BRIEF COMMUNICATION

SUCLA2 Arg407Trp mutation can cause a nonprogressive movement disorder – deafness syndrome

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Abstract

SUCLA2 is a component of mitochondrial succinate-CoA ligase and nucleotide diphosphokinase activities. Its absence results in Krebs cycle failure, mitochondrial DNA depletion, and a childhood-fatal encephalomyopathy. We describe a purely neurologic allelic form of the disease consisting of deafness, putamenal hyperintensity on MRI and a myoclonic-dystonic movement disorder unchanging from childhood into, so far, the late fourth decade. We show that succinate supplementation circumvents the Krebs cycle block, but does not correct the neurologic disease. Our patients' Arg407Trp mutation has been reported in children with (yet) no MRI abnormalities. It remains possible that early succinate supplementation could impact the disease.

Introduction

In 1977, Damasio, Antunes and Damasio described an autosomal recessive childhood-onset chorea-deafness syndrome. The movement disorder was remarkably constant and unchanging into adulthood.[1]

SUCLA2 encodes the β subunit of the ADP-specific succinate-CoA ligase, a Krebs cycle enzyme. SUCLA2 has a second function, namely to physically stabilize the mitochondrial nucleotide diphosphokinase (NDPK), which catalyzes formation of nucleotide triphosphates that constitute mitochondrial DNA.[2] The first *SUCLA2* mutation was discovered as part of the search for the cause of an unexplained encephalomyopathy with mitochondrial DNA depletion, and the disease therefore came to be known as mitochondrial DNA (mtDNA) depletion Type V.[3] The phenotype of patients with complete SUCLA2 absence is severe, including deafness and rapidly progressive wasting myopathy and Leigh syndrome-like encephalopathy, with death in childhood.[3,4,5,6] Over the years, milder phenotypes were reported. We describe three south-Lebanese siblings with a previously published missense mutation, but with follow-up to a greater age (37 versus 9 years), revealing this mutation to result in the mildest phenotype to date, namely autosomal recessive nonprogressive movement disorder with sensorineural hearing loss. The phenotype is neurologically distinctive, likely to be referred to neurologists, may not include mtDNA depletion, and with early diagnosis may have the potential to be treatable with anaplerotic succinate supplementation.

Methods

Genotyping was on Infinium HumanOmni2.5-Quad v1.0 BeadChip microarray and single nucleotide polymorphism (SNP) analysis on GenomeStudio (Illumina, San Diego,

USA); CNV Partition v.3.1.6 module was used for copy number variation and loss-of-heterozygosity analysis. Whole-exome sequencing (WES) was performed on SOLiD5500xL (Life Technologies, Foster City, USA) with target enrichment using SureSelect 50 Mb all-exon capture v4 (Agilent Technologies, Santa Clara, USA).

Fibroblast succinyl-CoA ligase and mitochondrial electron transport chain activities, skeletal muscle histology and electron microscopy, and fibroblast mtDNA quantification were performed through clinical laboratory testing, as described.[7,8] mtDNA from skeletal muscle biopsies was quantified by real-time PCR using primers for *D-loop* and *MT-ATP8* mitochondrial sequences and β-microglobulin (*B2M*).[9] Reaction mixtures (20 μL) containing 10 μL of PowerUp™ SYBR™ Green Master Mix (Thermo Fisher), 35 ng of forward and reverse primers (*B2M*: 5′-TGCTG TCTCCATGTTTGATGTATCT-3′, reverse 5′-TCTCTGCTC CCCACCTCTAAGT-3′, *D-loop*: 5′- CATCTGGTTCCTACT TCAGGG-3′, 5′- CCGTGAGTGGTTAATAGGGTG-3′ and *MT-ATP8*: 5′-ATGGCCCACCATAATTACCC-3′ 5′-GCAA TGAATGAAGCGAACAG-3′) and 10 ng genomic DNA were cycled at 95°C for 30s, 40 cycles of 95°C for 5s and 60°C for 20s and melting curve stage for 55°C for 30s and 95°C for 30s. The reactions were performed in triplicate. The *B2M* to *D-loop*, and *B2M* to *MT-ATP8* ratios were determined between patient ($n = 1$) and healthy controls ($n = 3$).

Results

Clinical presentation and workup

Two sisters and a brother born to second-degree cousins (Fig. 1A) had an identical course. Perinatal and early-postnatal development were normal. They could initially hear, responding to sounds, which they gradually lost by age two years, and have since been deaf. A movement disorder appeared insidiously after the first year of life, and developed until the present pattern which continues unchanged to their current ages of 29 to 37 years. It is a constant dystonia of all extremities and neck muscles, accentuated with myoclonic jerks, giving the appearance of a chorea (Video S1). It subsides with sleep. All three patients walked at two years and continue to ambulate independently, although frequently seeming about to fall due to the myoclonus. While cognitive testing has not been performed, they appear to be spared at least insofar as communicating with each other and their parents in sign language learned at school. Neurological examination is otherwise unremarkable.

Levels of serum amino acids, lactate and pyruvate, and urine organic acids, including methylmalonic acid, were obtained on all three patients between ages 3 and 5 years

and were then normal. Following mutation identification, repeat testing in all three at ages 26 to 34 revealed elevated serum lactate, acyl-carnitine, and urine methylmalonic acid. MRI brain (patient II:3) showed selective putamenal hyperintensity (Fig. 1B). MR spectroscopy was normal. Skeletal muscle histology and electron microscopy (patients II:1, II:2 and II:3) were normal, including mitochondrial morphology and absence of ragged-red fibers (Fig. 1C). Muscle mitochondrial respiratory chain enzyme activities were normal (patient II:3). There was no mtDNA depletion in fibroblasts (not shown) or skeletal muscle (patient II:3) (Fig. 1D).

Mutation identification and effect

SNP microarray testing revealed three regions of homozygosity shared between the three patients (Fig. S1), by far the largest a 19 Mb region in chromosome 13q14 (Hg19: Ch13: 45,245,670-64,202,461) (Fig. 1A). Whole-exome sequencing in individuals II:1 and II:3 revealed 30,803 single nucleotide variants. After excluding variants that were heterozygous, encoding amino acids synonymous to wild-type, predicted to be nondamaging, reported in dbSNP, 1000-genomes and in-house databases, and of low quality depth (QD) and high strand bias (SB), we were left with one variant, *SUCLA2* c.1219 C> T p.(Arg407Trp), in the 13q14 region (QD 33; SB −1,044). Sanger sequencing confirmed the mutation, its segregation with the disease (Fig. 1A) and its absence from 100 south-Lebanese. In the gnomAD database of variant frequencies in healthy adults, *SUCLA2* c.1219 C> T is present in heterozygous in three and homozygous state in no individuals. The mutated arginine is evolutionarily conserved from fish to humans. Finally, the mutation has previously been described in four patients with mtDNA depletion Type V[10,11] (Table 1).

SUCLA2 dimerizes with SUCLG1 to form the ADP-specific succinyl-CoA ligase, which predominates in brain and muscle. In liver, a dimer of SUCLG2 with SUCLG1 and GDP-specific succinyl-CoA ligase activity predominates.[12] Fibroblasts possess both activities.[12] In a fibroblast cell line from patient II:3, ADP-specific succinyl-CoA ligase activity was undetectable, while GDP-specific succinyl-CoA ligase was normal (Fig. 1E).

Treatment

To attempt to circumvent the Krebs cycle block at the ADP-specific succinyl-CoA ligase, we prescribed food-grade succinate 3 g orally twice per day to patient VI:1. Within four weeks, the key disease biochemical disturbances normalized or improved (Table 2). Unfortunately, the movement disorder did not change. The supplement

Table 1. Genotype-phenotype correlation in patients with two mild *SUCLA2* missense mutations[a].

Patient	Ethnic origin	Mutation (amino acid change); homozygous unless otherwise indicated	Neurological symptoms	Muscle pathology; muscle respiratory chain activities (<20% of control required to meet major criterion for mitochondrial disease)[7]	Muscle mitochondrial DNA (diagnostic threshold: <40% of control)[8,9]	Hearing impairment	Other findings	Neuroimaging	Last reported age
1	Swedish	p.Asp333Gly	Hypotonia, psychomotor delay, dystonia, hyperkinesias	Atrophy; I: 83%, II+III:118%, IV: 59%	N/A	-	Feeding problems, reflux, recurrent airway infections, postnatal GR, decreased spontaneous movement, inguinal hernia	N/A	31 y
2 (sibling of patient 1)	Swedish	p.Asp333Gly	Hypotonia, dystonia, psychomotor delay, choreoathetosis, dystonia	Atrophy, contractures, rhabdomyolysis; I: 35%, II+III:46%, IV: 37%	N/A	-	FTT, feeding problems, gastrostomy tube, lack of voluntary movement, reflux, abnormal breathing, hyperhidrosis, postnatal growth retardation (GR), acute deterioration	CT (1 y): normal	21 y
3	Swedish	p.Asp333Gly	Hypotonia, psychomotor delay, hyperkinesia	Atrophy; I: 36%, II: 91%, I+III: 33%, II+III: 70%, IV: 67%	Slightly decreased (likely not meeting diagnostic threshold)	+	FTT, feeding problems, dysarthria, moderate mental retardation, muscle weakness, muscular atrophy, walks w/o support, balance and coordination problems leukoencephalopathy, cerebral atrophy	MRI: basal ganglia hyperintensities,	27 y
4	Finnish	p.Asp333Gly	Psychomotor delay, lack of voluntary movement	N/A	N/A	?	FTT, feeding problems, gastrostomy tube, acute deterioration, slight postnatal GR, abnormal breathing, hypotonia, psychomotor retardation	MRI (8 months): cerebral atrophy, hemosiderin along wall of left ventricle	10 months
P1 (Matilainen et al 2015)	Finnish	p.Asp333Gly	Dystonia, ataxia, neuropathy	Type II atrophy; Respiratory chain activities normal	65%	+	Walks with a walker at 9 years-old, goes to school, communicates using signs and gestures	MRI: Normal at 5 months; basal ganglia atrophy at 2 y	7 y
P2	Finnish	(1) p.Asp333Gly (2) 1.54 Mb	Athetosis, epilepsy,	Essentially WNL;	N/A	+	Retinoblastoma related to the deletion mutation.		20 y

(Continued)

Table 1 Continued.

Patient	Ethnic origin	Mutation (amino acid change); homozygous unless otherwise indicated	Neurological symptoms	Muscle pathology; muscle respiratory chain activities (<20% of control required to meet major criterion for mitochondrial disease)[7]	Muscle mitochondrial DNA (diagnostic threshold: <40% of control)[8,9]	Hearing impairment	Other findings	Neuroimaging	Last reported age
(Matilainen et al 2015)		deletion; entire gene deleted	dystonia, neuropathy	Complex I+III activity: 9% of control			Walks with a walker at 20 years-old, goes to school, communicates using signs and gestures.	MRI: severe basal ganglia atrophy at 18 y	
5 + 6 (pair of twins)	Caucasian	p.Arg407Trp	Hypotonia, psychomotor delay, dystonia, choreoathetosis	N/A	N/A	+	Neonatal hypoglycemia, loss of speech MRS: lactate peak	MRI (26 months): basal ganglia lesions, mild cortical atrophy	3.5 y
7	Pakistani	p.Arg407Trp	Hypotonia, dystonia, psychomotor delay, hyperkinesias/choreoathetosis, epilepsy	Normal pathology; I: 92%, II: 67%, III: 65%, IV: 63%	31%	+	FTT, gastrostomy tube, reflux, episodes of acute deterioration, postnatal GR, no speech	MRI: basal ganglia hyperintensities	? y
8 (Garone et al 2017)	Brazilian	p.Arg407Trp	Psychomotor delay, myopathy, ataxia and Chorea.	Myopathy with ragged red fibers. Respiratory chain activities: II+III (4%), IV (12%); I+III (19%)	16%	+	Wheelchair-bound at 22 months. Growth retardation, gastrointestinal reflux, and incontinence.	MRI brain: normal. H-MRS: lactate peak in cerebral cortex and lateral ventricles.	9 y
9 (II:1)	Lebanese	p.Arg407Trp	Dystonia and myoclonus,	Normal	N/A	+	Communicates with signs and gestures	N/A	34 y
10 (II:2)	Lebanese	p.Arg407Trp	Dystonia and myoclonus	Normal	N/A	+	Communicates with signs and gestures	N/A	27 y
11 (II:3)	Lebanese	p.Arg407Trp	Dystonia and myoclonus	Normal	>80%	+	Communicates with signs and gestures	MRI: hyperintensities in basal ganglia with symmetric putamenal involvement	23 y

[a]Patients 9, 10 and 11 are from the present paper. Patient 8 is from Garone et al 2017 (ref. 10 in our reference list). The remaining patients are from the Carrozzo et al, 2016 review (ref. 11 in our reference list) for whom we maintained Carrozzo et al's patient numbering scheme; the original reference for patients P1 and P2 from the Carrozzo et al list is reference 13 in our references. N/A, not available.
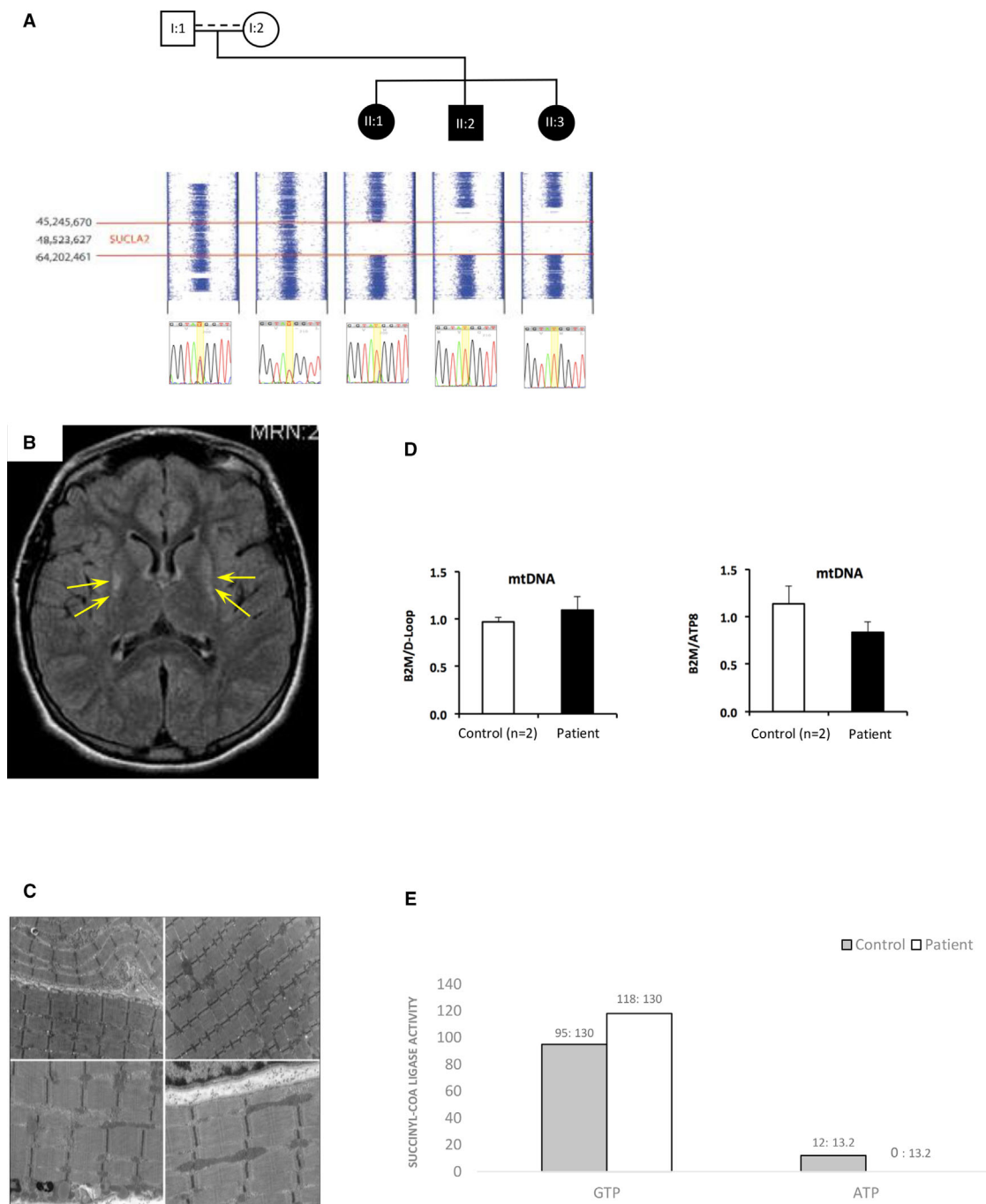
Figure 1. SUCLA2 p.Arg407Trp mutation in a sibship with nonprogressive movement disorder and sensorineural hearing loss. (A) Pedigree. Filled symbols, affected patients; beneath each symbol the patient's microarray result in the indicated region of chromosome 13, with absence of dots depicting homozygosity of SNP genotypes, and his or her *SUCLA2* c.1219 C> T mutation. (B) MRI brain in patient II:3 shows selective putamenal hyperintensity (arrows). (C) Muscle electron micrographs showing normal mitochondrial structure, and generally no myofiber pathology. (D) Mitochondrial DNA quantities at the D-Loop and ATP8 mitochondrial genome loci, relative to nuclear DNA at β-microglobulin (B2M); a value lower than 40% of control is required for a diagnosis of mitochondrial depletion[8,9]. (E) Relative succinyl-CoA ligase activity in fibroblasts in the presence of GTP (for SUCLG2-related activity) or ATP (for SUCLA2-related activity); activity is expressed as a ratio with the activity of another enzyme, short-chain 3- hydroxyacyl-CoA dehydrogenase (SCHAD).

was subsequently discontinued due to poor palatability and clinical inefficacy.

Discussion

Absent SUCLA2 is a major double-hit to mitochondria, directly interrupting the Krebs cycle and indirectly impairing the electron transport chain by depleting its mtDNA. Unsurprisingly, patients lacking SUCLA2 suffer a severe progressive encephalomyopathy with death in childhood. Since the discovery of the first mutations, milder phenotypes have emerged. Carrozzo and colleagues' exhaustive review of the literature until 2016 identified two missense mutations, p.Asp333Gly and p.Arg407Trp, as the mildest to that date.[11] The phenotype that is constant across all 13 patients with these two genotypes (including our three new patients) is the hyperkinetic-dystonic movement disorder. All other clinical features, including hearing loss, failure to thrive, psychomotor delay, postnatal growth retardation, feeding difficulty, and skeletal muscle atrophy were present variably (Table 1). In our patients, only hearing deficiency was present along with the movement disorder, both of which were constant at least into the late fourth decade. Clinical testing abnormalities, including serum amino acids, lactate and pyruvate, urine organic acids, brain MRI and skeletal muscle pathology and, deficiencies in electron transport chain activities and mtDNA were also variable. Taken together, these results indicate that *SUCLA2* mutations should be considered in children with a hyperkinetic-dystonic movement disorder even when none of the above associated findings is present.

Mutations Asp333Gly and Arg407Trp are located in the SUCLA2 ligase domain. We report for the first time that Arg407Trp inactivates the enzyme's succinyl-CoA ligase function. The same has not been reported yet for Asp333Gly, but is expected from structural modeling.[13] Is the phenotype associated with these two mutations milder because they might affect the protein's ligase but not NDPK-related funtion? Results from the patients give a mixed answer. Of six patients in whom skeletal muscle mtDNA and/or respiratory chain activities were quantified, three, including one of ours, had no deficiencies, but three others – patients P2, 8 and 11 in Table 1 – did. This indicates that these mutations can affect NDPK function and oxidative phosphorylation, but do so variably likely affected by extraneous, genetic or tissue-specific (e.g. different muscles biopsied in different patients) factors.

The phenotype shared by all patients with Asp333Gly and Arg407Trp–related SUCLA2 deficiency is the movement disorder, and the basal ganglia are therefore key. Possibly, the effect on NDPK is universal in this tissue, unvaryingly compounding the Krebs cycle failure with

Table 2. Summary of biochemical investigations in patient IV:1 at age 18 before and after succinate supplementation therapy.

Test (normal ranges in parentheses)	Before supplementation	Following one month of supplementation
Serum lactate (0-2.4 mmol/L)	5.7 mmol/L	2.1 mmol/L
Total blood carnitine (32.0-84.0 µmol/L)	24.1 µmol/L	33 µmol/L
Free blood carnitine (26.0-60.0 µmol/L)	10.7 µmol/L	20 µmol/L
Blood palmitoleoyl C16:1 (<0.09 µmol/L)	0.09 µmol/L	0.02 umol/L
C4DC carnitines (<0.13 µmol/L)	0.79 µmol/L	0.61 µmol/L
Urine methylmalonic acid	Elevated	Normal

respiratory chain insufficiency. The susceptibility of the basal ganglia is, however, likely multifactorial, including high energy demands.[14] Within the ganglia, it is likely neurons, and not astrocytes, that fail. This is because, remarkably, astrocytes do not express *SUCLA2* (nor *SUCLG2*) and do not complete their citric acid cycle,[15] in part because much of their glucose is transferred to neurons in the form of lactate.[16]

Since of the two functions of SUCLA2 the ligase activity is definitely affected by the Arg407Trp mutation, we attempted to circumvent that activity in one patient by provisioning succinate. This was successful at the metabolic level, at least in part and as could be measured in plasma and urine (Table 2) but did not alter the movement disorder. Noteworthy though that several patients with the Asp333Gly and Arg407Trp mutations had normal initial neuroimaging, with abnormalities appearing only later (Table 1). As such, succinate supplementation prior to neuronal injury could yet possibly prove beneficial.

Acknowledgments

Conflict of Interest

All authors have reviewed and approve the contents of the manuscript. The submission is not under review at any other publication. None of the authors report any conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Other observed shared homozygous regions in the genomes of the patients across chromosomes include a smaller region on chromosome 13 (98,258,673 – 104,845,473). A summary of all genes in the region is listed in the image.

Figure S2. Other observed shared homozygous regions in the genomes of the patients across chromosomes include a smaller region on chromosome 15 (56,982,357 – 57,486,997). A summary of all genes in the region is listed in the image.

Video S1. The video illustrates part of a neurological examination in patient II:2 and II: 1. Note, both are cooperative, comprehending and following commands. The evident movement disorder can be described as a dystonia interrupted by myoclonus of the arms, trunk and legs.