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A novel *PRDM13* gene duplication causing congenital North Carolina macular dystrophy phenotype in a Mexican family

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Purpose: North Carolina macular dystrophy (NCMD) is a rare autosomal dominantly inherited congenital maculopathy caused by either non-coding point mutations or tandem duplications in the DNase I hypersensitivity site DHS6S1, at chromosome 6q16 (MCDR1), or at chromosome 5 (MCDR3). To date, at least 30 NCMD pedigrees from different ethnicities have been genetically identified worldwide. Herein, we report the clinical and genetic features of a newly found NCMD family in Mexico with a novel tandem duplication involving both the DNASE1 site and the *PRDM13* gene.
Methods: Seven affected subjects from a Mexican family underwent a complete ophthalmic assessment that included dilated indirect ophthalmoscopy, fundus photography, optical coherence tomography (OCT), fundus autofluorescence (FAF), kinetic and chromatic perimetry, and electroretinography (ERG). Next-generation sequencing (NGS), followed by array-based comparative genomic hybridization (array-CGH) and quantitative polymerase chain reaction (qPCR) analyzes, were employed to demonstrate the causative molecular defect.
Results: All seven affected patients had a severe appearing phenotype characterized by symmetric excavated atrophic coloboma-like chorioretinal macular lesions. In addition, using OCT, lacunae in the inner retinal layers and inner retinal loss were observed in all patients. NGS identified a heterozygous tandem duplication of the entire coding sequence of the *PRDM13* gene in all seven affected individuals, whereas subsequent array CGH, NGS, and Sanger sequencing allowed for the identification of the precise boundaries of a ~148 kb MCDR1 duplication containing the whole *PRMD13* gene and the DNASE1 site.
Conclusions: The phenotypic features in this NCMD pedigree continue to support the concept that this disorder is a congenital macular malformation rather than a progressive dystrophic entity. Unlike most NCMD families, there was no variable expressivity found in this study, possibly due to the relatively small size of the family. The other hypothesis is that the duplication involves genomic segments that are more consistently or tightly bound to other regulatory regions of PRDM13. The identification of a novel causative tandem duplication involving the DNASE1 site and the *PRDM13* gene in this family allows for the expansion of the mutational spectrum of the disease.

North Carolina macular dystrophy (NCMD) is a rare autosomal dominant, fully penetrant disorder that affects macular development. The disease is a congenital, non-progressive maculopathy with highly variable expressivity ranging from scattered drusen to chorioretinal coloboma-like excavations, and it typically affects both eyes symmetrically [1,2]. NCMD is characterized by a non-progressive absence of the choroid, choriocapillaris, and the retinal pigment epithelial layer present at birth. Frequently, the patient's vision is better than what would be predicted based on the macular funduscopic appearance. Visual symptoms are variable, with approximately two-thirds of patients having mild or mild-to-moderate central vision impairment. Approximately 30% of patients are asymptomatic, and a few exhibit severe central vision impairment, typically due to choroidal neovascular membrane (CNVM) formation [1,3].

The name NCMD was given by Gass in his atlas [4] after the identification of a founder effect of the large family originating from North Carolina and first reported in 1971 [5]. This name is now considered a misnomer, since, as to 2024, over 70 families with NCMD have been reported worldwide

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[6-17]. Interestingly, the severity of the disease in the individuals affected in this family, spanning six generations, was highly variable [18]. In 1998, Small et al. re-ascertained the original NCMD family and classified the subjects into three grades: characterized by few drusen in the central macular region (Grade I), confluent drusen in the central macular region (Grade II), and severe macular coloboma (Grade III), with visual acuities ranging from 20/20 to 20/60 in Grades 1 and 2, and from 20/40 to 20/200 in Grade 3 [19]. Peripheral drusen were reported as variably present.

Using genetic linkage mapping, Small et al. mapped NCMD to chromosome 6 and the locus named MCDR1 by the Human Genome Organization (MC=macula, D=dystrophy, R=subtype retinal) to distinguish it from corneal macular dystrophy 1=1st macular dystrophy mapped in the human genome [20]. The causative mutations remained elusive until 2016, when Small et al. adopted a whole-genome sequencing approach and identified three non-coding point mutations and two tandem duplications in the DNase I hypersensitivity site DHS6S1, on chromosome 6q16, upstream of the *PRDM13* gene—a region known as MCDR1 [6]. Small et al. also found a tandem duplication on chromosome 5 involving another DNASE1 site and the *IRX1* gene (MCDR3). PRDM13 is a member of a large family of helix–loop–helix DNA binding proteins that plays a key role in controlling gene expression during retinal development [7]. To date, causal genetic defects, either single nucleotide variants (SNVs) or duplication structural variants, have been demonstrated in many NCMD families from different ethnicities [6-16].

Herein, we report the clinical and genetic findings of a novel NCMD family in Mexico. A novel tandem duplication, which also contained a small deletion involving the DNASE1 site and *PRDM13*, was identified in this Mexican pedigree, thus expanding the mutational spectrum of the disease.

METHODS

The study was approved by the Institutional Review Board of the Institute of Ophthalmology “Conde de Valenciana” (number CI-034–2021) in Mexico City. All procedures adhered to the tenets of the Helsinki Declaration, and written informed consent was obtained from all patients or their parents.

Clinical examination: Seven subjects from a single three-generation Mexican family were ascertained (I-2, II-1, II-2, II-5, III-1, III-2, and III-3; Figure 1). All patients underwent a complete ophthalmological examination, including best corrected visual acuity (BCVA), slit lamp examination, dilated indirect ophthalmoscopy, fundus photography, optical coherence tomography (OCT), fundus autofluorescence (FAF), kinetic and chromatic perimetry, and electroretinography (ERG).

DNA extraction and NGS: These seven affected subjects had their blood collected, and their genomic DNA (gDNA) was extracted using the QIAaMP DNA Blood Kit (Qiagen,

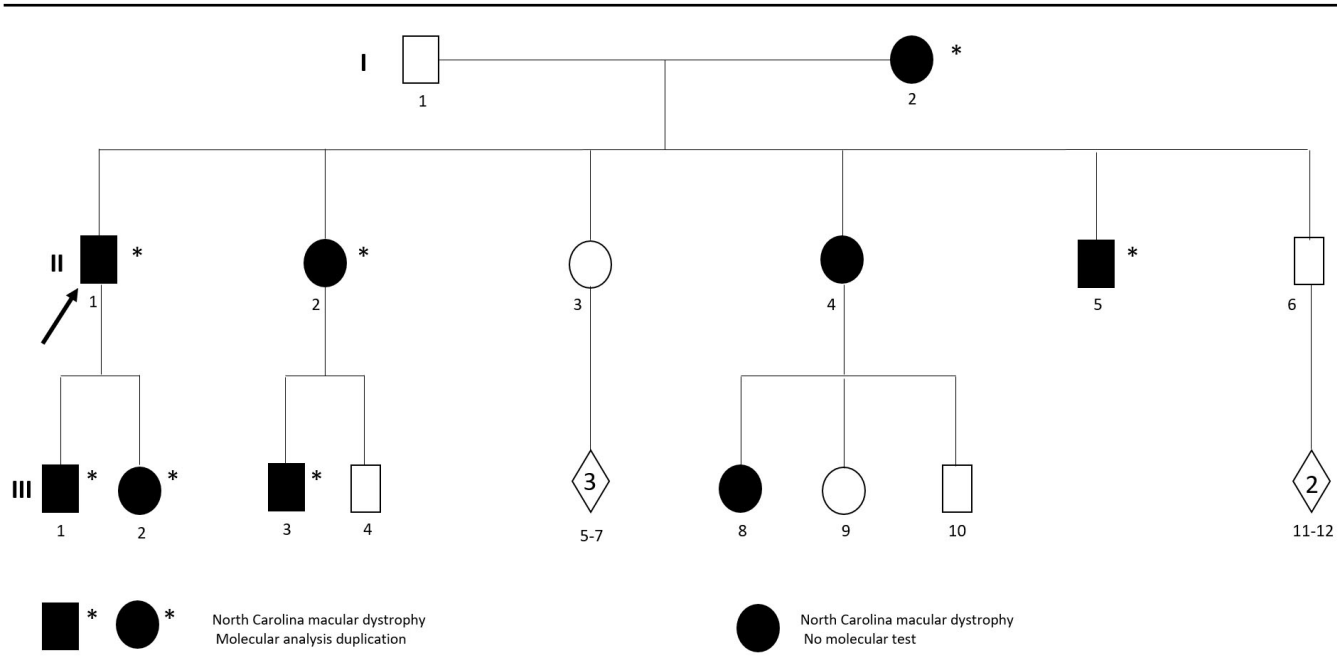

Figure 1. Pedigree of the Mexican family with North Carolina macular dystrophy (NCMD). The index case is indicated by an arrow.

Hilden, Germany), following standard procedures. Targeted genomic sequencing was performed using a hybridization-based protocol with the Illumina technology (Illumina, San Diego, CA). Sequence analysis and deletion/duplication testing were performed for the 328 genes included in the Invitae Inherited Retinal Disorders Panel (Invitae, San Francisco, CA). Targeted regions were sequenced with ≥50x depth, and reads were aligned to the GRCh37 (Hg19) human genome sequence. Exonic deletions and duplications were called using an in-house algorithm (Invitae), which determines the copy number at each target by comparing the read depth for each target in the proband sequence with both the mean read-depth and read-depth distribution obtained from a set of clinical samples.

Chromosomal microarrays: DNA was extracted from the peripheral blood of a NCMD patient (subject II-1) using the AS1010 cartridge in Maxwell 16 equipment (Promega Corp. Madison, WI). Copy number evaluation was performed using a SurePrint G3 Human Genome CGH+SNP (Single Nucleotide Polymorphism) 2x400K microarray (containing 292,097 CGH probes and 119,091 SNP probes with a median spacing of 7.2 kb, Agilent Santa Clara, CA). Eight informative probes were localized on the *PRMD13* gene, whereas two probes were localized on the *CCNC* gene. The results were analyzed using the hg19 human genome version as a reference and Agilent CytoScan software.

Sanger sequencing: Precise characterization of the duplication breakpoint was performed using pairs of primers designed to span the end of the first copy and the start of the second copy. Genotyping was performed through automated direct sequencing using the BigDyeTerminator Cycle Sequencing Kit (Applied Biosystems, Waltham, MA), and the obtained sequences were compared by using hg38 as a human reference genome. The primer sequences and polymerase chain reaction (PCR) conditions are available on request.

Quantitative polymerase chain reaction (qPCR): A quantitative PCR (qPCR) assay was designed to analyze the MCDR1 duplication in this pedigree. The qPCR reactions were performed using Rotor-Gene Q MDx (Qiagen, Germany) and the designed primers (available upon request) to amplify regions within or outside the identified MCDR1 duplication. SYBR Green (Bio-Rad, Hercules, CA) was used to detect qPCR products, and relative quantification analysis was performed by applying the $2^{-\Delta\Delta CT}$ method. The amplification was normalized to the *PIKFYVE* reference gene.

RESULTS

Ophthalmological examination: A total of seven affected subjects were available for study (I-2, II-1, II-2, II-5, III-1, III-2, and III-3, Figure 1), and they underwent ophthalmological evaluations at their first visit in 2017. During this evaluation, visual acuities, funduscopy, and OCT were recorded. Their visual acuities ranged from 20/50 (0.39 logMAR) to 20/200 (1 logMAR). The proband (II-1, Figure 1) was a 42-year-old man who was referred to our clinic due to blurred vision noticed at nine years of age. He was initially diagnosed with Best vitelliform macular dystrophy (BVMD) due to bilateral yellowish yolk-like lesions in the macula. His children, currently aged 11 and 9 years (III-1, and III-2), also complained of blurred vision at the age of 6 and 4 years, respectively. Two of the proband's siblings (II-2 and II-5) complained of blurred vision before the age of 10 years. All patients presented with similar and symmetric bilateral funduscopic lesions as well as excavated atrophic coloboma-like chorioretinal central macular lesions with a protruding rim of the retina partially surrounding the lesion temporally (Figure 2A,D,G). They were classified as having small Grade III lesions. On FAF, a macular hyperfluorescent ring surrounding the foveolar and parafoveolar hypoautofluorescence was observed in all patients (Figure 2B,E,H). In addition, OCT showed collapsed inner retinal layers and external retinal loss in all patients (Figure 2C,F,H). Three patients were reexamined five years later; two of them (III-1, III-2) had stable visual acuity, whereas the proband (II-1) had decreased vision.

The proband's chromatic perimetries showed peripheral islands of vision with reduced response and central scotoma with scotopic stimuli, as well as macular scotoma during photopic stimuli. Subject III-1 showed a mild response at the peripheral retina and central scotoma during scotopic stimuli, whereas photopic stimuli evidenced peripheral islands with no response and central scotoma. Chromatic perimetry in Subject III-2 demonstrated a general reduced response and central scotoma during scotopic stimuli and peripheral islands of reduced response and central scotoma with photopic stimuli. ERG showed normal scotopic and photopic responses in all patients. Kinetic perimetry demonstrated normal peripheral visual fields (V4 stimuli) and central scotoma (I4 stimuli) in all patients.

Molecular findings: Next-generation DNA sequencing of a retinal disease gene panel identified a copy number gain (heterozygous duplication) of the entire coding sequence of the *PRDM13* gene in all seven affected individuals (I-II, II-1, II-2, II-5, III-1, III-2, and III-3). Array-CGH analysis of DNA from an affected subject (II-1) disclosed

a heterozygous duplication NC_000006.11: g.(10,000,828-99577641)_(99,679,713-99697484)dup (Hg38), minimally spanning ~102 kb at 6q16.2 and involving eight probes with a mean log ratio of 0.654; this region contained the whole *PRMD13* gene and the DNASE1 site harboring the original NCMD SNVs, but it did not involve the *CCNC* gene (Figure 3). Targeted next-generation sequencing (NGS) of the region refined the duplication size to 148,178 bp (Hg38 DUP:chr6:99,571,553–99,719,731) and allowed for the characterization of the duplication breakpoints by designing primers across the predicted breakpoints to generate a unique junction fragment sequence (Figure 4 and Appendix 1). Furthermore, qPCR assay results confirmed MCDR1 duplication in clinically affected individuals (Appendix 2). Three unaffected individuals (II-6, III-9, and III-4) were shown to be negative for duplication by qPCR (Appendix 2).

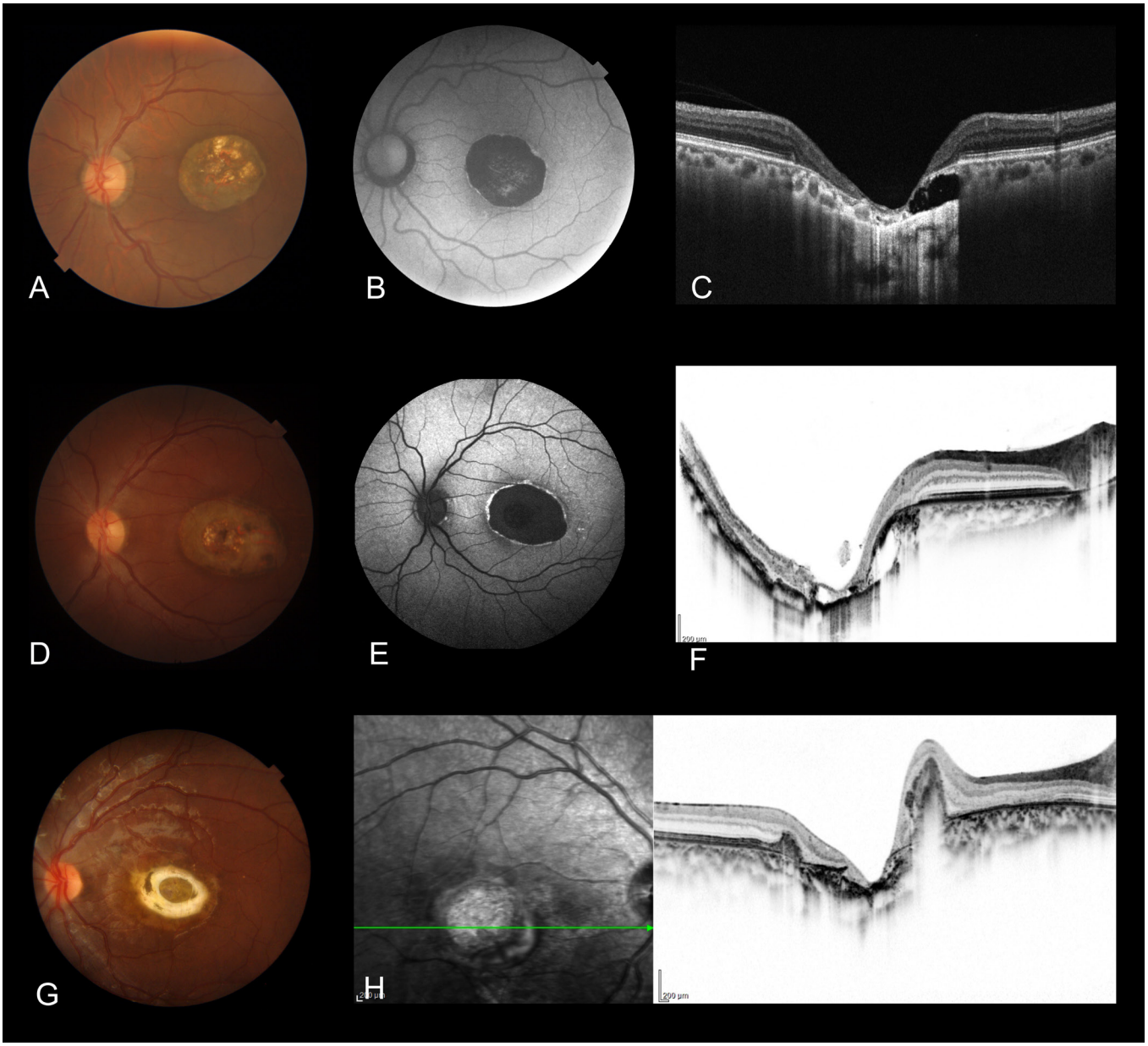

Figure 2. Imaging studies from three subjects with NCMD. **A**-**C** images correspond to a female patient from the first affected generation (I-2). **D**-**F** images correspond to a male patient from the second affected generation (II-1). **F**-**H** images correspond to a child from the third affected generation (III-1). **A, D,** and **G**: Fundus photographs showing colobomatous-like atrophic chorioretinal central macular lesions. **B**, **E**: Autofluorescence imaging showing central macular hypoautofluourescence with a surrounding hyperautofluorescence halo. **C**, **F,** and **H**: Optical coherence tomography (OCT) images showing loss of organization of internal and external retinal layers, with lacunae in **C** and **F**.

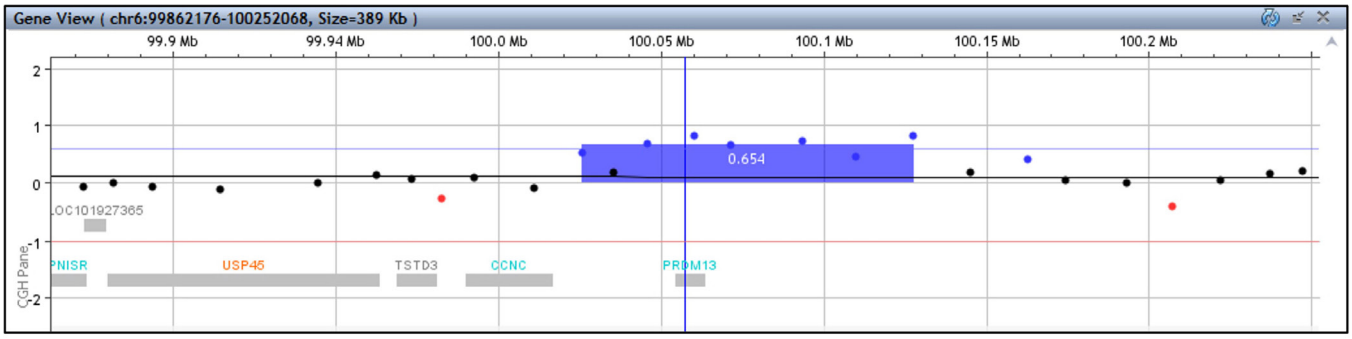

Figure 3. Comparative genomic hybridization (CGH) microarray analysis. A novel MCDR1 duplication of a ~102 kb region containing the *PRDM13* was identified in DNA from an NCMD-affected individual (II-1) in the Mexican pedigree. The duplication nomenclature is NC_000006.11: g.(10,000,828-99577641)_(99,679,713-99697484)dup (Hg38) and includes the maximum and minimum duplication sizes at 6q16.2.

DISCUSSION

Macular dystrophies are a group of hereditary retinal diseases that typically exhibit bilateral symmetry and predominantly affect central vision. In the last decade, knowledge about the molecular causes of macular dystrophies has rapidly evolved owing to technological improvements and improved access of patients to molecular diagnosis.

Herein, we describe a Mexican family comprising nine affected subjects from three generations with NCMD. Seven available subjects showed a severe phenotype (small Grade III), including three children, with all presenting a "coloboma-like malformation," which is characterized by a lack of laminations of the inner and outer retinal layers and outer retinal layer loss. Unlike most families with NCMD in which intrafamilial variable expressivity has been observed, the members of the family studied herein showed a high degree of clinical consistency. A similar homogeneous NCMD phenotype was recently reported in a Turkish NCMD family carrying a large *PRDM13* duplication [10]. Both of these families are relatively small and might not be of sufficient size to, by chance, observe the variable expressivity. Three of the seven affected subjects underwent subsequent ophthalmologic evaluations five years later. Two (III-1 and III-2) remained stable in their visual acuities (III-1 and III-2), whereas the proband (II-1) showed a decline in his visual acuity, probably due to CNVM. Gradual visual loss is uncommon in NCMD, considering its generally non-progressive nature. After revaluating the original NCMD family studied in 1971 [5], Small et al. (1989) reported that only one patient suffered visual loss due to the development of CNVM. This finding was confirmed in several reports, including a recent longitudinal study in which Small et al. reexamined some members of the original family described in the 1970s and identified that only four eyes from four patients (11%, 4/34 eyes) showed a decrease in visual acuity, which was invariably associated with the development of CNVMs [2].

To date, 18 distinct NCMD/MCDR1/PRDM13/MCDR3/IRX1 causal mutations have been reported, including nine

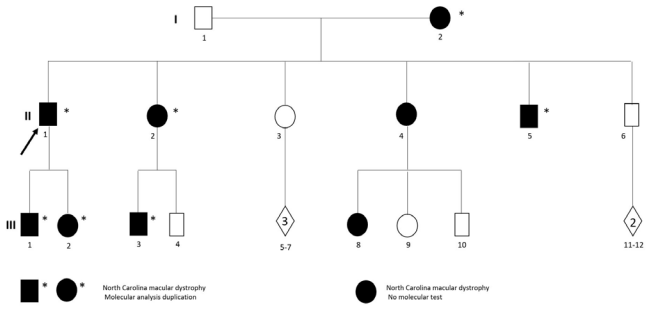

Figure 4. Sanger sequencing validation of duplication breakpoints at 6q16.2. The proband (II-1) and all available affected relatives (Appendix 1) were tested with primers designed across the predicted breakpoints to generate a unique junction fragment sequence. The exact breakpoint is marked with black vertical arrows; the location of polymerase chain reaction (PCR) primers is represented with red arrows. Genomic coordinates (Hg38) indicating the end of the first copy and the start of the second copy are indicated. An additional 15-bp sequence is inserted into the breakpoint of the duplicated sequence.

single nucleotide substitutions (SNPs) and nine tandem duplications, including the novel duplication described here (Table 1; Appendix 3) [7-15]. All SNPs are in non-coding regions involving one of two DNASE1 sites, 12 kb and 7.5 kb, upstream of *PRDM13*. The first three SNPs that Small et al. identified were in a DNase 1 hypersensitivity site (DNASE1), a regulatory region 12 kb upstream from *PRDM13* (Table 1) [6]. The V1 variant (chr6:99593030 G>T; Hg38) has been found exclusively in the USA [6]. The V2 variant (chr6:99593111 G>C), found by Small et al., has been identified in both American and Europeans [6,21]. Finally, the V3 variant (chr6:99593164 C>T), reported by Small et al. in 2016, was found in two families from China [6]. All three SNP variants are located in the same DNASE1 site 12 kb upstream. More recently, a novel SNV named V12 (chr6:99593098 A>C) was suggested as a mutation in a Georgian family with NCMD [9]. Of note, two different SNPs (V10, chr6:99598928 T>C; V11, chr6:99598907 A>C) were recognized in three additional families, two of which were diagnosed with progressive bifocal chorioretinal atrophy (PBCRA) [22]. PBRCA (OMIM 600790) is a rare disease characterized by progressive macular and nasal retinal atrophic lesions, nystagmus, myopia, and poor vision and mapping to a larger genomic region overlapping the MCDR1 locus at 6q14–16.2 [23]. One such family (mother and daughter) had non-progressive non-bifocal chorioretinal atrophy resembling more an extremely severe NCMD phenotype. This same SNP was identified in a moderate-sized Egyptian family with "congenital posterior polar chorioretinal hypoplasia." This SNP is located in the DNASE1 non-coding site, 7.5 kb upstream of PRDM13 [13]. More recently, Seo et al. found two more SNPs causing the NCMD phenotype in the same DNASE1 site in two Korean families (V17, V18) [16].

In addition to SNVs, a total of nine duplications in both NCMD loci (6q16.2 and 5p15.32), including the one reported herein, have been demonstrated as causative of NCMD (Appendix 3). The first, described by Small et al. in a Belizean family (V4, chr6:99572329–99695430), spans 123,101 bp and is the second largest MCDR1 duplication identified to date [6]. The macular phenotype of this family, which had at least 17 affected individuals, was highly variable, with those affected presenting different grades of severity [1]. This duplication encompassed the DNASE1 site and *PRDM13*, which was one piece of data that led Small et al. to propose that overexpression of this gene causes NCMD [6]. In a Danish family that was mapped to chromosome 5 (MCDR3), Small et al. found a large tandem duplication at another DNASE1 site and the *IRX1* gene. Two other smaller tandem duplications overlapping the original one were later found in Europeans, but they only included the DNASE1 site [17]. The V13 duplication identified in seven affected members of a Turkish family is the smallest MCDR1 duplication reported to date, and all patients in this pedigree had large Grade III coloboma-like lesions [10]. Variable expressivity was not observed, unlike in most NCMD families [10]. The novel MCDR1 duplication described in the present family (V19) spans ~148 kb (as determined by NGS and the Sanger sequencing of breakpoints) and is the largest MCDR1-related duplication reported to date (see Table 1 and Appendix 3).

In conclusion, we expand the existing knowledge on the molecular spectrum of defects leading to NCMD. The phenotypic assessment of the family described here supports the notion that the disorder is a congenital macular malformation rather than a progressive dystrophic entity. Although preliminary, our data support a genotype–phenotype correlation wherein smaller duplications upstream of *PRDM13* are associated with more severe macular defects.

APPENDIX 1. SUPPLEMENTARY FIGURE 1.

To access the data, click or select the words "Appendix 1." Sanger sequencing validation of duplication breakpoints. Available NCMD patients (symbols in genealogy are indicated) were tested with primers designed across the predicted breakpoints to generate a unique junction fragment sequence.

APPENDIX 2. SUPPLEMENTARY FIGURE 2.

To access the data, click or select the words "Appendix 2." qPCR results using primers located within and outside the duplicated MCDR1 region.

APPENDIX 3. SUPPLEMENTARY FIGURE 3.

To access the data, click or select the words "Appendix 3." NCMD causing variants identified to date. A partial chromosome 6 view is shown in the upper part of the figure. MCDR1 locus (6q16.2) is delimited in a red box. Below, there is a zoom of the 6q16.2 region spanning approximately 800 kb. The light blue regions of each gene represent the introns and the vertical lines within each gene represent the exons of the gene; thicker vertical lines represent multiple exons. The white regions between the genes represent the intergenic regions, the most important of which is the one found between the *CCNC* and *PRDM13* genes, which is called the DNA I hypersensitive region. Within this, NCMD-causing SNVs are located (yellow rectangle), in a region covering approximately 6 kb (enlarged on the right side of the image). On the left side of the image, all MCDR1 duplications reported on the date, including their genomic coordinates and their length are presented. The white boxes in the duplications represent

Table 1. Known genetic defects in the (MCDR1) *PRDM13* and (MCDR3) *IRX1* regions found in NCMD and possibly related diseases.

Variant number	Type of variant	Chromosomal position (hg19)	Chromosomal position (hg38)	Nucleotide change	Phenotype	Reference #
MCDR1 locus (*PRDM13*), chromosome 6q16.2						
V1	SNV	chr6:100040906	chr6:99593030	G>T	NCMD	6
V2	SNV	chr6:100040987	chr6:99593111	G>C	NCMD	6
V3	SNV	chr6:100041040	chr6:99593164	C>T	NCMD	6
V4	Tandem DUP	chr6:100020205-100143306	chr6:99572329-99695430	123,101 bp DUP	NCMD	6
V6	Tandem DUP	chr6:99996226-100065137	chr6:99548350-99617261	69,912 bp DUP	NCMD	7
V7	Tandem DUP	chr6:99984309-100082698	chr6:99536433-99634822	98,389 bp DUP	NCMD	8
V10	SNV	chr6:100046804	chr6:99598928	T>C	PBCRA	22
V11	SNV	chr6:100046783	chr6:99598907	A>C	NCMD PBCRA	22 13
V12	SNV	chr6:100040974	chr6:99593098	A>C	Possible NCMD*	9
V13	Tandem DUP	chr6:100008141-100064368	chr6:99560265-99616492	56,228 bp DUP	NCMD	10
V14	Tandem DUP	chr6:99932464-100067110	Chr6:99484588-99619234	134,646 bp DUP	NCMD	11
V15	SNV	Chr:100040970	Chr6:99599064	A>G	NCMD	12
V16	SNV	chr6:100040906	chr6:99593030	G>C	NCMD	12
V17	SNV	chr6:100046790	chr6:g.99598914	T>C	NCMD	16
V18	SNV	chr6:100046802	chr6:g.99598926	G>A	NCMD	16
V19	**Tandem DUP**	**chr6:100019429-100167607**	**chr6:99571553-99719731**	**148,178bp DUP**	**NCMD**	**Present report**
MCDR3 locus (*IRX1*), chromosome 5p15.32						
V5	Tandem DUP	chr5:3587901-4486027	chr5:3587787-4485914	898,126 bp DUP	NCMD	6
V8	Tandem DUP	chr5:4391377–4436535	chr5:4391264-4436422	45,158 bp DUP	NCMD	17
V9	Tandem DUP	chr5:4396927–4440442	chr5:4396814-4440329	43,515 bp DUP	NCMD	17

Abbreviations used: bp: base pair, chr: chromosome, DUP: duplication, SNV: single nucleotide variation. A: adenine, C: cytosine, G: guanine; T: thymine. NCMD: North Carolina Macular Dystrophy. PBCRA: progressive bifocal chorioretinal atrophy. *This reported SNV occurred in a small family in an isolated population with diagnostic inconsistencies and molecular confounding factors and needs to be corroborated.

intergenic regions. The novel duplication reported in the present work is indicated as V19.

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