**INTRODUCTION**

North Carolina Macular Dystrophy (NCMD) is an autosomal dominant disease with a congenital or infantile onset. It was first described in 1971 in a large family from the mountains of North Carolina and given the name “dominant macular degeneration and aminoaciduria”. Aminoaciduria was later determined to be unrelated after aminoaciduria was also found in family members without macular disease. Additional terms have been used to describe the disease due to vastly different phenotypes among affected family members. Dominant progressive foveal dystrophy reflected the progressive vision loss found in some patients, typically due to a few cases of choroidal neovascularization. Central areolar pigment epithelial dystrophy described a second family located in Iowa with many of the same characteristics. Central pigment epithelial and choroidal degeneration was used to describe yet another family with fundus appearances identical to the original North Carolina family. Genetic linkage has been confined to a location on chromosome 6q16, called MCDR1 because it was the first to be linked to macular degeneration. Additional studies refined the MCDR1 interval to 1.8 million base pairs between locus D6S1716 and D6S1671. Several of the different families identified with NCMD were found to have common ancestral alleles, linking them to the original Irish immigrants that settled in North Carolina in the 1800’s. However, other non-related families also linking to MCDR1 have been found in Europe and South America. For additional explanation on genetics, see “Genetics Overview” at the end of this article.

The goal of the present study was to utilize fourier domain optical coherence tomography (fd-OCT), fundus auto-fluorescence (FAF) and fundus color images to determine the relationship between anatomical abnormalities and loss of visual function in these patients.

**METHODS**

**Patients:** Data were obtained from six affected members of a Caucasian family, spanning three generations (Figure 1). All procedures received Institutional Review Board approval and informed consent was obtained from each patient after the procedures were explained.

The retinas of each patient were characterized as Gass’s Grades 1, 2 or 3 based on fundus examination and color fundus photographs.
**Grade 1:** Limited to small (<50µm) yellow specks (drusen) in the macula with possible mild retinal pigment epithelium (RPE) disturbances. Vision deficit is mild (20/30 or better).

**Grade 2:** Larger elevated confluent yellow drusen with RPE atrophy and/or disciform scars with pigment clumping. Vision changes can range from mild to moderate (20/25 to 20/60).

**Grade 3:** Large central atrophic excavation of the retinal and choroidal layers, resembling a staphyloma, but now referred to as a macular caldera. Vision ranges from 20/40 to 20/200.

Any of the three grades can also display peripheral retinal drusen-like lesions at varying degrees. A few cases of choroidal neovascularization (CNV) have been reported to cause progressive vision loss (20/100 to 20/400), but for the most part this condition is considered stationary.

**PROCEDURES**

Electronic visual acuity (EVA) was measured with a computerized version of the E-ETDRS, where letters, derived from the ETDRS chart, are displayed individually, surrounded by crowding bars.

Fd-OCT line and volume scans were obtained with a Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany). The confocal scanning laser ophthalmoscope (SLO) uses an infrared reflectance (820 nm) light source and has an optical resolution of about 10 µm. The fd-OCT is generated with a second pair of scanning mirrors.

The wavelength of the OCT is 870 nm, with an optical depth resolution of about 7 µm. Fundus measurements were done with the software measuring tool included with the Spectralis program. Measurements included height, width and depth of macular calderas and distance of the caldera edge to the edge of the optic nerve (papillo-macular bundle). The edge of the caldera was defined as the location where retinal layers appeared normal on OCT. The depth of the caldera was obtained by measuring from an imaginary “bridge” across the lesion, anchoring each end at the location where the reflective line of the photoreceptor inner and outer segments dropped out to the bottom of the OCT scan (Figure 2). On all fd-OCT scans where the retinal structures were severely distorted or missing, the automatic retinal thickness measurement function could not correctly identify the location of the inner limiting or Bruch’s membranes. To see the true retinal thickness, the lines had to be manually corrected for proper measurements.

Color photography utilized a 60° digital fundus camera (CF-60UD, Canon USA). Three subjects were also imaged through auto-fluorescence using the fluorescein angiography setting on the SLO portion of the Spectralis. The four subjects with Grade 3 in both eyes also had an ultrasound (b-scan) examination.

DNA samples from all six living affected family members were included in genetic mapping, which was performed using STR markers D6S300, D6S1671, and D6S434. Fluorescently labeled primer pairs for each marker were obtained from Applied Biosystems. Genomic DNA from available family members was amplified according to the manufacturer’s protocol, separated on a 3100-Avant (Applied Biosystems) Genetic Analyzer, and analyzed with GeneMapper V3.7 (Applied Biosystems), (See “Genetics Overview” at the end of this article).

**Figure 1:** Family pedigree. Squares represent males and circles females. A diagonal line indicates a deceased member. Based on family interviews and process of elimination we have concluded that the NCMD is most likely passed down from I/2 (see arrow).

**Figure 2:** The edge of the caldera was defined as the location where retinal layers appeared intact on OCT (red lines). The depth of the caldera was obtained by measuring from an imaginary “bridge” across the lesion (blue line) anchored at the red lines, to the bottom of the OCT scan (green line).
RESULTS

The family pedigree is shown in Figure 1. None of the patients in this pedigree had been diagnosed with NCMD previously. None were aware of any members of the family that had been reported previously, but the paternal ancestors of patient #8688, can be traced to North Carolina as far back as the mid 1700s.

**Generation III:** A 68 year old female (III, #8688) with Grade 3 in the right eye and Grade 2 in the left.

**Generation IV:**
- Daughter of #8688, age 44 (IV, #9395) with Grade 3 in the right eye and Grade 1 in the left.
- Son of #8688, age 39 (IV, #8602) with Grade 3 in both eyes.
- Nephew of #8688, age 39 (IV, #8686) with Grade 3 in both eyes.

**Generation V:**
- Son of #8602, age 5 (V, #8603) with Grade 3 in both eyes.
- Son of #8686, age 7 (V, #8687) also with Grade 3 in both eyes.

Visual acuity and other demographic data are listed in Table 1.

<table>
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<tr>
<th>Pt. ID</th>
<th>Age</th>
<th>Gender</th>
<th>RPE Visual Appearance</th>
<th>Eye</th>
<th>Visual Acuity</th>
<th>OCT Focus Diopter</th>
<th>Caldera (Grade 3)</th>
<th>Papillo-Caldera Distance</th>
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</tbody>
</table>

N/A = data not available.

$^*$ The + sign indicates an approximate measurement as both edges and bottom of the caldera could not be visualized at the same time.

$^{**}$ Distance measured on the fundus color image as no OCT scan was available of the papillo-macular bundle area.

Table 1: Summarizes demographics of age, gender, retinal appearance and visual acuity. To document refractive status, the focus setting on the OCT is listed in the table together with the caldera measurements. The biggest variation in measurements between patients is seen in the width and depth of the caldera (red numbers). The smallest difference is distance between lesion and the optic disk.

**Figure 3:** Patient #9395 with grade 1 in the left eye. (a) color fundus photo taken at 60°. (b) autofluorescence from an SLO taken at 30°. (c) fd-OCT 9mm line scan horizontal through the fovea. The green line on the infra-red image shows the OCT scan location on the retina. The fd-OCT image consists of 100 scans averaged together with auto retinal tracking. The top and bottom red lines mark the internal limiting membrane and Bruch’s membrane respectively.

**Figure 4:** Subject #8688 with grade 2 in the left eye. Descriptions of a, b and c are the same as for Figure 5. The 9mm OCT line scan is located about ½ disk diameter below the fovea, at the highest point of the retinal scar.
Grade 1

A single eye presented with this grade (Figure 3a). The left eye of subject #9395 (IV) had a small area of minute drusen with RPE disturbances centered in the macular. A few scattered drusen were also seen in the temporal macula. The remaining retina and optic nerve were within normal limits.

The fundus autofluorescence image of subject #9395 showed a mottled appearance in the area of RPE disturbance with both hypo- and hyper-autofluorescence spots. A concentrated area of decreased fluorescence was localized to the more severely affected RPE. Multiple hyper-autofluorescence spots could be seen in the temporal macula (Figure 3b).

The fd-OCT line scan through the macula had all retinal layers mostly intact with only local scattered areas of irregular RPE and outer segment disturbances. The retinal contour of the temporal side of the fovea has a slight hint of a depression at the level of the RPE, but with all retinal layers accounted for. (Figure 3c).

Grade 2

A single eye of subject #8688 (III) had Grade 2 (Figure 4a). The left eye had an old disciform scar with a large area of atrophy and pigment clumping covering the central macula and extending inferior-temporal towards the vessel arcade. The surrounding retina looked relatively healthy with a moderate amount of small drusen. The optic nerve and retinal vessels looked normal but with minor RPE atrophy at the temporal edge of the disk.

The fundus AF confirmed a large area of atrophy with a corresponding hypo-autofluorescence, surrounded by a ring of hyper-autofluorescence. The border between the two showed a “salt and pepper” looking band with both dark and light fluorescent spots (Figure 4b).

The fd-OCT line scan placed horizontally across the macular lesion showed the breakdown of the RPE/outer segment and outer nuclear/plexiform layers. An area of elevated retina with pigment clumping suggest an old CNV event. Only the inner retinal layers looked intact across the lesion, while all retinal layers beyond the macular lesion appeared normal (Figure 4c).

Grade 3

Ten out of the twelve eyes examined were categorized as Grade 3 with medium to large calderas centered in the macula, ranging in size from one and a half to four disk diameters. The distance from the temporal edge of the disk to the edge of the caldera was close to identical in all eyes, causing the lesions to stretch temporally at varying degrees. For the most part there were sharp borders between the lesions and the surface of the retina. Scattered pigment clumps and remnants of major choroidal vessels could be seen on a background of what appeared to be the scleral wall of the eye. Representative sample color fundus photographs are presented in Figure 5. A summary of fd-OCT measurements are in Table 1.

The fundus optical coherence tomography demonstrated a variety of caldera shapes and depths. The transition from a normal retinal surface to the plummeting wall of the caldera was abrupt as seen on the fd-OCT generated image of the retinal surface (Figure 6).

For the larger calderas, the retinal atrophy inside the lesions was complete with total loss of RPE and chorio-
capillaris, with only a few major choroidal vessels present at the bottom of the pit. The neatly arranged retinal layers on the surface of the retina were lost as the walls of the caldera dropped down. The outer segments and outer nuclear/plexiform layers disappeared first with a thin layer of RPE remaining in patches (Figure 7a). In a few of the shallow calderas, intact inner retinal layers were evident lining the depression on a background of Bruch’s membrane and the choroid (Figure 7b). The slightly deeper calderas had inner retinal layers that were occasionally intact in the shallower part of the pit, but in other places drop out completely (Figure 7c). Epi-retinal membranes were evident in three of our Grade 3 eyes (Figure 8).

The very large and deep calderas were particularly challenging to image with the fd-OCT. Patient #8687 (V) had the largest caldera in our family and an fd-OCT scan placed horizontally across the caldera could not display the retinal surface at the same time as the bottom of the caldera. Thus, two separate scans were required to view the full extent of the lesion (Figure 9).

The left eye of subject #8686 (IV) was given a Grade 3 due to a small caldera but an old disciform scar was present at the inferior temporal edge of the lesion, possibly from a CNV event, which is more often associated with Grade 2 (Figure 10a). Fundus auto-fluorescence imaging was done in this eye and showed areas of hypo-auto-fluorescence surrounded superior-nasally by a rim of hyper-fluorescence with a normal auto-fluorescence on the surface of the retina (Figure 10b). Inferior-temporally were patches of hyper-auto-fluorescence corresponding to the disciform scar seen in the color photograph. The fd-OCT line scan over the caldera showed a shallow depression with outer retina/RPE atrophy, where as the disciform area was elevated with partly intact inner retina and thickening RPE (Figure 11).

Of the subjects that were imaged with ultrasound, the calderas were visible as a retinal depression in seven out of the eight eyes tested. Even in the largest and deepest caldera of the 7 year old subject (V, #8687), no evidence of an abnormal scleral reflection line was noted (Figure 12). This supports the renaming of the Grade 3 lesion to macular caldera instead of staphyloma.

The DNA genetic analysis in the family is consistent with the previously reported North Carolina macular dystrophy locus (MCDR1) on chromosome 6.10,15
We examined six members of a family with NCMD with predominantly Grade 3 macular calderas. The fd-OCT was able to image the shallow to moderately deep calderas in a single scan, but to image the very deep lesions, the scan had to be split into two scans as the bottom could not be seen at the same time as the surface of the surrounding retina. The spectral domain resolution of the OCT showed the extent of retinal layer breakdown within the depth of the calderas.

Many of these patients have been able to maintain surprisingly good visual acuity. Interestingly, one observation made in our family was that size of the calderas appeared to be more strongly correlated with the pigment density of the RPE. The two subjects with the largest lesions were the seven year old and his 39 year old cousin, once removed; both with a blond-looking fundus appearance (see red numbers in Table 1). On the other hand, the smallest lesions were found in the retinas with darker looking RPE. Whether or not this was just a coincidence in our small sample size, or if this holds true in other families, will require further research.

The epi-retinal membranes seen in three of our patients have, to our knowledge, not been reported previously. However, it is only since the arrival of fd-OCT that subtle ERMs could be readily identified. It is tempting to speculate that the shallow depression see in patient (IV, #9395) with Grade 1 could be an arrested state of a potential caldera that never developed. The pathogenesis of a Grade 3 caldera is not known. It has been suggested, mainly due to the presence of sub-retinal fibrous scar tissue at the rim of some calderas, that it may be secondary to congenital retinal hemorrhage. However, only four out of the ten Grade 3 eyes in our family had evidence of fibrotic tissue at the rim. There are many genes and factors involved in the formation of the foveal pit during fetal development. The macula has a different developmental path than the surrounding retina in order to keep the retinal vessels away from the center. It is thought that once the avascular zone has been established, the foveal pit is created as a result of growth-induced stretch. None of the genes previously identified in the development of the macula are located on chromosome 6, but an indirect influence is still possible. A variable expression mutation in a gene involved in this process could explain the variations of phenotype in these patients.

Two of our subjects had signs of an old CNV event whereas his second cousin (age 5) had lesions smaller than his own father. Interestingly, one observation made in our family was that size of the calderas appeared to be more strongly correlated with the pigment density of the RPE. The two subjects with the largest lesions were the seven year old and his 39 year old cousin, once removed; both with a blond-looking fundus appearance (see red numbers in Table 1). On the other hand, the smallest lesions were found in the retinas with darker looking RPE. Whether or not this was just a coincidence in our small sample size, or if this holds true in other families, will require further research.

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are 22 pair of autosomal chromosomes (non-sex chromosome) within the nucleus, thus each parent provides 50% of their genetic makeup. One set of chromosomes is inherited from each parent, giving the genetic makeup of the human body is composed of 46 chromosomes or 23 chromosome pairs (2 sets of 23).

The genetic eye diseases can be passed on or inherited from past generations in several different patterns; autosomal dominant, autosomal recessive, or X-linked. The designation of isolate or sporadic is assigned to cases in which no previous family history of disease can be determined.

**Autosomal Dominant Inheritance:**
Autosomal dominant refers to a gene on a non-sex chromosome that expresses itself in a dominant manner. A dominant mutation is capable of causing disease even when paired with a normal gene (one normal copy, one faulty copy). Each time an affected person has children there is a 50% risk of passing on the disease-causing gene, regardless of gender. For the inheritance to be considered dominant, there should be at least 3 consecutive generations of affected individuals with males and females equally affected. Some autosomal dominant gene mutations are known to have reduced penetrance, which may cause a generation to appear “skipped” or vary the severity of the disease.

**Autosomal Recessive Inheritance:**
Autosomal recessive disease is caused by inheriting two faulty copies of a gene bearing a disease-causing mutation. A recessive mutation is capable of passing through several generations without causing the disease to appear, as long as it is paired with a normal copy of the gene (i.e., carrier state). For the disease to manifest, two recessive mutations, one from each “carrier” parent must be transmitted to the offspring. When two known carriers have children, there is a 25% risk of disease for each child (male and female). The risk of passing on the carrier state is 50% and there is a 25% chance of not passing the mutation to the next generation. All offspring of an individual affected with autosomal recessive disease will be carriers.

**X-linked Inheritance:**
X-linked inheritance is caused by a mutation in a gene located on the X-chromosome. The disease is carried by females and passed on to fully manifest in males. Female carriers (one normal X, one faulty X) may be non-symptomatic, have mild disease or be completely affected at a similar level as the males. Thus, there is a gender differential in the level of symptomatic expression in X-linked disease. Each female carrier has a 50% risk of passing on the faulty X to each offspring, regardless of gender; 50% risk for affected sons, 50% risk for carrier daughters. An affected male is obligated to pass on the faulty X to each
of his daughters (100% obligate carriers) but will never pass on the disease to a male offspring.

**Gene Sequencing:**
Once a gene has been identified to cause a particular disease, it can be sequenced to examine the internal code for mutations. Gene sequencing consists of identifying the sequence or order of the four compounds/letters (A, G, C, & T) that makes up the DNA. Gene sequencing is accomplished with the aid of DNA primers, enzymes and chemically-labeled nucleotides. Once the sequenced code is determined, it is compared to other family members or known unaffected people.

**Linkage Analysis:**
Linkage analysis is a method that can be used when a gene has not yet been identified to account for a particular disease. Access to DNA samples from multiple patients from the same family with the same disease allows for a method of indirect gene tracking, called linkage. Linkage analysis works by comparing DNA markers from affected patients with their unaffected family members. The linkage analysis can greatly narrow down the location of a potential gene and make it easier to find the responsible mutation. Linkage analysis for NCMD has so far only narrowed down the location of the disease to an area on chromosome 6 (i.e., 6q16). This region is over 1.8 million “letters” or basepairs in length and contains nine genes. To date, no mutations have been identified in this region which is believed to be the cause of NCMD.

**Genetic Nomenclature:**
6q16 is the name that describes the “address” location or locus within the DNA:

6 = the first number designates the chromosome.

q = the q stands for the long arm of the chromosome. Each diploid chromosome is shaped somewhat like an X with two long and two short arms. The center of the chromosome where the arms meet is called the centromere. The short arms are called the p-arms after the French “petit” and the long arms were given the name q-arms because q comes after p in the Latin alphabet.

16 = each arm has visible bands that can be seen under a microscope by applying a suitable stain. The bands are numbered, starting with 1 at the centromere. Each band has sub-bands that can be seen in higher magnifications and resolution.

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**References**