

The m.5650G > A mitochondrial tRNA^{Ala} mutation is pathogenic and causes a phenotype of pure myopathy

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Abstract

We report a family where a predominantly proximal myopathy has become increasingly severe with successive generations of the maternal lineage. This pure myopathy has been caused by a mutation (m.5650G > A) in the mt-tRNA^{Ala} gene that has been reported only once previously in a patient with CADASIL where the phenotype was dominated by neurological complications. This report is therefore the first description of the phenotype associated solely with this mutation and confirms its pathogenicity.

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1. Introduction

Point mutations of mitochondrial DNA (mtDNA) are responsible for a variety of different, but occasionally overlapping clinical syndromes [1]. Tissues heavily reliant on oxidative metabolism, such as skeletal muscle, are often worst affected and myopathy is a common clinical manifestation of mitochondrial disease. In these circumstances myopathy is usually only one of a number of clinical features comprising a ‘mitochondrial syndrome’ such as MELAS or Kearns-Sayre syndrome. Isolated or ‘pure’ myopathy as the solitary manifestation of mitochondrial disease is relatively rare. Nevertheless, it has been associated with several point mutations of mtDNA in both the cytochrome *b* [2] and mitochondrial tRNA (mt-tRNA) genes [3], as well as recessive mutations in the nuclear-encoded *TK2* gene [4]. Maternal inheritance of this pure myopathy phenotype is even less common, being limited

to a few mt-tRNA mutations [5]. We report a family where the m.5650G > A mutation in mt-tRNA^{Ala} has caused an increasingly severe proximal (limb-girdle) myopathy in successive generations. We detail the phenotype associated with this mutation and present evidence from single muscle fibre analysis that confirm its pathogenicity.

1.1. Case report

The proband first presented at the age of 11 years following difficulties during physical education classes at school. She experienced great difficulty rising from the floor, found it impossible to run and complained of fatigue after minimal exercise. She was born at full-term, the result of a normal pregnancy and delivery and had no congenital dislocations or contractures. Motor development was good and she was walking independently by 1 year. Running was not difficult in early childhood, but from the age of 6 years onward she endured increasing difficulty with inclines and stairs, which she presently manages to climb only by using both arms to haul herself upward. She did not suffer from

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cardiac or respiratory problems other than mild asthma and she did not complain of dysphagia. Examination revealed a waddling gait and full Gower's manoeuvre on rising from the floor. She had scapular winging and a mild scoliosis, but her spine remained mobile. Power was reduced around the shoulder and hip girdles with shoulder abduction and flexion and all hip movements being MRC grade 3. Cervical spine flexion was also weak (MRC grade 3) as was knee extension (MRC grade 4) and flexion (MRC grade 3). Distal movements in the upper and lower limbs were of normal power and she could stand on her heels and toes without difficulty. She had mild bilateral ptosis but no ophthalmoplegia or weakness of other facial muscles. Ophthalmoscopy revealed no retinal or optic nerve abnormalities and audiometry, ECG and echocardiogram were also normal. Serum creatine kinase (CK) was elevated at 374 (U/L) and measurements of respiratory function indicated diaphragmatic weakness with a supine forced vital capacity (FVC) of 46% predicted and seated FVC of 66% predicted. Enquiry about the family history found that although there was no formal diagnosis of any neuromuscular disorders in the family, the mother and grandmother of the index case were both aware of some problems with their muscles.

The mother of the index case is a 42-year-old female who had variable symptoms of fatigue. She could manage to climb one flight of 12 stairs without difficulty and walking distance on level ground was not impaired. However, anticipation of post-exercise fatigue had led her to minimise her physical activities and she had chosen a sedentary career as a secretary. She had noticed difficulty in rising from a squat and from a chair, but otherwise weakness was not a major feature. She did however avoid physical education at school and describes herself as having been a 'clumsy' child. Examination confirmed weakness of hip girdle musculature (MRC grade 4) and of shoulder abduction and flexion (MRC grade 4+), but she had no evidence of ptosis or facial weakness. She had normal ophthalmologic and cardiovascular examinations and no abnormalities were detected on either ECG or echocardiogram. As with her daughter, serum CK level was elevated at 610 U/L but she demonstrated only mild diaphragmatic weakness with a supine FVC of 75% predicted and seated FVC of 80% predicted.

The grandmother of the index case is a 63-year-old female who had no complaints of weakness or fatigue but had noticed over the previous 10 years some progressive difficulty in rising from a squat. Examination confirmed weakness of hip girdle musculature (MRC grade 4+) but was otherwise normal.

Muscle biopsy was performed in the index case to investigate her clinical presentation of possible autosomal dominant limb-girdle muscular dystrophy (LGMD) or myopathy. Her mother subsequently underwent muscle biopsy to confirm that she had the same disorder as her daughter. The grandmother refused muscle biopsy but consented to non-invasive samples being taken for genetic analysis.

2. Methods

Standard histological and histochemical (including sequential cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH)) analyses were performed on frozen sections (10 µm) of quadriceps muscle biopsies taken from the index case and her mother. The activities of the respiratory chain complexes and the matrix marker citrate synthase were determined as previously described [6].

Total DNA was extracted from several tissues by standard procedures. Rearrangements of mtDNA were investigated by Southern-blot analysis and long-range PCR of muscle DNA. The entire sequence of the mitochondrial genome was amplified using 36 M13-tailed oligonucleotide primer pairs with DNA isolated from skeletal muscle as template, sequenced using BigDye[®] Terminator v3.1 chemistries (Applied Biosystems) and directly compared to the revised Cambridge reference sequence [7].

To determine the level of heteroplasmy, a 150 bp PCR product spanning the mutation site was amplified using a modified forward primer (nt 5549–5569) 5'-GCCGTCAGTAAGTTGCAATAC-3' and a modified reverse primer (nt 5698–5676) 5'-CTGTAAAGTAAGTGTGGTGGT-3' with the mismatch nucleotides shown in bold in each primer sequence; the mismatches in each primer abolish naturally-occurring DdeI sites. Two DdeI restriction sites in the wild-type PCR product cut the 150 bp amplicon into three fragments of 38, 60 and 52 bp. In the presence of the m.5650G > A transition, one of these sites is lost, resulting in restriction fragments of 38 and 112 bp. Prior to the last cycle of PCR, 5 µCi [α -³²P]dCTP (3000 Ci/mmol) was added. Labelled products were precipitated, digested with 10 U DdeI, separated through a 12% non-denaturing polyacrylamide gel, and the radioactivity in each fragment quantified using ImageQuant software (Amersham Biosciences/GE Healthcare).

3. Results

Histological examination of the proband's muscle biopsy showed marked variation in fibre size, with evidence of inflammatory changes on H&E histology (Fig. 1a), and upregulation of MHC Class I antigens on immunohistochemistry (data not shown). Enzyme histochemistry revealed a marked number (>50%) of COX-deficient fibres (Fig. 1b), some of which showed evidence of subsarcolemmal mitochondrial accumulation. Respiratory chain enzyme analysis of a skeletal muscle homogenate showed a marked decrease in the activities of both complex I (20% of controls) and complex IV (15% of controls) when related to citrate synthase activity, with normal activities of complexes II and III. Muscle biopsy of her mother showed none of the histological changes seen in the index case, but the COX defect was even more severe (>80% fibres affected). Similar to the proband, respiratory chain enzyme studies revealed a combined defect involving complexes I and IV (20% and 10% of controls, respectively).

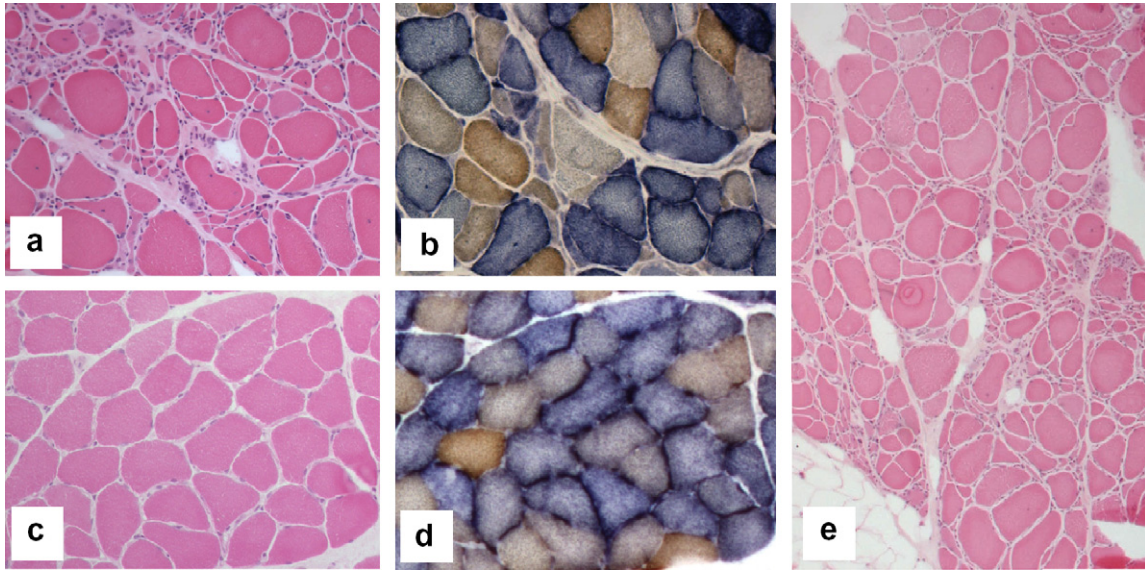


Fig. 1. Muscle biopsy analysis. (a) H&E-stained section of quadriceps muscle from the index case revealing a marked variation in fibre size and the presence of inflammatory infiltrate. Some fibres clearly show central nuclei. (b) Histochemical demonstration of combined COX and succinate dehydrogenase (SDH) activities in the patient's muscle biopsy, revealing significant numbers of COX-deficient fibres with subsarcolemmal accumulation of abnormal mitochondria. (c and d) Show H&E and COX/SDH sections of the mother's muscle biopsy, respectively, which does not exhibit dystrophic changes but has very high levels of respiratory-deficient fibres. (e) Is a low magnification H&E-stained section of quadriceps from the index case illustrating the extent of the inflammatory cell infiltrate.

Southern blot and long-range PCR analysis excluded the presence of large-scale mtDNA rearrangements in the proband's muscle DNA. Direct sequencing of the entire mitochondrial genome revealed a previously reported m.5650G > A transition [8] in the *MTTA* gene encoding mitochondrial tRNA^{Ala} (Fig. 2). PCR-RFLP analysis showed that the mutation was heteroplasmic, but present at very high levels (>95% mutant load) in all available tissues (muscle, blood, urine and buccal epithelia) with the exception of hair follicles (10%) (Fig. 2). Analysis of several tissues from her mother revealed similarly high levels of the mutation (Fig. 2), confirming maternal transmission of this mutation, whilst the grandmother harboured detectable levels only in urinary epithelial cells (2% mutant load) (Fig. 2).

Single muscle fibre analysis was performed to determine whether the amount of mutated mtDNA correlated with

the observed biochemical phenotype in individual fibres. We detected higher levels of the m.5650G > A mutation in the proband's COX-deficient fibres ($99.0 \pm 0.29\%$ ($n = 9$)) than in her COX-positive fibres ($87.6 \pm 2.26\%$ ($n = 15$)) ($P < 0.0008$, two-tailed Student's *t* test), confirming segregation of the m.5650A genotype with respiratory chain dysfunction (Fig. 3a).

4. Discussion

The m.5650G > A mutation in mt-tRNA^{Ala} has been reported once previously in a patient with cerebral autosomal dominant arteriopathy subcortical infarcts and leukoencephalopathy (CADASIL) [8]. This patient also harboured a concomitant R133C mutation in the *Notch 3* gene and although myopathy was described, the phenotype

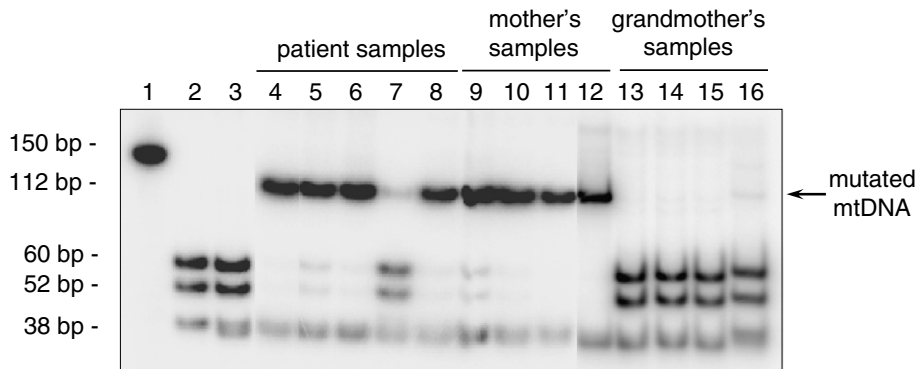


Fig. 2. m.5650G > A mutation analysis. Determination of the relative amounts of mutant and wild-type mtDNA in tissues from the patient, her mother and her grandmother by PCR-RFLP analysis. Lane 1, uncut sample; lanes 2 and 3, control samples; lanes 4 and 9, skeletal muscle; lanes 5, 10 and 13, circulating lymphocytes; lanes 6, 11 and 14, buccal epithelia; lanes 7 and 15, hair shafts; lanes 8, 12 and 16, urinary epithelia.

function in mt-tRNA^{Ala} aminoacylation, an essential prerequisite for protein translation [12]. The m.5650G > A mutation may disrupt this interaction between synthetase and acceptor stem, decreasing the efficiency of aminoacylation. The mutation does however fulfil other canonical criteria for pathogenicity – it is heteroplasmic, segregates with clinical disease, produces an observable biochemical defect and is absent from the control population (not present on either of the publicly available databases [13,14]). Interestingly, a previously reported mt-tRNA^{Ala} mutation (m.5591G > A) which also caused a limb-girdle myopathy resides within the aminoacyl acceptor stem of the tRNA molecule and was also associated with a marked histochemical COX deficiency [3].

In conclusion, the investigation of a possible autosomal dominant LGMD or myopathy in this family with increasingly severe disease through successive generations has revealed a maternally inherited mt-tRNA point mutation. We have been able to confirm the pathogenicity of this m.5650G > A mutation and ascribe a definitive phenotype where previously this has not been possible. The pure myopathic features seen in this family illustrate the remarkable tissue specificity of some mtDNA point mutations and indicate the need to consider mitochondrial disease in any family presenting with an apparently autosomal dominant limb-girdle syndrome.

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References

- [1] Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. *Nat Rev Genet* 2005;6:389–402.
- [2] Andreu AL, Hanna MG, Reichmann H, et al. Exercise intolerance due to mutations in the cytochrome *b* gene of mitochondrial DNA. *N Engl J Med* 1999;341:1037–44.
- [3] Swalwell H, Deschauer M, Hartl H, et al. Pure myopathy associated with a novel mitochondrial tRNA gene mutation. *Neurology* 2006;66:447–9.
- [4] Mancuso M, Salviati L, Sacconi S, et al. Mitochondrial DNA depletion: mutations in thymidine kinase gene with myopathy and SMA. *Neurology* 2002;59:1197–202.
- [5] Hadjigeorgiou GM, Kim SH, Fischbeck KH, et al. A new mitochondrial DNA mutation (A3288G) in the tRNA(Leu(UUR)) gene associated with familial myopathy. *J Neurol Sci* 1999;164:153–7.
- [6] Kirby DM, Thorburn DR, Turnbull DM, et al. Biochemical assays of respiratory chain complex activity. *Methods Cell Biol* 2007;80:93–119.
- [7] Andrews RM, Kubacka I, Chinnery PF, et al. Reanalysis and revision of the Cambridge Reference Sequence. *Nat Genet* 1999;23:147.
- [8] Finnila S, Tuisku S, Herva R, et al. A novel mitochondrial DNA mutation and a mutation in the *Notch3* gene in a patient with myopathy and CADASIL. *J Mol Med* 2001;79:641–7.
- [9] Annunen-Rasila J, Finnila S, Mykkanen K, et al. Mitochondrial DNA sequence variation and mutation rate in patients with CADASIL. *Neurogenetics* 2006;7:185–94.
- [10] Kiyomoto BH, Tengan CH, Costa CK, et al. Frequency of dystrophic muscle abnormalities in chronic progressive external ophthalmoplegia: analysis of 86 patients. *J Neurol Neurosurg Psychiatry* 2006;77:541–3.
- [11] Olsen DB, Langkilde AR, Orngreen MC, et al. Muscle structural changes in mitochondrial myopathy relate to genotype. *J Neurol* 2003;250:1328–34.
- [12] Park SJ, Schimmel P. Evidence for interaction of an aminoacyl transfer RNA synthetase with a region important for the identity of its cognate transfer RNA. *J Biol Chem* 1988;263:16527–30.
- [13] Ingman M, Gyllenstein U. mtDB: Human Mitochondrial Genome Database, a resource for population genetics and medical sciences. *Nucleic Acids Res* 2006;34:D749–51.
- [14] Brandon MC, Lott MT, Nguyen KC, et al. MITOMAP: a human mitochondrial genome database – 2004 update. *Nucleic Acids Res* 2005;33:D611–3.