Genetic and clinical landscape of ARR3-associated MYP26: the most common cause of Mendelian early-onset high myopia with a unique inheritance

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ABSTRACT

Aims To elucidate genetic background of early-onset high myopia (eoHM) and characteristics of ARR3-associated MYP26.

Methods Variants in 14 genes reported to contribute to eoHM, including ARR3, were selected from exome sequencing data set and classified into different categories following American College of Medical Genetics and Genomics guidelines based on in silico prediction, associated phenotypes, confirmation and cosegregation analysis. The available clinical data of individuals were summarised.

Results Pathogenic and likely pathogenic variants in three of 14 genes were identified in 52 of 928 families with eoHM, including 29 in ARR3, 22 in OPHN1W and 1 in LRPA1. For ARR3, 24 pathogenic variants (16 truncation and 8 missense) were identified in 66 women and 12 men, in whom 64 women and 4 men had eoHM by X-linked female-limited inheritance. Refraction ranged from −5.00 to −28.75 diopter (−12.58±4.83). Mild-to-moderately reduced cone responses were recorded in 76.9% (10/13) of patients with electroretinogram recordings. Most patients (75.9%, 41/54) had mild myopic fundus changes (C0 to C1). Genotype-phenotype analysis suggested that the myopic retinopathy degree was correlated with age and the variant’s nature. Peripheral retinal degeneration was observed in 38.5% (5/13) patients using wide-field examinations.

Conclusion This study reveals ARR3 as the most frequently implicated gene for Mendelian eoHM. Truncations and highly scored missense variants in ARR3 are pathogenic. Myopia due to ARR3 mutations is transmitted in X-linked female-limited inheritance, manifests with mild cone impairment and slowly progresses to pathologic myopia. Identification of the most common cause for Mendelian eoHM provides a valuable starting point into the molecular mechanism of myopia.

INTRODUCTION

Pathologic ocular changes associated with high myopia (HM) have become a leading cause of blindness and low vision.1 2 Genetic studies on HM, especially early-onset high myopia (eoHM), provide an important avenue for elucidating its underlying molecular basis. In the background of a major contribution of polygenic inheritance, clarifying the contribution of reported monogenic genes in eoHM was also valuable.3 4 In particular, mutations in ARR3 were suggested to be the most common cause of Mendelian eoHM based on our in-house data and a recent study.5 6

Our previous study first reported that MYP26—an eoHM transmitted in a unique X-linked female-limited inheritance—is caused by heterozygous variants of ARR3 (HGNC: 710, OMIM: 301770),5 ARR3 locates at Xq13.1 and encodes a 388-amino acid cone arrestin, which is mainly expressed in cones.7 To date, X-linked female-limited inheritance has been only observed in two diseases—MYP26 due to ARR3 mutations and epilepsy and mental retardation restricted to women due to PCDH19 mutations.8 10 ARR3-associated MYP26 has been further confirmed in other studies.7 11 12 Understanding the genetic and clinical landscape of ARR3-associated MYP26 is interesting considering that it is a potential most frequently implicated gene for Mendelian eoHM with a unique pattern of inheritance.

In the current study, potentially pathogenic variants (PPVs) in known genes associated with eoHM were selected from 928 families with eoHM collected in our clinic and thoroughly evaluated. Specifically, ARR3 variants were systematically and strictly analysed. Comparative analysis was performed using three data sets: (1) ARR3 variants in 6386 subjects with other eye conditions and permissions. Published by BMJ.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Heterozygous variants in ARR3 lead to early-onset high myopia (eoHM) in a unique X-linked female-limited inheritance.

WHAT THIS STUDY ADDS

⇒ Variants in ARR3 are the most frequent cause of Mendelian eoHM. Truncation and highly scored missense variants in ARR3 contributed to eoHM, which is characterised by mild cone impairment.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Revealing the most frequently implicated genes responsible for Mendelian eoHM might shed light on the myopic aetiology and potential targets for interventions.
ARR3 variants in gnomAD and (3) reported ARR3 variants. Clinical data of patients with PPVs in ARR3 were summarised and analysed. A total of 24 disease-causing mutations in ARR3 were identified in 29 families. Genotype and phenotype analysis of data of 78 subjects provided us with a brief genetic and clinical landscape of MYP26. Our data confirmed ARR3 as the most frequently implicated gene for Mendelian eoHM. Further study on the molecular pathogenesis of eoHM caused by mutant cone-specific arrestin may shed light on the mechanisms of myopia related to abnormal cone signals, especially common myopia.

METHOD

Subjects

This study was approved by the institutional review board of the Zhongshan Ophthalmic Center. In total, data of 928 families with eoHM were collected in the Pediatric and Genetic Clinic at the Zhongshan Ophthalmic Center, Guangzhou, China. All probands from these families were initially considered as isolated eoHM based on the initial complaint and routine examination at the out-patient clinic (some of them have been shown to be at the early stage of other diseases, such as syndromic high myopia, revealed by longitudinal observation or further specific examinations, as shown in our previous studies). An additional 6386 individuals with different eye conditions, including retinitis pigmentosa, glaucoma and others with normal eyes, served as controls. Written informed consent consistent with the tenets of the Declaration of Helsinki was obtained from the participants or their guardians before the collection of peripheral venous blood samples and clinical data. The genomic DNA was obtained using a previously described method.

Sequencing analysis

Whole-exome sequencing (WES) and targeting exome sequencing (TES) of genomic DNA samples were performed as previous study. Of the 928 families with eoHM and 6386 control families, at least the proband of each family underwent WES or TES, and some families had trio-based WES/TES or linkage analysis. Variants in the 14 genes reported to control families, at least the proband of each family under -

RESULTS

Genetic spectrum and variants identification

PPVs were identified in 52 families with eoHM, including 29 families in ARR3, 22 in OPN1LW (18 with LVAVA haplotype, 4 with c.739C>T/p. Arg247*, c.417_418insGCTGCT, c.519_520insCCCTG and c.617_620dup, respectively), and one in LRPA1. All were significantly clustered in families with eoHM. No specific variants in the remaining 11 genes were clustered in families with eoHM, including a specific class of variants, variants in a specific region or variants with overall high predicted scores based on multiple tools. Some known variants in the 11 genes were classified as likely benign or variants of uncertain significance for their equal distribution in controls and the general population based on comparative analysis of large data sets or they were tolerable in the general population without any significant enrichment in the large data sets (figure 1, online supplemental tables s1 and s2).

A total of 72 rare variants (29 missense, 15 synonymous, 14 splicing regions’ changes, 6 nonsense, 6 frameshift, 1 start loss and one 5’-UTR alternation) in ARR3 were detected. Among
the 72 variants, 24 were classified as PPVs (online supplemental table s3, figure 2A), including eight missense and 16 truncation variants (six nonsense, six frameshift, three splice-site acceptor and one start loss). Of the 24, 21 were novel. Of the eight missense variants, six were predicted to be damaged by at least four in silico tools, and two (c.239T>C and c.345G>C) were predicted to be damaged by one or two tools and also affect splicing. Two-thirds of the PPVs in ARR3 (66.7%, 16/24), were truncations, distributed across the entire coding frame without significant clustering in individual exons. All 16 truncation variants were detected in 21 of 928 families with eoHM but in none of the 6386 control families (p=1.84E-19). Compared with the relatively rare frequency of ARR3 truncation variants in the gnomAD database, truncation variants are significantly clustered in eoHM families (p=1.43E-45) (online supplemental figure s1).

Of the 29 families with ARR3 variants, an average of four individuals was sequenced per family. Totally, 78 individuals in the 29 families (26 new families) harboured the 24 PPVs in ARR3 (figure 3, online supplemental figure s2), including 66 heterozygous women and 12 hemizygous men, in whom eoHM was presented in 97.0% (64 women and 4 men), and 10 individuals (8 men and 2 women) were unaffected. All the 68 affected patients complained about near vision, but no photophobia. Sixty-two patients (58 women and 4 men) had available refraction data, the other 5 women only had axial length, and one complained about eoHM without detailed clinical information (online supplemental table s5).

The bilateral refraction recordings showed a strong correlation (ICC>0.75), which could be averaged by following conventional statistics. The spherical equivalent refraction ranged from −5.00 D to −28.75 D (−12.58D±4.83D) (online supplemental figure s3A). Based on follow-up data of eight affected women and the trend line fitted out by all patients’ refraction data, the ARR3-associated MYP26 presented with the trend of rapid progression at first followed by slow increase (online supplemental figure s3A,B). Astigmatism ranged from −0.25 D to −6.00 D (−2.63 D±1.32D) (online supplemental figure s3C). BCVA, measurable in 57 patients (53 women and 4 men), ranged from 0.01 to 1.20 (median 0.40, decimal). Approximately, 82.5% (47/57) of unknown origin in six families (F9, F13, F14, F16, F18 and F24). The transmission of eoHM in these families conformed to X-linked female-limited inheritance, contrary to classic X-linked traits (online supplemental table s4).

Clinical characterisation of patients with ARR3 variants

Among the 78 individuals with variants in ARR3, 68 were affected (64 women and 4 men), and 10 individuals (8 men and 2 women) were unaffected. All the 68 affected patients complained about near vision, but no photophobia. Sixty-two patients (58 women and 4 men) had available refraction data, the other 5 women only had axial length, and one complained about eoHM without detailed clinical information (online supplemental table s5). The bilateral refraction recordings showed a strong correlation (ICC>0.75), which could be averaged by following conventional statistics. The spherical equivalent refraction ranged from −5.00 D to −28.75 D (−12.58D±4.83D) (online supplemental figure s3A). Based on follow-up data of eight affected women and the trend line fitted out by all patients’ refraction data, the ARR3-associated MYP26 presented with the trend of rapid progression at first followed by slow increase (online supplemental figure s3A,B). Astigmatism ranged from −0.25 D to −6.00 D (−2.63 D±1.32D) (online supplemental figure s3C). BCVA, measurable in 57 patients (53 women and 4 men), ranged from 0.01 to 1.20 (median 0.40, decimal). Approximately, 82.5% (47/57) of unknown origin in six families (F9, F13, F14, F16, F18 and F24). The transmission of eoHM in these families conformed to X-linked female-limited inheritance, contrary to classic X-linked traits (online supplemental table s4).
patients had BCVA of no less than 0.3 at initial. Of the remaining 10 patients with BCVA of less than 0.3, four had vitreous opacity or age-related cataract and three were too young to get BCVA examined (online supplemental figure S3D).

Fundus images, available for 50 affected women and four affected men, were classified into four categories (C0, C1, C2 and C3) (figure 4).23 Relatively normal posterior fundus without myopic retinal degeneration (C0) was observed in six women (11.1%, 6/54). Almost two-thirds of patients (32 women and 3 men; 64.8%, 35/54) showed high myopic tessellated fundus with clearly visible choroidal vessels in the posterior area (C1). Yellowish white fundus due to diffuse chorioretinal atrophy (C2) was observed in five women and one man (11.1%, 6/54), whose atrophic area was restricted to the optic disc in three and extended to the macula area in three. Well-defined patchy white atrophy (C3) was observed in seven women (13.0%). None had the macular atrophy lesions (C4) involving the central fovea.

Apart from macular changes, peripapillary crescent enlargement was observed in 40 affected women and three affected men (79.6%, 43/54) and was measurable in 36 patients, in whom 18 patients (16 women and 2 men) had crescent shorter than 1 papillary diameter (PD) and 18 patients (17 women and 1 man) had crescent larger than 1 PD. Posterior staphyloma was observed in 33 of 55 patients, including 30 affected women and three affected men. According to Curtin’s classification,24 posterior staphyloma in 16 women and 2 men (18/33, 54.5%) involved the macula area, including six women with type I and 12 with type II. Posterior staphyloma not including the macula was observed in 14 women and 1 man (15/33, 45.5%), in which 11 women and 1 man showed peripapillary (type III) and two involved the inferior area (type V).

Wide-field fundus examinations were available in 13 affected women, among whom 5 women older than 20 years old had various peripheral retinal degenerations; three patients had

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**Figure 2** Comprehensive genetic and phenotypic analysis of ARR3-associated MYP26. (A) The distribution and allele count of the 24 pathogenic variants in ARR3 identified in our cohort. To get a bird’s-eye view of variant effects across the entire ARR3 gene, possible changes of every residue (NM_004312.2; UniProt: P36575) were sequentially predicted by the five in-silico missense prediction tools. The tolerance of mutation at each amino acid was calculated by summing up the score of each potential change and then ranked to draw a tolerance landscape. The tolerance landscape was used as a reference to assess variants in ARR3. The position and allele number of all in-house pathogenic variants are located above the mRNA structure diagram. Truncating mutations are presented as red dots, while missense variants are blue. Two missense variants (c.239C>T and c.345G>C) were predicted to cause splicing site changes and are marked with both colours. The bar colours for each missense variant represent the residue pathogenicity and tolerance according to the heatmap below, and all truncation variants are shown as red bars. (B) Genotype-phenotype correlation matrix based on the fundus images of 54 in-house patients with ARR3 variants. All individuals younger than 18 presented with C0 or C1 fundus changes, and patients with truncation variants were more likely to exhibit C1 compared with those with missense variants (p=0.01). With progression, pathogenic myopic lesions could be observed in patients older than 18, in whom truncation variants led to severer consequences (C2 and C3) than missense variants with statistical differences (p=0.02). SSC, splicing site change.
Figure 3  The pedigrees of 26 newly identified families with pathogenic variants in ARR3. Male individuals are represented with squares, while female individuals are shown as circles. The shading indicates an affected patient. The proband in each family is indicated by arrows. Successive family numbers are on the top of the pedigrees while variants are listed under the pedigrees.
myopic holes in the periphery and two with lattice degeneration. The remaining eight children had preserved peripheral retinas. No conspicuous hyper-or-hypo-autofluorescence lesions were observed in the whole retina (Figure 5). Normal foveal structure was observed in 21 affected women and two affected men (85.2%, 23/27) with OCT. Myopic maculopathy change was found in the remaining four, in whom one woman had atrophy, two showed tractions and one had neovascularisation change (online supplemental figure s4). Twelve affected women and one affected man had ERG, including three women (23.1%, 3/13) had normal photopic and scotopic responses, five women and one man (46.2%, 6/13) showed normal scotopic responses but mild-to-moderately decrease a-wave and b-wave amplitude in cones, four women (30.8%, 4/13) showed mild-to-moderately decreased b-wave amplitude in both rods and cones and decreased a-wave amplitude in cones (online supplemental figure s5). Nine affected women and one man from four families had colour vision tests. All had a normal colour vision. No significant difference was observed for phenotypes, including refraction, BCVA, fundus manifestation, structural and functional changes, between affected women and men (p>0.05).

Genotype–phenotype correlation of ARR3
Fundus images of the 54 patients were split into two groups by age: ages below or over 18 years old (Figure 2B). Advanced categories (C2 and C3) were only observed in patients older than 18 years old, whereas early categories (C0 and C1) were more common among patients younger than 18 compared with those over 18, with statistical significance (p<0.0001). Of the 26 patients with age equal to or less than 18 years, all had milder categories of maculopathy (C0 or C1), and most patients with truncating mutation (94.7%, 18/19) had C1, while the majority with missense (57.1%, 4/7) had C0 (p=0.01). Among the 28 patients with ages older than 18 years, almost half of them (13/28, 46.4%) presented with advanced myopic macular change, in whom majority with truncating mutation (71.4%, 10/14) showed severer categories of maculopathy (C2 or C3).
whereas most patients with missense variants (78.6%, 11/14) had relatively milder maculopathy (C0 or C1) (p=0.02).

**DISCUSSION**

This study sheds light on the genetic background of eoHM based on a large data set of 928 families with eoHM, of which ARR3 is the most frequently implicated gene by a unique X-linked female-limited inheritance, out of the 14 genes investigated. The large case series of 29 families with ARR3 mutations provide valuable recognition of the special ARR3-associated MYP26. All truncations and highly scored missense mutations in ARR3 were intolerant and caused eoHM with cone impairment in unique X-linked female-limited mode. Overall, our study largely expanded our knowledge of eoHM, especially ARR3-associated MYP26, which will bring a strong impact on a broad audience.

Non-syndromic HM is diagnosed by a high degree of refraction (<−6.0D) and an exclusion process of abnormality involving other ocular segments or other systems, which is the diagnosis requirement of syndromic HM. Precise ophthalmology and whole physical check-up are essential in the differential diagnosis of syndromic and non-syndromic HM. Pathologic myopia is not always associated with HM and is diagnosed by the presence of posterior structural changes (posterior staphyloma or myopic maculopathy). It has been identified that eoHM might be accompanied by reduced scotopic and photopic amplitudes, which was strongly correlated with the degree of myopia or myopic maculopathy in the late stage.25 26 Different from cone dystrophy, patients with ARR3-associated MYP26 usually presented with reports of near vision and myopia diagnosis by routine examinations, but no photophobia or colour blindness, which is characteristics of cone dystrophy. Macular involvement earlier occurred in cone dystrophy than ARR3-related myopic maculopathy. The cone involvement in ERG of patients with MYP26 and the strong association between the underlying pathogenic mechanism of ARR3 and cones suggested that ARR3-associated MYP26 and cone dystrophy are distinct but not completely separate. For the absence of completely ophthalmic examinations from some patients and not enough follow-up data for each patient, considering disease progression and phenotype variability, whether the MYP26 is non-syndromic or syndromic is eager to be clarified in more patients with detailed tests.

HM has drawn wide public attention for its increasing prevalence, poor understanding of pathogenesis mechanism and complex interaction.1 Identification of causative genes and exploration of underlying pathogenesis mechanism will open new avenues for potential intervention. A recent study found that ARR3 mutations were responsible for 5% of cases and concluded as the most common cause of eoHM.7 Based on our knowledge, eoHM could be a heterogeneous disease that is affected by many factors.
cohort of eoHM, ARR3 was the most frequently implicated gene for Mendelian eoHM (~3.1%), and OPN1LW ranked the second (~2.4%). Cone-specific expression of both ARR3 and OPN1LW reminds cone to play an important role in the development of eoHM. Complex interactions involving numerous pathways for myopia development have been raised. A meta-analysis revealed that the light-dependent retina-to-sclera signalling cascade is an essential trigger of refractive error. A ‘contrast hypothesis’ of OPN1LW though that mosaics status of cones with variable amount of photopigment leads to abnormal contrast and stimulates of eyeball growth. Recently, an ARR3-related cone-associated hypothesis postulated that X-arrestin dystrophy in long and medium (LM) cones results in more sensitive function-to-colour stimuli, leading to higher luminance contrast and elongation of eyeballs. The exact underlying mechanisms of both ARR3 and OPN1LW are unknown. Functional studies that provide insights into the molecular pathogenesis of cone-dysfunctional-related eoHM may shed light on effective intervention for eoHM. The absence of evidence of the other 11 genes with eoHM was based on updated criteria at the individual gene level, which might be explained by interaction effect with other factors, susceptible genetic contribution, incomplete penetrance and polygenic inheritance patterns of high myopia, which need to be uncovered in further research.

Recently, a man with a nonsense variant in ARR3 was found to display eoHM, which reminded the non-zero penetrance of MYP26 in men. Four affected men and two unaffected women in this study revealed the non-zero penetrance of MYP26 in hemizygous men (~33.3%) and not the 100% penetrance in women (~97.0%). The female-to-male sex ratio could be up to ~20:1, considering affected members without genotype. Female carriers of several genes in X-linked recessive traits exhibit the identical or milder phenotype than affected men, such as RPGR, FRMD7 and GPR143. These men with ARR3 variants and eoHM might provide insights into the mechanisms of the unique X-linked female-limited inheritance. The X-inactivation mechanism results in a somatic mosaicism cell status, in which mutant and wild-type cells coexist, mutually compete, and lead to unique female-limited inheritance. Affected men with mental retardation restriction to women due to PCDH119 mutations were also identified and were explained by the mosaicism status mimicking the cellular interference pathogenic mechanism of women, which has been tested by sophisticated mouse models. A few unaffected women might be explained by the irregular dominance, defects in other unknown hyperopia-associated genes or bidirectional regulation mechanisms. Ten unaffected individuals (8 men and 2 women) with mutations did not report of other clinical symptoms, had refraction no more than ~6.0D, and normal fundus manifestations as well as retinal structure. It is unknown whether they had functional impairment even though the normal fundus manifestation and whether there are abnormalities in far-periphery that is hard to find in routine posterior photography, which needs more attention in future studies.

In conclusion, our study enriched our knowledge regarding eoHM, especially ARR3-associated MYP26 with cone involvement, which develops into pathologic myopia with age. The nature of variants might affect the progression and be an important prognostic decoder, in which truncation variants result in a severer phenotype. The potential pathogenesis mechanism of ARR3, the most frequently implicated genes responsible for Mendelian eoHM, might provide new insights into the myopic aetiology as well as additional underlying targets for therapeutic interventions. Furthermore, confirmation of the unique X-linked female-limited inheritance highlights the underlying genetic defects for additional hereditary diseases and may be a significant breakpoint to solve more problems in inherited diseases of unknown genetic defects.

Contributors XX, SL, XJ, FZ, LZ, VS and QS recruited the individuals diagnosed with different forms of ocular conditions and collected the clinical records. SL, XX and XJ prepared the genomic DNA from venous blood. XX, SL, PW and QZ performed whole exome analysis and targeted exome sequencing. YW, XL, ZY, YJ, PW and QZ participated in the bioinformatics analysis of exome sequencing data and in review and classification of clinical data. XX, YW and XL performed Sanger sequence. QZ designed the study. YW wrote the manuscript and QZ critically revised the manuscript. All authors reviewed and approved the manuscript. YW and XX contributed equally to this work. QZ is the guarantor.

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