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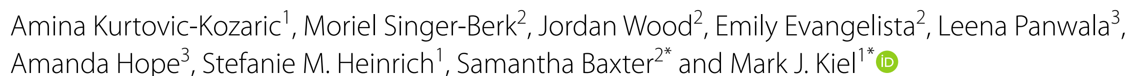
An estimation of global genetic prevalence of PLA2G6-associated neurodegeneration



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Abstract

Background PLA2G6-associated neurodegeneration (PLAN) comprises three diseases with overlapping features: infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (atypical NAD), and PLA2G6-related dystonia-parkinsonism. INAD is an early onset disease characterized by progressive loss of vision, muscular control, and mental skills. The prevalence of PLA2G6-associated diseases has not been previously calculated.

Methods To provide the most accurate prevalence estimate, we utilized two independent approaches: database-based approach which included collecting variants from ClinVar, Human Gene Mutation Database (HGMD) and high confidence predicted loss-of-function (pLoF) from gnomAD (Rare Genomes Project Genetic Prevalence Estimator; GeniE), and literature-based approach which gathered variants through Mastermind Genomic Search Engine (Genomenon, Inc). Genetic prevalence of PLAN was calculated based on allele frequencies from gnomAD, assuming Hardy–Weinberg equilibrium.

Results In the PLA2G6 gene, our analysis found 122 pathogenic, 82 VUS, and 15 variants with conflicting interpretations (pathogenic vs VUS) between two approaches. Allele frequency was available for 58 pathogenic, 42 VUS, and 15 conflicting variants in gnomAD database. If pathogenic and/or conflicting variants are included, the overall genetic prevalence was estimated to be between 1 in 987,267 to 1 in 1,570,079 pregnancies, with the highest genetic prevalence in African/African-American (1 in 421,960 to 1 in 365,197) and East-Asian (1 in 683,978 to 1 in 190,771) populations.

Conclusion Our estimates highlight the significant underdiagnosis of PLA2G6-associated neurodegeneration and underscores the need for increased awareness and diagnostic efforts. Furthermore, our study revealed a higher carrier frequency of PLA2G6 variants in African and Asian populations, stressing the importance of expanded genetic sequencing in non-European populations to ensure accurate and comprehensive diagnosis. Future research should focus on confirming our findings and implementing expanded sequencing strategies to facilitate maximal and accurate diagnosis, particularly in non-European populations.

Keywords PLA2G6-associated neurodegeneration, INAD, Genetic prevalence

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Background

PLA2G6-Associated Neurodegeneration (PLAN) comprises three autosomal recessive diseases with overlapping features: infantile neuroaxonal dystrophy (INAD, OMIM #256600, ORPHA: 35069), atypical neuroaxonal dystrophy (atypical NAD, OMIM #610217)), and PLA2G6-related dystonia-parkinsonism (PARK14, OMIM #612953), which are caused by pathogenic

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variants in the PLA2G6 gene [1–4]. PLA2G6 encodes an enzyme called phospholipase A2 group VI, which plays a vital role in lipid metabolism and maintaining the integrity of cell membranes, especially in nerve cells [2]. Pathogenic variants in the *PLA2G6* gene lead to the accumulation of abnormal lipids in neuronal tissues. This lipid accumulation, particularly in axons, can cause structural damage and impair the transmission of nerve signals.

INAD is an early onset disorder and is associated with severe symptoms including ataxia, mental and motor deterioration, hypotonia, progressive spastic tetraparesis, visual impairments, bulbar dysfunction, and extrapyramidal signs [4]. These symptoms are progressive and INAD patients usually succumb to the disease before their 10th birthday. Atypical cases, such as aNAD and PARK14, have a later onset of symptoms, including progressive dystonia and parkinsonism. Cerebellar atrophy is a characteristic symptom in both INAD and aNAD [5, 6].

Iron accumulation in the basal ganglia has been observed in some INAD, aNAD, and PARK14 patients, leading to the classification of these diseases as neurodegeneration with brain iron accumulation 2 (NBIA2) [7]. The neuropathological hallmarks of PLAN include the formation of spheroid structures containing membranes, α-synuclein, and ubiquitin, known as tubulovesicular structures (TVSs) [8]. Additionally, Lewy bodies and phosphorylated tau-positive neurofibrillary tangles have been observed in the nervous system of PLAN patients [9].

The diagnosis of INAD includes a combination of clinical evaluation, neurological examination, electrophysiology, imaging tests, as well as skin biopsies revealing axonal swellings and spheroid bodies in pre-synaptic terminals within the central or peripheral nervous system [10, 11]. Confirmatory biopsies often require multiple attempts, resulting in prolonged waiting periods for families seeking a diagnosis. Advancements in genetic testing paired with decreasing cost of gene and genome sequencing have expedited the diagnostic process. Families now receive diagnoses more quickly, sometimes within a year of initial symptom onset [12], but challenges remain. Although genome sequencing (GS) has the capacity to identify large and complex variants, interpreting these variants continues to pose difficulties, particularly when dealing with non-coding variants [13]. Challenges associated with identifying the causative variants from exome sequencing (ES)/GS can be broadly categorized into two groups: 1) interpretation issues, such as variants of uncertain significance (VUS) in known or novel disease genes and non-coding variants, and 2) detection challenges, including the absence of a second variant in recessive disorders or the presence of causative variants in regions that are difficult to sequence or are masked due to biases in reference genomes and genomic datasets. Some very rare variants may be unobserved in large population databases and do not have associated MAF (minor allele frequency) [14].

These challenges in variant identification and interpretation affect the diagnosis and the estimation of prevalence of PLA2G6-related disorders, which is currently unknown, but ranges from ~1 in 1,000,000 from the Rare Genomics Institute to 1 in 1-2 million children [15–18].

The goal of this study was to improve previous estimates by performing two independent approaches to identify pathogenic variants associated with PLA2G6-associated neurodegeneration: 1) an extensive literature review utilizing Mastermind Genomic Search Engine [19] to collect additional variants and supporting evidence, and 2) extracting data from publicly existing databases such as ClinVar, HGMD and gnomAD [18]. For both approaches, we employ standard variant interpretation to evaluate the level of evidence supporting the inclusion of variants in the estimate of disease prevalence [20–22].

Methods

Study design and participants

To evaluate the genetic spectrum of the PLA2G6 variants, a comprehensive search was performed to identify all previously reported disease-causing *PLA2G6* gene variants using two independent approaches. One approach used Mastermind Genomic Search Engine (Genomenon, Inc.) exclusively, while the second approach developed by the Rare Genomes Project's Genetic Prevalence Study combined several publicly available resources: gnomAD, HGMD, and ClinVar.

The inclusion criteria for targeted literature review conducted through Mastermind were: 1) studies published as full-text publications; 2) English or other languages were included; 3) single nucleotide variants and indels; 4) published until 30 April 2023. The terms included in the search strategy in Mastermind were defined by automated indexing using genomic language processing [18]. Mastermind search for published variants in *PLA2G6*, including single-nucleotide variants (SNVs) and indels, yielded 379 articles. Once articles were collected, variants were extracted and duplicate variants removed. Benign variants were removed from further analysis (Fig. 1). The remaining variants were curated and classified following current ACMG/AMP Guidelines for Sequence Variant Interpretation and ClinGen specifications [21, 22].

The Rare Genomes Project's Genetic Prevalence Study approach combined variants found in several databases: ClinVar (pathogenic, likely pathogenic and conflicting where one of the submissions listed the variant as pathogenic or likely pathogenic), HGMD (DM) and gnomAD

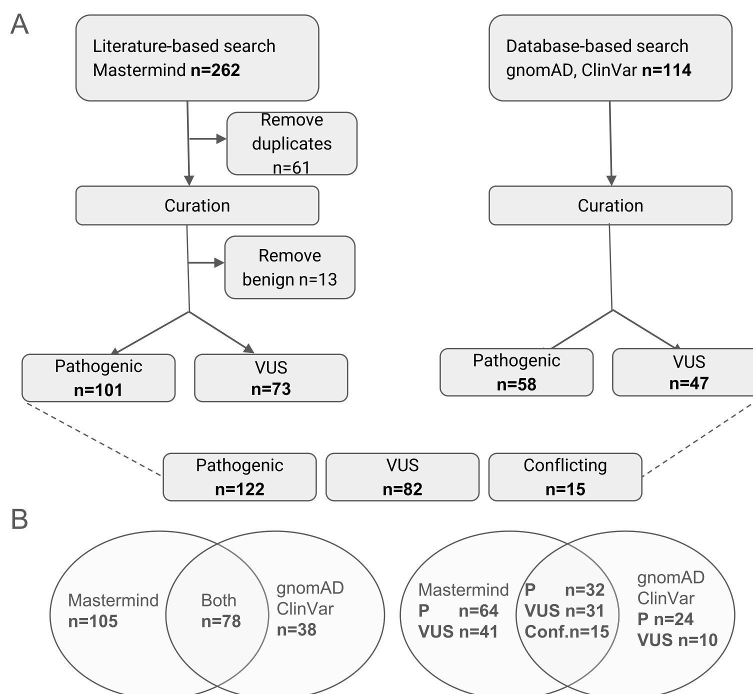

Fig. 1 **A** To estimate genetic prevalence of PLA2G6-associated neurodegeneration, we have utilized a literature-based approach (left) and database-based approach (right). In total, 122 pathogenic, 82 VUS, and 15 conflicting variants were curated and classified. **B** Pathogenic and VUS variants recorded by two independent approaches

high confidence (HC) predicted loss-of-function (pLoF). Benign (B) and likely benign (LB) variants were removed. Variants that were labeled as conflicting were removed if the conflict was LB vs L, VUS vs LB/B, while conflicting LP (likely pathogenic) vs P (pathogenic) vs VUS were kept for further classification following current ACMG/AMP Guidelines for Sequence Variant Interpretation and ClinGen specifications [20]. Additionally, high confidence predicted loss-of-function (pLoF) variants in gnomAD, including those in the list above, underwent a pLoF curation [15]. The pathogenic and likely pathogenic variants were further selected, as illustrated in Fig. 1. The variants were extracted from gnomAD v2.1.1 along with their overall and population-specific allele frequencies [23]. gnomAD v2.1.1 contains 125,748 exome sequences and 15,708 whole-genome sequences from 141,456 unrelated individuals. It is of note that gnomAD attempts to remove cohorts that were recruited for pediatric disease. Given the substantial morbidity associated with PLA2G6-associated neurodegeneration, we made the assumption that the gnomAD dataset does not include any individuals with this disease. If homozygotes were detected, they were removed during the curation process, as described below.

Each variant was standardized using the GRCh37/hg19 genome build and the canonical transcript— NM_003560.4—as well as the nomenclature guidelines set by the Human Genome Variation Society (HGVS) [21]. As described in Fig. 1, the curation process involved manually curating selected variants according to the standards set by the American College of Medical Genetics and Association of Molecular Pathologists (ACMG/AMP) into benign (B), likely benign (LB), variant of unknown significance (VUS), likely pathogenic (LP) and pathogenic (P) [22]. This interpretation process considered clinical and functional studies from the literature, population frequencies derived from gnomAD v2.1.1, computational predictions of the effect of missense variants derived from REVEL, PolyPhen-2, MutationTaster2, and SIFT, and computational predictions of splicing defects for single nucleotide variants derived from dbscSNV [24–28]. Once variants were identified and curated from two independent approaches, they were combined into a single database.

Allele frequency and prevalence calculations

Genetic prevalence estimates were produced according to a previously described method [29]. The disease prevalence was estimated by using the observed allele frequency of a pathogenic/likely pathogenic variant in the gnomAD database as the direct estimator for qi, as described previously in Shourick et al [30]. Genetic and

disease prevalences were calculated for all variants, pathogenic, and pathogenic plus conflicting variants. The overall (or population-specific) allele frequency was summed across all selected variants and then used within the Hardy–Weinberg equation to calculate the carrier frequency (2pq) and the frequency of a disease-causing genotype (q^2) [24].

Results

Two independent approaches were utilized to improve yield of pathogenic PLA2G6 variants to estimate genetic and disease prevalence of PLA2G6-associated neurodegeneration. Comprehensive retrieval of pathogenic variants using Mastermind resulted in the identification of 379 articles, which yielded 262 variants. As shown in Fig. 1A, 61 variants were removed because they were duplicates. The remaining 201 variants were curated and classified according to ACMG/AMP guidelines [22], which resulted in removal of 13 benign variants. After the curation, 101 pathogenic variants and 73 VUS were identified and added to the database for further analysis. Using RGP's Genetic Prevalence Study approach based on publicly available databases, 114 variants were identified (Fig. 1A). The variants were curated according to ACMG/AMP guidelines and pLoF curation framework, yielding 58 likely pathogenic/ pathogenic variants and 47 VUS, which were added to the database of PLA2G6 variants. Once the curation process was completed In summary, the variants collected using the two approaches were combined, yielding a total of 279 variants (i.e. 159 (101 + 58) pathogenic variants and 120 (73 + 47) VUS). When duplicate variants were removed, a single database was formed with 122 pathogenic, 82 VUS, and 15 variants with conflicting interpretations between the approaches. This includes all variants, including those variants that have zero allele frequency in gnomAD (Supplementary Table 1). In total, 115 variants had allele frequency reported in gnomAD. The overlapping variants between two approaches are presented in Fig. 1B.

Assessing the contribution of the most common pathogenic and VUS PLA2G6 variants to the genetic prevalence estimate with non-zero allele frequency in gnomAD, revealed that 15 variants accounted for 50% of the total allele frequency, namely p.Ala147Thr, p.Arg6Cys, p.Arg677Leu, p.Tyr790*, p.Arg741Gln, p.Arg301Cys, p.Arg70Gln, p.Val421AlafsTer26, p.Asp331Tyr, p.Leu129Pro, p.Arg710His, p.Arg635*, p.Arg635Gln, and p.Arg37* (Fig. 2A). The top five

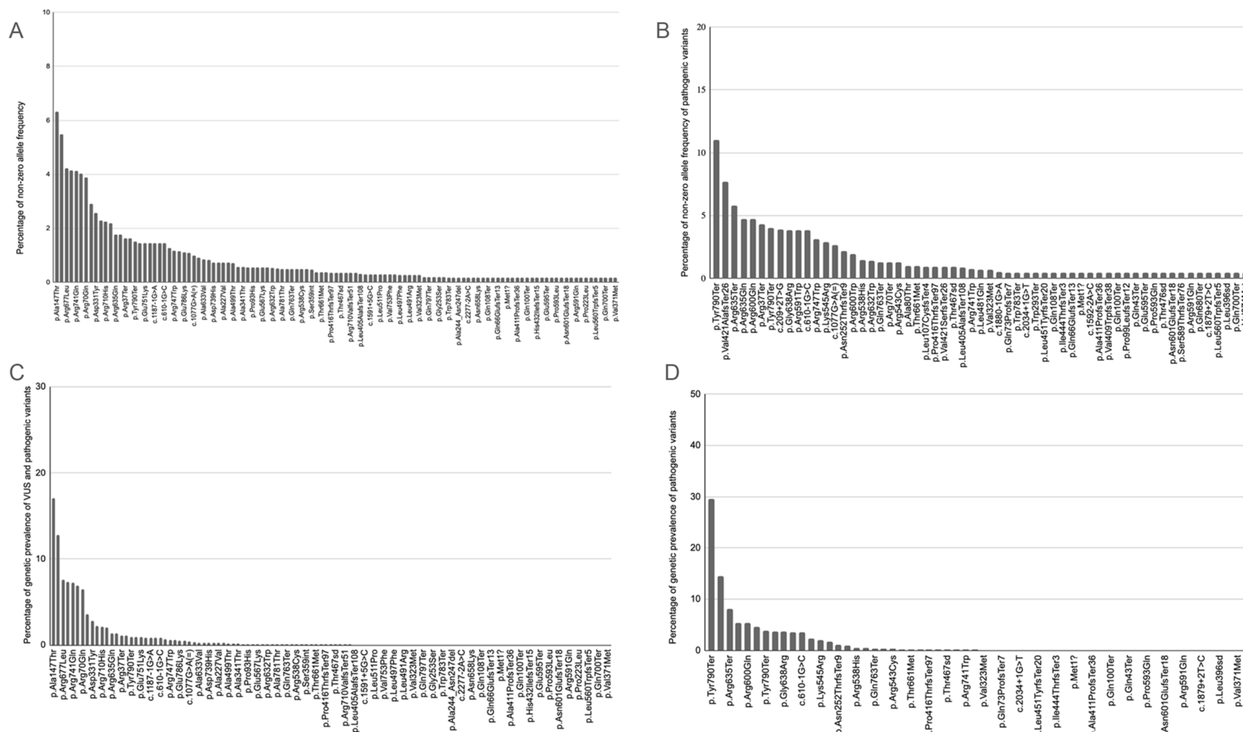

Fig. 2 Percentage of allele frequency in PLA2G6 gene. **A** Percentage of allele frequency of all pathogenic and VUS variants in PLA2G6 with non-zero allele frequencies. **B** Percentage of allele frequency of all pathogenic variants in PLA2G6 with non-zero allele frequencies. **C** Percentage of genetic prevalence of VUS and pathogenic variants in PLA2G6 with non-zero allele frequencies. **D** Percentage of genetic prevalence (q^2) of pathogenic variants in PLA2G6 with non-zero allele frequencies

most frequent variants alone (p.Ala147Thr, p.Arg6Cys, p.Arg677Leu, p.Tyr790*, and p.Arg741Gln) accounted for 24% of all variants. If pathogenic variants with non-zero allele frequency are analyzed (*n*=58), 9 variants accounted for 50% of the total allele frequency, namely p.Tyr790* (c.2370T>G), p.Val421AlafsTer26, p.Arg635Ter, p.Arg635Gln, p.Arg600Gln, p.Arg37Ter, p.Tyr790* (c.2370_2371del), c.209+2T>G, and Gly638Arg (Fig. 2B). Besides allele frequencies, cumulative percentage of genetic prevalence of VUS and pathogenic variants (Fig. 2C) and pathogenic variants only (Fig. 2D) with non-zero allele frequencies has also been presented to show the share of each variant in genetic prevalence of PLA2G6-associated neurodegeneration and neuroaxonal dystrophy.

The most common pathogenic variant, p.Tyr790*, accounted for 11% of all non-zero allele frequency pathogenic variants; it is a truncating variant that has been identified by both ClinVar and Mastermind databases. The second most common variant, p.Val421AlafsTer26, with 7.7% allelic frequency, is a frameshift mutation leading to a truncated protein, which was an unpublished variant found in ClinVar only. The third most common variant , p.Arg635*, with 5.8% allele frequency, is also a truncated variant found in both literature and ClinVar. The fourth and fifth most common variants, p.Arg635Gln and p.Arg600Gln, are both found at 4.7% allele frequency and are missense variants located in the Patatin-like phospholipase domain.

Previously it has been shown that disease prevalence of rare diseases is directly related to the number of publications [25]. Hence, we plotted the allelic frequencies of PLA2G6 pathogenic variants with the number of articles in Mastermind (Fig. 3). We found a positive correlation between the number of publications and PLA2G6 allele frequencies. The seven most common pathogenic variants are labeled, showing a positive correlation between allele frequency and the number of articles published.

The estimated heterozygous carrier frequency of PLA2G6 variants was found to vary between specific populations in gnomAD, as shown in Fig. 4, with the African/African American population having a significantly higher carrier frequency than other populations. The lowest carrier frequency was observed in Ashkenazi Jewish and European populations.

The overall genetic prevalence of pregnancies with recessive pathogenic variants was estimated to be from 6 to 10 pregnancies per 100,000, with the highest genetic prevalence in African populations between 24 to 27 pregnancies per 100,000 and East Asian between 15 to 52 pregnancies per 100,000 (Table 1). As shown in Table 1, the number of VUS variants varies significantly among populations, especially between East Asian and South Asian populations. If there are 128 million births per year worldwide, 82 (pathogenic only) to 127 (pathogenic and conflicting) births with PLA2G6-associated neurodegeneration are expected annually. If we estimate the world population to be 8 billion, then the conservative estimate is that 5,1590 individuals are expected to have PLA2G6-associated neurodegeneration worldwide, not accounting for the shortened life expectancy of the INAD phenotype. Due to the lack of genotype-phenotype correlation between the variants and the specific subtypes of PLA2G6-related diseases, we could not use variant specific AFs to calculate the approximate number of individuals with INAD, aNAD and PARK14. Instead we used the percentage of the subtypes in the INADcure Foundation's contact registry as a proxy for the percentage breakdown of disease spectrum. Using the rates of disease in their registry INAD (80.7%), aNAD (16.1%) and PARK14

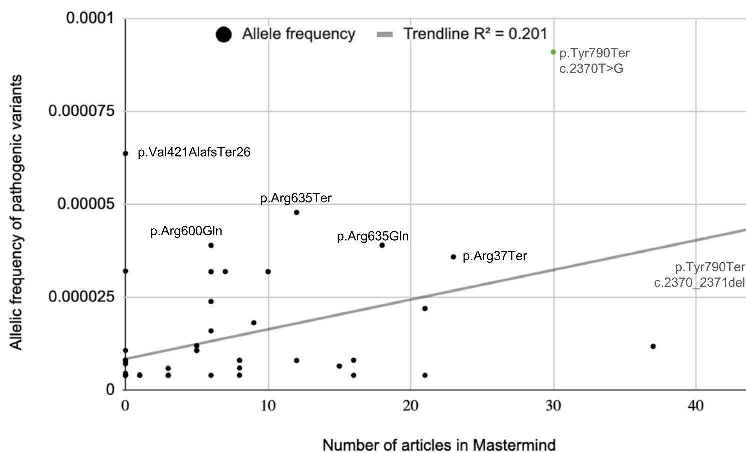

Fig. 3 Relationship between allele frequency and the number of articles associated with the variant in Mastermind

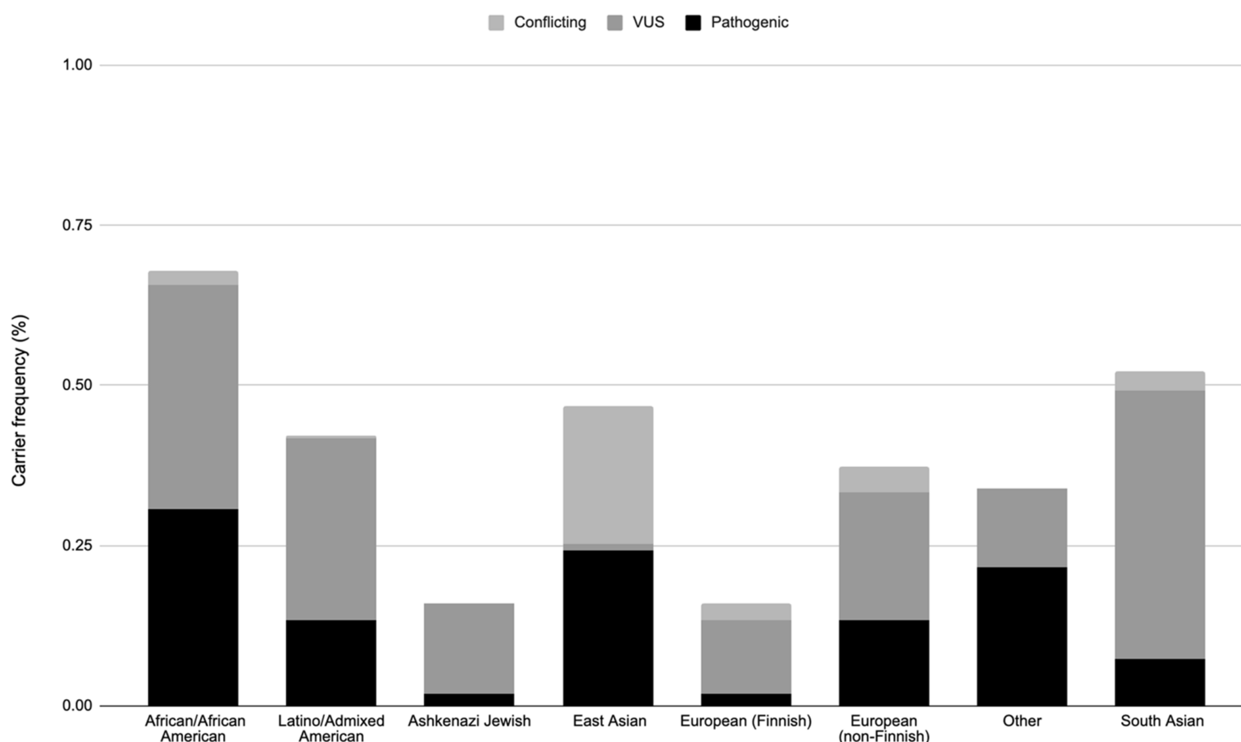

Fig. 4 Population-Specific Carrier Frequencies of *PLA2G6* Variants. The carrier frequency was calculated using the Hardy–Weinberg equilibrium equation and the sum of the allele frequencies of the specified variants. Variants that had conflicting interpretations of pathogenicity (P vs VUS) between database and literature approach are also included in the chart

(3.2%) and the conservative estimate of 5,150 PLA2G6-related disease, we expect there to be 4,160 individuals with INAD, 832 individuals with aNAD and 166 individuals with PARK14 worldwide.

Discussion

INAD is a rare autosomal recessive disorder caused by homozygous or compound heterozygous variants in phospholipase A2 group VI gene (*PLA2G6*) gene. Disorders associated with pathogenic variants in the PLA2G6 gene are: INAD, atypical NAD (aNAD) and PLA2G6-related dystonia-parkinsonism, also called Parkinson disease 14 (PARK14) [1]. INAD patients have early-onset progressive symptoms including severe symptoms including ataxia, mental and motor deterioration, hypotonia, progressive spastic tetraparesis, visual impairments, and bulbar dysfunction. aNAD and PARK14 have a later onset of symptoms, including progressive dystonia, parkinsonism and cerebellar atrophy is a characteristic symptom [2–4]. Since INAD is a rare disease, prevalence calculations have not been conducted previously. The current estimates of 1 in 1,000,000 to 1 in 2,000,000 pregnancies emphasize the urgent need for improved detection and care for individuals affected by this devastating condition.

Interestingly, we found a correlation between the allele frequency and the number of literature citations, as has been previously suggested [30].

Our approach allowed us to identify variants and gather supporting evidence, leading to an improved interpretation of these variants in line with current clinical guidelines. By providing a more accurate genetic prevalence estimate through our comprehensive approach, which involves leveraging advanced tools and databases, we contribute valuable insights into the understanding and management of PLAN. Based on our analysis, we determined that the carrier frequency for PLA2G6 variants in the general population is approximately from 1 in 497 to 1 in 627 individuals, corresponding to a genetic prevalence of from 1 in 987,267 to 1 in 1,570,079 pregnancies. This estimate highlights the underdiagnosis of PLA2G6-associated neurodegeneration and underscores the need for increased awareness and diagnostic efforts. Furthermore, our study revealed a higher carrier frequency of PLA2G6 variants in African and Asian populations, stressing the importance of expanded genetic sequencing in non-European populations to ensure accurate and comprehensive diagnosis. Future research should focus on confirming our findings and implementing expanded sequencing

Table 1 Carrier frequencies (%), disease prevalence per million, and genetic prevalence per million for different populations based on allelic frequencies derived from gnomAD

		Total AF	African/ African American	Latino/ Admixed American	Ashkenazi Jewish	East Asian	European (Finnish)	European (non-Finnish)	Other	South Asian	Total 1 in
Carrier frequency (%)	P+VUS+Conflicting*	0.43	0.68	0.42	0.16	0.47	0.16	0.37	0.34	0.48	235
	P only	0.16	0.31	0.13	0.02	0.24	0.02	0.13	0.22	0.07	627
	P+Conflicting**	0.20	0.33	0.14	0.02	0.46	0.04	0.17	0.22	0.10	497
Genetic prevalence/million	P+VUS+Conflicting*	4.54	11.63	4.48	0.63	5.47	0.63	3.49	2.86	5.73	220,322
	P only	0.64	2.37	0.45	0.01	1.46	0.01	0.45	1.16	0.13	1,570,079
	P+Conflicting**	1.01	2.74	0.49	0.01	5.24	0.05	0.76	1.16	0.27	987,267
Disease prevalence/million	P+VUS+Conflicting*	0.11	0.77	0.56	0.27	0.87	0.18	0.14	0.29	0.92	9,012,045
	P only	0.03	0.21	0.08	0.01	0.18	0.00	0.04	0.20	0.02	36,542,814
	P+Conflicting**	0.03	0.21	0.08	0.01	0.86	0.01	0.05	0.20	0.03	30,779,545

* All variants including conflicting interpretations between two approaches (VUS vs P)

** All pathogenic (n=58) and conflicting variants (n=15). Conflicting variants between two approaches: P vs. VUS

strategies to facilitate maximal and accurate diagnosis, particularly in non-European populations.

Conclusions

The genetic prevalence of PLA2G6-associated neurodegeneration was estimated using a literature-based approach which gathered variants through Mastermind Genomic Search Engine (Genomenon, Inc) and a database-based approach which included collecting variants from ClinVar and gnomAD. In the PLA2G6 gene, our analysis found 122 pathogenic, 82 VUS, and 15 variants with conflicting interpretations (pathogenic vs VUS) between two approaches. If pathogenic and/or conflicting variants are included, the overall genetic prevalence was estimated to be between 1 in 987,267 to 1 in 1,570,079 pregnancies, with the highest genetic prevalence in African/African-American (1 in 421,960 to 1 in 365,197) and East-Asian (1 in 683,978 to 1 in 190,771) populations. Our study revealed a higher carrier frequency of PLA2G6 variants in African and East Asian populations, stressing the importance of expanded genetic sequencing in non-European populations to ensure accurate and comprehensive diagnosis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13023-024-03275-x.

Supplementary Material 1.

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Authors' contributions
AK-K has analyzed the data and written the manuscript; M.S-B has extracted and analyzed data, JW extracted and analyzed data, EE has extracted and analyzed data, SMH has analyzed data and written the manuscript, LP and AH have analyzed data, SB and MJK designed the study, has analyzed the data and written the manuscript. All authors approved the final Manuscript.

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Availability of data and materials
All data analyzed during this study are available in gnomAD (https://gnomad.broadinstitute.org/) and this published article/supplementary files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors have consented to the final version of the manuscript.

Competing interests
Authors declare no competing interests.

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