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GSTM1 and *GSTM5* Genetic Polymorphisms and Expression in Age-related Macular Degeneration

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Abstract

Purpose—Previously, two cytosolic antioxidant enzymes, Glutathione S-transferase Mu 1 (*GSTM1)* and Mu 5 (*GSTM5*), were reduced in retinas with Age-related Macular Degeneration (AMD). This study compared genomic copy number variations (gCNV) of these two antioxidant enzymes in AMD vs. controls.

Methods—Genomic copy number (gCN) assays were performed using Taqman Gene Copy Number Assays (Applied Biosystems) in technical quadruplicate for both *GSTM1* and *GSTM5*. Peripheral leukocyte RNA levels were compared to controls in technical triplicates. Statistical comparisons were performed in SAS v9.2 (SAS Institute Inc. Cary, NC).

Results—A large percentage of patients in both AMD and age-matched control groups had no copies of *GSTM1* (0/0). The mean gCN of *GSTM1* was 1.40 (range 0 – 4) and 1.61 (range 0 – 5) for AMD and control, respectively ($p = 0.29$). A greater percentage of control patients had > 3 gCNs of *GSTM1* compared to AMD, respectively (15.3% vs. 3.0%, $p = 0.004$). The gCN of *GSTM5* was 2 in all samples except one control sample. The relative quantification of *GSTM1* and *GSTM5* mRNA from peripheral blood leukocytes in patients showed significant differences in relative expression in AMD vs. control ($p < 0.05$). Peripheral blood leukocyte mRNA and gCN were not significantly correlated ($p = 0.27$).

Conclusion—Since high copy numbers of *GSTM1* are found more frequently in controls than in AMD, it is possible that high copy number leads to increased retinal antioxidant defense. Genomic polymorphisms of *GSTM1* and *GSTM5* do not significantly affect the peripheral blood leukocyte mRNA levels.

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Keywords

Age-related macular degeneration (AMD); oxidative stress; glutathione S-transferase; genomic copy number; expression repression

INTRODUCTION

Although the cause of age-related macular degeneration (AMD) is not completely understood, there is evidence that oxidative stress is involved. The retina's high oxygen saturation and easily oxidized polyunsaturated fatty acids (e.g. docosahexaenoate, DHA) impart a great oxidative burden (1–3). Patients with AMD appear to have a reduced serum antioxidant potential that is partially alleviated by vitamin supplementation (4, 5). Indeed, antioxidant vitamins can slow the progression of vision loss in eyes with moderate to advanced AMD (6). The high concentration of vitamin C in the retina, a natural antioxidant, which has been shown to decrease after intense light exposure, presumably is used to quench oxidative stress (7, 8).

Oxidative insults can cause irreversible damage to the retina (4, 5, 9, 10). There is an increase in ω-(2-carboxyethyl) pyrrole (CEP) protein adducts secondary to oxidation of DHA-containing lipids in the retinas in AMD vs. control eyes (11, 12). Mechanisms employed to counter oxygen toxicity include compartmentalization, repair, removal of damaged macromolecules, and free radical elimination by 'scavenger' molecules, such as vitamins or antioxidant enzymes or compounds (13, 14). We have recently reported that two cytosolic antioxidant enzymes, Glutathione S-transferase Mu 1 (*GSTM1)* and Mu 5 (*GSTM5*), located within the neurosensory retina (NSR) and Retinal Pigment Epithelium (RPE)/choroid are greatly reduced in AMD vs. control eyes (15). There are four known allelic variants of *GSTM1 (*>+/+, +/+, +/0, 0/0), one of which is a deletion resulting in loss of function, which has been reported in up to 50% of the human population (16, 17). Information regarding allelic variants of the *GSTM*s is available in the ophthalmic literature but there is no consensus if any *GSTM1* polymorphism is associated with AMD vs. controls (18–23).

To determine if allelic variants of *GSTM1* and/or *GSTM5* are responsible for the decreased presence of both genes' mRNA and protein, we studied two separate cohorts of patients. The first cohort (Cohort 1) was used to compare the genotypes of *GSTM1* and *GSTM5* (≥+/+, +/0, 0/0) to AMD status to determine if polymorphisms in these genes results in an increase risk of AMD. The second, separate cohort (Cohort 2) genotype was compared to peripheral blood leukocyte mRNA quantification by real time-polymerase chain reaction (RT-PCR) to categorize the genetic polymorphisms affect on expression in peripheral blood leukocytes. A final analysis combining both cohort 1 and 2 (Combined Cohort) was used to determine genomic polymorphism risk for AMD in a large sample size.

METHODS

This is a prospective observational case control study of two independent cohort of patients diagnosed with AMD by a retinal specialist (JD) at the University of Pennsylvania (cohort

1), and by retinal specialists (SP, DT, LM) at the University of California Davis Eye Clinic (cohort 2). All patient underwent a comprehensive eye examination including a dilated fundoscopic examination. Patients were categorized as 'late AMD' vs. 'early AMD' if geographic atrophy (GA) or active exudative AMD was diagnosed in at least one eye by fluorescein angiography (FA) and spectral-domain optical coherence tomography (Cirrus or Spectralis) (Table 1). Age-match control (>60 years of age and within 3 years-of-age of AMD patients) subjects were identified and enrolled among patients (evaluated by the same retina providers) and determined to have a macula without any pathology or drusen. The study was conducted according to a protocol approved by the Office of Human Research Protection of the University of Pennsylvania and the University of California-Davis (UC Davis) School of Medicine and adhered to the tenets of the Declaration of Helsinki. The charts and electronic medical records were reviewed from both institutions at the time of each study enrollment from June 2010, to June 2013.

GSTM1 and *GSTM5* copy number assays

DNA was isolated from saliva samples using Buccal Amp DNA extraction kits per manufacturer's protocol (Epicenter Biotechnologies, Madison, Wisconsin). The DNA underwent a glycogen cold-precipitation and re-suspension in ddH_2O and was stored at −80 C. The final DNA concentration was determined by nanodrop and diluted to 10ng/μL. Copy number was determined by using Taqman Gene Copy Number Assays designed by Applied Biosystems. Multiplex real-time PCR reactions were run on the Applied Biosystems 7900HT Real-Time PCR system by using gene-specific primers with a FAM-MGB probe [GSTM1 (Hs04207352_cn) and GSTM5 (Hs06525674_cn)] along with primers for the RNase *P* gene and VIC/Tamra probe as the reference gene. Samples were run in quadruplicate in a 384-well format by using 10 ng of genomic DNA per reaction, along with a well-characterized reference sample (Coriell Institute for Medical Research, Camden, NJ, USA) with two copies of *GSTM1* as a calibrator. We used Sequence Detection Software (Applied Biosystems) to quantify the gene copy number of each sample. Concordance for blinded samples was more than 90%.

RNA Isolation and Quantitative Real-Time RT-PCR

RNA was isolated from peripheral blood leukocytes collected with PAXgene Blood RNA tubes (PreAnalytiX GmbH, Switzerland) per manufacturer's instructions. Tubes were stored at room temperature for 6 hours and then stored at −80C until RNA isolation (within two weeks after specimen collection). RNA was isolated with the Qiagen QIAcube (Qiagen, Valencia, CA) and PAXgene Blood miRNA Kit (PreAnalytiX GmbH, Switzerland) per manufacturer's instruction. cDNA was synthesized with reverse transcription reagents (TaqMan; Applied Biosystems, Darmstadt, Germany) according to the manufacturer's protocol. The final cDNA concentration was determined by nanodrop and diluted to 50ng/μL.

Gene expression assays (TaqMan; Applied Biosystems) were obtained and used for PCR analysis. Probes used were GSTM1 (hs01683722_gh) and GSTM5 (hs00757076_m1). Eukaryotic 18S rRNA (Hs99999901_s1) served as an internal control because of its constant expression level across the studied sample sets. Real-time RT-PCR (TaqMan; Applied

Biosystems) was performed (ABI Prism 7900 Sequence Detection System; Applied Biosystems) using the $\Delta\Delta C_T$ method, which provides normalized expression values. The amount of target mRNA was compared among the groups of interest. All reactions were performed in technical triplicates.

Statistical Analysis

Comparison of demographic characteristics between AMD cases and normal controls were performed by Fisher's exact test for categorical characteristics, and two-group t-test.

Genomic copy number data in AMD and normal control groups were summarized by mean ± standard deviation (SD) and compared using the two-group t-test. Because of the similarity of results between the two cohorts, the analysis of data from two cohorts combined was also performed. mRNA data were summarized by median (minimum, maximum) and compared between AMD and normal controls using Wilcoxon rank sum test due to the skewed distribution of data. Spearman correlation analysis was used for assessing the correlation between mRNA and copy number. Two-sided $p < 0.05$ was considered statistically significant. These analyses were performed in SAS v9.2 (SAS Institute Inc. Cary, NC).

RESULTS

In cohort 1 (Table 1), there were 50 AMD subjects and 37 control subjects enrolled. The mean age of patients in the AMD group was 3 years older than controls (p=0.06). Gender is evenly distributed between the two groups. Of 50 AMD cases, 30% had early AMD (drusen and pigmentary changes) and 70% had late AMD (geographic atrophy or exudation). The mean *GSTM1* gCN was 1.24 in the AMD group compared to 1.54 in the control group (p = 0.34, Table 2). A large percentage of patients had no copies of *GSTM1* (0/0) in both AMD vs. controls, 44% vs. 40.5% (Table 2, p = 0.66). More control patients had 4 or 5 copies of *GSTM1* compared to AMD, respectively (16.2% vs. 2.0%, p = 0.04, Table 2). *GSTM5* gCN was 2 in all samples but one control (from cohort 1) that had a copy number of 1 (data not shown).

The demographics for cohort 2 did not show any statistically significant difference between AMD vs. control (p = 0.28, Table 1). Of the 50 AMD cases in cohort 2, 24% had early AMD vs. 74% with late AMD (Table 1). *GSTM1* gCN ranged from 0 to 5 and the mean *GSTM1* gCN was not significantly different between AMD vs. controls (p=0.69), similar to the first cohort (Table 2). The presence of null genotype was noted for *GSTM1* that was not statistically significantly different between AMD (28%) vs. control (33.3%) (p = 0.76, Table 2). A non-significant, larger percentage of control patients had 4 or 5 copies of *GSTM1* compared to AMD, respectively (14.6% vs. 4.0%, p = 0.09, Table 2). *GSTM5* had two copy numbers in all samples (data not shown).

In the analysis of data from the combined cohort, the age in patients with AMD was significantly older than controls (80.2 vs. 77.5, p = 0.04, Table 1). The combined cohort's *GSTM1* gCN was still not significantly different, with a mean of 1.40 (range 0 – 4) in the AMD group compared to 1.61 (range 0 – 5) in the control group (p = 0.29, Table 2). Fewer

AMD vs. controls patients had 4 or 5 copies of *GSTM1*, respectively (3.0% vs. 15.3%, p = 0.004, Table 2). Restricting the AMD cohort to only late AMD (in either cohort 1, 2 or the combined cohort), did not increase any *GSTM1* gCN variation to statistical significance (data not shown).

The relative quantification of *GSTM1* mRNA (from patients with *GSTM1* gCN > 0/0) from peripheral blood leukocytes was significantly increased in AMD vs. control (−15 vs. −19, p = 0.02, Table 3). The relative quantification of *GSMT5* mRNA was significantly increased in AMD vs. control (−2351 vs. −4859, p = 0.001, Table 3). *GSTM1* mRNA from peripheral blood and gCN were not significantly correlated (Spearman correlation coefficient = 0.13, p = 0.27).

DISCUSSION

The human genome project first highlighted the large degree of gCNV (24, 25). Approximately, 0.4% of a person's genomic 'signature' is accounted for by copy number variance (26). Even identical twins have been shown to have *de novo* gCNVs not shared by their sibling (27–29).

Structural variation of our genes can be associated with disease or can be a benign variation (30, 31). *GSTM1* and *GSTM5* have been localized within the NSR and RPE/choroid of the human eye, and both genes had reduced mRNA and protein in AMD eyes (without null genotype 0/0) in the same pan-retinal studies (15). Piacentini et al analysis of *GSTM1* polymorphisms have recently emphasized the importance of using genotype association (+/+, +/0, 0/0) over phenotype (present, null) when looking at *GSTM1* protein function (32). Oz et al. performed phenotype analysis with PCR of peripheral blood leukocytes for *GSTM1*, *GSTT1* and *GSTP1* in exudative AMD vs. controls and found that paired homozygous deletions for *GSTM1*/*GSTT1* or *GSTM1*/*GSTP1* increase AMD risk (33). Possible loss of redundant or supportive antioxidant functions could cause an accumulative insult necessary for significant AMD risk. Interestingly, oxidative stress in AMD is likely still 'underreported' if a percentage of present phenotypes (≥+/0) have significantly reduced *GSTM1* or *GSTM5* protein in the NSR or RPE due to expression repression (15). Accordingly, we performed sample cohort studies using gCN analysis to see if copy number variations of *GSTM1* and *GSTM5* could be an independent risk factor for AMD. Guven et al. reported *GSTM1* homozygous null (determined by PCR from peripheral leukocytes) associated with AMD and dry AMD (after subtype stratification) in a Turkish population (34). Our results failed to show such an association for AMD or with AMD subtype stratification (e.g., early vs. late, neovascular or atrophic AMD), (data not shown).

Independent cohort analysis (cohort 1 and 2) did not show an age difference. In the larger combined cohort analysis a significant age discrepancy became evident. The age distribution was similar in the combined cohort (60 – 93 vs. 62 – 99) AMD vs. control, respectively. This suggests an age distribution curve bias toward younger ages in controls in the combined cohort (Table 1). All patients were within the age limits of AMD disease status (>60 years old) and the youngest control patient was two years older than the AMD combined cohort. The enrollment criteria of a normal macula (without evidence of any other pathology) for

controls may have caused a selection bias for younger individuals that only became evident when the two cohorts were combined. Patients with AMD and additional maculopathies (e.g., epiretinal membrane, history of pseudophakic cystoid macular edema), however, were included. The selection criteria prohibiting any maculopathy (including drusen or RPE change) was chosen to hope minimize misclassification of subclinical AMD given the exponential increase in AMD incidence with each decade of senior life.

GSTM5 had 2 gCNs in all but one control sample (data not shown). Several models plausibly explain the large degree of polymorphisms of *GSTM1* and the relative stable allelic configuration of *GSMT5* downstream on chromosome 1p13. Recurrent rearrangements of the human genome have been accounted for by different meiotic recombination mechanisms while non-recurrent rearrangements (including deletions) can be understood by errors of DNA replication models (30, 31). A genome wide analysis of copy number variation in AMD would be helpful in further elucidating where and how these *GSTM1* polymorphisms occur.

Retinal GSTs are likely to have a significant role in protection against oxidative insult (35–43). Pharmacologic augmentation of the body's antioxidant defenses, therefore, may prove to be protective in AMD pathogenesis, especially in people with low *GSTM1* and *GSTM5* expression in the eye. The increased presence of 4 or 5 gCN of *GSTM1* in control vs. AMD (Table 2) could potentially protect against AMD pathogenesis in the eye, however direct evidence linking increased copy number of antioxidant genes to reduced oxidative stress is sparse in the literature. For example, Wang et al showed that mitochondrial DNA copy numbers in peripheral blood leukocytes were increased in patients with higher plasma oxidation indicators (44). Like AMD, asthma is partially driven by inflammation, of which oxidative stress is believed a part. The incidence of asthma was greatly reduced in patients with two copy numbers of *GSTM1* (vs. +/0 or 0/0) in a Danish atopic cohort (45). In our study, interestingly, there was greater relative expression of *GSTM1* (for patients with gCNV > 0) in peripheral blood leukocytes in AMD vs. control (Table 3). *GSTM5* had a significant increased expression in AMD vs. control (Table 3). This highlights the tissue-specific nature of gene expression control. Future, larger studies would need to be conducted to corroborate these findings.

The lack of association of *GSTM1* null with AMD in two, independent cohort of patients (and in combined cohort analysis), highlights another form of expression repression to explain the low amounts of *GSTM1* mRNA and protein in human retinas with AMD vs. control previously reported. Additionally, given the lack of a *GSTM1* null being a single putative genomic risk factor for AMD incidence, possible additional models need to be examined. Given the relative high degree of homology between different isoforms, 'duplication of function' by the mu-5 isoform to *GSTM1*, for example, could additionally reduce the retina's anti-oxidative ability below a 'disease causing threshold'. Both *GSTM1* and *GSTM5* have been shown to be co-localized within the same areas of the NSR and RPE/choroid by immunofluorescence. Given the lack of polymorphisms of *GSTM5*, a co-expression repression of both enzymes may be antecedent to disease incidence.

In conclusion, we report here the first evidence showing a lack of a *GSTM1* null (0/0) or genomic polymorphisms of *GSTM5* (+/0, 0/0) (as determined by copy number determination) in AMD. The function and location of these isoenzymes suggest that this decline in antioxidant function could lead to increased oxidative stress in AMD eyes and contribute to AMD pathogenesis.

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References

1. Gaillard ER, Atherton SJ, Eldred G, Dillon J. Photophysical studies on human retinal lipofuscin. Photochem Photobiol. 1995; 61(5):448–53. Epub 1995/05/01. [PubMed: 7770505]
2. Rozanowska M, Jarvis-Evans J, Korytowski W, Boulton ME, Burke JM, Sarna T. Blue light-induced reactivity of retinal age pigment. In vitro generation of oxygen-reactive species. J Biol Chem. 1995; 270(32):18825–30. Epub 1995/08/11. [PubMed: 7642534]
3. Martinez-Lara E, Siles E, Hernandez R, Canuelo AR, Luisa del Moral M, Jimenez A, et al. Glutathione S-transferase isoenzymatic response to aging in rat cerebral cortex and cerebellum. Neurobiology of aging. 2003; 24(3):501–9. Epub 2003/02/26. [PubMed: 12600725]
4. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol. 2000; 45(2):115–34. Epub 2000/10/18. [PubMed: 11033038]
5. Mirza S, Plafker KS, Aston C, Plafker SM. Expression and distribution of the class III ubiquitin-conjugating enzymes in the retina. Mol Vis. 2010; 16:2425–37. Epub 2010/12/09. [PubMed: 21139979]
6. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol. 2001; 119(10):1417–36. Epub 2001/10/23. [PubMed: 11594942]
7. Organisciak DT, Bicknell IR, Darrow RM. The effects of L-and D-ascorbic acid administration on retinal tissue levels and light damage in rats. Curr Eye Res. 1992; 11(3):231–41. Epub 1992/03/01. [PubMed: 1587146]
8. Petrova LI, Molodova GA, Burtseva NN. Conditions of dextranase formation by Penicillium funiculosum 15. Prikl Biokhim Mikrobiol. 1975; 11(1):63–6. Epub 1975/01/01. Usloviia obrazovaniia dekstranazy Penicillium funiculosum 15. [PubMed: 236553]
9. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J. 1984; 219(1):1–14. Epub 1984/04/01. [PubMed: 6326753]
10. Peinado MA, Quesada A, Pedrosa JA, Martinez M, Esteban FJ, Del Moral ML, et al. Light microscopic quantification of morphological changes during aging in neurons and glia of the rat parietal cortex. Anat Rec. 1997; 247(3):420–5. Epub 1997/03/01. [PubMed: 9066920]
11. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci U S A. 2002; 99(23):14682–7. Epub 2002/10/23. [PubMed: 12391305]
12. Gu X, Meer SG, Miyagi M, Rayborn ME, Hollyfield JG, Crabb JW, et al. Carboxyethylpyrrole protein adducts and autoantibodies, biomarkers for age-related macular degeneration. J Biol Chem. 2003; 278(43):42027–35. Epub 2003/08/19. [PubMed: 12923198]
13. Sies H. Oxidative stress: from basic research to clinical application. Am J Med. 1991; 91(3C):31S–8S. Epub 1991/09/30. [PubMed: 1928209]

14. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev. 1994; 74(1): 139–62. Epub 1994/01/01. [PubMed: 8295932]
15. Hunter A, Spechler PA, Cwanger A, Song Y, Zhang Z, Ying GS, et al. DNA methylation is associated with altered gene expression in AMD. Invest Ophthalmol Vis Sci. 2012; 53(4):2089–105. Epub 2012/03/14. [PubMed: 22410570]
16. Nakajima T, Elovaara E, Anttila S, Hirvonen A, Camus AM, Hayes JD, et al. Expression and polymorphism of glutathione S-transferase in human lungs: risk factors in smoking-related lung cancer. Carcinogenesis. 1995; 16(4):707–11. Epub 1995/04/01. [PubMed: 7728947]
17. Strange RC, Jones PW, Fryer AA. Glutathione S-transferase: genetics and role in toxicology. Toxicol Lett. 2000; 112–113:357–63. Epub 2000/03/18.
18. Jourenkova-Mironova N, Voho A, Bouchardy C, Wikman H, Dayer P, Benhamou S, et al. Glutathione S-transferase GSTM3 and GSTP1 genotypes and larynx cancer risk. Cancer Epidemiol Biomarkers Prev. 1999; 8(2):185–8. Epub 1999/03/06. [PubMed: 10067818]
19. Jourenkova N, Reinikainen M, Bouchardy C, Dayer P, Benhamou S, Hirvonen A. Larynx cancer risk in relation to glutathione S-transferase M1 and T1 genotypes and tobacco smoking. Cancer Epidemiol Biomarkers Prev. 1998; 7(1):19–23. Epub 1998/02/10. [PubMed: 9456238]
20. Jahnke V, Matthias C, Fryer A, Strange R. Glutathione S-transferase and cytochrome-P-450 polymorphism as risk factors for squamous cell carcinoma of the larynx. Am J Surg. 1996; 172(6): 671–3. Epub 1996/12/01. [PubMed: 8988674]
21. Lafuente A, Pujol F, Carretero P, Villa JP, Cuchi A. Human glutathione S-transferase mu (GST mu) deficiency as a marker for the susceptibility to bladder and larynx cancer among smokers. Cancer Lett. 1993; 68(1):49–54. Epub 1993/01/15. [PubMed: 8422649]
22. Olshan AF, Weissler MC, Watson MA, Bell DA. GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 polymorphisms, tobacco use, and the risk of head and neck cancer. Cancer Epidemiol Biomarkers Prev. 2000; 9(2):185–91. Epub 2000/03/04. [PubMed: 10698480]
23. Kihara M, Kihara M, Kubota A, Furukawa M, Kimura H. GSTM1 gene polymorphism as a possible marker for susceptibility to head and neck cancers among Japanese smokers. Cancer Lett. 1997; 112(2):257–62. Epub 1997/01/30. [PubMed: 9066737]
24. Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, et al. Large-scale copy number polymorphism in the human genome. Science. 2004; 305(5683):525–8. Epub 2004/07/27. [PubMed: 15273396]
25. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, et al. Detection of large-scale variation in the human genome. Nat Genet. 2004; 36(9):949–51. Epub 2004/08/03. [PubMed: 15286789]
26. Kidd JM, Cooper GM, Donahue WF, Hayden HS, Sampas N, Graves T, et al. Mapping and sequencing of structural variation from eight human genomes. Nature. 2008; 453(7191):56–64. Epub 2008/05/03. [PubMed: 18451855]
27. Michaelson JJ, Shi Y, Gujral M, Zheng H, Malhotra D, Jin X, et al. Whole-genome sequencing in autism identifies hot spots for de novo germline mutation. Cell. 2012; 151(7):1431–42. Epub 2012/12/25. [PubMed: 23260136]
28. Dumanski JP, Piotrowski A. Structural genetic variation in the context of somatic mosaicism. Methods Mol Biol. 2012; 838:249–72. Epub 2012/01/10. [PubMed: 22228016]
29. Grayson BL, Smith ME, Thomas JW, Wang L, Dexheimer P, Jeffrey J, et al. Genome-wide analysis of copy number variation in type 1 diabetes. PLoS One. 2010; 5(11):e15393. Epub 2010/11/19. [PubMed: 21085585]
30. Lee JA, Lupski JR. Genomic rearrangements and gene copy-number alterations as a cause of nervous system disorders. Neuron. 2006; 52(1):103–21. Epub 2006/10/04. [PubMed: 17015230]
31. Lupski JR. Structural variation in the human genome. N Engl J Med. 2007; 356(11):1169–71. Epub 2007/03/16. [PubMed: 17360997]
32. Piacentini S, Polimanti R, De Angelis F, Iorio A, Fuciarelli M. Phenotype versus Genotype Methods for Copy Number Variant Analysis of Glutathione S-Transferases M1. Annals of human genetics. 2013 Epub 2013/06/05.

33. Liu MM, Chan CC, Tuo J. Genetic mechanisms and age-related macular degeneration: common variants, rare variants, copy number variations, epigenetics, and mitochondrial genetics. Human genomics. 2012; 6:13. Epub 2012/12/19. [PubMed: 23244519]
34. Guven M, Gorgun E, Unal M, Yenerel M, Batar B, Kucumen B, et al. Glutathione S-transferase M1, GSTT1 and GSTP1 genetic polymorphisms and the risk of age-related macular degeneration. Ophthalmic research. 2011; 46(1):31–7. Epub 2011/01/08. [PubMed: 21212706]
35. Keys SA, Zimmerman WF. Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. Exp Eye Res. 1999; 68(6):693–702. Epub 1999/06/22. [PubMed: 10375433]
36. Balin AK, Allen RG. Mechanisms of biologic aging. Dermatol Clin. 1986; 4(3):347–58. Epub 1986/07/01. [PubMed: 3521983]
37. Singh SV, Dao DD, Srivastava SK, Awasthi YC. Purification and characterization of glutathione S-transferases in human retina. Curr Eye Res. 1984; 3(11):1273–80. Epub 1984/11/01. [PubMed: 6510009]
38. De La Paz MA, Zhang J, Fridovich I. Red blood cell antioxidant enzymes in age-related macular degeneration. Br J Ophthalmol. 1996; 80(5):445–50. Epub 1996/05/01. [PubMed: 8695567]
39. Nicolas MG, Fujiki K, Murayama K, Suzuki MT, Shindo N, Hotta Y, et al. Studies on the mechanism of early onset macular degeneration in cynomolgus monkeys. II. Suppression of metallothionein synthesis in the retina in oxidative stress. Exp Eye Res. 1996; 62(4):399–408. Epub 1996/04/01. [PubMed: 8795458]
40. Cohen SM, Olin KL, Feuer WJ, Hjelmeland L, Keen CL, Morse LS. Low glutathione reductase and peroxidase activity in age-related macular degeneration. Br J Ophthalmol. 1994; 78(10):791–4. Epub 1994/10/01. [PubMed: 7803358]
41. Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J. 2001; 360(Pt 1):1–16. Epub 2001/11/07. [PubMed: 11695986]
42. Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev. 1997; 6(9):733–43. Epub 1997/09/23. [PubMed: 9298582]
43. Joshi PMFM, Dubovy S, Bhattacharya SK, Lee W. Decreased Expression of GSTP1 in the Macula Leads to AMD Pathogenesis. Invest Ophthalmol Vis Sci. 2009 E-Abstract(2346).
44. Wang YC, Lee WC, Liao SC, Lee LC, Su YJ, Lee CT, et al. Mitochondrial DNA copy number correlates with oxidative stress and predicts mortality in nondiabetic hemodialysis patients. Journal of nephrology. 2011; 24(3):351–8. Epub 2010/10/19. [PubMed: 20954133]
45. Brasch-Andersen C, Christiansen L, Tan Q, Haagerup A, Vestbo J, Kruse TA. Possible gene dosage effect of glutathione-S-transferases on atopic asthma: using real-time PCR for quantification of GSTM1 and GSTT1 gene copy numbers. Human mutation. 2004; 24(3):208–14. Epub 2004/08/10. [PubMed: 15300848]

Table 1

Comparison of demographic characteristics between AMD cases and controls

	Cohort 1			Cohort 2			Cohorts 1 and 2 Combined		
	AMD cases (N=50)	**Controls (N=37)**	**P-value**	**AMD cases (N=50)**	**Controls (N=48)**	**P-value**	**AMD cases (N=100)**	**Controls (N=85)**	**P-value**
Age in years									
Mean (SD)	80.9 (6.5)	77.8 (8.4)	0.06*	79.4 (8.0)	77.3 (10.5)	0.28*	80.2 (7.3)	77.5 (9.5)	0.04*
Gender			0.83†			0.67†			0.56†
Female	29(58.0%)	20 (54.1%)		24(48.0%)	27 (56.3%)		53 (53.0)	41 (48.2)	
Male	21 (42.0%)	17 (46.0%)		26(52.0%)	21 (43.8%)		47 (47.0)	44 (51.8)	
AMD type									
Early	15 (30.0%)			12(24.0%)			27(27.0%)		
Late	35 (70.0%)			37(74.0%)			72(72.0%)		
Unknown				1 (2.0%)			1 (1.0%)		

*From two group ttest.

†From Chi-squared test.

Table 2

Comparison of *GSTM1* copy number between AMD cases and controls

	Cohort 1			Cohort 2			Cohort 1 and 2 combined		
	AMD cases (N=50)	**Controls (N=37)**	**P-value**	**AMD cases (N=50)**	**Controls (N=48)**	**P-value**	**AMD cases (N=100)**	**Controls (N=85)**	**P-value**
GSTM1 copy number			0.29†			0.63†			0.24†
+/+	21 (42.0%)	11 (29.7%)		24 (48.0%)	17(35.4%)		45 (45.0%)	28(32.9%)	
+/0	1 (2.0%)	3 (8.11%)		4 (8.0%)	4 (8.33%)		5 (5.0%)	7 (8.24%)	
0/0	22 (44.0%)	15 (40.5%)		14 (28.0%)	16(33.3%)		36 (36.0%)	31(36.5%)	
Duplicate (3, 4, 5)	6 (12.0%)	8 (21.6%)		8 (16.0%)	11(22.9%)		14 (14.0%)	19(22.4%)	

†From Fisher exact test.

Table 3

Comparison of mRNA between AMD cases and controls in Cohort 2

	AMD cases	Controls	P-value
GSTM1 mRNA among all subjects			
N	50	48	
Median (min, max)	−9.3 (−59, 0)	−12 (−101, 0)	0.36**
GSTM1 mRNA among subjects with GSTM1 copy number >0			
N	36	32	
Median (min, max)	−15 (−59, −2.6)	−19 (−101, −4.1)	0.02**
GSTM5 mRNA			
N	50	48	
Median (min, max)	−2351 (−32054, −251)	−4859 (−28977, −463)	0.001**

** From Wilcoxon rank sum test.