Novel Imaging and Clinical Manifestations of Ndmsba Disorder Caused by a Homozygous Missense Variant of Plaa

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Research

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Abstract

Background

Phospholipase A-2-activating protein (PLAP) has essential roles in biological pathways. Neurodevelopmental disorder with progressive microcephaly, spasticity, and brain anomalies (NDMSBA) is a complex neurodevelopmental disease caused by defects in the *PLAA* gene (MIM: 603873). Herein, we aimed to detect the potential genetic factors contributing to the NDMSBA phenotype in a 2.5-year-old affected male in an Iranian consanguineous family.

Results

After meticulously performing neuroimaging and clinical examinations, due to heterogeneity of neurodevelopmental diseases, the proband was subjected to paired-end whole-exome sequencing (WES). The brain magnetic resonance imaging (MRI) revealed lissencephaly, polymicrogyria, severe subcortical, deep and deep white matter signal abnormalities, thinning of the corpus callosum, and severe vermis atrophy. Interestingly, we detected a novel homozygous missense variant, NM_001031689.3:c.2264A>G;p.(Asp755Gly) in the *PLAA* gene. To the best of our knowledge, this variant is the second one identified within the PUL domain (PLAP, Ufd3p, and Lub1p) of PLAP and also the sixth reported variant throughout the *PLAA* gene. *In silico* analyses underscored the pathogenicity of the variant.

Conclusions

The present study demonstrated severe cerebral and cerebellar white matter signal abnormalities, hypertelorism, strabismus, and drooling in the proband as the novel manifestation of NDMSBA that in turn caused by a novel likely pathogenic missense variant. Further studies are required to confirm how this variant contributes to NDMSBA.

Background

Neurodevelopmental disorder with progressive microcephaly, spasticity, and brain anomalies (NDMSBA) is an autosomal recessive disorder. Affected individuals are characterized by spastic quadriparesis, the infantile-onset of progressive microcephaly [1], failure to thrive, cognitive, and motor impairments [2]. Other clinical manifestations may include seizure that has been reported in 73.3% of affected patients and optic atrophy [3]. Furthermore, brain imaging may reveal impaired myelination and white matter signal lesions, and structural anomalies such as gyral abnormalities (lissencephaly and polymicrogyria), thin corpus callosum, cerebral or cerebellar atrophy [2]. Like almost any other neurodevelopmental disorder, NDMSBA is diagnosed by evaluating the presence of manifestations or abnormal behaviors in patients and may also be confirmed by genetic analysis.

NDMSBA is a rare genetic condition associated with homozygous loss of function mutations in the *Phospholipase A2-activating* (*PLAA*; OMIM: 603873) that encodes ubiquitin-binding protein [1]; up to now, only six mutations have been recorded in *PLAA* that are associated with this disorder. The *PLAA* gene, also known as *DOA1*, contains 14 exons and extends about 44 kbp on chromosome 9p21.2. According to the Human Protein Atlas [4], extensive-expression of *PLAA* is detectable within the cytoplasm and nucleus, underscoring the potential vital roles of the encoded protein—Phospholipase A-2-activating protein (PLAP)—in cellular events. PLAP plays a pivotal role in regulating the inflammatory response through activating the phospholipase A2, which catalyzes the release of arachidonic acid from membrane phospholipids [5]. PLAP is also a contributing factor to ubiquitin-dependent protein degradation via the ubiquitin-proteasome system and lysosomal degradation, and the turnover of synaptic membrane proteins important in neurotransmission and synaptic function [6]. Indeed, the PLAA family ubiquitin-binding domain (PFU) in the central part of PLAP binds to ubiquitin and a C-terminal PUL (PLAP, Ufd3p, and Lub1p) domain, which consists of six armadillo

domains that interact with the p97 enzyme which is involved in protein turnover and degradation [7, 8]. WD40 beta-propeller and PFU domain bind to ubiquitin with high-affinity and low-affinity, respectively [9, 10].

Ubiquitin signaling has been identified to play a critical role in synapse development and plasticity [3, 11]. Interruption of the Ub-mediated signaling pathway causes a variety of diseases such as cancer [12], immune deficiency [13], diabetes [14], and neurodegeneration [15]; the proteotoxic aggregation of ubiquitinylated proteins can result in neurodegenerative disorder [16]. It has been properly documented that PLAP is essential for neural function using two different mechanisms: firstly, the adjustment of post-endocytic trafficking of signaling receptors, which is imperative for neural development, and secondly sorting synaptic vesicle components during recycling that is essential for synaptic function [3]. Ubiquitin-dependent endolysosomal proteostasis at the synapse is also an indispensable factor for neuronal activity [3].

Herein, we analyzed the clinical features of a male patient from a consanguineous Iranian family, affected by NDMSBA disease. Using Whole-exome sequencing (WES), we introduced a novel variant, NM_001031689.3:c.2264A > G;p.(Asp755Gly), in the *PLAA* gene associated with NDMSBA. To the best of our knowledge, this variant is the second identified variant within the PUL domain (PLAP, Ufd3p, and Lub1p) of PLAP and also the sixth reported one throughout the *PLAA* gene. Moreover, novel neuroimaging and clinical features are reported in association with NDMSBA.

Results

Clinical presentation

A 2.5-year-old male born to a consanguineous family was referred to Myelin Disorder Clinic, Tehran, Iran due to neurodevelopmental delay in association with strabismus and esotropia; these conditions were since birth-time. According to initial neurological, imaging, and metabolic evaluations, a genetically-based intellectual disability was suspected. The proband (IV.2) was the second child of a family in which the family history of seizure was obvious, i.e. his aunt (III.1) suffered from seizure without remarkable details (Fig. 1a). However, the family never had any history of abnormal movements and seizures. His seven-year-old brother was apparently healthy.

The proband was born in a full-term pregnancy without any significant complications during prenatal and perinatal periods. His birth-time weight was reported 3.85 ± 0.01 kg that was in the normal range [17], while his head circumference (HC) was measured at 36.5 ± 0.1 cm (in comparison to 35.13 ± 1.45 cm as the average in the Iranian population [18]). Regarding the developmental milestones, he had a global developmental delay at 10 months of age as severe as he did not have complete neck holding, unable to sit without any aids, had limited fix and follow, got poor cognition inappropriate for his chronological age using Denver Developmental Screening Test (DDST), and had drooling. Besides that, on the first physical examination at this age, his weight was determined 7.0 kg (percentile 0.7; z-score: -2.4) and HC was 43 cm (3rd percentile; z-score: -1.9); and he was manifesting somehow dysmorphic features including protruding ears, micrognathia, high arch palate, and hypertelorism.

On the last physical examination era—that was performed at the age of 2 years—head circumference and weight were measured 47.0 ± 0.1 cm (14th percentile) and11.0 kg (percentile 23.33; z-score: -0.7), respectively. It seemed that the gross motor function milestones had been improving; although the patient was able to sit for a few minutes with assistance, he had no parachute reflex. In sum, the disease course was developed slowly without any neurologic regression. The patient's gross motor ability was calculated 3/5 based on the Gross Motor Function Classification System (GMFCS) score. Ophthalmic examination revealed persistent strabismus with remarkable esotropia, limited fix and follow and end gaze nystagmus, and normal pupils' reaction to light. His gag reflex was not satisfactory as he had drooling. Regarding the level of the patient's cooperation, further cranial nerves examination was normal. No abnormal movement or spasticity was recognized. Deep tendon reflexes were normal and plantar reflexes were bilaterally

downward. The skin was normal without any congenital or acquired rash. In general, the proband had poorly achieved developmental milestones at the age of 2 years. By the time the proband was subjected to be prescribed carnitine solution, omega 3 syrup, and supplementary vitamin E and D in addition to rehabilitation program during the follow-up period resulted in some progression in motor milestone.

All basic metabolic screening tests including thyroid function tests, urine organic acids profile, metabolic screen (MS/MS), serum ammonia, and lactate as well as additional ophthalmologic examinations including retinal, anterior, and posterior segments were normal. The first brain magnetic resonance imaging (MRI) that was done at the age of 18 months showed lissencephaly, polymicrogyria, extensive subcortical, periventricular and deep white matter signal abnormalities, thinning of the corpus callosum, and severe vermis atrophy (Fig. 1b-g).

Genetic analysis

Paired-end WES was performed for the DNA sample of the proband. After alignment and SNV calling, in total, 81,716 variants were found in the proband. To decrease the number of the variants as much and accurate as possible, the alleles with *minor allele frequency* > 1% were excluded using dbSNP150 [19, 20], 1000 Genomes Project [21], Exome Sequencing Project [22], and the genome aggregation database (gnomAD) [23]. According to variant functionality scores provided by Sorting Intolerant from Tolerant (SIFT) [24], PolyPhen-2 [25], and MutationTaster [26], the remained variants were prioritized. Furthermore, to this end, we used the important clinical features observed in the proband (e.g. lissencephaly and other brain anomalies) using ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) [27], Phenolyzer [28], and Face2Gene [29], only one relevant variant, NM_001031689.3:c.2264A > G;p.(Asp755Gly), was identified in the *PLAA* gene (Fig. 2a). We also checked the allele frequency in the Iranian population employing Iranome (http://www.iranome.ir/) [30] as a local database. The variant had not been reported in the Iranian population or any other sub-populations.

We analyzed the DNA samples from other available family members by Sanger sequencing that in turn confirmed the co-segregation of the missense variant of the *PLAA* gene. By assuming that the contributing genetic factor can be traced in the autosomal dominant mode of inheritance, we reanalyzed WES data, but no variant resulted from this supposition though (Fig. 2b).

In silico predictions

To evaluate the possible pathogenicity of the detected variant, locating on exon 14 of the *PLAA* gene, different databases were used including SIFT, PolyPhen-2, MutationTaster, and Provean (Fig. 3a) [31]. The conservation analyses performed at the nucleotide level by using the '2-Way Pseudogene Annotation Set' from the UCSC genome browser database [32] indicated that the variant was located in a highly conserved area in a wide range of species, specifically advanced cognitive animals and primates. Also, Consurf revealed that the variant was in a variable region in protein level (Consurf score ~ 1.0) (Fig. 3b). PDB file was built using protein structure homology-modeling online servers Phyre2 [33] and SWISS-MODEL [34]. The difference in flexibility and stability of wild-type and mutant residues suggest an unstable PLAP, which was verified and obtained using I-Mutant 3.0 sever [35] ($\Delta G < 0$). Finally, the possible effects of novel variant on PLAP and the protein structures were depicted by PyMOL [36] (Fig. 3c). Based on the American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG-AMP) guidelines [37], we also reclassified the variant into the "Likely Pathogenic" variant. The human variant and phenotypes were reported to ClinVar (accession number: SUB8886761) and Leiden Open Variation Database [38] (LOVD; individual ID: 00326505).

Discussion

Neurodevelopmental disorders can affect the growth and development of the central nervous system, resulting in abnormal brain function that in turn may impact learning ability, self-control, emotion, memory, and motor skills [39]. Both genetic and environmental factors may take part in a substantial role in these sorts of conditions [39].

Neurodevelopmental disorders could have great intellectual, emotional, physical, and economic consequences for affected individuals, their families, social groups, and communities [40].

Consistently, it has been identified that the *PLAA* mutations are responsible for NDMSBA which is a rare neurodevelopmental disorder wherein the patients experience many neurological and non-neurological symptoms [3] (Table 1).

Table 1
summary of clinical features

Group	Most important features	Hall *et al.*	Zaccai *et al.*	Dai *et al.*	Present case	Total
Demographic features	origin	Pakistani	Israeli	Chinese	Iranian	-
	No. affected individuals	8	7	2	1	18
	Male: female	6:2	5:2	1:1	M	13:5
	PLAA mutant genotype	c.68G > T p. (Gly23Val) c.68dupG p. (Leu24profs*55)	c.2254C > T p. (Leu754Phe)	c.829T > C p. (Cys277Arg) c.1049A > T p. (Glu350Val)	c.2264A > G p. (Asp755Gly)	-
	Age of onset	At birth (1st week)	2–4 month	22–24 days	At birth	-
	Age of death	1 2 days-6 years	Live at 2–34 years	Live at 30 month-3 years	Alive (3 years)	-
Brain abnormalities	Progressive microcephaly	8/8	7/7	NK/YES	YES	17/18(94.4%)
	Enlargement of ventricles	1/1	3/3	1/1	YES	6/6(100%)
	White matter atrophy	UK	5/5	1/1	YES	6/6(100%)
	Abnormal cortical gyration	6/6	1/1	1/1	YES	9/9(100%)
	Thin corpus callosum	5/5	7/7	UK	YES	13/13(100%)
	Lissencephaly	UK	UK	UK	YES	1/1(100%)
	seizure	6/6	3/7	2/2	NO	11/16(68.7%)
	pachygyria	1/8	UK	UK	YES	2/8(25%)
Structural abnormalities	Craniofacial deformity	8/8	1/1	1/1	YES	10/11(90%)
	Micrognathias	1/8	UK	UK	YES	2/9(22.2%)
	High palate	2/5	UK	1/1	YES	3/8(37.5%
	Low set ears	3/5	UK	1/1	YES	4/7(57%)
Eye abnormalities	Nystagmus	4/4	1/1	1/1	YES	7/7(100%)
	strabismus	UK	UK	UK	YES	1/1(100%)
	Hypertelorism	UK	UK	UK	YES	1/1(100%)
	Optic atrophy	4/5	1/1	UK	YES	6/7(85%)

Group	Most important features	Hall *et al.*	Zaccai *et al.*	Dai *et al.*	Present case	Total
Behavioral problems	Failure to thrive	8/8	7/7	2/2	YES	18/18(100%)
	Cognitive and motor impairment	8/8	7/7	2/2	YES	18/18(100%)
	Poor sucking and swallowing	4/4	UK	2/2	YES	7/7(100%)
	Could not fix and follow	3/3	UK	2/2	YES	6/6(100%)
	drooling	UK	UK	UK	YES	1/1(100%)

The *PLAA* gene encodes PLAP protein that is highly expressed in the brain, nerve, and skeletal tissues [41]. The *PLAA* gene contains 14 coding exons, which yields to 2 coding transcripts that the canonical transcript translated to a 408 amino acid protein. PLAP has been shown as a contributing factor to various important biological mechanisms. This protein consists of three conserved domains including a seven-bladed WD40 beta-propeller, PLAA family ubiquitin-binding domain (PFU) in central, and a PUL domain [42]. Ubiquitin-dependent molecular chaperone p97, also known as Transitional Endoplasmic Reticulum ATPase (TER ATPase) or Valosin-Containing Protein (VCP), is an AAA ATPase that is critical for protein turnover and degradation [43]. The C-terminus of the p97 chaperone, which interacts with the PUL domain, has a crucial role in folding/unfolding substrate proteins [44]. PLAP, e.g. PLA1 and PLA2, also functions as a regulator for the activation and production of phospholipases [45]. Activated PLA2 hydrolyses membrane phospholipids into arachidonic acid that is *per se* used as a substrate to produce leukotrienes and prostaglandins [46].

Whole-exome Sequencing has been developed into an efficient and cost-effective tool to identify new variants and genes for rare Mendelian disorders [47]. A shred of accumulating evidence about the clinical and mutational spectrum of known and unknown diseases can be ascribed to these techniques [47]. In this study, we subjected a 2.5-year-old Iranian boy, who was referred due to neurological and developmental delay, to the paired-end WES that revealed a novel homozygous missense variant— NM_001031689.3:c.2264A > G;p.(Asp755Gly)—in the PUL domain of the *PLAA* gene in association with the NDMSBA disease. Using different *in silico* tools, we also provided evidence approving the contributory role of this variant to NDMSBA. To date, only 5 families (17 patients) and a total of 5 mutations in the *PLAA* gene have been reported [48–50]. Our reported variant is the 6th one that has been identified in this gene in association with NDMSBA and also the second one in the PUL domain of PLAP.

PUL domain consists of 15 tightly packed a-helices that form a structure called ‘Armadillo domain’ that creates a single rigid structure found in several proteins such as importin-a, β-catenins, and Hsp70 [51]. Such Armadillo repeats form banana-shaped domains that are important for the binding of other molecules [51]. The C-terminus of p97 binds to such Armadillo repeats, which is important for releasing the denatured proteins in the endoplasmic reticulum-associated protein degradation from ER and bring them to proteasome [52]. Both mutations in Hall's [50] and Dai's [48] reports were located in the ubiquitin-biding domain of PLAP that plays a crucial role in *PLAA's* ubiquitin signaling functions during synapse development and plasticity, known as the WD40 domain. However, Zaccai *et al.* showed that the mutation was located in the PUL domain [49]. In our case, the mutation is also located in such a domain that mediates binding to p97. In addition to the common phenotype of the NDMSBA condition, we observed some novel clinical features like hypertelorism, strabismus and end-gaze nystagmus in the patient; the result of the brain MRI also indicated extensive secondary deep white matter signal changes, which can be considered as the novel imaging manifestation regarding the

severity of white matter signal changes. We believe that the intensity of clinical manifestations is due to the mutated domain—PUL domain.

Although the case reported in 2017 by Hall *et al.* had the most severe clinical symptoms, the MRI result did not reveal any abnormality in the brain white matter. By literature reviewing, we found that in cases affected by NDMSBA, brain white matter atrophy was prominent in the MRI, while secondary deep and subcortical white matter signal changes had not been observed in any affected individuals. Furthermore, in the last case reported by Dai *et al.* in 2019, one Chinese family with two affected individuals was studied [2]; using brain MRI that had been performed on the proband at the age of 23 days, no abnormality in the brain white matter was detected. Regarding our case, brain white matter signal changes were a novel finding in brain MRI findings with *PLAA* gene mutation, which has not been reported yet. Beyond that, strabismus, hypertelorism, were detected that had not been identified in any NDMSBA cases.

In silico analysis showed that the novel variant—p.(Asp755Gly)—may make PLAP unstable, a hypothesis which was postulated using I-Mutant 3.0 sever ($\Delta\Delta G < 0$). The size difference between wild-type and mutant residue increase this notion that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did; also, Glycine is very flexible and can disturb the required rigidity of the protein at this position which may affect the function of the protein. Furthermore, conservational analysis in both nucleotide and amino acid levels, showed that the affected residue is highly conserved in primates—the animals with a higher rate of cognitive and brain function [47].

Taken together, it can be suggested that p.(Asp755Gly) may reduce the stability or proper function of the PLAP. Regarding the importance of neurodevelopmental disorders, however, more functional studies are needed to understand the exact impact of p.(Asp755Gly) on NDMSBA. We are optimistic that such studies will help us to know better brain development in the future.

Conclusions

In sum, by using paired-end WES, we reported *PLAA* as the gene implicated in NDMSBA in a male Iranian patient. To our knowledge, this study reports the 6th variant of the *PLAA* gene worldwide which shows some novel clinical features such as, secondary deep white matter changes, strabismus, hypertelorism, and drooling. Before being used in genetic counseling, we strongly recommend doing functional studies by using animal models to show the molecular mechanisms underlying the pathogenesis of this novel variant. We believe that the identification of novel variants in PLAA may also yield new insights into the etiology of NDMSBA.

Methods

Subjects and Ethics consideration

We enrolled three members of a consanguineous Iranian family. The proband was a 2.5-year-old boy suspected to NDMSBA with initial complaints of neurological and developmental delay. His parents were first-cousins who had 2 offspring one healthy and an affected sibling. The study protocol was approved by the local medical ethics committee of Tarbiat Modares University, Tehran, Iran. Written consent was achieved from all individuals present in this study and their legal representatives. They also were informed that all clinical, neuroimaging, and WES data would be used only for scientific research and not for any other purposes. All of the patient's clinical information and medical histories were collected at the Department of Medical Genetics, DeNA Laboratory, Tehran, Iran and Myelin Disorders Clinic, Pediatric Neurology Division, Tehran University of Medical Sciences, Tehran, Iran.

DNA extraction and Whole-exome sequencing

Genomic DNA was extracted from the whole blood of the proband and available family members using the standard phenol-chloroform method [53]; the concentration of DNA was measured by Thermo Scientific™ Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA). Subsequently, around 2 μg of proband's genomic DNA was subjected to paired-end WES as previously reported [47]. The DNA sample was sequenced on an Illumina HiSeq 4000 to obtain an average coverage depth of ~ 100×. Bioinformatics analyses and filtering steps were taken forward according to the previous studies [47, 54].

In order to validate the candidate variant and survey the segregation, Sanger sequencing in forward and reverse direction was carried out. To this end, the exon and the flanking intronic regions were amplified using PCR between the primers 5̄-GCCAGGCAGGACAAAAACTC-3' and 5'-TTTCTGTTTCCCCTCCCCAC-3', designed using Primer3.0 plus online tool [55]. Finally, all sequences were analyzed using CodonCode aligner software version 6.0.2 (CodonCode Corp., Centerville, MA).

Prediction of single point variation on protein stability

The non-synonymous substitutions do not always have an equal effect on protein folding and function [56]. The impact can be defined by the effects of variants on the protein folding structure and then stability [57]. In order to predict how the reported variant—p.(Asp755Gly)—affects protein stability, the I-Mutant 3.0 was utilized. This web-based tool (http://gpcr2.biocomp.unibo.it/~emidio/I-Mutant3.0) predicts the effect of a single nonsynonymous point mutation on protein stability from protein sequence by computing the ΔΔG values of protein variant [35]. Moreover, the I-Mutant3.0 is trained to predict the thermodynamic free energy change upon single point variations in protein sequences. Furthermore, the DynaMut server [58] was used to predict the impact of the mutation on protein flexibility, conformation, and stability.

Three-dimensional structure modeling

The identified variant, p. (Asp755Gly), is located within the PUL domain of PLAP. Using ScanProsite [59], the protein families and domains were analyzed, and also sequence alignments were recruited using ClustalW (http://www.ebi.ac.uk/clustalw). The PDB structure file was built based on the Phyre2 [33] and SWISS-MODEL [34], consequently, the novel variant's possible effects on PLAP and the protein structures were depicted by PyMOL [36, 60].

Abbreviations

NDMSBA: Neurodevelopmental disorder with progressive microcephaly, spasticity, and brain anomalies

PLAA*: Phospholipase A2-activating*

PLAP: Phospholipase A-2-activating protein

WES: Whole exome sequencing

HC: head circumference

DDST: Denver Developmental Screening Test

MRI: magnetic resonance imaging

gnomAD: genome aggregation database

SIFT: Sorting Intolerant from Tolerant

ACMG-AMP: American College of Medical Genetics and Genomics-Association for Molecular Pathology

PFU: PLAA family ubiquitin-binding domain

TER ATPase: Transitional Endoplasmic Reticulum ATPase

Declarations

Ethics approval and consent to participate

The study protocol was approved by the local medical ethics committee of Tarbiat Modares University, Tehran, Iran. The written informed consent was received from each patient and guardian and they also provided a signed written consent form to publish all personal and medical details. All methods were performed in accordance with the relevant guidelines and regulations of the Declaration of Helsinki.

Consent for publication

The informed consent to publish was obtained from each patient and guardian.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The variant and pertinent phenotypes caused by a mutation in *RAB3GAP1* are accessible at ClinVar (SUB8886761) and Leiden Open Variation Database (LOVD; individual number: 00326505).

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

ART and MG conceived and planned the experiments. AD carried out the experiments. MG, AD, and ER planned and carried out the data analyses. FB contributed to sample preparation. AD, ER, and MG contributed to the interpretation of the results. AD took the lead in writing the manuscript. ART and MR carried out clinical analyses. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Figures

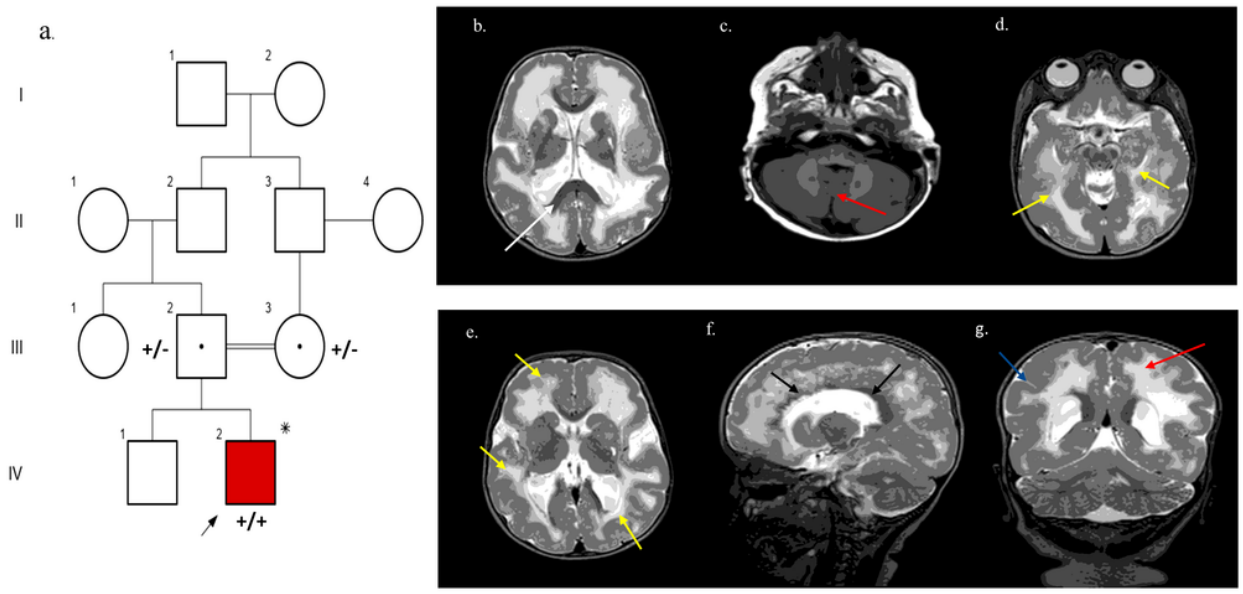

Figure 1

Pedigree and MRI findings in the family. a) Pedigree of the consanguineous Iranian family consists 4 generations. In this figure, the squares and circles indicate males and females, respectively; the black arrow shows the proband. The heterozygosity for the variant is shown by +/−, while homozygosity for the novel variant is shown by +/+. An asterisk (*) indicates the sample which was selected for applying whole-exome sequencing. b) Axial T2-Weighted image of brain MRI at the age of 18 months, shows extensive deep, periventricular and subcortical white matter signal changes, Lissencephay and abnormal disorganized gyral pattern and colpocephaly (white arrow). c) Axial T1-Weighted image shows mild vermis atrophy (red arrow). d and e) Axial T2-Weighted images represent anomalies in myelination and deep/subcortical white matter signal changes (yellow arrows). f) Sagittal T2-Weighted image shows thin corpus callosum (black arrows). g) Coronal T2-Weighted image of brain MRI shows pachygyria/polymicrogyria (blue arrow-right side) and confirms peri-dentate white matter signal changes (red arrow).

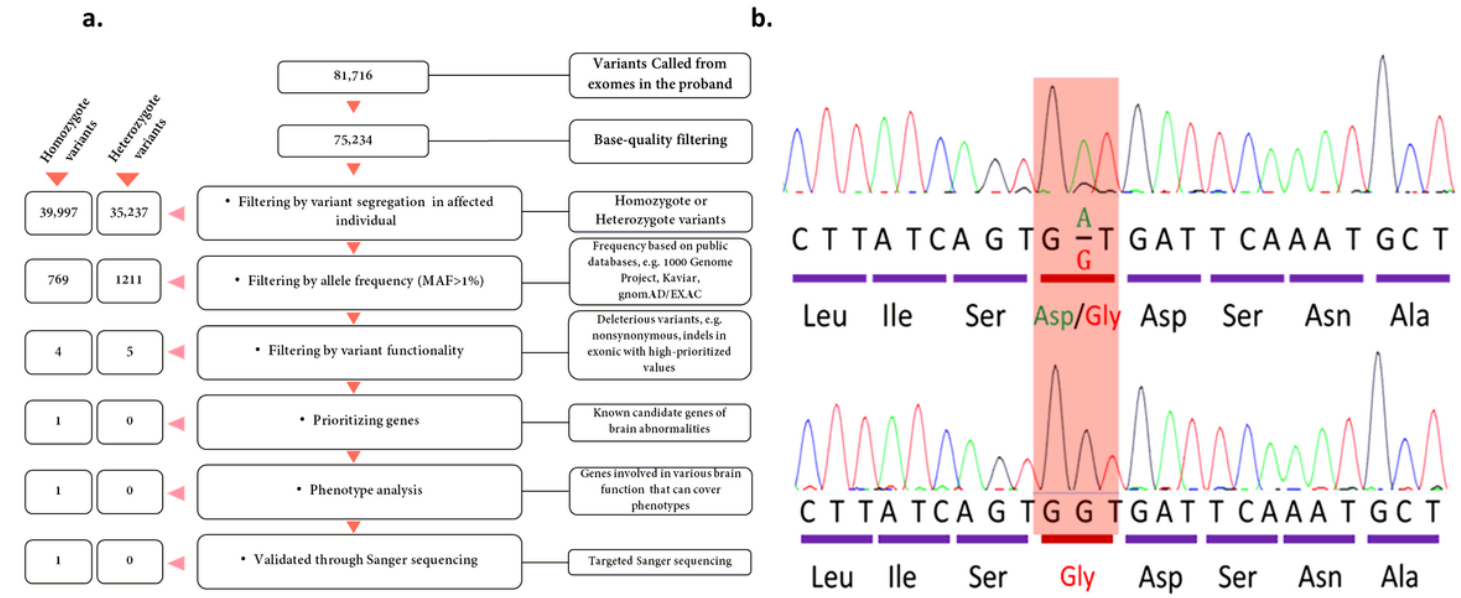

Figure 2

whole-exome sequencing data analyses and also the chromatograms. a) filtering steps that had been taken forward to analyze/filter the derived data. the data were reanalyzed by assuming the autosomal dominant inheritance, but no convincing finding was resulted in. b) chromatograms confirmed the homozygosity and heterozygosity for the novel variant in the family.

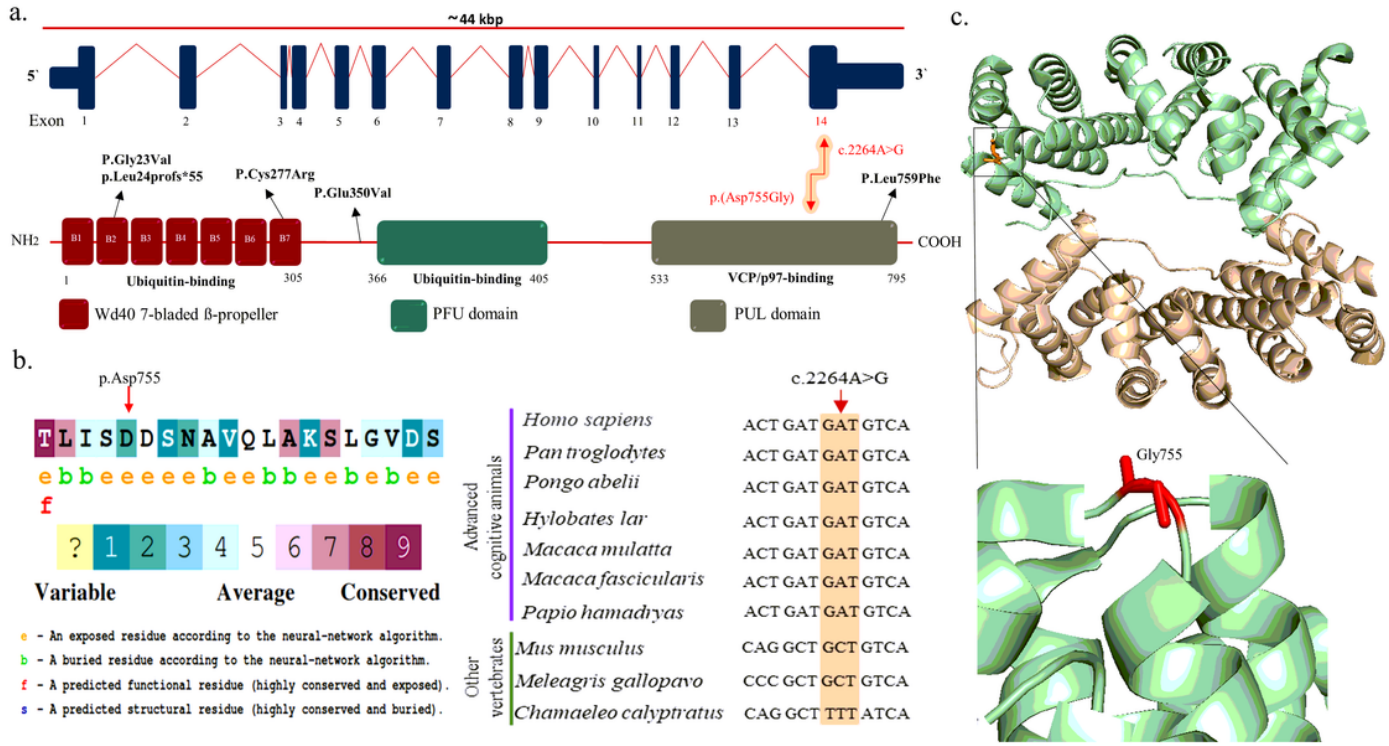

Figure 3

a) schematic structure of the PLAA gene and the encoded protein. This gene spans ~44 kbp and the encoded protein is comprised of 795 amino acids and three different domains (are shown by different colors). The red arrow indicates the position of the variant detected in this study; the variant is located in exon 14 that per se encodes some parts of the PUL domain region of PLAP. PLAP involves three different parts: WD40 domain contains in seven-bladed beta-propeller WD40 repeats in N-terminus, the PFU domain which is in the central part of PLAP and appears to be unique to the PLAA family of proteins, and C-terminus part of the protein which directly interacts with p97, an enzyme from the AAA-ATPase family of molecular chaperones. b) The amino acid sequence of PLAP is colored based on conservation scores by the ConSurf database. ConSurf is a repository for evolutionary conservation analysis of the known/unknown protein structure according to the phylogenetic relations between homologous sequences as well as amino acid's structural and functional importance. The results show that Gly755 is located in a variable exposed region based on the comparison performed among the candidate vertebrae and invertebrates. In the nucleotide level, the '2-Way Pseudogene Annotation Set' from the UCSC genome browser indicates the high conservation of the region embarrassing the variant site in advanced cognitive animals, i.e. primates. c) The three-dimensional structure of the PLAP is shown. The structure was made using PyMOL. The mutant residue is shown by red.