Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Genotype–Laterality Associations in DNAAF11-Related Primary Ciliary Dyskinesia: Functional Characterization of the p.Ile100Thr Variant

Feng Zhu
Shanghai General Hospital
Hongkun Wang
Shanghai General Hospital
Weijiao Feng
Shanghai General Hospital
Yanling Zhao
Shanghai General Hospital
Yue Sun
Shanghai General Hospital
Yuqing Chen
Shanghai Chest Hospital
Meihua Chen
meihua.chen@shgh.cn

Shanghai General Hospital

Research Article

Keywords: Primary ciliary dyskinesia, DNAAF11, LRRC6, p.Ile100Thr, Situs inversus totalis, Laterality defects

Posted Date: November 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11317218/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Defects in motile ciliary function can affect respiratory health and embryonic left–right patterning. *DNAAF11*, formerly known as *LRRC6*, encodes a protein required for normal axonemal dynein arm assembly. The effects of individual rare missense variants and their relationships with laterality phenotypes remain incompletely characterized. We investigated the *DNAAF11* c.299T > C (p.Ile100Thr) variant and reviewed the laterality spectrum of *DNAAF11*-associated PCD.

Methods

A patient with chronic respiratory disease and situs inversus totalis underwent whole-exome sequencing and Sanger validation. Structural modeling was used to explore the potential effects of p.Ile100Thr. Wild-type and variant *DNAAF11* constructs were expressed in HEK293T cells, and mRNA and protein abundance were assessed by quantitative PCR and Western blotting, respectively. Published *DNAAF11* genotypes and laterality phenotypes were reviewed.

Results

A homozygous *DNAAF11* c.299T > C (p.Ile100Thr) variant was identified in an adult woman with bronchiectasis, chronic productive cough, and situs inversus totalis; both parents were heterozygous carriers. Structural modeling suggested possible local changes around residue 100. Compared with the wild-type construct, the variant construct showed a modest reduction in mRNA abundance and a more pronounced reduction in protein abundance. The literature-based analysis included 63 patients, including the present case. Laterality information was available for 51 patients, of whom 29 had laterality abnormalities. Several recurrent variants occurred in individuals with different laterality phenotypes. After transcript harmonization, p.Ile100Thr was identified in three patients: the present homozygous case and two previously reported compound-heterozygous cases.

Conclusions

Reduced protein abundance provides additional experimental evidence relevant to the interpretation of *DNAAF11* p.Ile100Thr. The reviewed cases demonstrate variable laterality phenotypes among individuals carrying recurrent *DNAAF11* variants, but do not establish variant-specific associations with organ situs. Further studies in motile-ciliated cells are needed to determine the effects of p.Ile100Thr on dynein arm assembly and ciliary function.

1 Introduction

Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder of motile cilia, predominantly inherited in an autosomal recessive manner, that causes chronic respiratory disease and may be accompanied by laterality defects and male infertility [1]. PCD is a clinically and genetically heterogeneous disorder caused by pathogenic variants in more than 55 genes, and its diagnosis relies on clinical evaluation supported by genetic, ultrastructural, and complementary functional investigations [2]. Approximately 50% of patients with PCD exhibit

laterality abnormalities, including situs inversus totalis(SIT) or heterotaxy, while some patients with heterotaxy also present with respiratory manifestations consistent with ciliary dysfunction [3, 4]. This combination of respiratory and laterality manifestations reflects the roles of motile cilia in both airway clearance and embryonic left–right patterning.

Axonemal dynein arms provide the motor activity required for ciliary movement. Motile cilia at the embryonic left–right organizer generate directional fluid flow that establishes left–right asymmetry[5]. Impaired ciliary motility disrupts asymmetric signaling and can alter organ laterality. To date, pathogenic variants in more than 50 genes involved in ciliary assembly, axonemal structure, and dynein arm function have been identified in PCD [1].

Dynein axonemal assembly factor 11 (*DNAAF11*), formely known as Leucine-rich repeat-containing protein 6 (*LRRC6*), is associated with autosomal recessive primary ciliary dyskinesia 19 (OMIM: 614935). Biallelic loss-of-function variants in *DNAAF11* have been associated with PCD characterized by defects in both outer and inner dynein arms [6]. It encodes a cytoplasmic assembly factor required for axonemal dynein arm preassembly and normal ciliary motility[7]. Biallelic pathogenic variants in *DNAAF11* disrupt ciliary motility and lead to PCD, characterized by chronic respiratory disease, impaired mucociliary clearance, male infertility, and variable laterality defects, including situs inversus and heterotaxy [7]. *DNAAF11* is also required for normal sperm flagellar structure and motility [8].

In this study, we investigated the homozygous *DNAAF11* c.299T > C (p.Ile100Thr) variant identified in a patient with SIT, bronchiectasis, and chronic productive cough. The variant is classified as variant of uncertain significance (VUS) and is rare in population databases such as gnomAD. Variants of uncertain significance (VUSs) remain a major challenge in clinical genomic interpretation because insufficient evidence precludes definitive assessment of their pathogenicity, thereby limiting accurate molecular diagnosis, genetic counseling, and risk assessment [9]. We combined clinical and genetic characterization with structural modeling and in vitro expression assays to examine the variant's effects on mRNA and protein abundance. These findings were evaluated alongside the available genetic evidence within the ACMG/AMP framework to inform variant interpretation. We also reviewed published *DNAAF11* genotypes and laterality phenotypes to characterize the reported spectrum of organ situs and explore phenotypic variation among individuals carrying recurrent variants.

2 Materials and methods

2.1 Editorial policies and ethical considerations

This study had been reviewed by the Human Trial Ethics Review Committee of Shanghai General Hospital (No. [2023] 336), and the use of genetic samples had been approved by the Human Genetic Resources Department of Shanghai General Hospital. This study was conducted in accordance with the principles of the Declaration of Helsinki. Patients had signed informed consent forms.

2.2 Patient recruitment and sample collection

We recruited a patient with a phenotype of Situs Inversus Totalis. The patient, a 31-year-old female, suffers from Situs Inversus Totalis and bronchiectasis, and has been coughing up phlegm for over 20 years.

2.3 Whole Exome Sequencing (WES) and data analysis

Genomic DNA (gDNA) was extracted from EDTA-anticoagulated peripheral blood using a Qiagen DNA extraction kit. Exome libraries were prepared and enriched using the GenCap Whole Exome Capture Probe V6.0, followed by paired-end sequencing.

The quality of raw data was checked by FASTQC. After removing the low-quality reads, the adaptor reads were mapped to the reference genome (GRCh37/hg19) with Burrows-Wheeler Aligner (BWA), Picard, and SAMtools. GATK was also used for insertion and deletion realignment, quality recalibration, and variant calling. Detected variants were annotated using ANNOVAR. The detected variants were filtered with minor allele frequencies (MAFs) for dbSNP, 1000 Genome Project (1000G, http://www.internationalgenome.org/data), the Exome Aggregation Consortium Browser (ExAC, http://exac.broadinstitute.org), and the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/). Multi-bioinformatic tools were used to assess the pathogenicity of variants, including SIFT, Polyphen-2, MutationTaster, MutationAccesser, CADD, DANN, SpliceAI, REVEL, *etc.* Candidate variants were validated by Sanger sequencing (primers: 5'-TGGAAGAGTTGCTACCACGA-3' and 5'-ACTTCATGGTGAGCAATGCA-3').

2.4 Protein structure modeling

Structural models of wild-type DNAAF11 and the p.Ile100Thr variant were generated using AlphaFold v2.2.1. Model quality was assessed using Ramachandran plots to evaluate backbone stereochemistry and ERRAT analysis to examine nonbonded atomic interactions. These assessments were used to evaluate the structural plausibility of the predicted models, rather than to establish mutation-induced changes in protein stability.

2.5 Plasmid construction and in vitro expression analysis

The wild-type *DNAAF11* gene fragment was amplified by PCR, and the c.299T > C variant was introduced by overlap-extension PCR using the primers listed in Supplementary Table 1. The resulting inserts were cloned into the corresponding expression vectors using KpnI/HindIII or SalI/NotI. Recombinant plasmids were screened by colony PCR, purified using the Rapid Plasmid DNA Mini Kit, and verified by Sanger sequencing (Supplementary Figure S1).

HEK293T cells obtained from the China Center for Type Culture Collection were seeded in six-well plates and transfected with wild-type or mutant *DNAAF11* expression plasmids using Lipofectamine in Opti-MEM medium. Cells were harvested 48 h after transfection for RNA and protein analyses. Total RNA was extracted using TRIzol reagent and reverse-transcribed into cDNA using the PrimeScript™ RT kit. Quantitative real-time PCR (qPCR) was performed using SYBR Green chemistry, with *ACTB* as the reference gene and the primers listed in Supplementary Table 2. Three independent experiments were performed, each with three technical replicates.

For Western blot analysis, cells were lysed in RIPA buffer, and protein concentrations were determined using a BCA assay. Equal amounts of protein (20 μg per lane) were separated by 10% SDS–PAGE and transferred onto nitrocellulose membranes at 300 mA for 90 min. Membranes were blocked with 5% non-fat milk for 90 min, incubated overnight with the indicated primary antibodies (1:1,000), and subsequently incubated with the corresponding secondary antibodies (1:20,000) for 90 min before signal detection. Details of the reagents and antibodies are provided in Supplementary Tables 3 and 4.

2.6 *DNAAF11* genotype phenotype analysis

PubMed was searched through August 30, 2026, using the terms “*DNAAF11*,” “*LRRC6*,” and “Primary Ciliary Dyskinesia 19 (CILD19)” .The reference lists of eligible articles were also screened to identify additional reports. Studies describing human patients with *DNAAF11/ LRRC6* variants and individual-level genotype or phenotype data were included. Reported variants were standardized to the NM_012472.6 reference transcript, where possible. Patient characteristics, allelic status, respiratory manifestations, reproductive phenotypes, and laterality findings were extracted. A total of 63 patient records were included (see Supplementary Table 5) ; potentially overlapping reports were retained but flagged during data synthesis. The genotypes and clinical phenotypes of patients carrying c.299T > C (p.Ile100Thr) were compared descriptively according to allelic status.

3 Result

3.1 Genetic testing results

Trio-WES was conducted on the proband and her parents, and a homozygous mutation, c.299T > C (reference transcript: NM_012472.6), was detected in *DNAAF11*. This variation was inherited from both the father and mother, The pedigrees are shown in Fig. 1A. Sanger validation of the c.299T > C variant is presented in Fig. 1B.

No large fragment deletion repeats (CNVs) were found to be highly correlated with clinical phenotypes and have sufficient evidence of pathogenicity.

According to the ACMG/AMP standards and guidelines for the interpretation of sequence variants (Richards et al., 2015 [10]), a pathogenicity analysis was conducted on *DNAAF11*:c.299T > C: (1) The gnomAD database shows that this mutation has been included, with a population mutation frequency of 0.00007430 (supportive evidence for pathogenicity, PM2_Supporting); (2) Boaretto et al. (2016) reported a pathogenic variant, c.598_599delAA (p.Lys200Glufs), in trans in an affected family member, which provides moderate evidence for pathogenicity (PM3). In addition, Zhao et al. (2021) reported another variant, c.567_570delinsCATGGAAAA. ; (3) The proband's clinical manifestation of Situs Inversus Totalis, bronchiectasis, and productive cough, is highly consistent with the PCD phenotype induced by *DNAAF11* gene variations (supportive evidence for pathogenicity, PP4). (4) In vitro functional experiments confirmed that the mutation leads to downregulated protein expression (supportive evidence for pathogenicity, PS3 [11]). In summary, the evidence strength for the c.299T > C variant is “PS3_Supporting +PM2_Supporting+PM3 + PP4”, and it is classified as a likely pathogenic variant.

Based on our updated literature review, 63 non-overlapping patient records with reported *DNAAF11* variants were identified, including the present case. The c.299T > C variant was identified in three patients (3/63, 4.76%): the homozygous Chinese patient described here, an Italian patient carrying c.299T > C in compound heterozygosity with c.598_599del, and a Chinese patient reported by Zhao et al. carrying the corresponding variant in compound heterozygosity with c.567_570delinsCATGGAAAA. Zhao et al. originally described this variant as c.53T > C (p.Ile18Thr) using transcript(NM_001321962); it corresponds to c.299T > C (p.Ile100Thr) on NM_012472.5/6. Frameshift variants were the most common variant type, occurring in 35 patients (57.4%), including one patient with a large deletion resulting in a frameshifting protein consequence, followed by missense variants in 19 patients (31.1%) and nonsense variants in 10 patients (16.4%). Splice-site variants were reported in five patients (8.2%). A start-loss variant, c.1A > G, was identified in one patient (1.6%). The most frequently reported variants were c.630delG (18 patients), c.436G > C (9 patients), and c.598_599del (6 patients). These findings indicate recurrent *DNAAF11* variants across the reported cohort, alongside substantial allelic heterogeneity.

3.2 Mutation result prediction

The mutation c.299T > C p.Ile100Thr occurs at the 100th amino acid position and is located within the identified LRR_9 functional domain. The gnomAD database indicates that this mutation has been included, with a population mutation frequency of 0.00007430. The comprehensive protein function prediction software REVEL predicts an uncertain outcome, while SIFT, PolyPhen_2, MutationTaster, and GERP+ predict harmful, harmful, harmful, and harmful outcomes, respectively. Being isoleucine in all 8 species, as shown in Supplementary Fig. 2. FF, SpliceAI, and RDDCSC all suggest that the mutation does not affect splicing.

Ramachandran plot analysis showed that 99.1% of residues were in favored or allowed regions, and the model achieved an ERRAT score of 89.914, supporting the structural plausibility of the AlphaFold-predicted model, Refer to Supplementary Material 1. Wild-type Ile100 forms hydrogen bonds and hydrophobic interactions with nearby residues (Fig. 2A). The c.299T > C mutation replaces Ile100 with Thr, which forms new hydrogen bonds with adjacent residues (Fig. 2B). Altered intramolecular interactions and amino acid properties disrupt protein structure and function, suggesting that this mutation reduces protein stability.

3.3 qPCR detection results

The expression levels of wild-type and mutant transcripts in the pCMV-3XFlag-Neo(EGFP)- *DNAAF11* vector were detected using primers *DNAAF11*-qPCR-F and *DNAAF11*-qPCR-R. The results from three independent replicates demonstrated a slight decrease in the mutant mRNA expression level relative to that of the wild type. (Fig. 3A).

3.4 Western blot detection results

Use flag tag antibody to detect the expression levels of wt and mut proteins in the pCMV-3XFlag-Neo(EGFP)-*DNAAF11* vector. Use GFP antibody to detect the transfection efficiency of the plasmid. In the pCMV-3XFlag-Neo(EGFP)- *DNAAF11* series of vectors, the theoretical size of the wild-type protein is 54 kDa, and the theoretical size of the mutant proteins is also 54 kDa. The results from three repetitions all showed that the protein size was slightly larger, but the bands were single. The protein expression levels of the mutants were significantly downregulated compared with that of the wild type (Fig. 3B,C).

3.5 Phenotypic comparison of *DNAAF11* c.299T > c mutation status in different patients

Comparison of the homozygous *DNAAF11* c.299T > C patient with the compound heterozygous c.299T > C / c.598_599delAA patient reported by Boaretto et al [12] and the compound heterozygous c.299T > C / c.567_570delinsCATGGAAAA patient reported by Zhao et al [13], revealed three cases presented with laterality defects and respiratory symptoms (bronchiectasis, cough, etc.), suggesting that the c.299T > C variant is pathogenic and acts in an autosomal recessive manner, with both homozygous and compound heterozygous states capable of producing the core clinical phenotype.

3.6 Genotype–Laterality associations in *DNAAF11* variants

We reviewed the clinical features of 63 patien absent from population databasests with reported *DNAAF11* variants, including the patient described in the present study(Figs. 4–5; see Supplementary Table 3 for detailed data). Two additional patient records were excluded from the primary analysis because they may overlap with

previously reported founder-family cases. Among the 63 patients, 32 were male, 24 were female, and seven had an unreported sex. Laterality abnormalities were documented in 29 patients and were absent in 22, whereas laterality status was unavailable or insufficiently described in 12. Among the 51 patients with available laterality information, 29 (56.86%) had a laterality abnormality. Respiratory manifestations were documented in at least 47 patients, while patient-level respiratory information was unavailable or could not be attributed individually in the remaining 16.

4 Discussion

In this study, we identified the homozygous *DNAAF11* c.299T > C (p.Ile100Thr) variant in a patient with chronic respiratory disease and situs inversus totalis. The variant was inherited from heterozygous parents, consistent with an autosomal recessive model, and in vitro expression assays showed reduced abundance of the variant protein. Our literature review also identified different laterality phenotypes among individuals carrying recurrent *DNAAF11* variants. These findings provide additional clinical and functional evidence to support variant interpretation and highlight the heterogeneity of laterality phenotypes in *DNAAF11*-related disorders.

Previous studies provide a biological context for the expression findings. Horani et al. reported reduced DNAAF11 expression, loss of axonemal dynein arms, and impaired ciliary motility in patient-derived airway epithelial cells and cells with *DNAAF11* knockdown [7]. Studies in mice further demonstrated a requirement for Lrrc6 in the axonemal delivery of outer dynein arm complexes [14]. Other *DNAAF11* variants have been associated with reduced protein expression and abnormalities of sperm dynein arms and flagellar motility [8, 15]. Together, these observations support the importance of *DNAAF11* for normal dynein arm assembly and function. However, findings from other variants and experimental systems do not directly establish the effect of p.Ile100Thr.

Previous research demonstrated a physical interaction between DNAAF11 and ZMYND10, highlighting the importance of protein–protein interactions in dynein-arm assembly [16]. The p.Ile100Thr substitution replaces a conserved nonpolar isoleucine with a polar threonine. Structural modeling suggested possible local changes around residue 100 but did not establish altered protein stability or function. In HEK293T cells, the variant construct showed a modest reduction in mRNA abundance and a more pronounced reduction in protein abundance compared with the wild-type construct. This pattern raises the possibility of effects on translation, protein folding, or degradation, although these mechanisms were not directly tested. Reduced steady-state protein abundance should therefore be interpreted as an expression phenotype rather than direct evidence of decreased protein stability or impaired ciliary function.

The c.299T > C (p.Ile100Thr) variant was previously reported by Boaretto et al. in a patient carrying it together with c.598_599delAA [12]. Zhao et al. reported the same variant as c.53T > C (p.Ile18Thr) using an alternative transcript; after harmonization to NM_012472.6, it corresponds to c.299T > C (p.Ile100Thr) [13]. These previous reports, together with the present homozygous case, add to the clinical evidence relevant to variant interpretation. However, the small number of reported cases precludes firm conclusions regarding a variant-specific association with laterality defects. Its identification in the homozygous state in the present patient adds to the available clinical observations. Nevertheless, occurrence in affected individuals, familial segregation, and altered expression must be evaluated together rather than treated as independent proof of pathogenicity. Within the ACMG/AMP framework, the contribution of experimental findings depends on the validity of the assay and its relevance to the disease mechanism [10, 11]. The present expression assays contribute evidence for interpretation,

but do not directly assess the downstream consequences of p.Ile100Thr for dynein arm assembly or ciliary motility.

The laterality findings are consistent with the developmental role of motile cilia. At the embryonic left–right organizer, directional ciliary flow initiates asymmetric signaling, and disruption of this process can alter organ situs [17]. In our review, laterality abnormalities were reported in 29 of 51 patients with available laterality information. This proportion describes the published cases and should not be interpreted as a population prevalence estimate. Different laterality phenotypes were observed among individuals carrying several recurrent *DNAAF11* variants. Importantly, sharing one variant does not necessarily imply an identical biallelic genotype, particularly in compound-heterozygous patients. The available observations therefore indicate phenotypic variability but do not establish a deterministic relationship between an individual variant and situs inversus totalis.

The international cohort reported by Raidt et al. also demonstrated allelic heterogeneity and regional clustering of variants in PCD [18]. Such data provide useful context for the recurrent *DNAAF11* variants identified in our review. However, direct frequency comparisons are limited by differences in ascertainment, population composition, and the completeness of genotype and phenotype reporting. Literature-derived series may also be influenced by preferential reporting of unusual phenotypes or recurrent founder variants. In addition, variant-level observations are not equivalent to mutually exclusive patient groups, because individuals with compound-heterozygous genotypes may contribute to more than one variant category. These considerations limit conclusions about variant enrichment and genotype–laterality associations.

This study has several limitations. The single-patient design limits generalizability, and comprehensive ciliary functional and ultrastructural assessments were unavailable. HEK293T expression assays do not reproduce the context of motile-ciliated cells and cannot directly evaluate dynein arm assembly, axonemal localization, or ciliary motility. The literature analysis was further limited by incomplete phenotype reporting, transcript annotation differences, possible overlap between published cases, and small numbers of individuals carrying particular variants. Studies in patient-derived motile-ciliated cells are needed to assess downstream effects on ciliary structure and function, while protein turnover experiments would help determine whether reduced abundance reflects altered stability.

5 Conclusions

This study identified the homozygous *DNAAF11* c.299T > C (p.Ile100Thr) variant in a patient with chronic respiratory disease and situs inversus totalis. In vitro expression assays showed reduced abundance of the variant protein, providing additional evidence relevant to its interpretation. The literature review highlighted variable laterality phenotypes among individuals carrying recurrent *DNAAF11* variants, without establishing a variant-specific association with organ situs. Further studies in motile-ciliated cells are needed to determine the effects of p.Ile100Thr on dynein arm assembly and ciliary function.

Declarations

Acknowledgments

We are deeply grateful to the patient and their family for their understanding and support of this study. We also sincerely thank Dr. Yu An and Dr. Bin Hu for their valuable assistance with this study.

Ethics approval and consent to participate

The study had been approved by the Ethics Review Committee of Shanghai General Hospital (No: Yuanlun Kuai [2023] 336), and the use of genetic samples has been approved by the Human Genetic Resources of the Shanghai General Hospital. Written informed consent to participate in this study was provided by the participants or the participants' legal guardian. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Consent for publication

Written informed consent for publication was obtained from the participants involved in this study. All authors have read and approved the final manuscript.

Availability of data and materials

The *DNAAF11* variant c.299T>C (p.Ile100Thr) identified in this study is listed in the NCBI ClinVar database (Variation ID: 540329; accession number: VCV000540329.58). The raw sequencing data generated during this study are not publicly available due to patient privacy concerns and applicable ethical and regulatory restrictions. Requests for access to these data may be directed to the corresponding author and will be considered subject to the necessary ethical approvals, informed consent requirements, and applicable laws and regulations.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This study was funded by the Songjiang District Science and Technology Public Relations Project (20FJKGG356).

Author contributions

Conceptualization, FZ and MC; Methodology, FZ, WF, YZ, YS, YC and MC; Investigation, FZ, WF, YS, YC and MC; Formal analysis, FZ and MC; Data curation, FZ and HW; Validation, HW and YZ; Resources, WF and YZ; Supervision, YC and MC; Project administration, HW; Funding acquisition, HW; Writing – original draft, FZ and HW; Writing – review and editing, WF, YZ, YS, YC and MC. All authors have read and agreed to the published version of the manuscript.

Clinical trial number

Not applicable.

References

1. DESPOTES K A, ZARIWALA M A, DAVIS S D et al. Primary Ciliary Dyskinesia: A Clinical Review [J]. Cells, 2024, 13(11).
2. SHOEMARK A, GOUTAKI M. KINGHORN B, European Respiratory Society and American Thoracic Society guidelines for the diagnosis of primary ciliary dyskinesia [J]. Eur Respir J, 2025, 66(6).
3. EITLER K, BIBOK A. Situs Inversus Totalis: A Clinical Review [J]. Int J Gen Med. 2022;15:2437–49.
4. COREN M E, MEEKS M. Primary ciliary dyskinesia: age at diagnosis and symptom history [J]. Acta Paediatr. 2002;91(6):667–9.
5. SATIR P, CHRISTENSEN ST. Overview of structure and function of mammalian cilia [J]. Annu Rev Physiol. 2007;69:377–400.
6. KOTT E, DUQUESNOY P. Loss-of-function mutations in LRRC6, a gene essential for proper axonemal assembly of inner and outer dynein arms, cause primary ciliary dyskinesia [J]. Am J Hum Genet. 2012;91(5):958–64.
7. HORANI A, FERKOL T W SHOSEYOVD, et al. LRRC6 mutation causes primary ciliary dyskinesia with dynein arm defects [J]. PLoS ONE. 2013;8(3):e59436.
8. LI Y, JIANG C, ZHANG X, et al. The effect of a novel LRRC6 mutation on the flagellar ultrastructure in a primary ciliary dyskinesia patient [J]. J Assist Reprod Genet. 2021;38(3):689–96.
9. ZHENG H, CHENG C, HE M, et al. Interpreting Variants of Uncertain Significance in PCD: Abnormal Splicing Caused by a Missense Variant of DNAAF3 [J]. Volume 13. Molecular genetics & genomic medicine; 2025. p. e70036. 1.
10. RICHARDS S, AZIZ N. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [J]. Genet Med. 2015;17(5):405–24.
11. BRNICH S E, ABOU TAYOUN A N, COUCH F J, et al. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework [J]. Genome Med. 2019;12(1):3.
12. BOARETTO F, SNIJDERS D, SALVORO C, et al. Diagnosis of Primary Ciliary Dyskinesia by a Targeted Next-Generation Sequencing Panel: Molecular and Clinical Findings in Italian Patients [J]. J Mol Diagn. 2016;18(6):912–22.
13. ZHAO X, BIAN C, LIU K, et al. Clinical characteristics and genetic spectrum of 26 individuals of Chinese origin with primary ciliary dyskinesia [J]. Orphanet J Rare Dis. 2021;16(1):293.
14. INABA Y, SHINOHARA K, BOTILDE Y, et al. Transport of the outer dynein arm complex to cilia requires a cytoplasmic protein Lrrc6 [J]. Genes Cells. 2016;21(7):728–39.
15. SHI S, TANG X. A novel homozygous LRRC6 mutation causes male infertility with asthenozoospermia and primary ciliary dyskinesia in humans [J]. Andrology. 2025;13(3):459–72.
16. ZARIWALA M A, GEE H Y, KURKOWIAK M, et al. ZMYND10 is mutated in primary ciliary dyskinesia and interacts with LRRC6 [J]. Am J Hum Genet. 2013;93(2):336–45.
17. HIROKAWA N, TANAKA Y, OKADA Y, et al. Nodal flow and the generation of left-right asymmetry [J]. Cell. 2006;125(1):33–45.
18. RAIDT J, RIEPENHAUSEN S, PENNEKAMP P et al. Analyses of 1236 genotyped primary ciliary dyskinesia individuals identify regional clusters of distinct DNA variants and significant genotype-phenotype correlations [J]. Eur Respir J, 2024, 64(2).

Figures

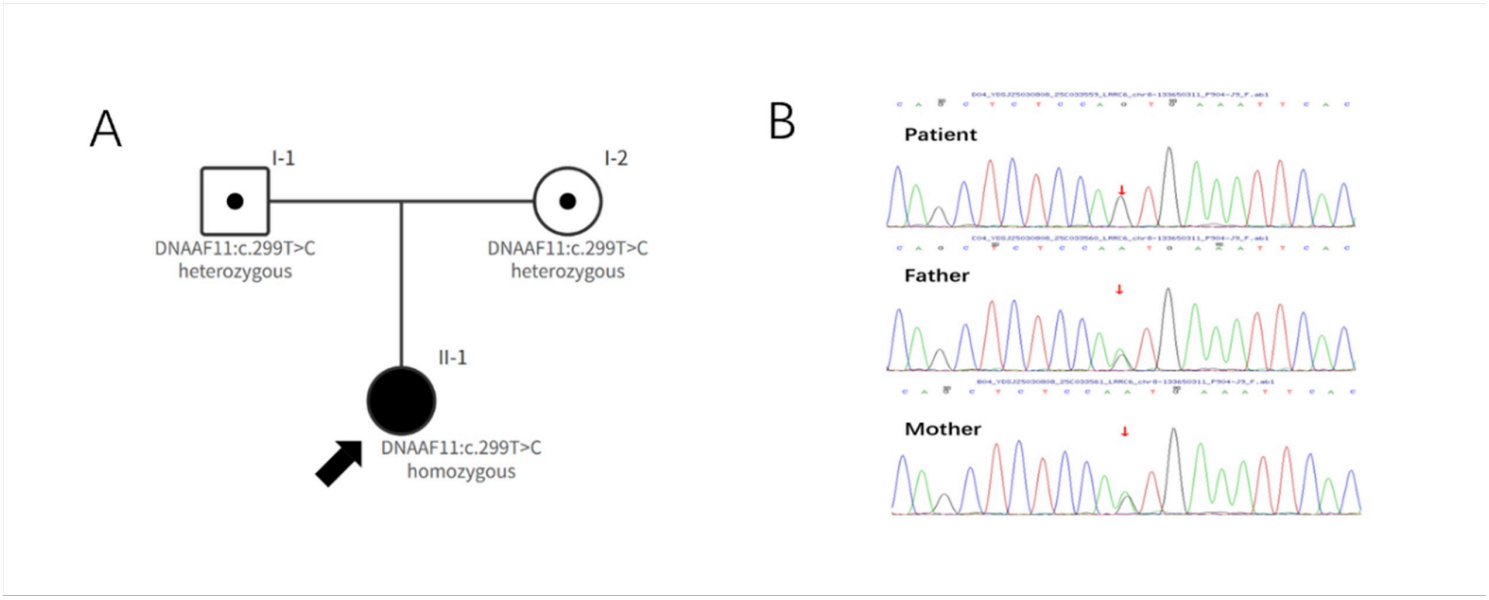


Figure 1

Genetic analysis results. Family pedigree and pathogenic variations in the *DNAAF11* gene.

(A).Pedigree of the family with the *DNAAF11*:c.299T>C variant. (B).Sanger sequencing validation of the *DNAAF11*:c.299T>C variant.

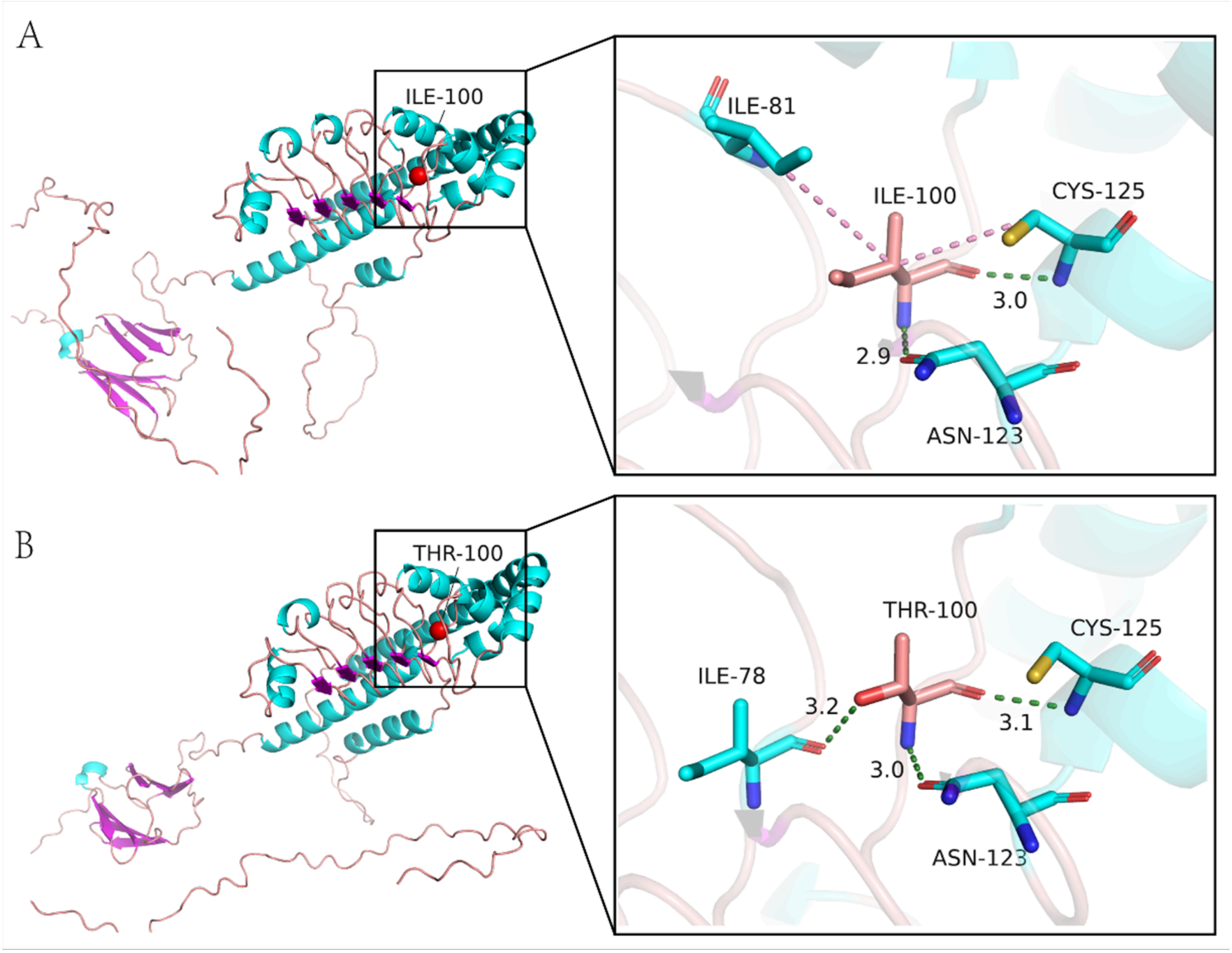


Figure 2

***DNAAF11* gene c.299T>C mutation three-dimensional protein prediction map.**

(A) The 100th position of the wild-type protein is non-polar valine; (B) c.299T>C mutation prediction p.Ile100Thr leads to the replacement of non-polar isoleucine at position 100 with polar threonine.

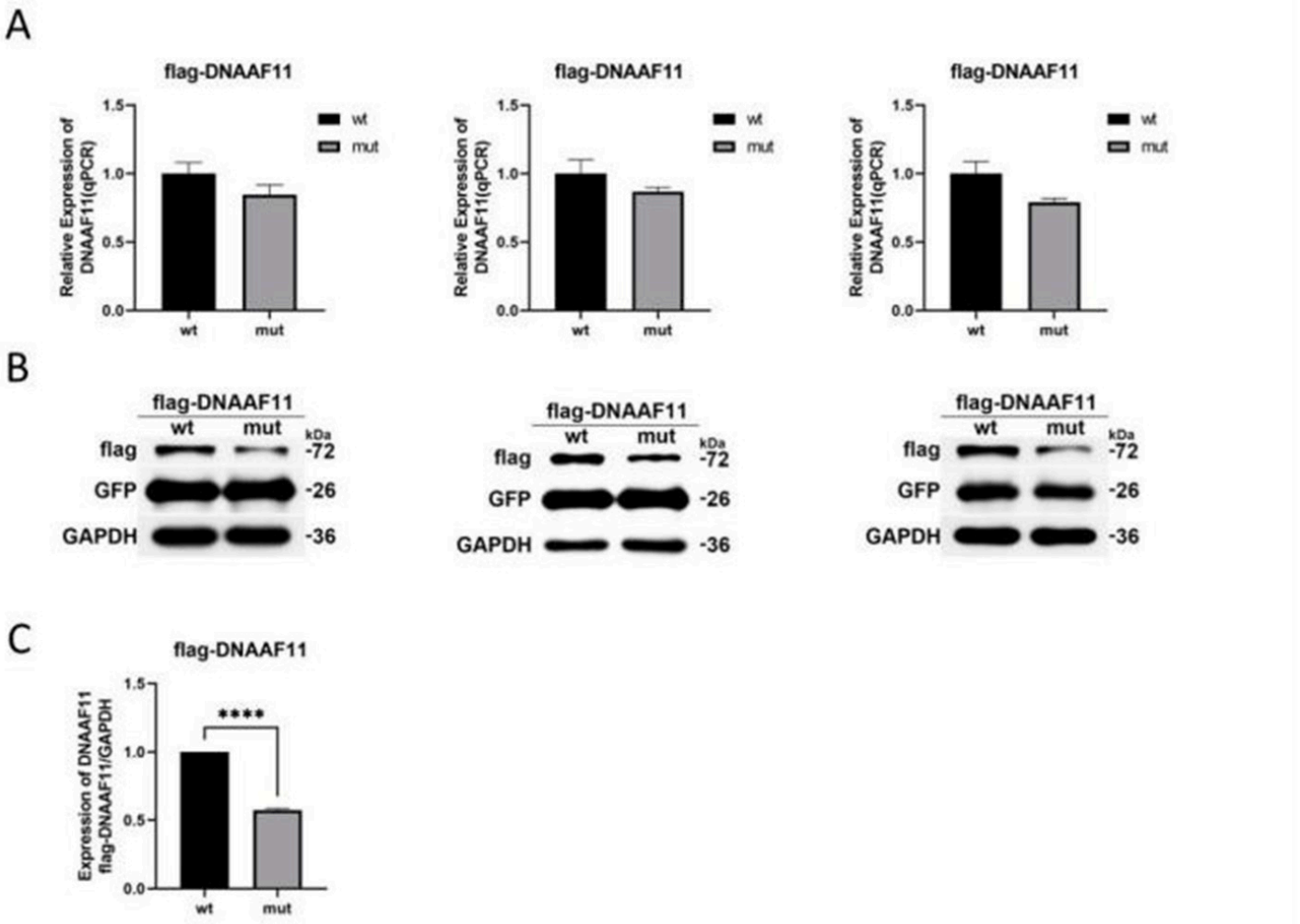


Figure 3

The impact of mutations on *DNAAF11* expression levels.

(A) Relative expression levels of *DNAAF11* mutant and wild-type mRNA (qPCR); (B) Relative expression levels of *DNAAF11* mutant and wild-type proteins (WB); (C) Bar graph of grayscale scanning statistics for WB results.

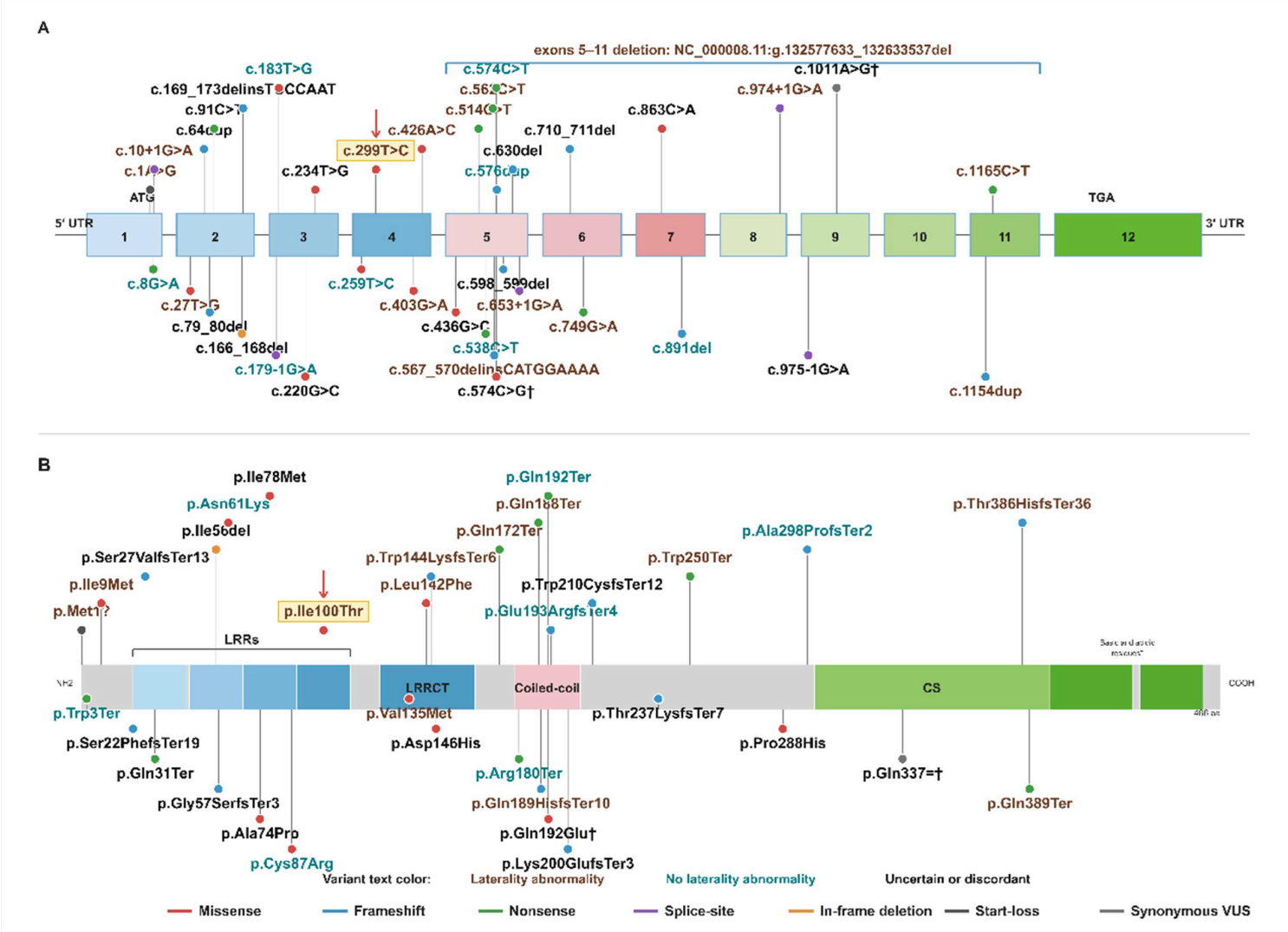


Figure 4

Distribution of reported *DNAAF11/ LRRC6* variants across the gene and encoded protein.
(A) Schematic representation of the 12-exon *DNAAF11/ LRRC6* transcript (NM_012472.6), showing the locations of reported coding and splice-site variants. The large genomic deletion spanning exons 5–11 is indicated by the horizontal bracket. (B) Distribution of the corresponding protein variants across the 466-amino-acid DNAAF11 protein (NP_036604.2) and its reported functional domains, including the leucine-rich repeats (LRRs), leucine-rich repeat C-terminal domain (LRRCT), coiled-coil region, CS domain, and C-terminal region enriched in basic and acidic residues. Missense variants are shown in red, frameshift variants in blue, nonsense variants in green, splice-site variants in purple, in-frame deletions in orange, and start-loss or synonymous variants in gray. The *DNAAF11* c.299T>C (p.Ile100Thr) variant investigated in the present study is indicated by red arrows. Variants marked with a dagger (†) were reported as cohort-level variants of uncertain significance without patient-level mapping in the main article and were not considered confirmed pathogenic alleles. Exon and domain sizes are shown schematically and are not drawn to scale.

Variants in blue font, no heterotaxy; red font, heterotaxy; orange font, both; black font, unknown. Heterotaxy here is a broad concept.

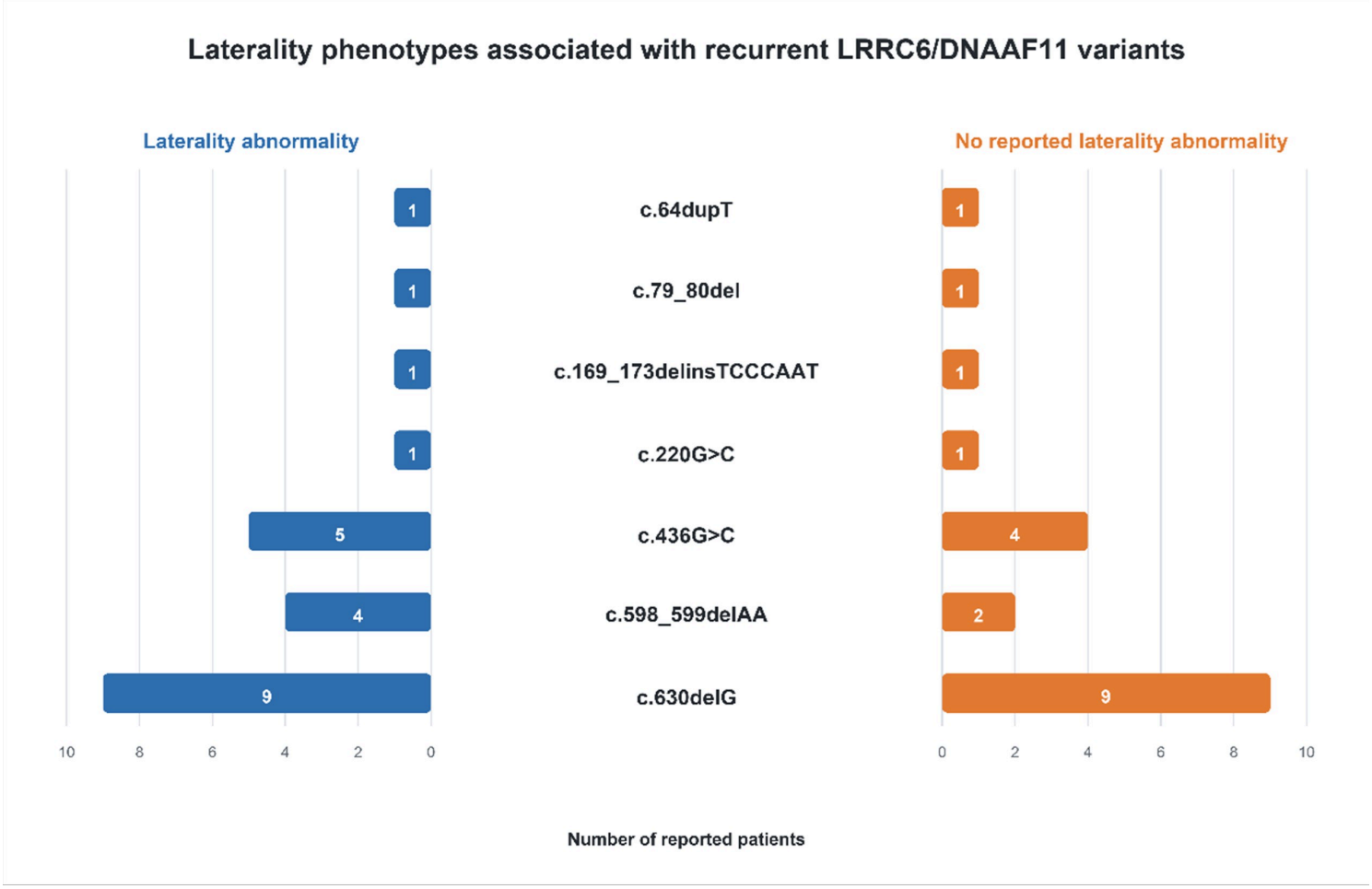


Figure 5

Laterality phenotypes associated with recurrent *LRRC6/DNAAF11* variants.Recurrent variants were defined as those reported in at least two patients. Bars indicate the number of patients with a laterality abnormality (blue) or no reported laterality abnormality (orange). Variant nomenclature was standardized to the *DNAAF11*reference transcript NM_012472.6. Counts represent variant-level patient observations; therefore, a patient carrying two recurrent variants may contribute to more than one variant category.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable14.docx
- SupplementaryTable5OverviewofthegenotypesandphenotypesofLRRC6reportedintheliterature20260830.docx
- WESSampleDataQualityControl.xlsx
- SupplementaryFigure12.docx
- SupplementaryMaterial1Modelevaluationofalphafold2.2.1modeling.docx
- FulllengthuncroppedWesternblotimages.pdf