**in vivo** *30*: 473-478 (2016)

# Polymorphism Analysis of *GSTM1* and *OPA1* Genes in Greek Patients with Primary Open-angle Glaucoma

ANASTASIOS LAVARIS[1], MARIA GAZOULI[2], DIMITRIOS BROUZAS[1] and MARILITA M. MOSCHOS[1]

[1]*Electrophysiology Laboratory, First Department of Ophthalmology, University of Athens, Athens, Greece;*
[2]*Biology Laboratory, Department of Basic Medical Science, School of Medicine, University of Athens, Athens, Greece*

**Abstract.** *Background: Glaucoma is a heterogenous group of optic neuropathies leading to progressive degeneration of the optic nerve and vision loss. Over the past decades, disease-causing genes have been identified and multigenic inheritance theory has-beens investigated for many cases of glaucoma. The purpose of this study was to investigate the distribution of mutations in glutathione S-transferase M1 (GSTM1) and optic atrophy 1 (OPA1) genes in a series of patients of Greek origin. Patients and Methods: This was a case–control study of 106 patients with primary open-angle glaucoma (POAG) and 120 healthy controls of Greek origin, surveyed for polymorphisms with potential correlation to POAG. A DNA sample from each individual was genotyped for GSTM1 and OPA1 (rs166850, rs10451941) polymorphisms. Results: GSTM1 null genotype carriers seem to have an increased risk of developing POAG (odds ratio=1.86, 95% confidence interval=1.07-3.21; p=0.03). The results indicate that the OPA1 genotype (rs166850 and rs10451941 polymorphisms) is not significantly associated with POAG. Conclusion: The GSTM1 null genotype might be associated with increased risk of development of POAG in the Greek population. No significant correlation was found between OPA1 polymorphisms and POAG.*

The term glaucoma corresponds to a heterogeneous group of diseases characterized by degeneration of the optic nerve and retinal ganglion cell death. Glaucoma is categorized according to whether it is congenital or acquired, whether the configuration of the iridocorneal angle is open or closed, and whether a direct cause is recognized (secondary) or not (primary). Primary open-angle glaucoma (POAG) is the most common form of glaucoma in the Western world. By 2020, there will be 79.6 million people with glaucoma and 74% of those will develop open-angle glaucomas (1).

POAG is mainly characterized by progressive optic neuropathy with characteristic visual field changes. The main risk factors are elevated intraocular pressure (IOP), smoking, myopia, aging, vascular factors (hypertension, diabetes, ischemia) and family history (2). POAG is classified into high-tension glaucoma (HTG) and normal-tension glaucoma (NTG). The main feature of HTG is a raised IOP. In NTG, IOP is within the statistically normal range and NTG cases account for approximately 20% of all POAG (3).

Even if POAG is considered to be a multifactorial disease, a significant heritable component has been suggested since the beginning of the 20th century (4-7). Genetic studies have defined three main causative genes in POAG, the optineurin (*OPTN*) (10p14-15) (8), myocilin (*MYOC*) (1q24-25) (9), and WD repeat domain 36 (*WDR36*) (5q21-22) (10). These genes are responsible only for a small proportion (approximately 10%) of POAG cases worldwide (11). However, recent genome-wide association studies have identified several new genetic variations that might contribute to POAG pathogenesis, including neurotrophin-4 (*NTF4*) (12, 13), caveolin 1 (*CAV1*) and caveolin 2 (*CAV2*) (14), S1 RNA binding domain 1 (*SRBD1*) (15, 16), transmembrane and coiled-coil domains 1 (*TMCO1*) (17), cyclin-dependent kinase inhibitor 2B–antisense RNA 1 (*CDKN2B–AS1*) (18) and more recently transforming growth factor, beta receptor 3 (*TGFBR3*) (19), variants near ATP-binding cassette, sub-family A (*ABCA1*) and in phosphomannomutase 2 (*PMM2*) (20). Among them polymorphisms in glutathione S-transferase M1 (*GSTM1*) and optic atrophy 1 (*OPA1*) genes are also believed to contribute in the pathogenesis of POAG (21-23).

*GSTM1* polymorphism is considered to be risk factor for POAG and other types of glaucoma. GST is a multigenetic group of enzymes playing important roles in antioxidative processes and elimination of carcinogens, toxins and oxidants (24). Studies have been conducted in various populations, however, results remain controversial. Several studies

*Correspondence to:* Marilita M. Moschos, MD, Ph.D., 6, Ikarias street, Ekali, 14578, Athens, Greece. Mobile: +30 6944887319, e-mail: moschosmarilita@yahoo.fr

*Key Words: GSTM1*, *OPA1*, primary open-angle glaucoma, POAG.

revealed possible correlation between *GSTM1* polymorphisms and POAG pathogenesis (25-30), although in some, results were insignificant probably due to small subject numbers (25); yet others studies showed no association between POAG and *GSTM1* polymorphisms (31, 32). However, it is important to notice that Yang *et al*. reported that a GST antigen was present at significantly higher levels in patients with glaucoma compared to controls. Their findings suggested that people expressing *GSTM1* are at an increased risk of developing auto-antibodies against GST and may have an increased risk of glaucoma (33).

The *OPA1* gene on chromosome 3q28 is responsible for autosomal dominant optic atrophy (ADOA) (34). Its product is a dynamin-related guanosine triphosphatase and has an important role in mitochondrial biogenesis and membrane integrity. As it is expressed in retinal ganglion cells, mutations of *OPA1* are suspected to lead to their apoptosis and optic neuropathy (35,36). Concerning POAG, although *OPA1* is a candidate genetic risk factor, results so far remain controversial. Mabuchi *et al*. reported that *OPA1* IVS 8 +32 T/C polymorphism may be genetic risk factor for both NTG and HTG (37). Several studies presented association of *OPA1* polymorphisms with NTG (38-40) but not with HTG (41-43) as well; therefore it is still debated whether *OPA1* polymorphisms are involved in POAG pathogenesis.

Given these considerations, and since it is known that there are variations between ethnic populations, the purpose of our study was to investigate the potential association between *OPA1* (rs166850, rs10451941) and *GSTM1* gene poly-morphisms and POAG susceptibility in a well-defined Greek cohort. To our knowledge, this is the first study conducting such genotyping in population of Greek patients with POAG.

## Materials and Methods

*Patients*. Familial (n=44) and sporadic (n=62) cases of POAG, primarily diagnosed at least 10 years before recruitment into this study, as well as 120 unaffected healthy controls, with a history free of other ophthalmological or systematic diseases were recruited from the Glaucoma Unit of the First Department of Ophthalmology, University of Athens. All participants gave their written consent and had a blood sample taken for genetic analysis.

The clinical examination protocol included a full medical and ophthalmologic disease history. The anterior segment of the eye was examined by slit lamp, including gonioscopy; fundus examination including evaluation of the appearance of the optic disc and cup/disc ratio measurement were assessed with a stereoscopic fundus lens (Volk Optical Inc., Mentor, OH, USA). IOP was measured with Goldmann applanation tonometer (Haag Streit AG, Bern, Switzerland); ocular hypertension was defined as ≥21 mmHg. Automated perimetry was performed with a Humphrey Automated Field Analyzer (Humphrey Inc., San Leandro, CA, USA) using 30-2 SITA protocol in order to determine visual field defects in the POAG patient group (44, 45). Both patients and controls underwent automated perimetry. No glaucomatic defects were detected in controls.

*Genotyping*. Genomic DNA was prepared from blood samples using the PureLink™ Genomic DNA kit (Invitrogen Inc., Rockville, MD, USA). The GSTM1 polymorphism was evaluated by polymerase chain reaction (PCR) as previously described (46). Primer sequences for *GSTM1* were 5'-GAACTCCCTGAAA AGCTAAAGC-3' (forward primer) and 5'-GTTGGGCTCAAATAT ACGGTGG-3' (reverse primer), which produced a 219 bp band. *GSTM1* null genotype (–) is indicated by the absence of a 219 bp band. Genotyping for *OPA1* rs166850 and rs10451941 was performed by restriction fragment-length polymorphism analysis as previously described with specific primers for both genotypes (forward primer, 5'-CCC TTT TAG TTT TTA CGA TGA AGA-3'; reverse primer, 5'-TTG CTT AAG ACA TTA CTT GGA ACA-3'). The restriction enzymes *Rsa*I and *Fsp*BI (Fermentas, York, UK) discriminated the respective genotypes (40). In all cases, as an internal positive control for successful PCR, β-globin (268 bp) was amplified with the primers 5'-CAACTTCATCCACGTTCACC-3' (forward primer) and 5'-GAAGAGCCAAGGACAGTTAC-3' (reverse primer).

*Statistical analysis*. Frequency and susceptibilities of mutations were compared using the $\chi^2$ test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated with the corresponding $\chi^2$ distribution test. The *p*-values obtained were two-tailed and determined to be significant when $p<0.05$. Hardy–Weinberg equilibrium was verified by calculating the expected frequencies and numbers and was tested separately in patients and controls using the goodness-of-fit $\chi^2$ test. All the comparisons were performed using GraphPad version 3.00 (GraphPad Software Inc., San Diego, CA, USA).

## Results

The demographic characteristics of patients and controls are presented in Table I. The genotyping results are presented in Table II. The patients with POAG had a significantly higher IOP than the healthy controls ($p<0.0001$). When analyzing the Hardy–Weinberg equilibrium, we observed no significant deviation from expected numbers of both cases and controls. The *GSTM1* null genotype frequency was significantly higher in patients with POAG (44.34%) than in controls (30%). The carriers of the *GSTM1* null genotype seemed to have an increased risk of developing POAG ($p=0.03$). As illustrated in Table II, there was no significant difference in OPA1 genotypic frequencies between patients and controls for the rs10451941 and rs166850 polymorphisms. No significant association was found between IOP and the genotypes of the *GSTM1* and *OPA1* genes tested.

## Discussion

Even if the pathophysiology of POAG is not well understood, it has been supported that genetic factors might play a crucial role in POAG development (8-11,47-48). This study aimed to determine whether previous findings of *OPA1* (rs166850, rs10451941) and *GSTM1* gene polymorphisms could be replicated in a Greek cohort of patients with POAG.

Table I. *Demographic characteristics of patients and controls.*

| Characteristic | POAG (n=106) | Controls (n=120) | *p*-Value |
|---|---|---|---|
| Sex, male/female | 42/64 | 56/64 | 0.857 |
| Age (mean±S.D.), years | 67.06±11.15 | 69.99±13.76 | 0.08 |
| Family history, n | 44 | 0 | - |
| Smoking, n (%) | 24 (22.64) | 21 (17.5) | 0.404 |
| IOP (mean±S.D.), mmHg | 30.13±5.71 | 16.21±3.18 | <0.0001 |

POAG: Primary open-angle glaucoma; IOP: intraocular pressure.

Table II. *Genotype distribution of optic atrophy 1 (OPA1) (rs166850, rs10451941) and glutathione S-transferase M1 (GSTM1) polymorphisms in primary open-angle glaucoma (POAG) patients and controls.*

| Polymorphism | Genotype | Controls (n=120) | POAG (n=106) | OR (95% CI) | *p*-Value |
|---|---|---|---|---|---|
| *OPA1* rs166850 | CC | 108 | 94 | 1.00 (Reference) | |
| | CT | 12 | 11 | 1.05 (0.44-2.49) | 1.00 |
| | TT | 0 | 1 | 3.44 (1.14-85.63) | 0.47 |
| rs10451941 | TT | 66 | 55 | 1.00 (Reference) | |
| | TC | 40 | 38 | 1.14 (0.64-2.02) | 0.66 |
| | CC | 14 | 13 | 1.11 (0.48-2.57) | 0.83 |
| *GSTM1* | Wild-type | 84 | 59 | 1.00 (Reference) | |
| | Null | 36 | 47 | 1.86 (1.07-3.21) | 0.03 |

OR: Odds ratio; CI: confidence interval.

Concerning the *GSTM1* gene, several studies supported that there is a significant association of *GSTM1* null genotype with POAG in several ethnic populations (25-30), indicating that oxidative damage, which is significantly increased in the trabecular meshwork of patients with glaucomas, as stated by Izzotti *et al*. (49-50), may be implicated in POAG development (27). Conversely, Jansson *et al*. found no evidence of association between *GSTM1* genotype and POAG development in a Swedish population (31), and Barbosa *et al*. (25) stated that although patients with POAG had a higher frequency of *GSTM1* null genotype, the difference was not significant ($p$=0.0874).

Our results confirm the association of *GSTM1* null genotype with POAG in this Greek population. Rocha *et al*. demonstrated correlation between *GSTM1* null genotype and POAG in a Brazilian population (27); their study also suggested that *GSTM1* null genotype was associated with higher IOP. However, even if Greek patients with POAG had increased IOPs compared to healthy controls, no association of IOP with a specific genotype was found. Regarding the association of the rs166850, rs10451941 polymorphisms of the *OPA1* gene with POAG risk, in agreement with previous studies (40, 42-43), our data could not support an association of these polymorphisms with the development of POAG in this Greek population. Furthermore, there was no association between those polymorphisms and elevated IOP.

In conclusion, our study did not find any significant association between *OPA1* polymorphisms and POAG, however, our results suggest that *GSTM1* null genotype might be a risk factor for POAG in the Greek population. It is hypothesized that several genes and lifestyle/ environmental factors may contribute to the development of POAG. However, the identity and number of these genes has yet to be defined. We hope that further studies will be directed towards investigation of *GSTM1* and *OPA1* polymorphisms as they might lead to better understanding of the pathophysiology of this important cause of irreversible blindness.

## Conflicts of Interest

The Authors declare that they have no competing interests in regard to this study.

## References

1 Quigley HA and Broman AT: The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol *90*: 262-267, 2006.

2 Worley A and Grimmer-Somers K: Risk factors for glaucoma: what do they really mean? Aust J Prim Health *17*: 233-239, 2011.

3 Tielsch JM, Katz J, Singh K, Quigley HA, Gottsch JD, Javitt J and Sommer A: population-based evaluation of glaucoma screening: The Baltimore Eye Survey. Am J Epidemiol *134*: 1102-1110, 1991.

4 Biró I: Notes on the heredity of glaucoma. Ophthalmologica *98*: 43-50, 1939.

5 Westerland KE: Clinical and genetic studies on the primary glaucoma diseases. Busck: Copenhagen, 1947.

6 Charliat G, Jolly D and Blanchard F: Genetic risk factor in primary open-angle glaucoma: a case–control study. Ophthalmic Epidemiol *1*: 131-138, 1994.

7 Fan BJ, Leung YF, Wang N, Lam SC, Liu Y, Tam OS and Pang CP: Genetic and environmental risk factors for primary open-angle glaucoma. Chin Med J *117*: 706-710, 2004.

8 Alward WL, Kwon YH, Kawase K, Craig JE, Hayreh SS, Johnson AT, Khanna CL, Yamamoto T, Mackey DA, Roos BR, Affatigato LM, Sheffield VC and Stone EM: Evaluation of optineurin sequence variations in 1,048 patients with open-angle glaucoma. Am J Ophthalmol *136*: 904-910, 2003.

9 Fingert JH, Héon E, Liebmann JM, Yamamoto T, Craig JE, Rait J, Kawase K, Hoh ST, Buys YM, Dickinson J, Hockey RR, Williams-Lyn D, Trope G, Kitazawa Y, Ritch R, Mackey DA, Alward WL, Sheffield VC and Stone EM: Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. Hum Mol Genet *8*: 899-905, 1999.

10 Hewitt AW, Dimasi DP, Mackey DA and Craig JE: A glaucoma case–control study of the WDR36 Gene D658G sequence variant. Am J Ophthalmol *142*: 324-325, 2006.

11 Fan BJ, Wang DY, Lam DS and Pang CP: Gene mapping for primary open-angle glaucoma. Clin Biochem *39*: 249-258, 2006.

12 Pasutto F, Matsumoto T, Mardin CY, Sticht H, Brandstätter JH, Michels-Rautenstrauss K, Weisschuh N, Gramer E, Ramdas WD, van Koolwijk LM, Klaver CC, Vingerling JR, Weber BH, Kruse FE, Rautenstrauss B, Barde YA and Reis A: Heterozygous *NTF4* mutations impairing neurotrophin-4 signaling in patients with primary open-angle glaucoma. Am J Hum Genet *85(4)*: 447-456, 2009.

13 Vithana EN, Nongpiur ME, Venkataraman D, Chan SH, Mavinahalli J and Aung T: Identification of a novel mutation in the *NTF4* gene that causes primary open-angle glaucoma in a Chinese population. Mol Vis *16*: 1640-1645, 2010.

14 Loomis SJ, Kang JH, Weinreb RN, Yaspan BL, Cooke Bailey JN, Gaasterland D, Gaasterland T, Lee RK, Lichter PR, Budenz DL, Liu Y, Realini T, Friedman DS, McCarty CA, Moroi SE, Olson L, Schuman JS, Singh K, Vollrath D, Wollstein G, Zack DJ, Brilliant M, Sit AJ, Christen WG, Fingert J, Kraft P, Zhang K, Allingham RR, Pericak-Vance MA, Richards JE, Hauser MA, Haines JL, Pasquale LR and Wiggs JL: Association of *CAV1/CAV2* genomic variants with primary open-angle glaucoma overall and by gender and pattern of visual field loss. Ophthalmology *121(2)*: 508-516, 2014.

15 Mabuchi F, Sakurada Y, Kashiwagi K, Yamagata Z, Iijima H and Tsukahara S: Association between *SRBD1* and *ELOVL5* gene polymorphisms and primary open-angle glaucoma. Invest Ophthalmol Vis Sci *52(7)*: 4626-4629, 2011.

16 Meguro A, Inoko H, Ota M, Mizuki N and Bahram S: Genome-wide association study of normal tension glaucoma: common variants in *SRBD1* and *ELOVL5* contribute to disease susceptibility. Ophthalmology *117(7)*: 1331-1338, 2010.

17 Chen Y, Hughes G, Chen X, Qian S, Cao W, Wang L, Wang M and Sun X: Genetic variants associated with different risks for high-tension glaucoma and normal-tension glaucoma in a Chinese population. Invest Ophthalmol Vis Sci *56(4)*: 2595-600, 2015.

18 Pasquale LR, Loomis SJ, Kang JH, Yaspan BL, Abdrabou W, Budenz DL, Chen TC, Delbono E, Friedman DS, Gaasterland D, Gaasterland T, Grosskreutz CL, Lee RK, Lichter PR, Liu Y, McCarty CA, Moroi SE, Olson LM, Realini T, Rhee DJ, Schuman JS, Singh K, Vollrath D, Wollstein G, Zack DJ, Allingham RR, Pericak-Vance MA, Weinreb RN, Zhang K, Hauser MA, Richards JE, Haines JL and Wiggs JL: CDKN2B-AS1 genotype-glaucoma feature correlations in primary open-angle glaucoma patients from the United States. Am J Ophthalmol *155(2)*: 342-353, 2013.

19 Li Z, Allingham RR, Nakano M, Jia L, Chen Y, Ikeda Y, Mani B, Chen LJ, Kee C, Garway-Heath DF, Sripriya S, Fuse N, Abu-Amero KK, Huang C, Namburi P, Burdon K, Perera SA, Gharahkhani P, Lin Y, Ueno M, Ozaki M, Mizoguchi T, Krishnadas SR, Osman EA, Lee MC, Chan AS, Tajudin LS, Do T, Goncalves A, Reynier P, Zhang H, Bourne R, Goh D, Broadway D, Husain R, Negi AK, Su DH, Ho CL, Blanco AA, Leung CK, Wong TT, Yakub A, Liu Y, Nongpiur ME, Han JC, Hon do N, Shantha B, Zhao B, Sang J, Zhang N, Sato R, Yoshii K, Panda-Jonas S, Ashley Koch AE, Herndon LW, Moroi SE, Challa P, Foo JN, Bei JX, Zeng YX, Simmons CP, Bich Chau TN, Sharmila PF, Chew M, Lim B, Tam PO, Chua E, Ng XY, Yong VH, Chong YF, Meah WY, Vijayan S, Seongsoo S, Xu W, Teo YY, Cooke Bailey JN, Kang JH, Haines JL, Cheng CY, Saw SM, Tai ES; ICAARE-Glaucoma Consortium; NEIGHBORHOOD Consortium, Richards JE, Ritch R, Gaasterland DE, Pasquale LR, Liu J, Jonas JB, Milea D, George R, Al-Obeidan SA, Mori K, Macgregor S, Hewitt AW, Girkin CA, Zhang M, Sundaresan P, Vijaya L, Mackey DA, Wong TY, Craig JE, Sun X, Kinoshita S, Wiggs JL, Khor CC, Yang Z, Pang CP, Wang N, Hauser MA, Tashiro K, Aung T and Vithana EN: A common variant near *TGFBR3* is associated with primary open-angle glaucoma. Hum Mol Genet *24(13)*: 3880-3892, 2015.

20 Chen Y, Lin Y, Vithana EN, Jia L, Zuo X, Wong TY, Chen LJ, Zhu X, Tam PO, Gong B, Qian S, Li Z, Liu X, Mani B, Luo Q, Guzman C, Leung CK, Li X, Cao W, Yang Q, Tham CC, Cheng Y, Zhang X, Wang N, Aung T, Khor CC, Pang CP, Sun X and Yang Z: Common variants near ABCA1 and in PMM2 are associated with primary open-angle glaucoma. Nat Genet *46(10)*: 1115-1119, 2014.

21 Liu T, Xie L, Ye J, Liu Y and He X: Screening of candidate genes for primary open angle glaucoma. Mol Vis *18*: 2119-2126, 2012.

22 Silva CT, Costa NB, Silva KS, Silva RE and Moura KK: Association between primary open-angle glaucoma and genetic polymorphisms *GSTM1/GSTT1* in patients from Goiânia Central-West Region of Brazil. Genet Mol Res *13(4)*: 8870-8875, 2014.

23 Guo Y, Chen X, Zhang H, Li N, Yang X, Cheng W and Zhao K: Association of OPA1 polymorphisms with NTG and HTG: a meta-analysis. PLoS One *7(8)*: e42387, 2012.

24 Eaton DL and Bammler TK: Concise review of the glutathione S-transferases and their significance to toxicology. Toxicol Sci *49*: 156-164, 1999.

25 Barbosa AM, Frare AB, Costa NB, Silva RE and Moura KK: *GSTM1* polymorphism in patients with primary open-angle glaucoma. Genet Mol Res *11*: 3256-3262, 2012.

26 Safa FK, Shahsavari G and Abyaneh RZ: Glutathione s-transferase M1 and T1 genetic polymorphisms in Iranian patients with glaucomaIran J Basic Med Sci *17(5)*: 332, 2014.
27 Rocha AV, Talbot T, Magalhães da Silva T, Almeida MC, Menezes CA, Di Pietro G and Rios-Santos F: Is the *GSTM1* null polymorphism a risk factor in primary open angle glaucoma? Mol Vis *17*: 1679-1686, 2011.
28 Unal M, Güven M, Devranoğlu K, Ozaydin A, Batar B, Tamçelik N, Görgün EE, Uçar D and Sarici A: Glutathione S-transferase M1 and T1 genetic polymorphisms are related to the risk of primary open-angle glaucoma: a study in a Turkish population. Br J Ophthalmol *91*: 527-530, 2007.
29 Abu-Amero KK, Morales J, Mohamed GH, Osman MN and Bosley TM: Glutathione S-transferase M1 and T1 polymorphisms in Arab glaucoma patients. Mol Vis *14*: 425-430, 2008.
30 Rasool HA, Nowier SR, Gheith M, Saif AS and Ismail S: The risk of primary open angle glaucoma and glutathione s transferase M1 and T1 polymorphism among Egyptians. J Am Sci *6*: 375-381, 2010.
31 Jansson M, Rada A, Tomic L, Larsson LI and Wadelius C: Analysis of the glutathione *S*-transferase M1 gene using pyrosequencing and multiplex PCR–no evidence of association to glaucoma. Exp Eye Res *77(2)*: 239-243, 2003.
32 Izzotti A and Sacca SC: Glutathione S-transferase M1 and its implications in glaucoma pathogenesis: a controversial matter. Exp Eye Res *79*: 141-142, 2004.
33 Yang J, Tezel G, Patil RV, Romano C and Wax MB: Serum autoantibody against glutathione S-transferase in patients with glaucoma. Invest Ophthalmol Vis Sci *42(6)*: 1273-1276, 2001.
34 Thiselton DL, Alexander C, Taanman JW, Brooks S, Rosenberg T, Eiberg H, Andreasson S, Van Regemorter N, Munier FL, Moore AT, Bhattacharya SS and Votruba M: A comprehensive survey of mutations in the *OPA1* gene in patients with autosomal dominant optic atrophy. Invest Ophthalmol Vis Sci *43*: 1715-1724, 2002.
35 Buono LM, Foroozan R, Sergott RC and Savino PJ: Is normal tension glaucoma actually an unrecognized hereditary optic neuropathy? New evidence from genetic analysis. Curr Opin Ophthalmol *13*: 362-370, 2002.
36 Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquellec L, Arnaud B, Ducommun B, Kaplan J and Hamel CP: Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nat Genet *26*: 207-210, 2000.
37 Mabuchi F, Tang S, Kashiwagi K, Yamagata Z, Iijima H and Tsukahara S: The OPA1 gene polymorphism is associated with normal-tension and high-tension glaucoma. Am J Ophthalmol *143*: 125-130, 2007.
38 Aung T, Ocaka L, Ebenezer ND, Morris AG, Krawczak M, Thiselton DL, Alexander C, Votruba M, Brice G, Child AH, Francis PJ, Hitchings RA, Lehmann OJ and Bhattacharya SS: A major marker for normal tension glaucoma: association with polymorphisms in the OPA1 gene. Hum Genet *110*: 52-56, 2002.
39 Powell BL, Toomes C, Scott S, Yeung A, Marchbank NJ, Spry PG, Lumb R, Inglehearn CF and Churchill AJ: Polymorphisms in *OPA1* are associated with normal tension glaucoma. Mol Vis *9*: 460-464, 2003.
40 Woo SJ, Kim DM, Kim JY, Park SS, Ko HS and Yoo T: Investigation of the association between *OPA1* polymorphisms and normal-tension glaucoma in Korea. J Glaucoma *13*: 492-495, 2004.
41 Aung T, Ocaka L, Ebenezer ND, Morris AG, Brice G, Child AH, Hitchings RA, Lehmann OJ and Bhattacharya SS: Investigating the association between *OPA1* polymorphisms and glaucoma: comparison between normal tension and high tension primary open angle glaucoma. Hum Genet *110*: 513-514, 2002.
42 Yu-Wai-Man P, Stewart JD, Hudson G, Andrews RM, Griffiths PG, Birch MK and Chinnery PF: *OPA1* increases the risk of normal- but not high-tension glaucoma. J Med Genet *47*: 120-125, 2010.
43 Liu Y, Schmidt S, Qin X, Gibson J, Munro D, Wiggs JL, Hauser MA and Allingham RR: No association between *OPA1* polymorphisms and primary open-angle glaucoma in three different populations. Mol Vis *13*: 2137-2141, 2007.
44 Parekh AS, Tafreshi A, Dorairal SK and Weinreb RN: Clinical applicability of the International Classification of Disease and Related Health Problems (ICD-9) Glaucoma Staging Codes to predict disease severity in patients with open-angle glaucoma. J Glaucoma *23(1)*: 18-22, 2014.
45 Mao LK, Stewart WC and Shields MB: Correlation between intraocular pressure control and progressive glaucomatous damage in primary open-angle glaucoma. Am J Ophthalmol *111(1)*: 51-55, 1991.
46 Buentello-Volante B, Elizondo-Olascoaga C, Miranda-Duarte A, Guadarrama-Vallejo D, Cabral-Macias J and Zenteno JC: Association study of multiple gene polymorphisms with the risk of adult-onset primary open-angle glaucoma in a Mexican population. Exp Eye Res *107*: 59-64, 2013.
47 Fingert JH: Primary open-angle glaucoma genes. Eye *25(5)*: 587-595, 2011.
48 Fuse N: Genetic bases for glaucoma. Tohoku J Exp Med *221(1)*: 1-10, 2010.
49 Izzotti A, Saccà SC, Cartiglia C and De Flora S: Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. Am J Med *114(8)*: 638-646, 2003.
50 Izzotti A, Bagnis A and Saccà SC: The role of oxidative stress in glaucoma. Mutat Res Rev Mutat Res *612(2)*: 105-114, 2006.

*Received January 12, 2016*
*Revised March 28, 2016*
*Accepted March 31, 2016*