"DOUBLE TROUBLE" OR DIGENIC DISORDER IN COMPLEX I DEFICIENCY

Abstract nº: 97

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Background

Complex I (CI) deficiency is a defect of OXPHOS caused by mutations in the mitochondrial or nuclear genomes. To date disease-causing mutations have been reported in all mitochondrial-encoded subunits and 22 nuclear genes. In about 50% of the patients no mutations are found, suggesting that undiscovered factors are an important cause of disease.

In this study we report a consanguineous family from Southern Portugal with three affected children presenting with CI deficiency and 3-methylglutaconic aciduria type IV.

Patients and Methods

Patients:

The family presented here is from Southern Portugal (Fig.1). Patient II.1 is the youngest daughter born from healthy consanguineous parents. Her older siblings (two sisters and one brother) are normal. Patient III.1 is the first son born to the eldest sister of case II.1. Patient III.2, is sister of patient II.2.

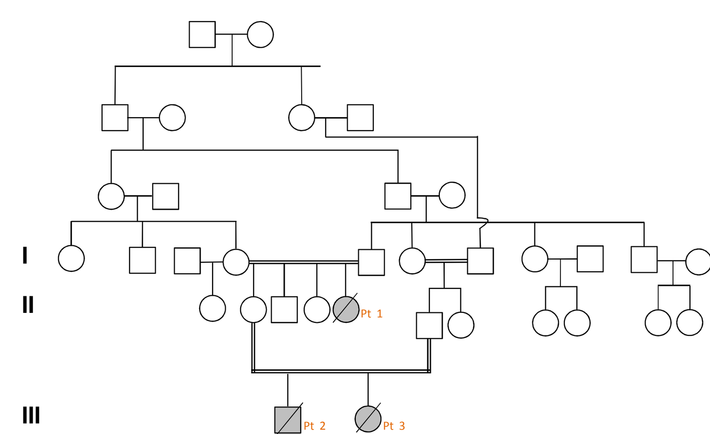

Figure 1. Pedrigree of the family studied

Blue Native- and Sodium Dodecyl Sulfate-PAGE analysis:

For BN-PAGE, mitochondria-enriched fractions were isolated from a 20-100 mg muscle specimen (Nijtmans *et al. 2002).* For the (SDS)-PAGE, protein fractions were prepared either from muscle homogenate or total intact cells.

Western blot:

Protein immunodetection was performed with primary antibodies directed against several subunits of the mitochondrial complexes. Peroxidase-conjugated anti-mouse IgG was used as secondary antibody and the signal detected using the *Immobilon Western Chemiluminescent HRP Substrate detection kit (Millipore Corporation*) and the fluorescence were quantified using the software *Quantity One (Bio-Rad).*

Molecular analysis:

Total genomic DNA was purified from either blood or muscle using commercially available kits (*EZ1 DNA Blood 350 µl Kit (QIAGEN®)*; *Puregene Tissue kit* (Gentra)*).* The mitochondrial genes encoding subunits of complex I and the whole mtDNA were amplified and sequenced using a commercially available kit, *mitoSEQr™ Resequencing System for the Human Mitochondrial Genome* (*Applied Biosystems*), according to the procedure recommended by the manufacturer. Several nuclear genes that encodes subunits of complex I, as well as assembly factors were amplified by PCR, purified with Exo-SAP (GE Healthcare, USA), and directly sequenced using the BigDye1.1 chemistry on an ABI Prism 3130 Genetic Analizer (Applied Biosystems, Foster City, CA).

Results

A reduced activity of CI was detected in two of the patients. SDS and BN-PAGE showed a global reduction of the protein expression levels of complex I subunits NDUFA9, NDUFB8, NDUFB4 (ranging from 75 to 60% reduction) and a decreased amount of the fully assembled complex I (approximately 30%) in (skeletal muscle from) patient II-1 (Fig. 2 A and B). Through SDS-PAGE followed by Western blotting using NDUFA9, NDUFB4 and NDUFS3 monoclonal antibodies it was also possible to observe a decreased amount of complex I subunits in skeletal muscle from patient II-3 (Fig. 2 C). Due to sample limitation it was not possible to perform BN-PAGE for this patient.

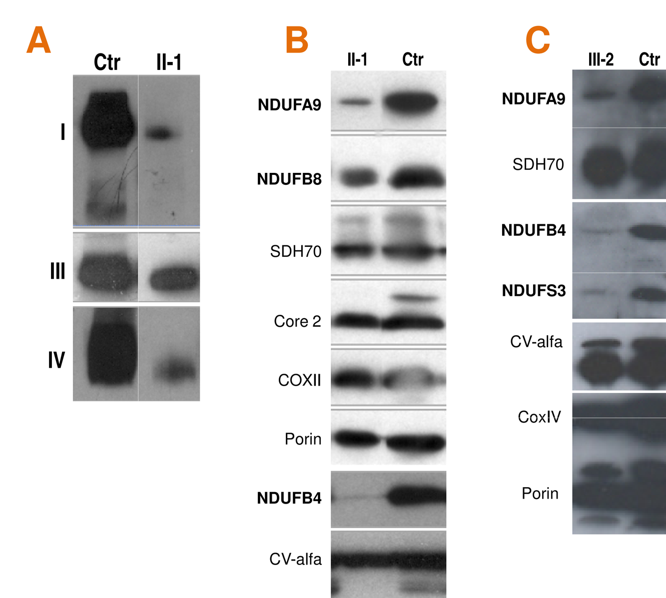

Figure 2. Complex I assembly and steady-state levels of mitochondrial proteins
(A) SDS-PAGE WB analysis in control (Ctr) and patient II-1 skeletal muscle. Loaded samples correspond to 30 µg of total protein. Specific antibodies, against subunits of complex I (NDUFA9, NDUFB8, NDUFB4), complex II (SDH 70kDa), complex III (Core2), complex IV (COX II) and porin were used. (B) BN-PAGE WB analysis of mitochondria from muscle of patient II-1 and Ctr. Complex I was immunovisualized using antibodies against the CI NDUFA9 (39 kDa) subunit; CIII core2 subunit and CIV, COXII subunit. **(C)** SDS-PAGE WB analysis in Ctr and patient III-2 skeletal muscle. Loaded samples correspond to 30 µg of total protein. Specific antibodies, against subunits of complex I (NDUFA9, NDUFB4, NDUFS3), complex II (SDH 70kDa), complex III (Core2), complex IV (CoxIV) and porin were used.

A number of CI associated genes were investigated at molecular level however, no mutations were detected. Besides CI deficiency the three patients excreted high amounts of 3-methylglutaconic acid. The presence of 3-methylglutaconic acid prompt us to study *POLG* gene, known to be involved in the etiology of 3-methylglutaconic aciduria type IV. In only one of the patients (II-1), that also showed mtDNA depletion, the p.G848S/p.Q1236H mutations were found. The mother of II-1 is carrier of p.Q1236H whereas the father is carrier of p.G848S. In patients III-1 and III-2 no *POLG* mutations were found.

Discussion

Molecular diagnosis of patients with complex I deficiency is a challenging task not only due to the clinical heterogeneity of the patients but also due to the large number of candidate genes (both nuclear and mitochondrial encoded). Besides all the advances in molecular technology it is known that a number of cases remain unsolved (Calvo *et al*., 2010, Tucker *et al*., 2011).

The molecular basis of complex I deficiency in this family is still not completely elucidated. It remains unanswered whether this family, due to the high consanguinity, could have two different disorders ("double trouble") or if an yet unknown gene leading to complex I deficiency could be involved. Patient II.1 carries two mutations in the POLG gene however none of the other patients carries any of the mutations. Due to its function, it is tempting to speculate that variations in *POLG* might contribute or modulate the clinical and biochemical phenotype, or both. Under this assumption, POLG will impair mtDNA replication, affecting the synthesis of ND subunits. This ultimately could affect the ND subunits pools, that are believed to exist, and consequently affect complex I assembly due to its dependence on ND subunits to form the membrane arm (Ugalde, 2004). Additionally the three patients present 3-methylglutaconic aciduria type IV, which comprise a very heterogeneous group both in presentation as in molecular etiologies, that adds up to the riddle in this family. The advances in technology available such as exome sequencing will certainly shed light for the molecular basis of CI deficiency and 3 methylglutaconic aciduria in this family.

Table 1. Summary of the clinical and laboratory findings

Patient	Age	Clinical, Histological and Biochemical Data	CI residual activity	mtDNA	Nuclear genes	SDS-PAGE
II.1	4m	Psychomotor delay, hypotonia which evolved to spasticity, neurosensorial deafness and epilepsy. Urinary organic acids showed presence of 3-methylglutaconic acid (208 µmol/mmol creatinine). Increased levels of plasma and CSF lactate and pyruvate. Morphology – predominance of type I fibers. Deceased at 10 months	37%	ND1-ND6, ND4L: negative	NDUFS2,8; NDUFV1-2; NDUFAF1,2,4: negative	NDUFA9, NDUFB8, NDUFB4: decreased
III.1	1m	Nephew of patient II-1. Hypotonia, nystagmus and feeding difficulties. Urinary organic acids showed the presence of 3-methylglutaconic acid. Increased levels of plasma and CSF lactate and pyruvate. Deceased at 2 months. Muscle biopsy not performed	Not done	Not done	Not done	Not done
III.2	1m	Sister of patient III-1. Similar clinical phenotype of previous cases. Urinary organic acids showed the presence of 3-methylglutaconic acid (1363 µmol/mmol creatinine). Deceased at 12 months	38%	ND1-ND6, ND4L: negative	NDUFS1,2,4: negative	NDUFA9, NDUFB4, NDUFS3: decreased

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