Phenotypic Marker for Early Disease Detection in Dominant Late-Onset Retinal Degeneration

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Purpose. To define early disease expression in autosomal dominant late-onset retinal degeneration (L-ORD), a retinopathy that becomes symptomatic after age 50 and is characterized histopathologically by sub-RPE deposits.

Methods. Three families with L-ORD were included; two families had postmortem eye donors with retina-wide sub-RPE deposits. Six patients with severe visual loss (ages 62–93) were examined clinically, and 17 available individuals (ages 35–60) at a 50:50 risk to inherit L-ORD were also studied with dark adaptometry. A short-term trial of vitamin A at 50,000 IU/day was conducted in three members. Three-year follow-up examinations were performed in a subset of members.

Results. Family 1 had 12 available members at risk. On initial examination, only one member had fundus abnormalities: yellow-white punctate lesions in the midperipheral fundus. Dark-adaptation kinetics were abnormal in 6 of 12. The youngest age with an abnormality was 35. Family 2 had two available members at risk, both of whom had punctate fundus lesions and abnormal dark adaptation. Family 3 had three available members at risk. One had fundus lesions and abnormal dark adaptation, whereas the others had normal fundi and normal adaptometry. Vitamin A accelerated adaptation kinetics but not to normal rates. Three-year follow-up examinations demonstrated further slowing of adaptation kinetics, whereas rod and cone thresholds remained unchanged.

Conclusions. Dark-adaptation abnormalities can precede symptoms and funduscopic signs of L-ORD by at least a decade. Short-term, high-dose vitamin A accelerates the kinetics of dark adaptation to a limited degree. The results contribute clues about early pathophysiology of this retinal degeneration and provide additional power for genetic mapping of the L-ORD locus. (*Invest Ophthalmol Vis Sci.* 2001;42:1882–1890)

Late-onset retinal degeneration (L-ORD) is an autosomal dominant retinal degeneration that becomes symptomatic after age 50.[1] Initially, there are no ophthalmoscopic findings. Clusters of punctate yellow-white lesions are the first funduscopic evidence of disease. The disease progresses to loss of central and peripheral vision and, in advanced stages, the fundus can show a disciform scar (suggesting previous choroidal neovascularization) and retina-wide pigmentary retinopathy indistinguishable from many forms of retinitis pigmentosa (RP). The striking finding by histopathology is extensive sub-RPE deposits throughout the retina.[1,2] The deposits are very similar to those reported in Sorsby fundus dystrophy[3–5] and to some examples of age-related macular degeneration (ARMD).[6–11]

In the present study, we extended our research into L-ORD with the long-term purpose of mapping the disease-causing gene(s) and further elucidating pathophysiological mechanisms. Three families (two identified by histopathology of deceased donor retinas[12,13]) were investigated with noninvasive tests to attempt to identify the disease before it became symptomatic, thereby increasing the number of known affected members and enabling linkage analysis.

Methods

Subjects and Clinical Examinations

Subjects were from three different families, all of Scottish origin (Fig. 1). The diagnosis of L-ORD in Family 1 was based on autosomal dominant inheritance with a history of late-onset and progressive severe retinal degeneration. In Families 2 and 3, there was the same history but also published histopathology in affected members indicating retina-wide, thick sub-RPE deposits.[12,13] All subjects had routine clinical ocular examinations and kinetic perimetry. Some of the subjects had fundus photography. The proband of Family 1 (VI-8) also had ERG testing using published methods.[14–16] All research procedures were in accordance with institutional guidelines and the Declaration of Helsinki.

Dark Adaptometry

Dark adaptometry was performed with two different instruments. In Philadelphia, extensive studies were performed on the proband of Family 1, by using instrumentation, techniques, and protocols that have been described.[17] In Edinburgh, the proband and other members of Family 1 and members of Families 2 and 3 were studied with a portable adaptometer connected to a laptop computer with a docking station (Solo 5100; Gateway, N. Sioux City, SD) and a data acquisition board (DT3100; Data Translation, Marlboro, MA). The stimulus was either a blue (LNG992CF9, 450 nm; Panasonic, Osaka, Japan) or red (LN261CAL, 665 nm; Panasonic) LED illuminating an opal diffuser (1.7° diameter). Under software control, LEDs were driven directly from the digital-to-analog channel with amplitude and pulse-width modulation to achieve a more than 6 log unit dynamic range.[18,19] Thresholds were determined using a staircase procedure and specified as the mean of two threshold crossings at each time point. The test eye was dilated and dark adapted for more than 1 hour and prebleach thresholds obtained. A flash unit (Sunpak 622; ToCad America, Inc., Parsippany, NJ) mounted at the top of a 150-mm-diameter sphere with a white inner coating and an opening for the subject's eye delivered the adapting light exposure (7.5 log scot-td · sec; 99th percentile at 11 msec). Approximately 97% of the available rhodopsin molecules would be expected to absorb a primary quantum with this flash.[20] We re-

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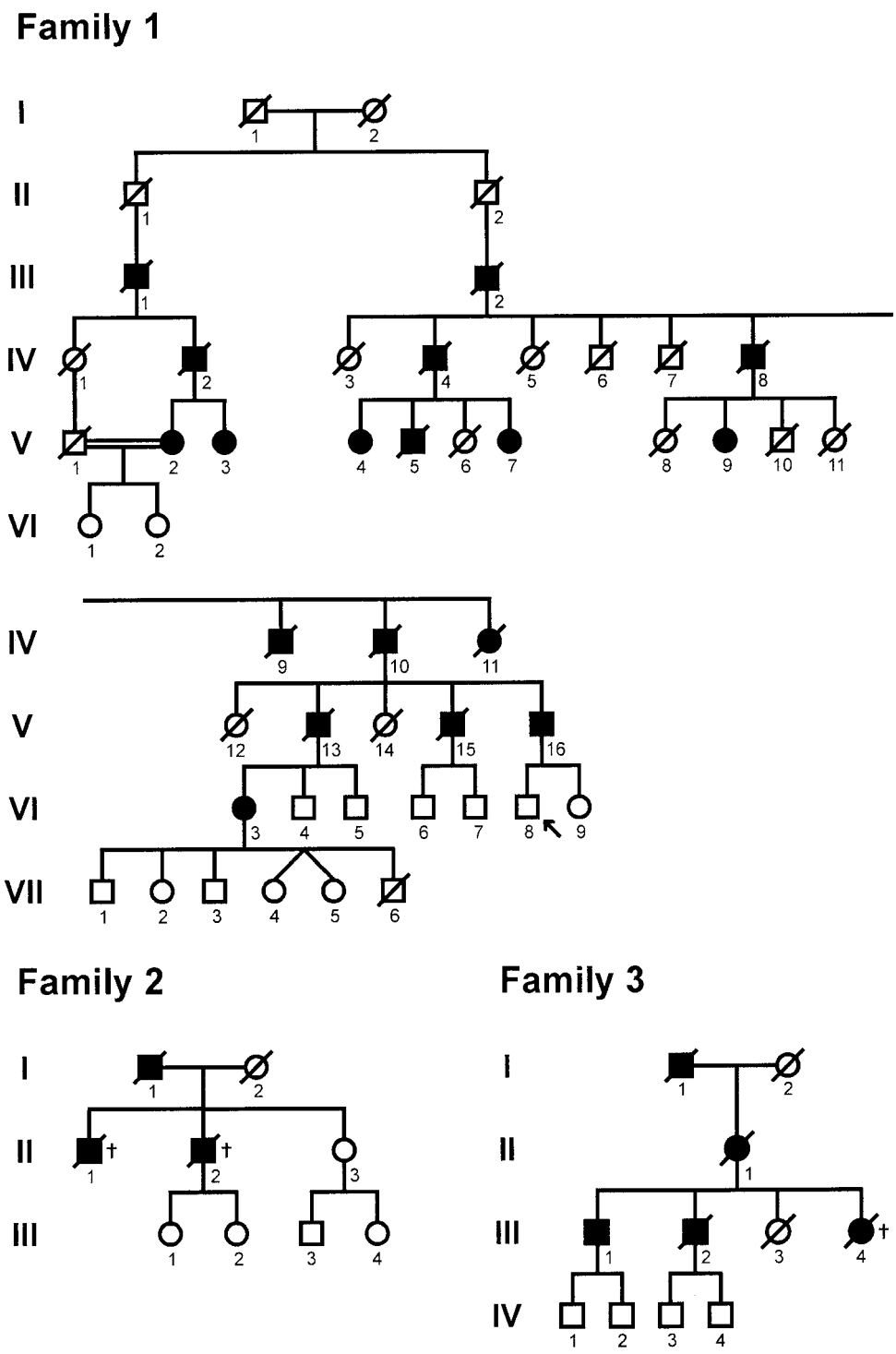

FIGURE 1. Pedigrees with L-ORD. *Arrow*: proband of Family 1 (patient VI-8). *Daggers*: previously studied eye donors in Family 2 (Patients II-1, II-2) and Family 3 (Patient III-4). *Filled symbols*: affected by history or by our examination. *Slashed symbols*: deceased.

ferred to this light exposure as a "97% bleach," or "full bleach." The bleach was delivered under infrared (IR) viewing of the subject's pupil to avoid reduction in retinal exposure due to partially closed eyelids. During testing, IR LEDs illuminated the pupil and an IR-sensitive camera continuously monitored pupil position. Other experimental details were comparable to those in our previous work in which we used dark adaptometry.[17,21]

The kinetics of dark-adaptation functions were quantified in two ways. A line was fitted (by eye) to the major section (second component) of linear recovery of log rod threshold,[22] although other methods are available.[23] When multibleach data were available, parallel lines were fitted simultaneously to all recovery functions. Otherwise, only 97% bleach data were used.[17,22,24] The slope of this line is specified as a time constant under the assumption that the log-linear decrease in thresholds represents the decay of a photoproduct.[25] Kinetics of recovery were also determined as the time to reach a criterion threshold. The criterion (3 log) was chosen to correspond to the approximate midpoint of the second component of recovery, to minimize variability.[2] The traditional measure of cone–rod break time was not used because of the complex interaction between rod recovery kinetics and cone plateau thresholds and the difficulty of determining this time point reliably in patients with very prolonged cone plateaus and ensuing slow rod adaptation. Dark adaptation was measured in the proband of Family 1 and both members of Family 2 before and after a 1-month

Table 1. Clinical Characteristics of the Subjects

Pedigree Number	Age at Visit (y)*	Gender	Visual Acuity†	Kinetic Field†	Fundus Appearance†
Family 1					
V-9	93	F	LP	np	PR
V-3	79	F	HM	Abnormal	PR
V-2	76	F	20/400	Abnormal	PR
V-16	74	M	CF	Abnormal	PR
VI-3	62	F	HM	Abnormal	PR
VI-4	58	M	20/20	Normal	Normal
VI-6	57	M	20/20	Normal	Normal
VI-5	52	M	20/20‡	Normal‡	Normal‡
VI-8	45	M	20/20	Normal	Yellow dots
	48		20/20	Normal	Yellow dots
VI-1	43	F	np	np	np
	44		20/20	Normal	Normal
VI-9	43	F	20/20	Normal	Normal
VII-1	41	M	20/20	Normal	Normal
	44		20/20	Normal	Normal
VI-2	40	F	np	np	np
	41		20/20	Normal	Normal
VII-2	39	F	20/20	Normal	Normal
	42		20/20	Normal	Normal
VII-3	38	M	20/20	Normal	Normal
	41		20/20	Normal	Yellow dots
VII-4	35	F	20/20	Normal	Normal
	38		20/20	Normal	Normal
VII-5	35	F	20/20	Normal	Normal
	38		20/20	Normal	Normal
Family 2					
III-1	49	F	20/30	Normal	Yellow dots
	52		20/30	Normal	Yellow dots
III-2	47	F	20/30	Normal	Yellow dots
	50		20/30	Normal	Yellow dots
Family 3					
III-1	90	M	LP	np	PR
IV-1	60	M	20/20	Normal	Normal
IV-2	56	M	20/20	Normal	Normal
IV-3	55	M	20/20	Normal	Yellow dots

LP, light perception; HM, hand motions; CF, counting fingers; PR, pigmentary retinopathy; np, not performed.

* Rounded to nearest year.

† Similar in the two eyes, unless specified.

‡ Left eye results; right eye vision complicated by ocular trauma and surgery.

course of oral vitamin A (50,000 IU/day; Aquasol A; Astra, Westborough, MA).

Results

Clinical characteristics of 23 members of Families 1, 2, and 3 (Fig. 1) are listed in Table 1. Common ancestry among the three Scottish families was suspected and genealogical investigation conducted, but no evidence of a founder effect could be obtained. The six oldest patients (ages 62–93) were markedly affected. Visual acuities were severely impaired, as were visual fields (small peripheral islands of function detectable only). Degenerative retinal disease evident on ophthalmoscopy included attenuated retinal vessels, peripheral pigmentary retinopathy with patches of chorioretinal atrophy, and atrophic macular lesions (Fig. 2). Seventeen other members (ages 35–60) were at a 50:50 risk of inheriting the disease (defined as having an affected parent by examination or by history).

On their first visits, only one of the 12 Family 1 members at risk reported visual symptoms (VI-8, age 45). Specifically, he described a longer time for his vision to adjust when moving from brightly lit to dimmer environments. Another member of this family who was not symptomatic at age 41 reported this same specific symptom at age 44 (VII-1). Of the two Family 2 members, one (III-1, age 49) described decreased vision at night (but not adjustment problems as above) and this continued unchanged. Her sister (III-2) at age 47 reported no visual symptoms, but at age 50 she described difficulty with adjustment to darkness from light. None of the three Family 3 members at risk had visual symptoms. All 17 subjects had normal visual acuities and normal kinetic visual fields in both eyes (with the exception of VI-5 of Family 1, who had lost vision from trauma in one eye). On their initial examination, four of these individuals (Family 1, VI-8; Family 2, III-1, III-2; Family 3, IV-3) had patches of yellow-white dots in the pericentral and/or midperipheral retina of one or both eyes (Fig. 2), whereas 13 showed no ophthalmoscopic abnormalities.

A composite of visual function data in the proband of Family 1 (VI-8) at age 45 is shown in Figure 3. Standard ERGs (Fig. 3A) to rod, mixed cone and rod, and cone stimuli were within normal limits for amplitude and timing parameters.[14] Rod- and cone-isolated photoresponses (Fig. 3B) were also normal for maximum amplitude and sensitivity.[15,16] Kinetic visual fields (V-4e and I-4e targets) and dark- and light-adapted static perimetry[26] were normal (data not shown). Ophthalmoscopy revealed a cluster of yellow-white punctate lesions in the near midperipheral retina (Fig. 2). Dark adaptometry to a full bleach was performed at three loci, and there were abnormal kinetics

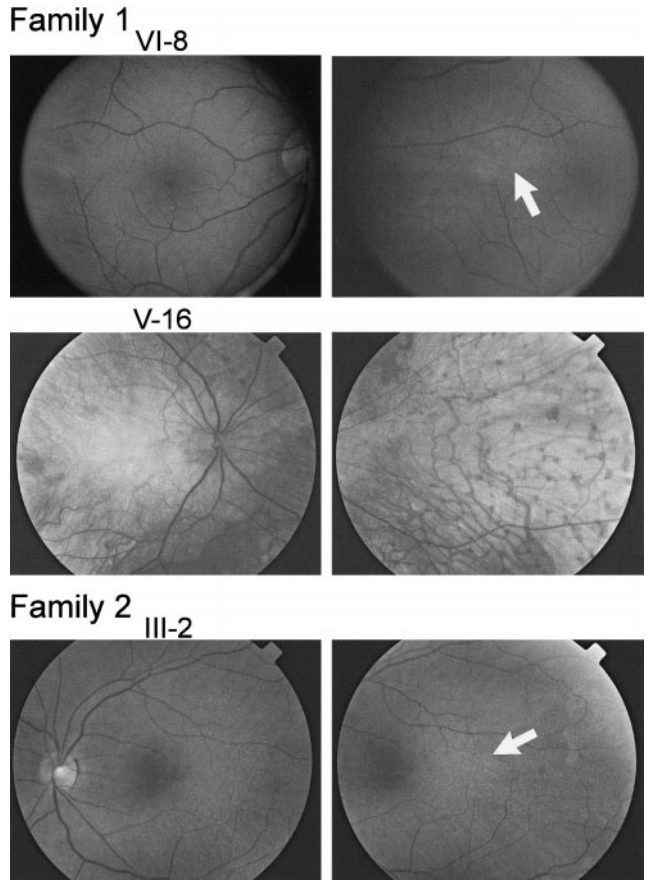

Figure 2. Fundus appearance in L-ORD family members at early and late stages. *Top*: Family 1, VI-8 (age 45), right eye. Yellow-white punctate lesions were in the temporal retina (*arrow*) of an otherwise normal fundus. *Middle*: Family 1, V-16 (age 74), right eye. Chorioretinal atrophy and pigmentary retinopathy throughout the fundus. *Bottom*: Family 2, III-2 (age 47), left eye. Yellow-white punctate lesions were visible, mainly temporal to the fovea (*arrow*).

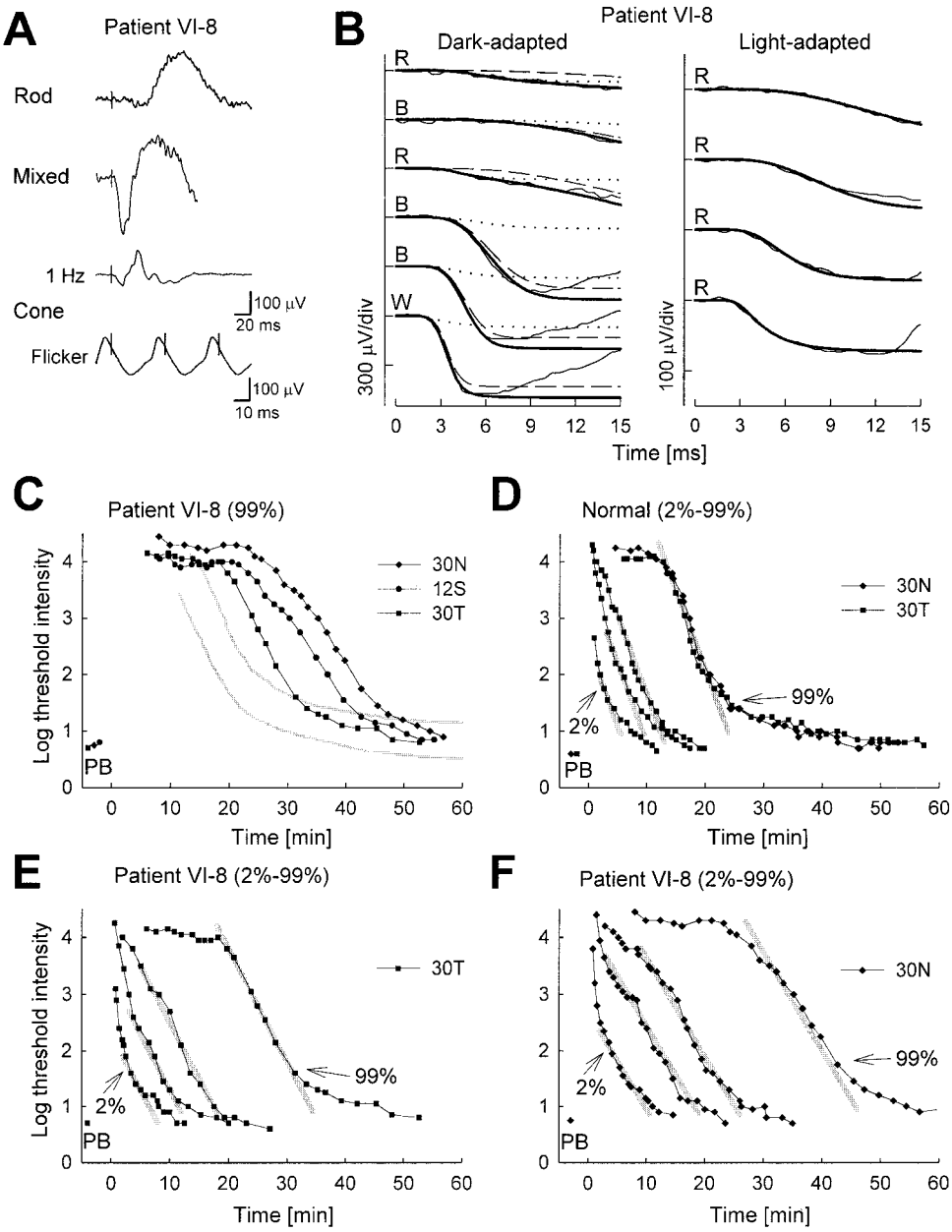

FIGURE 3. Visual function in the proband of Family 1 (VI-8) at age 45. (**A**) Standard rod, mixed cone-rod, cone (1-Hz and 29-Hz) ERGs. Stimulus onset denoted by small *vertical lines*. (**B**, *left*) Dark-adapted ERG photoresponses (*thin noisy lines*) evoked by red (R), blue (B), and white (W) flashes (1.9–5.4 log scot-td · sec; 1.4–5.1 log phot-td · sec). Waveforms are fitted simultaneously with a phototransduction model (*thick lines*) that is the sum of rod (*dashed lines*) and cone (*dotted lines*) components. (**B**, *right*) Light-adapted (3.2 log phot-td) ERG photoresponses (*thin lines*) evoked by red flashes (2.2–4.1 log phot-td · sec) fitted simultaneously with a cone phototransduction model (*thick lines*). (**C**) Dark-adaptation functions after a 99% bleach at three different locations (30° nasal, 30N; 12° superior, 12S; 30° temporal, 30T) in the visual field (*filled symbols*) of Family 1, VI-8. *Gray lines*: normal range for 12° eccentricity. (**D**) Dark-adaptation functions in a normal subject (age 34) after a 99% bleach (at 30N and 30T) and partial (2%, 6%, and 15%) bleaches (at 30T). (**E**, **F**) Dark-adaptation functions after the same bleaching regimen in Family 1, VI-8 at 30T and 30N. *Gray parallel lines* have been fitted to the second component of rod recovery. PB, prebleach.

at all locations (Fig. 3C). The most prolonged recovery of adaptation was at the 30° nasal field locus, and this prompted the decision to use this test location in our examinations of other family members at risk. We further investigated with multiple bleaches in Patient VI-8 the kinetics of adaptation at two loci that differed in rate (30° nasal and 30° temporal). At the 30° temporal locus (Fig. 3E), the major component of linear recovery of log threshold (or second component)[17,22,24] had a time constant of 130 seconds (equivalent to a slope of −0.2 log units/min), which is longer than that expected in a normal subject[17] (93 seconds; Fig. 3D). The time constant at 30° nasal (Fig. 3F) was even longer at 148 seconds.

Dark-adaptation results to full bleach at 30° nasal field in 11 members from the three families are illustrated in Figure 4. In Family 1, representative results from three members showing normal adaptation kinetics (Fig. 4A) are contrasted with results from three other members showing different degrees of adaptation abnormality (Fig. 4B). Of the 12 members examined in Family 1, 6 had adaptation abnormalities, defined as a prolongation of the time to reach a 3-log criterion threshold (normal mean, 21.3 ± 1.4 [SD] minutes) and/or a longer time constant (normal mean, 88.1 ± 9.2 seconds) than that of our group of control subjects without eye disease (n = 11; ages 20–53). Both members of Family 2 had abnormal dark adaptation (Fig. 4C). Of the three members examined in Family 3, one had abnormal dark adaptation and the other two were normal (Fig. 4D).

We tested the hypothesis that in L-ORD the sub-RPE deposits (noted in earlier histopathologic studies[12,13]) can act as a barrier to normal transport of nutrients to the retina and cause a chronic photoreceptor vitamin A deficiency.[1,27] High doses of vitamin A (50,000 IU/day, orally) were administered on a short-term basis to three patients with pronounced abnormalities in adaptometry (Family 1, VI-8; Family 2, III-1 and III-2) and the time course of dark adaptation was determined at baseline and after 1 month of treatment with the supplement (Fig. 5). Patients had normal serum vitamin A levels before trial onset. Results indicate that rod sensitivity levels (blue stimulus, prebleach dark-adapted) were unchanged in the month of supplementation, but dark-adaptation kinetics (for two bleaching

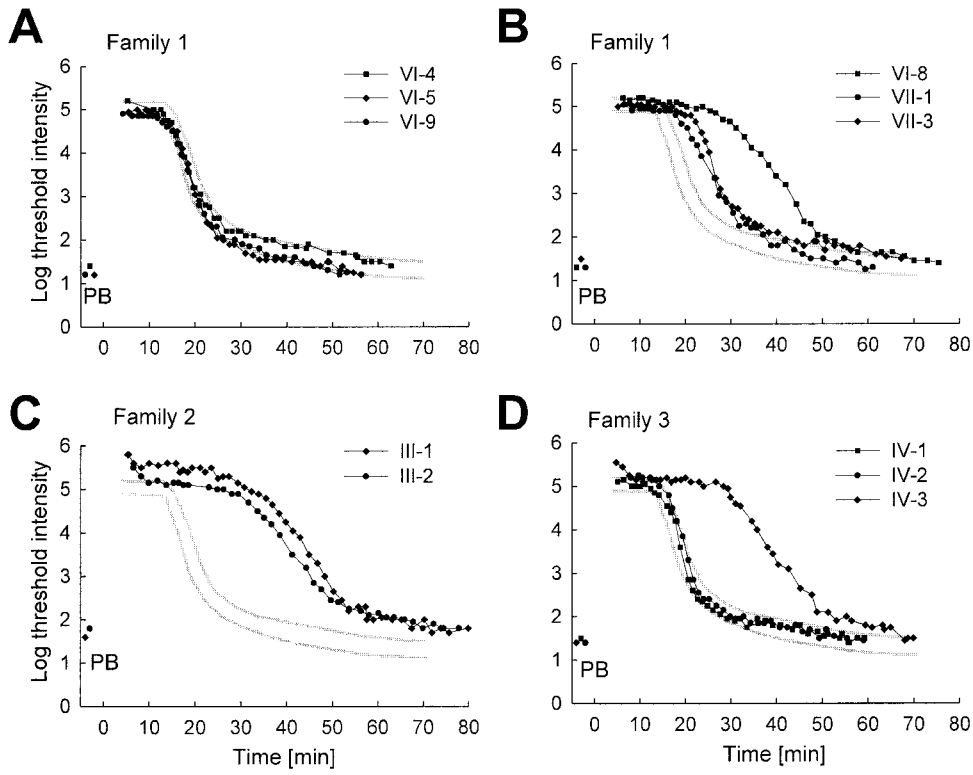

FIGURE 4. Dark-adaptation functions at 30° nasal field after a 97% bleach in representative at-risk members of (**A**, **B**) Family 1, (**C**) Family 2, and (**D**) Family 3. PB, prebleach.

conditions) were altered by the intervention. Criterion threshold times shortened by between 3.6 and 6.1 minutes, whereas variation was less than 1.7 minutes in normal subjects at a 3-year interval and in two members of Family 1 with normal kinetics of adaptation at an interval of 1 year (see natural history description below and Fig. 6F,G). Changes in the time constant of the major rod recovery phase were no different from normal variation.

During the period of supplementation, the rate of recovery of cones appeared to accelerate in the two members of Family 2 (Figs. 5B, 5C), but there was no obvious effect in VI-8 of Family 1 (Fig. 5A). Although the post–vitamin A adaptation results in the patients accelerated in kinetics, they did not become normal. Major abnormalities remained after the supplementation in all three individuals. The two patients from Family 2 discontinued the supplement, according to the study design. Patient VI-8 from Family 1 reported (at a 3-year follow-up examination) that he had not discontinued supplementation but had continued to self-treat with various sources of supplemental vitamin A (see natural history data below).

A part of the natural history of visual dysfunction in L-ORD was documented in data from a 3-year follow-up evaluation in six subjects from Family 1 and the two subjects in Family 2. To estimate variability, four normal subjects of comparable age to the patients (ages 34–53 at first visit) were also tested on two visits with an interval of 3 years, using the same test apparatus. Figure 6A shows the two adaptometry functions in a normal subject (at ages 48 and 51). Both functions fall within the normal range for blue and red stimuli and are very similar to one another. The same was true for the other three sets of normal data. Results from three siblings in Family 1 are also illustrated (Figs. 6B–D). Patient VII-5, at age 35, had a relatively mild abnormality in adaptation kinetics. The criterion threshold time was 25 minutes and thus was delayed beyond normal limits. The time constant of the major rod-mediated phase of recovery after the break was within normal limits (τ, 93 seconds). At age 38, the dark-adaptation defect was more pronounced as the criterion time extended to 27 minutes. The time constant of the second phase of recovery became longer but was still within normal limits at 99 seconds. It is notable that final dark-adapted thresholds were similar on the two visits.

Cone adaptation (red stimulus) remained relatively unchanged in threshold and kinetics on the two visits. Patient VII-3, at age 38, showed abnormal rod kinetics, but the abnormality became more pronounced by age 41. Initially, the criterion time was 29 minutes; after 3 years it extended to 36 minutes. There was an abnormal time constant of the second phase (τ, 107 seconds) and this became even slower (τ, 143 seconds). Final dark-adapted thresholds were unchanged in the interval. Although threshold for the red stimulus was also unchanged between sessions, the kinetics of cone adaptation appeared slower on the later visit. Yellow punctate lesions were also present on fundus examination of this patient on the second visit, whereas they were not evident on the first visit (Table 1). Patient VII-1 showed adaptation abnormalities to blue and red stimuli at ages 41 and 44. The criterion time was 28 minutes initially and extended to 47 minutes. The time constant of the second phase was 138 seconds at the earlier age and subsequently became 219 seconds, the latter representing greater than a twofold slowing of kinetics compared with normal. Thresholds for the two stimuli remained similar between sessions. Fundus appearance was normal on both examinations (Table 1).

To summarize the results of serial dark adaptometry, parameters of the functions were plotted against age of study participants who had two visits (Figs. 6E–G). Final dark-adapted rod thresholds (blue stimulus, prebleach) and cone thresholds (blue stimulus, cone plateau) at the 30° nasal locus showed variation between visits, but there was no systematic increase in threshold (Fig. 6E). Time to reach a criterion threshold after the bleach (Fig. 6F) increased with age in the six subjects at risk in Family 1 with abnormal dark adaptation at initial examination and in both subjects in Family 2. A similar pattern was evident when the time constant of the major rod recovery

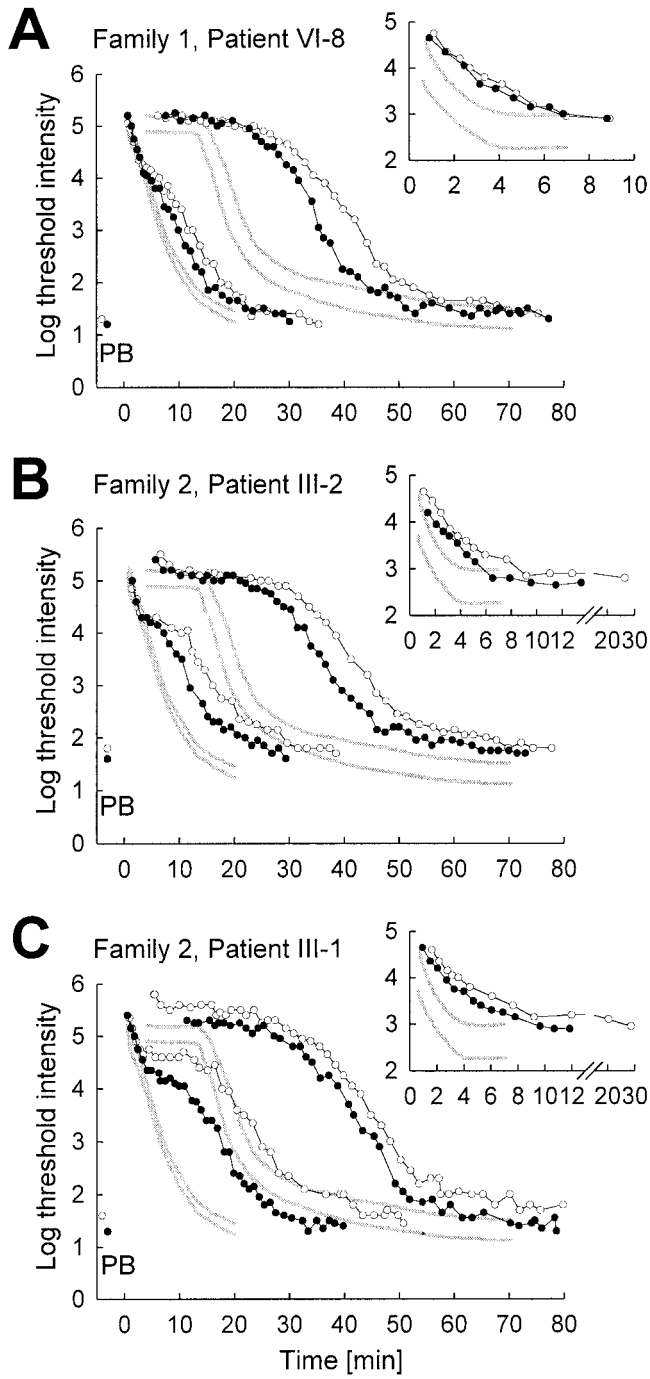

FIGURE 5. Effect of short-term high-dose supplemental vitamin A on dark adaptation at 30° nasal (30N) in an at-risk member of Family 1 (**A**) and two members of Family 2 (**B**, **C**). Two bleaches (97% and 14%) were performed at baseline (*unfilled symbols*) and after 1 month of vitamin A supplementation (*filled symbols*). *Gray lines*: normal limits for the 97% (*rightmost*) and 14% (*leftmost*) bleaches. *Larger panels*: adaptation functions to a blue stimulus; *insets*: cone adaptation to a red stimulus. PB, prebleach.

phase after the break was plotted as a function of age (Fig. 6G). In the data of Patient VI-8 (Family 1), the criterion time and time constant changed between visits but not as dramatically as did some of the others of his age group (in Family 2) or younger members of his family. This was the patient who self-treated during the time between examinations. Normal subjects showed no marked changes over the 3-year interval. Two members of Family 1 at 50:50 risk to inherit L-ORD (VI-1 and VI-2, Table 1) were examined 1 year apart and they had normal results both times.

DISCUSSION

We hypothesized that abnormal dark adaptation may be a phenotypic marker for presymptomatic detection of L-ORD, based on clues from our earlier psychophysical and histopathologic studies of two families with an autosomal dominant retinal degeneration characterized by late-onset of retinopathy and extensive sub-RPE deposits across most of the retina.[1,2] The hypothesis was then tested in three families not previously investigated by dark adaptometry: descendants of eye donors with extensive sub-RPE deposit[12,13] and members of a large family who were given the presumptive diagnosis of L-ORD on historical and clinical criteria. Among 17 tested members at 50:50 risk to inherit L-ORD, 9 showed abnormal dark adaptation. The earliest age with a detectable defect was 35 years. There were opportunities for clinical–psychophysical correlation. Evidence of early disease by ophthalmoscopy (i.e., yellow-white punctate lesions) was present in four individuals, all of whom showed abnormal adaptation. Follow-up of 3 years in a group of the subjects indicated progression of the adaptometry abnormalities and, in one case, fundus lesions became evident on the later visit.

Based on the results of this study, the L-ORD disease sequence can be thought of as having three overlapping stages: an early stage (first three decades of life) without symptoms, dark-adaptation abnormalities, or ophthalmoscopic findings; a middle stage (next two decades) with neither symptoms nor ophthalmoscopic change in most individuals, but with detectable and progressive abnormality in dark adaptation; and a final stage (sixth decade and thereafter) of visual symptoms, markedly abnormal visual function, and clinically overt retinal degeneration. The differences in degree of adaptation kinetic abnormality we found in Patient VI-8 of Family 1 at the different retinal loci tested suggest that disease stage may not be exactly the same across the retina at any given time.

What are the pathogenetic mechanisms at play in L-ORD? There is likely to be slow (decades-long) accumulation of sub-RPE deposits that theoretically would disrupt exchange of nutrients and metabolites between the RPE and choriocapillaris[1–3,8,27–32] and cause RPE dysfunction. It has been suggested that Bruch's membrane may have reserve permeability early in life. This reserve may be able to compensate for some level of genetically induced abnormal transport. With aging, the hydraulic conductivity of Bruch's membrane decreases significantly, correlated with age-related accumulation of lipids in that region. Normal aging changes in Bruch's membrane thus may trigger the onset of manifest retinal disease in L-ORD by even further reducing tolerance for this defective transport.[7,31,33] Eventually, there would be RPE degeneration and secondary photoreceptor dysfunction and death. The molecular basis of the L-ORD disease(s), such as Sorsby fundus dystrophy (SFD) caused by mutations in the *TIMP3* gene (*TIMP3-SFD*),[27,34] may relate to extracellular matrix regulation.

The abnormal dark-adaptation kinetics at the middle stage of L-ORD presumably result from a disturbance in the visual cycle, and this defect in retinal biochemistry occurs at a time when photoreceptors are likely to be normal in number and outer segment length. Defects in the visual cycle are known to cause such dark-adaptation abnormalities in relatively stationary retinal diseases. Examples would be mutations in the *RDH5*

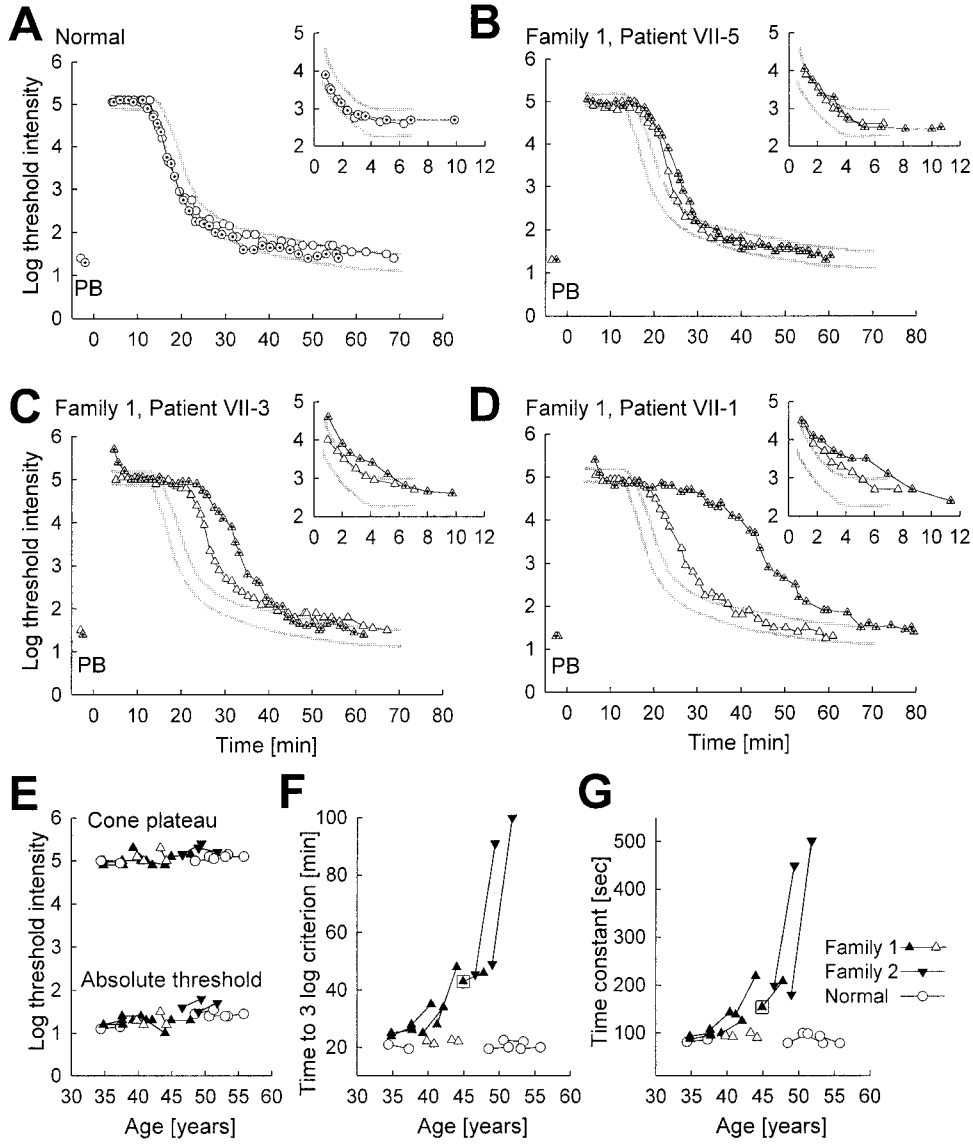

FIGURE 6. Natural history of dark-adaptation abnormalities in Families 1 and 2. (**A–D**) Dark-adaptation functions to a 97% bleach (30° nasal field) in a normal subject and in at-risk members of Family 1 at two sessions separated by approximately 3 years (earlier visit, *open symbols*; later visit, *symbols with crosses*). *Larger panels*: adaptation functions to a blue stimulus; *insets*: cone adaptation to a red stimulus. (**E**) Dark-adapted prebleach rod (absolute threshold, blue stimulus) and cone (cone plateau, blue stimulus) thresholds at different visits in at-risk members of Family 1 (*up triangles*) and Family 2 (*down triangles*) and normal subjects (*circles*). *Lines* connect the different visits of the same individuals. *Filled symbols*: those individuals with abnormal dark adaptation at the initial visit. (**F**, **G**) Time to reach a criterion threshold (3 log units) and time constant of the major rod recovery (second) component of the dark-adaptation functions at different visits in at-risk members of both families compared with normal subjects. Symbols as in (**E**), except for Patient VI-8 (Family 1 proband) whose data from an earlier visit are marked by a *square* surrounding the *up triangle*.

gene[21] and early systemic vitamin A deficiency.[17,35] The normal standard ERG with normal photoresponse parameters in the proband of Family 1 (VI-8), the normal ERGs in our previous studies of patients with L-ORD who had only the adaptation defect,[1,2] and the normal rod and cone psychophysical thresholds in nearly all at-risk patients in the current study, taken together with the absence of pigmentary retinopathy by clinical examination, suggests that the outer retina retains most of its functional and structural integrity for decades, despite a possible increasing barrier between RPE and choriocapillaris from the deposit.

The stereotypical sequence of change in adaptation kinetics in L-ORD begins with subtle delay of the major recovery component for rods and then for cones. These visual cycle abnormalities become progressively more and more extreme, and, finally, thresholds for rods and cones elevate. The latter psychophysical change may signal that there is no remaining tolerance by the retina for the chronic stress induced by increasing sub-RPE deposit. Cells then begin to die, visual thresholds and ERGs become abnormal, and retinal degeneration becomes obvious on clinical examination. Similar pathogenetic sequences but with different time courses have been proposed for *TIMP3-SFD*,[27,32] forms of ARMD,[29] and membranoproliferative glomerulonephritis type II[36,37]—all conditions sharing the same type of dark-adaptation abnormality and histologically shown thickening of Bruch's membrane with deposits between RPE and choriocapillaris.

The L-ORD visual cycle abnormality was responsive to oral vitamin A, but only minimally. The degree of response was more similar to that in *TIMP3-SFD* than in systemic vitamin A deficiency.[17,27,35] Normal kinetics were not attained in the three patients after 1 month of supplementation at the high oral dose of vitamin A. Thus, the dark-adaptation abnormality in these patients seems to have two components: a vitamin A–responsive one and an unresponsive one. The component responsive to vitamin A may result from depletion of stores within the RPE, a form of chronic ocular nutritional deprivation.[27] The second and apparently unresponsive component of the dark-adaptation abnormality may simply be a dose- or time-dependent effect, or supplementation of other nutrients may be needed for complete return of normal function.[38,39] Also, the stage of the disease of the three patients tested may have been too late for more reversal than we documented. It could be postulated that

the chronic nature of the defect may lead to long-term compensatory changes in visual cycle regulation or may differentially affect the multiple 11-*cis*-retinal production pathways thought to exist in the RPE.[21] In patients with *TIMP3-SFD* and vitamin A deficiency whom we have studied to date, an unresponsive component has also been noted.[17,27] If continued slowing of dark-adaptation kinetics is the prelude to visual loss in the pathogenetic sequence of L-ORD, there may be value in long-term administration of supplemental vitamin A, but at a level that would not compromise general health.[40] The anecdote of less profound change in dysfunction in a 3-year interval in Patient VI-8 of Family 1, who admitted to self-treatment with various forms of vitamin A, may deserve attention and warrant further study in this otherwise incurable disease.

The dark-adaptation abnormality described in the current work is a phenotypic marker for future disease expression of L-ORD, preceding symptoms by at least a decade in some at-risk individuals. Longer term molecular testing will permit validation of the conclusions in this study and eclipse phenotypic detection of individuals at risk. The dark-adaptation abnormality will retain value for monitoring disease progression or change with intervention such as we attempted using vitamin A supplementation in this study. The L-ORD gene (or genes) may be a worthy candidate for screening patients with ARMD, considering some of the histopathologic parallels between the diseases,[2] and for those patients with the diagnosis of RP that claim onset of disease late in life.

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