Molecular and Biochemical Diagnosis (MBD)
Vol 1, No 2, 2014
Original Article

Association between Genetic Polymorphism of Catalase (CAT) C-262T, Cu/Zn Superoxide Dismutase (SOD1) A251G and Risk of Age-related Macular Degeneration

Saghar Yousefnia[1]

1. Department of Biology, School of Sciences, Shiraz University, 71454, Shiraz, Iran

Abstract

Background: Cells have complex network of antioxidant enzymes that protect cells from induced damages by reactive oxygen species (ROS). Catalase and superoxide dismutase are known for their role as primary protection against oxidative stress. Oxidative damage is an important risk factor in age-related macular degeneration disease (AMD). For the first time in this study the impact of genetic polymorphisms of SOD1 and CAT with AMD has been examined. Hence, the association between genetic polymorphisms of catalase (CAT) C-262T, Cu/Zn superoxide dismutase (SOD1) A251G and risk of exudative AMD has been investigated.
Methods: This study was carried out on blood samples collected from 112 exudative AMD patients and 112 healthy individuals. Genotyping of CAT C-262T and SOD1A251G was done by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. Differences in the frequencies were estimated using the $\chi 2$ test and risk was estimated with a logistic regression after adjusting for smoking, working place and age status.
Results: There was significant difference between CAT CT+TT genotype and AMD disease (P=0.009, OR=0.38, 95%CI=0.18-0.78). Also T-allele has a significant association with risk of AMD and decreases risk of disease (P=0.036, OR=0.59, 95%CI=0.36-0.96), but there was no significant differences between SOD1A251G and variant homozygous and heterozygous frequencies in patients compared to controls (P=0.589, OR=0.77, 95%CI=0.3-1.96).
Conclusions: The data presented suggest that the T-allele in CAT genotypes can increase catalase expression and activity, as a result of which generation of reactive oxygen species (ROS) can be decreased. Therefore it is suggested that increased expression of CAT as a result of T-allele in CAT genotypes and existence of T-allele in CAT genotypes is associated with decreased risk of AMD.

Keywords: Age-related macular degeneration (AMD); Catalase (CAT); Cu/Zn Superoxide dismutase (SOD1); Polymorphism

Introduction

Age-related macular degeneration (AMD) is one of the reasons of losing vision in patients over the age of 60 years because of decayed retina. In some people, AMD develops slowly with ageing that gradually can cause losing vision in one or two eyes but in the others, this process is faster (Gehrs et al.,2006). Smoking and exposing by sunlight are some environmental risk factors that have been associated with an increased risk of AMD (El Matri et al.,2012). These factors can cause reactive oxygen species (ROS)-induced

*Corresponding author. SagharYousefnia
Department of Biology, School of Sciences, Shiraz University, 71454, Shiraz, Iran
Tel., 09132664157; Fax, 031-32320012
Email: saghar_yousefnia@yahoo.com

oxidative stress in retinal pigment epithelium (RPE) cells (Coleman et al., 2008). The high level of ROS causes macromolecular (DNA, Protein, lipid) damages (Dufour et al., 2000). The other risk factors are family history (Smith et al.,1998), sex, race (Clemons et al., 2005), high blood pressure and cholesterol.

Genetic factors especially genes coding complement system proteins have been identified to be associated with AMD disease (Hughes et al.,2006). Also there is a Mendelian inherited kind of disease that occurs in the early ages and can be either autosomal dominant or recessive (El Matri et al.,2012). Because of retina's high metabolic activity, high oxygen pressure and concentration of oxidized poly unsaturated fatty acids (PUFAs), the retina is susceptible to oxidative damage. Also retinal pigments generate ROS while exposing by sunlight (Beatty et al.,2000). Ageing induces retinal changes caused by ROS and oxidative stress in RPE cell shape and size (Gao et al.,1992).

There are two forms of advanced AMD; the "dry form" known as 'geographic atrophic' and the "wet form" or neovascular/exudative form. In dry form, ruined cells called drosen are accumulated between the retina and the choroid and the retina removed. In wet form, new blood vessels are formed from the choroid which known as neovascularization (CNV). This form of AMD is called exudative or neovascular AMD (Coleman et al.,2008; Spraul et al.,1997).

CAT is one of the most important antioxidant enzymes that protects organisms from oxidative damages induced by ROS (Chandrasena et al.,2006). This enzyme consists of four identical subunits; its gene is located on chromosome 11P13. CAT is mainly located in peroxisome of tissues such as liver, kidney and in the mature red blood cell in its cytoplasmic form. CAT, like superoxide dismutase and glutathione peroxidase contribute to the cell protection against oxidative stress. CAT catalyzes the hydrogen peroxide into water and oxygen (Halliwell et al.,1989). Many mutation and polymorphism in *CAT* gene has been associated with thalassemia (Halliwell et al., 1989; Quan et al.,1989; Ogata, 1991). Genetic polymorphisms in *CAT* have been associated with cancer and type 1 diabetes (Tang et al.,2010; Cebrian et al.,2006, Chistiakov et al.,2004).

One of the most important enzymes of superoxide dismutase (SOD) family is Cu/Zn SOD (SOD1) enzyme in cytoplasm. Along with CAT enzyme, SOD1 protects macromolecules from ROS induced damages that are generated by environmental factors such as, UV and X ray exposure, pressure and heat. SOD1which is leaked from mitochondria into cytoplasm (Tilak et al., 2004) is a

homodimer protein and its gene is located on chromosome 21q22. Many mutations in *SOD1* gene has been described with familial amyotrophic lateral sclerosis (FALS). The level of enzyme activity decreased significantly in most of the mutations (McCord et al.,1969; Forsberg et al.,2001b).

Previously, the association between the *CAT* and *SOD1* gene polymorphism and other age-related diseases such as breast cancer (Oestergaard et al.,2006), glioma (Rajaraman et al.,2010), cataract (Zhang et al.,2011), Alzheimer (Capurso et al.,2008), pseudoxanthoma elasticum (Zarbock et al.,2007), colorectal cancer (Funke et al.,2009), skin cancer (He et al.,2010), pancreatic cancer (Tang et al.,2010) and type 2 diabetes (Hong Chen et al.,2011) has been reported. However, to our knowledge there are no reports on the association of polymorphisms in antioxidant genes such as *CAT* and *SOD1* with AMD disorder. Based on this, the present study is aimed to investigate the possible association between genetic polymorphisms of CAT *CAT*C-262T, *SOD1*A251G and risk of AMD development.

Materials and Methods

Subjects

This case–control study consisted of 112 subjects (44 female and 68 male) diagnosed with exudative AMD in one or two eyes, referred by vitreoretinal surgeon. All the patients were admitted to the Ophthalmic Clinic at the Khalili hospital in Shiraz, Iran and blood sample was collected from each subject. Besides, blood samples from 112 sex-matched controls were obtained from volunteers in the same clinic and considered as control group. The study excluded subjects with diseases such as cataract, asthma, past history of malignancy, cardiovascular and glaucoma. Because these diseases have been associated with *CAT* and other antioxidant enzymes polymorphisms (Zhang et al.,2011; Fan et al.,2010; Islam et al.,2007; Rajić et al.,2009).

The subjects with exudative AMD in one or both eyes had severe visual disturbance and their corrected visual acuity were under 0.1. Exudative AMD (which is associated with hemorrhage) will lead to sudden sever decrease in vision. All of the patients were referred to ophthalmologist due to severe visual deterioration. After fundus examination by vitreoretinal surgeon, the event is confirmed by flourescine angiography. General characteristics of the patients and controls have been given in table 1. Average age of patients and controls were 69.5±8.8 (range: 42 to 87) and 63.2±9.9 years (range: 40 to 85), respectively. Iranian population is a heterogeneous population with variety ethnics (Rafiee et al.,2010). Therefore to minimize the genetic differences among the ethnic groups

both the patients and controls were selected from the same ethnical group (Persian living in Fars province, southern Iran). Information about working place and smoking status was gathered from patient and control groups. This study was approved by the local Ethics Committee. Informed written consent was obtained from all participants.

DNA extraction and genotyping analysis

The collected blood samples from both of groups were stored at -80°C until use. Genomic DNA was extracted from whole blood for polymerase chain reaction (PCR) using standard procedure (Newton,1995). Briefely, rotating 200µl blood sample and 800µl NH4Cl for 20 min, washing created sediment after centrifugation in 400µl NaCl-EDTA, adding 500µl NaOH and placing in boiling water for 5 min, adding 100µl Tris-HCl to above solution, extracting of DNA from supernatant solution.

Genotyping of *CAT* C-262T: *CAT* genotyping was performed by using PCR–RFLP method. The C>T conversion in the promoter region in -262 was amplified to form undigested fragment of 190 bp by using primers 5'-CTGATAACCGGGAGCCCCGCCCTGGGTTCGGATAT-3' and 5'-CTAGGCAGGCCAAGATTGGAAGCCCAATGG-3' as forward and reverse primers, respectively (Sinagene Company) (Zarbock et al.,2007). The PCR mixture was 13.95µl water, 2.5µl buffer10X, 0.75µl MgCl2, 0.5µl dNTP, 1µl forward and reverse primers and 0.3µl Taq DNA polymerase for every 5µl DNA sample. The PCR condition was; 95°C for 15 min, followed by 35 cycles of 94°C for 1 min, 68°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 15 min. The 190-bp fragment as a PCR product was digested by 5U EcoRV restriction enzyme at 37 °C overnight and separated on a 1.5% agarose gel.

Genotyping of *SOD1* A251G: *SOD1* genotyping was performed by using a PCR–RFLP method. An A>G in intron was amplified to form undigested fragments of 570 bp using primers 5′-AGTACTGTCAACCACTAGCA-3 ′and 5′-CCAGTGTGCGGCCAATGATG-3′ as forward and reverse primers, respectively (synthesized by Sinagene Company) (Zhang et al.,2011). The PCR mixture comprised of 13.95µl water, 2.5µl buffer10X, 0.75µl MgCl2, 0.5µl dNTP, 1µl forward and reverse primers and 0.3µl Taq DNA polymerase for every 5µl DNA sample. PCR conditions were 94°C for 4 min, followed by 33 cycles of 94°C for 50 s, 63°C for 50 s, 72°C for 50 s, and a final extension step at 72°C for 7 min. The 570 bp PCR products were digested at 37 °C overnight with 5U MspI and separated on a 2% agarose gel.

Statistical analysis

Statistical analysis was performed by SPSS for

Windows (version 16.0; SPSS Inc., Chicago, IL). Logistic regression model was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for risk of AMD disease associated with *CAT* and *SOD1* polymorphisms and χ^2 test was used to estimate Hardy–Weinberg equilibrium for studied population, (χ^2=0.03, P>0.05) for *CAT* genotypes and (χ^2=0.015, P>0.05) for *SOD1* genotypes. Also t-test was employed to evaluate statistical differences for age between case and control. *P* values less than 0.05 were considered as statistical significant differences. There were significant differences for age, working place and smoking between cases and controls. Therefore ORs were adjusted for these three factors. Variants of homozygotes and heterozygotes were combined to evaluate the dominant effect.

Results

As shown in figure 1A, the EcoRV digestion resulted in one fragment of 190 bp for homozygous (TT); two fragments of 157bp and 33bp for wild-type (CC); and three fragments of 190bp, 157bp and 33bp for heterozygous (CT). MspI digestion also resulted in one fragment of 570 bp for wild-type (AA); two fragments of 369 and 201 bp for homozygous (GG); and three fragments of 570, 369, and 201 bp for heterozygous (AG) (Fig.1,sectionB).

In this study both patients and controls were divided into sub-groups, according to sex (male/female), working place (indoor/outdoor), smoking (yes/no) and refractive status (hyperopic, myopic or normal). The subjects were further divided into other illness groups (fatness, hypertension and diabetes mellitus). The patient group was divided into two sub-groups of patient with AMD in one and two eyes.

Based on statistical analysis, the frequency of smoking patients was significantly higher than control group (OR=2.66, 95% CI=1.35-5.25, P=0.004). Also the number of outdoor patients was significantly higher than control group (OR=1.96, 95%CI=1.08-3.55, P=0.025). There was a significant difference in terms of age between cases and controls (t = 5.12, df = 222, P<0.001). Whereas, there was no significant association between hyperopia (OR=1.67, 95%CI=0.8-3.49, P=0.172) and myopia (OR=2.48, 95%CI=1.13-5.46, P=0.074) and exudative AMD. Also there was no significant association among the patients diagnosed with other diseases (obese, hypertension and diabetes mellitus) and AMD (Table-1).

To reduce the influence of environmental factors on the genotypes, the ORs were adjusted for age, working place and smoking. After adjusting, binary logistic regression showed significant association between CT genotype of *CAT* C-262T polymorphism and AMD disease (OR=0.37, 95%CI=0.17-0.81,

P=0.012), because of few numbers of TT genotypes, CT and TT genotypes of *CAT* C-262T polymorphism were considered together to evaluate the dominant effect. Data analysis showed significant association between CT+TT genotypes in contrast with CC genotype of *CAT* C-262T polymorphism and AMD (OR=0.38, 95%CI=0.18-0.78, P=0.009) (Table-2). Likewise, the T-allele showed significant association with risk of AMD and decreases risk of disease (OR=0.59, 95%CI=0.36-0.96, P=0.036). But there was no significant association between polymorphism of *SOD1* A251G (OR=0.77, 95%CI=0.3-1.96, P=0.589) and AMD disease. Also data analysis showed no significant association between polymorphisms of *CAT* C-262T (OR=1, 95%CI=0.41-2.44, P=0.9), *SOD1* A251G (OR=1.06, 95%CI=0.38-2.95, P=0.91) and bilateralities of the eyes related to AMD.

Table 1 Demograhic data of the AMD patients and controls.

Demographic data	AMD group	Control group	OR (95%CI)	P-value
Number of participation	112	112		
Smoking status				
Yes, n (%)	42 (37.5%)	16 (14.3%)		
No, n (%)	60 (53.6%)	61 (54.5%)	2.66 (1.35-5.25)	0.004
Missing	10 (8.9%)	35 (31.3%)		
Work place				
Indoor, n (%)	60 (53.65%)	59 (52.7%)		
Outdoor, n (%)	52 (46.4%)	26 (23.2%)	1.96 (1.08-3.55)	0.025
Missing	0	27 (24.1%)		
Refractive status				
Normal	15(13.4%)	26(23.2%)		
Hyperopic	54(48.2%)	52(46.4%)	1.67(0.8-3.49)	0.172
Myopic	43(38.4%)	34(30.4%)	2.48(1.13-5.46)	0.074
Other diseases				
None	26(23.2%)	32(28.6%)		
Fatness*	4(3.6%)	5(4.5%)	0.98(0.24-4.04)	0.983
Hypertension	7(6.3%)	3(2.7%)	2.87(0.67-12.22)	0.153
DM**	10(8.9%)	4(3.5%)	3.07(0.86-10.95)	0.083
Fatness*+DM**	37(33%)	30(26.8%)	1.52(0.75-3.07)	0.247
Fatness*+Hypertension	18(16.1%)	23(20.5%)	0.96(0.43-2.15)	0.927
Hypertension+DM**	10(8.9%)	15(13.4%)	0.82(0.32-2.13)	0.684

*Based on Body Mass Index (BMI), BMI=(Weight(Kg)/Height(m))^2 , BMI >30 means Fatness
**DM= Diabetes Mellitus

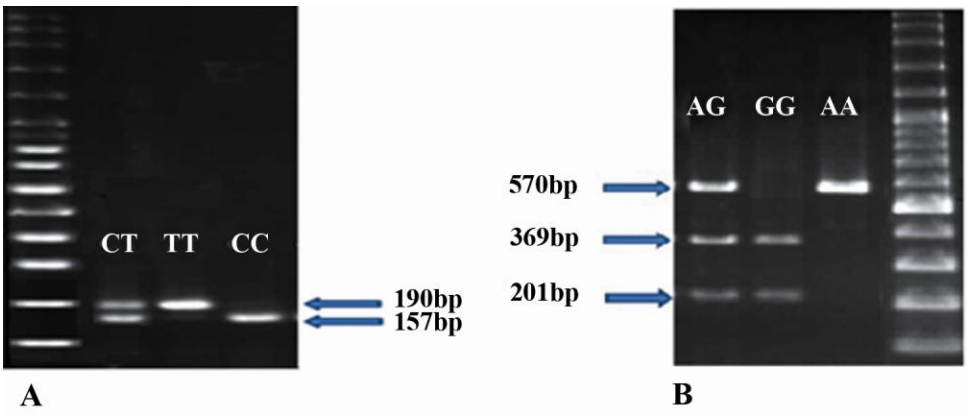

Figure 1. A:PCR-RFLP data after enzyme digestion by EcoRV on electrophoresis gel for *CAT* gene. B: PCR-RFLP data after digestion by MspI on electrophoresis gel for *SOD1* gene.

Table 2 Polymorphism in antioxidant genes; *CAT* C-262T, *SOD1* A251G and risk factor of AMD development.

Genotype	Patients (%)	Controls (%)	OR (95% CI)	p-value
***CAT* C-262T**				
CC	84 (75%)	69 (61.6%)	1	
CT	25(22.4%)	38(34%)	0.37(0.17-0.81)	0.012
TT	3(2.6%)	5(4.4%)	0.39(0.06-2.52)	0.323
CT+TT	28 (25%)	43 (38.4%)	0.38(0.18-0.78)	0.009
***SOD1* A251G**				
AA	93(83%)	91(81.3%)	1	
AG	19(17%)	20(17.8%)	0.83(0.32-2.15)	0.697
GG	0	1(0.9%)	0	1
AG+GG	19(17%)	21(18.7%)	0.77(0.3-1.96)	0.589

Discussion

In this study we used a genetic polymorphism of C-262T in promoter region of *CAT* gene because the impact of this genotype on the level of gene expression and enzyme activity is well established. Also we selected a genetic polymorphism A251G in intron of *SOD1* gene because a genetic polymorphism in intron of *SOD1* gene can make *SOD1* mRNA unstable. CAT is one of the most important antioxidant enzymes that protects organisms from oxidative stress and modulate ROS generation which is responsible for cellular damages by converting H2O2 to H2O and O2 (Halliwell et al., 1989). Every change in detoxification genes such as *CAT* causes changes in enzyme activity, quantity and ROS detoxification (Forsberg et al., 2001(a)). Also every change in the antioxidant enzymes such as CAT and SOD can interfere in age at onset disease (Muller et al., 2007; Arsova-Sarafinovska1y et al.,2008). AMD is a kind of age of onset disease (Gehrs et al., 2006) and the pathogenic role of ROS in AMD has been reported by

others (Coleman et al .,2008). The retina is a main region of eye that is damaged in AMD, which is susceptible to the deleterious effects of oxidative stress (Beatty et al.,2000). Hence it was assumed that the polymorphisms *CAT* and *SOD1* can play a role in modulation of ROS production in AMD disorder.

This study showed a good association of smoking (OR=2.66, 95% CI=1.35-5.25, P=0.004) and working place (OR=1.96, 95%CI=1.08-3.55, P=0.025) status and AMD disease. This finding is in agreement with other reports showing that smoking habit and the amount of leisure time spent outdoors are significantly associated with AMD (Newton,1995; Muller et al.,2007). Also the information about the role of work place agrees with reports from other laboratories.

Hyperopia has been reported to be associated with wet AMD in several case–control studies (Sandberg et al.,1993; Boker et al.,1993), but current study showed no significant association between hyperopia (OR=1.67, 95%CI=0.8-3.49, P=0.172) and myopia (OR=2.48, 95%CI=1.13-5.46, P=0.074) and wet AMD. After adjusting smoking, working place and age status there was a significant association between *CAT* CT+TT genotype (P=0.009, OR=0.0.38, 95%CI=0.18-0.78) and AMD. Our data showed that the T-allele decreases the risk of AMD (P=0.036, OR=0.59, 95%CI=0.36-0.96). A recent study performed in Sweden, showed that the *CAT* C-262T gene polymorphism leads to increased expression of CAT in erythrocytes (Forsberg et al.,2001a). According to this study, existence of T-allele can improve antioxidant status and lower the rate of ROS, suggesting that the T-allele can decrease risk of susceptible diseases such as AMD to oxidant. Other reports showed an association between *CAT* polymorphism and other age onset disease was performed in Germany (Zarbock et al.,2007), by showing that *CAT* C-262T gene polymorphism is associated with Pseudoxanthoma Elasticum (an early onset disease). The C allele in *CAT* C-262T has also been reported to be linked to the risk of type 2 diabetes (Hong Chen et al.,2011). The *CAT* C-262T and some age-related diseases is probably differs in different diseases and the ethnic groups. In this connection Cristiano Capurso and co-workers *(2008)* reported no significant association between *CAT* C-262T and Alzheimer in an Italian population. Lack of association between CAT C-262T and other diseases has been reported from other countries (Funke et al., 2009; He et al.,2010).

Studies focused on *SOD1* A251G polymorphism and age-related diseases also failed to show a major association In Studies by Oestergaard and *et al*. (2006) in Britain showed no significant association between *SOD1* A251G and breast cancer. Similar

results were reported by Rajaraman *et al*., 2010 by reporting no significant association between *SOD1* A251G and glioma. Studies on a Chinese population, however showed a significant association between *SOD1* A251G and risk of age-related cataract and G allele increases risk of cataract but showed no significant association with *CAT* A-21T polymorphism (Zhang et al.,2011).

In conclusion, the results presented in this study in favor of the reports showing no significant association between *SOD1* A251G and AMD. There are several factors which may influence the relationship between the clinical manifestation of the disease and polymorphism in one or more antioxidant enzyme genotypes. Further studies with a larger population with different isoforms of *SOD* such as *SOD2* and *SOD3* and other polymorphisms in these genes may help understanding the contribution of these antioxidant enzymes during different stages of AMD development.

Acknowledgments

The author gratefully acknowledges the Shiraz University for providing funds for this research work. The author is indebted to the participants for their close cooperation and help in the procurement of AMD blood samples.

Conflict of Interest

None declared.

References:

[1] Arsova-Sarafinovska1y Z, Matevska N, Petrovski D, Banev S, Dzikova S, et al. 2008. Manganese superoxide dismutase (MnSOD) genetic polymorphism is associated with risk of early-onset prostate cancer. Cell BiochemFunct 26:771-7.

[2] Beatty S, Koh H, Phil M, Henson D, Boulton M. 2000.The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 45: 115-34.

[3] Boker T, Fang T, Steinmetz R. 1993. Refractive error and choroidal perfusion characteristics in patients with choroidal neovascularization and age-related macular degeneration.Ger J Ophthalmol 2:10-3.

[4] Capurso C, Solfrizzi V, D'Introno A , Colacicco A , Capurso S, et al. 2008. Short arm of chromosome 11 and sporadic Alzheimer's disease:CAT and cathepsin D gene polymorphisms. Neuroscience Letters. 432:237–242.

[5] Cebrian A, Pharoah PD, Ahmed S, Smith PL, Luccarini C, et al. 2006. Tagging Single-Nucleotide Polymorphisms in Antioxidant Defense Enzymes and Susceptibility to Breast Cancer. Cancer Res 66:1225-33.

[6] Chandrasena LG, Chackrewarthy S, Perera PT, de Silva D. 2006. Erythrocyte antioxidant enzymes in patients with

cataract. Ann Clin Lab Sci 36: 201-4.

[7] Chistiakov DA, Savost'anov KV, Turakulov RI, Titovich EV, Zilberman LI, et al. 2004.A new type 1 diabetes susceptibility locus containing the CAT gene (chromosome 11p13) in a Russian population. Diabetes Metab Res Rev 20: 219-24.

[8] Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL. 2005. Risk factors for the incidence of advanced age-related macular degeneration in the age-related eye disease study. Ophthalmology 112:533-9.

[9] Coleman HR, Chan CC, Ferris FL III, Chew EY. 2008. Age-related macular degeneration. Lancet, 372:1835-45.

[10] Dufour E, Boulay J, Rincheval V, Sainsard-Chanet A. 2000.A causal link between respiration and senescence in Podosporaanserina. Proc Natl Acad Sci USA 97:4138-43.

[11] El Matri L, Bouraoui R, Chebil A, Kort F, Limaiem R, Bouladi M, et al. Prevalence and risk factors of age-related macular degeneration (AMD) in a Tunisian hospital population. Bull SocBelgeOphtalmol 2012; 319:35-41.

[12] Fan AZ, Yesupriya A, Chang Mh, House M, Fang J, et al. 2010. Gene polymorphisms in association with emerging cardiovascular risk markers in adult women. BMC Med Genet 11:6.

[13] Forsberg L, de Faire U, Morgenstern R. 2001(b). Oxidative stress, human genetic variation, and disease. Arch. Biochem. Biophys 389:84-93.

[14] Forsberg L, Lyrenäs L, Faire UD, Morgenstern R. 2001(a). A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. Free Rad Biol Med 30:500-5.

[15] Funke S, Hoffmeister M, Brenner H, Chang-Claude J. 2009. Effect modification by smoking on the association between genetic polymorphisms in oxidative stress genes and colorectal cancer risk. Cancer Epidemiology Biomarkers & Prevention 18:2336-8.

[16] Gao H, Hollyfield JG. 1992. Aging of the human retina. Differential loss of neurons and retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 33:1-17.

[17] Gehrs KM, Anderson DH, Johnson LV, Hageman GS. 2006. Age-related macular degeneration-emerging pathogenetic and therapeutic concepts. Ann.Med 38:450-71.

[18] Halliwell B, Gutteridge J. M .C. 1989. Free radicals in biology and medicine. Oxford: Clarendon Press, 12:93-5.

[19] He C, Qureshi AA, Han J. 2010. Polymorphisms in genes involved in oxidative stress and their interactions with

life style factors on skin cancer risk. Journal of Dermatology science 60:54-6.

[20] Hong Chen, Ming Yu, Ming Li, RuieZha, Qihan Zhu, et al. 2011. Polymorphic variations in manganese superoxide dismutase (MnSOD), glutathione peroxidase-1 (GPX1), and catalase (CAT) contribute to elevated plasma triglyceride levels in Chinese patients with type 2 diabetes or diabetic cardiovascular disease. Molecular Cell Biochemistry 22:610-14.

[21] Hughes AE, Orr N, Esfandiary H, Diaz-Torres M, Goodship T, Chakravarthy U. 2006. A common CFH haplotype, with deletion of CFHR1 and CFHR3, is associated with lower risk of age-related macular degeneration. Nat Genet 38:1173-7.

[22] Islam T, McConnell R, GaudermanW.j, Avo Ed M, Peters J D, Gilliland F. 2007. Ozone, Oxidant Defense Genes, and Risk of Asthma during Adolescence. Am J RespirCrit Care Med 177:388-95.

[23] McCord J M, Fridovich I. 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem 244:6049-55.

[24] Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H. 2007.Trends in oxidative aging theories. Free Rad Biol Med 43:477-503.

[25] Newton CR. 1995. Mutational analysis: known mutations. In: McPherson MJ, Hames D, Taylor GR (eds) PCR2: a practical approach. IRL-Press, Oxford, p. 219-22.

[26] Oestergaard MZ, Tyrer J, Cebrian A, Shah M, Dunning AM, et al. 2006. Interactions between genes involved in the antioxidant defence system and breast cancer risk. British Journal Cancer 95:525-31.

[27] Ogata M. 1991. Acatalasemia. Hum. Genet, 86:331-40.

[28] Quan F, Korneluk R, Tropak M, Gravel R. 1989. Isolation and characterization of the human catalse gene. Nucleic Acids Res 14:5321-35.

[29] Rafiee L, Saadat I, Saadat M. 2010. Glutathione S-transferase genetic polymorphisms (GSTM1, GSTT1 and GSTO2) in three Iranian populations. Mol Biol Rep 37:155-8.

[30] Rajaraman P, Hutchinson A, Rothman N, Black PM, Fine HA, et al. 2010. DNA repair gene polymorphisms and risk of adult meningioma, glioma and acoustic neuroma. Neuro Oncology 12:37-48.

[31] Rajić V, Aplenc R, Debeljak M, Prestor VV, Karas-Kuzelicki N, et al. 2009. Influence of the polymorphism in candidate genes on late cardiac damage in patients treated due to acute leukemia in childhood. Leuk Lymphoma 50:1693-8.

[32] Sandberg MA, Tolentino MJ, Miller S, Berson EL, Gaudio AR. 1993. Hyperopia

and neovascularization in age-related macular degeneration. Ophthalmology 100:1009-13.

[33] Smith W, Mitchell P. 1998. Family history and age-related maculopathy: the blue mountains eye study. Aust. N.Z.J. Ophthalmol 26:203-6.

[34] Spraul CW, Grossniklaus HE. 1997. Characteristics of Drusen and Bruch's membrane in postmortem eyes with age-related macular degeneration. Arch Ophthalmol 115:267-73.

[35] Tang H, Dong X, Day RS, Hassan MM, Li D. 2010. Antioxidant genes, diabetes and dietary antioxidants in association with risk of pancreatic cancer. Carcinogenesis 31:607-13.

[36] Tilak JC, Boloor KK, Sane Ketaki S, GhaskadbiSaroj S, Lele RD. 2004. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. J Assoc Physicians India 52: 796.

[37] Zarbock R, Hendig D, Szliska C, Kleesiek K, Götting C. 2007. Pseudoxanthoma Elasticum: genetic variations in antioxidant genes are risk factors for early disease onset. ClinChem 53:1734-40.

[38] Zhang Y, Lan Z, DongL Is, ZhiSheng Li, Lin W, Ping L. 2011. Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract.Mol Vis 17.