Association of Pattern Dystrophy With an HTRA1 Single-Nucleotide Polymorphism

Tareq Jaouni, MD; Edward Averbukh, MD; Tal Burstyn-Cohen, PhD; Michelle Grunin, BSc; Eyal Banin, MD, PhD; Dror Sharon, PhD; Itay Chowers, MD

Objective: To evaluate if adult-onset foveomacular vitelliform dystrophy (AOFVD) and butterfly-shaped pigment dystrophy (BSPD) are associated with risk single-nucleotide polymorphisms (SNPs) for age-related macular degeneration (AMD).

Methods: This was a tertiary referral center–based cross-sectional study including 35 consecutive patients with BSPD and AOFVD, 317 patients with AMD, and 159 unaffected individuals. Demographics, clinical information, and ophthalmic imaging studies were collected. Sequencing was performed for the peripherin/RDS and BEST1 genes, and genotyping was performed for SNPs in the genes for complement factor H (CFH) (rs1061170), HTRA1 (rs11200638), and complement component 3 (C3) (rs2231099).

Results: Adult-onset foveomacular vitelliform dystrophy and BSPD were diagnosed in 24 (68.6%) and 11 (31.4%) of the 35 patients, respectively. The mean (SD) age of patients with pattern dystrophy (PD) was 75.3 (10) years and median visual acuity was 0.7. Pattern dystrophy was associated with the HTRA1 risk allele compared with unaffected individuals (odds ratio, 1.72; 95% CI, 1.11-2.66; \( P = .03 \)). The HTRA1 SNP showed similar prevalence in patients with AMD and PD. The CFH risk allele was significantly less common in patients with PD compared with patients with AMD (odds ratio, 0.47; 95% CI, 0.28-0.76; \( P = .002 \)). No mutations in peripherin/RDS or BEST1 were detected.

Conclusions: The AOFVD and BSPD phenotypes are associated with an HTRA1 risk SNP. These phenotypes often present in elderly individuals who do not carry peripherin/RDS gene mutations and are associated with retinal pigment epithelium alterations and increased risk for choroidal neovascularization. Further research is required to evaluate if AOFVD and BSPD phenotypes in aged individuals are associated with AMD.

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Pattern dystrophy (PD) of the retinal pigment epithelium (RPE) is a common form of macular degeneration. In ophthalmoscopy, PD is characterized by the accumulation of a yellowish-brown material at the level of the RPE and by RPE alterations in the macular area.

This process can lead to a wide variety of clinical presentations and patterns that were classified by Gass into 5 major forms including adult-onset foveomacular vitelliform dystrophy (AOFVD), butterfly-shaped pigment dystrophy (BSPD), reticular dystrophy, multifocal dystrophy, and fundus pulverulentis (coarse mottling). Yet, Gass and others also noted that additional forms of PD are observed and that eyes of the same patient may manifest different forms of PD. While patients with PD often retain better than 20/40 acuity, as the disease progresses, patients may encounter visual loss secondary to RPE atrophy or due to the development of choroidal neovascularization. Autosomal dominant inheritance mode was reported in PD, and several mutations in the peripherin/RDS gene were associated with the PD phenotype. Infrequently, patients showing a PD-like phenotype carry a BEST1 gene mutation. Yet, many patients with the clinical diagnosis of PD carry neither a peripherin/RDS nor a BEST1 gene mutation. Vitelliform macular lesion may also be associated with cuticular drusen. Cuticular drusen phenotype by itself was associated with the Tyr402His variant of the complement factor H (CFH) gene, an established risk single-nucleotide polymorphism for age-related macular degeneration (AMD). This CFH variant was not detected in patients manifesting the combination of vitelliform lesion and cuticular drusen.
Although PD phenotypes such as AOFVD and BSPD may be distinct from typical non-neovascular AMD, both conditions have variable appearances that have some common features including RPE alterations and yellowish sub-retinal deposits that appear as vitelliform lesions in PD and as pseudodrusen in association with AMD. 10 Another characteristic common for both diseases is presentation in elderly individuals who often have a positive family history. This study aims to further evaluate the genetic basis of AOFVD and BSPD and to assess its genetic similarity with AMD. To that end, we have studied patients with the clinical diagnosis of AOFVD and BSPD. All patients had a negative family history for maculopathy. Genotyping was performed for the major risk single nucleotide polymorphisms (SNPs) for AMD in the genes for CFH (rs1061170, Y402H variant), HTRA1 (rs11200638, +504G>A), and complement component 3 (C3) (rs2230199, R102G). These SNPs were previously associated with AMD in several populations including the Israeli population. 17-20 The peripherin/RDS and BEST1 genes were also genotyped to exclude mutations in these genes as a cause for the PD phenotype.

**METHODS**

**PATIENTS**

A sequential group of 35 patients who were diagnosed with AOFVD or BSPD by retina specialists (E.A., I.C., and E.B.) was included in the study. To limit the bias that might be introduced by incorporating variable phenotypes of PD that potentially overlap with the phenotype of AMD, we limited this study to typical AOFVD and BSPD phenotypes. Diagnosis was based on ophthalmoscopy according to the classification of Gass1 and was assisted by optical coherence tomography (OCT) to identify the vitelliform lesions. All patients were referred for evaluation of maculopathy in the retina service of the Hadassah–Hebrew University Medical Center between July 2010 and September 2011. Data including demographics, family history for maculopathy, and ophthalmic findings were collected. Ophthalmic imaging including OCT images (Spectralis [Heidelberg Engineering] or Stratus [Carl Zeiss Meditec]), autofluorescence (HRA), and fluorescein angiography images (Figure 1). Optical coherence tomography (n=20 patients) showed either central focal or multifocal linear hyperfluorescent patterns in the macular area (Figure 2). Optical coherence tomography (n=15 patients) showed subretinal dome-shaped deposits (18 patients) and RPE atrophic changes (4 patients, 2 of them had both subretinal deposits and atrophic changes) in both AOFVD and BSPD (Figure 1 and Figure 2). Subretinal dome-shaped deposits and RPE atrophic changes (4 patients, 2 of them had both subretinal deposits and atrophic changes) in both AOFVD and BSPD (Figure 1 and Figure 2). Choroidal neovascularization was diagnosed in 8 eyes (5 with AOFVD, 3 with BSPD) of 8 patients (22.8%) based on clinical, fluorescein angiography, and OCT findings (Figure 1).

**GENETIC TESTING**

DNA was extracted from whole blood using the FlexiGene DNA Kit (QIAGEN); DNA was then used as a template for polymerase chain reaction (PCR) amplification and sequencing for the peripherin/RDS and BEST1 genes. All 3 encoding exons of the peripherin/RDS gene were sequenced. Because of the exons’ length and composition, exon 1 was divided into 2 overlapping fragments and exon 3 was divided into 2 fragments of which only the first fragment, composed of 647 base pairs, was studied. The remaining region of the exon is noncoding and, thus, was not sequenced. The encoding exons of the BEST1 gene were sequenced, and exon 10 was divided into 2 overlapping fragments (eTable, http://www.archophthalmol.com). Exon boundaries were included in the analysis.

Polymerase chain reaction was performed for the fragments containing the SNPs in the CFH (rs1061170), HTRA1 (rs11200638), and C3 (rs2230199) genes using specific pre-designed primers (eTable) and ReadyMix PCR reaction mixture (Sigma-Aldrich), for a total volume of 25 µL. The PCR reactions were performed with an annealing temperature of 58°C to 60°C, elongation temperature of 72°C, and melting temperature of 94°C (eTable). The PCR products were evaluated on a 1.5% agarose gel to confirm the success of the PCR reaction, followed by automatic sequencing (Macrogen), where a preprovided primer (eTable) was used to extend the PCR product with fluorescent nucleotides to provide the sequence of the targeted area.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using SPSS (IBM SPSS) and Instat software (GraphPad), as we have previously described. 25-26 Briefly, logistic regression, Fisher exact, and χ2 tests were applied to assess odds ratios, confidence intervals, and significance.

**RESULTS**

**CLINICAL CHARACTERISTICS**

Thirty-five patients (70 eyes) were evaluated (20 women; 15 men); the mean (SD) age at presentation was 75.3 (10) years (range, 46-93 years), and this was similar to the mean (SD) age of patients with AMD (78.1 [7.6]; P=.22). Both patients with AMD and PD were older than the controls (mean [SD] age, 70.8 [8.2]; P<.05). None of the patients with PD had a positive family history for maculopathy. Mean visual acuity was 0.7 (range, counting fingers from 10 cm to 1.25 decimals). Patients showed BSPD (11 of 35 [31.4%]) or AOFVD (24 of 35 [68.6%]) (Figure 1 and Figure 2) phenotypes. Autofluorescence imaging (n=15 patients) showed either central focal or multifocal linear hyperfluorescent patterns in the macular area (Figure 2). Optical coherence tomography (n=20 patients) showed subretinal dome-shaped deposits (18 patients) and RPE atrophic changes (4 patients, 2 of them had both subretinal deposits and atrophic changes) in both AOFVD and BSPD (Figure 1 and Figure 2). Choroidal neovascularization was diagnosed in 8 eyes (5 with AOFVD, 3 with BSPD) of 8 patients (22.8%) based on clinical, fluorescein angiogram, and OCT findings (Figure 1).

**GENOTYPING**

No known or novel mutations in the peripherin/RDS or the BEST1 genes were detected in this group of patients. Several SNPs that were previously reported in unaffected individuals from other populations were also found in the Israeli patients with PD. The prevalence of these SNPs was similar to that reported in other unaffected populations (data not shown).
Risk-associated SNPs in \textit{CFH}, \textit{C3}, and \textit{HTRA1} were identified in 42.8%, 39.4%, and 52.4% of patients with PD, respectively (Table). The AMD risk alleles in \textit{CFH} and \textit{C3} were not associated with PD, and their prevalence was similar in the patients with PD and controls. In fact, the \textit{CFH} risk allele was significantly less common in patients with PD compared with patients with AMD (odds ratio, 0.47; 95% CI, 0.28-0.76; \(P = .002\)). The \textit{HTRA1} risk allele was associated with patients with PD compared with unaffected individuals (odds ratio, 1.72; 95% CI, 1.11-2.66; \(P = .03\)) and showed similar prevalence in patients with AMD and PD (odds ratio, 0.84; 95% CI, 0.52-1.34; \(P = .54\)). There was no significant difference in the distribution of the \textit{C3} SNP between patients with PD and AMD and between patients with PD and unaffected individuals. Analysis according to genotypes showed similar results to allele-based analysis (Table).

**GENOTYPE-PHENOTYPE CORRELATION**

Mean (SD) age at presentation of patients with PD who were positive (\(n = 18\)) or negative (\(n = 17\)) for the \textit{HTRA1} SNP was 74.7 (10.8) and 75.9 (11.4) years, respectively (\(P = .74\)). While the \textit{HTRA1} SNP was associated with PD, the phenotype of carriers (either homozygotes or heterozygotes) of the \textit{HTRA1} risk SNP was similar to \textit{HTRA1}-negative patients with PD (Figure 1 and Figure 2). There was no difference in the prevalence of \textit{HTRA1} and \textit{CFH} alleles between the AOFVD and BSPD phenotypes, respectively (data not shown). There was also no association between the development of choroidal neovascularization and the SNPs that were evaluated.

**COMMENT**

We have characterized the genotype of patients with AOFVD or BSPD who had a negative family history for maculopathy. None of the cases showed a peripherin/RDS or \textit{BEST1} gene mutation. Yet, these phenotypes of PD were associated with an \textit{HTRA1} SNP. This SNP was associated with AMD in several populations including in Israel.\textsuperscript{20-22,26} Since the \textit{HTRA1} SNP is in complete linkage disequilibrium with an \textit{ARMS2} SNP, it is not possible to determine which of the 2 genes, \textit{HTRA1} or \textit{ARMS2}, has a functional role in PD.\textsuperscript{21,26} In this group of patients, there was no association between PD phenotypes that we evaluated and the major risk SNPs for AMD in the \textit{CFH} and \textit{C3} genes. In fact, the \textit{CFH} SNP was less common in PD compared with AMD and its prevalence was similar in patients with PD and unaffected individuals.
 Associated with disease duration. Francis and colleagues found
ported to be common in PD and is thought to be asso-
development of choroidal neovascularization was re-

yet affected and variable genetic backgrounds for simi-
lar PD phenotypes that may be associated with variable
age at onset.

Several weaknesses of this research should be ac-
nowledged. First, while none of the patients included
in the study had mutations in the peripherin/RDS gene
and none had a positive family history for the disease, it
is still possible that other family members of our pa-
tients had PD. Conceivably, relatives of patients with PD
who also carry the HTRA1 risk SNP have increased risk
for the disease. Second, while the CFH SNP is less com-

Substantial visual loss due to RPE atrophy or the de-
velopment of choroidal neovascularization was re-
ported to be common in PD and is thought to be asso-
ciated with disease duration. Francis and colleagues found
visual acuity of 20/200 or less in both eyes in 44% of 16
patients with PD carrying the peripherin/RDS mutation
who were 70 years or older. In comparison, none of the
patients in our study had such poor visual acuity in both
eyes, including none of our 16 patients who were older
than 70 years. Further research is required to compare visual
consequences of patients with PD with and without
peripherin/RDS mutations and to correlate it with age at onset.

The peripherin/RDS gene has a structural role in photoreceptor outer segment discs and is known to interact with rod outer segment membrane protein 1 (ROM1) and to be associated with several types of retinal degenerations.4,6,11,28,29 The retinal function of the HTRA1 and ARMS2 genes is poorly understood, and neither of these genes is known to interact with the peripherin/RDS or BEST1 gene products. Recently, the ARMS2 gene was sug-
gested to be a component of the extracellular matrix. Such
function, if validated, may potentially underlie the asso-
ciation of HTRA1/ARMS2 with both drusen and vitelliform lesion formation.26

Several weaknesses of this research should be ac-

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Correspondence: Itay Chowers, MD, Department of Oph-
thalmology, Hadassah–Hebrew University Medical Cen-
ter, POB 12000, Jerusalem, Israel 91120 (chowers@hadassa-
.org.il).
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Table. Genotyping Data

<table>
<thead>
<tr>
<th>No. (%)</th>
<th>PD</th>
<th>AMD</th>
<th>OR (95% CI)</th>
<th>P Value</th>
<th>Control, No. (%)</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild (TT)</td>
<td>20 (57.1)</td>
<td>73 (23)</td>
<td>&lt;.001</td>
<td>79 (49.7)</td>
<td>.22</td>
<td></td>
<td></td>
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<tr>
<td>Het (T/C)</td>
<td>9 (25.7)</td>
<td>172 (54.3)</td>
<td>1.9 (0.08-0.40)</td>
<td>63 (39.6)</td>
<td>0.56 (0.24-1.32)</td>
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<tr>
<td>Risk (CC)</td>
<td>6 (17.1)</td>
<td>72 (22.7)</td>
<td>0.3 (0.12-0.80)</td>
<td>17 (10.7)</td>
<td>1.39 (0.49-4.00)</td>
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</tr>
<tr>
<td>Risk/wild alleles</td>
<td>21/49 (30/70)</td>
<td>316/318 (49.8/50.2)</td>
<td>0.47 (0.28-0.76)</td>
<td>.002</td>
<td>97/221 (30.5/69.5)</td>
<td>0.98 (0.62-1.56)</td>
<td></td>
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<tr>
<td>HTRA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Wild (GG)</td>
<td>17 (48.6)</td>
<td>139 (43.8)</td>
<td>.79</td>
<td>101 (63.5)</td>
<td>.02</td>
<td></td>
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<tr>
<td>Het (G/A)</td>
<td>13 (37.1)</td>
<td>119 (37.5)</td>
<td>0.89 (0.42-1.92)</td>
<td>53 (33.3)</td>
<td>1.46 (0.66-3.23)</td>
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<tr>
<td>Risk (AA)</td>
<td>5 (14.3)</td>
<td>59 (18.6)</td>
<td>0.69 (0.24-1.96)</td>
<td>5 (3.1)</td>
<td>5.95 (1.55-22.73)</td>
<td></td>
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</tr>
<tr>
<td>Risk/wild alleles</td>
<td>23/47 (32.9/67.1)</td>
<td>237/397 (37.4/62.6)</td>
<td>0.84 (0.52-1.34)</td>
<td>.54</td>
<td>63/255 (19.8/80.2)</td>
<td>1.72 (1.11-2.66)</td>
<td></td>
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<tr>
<td>C3</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild (CC)</td>
<td>20 (60.6)</td>
<td>175 (55.2)</td>
<td>.46</td>
<td>100 (62.9)</td>
<td>.37</td>
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<tr>
<td>Het (C/G)</td>
<td>13 (39.4)</td>
<td>123 (38.8)</td>
<td>0.95 (0.44-1.93)</td>
<td>50 (31.4)</td>
<td>1.30 (0.60-2.82)</td>
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<tr>
<td>Risk (G/G)</td>
<td>0 (0)</td>
<td>19 (6)</td>
<td>. . .</td>
<td>9 (5.7)</td>
<td>. . .</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk/wild alleles</td>
<td>13/53 (19.7/80.3)</td>
<td>161/473 (25.4/74.6)</td>
<td>0.74 (0.42-1.33)</td>
<td>.39</td>
<td>68/250 (21.4/78.6)</td>
<td>0.92 (0.53-1.60)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; C3, complement component 3; CFH, complement factor H; Het, heterozygote; PD, pattern dystrophy; OR, odds ratio.

a For the PD vs control comparison.

b For the PD vs AMD comparison.

c For the PD vs control comparison. C3 genotyping was successful in 33 patients with PD only.