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Abstract

ADNP syndrome, also known as the Helsmoortel-Van der Aa syndrome (HVDAS), is a neurodevelopmental disorder characterized by hypotonia, developmental delay, and intellectual disability. Diagnosis is typically made postnatally, and little is known about prenatal presentation of the disorder. We report a child who presented with intrauterine growth restriction, proportionate microcephaly, and an abnormal skull shape on fetal ultrasound. Whole exome sequencing performed on amniotic fluid cells showed a *de novo* pathogenic variant in the *ADNP* gene, corresponding to a diagnosis of ADNP syndrome.

Keywords: ADNP syndrome, Prenatal diagnosis, Intrauterine growth restriction, Microcephaly, Abnormal skull shape

Introduction

The *ADNP*-gene encodes the activity-dependent neuroprotector homeobox protein (ADNP), was first discovered as a vasoactive intestinal peptide (VIP)-responsive gene (Bassan et al. 1999). The gene is now known to be involved in chromatin remodeling by interacting with several different chromatin remodeling protein complexes, as reviewed extensively by d'Incal et al. (D'Incal et al. 2023), and is an important regulator of gene activity. In mice, complete knock-out of the *ADNP* gene has shown to cause dysregulation of over 400 different genes (Mandel and Gozes 2007).

The *ADNP* gene is highly expressed in brain tissue, and plays an essential role in brain development (Zamostiano et al. 2001; Mandel and Gozes 2007; D'Incal et al. 2023). *ADNP* knock-out mice show defects in cranial neural tube closure and lethality in the embryonic stage, with downregulation of *PAX6* and upregulation of *POU5F1* (*OCT4*) (Pinhasov et al. 2003). *PAX6* is a transcription factor known to play an essential role in brain development and function (Kikkawa et al. 2019).

A monogenic neurodevelopmental syndrome caused by variants in the *ADNP* gene was first described in 2014 by Helsmoortel and Van der Aa (Helsmoortel et al. 2014). Many more affected individuals have come to light since this first publication, and the condition is considered one of the most frequent causes of syndromic autism spectrum disorder (ASD) (D'Incal et al. 2023). The ADNP syndrome, also called Helsmoortel-Van der Aa syndrome (HVDAS), is characterized by hypotonia, severe developmental delay, mild to severe intellectual disability, and characteristic facial features. There is a wide range of associated features including structural brain abnormalities, seizures, ASD and other behavioral problems, sleep disturbances, visual and hearing abnormalities, feeding problems, gastrointestinal

symptoms, endocrine issues, musculoskeletal anomalies, and cardiac and urinary tract malformations (Van Dijck, Vandeweyer, and Kooy 2016, Updated 2022).

The condition is often diagnosed in light of developmental problems in the first years of life. A prenatally ascertained congenital diaphragmatic hernia resulting in the diagnosis of ADNP syndrome has recently been reported (Asegaonkar et al. 2023). In this report, we describe an additional individual with a prenatal presentation of *ADNP* syndrome.

Patient Data

A 33-year-old primigravida woman was first seen at our tertiary gynecology department at a gestational age of 27 weeks and 5 days. She had undergone Large Loop Excision of the Transformation Zone (LLETZ) for a cervix carcinoma (cervical intra-epithelial neoplasia 3 (CIN3)) eight years prior to this pregnancy. Further medical history was unremarkable. The family history was non-contributory. The woman and her 42-year old partner were non-consanguineous and both of Caucasian Belgian descent. IgM and IgG antibodies for Toxoplasmosis and for CMV were negative. Rubella IgG antibodies were positive with negative IgM antibodies, indicating immunity. Treponema pallidum haemagglutination testing was negative.

She was referred because of a short cervical length, diagnosed a week earlier, as well as uterine contractions on cardiotocography. Additionally, fetal growth restriction under the first percentile was seen on ultrasound. This was initially attributed to a placental chorioangioma of approximately 5x6 cm. Progesterone treatment had been initiated to prevent preterm birth, and she received Atosiban at our hospital for the same indication. According to standard protocol, she received corticoids to accelerate fetal lung maturation.

The patient was closely monitored. Ultrasound at 31 weeks and 5 days showed a substantially reduced fetal growth (less than first percentile) with proportionate microcephaly, as well as previously unobserved hypertelorism. Furthermore, the fetus had an aberrant skull shape,

somewhat resembling a strawberry skull (See Figure 1). On ultrasound, the skull sutures appeared to be open. Fetal and umbilical blood vessels showed normal Doppler indices.

Fetal magnetic resonance imaging (MRI) confirmed an abnormally appearing skull, with a rather pointy shape frontally, and a suspicion of an emerging cloverleaf skull. Abnormal white matter intensity was also noted, with heterogeneous hyperintensities in the periventricular and subcortical regions (See figure 2).

At a gestational age of 35 weeks and 4 days, cardiotocography showed fetal distress and preterm labor, for which an emergency cesarean section was performed. A girl was born, with a birth weight of 1.575 kg (percentile 0.91; -2.36 SD), length of 40.5 cm (percentile 1.74; -2.11 SD), and head circumference of 28.6 cm (percentile 1.2; -2.26 SD). Apgar score was 3/9/9 at 1, 5, and 10 minutes respectively.

There was transient thrombocytopenia which spontaneously resolved, as well as transient neonatal hypoglycemia. Additionally, the girl was diagnosed with early-onset sepsis. There was an initial need for total parenteral feeding, followed by nasogastric feeding after four days. Nasogastric feeding could be fully ceased after eighteen days. Postnatal Cytomegalovirus (CMV) polymerase chain reaction (PCR) on sputum was negative.

Physical examination showed a broad anterior fontanel and a rather high anterior hairline. The corners of the mouth were somewhat downturned. The examination was slightly hindered by an intravenous line, nasogastric tube, and monitoring fixing material. Neurological examination was normal. Postnatal brain MRI showed no structural abnormalities. The deep white matter appeared rather hyperintense on T2-weighted images, but this was considered within physiological ranges by two radiologists. Renal ultrasound and cardiac ultrasound were normal.

On clinical reevaluation at the age of ten months the girl was sitting independently and standing with support. She was able to push herself back when lying on her belly, but was not crawling forward yet. Some stereotypical behaviors were observed. Physical examination showed a

large forehead, and deep-set, but prominent eyes, and a narrow nose with relatively small nostrils. She was a cheerful baby. The microcephaly had normalized (44.3 cm, -0.33 SD), but the small stature remained; height was 64.2 cm (-3.22 SD) and weight was 7.08 kg (-1.93 SD).

Results

Prenatally, an amniocentesis was performed for genetic testing. Chromosomal microarray (Illumina Human Cyto SNP-12v2.1 beadchip on an iScan system, following manufacturer's protocols; CNV analysis through GenomeStudio software (Illumina) and CNV WebStore version 2.0 (Vandeweyer et al. 2011)) showed no pathogenic copy number variants (CNV). Urgent whole exome sequencing was initiated (Illumina HiSeq4000 instrument after enrichment with the Twist Human Core Exome kit (Twist Bioscience); data analysis with an in-house developed pipeline following GATK Best Practice Guidelines; variant annotation and filtering using VariantDB (Vandeweyer et al. 2014)). Eleven days after birth, a *de novo* heterozygous pathogenic c.3069_3072del p.(Arg1023Serfs*3) variant in *ADNP* (NM_015339.4) was diagnosed. In light of the skull abnormality, WES variant filtering was first performed using a panel of genes involved in skeletal dysplasia. An overview of the genes included in this WES-based gene panel can be found in the supplemental information (Supplement 1). This analysis did not show any (likely) pathogenic variants or variants of unknown significance. However, by using an additional genome-wide Human Phenotype Ontology (HPO)-based software package (MOON software), the pathogenic variant in the *ADNP* gene was detected.

Discussion

We describe a prenatal presentation of ADNP syndrome, characterized by intrauterine growth restriction (IUGR), proportionate microcephaly, and an abnormal skull shape. The *ADNP* variant that was found in our patient has previously been reported by Van Dijck et al. (Van Dijck et al. 2019).

Until now, IUGR has not been associated with ADNP syndrome. In a large cohort of 78 individuals, overall birth weight, height, and head circumference were reported to be normal, although some patients had birth parameters < -2 SD below the mean (Van Dijck et al. 2019). Short stature, however, is quite commonly associated with the disorder (Van Dijck, Vandeweyer, and Kooy 2016, Updated 2022).

The presence of a chorioangioma could by itself explain the IUGR. However, brain sparing is typically observed in IUGR secondary to a placental abnormality. In this instance, microcephaly was observed, which would generally not be expected in a non-fetal etiology of IUGR. The abnormal skull shaped can likewise not be attributed to the chorioangioma.

Skull deformities are reported in 14% of patients with an ADNP-related disorder, and include plagiocephaly, trigonocephaly, and brachycephaly (Van Dijck et al. 2019).

Prenatal ultrasound and MRI showed a clearly abnormal skull shape, as well as white matter abnormalities. This finding guided prenatal counseling and was the strongest argument for initiating additional prenatal genetic testing. It remains unclear why the postnatal brain MRI did not confirm these abnormalities.

Recently, the first instance of ADNP syndrome being detected prenatally was reported, following the diagnosis of congenital diaphragmatic hernia. This patient had an additional pathogenic variant in *GARS1* and a variant of unknown significance in *NRIP1*. There was no mention of abnormal growth parameters or cranial anomalies (Asegaonkar et al. 2023). There has been one report of a prenatal diagnosis in a patient with a heterozygous multigene deletion encompassing the *ADNP* gene. There was no IUGR, microcephaly, or skull abnormality in this fetus (Stipoljev et al. 2017). However, no instances of ADNP syndrome have been attributed to heterozygous whole-gene deletions of the ADNP gene to date. Additionally, contribution of other deleted genes to the phenotype in this fetus cannot be excluded.

Interestingly, there are no known accounts of homozygous deletions of the *ADNP* gene in humans in literature or on the DECIPHER platform. This would agree with the essential role of

ADNP in brain formation, and the fact that complete *ADNP* knock-out is lethal in mice (Pinhasov, 2003).

For many individuals with ADNP syndrome reported in literature the age at diagnosis is not mentioned. However, no prenatal abnormalities were reported, suggesting these were postnatal diagnoses.

In conclusion, we present a prenatal presentation of ADNP-syndrome with aberrant skull shape and microcephaly as major findings, hereby broadening the phenotypic spectrum of the syndrome. With this report, we want to illustrate that whole exome sequencing in prenatal setting can sometimes lead to unexpected diagnoses with important consequences for counselling of postnatal outcome and prognosis.

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Data availability

Data will be made available upon personal request.

Figure Titles and Legends

Fig. 1: Fetal ultrasound at a gestational age of 31 weeks and 5 days (A, B) and 33 weeks and 5 days (C) showing an abnormal skull shape.

Fig. 2: Fetal MRI at a gestational age of 33 weeks and 5 days. T2-weighted in the axial (A, B) and coronal (C) plane and diffusion weighted image (DWI) in the axial plane (D).
The abnormal skull configuration with pointed configuration of the frontal bones anteriorly (arrowheads in A and B) are seen in the axial plane. The hyperintense appearance of the periventricular white matter is evident in all planes and on both sequences, with a sharp delineation seen in the axial and coronal plane (white arrows in A – D). The diffusion apparent coefficient (DAC) value of the white matter in the parietal region was 1950 x 10^-3 m^2/s.

References

Asegaonkar, P., U. Kotecha, M. Dongre, M. Mistri, and S. Sharda. 2023. 'Expanding the spectrum of ADNP-related disorder-Antenatally diagnosed congenital diaphragmatic hernia and a novel de novo mutation in ADNP gene', *Am J Med Genet A*, 191: 275-79.

Bassan, M., R. Zamostiano, A. Davidson, A. Pinhasov, E. Giladi, O. Perl, H. Bassan, C. Blat, G. Gibney, G. Glazner, D. E. Brenneman, and I. Gozes. 1999. 'Complete sequence of a novel protein containing a femtomolar-activity-dependent neuroprotective peptide', *J Neurochem*, 72: 1283-93.

D'Incal, C. P., K. E. Van Rossem, K. De Man, A. Konings, A. Van Dijck, L. Rizzuti, A. Vitriolo, G. Testa, I. Gozes, W. Vanden Berghe, and R. F. Kooy. 2023. 'Chromatin remodeler Activity-Dependent Neuroprotective Protein (ADNP) contributes to syndromic autism', *Clin Epigenetics*, 15: 45.

Helsmoortel, C., A. T. Vulto-van Silfhout, B. P. Coe, G. Vandeweyer, L. Rooms, J. van den Ende, J. H. M. Schuurs-Hoeijmakers, C. L. Marcelis, M. H. Willemsen, L. E. L. M. Vissers, H. G. Yntema, M. Bakshi, M. Wilson, K. T. Witherspoon, H. Malmgren, A. Nordgren, G. Anneren, M. Fichera, P. Bosco, C. Romano, B. B. A. de Vries, T. Kleefstra, R. F. Kooy, E. E. Eichler, and N. Van der Aa. 2014. 'A SWI/SNF-related autism syndrome caused by de novo mutations in ADNP', *Nature Genetics*, 46: 380-+.

Kikkawa, T., C. R. Casingal, S. H. Chun, H. Shinohara, K. Hiraoka, and N. Osumi. 2019. 'The role of Pax6 in brain development and its impact on pathogenesis of autism spectrum disorder', *Brain Res*, 1705: 95-103.

Mandel, S., and I. Gozes. 2007. 'Activity-dependent neuroprotective protein constitutes a novel element in the SWI/SNF chromatin remodeling complex', *Journal of Biological Chemistry*, 282: 34448-56.

Pinhasov, A., S. Mandel, A. Torchinsky, E. Giladi, Z. Pittel, A. M. Goldsweig, S. J. Servoss, D. E. Brenneman, and I. Gozes. 2003. 'Activity-dependent neuroprotective protein: a novel gene essential for brain formation', *Developmental Brain Research*, 144: 83-90.

Stipoljev, F., D. Miric-Tesanic, T. Hafner, M. Barbalic, M. Logara, R. Lasan-Trcic, A. Vicic, and R. Gjergja-Juraski. 2017. 'Prenatal diagnosis of complex phenotype in a 13-week-old fetus with an interstitial multigene deletion 20q13.13.-q13.2 by chromosomal microarray', *Eur J Med Genet*, 60: 589-94.

Van Dijck, A., G. Vandeweyer, and F. Kooy. 2016, Updated 2022. 'ADNP-Related Disorder.' in M. P. Adam, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. W. Gripp and A. Amemiya (eds.), *GeneReviews((R))* (Seattle (WA)).

Van Dijck, A., A. T. Vulto-van Silfhout, E. Cappuyns, I. M. van der Werf, G. M. Mancini, A. Tzschach, R. Bernier, I. Gozes, E. E. Eichler, C. Romano, A. Lindstrand, A. Nordgren, Adnp Consortium, M. Kvarnung, T. Kleefstra, B. B. A. de Vries, S. Kury, J. A. Rosenfeld, M. E. Meuwissen, G. Vandeweyer, and R. F. Kooy. 2019. 'Clinical Presentation of a Complex Neurodevelopmental Disorder Caused by Mutations in ADNP', *Biol Psychiatry*, 85: 287-97.

Vandeweyer, G., E. Reyniers, W. Wuyts, L. Rooms, and R. F. Kooy. 2011. 'CNV-WebStore: online CNV analysis, storage and interpretation', *BMC Bioinformatics*, 12: 4.

Vandeweyer, G., L. Van Laer, B. Loeys, T. Van den Bulcke, and R. F. Kooy. 2014. 'VariantDB: a flexible annotation and filtering portal for next generation sequencing data', *Genome Med*, 6: 74.

Zamostiano, R., A. Pinhasov, E. Gelber, R. A. Steingart, E. Seroussi, E. Giladi, M. Bassan, Y. Wollman, H. J. Eyre, J. C. Mulley, D. E. Brenneman, and I. Gozes. 2001. 'Cloning and characterization of the human activity-dependent neuroprotective protein', *Journal of Biological Chemistry*, 276: 708-14.