



Expanding the genetic cause of multiple sulfatase deficiency: A novel *SUMF1* variant in a patient displaying a severe late infantile form of the disease

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ABSTRACT

Multiple sulfatase deficiency (MSD) is a rare inherited metabolic disease caused by defective cellular sulfatases. Activity of sulfatases depends on post-translational modification catalyzed by formylglycine-generating enzyme (FGE), encoded by the *SUMF1* gene. *SUMF1* pathologic variants cause MSD, a syndrome presenting with a complex phenotype. We describe the first Polish patient with MSD caused by a yet undescribed pathologic variant c.337G>A [p.Glu113Lys] (i.e. p.E113K) in heterozygous combination with the known deletion allele c.519+5_519+8del [p.Ala149_Ala173del]. The clinical picture of the patient initially suggested late infantile metachromatic leukodystrophy, with developmental delay followed by regression of visual, hearing and motor abilities as the most apparent clinical symptoms. Transient signs of ichthyosis and minor dysmorphic features guided the laboratory workup towards MSD. Since MSD is a rare disease and there is a variable clinical spectrum, we thoroughly describe the clinical outcome of our patient. The FGE-E113K variant, expressed in cell culture, correctly localized to the endoplasmic reticulum but was retained intracellularly in contrast to the wild type FGE. Analysis of FGE-mediated activation of steroid sulfatase in immortalized MSD cells revealed that FGE-E113K exhibited only approx. 15% of the activity of wild type FGE. Based on the crystal structure we predict that the exchange of glutamate-113 against lysine should induce a strong destabilization of the secondary structure, possibly affecting the folding for correct disulfide bridging between C235–C346 as well as distortion of the active site groove that could affect both the intracellular stability as well as the activity of FGE. Thus, the novel variant of the *SUMF1* gene obviously results in functionally impaired FGE protein leading to a severe late infantile type of MSD.

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1. Introduction

Multiple sulfatase deficiency (MSD) is an ultra-rare metabolic disease (OMIM #272200). It is caused by defective activity of all sulfatases, most of them with lysosomal localization. Non-degraded sulfatase substrates, mainly glycosaminoglycans and sulfolipids, lead to a lysosomal

storage disorder in affected individuals. To gain enzymatic activity all human sulfatases require an essential post-translational modification of a cysteine residue to C α -formylglycine present in the catalytic domain [1]. This modification is catalyzed by the formylglycine-generating enzyme (FGE; EC 1.8.99), which is encoded by the *SUMF1* gene located on chromosome 3p26.2. Impaired FGE stability and/or activity result in MSD. More than 40 different pathologic variants in the *SUMF1* gene have been identified so far, the majority of which are missense mutations (Human Gene Mutation Database, June 2016). MSD is inherited in an autosomal recessive manner. Its prevalence has been estimated to be 1 in 1.4 million live births [2].

According to the age at onset and the dynamics of the clinical outcome there are four types of MSD: neonatal, severe (onset in the first

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year of life), mild late infantile (onset after 2 years of life), and juvenile type (late onset of the disease). The clinical findings in MSD form a wide spectrum and combine symptoms of single sulfatase deficiencies. Among others, neurological symptoms resembling metachromatic leukodystrophy, dysmorphic features like in mucopolysaccharidoses and ichthyosis caused by steroidsulfatase deficiency are characteristics of MSD. The presence of clinical symptoms in patients younger than 2 years and concurrent psychomotor regression result in rapid progression of the disease and a bad prognosis [3]. Here, we report a first case of MSD detected in Poland, who presented with typical symptoms of a severe late infantile type of the disease caused by a yet undescribed missense mutation. Additionally, a thorough biochemical, functional, and bioinformatic analysis of the novel mutation is provided.

2. Materials and methods

2.1. Molecular genetic analysis

In order to elucidate the molecular background of the disease and to provide the genetic counseling for the family the molecular genetic analysis was performed in proband, his parents and sisters. Genomic DNA was isolated from peripheral blood leukocytes using standard protocol for the phenolic method. The coding exons together with adjacent intronic regions were amplified by PCR and sequenced with the Sanger method (primers sequences are available on request). Mutation nomenclature is according to the mRNA transcript variant 1 sequence available at GeneBank accession number NM_182760.3.

2.2. Cell lines and expression constructs

Cell culturing of HT1080 fibrosarcoma cells and immortalized MSD (MSDi) patient skin fibroblasts, generation of respective Tet-On cell lines and pBI expression constructs encoding FGE and steroid sulfatase (STS) were described earlier [4]. The FGE-E113K mutation was created by site-directed mutagenesis PCR with pBI-FGE plasmid as template and complementary primers (coding sequence only: FGE-E113K_Fwd, 5' ATAAAGCAGGATGGGAAAGCACCTGCGAGGAGA-3'). The resulting construct pBI-FGE-E113K was verified by sequencing of the entire coding region to exclude any undesired PCR-derived errors.

2.3. Steroid sulfatase (STS) activity assay and western blotting

The STS activity assay in MSDi cells were performed as described earlier [4]. Briefly, the activity assay was performed in lysates of cells that transiently express either STS alone or STS together with FGE. For western blot analysis, rabbit polyclonal antisera against FGE or STS were used as primary antibodies and HRP-conjugated goat anti-rabbit antibodies were used as secondary antibodies [4]. Western blot signals were quantified using the AIDA 2.1 software (Raytest). The specific activity of STS was calculated by dividing the activity in lysates by the western blot signal (arbitrary units).

2.4. Indirect immunofluorescence analysis

HT1080 cells transiently expressing FGE were fixed in 4% paraformaldehyde, permeabilized with saponin and incubated with rabbit polyclonal antisera against FGE and mouse monoclonal anti-Protein disulfide isomerase antibody, PDI (Abcam). This was followed by incubation with fluorescently tagged secondary antibodies, goat anti-rabbit Alexa488 and goat anti-mouse Alexa546 (Molecular Probes). Confocal images were acquired using Zeiss-LSM700 confocal microscope and images were processed using the manufacturer's software.

2.5. Sequence logo generation

The sequence logo for the conservation of amino acid residue E113 (Glu113) were generated using the WebLogo 3.0 program as described earlier [5]. FGE protein sequences (corresponding to residues 100–140 of human FGE) from various higher mammalian species comprising Euarchontoglires, Laurasiatheres and Atlantogenata were selected based on a ClustalW alignment. The residues were selected in such a way that the conservation of E113 can be compared to the conservation of other known MSD-causing mutations in the vicinity of E113. The sequences from 29 species that were used for the logo generation are given in Table S1.

2.6. Bioinformatic analysis

The potential pathogenicity of novel mutation was determined using PolyPhen-2, Provean and Mutation Taster software.

To predict the effect of Glu113 mutation to Lys on the possible structural perturbations, based on the crystal structure of FGE [1], we performed an *in-silico* analysis of the interactions of Glu113 with other residues using PyMOL software with X-ray coordinates of FGE crystal structure (PDB: 1Y1E).

3. Results

3.1. Patient description

The male patient is the second child of young, non-consanguineous parents. He has one older sister with epilepsy and one healthy younger sister. The patient was born at 39 weeks of gestation via cesarean section after a pregnancy marked by intrauterine growth restriction (IUGR) from 28th week, probably caused by placental dysfunction, birth weight 2780 g, birth length 52 cm, head circumference 33 cm, Apgar scores 6/6. The newborn presented with a poor sucking reflex and weak spontaneous activity. CT scans at the age of 1 month revealed agenesis of corpus callosum and dilated lateral brain ventricles (especially widening of the distance between both cornu anterii and cornu inferii ventriculi lateralis).

At the age of 3 months first symptoms were observed such as psychomotor delay, lack of speech development, poor environmental contact, lack of social smile, strange smell, salty sweat, white tears and dry skin, frequent vomiting and feeding problems. The patient was hospitalized five times during the first year of life because of failure to thrive and delayed neurological development with axial hypotonia and limb hypertonia, and recurrent respiratory tract infections. At the age of 3 months tracheomalacia was diagnosed. Dysmorphic features included: dolichocephaly, bilateral narrowing of the face, hypertelorism, long nasal bridge, retrognathism, high arched palate, clinodactyly of the right fifth finger. Left sided cryptorchidism and talipes varus were noted as well.

At 5 months of age, the child displayed signs of gastroesophageal reflux, high blood pressure, hypothyreosis and skeletal deformities (funnel chest, thoracolumbar kyphosis). Hearing impairment was confirmed. The ultrasound scan of the abdomen revealed a hepatosplenomegaly. At the age of 6 months the baby presented with a transient ichthyosis, appearing only in winter. At 9 months the psychomotor development clearly deteriorated with rapid regression. He was barely able to roll and to sit without support. Head coordination was rather weak, hands were not used for playing. Brain MRI confirmed the agenesis of the corpus callosum and revealed agenesis of the septum pellucidum, enlargement of the lateral ventricles, cisterna magna and subdural spaces, and progressive leukodystrophy. Pale optic discs appeared bilaterally.

The patient's current age is 4 years. He suffers from epilepsy and recurrent infections. Reaction to visual or hearing stimuli has been lost. He needs a respirator and special parenteral feeding. Sitting, even with

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