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NOVEL LOW-FREQUENCY MATERNAL MOSAICISM MUTATION OF *CHRDL1* GENE RESULTED IN THE X-LINKED MEGALOCORNEA IN THE OFFSPRING

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ABSTRACT

X-linked megalocornea (MGC1) is an inherited disorder resulting from mutations of the *CHRDL1* gene. Common symptoms of megalocornea include oversized cornea, deeper fluid-filled space between the cornea and iris, blurred or distorted vision at every distance, leading to mild vision issues. In this report, we present the first cases of megalocornea caused by the maternal low-frequency somatic mosaicism of the novel *CHRDL1* mutation. Two Vietnamese siblings, 5 years old (MG001) and 13 years old (MG002) visited the hospital for regular check-ups with manifestations such as myopic, exophthalmia, and enlarged cornea. They were suspected of having inherited megalocornea disorder without glaucoma symptoms. The DNA genome of the proband (MG001) was applied for whole exome sequencing (WES) analysis, and the result revealed a hemizygous pathogenic variant c.943dupA in the *CHRDL1* gene, which results in a frameshift mutation (p.Ile315fs) in protein. This mutation was also identified in the proband (MG002) by Sanger sequencing. Notably, the *CHRDL1* c.943dupA mutation was recognized at a very low fraction in the maternal DNA after triplicate Sanger sequencing, indicating that the mother has a mosaicism mutation of *CHRDL1* c.943dupA. This study reported a rare case of X-linked megalocornea in a family and provides a novel genetic factor relating to an eye disorder in Vietnam.

Keywords: megalocornea, anterior segment dysgenesis, *CHRDL1* gene, whole exome sequencing, hemizygous variant.

INTRODUCTION

Nowadays, there are hundreds of different eye disorders and vision problems. In which, hereditary eye disease is a common group of vision abnormalities caused by genetic mutations. These changes can have a

negative effect on human's eyesight, and they can vary at various periods of life. Eye disorders have severe consequences since they are frequently linked to many comorbidities that lower the quality of life (Yu et al., 2021). The mutations are significantly involved with the eye's development, especially structural or functional eyes. Anterior segment dysgenesis is one of the ocular developmental disorders in which the structure in the front of the eye (cornea, lens, iris…) is affected. This presents specific challenges in patients due to complex symptoms of the cornea or lens which can result in vision loss, higher intraocular pressure (IOP), or even retinal ganglion cell death (Mao et al., 2017). Therefore, the understanding of genetic predisposition is a necessary thing that enables the patient to seek early diagnosis and treatment and manage their disorder better.

Megalocornea (MGC1, OMIM: #309300) is a rare inherited disorder that causes either the cornea to be bilaterally enlarged with a horizontal diameter greater than 13 mm or a deeper anterior chamber with normal intraocular pressure (Davidson et al., 2014). The incidence of megalocornea has been unknown, and it is usually present in children and more common in males than in females (Tuft, 2016). This condition is transmitted in an X-linked recessive inheritance, which occurs due to a mutation in the Chordin-like 1 (*CHRDL1*) gene during fetal development. *CHRDL1* contains 12 exons located in chromosome q23 and encodes for an antagonist (ventropin) to bone morphogenetic protein 4 (BMP-4). *CHRDL1* helps control eye development by the interaction between BMP-4 and ventropin according to the progress of corneal stroma and endothelium (Sakuta et al., 2001; Webb et al., 2012). The primary megalocornea affects only the cornea, while the anterior megalophthalmos is associated with an underlying disease and systemic abnormalities (Moshirfar et al., 2023). Additionally, patients who get megalocornea can have no symptom or suffer from gradual vision changes, photophobia or myopia. Patients can be diagnosed by specific clinical assessment of the anterior segment and cornea and by analysis of genetic. The genetic testing would benefit differentiating megalocornea from other diseases and understanding of mutations and megalocornea mechanisms which result in better treatment (Ong et al., 2021).

In the present study, we report the genetic findings of two brothers with megalocornea phenotypes and low vision. It also provides more knowledge about genetic etiology in the ophthalmology field in Vietnam.

MATERIALS AND METHODS

Study subjects and clinical examination

Two patients in this study are siblings from a family. The younger brother (MG001) is 5 years old, and the older brother (MG002) is 13 years old. Both were diagnosed with congenital, bulging eyes, and congenital enlarged cornea at the Vietnam National Eye Hospital. The younger brother's examination results also showed a corneal diameter of 17 mm, an axial length of 25.9 mm, a transparent cornea, and a pink optic disc. The older brother, in addition to these conditions, was also diagnosed with short-sightedness and had a normal central cornea. This information has been published with the consent of the patients' family.

Genomic DNA extraction

Peripheral blood samples were obtained with the permission of the patient's parental guardians on behalf of the minors enrolled. Then, the genomic DNA of patients was extracted by Exgene ™ Blood SV mini 250p Kit (South Korea). Concentration of the DNA was determined by Qubit™ dsDNA Quantification Assay Kits (Thermo Fisher Scientific, USA).

Whole exome sequencing (WES)

Genomic DNA was analyzed by whole exome sequencing (WES). Reads were aligned to the hg19 human reference sequence. Then they were performed by screening of variants in the Anterior segment dysgenesis including glaucoma (ASD) gene panel. The variants that were reported as benign in the ClinVar database, the minor allele frequency (MAF) > 0.1% in 1000 Genomes Project (www.1000genomes.org/) and/or variants in the non-coding regions were removed. The in-house database of 500 Vietnamese individuals without ocular problems was used as the control population data. The novel and rare variants whose apparent fit to any genetic models were considered as candidates' causative variants and were selected for further evaluation. The pathogenicity of variants was classified based on the American College of Medical Genetics and Genomics (ACMG) recommendations.

A panel of 59 genes related to anterior segment dysgenesis including glaucoma (ASD): *ADAMTS18, AGBL1, ALDH18A1, ATOH7, B3GLCT, BEST1, BMP7, CHRDL1, CHST6, COL4A1, COL8A2, CRIM1, CRYGC, CYP1B1, DCN, EYA1, FBN1, FOXC1, FOXE3, FOXL2, GJA1, GNPTG, GSN, KERA, KRT12, KRT3, LAMB2, LCAT, LMX1B, LTBP2, MIR184, MYOC, NOTCH2, OPTN, PAX3, PAX6, PEX2, PIKFYVE, PITX2, PITX3, PRDM5, PXDN, RAB18, RAB3GAP1, RAB3GAP2, SEC23A, SH3PXD2B, SIX3, SLC16A12, SLC38A8, SLC4A11, SLC4A4, TACSTD2, TBC1D20, TGFBI, UBIAD1, VSX1, WDR36, ZEB1*.

Sanger sequencing

Sanger sequencing was used to confirm the presence of candidate pathogenic variants in probands and the family members.

The primer pair (CHRDL_E9F: 5'–AGTAGTTCAGGGCTTGGGTT - 3' and CHRDL_E9R: 5' - TCAGTCAACCAAAAGCAGGG - 3') to amplify an amplicon with 329 bp containing the exon 9 of the *CHRDL1* gene was designed and synthesized by PHUSA Genomics (Can Tho, Vietnam). PCR was performed with a total volume of 20 µL containing 10-40 ng genomic DNA, 1X DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA), and 10 pmol of each primer. The thermocycle was followed as 95^{o}C / 5 min; 40 cycles (95^{o}C / 30 sec; 58^{o}C/ 30 sec; 72^{o}C /30 sec); final extension at 72^{o}C/ 8 min. The PCR products were then purified by MultiScreen PCR 96 Plate (Merck KGaA, Darmstadt, Germany) and subsequently sequenced in a single direction using ABI Prism BigDye Terminator Cycle Sequencing Kit Version 3.1 on an ABI genetic analyzer 3500 (Applied Biosystems, Waltham, Massachusetts, USA). Sequencing data were analyzed with BioEdit software and using the reference sequence of the *CHRDL1* gene (NM_001143981.1) in the NCBI.

RESULTS AND DISCUSSION

Clinical characteristics of probands

In July 2023, two male siblings visited the National Institute of Ophthalmology in Hanoi, Vietnam for regular check-ups due to their abnormal eye phenotypes (Figure 1). The older brother, aged 13 years old, presented with myopic, exophthalmia, and enlarged corneas with a normal central retina. Similarly, the younger brother, aged 5 years old, revealed the same symptoms as his brother. He was 17 mm in corneal horizontal diameter, 25.9 mm of the visual axis with a clear cornea and normal optic nerve. Two siblings were diagnosed to have congenital megalocornea disorder. The younger patient was even suspected of glaucoma at ages 2-3. However, he had no symptoms of glaucoma at the time of check-ups in 2023. After getting genetic counseling, their mother agreed to participate in genetic testing to find the exact cause of these symptoms.

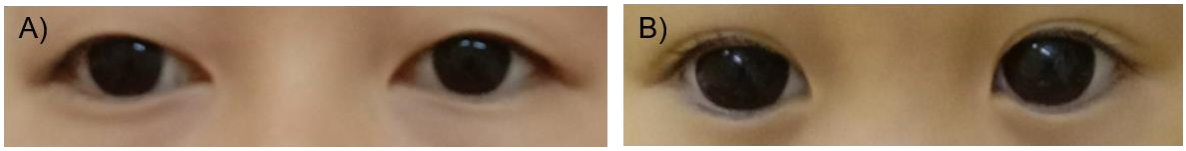

Figure 1. Eyes phenotype of two siblings. A) Older brother's eyes B) Younger brother's eyes.

Genetic screening by WES analysis

WES followed by anterior segment dysgenesis gene panel screening was performed for the MG001 sample at first. As a result, a hemizygous variant c.943dupA (p.Ile315fs) in the *CHRDL1* gene was identified in patient MG001. According to the OMIM database, the *CHRDL1* variants may involve X-linked megalocornea, affecting corneal growth. The found variant c.943dupA (p.Ile315fs) results in a frameshift in the reading frame of the protein translation process, and it has not been reported in the 1000G, Clinvar, LOVD *CHRDL1* databases, and in-house databasse. According to the guidelines of ACMG, the *CHRDL1*c.943dupA (p.Ile315fs) could be classified as a likely pathogenic variant.

Segregation of *CHRDL1* variant in the family's members

Since *CHRDL1* c.943dupA was identified as a likely pathogenic variant, Sanger sequencing was used to validate the existence of the c.943dupA variant in the MG001 in the next step. The duplication of alanine at nucleotide 943 has been observed clearly in the *CHRDL1* of both the affected MG001 and MG002 patients. However, the mother's Sanger chromatogram peak was almost a wild-type genotype, only a very low signal of alanine duplication could be recognized. This phenomenon is likely due to the mother's mosaicism of *CHRDL1* c.943dupA (Figure 2A).

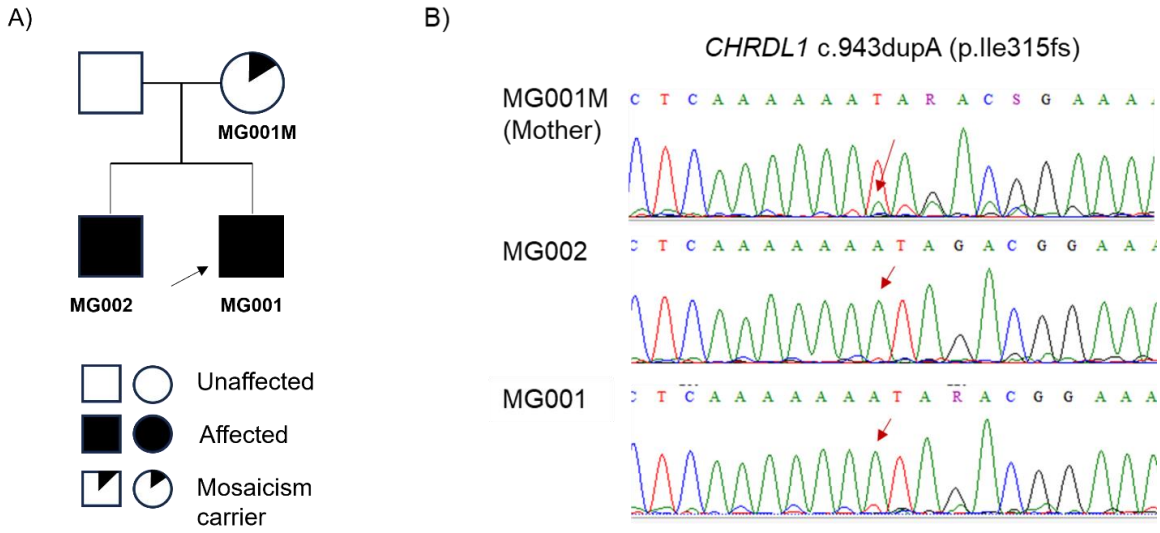

Figure 2. A) Patient's family pedigree in which the patient performed whole exome sequencing is denoted with an arrow symbol. B) Sanger sequencing chromatograms of the variant of *CHRDL1* c.943dupA (p.Ile315fs) in two siblings and their mother, the mutated nucleotide position was indicated with a vertical line (red).

As shown, two related children harbored the hemizygous *CHRDL1* c.943dupA (p.Ile315fs) variant, which inserted Alanine at position 943 in exon 9 of the gene, shifting the reading frame of the codons. This change results in a premature stop codon at position 315 of the protein, which truncates the protein and makes it non-functional. In addition, this variant is novel because it has not been reported in any surveyed database. The molecular mechanism of CHRDL1-mediated X-linked megalocornea has been demonstrated in humans and Xenopus models (Pfirrmann et al., 2015). The *CHRDL1* gene encodes for protein ventropin, which helps regulate the production of bone morphogenetic protein 4 (BMP-4). These proteins are involved in controlling eye development by the interaction between BMP-4 and ventropin according to the progress of corneal stroma and endothelium. The variants in the *CHRDL1* gene cause ventropin deficiency and lead to a delay in the optical cup fusion process occurring during embryonic development (Webb et al., 2012). The delay in fusion gives more time for corneal growth, resulting in a larger-than-average cornea and anterior segment. Furthermore, *CHRDL1* is expressed in the human corneal stroma, corneal endothelium, and developing periocular mesenchyme, characterizing MGC1 as an anterior segment disorder arising from the neural crest (Walker et al., 2020). The possibility of an asymptomatic retinal or visual pathway phenotype in MGC1 patients was investigated because *CHRDL1* is expressed in the developing human retina and ventropin has a suggested involvement in retinotectal patterning (Webb et al., 2012). The *CHRDL1* mutation most likely has a different phenotype that goes beyond the eye. Researchers predicted that additional common alleles in crucial genes expressed in

the brain may be linked to the complicated morphological and neuropsychological aspects (Webb et al., 2012).

Several reports showed that MGC1 overlapped with other eye disorders. In 2019, Scuderi et al. showed that some MGC1 symptoms such as elevated IOP and extensive visual field loss can be possible signs of glaucoma. Pigment deposition onto the trabecular meshwork may predispose the eye to aqueous outflow blockage and subsequent glaucoma, while the exact mechanism is unknown (Scuderi et al., 2019). In 2005, Mohamed et al. reported that an individual suffered from Fuchs heterochromic iridocyclitis. This disorder is considered to share overlapping features with MGC1 like early-onset cataracts (Mohamed & Zamir, 2005). One patient in this study was also suspected of glaucoma at age 2, and a recent examination showed that he had normal intraocular pressure.

Furthermore, a likely mosaic presence of this variant in the mother was found for the first time; hence, it was slightly surprising while it was predicted to be heterozygous. With the germline *CHRDL1* mutation, patients can transmit the MGC1-causing genes to their daughter in a heterozygous form in the next generation, which does not manifest the disease. Patients need to be involved in genetic counseling when getting married and planning to give birth in the future. The detection of this mutation in two MCC1 patients has provided more understanding of inherited eye conditions in Vietnam and around the world.

CONCLUSION

Applying WES technology, we identified the novel hemizygous variant, c.943dupA (p.Ile315fs) in the *CHRDL1* gene as the cause of MCC1 conditions in two sibling patients. This variant likely originated from a mosaicism variant in their unaffected mother. This finding provided knowledge of the inherited anterior segment abnormalities in Vietnamese children.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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