

Novel Mutation in the *CHRDLI* Gene Detected in Patients With Megalocornea

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Purpose: The aim of this study was to determine the mutation associated with X-linked megalocornea (MGC1) found in 2 patients from the same area in southern Italy.

Methods: Diagnosis of megalocornea was confirmed by detailed ophthalmic examination in 2 probands from independent families and in another 3 affected family members. Genomic DNA of the probands was used to amplify and sequence all the coding regions of *CHRDLI*.

Results: Megalocornea diagnosis was associated with a novel mutation found in the probands and affected kindreds (5 subjects). The mutation is an 11-base pair deletion that leads to a stop codon in the second coding exon of the *CHRDLI* gene. Research on the *CHRDLI* mutation was also performed on other family members (11 subjects) not affected by MGC1, and the mutation was not detected in unaffected male family members.

Conclusions: The detection of mutations in the *CHRDLI* gene is useful for differential diagnosis with different forms of megalocornea.

Key Words: cornea, megalocornea, *CHRDLI* mutation, congenital glaucoma

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Megalocornea is a rare ocular disorder (frequency of 1/50,000) characterized by bilateral enlargement of the corneal diameter (with a horizontal diameter of >13 mm, measured after the age of 2 years) and reduced central corneal thickness (CCT) in the absence of raised intraocular pressure.¹ It may be an isolated finding or may be associated with several conditions, which include Down syndrome,² mental retardation,³ Marfan syndrome,⁴ oxycephaly,⁵ poikiloderma congenital,⁶ and albinism.⁷

X-linked megalocornea (MGC1; MIM 309300) is associated with distinctive secondary changes in the posterior crocodile shagreen and corneal arcus juvenilis. Later in life, pathological changes may also include mild iris atrophy with pigment dispersion, lens dislocation, and cataract.^{8,9}

MGC1 has been mapped to Xq12-q26, and *CHRDLI* (MIM 300350) has been identified as the causative gene.¹⁰ The gene codes for the protein ventroptin, which plays a crucial role in anterior segment development. *CHRDLI* mutation screening of MGC1 families led to the identification of frame-shift, nonsense, missense, splice-site mutations, and segmental deletions.^{10,11} In this article, we describe a novel mutation in the *CHRDLI* gene detected in 2 patients from 2 independent pedigrees from Calabria, a region in southern Italy.

PATIENTS AND METHODS

Two patients affected by megalocornea were referred to the Ophthalmology Unit of Magna Graecia University. The patients underwent a complete ocular examination, including slit-lamp examination, Snellen uncorrected and best spectacle-corrected visual acuities, refraction, tonometry, and funduscopy. The white-to-white distance (WTW), anterior chamber depth, and CCT were calculated by anterior segment optical coherence tomography (AS-OCT, SS-1000 CASIA; Tomey, Kyoto, Japan). After completing an ophthalmologic evaluation, the patients were referred to the Genetic Unit for counseling. The study was approved by the ethics committee of the University of Magna Graecia; a detailed informed consent form for genetic testing was signed by all patients and their family members during the genetic counseling.

Mutation Analysis

Total DNA was extracted from whole blood using a commercial kit following the manufacturer's instructions (Nuclear Laser, Settala, Italy). All the exons and flanking sequences of the *CHRDLI* gene were amplified by polymerase chain reaction; primer sequences are reported in Table 1. Polymerase chain reaction was performed with the following conditions: initial denaturation at 94°C for 3 minutes, 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute and final extension at 72°C for 7 minutes. Amplified products were checked using

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TABLE 1. Primers for the Amplification of *CHRD1* Exons

Exon	Forward Primer	Reverse Primer	Length, bp
2	5'-GGTTAGAAGCCTGAGCATC-3'	5'-ACGGCCACATTGGGCTA-3'	308
3	5'-TGGATGGAACCTGTGATTGC-3'	5'-GGCATGCTGAGGAGCAAAC-3'	246
4	5'-GTTTCCCAAACCTGGCTTGAT-3'	5'-CTGGAAGTTTCCCTTCTGG-3'	267
5	5'-CCGTCTGCTGTTGGGATTTA-3'	5'-TGCAGGCACAAATGAAAGTA-3'	311
6	5'-TTGAGTCCCTGCTCTATTTCC-3'	5'-CCCTAGGACATTAGACAGGTAT-3'	273
7	5'-CACAAAGGGTGATAACTTTCC-3'	5'-TGATGCTGTGGAGTGTGA-3'	177
8	5'-CTGTGATCTCATTGGTGGCTTT-3'	5'-TGCTGAAGCCTTGTGTGGA-3'	268
9	5'-GTAGTTCAGGGCTTGGGTT-3'	5'-GAAAGACTAGGTGAGAATCTCAG-3'	347
10	5'-GCAGAAGTTCACCTCTAGC-3'	5'-GTGAGTCTCTCATCCTTTTCTG-3'	295
11	5'-AGGAGTTGATGGTCCAGATTG-3'	5'-TTGCGGAGGGACTTTAGC-3'	235
12	5'-CTAATTTGCTCTGCTTCTC-3'	5'-CAAATAAGCCTGCAGTCCA-3'	263

Primers used and the length of the corresponding amplified product. The *CHRD1*-coding region starts from exon 2.

agarose gel electrophoresis, and were then purified and directly sequenced with an ABI BigDye terminator cycle sequencing kit on a 310 ABI PRISM Genetic Analyzer (Applied Biosystems, Foster City, CA). NM_145234.3 was used as the reference sequence.

RESULTS

Biometric ocular measurements for both patients confirmed the diagnosis of megalocornea. In both probands, the WTW, anterior chamber depth, and CCT were found beyond the range of normality compared with the corresponding reference mean values of the general population (Table 2). None of the patients showed signs of mental retardation.

Pedigrees of both families were delineated, and other family members were suspected to be affected by megalocornea (Fig. 1). The diagnosis was then confirmed by complete ocular examination performed at the Ophthalmology Unit. Both the pedigrees were compatible with the hypothesis of X-linked heredity. The *CHRD1*-coding sequence revealed a small deletion spanning from the end of exon 2 to the beginning of intron 2 (c.90_100delAAAACGTAAGT) in both

probands (Fig. 2). This deletion could generate a frame shift with a consequent premature stop (pLys31PhefsX15) of the *CHRD1*-coding sequence.

In pedigree A (Fig. 1), genetic testing was also performed for the parents of the proband and for his brother and sister. The brother of the proband underwent ocular examination and was found to be affected by megalocornea. In addition, the grandfather of the proband (III 6), who was suspected to be affected by megalocornea and underwent both ocular examination and genetic testing, was found to be affected and to be a heterozygous carrier of the *CHRD1* mutation. In the left branch of the pedigree A, there is another individual affected by megalocornea (IV 1) carrying the mutation, and his mother and sister were found to be heterozygous carriers of the mutation, whereas his father was found to be wild type for the *CHRD1* sequence.

In pedigree B, all individuals were subjected to genetic testing. The mother and the grandmother of the proband were both heterozygous for the mutation. Thus, the mothers of both probands were found to be heterozygous carriers of the *CHRD1* mutation, whereas the unaffected fathers had a wild-type sequence of the gene.

TABLE 2. Phenotypic Data of the Affected Patients

Patients*	Sex/Age, yrs	Eye	IOP, mm Hg	WTW, mm	ACD, mm	KRs, Diopters	CCT, μ m	BSCVA, logMAR
A, V:1	M/11	OD	9	13.8	5.7	52.0	493	0.3
		OS	11	14.4	6.1	51.0	488	0.1
A, V:3	M/5	OD	9	15.5	6.0	48.0	475	0.0
		OS	10	16.0	6.0	46.7	462	0.0
A, III:6	M/50	OD	16	13.3	5.5	50.6	472	0.0
		OS	15	13.3	5.4	52.3	475	0.0
A, IV:1	M/8	OD	11	15.5	6.5	48.5	362	0.2
		OS	10	14.7	6.4	50.8	451	0.2
B, III:1	M/7	OD	12	13.4	4.7	51.2	410	0.1
		OS	10	13.7	4.6	51.8	410	0.1

*Refer Figure 2 for pedigree collocation of the patients.

ACD, anterior chamber depth; BSCVA, best spectacle-corrected visual acuity; IOP, intraocular pressure; KRs, average keratometric readings; WTW, white-to-white distance.

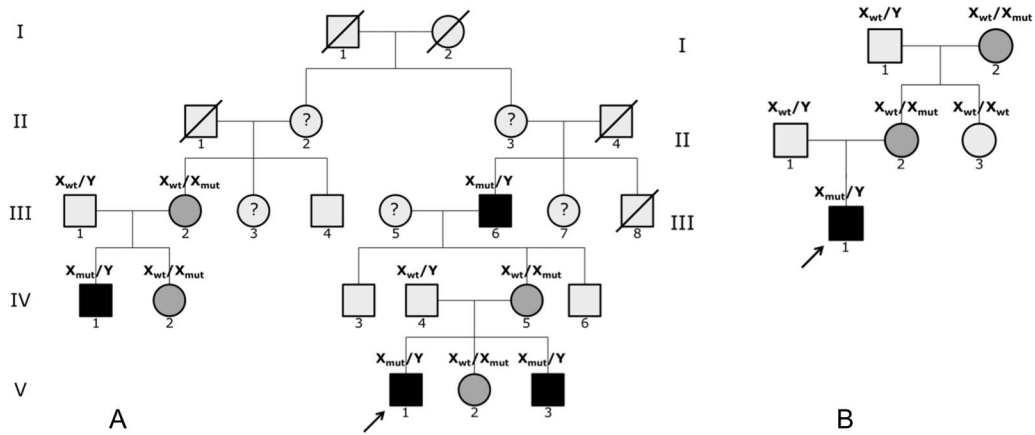


FIGURE 1. Pedigree of families: black squares indicate affected males confirmed by genetic testing, white squares indicate unaffected males, gray circles indicate heterozygous female carriers confirmed by genetic testing, the white circle indicates the unaffected noncarrier female confirmed by genetic testing, and the “?” in circles indicate females for whom genetic testing was not performed. Genotypes are assigned to all subjects who underwent genetic testing.

c.90_100delIAAAACGTAAGT

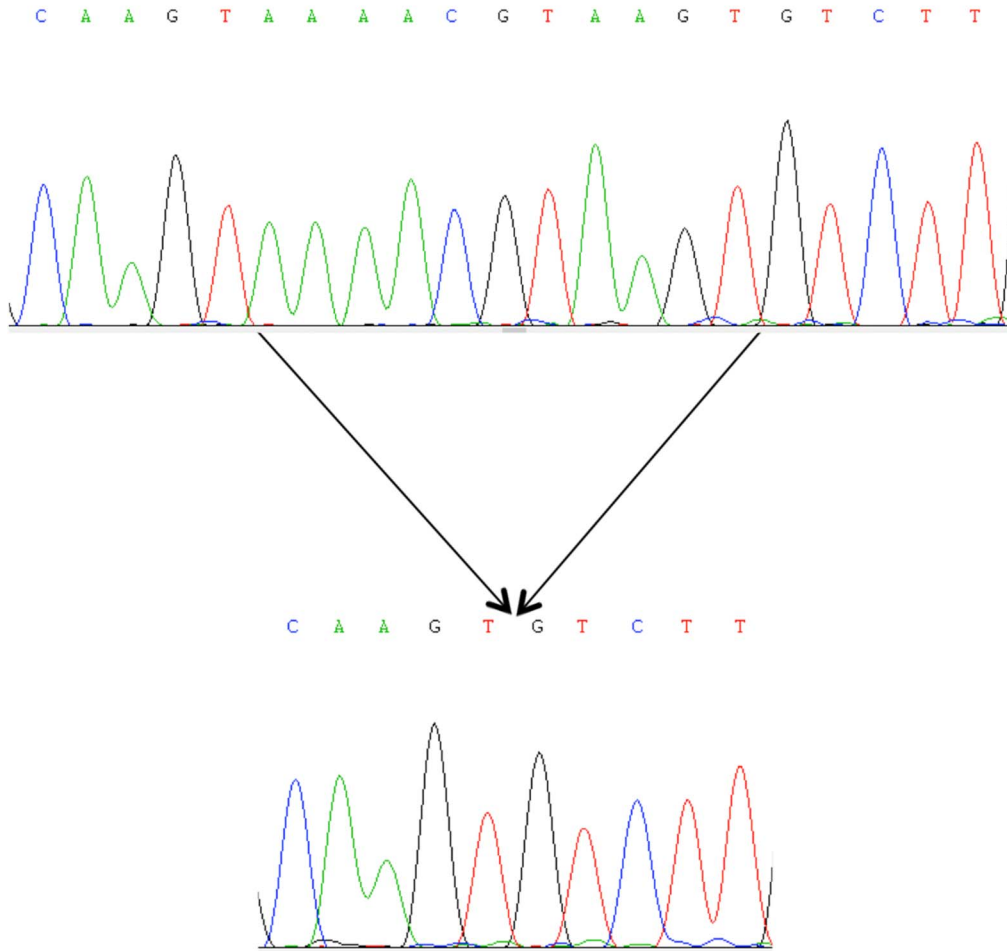


FIGURE 2. Electropherograms of the wild-type sequence and the pathological mutation of *CHRD1*.

DISCUSSION

In this study, we report evidence of a new mutation of *CHRD1* that causes MGC1. The involvement of *CHRD1* mutations in the pathogenesis of MGC1 has recently been demonstrated.¹⁰

Ventropin, the protein product of the *CHRD1* gene, is characterized by the presence of 3 cysteine-rich domains, which are necessary for the physical interaction with bone morphogenic proteins (BMPs), a group of secreted proteins that belong to the superfamily of transforming growth factor beta. The interaction results in the regulation of BMP signaling. One of those proteins, the BMP4, is very important in the development of the eye because loss-of-function mutations in the gene coding for BMP4 (MIM112262) cause a range of syndromic anophthalmia/microphthalmia phenotypes in humans.^{12,13} However, *Bmp4* heterozygous knockout mice have anterior segment dysgenesis and elevated intraocular pressure. Interestingly, the knockdown of *CHRD1* in *Xenopus laevis* recapitulates MGC1 human disease and leads to *bmp4* downregulation in eyes.¹⁴ The mutation described in our pedigrees interrupts the coding sequence for ventropin at codon 31, just before the first of the cysteine-rich domains, and probably abrogates the interaction of ventropin and BMPs causing a possible deregulation in BMP signaling.

To date, 18 mutations in *CHRD1* have been associated with MGC1; however, the great majority (15/18, 83.3%) are loss-of-function mutations because they are nonsense, frame shift, or large deletions of *CHRD1*.^{10,11,14,15} The mutation reported in our study could also probably generate a frame shift with a premature stop codon; however, this point needs to be experimentally verified. The identification of mutations in the *CHRD1* gene is useful for the early detection of the disorder and the differential diagnosis with other diseases, with a completely different genetic basis, such as primary congenital glaucoma characterized by high intraocular pressure and megalocornea-mental retardation syndrome (MMR). It is important to underline that both families came from the same geographical region, southwest Calabria in Italy. Although we were unable to establish a relation between the 2 families, a founder effect of the *CHRD1* mutation detected is possible.

Megalocornea is also a feature of Neuhäuser or MMR syndrome, a rare condition of unknown etiology. Although

a missense mutation in *CHRD1* has been found in 1 patient with MMR,¹⁵ careful analysis of the pedigree failed to demonstrate that this mutation accounted for nonocular features, suggesting that MMR can be a digenic or multigenic disease.

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