD) syndrome. This syndrome is characterized by diabetes insipidus (DI), diabetes mellitus (DM), optic atrophy (OA) and deafness (D) (22,23). Its mode of inheritance seems autosomal recessive (24). Diseases linked to mtDNA changes can be found in sporadic cases or are inherited in a maternal or autosomal way (25). In view of both the diverse genetics of mitochondrial diseases, and the relation in clinical phenotype, we have sequenced all tRNA genes of mtDNA from two Wolfram patients. In each patient a different mutation was identified: A to G at np 12,308 in $tRNA^{Leu(CUN)}$ and C to T at np 15,904 in $tRNA^{Thr}$.

METHODS

Patients

Patients were diagnosed as having Wolfram syndrome if they exhibited the classical symptoms (22,23).

* To whom correspondence should be addressed

DNA preparation

Total DNA was isolated from blood and cultured primary fibroblasts by standard procedures (26,27).

DNA sequencing

MtDNA fragments encompassing tRNA genes were amplified from total DNA derived from fibroblasts by the standard PCR procedure (28) using following sets of 20-mer oligonucleotides (nucleotide positions (np) as in Cambridge mtDNA sequence (29)): np 245–264 (forward:f) and np 706–687 (reverse:r) for $tRNA^{Phe}$; np 1,412–1,431 (f) and np 1,856–1,837 (r) for $tRNA^{Val}$; np 3,029–3,048 (f) and np 3,456–3,437 (r) for $tRNA^{Leu(UUR)}$; np 4,211–4,230 (f) and np 4,668–4,649 (r) for $tRNA^{Ile}$, $tRNA^{Gln}$ and $tRNA^{fMet}$; np 5,464–5,483 (f) and np 5,955–5,936 (r) for $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$ and $tRNA^{Tyr}$; np 7,048–7,067 (f) and np 7,865–7,846 (r) for $tRNA^{Ser}$ and $tRNA^{Asp}$; np 8,134–8,154 (f) and np 8,626–8,607 (r) for $tRNA^{Lys}$; np 9,774–9,793 (f) and np 10,273–10,254 (r) for $tRNA^{Gly}$; np 10,166–10,185 (f) and np 10,604–10,585 (r) for $tRNA^{Arg}$; np 12,114–12,133 (f) and np 12,585–12,566 (r) for $tRNA^{Leu(CUN)}$, $tRNA^{Ser}$ and $tRNA^{His}$; np 14,544–14,563 (f) and np 15,042–15,023 (r) for $tRNA^{Glu}$; np 15,788–15,807 (f) and np 16,259–16,240 (r) for $tRNA^{Thr}$ and $tRNA^{Pro}$. The double stranded PCR products were purified by electrophoresis on low melting temperature agarose gel (Nusieve) and used directly for DNA sequencing with the same sets of primers by an adapted dideoxy chain termination method (30).

Restriction enzyme analysis

A 471 bp fragment encompassing the $tRNA^{Thr}$ mutation site, located at np 15,904, was amplified by PCR (primers np 15,788–15,807 (f) and np 16,259–16,240 (r)). The mtDNA fragment was digested with *Ase* I for one hour at 37°C and the fragments (355 and 116 bp) were separated on a 1.5% agarose gel. Bands were visualized by ethidium bromide staining.

A 144 bp fragment encompassing the $tRNA^{Leu(CUN)}$ mutation site, located at np 12,308, was amplified by mispairing PCR (1) (primers np 12,190–12,209 (f) and np 12,338–12,309 (r)). The reverse 30-mer oligonucleotide (5′-ATTACTTTTATTTGGA-GTTGCACCAGAATT) contains a nucleotide that differs from the Cambridge mtDNA sequence (29) (A to G substitution; underlined in oligonucleotide sequence). With this modification an *Eco* RI site is created. The mtDNA fragment was digested with *Eco* RI for one hour at 37°C and the fragments (119 and 25 bp) were separated on a 2.5% agarose gel. Bands were visualized by ethidium bromide staining.

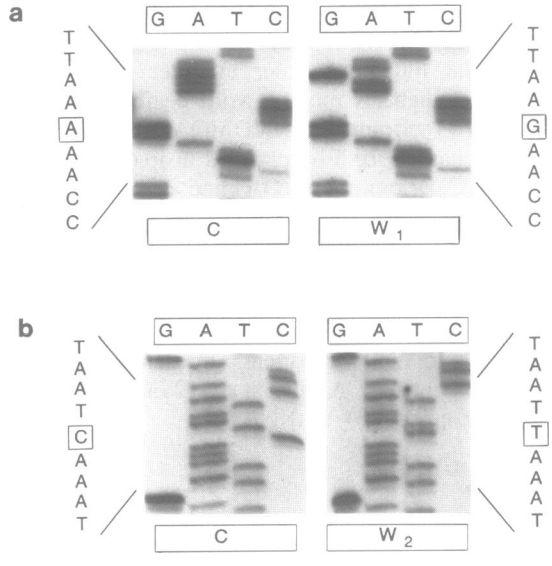

Figure 1. Mt DNA sequences surrounding the mutated site in mitochondrial $tRNA^{Leu(CUN)}$ in Wolfram patient W_1 (a) and $tRNA^{Thr}$ in Wolfram patient W_2 (b). Corresponding sequences from a control individual (C) are shown.

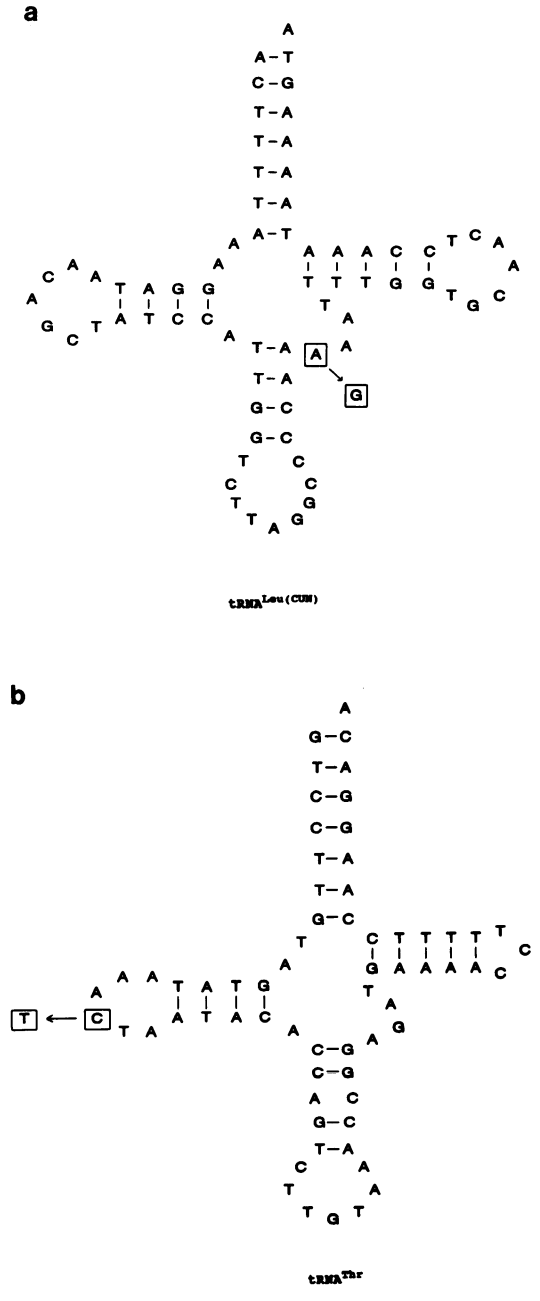

Figure 2. Proposed secondary structures of mitochondrial $tRNA^{Leu(CUN)}$ (a) and $tRNA^{Thr}$ (b) genes. Mutations at np 12,308 in the variable loop of $tRNA^{Leu(CUN)}$ (a) and at np 15,904 in the DHU loop of $tRNA^{Thr}$ (b) are indicated.

RESULTS

DNA sequencing of all mitochondrial tRNA genes from one patient with Wolfram syndrome (W_1) revealed an A to G transition mutation at np 12,308 (Fig. 1a), being located in the variable loop of $tRNA^{Leu(CUN)}$ (Fig. 2a). This position is highly conserved between species during evolution (Fig 3a). Homoplasmy was seen in fibroblast and leucocyte DNA. To determine whether this mutation was specific for patients with Wolfram syndrome, we analyzed mtDNA from blood of three other Wolfram patients by mispairing PCR (ref). A mtDNA fragment encompassing the $tRNA^{Leu(CUN)}$ mutation site (144 bp) was amplified and digested by *Eco* RI. This restriction enzyme cleaves the mutant sequence only, creating 119 and 25 bp fragments. The $tRNA^{Leu(CUN)}$ mutation could not be detected in these Wolfram patients (result not shown), which indicated that this mutation was either specific for the particular patient or reflects a neutral polymorphism. Subsequent analysis showed that the mutation was present in homoplasmic state in blood from the patient's mother and his sister (both healthy individuals) and in 12 out of 75 healthy controls from the general population (Fig. 4a).

We also sequenced all mitochondrial tRNA genes from Wolfram patient W_2. This resulted in the identification of a homoplasmic C to T transition mutation at np 15,904 (Fig. 1b), being located in the DHU loop of $tRNA^{Thr}$ (Fig. 2b). This mutation is in a region which is poorly conserved between species during evolution (Fig. 3b). Then, mtDNA of three other Wolfram patients was subsequently analyzed for the presence of this mutation. A mtDNA fragment encompassing the $tRNA^{Thr}$ mutation site (471 bp) was amplified by PCR and digested by *Ase* I. This restriction enzyme cleaves the mutant sequence, resulting in 355 and 116 bp fragments. The $tRNA^{Thr}$ mutation could not be detected in any of these patients (result not shown). In blood from the patient's mother and his brother (both healthy individuals) the mutation was present in homoplasmic form as it was in 1 out of 66 healthy controls (Fig. 4b).

DISCUSSION

Sequencing analysis of all 22 mitochondrial tRNA genes from two patients with Wolfram syndrome resulted in the identification of a single different point mutation in each patient. One is an A to G transition mutation at np 12,308 in the $tRNA^{Leu(CUN)}$ gene. This mutation is in a region which is highly conserved between species during evolution and has been found to associate with Chronic Progressive External Ophthalmoplegia (CPEO)(1). Using DNA from other Wolfram patients, distant relatives and a large panel of controls, we have shown that this mutation is neither specific for patients with Wolfram syndrome nor specific for patients with CPEO, but is a polymorphism that occurs with a high frequency (0.16) in the general population. The other point mutation we have identified is a C to T transition at np 15,904 in $tRNA^{Thr}$ gene. Similarly, using distant relatives and a large number of controls, we could show that this mutation is a polymorphism and occurs in the general population with low frequency (0.015). This report shows that mutations in evolutionarily conserved regions can occur without having a direct effect on the clinical phenotype. It remains to be established whether these mutations are risk factors for particular diseases.

In the last year more than 15 different mitochondrial tRNA gene mutations have been described as associating with neuromuscular diseases (1,14–20). A $tRNA^{Lys}$ mutation was present in several pedigrees with MERRF, but it was absent in two other cases of MERRF (14,32), a $tRNA^{Leu(UUR)}$ mutation was identified in 26 of 31 patients with MELAS (15) and in a large pedigree with diabetes mellitus (21) and another $tRNA^{Leu(UUR)}$ mutation segregated with cardiomyopathy in a single large pedigree (16). All three mutations show heteroplasmy, and were absent in a large panel of controls. Heteroplasmy has been suggested to be an indicator for pathogenetic mutation, since it is generally absent in normal individuals (16). All other tRNA gene mutations were homoplasmic and were identified in single patients. No controls, or only small numbers, were analyzed in these cases.

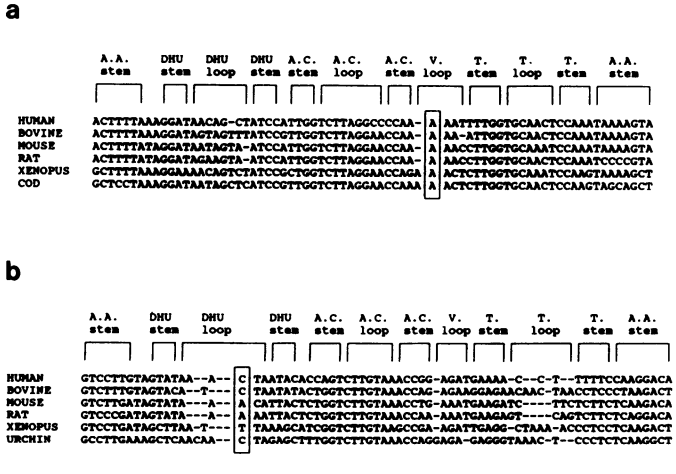

Figure 3. Interspecies homology of $tRNA^{Leu(CUN)}$ (a) and $tRNA^{Thr}$ (b) genes. The boxed nucleotides indicate the positions of the mutations in the Wolfram patients W_1 and W_2 and the evolutionary conservation between human, bovine, mouse, rat, xenopus and sea urchin (31). (A.A., amino acid; DHU, dihydrouridine; A.C., anticodon; V., variable; T., TψC)

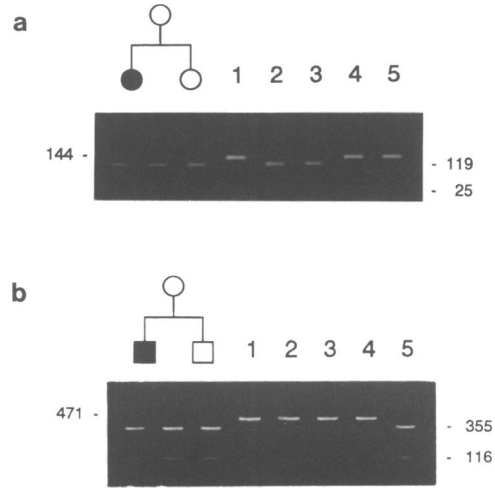

Figure 4. Detection of $tRNA^{Leu(CUN)}$ mutation by *Eco* RI digestion in Wolfram patient W_1, her mother and sister (a) and $tRNA^{Thr}$ mutation by *Ase* I digestion in Wolfram patient W_2, his mother and brother (b). 5 of 75 controls for the $tRNA^{Leu(CUN)}$ mutation and 5 of 66 controls for the $tRNA^{Thr}$ mutation are shown. Fragment sizes in basepairs are indicated.

As yet little is known about the effects of the tRNA gene mutations on the biological properties of the tRNA molecule and the way they affect the functioning of the mitochondrion. The $tRNA^{Lys}$ mutation in MERRF patients results in severe mitochondrial protein synthesis defect (14,33) and the $tRNA^{Leu(UUR)}$ mutation in MELAS and diabetes patients gives rise to severe impairment of 16S ribosomal RNA transcription termination, resulting in an imbalance between the amounts of ribosomal and other RNA transcripts (34). The effect of all other tRNA gene mutations on the transcription and translation of mitochondrial genes remains to be investigated.

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