Association of Pattern Dystrophy With an \textit{HTRA1} Single-Nucleotide Polymorphism

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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 \textit{BEST1} genes, and genotyping was performed for SNPs in the genes for complement factor H (\textit{CFH}) (rs1061170), \textit{HTRA1} (rs11200638), and complement component 3 (\textit{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 \textit{HTRA1} risk allele compared with unaffected individuals (odds ratio, 1.72; 95% CI, 1.11-2.66; \(P = .03\)). The \textit{HTRA1} SNP showed similar prevalence in patients with AMD and PD. 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; \(P = .002\)). No mutations in peripherin/RDS or \textit{BEST1} were detected.

Conclusions: The AOFVD and BSPD phenotypes are associated with an \textit{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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This process can lead to a wide variety of clinical presentations and patterns that were classified by Gass\textsuperscript{1} into 5 major forms including adult-onset foveomacular vitelliform dystrophy (AOFVD), butterfly-shaped pigment dystrophy (BSPD), reticular dystrophy, multifocal dystrophy, and fundus pulverulentis (coarse motting). 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.\textsuperscript{1,3} While patients with PD often retain better than 20/40 acuity, as the disease progress, patients may encounter visual loss secondary to RPE atrophy or due to the development of choroidal neovascularization.\textsuperscript{4}

Autosomal dominant inheritance mode was reported in PD, and several mutations in the peripherin/RDS gene were associated with the PD phenotype.\textsuperscript{3,9} Infrequently, patients showing a PD-like phenotype carry a \textit{BEST1} gene mutation.\textsuperscript{10,11} Yet, many patients with the clinical diagnosis of PD carry neither a peripherin/RDS nor a \textit{BEST1} gene mutation.\textsuperscript{8} 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 (\textit{CFH}) gene, an established risk single-nucleotide polymorphism for age-related macular degeneration (AMD). This \textit{CFH} variant was not detected in patients manifesting the combination of vitelliform lesion and cuticular drusen.\textsuperscript{13,15}
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 subretinal 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.

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, when available, were analyzed.

Blood samples were collected from each patient and DNA was extracted for genetic analysis. The study was approved by the institutional ethics committee, and each patient signed an informed consent form. Genotyping for the major risk SNPs for AMD in CFH, C3, and HTRA1 was compared with data from a group of 159 unaffected controls and 317 patients with AMD whose data were sequentially collected from the same retina clinic. Inclusion criteria for the control group included age older than 60 years, clear media that enabled ophthalmoscopy, and absence of intermediate-size drusen, multiple small drusen, or retinal pigment epithelial abnormalities (Age-Related Eye Disease Study category 1). Age-related macular degeneration was diagnosed according to the Age-Related Eye Disease Study criteria.

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.

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. Median 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 depositions 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).

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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 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).

\textbf{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.

\textbf{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.\(^{20-22,26}\) 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.\(^{21,26}\) 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 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.
ciated with disease duration. Francis and colleagues found that retinal abnormalities, including drusen and vitelliform lesion formation, are common in PD and are thought to be associated with several types of retinal degenerations.

A comparison of genotype and allele frequency of the CFH (rs1061170), HTRA1 (rs11200638), and C3 (rs2231099) variants among patients with PD, patients with AMD, and unaffected controls is shown in Table 1. The study was supported by a grant from the Israel Science Foundation (Dr Chowers) and from the Hadassah Medical Center (Dr Jaouni).

Several weaknesses of this research should be acknowledged. 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 patients 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 common in patients with PD compared with patients with AMD, we cannot exclude a low-magnitude association between the CFH or C3 SNPs and PD phenotypes. Yet, our data show that such an association, even if present, is of low magnitude compared with the association of typical AMD with these SNPs.

In conclusion, our data show that the AOFVD and BSDP phenotypes with a negative family history for the disease are a relatively common clinical diagnosis in the retina clinic. It is often diagnosed in elderly individuals and is associated with an HTRA1 SNP. Further research is required to evaluate if such PD phenotypes have variable visual outcome and age at onset in association with RDS mutation status, as well as to test the association of these phenotypes with AMD.

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Online-Only Material: The eTable is available at http://www.archophthalmol.com. This article is featured in the Archives Journal Club. Go to http://www.archophthalmol.com to download teaching powerpoint slides.

REFERENCES