European Journal of Human Genetics (2010) 18, 324–329

www.nature.com/ejhg

ARTICLE

Functional consequences of mitochondrial tRNA^{Trp} and tRNA^{Arg} mutations causing combined OXPHOS defects

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Combined oxidative phosphorylation (OXPHOS) system deficiencies are a group of mitochondrial disorders that are associated with a range of clinical phenotypes and genetic defects. They occur in approximately 30% of all OXPHOS disorders and around 4% are combined complex I, III and IV deficiencies. In this study we present two mutations in the mitochondrial tRNA^{Trp} (*MT-TW*) and tRNA^{Arg} (*MT-TR*) genes, m.5556G>A and m.10450A>G, respectively, which were detected in two unrelated patients showing combined OXPHOS complex I, III and IV deficiencies and progressive multisystemic diseases. Both mitochondrial tRNA mutations were almost homoplasmic in fibroblasts and muscle tissue of the two patients and not present in controls. Patient fibroblasts showed a general mitochondrial translation defect. The mutations resulted in lowered steady-state levels and altered conformations of the tRNAs. Cybrid cell lines showed similar tRNA defects and impairment of OXPHOS complex assembly as patient fibroblasts. Our results show that these tRNA^{Trp} and tRNA^{Arg} mutations cause the combined OXPHOS deficiencies in the patients, adding to the still expanding group of pathogenic mitochondrial tRNA mutations.
European Journal of Human Genetics (2010) **18,** 324–329; doi:10.1038/ejhg.2009.169; published online 7 October 2009

Keywords: combined OXPHOS defects; mitochondrial DNA; tRNA^{Trp}; tRNA^{Arg}; molecular mechanism

INTRODUCTION

Combined oxidative phosphorylation (OXPHOS) system deficiencies, in which two or more enzyme complexes show a decrease in activity, occur in around 30% of all OXPHOS disorders diagnosed at our center, and approximately 4% consist of combined complex I, III and IV defects.[1] Candidate genes for these deficiencies are, among others, all genes encoding proteins and RNAs involved in mitochondrial translation. Protein synthesis in the mitochondrion, which is responsible for the production of 13 polypeptides of the OXPHOS complexes I, III, IV and V, is executed by mitochondrial DNA (mtDNA)-encoded rRNAs and tRNAs and a multitude of nuclear-encoded factors. Although tRNA-encoding genes make up only 9% of the entire mitochondrial genome, over 40% of all point mutations reported in the mtDNA are located in tRNA genes.[2] In contrast, rRNA sequences cover 15% of the mtDNA, but mutations in the two rRNAs account for only 4% of all mtDNA mutations. In addition, in recent years mutations in various nuclear-encoded proteins involved in the mitochondrial translation process have been found and this group of gene defects is expanding rapidly.[3]

This study describes two mutations, m.5556G>A in the tRNA^{Trp} and m.10450A>G in the tRNA^{Arg} gene, that lead to combined OXPHOS deficiencies and shows their pathogenicity.

MATERIALS AND METHODS

Case reports

Patient T. This female child was born at term as the first child of healthy unrelated parents. She had feeding problems from birth and gastroesophageal reflux disease was suspected. From the age of 7 months, a regression of psychomotor functioning was noted and she became somnolent. In addition, she developed a severe constipation. There was failure to thrive; weight decreased from 0 to −2.5 SD, length from 0 to −1 SD and head circumference from +1 to −1 SD from the age of 1 month to the age of 10 months. Physical examination at the age of 10 months showed an irritable child with marked generalized hypotonia. She could not sit without support and showed no intentional hand function. Fundoscopy was normal. Gavage feeding was initiated. Laboratory studies revealed a metabolic acidosis. Plasma lactate was 12.3 mmol/l (N: <2.1 mmol/l) with an increased lactate/pyruvate ratio and CSF lactate was 12.5 mmol/l (N: <1.4 mmol/l). Additional laboratory studies, including liver function tests, were normal. A muscle biopsy was performed at the age of 11 months. Immunohistochemistry and electron microscopy were normal. She became comatose and died at the age of 13 months because of respiratory insufficiency. Autopsy or additional studies were not performed.

Patient A. This male patient was born at 37 weeks of gestation with a birth weight of 2.118 kg (<−2.5. SD), length 44.5 cm (<−2.5 SD) and head circumference 32.4 cm (−2 SD), as a first child of healthy, non-consanguineous parents. He was admitted to the neonatal unit for severe generalized hypotonia, respiratory insufficiency, feeding difficulty and anemia. He was further evaluated at the age of 4 months for severe psychomotor retardation, growth retardation, visual dysfunction, epilepsy (West syndrome) and chronic lactic acidemia. He received a gastric tube placement and started on nutritional intervention. A cranial MRI reported hypoplastic corpus callosum and abnormal signal intensity of the basal ganglia, including the globus pallidus and the nucleus caudatus. A follow-up cranial MRI showed progressive cerebral and cerebellar atrophy. EEG and VEP were severely abnormal. EEG showed generalized seizure activity; no burst suppression pattern was present. The VEP showed severe central visual perception loss. Metabolic studies showed markedly elevated lactate in both blood (3.6–7 mmol/l, *N*: <2.1 mmol/l) and

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Received 28 May 2009; revised 16 July 2009; accepted 11 August 2009; published online 7 October 2009

CSF (3.8 mmol/l, *N*: <1.4 mmol/l), and hyperalaninemia (860 μmol/l, *N*: <460 μmol/l) and increased urinary lactate and Krebs cycle intermediates. Additional laboratory studies were normal. At the age of 8 months, at the time of the surgical muscle biopsy, he had severe microcephaly; other growth parameters were normal. No internal organ dysfunction was noted. He had severe generalized hypotonia and hyporeflexia. The psychomotor development of the patient remained at the level of a 3-month-old baby. He had no visual contact with the surroundings and was suffering from intractable Blitz-Nick-Salaam (BNS)-type seizures. Using histological analysis, a homogenously decreased staining of COX activity was confirmed with a residual activity of 15–25%. No fiber-type disproportion, lipid storage or ragged red fibers were detected. The patient is currently 4.5 years old, has no acoustic or visual contact, is severely retarded and fully tube fed.

Cell culture

Skin fibroblasts were cultured in M199 medium (Gibco, Breda, The Netherlands) supplemented with 10% fetal calf serum (FCS) and antibiotics. Transmitochondrial cybrid cell lines were constructed as described elsewhere.[4] The cybrids were grown in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 mg/ml glucose and supplemented with 10% FCS, 110 μg/ml pyruvate and antibiotics; for the mtDNA-less 143B206 cells this medium was additionally supplemented with 50 μg/ml uridine and 100 μg/ml bromodeoxyuridine. The human osteosarcoma cell line 143B (TK−) was cultured in Eagle's minimum essential medium (EMEM) with Earle's BSS supplemented with 10% FCS and 15 μg/ml bromodeoxyuridine.

Enzyme measurements

The activities of the OXPHOS enzyme complexes were measured in skin fibroblasts and muscle tissue of the patients as described previously.[5,6]

Molecular genetic analysis

DNA was isolated from cultured skin fibroblasts, muscle tissue or blood using salt extraction.[7] The entire mtDNA of the patients was amplified and sequenced according to the protocol of Taylor *et al*[8] with minor adaptations.

Percentage heteroplasmy was quantified using the Pyrosequencing technology (Biotage AB, Uppsala, Sweden). Pyrosequencing was performed on the PSQ96 MA platform according to the protocol of the manufacturer. Primer sequences are available upon request.

Analysis of mitochondrial protein synthesis

In vitro pulse labeling of mitochondrial translation was performed as described elsewhere,[9] with a few adaptations. In short, cells were labeled for 60 min at 37 °C in methionine-free DMEM with 10% dialyzed FCS, 200 μCi/ml [^{35}S]methionine (Tran^{35}S-Label, MP Biomedicals, Eindhoven, The Netherlands) and 100 μg/ml emetine, and subsequently chased for 10 min in regular DMEM with 10% FCS. Total cellular protein (100 μg) was resuspended and incubated for 10 min in PBS containing 2% lauryl maltoside. Unsolubilized material was removed by centrifugation at 10 000 *g* for 10 min and loading buffer was added to the supernatant to a final concentration of 100 mM Tris-HCl (pH 6.8), 20% glycerol, 1% SDS, 0.02% Coomassie blue G-250 and 1% mercaptoethanol. Samples were run on 16% polyacrylamide gels,[10] which were subsequently scanned on a FLA5100 (Fujifilm Life Science, Düsseldorf, Germany) and analyzed using ImageQuant software (GE Healthcare, Diegem, Belgium). Equal protein loading was confirmed using Coomassie blue staining (data not shown).

Blue-native PAGE and complex I in-gel activity assay

Blue-native PAGE was used for separation of the OXPHOS complexes on 5–15% polyacrylamide-gradient gels as described before.[11] After electrophoresis of 40 μg protein from fibroblast and cybrid cells, gels were further processed for in-gel activity assays and western blotting. Assembly of the OXPHOS complexes was analyzed using monoclonal antibodies against subunits of complexes I, III, V (Molecular Probes, Leiden, The Netherlands), II, and IV (MitoSciences, Eugene, OR, USA).

Northern blot analysis

Total RNA samples (10 μg) isolated from cultured fibroblast and cybrid cell lines using RNA-Bee (Tel-Test) were separated on a 15% denaturing UREA-PAGE gel and transferred to a Hybond-N membrane (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) using electroblotting. For the conformational analysis, total RNA samples were separated for 24 h at 4 °C on a non-denaturing 15% polyacrylamide gel. Denaturing was performed by placing the gel for 10 min in 0.2 M NaOH/ 0.5 M NaCl. Before electroblotting to a Hybond-N membrane (Amersham Pharmacia Biotech), neutralization was carried out by washing twice for 10 min in 5×TBE and twice for 10 min in 1×TBE. After hybridization with the tRNA^{Arg} probe, the membrane was washed and subsequently rehybridized with the tRNA^{Trp} probe.

For the detection of tRNA^{Trp} and tRNA^{Arg}, templates for hybridization probes were generated by PCR using oligonucleotides containing the T7 RNA polymerase promoter sequence in the reverse primer. Primer sequences are available upon request. For *in vitro* transcription, the gel-purified PCR template was incubated for 2 h at 37 °C in 60 ml of buffer containing 40 mM Tris-HCl (pH 7.9), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM dithiotreitol, 1 mM each of ATP, CTP and UTP, 0.1 mM GTP, 60mCi [a-32P]GTP and 45 U T7 RNA polymerase.

Imaging was performed using a PhosphorImager (Molecular Imager FX, Bio-Rad, Veenendaal, The Netherlands); signal intensities for tRNA^{Trp} and tRNA^{Arg} were normalized to each other for each sample.

RESULTS

Enzyme measurements

The biochemical assays of OXPHOS enzyme activities showed clear combined complex I, III and IV deficiencies in patients T and A (Table 1). The activities measured as percentage of the lowest control value ranged from 15 to 59% in patient T and from 19 to 44% in patient A, excluding complex III activity, which was not decreased considerably in patient A.

Molecular genetic analysis

Sequence analysis of the entire mitochondrial genome revealed 18 (patient T) and 39 (patient A) known polymorphic variants compared with the revised Cambridge Reference Sequence,[12] and two mutations: a G-to-A transition at position m.5556 in the tRNA^{Trp} gene (*MT-TW*) in patient T and an A-to-G transition at position m.10450 in the tRNA^{Arg} gene (*MT-TR*) in patient A. The levels of the mutations were nearly homoplasmic in both patients, in muscle tissue as well as cultured fibroblasts (Table 2). In the blood of the mothers the mutations could not be detected, whereas in fibroblasts of the mother of patient A the m.10450A>G mutation was present with 18% heteroplasmy. The two mutations were not reported before and were found neither in 84 healthy control subjects and in 31 patients with a combined OXPHOS complex I, III and IV deficiency (tested by PCR) nor in haplogroup-matched controls, 499 controls of haplogroup H for tRNA^{Trp} and 121 controls of haplogroup T for tRNA^{Arg} (database search).[13] Position m.5556 is located in the variable region of the tRNA^{Trp} structure and position m.10450 is located in the T stem of tRNA^{Arg} (Figures 1a and b). The nucleotide mutated in tRNA^{Trp} is entirely conserved in mammals[14] and also in several lower species (Figure 1c). The mutation in tRNA^{Arg}, in contrast, affects a base that is conserved in many mammalia,[14] but not in lower species (Figure 1d).

Analysis of mitochondrial protein synthesis

As shown in Figure 2, both patients showed a marked reduction in radioactive labeling as compared with controls, indicating that the rate of mitochondrial protein synthesis is decreased. The total incorporation of [^{35}S]methionine was 45% (patient T) and 57% (patient A) of that in controls; however, different polypeptides were affected to

Table 1 OXPHOS enzyme activities in skeletal muscle and fibroblasts

	Activities (mU/U citrate synthase) in					
	Skeletal muscle			*Fibroblasts*		
Complex	*Patient T*	*Patient A*	*Control range*	*Patient T*	*Patient A*	*Control range*
I	**26**	**31**	70–251	**39**	**39**	100–310
II	387	455	335–749	774	544	520–845
III	**1306**	**1870**	2200–6610	**571**	1383	1320–2610
II+III	386	420	300–970	140	218	110–470
IV	**322**	**157**	810–3120	**102**	**223**	680–1190

Samples were supernatants obtained after 600 *g* centrifugation of skeletal muscle or mitochondrial-enriched fractions from fibroblasts. The decreased activities as compared with controls are in bold.

Table 2 Percentages of mutant mtDNA as determined using pyrosequencing

Subject	*Sample*	*m.5556G>A*	*m.10450A>G*
Patient	Fibroblasts	92	92
	Skeletal muscle	93	93
	Cybrid clone 1	90	91
	Cybrid clone 2	93	90
	Cybrid clone 3	96	88
Mother	Blood	0	0
	Fibroblasts	ND	18

Abbreviation: ND, not determined.

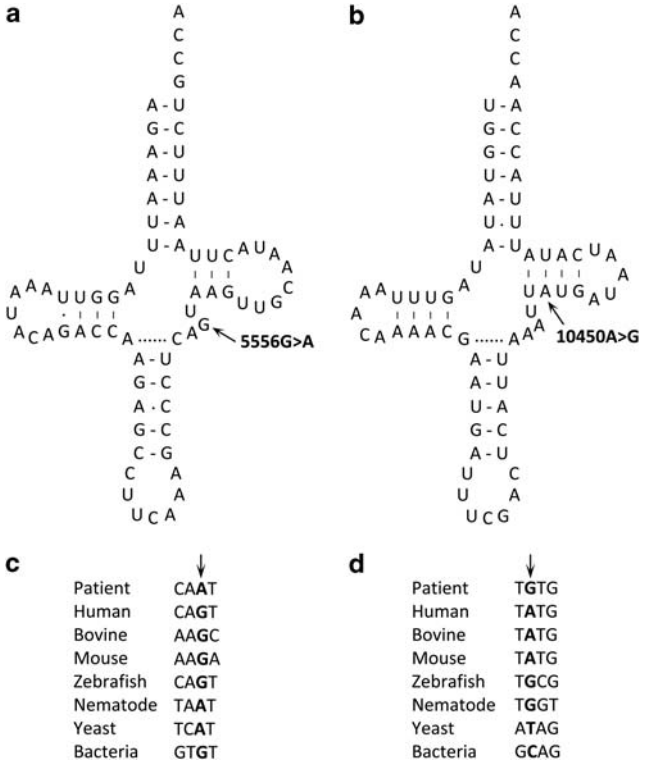

Figure 1 Schematic representation of the tRNA^{Trp} (**a**) and tRNA^{Arg} (**b**) cloverleaf structures, showing the location of the mutations. Comparison of the sequences of the variable region of the tRNA^{Trp} gene (**c**) and the T-stem of the tRNA^{Arg} gene (**d**) across different species; the mutated bases are depicted in bold.

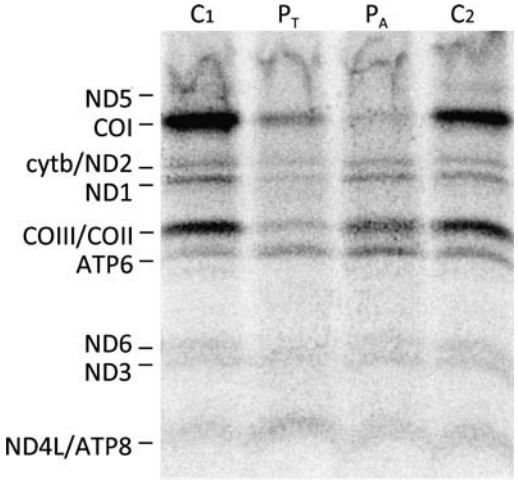

Figure 2 *In vitro* labeling of mitochondrial translation products. Fibroblasts from the patients (P_T and P_A) and controls (C1 and C2) were labeled with [^{35}S]methionine in the presence of emetine. The mitochondrial translation products are indicated on the left of the autoradiogram. COI–COIII, subunits of cytochrome *c* oxidase; cyt b, cytochrome *b* subunit; ND1–6, subunits of NADH CoQ oxidoreductase; ATP6–8, ATP synthase subunit 6 and 8.

variable extents (Table 3). In patient T, the rate of mitochondrial translation ranged from 22 to 126% and the three subunits of complex IV (COI–COIII) were most severely affected. In patient A, synthesis of COI showed by far the strongest decrease (12%) and the other polypeptides were affected only minimally with incorporation rates of 68% or higher. In both patients, the synthesis levels of the two ATP synthase subunits (ATP6 and ATP8; the latter migrated in a band together with ND4L) were comparable to or even higher than the levels in controls.

Blue-native PAGE and complex I in-gel activity assay

To determine whether the combined OXPHOS deficiencies in the patients were indeed caused by an mtDNA defect, we analyzed the assembly of all OXPHOS complexes in fibroblasts and cybrid cell lines using blue-native PAGE analysis. Patient fibroblasts showed reduced levels of fully assembled complexes I, III (only in patient T), IV and V as well as decreased complex I activities (Figure 3), which is consistent with the deficiencies detected in the enzyme measurements (Table 1). This pattern was also found in the different cybrid clones, proving the mitochondrial origin of the OXPHOS defect. In addition to the fully assembled complex V, two subcomplexes were observed in most samples with raised levels in the patient samples. These subcomplexes are often increased in patients with a defect in the mtDNA, but sometimes they are entirely absent. This was the case for the experiment with fibroblasts from patient A and the control, which was probably caused during the preparation of the samples.

Northern blot analysis

To analyze the effect of both mutations on tRNA stability, steady-state levels of tRNA^{Trp} and tRNA^{Arg} were determined using northern blot hybridization in cultured fibroblasts and cybrid cell lines of the two patients. The amounts of mutant tRNA were markedly decreased (Figure 4): in patient T, the tRNA^{Trp} levels were reduced to 29% (fibroblasts) and 33% (cybrids), and in patient A the tRNA^{Arg} levels

were reduced to 29% (fibroblasts) and 50% (cybrids) (after normalization to the tRNA^{Arg} or tRNA^{Trp} levels, respectively, and relative to average control values).

In addition, native gel electrophoresis provided insights into whether the mutations affect tRNA structure. Whereas on a denaturing gel the tRNA mutants migrated at the same rate as the wild type (see steady-state data, Figure 4), both tRNA mutations resulted in aberrant migration patterns under non-denaturing conditions (see conformation data, Figure 4), indicating that they primarily affect the secondary or tertiary structure of the tRNAs. The mutation in tRNA^{Trp} led to retardation of the electrophoretic mobility of the residual tRNA^{Trp} as compared with controls. The analysis of tRNA^{Arg} revealed the presence of two different conformations with accelerated mobility.

As the cybrid clones showed similar steady-state levels and migration patterns as found in fibroblasts of the patients, these results are again consistent with the mitochondrial origin of the OXPHOS defects.

DISCUSSION

In this study we clearly show that the m.5556G>A and m.10450A>G mutations are pathogenic and are the cause of the combined OXPHOS disorders found in the two patients. This conclusion is based on the following evidence: (1) the mutations are not present in healthy controls or in asymptomatic maternal relatives (or with a low mutant load), (2) the mutations affect evolutionary conserved bases (applies more to the tRNA^{Trp} mutation than the mutation in the tRNA^{Arg} gene), (3) patient fibroblasts have a general mitochondrial translation defect, (4) tRNA steady-state levels and conformation are affected by the mutations and (5) transmitochondrial cybrids show similar OXPHOS complex and tRNA defects as patient fibroblasts.

Although the mutation in the tRNA^{Trp} gene was not found in the blood of the patient's mother, we cannot conclude that it is a *de novo* mutation. Mutation load can vary greatly between tissues causing the mutation to be undetectable in, for instance, blood, as was the case for the tRNA^{Arg} mutation. In patient A the mutation was present at 92% heteroplasmy, whereas his mother had a mutant load of only 18% in fibroblasts, which is apparently below the threshold level for this mutation and therefore does not result in clinical disease.

Approximately 70% of pathogenic mutations occur in the stems of the tRNA cloverleaf structure.[15,16] Furthermore, the disruption of Watson–Crick base pairing is an important characteristic of pathogenic mutations located in the stem structures.[15] The mutation in the tRNA^{Arg} gene affects base 50 in the T stem and disrupts Watson–Crick base pairing, which makes it a likely candidate for the disease-causing mutation. However, a wobble base pair can still be formed with the mutant base. The disturbance of Watson–Crick base pairing could affect the secondary structure of the tRNA^{Arg}, leading to misfolding and instability of the mutant tRNA. This was confirmed using northern blotting, which showed reduced tRNA^{Arg} steady-state levels and the existence of two aberrant conformations in the absence of the normal tRNA. In contrast, the site that is mutated in the tRNA^{Arg} gene is conserved merely in mammalia. Although most pathogenic tRNA mutations are found at highly conserved sites, there are exceptions, indicating that evolutionary conservation is not required for a mutation to be pathogenic.[16,17]

The m.5556G>A mutation is located at position 46 in the variable region of tRNA^{Trp}, which is not a common site for pathogenic mutations.[15] However, this base is highly conserved. In addition, a potential tertiary interaction between nucleotides 13-22-46 in tRNA^{Trp} has been described.[14] This interaction is present in all mammalian tRNA families and is part of a set of three tertiary interactions that are thought to be minimally required for proper functioning of the mitochondrial tRNAs. Nucleotide 46 is N^7-methylated in many

Table 3 Level of mitochondrial protein synthesis

Polypeptide	*Patient T*	*Patient A*
COI	28	12
Cytb/ND2	56	86
ND1	35	82
COIII/COII	22	68
ATP6	124	163
ND6/ND3	82	89
ND4L/ATP8	126	94

The values represent percentages of the average incorporation of [^{35}S]methionine measured in two controls.
Abbreviations: COI–COIII, subunits of cytochrome *c* oxidase; cyt b, cytochrome *b* subunit; ND1–6, subunits of NADH CoQ oxidoreductase; ATP6–8, ATP synthase subunits 6 and 8.

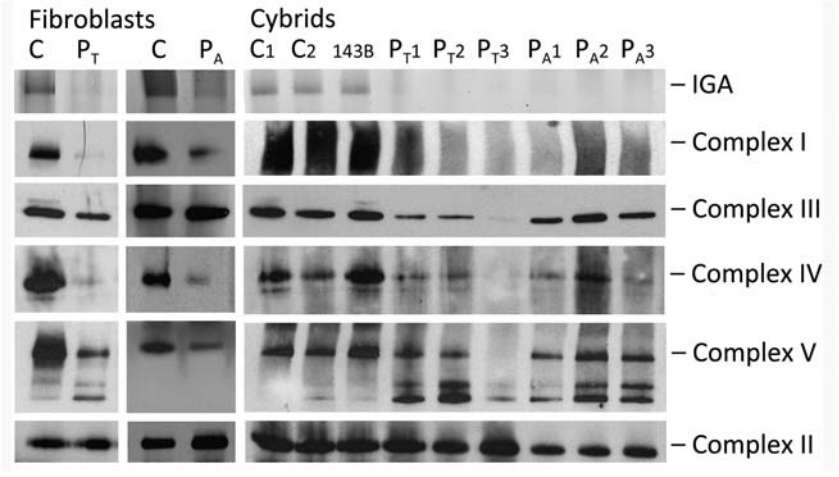

Figure 3 Blue-native PAGE and complex I in-gel activity assay. Fibroblasts and cybrid clones from patient T (P$_T$) and A (P$_A$) and controls (C1–3 and 143B) were analyzed. The blue-native PAGE gels were immunoblotted with antibodies directed against specific individual subunits to assess the amount of each of the five fully assembled OXPHOS complexes. Complex II was used as a loading control. IGA, in-gel activity.

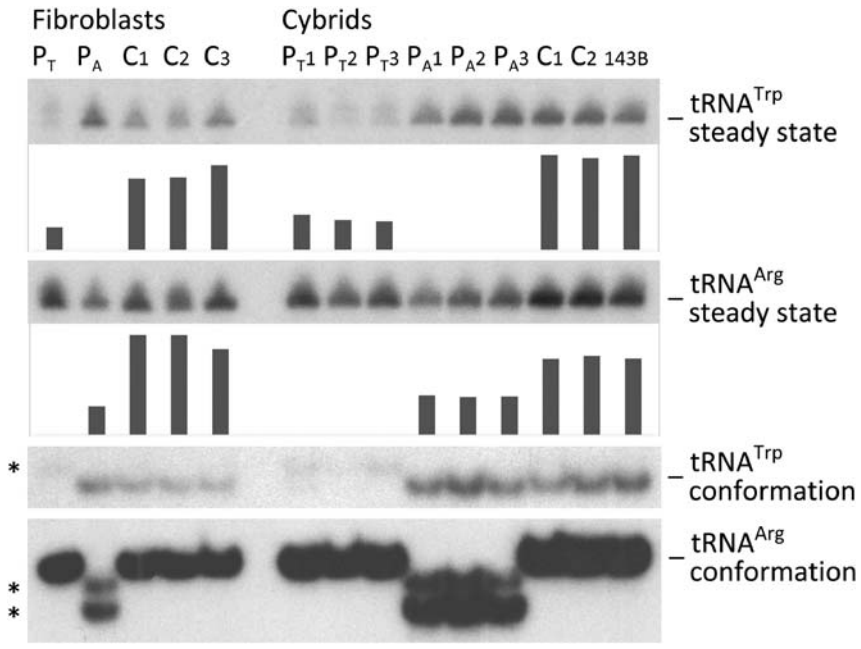

Figure 4 Steady-state levels and conformation of tRNA^{Trp} and tRNA^{Arg}. Fibroblasts and cybrid clones from patient T (P_T) and A (P_A) and controls (C1–3 and 143B) were analyzed using high-resolution northern blot under denaturing (steady-state) and native (conformation) conditions. Signal intensities for tRNA^{Trp} and tRNA^{Arg} were normalized to each other for each sample, as shown in the column charts (only relevant steady-state levels are depicted). The asterisks indicate the position of altered conformations of mutant tRNA^{Trp} and mutant tRNA^{Arg}.

tRNAs of different species.[18] This post-transcriptional modification, together with hydrogen bonding to the bases G22 and C13, results in a positive charge, which could be necessary for the L-shaped three-dimensional tRNA structure and/or for protein recognition.[19] In general, the role of modified bases for the maintenance of the tRNA tertiary structure is of higher importance in mitochondrial tRNAs than in their cytosolic counterparts.[20] Certain mitochondrial tRNAs will consequently be completely non-functional when lacking the post-transcriptional modification. Northern blot analysis revealed a clear decrease in the amount of mutant tRNA^{Trp} as well as an altered conformation, indicating that the mutation might indeed interfere with the tertiary interaction and thereby disturb the three-dimensional tRNA structure and tRNA stability.

Although the MITOMAP database lists the m.5556G>A transition as an unpublished polymorphism,[21] our results clearly show that this nucleotide change is a pathogenic mutation in our patient. This underscores the importance of functional studies in defining the pathogenicity of tRNA mutations. Seven pathogenic mutations have been reported in the mitochondrial tRNA^{Trp} gene.[2] None of these mutations are located near the m.5556G>A mutation that we found. However, the m.5532G>A mutation[22] affects base 22, which is supposedly involved in the tertiary interaction with base 46 (m.5556) mentioned above. Unfortunately, the effect of this point mutation on tRNA stability and conformation has not been analyzed. A common feature of most tRNA^{Trp} mutations is a profound complex IV defect.[22–28] In some cases complex IV is selectively decreased, in other cases complex I is affected to a similar extent and occasionally complex III is involved as well. Biochemical analysis revealed a combined complex I, III and IV deficiency in patient T, in which complex IV was most severely affected (on average, 28% of control levels), followed by complex I (38%) and last by complex III (51%). The results of the *in vitro* translation assay also supported this as the synthesis rates of COI and COIII/COII showed the strongest decrease as compared with control rates. The fact that complex IV is more susceptible to tRNA^{Trp} mutations could be explained by the higher percentage of tryptophan in the subunits COI and COIII as compared with other complex subunits, as has been proposed before.[26,27] Nonetheless, a correlation between the relative amount of tryptophan in individual subunits and the reduction of their mitochondrial translation rates in our patient was not evident, which has also been observed by Sacconi *et al* for the m.5545C>T mutation.[24] They found COI and COIII translation to be most severely disturbed, but in contrast to our results ATP6 showed a profoundly decreased synthesis as well. Increased synthesis of ATP6 and ATP8, as we observed for our tRNA^{Trp} and tRNA^{Arg} mutations, has been reported before[29–32] and has been suggested to be caused by the fact that bicistronic mRNAs can be translated more efficiently than monocistronic mRNAs, thereby giving the translation of ATP6 and ATP8 an advantage.[32] Many patients with a tRNA^{Trp} mutation, including our patient, show developmental delay, gastrointestinal symptoms and failure to thrive.[22,24,25,28] In addition, visual defects, deafness, epilepsy and neurodegenerative disease are frequently observed in other patients.[22–28,33]

In the mitochondrial tRNA^{Arg} gene only two pathogenic mutations have been reported so far: m.10406G>A and m.10438A>G.[2] In the patient with the m.10438A>G mutation, no clear OXPHOS deficiency was found, only a minor decrease in complex IV activity.[34] In contrast, the m.10406G>A mutation caused a marked reduction in complex IV activity, and complex I and III were also affected.[35] This is consistent with our findings, except that complex III was hardly impaired in patient A. The clinical phenotypes of the three patients carrying tRNA^{Arg} mutations are variable; the only common features are muscle weakness and brain abnormalities. Furthermore, visual dysfunction and progressive psychomotor retardation were observed both in our patient and for the m.10438A>G mutation.[34] The clinical presentation of the m.10406G>A mutation is considerably different with Asperger syndrome and normal cognitive function.[35] Our patient manifested the most severe symptoms in a progressive multisystem

disorder. The variability in clinical phenotypes could be explained by the fact that all three mutations are located in different domains of the tRNA cloverleaf structure. A distinct correlation seems not to exist between clinical presentation and the specific tRNA that is mutated.[16,36] For our m.10450A>G mutation, the disproportionate decrease in synthesis of some polypeptides (mainly COI synthesis was impaired) is not correlated with the arginine content of the polypeptides. The effects on translation have not been analyzed for the other tRNA^{Arg} mutations.

In conclusion, we show that the mutations in the mitochondrial tRNA^{Trp} and tRNA^{Arg} genes are pathogenic. The effect of the mutations on the tertiary (tRNA^{Trp}) or secondary (tRNA^{Arg}) structure will likely lead to misfolding and instability, resulting in increased degradation and possibly also in impaired aminoacylation. Consequently, the scarcity and malfunctioning of the tRNAs cause a combined OXPHOS deficiency through reduced rates of mitochondrial protein synthesis. This gives rise to a progressive multisystem disease in both patients, marked by failure to thrive, lactic acidosis, feeding difficulties, gastrointestinal problems, generalized muscle hypotonia, progressive psychomotor retardation and microcephaly.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the European Union's Sixth Framework Program, contract number LSHMCT-2004-005260 (MITOCIRCLE), and in part by the Council for Chemical Sciences (NWO-CW) of the Netherlands Organization for Scientific Research. We thank M Zeviani for providing one of the cybrid control cell lines.

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