Objective: To determine the histidine frequency in patients with the cuticular drusen phenotype of age-related macular degeneration (AMD).

Methods: Fifty individuals were identified who met the criteria for the cuticular drusen phenotype using a standard threshold photograph. We genotyped DNA samples using a polymerase chain reaction–based restriction digest assay. Seven hundred individuals with typical AMD and 252 controls were also genotyped. Fisher exact test was used to analyze the significance of allele frequency differences.

Results: The histidine variant was present in 70% (frequency±SE, 0.70±0.05) of the cuticular cohort, 55% (frequency±SE, 0.55±0.01) of the more typical AMD cases, and 34% (frequency±SE, 0.34±0.02) of controls. The association between the cuticular drusen phenotype and the histidine allele was highly significant (P = .003; odds ratio, 2.0; 95% confidence interval, 1.21-3.07; vs AMD cases P <.001; odds ratio 4.54; 95% confidence interval, 2.79-7.50; vs controls). Genotype distribution between the 3 groups was similarly significant (P <.001).

Conclusion: The cuticular drusen phenotype is highly associated with the Tyr402His variant of the complement factor H (CFH) gene. The significantly higher histidine allele frequency in this group compared with the typical AMD cohort suggests that the complement cascade may play a greater role in the pathogenesis of the cuticular drusen subtype than in AMD as a whole.

Clinical Relevance: The c.1204T>C, p.Tyr402His allelic variant in the CFH gene is associated with a 3-fold increased risk for AMD. A high frequency of the histidine allele has also been noted in patients with membranoproliferative glomerulonephritis type II.

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population might be similarly increased when compared with a cohort of AMD patients and controls.

**METHODS**

**PATIENTS**

The recruitment and research protocols were reviewed and approved by the University of Iowa institutional review board. Informed consent was obtained from all study participants. All patients were examined by an ophthalmologist and were found to have signs consistent with the clinical diagnosis of AMD. Only those individuals with normal examination findings were enrolled as controls. All patients were ascertained from the University of Iowa’s Department of Ophthalmology. The AMD patients and controls were all more than 50 years old (average age of controls, 75.5 years). Only Caucasian individuals were enrolled in this study. All participants were ascertained during the same period by the same clinic.

Two of us (M.A.G. and J.C.F.) assigned individuals to the cuticular drusen cohort. Both reviewers independently graded the ophthalmoscopic images of more than 1000 individuals with AMD to identify patients who exceeded the threshold for the cuticular drusen phenotype as defined by a standard photo (Figure 1D). Irreconcilable disagreement between the 2 grad-

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**Figure 1.** Ophthalmoscopic and angiographic features that characterize the cuticular drusen phenotype. A, Thirty-degree color fundus photograph centered on the macula demonstrating classic features of cuticular drusen, including a vitelliform macular detachment. B, Negative fluorescein angiogram of the same patient taken at 55.2 seconds revealing multiple pinpoint areas of hyperfluorescence corresponding to drusen in a “starry sky” distribution. Note the fluorescein blockage and associated early leakage due to a drusenoid pigment epithelial detachment. C, Sixty-degree fundus photograph centered on the macula of a patient with membranoproliferative glomerulonephritis type II demonstrating diffuse cuticular drusen. D, Threshold photograph used for grading the cuticular drusen phenotype. Classification required the presence of cuticular drusen on fluorescein angiography of equal or greater number and extent than that visualized in this frame.
ers disqualified the patient from inclusion in the study. The reviewers were masked for patient genotype. Inclusion criteria included the presence of all of the following: diffuse small, circular, uniform drusen distributed throughout the macula extending to the arcades of greater number and extent than that visualized in Figure 1D; characteristic clustering or clumping of the drusen into groups of 15 to 203; and early hyperfluorescence of drusen on fluorescein angiography with greater than one half the fundus area revealing pinpoint hyperfluorescence. Fundus photos were also analyzed for the presence of geographic atrophy and choroidal neovascularization.

**GENOTYPING**

We extracted DNA from peripheral blood according to a previously described protocol.22 We used 12.5 ng of each patient’s DNA as template in a 8.35-µL polymerase chain reaction containing 1.25 µL 10X buffer (100 mM Tris-hydrochloric acid, pH 8.3; 500 mM potassium chloride; 15 mM magnesium chloride); 300 µM each of dCTP, dATP, dGTP, and dTTP; 1 pmol of each primer; and 0.25 units of polymerase (Biolase, Irvine, Calif). Samples were denatured for 5 minutes at 94°C and incubated for 35 cycles under the following conditions: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds in a Peltier DNA thermocycler (PTC-225; MJ Research, Johannesburg, South Africa). Primers were generated based on the GenBank sequence of the CFH gene (NM_000186.2):

**F:** 5’/H11032 TCATTGTATGGTCCTTAGGAAA 3’

**R:** 5’/H11032 ACTGTGGTCTGCGCTTTTG 3’

We added restriction endonuclease NlaIII (New England Biolabs, Ipswich, Mass) to the polymerase chain reaction products in the following reaction per well: 1.2 µL 10X bovine serum albumin, 1.2 µL NEBuffer 4 (New England Biolabs), 0.2 µL NlaIII (10,000 U/mL), and 1.05 µL nuclease-free water. The samples were incubated at 37°C for 2 hours in a DNA thermocycler (Omnigene; Hybaid, Middlesex, England). After digestion, 5 µL of stop solution (95% formamide, 10 mM sodium hydroxide, 0.05% bromophenol blue, 0.05% xylene cyanol) was added to each sample. Digested amplification products underwent electrophoresis on 2% agarose E-Gels (Invitrogen, Carlsbad, Calif). All gels were stained with ethidium bromide for 15 minutes in the Invitrogen E-Gel Powerbase version 4, and the presence of the CFH C allele was determined by inspection of the restriction pattern (Figure 2). Genotype correlation with the restriction pattern was confirmed in a small subset of subjects by automated sequencing.

**STATISTICS**

Standard error for allele and genotype frequency was calculated using the formula:

\[
SE = \sqrt{\frac{p(1-p)}{n}}
\]

where \( p \) is the frequency, and \( n \) is the total number of alleles for allele frequency or the total number of individuals for genotype frequency. Significance of the genotype and allele frequencies was analyzed between the groups using a 2-tailed Fisher exact test.

**RESULTS**

**CONTROLS**

The prevalence of the histidine alteration was assessed in 252 unrelated subjects at the University of Iowa. The frequency of the risk allele in the University of Iowa control cohort is 34% (frequency±SE, 0.34±0.02) (Table 1). The prevalence of the histidine allele ranges from 34% to 39% in the Caucasian population unaffected by AMD.18-21

**AMD CASES**

We analyzed 700 unrelated individuals with AMD for the presence of the histidine allele at the Tyr402His locus. Risk allele frequency in the Iowa AMD cohort is 55% (frequency±SE, 0.55±0.01) (Table 1), which corresponds well with prior studies where the reported frequency ranged from approximately 46% to 59%.18-21
The cuticular drusen phenotype has been given many different names. Initially called *diffuse drusen*, it was later termed *cuticular drusen* followed by *basal laminar drusen* and more recently *early adult onset grouped drusen*. Because of its striking ophthalmoscopic and angiographic appearance as well as an earlier age at onset, this phenotype appears to be a distinct clinical entity within the heterogeneous spectrum of fundus findings that compose *AMD*.

There are at least 2 reasons that this phenotype is worthy of study from a genetic perspective. First, since the location and composition of cuticular drusen are identical to that found in *AMD*, it is likely that these 2 conditions share a common mechanism of drusen biogenesis. Second, the characteristic early onset of this disorder suggests that there may be a greater genetic predisposition to this phenotype than *AMD* in general. Almost half of the patients in our cuticular cohort had a positive family history of *AMD*. In addition, individuals with the cuticular phenotype demonstrate a 2-times greater frequency of the histidine allele (c.1204T>C, p.Tyr402His) than patients with typical *AMD*. It is notable that cuticular drusen are also present in patients with *AMD* who harbor missense changes in the fibulin 5 gene (*FBLN5* (OMIM #604580)).

Of the 50 individuals identified with the cuticular phenotype, 5 were homozygous for the protective tyrosine allele. To determine whether these patients were outliers, the clinical data for these patients were reviewed following unmasking of the graders. We sought to determine whether these patients were significantly older than those found in the remainder of the cuticular cohort. In addition, fundus photos for these patients were reanalyzed to further unmasking of the graders. We sought to determine whether there was a discernable ophthalmoscopic difference in the appearance of these patients. We speculated that on the phenotypic spectrum of cuticular drusen, these patients might be clustered to the left near the standard threshold image (Figure 1D). We found no difference in the age distribution of these patients. Moreover, of the 5 patients, 3 were considered to have classic, exemplary features of the phenotype that far exceeded the threshold criteria.

It has been suggested that the natural history associated with this phenotype may be better than that of *AMD* as a whole. In our study, the prevalence of choroidal neovascularization was lower in the cuticular cohort compared with the overall rate in the *AMD* population (0.21 vs 0.40, respectively) (Table 2). The lower rate of cho-

### Table 1. Comparison of Genotype and Allele Frequencies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>AMD</th>
<th>Cuticular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosine allele frequency, No. (F ± SE)†‡</td>
<td>171 (0.34 ± 0.02)</td>
<td>770 (0.55 ± 0.01)</td>
<td>70 (0.70 ± 0.05)</td>
</tr>
<tr>
<td>Thymine allele frequency, No. (F ± SE)</td>
<td>333 (0.66 ± 0.02)</td>
<td>630 (0.45 ± 0.01)</td>
<td>30 (0.30 ± 0.05)</td>
</tr>
<tr>
<td>CC genotype frequency, No. (F ± SE)</td>
<td>20 (0.08 ± 0.02)</td>
<td>231 (0.33 ± 0.02)</td>
<td>25 (0.50 ± 0.07)</td>
</tr>
<tr>
<td>CT genotype frequency, No. (F ± SE)</td>
<td>120 (0.50 ± 0.03)</td>
<td>315 (0.45 ± 0.02)</td>
<td>20 (0.40 ± 0.07)</td>
</tr>
<tr>
<td>TT genotype frequency, No. (F ± SE)</td>
<td>106 (0.42 ± 0.03)</td>
<td>154 (0.22 ± 0.02)</td>
<td>5 (0.10 ± 0.04)</td>
</tr>
<tr>
<td>Total, No.</td>
<td>252</td>
<td>700</td>
<td>50</td>
</tr>
<tr>
<td>Odds ratio (95% CI)‡</td>
<td>1</td>
<td>1.91 (1.21-3.07)</td>
<td>4.54 (2.79-7.50)</td>
</tr>
<tr>
<td>P value‡</td>
<td>1</td>
<td>.003</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**Abbreviations:** AMD, age-related macular degeneration; CC, homozygous cytosine genotype; CI, confidence interval; CT, heterozygous genotype composed of a cytosine allele and a thymine allele; F, frequency; TT, homozygous thymine genotype.

*All allele frequencies are in Hardy-Weinberg equilibrium.*

†The single nucleotide polymorphism c.1204T>C, p.Tyr402His evaluated in this study.

‡Odds ratios and *P* values calculated based on comparison with allele frequencies in the cuticular cohort.

### Table 2. Clinical Characteristics of the Cuticular Drusen Cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choroidal neovascularization</td>
<td>0.21*</td>
</tr>
<tr>
<td>Geographic atrophy</td>
<td>0.11*</td>
</tr>
<tr>
<td>Vision in better eye worse than 20/100</td>
<td>0.22</td>
</tr>
<tr>
<td>Positive FH of AMD</td>
<td>0.44</td>
</tr>
</tbody>
</table>

**Abbreviations:** AMD, age-related macular degeneration; FH, family history.

*Numbers calculated based on number of total eyes involved from cohort.*

### CUTICULAR CASES

Fifty individuals met the criteria for inclusion into the cuticular drusen cohort. The histidine allele frequency in this population is 70% (frequency ± SE, 0.70 ± 0.05) (Table 1), which closely approximates the prevalence reported in patients with MPGN II. When the risk allele (Table 1), which closely approximates the prevalence reported in patients with MPGN II. When the risk allele frequency in this group is compared with that observed in controls, a statistically significant difference is apparent (*P* < .001; odds ratio, 4.54; 95% confidence interval, 2.79-7.50). Moreover, when allele frequencies are compared with the AMD cohort, this trend remains significant (*P* = .003; odds ratio, 1.91; 95% confidence interval, 1.21-3.07). Genotype frequencies between the groups were also analyzed and found to be similarly significant (*P* < .001).

Clinical characteristics of the cuticular cohort are shown in Table 2. Sixty percent of the group was composed of women, and the mean age at presentation was 62.7 years (range, 34-98 years). Natural history was assessed by evaluating the rate of choroidal neovascularization (21%; 21/100 eyes); geographic atrophy (11%; 11/100 eyes); and vision in the better eye equal to or worse than 20/100 (22%; 11/50 patients).
oidal neovascularization in the cuticular cohort may simply reflect a bias of ascertainment as the phenotype may be partially masked in those individuals harboring large disciform scars. Moreover, our data suggest that the course of cuticular patients is not benign with more than 1 in 5 patients developing vision of 20/100 or worse in their better eye.

In summary, our findings clearly implicate the CFH gene as an important determinant of expression of the cuticular drusen phenotype. To our knowledge, this is the largest cohort of patients with cuticular drusen reported to date. These data support the observation that cuticular drusen is a unique and identifiable phenotype within the spectrum of AMD.

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Author Contribution: Dr Grassi had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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