Metabolic Brain Disease (2019) 34:1515–1518
https://doi.org/10.1007/s11011-019-00453-w

SHORT COMMUNICATION

Novel mutations in two unrelated Italian patients with SSADH deficiency

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Received: 29 March 2019 / Accepted: 17 June 2019 / Published online: 2 July 2019

Abstract

Succinic semialdehyde dehydrogenase deficiency (SSADHD) is a rare autosomal recessive disorder of γ-aminobutyric acid (GABA) catabolism caused by mutations in the gene coding for succinic semialdehyde dehydrogenase (*ALDH5A1*). The abnormal levels of GHB detected in the brain and in all physiological fluids of SSADHD patients represent a diagnostic biochemical hallmark of the disease. Here we report on the clinical and molecular characterization of two unrelated Italian patients and the identification of two novel mutations: a 22 bp DNA duplication in exon 1, c.114_135dup, p.(C46AfsX97), and a non-sense mutation in exon 10, c.1429C > T, p.(Q477X). The two patients showed very different clinical phenotypes, coherent with their age. These findings enrich the characterization of SSADHD families and contribute to the knowledge on the progression of the disease.

Keywords SSADHD (succinic semialdehyde dehydrogenase deficiency) · GABA (γ-aminobutyric acid) · GHB (γ-hydroxybutyric acid) · ALDH5A1 gene · 4-HBA (4-hydroxybutiric aciduria)

Introduction

Succinic semialdehyde dehydrogenase deficiency (SSADHD) (OMIM #271980) is a rare autosomal recessive disorder of γ-aminobutyric acid (GABA) catabolism caused by mutations in the gene coding for succinic semialdehyde dehydrogenase (*ALDH5A1*). The first step of GABA degradation involves GABA transaminase which converts GABA to succinic semialdehyde (SSA) later oxidized by SSADH (E.C.1.2.1.24) to succinic acid and, ultimately, entering the tricarboxylic acid cycle. As for SSADHD, SSA is reduced to γ-hydroxybutyrate (GHB) by either specific or non-specific cytosolic oxidoreductases. The abnormal levels of GHB in urine and in all physiological fluids can be detected by GC/MS technique (Jakobs et al. 1981; Gibson et al. 1990) and represent a diagnostic biochemical hallmark of SSADHD (Pearl et al. 2009)

SSADHD is significantly under-diagnosed due to highly variable non-specific neurological symptoms including psychomotor retardation, language delay, hypotonia, seizures and non-progressive ataxia. Magnetic resonance imaging (MRI) shows symmetric involvement of cerebral dentate, globus pallidus and subthalamic nuclei (Pearl et al. 2003).

Several studies on SSADHD patients and on a murine model have highlighted that supraphysiological levels of GHB in the Central Nervous System (CNS) represent the main pathophysiological factor, which may act as neurotransmitter/neuromodulator (Jansen et al. 2008). Besides, mitochondrial dysfunction and/or redox imbalance have been suggested to play a role in the clinical manifestations (Niemi et al. 2014).

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The *ALDH5A1* gene includes 10 exons (1605 bp) encoding 535 amino acids, with the first 47 residues acting as mitochondrial targeting peptide (Chambliss et al. 1998; Kim et al. 2011; Malaspina et al. 2016).

In the present study, we report on the clinical and molecular characterization of two unrelated Italian patients leading to the identification of novel mutations in the *ALDH5A1* gene. Patient 1 is compound heterozygote carrying the pathological mutation c.278G > T, p.(C93F) (Akaboshi et al. 2003) and a new coding duplication c.114_135dup, p.(C46AfsX97). Patient 2 turned out to be homozygous for the novel nonsense mutation c.1429C > T, p.(Q477X).

Materials and methods

Clinical report and biochemical findings

Patient 1 is a born at term woman who, due to a clinical picture of developmental delay and aggressive behavior associated with a progressive motor/coordination/attention disorder, underwent a brain MRI at the age of 14, which reported the presence of a bilateral basal ganglia symmetrical alteration at the pale nuclei level. A urine organic acid profile was performed and revealed increased GHB levels (398 mmol/mol of urinary creatinine vs normal range 0–7). Vigabatrin treatment (1000 mg/day) was started at age of 14 with a very good effect on the behavior. When she was 31, Vigabatrin was lowered (500 mg/day) due to a dubious mild reduction of the visual field and obesity. Finally, the treatment was suspended at 32 years even considering that it was probably no longer necessary to control her behavior. At the age of 34 she developed a growing state of anxiety, hallucinations, insomnia and delirium, for which she was admitted to S. Paolo Hospital (Milan, Italy) for further analyses. High GHB excretion was confirmed (350 mmol/mol) and at her last eye assessment, a non-specific reduction of the retinal sensitivity in her right eye temporal hemifield and in the left eye lower hemifield could be observed. Currently, the patient is treated with Risperidone and Clonazepam. Primary hypothyroidism was diagnosed at the age of 30 years and oral administration of L-Thyroxine was started.

Patient 2 is a young girl who underwent clinical and biochemical evaluation at Antonio Cao Hospital (Cagliari, Italy) at the age of 16 months. During the first year of life, the patient showed feeding difficulties and psychomotor retardation, with hypotonia and ligamentous hyperlaxity. Urine organic acid analysis revealed high levels of GHB (852 mmol/mol of urinary creatinine) and a small amount of 4,5-dihydroxyhexanoic and 3-hydroxypropionic acids. The Griffith Mental Development Test (E.R.) was administered to the patient at the age of 2.5 years, showing a developmental age of 15 months. The scores obtained in the different sub-scales overall indicate severe cognitive impairment and moderate motor disability.

EEG performed when the patient was 1.9 years revealed severe disorganization without epileptiform anomalies that were documented later at the age of 2.8 years. Subsequently, at the age of 3.3 years, epilepsy occurred in the form of atypical absence and generalized tonic-clonic seizures. The patient is now effectively treated with levetiracetam (15 mg/kg/day).

The informed consent for performing the genetic analyses and publication of the case report was obtained from patient 1 and the parents of patient 2, using the forms approved by the S. Paolo and A. Cao Hospitals, respectively.

ALDH5A1 mutation analysis

Genomic DNA was extracted from peripheral blood of the two patients and their parents using standard procedures. The *ALDH5A1* coding region and all exon-intron boundaries were amplified by PCR (Blasi et al. 2002). PCR products were sequenced on both strands with BigDye Terminator kit (Applied Biosystem, USA) and analysed with an ABI PRISM® 3100-*Avant*™Genetic Analyzer.

To determine the exact size of the duplication detected in patient 1, PCR amplicons of exon 1 were cloned into pGEM®-T Easy Vector (Promega, USA) to transform *DH5α E. coli* competent cells. Plasmid DNA from recombinant clones was purified by standard techniques and sequenced. Clones harbouring the *wild-type* and the mutated sequence were both identified.

Results and discussion

Molecular analysis of *ALDH5A1* gene in proband 1 revealed the presence of heterozigosity for the c.278G > T, p.(C93F) (paternal origin) and the c.114_135dup, p.(C46AfsX97) (maternal origin) mutations, both located in exon 1. The c.278G > T mutation has already been reported in SSADHD families, mainly of European origin (Akaboshi et al. 2003; Leuzzi et al. 2007). The c.114_135dup is a novel mutation consisting of a 22 bp duplication (Fig. 1) that causes a frameshift with the production of a premature downstream stop codon. This event would probably cause the total absence of the polypeptide chain, due to the mechanism known as Nonsense-Mediated Decay that leads to the degradation of mRNAs containing highly premature stop codons. Among the numerous pathological mutations identified so far, insertions/duplications have been reported in six patients only (Akaboshi et al. 2003; Li et al. 2015; Lin et al. 2015).

Proband 2 showed homozygosity for the novel c.1429C > T mutation located in exon 10. This variation results in the substitution of a codon for Glutamine in position 477 with a stop codon, p.(Q477X), thus determining the truncation of the

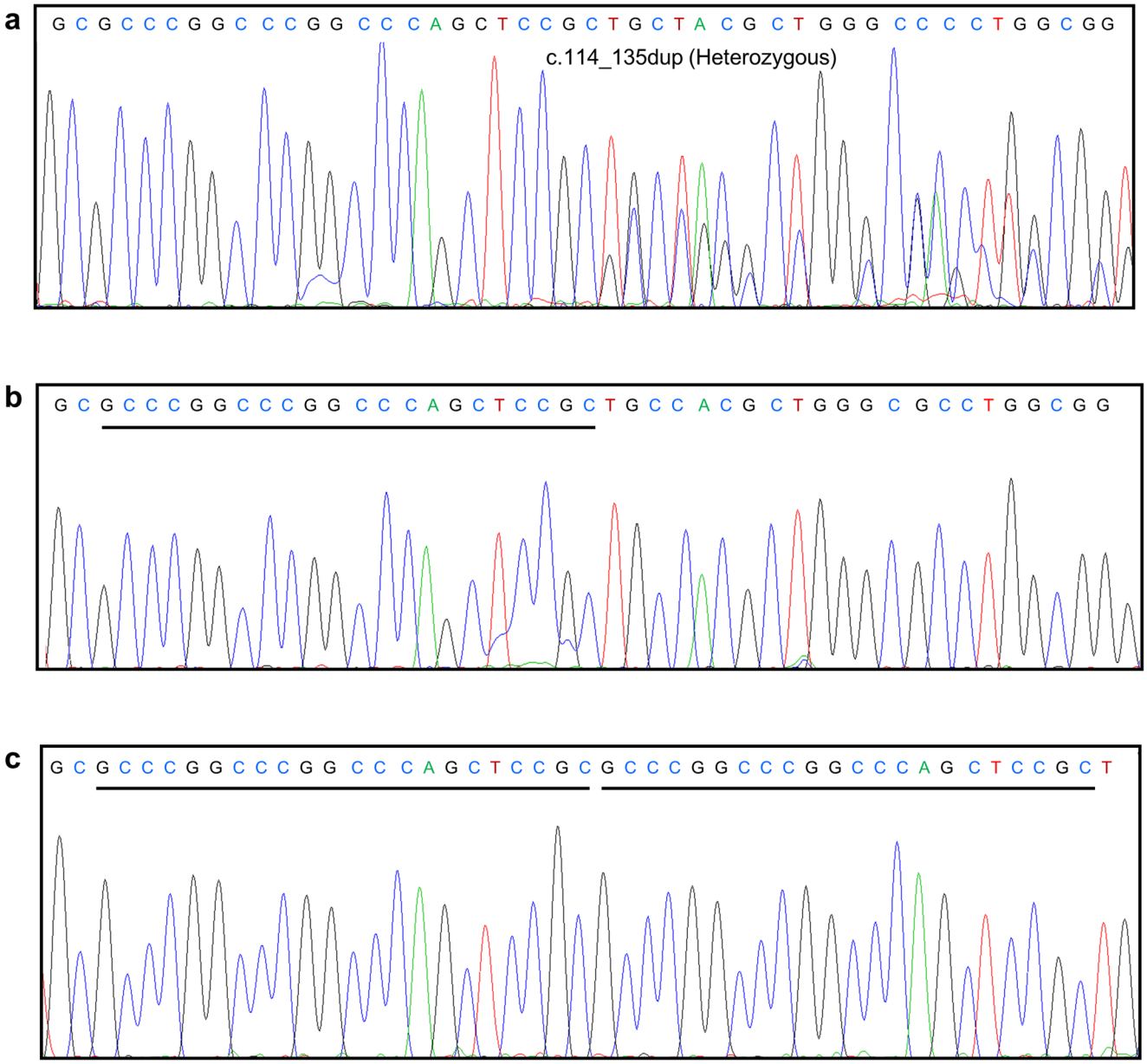

Fig. 1 Sequencing results for the novel mutation in patient 1. **a** Electropherogram showing the heterozygosity for the duplication detected in exon 1. **b** The *wild type* allele and **c** the mutant allele (c.114_135dup) identified in recombinant plasmids after cloning of exon 1 PCR product. In the last two panels, the sequence involved in the duplication is underlined. Numbering of nucleotides and amino acids refers to the *ALDH5A1* cDNA sequence (GenBank: Y11192)

last 59 amino acids of the polypeptide and the disruption of the catalytic domain (Kim et al. 2009). Due to the known geographic isolation of the Sardinian region where the family originated (Ogliastra), it is likely that the heterozygous parents would share a common ancestry.

The two patients are very different in age (35 years old vs 4 years old) and show clinical manifestations in agreement with the phase of their life. Following the discovery of SSADHD in 1981, the institution of a patient registry has allowed the collection of follow-up data and provided information on the natural history of the disease (Lapalme-Remis et al. 2015). Evolving clinical features on the course of the disease have been reported, and were particularly significant for the pediatric vs adolescent/adult groups. There is an age-dependent association with worsening of epilepsy, behavioral and sleep disturbances. On the contrary, hypotonia affects a smaller percentage of older patients. It has been found that GABA and GHB concentrations change through lifetime, with higher levels limited to the first decade. Most relevant, the finding of an age-dependent negative correlation with both plasma GABA and thyroid function levels can explain the onset of hypothyroidism in adulthood (DiBacco et al. 2018).

In conclusion, we report the identification of two novel disease-causing mutations in *ALDH5A1* gene, which enriches the characterization of Italian SSADHD patients (Leo et al. 2017; Menduti et al. 2018). Our report confirms an age-related clinical phenotype thus contributing to the knowledge on the progression of the disease over the patient's lifetime. Since no successful targeted therapy has been developed yet, treatments of the disease remain symptomatic. Therefore, a consolidated information on the evolution of the disorder would allow the application of age-tailored therapeutic strategies to improve the life quality of SSADHD patients.

Acknowledgements The authors would like to thank Cometa Sardegna Onlus Association for its support to the research. We also thank Dr. M. Lo Ponte for the English revision of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interests.

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