Missense Mutations in *MAB21L1*: Causation of Novel Autosomal Dominant Ocular BAMD Syndrome

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Purpose. Biallelic *MAB21L1* variants have been reported to cause autosomal recessive cerebellar, ocular, craniofacial, and genital syndrome (COFG), whereas only five heterozygous pathogenic variants have been suspected to cause autosomal dominant (AD) microphthalmia and aniridia in eight families. This study aimed to report an AD ocular syndrome (blepharophimosis plus anterior segment and macular dysgenesis [BAMD]) syndrome based on clinical and genetic findings from patients with monoallelic *MAB21L1* pathogenic variants in our cohort and reported cases.

Methods. Potential pathogenic variants in *MAB21L1* were detected from a large in-house exome sequencing dataset. Ocular phenotypes of the patients with potential pathogenic variants in *MAB21L1* were summarized, and the genotype-phenotype correlation was analyzed through a comprehensive literature review.

Results. Three heterozygous missense variants in *MAB21L1*, predicted to be damaging, were detected in 5 unrelated families, including c.152G>T in 2, c.152G>A in 2, and c.155T>G in one. All were absent from gnomAD. The variants were de novo in two families, transmitted from affected parents to offspring in two families, and with an unknown origin in the other family, demonstrating strong evidence of AD inheritance. All patients revealed similar BAMD phenotypes, including blepharophimosis, anterior segment dysgenesis, and macular dysgenesis. Genotype-phenotype analysis suggested that patients with monoallelic *MAB21L1* missense variants had only ocular anomalies (BAMD), whereas patients with biallelic variants presented both ocular and extraocular symptoms.

Conclusions. Heterozygous pathogenic variants in *MAB21L1* account for a new AD BAMD syndrome, which is completely different from COFG caused by homozygous variants in *MAB21L1*. Nucleotide c.152 is likely a mutation hot spot, and the encoded residue of p.Arg51 might be critical for *MAB21L1*.

Keywords: *MAB21L1*, autosomal dominant (AD), pathogenic variant, blepharophimosis plus anterior segment and macular dysgenesis (BAMD) syndrome, hotspot

Microphthalmia is a severe ocular developmental defect characterized by a marked reduction in eye globe volume, which is usually associated with severe sight impairment.[1–3] The prevalence of microphthalmia has been reported to be 2 to 17 of 100,000 live births.[4–7] Ocular coloboma is caused by an abnormal or incomplete optic fissure closure.[8,9] As many as 39 coloboma disease genes have been reported; and 70% (27/39) of them involve microphthalmia, and 82% (32/39) of them are associated with anterior segment dysplasia.[10] Whereas anterior segment dysplasia is often observed in association with microphthalmia, reports of blepharophimosis with microphthalmia are more limited and in most cases are associated with additional facial or skeletal anomalies.[11] The etiology of microphthalmia is diverse and includes both environmental and heritable factors. Although more than 83 genes have been reported as causative of this disease,[9,12,13] most cases with microphthalmia still do not have a genetic explanation.

The Male-Abnormal 21-Like (*MAB21L1*; HGNC: 4081; OMIM: 601280) gene resides at 13q13 and is a member of the conserved male abnormal gene family 21, which was initially described as a transcription factor determining cell fate in the nematode *Caenorhabditis elegans*.[14] Since its identification, an essential role of the *MAB21L1* gene has been confirmed in embryonic development by regulating both eye and brain development.[15–18] In addition, it is also developmentally important for regulating axis and dorsal-ventral patterning, as well as cardiac and hepatic development.[16,17,19] Variants in *MAB21L1* were first reported to cause a global development delay, facial dysmorphism, scrotal agenesis, and cerebellar malformation in autosomal-recessive mode.[20] Further evidence was found that biallelic variants in *MAB21L1* may lead to an autosomal recessive condition, cerebello-oculo-facio-genital syndrome (COFG syndrome). Congenital ocular anomalies, including nystagmus, strabismus, and corneal dystrophy/opacities,

were also observed in most of these patients.[21] In contrast, five heterozygous variants (c.152G>T [p. Arg51Leu], c.152G>A [p.Arg51Gln], c.152G>C [p.Arg51Pro], c.155T>G [p.Phe52Cys], and c.156C>G [p.Phe52Leu]) were identified in eight families with ocular abnormalities as being autosomal dominant.[18,22,23] These patients presented with various phenotypes, including eyelid abnormality, microphthalmia, microcornea, strabismus, nystagmus, iris coloboma, aniridia, cataract, lens luxation, and retina dystrophy. Among them, only two cases manifested as syndromic disorders. The mechanism of pathogenic dominant and recessive *MAB21L1* pathogenic variants remains unknown. More evidence is required to support the gain of function and the autosomal dominant inheritance mode of the *MAB21L1* gene.

In this study, we focused on the clinical manifestations of patients with *MAB21L1* monoallelic missense pathogenic variants. We summarized these phenotype characteristics from our patients and reported cases,[18,20–23] and named the new ocular syndrome (blepharophimosis, anterior segment, and macular dysgenesis [BAMD]), caused by monoallelic pathogenic variants in *MAB21L1*.

Methods

Patient Recruitment and Data Collection

This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. The ocular phenotypes and venous blood samples for the probands and their accessible family members were collected from our Pediatric and Genetic Clinic Department, Zhongshan Ophthalmic Center, Guangzhou, China. Written informed consent consistent with the tenets of the Declaration of Helsinki was acquired from the probands or their guardians before they participated in this study. Genomic DNA was prepared from the leukocytes of peripheral venous blood with a previously described method.[24]

Each participating individual underwent routine ophthalmologic examinations. Additional ophthalmologic examinations, including photography of the anterior segment and/or fundus, refraction examination, scanning laser ophthalmoscopy, optic coherence tomography (OCT), and ultrasound biomicroscopy (UBM), were performed when needed. The ocular axial length was measured with an IOL master or A-scan ultrasonography. When visual axis opacification occurred, B-scan ultrasonography was performed.

Variant Detection and Evaluation

Variant data on *MAB21L1* were retrieved from our in-house database, including 7538 probands with different kinds of hereditary eye disease who underwent genetic testing whole-exome sequencing and whole-genome sequencing.[25,26] The partial pooled analysis from the in-house database indicated that among the individuals who performed target sequencing, patients with anterior segment dysgenesis accounted for 4.05%.[27] After the detection of variants in *MAB21L1* from the exome sequencing data, the variants were analyzed through multistep bioinformatics analysis, as previously described.[28] First, low-sequencing quality variants with a coverage of less than five were excluded. Second, synonymous and noncoding variants that did not alter any splicing site predicted by the Berkeley Drosophila Genome Project (https://www.fruitfly.org/) were excluded. Third, based on the gnomAD database (https://gnomad.broadinstitute.org/), variants with minor allelic frequencies (MAFs) ≥0.01 were excluded. In silico tools were used to predict the remaining variants, such as SIFT (http://sift.jcvi.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), PROVEAN (http://provean.jcvi.org/seq_submit.php), CADD (http://cadd.gs.washington.edu), and REVEL (https://sites.google.com/site/revelgenomics/). Fourth, potentially pathogenic variants were further evaluated by co-segregation in available family members through Sanger sequencing. The primer sequences are available on request. Sanger sequencing validation, including amplification, sequencing, and target sequence analysis, was performed using a BigDye Terminator cycle sequencing kit version 3.1 and ABI 3130 Genetic Analyzer (both from Applied Biosystems, Foster City, CA, USA). The resultant sequences were further compared to consensus sequences using Seqman software (Lasergene 8.0; DANSTAR, Inc., Madison, WI, USA). Finally, the allele frequency of the detected variants was compared between this patient cohort and our in-house data, as well as the gnomAD database. The variants were reclassified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG). Two sets of criteria were applied to classify the variants: one for the classification of pathogenic (P) or likely pathogenic (LP) and one for the classification of benign or likely benign. If a variant did not fulfill criteria using either of these sets (pathogenic or benign), or the evidence for benign or pathogenic was conflicting, the variant defaulted to uncertain significance (VUS).[29]

Structure of Proteins

Protein structure prediction was performed with the SWISS-MODEL online server (https://swissmodel.expasy.org).[30] The molecular visualization of protein structures was conducted using PyMOL software (https://pymol.org/2/). A comparative analysis was undertaken based on the 3D structures of the wild-type and mutant proteins.

Statistical Analysis

Statistical significance was calculated using GraphPad Prism (Prism 8, GraphPad Software, La Jolla, CA, USA). Fisher's exact test was used to compare the frequency of different phenotypes of patients with heterozygous pathogenic variants between our data and reported cases, as well as that of different phenotypes between monoallelic and biallelic groups based on available data. Statistical significance was defined as $P < 0.05$.

Results

MAB21L1 Variants Identified in In-House Data

In total, 3 heterozygous missense variants in *MAB21L1* were identified in 5 out of the unrelated probands with different eye conditions, including c.152G>T/p.Arg51Leu, c.152G>A/p.Arg51Gln, and c.155T>G/p.Phe52Cys (Table 1, Fig. 1). All three variants were absent from gnomAD (Figs. 2A–2C) and were predicted to be deleterious by five in silico tools. Both heterozygous variants (c.152G>T and c.152G>A) were detected in 2 probands, whereas variant c.155T>G was identified in one proband. Variants c.152G>T, c.152G>A, and c.155T>G in our cohort were further confirmed by Sanger sequencing and

Table 1. Potential Pathogenic Variants in *MAB21L1* Identified in This Study

No.	Position chr 13	Ref	Alt	NM_005584.4 Change	Effect	Family Count	ACMG Rank	ACMG Evidence	REVEL	CADD	SIFT	Polyphen-2	PROVEAN	HGMD	Reference
1	36050124	C	A	c.152G>T	p.Arg51Leu	2	P	PS1+PS2+PS3+PM1+PM2+PP1+PP3+PP4	0.682	32	D (0)	D (0.999)	D (−5.36)	DM	18,23
2	36050124	C	T	c.152G>A	p.Arg51Gln	2	P	PS2+PM1+PM2+PM5+PP1+PP3+PP4	0.542	32	D (0)	D (0.999)	D (−2.96)	/	23
3	36050121	A	C	c.155T>G	p.Phe52Cys	1	LP	PM1+PM2+PP1+PP3+PP4	0.745	32	D (0)	D (0.969)	D (−6.1)	/	23

Ref, reference; Alt, alteration; P, pathogenic; LP, likely pathogenic; D, damaging; N, neutral; HGMD, human genome mutation database; DM, disease-causing mutation; "/" indicates no case reported.

completely co-segregated. These missenses were verified as de novo in two families, as transmitted from affected parents to offspring in two families, and as having an unknown origin in the other family, demonstrating a strong inheritance pattern for autosomal dominant (AD), which was similar to previous reports.[18,22,23] Potential pathogenic variants (PPV), c.152G>T and c.152G>A were classified as pathogenic, and c.155T>G was classified as likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines. Additionally, variants c.510C>G, c.348C>G, and c.218A>G were identified in our cohort and classified as VUS (Supplementary Table S1).

Structure-based analyses demonstrate that the amino acid substitution at position p.51 disrupts the hydrogen bonding among R51, E49, and E115, which is fundamental to the stabilization of the MAB21L1 protein (Fig. 2D),[31] thus substantially altering the protein structure and leading to function damage. The alteration of the amino acid at position p.52 also has a considerable influence on the protein's secondary structure (see Fig. 2D). Comparative amino acid sequence analyses indicated the conservation of the position of MAB21L1 among species (Fig. 2E).

Phenotypic Features of Patients With *MAB21L1* Variants

Typical symptoms were observed in 7 Chinese patients from our cohort (Figs. 3A-3H, Supplementary Fig. S1), including blepharophimosis 100% (7/7), microphthalmia 86% (6/7), microcornea 100% (7/7), cornea dystrophy 14% (1/7), aniridia 67% (4/6), iris coloboma 33% (2/6), cataract 100% (6/6), lens luxation 50% (3/6), macular dysgenesis 100% (3/3), choroidal coloboma 67% (2/3), optic dysplastic 33% (1/3), nystagmus 86% (6/7), and strabismus 86% (6/7). We summarized these phenotypes and named the new ocular syndrome (BAMD). Partial and milder abnormalities, including ptosis, shorter axial length, iris hypoplasia, and macular dysgenesis, were identified in another proband with the VUS c.510C>G. Unlike the typical iris coloboma, which was inferonasal in position and displayed a "keyhole" appearance,[4,32] an atypical supranasal iris coloboma was observed in the patients from family 5. Compared with the proband, the supranasal iris deficiency of her father (family 5 II:3) was less severe. Variable phenotypes could arise despite an identical variant in one pedigree (see Figs. 3C, 3D). In contrast with the phenotype of family 5 III:1 with variant c.155T>G, family 5 II:3 presented as right corneal dystrophy and left buphthalmia, which was likely related to glaucoma with elevated intraocular pressure. The phenotypic difference could have been a result of age. A varying degree of retinal dysplasia, from macular dysgenesis to retinochoroidal coloboma, was observed in patients with *MAB21L1* pathogenic variants (see Figs. 3F–3H).

The correlation between genotype and phenotype was subsequently analyzed based on the patients from our in-house database and previously reported literature. The clinical features of 7 patients in our cohort, 18 patients from reports with monoallelic *MAB21L1* missense variants, and 11 other patients reported to have biallelic *MAB21L1* variants are summarized in Table 2 and Supplementary Tables S2, S3.[18,20–23] Because there were no significant differences in different proportions of symptoms in patients with monoallelic *MAB21L1* variants between our

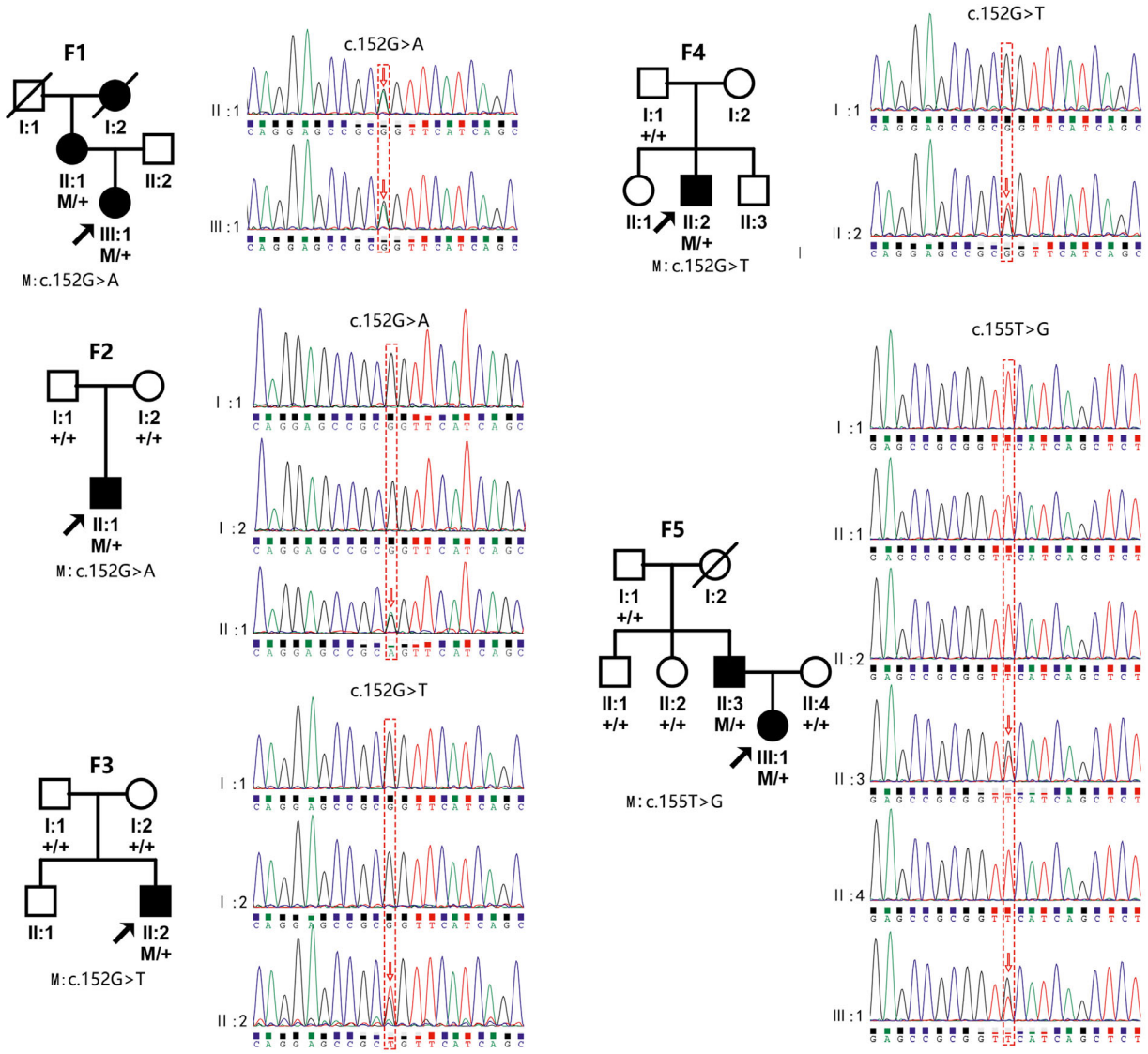

Figure 1. The pedigrees of 5 families with the sequencing of heterozygous pathogenic variants in *MAB21L1*. The pathogenic variant is represented by "M" and indicated by a *red arrow*. Wild-type alleles are represented by "+." NC, normal control.

cohort and published literature, we combined all data and summarized the frequency of BAMD phenotypes as follows: blepharophimosis 100% (7/7), microphthalmia 84% (21/25), microcornea 67% (12/18), corneal dystrophy 20% (5/25), aniridia 50% (12/24), iris coloboma 42% (10/24), cataract 86% (18/21), lens luxation 57% (12/21), macular dysgenesis 53% (9/17), choroidal coloboma 26% (5/19), optic dysplastic 26% (5/19), nystagmus 79% (19/24), and strabismus 52% (13/25). In addition, extraocular abnormalities were observed in only two cases (8%, 2/25), including hyperthyroidism and sensorineural hearing loss (Fig. 3J).[18,22,23] Although the phenotype of blepharophimosis was not described in the reported literature, abnormalities in the eyelid could be observed in the anterior segment photograph of two patients in family 511 (III:1 and III:2), including small palpebral fissures and epicanthus inversus.[23] As there was insufficient evidence to support a diagnosis of blepharophimosis, we did not include these two cases with eyelid malformations when calculating the frequency of the blepharophimosis phenotype; instead, these cases are included in Supplementary Table 2. We expect that more details on the eyelid in patients harboring monoallelic pathogenic variants in *MAB21L1* will be described in future studies.

In contrast, all 11 patients with biallelic variants from the published literature manifested global abnormalities as COFG syndrome. The ocular abnormalities consisted of corneal dystrophy (100%, 11/11), nystagmus (91%, 10/11), and strabismus (55%, 6/11).[20,21] The frequency of corneal dystrophy and global abnormalities in patients with biallelic *MAB21L1* mutations was significantly higher than that in patients with monoallelic mutations ($P < 1 \times 10^{-4}$). The frequency of cataract and retinal dystrophy was not calculated because these phenotypes were documented in only one patient separately. Other BAMD phenotypes were not described in 10 of the 11 patients (Fig. 3J). Ocular abnormalities in patients with monoallelic missense pathogenic variants were BAMD phenotypes, whereas ocular or syndromic abnormalities were not reported in 18 individuals with monoallelic truncating variants according to previous

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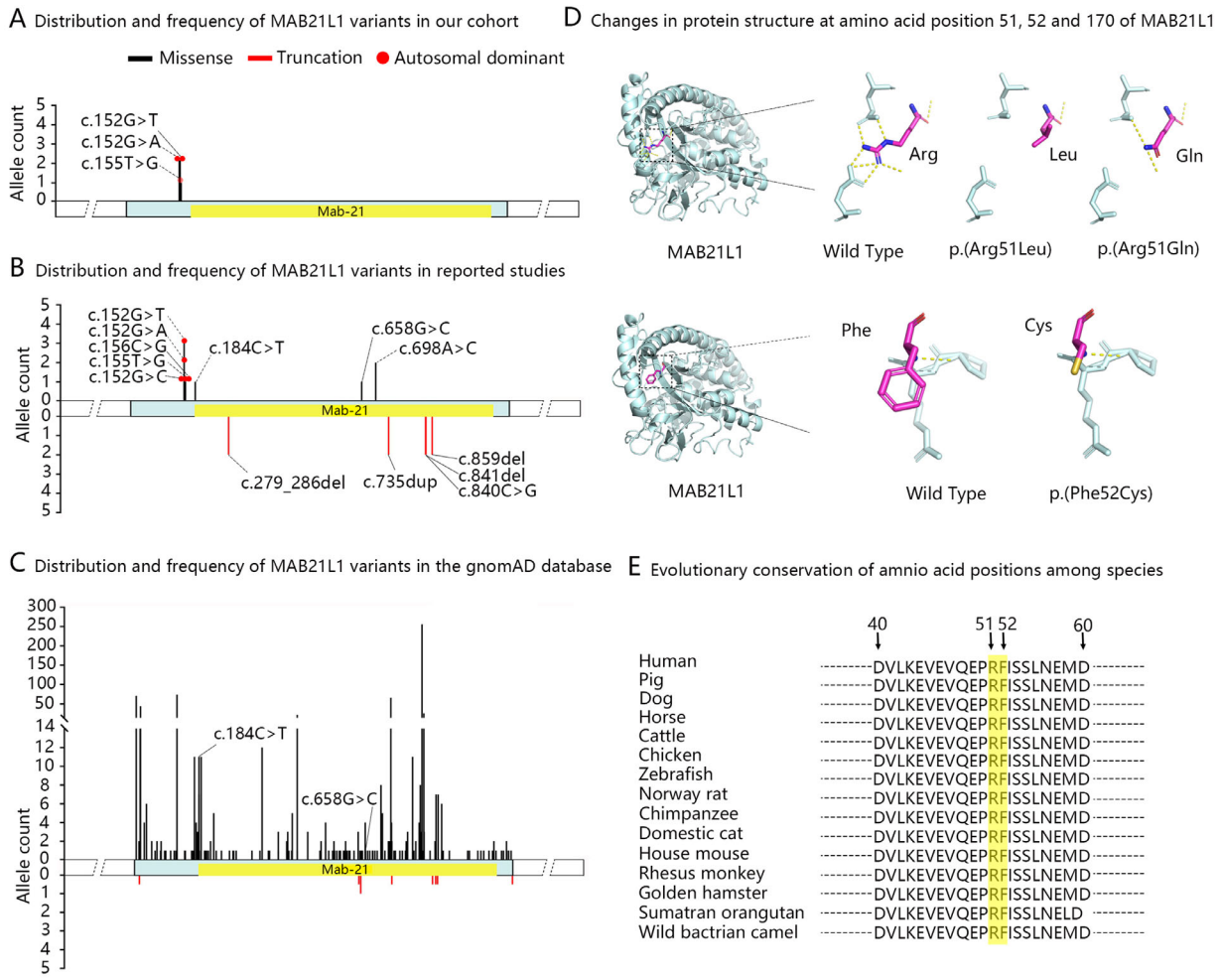

Figure 2. Pathogenic analysis of *MAB21L1* variants identified in our cohort and reported studies. (**A**) Distribution and frequency of monoallelic *MAB21L1* potential pathogenic variants in our cohort. (**B**) Distribution and frequency of *MAB21L1* variants in reported studies. (**C**) Distribution and frequency of *MAB21L1* variants in the gnomAD database. The potential pathogenic variants from the published literature that could be observed in gnomAD are marked with the nucleic acid position. The *red solid circles* indicate that these variants are autosomal dominant inherited. The *black and red lines* represent missense and truncation variants, respectively. The missense variants are shown above and the truncation variants are shown below. (**D**) Changes in protein structure at positions 51 and 52 of *MAB21L1*. The structural models on the left represent the wild-type protein. The alteration of the protein secondary structure and disruption of the hydrogen band between 51Arg and 49Glu can be observed. (**E**) Evolutionary conservation of amino acid positions among different species. Mutants of amino acids are highlighted in *yellow*.

studies.[20,21] The phenotype varied depending on the type of pathogenic variants in *MAB21L1*.

Discussion

MAB21L1 is a gene of growing interest to the ophthalmic community that plays a pivotal role in congenital ocular malformations by regulating lens, cornea, and iris development.[18,33–36] Here, we identified heterozygous variants in *MAB21L1* in 7 affected members of 5 families who were phenotypically similar to individuals with congenital ocular anomalies. Typical BAMD syndromes were observed in patients with potential pathogenic variants in *MAB21L1*. Genetic analysis revealed that patients harboring variants at c.152 or c.155 presented similar symptoms. Our study demonstrates that *MAB21L1* is a new causative gene for autosomal dominant BAMD syndrome and provides genetic evidence for the detrimental function of pathogenic variants in *MAB21L1*.

Heterozygous pathogenic variants near the hotspot region (c.152 in *MAB21L1*) were identified in patients with BAMD syndrome. *MAB21L1* and *MAB21L2* both belong to the male-abnormal 21-like family and share an open reading frame of 1080 nucleotides with 94% similarity, which suggests similar functions.[34] Interestingly, parts of BAMD phenotypes, including microphthalmia, microcornea, cataracts, and ocular coloboma, were also observed in patients with heterozygous variants affecting the Arg 51 residue in *MAB21L2* in an autosomal dominant inheritance mode.[37–39] The Arg 51 residue in MAB21L1 or MAB21L2 might play a special role when the 2 proteins participate in the eyeball development, which was supported by the *Mab21l2*$^{R51C/+}$ mice without eyeballs.[40] *MAB21L1* is a globular protein with 2 lobes connected by a long α-helix.[31] A subdomain structurally similar to the NTase domain can

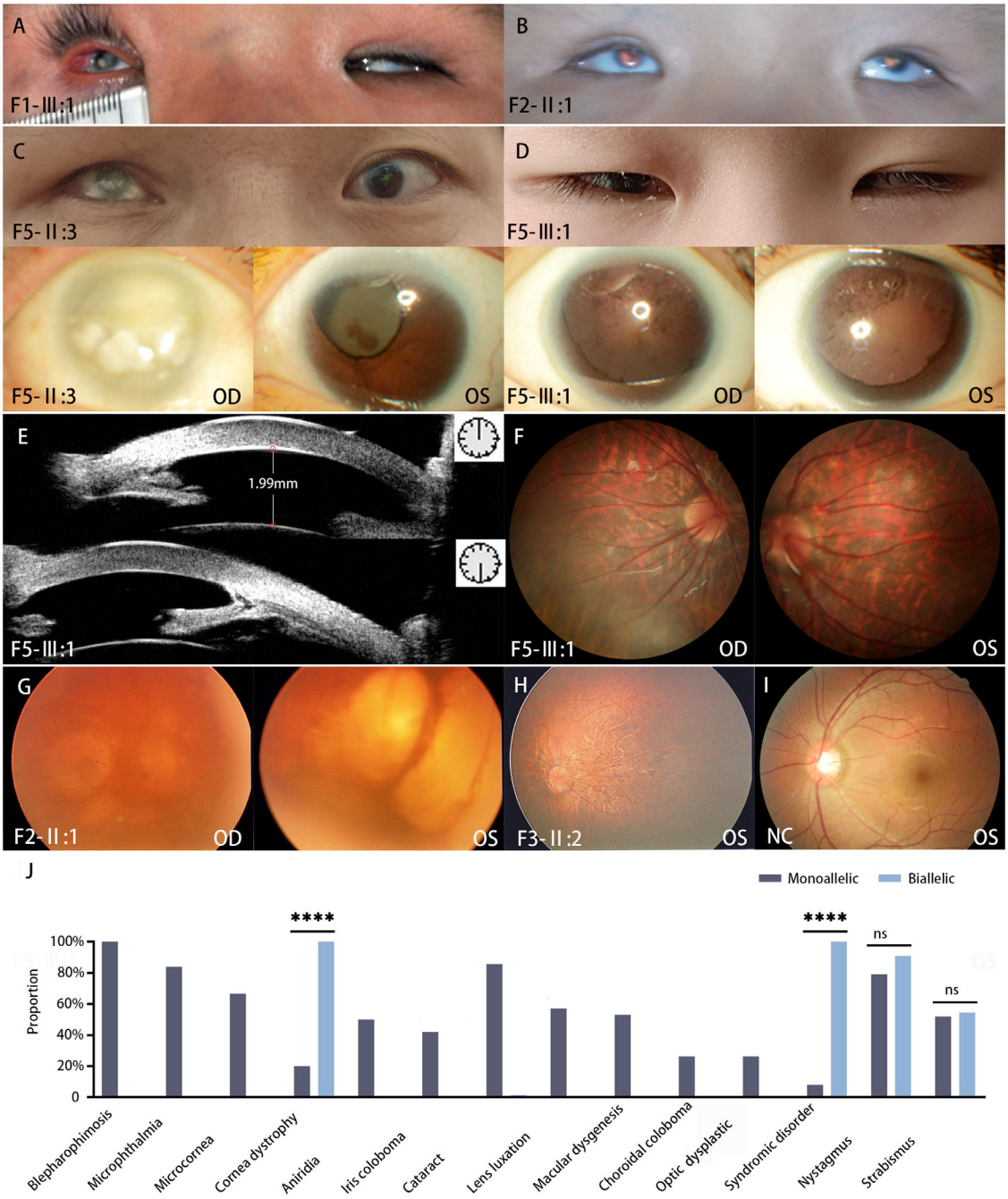

Figure 3. Phenotypes of patients with *MAB21L1* in our cohort. (**A–D**) Typical ocular abnormalities involving blepharophimosis, microcornea, iris coloboma, and lens abnormalities were observed in patients with *MAB21L1* pathogenic variants. (**C, D**) Different degrees of ocular phenotypes were observed in family 5. Corneal dystrophy was observed in the right cornea of family 5 II:3, whereas a transparent cornea was observed in his daughter family 5 III:1. Atypical supranasal iris coloboma is observed in family 5 II:3 and family 5 III:1. (**E**) UBM indicated that bilateral eyes had significantly shallower anterior chambers with partial iridocorneal adhesion and anterior chamber angle closure in family 5 III:1. (**F–H**) Fundus changes were present in patients with *MAB21L1* pathogenic variants. Macular dysplasia for both eyes without normal macular foveal reflection was observed in **F** and **H** compared with a normal fundus in **I**. Retinochoroidal coloboma was observed in **G**. (**J**) Comparison of the frequency of different symptoms between monoallelic and biallelic groups from our cohorts and reported cases. AD, autosomal dominant; AR, autosomal recessive; $^{****}P < 0.0001$, ns: no statistical significance.

be found at the N-terminal lobe, which is largely surface-accessible and contains several positively charged amino acid residues. The N-terminal region is responsible for the functional specificities of the MAB21L1 protein, as it is involved in the recognition of cytosolic nucleic acids and the subsequent production of 2′,3′-cGAMP.[41,42] Arg51 of MAB21L1 is located at the beginning of the $\alpha 1$ - $\beta 1$ subunit within the N-terminal DNA-binding domain and plays a pivotal role in the formation of salt bridges along with Glu49 and Glu115, which is crucial to protein stability, by anchoring the loop between $\beta 4$ and $\alpha 4$.[31] Pathogenic variants at this hotspot are more likely to destabilize the protein, leading to

Table 2. Clinical Data of Patients Identified With Monoallelic *MAB21L1* Variants in Our Cohort.

Family ID	Family 1-II:1	Family 1-III:1	Family 2-II:1	Family 3-II:2	Family 4-II:2	Family 5-II:3	Family 5-III:1	Proportion*	Proportion†	*P* Value
DNA change	c.152G>A	c.152G>A	c.152G>A	c.152G>T	c.152G>T	c.155T>G	c.155T>G	/	/	
Het or homo	het	het	het	het	het	het	het	/	/	
Protein change	p.Arg51Gln	p.Arg51Gln	p.Arg51Gln	p.Arg51Leu	p.Arg51Leu	p.Phe52Cys	p.Phe52Cys	/	/	
Age (y)	25	4.4	2.7	1.7	1.3	38	0.3	/	/	
Ancestry	CN	CN	CN	CN	CN	CN	CN	/	/	
BCVA (OU)	LP	NA	CL	NA	CL	NLP(OD), 0.02(OS)	0.15	/	/	
Blepharophimosis	+	+	+	+	+	+	+	100%	NA	NA
Nystagmus	+	+	+	+	+	+	/	86%	76%	1.00
Strabismus	+	+	+	+	+	+	/	86%	39%	0.07
Microphthalmia	OU	OU	OU	OU	OU	/	OU	86%	83%	1.00
Axial length (mm)										
OD	NA	14.4	NA	18	17.7	NA	18.21	/	NA	
OS	NA	15.1	NA	19	17.6	29.16	18.24	/	NA	
Corneal diameter (OU, mm)	6	4	5	7	6	7	7	100% (MC)	45% (MC)	1.00
Corneal dystrophy	/	/	/	/	/	OD	/	14%	22%	1.00
Aniridia	OU	OU	NA	OU	OU	/	/	66%	44%	0.64
Iris coloboma	/	/	NA	/	/	OS, NA (OD)	OU	33%	44%	1.00
Cataract	OU	OD	NA	OU	OU	OU	PALC (OU)	100%	80%	0.53
Lens subluxation	OU	MS (OS)	NA	/	/	OS	/	50%	60%	1.00
Choroidal coloboma	NA	NA	OU	OU	NA	NA	/	67%	19%	0.21
Macular dysgenesis	NA	NA	OU	OU	NA	NA	OU	100%	43%	0.16
Optic dysplastic	NA	NA	Hypoplastic (OU)	/	NA	NA	/	33%	25%	1.00
Origin	This study	This study	This study	This study	This study	This study	This study	/	/	

Het, heterozygous; homo, homozygous; y, years; CN, Chinese; HIS, Hispanic; LP, light perception; CL, chasing light; NLP, no light perception; "+", positive; "/", negative; NA, not available; MC, microcornea; IC, iris coloboma; PALC, pigmented anterior lens capsule; MS, microspherophakia.

P value: Fisher's exact test was used to compare the frequency of different phenotypes of patient with heterozygous pathogenic variants between our data and the reported cases. No significant differences were observed.

* Proportion, the proportion of each symptom in our cohort.

† Proportion: the proportion of each symptom in the reported literature.

dysfunction of the MAB21L1 protein and resulting in more similar clinical symptoms in patients with different ethnic backgrounds.

Blepharophimosis is most commonly associated with blepharophimosis-ptosis-epicanthus-inversus syndrome, also known as isolated BPES.[43] In addition, "blepharophimosis-plus" disorders, including Dubowitz syndrome, Ohdo syndrome, congenital contractural arachnodactyly, Van den Ende-Gupta syndrome, 22q11.2 duplication, and 3q22 deletion, have also been reported.[43,44] To date, for the symptoms of colobomatous microphthalmia accompanied by blepharophimosis, only a few case reports have been described in the literature, and the genetic cause remains unclear.[45] Blepharophimosis has been reported to be an associated symptom caused by the deficiency of eye globe stimuli during development.[46–48] There is still a lack of an entry that contains all the typical symptoms. Referring to the 18 cases with heterozygous P/LP variants in *MAB21L1* in the reported literature,[18,22,23] we summarized the phenotype characteristics from 25 patients with heterozygous missense pathogenic variants in *MAB21L1* around hotspots in this study. Blepharophimosis was observed in all 7 patients from 5 different pedigrees in this study. Although previous studies did not well describe this phenotype,[18,22,23] abnormalities in the eyelid (including small palpebral fissures and epicanthus inversus) could be observed in the image of 2 patients in family 511.[23] Other global malformations, including microphthalmia, anterior segment (microcornea, aniridia, iris coloboma, and cataract), and macula dysgenesis, were highly overlapped in our patients and reported cases. Therefore, we summarized these findings and reported a novel autosomal dominant ocular BAMD (blepharophimosis, anterior segment, and macular dysgenesis) syndrome based on clinical and genetic findings and hotspot pathogenic variants in *MAB21L1* should be considered for patients with a similar spectrum of eye malformations, including blepharophimosis or coloboma.

Coloboma, a segmental ocular defect typically in the inferonasal quadrant (a "keyhole" deficiency), is mostly caused by failure of the fetal or choroidal fissure to close.[4,32] A coloboma that occurs anywhere other than the inferonasal quadrant of the eyeball is termed "atypical," and its embryologic basis is still unknown. Interestingly, the two patients with c.155T>G in *MAB21L1* from family 5 in our cohort presented atypical iris coloboma in the supranasal region. A similar situation was previously observed in a patient harboring c.152G>T in *MAB21L1* (family 1434),[23] in which the iris defect also occurred in the supranasal region. These findings shed new light on the role of *MAB21L1* in the formation of atypical colobomas and additional studies are required to discover the possible mechanism.

Homozygous or compound heterozygous pathogenic variants in *MAB21L1* were first reported in patients diagnosed with COFG and loss-of-function was the causative mechanism.[20,21] To date, 5 autosomal dominant missense pathogenic variants in the hotspot of *MAB21L1* have been observed in 25 cases from 13 families,[18,22,23] and the gain of function may be the cause of BAMD. Functional trials indicated that Arg51Leu exerted a dominant-negative effect by increasing stability and exhibited the highest degree of functional deficiency in the zebrafish *mab21l1* mutant rescue test.[18] The different causative mechanisms might be addressed by the phenomenon in which *MAB21L1* deficiency caused COFG in the autosomal recessive (AR) pattern and BAMD in the AD pattern. In this study, ocular symptoms were autosomal dominantly inherited in four patients from two unrelated families harboring monoallelic missense variants. In addition, two variants were de novo, which was confirmed by co-segregation. Solid genetic evidence supports the idea that heterozygous pathogenic variants in *MAB21L1* are the cause of BAMD with autosomal dominant inheritance.

Patients harboring the same nucleic acid alteration in *MAB21L1* may differ regarding their severity of BAMD, varying from iris coloboma limited to the supranasal quarter (family 5 II:3), to three quarters (family 5 III:1), from buphthalmos (family 5 II:3) to microphthalmos (family 5 III:1), and from corneal dystrophy (family 5 II:3) to microcornea (family 5 III:1). This is similar to previously reported cases.[18,22,23] Further studies are required to elucidate the molecular genetic mechanism for the phenotypic difference with the same variant in *MAB21L1*.

In our current study, integrative analysis was performed to elucidate the genotypic and phenotypic spectra of *MAB21L1* according to our exome-sequencing data and the previously reported literature.[20–23,34] An ethnic-specific spectrum of *MAB21L1* variants was reported, which simplified the interpretation of variants in different populations. Isolated BAMD syndromes were revealed to be caused by monoallelic variants in *MAB21L1* by autosomal dominant inheritance. The patients carrying nucleic acid changes near the hotspot region c.152 were likely to present BAMD syndromes. This study provides a new reference for the genetic diagnosis of patients harboring *MAB21L1* pathogenic variants by assessing genetic variants and associated phenotypes in diverse populations.

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Investigative Ophthalmology & Visual Science

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