Role of cholesterol 24S-hydroxylase gene polymorphism (rs754203) in primary open angle glaucoma

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Purpose: The enzyme cholesterol 24S-hydroxylase (Cyp46A1) is responsible for the conversion of cholesterol to its more polar metabolite 24S-hydroxycholesterol, thereby enabling the intracerebral elimination of cholesterol. An intronic single nucleotide polymorphism in the gene CYP46A1 (IVS2 −150 T>C; rs754203) has recently been associated with primary open angle glaucoma (POAG). This association, however, lacks confirmation in other studies. The purpose of the present study was to investigate a hypothesized association between rs754203 and the presence of POAG in a Central European population of Caucasian descent.

Methods: The present institutional study comprised a total of 581 unrelated subjects: 330 patients with POAG, and 251 control subjects. Main outcome measures are genotype distributions and allelic frequencies determined by polymerase chain reaction.

Results: No significant differences in either genotype distribution or allelic frequencies were found between patients with POAG and control subjects (p>0.05). The presence of the rs754203 T-allele was associated with a nonsignificant odds ratio of 0.81 (95% CI: 0.63–1.04; p=0.11) for POAG.

Conclusions: Our data suggest that the rs754203 polymorphism itself is unlikely a genetic risk factor for POAG in Caucasian individuals.

The glaucomas are the most frequent cause of irreversible blindness worldwide [1]. They are defined as progressive optic neuropathies with apoptotic retinal ganglion cell death leading to cupping of the optic nerve with typical visual field defects. Although much effort has been made, the exact pathomechanisms are still elusive [2]. Noteworthy, a positive family history for glaucoma is known to increase the individual risk substantially, albeit most of glaucoma cases are likely to be multifactorial and presumably polygenetic with a combination of distinct genetic variants exerting small effects [3-6].

Some debate has been evoked by reports that cholesterol lowering statins may decrease the risk of developing and progression of glaucoma [7-9]. As cholesterol can not cross the blood-brain barrier in significant amounts it has to be synthesized de novo in the central nervous system [10]. For elimination of cholesterol from the central nervous system, it has to be converted into its more polar metabolite 24S-hydroxycholesterol, which is catalyzed by the enzyme cholesterol 24S-hydroxylase (Cyp46A1) [11]. Cyp46A1 is a member of the cytochrome P-450 family and has also been detected in rodent and bovine retinal ganglion cells, indicating a physiologic role in the cholesterol metabolism of the mammalian retina [12,13].

It can be speculated that an altered function of Cyp46A1 may either lead to increased intracerebral cholesterol concentration or accumulation of 24S-hydroxycholesterol. In a rodent glaucoma model hypercholesterolemia has been shown to cause oxidative damage of the retina via induction of nitric oxide synthase 2 [14]. Furthermore, in an in vitro study 24S-hydroxycholesterol has been found to possess neurotoxic properties in physiologic concentration range [15]. Interestingly, in a recent study a positive association between an intronic single nucleotide polymorphism (SNP) in the gene of Cyp46A1 (IVS2 −150 T>C; rs754203) and POAG was reported in a French population [16]. This association, however, lacks confirmation in other studies.

Our study was thus set to investigate a hypothesized association between rs754203 of CYP46A1 and POAG.

METHODS

In the present institutional, retrospective case-control study we investigated a total of 581 unrelated Caucasian subjects comprising 330 patients with POAG, and 251 control subjects. All participants were of Caucasian origin, living in the same geographical area and were seen at the local Department of Ophthalmology, Medical University of Graz, Austria. The study was approved by the Institutional Review Board of the Medical University of Graz and followed the...
Continuous data are given as mean±standard deviation (SD). Means were compared using Mann–Whitney test. Proportions of groups were compared by the χ² test. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated by logistic regression. The criterion for statistical significance was p≤0.05. Hardy–Weinberg equilibrium has been calculated using HW Diagnostics-Version 1.beta (Fox Chase Cancer Center, Philadelphia, PA). Statistical analysis was done using the SPSS statistical package (SPSS, version 17.0, Chicago, IL). Power calculation was done using PS Power and Sample Size Calculation software version 2.1.30 [18].

RESULTS
Our study included 330 patients with POAG (196 female and 134 male), and 251 control subjects (126 female and 125 male). The mean age of patients with POAG was 73.5±10.0 years, and 74.2±7.2 years in control subjects, respectively. 59 (17.9%) patients had early (MD above −6dB), 87 (26.4%) had moderate (MD between −6 and −12 dB), and 127 (38.5%) had severe (MD below −12dB) defect.

No significant differences in either genotype distribution or allelic frequencies of rs754203 were found between patients with POAG and control subjects (Table 1). Presence of the rs754203 C-allele was associated with a nonsignificant odds ratio of 1.23 (95% CI: 0.96–1.58; p=0.11) for POAG. Furthermore, no significant differences in either genotype distribution or allelic frequencies of rs754203 were found between patients with early, moderate or severe defect and control subjects (Table 2).

The present study had a statistical power of 0.80 to detect an odds ratio of 1.42 for the rs754203 C-allele in patients with POAG.

The observed genotype distributions did not deviate from those predicted by the Hardy–Weinberg equilibrium, and for control subjects were similar to those reported for Caucasian populations [19,20].

DISCUSSION
Conversion of cholesterol to 24S-hydroxycholesterol, catalyzed by Cyp46A1, is the critical step for the elimination of cholesterol from the central nervous system [10]. Alteration of this enzyme may therefore lead to a dysbalance of the intracerebral cholesterol/24S-hydroxycholesterol homeostasis, which may contribute to neurodegenerative diseases like POAG [14,15]. Indeed, a positive association between an intronic SNP in the gene CYP46A1 (CYP46A1 IVS2 –150 T>C; rs754203) and POAG has been reported [16].

In the present study genotypes of the rs754203 polymorphism were determined in 330 patients with POAG, and 251 control subjects. Allelic frequencies as well as genotype distributions did not significantly differ between patients with POAG and control subjects. This finding is in
contrast to data obtained from 150 POAG patients and 118 control subjects in a French study, in which an OR of 1.26 (95% CI: 1.006–1.574; p<0.05) for the TT genotype was reported [16]. The controversial results may be explained by different sample sizes or varying genotype distributions among different populations.

Interestingly, previous studies linked the rs754203 polymorphism to late onset Alzheimer disease (LOAD), a chronic neurodegenerative disease mostly of the elderly like POAG [19-27]. The results of these studies, however, have been controversial and the risk-bearing allele and genotype has not been the same in the positive studies. Of the positive studies, three studies linked the T-allele to an increased risk for LOAD, while five studies suggested the C-allele as risk-bearing allele [19-27].

Generally, intronic SNPs may affect RNA splicing (i.e., exon skipping, intron retention, or introduction of ectopic or cryptic splice sites) or RNA stability, thereby modifying functionality or synthesis rate of a gene’s product [28]. Papassotriopoulos and coworkers [16,19] provided evidence that the brain β-amyloid load and the cerebrospinal fluid concentration of β-amyloid are increased in individuals with the rs754203 TT genotype, whereas serum as well as cerebrospinal fluid levels of cholesterol and 24S-hydroxycholesterol appeared to be unaffected by the rs754203 genotype. Interestingly, most studies reported decreased levels of β-amyloid in the cerebrospinal fluid of patients with LOAD and likewise, Yoneda and coworkers [29,30] found decreased levels of β-amyloid in the vitreous fluid of patients with glaucoma. As mentioned above, an unambiguous genetic impact for rs754203 has not yet been established indicating that it may be in linkage disequilibrium with a causative locus.

In conclusion, in the present study no statistically significant difference in the genotype and allele distribution of the rs754203 polymorphism was found between patients with POAG and control subjects, suggesting that this polymorphism itself is unlikely a major risk-factor for POAG in a Caucasian population. Further studies are clearly warranted to elucidate the functionality of this polymorphism and its role in POAG.

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REFERENCES

1. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP. Global data on visual impairment in the year 2002. Bull World Health Organ 2004; 82:844-51. [PMID: 15640920]
2. Kwon YH, Fingert JH, Kuehn MH, Alward WL. Primary open-angle glaucoma. N Engl J Med 2009; 360:1113-24. [PMID: 19279343]

### Table 1. Genotype distribution and allele frequency of the CYP46A1 IVS2 −150 C>T polymorphism (rs754203) in patients with primary open angle glaucoma (POAG).

| Genotype   | Patients with POAG (n=330) | Control subjects (n=251) | Significance p-value |
|------------|-----------------------------|--------------------------|----------------------|
| rs754203 TT | 136 (41.2)                  | 120 (47.8)               | 0.20                 |
| TC         | 161 (48.8)                  | 112 (44.6)               |                      |
| CC         | 33 (10.0)                   | 19 (7.6)                 |                      |
| rs754203 C-allele frequency | 0.344                      | 0.299                    | 0.10                 |

Numbers for genotypes are n (%).

### Table 2. Genotype distribution and allele frequency of the CYP46A1 IVS2 −150 C>T polymorphism (rs754203) in patients with primary open angle glaucoma (POAG) with early (mean defect [MD] above −6 dB), moderate (MD between −6 and −12 dB) and severe (MD below −12 dB) defect.

| Genotype   | Patients with early defect (n=59) | Patients with moderate defect (n=87) | Patients with severe defect (n=127) | Control subjects (n=251) | Significance p-value |
|------------|-----------------------------------|-------------------------------------|------------------------------------|--------------------------|----------------------|
| rs754203 TT | 26 (44.1)                         | 34 (39.1)                           | 52 (40.9)                          | 120 (47.8)               | 0.60; 0.16; 0.21*     |
| TC         | 26 (44.1)                         | 43 (49.4)                           | 63 (49.6)                          | 112 (44.6)               |                      |
| CC         | 7 (11.8)                          | 10 (11.5)                           | 12 (9.4)                           | 19 (7.6)                 |                      |
| rs754203 C-allele frequency | 0.339                         | 0.362                              | 0.343                             | 0.299                    | 0.39; 0.12; 0.22      |

Numbers for genotypes are n (%); *=early, moderate, severe.
3. Tielsch JM, Katz J, Sommer A, Quigley HA, Javitt JC. Family history and risk of primary open angle glaucoma. The Baltimore Eye Survey. Arch Ophthalmol 1994; 112:69-73. [PMID: 8285897]

4. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. Arch Ophthalmol 1998; 116:1640-5. [PMID: 9869795]

5. Allingham RR, Liu Y, Rhee DJ. The genetics of primary open-angle glaucoma: a review. Exp Eye Res 2009; 88:837-44. [PMID: 19061866]

6. Mossböck G, Faschinger C, Weger M, Renner W. Role of genetic factors in glaucomas. Spektrum Augenheilkd 2008; 22:227-32.

7. McGwin G Jr, McNeal S, Owsley C, Girkin C, Epstein D, Lee AF, de Jong PT. Genetic risk of primary open-angle glaucoma. Arch Ophthalmol 2004; 122:822-6. [PMID: 15197056]

8. Leung DY, Li FC, Kwong YY, Tham CC, Chi SC, Lam DS. Simvastatin and Disease Stabilization in Normal Tension Glaucoma: A Cohort Study. Ophthalmology 2010; 117:471-6. [PMID: 20045568]

9. De Castro DK, Punjabi OS, Bostrom AG, Stamper RL, Lietman TM, Ray K, Lin SC. Effect of statin drugs and aspirin on progression in open-angle glaucoma suspects using confocal scanning laser ophthalmoscopy. Clin Experiment Ophthalmol 2007; 35:506-13. [PMID: 17760631]

10. Leoni V. Oxysterols as markers of neurological disease – a review. Scand J Clin Lab Invest 2009; 69:22-5. [PMID: 19199127]

11. Björkhem I, Lütjohann D, Diczfalusy U, Stähle L, Ahlborg G, Wahren J. Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. J Lipid Res 1998; 39:1594-600. [PMID: 9717719]

12. Bretillon L, Diczfalusy U, Björkhem I, Maire MA, Martine L, Joffre C, Acar N, Bron A, Creuzot-Garcher C. Cholesterol-24S-hydroxylase (CYP46A1) is specifically expressed in neurons of the neural retina. Curr Eye Res 2007; 32:361-6. [PMID: 17453958]

13. Ramirez DM, Andersson S, Russell DW. Neuronal expression and subcellular localization of cholesterol 24-hydroxylase in the mouse brain. J Comp Neurol 2008; 507:1676-93. [PMID: 18241055]

14. Yücel I, Akar Y, Yücel G, Ciftçioğlu MA, Keleş N, Aslan M. Effect of hypercholesterolemia on inducible nitric oxide synthase expression in a rat model of elevated intraocular pressure. Vision Res 2005; 45:1107-14. [PMID: 15707919]

15. Kölsch H, Lütjohann D, Tulke A, Björkhem I, Rao ML. The neurotoxic effect of 24-hydroxycholesterol on SH-SY5Y human neuroblastoma cells. Brain Res 1999; 818:171-5. [PMID: 9914453]

16. Fourgeux C, Martine L, Björkhem I, Diczfalusy U, Joffre C, Acar N, Creuzot-Garcher C, Bron A, Bretillon L. Primary open-angle glaucoma: association with cholesterol 24S-hydroxylase (CYP46A1) gene polymorphism and plasma 24-hydroxycholesterol levels. Invest Ophthalmol Vis Sci 2009; 50:5712-7. [PMID: 19553612]

17. Hoddap E, Parrish R, Anderson D. Clinical decisions in glaucoma. Saint Louis (MO): Mosby Year Book; 1993.

18. Dupont WD, Plummer WD. PS power and sample size program available for free on the Internet. Control Clin Trials 1997; 18:274.

19. Papasotiriopoulos A, Streffer JR, Tsolaki M, Schmid S, Thal D, Nicosia F, Iakovidou V, Maddalena A, Lütjohann D, Ghebrmedhin E, Hegi T, Pasch T, Träxler M, Brühl A, Benüssi L, Binetti G, Braak H, Nitsch RM, Hock C. Increased brain beta-amyloid load, phosphorylated tau, and risk of Alzheimer disease associated with an intronic CYP46 polymorphism. Arch Neurol 2003; 60:29-35. [PMID: 12533085]

20. Golanska E, Hulas-Bigoszewska K, Wojciech I, Rieske P, Styczynska M, Peplonska B, Pfeffer A, Wiesiak B, Gabryelewicz T, Religa D, Chodakowska-Zebrowska M, Barcikowska M, Sobow T, Liberski PP. CYP46: a risk factor for Alzheimer's disease or a coincidence? Neurosci Lett 2005; 383:105-8. [PMID: 15936520]

21. Garcia AN, Muniz MT, Souza e Silva HR, da Silva HA, Athayde-Junior L. Cyp46 polymorphisms in Alzheimer's disease: a review. J Mol Neurosci 2009; 39:342-5. [PMID: 19705089]

22. Wang B, Zhang C, Zheng W, Lu Z, Zheng C, Yang Z, Wang L, Jin F. Association between a T/C polymorphism in intron 2 of cholesterol 24S-hydroxylase gene and Alzheimer's disease in Chinese. Neurosci Lett 2004; 369:104-7. [PMID: 15450677]

23. Fernández Del Pozo V, Alvarez Alvarez M, Fernández Martinez M, Galdós Alcelay L, Gómez Busto F, Peña JA, Alfonso-Sánchez MA, Zarranz Irizarialdu JJ, de Pancorbo MM. Polymorphism in the cholesterol 24S-hydroxylase gene (CYP46A1) associated with the APOEpsilon3 allele increases the risk of Alzheimer's disease and of mild cognitive impairment progressing to Alzheimer's disease. Dement Geriatr Cogn Disord 2006; 21:81-7. [PMID: 16340204]

24. Borroni B, Archetti S, Agosti C, Akkawi N, Brambilla C, Caimi L, Caltagirone C, Di Luca M, Padovani A. Intronic CYP46 polymorphism along with ApoE genotype in sporadic Alzheimer Disease: from risk factors to disease modulators. Neurobiol Aging 2004; 25:747-51. [PMID: 15165699]

25. Kölsch H, Lütjohann D, Ludwig W, Schulte A, Ptok U, Jessen F, von Bergmann K, Rao ML, Maier W, Heun R. Polymorphism in the cholesterol 24S-hydroxylase gene is associated with Alzheimer's disease. Mol Psychiatry 2002; 7:899-902. [PMID: 12232784]

26. Combarros O, Infante J, Llorca J, Berciano J. Genetic association of CYP46 and risk for Alzheimer's disease. Dement Geriatr Cogn Disord 2004; 18:257-60. [PMID: 15286456]

27. Li Y, Chu LW, Chen YQ, Cheung BM, Leung RY, Yik PY, Ng KM, Mak W, Jin DY, St George-Hyslop P, Song YQ. Intron 2 (T/C) CYP46 polymorphism is associated with Alzheimer's disease in Chinese patients. Dement Geriatr Cogn Disord 2006; 22:399-404. [PMID: 16960449]

28. Jensen CJ, Oldfield BJ, Rubio JP. Splicing, cis genetic variation and disease. Biochem Soc Trans 2009; 37:1311-5. [PMID: 19909267]

29. Sjögren M, Andreassen N, Blennow K. Advances in the detection of Alzheimer's disease-use of cerebrospinal fluid biomarkers. Clin Chim Acta 2003; 322:1-10. [PMID: 12763273]
30. Yoneda S, Hara H, Hirata A, Fukushima M, Inomata Y, Tanihara H. Vitreous fluid levels of beta-amyloid((1–42)) and tau in patients with retinal diseases. Jpn J Ophthalmol 2005; 49:106-8. [PMID: 15838725]