Title: New missense variants of *NDUFA11* associated with late onset myopathy

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Running title: Adult myopathy due to *NDUFA11* variant

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

Conflict of interest

None of the authors has any conflict of interest to disclose

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Introduction

Human mitochondrial respiratory chain complex I (CI) deficiency (MIM 252010) is the most common and challenging biochemical indicator of mitochondrial disease. Mitochondrial CI, or NADH-ubiquinone oxidoreductase, is a heteromeric structure composed of 45 subunits, seven encoded by mitochondrial DNA (mtDNA) genes and the remaining by nuclear genes.[1,2] Several additional gene products contribute to its formation and activity. This genetic, structural and functional complexity can explain the extraordinary clinical heterogeneity of CI defects that range from mild muscle involvement to Leigh syndrome or fatal neonatal multiorgan disease.[1] The nuclear gene *NDUFA11* encodes one of the CI subunits, which is believed to have a role in assembly and/or stabilizing the membrane domain of CI.[3] Only one report has been published of a disease caused by a mutation in this gene. The identified nucleotide change was at a splicing site and was inherited as a homozygous trait in affected individuals from three families of Israeli Bedouin origin. This mutation was associated with an early, often fatal, encephalocardiomyopathy phenotype and CI deficiency.[4]

Here we describe a patient who is a compound heterozygote for two novel rare variants of *NDUFA11* and affected by an adult onset neuromuscular phenotype, with a severe reduction of CI activity in muscle.

Case Presentation

The patient was a 72-year-old man with two healthy daughters and no family history of any

neuromuscular disease. At 65 years of age, he noted mild muscle weakness in his legs along with moderate leg stiffness. Examination revealed saccadic eye movements and bilateral hearing loss. Mild, proximal leg weakness was present (Medical Research Council [MRC] scale: 4.5) along with a reduction in the muscle bulk of the thighs. Deep tendon reflexes were symmetrically reduced in the lower limbs. No dysmetria or other cerebellar involvement was observed. No heart involvement was reported. Elevated creatine kinase (CK) levels (900 U/l) were detected.

Needle electromyography examination revealed short duration, low amplitude motor unit potentials, absence of abnormal spontaneous activity, and an early recruitment pattern in the muscles of the arms and legs. Sensory and motor nerve conduction studies were normal. A brain MRI showed diffuse white matter involvement due to cerebrovascular chronic lesions.

Informed consent was obtained for biochemical and genetic studies. Histological and histochemical analyses of a muscle biopsy from the quadriceps showed diffuse mitochondrial alterations (Figure 1A-D). The biochemical assessment of the mitochondrial respiratory chain revealed an isolated and severe deficiency of CI (Figure 1E-F).

The whole mtDNA sequence was normal. A genetic panel screening for nuclear genes encoding CI subunits and assembly factors was used and led to the identification of two heterozygous variants in *NDUFA11* (c.317C>T, p.Thr106Ile and c.394G>C, p.Ala132Pro; Figure 2A-B). Next generation sequencing revealed that the two variants were on different alleles (Figure 2C). Notably, the two variants present in our proband were located in the C-terminal exon, present only in the alternative isoform 2 of *NDUFAF11* (NM_001193375.1; NP_001180304.1) (Figure 2D). The variant c.317C>T was not reported in public SNP databases, while prediction programs gave contrasting results (Polyphen: benign; SIFT: deleterious) about the amino acid change p.Thr106Ile. However, the mutant nucleotide is located four positions from the intron 3-exon 4 junction and thus

could affect the splicing process. *In silico* prediction analysis (Human Splicing Finder) suggested that the nucleotide change c.317C>T impairs splicing (acceptor splice site: -9.22%), disrupting a splicing enhancer motif (site broken for enhancer motif SRp40). The variant c.394G>C is reported in dbSNPs as rs192066309 and has a frequency of 0.07% and 0.19% in the ExAC and gnomAD databases, respectively. The corresponding p.Ala132Pro missense change is predicted to be probably damaging by different bioinformatics tools (Polyphen2 and SIFT).

Idebenone therapy (5 mg/kg per day) was started after the identification of the genetic cause and we observed a mild improvement in muscle strength as measured on the MRC scale and reported by the patient.

No specimen was available from family members.

Discussion

This case represents the second report of biallelic mutations in *NDUFA11*. To date, only one mutation has been detected in this gene, associated with an extremely severe, early onset phenotype characterized by fatal infantile metabolic acidosis or severe encephalocardiomyopathy.[4]

As previously mentioned the two identified variants affect only isoform 2 of *NDUFA11*, and do not impair the production of isoform 1 (NM_175614). This may explain the mild phenotype observed in the present patient. Moreover, we speculate that the observed tissue specificity of the clinical presentation may be due to a specific or predominant expression of isoform 2 in skeletal muscle tissue. Transcriptomic data in a public dataset (e.g. GTex) cannot confirm this hypothesis, because of the extremely low expression level of isoform 2 in all the reported tissues.

This case further improves our understanding of the clinical disease phenotype associated with *NDUFA11*. We think that in the era of next generation sequencing it is extremely important to

describe all previously unreported variants and their corresponding clinical features to widen the clinical disease spectra and try to identify better genotype-phenotype correlations.

Furthermore, CI-associated diseases could benefit from therapy with specific "CI bypass" agents, such as idebenone and menadione.[5] The therapeutic effectiveness of idebenone in Leber hereditary optic atrophy, is well known.[6] Although clear beneficial effects have been demonstrated in only a few other mitochondrial disorders,[7] idebenone and coenzyme Q10 are currently used in clinical practice to treat mitochondrial dysfunction.

Additional *NDUFA11*-mutant subjects are needed to confirm the possible role of this gene, or of its specific isoform 2, as a cause of mitochondrial myopathy and to better evaluate their potential responsiveness to idebenone treatment.

Abbreviations

CI: Complex I

CK: Creatine Kinase

COX: Cytochrome C Oxidase

MRC: Medical Research Council

mtDNA: mitochondrial DNA

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Figure 1: Histological, histochemical and biochemical studies on patient's muscle biopsy

A-B-C-D: Histological examination of the quadriceps muscle showed a ragged red fiber (RRF), numerous cytochrome c oxidase (COX) negative fibers along with scattered necrosis and a mild increase in connective tissue. Control samples for each staining are shownin the inserts. Scale bar: 1cm:50µm

A: Gomori trichrome staining (GT). The star (*) indicates a RRF, present in all panels. RRFs are expression of compensatory mitochondrial proliferation.

B: COX staining and **C:** Succinate dehydrogenase staining (SDH) showing scattered COX negative and SDH hyperchromic fibres (*), expression of impairment of the respiratory complexes.

D: COX/SDH double-staining highlights the presence of some COX-negative fibres, hyperchromic on SDH staining (*).

E-F: Biochemical analysis of mitochondrial respiratory chain complex activities in muscle from the proband. CI, CII, CIII, CIV: complex I, II, III, IV. All activities are normalized for citrate synthase (CS) activity.

E: Values of specific activities for each complex are reported for the patient (*) and controls (grey boxes represent control ranges).

F: Enzyme activities are expressed as percentages of the control mean. The dotted line indicates the lower values in the control range

Figure 2. Genetic findings

A. Snapshot from IGV software of region containing the variants identified in the proband. *NDUFA11* is on the (−) strand, thus IGV reports the reverse complementary nucleotides compared to cDNA.

B. Electropherograms of the *NDUFAF11* region containing the c.317C>T and c.394G>C variants.

C. A detail of the panel A showing that each read contains either one or the other variant, thus indicating that they are in trans.

D. Schematic structure of *NDUFA11* transcripts: isoform1 (upper panel) and isoform 2 (lower panel). Blue boxes correspond to exons, white boxes to UnTranslated Regions. Black arrows indicate the position of the variants identified in the proband, affecting only isoform 2. The grey arrow indicates the position of the intronic mutation in *NDUFA11* as previously reported[4].