Associations between CYP2C8 rs10509681 and rs11572080 gene polymorphisms and age-related macular degeneration

Rasa Liutkevičienė¹,²,
Ramunė Sungailienė¹,
Alvita Vilkevičiūtė²,
Lorea Kriauciūnienė¹,²,
Paulina Vaitkiene³,
Romanas Chaleckis³,
Vytenis Pranas Deltuva²

¹Department of Ophthalmology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
²Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
³Gunma University Initiative for Advanced Research, Maebashi, Gunma, Japan

Background. Age-related macular degeneration (AMD) is the most common cause of irreversible visual loss in industrialized countries. Early symptoms of AMD include drusen and changes in retinal pigment epithelium. However, the etiology of AMD and drusen formation is not fully understood. Recent studies suggest that CYP2C8-related metabolic processes might play an important role in the development of AMD. The aim of our study is to investigate CYP2C8 rs10509681 and CYP2C8 rs11572080 genotype frequencies in patients with early AMD and to compare them with healthy controls.

Materials and Methods. The study enrolled 305 patients with early AMD and 300 healthy controls. The genotyping of CYP2C8 rs10509681 and CYP2C8 rs11572080 were carried out using the real-time PCR method.

Results. The analysis of studied CYP2C8 polymorphisms did not reveal any statistically significant differences between the AMD and the control groups. For the CYP2C8 rs10509681 gene polymorphism the distribution of T/T, T/C, and C/C genotypes was 83.3%, 16.7%, and 0% vs. 83.7%, 15.7%, and 0.7%, p = 0.343. For the CYP2C8 rs11572080 gene polymorphism the distribution of C/C, T/C and T/T genotypes was 84.9%, 15.1%, and 0% vs. 82.3%, 17.3%, and 0.3%, p = 0.447.

Conclusion. The study revealed that there were no statistically significant differences in the distribution of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes in patients with early AMD and in healthy controls.

Keywords: Age-related macular degeneration, cytochrome P450, rs10509681, rs11572080, gene polymorphisms
INTRODUCTION

Age-related macular degeneration (AMD) is a degenerative disease that affects the central part of the retina. Specific changes in the macular area such as drusen formation and changes in retinal pigment epithelium (RPE) in the absence of the other ophthalmological pathologies characterize AMD (1). It is the most common cause of blindness in the developed countries (1) and usually affects persons older than 50 years of age (2).

In addition to age, AMD has been reported to have multiple risk factors including gender, race, obesity, hypercholesterolemia, hyperglycaemia, heart diseases, hypertension, iris colour, hyperopia, lifestyle (smoking, alcohol abuse, eating disorders, physical inactivity, stress), and environmental factors such as ultraviolet radiation (3–6). For example, it was found that the more cigarettes were smoked per day, the greater was the likelihood of developing AMD, and vice versa – quitting smoking reduced the risk (2, 7, 8). Individuals who did not smoke for more than 20 years had the same probability of developing AMD as people who had never smoked (2). Also, the risk of AMD development is increased by a diet low in antioxidants, namely, vitamins C and E, carotenoids (lutein and zeaxanthin), and zinc. Healthier food or dietary supplements correlate with a slower progression of AMD (2). The best food for AMD prevention contains omega-3 polyunsaturated fatty acids, which are mostly found in oily fish (salmon, herring). In contrast, food containing saturated fats increases the risk of AMD (2).

Composed mostly of lipids, the extracellular deposits or drusen are the hallmark of AMD (9). These deposits build up beneath RPE and Bruch’s membrane (1) resulting in damage and redistribution of RPE cells (10). Disruption of the oxygen metabolism leads to photoreceptor degeneration associated with the impairment of visual functions (11).

Drusen develop during immune-mediated metabolic processes. Genetic factors related to lipid metabolism are thought to play a role in the progression of the AMD. However, the exact mechanism by which the lipids accumulate in RPE is not fully understood. Some authors speculate that the origin of the lipids in the drusen is the circulatory system associated with cholesterol changes in the blood (11); others speculate that the deposits are a consequence of the locally produced lipoproteins of the Bruch’s membrane with different density and structure (12). The latter hypothesis is supported by the observation that an oxidative stress leads to the impairment of barrier properties and increased permeability of Bruch’s membrane (13). It is believed that genetic factors may have a significant impact on the development of the disease. One of the candidates is the Cytochrome P450 2C8 (CYP2C8) gene, a member of the multifunctional oxidase system, involved in the metabolism of xenobiotics. It is also responsible for the activity of the epoxygenases involved in the metabolism of the long chain polyunsaturated fatty acids, such as eicosapentaenoic acid and docosapentaenoic acid – the omega 3 fatty acids found in fish oil (14, 15). As unsaturated fatty acids have a beneficial impact on AMD, the polymorphisms of the CYP2C8 gene may be involved in the development of AMD.

In addition, a comparison of AMD and Alzheimer’s disease (reviewed in Çerman et al.) indicates common pathological pathways. Both AMD and Alzheimer’s disease share common features such as vitronectin and amyloid-β accumulation, increased oxidative stress, apolipoprotein and complement activation pathways (16). A study by Yan et al. identified CYP2J2 rs890293 being a possible predisposing genetic factor for late-onset Alzheimer’s disease progression in the Chinese Han population (17). These findings have led us to the hypothesis that the genes involved in the pathogenesis of Alzheimer’s disease and lipid metabolism, such as genes of the cytochrome P450 family, may be associated with AMD. Therefore, CYP2C8 rs10509681 and CYP2C8 rs11572080 single nucleotide polymorphisms were chosen to be investigated in patients with early AMD.

Here we report the frequencies of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes in patients with early AMD in the Lithuanian population.

MATERIALS AND METHODS

Ethics statement
Permission to conduct the study was obtained from the Ethics Committee for Biomedical Research (No. BE-2-/13). All donors provided written
informed consent in accordance with the Declaration of Helsinki.

**Study population**
A total of 305 patients with the diagnosis of early AMD were enrolled in the study, based on exclusion criteria described below. The control group comprised 300 persons who had no ophthalmologic pathology on examination and agreed to participate in the study. The AMD patients and the controls were matched by age and gender ($p > 0.05$) (Table 1).

| Table 1. Demographic characteristics of the study population |
|------------------|------------------|------------------|
| Characteristic   | Group n = 305   | Group n = 300   |
|------------------|------------------|------------------|
| Men, n (%)       | 91 (29.84)       | 77 (25.67)       |
| Women, n (%)     | 214 (70.16)      | 223 (74.33)      |
| Age, mean        | 67.52            | 66.42            |
| p value          | 0.252*           | 0.9994*          |

* not significant – $p > 0.05$

For the analysis, the study population was divided into two groups according to their age: younger than 65 years; and 65 years and older.

**Ophthalmological evaluation**
Ophthalmological evaluation for all subjects in our study was carried out as described above (18).

**DNA extraction and genotyping**
DNA was extracted from leucocytes using a GeneJET Genomic DNA Purification Kit (Thermo Scientific). The polymorphisms (rs10509681 and rs11572080) in the CYP2C8 gene were analyzed using TaqMan® Drug Metabolism assays (Applied Biosystems, USA) on a Rotor-Gene Q RT-PCR system (Qiagen, USA). DNA extraction and genotyping were performed following the same procedure described earlier (18).

**Statistical analysis**
Statistical analysis was performed using SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA). The data are presented as absolute numbers with percentages and mean values. The frequencies of genotypes (in percentage) are presented in Table 2.

| Table 2. Frequency of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes in all patients with early AMD and in control group |
|------------------|-------------|-------------|-------------|
| Gene marker      | Genotype/Allele | Control n (%) | p value HWE | AMD n (%) | p value HWE | p value |
|------------------|----------------|--------------|-------------|-----------|-------------|---------|
| CYP2C8 rs10509681| T/T            | 251 (83.7)   | 0.901       | 254 (83.3) | 0.112       | 0.343   |
|                  | C/C            | 47 (15.7)    |             | 51 (16.7)  |             |         |
|                  | Total          | 2 (0.7)      |             | 0 (0)      |             |         |
| Allele           |                | 300 (100)    |             | 305 (100)  |             |         |
| T                | 495 (83.62)    |              |             | 264 (88)   |             |         |
| C                | 97 (16.38)     |              |             | 36 (12)    |             |         |
| CYP2C8 rs11572080| C/C            | 247 (82.3)   | 0.313       | 259 (84.9) | 0.154       | 0.447   |
|                  | C/T            | 52 (17.3)    |             | 46 (15.1)  |             |         |
|                  | T/T            | 1 (0.3)      |             | 0 (0)      |             |         |
| Total            |                | 300 (100)    |             | 305 (100)  |             |         |
| Allele           |                |              |             |           |             |         |
| C                | 546 (91)       |              |             | 564 (92.46)|             |         |
| T                | 54 (9)         |              |             | 46 (7.54)  |             |         |
Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of CYP2C8 rs10509681 and CYP2C8 rs11572080 using the $\chi^2$ test in all groups. The distribution of both (rs10509681 and rs11572080) SNPs in AMD and control groups were compared using the $\chi^2$ test or the Fisher exact test. Binomial logistic regression analysis was performed to estimate the impact of genotypes on the development of AMD. Odds ratios and 95% confidence intervals were calculated but did not show any statistical significance. Differences were considered statistically significant when $p < 0.05$.

RESULTS

The frequencies of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes in all healthy controls and patients with early AMD are shown in Table 2. The distribution of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes and alleles in all patients with early AMD and in the control group was consistent with the Hardy-Weinberg equilibrium. The analysis of CYP2C8 rs10509681 and CYP2C8 rs11572080 gene polymorphisms including all subjects did not reveal any differences in the distribution of genotypes and between the patients with AMD and the controls (CYP2C8 rs10509681 gene polymorphism genotypes T/T, T/C, and C/C (83.3%, 16.7%, and 0% vs. 83.7%, 15.7%, and 0.7%, $p = 0.343$); and CYP2C8 rs11572080 gene polymorphism genotypes C/C, T/C and T/T (84.9%, 15.1%, and 0% vs. 82.3%, 17.3%, and 0.3%, $p = 0.447$).

The comparison of the frequency of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes in age groups did not show any significant differences (Table 3).

The comparison of the frequency of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes by gender did not show any significant differences either (Table 4).

Binomial logistic regression analysis in the patients with AMD and in the control group did not reveal any significant variables either.

Table 3. Frequency of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes in patients with early AMD and control subjects by age

| Gene     | Genotype/Allele | <65 years | p value | ≥65 years | p value |
|----------|-----------------|-----------|---------|-----------|---------|
|          | AMD n (%)       | Control n (%) |         | AMD n (%) | Control n (%) |
| CYP2C8  | Genotype        |            |         |           |         |
| rs10509681 | T/T             | 78 (87.6) | 184 (83.6) | 0.510 | 176 (81.5) | 67 (83.3) | 0.734 |
|          | T/C             | 11 (12.4) | 34 (15.5) |         | 40 (18.5) | 13 (16.2) |       |
|          | C/C             | 0 (0)     | 2 (0.9)  |         | 0 (0)     | 0 (0)     |       |
|          | Allele          |            |         |           |         |
|          | T               | 167 (93.82)| 402 (31.36) | 184 (87.71)| 147 (91.875)|       |
|          | C               | 11 (6.18) | 38 (8.64)  | 29 (12.29) | 13 (8.125) |       |
| CYP2C8  | Genotype        |            |         |           |         |
| rs11572080 | C/C             | 80 (89.9) | 183 (82.8) | 0.160 | 179 (82.9) | 64 (81.0) | 0.250 |
|          | C/T             | 9 (10.1)  | 38 (17.2)  | 37 (17.1)  | 14 (17.7)  |       |
|          | T/T             | 0 (0)     | 0 (0)     |         | 0 (0)     | 1 (1.3)  |       |
|          | Allele          |            |         |           |         |
|          | C               | 169 (94.94)| 404 (91.4)  | 395 (91.44) | 142 (89.87) |       |
|          | T               | 9 (5.06)  | 38 (8.6)   | 37 (8.56)  | 16 (10.13) |       |
**DISCUSSION**

We observed no statistically significant differences between the AMD group \((n = 305)\) and the control group \((n = 300)\) in the distribution of CYP2C8 gene rs10509681, rs11572080 polymorphism genotypes. Neither did we find any statistically significant differences in polymorphisms according to the age or gender. To the best of our knowledge, we are the first to investigate the CYP2C8 gene polymorphisms in AMD patients. On the basis of our research results, we conclude that CYP2C8 gene rs10509681 and rs11572080 polymorphisms are not associated with the manifestation of early AMD.

Similar to our results, no differences between disease and control groups for the CYP2C8 were reported in colorectal cancer and coronary heart disease. Ladero et al. investigated CYP2C8 gene polymorphism rs11572080 in patients with colorectal cancer \((n = 153)\) and in healthy individuals \((n = 298)\) (19). Craig et al. investigated CYP2C8 rs10509681 gene polymorphism in 980 patients with coronary heart disease and 1085 healthy individuals (20). The C/C genotype was observed in 81.3% of patients, while the results of C/T and T/T genotypes were summed up and made up 18.7%, which is similar to our findings \(\text{CC} 83.7\%, \text{C/T} 15.7\%, \text{T/T} 0.7\% – \text{Table 2}\). Our reported CYP2C8 gene polymorphism distribution in healthy Lithuanian population is consistent with previous reports.

Spanish subjects were: T/T 67.8%, T/C 30.2%, C/C 2.0% (19), while the genotype distribution in our group of healthy subjects was: the T/T genotype was dominant and was observed in 82.3% of patients, the T/C genotype in 17.3% of patients, and the C/C in 0.3%. CYP2C8 gene polymorphism distribution in healthy Lithuanian population is consistent with previous reports.

CYP2C8 gene polymorphisms were found to be associated with essential hypertension (EH) (21). CYP2C8*3 allele was statistically significantly more frequent in men with EH (21). Arterial hypertension (AH) is one of the risk factors that would provoke AMD, regardless of gender (22). AH can damage

### Table 4. Frequency of CYP2C8 rs10509681 and CYP2C8 rs11572080 genotypes in patients with early AMD and control group by gender

| Gene         | Genotype/Allele | Men | Women | p value | Men | Women | p value |
|--------------|-----------------|-----|-------|---------|-----|-------|---------|
| CYP2C8       | rs10509681      |     |       |         |     |       |         |
|              | T/T             | 81  | 64    | 0.368   | 173 | 187   | 0.452   |
|              | T/C             | 10  | 12    | 0.492   | 41  | 35    | 0.378   |
|              | C/C             | 0   | 1     | 0.458   | 0   | 1     | 1.00    |
|              | Allele          |     |       |         |     |       |         |
|              | T               | 172 | 140   | 0.176   | 106 | 409   | 1.00    |
|              | C               | 10  | 12    | 0.251   | 37  | 39    | 1.00    |
| CYP2C8       | rs11572080      |     |       |         |     |       |         |
|              | C/C             | 82  | 63    | 0.176   | 177 | 184   | 1.00    |
|              | C/T             | 9   | 13    | 0.251   | 37  | 39    | 1.00    |
|              | T/T             | 0   | 1     | 0.458   | 0   | 0     | 1.00    |
|              | Allele          |     |       |         |     |       |         |
|              | C               | 173 | 139   | 0.176   | 391 | 407   | 1.00    |
|              | T               | 9   | 14    | 0.251   | 37  | 39    | 1.00    |
arteries and arteriolars of the rear submacular area, causing local ischemia and impeding the removal of metabolic products, which leads to AMD development (23). In 2003, Klein et al. suggested that the risk of the development of exudative AMD increases 1.5-fold in men with hypertension (24). Therefore, we assumed that CYP2C8 (rs10509681 and rs11572080) polymorphisms may be associated with the development of exudative AMD in men, especially in patients with arterial hypertension. However, our results do not corroborate such assumptions.

CYP2C8 gene (rs10509681 and rs11572080) polymorphism has been reported to be associated with drug metabolism (reviewed in Daily E et al.). For example, the association between the CYP2C8 gene and serum lipid-lowering drugs was noticed, particularly with cerivastatin and, to a lesser extent, with fluvatstatin and simvastatin (25). In the review, a close relationship was concluded not only between CYP2C8 and AMD risk factors such as hyperglycaemia, hypercholesterolemia, obesity, hypertension, and heart disease, but also between the medications that control risk factors and metabolism.

The main limitations of this study are that it did not investigate the patients with late forms of AMD (exudative and atrophic). Neither did we take into consideration information on the usage of medications. Our results should be confirmed in a larger sample size study involving patients with exudative and atrophic AMD as well as including usage of medication. Ideally patients should be followed up in order to find which form of AMD (wet or dry) will manifest in later years.

CONCLUSIONS

To the best of our knowledge, this is the first study to examine CYP2C8 rs10509681 and CYP2C8 rs11572080 genotype frequencies in patients with early AMD. While we could not find differences in genotype frequencies between the healthy controls and patients with early AMD, we think that larger and detailed studies including more aspects of analysis, such as medications and lifestyle, might still elucidate the role of CYP2C8 in the development of AMD.

The CYP2C8 gene polymorphisms we examined are only part of studies (18, 26) performed at our laboratory to better understand the roles of genetic markers in AMD development.

ACKNOWLEDGEMENTS

This work was funded by a grant (No. SEN-11/2015) from the Research Council of Lithuania.

Received 23 February 2017
Accepted 1 June 2017

References

1. Bowling B. Kanski’s clinical ophthalmology, a systematic approach. 8th ed. Elsevier; 2016.
2. American Academy of Ophthalmology Retinal/Vitreous Panel. Preferred practice pattern guidelines. Age-related macular degeneration. San Francisco: American Academy of Ophthalmology; 2015.
3. Neverauskiene J, Chaleckiene G, Baniuliene D, Kalasauskiene A. Blindness incidence in Lithuania. Int J of Ophthalmol. 2003; 3: 225–8.
4. Curcio CA, Johnson M, Huang JD, Rudolf M. Aging, age-related macular degeneration, and the response-to-retention of apolipoprotein B-containing lipoproteins. Prog Retin Eye Res. 2009; 28(6): 393–422.
5. Bultel-Brienne S, Lestavel S, Pilon A, Laffont I, Tailleux A, Fruchart JCh, et al. Lipid free apolipoprotein E binds to the class B type I scavenger receptor I (SR-BI) and enhances cholesteryl ester uptake from lipoproteins. J Biol Chem. 2002; 277: 36092–9.
6. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, Omega-3 fatty acid intake, and associations with age-related macular degeneration. The US twin study of age-related macular degeneration. Arch Ophthalmol. 2006; 124(7): 995–1001.
7. Thornton J, Edwards R, Mitchell P, Harrison RA, Buchan I, Kelly SP. Smoking and age-related macular degeneration: a review of association. Eye. 2005; 19: 935–44.
8. Martínez K, García-Martín E, Blanco G, Gamito FJ, Ladero JM, Agúndez JA. The effect of the cytochrome P450 CYP2C8 polymorphism on...
the disposition of (R)-ibuprofen enantiomer in healthy subjects. BJCP. 2004; 10: 1365–2125.

9. Wang L, Clark ME, Crossman DK, Kojima K, Messinger JD, Mobley JA, et al. Abundant lipid and protein components of drusen. PLoS One. 2010; 5: e10329.

10. Khan JC, Thurlby DA, Shahid H, Clayton DG, Yates JR, Bradley M, et al. Smoking and age-related macular degeneration: the number of pack years of cigarette smoking is a major determinant of risk for both geographic atrophy and choroidal neovascularisation. Br J Ophthalmology. 2006; 90: 75–80.

11. Tomany S, Wang J, Van Leeuwen R, Klein R, Mitchell P, Vingerling JR, et al. Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. Ophthalmology. 2004; 111: 1280–7.

12. Delcourt C, Michel F, Colvez A, Lacroux A, Delage M, Vernet MH. Associations of cardiovascular disease and its risk factors with age-related macular degeneration: the POLA Study. Ophthalmic Epidemiology 2001; 8: 237–49.

13. Stanislovaitytienė D. Klinikinių veiksnių, oksidacinio streso žymens N – karboksimetilizino ir SCARB1 geno polimorfizmo sąsajos su amžine geltonosios dėmės degeneracija ir išeminė širdies liga [doctoral dissertation]. Lietuvos sveikatos mokslo universitetas, 2013. Lithuanian.

14. Cimbalas A, Paunksnis A, Cerniauskiene RL, Domarkiene S. Prevalence and risk factors of age-related maculopathy among middle-aged people. Medicina (Kaunas). 2003; 39(12): 1237–43.

15. Owsley C, Jackson GR, Cideciyan AV, Huang Y, Fine SL, Ho AC, et al. Psychophysical evidence for rod vulnerability in age-related macular degeneration. Invest Ophthalmol Vis Sci. 2000; 41: 267–73.

16. Çerman E, Eraslın M, Çekić O. Age-related macular degeneration and Alzheimer disease. Turk J Med Sci. 2015; 45(5): 1004–9.
CYP2C8 RS10509681 IR RS11572080 POLIMORFIZMU SĄSAJOS SU AMŽINĖS GELTONOSIOS DĖMĖS DEGENERACIJOS PASIREIŠKIMU

Santrauka

Įvadas. Amžinė geltonosios dėmės degeneracija (AGDD) – dažniausiai pasitaikanti negrįžtantį aklumo priežastis įsivysčiusiose šalyse. Pradiniai ligos požymiai yra drūzų formavimas ir tinklainės pigmentinio epitelio pakitimai. AGDD etiologija ir drūzų formavimosi priežastys nėra visiškai aiškios. Naujųjų studijų rodo, kad metaboliniai procesai yra vieni iš svarbiausių AGDD vystymosi priežasčių, o CYP2C8 genas gali dalyvauti šiame procese. Tyrimo tikslas – nustatyti CYP2C8 geno (rs10509681 ir rs11572080) polimorfizmų dažnį pacientams, sergantiems ankstyva AGDD ir palyginti rezultatus su kontroline sveikų asmenų grupe.

Metodai. Tyrimo dalyvavo 305 pacientai, sergantys pradine AGDD, ir 300 sveikų asmenų. Genotypai išskirti naudojant tikro laiko polimerazės grandinės reakciją.

Rezultatai. CYP2C8 rs10509681 geno polimorfizmo analizė neatskleidė T/T, T/C ir C/C genotipų pasiskirstymo skirtumo lyginant AGDD sergančius bei sveikus asmenis (83,3; 16,7 ir 0 %, palyginti su 83,7; 15,7 ir 0,7 %, p = 0,343). CYP2C8 rs11572080 geno polimorfizmo analizė taip pat neatskleidė statistiškai reikšmingų C/C, T/C ir T/T genotipų pasiskirstymo lyginant AGDD sergančius ir kontrolinės grupės sveikus asmenis (84,9; 15,1 ir 0 %, palyginti su 82,3; 17,3 ir 0,3 %, p = 0,447).

Išvados. Palygintų CYP2C8 geno (rs10509681 ir rs11572080) polimorfizmų genotipų pasiskirstymą AGDD sergančių ir kontrolinėje sveikų asmenų grupėse, statistiškai reikšmingų skirtolemų nenustatėme.

Raktažodžiai: amžinė geltonosios dėmės degeneracija, citochromas P450, rs10509681, rs11572080, genų polimorfizmai