Received: 7 July 2023 | Revised: 27 February 2024 | Accepted: 19 March 2024

DOI: 10.1002/mgg3.2424

Molecular Genetics & Genomic Medicine Open Access WILEY

CLINICAL REPORT

A novel variant in *ASNS* gene responsible for syndromic intellectual disability and microcephaly: Case report and literature review

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Abstract

Background: The *ASNS* (ASNS, MIM 108370) gene variations are responsible for asparagine synthetase deficiency (ASNSD, MIM 615574), a very rare autosomal recessive disease characterized by cerebral anomalies. These patients have congenital microcephaly, progressive encephalopathy, severe intellectual disability, and intractable seizures.

Method: Clinical characteristics of the patient were collected. Exome sequencing was used for the identification of variants. Sanger sequencing was used to confirm the variant in the target region. The structure of the protein was checked using the DynaMut2 web server.

Results: The proband is an 11-year-old Iranian-Azeri girl with primary microcephaly and severe intellectual disability in a family with a consanguineous marriage. Symptoms emerged around the 10–20th days of life, when refractory epileptic gaze and unilateral tonic–clonic seizures initiated without any provoking factor such as fever. A brain MRI revealed no abnormalities except for brain atrophy. The karyotype was normal. Using exome sequencing, we identified a novel homozygous variant of thymine to adenine (NM_001673.5:c.538T>A) in the *ASNS* gene. Both parents had a heterozygous variant in this location. Subsequently, Sanger sequencing confirmed this variant. We also reviewed the clinical manifestations and MRI findings of the previously reported patients.

Conclusion: In the present study, a novel homozygous variant was recognized in the *ASNS* gene in an Iranian-Azeri girl manifesting typical ASNSD symptoms, particularly intellectual disability and microcephaly. This study expands the mutation spectrum of ASNSD and reviews previously reported patients.

KEYWORDS

ASNS gene, ASNSD, exome sequencing, intellectual disability, microcephaly, Sanger sequencing

1 | BACKGROUND

About 3% of the overall population suffers from an intellectual disability (ID), which is defined by a wide variety of cognitive deficits. Based on other abnormalities, it is typically divided into syndromic and non-syndromic types (Ruzzo et al., 2013). Microcephaly, which is a condition in which the occipital-frontal head circumference is two standard deviations (SD) less than the average expected for age and gender, is frequently present in patients with syndromic ID. Groups with a high level of consanguinity have higher rates of microcephaly (Mahmood et al., 2011). Causes of congenital microcephaly include metabolic disorders, chromosomal anomalies, and intrauterine infections; however, the genetic etiology of most congenital microcephaly cases is still unknown (Ruzzo et al., 2013).

Asparagine synthetase deficiency (ASNSD, MIM 615574) is a very rare autosomal recessive disease characterized by cerebral anomalies. These patients have congenital microcephaly, progressive encephalopathy, severe intellectual disability, and intractable seizures (Ben-Salem et al., 2015). The *ASNS* gene contains instructions for producing the asparagine synthetase enzyme. This enzyme consists of two domains that contribute to the main reaction. The glutamine amidotransferase domain hydrolyzes glutamine to form glutamate and ammonia. Glutamine acts as an ammonia group donor in this reaction, and the asparagine synthetase domain generates the asparagine using ammonia and aspartate through an ATP-dependent reaction (Richards & Kilberg, 2006).

We have identified one novel variant in one consanguineous family with a distinct type of severe ID associated with congenital microcephaly. This variant has not been reported in the current human genome databases, suggesting that this is a new genetic variant causing this syndromic ID.

2 | METHODS

2.1 | Editorial policies and ethical considerations

Written informed consent was obtained from the patient's parents for collecting samples and the publication of this case report and any accompanying images. A copy of the written consent is available for review. This study was approved by the ethics committee of Ardabil University of Medical Sciences (IR.ARUMS.REC.1402.035).

2.2 | DNA extraction and quality control

The peripheral blood sample was obtained, and then DNA extraction was performed using the standard salting-out method. Finally, the quality and purity of DNA were analyzed by agarose gel electrophoresis and a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 | Exome sequencing

Sample preparation was performed on 1.0 μg of the proband's extracted genomic DNA. Then, it was captured by the SureSelect Human All ExonV6 kit (Agilent Technologies, CA, USA). DNA fragments were enriched by adding ligated adapter molecules throughout a PCR reaction. Following enrichment, the captured library was sequenced using a NovaSeq 6000 Illumina sequencer with an average coverage of 100X.

2.4 | Sanger sequencing for the validation of the variant

After exome sequencing, we used Sanger sequencing to validate the variant through specific primers, according to the reference genomic sequences from the Human Genome from GenBank in NCBI (GI:1519243973), primer pairs designed for the candidate loci.

2.5 | In silico analysis of the protein structure

The DynaMut2 web server was used to determine the effect of mutation on the 3D structure of the mutant protein compared with the wild-type protein.

3 | RESULTS

3.1 | Case presentation

The proband is an 11-year-old Iranian-Azeri female who presented to the pediatric neurologist for her intermittent seizures accompanied by progressive microcephaly and ID. She was born by normal vaginal delivery in a family with a consanguineous marriage and without any perinatal insults (Figure 1a). At birth, she was 3.2 kg (21st percentile, −0.81 SD), with a head circumference of 33 cm (11th percentile, −1.22 SD) and a height of

50 cm (58th percentile, +0.20 SD) in length. Symptoms emerged around the 10–20th days of life, when refractory epileptic gaze and unilateral tonic–clonic seizures initiated without any provoking factor such as fever. At the age of two, with the initial diagnosis of Rett syndrome, she underwent further evaluations. No family member had the same symptoms, so a disorder of recessive inheritance came to mind. She started to sit, crawl, and walk at the ages of 12, 18, and 36 months, respectively. In the course of her disease, she showed bizarre behaviors, including hyperactivity and agitation, so the diagnosis of attention deficit and hyperactivity disorder (ADHD) was also made. She has been under treatment with risperidone (1 mg/night) and Tegretol (Carbamazepine) suspension 100 mg twice a day.

At physical examination, microcephaly was detected with a head circumference measuring 44 cm (<1st percentile, −7.03 SD), weight 33 kg (23rd percentile, −0.75 SD), and height 146 cm (61st percentile, +0.28 SD). Other apparent features were a sloping forehead, a small jaw, open-mouth posture, and macrotia (Figure 1b). Delayed psychomotor development was detected, and intellectual disability was categorized as severe to profound. Laboratory tests, including hematologic indices, electrolytes, and renal and liver function, were all within normal limits. Metabolic analysis, plasma and CSF amino acids, lactate, and ammonia were also normal (Table 1). A brain 3D CT-scan suggested turricephaly, and an MRI revealed no abnormalities except brain atrophy. The karyotype was normal. The *MECP2* gene was unremarkable, and the diagnosis of Rett syndrome was ruled out.

3.2 | Exome and Sanger sequencing analysis

We identified a novel biallelic missense homozygote alteration of thymine to adenine (NM_001673.5:c.538T>A) using exome sequencing analysis in the *ASNS* gene. Sanger sequencing subsequently confirmed this homozygous variant identified by exome sequencing in this individual. According to the NCBI database, NM_001673 is a transcript variant of the *ASNS* gene and is responsible for the formation of asparagine synthetase (glutamine-hydrolyzing) isoform A, with 561 amino acids. This variant has 13 exons (Gene [Internet], 2004). The results of sequence analysis revealed that the c.538T>A: p.Phe180Ile transition at codon 180 was a variant in exon 5 of this transcript in which isoleucine amino acid replaces phenylalanine in the glutamine amidotransferase domain of *ASNS* protein. A heterozygous variant was detected in both parents (Figure 1c,d).

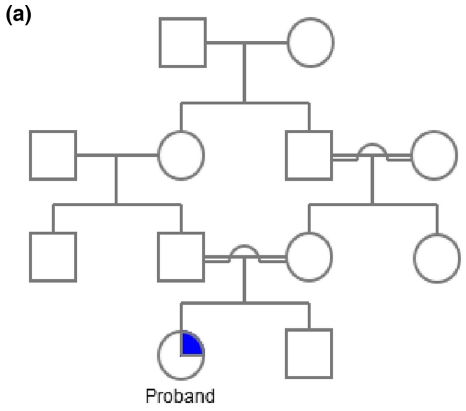
3.3 | Bioinformatics analysis

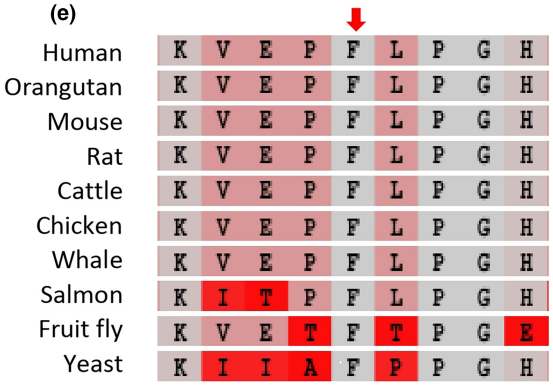
Evolutionary conservation analysis was performed using the wild-type sequence of *ASNS* protein in 11 different species and showed that the phenylalanine amino acid in position 180 is highly conserved across different species (Figure 1e). This variant is predicted to be pathogenic by PolyPhen-2 (probably damaging) and SIFT (affecting protein function). This alteration (p.Phe180Ile) replaces phenylalanine with isoleucine. In the wild-type protein, phenylalanine has four hydrogen bonds with arginine, leucine, glutamic acid, and histidine. However, in the mutant protein, hydrogen bonds with histidine and glutamic acid are absent, and there is an additional bond with leucine. According to DynaMut2, these changes destabilize the protein structure (Figure 1f). This variant has not been reported in variant databases, including ClinVar, gnomAD, and HGMD.

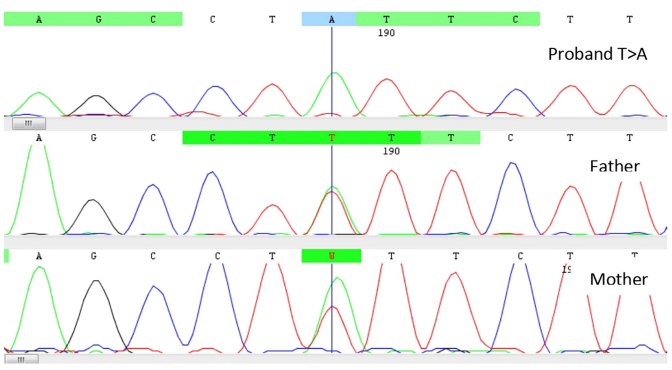
4 | DISCUSSION

Asparagine synthetase deficiency (ASNSD, OMIM# 615574) is a severe neurologic, autosomal recessive condition (Ruzzo et al., 2013). To the best of our knowledge, there are 51 described unique variants in 75 patients reported in the literature [Table 2]. Consanguinity was reported in 61% of the families. All of these patients had developmental delays and microcephaly; 82% experienced seizure attacks, and 80% had spasticity. About 70%, 55%, and 40% of patients had hyperreflexia, axial hypotonia, and visual impairment, respectively. MRI findings showed that near to 100% of the patients had brain atrophy, and 75%, 62%, and 61% of patients reported to have delayed myelination, a gyral simplification pattern, and a decreased size of the pons, respectively (Abdel-Salam & Abdel-Hamid, 2021; Abhyankar et al., 2017; Akesson et al., 2020; Alfadhel et al., 2015; Alharby et al., 2020; Altıntaş et al., 2023; Ben-Salem et al., 2015; Chen et al., 2019; Churchill et al., 2020; Faoucher et al., 2019; Galada et al., 2018; Gataullina et al., 2016; Gupta et al., 2017; Liu et al., 2022; Monies et al., 2019; Palmer et al., 2015; Radha Rama Devi & Naushad, 2019; Reed, 2016; Ruzzo et al., 2013; Sacharow et al., 2018; Saini et al., 2023; Schleinitz et al., 2018; Seidahmed et al., 2016; Shaheen et al., 2019; Sprute et al., 2019; Staklinski et al., 2022, 2023; Sun et al., 2017; Wang et al., 2020; Yamamoto et al., 2017; Yingjun et al., 2019; Zhu et al., 2023).

The c.1193A>G, p.Tyr398Cys variant has been reported in 10 patients and is the most frequent pathogenic variant in the literature. This variant is reported mainly in Saudi families (Alfadhel et al., 2015;







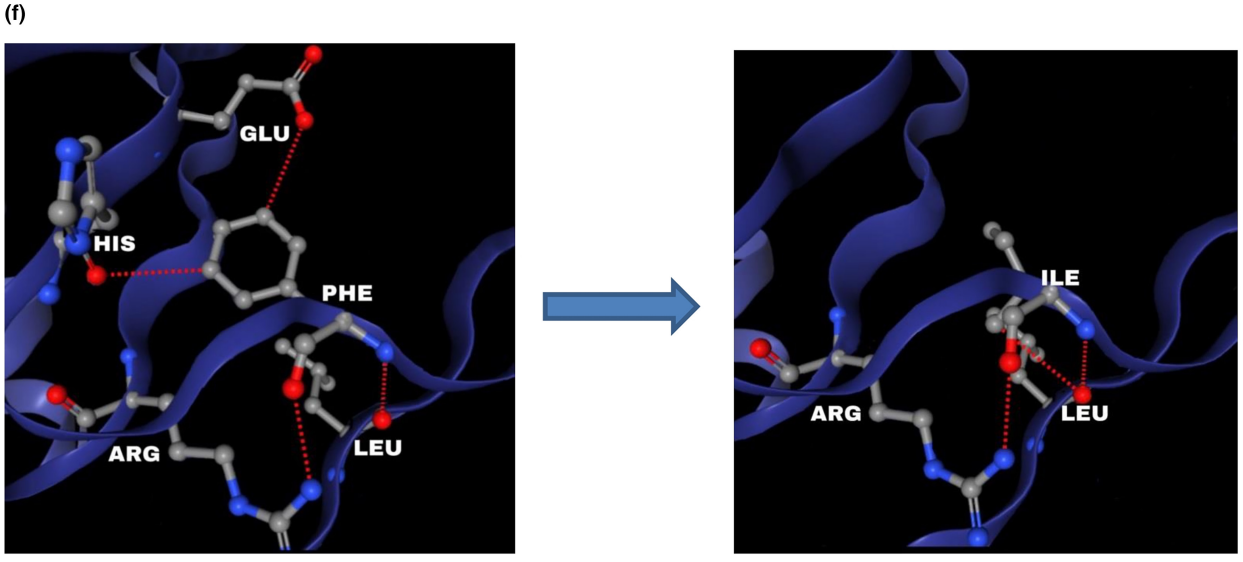


FIGURE 1 (a) A pedigree illustrating an affected patient. Double lines show a consanguineous marriage. (b): Photograph of proband showing microcephaly, sloping forehead, small jaw, macrotia, and open-mouth posture. (c) A diagram showing *ASNS* protein domains and the location of the Phe180Ile variant. (d) Sequencing results showing c.538T>A variant in the proband. (e) Evolutionary conservation analysis for the human Phe180 amino acid in different species: *Homo sapiens* (Human), *Pongo abelii* (Sumatran orangutan), *Mus musculus* (Mouse), *Rattus norvegicus* (Rat), *Bos taurus* (Cattle), *Gallus gallus* (Chicken), *Physeter macrocephalus* (Sperm whale), *Salmo salar* (Atlantic salmon), *Drosophila melanogaster* (Fruit fly), and *Schizosaccharomyces pombe* (Fission yeast). (f) In silico analysis of the protein structure of the humanized model of wild-type *ASNS* protein (left) and its Phe180Ile variant (right) created by DynaMut2 web server.

TABLE 1 CSF amino acid levels.

Amino acid	Result (μmol/L)	Reference
Aspartic acid	0.7	0–1
Glutamic acid	1.2	0–11
Serine	26.1	15–62
Asparagine	3.7	0–25
Glycine	3.6	0–13
Glutamine	385.8	377–1738
Histidine	8.9	7–25
Citrulline	1.8	1–2
Arginine	17.9	9–31
Threonine	21.8	8–85
Alanine	14.7	5–62
Alfa-amino butyric acid	4.6	1–11
Tyrosine	9.8	5–32
Valine	11.1	2–37
Methionine	0.8	0–9
Isoleucine	5.5	2–13
Leucine	10.5	8–27
Phenylalanine	5.3	0–25
Tryptophan	3.1	1–5
Ornithine	2.9	0–5
Lysine	15.2	9–58

Ben-Salem et al., 2015; Seidahmed et al., 2016; Shaheen et al., 2019). The c.1649C>T variant is in the second place reported in 8 patients; interestingly, this variant is highly lethal. All patients have passed away within the first year of their life (Gataullina et al., 2016; Ruzzo et al., 2013). This variant replaces arginine with cysteine in the C-terminal of the *ASNS* protein (p.Arg550Cys). Wang et al. investigated arginine amino acid in this location and found that it is highly conserved among different species; they also mentioned that this amino acid might be essential in the tertiary structure of the *ASNS* protein (Wang et al., 2020).

The *ASNS* protein encompasses two functional domains: glutamine amidotransferase type-2 and asparagine synthetase domains (Richards & Kilberg, 2006). About 62% of the unique variants reported in the literature reside in the asparagine synthetase domain and 30% in the glutamine amidotransferase type-2 domain. The remaining 8% is located in neither of the functional domains. 35% of all unique variants reside in exons 3 (9 unique variants) and 10 (8 unique variants). Interestingly, unlike exon 3, exon 10 is relatively short, with only 101 nucleotides. We report a novel variant in exon 5 (186 nucleotides) of the *ASNS* gene. Previous studies reported three mutations in exon 5; however, these variants were compound heterozygous (Chen et al., 2019; Schleinitz et al., 2018; Staklinski et al., 2023), and our case is the first homozygous pathogenic variant in exon 5. One reason that might explain the high frequency of pathogenic unique variants in exon 10 compared to exon 5 is the higher proportion of conserved amino acids in this exon. Exon 10 encodes 34 amino acids, 62% of which are conserved across the 10 species mentioned earlier. This percentage drops to 13% in exon 5, which encodes 63 amino acids. Considering the fact that less pathogenic and benign variants are underreported, this result is to be expected.

We report a novel variant in the *ASNS* gene in an Iranian-Azeri girl from a family with a consanguineous marriage (first cousin). This is an independent SNV within a region in which an indel has previously been identified (rs866033169). The clinical manifestations of the patient are syndromic intellectual disability with microcephaly, delayed psychomotor symptoms, and seizures; they are similar to what we mentioned above. According to previous reports, the underlying mechanism behind the clinical manifestations of ASNSD is not understood. Evolutionary conservation analysis revealed that Phe180 is highly conserved across different species, from yeast to humans. This shows that this amino acid has a vital role in *ASNS* protein function. The Phe180Ile mutation is located in the glutamine amidotransferase domain. Previous studies showed that this domain is essential for the synthesis of asparagine (Van Heeke & Schuster, 1989). Although asparagine is a non-essential amino acid, because of the poor transportation of asparagine through the blood–brain barrier, the brain is mainly dependent on asparagine synthetase activity, which might explain the neurologic dominance in clinical features (Ruzzo et al., 2013). It has been shown that in both embryonic and postembryonic early neurological and cerebral development, asparagine has an essential

TABLE 2 Previously reported ASNSD patients.

				Clinical presentation						
No.	**Authors**	**Sex & age**	**Consanguinity & Ethnicity**	**DD**	**E**	**AH**	**S**	**HR**	**VI**	**HC at birth**
1	Ruzzo et al.	Male, 14 Y	Yes & Iranian Jewish	+	+	−	+	+	+	31.5
2		Male, 14 Y	No & Iranian Jewish	+	+	−	+	+	+	31
3		Female,12 Y	No & Iranian Jewish	+	+	−	+	+	+	31
4		Male, 4 M	Yes & Bangladeshi	+	−	−	+	+	−	30.5
5		Male, 4 M	Yes & Bangladeshi	+	−	+	+	+	−	33
6		Male, 6 M	Yes & Bangladeshi	+	−	+	+	+	−	32
7		Male, 9 D	No & French Canadian	+	+	+	+	+	NA	31.5
8		Male, 11 M	No & French Canadian	+	+	+	+	+	+	31
9		Male, 12 M	No & French Canadian	+	+	+	+	+	−	28.5
10	Ben Salem et al.	Male, 5 Y	Yes & Emirati	+	+	−	+	+	+	29.5
11	Alfadhel et al.	Male, 4 Y	Yes & Saudi Arabian	+	+	+	+	+	−	30
12		Female, 2 Y	Yes & Saudi Arabian	+	+	+	+	+	−	26.5
13	Palmer et al.	Male, 8 Y	No & Chinese Brunei	+	+	+	+	+	+	32.5
14	Gataullina et al.	Male, 8 M	No & NA	+	+	NA	+	NA	−	34
15		Female, 8 M	No & NA	+	+	NA	+	NA	−	31
16	Seidahmed et al.	Male, 1 M	Yes & Saudi Arabian	NA	+	−	−	+	NA	29
17		Male, 4 Y	Yes & Saudi Arabian	+	−	−	+	−	+	30
18	Sun et al.	Female,6 M	Yes & Indian	+	−	+	+	+	NA	30.5
19		Female,11 D	Yes & Indian	+	−	+	+	+	NA	28.5
20	Yamamoto et al.	Male, 2 Y	No & Japanese	+	+	+	+	+	+	29
21		Male, 19 M	No & Japanese	+	+	+	+	+	+	33.5
22	Reed et al.	Female, 3 M	Yes & Yemeni	+	−	+	+	+	−	31
23	Gupta et al.	Female, 2.5 Y	No & Indian	+	+	+	+	+	+	MC
24	Abhyankar et al.	NA, 15 M	NA & NA	+	+	NA	+	NA	+	MC
25	Galada et al.	Male, 10 D	No & Indian	NA	+	−	+	+	NA	29.5
26		Female, 2 M	No & Indian	NA	−	−	+	+	NA	MC
27		Male, 1 Y	No & Indian	+	+	−	+	+	NA	30
28	Shaheen et al.	Male, NA	Yes & NA	+	NA	NA	+	NA	NA	MC
29		Female, NA	No & NA	+	+	NA	NA	NA	NA	MC
30		Female, NA	Yes & NA	+	NA	NA	NA	NA	NA	MC
31		Male, NA	Yes & NA	+	NA	NA	NA	NA	NA	MC
32		Male, NA	Yes & NA	+	+	+	+	+	NA	MC
33		Female, NA	Yes & NA	+	NA	NA	NA	NA	NA	MC
34		Female, NA	Yes & NA	+	+	NA	NA	NA	NA	MC
35		Female, NA	Yes & NA	+	NA	+	NA	NA	NA	MC
36	Sacharow et al.	Male, 7 Y	Yes & Emirati	+	+	−	−	−	+	34
37		Female, 3 Y	Yes & Emirati	+	+	+	−	+	−	NL
38	Schleinitz et al.	Female, 19 Y	No & German	+	+	−	+	−	+	29.5
39		Female, 16 Y	No & German	+	+	−	+	−	+	30

MRI					Nucleotide & protein change NM_001673.5	Exon	Genotype	Alive at the time of report
MC	BA	GS	DP	DM				
+	+	−	−	+	c.1084T>G, p.Phe362Val (1)	9	Homozygous	Yes
+	+	−	−	+				Yes
+	+	−	−	+				Yes
+	+	+	+	+	c.1648C>T, p.Arg550Cys (2)	13	Homozygous	No
+	+	+	+	+				No
+	+	+	+	+				No
+	+	+	+	+	c.17C>A, p.Ala6Glu (3)	3/13	Compound Heterozygous	No
+	+	+	+	+	c.1648C>T, p.Arg550Cys			No
+	+	+	+	+				No
+	+	+	+	NA	c.1193A>G, p.Tyr398Cys (4)	10	Homozygous	Yes
+	+	+	−	+	c.1193A>G, p.Tyr398Cys	10	Homozygous	Yes
+	+	+	−	+				Yes
+	+	−	−	−	c.866G>C, p.Gly289Ala (5) c.1010C>T, p.Thr337Ile (6)	7/8	Compound heterozygous	Yes
+	+	+	+	+	c.1439C>T, p.Ser480Phe (7)	12/13	Compound heterozygous	No
+	+	+	+	+	c.1648C>T, p.Arg550Cys			No
+	+	+	+	+	c.1219C>T, p.Arg407Ter (8)	10	Homozygous	No
+	+	+	+	+	c.1193A>G, p.Tyr398Cys	10	Homozygous	Yes
+	+	+	+	+	c.1019G>A, p.Arg340His (9)	8	Homozygous	No
+	+	+	+	−				No
+	+	+	+	+	c.434T>C, p.Leu145Ser (10) c.740T>G, p.Leu247Trp (11)	4/6	Compound heterozygous	Yes
+	+	+	+	+	c.1466T>A, p.Val489Asp (12) c.1623_1624del, p.Trp541CysfsTer5 (13)	12/13	Compound heterozygous	Yes
+	−	−	−	−	c.198_202delATAT, p.Lys66AsnfsTer10 (14)	3	Homozygous	No
+	+	−	+	−	c.1138G>T, p.Ala380Ser (15)	10	Homozygous	Yes
+	+	+	+	+	c.728T>C, p.Val243Ala (16) c.1097G>A, p.Gly366Glu (17)	6/9	Compound heterozygous	No
+	+	+	+	+	c.1211G>A, p.Arg404His (18)	10	Homozygous	No
+	+	+	+	+	c.224A>G, p.Asn75Ser (19) c.413A>C, p.Asp138Ala (20)	3/4	Compound heterozygous	No
+	+	+	+	−	c.1649G>A, p.Arg550His (21)	13	Homozygous	No
+	NA	NA	NA	NA	c.1193A>G, p.Tyr398Cys	10	Homozygous	NA
NA	NA	NA	NA	NA				NA
+	+	NA	NA	+	c. 224A>T, p.Asn75Ile (22)	3	Homozygous	NA
+	+	NA	NA	NA	c.1211G>A, p.Arg404His	10	Homozygous	NA
+	+	NA	NA	NA	c.1219C>T, p.Arg407Ter	10	Homozygous	NA
+	+	NA	NA	NA	c.1137+1G>A, p.? (23)	NA	Homozygous	NA
+	NA	NA	NA	NA	c.1354T>A, p.Phe452Ile (24)	12	Homozygous	NA
+	+	NA	NA	NA	c.28A>C, p.Ser10Arg (25)	3	Homozygous	NA
+	+	NA	NA	+	c.146G>A, p.Arg49Gln (26)	3	Homozygous	Yes
+	+	NA	NA	+				Yes
+	+	NA	NA	NA	c.1165G>C, p.Glu389Gln (27)	10/5	Compound heterozygous	Yes
NA	NA	NA	NA	NA	c.601delA, p.Met201TrpfsTer28 (28)			Yes

(Continues)

TABLE 2 (Continued)

No.	Authors	Sex & age	Consanguinity & Ethnicity	Clinical presentation						HC at birth
				DD	E	AH	S	HR	VI	
40	Sprute et al.	Male, 5 Y	Yes & Turkish	+	+	+	+	+	−	33
41		Male, 4 Y	Yes & Turkish	+	+	+	+	+	−	31
42	Devi et al.	Male, 1 M	Yes & Indian	+	+	NA	−	−	−	30
43		Female, 1 M	Yes & Indian	+	−	NA	+	+	−	30
44		Female, 3 Y	Yes & Indian	+	+	NA	−	−	−	MC
45	Chen et al.	Male, 1 Y	NA & NA	+	+	−	−	−	+	32
46	Monies et al.	Male, 1 Y	Yes & NA	+	−	−	−	−	+	MC
47		Female, 6 M	NA & NA	+	+	+	+	−	−	MC
48	Wang et al.	Female, 2 M	No & Chinese	+	+	+	+	+	+	NA
49	Yingjun et al.	Male, 10 Y	NA & Chinese	NA	NA	NA	NA	NA	NA	NA
50		Female, 6 Y	NA & Chinese	NA	NA	NA	NA	NA	NA	NA
51	Faoucher et al.	Female, 4 Y	No & French	+	+	+	+	+	−	30
52	Alharby et al.	Male, 6 Y	Yes & Saudi Arabian	+	+	+	+	NA	−	MC
53		Male, 1 Y	Yes & Saudi Arabian	+	+	+	+	NA	−	MC
54		Female, 4 Y	Yes & Saudi Arabian	+	+	+	−	NA	−	MC
55		Male, 2 Y	Yes & Saudi Arabian	+	+	+	+	NA	+	MC
56		Female, 3 Y	Yes & Saudi Arabian	+	+	−	+	NA	−	MC
57		Male, 3 Y	Yes & Saudi Arabian	+	+	−	+	NA	+	MC
58		Female, 3 Y	Yes & Saudi Arabian	+	+	−	+	NA	−	MC
59		Male, 2 Y	Yes & Saudi Arabian	+	+	+	+	NA	−	MC
60		Female, 3 Y	Yes & Saudi Arabian	+	+	+	+	NA	−	MC
61		Female, 7 Y	Yes & Saudi Arabian	+	+	+	+	NA	−	MC
62		Male, 22 D	Yes & Saudi Arabian	+	+	+	+	NA	−	MC
63		Female, 8 D	Yes & Saudi Arabian	+	−	−	−	NA	−	MC
64		Male, 3 Y	Yes & Saudi Arabian	+	+	−	+	NA	+	MC
65		Male, 3 M	Yes & Saudi Arabian	+	+	+	+	NA	−	MC
66	Abdel-Salam et al.	Female 8 M	Yes & Egyptian	+	+	−	+	+	+	30
67	Akesson et al.	Male, 1 M	No & NA0	+	−	NA	NA	NA	NA	MC
68	Churchill et al.	Fetus	No & Caucasian	NA	NA	NA	NA	NA	NA	NA
69	Liu et al.	Male-7 Y	No & Chinese	+	+	−	−	−	+	NA
70		Male, 5 Y	No & Chinese	+	+	−	−	−	−	NA
71	Saini et al.	Female, 2 Y	No & NA	+	+	−	+	+	+	MC
72	Staklinski et al.	Female, 9 Y	No & NA	+	+	−	−	−	−	32
73	Staklinski et al.	Male, 4 Y	No & Cuban Italian	+	+	+	−	+	−	34
74	Altintas et al.	Male, 7 Y	No & Turkish	+	+	+	+	NA	NA	MC
75	Zhu et al.	Fetus	No & Chinese	NA	NA	NA	NA	NA	NA	NA
76	Our patient	Female, 11 Y	Yes & Iranian Azeri	+	+	−	+	+	−	33

Note: Numbers inside the brackets indicate the count of unique variants.

Abbreviations: AH, axial hypotonia; BA, brain atrophy; DD, developmental delay; DM, delayed myelination; DP, decreased size of pons; E, epilepsy; GS, gyral simplification; HR, hyperreflexia; MC, microcephaly; NA, not available; NL, normal; S, spasticity; TP, terminated pregnancy; VI, visual impairment.

MRI					Nucleotide & protein change			Alive at the time of report
MC	BA	GS	DP	DM	NM_001673.5	Exon	Genotype	
+	+	+	−	+	c.1108C>T, p.Leu370Phe (29)	9	Homozygous	Yes
+	+	+	−	+				Yes
+	+	NA	NA	+	c.788C>T, p.Ser263Phe (30)	7	Homozygous	No
+	+	NA	NA	NA				No
+	+	NA	NA	NA	c.146G>A, p.Arg49Gln	3	Homozygous	Yes
+	+	+	NA	+	c.1424C>T, p.Thr475Ile (31) c.666_ 667delCT, p.Leu222LeufsTer5 (32)	12/5	Compound heterozygous	Yes
NA	NA	NA	NA	NA	c. 224A>T, p.Asn75Ile	3	Homozygous	NA
NA	NA	NA	NA	NA				NA
+	+	+	NA	NA	c.1649G>A, p.Arg550His c.368T>C, p.Phe123Ser (33)	13/4	Compound heterozygous	NA
NA	NA	NA	NA	NA	c.1193A>C, p.Tyr398Ser (34)	10/7	Compound heterozygous	Yes
NA	NA	NA	NA	NA	c.800C>T, p.Ala267Val (35)			Yes
+	+	−	−	+	c.144C>A, p.His48Gln (36)	3	Homozygous	Yes
+	+	+	NA	+	c.1193A>G, p.Tyr398Cys	10	Homozygous	No
+	+	−	NA	+				No
+	+	+	NA	+				No
+	+	+	NA	+				Yes
+	+	−	NA	−	c. 224A>T, p.Asn75Ile	3	Homozygous	No
+	+	−	NA	−				Yes
+	+	−	NA	−				Yes
+	+	−	NA	+	c.1424C>A, p.Thr475Asn (37)	12	Homozygous	Yes
+	+	−	NA	−				Yes
+	+	−	NA	+				Yes
+	+	+	NA	+	c.1211G>A, p.Arg404His	10	Homozygous	No
NA	NA	NA	NA	NA	c.1219C>T, p.Arg407Ter	10	Homozygous	No
+	+	+	NA	+	c.1137+1G>A, p.?	NA	Homozygous	No
+	+	−	NA	+	c.674-1G>A, p.? (38)	NA	Homozygous	No
+	−	−	+	+	c.397_398GT>CA, p.Val133Gln (39)	4	Homozygous	No
+	+	−	−	−	c.1476+1G>A, p.? (40)	NA	Homozygous	No
+	+	NA	+	NA	c.478delG, p.Glu160LeufsTer8 (41) c.1283A>G, p.Tyr428Cys (42)	4/10	Compound heterozygous	TP
+	+	NA	NA	NA	c.224A>G, p.Asn75Ser	3/13	Compound heterozygous	Yes
+	+	NA	NA	NA	c.1612A>G, p.Met538Val (43)			Yes
+	+	+	−	+	c. 1138G>T, p.Ala380Ser	10	Homozygous	NA
+	+	NA	NA	NA	c.1118G>T, p.Gly373Val (44) c.1556G>A, p.Arg519His (45)	9/13	Compound heterozygous	Yes
+	+	−	−	−	c.614A>C, p.His205Pro (46) c.1192dupT, p.Tyr398LeufsTer4 (47)	5/10	Compound heterozygous	Yes
+	+	−	−	−	c.83G>A, p.Arg28Gln (48) c.1082A>T, p.Glu361Val (49)	3/9	Compound heterozygous	Yes
+	+	NA	NA	NA	c.97C>T, p.arg33Cys (50) c.1031-2_1033del (51)	3/9	Compound heterozygous	TP
+	+	−	−	−	c.538T>A, p.Phe180Ile (52)	5	Homozygous	Yes

role (Ruzzo et al., 2013; Wang et al., 2020). In this case, CSF amino acid levels were investigated (asparagine: 3.7 μmol/L); however, because the reference value was 0–25, it was not possible to identify low asparagine levels.

5 | CONCLUSION

In the present study, a novel homozygous mutation was recognized in the *ASNS* gene in an Iranian-Azeri girl manifesting typical ASNSD symptoms, particularly intellectual disability and microcephaly. This study expands the mutation spectrum of ASNSD and reviews previously reported patients. Like previous studies, this case was diagnosed by exome sequencing and validated by Sanger sequencing, showing the importance of sequencing for diagnosing rare diseases.

AUTHOR CONTRIBUTIONS

BD and FA conceived and supervised the study, and conceptualized and revised the manuscript. BD, MJ, and DM contributed to the laboratory work. MJ, DM, HM, and SA prepared the manuscript, images, and review table. FA was in charge of the patient's clinical management. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank the patient's family for their participation in this study.

FUNDING INFORMATION

This research received no specific grant, funding, equipment, or supplies from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Please get in touch with the corresponding author for data requests.

ETHICS STATEMENT

This study was approved by the ethics committee of Ardabil University of Medical Sciences (IR.ARUMS.REC.1402.035).

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How to cite this article: Jahanpanah, M., Mokhtari, D., Mokaber, H., Arish, S., Ahmadabadi, F., & Davarnia, B. (2024). A novel variant in *ASNS* gene responsible for syndromic intellectual disability and microcephaly: Case report and literature review. *Molecular Genetics & Genomic Medicine*, *12*, e2424. https://doi.org/10.1002/mgg3.2424