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A novel variant in the *SUOX* gene in the oldest individual with late-onset isolated sulfite oxidase deficiency

Susanna Rizzi[1], Carlo Alberto Cesaroni[1]*, Carlotta Spagnoli[1], Anna Cavalli[1], Marzia Pollazzon[2], Stefano Giuseppe Caraffi[2], Claudia Dittadi[1], Daniele Frattini[1], Livia Garavelli[2], Carlo Fusco[1]

[1]Child Neurology and Psychiatry Unit, Pediatric Neurophysiology Laboratory, Mother-Child Department, Azienda USL-IRCCS di Reggio Emilia, 42123, Reggio Emilia, Italy

[2]Medical Genetics Unit, Azienda USL-IRCCS di Reggio Emilia, 42123, Reggio Emilia, Italy.

***Corresponding Author:** Carlo Alberto Cesaroni, carloalberto.cesaroni@ausl.re.it

Isolated sulfite oxidase deficiency (ISOD, OMIM #272300) is a rare neurometabolic autosomal recessive disorder, caused by pathogenic homozygous or compound heterozygous variants in the *SUOX* gene. *SUOX* encodes the mitochondrial enzyme sulfite oxidase, responsible for catalyzing the oxidation of sulfites to non-neurotoxic sulfates in the final step of the degradation of amino acids cysteine and methionine. The clinical presentation varies from typical severe disease with prenatal-neonatal onset to rarer, atypical, mild to moderate disease with post-neonatal onset. Among atypical presentations, the onset of symptoms starting from 6 months is defined as late-onset ISOD. Late-onset ISOD occurs in children who were mostly previously asymptomatic and is often precipitated by intercurrent illness or trivial head trauma. The most common clinical manifestation is acute encephalopathy characterized by developmental regression (transient or permanent), seizures, abnormal muscle tone and/or movement disorder [1, 2, 3]. However, minor signs and symptoms such as occasional unsteady gait or slight motor delay and/or hypotonia may be reported.

ISOD may be suspected in case of increased urinary S-sulfocysteine, taurine and thiosulfate, increased plasma S-sulfocysteine and taurine, decreased plasma cystine and markedly reduced plasma homocysteine. The urinary sulfite test is often positive, while enzymatic activity in fibroblasts is absent. Concerning therapy, first-line agents are symptomatic: antiepileptic, antidystonic and muscle-relaxant drugs. Additional option is low-protein diet

without cystine and methionine in an attempt to reduce metabolites prior to enzyme blockade and thus prevent accumulation. [4].

Here we describe the oldest reported patient affected by late-onset ISOD, due to compound heterozygosity for a novel and a known pathogenic *SUOX* variant.

We evaluated a 16-year-old boy due to a phenotype characterized by disruptive, impulse-control and conduct disorders associated with moderate intellectual disability. He achieved his psychomotor milestones on time and did not show neurological symptoms until 18 months of age, when after a febrile episode he showed ataxia and subsequently a drowsy state. He was admitted to hospital and treated as a possible cerebellitis. Brain CT scan and EEGwere normal. He was discharged with the diagnosis of "post-flu drowsy state". After this episode, focal seizures appeared, associated with interictal mid-posterior EEG abnormalities, managed with valproic acid. At the time of our evaluation he showed moderate intellectual disability (Wechsler Intelligence Scale for Children IV: total IQ 45), stable over the subsequent years, an oppositional defiant disorder, intermittent explosive disorder with heteroaggressiveness, and obsessive-compulsive traits. The behavioral disorder was the most debilitating aspect and required pharmacological treatment. Neurological examination revealed dysarthria with slurred speech, slight dysmetria and action tremor.

Brain MRI at 9 years showed enlarged cerebrospinal fluid spaces in the cerebellar, hemispheric and vermian regions with inferior vermis hypoplasia, slightly dilated fourth ventricle widely communicating with the cisterna magna, and mild hyperintensity of the dentate nuclei (fig.1). Neuroradiological follow-up until the age of 15 years did not reveal significant changes.

Exome Sequencing showed two variants in the *SUOX* gene (NM_000456.2): c.1049_1052del, p.(Tyr350*) of paternal origin, never previously reported, and c.1096C>T, p.(Arg366Cys) of maternal origin. The truncating variant is expected to generate a shorter and possibly unstable protein. The missense variant affects the Moco-binding domain and could reduce the stability of the sulfite oxidase holoenzyme [1]. Contrary to the observation by Misko et al., this missense variant contributed to a late-onset phenotype in our individual as well as in three other reported individuals [2, 5]. Both variants were classified as likely pathogenic according to the ACMG/AMP recommendations [6]. Metabolic tests detected

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reduced plasma homocysteine and cystine, increased urinary sulfocysteine and positive urinary sulfite test, confirming the molecular diagnosis.

To our knowledge, only 13 individuals with late-onset ISOD have been reported in the literature so far (Supplementary table 1) [5, 7-13]. In our evaluation we have excluded three individuals due to the presence of neurological symptoms prior to 6 months of life (Supplementary table 2) [14-16].

The average age of onset was 12 months (range 6-23 months). Presentation consisted of acute encephalopathy with psychomotor regression variably associated with behavioral abnormalities in four individuals, acute encephalopathy associated with seizures in three, isolated seizures in two, seizures associated with hyperkinetic movement disorder in two, and acute hypotonia, occasional ataxia or mild motor delay in one individual each. In over half of the cases a factor precipitating the onset was identified: intervening illness in seven and trivial trauma in two. Psychomotor development was reported normal until the onset of neurological symptoms or until a few months afterwards in 71% of individuals. Muscle tone abnormalities were reported in 71 % of individuals and ataxia in 43%. More than half of the individuals had a movement disorder. 57% presented with seizures, four exclusively at onset, while four developed epilepsy (drug-resistant in two cases). EEG was performed in almost half showing a slowed background posteriorly with focal sharp-activity in one case and focal abnormalities in a second one. Aggressive behavior is persistently present in two individuals including our proband.

The majority underwent a neuroradiological examination (4 CT scans and 8 MRIs) and only 2 individuals had normal results (1 CT scan and 1 MRI). More than half of the individuals with available neuroimaging showed signal abnormalities at the level of the basal ganglia and/or cerebellum: three had hyperintensity of the globus pallidi and the substantia nigra, two hyperintensity of the globus pallidi and the dentate nucleus of the cerebellum, one hyperintensity exclusively of the globus pallidi bilaterally. One patient had hyperintensity of the nuclei dentate with associated vermian hypoplasia. A CT scan showed vermian hypoplasia, another one hypodensity of the white matter and frontal lobe and another one temporal and cerebellar atrophy.

Plasma homocysteine was significantly lower than the normal range in all individuals tested. Hypohomocysteinemia is defined by a value < 5 micromol/L [3]. The urinary sulfite test was

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carried out in almost all patients and was negative in only one. It's known that sulfite test can give false negative results due to the auto-oxidation of sulfites into sulfates [8, 10]. Six individuals undertook low-protein diet and in almost all a slight biochemical and/or clinical improvement was found.

Due to the rarity of the condition and the broad spectrum of neurological symptoms, late-onset ISOD is potentially misdiagnosed with infectious diseases, intoxications or other neurometabolic/neurogenetic disorders with similar clinical manifestation and onset. Since the onset is acute, it is useful to identify a reliable, minimally invasive and inexpensive diagnostic marker such as plasma homocysteine. Furthermore, the assay of plasma homocysteine is not subject to the risk of false negative results as the sulfite test is. However, it must be considered that some laboratories do not identify a normal range for plasma homocysteine but only a cut-off above which hyperhomocysteinemia is defined. In these cases, therefore, hypohomocysteinemia risks being missed and with it the diagnostic suspicion of ISOD. A correct early diagnosis allows us to avoid more invasive tests, to better understand the prognosis, to start low-protein diet early and finally to carry out genetic parental counseling. In accordance with the latest guidelines relating to ISOD management, the dietary sulfur restriction provides greater benefits precisely in individuals with atypical late-onset presentation [3]. It is therefore necessary not to miss the diagnosis of late-onset ISOD due to the therapeutic implications and the possibility of modifying the clinical picture.

Supplementary Information

Below is the link to the electronic supplementary material (see Supplementary Table 1).

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Authors' contributions

Conceptualization, CAC, SR; clinical data collection and data curation, CAC, SR, CS, CD, DF, MP, SC, MP, SC, LG, AC, CF; writing—original draft preparation, CAC, SR, CD; writing—review and editing, CAC, SR, SC; supervision, CF. All authors have read and agreed to the published version of the manuscript.

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Data availability

The authors take full responsibility for the data, the analysis, and interpretation of the research, and they have full access to all of the data.

Declarations

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical standards

All investigations were carried out according to the Declaration of Helsinki.

Consent to participate

Written informed consent was collected from the parents of the patient for the inclusion of de-identified clinical data in a scientific publication, in accordance with the Declaration of Helsinki.

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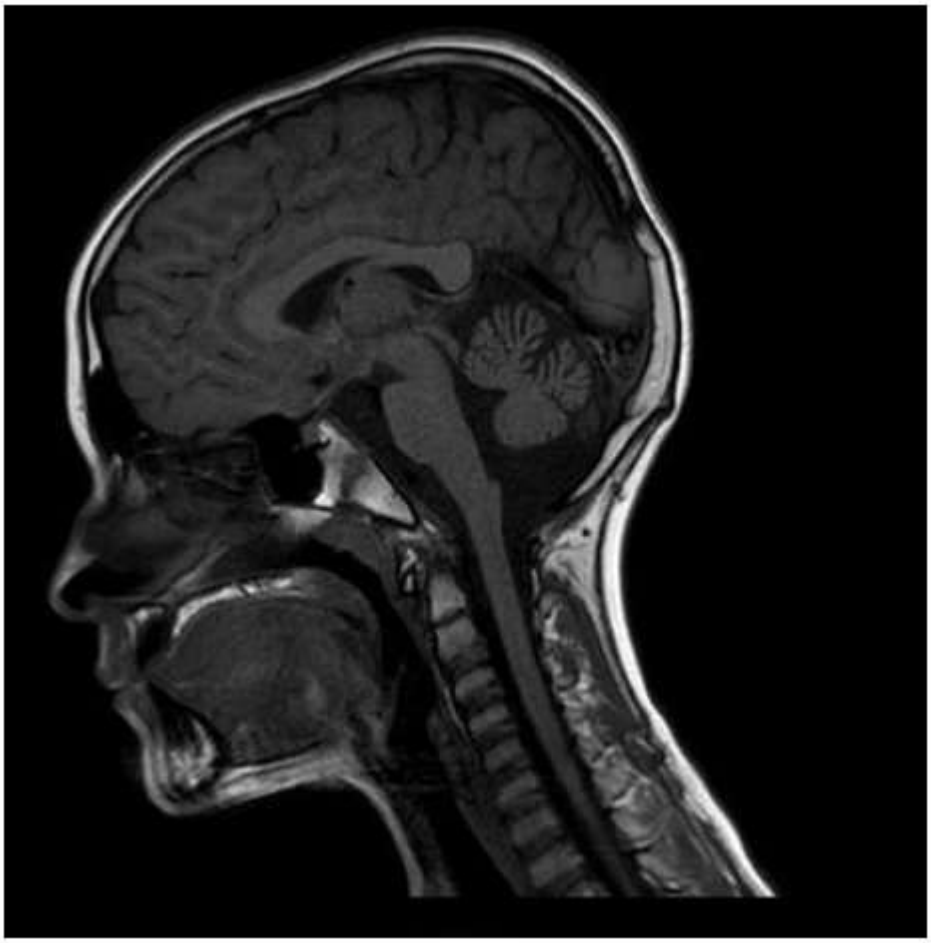

Figure 1. Brain MRI (1,5 t), sagittal T2 flair, show enlarged cerebrospinal fluid spaces in the cerebellar, hemispheric and vermian regions with inferior vermis hypoplasia, slightly dilated fourth ventricle widely communicating with the cisterna magna, and mild hyperintensity of the dentate nuclei.

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