A Novel Homozygous *ATP8A2* Variant in a Patient With Phenotypic Features of Dysequilibrium Syndrome

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Abstract- The ATP8A2 protein is mainly located in the brain and takes part in the lipid flipping process. Mutations in the *ATP8A2* gene and chromosomal translocations that interfere with the ATP8A2 gene product have been reported in association with global developmental delay and hypotonia. Here, we will report a three-year-old male presented with major phenotypic features of dysequilibrium syndrome (DES), including severe hypotonia, global developmental delay, speech problem, and strabismus. Whole exome sequencing revealed a homozygous in-frame deletion in the *ATP8A2* gene (c.1286_1288delAGA, p.Lys429del). This *ATP8A2* variant has not been reported yet and seems to be linked to the phenotypic features of dysequilibrium syndrome.

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Introduction

Maintaining the delicate asymmetry of biomembrane is vital to the life of all eukaryotic cells. It is mainly determined by the distribution of phospholipids within two lipid layers of the biological membranes; the cytoplasmic leaflet and the exoplasmic leaflet. To distribute lipids between the layers, there are two lipid flows; an inward flow (from the exoplasmic to the cytoplasmic leaflet) and an outward flow (from the cytoplasmic to the exoplasmic leaflet). These flows might generate transbilayer traffic unless floppase and flippase proteins function properly. Floppases catalyze the lipid flopping process or the outward lipid flow whereas flippases catalyze the process of lipid flipping where phospholipids are transported from the exoplasmic to the cytoplasmic leaflet of biomembranes. Both floppases and flippases and therefore both the inward and outward lipid flows function dependent on adenosine triphosphate (ATP).

The P4 ATPase (type 4 P-type ATPases) family of proteins takes part in the lipid flipping process. Fourteen human and fifteen mouse P4 ATPases have so far been identified. In general, they physiologically contribute to the biology and transport of intracellular vesicles. However, certain P4 ATPase proteins have been associated with particular pathologies (1). For example, variants in the *ATP8B1* gene are linked to cholestatic diseases. Moreover, since the chromosomal abnormalities that interfere with the *ATP10A* or *ATP10D* genes caused metabolic problems such as insulin resistance, scientists have established possible links between *ATP10A* or *ATP10D* genes and obesity (1).

The transmembrane protein, ATP8A2, consists of four protein-coding isoforms. The longest isoform (ENST00000381655) contains 37 exons and encodes a 112kDa protein. This protein is another member of the P4 ATPase which its biofunction has not been well understood yet. It is mostly localized in testis and to some extent in the brain and heart and seems to have a pro-tumorigenic role in human cells (2).

The *ATP8A2* gene (OMIM#605870, NM_016529, chromosomal location 13q12.13) gene belongs to the P(4)-ATPase subfamily of P-type ATPases, which are

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involved in the transport of aminophospholipids. P(4)-ATPases determine the curvature of the phospholipid bilayer by flipping aminophospholipids from the exoplasmic to the cytoplasmic leaflet (3).

Cacciagli and his colleagues (2010) reported a patient with profound neurological problems, including severe hypotonia, speech problems, and the inability to hold her head (4). They revealed a chromosomal translocation is interfering with the P4-ATPase *ATP8A2* gene as the only genetic evidence in the patient. Here we will report a male patient with a similar, however milder, neurological phenotype that resembles dysequilibrium syndrome (DES). Although clinical signs and symptoms associated with the syndrome can vary significantly according to the type of syndrome, the most frequent clinical features characterizing the syndrome include ataxia, gait disturbance, hyperreflexia, intellectual disability, and muscular hypotonia. We used whole-exome sequencing which revealed a homozygous in-frame deletion in exon 14 of the *ATP8A2* gene defined as c.1286_1288delAGA (p.Lys429del). To the best of our knowledge, this *ATP8A2* variant has not been reported yet.

Case Report

The patient is a three-year-old boy who was born by cesarean at term following an uncomplicated pregnancy. He is the second child of contagious, healthy parents. His six-year-old older brother is normal. At birth, his weight (3250 g), length (53 cm), and head circumference (36 cm) were normal. His Apgar scores were also normal. Hypotonia was the first clinical sign discovered by a nurse in health care services in the routine childhood immunization schedule. However, he was generally hypotonia and was not able to hold his head. At age 3 months he developed severe pneumonia requiring ICU admission. After recovering from pneumonia, he was referred to the Neurology Clinic at the Children Hospital Medical Center (CHMC), Tehran, Iran, for an inability to hold the head. Neonatal screening tests (hemoglobin, hemoglobins (S, D, C), TSH, 17-OH-Progesterone, galactose (GAL, GAL-1-p), Gal-1-P-uridyltransferase, succinylacetone, and biotinidase) was performed by the MVZ wagnerstibbe für Laboratoriumsmedizin und Pathologie (Göttingen, Germany) and results fall in the normal range. Investigations of amino acids metabolism disorders, as well as β-oxidation of fatty acids, organic acids, and urea cycle disorders, could not provide a metabolic diagnosis. From age 4 months to 1 year six months, he underwent comprehensive neurorehabilitation program for head lag. However, rehabilitation had no significant effect. Clinical signs and symptoms were made visible by aging, and at age 9 months more clinical and paraclinical examinations were requested for the patient due to a spectrum of clinical features, such as severe hypotonia, global developmental delay, speech problem, strabismus, and high-arched palate. In addition, he was unable to stand, walk, or even sit alone. The electromyography (EMG) and sensory nerve conduction studies revealed no evidence of lower motor neuron lesion. Muscle biopsy at age 1 year five months indicated no significant histochemical finding other than slight type I fibers predominance. MRI of the brain showed no abnormal findings at any time (age 5 months and 2 years).

Molecular genetic studies

The patient was investigated for SMN1 (Survival of Motor Neuron 1, Telomeric)-related spinal muscular atrophy. DNA sample from the patient was investigated for the common deletion of the exon 7 and 8 in the SMN1 gene by MLPA method. Analysis of this gene showed no deletion, so the patient was unlikely to suffer from spinal muscular atrophy.

Karyotype analysis for chromosomal abnormalities

Twenty metaphase spreads were studied on the basis of Trypsin and Giemsa produce G-banded chromosomes (GTG) technique at 450-500 band resolution. Chromosomal analysis indicated 46,XY karyotype and that no chromosomal abnormality was identified.

Whole exome sequencing**,** whole exome sequencing of this patient revealed a homozygous in-frame deletion in the ATP8A2 gene defined as c.1286_1288delAGA (p.Lys429del). This variant in ATP8A2 gene neither been published as a mutation nor as a benign polymorphism so far. This variant was not found in the Exome Aggregation Consortium (ExAC) and NHLBI Exome Variant Server and dbSNP databases. In silico prediction, program is in support of its probable pathogenicity and predicts this variant as disease-causing.

Discussion

Dysequilibrium syndrome (DES, OMIM#224050) is a heterogeneous disease characterized by autosomal recessive non-progressive cerebellar ataxia, mild to severe mental retardation, and delayed ambulation (3). Phenotypic features of dysequilibrium syndrome have been seen among patients with different predisposing genetic variants. Accordingly, dysequilibrium syndrome can be categorized into CAMRQ1 (OMIM#224050), CAMRQ2 (OMIM#610185), CAMRQ3

(OMIM#613227), and CAMRQ4 (OMIM#615268). Generally, CAMRQ is a very uncommon congenital condition characterized by cerebellar ataxia, mental retardation, and dysequilibrium syndrome. CAMRQ with quadrupedal locomotion is said to provide a living model that confirms the theory of punctuated equilibrium (4). There has been reported a few studies concerning the problem. However, the evidence is enough to conclude that quadrupedal locomotion is not ubiquitous in patients with CAMRQ. In fact, studies offer considering the quadrupedal locomotion as a recessive trait that is linked to the chromosome 17p (5). CAMRQ with quadrupedal locomotion has been reported from consanguineous families from Turkey and Brazil (5-7).

Dysequilibrium syndrome type 1 (CAMRQ1) has been seen in a patient with mutations in the *VLDLR* (Very Low-Density Lipoprotein Receptor) gene (3). The patient was an 18-month-old female whose parents were not consanguineous and had severe hypotonia, global developmental delay, and truncal and peripheral ataxia. More recently DES family with mutation detected in *VLDLR* gene in a large consanguineous Iranian family with eight patients who suffer from mental retardation, disturbed equilibrium, walking disability, strabismus and short stature has been reported (10).

Dysequilibrium syndrome type 2 (CAMRQ2) has been reported from a consanguineous Arab family (8) with a homozygous mutation in the *WDR81* (WD Repeat Domain 81) gene. The patients were two sisters aged three and seven years, presenting with global developmental delay. The authors of this study have also proved that the trait for hearing loss might affect patients with CAMRQ and that it is linked to mutation in the *LHFPL5* gene (8). Dysequilibrium syndrome type 3 (CAMRQ3) was detected in two consanguineous Arab families and linked to mutation in the *CA8* (carbonic anhydrase 8) gene. There were seven patients who had global developmental delay with borderline to mild intellectual functioning. Ozcelik *et al.*, (2008) have reported a consanguineous Turkish family with four subjects affected by cerebellar ataxia, mental retardation, and dysequilibrium syndrome type 4 (CAMRQ4) (9), in which identified a homozygous c.1128C-G transversion in exon 12 (p.Ile376Met) of the *ATP8A2* gene. Cacciagli *et al*., (2010) reported a female with moderate mental retardation, severe axial hypotonia, and abnormal movements apparent from early infancy associated with a *de novo* balanced translocation, t(10;13)(p12.1;q12.13), that disrupted exons 1–3 for the *WAC* gene, and exons 26–28 for the *ATP8A2* gene (4).

Here, we reported a male patient with a constellation of neurodevelopmental and neuromuscular features including severe hypotonia, microcephaly, global developmental delay, speech problem, and strabismus. The patient also experienced severe pneumonia requiring ICU admission at age 3 months. All paraclinical examinations failed to provide a diagnosis. However, muscle biopsy indicated a slight type I fiber predominance. Therefore, the patient was investigated for possible muscular pathologies associated with type I fiber predominance, including bulbospinal muscular atrophy and multisystem selenoprotein deficiency. These pathologies were less prominent on the basis of clinical features and paraclinical findings. Eventually, whole exome sequencing was performed for the patient. It could identify a novel homozygous in-frame deletion in exon 14 of the *ATP8A2* gene (p.Lys429del). Finally, dysequilibrium syndrome was the best diagnosis that can describe the clinical features and molecular diagnosis of our patient. Also, our finding suggests that *ATP8A2* could be critical for the developmental processes of the central nervous system, and alterations of this gene may lead to severe neurological phenotypes. Future studies must assess the incidence of this variant in healthy Iranian population. Additionally, animal studies are required to address whether this variant is pathogenic.

References

1. Folmer DE, Elferink RPJO, Paulusma CC. P4 ATPases-lipid flippases and their role in disease. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 2009;1791:628-35.
2. Sun XL, Li D, Fang JIN, Noyes I, Casto B, Theil K, et al. Changes in levels of normal ML-1 gene transcripts associated with the conversion of human nontumorigenic to tumorigenic phenotypes. Gene Exp1999;8:129-39.
3. Boycott KM, Bonnemann C, Herz J, Neuert S, Beaulieu C, Scott JN, et al. Mutations in VLDLR as a cause for autosomal recessive cerebellar ataxia with mental retardation (dysequilibrium syndrome). J Child Neurol 2009;24:1310-5.
4. Tan U. A new syndrome with quadrupedal gait, primitive speech, and severe mental retardation as a live model for human evolution. Int J Neurosci 2006;116:361-9.
5. Türkmen S, Demirhan O, Hoffmann K, Diers A, Zimmer C, Sperling K, et al. Cerebellar hypoplasia and quadrupedal locomotion in humans as a recessive trait mapping to chromosome 17p. J Med Genet 2006;43:461-4.
6. Gulsuner S, Tekinay AB, Doerschner K, Boyaci H, Bilguvar K, Unal H, et al. Homozygosity mapping and targeted genomic sequencing reveal the gene responsible

for cerebellar hypoplasia and quadrupedal locomotion in a consanguineous kindred. Genome Res 2011;21:1995-2003.

7. Garcias GDL, Roth MDGM. A Brazilian family with quadrupedal gait, severe mental retardation, coarse facial characteristics, and hirsutism. Int J Neurosci 2007;117:927-33.
8. Komara M, John A, Suleiman J, Ali BR, Al-Gazali L Clinical and molecular delineation of dysequilibrium syndrome type 2 and profound sensorineural hearing loss in an inbred Arab family. Am J Med Genet Part A 2016;170:540-3.
9. Onat OE, Gulsuner S, Bilguvar K, Basak AN, Topaloglu H, Tan M, et al. Missense mutation in the ATPase, aminophospholipid transporter protein ATP8A2 is associated with cerebellar atrophy and quadrupedal locomotion. Eur J Hum Genet 2013;21:281-5.