

# Single Nucleotide Polymorphism in the Cholesterol-24S-Hydroxylase (*CYP46A1*) Gene and Its Association with *CFH* and *LOC387715* Gene Polymorphisms in Age-Related Macular Degeneration

Cynthia Fourgeux,<sup>1-3</sup> Brice Dugas,<sup>4</sup> Florence Richard,<sup>5</sup> Ingemar Björkhem,<sup>6</sup> Niyazi Acar,<sup>1-3</sup> Alain M. Bron,<sup>1-4</sup> Jean-François Korobelnik,<sup>7</sup> Nicolas Leveziel,<sup>8</sup> Jennyfer Zerbib,<sup>8</sup> Nathalie Puche,<sup>8</sup> Catherine P. Creuzot-Garcher,<sup>1-4</sup> Eric Souied,<sup>8</sup> and Lionel Bretillon<sup>1-3</sup>

**PURPOSE.** We investigated the association of single nucleotide polymorphism (SNP) in the *cholesterol-24S-hydroxylase* (*CYP46A1*) gene, according to *CFH* and *LOC387715* SNPs, with age-related macular degeneration (AMD).

**METHODS.** We enrolled 1388 AMD patients with neovascular AMD or geographic atrophy and 487 unrelated control subjects. SNPs were genotyped in the *CYP46A1* (rs754203), *LOC387715* (rs10490924), and *CFH* (rs1061170) genes. Plasma 24S-hydroxycholesterol, the metabolic product of *CYP46A1*, was quantified by gas chromatography-mass spectrometry using an authentic deuterated internal standard in subgroups of patients and controls. The  $\chi^2$  test was used to compare categoric allelic and genotype distributions between cases and controls. The odds ratio (OR) with a 95% confidence interval (95% CI) was calculated for AMD risk, and adjusted for age and gender. Significance levels were set at  $P < 0.05$ .

**RESULTS.** The rs754203 SNP in the *CYP46A1* gene was not associated with AMD (crude OR = 1.2, 95% CI = 0.9–1.4,  $P = 0.2$ ). The crude OR for risk of AMD was 2.9 (95% CI = 2.4–3.4,  $P < 0.0001$ ) according to the number of rs10490924 T alleles

in the *LOC387715* gene, and 2.0 (95% CI = 1.7–2.3,  $P < 0.0001$ ) according to the number of rs1061170 C alleles in the *CFH* gene. After adjustment for age and gender, an OR of 2.2 (95% CI = 1.1–4.1,  $P = 0.04$ ) was obtained for AMD cases with the C allele in the *CYP46A1* gene, and carrying no risk alleles in the *CFH* and *LOC387715* genes.

**CONCLUSIONS.** The rs754203 C allele in the *CYP46A1* gene may confer a higher risk for exudative AMD in patients who carry no risk alleles in the *CFH* and *LOC387715* genes. Additional studies with larger sample sizes are needed in AMD subjects at no risk in *CFH* and *LOC387715*. (*Invest Ophthalmol Vis Sci*. 2012;53:7026–7033) DOI:10.1167/iovs.12-9652

Age-related macular degeneration (AMD) is the most common cause of irreversible visual loss in Western elderly populations. Its prevalence recently was estimated at 6.5% after 40 years of age in the United States.<sup>1</sup> AMD is a genetically complex disorder of the photoreceptor–RPE–Bruch's membrane (BrM)–choriocapillaris complex.<sup>2–5</sup> Early AMD is characterized histopathologically by retinal pigment abnormalities and drusen between the RPE and choroid.<sup>6,7</sup> Drusen are deposits of extracellular debris, enriched in neutral lipids, including cholesterol and cholesteryl esters (see the study of Curcio et al.<sup>8</sup> for review). Drusen are classified as hard or soft. Although occurring frequent in elderly human subjects and found mainly in the macula,<sup>9</sup> soft drusen at early stages are associated with a higher risk for late AMD.<sup>10,11</sup> Late AMD includes geographic atrophy (GA) and choroidal neovascularization (CNV). AMD is a complex disease derived from inherited and environmental factors, such as advanced age, smoking habits, lifestyle, and dietary status.<sup>12</sup> Familial aggregation<sup>13–16</sup> and twin studies<sup>17–20</sup> have shown that genetic propensity accounts for a large proportion in the prevalence of AMD.

Nevertheless, its etiology remains poorly understood. In the past few years, research into the genetics of AMD has been successful. Several studies have supported definitively the association between AMD and variants of genes coding for members of the complement cascade, including complement factor H (CFH).<sup>21</sup> A single nucleotide polymorphism (SNP), rs1061170 (Y402H), in the *CFH* gene results in a missense mutation, and significantly increases the likelihood of AMD in European populations.<sup>22,23</sup> Protective variants in the complement pathway subsequently were identified in the complement component 2/B factor locus.<sup>24</sup> Recently, two reports have indicated an association with variation in the complement component 3 locus.<sup>25,26</sup> Genome-wide scans also support

From the <sup>1</sup>INRA, UMR1324 Centre des Sciences du Goût et de l'Alimentation, Eye & Nutrition Research Group, Dijon, France; the <sup>2</sup>CNRS, UMR6265 Centre des Sciences du Goût et de l'Alimentation, Eye & Nutrition Research Group, Dijon, France; the <sup>3</sup>Université de Bourgogne, Centre des Sciences du Goût et de l'Alimentation, Eye & Nutrition Research Group, Dijon, France; the <sup>4</sup>CHU de Dijon, Service d'Ophtalmologie, Dijon, France; the <sup>5</sup>Institut Pasteur de Lille, Lille, France; the <sup>6</sup>Department of Laboratory Medicine, Division of Clinical Chemistry, Karolinska University Hospital Huddinge, Stockholm, Sweden; the <sup>7</sup>Service d'Ophtalmologie, CHU de Bordeaux, Université Bordeaux Segalen, Bordeaux, France; and the <sup>8</sup>Department of Ophthalmology, University Hospital, Créteil, France.

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Corresponding author: Lionel Bretillon, CSGA, Eye & Nutrition Research Group, INRA Research Center, 17 rue Sully, BP 86510, F-21065 Dijon, France; lionel.bretillon@dijon.inra.fr.

linkage to chromosome loci.<sup>27,28</sup> Two reports implicated *LOC387715* as a major contributor to AMD susceptibility,<sup>29,30</sup> and recently *HTRA1* (which lies 6 kb distal to *LOC387715*) emerged as a leading contender for the second AMD susceptibility gene.<sup>31,32</sup> The promoter SNP in *HTRA1*, a gene that encodes a heat-shock serine protease found in retinal tissues, produces a change in the expression level of the gene.<sup>31,32</sup> *HTRA1* expression has been reported to increase with advancing age in human fibroblasts.<sup>31</sup> In transgenic mice, overexpression of *HTRA1* induces changes in the elastic layer of Bruch's membrane, which appear fragmented with electron-dense spots<sup>33</sup> and polypoidal choroidal vasculopathy.<sup>34</sup> *LOC387715/ARMS2* has been suggested to encode a mitochondrial-associated protein that also is found in the retina. The common c.205G.T non-synonymous coding SNP (rs10490924), which changes alanine to serine at codon 69 of *LOC387715*, may result in misfolding of the protein.<sup>35</sup>

Cholesterol is a key component of the neural retina and retinal pigment epithelium.<sup>36,37</sup> In humans, it accounts for more than 10% of the lipids therein.<sup>38</sup> Similar to the brain,<sup>39–41</sup> the neural retina expresses a cytochrome P450 enzyme called cholesterol-24S-hydroxylase (*CYP46A1*),<sup>42,43</sup> which can convert cholesterol into 24S-hydroxycholesterol. This pathway has been suggested as a mechanism for controlling cholesterol homeostasis in neurons, at least in the brain.<sup>44</sup> In addition to 24S-hydroxycholesterol, the retina exhibits significant amounts of pregnenolone, which is produced by *CYP11A1*, 27-hydroxycholesterol, and 3 $\beta$ -hydroxy-5-cholestenic acid, an oxidation product of 27-hydroxycholesterol, that are formed by *CYP27A1*.<sup>45</sup> Interestingly, the amount of 3 $\beta$ -hydroxy-5-cholestenic acid exceeded that of 24S-hydroxycholesterol in the human retina and RPE, while 24S-hydroxycholesterol was absent from RPE.<sup>45</sup> Therefore, other mechanisms than 24S-hydroxylation of cholesterol likely may participate in the removal of cholesterol from the retina. Nevertheless, contrary to 27-hydroxycholesterol, which is produced mainly in photoreceptors and RPE, which are the primary sites of AMD, 24S-hydroxycholesterol appears to be neuron-specific.<sup>42,43,45</sup> Assuming these site- and cell-differences, 24S-hydroxycholesterol thus may be associated less likely with AMD than other oxysterols, such as 7-ketocholesterol.<sup>46</sup> However, a link between *CYP46A1*, 24S-hydroxycholesterol, and neurodegeneration in Alzheimer's has been suggested. Overexpression of *CYP46A1* has been reported to reduce amyloid deposits in the hippocampus of transgenic mice carrying the Swedish APP23 mutation and APP/Presenilin 1 mice, as murine models of Alzheimer's disease.<sup>47</sup> A knock out in *CYP46A1* gene in a mouse model of Alzheimer's disease resulted in a prolonged lifespan.<sup>48</sup> SNPs in the *CYP46A1* gene have been identified. In some,<sup>49–60</sup> but not all,<sup>56,61–67</sup> studies, this type of polymorphism was associated with increased risk for late-onset Alzheimer disease. Recently, we found that the T allele in the *CYP46A1* rs754203 SNP was associated with a higher risk for primary open-angle glaucoma.<sup>68</sup>

The aim of our investigation was to provide data on the possible involvement of the rs754203 SNP in *CYP46A1* as an additional risk factor for AMD. For that purpose, AMD patients with GA and CNV were genotyped for the rs754203 SNP in *CYP46A1*, rs10490924 in *LOC387715*, and rs1061170 in *CFH*.

## MATERIALS AND METHODS

### Subjects

The protocol adhered to the tenets of the Declaration of Helsinki. This case-control study was approved by the local Ethics Committee (Dijon,

Bordeaux, and Créteil, France). Informed written consent was obtained from all subjects before participation.

The population comprised 1388 unrelated patients with AMD in at least one eye and 487 unrelated control subjects recruited in the Department of Ophthalmology in Dijon, Créteil, and Bordeaux, France. All control subjects and patients were Caucasian. The diagnosis of AMD was confirmed in cases or infirmed in control subjects by clinical non-mydratric examination followed by fluorescein angiography. All patients included in our study were examined by a retina specialist. The control subjects were recruited among patients operated on for cataract or lid surgery, and willing to participate in the study. Stereoscopic digital fundus photographs were recorded at the time of examination, and images were graded using the definitions of the Wisconsin Age-Related Maculopathy Grading System.<sup>11</sup> The fundi of controls were free of any drusen. AMD patients were divided into two groups: CNV and GA. Blood samples for genotyping were obtained from all the patients with AMD and from control individuals. The brain weight and liver volume were calculated using size and weight data, and established formulas from the literature,<sup>69–71</sup> as used previously<sup>68,72</sup> in 71 control subjects and 128 AMD patients. Plasma was taken in a fasting state from these subjects for measurements of cholesterol and 24S-hydroxycholesterol (see below). The use of cholesterol-lowering drugs was recorded. In this subpopulation we evaluated whether interindividual differences in 24S-hydroxycholesterol levels would be explained by similar differences in the ratio of brain weight to liver volume. Given that the brain accounts for about 80% of 24S-hydroxycholesterol production in humans,<sup>39,41</sup> and the liver is responsible for its elimination,<sup>39</sup> we assumed that this ratio would be the best marker for the capacity of the organism to synthesize and metabolize 24S-hydroxycholesterol, and to reflect plasma 24S-hydroxycholesterol levels.<sup>72</sup>

### CYP46A1, CFH, and LOC387715 Genotyping

DNA samples were evaluated for the rs754203 SNP in *CYP46A1*, rs10490924 in *LOC387715*, and rs1061170 in *CFH* using either an Affymetrix 6.0 platform (Créteil, France) as previously described<sup>73</sup> or in our own facilities.<sup>68</sup>

### Quantification of Plasma 24S-Hydroxycholesterol Levels

Plasma 24S-hydroxycholesterol levels were assayed in a subset of 71 subjects and 128 AMD cases from the Department of Ophthalmology (University Hospital, Dijon, France) by isotope dilution mass spectrometry using racemic [23,23,24-<sup>2</sup>H<sub>3</sub>]-24-hydroxycholesterol, as described previously.<sup>39,41,72,74</sup> Briefly, deuterated 24-hydroxycholesterol (200 ng, [23,23,24-<sup>2</sup>H<sub>3</sub>]-24-hydroxycholesterol) was added to 0.5 mL of plasma. After alkaline hydrolysis with 1 N KOH in 90% ethanol for 2 hours, the solution was neutralized with 65  $\mu$ L of phosphoric acid and the sterols were extracted with 9 mL of chloroform in the presence of 3 mL of 0.9% sodium chloride. The organic phase was removed and the solvent was evaporated to dryness. The sterols were dissolved in 1 mL of toluene. A 100  $\mu$ L aliquot was removed for quantification of the cholesterol concentration. For this purpose, 30  $\mu$ g of 5 $\alpha$ -cholestane were added, the solvents were evaporated to dryness, and the sterols were derivatized to trimethylsilyl ethers by heating at 60°C after the addition of 200  $\mu$ L of pyridine and 200  $\mu$ L of BSTFA (Supelco, Bellefonte, PA). The solvents were evaporated under nitrogen gas, and the samples were resuspended in hexane and analyzed by gas chromatography: electron ionization mass spectrometry using an HP6890 series II plus a chromatograph combined with an HP mass selective detector operated in selected ion monitoring mode. A 1  $\mu$ L aliquot was injected by automated injection in a splitless mode at an injection temperature of 300°C on a DB-5MS fused silica capillary column (30 m  $\times$  0.25 mm id, 0.25  $\mu$ m film thickness; J&W Scientific, Agilent Technologies, Massy, France). The initial oven temperature was kept at 50°C for 1 minute, then it was increased at a rate of 20°C/min to

250°C and thereafter at 5°C/min to a final temperature of 300°C. The temperature of the transfer line was kept at 300°C. Electron impact ionization was used at 70 eV ionization energy. Trimethylsilyl-cholesterol and 5 $\alpha$ -cholestane were measured at m/z 368 and 372 amu, respectively. Absolute amounts of cholesterol were determined by interpolation from a standard curve generated in each experiment.

The remainder of the saponified sterols was purified on silica columns (Supelco) for quantification of 24S-hydroxycholesterol. Cholesterol was eluted with 8 mL of 0.5% isopropanol in hexane. Purified oxysterols subsequently were eluted with 5 mL of 30% isopropanol in hexane and derivatized to trimethylsilyl ether as described above. The samples were analyzed by gas chromatography-mass spectrometry as described above. 24S-Hydroxycholesterol and deuterated 24-hydroxycholesterol were measured at m/z 413 and 416 amu, respectively. Absolute amounts of 24S-hydroxycholesterol were determined by interpolation from a standard curve generated in each experiment.

### Statistical Analyses

The Hardy-Weinberg assumption was assessed by the standard method comparing the observed numbers of subjects in different genotype categories with the expected number under Hardy-Weinberg equilibrium for the estimated allele frequency, and testing with a Pearson goodness-of-fit statistic using the  $\chi^2$  with 1 degree of freedom. The  $\chi^2$  test was used to compare categorical allelic and genotype distributions between cases and controls. General linear models were used to compare means between cases and controls. Logistic regression models were used to estimate the odds ratio (OR) with a 95% confidence interval (95% CI) for AMD risk. ORs were adjusted for age and gender. Significance levels were set at  $P < 0.05$ . Analyses were performed with the SAS software release 9.02 (SAS Institute Inc., Cary, NC).

### RESULTS

The clinical characteristics of control subjects and AMD patients are presented in Table 1. The control group was significantly younger than the AMD cases ( $P < 0.01$ ). There was a preponderance of females over males in the AMD group, as expected for this age group.

**TABLE 1.** Characteristics of the Population of Control Subjects and AMD Cases, and Distribution of the Genotypes

	Controls	AMD Cases	P Value
<i>n</i>	487	1388	
Males (%)	39.8%	33.1%	0.008
Age (y $\pm$ SD)	68.3 $\pm$ 8.5	78.8 $\pm$ 7.4	<0.0001
<i>CFH</i> gene (rs1061170 SNP)			
TT	188 (38.6%)	291 (21.0%)	<0.0001
TC	234 (48.1%)	708 (51.0%)	
CC	65 (13.3%)	389 (28.0%)	
C allele frequency	0.374	0.535	<0.0001
<i>LOC387715</i> gene (rs10490924 SNP)			
GG	299 (61.4%)	449 (32.4%)	<0.0001
GT	171 (35.1%)	644 (46.4%)	
TT	17 (3.5%)	295 (21.2%)	
T allele frequency	0.211	0.444	<0.0001
<i>CYP46A1</i> gene (rs754203 SNP)			
TT	259 (53.2%)	691 (49.8%)	0.42
CT	188 (38.6%)	580 (41.8%)	
CC	40 (8.2%)	117 (8.4%)	
C allele frequency	0.280	0.293	0.45

The genotype and allelic frequencies for rs10490924 in *LOC387715*, rs1061170 in *CFH*, and rs754203 in *CYP46A1* are given in Table 1. The frequency of the rs10490924 risk T allele in the *LOC387715* gene and rs1161170 C allele in the *CFH* gene was significantly higher ( $P < 0.0001$ ) in AMD patients than in control subjects (Table 1). There was no statistically significant difference between the frequency of the rs754203 risk C allele in the *CYP46A1* gene in control subjects and AMD cases ( $P > 0.05$ , Table 1).

We then determined whether there was any association between the risk alleles of rs754203 in *CYP46A1*, rs10490924 in *LOC387715*, and rs1061170 in *CFH* genes and the clinical phenotype. The allele and genotype ORs of the SNPs in AMD and control patients are given in Table 2.

The rs10490924 SNP in the *LOC387715* gene was significantly ( $P < 0.0001$ ) associated with AMD patients. The age- and gender-adjusted OR was 13.9 (95% CI = 7.9-24.2) for the homozygous TT genotype and 2.7 (95% CI = 2.0-3.5) for the heterozygous GT genotype, compared to the GG genotype (Table 2).

The rs1061170 SNP in the *CFH* gene was associated significantly with increased risk of AMD ( $P < 0.0001$ , Table 2). Compared to the TT genotype, the age- and gender-adjusted OR for the risk of AMD was 4.8 (95% CI = 3.2-7.0) for the homozygous CC genotype and 2.4 (95% CI = 2.0-3.5) for the heterozygous CT genotype.

The rs754203 SNP in the *CYP46A1* gene was not associated with AMD (Table 2). No significant difference between *CYP46A1* genotypes in the AMD and control groups was observed.

We also assessed the joint effects of the rs754203 SNP in *CYP46A1* with the SNP in *LOC387715* and *CFH*. An OR of 2.2 (95% CI = 1.1-4.4,  $P = 0.04$ ) was obtained for carriers of the C allele in the *CYP46A1* gene and no risk allele in the *CFH* and *LOC387715* genes (Table 2).

Plasma cholesterol, 24S-hydroxycholesterol and 24S-hydroxycholesterol/cholesterol levels for the three genotypes of *CYP46A1* in 71 control subjects and 128 AMD patients are given in Table 3. AMD patients presented a significant difference in plasma cholesterol ( $P < 0.01$ ), 24S-hydroxycholesterol levels ( $P < 0.01$ ), and the 24S-hydroxycholesterol-to-cholesterol ratio ( $P = 0.04$ ) compared to control subjects (Table 3). A gender effect in plasma cholesterol levels was found in control subjects only. After adjusted by gender, SNP, and statin use, the difference remained at the limit of significance in controls ( $P = 0.0579$ ), women homozygous for the T allele in *CYP46A1* gene SNP had significantly lower plasma cholesterol levels than women with the C allele ( $P = 0.0261$ ) and men homozygous for the T allele ( $P = 0.0042$ ).

The Figure shows the plasma cholesterol-related levels of 24S-hydroxycholesterol in control subjects (part A of Figure) and AMD patients (part B of Figure) from the age of 50 years onward. The ratio between calculated brain weight and liver volume for the different ages, based on data from the literature<sup>69-71</sup> and calculated in each of the control subjects and patients, also is indicated in the Figure. As initially postulated<sup>72</sup> and verified in glaucoma patients,<sup>68</sup> this ratio can be used as an index for the overall capacity of the body to synthesize and metabolize 24S-hydroxycholesterol, especially in infancy and adulthood and marginal changes in the elderly.<sup>72</sup> The levels of 24S-hydroxycholesterol closely follow the brain weight-to-liver volume ratio during the different decades of life. No differences were found between control subjects and AMD patients, although a slight, but not significant increase in the plasma 24S-hydroxycholesterol level was observed in AMD patients at older ages, which is consistent with previous findings<sup>72</sup> and with the more advanced age of our AMD population compared to controls.

TABLE 2. Crude and Adjusted ORs for rs1061170 in *CFH*, rs10490924 in *LOC387715*, and rs754203 in *CYP46A1* Gene in AMD Cases

	Crude OR (95% CI)	P Value	Adjusted for Gender and Age	P Value
<i>CFH</i> (rs1061170)		<0.0001		<0.0001
TT	1 (ref)		1 (ref)	
TC	2.0 (1.6-2.5)		2.4 (1.8-3.2)	
CC	3.9 (2.8-5.3)		4.8 (3.2-7.0)	
/n of C allele	2.0 (1.7-2.3)	<0.0001	2.2 (1.8-2.7)	<0.0001
<i>LOC387715</i> (rs10490924)		<0.0001		<0.0001
GG	1 (ref)		1 (ref)	
GT	2.5 (2.0-3.1)		2.7 (2.0-3.5)	
TT	11.6 (6.9-19.3)		13.9 (7.9-24.2)	
/n of T allele	2.9 (2.4-3.4)	<0.0001	3.2 (2.6-3.9)	<0.0001
<i>CYP46A1</i> (rs754203)				
Total population		0.42		0.32
TT	1 (ref)		1 (ref)	
CT	1.2 (0.9-1.4)		1.2 (0.9-1.4)	
CC	1.1 (0.8-1.6)		1.2 (0.8-1.6)	
CT+CC vs. TT	1.2 (0.9-1.4)	0.20	1.2 (0.9-1.5)	0.13
Patients at no risk in <i>CFH</i> (rs1061170) and <i>LOC387715</i> (rs10490924)				
CT+CC (n = 87) vs. TT (n = 102)	1.7 (0.9-3.1)	0.08	2.2 (1.1-4.4)	0.04
AMD cases with choroidal neovascularization				
CT+CC (n = 40) vs. TT (n = 33)	1.2 (0.9-1.4)	0.17	1.2 (0.9-1.6)	0.12

## DISCUSSION

Familial aggregation studies, twin studies, and segregation analyses have provided strong evidence for the heritability of AMD.<sup>75</sup> Our data confirmed the association between exudative AMD and the rs10490924 SNP in the *LOC387715* gene and rs1061170 in the *CFH* gene. Consistent with previous findings in Caucasians and Chinese populations,<sup>32,76-78</sup> our study showed that the exudative AMD risk is higher with the T allele of rs10490924 in *LOC387715* and the C allele of rs1061170 in the *CFH* gene. The *LOC387715* T allele frequency was 0.211 in control subjects, similar to that reported for a population of European descent.<sup>79</sup> In multiple studies examining linkage disequilibrium in the 10q26 region, the *HTRA1*, and *LOC387715* SNPs have been reported to be in almost complete linkage disequilibrium.<sup>76,78,80</sup> *LOC387715* mRNA is detected in the human retina<sup>35</sup> and retinal pigment epithelium.<sup>35,81</sup> It encodes a 12 kilodalton (kDa) protein, which localizes to the extracellular matrix,<sup>82</sup> and mitochondrial outer membrane when expressed in mammalian cells.<sup>35</sup> The risk T allele of SNP rs10490924 maps to exon 1 of the hypothetical *LOC387715* gene and changes putative amino acid 69 from alanine to serine (A69S), affecting its function in mitochondria and increasing the susceptibility to ageing-associated degeneration of the macular photoreceptors.<sup>35</sup>

As reviewed recently, chronic local inflammation participates in drusen formation within Bruch's membrane, and consequently in the pathophysiology of AMD.<sup>21</sup> *CFH* is the gene encoding complement factor H and one of the members of the complement cascade that has a role in inflammatory processes. Laine et al. reported that the association of the *CFH* Y402H polymorphism with AMD could be due to reduced clearance of cellular debris and increased local inflammation.<sup>83</sup>

The main objective of our present study was to provide data on the *CYP46A1*-T/C polymorphism in patients with AMD. The frequency of the *CYP46A1* C allele in our control subject population (28%) was comparable with other Caucasian populations from Europe.<sup>50,64,67</sup> Our finding provided evidence that the *CYP46A1* rs754203 polymorphism per se is not associated with AMD, but may be associated with AMD in cases

carrying no risk alleles in the *CFH* and *LOC387715* genes. Although the association reached statistical significance, it is difficult to draw firm conclusions due to the relatively small size of the present population. Further studies with larger populations may be needed to confirm our findings. Cholesterol-24S-hydroxylase (*CYP46A1*) is a cholesterol-metabolizing enzyme involved in the removal of cholesterol from neuronal structures.<sup>39-41,72,84</sup> The TT genotype in intron 2 of *CYP46A1*, designated as the rs754203 SNP, has been identified as a risk factor for Alzheimer disease<sup>53,54,58,60</sup> and, more recently, for primary open-angle glaucoma.<sup>68</sup> *CYP46A1* and its metabolic product 24S-hydroxycholesterol are specific for the neural retina, and especially for retinal ganglion cells.<sup>42,43</sup> Since the loss of retinal ganglion cells is associated with glaucomatous retinopathy, it is, therefore, clearly understandable that we found a *CYP46A1* SNP association with glaucoma,<sup>68</sup> but not with AMD. Indeed, although there is neuronal degeneration in the course of AMD, it involves primarily photoreceptors and not retinal ganglion cells.

The relevance of circulating 24S-hydroxycholesterol levels has been questioned not only in neurodegenerative diseases,<sup>74,85</sup> but also with oxidative stress.<sup>86</sup> However, the clinical value of this still is under discussion.<sup>85</sup> Similar to our previous data in glaucoma patients,<sup>68</sup> we suggest that 24S-hydroxycholesterol cannot be used as a biomarker for AMD. Interestingly, the highest levels of 24S-hydroxycholesterol were observed in patients with the C-allele in the *CYP46A1* SNP. These data somehow are consistent with the finding that the highest risk for AMD in patients not carrying a risk allele in rs1061170 SNP in the *CFH* gene and rs10490924 SNP in the *LOC387715* gene was found in patients with the C-allele in rs754203 SNP in the *CYP46A1* gene. As discussed previously, the ratio of 24S-hydroxycholesterol to cholesterol may be a better marker for the production of this oxysterol than the absolute amount.<sup>72</sup> Based on this assumption, no difference was observed in plasma 24S-hydroxycholesterol between AMD and control subjects. The higher plasma cholesterol level and, as a consequence, the lower 24S-hydroxycholesterol-to-cholesterol ratio in AMD patients than in controls may be explained more likely by chance than a disease effect. Indeed, the association

TABLE 3. Plasma Cholesterol, 24S-Hydroxycholesterol and 24S-Hydroxycholesterol/Cholesterol Levels for the Genotypes in rs754203 SNP in CYP46A1 Gene in 71 Control Subjects and 128 AMD Cases

	n		Plasma Cholesterol (mg/mL)		24S-Hydroxycholesterol (µg/mL)		24S-Hydroxycholesterol/Cholesterol (ng/mg)		P Value	
	Control	AMD	Control	AMD	Control	AMD	Control	AMD		
All genotypes	71	128	0.81 ± 0.04	1.04 ± 0.04	<0.01	89.6 ± 4.9	102.0 ± 3.1	126.3 ± 6.7	115.0 ± 5.6	0.04
Subjects using cholesterol-lowering drugs (n, %)	13, 18.3%	29, 22.7%								
CYP46A1 genotypes										
CC+CT	37	65	0.78 ± 0.07	1.04 ± 0.06	0.0013	80.5 ± 4.6	103.1 ± 3.7	125.5 ± 9.1	119.2 ± 7.8	ns
TT	34	63	0.85 ± 0.06	1.04 ± 0.06	0.031	99.2 ± 8.6	100.7 ± 5.7	127.2 ± 9.9	110.4 ± 8.0	ns
			Control	AMD	All	Control	AMD	Control	AMD	All
Gender effect (P value)			0.0255	ns	ns	ns	ns	ns	ns	ns
SNP effect (P value)			ns	ns	ns	ns	ns	ns	ns	ns
Statin effect (P value)			ns	ns	ns	ns	ns	ns	ns	ns
Gender and SNP interaction (P value)			0.0593	ns	ns	ns	ns	ns	ns	ns
Gender and SNP and statin interaction (P value)			0.0579	ns	ns	ns	ns	ns	ns	ns

Mean ± SEM. ns, not significant (P > 0.05).

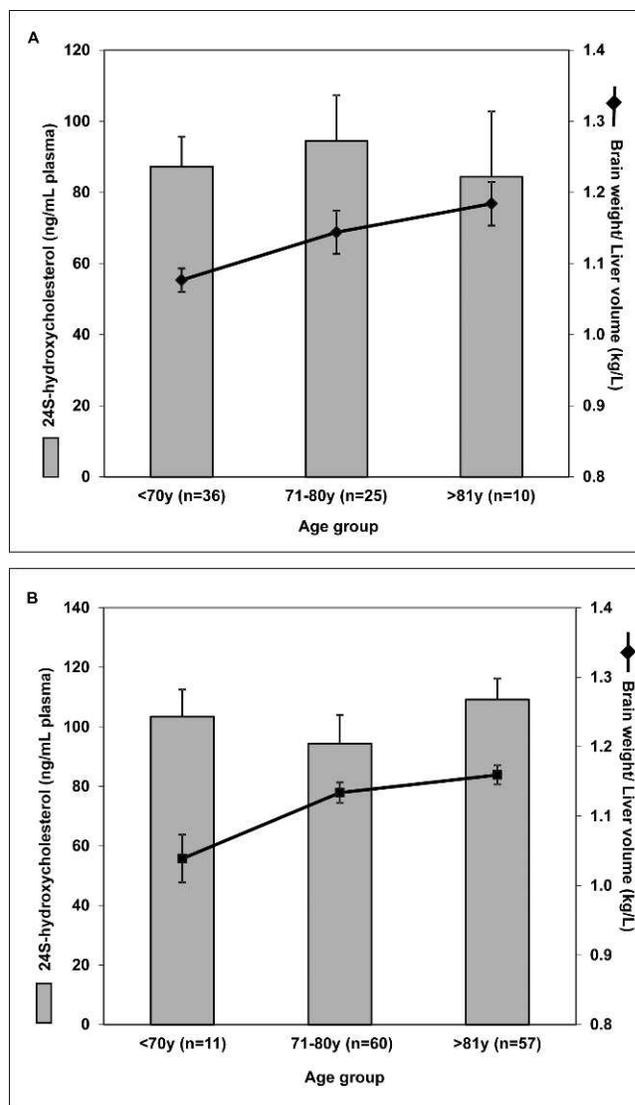


FIGURE. Plasma 24S-hydroxycholesterol levels (ng/mL) in normal subjects (A) and AMD cases (B) as a function of age. Results are expressed as means ± SEM. Estimated ratio between brain weight and liver volume is indicated.

of plasma cholesterol and AMD has not been established firmly.<sup>87,88</sup> Moreover, we must point out the low levels of plasma cholesterol in our controls and AMD patients, compared to the general population. Several biases in our study may participate in this finding. First, and probably more likely, we cannot exclude a niche effect accounting for the limited number of subjects. Secondly, the advanced age of controls and AMD patients (68 and 78 years, respectively) may contribute to the low values, accounting the trend for a reduction of total cholesterol in the elderly.<sup>89</sup> Lifestyle is one of the more potent environmental factors that modifies plasma lipids. Controls and AMD patients, in whom plasma cholesterol was quantified, have been selected in one single ophthalmology center (Dijon, France). Therefore, the value of plasma cholesterol would not be representative of the general population, but of a restricted part of the population from a middle-size city.

AMD is a multifactorial disease in which the risk can be explained in half of the population of AMD cases by five polymorphisms in three genes: *CFH*, *LOC387715*, and *CFB*.

C2.<sup>90</sup> Two of these five SNPs have been analyzed in our study. Gene polymorphism in *LIPC*<sup>91</sup> and *SCARB1*<sup>73</sup> recently have been identified as potential risk factors for AMD. Hepatic lipase and SRB1 are the products of *LIPC* and *SCARB1* genes, respectively. Hepatic lipase and SRB1 share the common property to modulate cholesterol metabolism by affecting HDL-cholesterol levels and cellular uptake of cholesterol. These two genes deserve further investigations in connection with cholesterol and AMD, independently from their effects on plasma HDL cholesterol levels. Indeed, variants in the *LIPC* gene have been associated with a greater risk for AMD.<sup>92,93</sup> To our knowledge, our study is the first report providing data on the possible, although minor, involvement of the *CYP46A1* polymorphism in AMD cases who do not carry two potent SNPs. The link between the SNP and disease remains unknown. *CYP46A1* expression, and possibly activity, is susceptible to epigenetic regulation.<sup>94</sup> Further work is warranted to evaluate whether the SNP in rs754203 would be associated with differences in the response to epigenetic regulation.

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