Retinal Crystals in Type 2 Idiopathic Macular Telangiectasia

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Purpose: To characterize the phenotype and investigate the associations of intraretinal crystalline deposits in a large cohort with type 2 idiopathic macular telangiectasia (MacTel).

Design: Case-control study.

Participants: Patients with and without retinal crystals from the Macular Telangiectasia Project, an international multicenter prospective study of type 2 MacTel.

Methods: Grading of stereoscopic 30-degree color fundus (CF), confocal blue light reflectance (CBR), red-free (RF), and infrared (IR) images was performed according to the MacTel Natural History Study protocol and staged using the classification system devised by Gass and Blodi. Spectral domain-optical coherence tomography (SD-OCT) and adaptive optics imaging were used for a finer analysis of the phenotype. Associations between crystals and other characteristics of the disease, as well as potential risk factors, were investigated.

Main Outcome Measures: Presence of crystals, fundus signs of MacTel, clinical characteristics, and presence of potential risk factors of MacTel.

Results: Of 443 probands enrolled in the MacTel study, 203 (46%) had crystalline deposits present; 60% of the cases were bilateral at baseline. Eyes with crystals had a mean letter score of 70.7 (standard deviation [SD] = 15.9), whereas those without crystals had a mean letter score of 66.5 (SD = 15.5, P < 0.001). Crystals were present at all stages of the disease and showed high reflectivity within a wide wavelength range. They were located at the anterior surface of the nerve fiber layer, arranged along the nerve fibers, within an annular area centered on the fovea. Significant associations of crystalline deposits were found with a loss of retinal transparency, macular pigment optical density (MPOD) loss, fluorescein leakage, retinal thickness, and a break in the inner segment/outer segment junction line. Associations with environmental risk factors were not found.

Conclusions: Intraretinal crystals are a frequent phenomenon associated with type 2 MacTel. They may appear at all stages and aid in the early diagnosis of the disease. Their morphology further implicates Müller cells in the pathogenesis of the disease. Insight into their physical and chemical properties may provide clues to the metabolic pathways involved in the pathogenesis of the disease.

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Idiopathic macular telangiectasia (MacTel) is a vascular anomaly affecting retinal capillaries in the juxtafoveal region.1 The cause and pathogenesis of the disease are little known.

According to a system originally devised by Gass and Blodi2 and subsequently simplified by Yannuzzi et al.,3 MacTel is classified into 2 main categories based on biomicroscopic and fluorescein angiographic features. Type 1 (aneurysmal telangiectasia) is a predominantly unilateral disease affecting mostly men, associated clinically with retinal thickening, prominent extensive telangiectasis, lipid exudates, and cystoid changes, mainly in the temporal part of the macula. Type 2 (perifoveal telangiectasia) is a bilateral disease that affects both sexes, starting in the 5th to 7th decades. Early clinical features are more subtle and include a loss of retinal transparency, retinal crystals, intraretinal cystoid spaces at the fovea, and a diffuse leakage in the fluorescein angiogram. The retina is thinner than normal. Subsequent vascular remodeling and fibrosis accompanied by pigment plaques and eventual subretinal neovascularization, scarring, and atrophy limit the prognosis for central vision. The benefit of therapies including anti-vascular endothelial growth factor agents has been explored in several case series, with variable, mostly limited, and transient success. To date, no known therapeutic modality has been proven effective in the long term.4–8

The presence of yellow-white refractile crystals in the superficial layers of the parafoveal retina is a characteristic and frequent feature of the disease phenotype.9,10 Their origin, composition, and significance are unknown. Our aim was to characterize the phenotype and associations of these intraretinal crystalline deposits using multiple imaging modalities in a large cohort with type 2 MacTel.
video rate. Confocal pinholes of 0.5 to 1.5 Airy disks improved near-IR reflectance with photoreceptors in the same retinal locations were acquired using (cSLOs) (Heidelberg Retina Angiograph 2 or Spectralis HRA color transparencies. Confocal scanning laser ophthalmoscopes images were recorded digitally or digitized at high resolution from Standard 30-degree stereo field 2 color fundus (CF) and red-free (RF) Imaging below the limits established by the American National Standards of the study. Maximum retinal irradiance of lasers used was well sent was obtained from each participant after explanation of the nature at each participating center. Before inclusion, written, informed consent was obtained from each participant after explanation of the nature of the study. Maximum retinal irradiance of lasers used was well below the limits established by the American National Standards Institute (Z136.1; 1993) and other international standards.

Materials and Methods

Patients

Patients with a diagnosis of type 2 MacTel with and without retinal crystals from the MacTel project, an international multicenter prospective study of type 2 MacTel, currently involving 28 research centers worldwide, were included in this analysis. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the respective institutional review board or local ethics committee at each participating center. Before inclusion, written, informed consent was obtained from each participant after explanation of the nature of the study. Maximum retinal irradiance of lasers used was well below the limits established by the American National Standards Institute (Z136.1; 1993) and other international standards.

Imaging

Standard 30-degree stereo field 2 color fundus (CF) and red-free (RF) images were recorded digitally or digitized at high resolution from color transparencies. Confocal scanning laser ophthalmoscopes (cSLOs) (Heidelberg Retina Angiograph 2 or Spectralis HRA+OCT, Heidelberg Engineering GmbH, Dossenheim, Germany) were used for fundus autofluorescence (FAF), confocal blue light reflectance (CBR), infrared (IR) imaging, and indocyanine green (ICG) angiography. For FAF and CBR imaging, the fundus was illuminated using an excitation light with a wavelength of 488 nm. Fundus autofluorescence was recorded through a filter with a short-wavelength cutoff at 520 nm. To reduce random noise, a master image was produced for IR imaging. Fluorescein angiography (FA) was performed using a standard excitation wavelength of 790 nm was used for ICG angiography. Infrared reflectance imaging of the retina was achieved with a 680-nm superluminescent diode source and a photomultiplier tube for light detection. The raw data were registered and frame averaged using custom Matlab software (MathWorks, Inc., Natick, MA), and individual frames were montaged using Adobe Photoshop (Adobe Systems Inc., San Jose, CA).

Phenotyping

Detailed phenotyping based on the Gass and Blodi\textsuperscript{2} classification was performed by grading of field 2 CF, RF, and FA images, according to the system defined in the MacTel Study protocol.\textsuperscript{14} Briefly, a grid (adapted from the standard grid for age-related maculopathy classification\textsuperscript{4,5,16} consisting of 3 concentric circles with 1000-, 3000-, and 6000-μm diameters and 4 radial quadrant spokes dividing the central macula into 9 subfields) was superimposed over the CF image, centered on the fovea, in Adobe Photoshop CS4 (Adobe Systems Inc.). Images were graded for image quality and fundus features of type 2 MacTel listed in Table 1, and for OCT and FAF characteristics as described previously.\textsuperscript{14} Grading was performed independently by 3 certified graders masked to the identity and clinical data of the patients. Gradings were compared for inter- and intraobserver reliability, and final copy was created by adjudication. Staging of disease severity was based on the criteria devised by Gass and Blodi\textsuperscript{2} using the categories shown in Table 2.

Registration of Retinal Crystals

In cases where grading detected the presence of crystals, composite images containing overlays of aligned CF, CBR, RF, and IR images were recorded digitally or digitized at high resolution from color transparencies. Confocal scanning laser ophthalmoscope (cSLO) images were recorded digitally or digitized at high resolution from Standard 30-degree stereo field 2 color fundus (CF) and red-free (RF) Imaging below the limits established by the American National Standards Institute (Z136.1; 1993) and other international standards.

\textsuperscript{1}Rase was compared with non-Caucasian race given the small numbers in each individual race group. Non-Caucasian races included American Indian, Asian, African American, Native Hawaiian or Pacific Islander, and Australian Aboriginal or Torres Strait Islander. Test: chi-square test.

| Table 1. Distribution of Probands with Retinal Crystals by Gender and Race |
|-----------------------------|-----------------------------|-----------------------------|
| Gender                     | No Crystals (n=232)         | Crystals (n=203)            | P Value |
| Male gender                | 87                          | 83                          | 0.47    |
| White race*                | 193                         | 159                         | 0.2     |

\*Rase was compared with non-Caucasian race given the small numbers in each individual race group. Non-Caucasian races included American Indian, Asian, African American, Native Hawaiian or Pacific Islander, and Australian Aboriginal or Torres Strait Islander. Test: chi-square test.

| Table 2. Associations with Fundus Autofluorescence and Optical Coherence Tomography Characteristics |
|--------------------------------------------------|-----------------------------------------------|-------------|
| No Crystals %                                   | Crystals %                                    | P value     |
| Abnormal foveal avascular zone                  | 89                                           | 85          | 0.4      |
| Inner empty spaces                              | 46                                           | 50          | 0.29     |
| Outer empty spaces                              | 18                                           | 24          | 0.09     |
| Inner empty spaces                              | 25                                           | 25          | 0.45     |
| Center not involved                            | 20                                           | 24          |          |
| Outer empty spaces                              | 10                                           | 17          | 0.13     |
| Center not involved                            | 7                                            | 7           |          |
| Inner segment/outer segment junction line break | 50                                           | 68          | <0.001   |
| Increased autofluorescence within 1 DD of the fovea | 80                                           | 83          | 0.47     |
| Fovea abnormal                                  | 85                                           | 83          | 0.72     |
| Large area of decreased autofluorescence        | 7                                            | 8           | 0.65     |

DD = disc diameter. Test: chi-square test.
images were created (in Adobe Photoshop CS4) and analyzed for the presence and location of crystals in images of respective modality. The number of crystals present per subfield within the grading grid was assessed in each imaging modality and graded using the categories 0, 1–5, 6–10, and >10.

Clinical Data Collection
Clinical information, including age, gender, self-reported race/ethnicity, and medical and ocular history, was collected through standardized questionnaires. Monocular best-corrected visual acuities were determined according to a standardized protocol using the Early Treatment Diabetic Retinopathy Study logarithm of the minimum angle of resolution visual acuity charts at a distance of 4 m. Scoring of the test was based on the number of letters read correctly. Possible scores ranged from 0 (Snellen equivalent 20/800) to 100 (Snellen equivalent 20/12). Participants also underwent an annual comprehensive ophthalmologic examination. Evaluation of vision-targeted health-related quality of life was measured using the National Eye Institute Visual Functioning Questionnaire-25.

Statistical Methods
Comparisons of various characteristics of interest between participants/eyes with and without crystalline deposits were performed using a chi-square test. Generalized linear models were used to compare continuous image grading characteristics across the crystalline deposit groups. Kappa statistics were used to assess the agreement of the crystalline deposit grading across the various image modalities. A P value of <0.05 was accepted as statistically significant. All analyses were conducted using commercially available statistical software (SAS version 9.1; SAS Inc., Cary, NC).

Results

Demographics
Of 443 patients enrolled in the MacTel study, 203 (46%) showed crystalline deposits. At enrollment, the mean age of patients with crystals was 60.0 years (standard deviation [SD] = 8.8), and the mean age of patients without crystals was 60.4 years (SD = 9.8).
Follow-up periods ranged from 0 to 4 years with a mean of 1.6 years (median 2, SD 1.2 years). The distributions by gender and race are shown in Table 1.

**Phenotype**

At baseline, of 203 participants showing retinal crystals, 121 (59.6%) were bilateral and 81 (39.9%) were unilateral. Retinal crystals were located within a band approximately 4000 μm in diameter centered on the fovea, with a sparing of approximately 700 μm around the center of the fovea. In 34 cases (16.8%), a pattern in the alignment of the crystals corresponding to that of the NFL was clearly distinguishable in fundus images (Figs 1A, B and 2B, C). In 78% of cases with an NFL pattern, a gap in the pattern temporal of the fovea corresponding to the raphe was apparent (Fig 1A, B). High-resolution imaging using AOSLO confirmed this pattern and the presence of the crystals within the same optical section as the nerve fiber bundles (Fig 2). Individual crystals were least 5 μm or larger. Crystals appeared to be angular with sharply demarcated edges and were distributed in a distinct to subconfluent pattern. On the basis of the analysis of stereoscopic fundus images, retinal crystals appeared to be located at the inner surface of the retina. This observation was confirmed by SD-OCT imaging; crystals appeared within the NFL (Fig 1C).

In CF images the crystals appeared highly reflective, white, or golden yellow. Crystals were also detectable in RF, CBR, and IR images. Agreement of crystal counts grading based on CF images (in the temporal zone 2 subfield) was substantial with RF $\kappa=0.80$ (0.77–0.83) and CBR $\kappa=0.69$ (0.63–0.75) images, and moderate with IR images $\kappa=0.47$ (0.41–0.54). Values shown are weighted kappa values and 95% confidence intervals in parentheses. Red-free and CBR imaging provided the highest detection rates, whereas only a subset of crystals was typically seen in IR images. Crystals were not detectable in FA, FAF, or ICG images.

Crystals were seen at all stages of the disease, most frequently in stages 3 and 4. The association of the amount of crystals with Gass and Blodi stage reached significance in zone 1 ($P=0.003$) and the temporal subfield of zone 2 ($P=0.035$, Fisher exact test).

**Associations with Features of the Phenotype**

The presence of crystals showed a strong association with the presence of a break in the inner segment/outer segment junction line but not with other FAF/OCT characteristics of the disease (Table 2).

The amount of crystals showed a statistically significant association with loss of retinal transparency and fluorescein leakage in all subfields and with retinal thickness except in zone 1 and temporal zone 2 (Tables 3 and 4, available at http://aaojournal.org). The association with visible telangiectatic vessels, dilated retinal vessels, and right-angled veins only reached significance in the nasal subfield of zone 2 ($P=0.002$, $P=0.04$, and $P=0.03$, respectively). No association was found with blunted veins. Associations with MPOD loss were significant in all subfields except the inferior subfield of zone 2 (zone 1 $P=0.04$, zone 2: superior $P=0.009$, nasal $P=0.02$, inferior $P=0.08$, and temporal $P=0.001$, n=52 eyes).

**Associations with Clinical Features**

Eyes with crystals (n=520) had a mean letter score of 70.7 (SD 15.9), and eyes without crystals (n=343) had a mean letter score of 66.5 (SD 15.5). The differences were significant ($P < 0.001$). Associations with diet or other clinical characteristics were not detectable (Table 5).
Retinal crystals may be present in a wide range of disorders from hereditary to drug-induced and from systemic to local retinal. In type 2 MacTel, retinal crystals have some distinguishing characteristics. In our study, retinal crystals were present in 46% of 443 patients with MacTel at baseline, and approximately two thirds of these cases were bilateral. Crystals appeared highly reflective at wavelengths ranging from 488 nm (blue) to 820 nm (IR). In IR images, typically only a subset of crystals showed a variation in the number and location of the crystals. In our study, retinal crystals did however also show a statistically significant association with the loss of retinal transparency, loss of MPOD, and fluorescein leakage.

The implications of these observations are not known, but they suggest further Müller cell involvement in the pathogenesis of type 2 MacTel. Müller cells are radially oriented support cells traversing the retina from the inner vitreal border to the distal end of the outer nuclear layer. They have an extended funnel shape and a higher refractive index than their surrounding tissue and are oriented along the direction of light propagation. Müller cells have been shown to act as optical fibers within the retina. A disruption of their structure may explain a loss of retinal transparency even without significant edema. In our study, retinal crystals did however also show a statistically significant association with a minimal level of retinal thickening in all subfields.

The cell processes of Müller cells enclose regions of the perikarya and fascicles of axons of the ganglion cells within the NFL; processes from their footplates surround the nerve fiber bundles. In our study, high-resolution retinal images obtained using adaptive optics confirmed our findings. On stereoscopic fundus images and SD-OCT imaging, crystals seem to be located superficially within the NFL, in close proximity of the nerve fibers. Individual ganglion cell axon diameters vary from 0.6 to 2.0 μm depending on the type of ganglion cell of their origin. Midget ganglion cells with thinner axons are found more frequently within the macular area. We estimate that individual crystals are ~5 μm or larger. This, together with their often confluent distribution, suggests that crystals are more likely to be located around the nerve fiber bundles, possibly within Müller cell footplates, rather than within individual axons. A similar conclusion was drawn in the case of tamoxifen crystals, and other crystals, such as canthaxanthin and beta-carotene, also form in the innermost retina.

Müller cells play a role in retinoid metabolism, including cone pigment regeneration, and show avid phagocytic activity. Although the origin and chemical composition of the crystals are not known, according to their distribution and reflective properties, we hypothesize that they may be composed of retinoids originating in the visual cycle and may be
located within the Müller cell footplates and processes surrounding the nerve fibers. We also found a significant association between the amount of crystals and the presence of a break in the inner segment/outer segment junction line, which may further support this hypothesis; however, significant associations with increased retinoid intake via the diet or supplementation were not demonstrated in our study.

We found an association between the amount of crystals and the fluorescein leakage. Macular pigment loss was also associated with the amount of retinal crystals in our study. Müller cells extend branches that interdigitate with retinal neurons and glial cells, and their processes ensheathe the retinal capillaries and may contribute to the blood–retina barrier.26 The role of Müller cells in the metabolism of retinal capillaries and may contribute to the blood–retina barrier. The Müller cells extend branches that interdigitate with retinal neurons and glial cells, and their processes ensheathe the retinal capillaries and may contribute to the blood–retina barrier.

The role of Müller cells in the metabolism of macular pigment is not clear; however, in a histologic study of a MacTel eye, Powner et al29 found a depletion of Müller cells and vascular abnormalities within an area of the same extent as the loss of macular pigment. The distribution and associations of retinal crystals found in our study thus further implicate Müller cells in the pathogenesis of MacTel.

The reflective properties of the crystals are compatible with those of retinoids but also with those of lipids. Retinal crystals arranged along the nerve fibers, central cystoid spaces, and a loss of luteal pigment seen in MacTel are also the ocular manifestations of SLS, which raises the possibility of similarities in the metabolic pathways responsible for the pathogenesis of the 2 diseases. Sjögren–Larsson Syndrome is an autosomal dominant hereditary systemic metabolic disorder characterized by deficient microsomal fatty alcohol dehydrogenase activity, resulting in an accumulation of fatty aldehydes and alcohols in body tissues; however, SLS is also associated with severe extracocular manifestations, including cognitive deficiencies, spastic diplegia, and congenital ichthyosis.22,23

In conclusion, further insight into the physical and chemical properties of retinal crystals in MacTel may provide clues to the metabolic pathways involved in the pathogenesis of the disease.

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References


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Footnotes and Financial Disclosures

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