Disease Expression in Autosomal Recessive Retinal Dystrophy Associated With Mutations in the *DRAM2* Gene

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PURPOSE. To determine the disease course of retinal dystrophy caused by recessive variants in the DRAM2 (damage-regulated autophagy modulator 2) gene.

METHODS. Sixteen individuals with *DRAM2*-retinopathy were examined (six families; age range, 19–56 years, includes one pre-symptomatic case). The change in visual acuity over time was studied, and electrophysiology ($n = 6$), retina-tracking perimetry ($n = 1$), fundus autofluorescence (FAF) imaging ($n = 6$), and optical coherence tomography (OCT; $n = 12$) were performed.

RESULTS. All symptomatic patients presented with central visual loss (15/15) unaccompanied either by nyctalopia or light-hypersensitivity; most (11/15) developed symptoms in the third decade of life. A granular macular appearance, often with associated white/yellow dots, was an early fundoscopic feature. There was an ill-defined ring of hyperautofluorescence on FAF. Optical coherence tomography revealed loss of the ellipsoid zone perifoveally in a 19-year-old pre-symptomatic individual. The central atrophic area enlarged over time and fundoscopy showed peripheral degeneration in seven of the nine individuals that were examined ≥ 10 years after becoming symptomatic; some of these subjects developed nyctalopia and light hypersensitivity. Electrophysiology revealed generalized retinal dysfunction in three of the five individuals that were tested ≥ 10 years after becoming symptomatic.

CONCLUSIONS. Patients with *DRAM2*-retinopathy are typically asymptomatic in the first two decades of life and present with central visual loss and a maculopathy. A faint hyperautofluorescent ring on FAF can be a suggestive feature. The retinal periphery is frequently affected later in the disease process. Photoreceptor degeneration is likely to be the primary event and future studies on *DRAM2*-retinopathy are expected to provide important insights into retinal autophagy.

Keywords: retinal dystrophy, autophagy, *DRAM2*

Genetic testing for retinal dystrophies has been revolutionized by the advent of high-throughput sequencing approaches such as multigene panel testing, exome sequencing, and genome sequencing.[1-3] As the results of genetic testing are increasingly being used in medical decision-making (e.g., genetic risk assessment and identification of subjects who would benefit from gene-based therapies), misinterpretation of variants can have important consequences for patients.[4] A comprehensive understanding of the natural history and phenotypic variability associated with each genetic disease subtype can often help avoid such misinterpretations. To achieve this, it is key to pool patient cohorts from multiple centers and to report clinical data to the fullest possible extent. The focus of this study is DRAM2, a gene recently associated with autosomal recessive retinal dystrophy.[5] Affected individuals from five families have been previously described; early macular involvement and, in some cases, panretinal degeneration was observed.[5]

The gene DRAM2 [MIM 613360] encodes a 266–amino acid transmembrane protein with a role in autophagy induction.[6] Autophagy is a recycling pathway for obsolete parts of cells such as organelles and long-lived or misfolded proteins. It involves delivering material to lysosomes for degradation and is often upregulated in response to organelle damage or metabolic stress.[7,8] Dysregulation of autophagy has been implicated in many disorders, including cancer and neurodegeneration.[9,10] Its role in the visual system, and especially in the photoreceptors and RPE, is currently an area of intense investigation.[11-13]

DRAM2 is expressed in a number of tissues, including heart, placenta and retina; the associated protein, like many other autophagy regulator molecules, is localized in the lysosome.[5,14,15] Immunofluorescence microscopy in murine retinae revealed Dram2 staining in the photoreceptor inner segments, where photoreceptor lysosomes are found, and in the apical surface of the RPE.[5]

The present study details retinal structure and visual function in a cohort of individuals with DRAM2-retinopathy, many with long-term follow-up. Genetic findings in a previously unreported case are also described.

SUBJECTS AND METHODS

Biallelic DRAM2 variants have previously been shown to be disease-causing in one large consanguineous pedigree (family ES1, 11 affected subjects) and four simplex cases (1325, gc17004, gc4728 and BL1).[5] All 15 affected individuals from these five families participated in the present study. An additional unrelated proband (subject PCI1) that was found by exome sequencing to harbor a homozygous DRAM2 variant was also included; exome sequencing was performed as previously described (SureSelectXT Human All Exon V5 capture, Illumina HiSeq2000 sequencer; Agilent, Santa Clara, CA, USA)[16] and segregation analysis in parental samples was conducted using Sanger sequencing. The Ghent University Hospital, University Hospital Bonn, Moorfields Eye Hospital and Leeds East Research Ethics Committees approved the study and all investigations were conducted in accordance to the tenets of the Declaration of Helsinki; informed consent was obtained from all participating individuals.

Clinical assessment included best-corrected visual acuity testing, dilated fundus examination, color fundus photography, fundus autofluorescence (FAF) imaging, retina-tracking perimetry and optical coherence tomography (OCT). The Spectralis HRA+OCT system (Heidelberg Engineering, Heidelberg, Germany) was used to acquire FAF images (over a 30° × 30° and/or a 55° × 55° field) in six unrelated subjects (age range, 25–46 years), and spectral-domain OCTs in nine subjects (age range, 19–46 years). A time-domain OCT system (Stratus OCT3; Carl Zeiss Meditec, Dublin, CA, USA) was used to image one of these patients plus three additional study subjects (age range, 24–47 years). The OCT protocol included volume scans centered on the fovea in each case. Retinal imaging was performed on more than one visit in six patients (intertest intervals of 1–8 years).

Electrophysiological assessment was performed in six unrelated subjects (age range, 29–47 years). The protocols used incorporated the standards of the International Society of Electrophysiology of Vision (ISCEV) for full-field and pattern ERG (PERG).[17,18] Longitudinal data were available in three study subjects (intertest intervals of 4, 9, and 13 years).

TABLE. Clinical Characteristics in Subjects With DRAM2-Related Retinal Disease

Case ID (Family ID)	Sex	Presenting Symptom, (Age of Onset, y)	Age at Last Examination, (y)	LogMAR Visual Acuity at Last Examination, Right/Left	DRAM2 Genotype (Reference Sequence NP_848549.3)
IV:10 (ES1)	M	Asymptomatic (19)	19	0.0/0.0	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
IV:7 (ES1)	M	Central visual loss (22)	23	0.2/0.2	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
IV:9 (ES1)	F	Central visual loss (22)	25	1.0/1.0	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
IV:8 (ES1)	F	Central visual loss (21)	24	0.8/0.8	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
IV:11 (ES1)	F	Central visual loss (27)	29	1.0/1.0	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
IV:6 (ES1)	F	Central visual loss (26)	32	0.8/0.8	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
III:4 (ES1)	M	Central visual loss (23)	56	2.0/2.0	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
III:13 (ES1)	M	Central visual loss (25)	46	2.0/2.0	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
III:5 (ES1)	F	Central visual loss (28)	51	1.0/2.0	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
III:6 (ES1)	M	Central visual loss (25)	37	1.3/1.3	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
III:1 (ES1)	M	Central visual loss (16)	48	2.3/2.3	p.[(Gly47Valfs*3)];[(Gly47Valfs*3)]
1325	F	Central visual loss (29)	47	1.0/1.0	p.[(Ser44Asn)];[(Trp165*)]
gc17004	F	Central visual loss (29)	39	1.3/2.0	p.[(Tyr27His)];[(Val73_Tyr75del)]
gc4728	M	Central visual loss (34)	47	1.3/1.3	p.[(His121Leu)];[(His121Leu)]
BL1	F	Central visual loss (30)	44	1.0/1.0	p.[(Ala22del)];[(Ala22del)]
PCI1	F	Central visual loss (35)	43	0.6/0.4	p.[(Gly57Arg)];[(Gly57Arg)]

As the disease progressed, subjects III:4, III:13, IV:9, IV:6, 1325, and PCI1 developed photophobia; subjects III:13 and 1325 also complained of night vision problems. On fundoscopy, subjects III:4, III:13, III:5, III:1, 1325, gc17004, and gc4728 had both central and peripheral retinal changes at last examination while IV:10 had an apparently normal fundus.
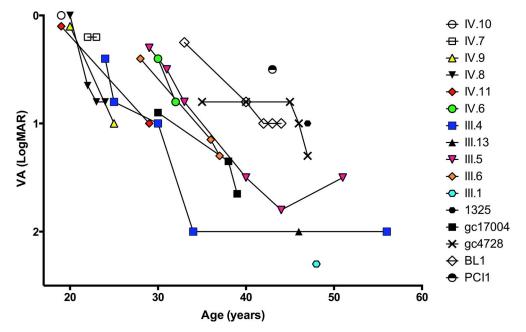
FIGURE 1. Diagram illustrating the visual acuity in 16 subjects with *DRAM2*-retinopathy and how this changed over time. The mean logMAR visual acuity between the right and left eye is plotted for each time point.
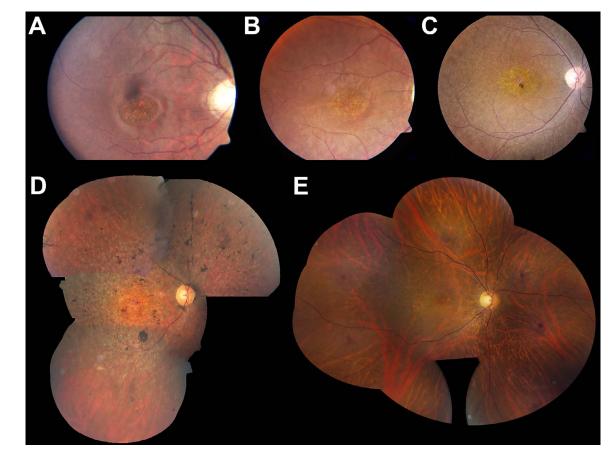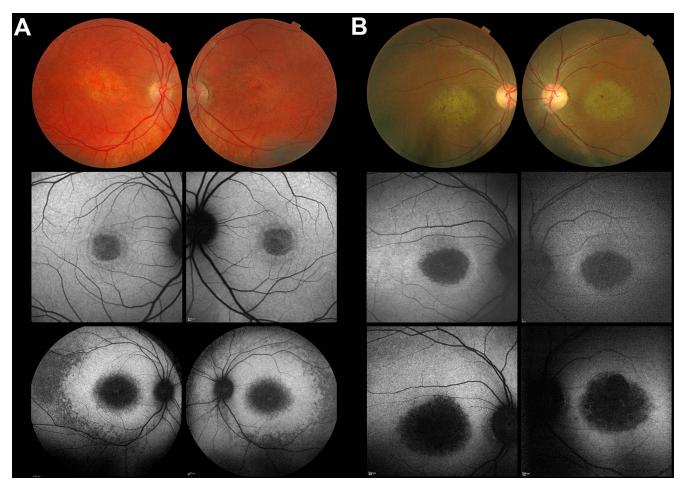

FIGURE 2. Color fundus photographs from five subjects with *DRAM2*-retinopathy. Images from the right eye of subject IV:8 at age 24 (**A**), subject IV:11 at age 29 (**B**), subject IV:6 at age 32 (**C**), subject III:4 at age 56 (**D**; all from family ES1), and subject BL1 at age 44 (**E**) are shown. A granular appearance with white/yellow dots in the central macula is present in (**A–C**) and (**E**). There is more widespread RPE irregularity and pigment clumping in (**C**) and (**D**). All cases show a high degree of interocular symmetry.

FIGURE 3. Color fundus photographs and FAF images from two unrelated individuals with *DRAM2*-retinopathy. (**A**) Subject gc17004: fundus photography at age 38 (*top row*), FAF imaging at age 30 (*middle row*) and 38 (*bottom row*). (**B**) Subject gc4728: fundus photography at age 45 (*top row*), FAF imaging at age 37 (*middle row*) and 45 (*bottom row*). Fundus autofluorescence imaging shows an area of decreased signal at the center surrounded by a faint, hyperautofluorescent ring that enlarges and becomes less well-defined over time. Midperipheral areas of irregular signal were also observed at the follow-up images of both study subjects (*bottom row*).

Retina-tracking mesopic perimetry (MAIA; CenterVue, Padova, Italy) was performed in subject PCI1 following pupil dilation. The examination was started after 20 minutes of adaptation to the red test background luminance at 1.27 cd/m². A custom perimetric test pattern (foveopapillary profile) examining 50 loci, mostly placed along the horizontal line through the foveal center covering 30° of the central visual field, was used. White test points (Goldmann III, 200 ms in duration) were presented using a 4-2 staircase strategy. Luminance of stimuli was scaled from 0 (318.5 cd/m²) to an attenuation of 36 dB (0.1 cd/m²; measurement range, 3.6 log units). Ceiling effects should be negligible as normal controls revealed retinal light sensitivity of 29.7 ± 1.14 dB in the macular area (see manufacturer's instructions available online at http://www.accessdata.fda.gov/cdrh_docs/pdf9/K092187. pdf). The patient was asked to maintain fixation on a red central circle of 1° diameter at a preferred retinal locus. One full perimetric test was performed with each eye before examination was executed to reduce learning effects. The inbuilt confocal scanning laser ophthalmoscope (830 nm) creates an infrared image of 36° × 36° field of view of the central retina, which is followed in real-time by a 25-Hz retinal landmark eye tracker, assuring precise topographic correlation between fundus details and light stimuli projection.

RESULTS

The clinical features of the 16 individuals with *DRAM2*-retinopathy are summarized in the Table and Supplementary Table S1. One study subject remained asymptomatic at age 19. All other patients presented with reduced central vision. The median age at onset for the cohort was 25 years (range, 16–35 years) with 11 of 15 patients becoming symptomatic in the third decade of life (Table). Most affected individuals developed significant visual impairment within a few years from presentation (Fig. 1). Three of the nine subjects that were seen at least a decade after becoming symptomatic reported light hypersensitivity; two of these subjects also reported difficulty seeing in dim illumination. Fundoscopic findings are illustrated in Figures 2, 3, and 4, and described in Supplementary Table S1. A granular macular appearance was present early in the disease. This was typically associated with fine white/yellow dots and often evolved into a well-defined central atrophic area. Peripheral bone-spicule pigmentation can develop later in the disease process; seven of the nine subjects that were seen at least a decade after becoming symptomatic developed midperipheral and/or peripheral retinal changes. All patients were phakic and none showed extraocular abnormalities suggestive of syndromic retinal disease.
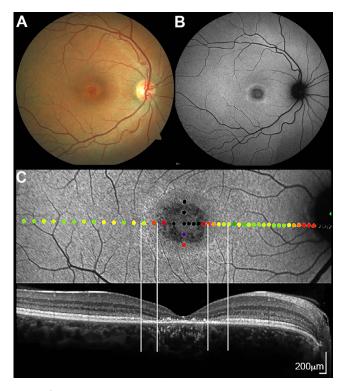
FIGURE 4. Color fundus photography (**A**), FAF imaging ([**B**] and *top row* of [**C**]), optical coherence tomography (*bottom row* of [**C**]), and retina-tracking perimetry (overlay in *top row* of [**C**]) in a 43-year-old individual with *DRAM2*-retinopathy (subject PCI1). There was a high degree of interocular symmetry and data from the right eye are only shown. In order to study the nature of the observed hyperautofluorescent ring, the FAF and OCT images were overlaid with the perimetry results (**C**). There was severe loss of retinal sensitivity over the central area of reduced FAF with less severe losses over a concentric parafoveal area; slightly eccentric to this, sensitivity was normal or relatively preserved over the narrow ring of increased FAF signal. Best corrected visual acuity was 0.6 logMAR and the preferred retinal locus was located about 5° superior to the foveal center. Complete disruption of the ellipsoid zone (corresponding to the photoreceptor outer segments) is noted at the outer/anterior border of the ring. The perimetry decibel scale is color-coded according to the manufacturer's normative studies with *green* representing normal values, *yellow* suspect, *red* abnormal, and *black* scotoma.
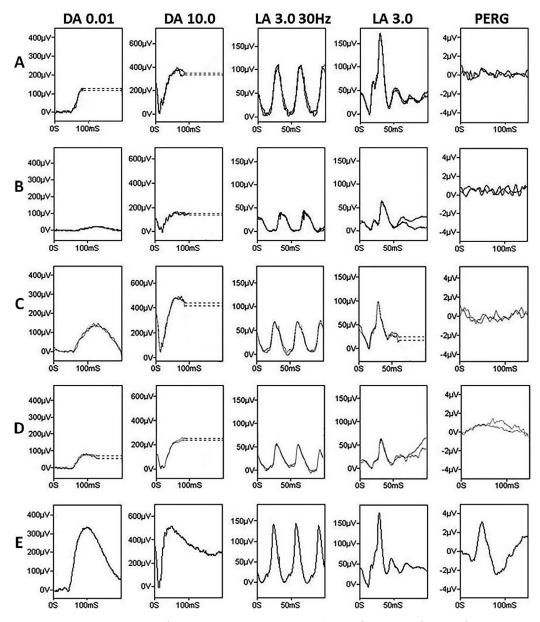
Fundus autofluorescence imaging was performed in six patients: subject IV:9 from family ES1 (images presented in Ref. 5) as well as subjects 1325, gc17004, gc4728, BL1, and PCI1. All tested individuals had a central area of irregular signal surrounded by a faint, ill-defined hyperautofluorescent ring (Figs. 3, 4). Notably, FAF imaging data from follow-up visits in three patients revealed progressive enlargement of the central defect; peripheral retinal involvement was present in two of these individuals (Fig. 3). Thinning of the hyporeflective band corresponding to the outer nuclear layer was observed on OCT imaging in 11 of 11 symptomatic individuals. Spectral domain OCT in the pre-symptomatic 19-year-old subject IV:10 (family ES1) revealed loss of the ellipsoid zone in a perifoveal area (Supplementary Fig. S1A). Subject IV:9 had OCT imaging 1 year before developing symptoms (age 21, using time-domain OCT) and 3 years after becoming symptomatic (age 25, using spectral domain OCT; data shown in Ref. 5). The central foveal thickness (mean thickness within the central 1000 μm diameter) in that subject was bilaterally reduced in the pre-symptomatic phase (100 μm right, 115 μm left).

Electrophysiology was performed in one individual from each family. Representative data are shown in Figure 5. Subject III:6 from family ES1 was assessed at age 37 (12 years after becoming symptomatic). He had normal full-field ERGs, but the PERGs were severely subnormal in keeping with macular dysfunction. Subject 1325 was also tested; full-field ERGs and PERGs were undetectable at age 46 (17 years after becoming symptomatic; data shown in Ref. 5). Both subjects gc17004 and gc4728 were tested soon after presentation (ages 30 and 34 years, respectively) and showed PERG evidence of severe macular dysfunction but normal full-field ERGs (Figs. 5A, 5C); subject gc17004 also had an electro-oculogram which was within normal limits. Both were retested 9 (for subject gc17004) and 13 (for subject gc4728) years later (10 and 13 years after becoming symptomatic). Full-field ERGs under both scotopic and photopic conditions were subnormal with additional delay in the flicker ERGs (Figs. 5B, 5D). Subject BL1 was tested at age 40 and 44 (10 and 14 years after becoming symptomatic): the PERGs were severely attenuated in keeping with macular dysfunction, but full-field ERGs were normal without evidence of progression. Subject PCI1 was tested at age 42 (7 years after becoming symptomatic) and was found to have normal full-field ERGs under scotopic conditions; the amplitude of the photopic ERG was borderline and there was no delay in the flicker ERG. Retina-tracking perimetry findings from this individual are shown in Figure 4. When the perimetry results were overlaid with the FAF and OCT images, relatively preserved visual function and retinal structure were noted outside the hyper-autofluorescent ring, although a slight functional deficit and thinning of the outer retina may be present.

The genetic data are summarized in the Table and Supplementary Table S2. The previously unreported subject PCI1, a female born to first cousin parents, was found to have a homozygous c.169G>C, p.(Gly57Arg) variant in *DRAM2*. This variant affects a glycine residue that is conserved from human to nematode and was not present in publicly available datasets (Exome Aggregation Consortium [ExAC] browser, NHLBI Exome Sequencing Project Exome Variant Server [EVS], dbSNP and 1000 Genomes Project database; all accessed June 22, 2015). In silico analysis using the SIFT (v1.03) and Polyphen-2 (v2.2.2r398) tools (both accessed June 22, 2015) predicted the change to be pathogenic (SIFT score of 0.0 and HumVar score of 1.0). She was the only affected family member and had two unaffected siblings and two unaffected children. Both her parents were heterozygotes for the c.169G>C change and when the exome sequencing data were inspected, no putatively disease-causing variants were detected in any other retinal dystrophy associated gene.

Robust genotype-phenotype correlations cannot be drawn as this is the first report detailing clinical findings in this molecular subtype of retinal dystrophy. However, it may be significant that affected individuals harboring at least one presumed loss-of-function variant in *DRAM2* (affected subjects from family ES1 and subject 1325) appear to manifest first symptoms earlier than patients harboring only missense changes or in-frame deletions. Subjects gc17004, gc4728, BL1 and PCI1, who fall in the latter category, became symptomatic at a later age than other subjects in the cohort. However, it cannot be excluded that this could in part be due to case ascertainment bias.

Intrafamilial variability was observed in family ES1 where the age of disease onset ranged from 16 to 28 years. Affected family members can be split into two groups: the five siblings IV:7, IV:8, IV:9, IV:10 and IV:11 (age range, 23–32 years), and their uncles and parents (III:1, III:4, III:5, III:6, III:13; age range, 37–56 years). In the sibling group, fundus appearance was consistent with a macular dystrophy and only the eldest member of the group (subject IV:6) had midperipheral changes at age 32 (Fig. 2C). In the older group, significant

FIGURE 5. Full-field and PERGs in subject gc17004 at age 30 (**A**) and 39 (**B**) and in subject gc4728 at age 34 (**C**) and 47 (**D**); a representative normal set of traces is also shown for comparison (**E**). Both subjects showed a high degree of interocular symmetry and data from only one eye are shown. Dark-adapted ERGs are shown for flash strengths of 0.01 and 10.0 cd.s/m² (DA 0.01; DA 10.0). Light-adapted recordings are shown for a flash strength of 3.0 cd.s/m² (LA 3.0 and LA 30 Hz). Horizontal lines replace eye movement/blink artefacts. Initially (**A, C**), full-field ERGs show no abnormality, but the PERG is severely reduced (**A**) or undetectable (**C**) in keeping with macular dysfunction. There is marked deterioration of both rod and cone-derived ERGs at follow-up (**B, D**).

central and peripheral retinal involvement was observed in subjects III:4 (age 56; Fig. 2D), III:13 (age 46), and III:1 (age 48; see Ref. 5). In contrast, subject III:6 had a healthy looking peripheral retina and preserved full-field electroretinograms at age 37, and subject III:5 had only subtle peripheral retinal changes at age 51. The latter individual developed symptoms at a later age and his retinopathy appears milder compared with other affected family members of similar age (subjects III:1, III:13, III:4).

DISCUSSION

Recessive variants in *DRAM2*, an autophagy regulator gene, have been recently identified as a cause of retinal dystrophy with early macular involvement.[5] Although limited imaging data have been previously presented,[5] the phenotypic variability and natural history of the disorder have not been described in detail. A combination of longitudinal and cross-sectional data from 16 affected individuals is reported here, providing important insights into *DRAM2*-retinopathy.

Patients typically become symptomatic in the third decade of life, describing increasing difficulty with close visual tasks. Light hypersensitivity and night-blindness are not significant early symptoms but they may be associated with advanced disease. Fundus examination at early disease stages typically reveals a granular appearance in the central macula, often with associated fine white/yellow dots. Fundus autofluorescence imaging shows a central area of abnormal signal surrounded by a faint perifoveal hyperautofluorescent ring; associated chang-

es are observed on OCT. Enlargement of the central atrophic area and, frequently, peripheral retinal involvement occur over time. Electrophysiology confirms that dysfunction is initially confined to the macula, but three of the five subjects that were tested at least a decade after becoming symptomatic, had developed full-field ERG abnormalities consistent with generalized dysfunction involving both rods and cones.

A similar but not identical pattern of visual loss has been previously reported in other retinal dystrophy subtypes. Progressive macular atrophy surrounded by a ring of hyperautofluorescence has been described in individuals with dominant variants in *CRX* [MIM 602225], *PRPH2* [MIM 179605], *GUCY2D* [MIM 600179], *RIMS1* [MIM 606629] and *PROM1* [MIM 604365]; X-linked variants in *RPGR* [MIM 312610]; and recessive variants in *CDH3* [MIM 114021], *TTLL5* [MIM 612268], *KCNV2* [MIM 607604], and *ABCA4* [MIM 601691].[19,20] The lack of light hypersensitivity and nightblindness at presentation can be a useful distinguishing feature. *ABCA4*-retinopathy[21] is perhaps the molecular subtype with the closest resemblance to *DRAM2*-related disease. The pattern of electroretinographic progression can be similar as one-fifth of patients with *ABCA4*-retinopathy and normal full-field ERGs at presentation, develop generalized retinal dysfunction over a decade.[22] The absence of retinal flecks is uncommon in *ABCA4*-retinopathy but can occur.

Dram2 is expressed both in photoreceptor and RPE cells and the location of the primary insult in *DRAM2*-retinopathy is unclear.[5] Optical coherence tomography data (Supplementary Fig. S1A) suggest loss of photoreceptor outer segments early in the disease process and support a primary photoreceptor etiology. That is also in keeping with the FAF and perimetry data. It can be speculated that the hyperautofluorescent ring is associated with reduced absorption of light in a zone of retina where outer segments are absent, and RPE fluorescence is otherwise normal. This would explain the OCT finding of an absent ellipsoid zone over the ring and an intact ellipsoid zone outside the ring. A similar mechanism has been proposed for the hyperautofluorescent ring observed in *MYO7A*-related retinitis pigmentosa.[23] Despite these observations, photoreceptor degeneration secondary to loss of RPE support cannot be excluded. Notably, autophagy within the RPE has been shown to contribute to both photoreceptor outer segment degradation and visual cycle chromophore regeneration.[24] Quantitative FAF imaging[25] in subjects with *DRAM2*-retinopathy and imaging of autophagic activity[26] in experimental models of *DRAM2*-related disease are expected to provide further insights.

Although the exact role of DRAM2 remains to be determined, previous work has suggested that it acts as a positive regulator of autophagy.[6] It is of interest that hydroxychloroquine, an autophagy inhibitor widely prescribed in the treatment of autoimmune diseases such as systemic lupus erythematosus, can be associated with retinal toxicity.[27,28] In particular, the early OCT findings of *DRAM2*-retinopathy (central retinal thinning and loss of the ellipsoid zone in the perifoveal area), are reminiscent of frequent observations in hydroxychloroquine toxicity.[29] A number of drugs with the capacity to induce autophagy have also been reported including rapamycin (also known as sirolimus), intravitreal administration of which has been attempted in subjects with age-related macular degeneration and posterior uveitis.[30,31] The role of these agents in *DRAM2*-retinopathy remains to be determined.

To conclude, this study details the clinical features of *DRAM2*-retinopathy and illustrates the natural history of photoreceptor loss in this disorder. Genetic data on a previously unreported case are described and the clinical utility of FAF imaging is demonstrated. The data presented have the potential to improve counseling on disease prognosis, to facilitate the identification of further individuals with this molecular diagnosis and to guide future therapeutic interventions.

References

1. Consugar MB, Navarro-Gomez D, Place EM, et al. Panel-based genetic diagnostic testing for inherited eye diseases is highly accurate and reproducible, and more sensitive for variant detection, than exome sequencing. *Genet Med*. 2015;17:253–261.

2. Belkadi A, Bolze A, Itan Y, et al. Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. *Proc Natl Acad Sci U S A*. 2015;112:5473–5478.

3. Watson CM, El-Asrag M, Parry DA, et al. Mutation screening of retinal dystrophy patients by targeted capture from tagged pooled DNAs and next generation sequencing. *PLoS One*. 2014;9:e104281.

4. MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. *Nature*. 2014;508:469–476.

5. El-Asrag M, Sergouniotis PI, McKibbin M, et al. Biallelic mutations in the autophagy regulator DRAM2 cause retinal dystrophy with early macular involvement. *Am J Hum Genet*. 2015;96:948–954.

6. Yoon JH, Her S, Kim M, et al. The expression of damage-regulated autophagy modulator 2 (DRAM2) contributes to autophagy induction. *Mol Biol Rep*. 2012;39:1087–1093.

7. Mariño G, Niso-Santano M, Baehrecke EH, Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. *Nat Rev Mol Cell Biol*. 2014;15:81–94.

8. Green DR, Galluzzi L, Kroemer G. Cell biology. Metabolic control of cell death. *Science*. 2014;345:1250256.

9. Vilchez D, Saez I, Dillin A. The role of protein clearance mechanisms in organismal ageing and age-related diseases. *Nat Commun*. 2014;5:5659.

10. Menzies FM, Fleming A, Rubinsztein DC. Compromised autophagy and neurodegenerative diseases. *Nat Rev Neurosci*. 2015;16:345–357.

11. Frost LS, Mitchell CH, Boesze-Battaglia K. Autophagy in the eye: implications for ocular cell health. *Exp Eye Res*. 2014;124:56–66.

12. Ferguson TA, Green DR. Autophagy and phagocytosis converge for better vision. *Autophagy*. 2014;10:165–167.

13. Yao J, Jia L, Shelby SJ, et al. Circadian and noncircadian modulation of autophagy in photoreceptors and retinal pigment epithelium. *Invest Ophthalmol Vis Sci*. 2014;55: 3237–3246.

14. O'Prey J, Skommer J, Wilkinson S, Ryan KM. Analysis of DRAM-related proteins reveals evolutionarily conserved and divergent roles in the control of autophagy. *Cell Cycle*. 2009;8: 2260–2265.

15. Park SM, Kim K, Lee EJ, et al. Reduced expression of DRAM2/TMEM77 in tumor cells interferes with cell death. *Biochem Biophys Res Commun*. 2009;390:1340–1344.

16. Elsayed SM, Phillips JB, Heller R, et al. Non-manifesting AHI1 truncations indicate localized loss-of-function tolerance in a severe Mendelian disease gene. *Hum Mol Genet*. 2015;24: 2594–2603.

17. McCulloch DL, Marmor MF, Brigell MG, et al. ISCEV Standard for full-field clinical electroretinography (2015 update). *Doc Ophthalmol*. 2015;130:1–12.

18. Bach M, Brigell MG, Hawlina M, et al. ISCEV standard for clinical pattern electroretinography (PERG): 2012 update. *Doc Ophthalmol*. 2013;126:1–7.

19. Roosing S, Thiadens AA, Hoyng CB, Klaver CC, den Hollander AI, Cremers FP. Causes and consequences of inherited cone disorders. *Prog Retin Eye Res*. 2014;42:1–26.

20. Sergouniotis PI, Chakarova C, Murphy C, et al. Biallelic variants in TTLL5, encoding a tubulin glutamylase, cause retinal dystrophy. *Am J Hum Genet*. 2014;94:760–769.

21. Cideciyan AV, Swider M, Aleman TS, et al. ABCA4 disease progression and a proposed strategy for gene therapy. *Hum Mol Genet*. 2009;18:931–941.

22. Fujinami K, Lois N, Davidson AE, et al. A longitudinal study of Stargardt disease: clinical and electrophysiologic assessment, progression, and genotype correlations. *Am J Ophthalmol*. 2013;155:1075–1088.

23. Lenassi E, Saihan Z, Cipriani V, et al. Natural history and retinal structure in patients with Usher syndrome type 1 owing to *MYO7A* mutation. *Ophthalmology*. 2014;121:580–587.

24. Kim JY, Zhao H, Martinez J, et al. Non-canonical autophagy promotes the visual cycle. *Cell*. 2013;154:365–376.

25. Duncker T, Tsang SH, Lee W, et al. Quantitative fundus autofluorescence distinguishes ABCA4-associated and non-ABCA4-associated bull's-eye maculopathy. *Ophthalmology*. 2015;122:345–355.

26. Tsukamoto S, Hara T, Yamamoto A, et al. Fluorescence-based visualization of autophagic activity predicts mouse embryo viability. *Sci Rep*. 2014;4:4533.

27. Marmor MF, Melles RB. Hydroxychloroquine and the retina. *JAMA*. 2015;313:847–848.

28. Cheng Y, Ren X, Hait WN, Yang JM. Therapeutic targeting of autophagy in disease: biology and pharmacology. *Pharmacol Rev*. 2013;65:1162–1197.

29. Cukras C, Huynh N, Vitale S, Wong WT, Ferris FL III, Sieving PA. Subjective and objective screening tests for hydroxychloroquine toxicity. *Ophthalmology*. 2015;122:356–366.

30. Petrou PA, Cunningham D, Shimel K, et al. Intravitreal sirolimus for the treatment of geographic atrophy: results of a phase I/II clinical trial. *Invest Ophthalmol Vis Sci*. 2014;56: 330–338.

31. Ibrahim MA, Sepah YJ, Watters A, et al. One-year outcomes of the SAVE study: sirolimus as a therapeutic approach for uveitis. *Transl Vis Sci Technol*. 2015;4:4.

Appendix

UK Inherited Retinal Disease Consortium

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