Novel variant (c.472_477del) in the MOCS2 gene

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Abstract

Molybdenum cofactor deficiency type B (MOCODB, #252160) is a rare autosomal recessive metabolic disorder characterized by intractable seizures of neonatal-onset, muscular spasticity, accompanying with hypouricemia, elevated urinary sulfite levels and craniofacial dysmorphism. Thirty-five patients were reported to date. Our paper aimed to delineate the disease genotype by presenting another patient, in whom novel, inframe variant within the MOCS2 gene was identified. Its clinical significance was supported by the medical history and analysis of the possible mutation consequences on a molecular level with the use of the available crystal structure of the human molybdopterin synthase complex. Moreover, potential pathomechanism resulting from molecular defect was presented, giving original insight into current knowledge on this rare disease, including treatment options.

Introduction

Molybdenum cofactor deficiency type B (MOCODB, #252160) is an autosomal recessive metabolic disorder characterized by intractable seizures of neonatal-onset, muscular spasticity, accompanying with hypouricemia, elevated urinary sulfite levels and craniofacial dysmorphism. It came to medical attention first in 1980 (Johnson, 1980). Affected children show severe neurologic complications, which may lead to early death, rarely (only three cases described to date) presented with a milder form with global developmental delay without seizures (Arican, 2019). The disorder results from decreased activity of sulfite oxidase (SUOX; EC 1.8.3.1) and xanthine dehydrogenase (XDH; EC 1.17.1.4 and 1.17.3.2), which are molybdenum cofactor-dependent for their activity.

Molybdenum cofactor (MOSC2) is encoded by the *MOCS2* gene, localized on chromosome 5q11.2. Its biosynthesis is, however, a multistep process, involving: synthesis of precursor Z by proteins encoded by *MOCS1* (603707), conversion of precursor Z to molybdopterin (MPT) by MPT synthase (MOCS2), attachment of molybdenum to the dithiolene moiety of MPT by gephyrin (GPHN; EC 2.10.1.1 and 2.7.7.75) (Reiss, 1999; Reiss and Hahnewald, 2011). MOCS2 is bicistronic, overlapping open reading frames (ORFs) which encode MOCS2A and MOCS2B, the two subunits of MPT synthase (Leimkuhler, 2003), what implicates its functioning and molecular diagnostics of MOSC deficiency.

In this paper, we like to present another patient, in whom novel, inframe variant within the *MOCS2* gene was identified. We aim to delineate the MOCS2 phenotype and give evidence of the clinical significance of the novel variant.

Method

Molecular analysis

Informed consent for genetic testing was obtained from the patient's parents. Whole exome sequencing (WES) was performed in 3billion, Inc (Seoul, South Korea), using genomic DNA isolated from the patient's whole blood. All exons of all human genes (approximately 22,000) were captured using a Twist Human Core Exome Kit (Twist Bioscience, San Francisco, CA, USA). The captured genomic regions were sequenced using a NovaSeq platform (Illumina, San Diego, CA, USA). Raw genome sequencing data analyses included alignment to the reference sequence (NCBI genome assembly GRCh37; accessed in February 2009). The mean depth of coverage was 100-fold, with 99.2% coverage higher than 10-fold. Variant calling, annotation, and prioritization were performed as previously described (Seo, 2019). Sanger sequencing of the variant identified by exome sequencing was performed for the patient.

Crystal structures analysis

The crystal structure of human molybdopterin synthase complex (PDB ID: 5MPO, (Kopec, 2016) and molybdopterin synthase from *Staphylococcus aureus* complexed with precursor Z (PDB ID: 2QIE, (Daniels, 2008) were downloaded from the Protein Data Bank (Rose, 2017). Protein structures were aligned and analyzed using PyMol software [PyMol Schrödinger LLC]. The potential effects of substitutions at 158 and 159 positions in the molybdopterin synthase catalytic subunit were examined by the PredictSNP webserver (Bendl, 2014).

Proband's history

Clinical course

The boy was born from second pregnancy, second birth, born by Caesarean section (due to polyhydramnios, fetal heart rate anomalies in CTG) in 38th week of pregnancy with birth weight 3960g, head circumference 37cm ($> 97^{\text{th}}$ percentile). In the Apgar scale, he received 7/7/10/10 points. Irregular breathing, pale grey skin, and increased muscle tone were noted; saturation of 65% and mild metabolic acidosis in umbilical cord blood. Mechanical ventilation was used to stabilize the child's condition. During pregnancy, the mother was diagnosed with hypothyroidism, treated with levothyroxine; fetal hiccups were noted. The family history for miscarriages, deaths in early childhood, and neurological and metabolic diseases were negative. The child's parents are unrelated; the proband has a healthy, 2-yr-old sister.

Physical examination revealed facial dysmorphism (as coarse features with puffy cheeks, bitemporal narrowing, deep-set eyes, long philtrum, dysplastic auricles), long and overlapping fingers, large feet, pectus excavatum, swelling of the eyelids and lumbosacral region. At 30 minutes after the birth, deterioration in the general condition, grunting drops in desaturation were observed with subsequent diagnosis of congenital pneumonia. Difficulties in feeding (lack of sucking), muscular tension disorders, epileptic seizures, and breathing difficulties with an abnormal crying further complicated perinatal period. Convulsions with drops in saturation occurred on the first day of life. Despite the phenobarbital implementation, polymorphic epileptic seizures, limb myoclonus, breathing disorders, sometimes with desaturation, were still observed. Within the following days, the child became inactive and flaccid. Abnormal spontaneous movements of the extremities were observed, as nonrhythmic waving, pedaling, and increased muscle tone, especially in upper extremities. Reduced muscular tension was accompanied by areflexia. Since seizures were still uncontrolled, levetiracetam was additionally introduced to the treatment with initially good effect. During the hospitalization, muscle tone gradually increased, and elbow and knee joints contractures appeared.

In the second month of life, the child gradually became more reactive, but still presented clearly an impoverished spontaneous motor activity. At times, the boy opened his eyes, but with no eye contact and did not follow with his eyes. He was fed with a nasogastric tube. Physically, the skin was pale, pasty, head circumference was still above 97^{th} percentile, and prominent cranial sutures were noted. The axial hypotonia and lower limb spasticity were observed; tendon hyperreflexia appeared. In the neurological examination, the traction test was negative, no sole reflex on both sides, no Babinski sign bilaterally, weak grasping reflexes, no Moro reflex. Periodically restless, crying, and hyperekplexia were observed, as well as epileptic seizures,

mainly myoclonic. Thus, in addition to phenobarbital and levetiracetam, valproic acid was later introduced. Due to tachycardia, propranolol was included in the treatment. Hypothyroidism requiring substitution with levothyroxine was also diagnosed. Because of severe and worsening clinical condition, the child was transferred to a home hospice.

Laboratory and imagine assessment.

These are summarized in Table 1.

Table 1. Abnormal results of laboratory and imagine assessment of our Proband.

Test	Result
uric acid/serum	not detectable
homocysteine/serum	<1.00 umol/L
Sulfite dipstick	positive
brain US	3rd day after birth - vague brain structure marginal widening of the occipital corners of both lateral ventricles (normal Evans index) slightly accelerated flows in the sagittal sinus 4th week of life – extensive malacic lesions within the frontal and parietal cortex symmetrically with prevalence on the right side and hyperechogenic areas around tegmentum and globus pallidus bilaterally 2nd month of life– extensively disseminated porencephaly within the hemispheres and the subcortical regions - extensive disseminated brain necrosis and low-pressure hydrocephalus
brain MR (5th day after birth, Figure 1)	cerebellar hypoplasia and dilated cerebellar fluid spaces slightly enlarged cerebral fluid spaces in the left temporal region a discrete band of enhanced signal in T1-dependent images and FLAIR within one of the grooves of the left frontal lobe (after the bleeding?) the supratentorial ventricular system symmetrically widened within the lateral ventricle (Evans index - 0.36)
EEG	neonatal period - localized changes with a tendency to generalize 2nd month of life– almost continuous right- or left-sided localized burst-suppression pattern
ECHO	hypertrophic cardiomyopathy, non-restrictive
other	elevated levels of troponin and NT-proBNP, hypothyroidism

Figure 1

Genetic result

WES led to the identification of a variant in the *MOSC2* gene - c.472_477del (Table 2, Suppl. 1). The results of the predicted effect of mutations at position 158 and 159 of the catalytic subunit of molybdopterin synthase complex obtained by PredictSNP server (Brendl, 2014).

Table 2. Genetic result of our patient. Nomenclature of the variant following the guidelines of the Human Genome Variation Society using NM_004531.4 as a reference cDNA sequence and NP_004522.1 as a protein sequence.

Gene	*MOCS2*
Variant nomenclature	Variant nomenclature
cDNA Level:	NM_004531.3: c.472_477del
gDNA Level:	Chr5(GRCh38):g.53100435_53100440del
Protein Level:	NP_004522.1: p.(Leu158_Lys159del)
Variant type	inframe deletion
Zygosity	homozygous
Allele frequency*	-
Classification	VUS
Disease	Molybdenum cofactor deficiency B (autosomal recessive)

* Allele frequency is based on genomes in the gnomAD database; VUS variant of unknown significance

Structural analysis

There is no crystal structure of the protein carrying described deletion; however, the analysis of the native conformation of the molybdopterin synthase and comparison with the structure from*Staphylococcus aureus* complexed with precursor Z shed light on the consequences of the deletion. Human molybdopterin synthase complex was crystallized as a dimer constituted of two heterodimers. Each heterodimer consists of molybdopterin synthase sulfur carrier subunit and molybdopterin synthase catalytic subunit. The Leu158 and Lys159 deletion occur in the catalytic subunit. The Leu158 and Lys159 residues are located at the end of the last helix, close to the C-terminus of the subunit, and together with the C-terminus, they participate in the molybdopterin synthase sulfur carrier subunit binding (Figure 2A). Moreover, the comparison of the structures of molybdopterin synthases from human and *Staphylococcus aureus* suggests, that the Lys159 residue is essential for precursor Z binding (Figure 2B).

Figure 2

Discussion

The molybdenum cofactor deficiency is an ultra-rare autosomal recessive disease, characterized by rapidly progressive and severe neurological damage, sometimes misdiagnosed as hypoxic-ischemic encephalopathy. The clinical and molecular characteristics of molybdenum cofactor deficiency due to *the MOCS2* variant have been recently extensively reviewed by Arican et al. (2019). The presented group encompassed 35 patients with proven molecular etiology and 29 different pathogenic variants identified in the *MOCS2* gene.

Our proband's history and clinical features are consistent with the previous descriptions. That prompted us to test toward a deficiency of molybdenum cofactor, while neonatal-onset, refractory epilepsy, feeding difficulties, facial dysmorphism, and brain images were the most suggestive phenotypic feature. This initial clinical diagnosis, supported by undetectable serum uric acid, very low serum homocysteine, and positive Sulfite dipstick results, was finally verified in whole-exome sequencing analysis. It revealed a variant of unknown significance in the *MOSC2* gene. The homozygous variant c.472_477del on *MOCS2* deletes nucleotides 'CTTTTA' from chromosome5 52396264, which does not change frameshift in the protein-coding sequence. It has been reported with an extremely low frequency in the large population cohorts (GenomAD). This inframe deletion in the non-repeat region can change the length of proteins and disrupt protein function. This variant is classified as uncertain significance according to the recommendation of the American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) guideline. The child's clinical features were, however, highly consistent with the disease. Additionally, taking into account the homozygous state of this variant, we suggest as causative.

We have performed an analysis of the crystal structure of the human molybdopterin synthase complex to support this statement. The Leu158, Lys159 are localized at the end of the last helix of the catalytic subunit,

just before the 13 aa of the C-terminus. This region of the molybdopterin synthase has dual functionality. It binds the sulfur carrier subunit and the precursor Z. The alignment of the crystal structure of human molybdopterin synthase with the one of*Staphylococcus aureus* complexed with precursor Z reflects that Lys159 corresponds to Leu116 of (*S. aureus*) is essential residue for proper binding of the precursor Z.

In consequence of the mutation, the Leu158 will be replaced by Ala160, and Lys159 by Lys161. Luckily, the deleted Lys159 should be replaced by Lys161, and thus it's role can be preserved (similar replacement of the key amino acid was observed for example in D-amino acid oxidase, where Tyr314 repossess the function of the Tyr55, eliminated by the Tyr55Ala mutation and modifies the enzyme-substrate specificity (Subramanian, 2014). Since the second one replaces the crucial lysine, one could expect, that the precursor Z binding can still occur, however, the protein-ligand affinity can be already modified. However, in this case, the situation is more complicated. The second lysine residue (123 in*S. aureus* and 166 in human) is essential for precursor Z binding, and this crucial residue is located at the C-terminus. It is highly probable that the deletion of two residues can modify the position of the second lysine.

Moreover, it will have consequences on the folding of the C-terminus and can result in changes in binding affinity of the sulfur carrier subunit to the catalytic one. Thus, the described deletion could have significant consequences for both, the heterodimer and the active complex formation, which may suggest another mechanism of pathogenicity of c.472_477del variant in the *MOCS2* gene. As results from predictSNP have shown (Suppl. 2), no deletion analysis is possible, but the report clearly indicates that any mutation in this area has consequences. However, to verify, which functionality is mostly disturbed, the precursor binding or carrier subunit binding, further study has to be considered.

Conclusions

In this paper, we give clinical and structural evidence of pathogenicity of the novel, inframe variant - c.472_-477del. We emphasize the need for rapid genetic diagnosis, next-generation sequencing (NGS), in metabolic diseases, especially in cases with unclear clinical picture or when the prognosis is poor. The described mutation modifies the binding cavity of the catalytic subunit, and the preliminary structural analysis shows two potential processes that can be distorted. We can speculate, that if the heterodimer formation is preserved, the deficiency caused by abnormal precursor Z binding could be perhaps limited, but a chemical modification of the precursor, whereas the changes in carrier subunit binding would be much more challenging to reverse.

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Data Availability Statement

Data available on request from the authors.

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