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Neuromuscular Disorders 15 (2005) 364–371

www.elsevier.com/locate/nmd

New phenotypic diversity associated with the mitochondrial tRNA$^{Ser(UCN)}$ gene mutation

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Received 22 November 2004; received in revised form 15 January 2005; accepted 21 January 2005

Abstract

We performed detailed clinical, histopathological, biochemical, in vitro translation and molecular genetic analysis in patients from two unrelated families harbouring the tRNA$^{Ser(UCN)}$ 7472C-insertion mutation. Proband 1 developed a progressive neurodegenerative phenotype characterised by myoclonus, epilepsy, cerebellar ataxia and progressive hearing loss. Proband 2 had a comparatively benign phenotype characterised by isolated myopathy with exercise intolerance. Both patients had the 7472C-insertion mutation in identical proportions and they exhibited a similar muscle biochemical and histopathological phenotype. However, proband 2 also had a previously unreported homoplasmic A to C transition at nucleotide position 7472 in the tRNA$^{Ser(UCN)}$ gene. This change lengthens further the homopolymeric C run already expanded by the 7472C-insertion. These data extend the phenotypic range associated with the 7472C-insertion to include isolated skeletal myopathy, as well as a MERRF-like phenotype.

Keywords: Exercise intolerances; MERRF; Respiratory chain disease; Mitochondrial DNA

1. Introduction

The mitochondrial encephalomyopathies are a genetically heterogeneous group of disorders associated with inborn errors of oxidative phosphorylation [1]. They commonly associate with mutations in mitochondrial DNA (mtDNA) but the mechanisms of phenotype–genotype correlation are still unclear [2,3]. Tiranti et al. [4] originally described an Italian family with maternally inherited sensorineural hearing loss (SNHL), progressive ataxia and myoclonus associated with a novel heteroplasmic 7472C-insertion (7472insC) mutation in the mitochondrial transfer RNA for Serine (UCN) (tRNA$^{Ser(UCN)}$) gene. Further families with similar phenotypes were reported to harbour this mutation indicating it is a common cause of a MERRF-like phenotype with SNHL [5,6]. It has also been reported with SNHL in isolation [7].

Here, we report patients from two unrelated families harbouring the 7472insC mutation in the tRNA$^{Ser(UCN)}$ gene. The first patient had a MERRF phenotype similar to patients previously reported, although there were notable differences in the histochemical findings. The second patient presented with early onset myopathy and exercise intolerance but without any central nervous system involvement. The second patient also harboured an additional unreported change in the mitochondrial tRNA$^{Ser(UCN)}$ gene (A7472C).

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doi:10.1016/j.nmd.2005.01.006

2. Patients and methods

2.1. Patients

Patient 1 was a normal full term delivery and had normal motor and cognitive milestones. At the age of 12 years he developed generalised seizures and myoclonus. By the age of 16 years he had developed drug resistant epilepsy. From age 17 years, he developed progressive hearing loss and cognitive impairment. There was no family history of neurological disease. On examination at the age of 23 years he had a marked cerebellar syndrome including dysarthria and gait and limb ataxia. He had bilateral SNHL. There were myoclonic jerks in all limbs. There was no limb weakness or fatigue. An electroencephalography showed generalised spike wave discharge with photosensitivity.

Patient 2 was a normal full term delivery with normal cognitive and motor milestones. She developed exercise intolerance from the age of 11 years. After this age walking even short distances produced muscle fatigue. There was no history of muscle pain or myoglobinuria. There was no family history. On examination at the age of 15 years she had mild proximal upper and lower limb weakness (MRC grade 4/5). Electromyography showed a myopathic pattern and nerve conduction studies were normal. A CT brain scan was normal at the age of 13 years. Six maternal family members were examined but had no abnormal neurological signs. Last assessed at age 19 she had myopathy alone without CNS involvement.

2.2. Methods

2.2.1. Muscle biopsies

Diagnostic muscle biopsies were obtained from the left vastus lateralis in both patients at the age of 23 years in patient 1 and 16 years in patient 2.

2.2.2. Biochemistry

Mitochondria were isolated from fresh skeletal muscle. Respiratory enzyme activity assays were performed as described [8].

2.2.3. In vitro mitochondrial protein synthesis

A mitochondrial fraction from patient 1 was incubated at a concentration of 1.8 mg/ml in 100 μl of buffer and salts containing 90 mM KCl, 10 mM $MgCl_2$, 10 mM KH_2PO_4, 1 mM EDTA, 50 mM Bicine buffer (adjusted to pH 7.6), 25 mM glutamate, 50 μM atractyroside, 50 μl amino acid mixture deficient in methionine (Amersham) and 50 μCi ^{35}S methionine (specific activity > 1000 Ci/mM, Amersham). Incubation was carried out in a shaking water bath at 30 °C for 60 min. The reaction was then stopped on ice and the mitochondria pelleted down and stored at −70 °C prior to electrophoresis. Labelled mitochondria were solubilised in 4% sodium dodecyl sulphate (SDS) buffer and run in a LKB 2000 vertical electrophoresis system using a 6 M urea SDS polyacrylamide gel as previously described [9]. Gels were stained with Coomasie blue, dried onto filter paper under vacuum and autoradiographed at room temperature for up to 1 week.

2.2.4. MtDNA sequencing

Total DNA was extracted from tissues by standard procedures. Large-scale rearrangements of mtDNA were screened for by Southern blotting and the common mtDNA point mutations (A3243G, A8344G and T8993G/C) were excluded by PCR-RFLP of DNA extracted from muscle. MtDNA sequencing was performed on DNA extracted from muscle. All mitochondrial tRNA and cytochrome *c* oxidase (COX) subunit genes were sequenced by direct automatic DNA sequencing [details of primers available from authors].

2.2.5. Mismatch PCR and RFLP analysis for quantitation of proportion of the mutant mtDNA

The presence of the 7472insC mutation was confirmed by using mismatch PCR reactions in which a restriction site for the endonuclease *Xcm*I was introduced for the mutant nucleotide as previously described [4]. In the presence of mutant nucleotide at position 7472, the 208 bp PCR product was cleaved into two fragments of 168 and 40 bp. Another mismatch PCR reaction was designed to screen for the A7472C transition, in which a restriction site for *Nla*III was introduced in the presence of 'C' at position 7472. The oligonucleotide primers were: for heavy strand, 7492–7473 GGTTGGCTTGAAACCAGC**A**T (mismatch nucleotide in bold and underlined) and for light strand, 7321–7340. In the presence of mutant nucleotide, the 172 bp fragment was cleaved into two fragments, 155 and 17 bp.

Quantitation of the proportion of the 7472insC mutation was performed by an adaptation of the mismatch PCR. Fluorescently labelled deoxynucleotide was added prior to the last cycle of the PCR. The PCR products were digested with the endonuclease *Xcm*I and separated on a 5% non-denaturing polyacrylamide gel in a 373A DNA sequencer (Applied Biosystems). The data was then quantified using Genescan software (Applied Biosystems).

2.2.6. C-tract heteroplasmic length variation analysis

In order to determine whether length variation in the C-tract was relevant to phenotypic diversity, we performed RT-PCR as described previously [10]. PCR products of approximately 529 bp were generated (using a primer pair consisting of a gel purified 7446–7465 oligonucleotide and a biotinylated 7975–7957 oligonucleotide) using standard PCR conditions and immobilised on streptavidin coated beads. A single stranded ^{32}P-labelled product of approximately 52 bp, in which length variation was representative of length variation in the poly C-tract, was released by digestion with *Hae*III and electrophoresed on 8% denaturing acrylamide gels.