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A novel homozygous mutation in *SUCLA2* gene identified by exome sequencing

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ABSTRACT

Mitochondrial disorders with multiple mitochondrial respiratory chain (MRC) enzyme deficiency and depletion of mitochondrial DNA (mtDNA) are autosomal recessive conditions due to mutations in several nuclear genes necessary for proper mtDNA maintenance.
In this report, we describe two Italian siblings presenting with encephalomyopathy and mtDNA depletion in muscle. By whole exome-sequencing and prioritization of candidate genes, we identified a novel homozygous missense mutation in the *SUCLA2* gene in a highly conserved aminoacid residue. Although a recurrent mutation in the *SUCLA2* gene is relatively frequent in the Faroe Islands, mutations in other populations are extremely rare. In contrast with what has been reported in other patients, methyl-malonic aciduria, a biomarker for this genetic defect, was absent in our proband and very mildly elevated in her affected sister.
This report demonstrates that next-generation technologies, particularly exome-sequencing, are user friendly, powerful means for the identification of disease genes in genetically and clinically heterogeneous inherited conditions, such as mitochondrial disorders.

1. Introduction

Multiple mitochondrial respiratory chain (MRC) enzyme deficiency is a biochemical signature, common to a number of mitochondrial disorders, which can be due to diverse gene defects, including mitochondrial DNA (mtDNA) single or multiple deletions, mtDNA point mutations affecting mitochondrial tRNA genes, or mutations in nuclear genes related to several mitochondrial pathways, such as mtDNA maintenance and translation, assembly/regulation of respiratory chain subunits, biosynthesis of mitochondrial inner membrane phospholipids or MRC cofactors, and import of mitochondrial proteins [1]. In childhood, multiple MRC deficiency is often associated with mtDNA depletion, i.e., reduced mtDNA copy number; this condition, known as mtDNA depletion syndrome (MDS, OMIM ID: 251880) comprises a clinically and genetically heterogeneous group of autosomal recessive diseases [1,2]. Several nuclear genes have been associated with MDS. Mutations in the deoxyguanosine kinase (*DGUOK*) and thymidine kinase 2 (*TK2*) genes have been reported in the hepatocerebral [3] and myopathic [4] forms of MDS, respectively; and mutations in the catalytic subunit of the mtDNA polymerase gamma (*POLG1*) are associated with Alpers' syndrome, a condition characterized by mtDNA depletion in the brain and liver [2,5]. Less frequently, MDS can be caused by mutations in *MPV17*, encoding a small protein of unknown function embedded in the inner mitochondrial membrane [6], *RRM2B*, encoding the p53-inducible ribonucleotide reductase subunit 2, [7], *C10orf2*, encoding the mtDNA-Twinkle helicase [8], and *SUCLG1* [9] or *SUCLA2* [10], encoding the a and b subunits of the succinate-CoA ligase, (EC 6.2.1.5), a Krebs-cycle enzyme that catalyzes the reversible formation of succinate and ATP from succinyl-CoA and ADP. Defects in *SUCLA2* cause an encephalomyopathic MDS with moderate methylmalonic aciduria (OMIM ID: 612073). Mutations in this gene seem exceptionally rare, although a recurrent mutation has been reported in several patients originating from the Faroe Islands.

For several years, linkage analysis has been the most powerful and widely used strategy to identify the gene defects responsible for mendelian inherited disorders. However, this approach is very time consuming, and requires the availability of cohorts of homogeneous and informative families. Homozygosity mapping (e.g., by SNP arrays) is a potentiated version of linkage analysis, which had made possible the identification of more than 100 genes responsible for autosomal recessive diseases, but it requires the availability of informative, possibly

Abbreviations: MDS, mtDNA depletion syndrome; MMA, methylmalonic acid; MRC, mitochondrial respiratory chain; mtDNA, mitochondrial DNA; NGS, next-generation sequencing; OXPHOS, oxidative phosphorylation.

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large, consanguineous families. Nowadays, Next-Generation Sequencing (NGS) can be performed at affordable cost and timescale to analyze the coding regions (exome) of the human genome in single individuals or small families, including patients in which a clear genotype–phenotype correlation is absent or for clinically and genetically heterogeneous conditions, such as mitochondrial disorders. In addition, NGS-based homozygosity mapping can also be applied to select candidate genes and eventually find the causative mutation in patients from small, or even nuclear, consanguineous families [11,12].

Here we illustrate the power of NGS-based homozygosity mapping to identify a novel homozygous mutation in *SUCLA2* in a girl affected by severe, progressive encephalomyopathy with multiple MRC defects, but hardly any methylmalonic aciduria. A second, affected sister was then found to carry the same mutation.

2. Patients and methods

2.1. Patients

The index patient (PI) was an Italian girl born at term after normal pregnancy and delivery. Her parents were first cousins (Fig. 1A). Since the first months of life, the mother reported muscle hypotonia, failure to thrive, poor weight gain, and frequent vomiting. At 8 months of age the neurological examination showed normal cranial nerves, good visual contact but marked axial hypotonia with the absence of head control, in association with distal hypertonia, poor active movements, and brisk tendon reflexes. Nasogastric tube feeding was started because of severe dysphagia. Neurosensory hearing loss was documented by abnormal Brain Audiometry Evoked Potentials. Liver and kidney functions were normal. Biochemical exams revealed increased levels of lactate and pyruvate in both plasma (lactate 3057 μM, pyruvate 193 μM; normal values (nv): <2000 and <140) and CSF (lactate 2262 μM, pyruvate 144 μM; nv: <1800 and <120). Plasma carnitine short chain esters were moderately elevated (20 nM; nv: 3.4–10 nM). Methylmalonic acid (MMA) in urine was not tested, as well as the whole acylcarnitine profile. A brain MRI performed at 8 months showed bilateral abnormal signals in the caudate and putamina nuclei; these lesions persisted in a second brain MRI performed at age 24 months. The clinical features progressively worsened; at 18 months the patient showed a dystonic tetraparesis associated with bilateral ptosis and ophthalmoparesis, and severe cognitive impairment with no verbal development. She required nasogastric tube feeding until a percutaneous gastrostomy was performed at 7 years of age. Despite the severe clinical features she never presented metabolic crisis and the EEG was always normal. She developed multiple tendon retractions and progressive scoliosis since 10 years of age. The neurological condition slowly deteriorated; at her last examination, at 14 years of age, she showed normal growth parameters, severe ptosis with almost complete ophthalmoparesis, poor response to visual and tactile stimuli, diffuse muscle wasting, axial and limb hypotonia, areflexia, poor active movements and severe dystonic

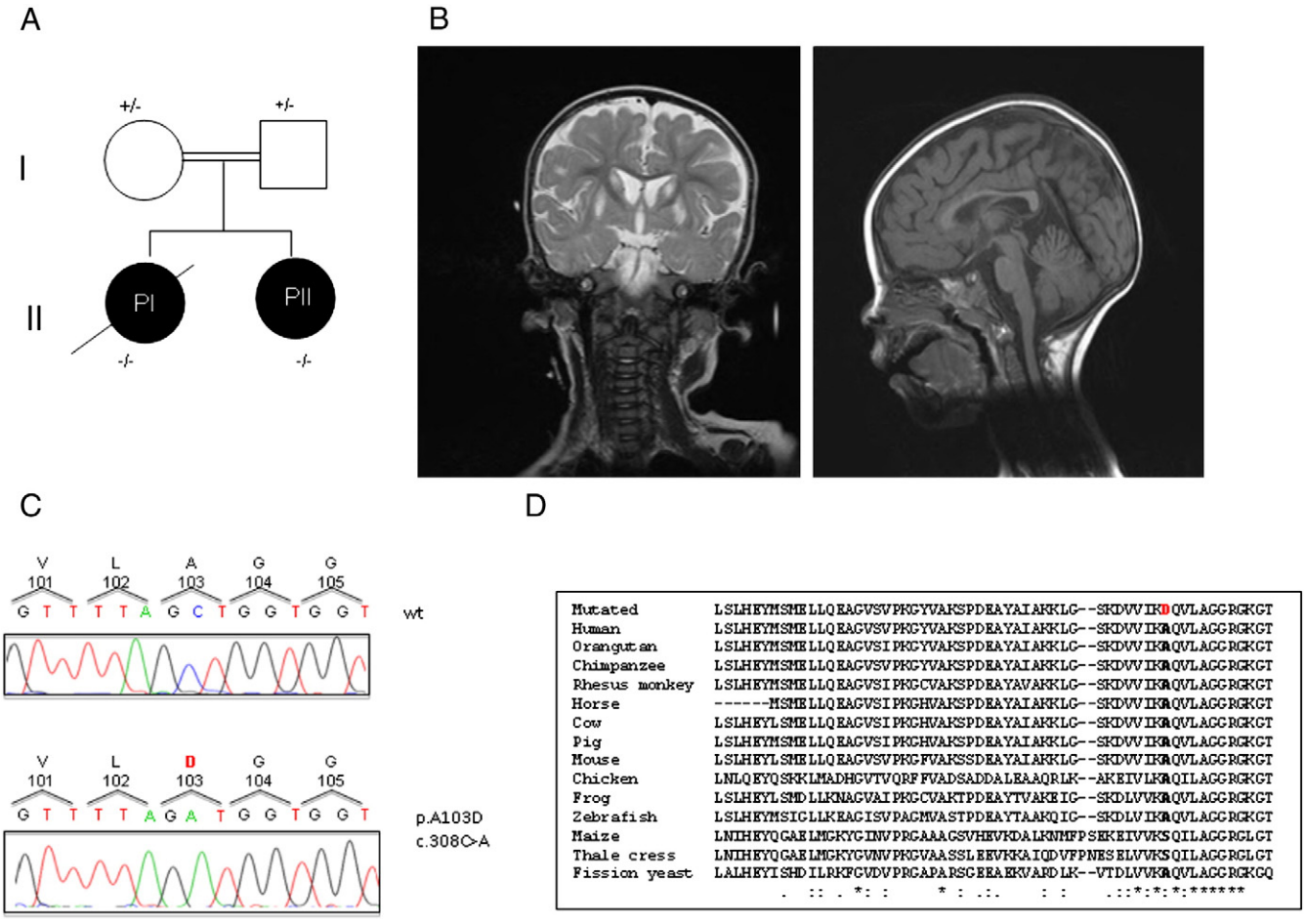

Fig. 1. A. Pedigree of the family. "−" corresponds to the allele with the Ala103Asp mutation; "+" corresponds to the wild-type allele. Black symbols indicate affected subjects. B. Brain MRI of patient II showing bilateral hyperintense lesions in caudate and putamina nuclei (*left panel*), and slight cerebellar and medullar atrophy (*right panel*). C. Electropherograms of the genomic region encompassing the c.308C>A substitution in a control wild-type subject (*upper panel*) and in patient I (*lower panel*). D. ClustalW multiple alignment of human SUCLA2 region containing the Ala103Asp mutation with aminoacid sequences from ortholog proteins: Orangutan (*Pongo pygmaeus*), Chimpanzee (*Pan troglodytes*), Rhesus monkey (*Macaca mulata*), Horse (*Equus caballus*), Cow (*Bos taurus*), Pig (*Sus scrofa*), Mouse (*Mus musculus*), Chicken Frog (*Xenopus laevis*), Zebrafish (*Danio rerio*), Maize (*Zea mays*), Thale cress (*Arabidopsis thaliana*), and Fission yeast (*Saccharomyces cerevisiae*).