"DOUBLE TROUBLE" OR DIGENIC DISORDER IN COMPLEX I DEFICIENCY

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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 firstborn to the eldest sister of case II.1. Patient III.2, is sister of patient II.2.

Figure 1. Pedigree of the family studied

Blue NativE- and Sodium DodecyI Sulfate-PAGE analysis:

For BN-PAGE, mitochondria-enriched fractions were isolated from a 20-100 mg muscle specimen (Nimemi 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 Amersham Western Chemiluminescent HRP Substrate detection kit (Milwaukee Corporation) and the fluorochrome units were quantified using the software Quantity One (Bio-Rad).

Molecular analysis:

Total genomic DNA was purified from either blood or muscle using commercially available kit (QIAGEN Blood 350 µl kit (QIAGEN)). Pangenie Tissue Kit (General). The mitochondrial genes encoding subunits of complex I and the whole mtDNA were amplified and sequenced using a commercially available kit, mtSEQ™ Resequencing System for the Human Mitochondrial Genome (Applied Biosystems), according to the procedure recommended by the manufacturer. Several nuclear genes that encode 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 BigDye™ chemistry on an ABI Prism 3130 Genetic Analyzer (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 NDUF49, NDUF8, NDUFB4 (ranging from 75% to 80% 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 SDSPAGE followed by Western blotting using NDUF49, NDUF84 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

Table 1. Summary of the clinical and laboratory findings

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.

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