

# X-linked heterozygous mutations in *ARR3* cause female-limited early onset high myopia

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**Purpose:** To identify genetic mutations in three families with early onset high myopia (eoHM) limited to female members.

**Methods:** Genomic DNA was collected from participating members of families XF1, XF2, and XF3. Genome-wide linkage scans were performed on the largest family (XF1). Whole exome sequencing was performed on seven samples, including five samples (four affected and one unaffected) from family XF1, as well as the two probands from family XF2 and XF3. Variants were analyzed with multistep bioinformatics analyses. Sanger-dideoxy sequencing was used to verify candidate variations in families and controls.

**Results:** The genome-wide linkage scans performed on family XF1 detected a candidate locus on chromosome Xp11.1-Xq13.3 with a maximum logarithm of the odds (LOD) score of 2.48 and 3.01 for markers DXS991 and DXS986, respectively. Parallel whole exome sequencing identified a novel c.893C>A (p.Ala298Asp) mutation in *ARR3* located on Xq13.1 in family XF1, which was shared by all four affected individuals but not the unaffected individual. Two other novel mutations in *ARR3*, c.298C>T (p.Arg100\*) and c.239T>C (p.Leu80Pro), were detected in families XF2 and XF3, respectively. These mutations were predicted to be damaging and were not present in the normal controls and existing databases. All three mutations cosegregated with eoHM in each of the three families, in which all heterozygous female members are affected whereas all hemizygous male family members are not affected. Transmission of the mutations and eoHM in the three families demonstrates an unusual pattern of X-linked female-limited inheritance.

**Conclusions:** These data suggest that heterozygous mutations in *ARR3* might be responsible for X-linked female-limited eoHM in the three families, a pattern contrary to the standard X-linked recessive trait. To our knowledge, eoHM is the first human disease associated with mutations in *ARR3* and the second X-linked female-limited disease identified thus far. Identification of *ARR3* associated with X-linked female-limited trait provides not only additional evidence of this unusual hereditary pattern but also an additional model for investigating the molecular mechanism responsible for female-limited phenotypes.

Some genetic characteristics are more commonly seen in men while others are seen more commonly in women. Many such characteristics are determined by genes located on the X or Y chromosome, that is, sex-linked traits. However, sex-limited traits may be present in men or women alone despite the same genotype in male and female individuals, such as breast cancer and prostate cancer [1,2]. Sex-limited traits outside sex-specific organs are rare [3-7], such as male-limited precocious puberty associated with mutations in the *LHCGR* gene (OMIM 152790) [4] and female-limited epilepsy and cognitive impairment associated with mutations in *PCDH19* (OMIM 300460) [5-7].

Degenerative changes in the retina associated with high myopia have become one of the most common causes of

irreversible blindness [8-11]. Mendelian and complex modes of inheritance have been suggested for high myopia [12-21]. Early onset high myopia (eoHM) [22], with minimum influence of the environment and different clinical characteristics, is a unique resource for the identification of genes responsible for high myopia [23-26]. During a genetic study on high myopia, we examined three large families with eoHM limited to female family members but without any affected male family members. Transmission of eoHM in the families demonstrates an unusual pattern of inheritance, which could hardly be explained by traditional X-linked traits or by sex-limited traits in sex-specific organs. Based on a genome-wide linkage scan and whole exome sequencing, novel mutations in *ARR3* (Gene ID: 407; OMIM 301770) are responsible for X-linked female-limited eoHM in the three families, the first human disease associated with *ARR3* and the second X-linked female-limited disease identified thus far.

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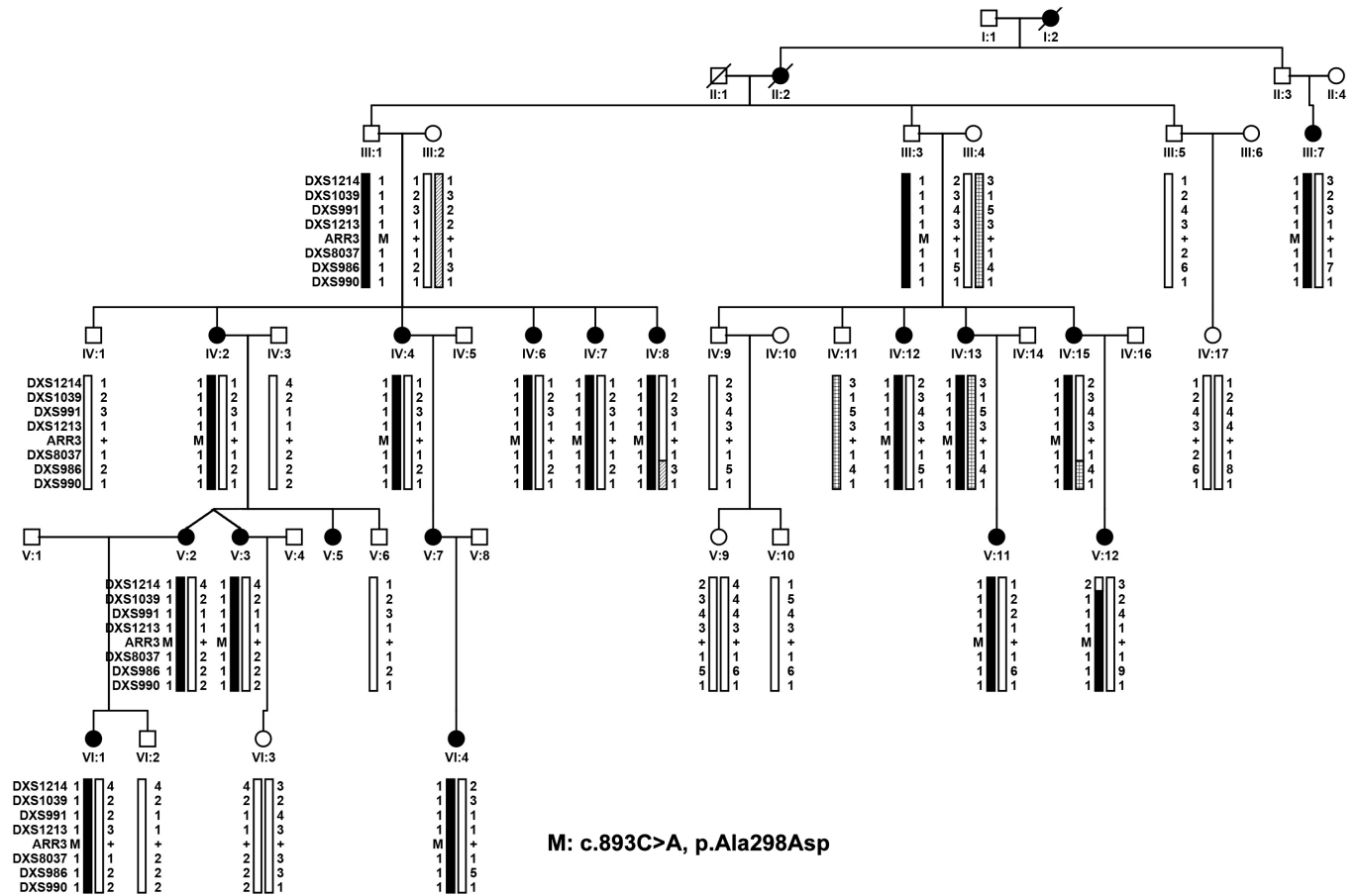


Figure 1. Family XF1 demonstrating haplotypes around *ARR3* and mutation segregation with eoHM. Filled circles represent female family members affected with early onset high myopia (eoHM). V:12 is the proband. The novel c.893C>A (p.Ala298Asp) mutation in *ARR3* was present in 15 female patients examined in this family but not in unaffected female family members. Two male family members with mutations (III:1 and III:3) and one obligate male carrier (II:3) did not have eoHM. M: mutation; +: Normal allele.

### METHODS

Three large families with eoHM limited to female family members and with no affected male family members were examined during a genetic study on eoHM (Figure 1, Figure 2, and Figure 3). Written informed consent in accordance with the tenets of the Declaration of Helsinki was obtained from the participants or their guardians. This study was approved by the institutional review board of the Zhongshan Ophthalmic Center. Venous blood for genomic DNA preparation was collected from 30 (15 affected; Figure 1), 12 (10 affected; Figure 2), and eight (four affected; Figure 3) individuals in the three families, respectively. All affected female family members had significant nearsightedness in early childhood and demonstrated typical tigroid fundus changes commonly seen in early onset high myopia (Figure 4). Refractive errors were measured with retinoscopy after mydriasis. eoHM was defined as axial length greater than 26.00 mm or spherical refraction in each meridian equal to

or greater than -6.00 diopter in both eyes developed before the age of 7 years, with the exclusion of other known ocular or related systemic diseases.

A genome-wide linkage scan on family XF1 was performed using panels 1–28 of the ABI PRISM linkage Mapping Set Version 2, which includes 400 markers spaced at intervals of about 10 cM. Genotyping for all participating family members was performed using 5'-fluorescently labeled microsatellite markers, as previously described [27]. Two-point linkage analysis was performed using the MLINK program of the FASTLINK implementation of the LINKAGE program package [28,29]. The eoHM in the family was analyzed as an autosomal dominant trait with incomplete penetrance for panels 1–27 markers or as X-linked inheritance limited to female family members for panel 28 markers.

Whole exome sequencing was performed on genomic DNA from five (four affected and one unaffected) individuals

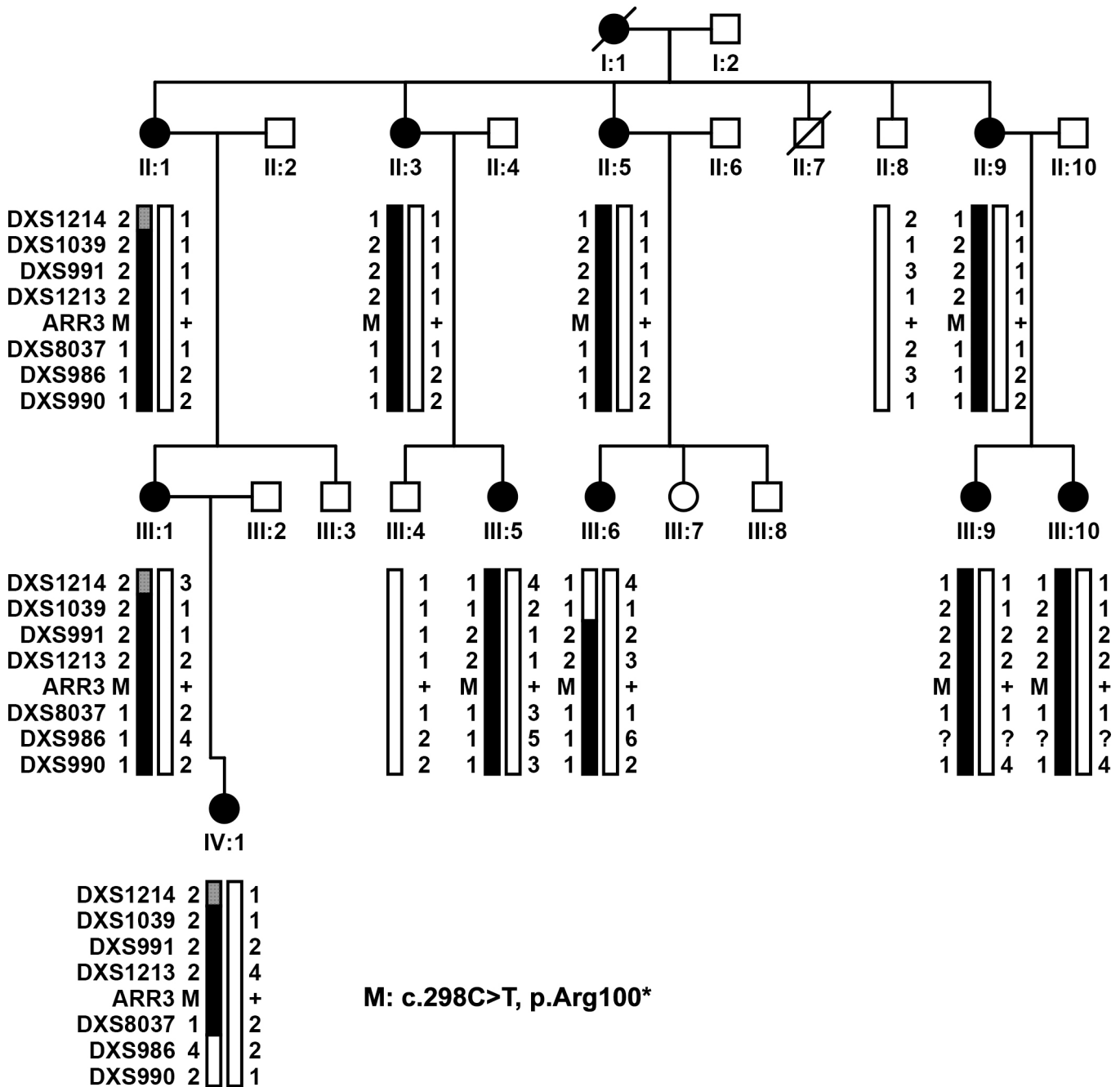


Figure 2. Family XF2 demonstrating haplotypes around *ARR3* and mutation segregation with eoHM. Filled circles represent female family members affected with early onset high myopia (eoHM). III:10 was the proband. The novel c.298C>T (p.Arg100\*) mutation in *ARR3* was present in all ten female patients examined. M: mutation; +: Normal allele.

of family XF1, one affected individual of family XF2, and one affected individual of family XF3, using a commercial service from [Macrogen](#), as described in our previous study [25,30]. Variants detected were initially filtered with multi-step bioinformatics analyses, as described in our previous study [25,30]. Then, variants shared by four affected individuals but not the unaffected individual in family XF1

were selected. Potential variants in the other two families were also analyzed. Candidate variants were also filtered by comparing with existing databases, including [HGMD](#), [EVS](#), and [ExAC](#). The possible impact of missense changes was predicted by using the [SIFT](#) [31] and [PolyPhen-2](#) [32] online tools. Sanger-dideoxy sequencing was used to confirm potential causative variants and to validate their cosegregation in

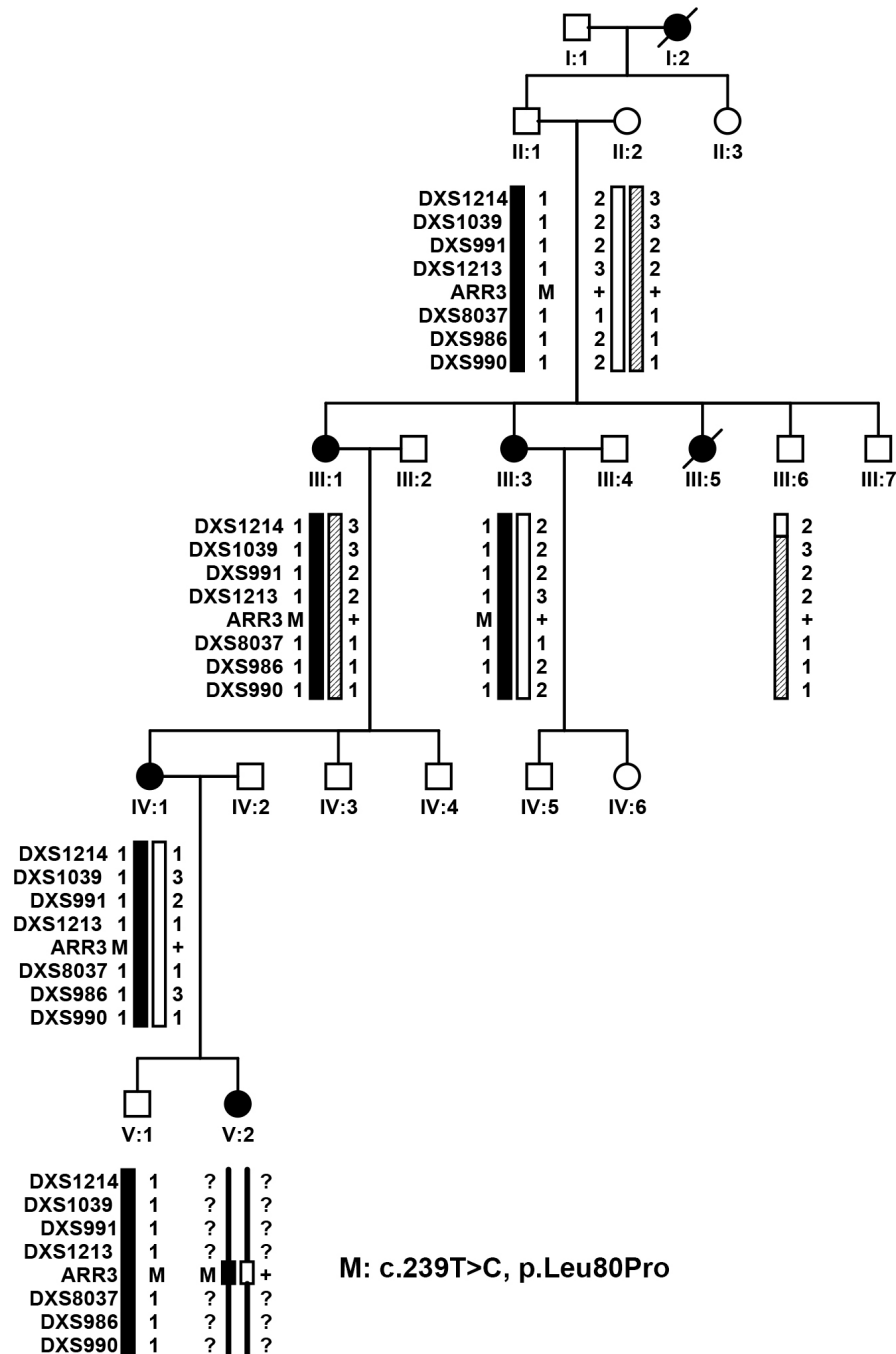


Figure 3. Family XF3 demonstrating haplotypes around *ARR3* and mutation segregation with eoHM. Filled circles represent female family members affected with early onset high myopia (eoHM). IV:1 is the proband. The novel c.239T>C (p.Leu80Pro) mutation in *ARR3* was present in all four female patients examined. One male family member with the mutation (V:1) did not have eoHM. Except the mutation, genotyping information for microsatellite markers around *ARR3* was not available for V:2 because she was recently added. M: mutation; +: Normal allele.

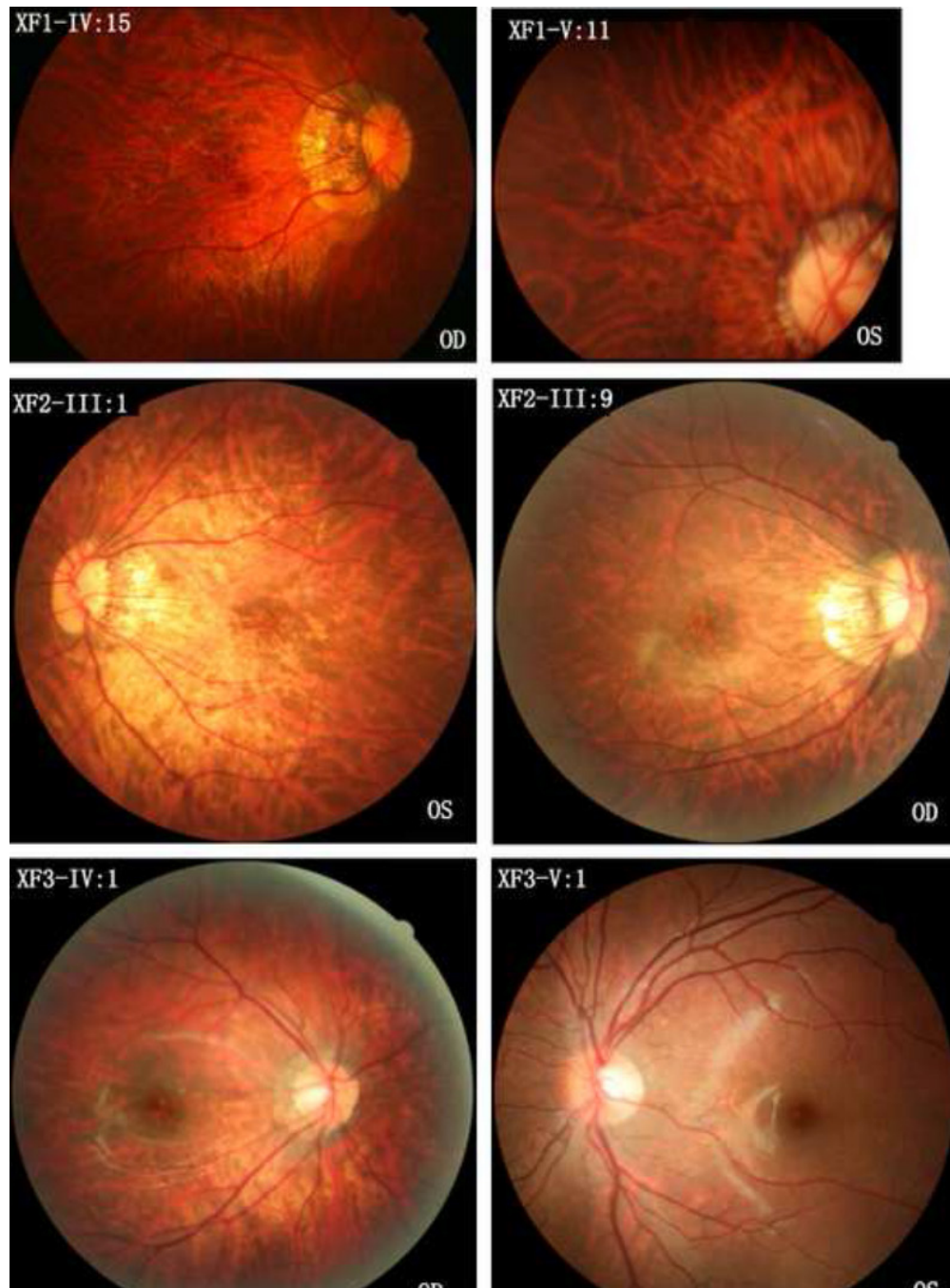


Figure 4. Fundus photographs of female patients with eoHM and different heterozygous mutations in *ARR3* and an unaffected male family member with a hemizygous mutation in *ARR3*. The top two photographs are from family members IV:15 and V:11 of family XF1, respectively. Both have the heterozygous c.893C>A mutation in *ARR3*. The middle two photographs are from family members III:1 and III:9 of family XF2, respectively. Both have the heterozygous c.298C>T mutation in *ARR3*. The lower two photographs are from family members IV:1 and V:1 of family XF3, respectively, in which the female patient (IV:1) has the heterozygous c.239T>C mutation in *ARR3* and has early onset high myopia (eoHM), but the male family member (V:1) has the hemizygous mutation in *ARR3* without eoHM. All five female patients (XF1-IV:15, XF1-V:11, XF2-III:1, XF2-III:9, and XF3-IV:1) with heterozygous mutations in *ARR3* demonstrated a temporal crescent of the optic nerve head and tigroid appearance of the posterior retina. However, XF3-V:1 is a 6-year-old boy with a hemizygous mutation in *ARR3* who did not have eoHM. OD: right eye. OS: left eye.

family members, as well as the novelty of the variants by analyzing controls.

### RESULTS

Genome-wide linkage scan on family XF1 resulted in logarithm of the odds (LOD) scores higher than 1.5 in only four markers: 1.54 for marker D2S206, 1.67 for D9S285, 2.48 for DXS991, and 3.01 for DXS986, suggesting a candidate locus on Xp11.1-Xq13.3 for eoHM in family XF1 (Figure 1).

Parallel whole exome sequencing identified only one novel candidate variant shared by four affected individuals (IV:4, IV:13, IV:15, and VI:1) but absent in the unaffected female member (VI:3) in family XF1 (Figure 1), that is, the

c.893C>A (p.Ala298Asp) mutation in the *AAR3* gene located at Xq13.1, a region within the linkage interval. Analyzing whole exome sequencing data of the two probands (III:10 in family XF2 and IV:1 in family XF3) from the other two families identified other novel mutations in *ARR3*: c.298C>T (p.Arg100\*) and c.239T>C (p.Leu80Pro; Figure 2 and Figure 3), respectively. These three mutations were confirmed with Sanger sequencing (Figure 5). Analysis of these mutations in available family members showed complete segregation of heterozygous mutations with affected female family members, in which unaffected female family members did not harbor the mutation (Figure 1, Figure 2, and Figure 3). The most striking phenomenon is that hemizygous male family members were not affected. The p.Ala298Asp

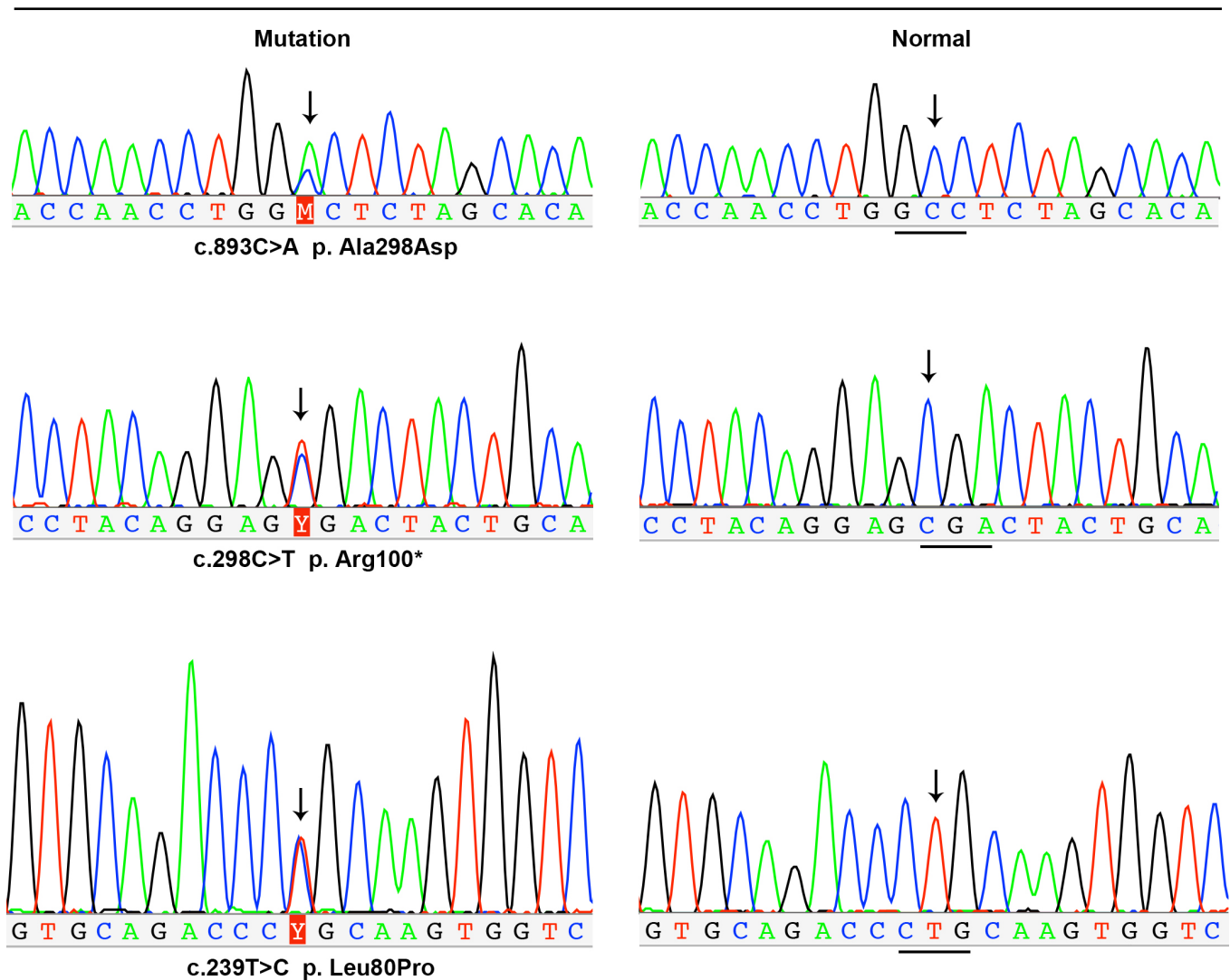


Figure 5. Sequence chromatography from Sanger sequencing. Mutations in *ARR3* identified with whole exome sequencing were further confirmed with Sanger sequencing and then validated in family members. The sequence with heterozygous mutations detected in female patients with early onset high myopia (eoHM) are shown on the left, whereas the corresponding normal sequences from controls are shown on the right. Arrows indicate the sites with and without mutations in the patients and controls, respectively.

mutation was predicted to be damaging with a SIFT score of zero and probably damaging with a PolyPhen2 score of 1.0. The p.Arg100\* mutation would result in truncation of most of the 388 residues but is more likely to be a null allele due to nonsense-mediated decay. The p.Leu80Pro mutation was predicted to be possibly damaging with a PolyPhen2 score of 0.523 but tolerated with a SIFT score of 0.07. All three mutations were not present in 192 normal controls (263 X chromosomes) or in existing databases (HGMD, EVS, ExAC, and 1000G). An analysis of the whole exome data of patients from these three families did not identify mutations in genes responsible for other forms of syndromic or nonsyndromic high myopia or in genes associated with other known retinal diseases [23,24].

## DISCUSSION

In this study, female-limited eoHM was mapped to a novel locus on Xp11.1-Xq13.3 and was associated with novel mutations in *ARR3*. This association is supported by linkage mapping, identification of novel mutations in *ARR3* in three families, segregation of heterozygous mutations with eoHM in the families, absence of the mutations in controls and existing databases, absence of known disease with *ARR3*, retinal-specific and highly enriched expression of *ARR3*, and exclusion of other potential mutations in the whole genome. To our knowledge, female-limited eoHM is the first human disease associated with mutations in *ARR3* and is the second X-linked female-limited disease identified thus far.

The patterns of disease transmission in the three families with eoHM with mutations in *ARR3* is highly likely to be X-linked female-limited [33]. Such an unusual pattern of inheritance has been rarely reported, except epilepsy and mental retardation limited to women (EFMR) that is caused by mutations in the *PCDH19* gene located at chromosome X [5,6,34], where female family members with heterozygous mutations are affected while hemizygous male family members are spared, a pattern contrary to the standard X-linked recessive trait. In addition, a similar but slight different pattern was also observed in ephrin-B1 (*EFNB1*, OMIM 300035)-related craniofrontonasal syndrome, where female family members are affected while male family members had no or only mild abnormalities [35,36]. In this unusual pattern of inheritance, it is unclear why heterozygous female family members are affected while hemizygous male family members are not affected. It has been postulated that an alternative pathway may compensate the complete loss of the functional products encoded by the mutant gene. In the heterozygous female family members, however, random inactivation of one X chromosome may create mosaic cells that

express either a normal or mutant gene so that these two types of cells may behave differently in cell interaction, migration, connection, metabolism, or even signal transmission [7,35,37-40]. Uncompromised behavior of these two types of cells may be harmful in development or in performing their natural function [6]. This proposed mechanism might also explain the unusual pattern of inheritance seen in families with eoHM with mutations in *ARR3*. Although diseases with X-linked female-limited inheritance are rare thus far, this mechanism may represent a special molecular pathological mechanism for a new class of diseases. This mechanism may be increasingly recognized in other diseases of unknown causes if it can be investigated further, especially in those with developmental anomalies or functional abnormalities of the neurosystems.

*ARR3*, located at Xq13.1 with 17 coding exons, encodes cone arrestin with retina-specific and retina-enriched expression [41-43]. *ARR3* has been speculated to play a role in as-yet undefined retina-specific signal transduction [44,45]. To date, mutations in *ARR3* have not been associated with any human disease. Based on a study of *Arr4* (the ortholog of human *ARR3*) knockout mice [46], 2-month-old *Arr4*-null mice had diminished visual acuity and contrast sensitivity but higher b-wave amplitudes, while 7-month-old *Arr4*-null mice had significantly reduced a-wave amplitudes compared with normal controls. The older *Arr4*-null mice had reduced cone numbers and cone opsin expression with normal thickness of the outer nuclear layer, suggesting a model of age-related cone dystrophy [46]. These data from *Arr4*-null mice suggest the involvement of cone arrestin in the structural and functional circuit of cones but do not explain why eoHM was present in heterozygous female family members but not in hemizygous male family members. Phenotypic differences in mice and humans have been observed in other genes, such as a null mutation in *LOXL3* (OMIM 607163) that causes embryonic lethality in mice [15] but eoHM in human beings [26]. In addition, mutations in genes with encoded products that participate in the cone signal pathway have previously been reported in patients with eoHM, such as those in *OPNILW* (OMIM 300822) [21,24] and *NYX* (OMIM 300278) [47,48]. Nevertheless, our results suggest that *ARR3* plays an important role in cone-related function. Further study of *ARR3* in suitable knockout animal models, such as the rat or even the monkey, may help to elucidate the underlying molecular mechanism related to female-limited expression of mutations in X-linked genes.

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