New VOUS in *CASK* Gene Correlating with the MICPCH Phenotype

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ABSTRACT

We present the case of a three-year-old girl with normal family history who was admitted to our hospital for medical recovery. The patient had microcephaly, pontocerebellar hypoplasia, slight facial dysmorphism, axial hypotonia, epileptic seizures, absent walking skills and severe speech delay. Genetic testing identified a heterozygous intronic variant in the CASK gene, namely CASK c.278 + 5G>A, which has never been reported in the medical literature or in other databases (gnomAD, ClinVar, HGMD). In mammals as well as more distant species, the G nucleotide is fully conserved at this position, suggesting it may not tolerate variation. In silico tools predict the substitution to be deleterious. Pathogenic mutations of these gene are responsible of mental retardation and microcephaly with pontine and cerebellar hypoplasia (MICPCH) syndrome, which overlaps completely with our patient's phenotype.

Keywords: MICPCH syndrome, pontocerebellar hypoplasia, *CASK*, VOUS.

INTRODUCTION

Mental retardation and microcephaly with pontine and cerebellar hypoplasia (MICPCH) syndrome (OMIM # 300749) is an X-linked dominant disease caused by heterozygous loss of function of *CASK*, a gene which plays an important role in brain development (Xp11.4) (1-6). This syndrome is characterized by congenital microcephaly, pontine and cerebellar hypoplasia, postnatal growth retardation, low weight for length, severe intellectual disability (language skills are usually never developed), marked motor retardation (only a quarter of the patients acquire walking abilities), axial hypotonia, limb hypertonia and spasticity, dystonic movements, autistic manifestations such as hand flapping or self-biting, seizures, ocular manifestations such as strabismus, optic nerve atrophy or hypoplasia, nistagmus, cataracts or retinopathy, sensorineural deafness, and minor

facial dysmorphism, such as broad forehead, hypertelorism, epicanthus, arched eyebrows, large ocular globes, macrotia with protruding ears, long philtrum, short nose with a wide bridge and a broad tip, microretrognathia and prominent maxilla (7-11). Because the syndrome is very rare, its frequency is still unknown (12). More than 50 female and a few male patients affected with MICPCH have been reported in the literature (12). ❏

CASE REPORT

A three-year-old girl was admitted in "Dr. Nicolae Robanescu" National Clinical Center for Children's Neurorecovery during the period July-August 2020 for evaluation and treatment. Family history was not significant, parents were healthy and denied consanguinity. The mother never had miscarriages. The patient had a nine-and-a-half-year-old brother who was healthy.

The patient came from a pregnancy with imminent miscarriage at 19 weeks for which the mother received progesterone throughout the entire pregnancy. The mother mentioned she had pelvic pain throughout the entire pregnancy. The pregnancy was carried to full-term (38 weeks and four days), and delivery was performed by C-section (although natural birth was possible, the mother opted for C-section). The baby was born with an Apgar score of 9. At birth, her weight was 2860 g (the 5th percentile), length 50 cm (the 50th percentile), and head circumference 29 cm (below 3rd percentile – microcephaly).

Craniostenosis was noticed at birth, the anterior fontanelle had an area of about 1/1 cm.

Anamnestic data from the mother revealed that the patient had epileptic seizures (mioclonic spasms of upper and lower limbs, more pronounced on the right side compared to the left side, with onset around the age of one, which were occuring for about 2-3 days at every awakening, with remission for about a month followed by the repeating of this cycle), which were confirmed following an EEG test performed in the pediatric neurology department. Since 2018, the patient has been receiving treatment for seizures (sodium valproate, topiramate, clobazam), which was successfull, with the last seizure in May 2020.

The patient could hold her head at about two months and sat with support at about seven months. After the onset of seizures, she lost these acquisitions, and at the moment of our consultation she could not either hold her neck or sit and presented severe axial hypotonia. The patient had severe language delay and could only pronounce one word, "mama", when seeing her mother.

According to the mother, from November 2019 to May 2020 the patient had frequent episodes of nausea and vomiting, and since October 2019 she started to have recurrent infections of the respiratory tract, which occurred very often. Also, according to her mother, the girl had deglutition problems and several episodes of aspiration pneumonia. The patient had also chronic constipation. Her mother told us her daughter was born with occipital hemangioma, which was not visible during the consultation.

Biochemical tests revealed normal liver enzymes, LDH and CK levels.

At the age of six months, a transfontanellar ultrasound was performed, revealing chronic intrauterine distress and megacisterna magna.

Two brain MRIs were performed in May 2018 and April 2019, and both revealed pontocerebellar hypoplasia associated with Dandy-Walker variant and a right parietal venous angioma.

SNP array (Louis Turcanu Hospital, Timisoara, Romania) was normal.

Her physical exam showed ponderal hypotrophy: weight 11.8 kg (> pc 3), a length of 93 cm (the 25th percentile), and head circumference with two standard deviations below the 50th percentile. The patient had severe mental disability, severe language delay with absent speech, and severe motor retardation with axial hypotonia. The girl could hold her head, or sit unaided, or walk. She had limb hypertonia (patient's axial hypotonia and limb hypertonia can be seen in Figure 2), dystonic movements, no sphincter control, ponderal hypotrophy, microcephaly, facial particularities with oval face, micrognathia, high and ogival palate, arched eyebrows, hypertelorism, long eyelashes, epicanthus at the left eye, large ocular globes, stellate iris, low set, prominent ears, long philtrum, curly hair (mother also had curly hair), short nose with a broad nasal bridge and tip (patient's facial dysmorphism can be seen in Figure 1), bilaterally absent patellar DTRs, bilateral Babinski reflex

FIGURE 1. Patient presented craniofacial dysmorphism with microcephaly, broad forehead, short nose with broad bridge and round tip, arched eyebrows, epicanthus of the left eye, large ocular globes, long philtrum, macrotia and micrognathia

FIGURE 2. Patient with axial hypotonia and limb hypertonia, who had not acquired the ability to walk

and could not perform dorsiflexion of the plant, bilaterally. Sight and hearing were present, and at the moment of our consultation she had not performed an ophthalmology exam, as the diagnosis of MICPCH had not been established yet.

In order to establish the etiology, 2 milliliters of blood on EDTA were sent for genetic testing (NGS sequencing, FLEX Microcephaly and Pontocerebellar Hypoplasia Panel Plus, Blueprint Genetics Lab, Helsinki, Finland). The patient was found heterozygous for *CASK* c.278 + 5G > A, a variant of unknown significance. Although this was a VOUS, there was a strong association between the detected gene and patient's phenotype. The variant is absent in control populations and in silico tools predict this substitution will

abolish the splice donor of intron 3 and may therefore lead to aberrant splicing.

The parents were informed about the result and genetic counselling was offered, with the recommendation of parental testing to establish the inheritance pattern and for further classification of the variant, and have decided for now not to carry on with further testing. ❑

DISCUSSION

MeNtal retardation and microcephaly with pontine and cerebellar hypoplasia is most often diagnosed in females with severe intellectual disability and progressive microcephaly, usually associated with epileptic seizures, a characteristic facial dysmorphism (broad forehead, oval face, arched eyebrows, hypertelorism, epicanthus, macrotia, long philtrum, short nose with a broad bridge and tip, large ocular globes, micrognathia) as well as ophthalmologic (nystagmus, optic nerve hypoplasia, coloboma, myopia) and auditory (sensorineural hearing loss in about 25% of the cases) manifestations. Neurologic manifestations such as seizures (in about 40% of patients), axial hypotonia, hypertonia of the limbs or dystonia can also be present. Only about 20 to 25% of patients develop the ability to walk, while language is nearly absent in most individuals (6, 7). The syndrome is caused by monoallelic mutations in *CASK* gene which encodes calcium/calmodulin dependent serine protein kinase, a multi-domain scaffold protein interacting with more than two dozen proteins (3, 13, 14).

There are currently 114 variants in *CASK* annotated as disease-causing in the HGMD Professional variant database (version 2020.1) (27), which include approximately 21% nonsense, 18% missense, 14% splicing, 16% small deletions/insertions, 24% gross deletions/insertions and 4% complex rearrangements. In the ClinVar database, as of June 2020 there were 81 likely pathogenic or pathogenic variants seen in clinical testing (16).

The *CASK* c.278 + 5G>A variant substitutes a nucleotide within the intronic splice region. It is absent in the Genome Aggregation Database control population cohorts (gnomAD, n>120 000 exomes and > 15 000 genomes) (6). In silico splice prediction tools (SSF, MaxEntScan, NNSpice) predict the substitution will abolish the

splice donor of intron 3 of *CASK*. All in silico splice prediction tools predict a cryptic donor site at position c.278+27. The variant may therefore disrupt splicing. The G-nucleotide is fully conserved at this position in mammals as well as more distant species suggesting that this position may not tolerate variation.

The variant has never been described in the medical literature or reported in disease-related variation databases such as ClinVar or HGMD. However, a hemizygous splice donor variant c.278+1G>A within the canonical donor splice site of the *CASK* intron 3 has been reported as *de novo* in a male patient with X-linked microcephaly with pontine and cerebellar hypoplasia (18). He had a very severe phenotype with nearly no development and refractory epilepsy.

Although *CASK* c.278 + 5G>A is classified as a variant of unknown significance and there is currently insufficient evidence to support its disease-causing role, given the strong association between pathogenic mutations in this gene and the patient's phenotype, which overlaps with the MICPCH syndrome phenotype, the fact that the variant is absent in control populations and it has been predicted by in silico studies to be deleterious, we presume that it is pathogenic. For now, the parents have opted not to be tested.

Management following diagnosis consists of neurological evaluation, including MRI and EEG, developmental assessment (motor, cognitive and speech function), psychiatric exam, nutrition evaluation, ophthalmologic and audiologic evaluation, cardiac ultrasound, abdominal ultrasound with focus on genitourinary abnormalities, as well as clinical genetics consultation (19).

The treatment is symptomatic and includes early management of seizures, kinetotherapy, logotherapy, nutritional support, management of the psychiatric manifestations as well as treatment of hearing loss and visual problems (19).

The disease has an X-linked inheritance pattern, with most cases appearing de novo (20). Nevertheless, prenatal testing should be performed to exclude germinal mosaicism. *In vitro* fertilization with preimplantational genetic diagnosis is also a viable option. Heterozygous females can manifest the phenotype. Up to now, females with MICPCH are not known to reproduce (19).

Differential diagnosis is very broad, including syndromic and non-syndromic X-linked mental retardation, PCH (pontocerebellar hypoplasia), Fragile X syndrome, Wilson-Turner syndrome, Aarskog-Scott syndrome, Prieto syndrome, MEHMO syndrome, Van Esch O'Driscoll syndrome, Raynaud-Claes syndrome, Borkeson-Forssman-Lehmann syndrome, Waisman syndrome, Tonne-Kalscheuer syndrome, Lujan-Fryns syndrome, and many others (19). ❑

CONCLUSION

Mental retardation and microcephaly with pontine and cerebellar hypoplasia syndrome is a very rare disease, with less than 100 cases of females affected reported so far. Our patient presented a never before reported variant in the *CASK* gene, overlapping with MICPCH phenotype. Due to the fact that in silico tools predict this variant to be deleterious and that the variant is absent from control population cohorts, we believe it is pathogenic. We consider that the description of this case could be useful in expanding the knowledge about MICPCH syndrome. ❑

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