Molecular Genetics and Metabolism Reports 5 (2015) 51–54

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/molecular-genetics-and-metabolism-reports/

Short Communication

Mitochondrial leukoencephalopathy and complex II deficiency associated with a recessive *SDHB* mutation with reduced penetrance

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ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form 13 October 2015
Accepted 13 October 2015
Available online 22 October 2015

Keywords:
SDHB
Mitochondrial complex II
Leukoencephalopathy
Mitochondrial disorder

ABSTRACT

Mitochondrial disease involving complex II is rare among respiratory chain deficiencies and its genetic cause remains often unknown. Two main clinical presentations are associated with this biochemical defect: mitochondrial encephalomyopathy and susceptibility to tumors. Only one homozygous *SDHB* mutation has been described in a patient with mitochondrial disorder. We report here two sisters, who presented highly different phenotypes (neurological impairment with leukoencephalopathy vs. asymptomatic status) and harbored the same homozygous *SDHB* mutation, suggesting reduced penetrance.

1. Introduction

SDHB encodes one of four structural subunits (*SDHA*, *SDHB*, *SDHC*, *SDHD*) forming complex II (cII) of the mitochondrial respiratory chain (MRC). cII, or succinate-ubiquinone oxidoreductase (E.C. 1.3.5.1), is the only membrane-bound member of the tricarboxylic acid cycle, where it functions as a succinate dehydrogenase (SDH). By coupling this reaction to the reduction of ubiquinone to ubiquinol, cII takes part in the MRC. At least four assembly factors (*SDHAF1*-4) assist the formation of the holocomplex and additional proteins are required for the iron-sulfur clusters incorporation into cII.

Several heterozygous mutations in *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2* are susceptibility factors for developing tumors of chromaffin-cells, such as paragangliomas (PGL) and phaechromocytomas, gastrointestinal stromal tumors and/or renal cell carcinoma [1]. On the contrary, only a few recessive mutations in *SDHA* [2,3] or in *SDHD* [4,5], have been reported in mitochondrial encephalomyopathy with (or without) cardiac involvement associated with cII deficiency, while *SDHAF1* mutations are the most common cause of mitochondrial leukoencephalopathy associated with cII deficiency [6,7]. The reasons determining whether cII defects lead to neurological disease or tumor are poorly understood, as well the possible link between mutations in specific cII genes and either one or the other clinical presentation.

There is only one report describing a homozygous *SDHB* mutation associated with mitochondrial disease in a child affected by leukoencephalopathy and cII deficiency [4]. Since no other *SDHB*-related mitochondrial diseases have been reported so far, this variant is classified as a variant of unknown significance because its contribution to mitochondrial complex II deficiency has not been confirmed (MIM*185470). We describe two sisters with the same homozygous mutation p.Asp48Val in *SDHB*, one presenting with severe hypotonia and psychomotor regression with leukoencephalopathy and the other one virtually asymptomatic.

2. Material and methods

2.1. Histochemical and biochemical analyses

Cryostatic cross sections from skeletal muscle biopsy were processed according to standard histochemical procedures. MRC complex activities were measured using standard spectrophotometric methods [8] in muscle homogenate and digitonin-treated skin fibroblasts.

2.2. Mutational analysis

Total genomic DNA was extracted by standard methods from peripheral blood of the patients and parents. A customized gene panel (TruSeq Custom Amplicon, Illumina) containing nuclear genes associated with cII deficiency (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF1*, *SDHAF2*,

Abbreviations: MRC, mitochondrial respiratory chain; cII, mitochondrial complex II; SDH, succinate dehydrogenase; PGL, paragangliomas; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.

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http://dx.doi.org/10.1016/j.ymgmr.2015.10.006

SDHAF3) was used for library preparation; then samples were analyzed by a Miseq system (Illumina), with 100X effective mean depth. The generated reads were aligned to human genome assembly hg19 and the identified variants were annotated (Variant-Studio, Illumina) and filtered, focusing on rare variants (minimum allele frequency <1% in 1000 Genome Project [www.1000genomes.org] and ExAc [http://exac.broadinstitute.org] databases), causing changes potentially damaging for the protein function (Polyphen2, SIFT). Since the pedigree was suggestive for a recessive trait, we searched for genes with a homozygous variant or two heterozygous variants. Sanger sequencing was used to confirm the mutation in the patient and the segregation in the family.

2.3. Western blot analysis

Fibroblasts were pelleted and solubilized in RIPA buffer with protease inhibitors. Lymphocytes were obtained from peripheral blood using Lympholyte-H (Cedarlane Laboratories) and treated as described above. 50 μg of proteins were loaded for each sample in 12% denaturing sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Monoclonal antibodies against cII subunits *SDHB* and *SDHA* (Mitosciences), mitochondrial porin/VDAC1 (Abcam) and GAPDH (Millipore) were used.

3. Case reports.

The proband (P, II-4) is a girl, fourth child of healthy related -first cousins- parents of Pakistani origin. Family and personal history were unremarkable. Psychomotor development was referred normal: head control at 3 months, sitting at 6 months, walking alone at 12 months. At 15 months, a few days after a febrile illness, she presented acute psychomotor regression, losing previously acquired psychomotor skills in about a week. She was admitted to our Institute one month later. She presented with generalized hypotonia, hyperreflexia, no postural control, poor voluntary movements, marked irritability with frequent crying. She did not present with seizures. Lactate and pyruvate were elevated in plasma: 3327 μmol/l (normal values, nv: 580–2100) and 151 μmol/l (nv: 55–145) respectively, and normal in CSF; 2-ketoglutaric aciduria (557 μg/mg creatinine; nv <140) was detected. Brain MRI showed diffuse hyperintensity of the hemispheric white matter and corpus callosum. The subcortical U-fibers are spared. Posterior deep white matter showed evidence of rarefaction and cystic degeneration. There were also small symmetric hyperintensites in the thalami. HNMR-spectroscopy demonstrated a peak of succinate and elevate lactate (Fig. 1a).

Fundus oculi, electroretinogram, brainstem auditory evoked potential and motor and sensory nerve conduction velocities were normal. Visual evoked potential showed central conduction abnormalities. Electroencephalography disclosed normal background activity, with a prevalence of slow activity in the right posterior regions. Electrocardiogram and echocardiogram were normal.

Informed consent for biochemical and genetic studies was obtained from patient's parents.

All her siblings were reported in good health. The older sister (II-1), now 11 years old, was born after uncomplicated pregnancy and delivery; her neonatal period was normal. Psychomotor development was referred normal. She was in good health and neurological history was negative. After the genetic analysis, she underwent a neurological examination that resulted normal. Routine exams, lactate and pyruvate serum levels, as well as hemogasanalysis were normal. Brain MRI showed very small symmetric lesions in the medial thalami (Fig. 1b); HNMR-spectroscopy was normal. Because of the healthy status of the girl, the parents did not agree on a skin or muscle biopsy.

4. Results

Histological analysis of proband's muscle biopsy showed few hypotrophic fibers, with normal lipids and glycogen content. Reduction of cII (succinate-ubiquinone reductase) and SDH activities were documented both on muscle tissue and skin fibroblasts (Fig. 2a). Complex I activity was at the lower range of control values in muscle. Sequence

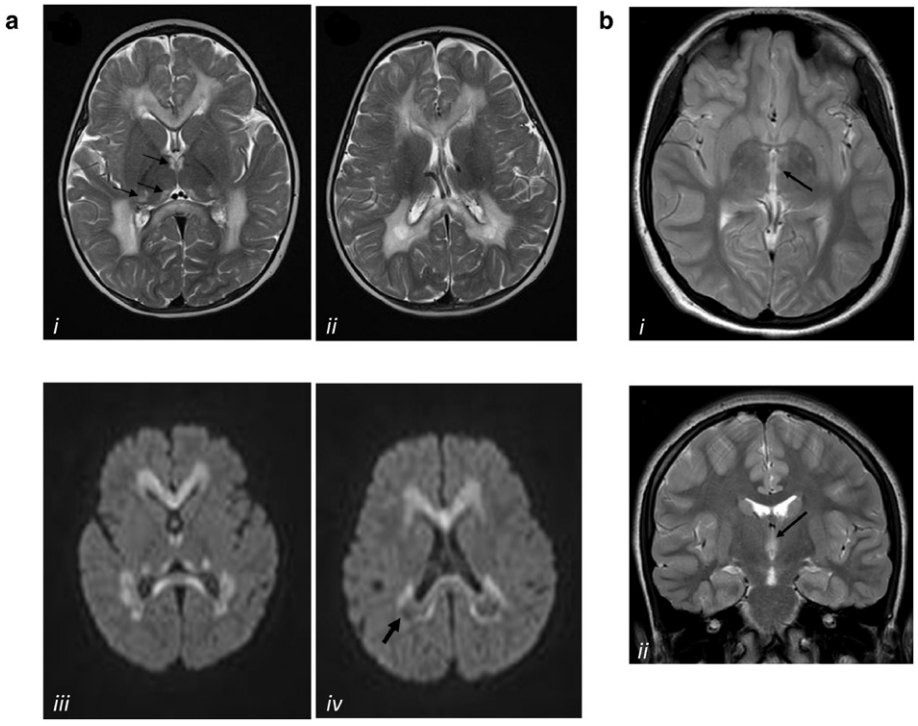

Fig. 1. Representative MRI images of our *SDHB*-mutant cases a. Brain MRI of the proband (II-4). Axial T2-weighted images (*i*, *ii*) show diffuse hyperintensities in the hemispheric white matter and corpus callosum. Small symmetric high signal abnormalities are also present in the medial and posterior thalami (arrows in panel *i*). Diffusion-weighted images (*iii*, *iv*) show restricted diffusion in the corpus callosum, thalami and, partially, in the white matter. Posterior white matter has a prevalent low signal (*iv*) reflecting partial cystic degeneration and cavitations (arrow). b. Brain MRI of the asymptomatic sister II-1. Axial (*i*) and coronal (*ii*) T2-weighted images show very small symmetric lesions in the medial thalami (arrows).