Autosomal Dominant Retinitis Pigmentosa–Associated *TOPORS* Protein Truncating Variants Are Exclusively Located in the Region of Amino Acid Residues 807 to 867

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Purpose. Heterozygous truncating variants of *TOPORS* have been reported to cause autosomal dominant retinitis pigmentosa (adRP). The purpose of this study was to investigate whether all heterozygous truncating variants, including copy number variants (CNVs), are pathogenic.

Methods. *TOPORS* truncating variants were collected and reviewed through an in-house dataset and existing databases. Individuals with truncating variants underwent ophthalmological evaluation.

Results. Six truncating variants were detected in seven families. Three N-terminus truncating variants were detected in three families without RP, and the other three were identified in four unrelated families with typical RP. Based on the in-house dataset and published literature, 17 truncating variants were identified in 47 families with RP. All RP-associated truncating alleles, except one, were distributed in the last exon of *TOPORS* and clustered in amino acid residues 807 to 867 (46/47, 97.9%). Conversely, in the gnomAD database, only one truncating allele (1/27, 3.7%) was in this region, and the others were outside (26/27, 96.3%), suggesting that the pathogenic truncating variants were significantly clustered in residues 807 to 867 ($\chi^2 = 65.6$, $P = 1.1 \times 10^{-17}$). Additionally, three CNVs involving the N-terminus of *TOPORS* were recorded in control populations but were absent in affected patients.

Conclusions. This study suggests that all pathogenic truncating variants of *TOPORS* were clustered in residues 807 to 867, whereas the truncating variants outside this region and the CNVs involving the N-terminus were not associated with RP. A dominant-negative effect, rather than haploinsufficiency, is speculated to be the underlying pathogenesis. These findings provide valuable information for interpreting variation in *TOPORS* and other genes in similar situations, especially for CNVs.

Keywords: *TOPORS*, truncating variants, copy number variants, retinitis pigmentosa, clinical interpretation

Notable progress has been made in genomic medicine in the past few decades. The detection of variants in a subset of or all functional genes has become easily accessible in clinical practice. However, interpretation of detected variants in individual genes, including single nucleotide variants, small indels, and copy number variants (CNVs), is challenging in the clinical setting,[1] especially when the detection of CNVs in many genes is performed in a prenatal genetic screening test.[2,3] Recently, a pregnant woman requested genetic counseling for a heterozygous deletion of the whole *TOPORS* gene (OMIM: 609507), for which truncating variants have been reported to cause autosomal dominant retinitis pigmentosa (adRP).[4–16] Based on these studies, haploinsufficiency has been suggested as the molecular mechanism, implying that most truncating variants (especially those at the N-terminus, large structural variants, or copy number deletions) are pathogenic. Therefore, these CNVs may be classified as potential pathogenic variants based on the recommended criteria in 2015 by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP),[17] as well as suggested by updated recommendations.[18] However, comparative analyses at the individual gene level based on large datasets have revealed that not all heterozygous truncating variants (including CNVs) are pathogenic for autosomal dominant diseases in genes that are well characterized and frequently studied, as demonstrated in our previous studies on other genes, such as *CRX*.[19] These studies emphasize that systemic analysis of variant pathogenicity at the individual gene level is important. Therefore, the potential pathogenicity of variants in *TOPORS* should be systemically evaluated based on comparative analysis of large datasets and genotype–phenotype analysis before performing genetic counseling on CNVs of *TOPORS* for pregnant women.

In this study, variants in *TOPORS* were collected and systematically analyzed by comparing the in-house exome sequencing dataset with the existing databases, which included the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/validate.php), Leiden Open Variation Database (LOVD, https://www.lovd.nl), Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org), Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/app/home), and Database of Genomic Variation and Phenotype in Humans using Ensembl Resources (DECIPHER, https://www.deciphergenomics.org). Potential candidate pathogenic variants were selected, specific ophthalmological examinations further characterized the phenotypes associated with these variants, and genotype–phenotype associations were assessed to determine the pathogenicity of individual variants. Based on comparative analysis of large datasets at the individual gene level, pathogenic truncating variants in *TOPORS* were only clustered in the 807 to 867 residues, whereas truncating variants (including CNVs) in other regions were likely nonpathogenic. The results from this study provide useful information for the interpretation of *TOPORS* variants. They may also serve as a warning sign in the interpretation of variants in other genes with similar situations as *TOPORS*, especially for CNVs and truncating variants in genes with autosomal dominant diseases.

Methods

Subjects

This study was approved by the institutional review board of Zhongshan Ophthalmic Center. Patients with different genetic eye conditions were recruited from the Pediatric and Genetic Clinic, Zhongshan Ophthalmic Center, Guangzhou, China. This study adhered to the tenets of the Declaration of Helsinki. After patients or their guardians signed informed consent forms, their clinical data and peripheral venous blood samples were collected. Genomic DNA of patients and available family members was prepared from peripheral blood according to the method described in our previous study.[20]

Variants' Evaluation of the In-House Dataset

TOPORS variants were collected from in-house exome sequencing, including whole-exome sequencing (WES) and targeted exome sequencing (TES). The procedures for WES and TES and the multistep bioinformatics analysis method were carried out as described in our previous studies.[21–23] The Human Genome Variation Society guidelines (https://www.hgvs.org/mutnomen/) were used for the variant nomenclature. Specific ophthalmological examinations further characterized the phenotypes associated with these variants, and, finally, genotype–phenotype associations were assessed to determine the pathogenicity of individual variants. These truncating variants were confirmed by Sanger sequencing, and co-segregation was performed in the available family members. Several previously reported pathogenic variants were also included in the current study to obtain an overview of the *TOPORS* variant landscape in our cohort.[24]

Review of *TOPORS* Variants

The terms "TOPORS" and "retinitis pigmentosa" were used as the keywords to search on PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) until January 2022. *TOPORS* variants in the HGMD database were systematically reviewed as of January 2021. In addition, as of December 16, 2021, *TOPORS* variants in the LOVD database were systematically analyzed, and variants without references and with clinical classification as "benign" or "likely benign" were excluded. The frequency of each variant and the related clinical data of patients with these variants were then summarized and analyzed.

Variants and CNVs Evaluation

Variants of *TOPORS* from the in-house dataset; the gnomAD, HGMD, and LOVD databases; and published literature were systematically compared and analyzed. The phenotypes of the patients with variants, allele frequency of variants in the control population, and distribution of variants in the coding region were compared. The allele frequencies of the *TOPORS* variants were annotated using the gnomAD v2.1.1 database. Five in silico tools were used to predict the possible impact of missense variants, including Rare Exome Variant Ensemble Learner (REVEL, https://sites.google.com/site/revelgenomics/),[25] Combined Annotation Dependent Depletion (CADD, https://cadd.gs.washington.edu/),[26] Sorting Intolerant From Tolerant (SIFT, http://sift.jcvi.org/),[27] Polymorphism Phenotyping v2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/),[28] and Protein Variation Effect Analyzer (PROVEAN, http://provean.jcvi.org/).[29] The CNVs were collected and analyzed from control populations, such as the gnomAD SVs v2.1 database and DGV Version 1 Structural Variants database, as well as from patients (e.g., DECIPHER database). CNVs listed in the gnomAD SVs v2.1 database and the DGV database are usually indicated as benign. The genome built for these chromosomal positions was the University of California, Santa Cruz GRCh37/hg19, and the transcript NM_005802 was used.

Statistical Analysis

SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. The truncating variant frequency of *TOPORS* in the gnomAD database, HGMD database, LOVD database, and published literature was statistically compared by using the χ^2 test. Statistical significance was defined as $P < 0.05$.

Results

TOPORS Variants in our Cohort

Six truncating variants in *TOPORS* were detected in seven unrelated families, including five frameshifts and one nonsense variant (Supplementary Table S1). Three of these six—namely, c.24dup/p.(Ser9Valfs*9), c.60dup/p.(Pro21Alafs*8), and c.121C>T/p.(Arg41*)—were located at the N-terminus of *TOPORS* (Fig. 1A) and were considered likely benign variants for the following reasons: (1) The phenotype of individuals with these variants was not RP or a related disease (Supplementary Fig. S1). (2) Variants were also present in healthy individuals (Supplementary Fig. S1). (3) Truncating variants at the N-terminus were frequently seen in the gnomAD database but were not clustered in patients with RP (Fig. 1A). (4) The N-terminus truncating variant was detected in individuals with a clear alternative genetic disease

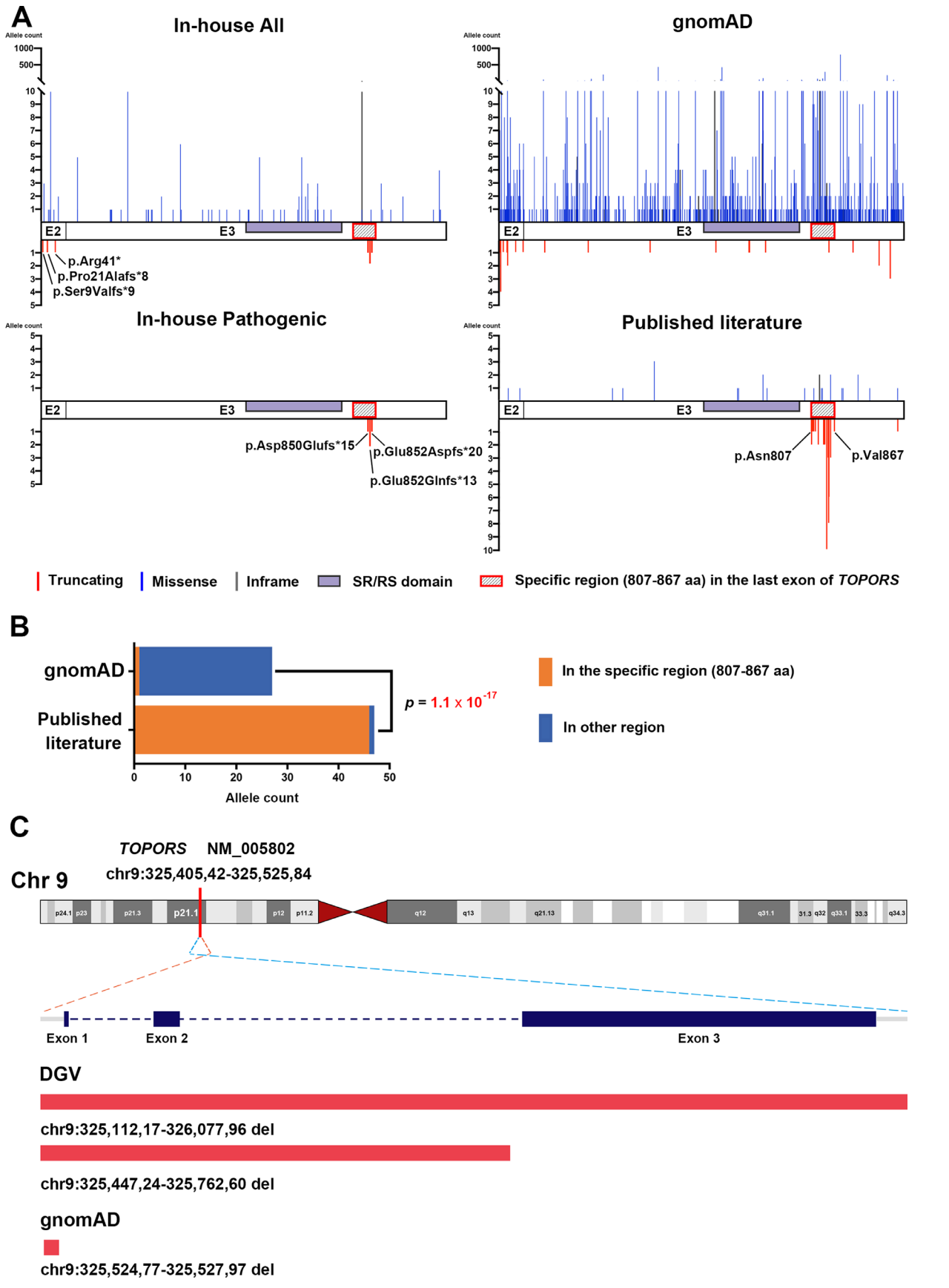

Figure 1. Distribution and frequency of *TOPORS* variants. (**A**) The distribution and frequency of *TOPORS* variants in our cohort, the gnomAD database, and the Human Gene Mutation Database. (**B**) There is a statistically significant difference between the gnomAD database and published literature with regard to the clustering of truncating variants in specific regions. (**C**) Copy number variants related to *TOPORS* in the gnomAD database and the database of genomic variants.

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etiology (Supplementary Fig. S1). The remaining three truncating variants located in the last exon and downstream of the arginine- and serine-rich (SR/RS) domains (Fig. 1A)—namely, c.2550_2553del/p.(Asp850Glufs*15), c.2554_2557del/p.(Glu852Glnfs*13), and c.2556_2557del/p.(Glu852Aspfs*20)—were detected in five patients with RP from four unrelated families (Supplementary Fig. S2), of which three families were described in our previous study.[24]

In our cohort, a total of 60 missense variants were detected in 113 unrelated families (Supplementary Table S2). These variants were distributed throughout the coding region and did not cluster in a specific region of *TOPORS* (Fig. 1A). Of the 60 missense variants, all had at least one allele present in the gnomAD database, and 50 (83.3%) were predicted to be benign by at least three of the five in silico prediction tools. Four of these variants—namely, c.2141A>G/p.(Asp714Gly), c.2047C>T/p.(Arg683Trp), c.74C>G/p.(Ser25Trp) and c.23G>C/p.(Gly8Ala)—were classified as "likely pathogenic" by the LOVD database; however, they were defined as non-causal in our cohort, mainly due to their non-RP phenotype and the prediction of benign and high allele frequencies in the gnomAD database. However, the c.671A>G/p.(Asp224Gly) variant was detected in families with Leber congenital amaurosis in our previous study.[30] Reanalysis revealed that five in silico prediction tools predicted the variant to be benign, and the variant had a high allele frequency in the gnomAD database; thus, it was considered benign. The c.1537G>C/p.(Val513Leu) variant was classified as a "variant of uncertain significance" by the LOVD database, and the variant did not segregate with disease in their family; moreover, individuals with this variant carry the more likely pathogenic variant c.[313G>A];[2047G>A]/p.[Glu105Lys];[Val683Met] of the *PDE6B* gene.[24] The in-frame deletion variant, c.(2484_2486del)/p.(Ser830del), was considered benign in our cohort because of its frequency of 40/282,760 in the gnomAD database, especially due to its frequency of 37/19,954 in the East Asian population.

Variants and CNVs in Existing Databases

Based on the HGMD database (as of January 2021), LOVD database (as of December 2021), and PubMed search (as of January 2022), 17 heterozygous truncating variants of *TOPORS* were reported to be causative for autosomal dominant retinopathy in 47 families, including 42 families with typical RP and five families with unclassified retinal dystrophy.[4–16,31–45] All except one variant were distributed in exon 3, which is the last exon of *TOPORS*. These variants were clustered in a specific region from coding residues 807 to 867 downstream of the SR/RS domain (Fig. 1A). Only the c.3090dup/p.(Thr1031Aspfs*10) variant, which was not in this specific region, was located at the C-terminus of *TOPORS* (Fig. 1A). The allele frequency of this variant in the gnomAD database is 2/282,804. This seems to be indicative of benignity, particularly in autosomal dominant disorders.

We analyzed 27 high-confidence truncating alleles in *TOPORS* present in the gnomAD v2.1.1 database (Fig. 1A). The 27 truncating alleles were spread throughout the coding region, including 20 alleles (20/266,870) from the start codon to residue 806, one allele between residues 807 and 867, and six alleles (6/251444) from residue 868 to the terminal codon. The variant c.2554_2557del/p.(Glu852Glnfs*13), located in the region of residues 807 to 867, has an allele frequency of 1/31,342 in the gnomAD database; however, this variant was clustered in patients with adRP.[10,13,31,37,39–41] When we compared the distribution of *TOPORS* truncating variants in a specific region (807–867 residues) in the published literature with the distribution of truncating variants overlapping with the same region in the gnomAD database, we found that disease-associated truncating variants were significantly clustered in the 807 to 867 residues ($\chi^2 = 65.6$, $P = 1.1 \times 10^{-17}$) (Fig. 1). Conversely, most truncating alleles (26/27, 96.3%) in the gnomAD database were located outside the 807 to 867 residues, which were not clustered in patients with RP and were likely nonpathogenic.

Large structural variants involving whole or partial deletions of *TOPORS* have been documented in related databases. For example, a 320-bp deletion variant was detected by whole-genome sequencing and was listed in the gnomAD SVs v2.1 database (Fig. 1C). In addition, two CNVs have been listed in the DGV database, one of which was an approximately 96.6-kb deletion variant that contained the whole *TOPORS* gene (Fig. 1C). The other one was an approximately 31.5-kb deletion variant that contained the N-terminus of *TOPORS* (Fig. 1C). These variants are not associated with any known ocular diseases. To date, no disease-related CNVs (deletions) of *TOPORS* have been included in the DECIPHER database.

Nineteen missense variants and one in-frame deletion variant were listed in the LOVD database (https://databases.lovd.nl/shared/variants/TOPORS/unique). These variants were considered as likely benign according to the allele frequency in the gnomAD v2.1.1 database and computational predictions (Supplementary Table S2). Assuming full penetrance of *TOPORS* variants, the threshold for causal autosomal dominant variants in *TOPORS* was set at 0.000 0013, based on the prevalence of adRP (0.00025), the *TOPORS* part of this (0.01),[4] and the heterozygosity in persons (0.5). In addition, missense variants were evaluated by using five in silico prediction tools (REVEL, CADD, SIFT, PolyPhen-2, and PROVEAN). Thus, the 20 variants are considered as likely benign based on the following points: (1) one in-frame deletion and seven missense variants had an allele frequency of at least 2/251,444 (8.0×10^{-6}) in the gnomAD database; (2) 11 missense variants were predicted as benign by at least three of the five tools; and (3) the remaining c.881A>G/p.(His294Arg) variant was identified in a case of RP in which a pathogenic variant in *PRPF3* was confirmed, as well.[46]

Multimodal Imaging Findings of *TOPORS*-Related RP

Patients with pathogenic truncating variants in *TOPORS* showed typical RP fundus changes, including pallor optic disc, attenuated retinal arterioles, and tapetoretinal degeneration highlighted around the retinal arcades, with no or minimal pigment deposits (Figs. 2A, 2B). Fundus images from a patient with RP with the heterozygous c.2550_2553del/p.(Asp850Glufs*15) variant demonstrated a typical change in retinal degeneration, consistent with a previous report by Chakarova et al.,[11] including retinal pigment epithelium (RPE) atrophy around the superior and inferior temporal vascular arcades and on the nasal side of the optic disc (Figs. 2A, 2B, 2E, 2F). The severity

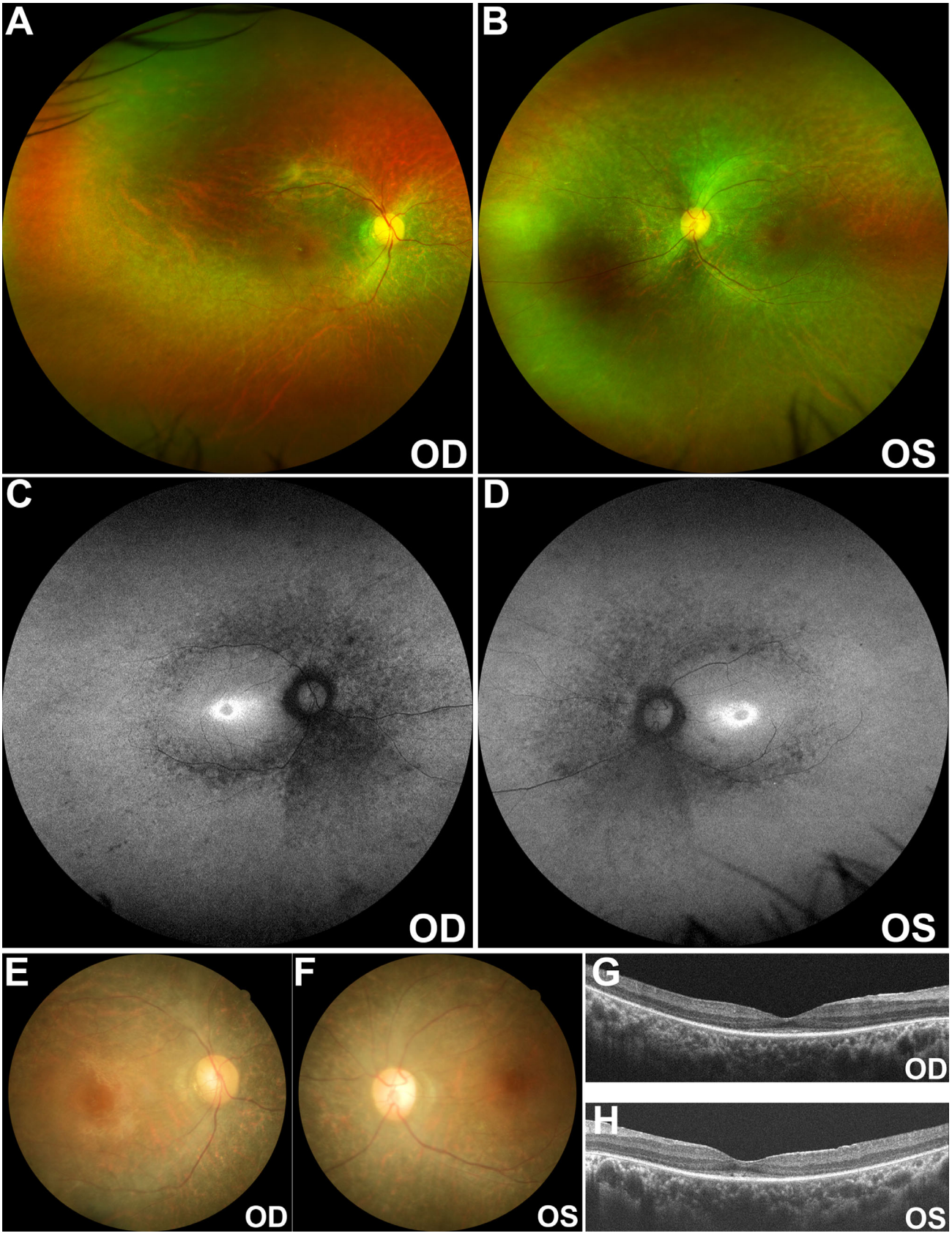

Figure 2. Multimodal imaging findings of *TOPORS*-related retinitis pigmentosa. (**A**, **B**) The bone spicule pigmentation was not evident from posterior pole to peripheral retinal through a scanning laser ophthalmoscopy examination. (**C–F**) RPE atrophy around the superior and inferior temporal vascular arcades and on the nasal side of the optic disk. (**G**, **H**) The transfoveal OCT scan showed that the structure of each layer of the retina was irregular, and the most evident changes were the thinning of the outer plexiform layer and outer nuclear layer, loss of the external limiting membrane and the ellipsoid zone, and atrophy of the RPE.

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and geographical features of retinal degeneration can be observed more clearly through widefield fundus autofluorescence (FAF) imaging, in which hypofluorescence represents RPE atrophy around the retinal vascular arcade (Figs. 2C, 2D). The FAF images also showed abnormal circular hyperfluorescence in the parafoveal region (Figs. 2C, 2D). Transfoveal optical coherence tomography (OCT) scans showed that the structure of each layer of the retina was irregular; the most notable changes were the thinning of the outer plexiform layer and outer nuclear layer, loss of the external limiting membrane and the ellipsoid zone, and RPE atrophy (Figs. 2G, 2H).

Discussion

A series of previous studies, including a prior study of ours, have demonstrated that heterozygous truncating variants of *TOPORS* are responsible for adRP.[4–16,24,31–44] Haploinsufficiency has been suggested to be an underlying mechanism of pathogenesis in previous studies. In this study, however, systematic comparative analysis at the individual gene level based on large datasets suggests that heterozygous causative truncating variants for adRP are clustered in the 807 to 867 residues. In contrast, heterozygous truncating variants outside the specific residues are likely nonpathogenic. Specifically, most truncating variants of *TOPORS* are likely nonpathogenic, including heterozygous truncating variants between the N-terminus and residue 806 and between residue 868 and the C-terminal, as well as large structural variants with similar effects (such as CNVs in the gnomAD or DGV databases). In such cases, a dominant-negative effect other than haploinsufficiency may be the underlying molecular mechanism. These new findings have not been reported before and are supported by the following evidence: (1) In our cohort, individuals with N-terminus truncating variants were not related to RP. (2) Compared with the control populations (the gnomAD database), the heterozygous causative truncating variants were significantly clustered in the 807 to 867 residues ($\chi^2 = 65.6$, $P = 1.1 \times 10^{-17}$). (3) In the gnomAD database, the frequency of truncating variants outside the special residues is close to the incidence of RP. Finally, (4) CNVs involving whole or partial deletion of *TOPORS* were documented in the control populations (e.g., gnomAD and DGV databases), whereas they were absent in the affected patients (e.g., DECIPHER database).

Clustering of pathogenic variants in specific regions has also been observed in splicing factor RP genes. For example, pathogenic variants in *PRPF8* and *SNRNP200* were clustered in their interaction regions, where defects disrupted PRPF8–SNRNP200 interactions.[47–49] Previously, situations similar to that of *TOPORS* have been reported in the *RP1* gene, in which causative heterozygous truncating variants are only located in a specific region in the middle of the gene.[50–52] Regarding *TOPORS*, products resulting from pathogenic truncating variants located in the last exon and downstream of the SR/RS domain may escape nonsense-mediated mRNA decay (NMD)[53] and reduce the activity of the wild-type allele via the dominant-negative effect. The normal function of *TOPORS* in photoreceptor cilia requires a complete SR/RS domain,[54] which promotes assembly and activation of the spliceosome by binding to the exonic splicing enhancer.[55–57] Thus, N-terminus truncating variants will likely trigger NMD and eliminate aberrant transcripts, whereas CNVs will lead to a null *TOPORS* allele. Protein expression from the wild-type *TOPORS* allele may be sufficient to maintain a normal phenotype. Therefore, it is speculated that the dominant-negative effect, rather than haploinsufficiency, is the underlying molecular mechanism; however, further functional studies are needed to confirm this. Recently, Weisschuh et al.[41] detected a C-terminal truncating variant, c.3090dup/p.(Thr1031Aspfs*10), by targeted next-generation sequencing in a cohort of 1785 families with inherited retinal degeneration. However, the allele frequency of this variant was 2/282,804 in the gnomAD database. This variant is located at the C-terminus of *TOPORS*, where truncating variants in the gnomAD database appear slightly clustered (Fig. 1A). Therefore, individuals with variants in the C-terminus should undergo a detailed clinical evaluation and co-segregation analysis.

The probability of being a loss-of-function (pLoF) intolerant (pLI) value is valuable for interpreting pLoF variants of a gene.[58] However, the pLI of *TOPORS* pLoF variants may be inappropriate when considered alone due to the following reasons: (1) For pLoF, the count of the observed single-nucleotide variants (SNVs) is also an important parameter apart from pLI. Sometimes, the presence of two or more observed SNVs of a gene with a pLI = 1 indicates tolerance of heterozygous truncation variants, such as *DST* (observed SNVs = 38), *ZNF516* (observed SNVs = 2), and *CDKL5* (observed SNVs = 3, in which c.2854C>T/p.Arg952* is a benign variant), which is considered to be similar to *TOPORS* (observed SNVs = 4). (2) The pLoF of the gnomAD database includes only nonsense and splice variants (acceptor or donor) caused by single nucleotide changes (http://gnomad-sg.org/help). Therefore, the pLI of *TOPORS* may be overestimated by missing frameshift variants that account for approximately half (12/27, 44.4%) of the *TOPORS* pLoF alleles, according to the gnomAD database. (3) The pLoF variants are frequently subjected to NMD and thus result in haploinsufficiency. For *TOPORS*, 55.6% (15/27) of the protein-truncating alleles in the gnomAD database were located in the last exon, which probably escaped NMD and produced a truncated protein with unexpected function. The involvement of these variants may also overestimates the pLI of *TOPORS*. This problem may not be limited to *TOPORS*; however, our study suggests that a systematic evaluation of variants at the individual gene level may address these problems, not only for *TOPORS* but also for other genes associated with autosomal dominant diseases. In the gnomAD database, the c.2554_2557del/p.(Glu852Glnfs*13) variant, a common pathogenic variant reported in this study and previous studies,[10,13,31,37,39–41] was identified in the 807 to 867 residues. A case with this variant could be a late-onset RP case or a non-penetrant person.

Recently, with the development of CNV-testing and bioinformatics analysis technologies, an increasing number of CNVs have been identified in Mendelian diseases. Approximately 9.8% of patients with various genetic disorders have pathogenic or likely pathogenic CNVs.[59] In addition, with the development of sequencing technologies, such as chromosome microarray analysis and next-generation sequencing, various types of CNVs have been detected. The ACMG and the Clinical Genome Resource have developed technical standards for interpreting and reporting CNVs to standardize the consistency of CNVs reports across different laboratories,[60] and the ACMG/AMP standards have been updated to better classify CNVs.[18] However, accurate clinical interpretation of CNVs and defining their clinical significance are still challenging, especially when used for prenatal evaluation.[2,61] In particular, it is difficult to compare the detected

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CNVs with the reported CNVs, due to breakpoint uncertainty.[18] Although the current evidence suggests that truncating variants and CNVs at the N-terminus of *TOPORS* are unlikely to be pathogenic, it is noteworthy that they may be associated with late-onset mild RP as *CRX* (Yahya S, et al. *IOVS* 2021;62:ARVO E-Abstract 1536) and or even non-RP diseases, which requires further investigation.

In our cohort, none of the missense and in-frame variants of *TOPORS* was considered to be causal with RP for the following reasons. First, individuals with these variants were not related to RP or even retinopathy. Second, variants were detected in individuals with other clear alternative genetic disease etiologies. Third, these variants have a high allele frequency in control populations, making them tolerable in autosomal dominant diseases. Fourth, these variants were predicted to be benign using at least three in silico prediction tools. Fifth, these missense and in-frame variants do not cluster in a specific region like truncating variants; however, based on the literature, a few missense and in-frame variants have been reported to be pathogenic (https://databases.lovd.nl/shared/variants/TOPORS/unique). In particular, the c.1205A>C/p.(Gln402Pro) variant was identified in a large Norwegian family with 10 affected individuals[62]; therefore, caution should be exercised when interpreting missense or in-frame variants in *TOPORS*.

In conclusion, for *TOPORS* variants, pathogenic truncating variants are located within residues 807 to 867 but are nonpathogenic for N-terminus truncating variants, including CNVs. Therefore, a dominant-negative effect, rather than haploinsufficiency, is presumed to be the underlying pathogenesis of RP caused by *TOPORS* variants; however, additional functional studies are required to validate this hypothesis. The results of this study provide a reference for the clinical interpretation of *TOPORS* variants and other genes in similar situations, especially providing insight on the interpretation of CNVs.

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