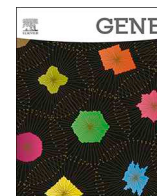




ELSEVIER

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short communication

Molecular diagnosis of asparagine synthetase (ASNS) deficiency in two Indian families and literature review of 29 ASNS deficient cases

Akella Radha Rama Devi^{a,b,*}, Shaik Mohammad Naushad^b^a Rainbow Children's Hospital, Banjara Hills, Road No. 2, Hyderabad, India^b Sandor Speciality Diagnostics Pvt Ltd, Banjara Hills, Road No.3, Hyderabad, India

ARTICLE INFO

Keywords:

Asparagine synthetase deficiency
Microcephaly
ASNS gene
In silico characterization

ABSTRACT

In the current study, we report three cases of Asparagine Synthetase (ASNS) Deficiency from two consanguineous families. Family 1 had two early neonatal deaths due to a novel mutation in the ASNS gene c.788C > T (p.S263F) and both the children presented with microcephaly and one of them had severe intracranial haemorrhage. The proband from the second family was homozygous for c.146G > A (p.R49Q) and manifested myoclonic seizures, developmental delay, coarse hair and diffuse cortical atrophy. Molecular docking studies of both the mutations revealed alteration in the ligand binding site. Till date, 26 mutations were reported in ASNS gene in 29 affected children indicating high degree of genetic heterogeneity and high mortality. Although asparagine depletion is not of diagnostic utility, multiple linear regression model suggested that asparagine levels vary to the extent of 20.6% based on glutamine and aspartate levels and ASNS deficiency results in depletion of asparagine synthesis. ASNS deficiency should be suspected in any neonate with microcephaly and epileptic encephalopathy.

1. Introduction

Asparagine synthetase (ASNS, OMIM 615574) deficiency is an autosomal recessive neurometabolic disorder of amino acid metabolism characterized by congenital microcephaly, intellectual disability, progressive cerebral atrophy and intractable seizures. ASNS deficiency was first described in four families with neurological symptoms (Ruzzo et al., 2013). Asparagine synthetase also known as aspartate-ammonia ligase, is an enzyme involved in the biosynthesis of asparagine from aspartate through an ATP-dependent transamination reaction in the presence of glutamine which acts as amino group donor (Zhang et al., 1989). Enzyme deficiency blocks asparagine synthesis resulting in low asparagine levels in body fluids. Since ASNS is highly expressed in the brain, the levels will be significantly low in CSF (Hongo et al., 1994).

This is a pan-ethnic disorder and has been identified within a variety of ethnic groups with reports from Iran, Bangladesh, Arabian, French Canadian and Indian families. Worldwide carrier frequency is reported to be 1 in 2049, highest among Sephardic - Iranian Jewish 1:80, South Asia 1 in 3072. (Asparagine Synthetase (ASNS) Deficiency– Sema4). The first report of Japanese patients with ASNS deficiency is reported

recently (Yamamoto et al., 2017).

Asparagine synthetase, the human ASNS enzyme, is a 65 kDa protein that has two primary domains, termed the N- and C-terminal domains. The human ASNS gene is located at chromosome 7q21.3 and is 35 kb long with 13 exons (Heng et al., 1994). Biochemically, plasma concentration of asparagine is variable with only a few manifesting lower levels of asparagine. Diagnosis is difficult in suspected cases and can only be diagnosed through DNA sequencing. Presence of microcephaly at birth suggests that brain ASNS activity is critical for the brain development. Four patients are identified in India till date, and our report adds three more affected infants from two families with novel mutations.

2. Materials and methods

The patients were recruited for the study after obtaining informed consent from the parents. The study protocol was approved by the Institutional Ethical Committee of Rainbow Children's Hospital, Hyderabad, India.

Abbreviations: ASNS, asparagine synthetase deficiency; SD, standard deviation; ExAC, Exome aggregation Consortium; TMS, Tandem mass spectrometry; EEG, electroencephalogram

* Corresponding author at: Rainbow Children's Hospital, Banjara Hills, Road No. 2, Sandor Speciality Diagnostics Pvt Ltd, Banjara Hills, Road No.3, Hyderabad, India.

E-mail address: radharamadevi@sandor.co.in (A. Radha Rama Devi).

<https://doi.org/10.1016/j.gene.2019.04.024>

Received 5 March 2019; Received in revised form 8 April 2019; Accepted 8 April 2019

Available online 09 April 2019

0378-1119/ © 2019 Published by Elsevier B.V.

2.1. Patient 1

The first male child, to a consanguineous couple, born by caesarean section with a birth weight of 3.0 kg and the head circumference was 30 cm and length was 50 cm. The child has been lethargic since birth with refusal to feeds and manifested seizures on the 5th day of life. Metabolic screening by Tandem mass spectrometry revealed normal acyl carnitine profile and amino acids. There were no dysmorphic features. The neonate was hemodynamically unstable and was ventilated. MRI brain revealed extensive intraventricular haemorrhage and in cisterna magna. Diffuse cerebral atrophy, incomplete sulcal formation, no myelin signal was seen on T2 weighed images in the posterior limb of the internal capsule. The child expired at one month of age.

2.2. Patient 2

Patient 2 was the sister of the 1st patient, was evaluated at one month, with microcephaly at birth, excessive irritability and refusal to feed. The baby was born by caesarean section at 40 weeks of gestation. Birth weight was 2.9 kg, head circumference of 30 cm and length of 52 cm. At evaluation weight was 3.6 kg. Head circumference was 32 cm (< 5th Centile) suggestive of severe microcephaly. The child was lethargic and not accepting feeds. No neurological signs were evident except for hyperreflexia. MRI brain showed diffuse cerebral atrophy, white matter cystic changes in the anterior frontal and temporal region with thinned out brain stem. There were no obvious seizures but the electroencephalogram (EEG) showed multifocal seizure disorder. The child became comatose and expired on the 5th day of admission.

2.3. Patient 3

A 3-yr-old girl born to consanguineous parents, first in birth order, presented with myoclonic seizures since birth. Developmental delay and seizures were present since birth with EEG showing diffuse seizure activity. She had coarse hair, microcephaly (head circumference < 3rd centile). MRI brain revealed diffuse cortical atrophy. TMS was normal with no metabolic cause. Fundus examination was normal. She was globally delayed in development. An exome sequencing was performed which revealed *ASNS* mutation along with a homozygous mutation in *ACT1* gene. In view of this, urine organic acid analysis was done which was normal with no increased excretion of triglyl glycine. Her urine ketones were negative. Hence the possibility of Beta ketothiolase was excluded. Further evaluation was not possible as the child was lost to follow up.

2.4. Molecular analysis

Exome Sequencing is performed using next generation sequencing. The genome is sequenced to mean > 80–100× coverage and a minimum of ~84% of bases are sequenced to at least 20× coverage. Paired-end 151 bp reads are aligned to the NCBI reference sequence (GRCh37.75) using the bowtie2.2.1 short read aligner, and variant calls are made using the Annovar. Variants are subsequently filtered to identify Variants with a minor allele frequency < 5%, Variants classified as disease causing mutations in public databases, Predicted loss-of-function variants with a minor allele frequency < 5% in the patient-specific phenotype-driven gene list.

2.5. In silico analysis

Using the crystal structure of asparagine synthetase B from *Escherichia coli* (c1ct9D) as a template, we have developed a homology model of asparagine synthetase (*Homo sapiens*, NM_133436.3) using Phyre2 web portal (Kelley et al., 2015). A total of 499 residues (89% of target sequence) were modeled with 100% confidence using this highest scoring template. The remaining 62 residues were modeled ab

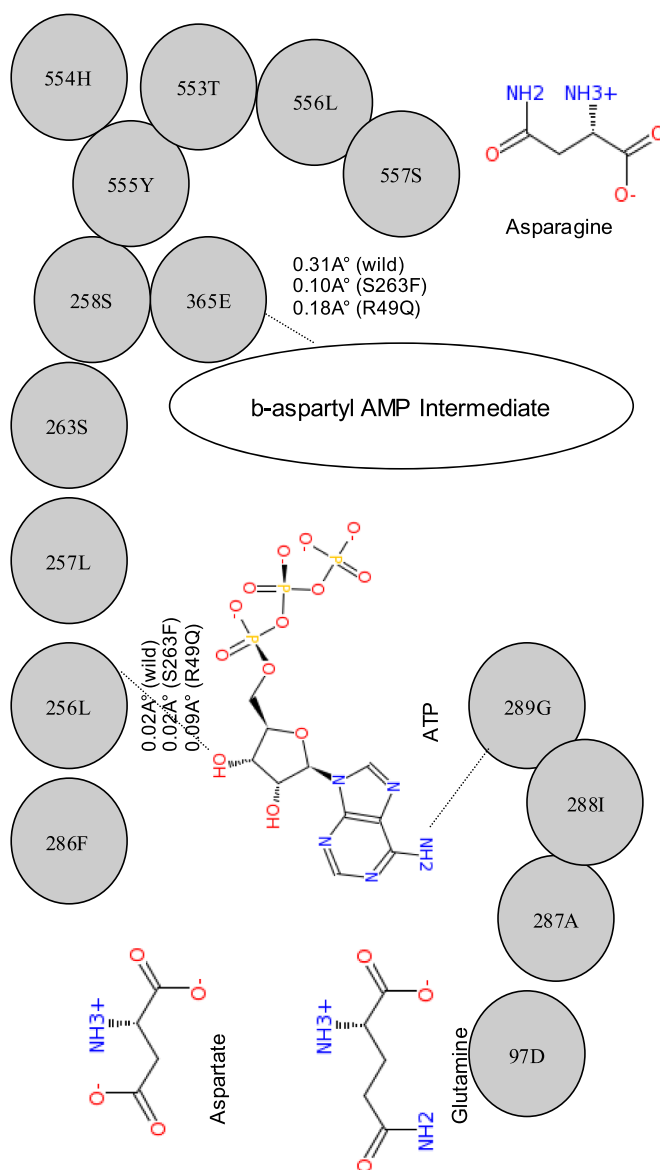


Fig. 1. Two dimensional representation of interactions of ASNS protein with its ligands.

The catalytic site of ASNS protein was represented in the form of individual amino acid residues interacting with various ligands. Aspartate, glutamate and ATP are the reactants required for the synthesis of asparagine. Beta aspartyl AMP intermediate is the reaction intermediate. We calculated bond length between 256L and ATP; and 365E with that of reaction of intermediate. We observed that R49Q mutant has lesser affinity towards ATP when compared to wild and S263F mutant. The reaction intermediate is strongly held in S263F and R49Q mutants than the wild protein. Hence, it is proposed that decreased clearance of the product from the catalytic site might be resulting in feedback inhibition of ASNS enzyme.

initio. The wild and mutant proteins (S180F and R49Q) were modeled (Fig. 1). The 3DLigandSite module (Wass et al., 2010) was used for the prediction of ligand binding site (Fig. 2). The impact of ASNS deficiency in reducing cortical thickening and in dilating lateral ventricles is schematically represented (See Fig. 3.)

2.6. Statistical analysis

Student *t*-test was used to compare the distribution of glutamine, aspartate and asparagine in cases and controls. In order to assess the interrelationships of asparagine with glutamine, aspartate and

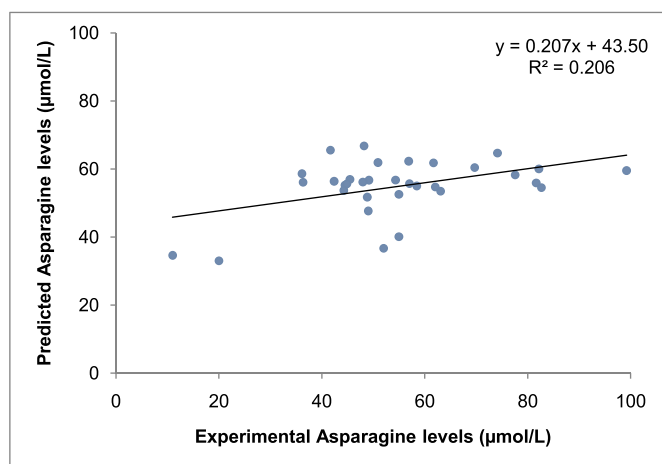


Fig. 2. Multiple linear regression model of asparagine synthesis. Using glutamine, aspartate and presence or absence of ASNS deficiency as input variables, we have predicted the asparagine levels. Glutamine and aspartate contribute 20.6% variability in asparagine levels. ASNS deficiency was associated with depleted asparagine synthesis. R: Spearman rank correlation coefficient.

asparagine synthase deficiency, a multiple linear regression equation was deduced. The data of seven ASNS deficiency cases and 26 healthy children comprising of glutamine, aspartate and asparagine levels was the basis of this model. All these values were expressed in $\mu\text{mol/L}$. The input variables are glutamine, aspartate and presence (1) or absence (0) of ASNS deficiency. The output variable is asparagine level. The resultant equation is expressed as $y = m_1x_1 + m_2x_2 + m_3x_3 + C$ where in 'y' corresponds to asparagine while x_1 , x_2 and x_3 correspond to glutamine, aspartate, ASNS deficiency (0/1). The m_1 , m_2 , m_3 values signify the impact of corresponding input variables and the '+' sign denotes positive association and '-' denotes inverse association. The actual vs. predicted values of asparagine were plotted against each other to calculate the spearman rank correlation coefficient 'r'.

3. Results

3.1. Molecular diagnosis

In the Family 1, the dried blood spot of the 1st child was retrieved from the new-born screening laboratory and DNA analysis was performed. Both the patients were homozygous for an unreported missense mutation NM_133436.3 (ASNS): c.788C > T (p.S263F). This variant is novel and not observed in 1000G database and ExAC database. This variant is predicted to be damaging by SIFT, Polyphen2, LRT_Pred, Mutation Taster, Mutation Assessor, RadialSVR and Phenolyzer. Parents were carriers for the same mutation seen in the children. The mutation is inherited as both the parents were carriers. Family 2, the affected girl was homozygous mutant for NM_133436.3 (ASNS): c.146G > A (p.R49Q). One carrier of this mutation was identified in ExAC database.

3.2. In silico analysis of functional implications

The wild ASNS protein was predicted to form ligand binding site using 256Leu, 257Leu, 258Ser, 263Ser, 286Phe, 287Ala, 288Ileu, 365Glu, 553Thr, 554His, 555Tyr, 556Lys and 557Ser residues. The 256Leu, 288Ileu are the binding sites for ATP via carbonyl oxygen and via amide nitrogen respectively. Through molecular docking studies we calculated the H-bond length between the 256Leu residue and carbonyl oxygen of ATP in wild vs. mutant proteins. The H-bond length was similar in wild and S180F mutant (0.02\AA) while the bond length is larger in R49Q mutant (0.09\AA). The 365Glu is the site for the formation of

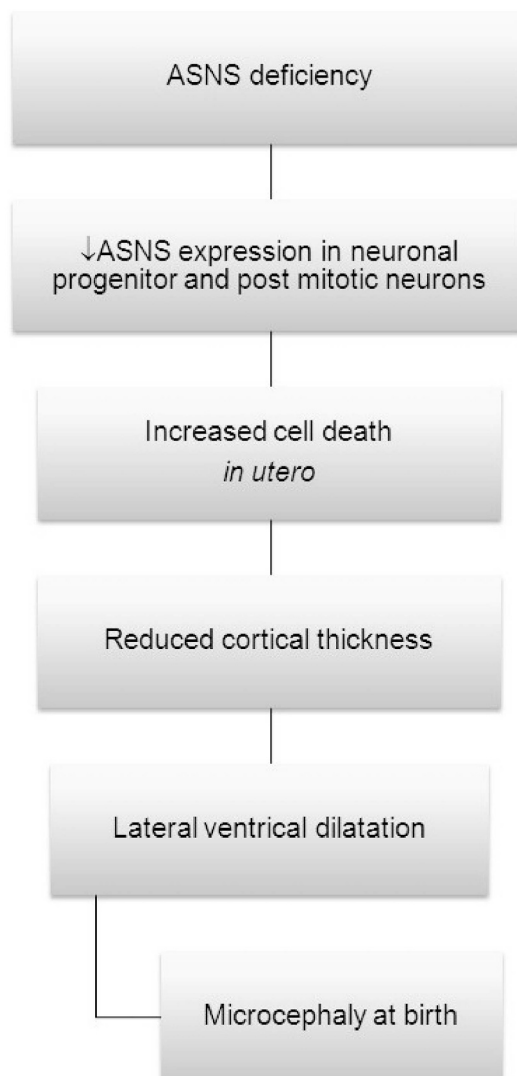


Fig. 3. Schema depicting the clinical implications of ASNS deficiency. In ASNS deficient subjects, as a result of reduced expression of ASNS in neuronal progenitor and post mitotic neurons, increased cell death happens in utero contributing to reduced cortical thickness and increased dilatation of lateral ventricles thus manifesting as microcephaly at birth.

beta aspartyl AMP intermediate. Molecular docking studies showed that wild protein has higher bond-length (0.31\AA) compared to S180F (0.10\AA) and R49Q (0.18\AA) mutants between 365Leu and beta aspartyl AMP intermediate. This indicates decreased clearance of the product in the mutants resulting in feedback inhibition of the enzyme (Fig. 1).

The S263F mutation corresponds to 263Ser in this transcript. As shown in Fig. 1, this mutation is altering the tertiary structure of the protein by uncoiling of one of the helical structures probably due to steric repulsion by 263Phe. As shown in Fig. 2, this mutation alters the ligand binding site, which contains 256Leu, 257Leu, 258Ser, 286Phe, 287Ala, 288Ileu, 365Glu intact while 363Ser, 404Arg and 546Asp residues being the new residues forming the site. The 553Thr, 554His, 555Tyr, 556Lys and 557Ser residues are not taking part in the binding site formation in the mutated protein.

As shown in Fig. 2, The R49Q mutation although not occurred in residues that form ligand binding sites, the mutated protein has 256Leu, 257Leu, 258Ser, 263Ser, 286Phe, 287Ala, 288Ileu, 365Glu residues as in the wild protein, 363Ser and 368Asp additional residues forming the ligand binding site. Due to the mutation, 553Thr, 554His, 555Tyr, 556Lys and 557Ser residues are not part of binding site.

Table 1
Comparison of cases from the current study with existing literature.

Variable	Value	Patient 1	Patient 2	Patient 3
Total number of cases	29			
Male: female	17:9	Male	Female	Female
Age group (yr)	0.01–14	0.08	0.08	3 yr
Consanguinity	12/26 (46.15%)	Yes	Yes	Yes
Developmental delay	28/29 (96.6%)	Yes	Yes	Yes
Epilepsy	23/29 (79.3%)	Yes	No	Yes
Microcephaly	28/29 (96.6%)	Yes	Yes	Yes
Spasticity	26/29 (89.7%)	No	Yes	No
Cortical blindness	8/29 (27.6%)	No	No	No
Mortality	13/26 (50.0%)	Yes	Yes	No
ASNS mutation	n = 26	c.788C > T (S263F)	c.788C > T (S263F)	c.146G > A (R49Q)

Note: In three cases, information on gender, consanguinity and mortality could not be obtained.

3.3. Review of all the reported cases

Including the three cases reported in the current study, there are 29 cases of ASNS deficiency reported till date. The age at presentation varied from 0.01 to 14 yr. Consanguinity was observed in 46.15% cases. Developmental delay, epilepsy, microcephaly and spasticity are the key features accounting for 96.6%, 79.3%, 96.6% and 89.7% of cases respectively. Cortical blindness was observed in 27.6% cases. High mortality (50.0%) is reported in the affected cases. The mean age of death was 0.42 ± 0.42 yr while the mean age of surviving children was 4.55 ± 4.74 yr ($p = 0.004$) (Table 1).

Plasma and CSF levels of asparagine levels could not be performed in the reported cases as they expired before the diagnosis. Few studies have explored asparagine (mean \pm SD: 29.4 ± 20.0 $\mu\text{mol/L}$, $n = 14$), glutamine (mean \pm SD: 663.6 ± 305.9 $\mu\text{mol/L}$, $n = 11$) and aspartate (mean \pm SD: 10.3 ± 9.9 $\mu\text{mol/L}$, $n = 9$) levels in ASNS deficient patients. When compared to control children, the asparagine levels were significantly lowered in ASNS deficiency ($p < 0.0001$) (Table 2).

In order to assess the extent of depletion of asparagine synthesis in ASNS patients, we have compared the data of ASNS patients with controls ($n = 26$). A multiple linear regression equation was deduced to predict asparagine levels as a function of glutamine and aspartate in cases as well as controls. Asparagine ($\mu\text{mol/L}$) = $(0.021 \times \text{glutamine}) + (0.374 \times \text{aspartate}) - (16.55 \times \text{ASNS deficiency}) + 39.29$.

This equation explains 20.6% variability in asparagine levels. There is a significant depletion of asparagine in ASNS deficiency cases (Fig. 2).

4. Discussion

Till date, 26 different mutations were reported in the ASNS gene, 23 missense and three are non-sense mutation (two frame shift mutations and one point mutation). Seven of these mutations occurred in exon 5. Till date, seven cases of ASNS deficiency were reported from India. The reported mutations in India were: c.146G > A, c.224A > G and c.413A > C, c.788C > T of exon 1; c.1019G > A of exon 4; c.1138G > T and c.1211G > A of exon 5; and c.1649G > A of exon 6

Table 2
Biochemical markers of ASNS deficiency.

Amino acid	Cases	Controls	P value
Asparagine ($\mu\text{mol/L}$)	29.4 ± 20.0 ($n = 14$)	57.9 ± 16.6 ($n = 26$)	$< 0.0001^a$
Aspartate ($\mu\text{mol/L}$)	10.3 ± 9.9 ($n = 9$)	10.7 ± 4.9 ($n = 26$)	0.87
Glutamine ($\mu\text{mol/L}$)	663.6 ± 305.9 ($n = 11$)	701.3 ± 323.4 ($n = 26$)	0.74

The values were represented in mean \pm standard deviation format.

^a Statistically significant.

(Gupta et al., 2017; Galada et al., 2018; Sun et al., 2016). Mutations in Japanese were: c.434T > C, c.740T > G, c.1466T > A and c.1623_1624del (Yamamoto et al., 2017). Arabians had c.932 A > G, c.1121A > G, c.1193A > C, c.1219C > T mutations (Seidahmed et al., 2016; Alfadhel et al., 2015; Ben-Salem et al., 2014). The c.1648C > T mutation appears to be an ancestral mutation for French population (Ruzzo et al., 2013; Gataullina et al., 2016). The c.1439C > T in French population and c.17C > A in French-Canadian population might have emerged after the divergence of these two population groups (Ruzzo et al., 2013; Gataullina et al., 2016). The c.866G > C and c.1010C > T mutations were reported among Australians (Palmer et al., 2015) while c.601delA and c.1165G > C mutations were frequent in German., USA exhibited c.728 T > C and c.1097G > A mutations (Schleinitz et al., 2018), Iranians had c.1084 T > G mutation (Ruzzo et al., 2013). Thus high degree of genetic heterogeneity is observed in ASNS deficiency despite identical phenotypic spectrum (Table 3).

Diffuse cerebral atrophy, incomplete sulcal formation, myelination defects are the hallmark features of ASNS deficiency. ASNS activity in the brain is crucial for organ development, and a tissue-specific dependence on asparagine for neural development (Ruzzo et al., 2013) could cause the characteristic brain changes seen in these patients at birth. Reviewing 29 individuals reported till now, a significant proportion (22/29) manifested microcephaly and abnormal gyral pattern. Although seizures are reported to be refractory in ASNS deficiency, both the sibs in one family did not present with clinical seizures whereas the third child had refractory seizures. Progression of the disease could be rapid as reported in an earlier study where in two sibs had ventration of the diaphragm resulting in severe respiratory complications and death (Sun et al., 2016). Although it is a rare coincidental association, it was the first abnormality reported outside the central nervous system in this disorder indicating the possibility of multiple organ involvements in ASNS deficiency. In the current study, extensive intraventricular haemorrhage occurred in one infant and none of the cases reported till now manifested cerebral haemorrhage. Asparagine is reported to be essential for endothelial cell growth which is important for angiogenesis. Consistently, fibroblasts proliferation was markedly reduced under conditions of asparagine deprivation. Studies on patients with Acute Lymphatic Leukaemia on Asparaginase treatment showed depletion of fibrinogen (Alqasim et al., 2018) and inhibition of the biosynthesis of hepatic coagulation factors (Rota et al., 2016). Clinical data suggest that asparagine depletion is effective in treating hematologic malignancies. However, coagulation defects and platelet dysfunction are noted in asparaginase treatment (Jaccard et al., 2010). This may be the possible cause of intracranial bleed in patients with asparagine deficiency.

The c.788C > T is a novel mutation in this family and predicted to be deleterious by different bioinformatics tools. The mutation segregating in the family with recurrence indicates the pathogenicity of the

Table 3
Frequency of different ASNS mutations reported till date.

Exon	Nucleotide change	Protein change	No of mutated alleles	Frequency (%)	Population
1	c.17C > A	A6E	3	5.17	French-Canadian
1	c.146G > A*	R49Q	2	3.45	Indian
1	c.224A > G	N75S	1	1.72	Indian
1	c.413A > C	D138A	1	1.72	Indian
1	c.434T > C	L145S	1	1.72	Japanese
3	c.601delA	M201Wfs*28	2	3.45	German
3	c.728T > C	V243A	1	1.72	USA
3	c.740T > G	L247W	1	1.72	Japanese
4	c.788C > T*	S263F	2	3.45	Indian
4	c.866G > C	G289A	1	1.72	Australian
4	c.932A > G	Y311C	2	3.45	Arabian
5	c.1010C > T	T337I	1	1.72	Australian
5	c.1019G > A	R340H	4	6.90	Indian
5	c.1084T > G	F362V	6	10.34	Iranian
5	c.1097G > A	G374E	1	1.72	USA
5	c.1121A > G	Y374C	4	6.90	Arabian
5	c.1138G > T	A380S	2	3.45	Indian
5	c.1165G > C	E389Q	2	3.45	German
6	c.1193A > C	Y398S	2	3.45	Arabian
6	c.1211G > A	R404H	2	3.45	Indian
6	c.1219C > T	R407*	2	3.45	Arabian
8	c.1439C > T	S480F	2	3.85	French
8	c.1466T > A	V489D	1	1.72	Japanese
9	c.1623_1624del	W541Cfs*5	1	1.72	Japanese
10	c.1648C > T	R550C	9	15.52	French, French-Canadian, Bangladeshi
10	c.1649G > A	R550H	2	3.45	Indian

*: Mutations identified in the current study.

novel mutation. In silico analysis revealed that this mutation occurred in the 263Ser residue that is crucial part of ligand binding site. The second mutation i.e. R49Q although distant to the key binding sites, there is conformational change leading to altered ligand binding site. The binding affinities of both mutants are presumed to be lower than the wild ASNS due to loss of interaction of ligand with 553Thr, 554His, 555Tyr, 556Lys and 557Ser residues. The R49Q has lesser affinity towards ATP compared to wild and S263F proteins. The beta aspartyl AMP intermediate has higher affinity towards 365Glu in S263F than the wild and R49Q mutant resulting in decreased clearance of the product and feedback inhibition of ASNS.

ASNSD is one of the defects in synthesis like creatine deficiency syndromes (Stöckler et al., 1994), glutamine synthetase deficiency (Häberle et al., 2006), and the serine synthetic defects (Crabben et al., 2013). Since there is a deficiency of end product in these cases, a therapeutic benefit of supplementation has been reported in serine biosynthetic disorders and glutamine synthetase deficiency. Worsening of seizures after asparagine supplementation in a child with asparagine synthetase deficiency is reported (Alrifai and Alfadhel, 2016). Value of supplementing asparagine in ASNS deficiency is questionable as the brain is affected at birth.

4.1. Conclusion

ASNS deficiency is a progressive disease with no cure ultimately leading to death. Biochemical testing is not always helpful as majority can have normal asparagine levels in plasma. DNA testing is the ultimate diagnosis and should be done in all patients with microcephaly for proper counselling and prenatal diagnosis in subsequent pregnancies. The reported variants in the current study i.e. S263F and R49Q are predicted to alter ligand binding site and product clearance thus contributing to ASNS deficiency.

Acknowledgements

We thank both the families who participated in this study. No specific funding was obtained from any agency.

Disclosure of potential conflicts of interest

Both the authors hereby declare no conflicts of interest.

Research involving human participants and/or animals

This study complied with the ethical principles outlined in the Declaration of Helsinki.

Informed consent

Informed consent was obtained from the parents or guardians of the patients.

References

- Alfadhel, M., Alrifai, M.T., Trujillano, D., et al., 2015. Asparagine synthetase deficiency: new inborn errors of metabolism. *JIMD Rep.* 22, 11–16.
- Alqasim, A.M.Z., Al-Hadithi, R.H., Al-Khalidi, A.N., 2018. Coagulopathic side effect of L-asparaginase on fibrinogen level in childhood acute lymphoblastic leukemia during induction phase. *Hematol. Oncol. Stem Cell Ther.* <https://doi.org/10.1016/j.hemonc.2018.01.003>.
- Alrifai, M.T., Alfadhel, M., 2016. Worsening of seizures after asparagine supplementation in a child with asparagine synthetase deficiency. *Pediatr. Neurol.* 58, 98–100.
- Ben-Salem, S., Gleeson, J.G., Al-Shamsi, A.M., et al., 2014. Asparagine synthetase deficiency detected by whole exome sequencing causes congenital microcephaly, epileptic encephalopathy and psychomotor delay. *Metab. Brain Dis.* 30, 687–694.
- Crabben, S.N.V.D., Verhoeven-Duif, N.M., Brilstra, E.H., et al., 2013. An update on serine deficiency disorders. *J. Inher. Metab. Dis.* 36, 613–619.
- Galada, C., Hebbar, M., Lewis, L., et al., 2018. Report of four novel variants in ASNS causing asparagine synthetase deficiency and review of literature. *Congenit. Anom.* 58, 181–182.
- Gataullina, S., Lauer-Zillhardt, J., Kaminska, A., Galmiche-Rolland, L., Bahi-Buisson, N., Pontoizeau, C., Ottolenghi, C., Dulac, O., Fallet-Bianco, C., 2016. Epileptic phenotype of two siblings with asparagine synthesis deficiency mimics neonatal pyridoxine-dependent epilepsy. *Neuropediatrics* 47 (6), 399–403.
- Gupta, N., Tewari, V.V., Kumar, M., et al., 2017. Asparagine synthetase deficiency-report of a novel mutation and review of literature. *Metab. Brain Dis.* 32, 1889–1900.
- Häberle, J., Görg, B., Rutsch, F., et al., 2006. Congenital glutamine deficiency with glutamine synthetase mutations. *N. Engl. J. Med.* 354, 1093–1094.
- Heng, H., Shi, X.-M., Scherer, S., et al., 1994. Refined localization of the asparagine synthetase gene (ASNS) to chromosome 7, region q21.3, and characterization of the somatic cell hybrid line 4AF/106/K015. *Cytogenet. Genome Res.* 66, 135–138.
- Hongo, S., Chiyo, T., Sato, T., 1994. Induction of asparagine synthetase by follicle-

- stimulating hormone in primary cultures of rat Sertoli cells. *Arch. Biochem. Biophys.* 313, 222–228.
- Jaccard, A., Gachard, N., Marin, B., et al., 2010. Efficacy of L-asparaginase with methotrexate and dexamethasone (AspaMetDex regimen) in patients with refractory or relapsing extranodal NK/T-cell lymphoma, a phase 2 study. *Blood* 117, 1834–1839.
- Kelley, L.A., Mezulis, S., Yates, C.M., et al., 2015. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* 10, 845–858.
- Palmer, E.E., Hayner, J., Sachdev, R., Cardamone, M., Kandula, T., Morris, P., Dias, K.R., Tao, J., Miller, D., Zhu, Y., Macintosh, R., Dinger, M.E., Cowley, M.J., Buckley, M.F., Roscioli, T., Bye, A., Kilberg, M.S., Kirk, E.P., 2015. Asparagine synthetase deficiency causes reduced proliferation of cells under conditions of limited asparagine. *Mol. Genet. Metab.* 116 (3), 178–186.
- Rota, E., Vallisa, D., Morelli, N., Scagnelli, P., 2016. Iatrogenic coagulopathy and the development of posterior reversible encephalopathy syndrome after L-asparaginase chemotherapy. *J. Clin. Imaging Sci.* 6, 7.
- Ruzzo, E.K., Capo-Chichi, J.-M., Ben-Zeev, B., et al., 2013. Deficiency of asparagine synthetase causes congenital microcephaly and a progressive form of encephalopathy. *Neuron* 80, 429–441.
- Schleinitz, D., Seidel, A., Stassart, R., Klammt, J., Hirrlinger, P.G., Winkler, U., Köhler, S., Heiker, J.T., Schönauer, R., Bialek, J., Krohn, K., Hoffmann, K., Kovacs, P., Hirrlinger, J., 2018. Novel mutations in the asparagine synthetase gene (ASNS) associated with microcephaly. *Front. Genet.* 9, 245.
- Seidahmed, M.Z., Salihi, M.A., Abdulbasit, O.B., Samadi, A., Al Hussien, K., Miqdad, A.M., Biary, M.S., Alazami, A.M., Alorainy, I.A., Kabiraj, M.M., Shaheen, R., Alkuraya, F.S., 2016. Hyperekplexia, microcephaly and simplified gyral pattern caused by novel ASNS mutations, case report. *BMC Neurol.* 16, 105.
- Stöckler, S., Holzbach, U., Hanefeld, F., et al., 1994. Creatine deficiency in the brain: a new, treatable inborn error of metabolism. *Pediatr. Res.* 36, 409–413.
- Sun, J., McGillivray, A.J., Pinner, J., et al., 2016. Diaphragmatic eventration in sisters with asparagine synthetase deficiency: a novel homozygous ASNS mutation and expanded phenotype. *JIMD Rep.* 34, 1–9.
- Wass, M.N., Kelley, L.A., Sternberg, M.J.E., 2010. 3DLigandSite: predicting ligand-binding sites using similar structures. *Nucleic Acids Res.* 38, W469–W473.
- Yamamoto, T., Endo, W., Ohnishi, H., et al., 2017. The first report of Japanese patients with asparagine synthetase deficiency. *Brain Dev.* 39, 236–242.
- Zhang, Y.P., Lambert, M.A., Cairney, A.L., et al., 1989. Molecular structure of the human asparagine synthetase gene. *Genomics* 4, 259–265.