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SHORT COMMUNICATION

Delineating the phenotype and genetic basis of AMPD2-related pontocerebellar hypoplasia

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Abstract

Pontocerebellar hypoplasia is a group of disorders with a wide range of presentations. We describe here the genetic and phenotypic features of PCH type 9 due to mutations in AMPD2. All patients have severe intellectual disability, and the vast majority manifest abnormal tone, cortical blindness, and microcephaly. Almost all have agenesis of the corpus callosum and severe cerebellar hypoplasia. The course is not progressive, however, few die in the first decade of life. Mutations are spread throughout the gene, and no hot spot can be identified. One of the mutations we report here is the most distal truncating variant known in this gene and is predicted to result in a truncated protein. The phenotype is severe in all cases; thus, no clear genotype–phenotype correlation can be established.

Keywords Pontocerebellar hypoplasia · AMPD2 · Microcephaly · Growth retardation

Introduction

Pontocerebellar hypoplasia type 9 (PCH9) is a rare neurodegenerative disorder caused by bi-allelic mutations in the AMP (adenosine monophosphate) deaminase 2 gene [1]. So far, only 31 patients have been reported worldwide, with a wide range of ethnic origins [1–8]. The primary clinical characteristics are severe intellectual disability, acquired microcephaly, epilepsy, and abnormal tone. Other manifestations may include facial dysmorphism, visual impairment, dysphagia, irritability, and extrapyramidal movement disorder. Death during childhood was reported in some [3, 9], but a few patients who reached young adulthood have been described [2].

The radiographic hallmarks are the "Fig. 8" appearance of the midbrain (due to hypoplastic midbrain and pons), along with hypoplasia of the cerebellar hemispheres and hypomyelination of the cerebral white matter with a thin or absent corpus callosum [6]. Sequential imaging does not support ongoing brainstem and cerebellar atrophy but is consistent with the progressive cerebral process, leading to ventriculomegaly and enlarged extra-axial fluid [5].

The adenosine monophosphate deaminase 2 (AMPD2) gene contains 19 exons encoding an important protein in purine metabolism, converting AMP into IMP (inosine monophosphate) as part of the purine salvage system. It acts as a homotetramer in the cytosol and uses zinc as a co-factor. Loss of function of AMPD2 leads to an excess of adenosine and deficiency in guanine nucleotides, thus leading to defective GTP-dependent protein translation initiation. Intracellular accumulation of adenosine and derived nucleotides and depletion of guanine nucleotides lead to neurotoxicity [1].

There are five known isoforms of AMPD2 formed by alternative splicing, which may explain some of the variability in the clinical presentation between patients [5]. A single family with a frameshift mutation in the 5′ part of the gene, which disrupts the N-terminal domain of the longest predicted AMPD2 isoform, has been reported, and the clinical presentation was spastic paraplegia [10].

We report here a compound heterozygous patient with likely pathogenic bi-allelic variants in the catalytic part of the AMPD2 gene, both not reported previously.

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Clinical description

The proband is a 17-year-old girl, an only child of parents who are nonconsanguineous and of Ashkenazi Jewish and Moroccan Jewish origin. She was born after an uneventful pregnancy at 38 weeks of gestation (2450 gr birth weight). Global developmental delay was noted in the first few months of life. She was diagnosed with epilepsy at age 18 months after presenting with multiple seizure types and multifocal epileptiform discharges. Extensive metabolic workup was normal, and no other organ involvement was noted. She failed to reach any developmental milestones; her current weight is 32 kg (< < 3%), and head circumference is 48 cm (< < 3%). She is bedridden, expresses occasional vocalic sounds, has limited eye tracking, and manifests pyramidal signs in the lower limbs, including increased tone and bilateral Babinski's sign. She suffers from abnormal sleep, with occasional periods of several days of no sleep at all.

Magnetic resonance imaging (MRI) scans at ages 7 months (Fig. 1a–d) and 12 years (Fig. 1e–h) revealed diffuse cerebral atrophy, agenesis of the corpus callosum, hypoplastic pons, and midbrain, as well as small and dysplastic cerebellar hemispheres, without evidence of progression.

Initial genetic workup included comparative microarray genomic hybridization (CGH, Affymetrix CytoScan HD array), which no copy number variants were reported. Later, whole exome sequencing (WES) was performed on DNA samples of the proband and her parents. Genomic DNA samples were enriched using the SureSelect Human All Exon 50 Mb V5 Kit (Agilent Technologies, Santa Clara, CA), DNA libraries were sequenced on HiSeq2500 platform (Illumina, San Diego, CA). The exome analyses yielded 54.3 million reads, with a mean coverage of 76.1X. After alignment to the reference genome (Hg19) and variant calling, we removed variants that were called less than X8, were off-target (> 7 intronic bp from splice-site) or synonymous (and > 3 exonic bp from splice site), or had minor allele frequency (MAF) > 0.01 in the GnomAD (https://gnomad.broadinstitute.org/) database.

The WES analysis revealed two novel variants in the AMPD2 gene: a maternally inherited frameshift variant (chr1:110,173,381 [hg19]; NM_001368809.2: c.2235dup, p.Asn746GlnfsTer27), and a paternally inherited missense variant (chr1:110,171,313 [hg19]; NM_001368809.2: c.1456C > T, p.Arg486Trp, rs1390825220) (Fig. 1j).

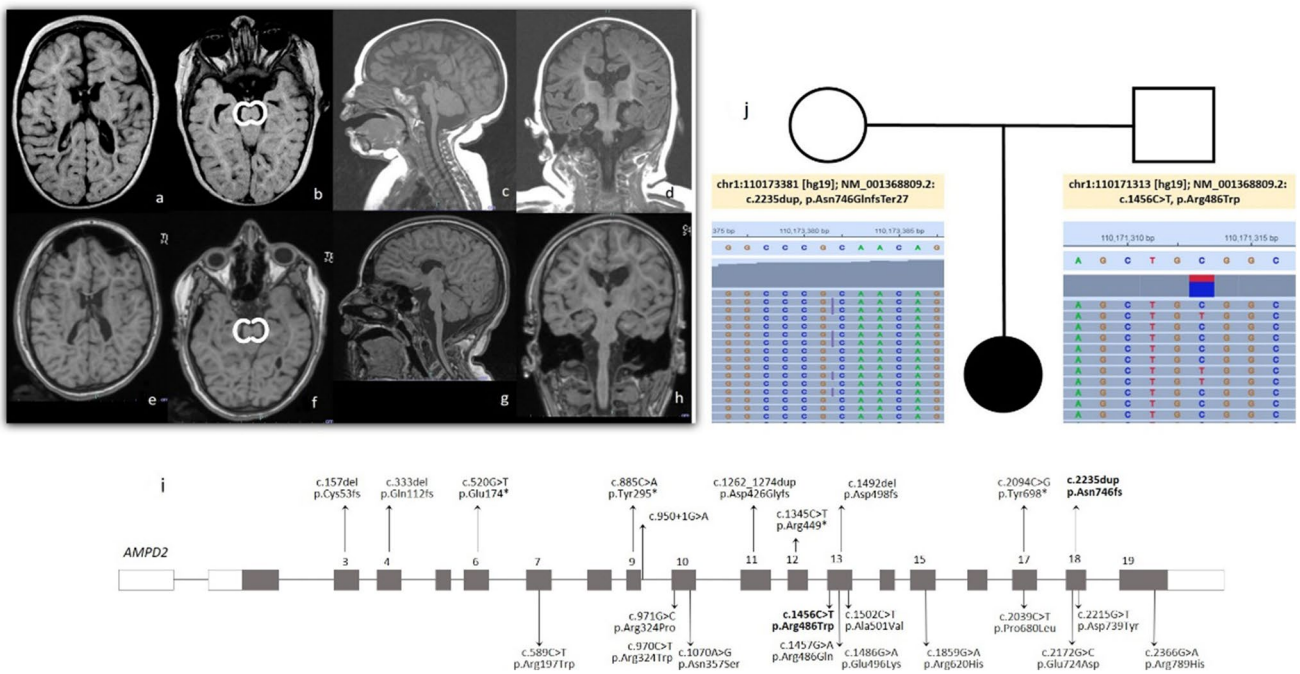

Fig. 1 **a**–**h** MRI of the patient at 7 months of age 1a-d and at 12 years 1e-h. Typical radiological findings shown—brainstem appearance of figure of 8 (b, f – white mark), absent corpus callosum (**a**, **c**, **d**, **e**, **g**, **h**). No significant change noted over 11 years. **i** Schematic representation of the *AMPD2* gene (NM_001368809.2). Boxes represent exons, with exon number indicated above. Grey boxes indicate coding regions, white boxes indicate untranslated regions. Lines represent introns. The known and novel *AMPD2* variants are mapped across the gene, novel in bold. Variants that are listed above are expected to result in a truncated protein (nonsense, frameshift, splice-site), and missense variants are listed below. Additional details for the variants are given in table S1. 1j proband and parental sequencing results

The frameshift variant is extremely rare; it is not carried by any of the ~ 140,000 individuals whose exome or genome sequences were deposited in the gnomAD database. The frameshift's effect begins at amino acid Asn746. It is predicted to produce a premature termination codon (PTC) 27 amino acids downstream, located in exon 19, which is the last exon of the gene. Therefore, this transcript is expected to escape nonsense-mediated decay pathways and result in the translation of a truncated protein. The altered amino acids and PTC occur within the C-terminal domain, causing an altered and shortened domain. This domain is highly conserved among all human AMP deaminase isoforms [11] and contains the AMP deaminase active site (amino acids 301–797), as predicted by the Conserved Domain Database [12]. Of note, this frameshift variant is the most distal truncating variant reported so far in patients with PCH9 (other truncating variants, predicted loss-of-function, were reported [1, 2, 4, 5, 8]. Based on ACMG classification criteria [13], this variant is classified as likely pathogenic (using the following lines of evidence: PM4_Strong, PM2).

The missense variant p.Arg486Trp alters an evolutionarily conserved amino acid with a GREP score of 5.0799 [14]. It is predicted to be damaging or disease-causing by various prediction tools (In franklin.genoox.com/, the calculated aggregated prediction score is 0.857. Ranges: benign 0–0.15, deleterious 0.7–1). The variant has an extremely low allele frequency in the gnomAD database (0.00001196), present only in three heterozygous individuals. Notably, a different amino acid change in the same codon (chr1:110,171,314 [hg19]; NM_001368809.2: c.1457G > A, p.Arg486Gln, rs192669225) was identified in a homozygous state in a patient with global developmental delay, epilepsy, and brain malformations which include agenesis of the corpus callosum, bilateral cerebellar hypoplasia, and bilateral colpocephaly [15]. Based on ACMG classification criteria [13], this variant is classified as a likely pathogenic variant (using the following lines of evidence: PM2, PM5, PM3, PP3).

Discussion

Pontocerebellar hypoplasia (PCH) is a heterogeneous group of autosomal recessive disorders affecting mainly the central nervous system with a wide variety of clinical and radiological phenotypes. So far, 11 types have been recognized, based on the clinical phenotype and the genetic basis. The most common are types 1 and 2 which are further subdivided into 4 and 6 subgroups respectively. Each group and subgroup are associated with a single causative gene [16]. The list of PCH causative genes is constantly growing thus, new types are expected to be recognized [17].

The major clinical features include movement disorders, such as dystonia, chorea, and ataxia, and severe cognitive impairment. The main radiological feature is congenital hypoplasia of the cerebellum and pons. Associated clinical features vary between the different clinical groups and may include intellectual disability, microcephaly, epilepsy, peripheral neuropathy, optic neuropathy, abnormal tone, cortical malformation, genital anomalies, and growth retardation. The clinical course and the radiographic features may be stable or progressive [18].

The different genes implicated in PCH are involved in several pathways, including mRNA degradation, tRNA splicing, regulation of synaptic protein, and vesicle formation, as well as intracellular vesicle transport [18]. AMPD2 is involved in the regulation of GTP synthesis and may alter protein synthesis due to insufficient energy supply [1]. Recently, two new genes, PPIL1 and PRP17, involved in pre-mRNA splicing by the spliceosome complex, were identified as the cause of PCH with microcephaly in 19 patients from 10 unrelated families [17].

PCH type 9 is a rare form of PCH caused by biallelic mutations in the AMPD2 gene. A distinctive radiological feature, known as the "figure of 8," which describes the appearance of the brainstem (Fig. 1b, f), is characteristic. The cerebellar hemispheres are hypoplastic, but the vermis is often preserved. Clinically, the phenotype varies, but there are some common features (Table 1). Symptoms are present in the first few months of life but are nonspecific. The developmental milestones are delayed and often not reached, leading to profound cognitive impairment, cortical visual impairment, and axial hypotonia with limb spasticity that are reported in almost all patients (Table 1). Facial dysmorphisms with dental abnormalities are reported in a few patients. Later in life, axonal neuropathy may develop [1, 2, 4, 18]; however, the overall course is stable. Despite the significant cerebellar involvement, nystagmus is not part of the clinical picture. Table 1 summarizes the published data on clinical and radiological findings.

In this paper, we report a patient with PCH type 9 who is compound heterozygous to two novel variants in the AMPD2 gene. Including our patient, 21 variants in the AMPD2 gene have been associated with PCH9 in 32 patients of multiple families. These include missense, nonsense, frameshift due to deletion and duplication, and splice site variants, all leading to severe neurological disease. They are located mainly within the catalytic domain other coding parts of the gene, with no known "hot spots" or founder mutations in specific populations [1–9].

Several isoforms of AMP deaminase are present in the human body, AMPD2 is the most common, and it is expressed in both the brain and liver. The predominant AMPD2 transcript in the cerebellum, unlike other brain regions, initiates with exon 1B, situated in the 5′-end of the gene, followed by exon 2. This configuration has several potential post-translational modification sites, including

Table 1 major clinical and radiological phenotype

	Akizu 2013	Marsh 2015	Marsh 2017	Accogli 2017	Severino 2017	Krotum 2018	Scola 2019	Abreu 2020	Holla 2021	Gilboa 2022	Total	%
Patients	8	5	1	3	1	8	3	1	1	1	32	100
Severe cognitive impairment	8	5	1	3	1	8	3	1	1	1	32	100
Appendicular spasticity	8	5	1	3	1	4	3	1	1	1	28	87.5
Axial hypotonia	3	5	1	3	1	6	3	1	1	1	25	78.13
Facial dysmorphism	ND	3	0	3	1	6	ND	ND	1	1	15	46.9
Axonal neuropathy	ND	2	0	ND	ND	0	ND	ND	0	0	2	6.3
Epilepsy	6	4	0	3	1	1	1	0	0	1	17	53.1
Extrapyramidal movement disorder	3	ND	ND	ND	1	4	1	ND	ND	0	9	28.15
Cortical blindness	5	5	1	3	1	5	1	1	ND	1	23	71.9
Death in early childhood	ND	3	1	ND	1	1	ND	ND	ND	NA	6	18.8
Figure 8 brainstem	ND	5	1	3	1	5	3	1	1	1	21	65.6
severe cerebellar hypoplasia	8	5	1	3	1	6	3	1	1	1	30	93.8
Thin/ACC	8	5	1	3	1	7	3	1	1	1	31	96.9
IUGR	0	ND	0	ND	ND	0	ND	ND	ND	1	1	3.1
FTT	ND	ND	ND	ND	1	3	ND	ND	ND	1	5	15.6
Congenital microcephaly	0	0	0	ND	ND	0	ND	ND	ND	0	0	0
Acquired microcephaly	8	5	1	3	1	7	1	1	1	1	29	90.6
Cerebral white matter hypomyelination	6	ND	0	ND	ND	5	ND	ND	1	1	13	40.6
Nystagmus	ND	ND	ND	ND	1	ND	ND	ND	ND	0	1	3.1
Sleep disorder										1	1	3.1
Oldest patient documented	ND	20y	2y2m	9y	3y	6y3m	7y	3y	8 m	17y		

ND not described, *NA* not applicable, *ACC* absent corpus callosum, *IUGR* intrauterine growth retardation, *FTT* failure to thrive

phosphorylation and glycosylation sites [19]. So far, no mutations have been reported in the first two exons (Fig. 1i).

Within the cerebellum, AMPD2 is expressed mainly in Purkinje cells, the sole source of output from the cerebellar cortex (http://www.proteinatlas.org/ENSG00000116337-AMPD2/brain/cerebellum). This may explain part of the clinical and radiographic characteristics of the patients. The cerebellum is involved in controlling muscle tone, coordination, language, and learning; thus, the clinical phenotype includes severe motor and cognitive impairment in all patients. However, the phenotype includes cortical and subcortical dysfunction, such as cortical blindness in most patients, as well as microcephaly and ACC, hinting that AMPD2 is required in multiple areas within the brain. The early clinical presentation may be due to the importance of AMPD2 in the developing brain. No clear genotype–phenotype could be established. This is due to the small number of patients, lack of individual clinical details in some of the reports, and the overall severe phenotype in all reported patients.

Since AMPD2 plays a role in the maintenance of cellular guanine nucleotide pools, a crucial part of the de novo purine synthesis [1], its dysfunction is deleterious in the early stages of brain development, leading to a severe clinical phenotype. Despite the laboratory evidence of potential treatment for AMPD2 deficient cells, shown by [1], no reports of clinical use have been published so far. Unfortunately, in real life, most children manifest a severe developmental impairment by the time of diagnosis, and the hope for reversing the damage is low. Nonetheless, in the era of genetic treatment, early diagnosis and intervention may alter the outcome.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10048-022-00706-4.

Data availability The authors confirm that the data supporting the findings of this study are available within the article and will be shared upon request.

Declarations

Ethics approval The patient's legal guardian consented to the publication.

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