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Lack of association between three common genetic variations of *XPC* and susceptibility to age-related macular degeneration, a preliminary study

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Abstract

Background: Numerous association studies have indicated that genetic alterations in genes involved in DNA repair processes are associated with the risk of age-related macular degeneration (ARMD). There is no published study on the relationship between common xeroderma pigmentosum complementation group C (*XPC*, MIM 613208) polymorphisms and susceptibility to ARMD. The aim of this study is to determine whether three common (Ala499Val, Lys939Gln, and PAT) genetic variants of *XPC* are associated with the risk of developing ARMD. A total of 120 ARMD patients and 118 healthy controls were included in the study. Genotyping analyses were carried out by PCR-based methods.

Results: Our analysis revealed that there was no relationship between the *XPC* polymorphisms and susceptibility to ARMD. In both case and control groups, strong linkage disequilibrium existed between three common (Ala499Val, Lys939Gln, and PAT) genetic polymorphisms of *XPC*. Statistical analysis showed no association between the haplotypes and the risk of ARMD.

Conclusions: The present data indicated that the common polymorphisms of *XPC* are not susceptible genetic variations for ARMD.

Keywords: DNA repair, Linkage disequilibrium, Macular degeneration, Polymorphism, *XPC*, Risk

Background

Age-related macular degeneration (ARMD) is a multifactorial complex disease. The photoreceptor degeneration in the central part of the retina leads to the complete loss of central vision. Although the etiology of ARMD is not understood, previous studies have indicated the reactive oxygen species (ROS)-induced damage in ARMD patients [1, 2]. The retina has high level of oxygen consumption. It is revealed that photochemical reactions between light and O_2 lead to the production of ROS [3, 4]. It is well established that ROS can damage cellular macromolecules including DNA. The impaired efficacy of cellular DNA repair might contribute to the pathogenesis of ARMD [5–7].

Xeroderma pigmentosum complementation group C (*XPC*, MIM 613208) encodes a protein which is involved in nucleotide excision repair by initially detecting the DNA damage [8]. In human populations, the *XPC* has several common polymorphisms including Ala499Val (rs2228000), Lys939Gln (rs2228001), and PAT [9, 10]. It should be noted that these polymorphisms are associated with cellular DNA repair capacity [11]. Studies have indicated that these polymorphisms are associated with the risk of several multifactorial traits such as cancers [12–15], schizophrenia [16], and dependency to drugs [17].

A meta-analysis of genome wide linkage studies has confirmed that the human chromosome 3p is a

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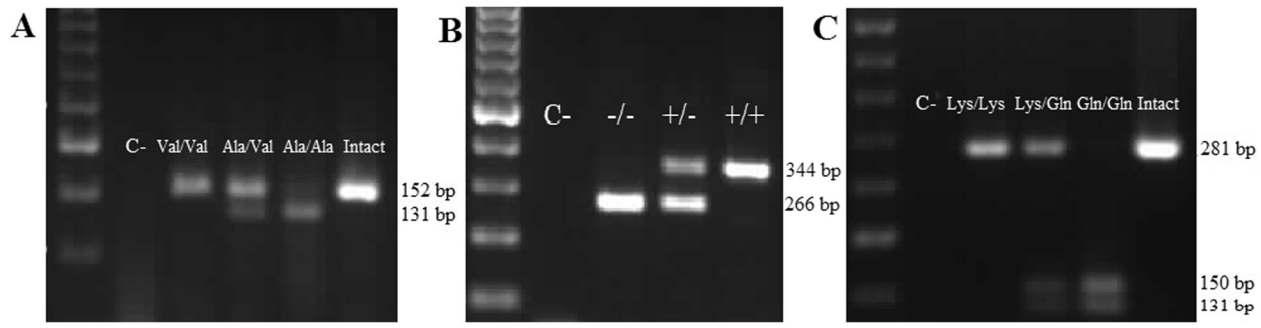

Fig. 1 Genotyping analyses for three common (Ala499Val, Lys939Gln, and PAT) genetic variants of *XPC*. **a**–**c** Showed the genotypes of the Ala499Val, PAT, and Lys939Gln polymorphisms, respectively. In each panel line, "C-" means "negative control"; lines "Intact" showed the PCR product before digestion with restriction enzyme

candidate chromosome segment associated with the ARMD [18]. It should be noted that the gene encoding *XPC* is located on human chromosome 3p25.1 [19]. Previously, the association between common genetic polymorphisms of genes involved in DNA repair processes and susceptibility to ARMD has been reported [20–25]. Taken together, it is concluded that the XPC might be associated with the susceptibility to ARMD. As there is no published study on the association between common *XPC* polymorphisms and susceptibility to ARMD, this case-control study was carried out.

Methods

This hospital-based case-control study consisted of 120 patients (75 males, 45 females) with exudative ARMD. The patients were recruited from the "Khalili Hospital Ophthalmic clinic" (Fars province, Iran), referred by a vitreoretinal surgeon. Moreover, 118 gender frequency-matched participants (68 males, 50 females) were randomly selected from unrelated volunteers in the same clinic and used as the control group. The mean age (SD) of the ARMD patient and the control groups was 69.6 (9.7) and 63.5 (10.0) years, respectively. There was significant difference in age distribution between the patients and the controls (t = 4.73, df = 236, P < 0.001). Based on the job titles, the participants were categorized into indoor (teachers, housewives, etc.) and outdoor (drivers, farmers, etc.) groups. The outdoor participants were occupationally exposed to sunlight. In the present study, the participants were selected from the same ethnical group (Muslims/Persians) living in Shiraz. This case-control study was approved by the local ethics committee. Informed consent was obtained from all participants.

Genotyping analyses for the Ala499Val (rs2228000) and Lys939Gln (rs2228001) polymorphisms were carried out using specific primers as described previously [9, 10] for the polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP). The PAT polymorphism is an insertion of 83 bases of A and T [poly (AT)] with a 5-base deletion (GTAAC at position 1457–1461, GenBank Accession No. AF076952) within intron 9 of the *XPC* gene [26]. Therefore, PCR products of the Ins and Del alleles have different lengths (Fig. 1). Genotyping for this polymorphism was carried out using specific primers as described previously in a simple PCR [10].

For each study polymorphisms, the observed genotypic frequencies were compared with the expected frequencies based on the Hardy-Weinberg equilibrium (HWE). Unconditional binary logistic regression analysis was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for ARMD risk associated with the genotypes of the study polymorphisms. Considering the significant age difference between the cases and the controls, in further analysis, logistic regression was used to estimate ORs and 95% CIs for the various genotypes after adjusting for age. Genetic linkage

Table 1 The general characteristics of age-related macular degeneration patients and the healthy control group

	Control	Case	χ^2 (df = 1)	P
Gender				
Females	50	45	0.58	0.443
Males	68	75		
Smoking habit				
Non-smoker	64	68	5.76	0.016
Smoker	18	42		
Missing	36	10		
Workplace				
Indoor	66	66	6.21	0.013
Outdoor	26	54		
Missing	26	0		
Hypertension				
No	105	112	1.03	0.309
Yes	4	8		
Missing	9	0		
Age	63.5 ± 10.0	69.9 ± 9.7	t = 4.76, df = 236	< 0.001

disequilibrium between the alleles of the *XPC* polymorphisms was estimated using the SNPAlyze (TM) software (ver. 6 Standard, Dynacom Co, Ltd. Kanagawa, Japan). A $P < 0.05$ was considered a statistically significant difference.

Results

The general characteristics of the ARMD patients and the control group are summarized in Table 1. In our study subjects, 45.0% (out of 120) of the patients and 28.3% (out of 92) of the healthy controls had outdoor jobs. This difference was statistically significant (χ^2 = 6.21, df = 1, P = 0.013). Among the ARMD patients and the controls, 38.2% (out of 110) and 22.0% (out of 82) were smokers (χ^2 = 5.76, df = 1, P = 0.016), respectively.

Table 2 summarized the genotypic frequency of the three common *XPC* polymorphisms among the ARMD

Table 2 The association between three common *XPC* polymorphisms and the risk of age-related macular degeneration

Polymorphisms/genetic models	Controls	Cases	OR	95% CI	*P*	OR*	95% CI	*P*
PAT								
Additive model								
-/-	33	31	1.0	–	–	1.0	–	–
-/+	57	60	1.12	0.61–2.06	0.714	1.11	0.58–2.11	0.746
+/+	28	29	1.10	0.54–2.25	0.789	0.92	0.44–1.95	0.846
Dominant model								
-/-	33	31	1.0	–	–	1.0	–	–
-/+ and +/+	85	89	1.11	0.62–1.97	0.711	1.04	0.57–1.91	0.881
Recessive model								
-/- and -/+	90	91	1.0	–	–	1.0	–	–
+/+	28	29	1.02	0.56–1.85	0.937	0.86	0.46–1.61	0.653
Minor allele frequency	0.4788	0.4917	1.05	0.73–1.50	0.779			
Ala499Val								
Additive model								
Ala/Ala	72	73	1.0	–	–	1.0	–	-
Ala/Val	41	39	0.93	0.54–1.62	0.819	1.02	0.57–1.81	0.947
Val/Val	5	8	1.57	0.49–5.05	0.442	1.48	0.45–4.92	0.515
Dominant model								
Ala/Ala	72	73	1.0	–	–	1.0	–	–
Ala/Val + Val/Val	46	47	1.01	0.59-1.69	0.977	1.07	0.62–1.86	0.791
Recessive model								
Ala + Ala/Val	113	112	1.0	–	–	1.0	–	–
Val/Val	5	8	1.61	0.51–5.08	0.413	1.47	0.45–4.80	0.517
Minor allele frequency	0.2161	0.2291	1.07	0.70–1.66	0.732			
Lys939Gln								
Additive model								
Lys/Lys	31	30	1.0	–	–	1.0	–	–
Lys/Gln	58	61	1.08	0.58–2.01	0.792	1.04	0.54–1.99	0.899
Gln/Gln	29	29	1.03	0.50–2.12	0.929	0.84	0.39–1.78	0.656
Dominant model								
Lys/Lys	31	30	1.0	–	–	1.0	–	–
Lys/Gln + Gln/Gln	87	90	1.06	0.59–1.91	0.822	0.97	0.52–1.79	0.927
Recessive model								
Lys/Lys + Lys/Gln	89	91	1.0	–	–	1.0	–	–
Gln/Gln	29	29	0.98	0.54–1.76	0.941	0.82	0.44–1.52	0.528
Minor allele frequency	0.4915	0.4958	1.01	0.71–1.45	0.925			

*Adjusted ORs for age of participants

cases and the control subjects. For the Ala499Val, PAT, and Lys939Gln polymorphisms, the minor alleles showed 0.2161, 0.4788, and 0.4915 in the control group, respectively. Our statistical analysis indicated that there was very high similarity between the observed genotypic frequencies and the expected frequencies according to the HWE distribution in the controls (For Ala499Val polymorphism: χ^2 = 0.07, df = 1, P = 0.781; For PAT polymorphism: χ^2 = 0.122, df = 1, P = 0.726; For Lys939Gln polymorphism: χ^2 = 0.03, df = 1, P = 0.856). Our present data revealed that there was no significant relationship between the *XPC* polymorphisms and the risk of ARMD (Table 2).

Statistical analysis demonstrated extremely high level of linkage disequilibrium between the *XPC* polymorphisms (Table 3). The haplotypic frequency in the ARMD cases and the controls are given in Table 4. The frequency of the haplotypes "Ala + Gln," "Ala – Lys," "Val – Lys," and "Ala - Gln" were 113, 69, 51, and 3 in the control group and were 118, 66, 55, and 1 in the ARMD group, respectively. The "Ala + Gln" haplotype was more common compared to the other haplotypes we used as reference group (OR = 1.0). Statistical analysis showed no relationship between the haplotypes and the susceptibility to ARMD (Table 4).

Discussion

Similar to numerous epidemiologic studies [27, 28], we found that the risk of ARMD has strong associations with cigarette smoking and outdoor workplace. When considering the occupationally sunlight exposure and smoking habit (risk factors for ARMD), it becomes apparent that these factors have well-documented effects on oxidative stress and its consequent inflammation. Moreover, oxidative stress has a variety of consequences that can affect disease progression through numerous avenues [29].

Table 3 Linkage disequilibrium between Ala499Val, PAT, and Lys939Gln polymorphisms of *XPC* among healthy controls and age-related macular degeneration patients

Polymorphisms	Ala499Val	PAT	Lys939Gln
Ala499Val	–	D' = 1.0 r^2 = 0.2533 χ^2 = 59.77 $P = 1.0 \times 10^{-14}$	D' = 1.0 r^2 = 0.2665 χ^2 = 62.89 $P = 2.1 \times 10^{-15}$
PAT	D' = 1.0 r^2 = 0.2875 χ^2 = 69.01 $P = 9.7 \times 10^{-17}$	–	D' = 1.0 r^2 = 0.9504 χ^2 = 224.28 $P = 1.0 \times 10^{-50}$
Lys939Gln	D' = 1.0 r^2 = 0.2924 χ^2 = 70.17 $P = 5.4 \times 10^{-17}$	D' = 1.0 r^2 = 0.9835 χ^2 = 236.03 $P = 2.8 \times 10^{-53}$	–

The upper and lower parts of the table showed parameters among healthy controls and age-related macular degeneration patients, respectively

Table 4 The association between the haplotypes of the studied *XPC* polymorphisms and the risk of age-related macular degeneration

Polymorphisms			Controls	Cases	OR	95% CI	*P*
499	PAT	939					
Ala	+	Gln	113	118	1.0	–	–
Ala	–	Lys	69	66	0.91	0.59–1.40	0.686
Val	–	Lys	51	55	1.03	0.65–1.63	0.891
Ala	–	Gln	3	1	0.31	0.03–3.11	0.326

The frequencies of the 499Val, PAT-, and 939Gln alleles in our control samples were similar to the Caucasian populations [12] and our previous reports from Iran [16, 17]. The *XPC* Ala499Val, PAT, and Lys939Gln polymorphisms showed linkage disequilibrium in both patient and control groups, as reported in previous reports [12, 17]. We observed only four haplotypes ("Ala + Gln," "Ala – Lys," "Val – Lys," and "Ala - Gln") out of nine expected haplotypes among our participants, due to linkage disequilibrium in Iranian gene pool. The prevalence of four haplotypes is similar to our previous reports from Iran [17].

Previous studies have indicated that the impaired efficacy of cellular DNA repair may contribute to the pathogenesis of ARMD [5–7]. The *XPC* is involved in the first step of global genome nucleotide excision DNA repair pathway [8]. Considering that the Ala499Val, Lys939Gln, and PAT polymorphisms are associated with cellular DNA repair capacity [11], we hypothesized that the above-mentioned genetic variations might be involved in pathogenesis of ARMD. However, our present findings indicate that the studied genetic polymorphisms are not significantly associated with the risk of ARMD.

As we know, there are two types of ARMD. The patients included in the present study had exudative AMRD. It has been reported that a genetic polymorphism may be associated with a specific type of a multifactorial trait. For example, several genetic polymorphisms affect the risk of early or late onset bipolar disorder type 1 in different manners [30–32]. It is suggested that there is the same story for association between *XPC* polymorphisms and dry or exudative AMRD. It should be noted that among the known genetic variations, the polymorphisms of complement factor H (*CFH*) contribute to more than 50% of disease risk, which is the first major disease-related gene for ARMD [33]. In the present study, we did not determine the *CFH* genotypes. It should be noted that this may mask the effect of *XPC* polymorphisms. Further studies are needed to investigate the synergistic effect of common polymorphisms in *XPC* and *CFH* on predisposition to ARMD.

Considering the limited sample size in the present case-control study, further larger scaled and well-

designed studies are needed to confirm our results, and before the final conclusion regarding the involvement of the common *XPC* polymorphisms in age-related macular degeneration can be drawn.

Conclusions

In the present case-control study, the association between three common *XPC* genetic variations and the risk of ARMD was evaluated. No significant relationship was found between the genotypes of each *XPC* polymorphisms and the risk of ARMD. There was also no association between the haplotypes of the study polymorphisms and the risk of ARMD.

Abbreviations

ARMD: Age-related macular degeneration; HWE: Hardy-Weinberg equilibrium; OR: Odds ratio; ROS: Reactive oxygen species; CFH: Complement factor H; CI: Confidence intervals; *XPC*: Xeroderma pigmentosum complementation group

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Authors' contributions

MS designed the research; SK performed genotyping; SK and MS performed the statistical analyses; SK and MS interpreted the results. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study is approved by the ethics committee of Shiraz University (Iran). A written informed consent form was obtained from each patient according to the Declaration of Helsinki.

Consent for publication

Consent to publish the data was obtained from all individual participants or their attendants included in the study.

Competing interests

The authors declare that they have no conflict of interest.

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References

1. Kaarniranta K, Pawlowska E, Szczepanska J, Jablkowska A, Blasiak J (2019) Role of mitochondrial DNA damage in ROS-mediated pathogenesis of age-related macular degeneration (AMD). Int J Mol Sci 20:10. https://doi.org/10.3390/ijms20102374
2. Bellezza I (2018) Oxidative stress in age-related macular degeneration: Nrf2 as therapeutic target. Front Pharmacol 9:1280
3. Gaillard ER, Atherton SJ, Dillon J (1995) Photophysical studies on human retinal lipofuscin. Photochem Photobiol 61:448–453
4. Strunnikova N, Zhang C, Teichberg D, Cousins SW, Baffi J, Becker KG, Csaky KG (2004) Survival of retinal pigment epithelium after exposure to prolonged oxidative injury: a detailed gene expression and cellular analysis. Invest Ophthalmol Vis Sci 45:3767–3777
5. Blasiak J, Glowacki S, Kauppinen A, Kaarniranta K (2013) Mitochondrial and nuclear DNA damage and repair in age-related macular degeneration. Int J Mol Sci 14:2996–3010
6. Lin H, Xu H, Liang FQ, Liang H, Gupta P, Havey AN, Boulton ME, Godley BF (2011) Mitochondrial DNA damage and repair in RPE associated with aging and age-related macular degeneration. Invest Ophthalmol Vis Sci 52:3521–3529
7. Szaflik JP, Janik-Papis K, Synowiec E, Ksiazek D, Zaras M, Wozniak K, Szaflik J, Blasiak J (2009) DNA damage and repair in age-related macular degeneration. Mutat Res 669:169–176
8. Sugasawa K, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker AP, Hanaoka F, Bootsma D, Hoeijmakers JH (1998) Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. Mol Cell 2:223–232
9. Hu Z, Wang Y, Wang X, Liang G, Miao X, Xu Y, Tan W, Wei Q, Lin D, Shen H (2005) DNA repair gene *XPC* genotypes/haplotypes and risk of lung cancer in a Chinese population. Int J Cancer 115:478–483
10. Liu Y, Wang H, Lin T, Wei Q, Zhi Y, Yuan F, Song B, Yang J, Chen Z (2012) Interactions between cigarette smoking and *XPC*-PAT genetic polymorphism enhance bladder cancer risk. Oncol Rep 28:337–345
11. Zhu Y, Yang H, Chen Q, Lin J, Grossman HB, Dinney CP, Wu X, Gu J (2008) Modulation of DNA damage/DNA repair capacity by *XPC* polymorphisms. DNA Repair (Amst) 7:141–148
12. Sankhwar M, Sankhwar SN, Bansal SK, Gupta G, Rajender S (2016) Polymorphisms in the *XPC* gene affect urinary bladder cancer risk: a case-control study, meta-analyses and trial sequential analyses. Sci Rep 6:27018
13. Jin B, Dong Y, Zhang X, Wang H, Han B (2014) Association of *XPC* polymorphisms and lung cancer risk: a meta-analysis. PLoS ONE 9:e93937
14. Zhang Y, Li Z, Zhong Q, Zhou W, Chen X, Chen X, Fang J, Huang Z (2014) Polymorphisms of the *XPC* gene may contribute to the risk of head and neck cancer: a meta-analysis. Tumour Biol 35:3917–3931
15. Liang XH, Yan D, Zhao JX, Ding W, Xu XJ, Wang XY (2018) Interaction of polymorphisms in xeroderma pigmentosum group C with cigarette smoking and pancreatic cancer risk. Oncol Lett 16:5631–5638
16. Taghipour N, Saadat I, Saadat M (2019) Association between polymorphisms of Xeroderma pigmentosum complementation group C gene (*XPC*) and susceptibility to schizophrenia. Gene 695:99–100
17. Qasemian-Talgard A, Saadat M (2020) Association between three common genetic polymorphisms of *XPC* and susceptibility to heroin dependency. Gene 724:144153. https://doi.org/10.1016/j.gene.2019.144153
18. Legerski RJ, Liu P, Li L, Peterson CA, Zhao Y, Leach RJ, Naylor SL, Siciliano MJ (1994) Assignment of xeroderma pigmentosum group C (*XPC*) gene to chromosome 3p25. Genomics 21:266–269
19. Fisher SA, Abecasis GR, Yashar BM, Zareparsi S, Swaroop A, Iyengar SK, Klein BE, Klein R, Lee KE, Majewski J, Schultz DW, Klein ML, Seddon JM, Santangelo SL, Weeks DE, Conley YP, Mah TS, Schmidt S, Haines JL, Pericak-Vance MA, Gorin MB, Schulz HL, Pardi F, Lewis CM, Weber BH (2005) Meta-analysis of genome scans of age-related macular degeneration. Hum Mol Genet 14:2257–2264
20. Chu XK, Meyerle CB, Liang X, Chew EY, Chan CC, Tuo J (2014) In-depth analyses unveil the association and possible functional involvement of novel *RAD51B* polymorphisms in age-related macular degeneration. Age (Dordr) 36:9627
21. Synowiec E, Wysokinski D, Zaras M, Kolodziejska U, Stoczynska-Fidelus E, Janik K, Szaflik J, Blasiak J, Szaflik JP (2014) Association between polymorphism of the DNA repair *SMUG1* and *UNG* genes and age-related macular degeneration. Retina 34:38–47
22. Saadat I, Vakili-Ghartavol R, Farvardin-Jahromi M, Saadat M (2012) Association between exudative age-related macular degeneration and the G6721T polymorphism of *XRCC7* in outdoor subjects. Korean J Ophthalmol 26:423–427
23. Blasiak J, Synowiec E, Salminen A, Kaarniranta K (2012) Genetic variability in DNA repair proteins in age-related macular degeneration. Int J Mol Sci 13:13378–13397
24. Görgün E, Güven M, Unal M, Batar B, Güven GS, Yenerel M, Tatlipinar S, Seven M, Yüksel A (2010) Polymorphisms of the DNA repair genes *XPD* and *XRCC1* and the risk of age-related macular degeneration. Invest Ophthalmol Vis Sci 51:4732–4737
25. Tuo J, Ning B, Bojanowski CM, Lin ZN, Ross RJ, Reed GF, Shen D, Jiao X, Zhou M, Chew EY, Kadlubar FF, Chan CC (2006) Synergic effect of polymorphisms in *ERCC6* 5'flanking region and complement factor H on

age-related macular degeneration predisposition. Proc Natl Acad Sci U S A 103:9256–9261
26. Khan SG, Metter EJ, Tarone RE, Bohr VA, Grossman L, Hedayati M, Bale SJ, Emmert S, Kraemer KH (2000) A new xeroderma pigmentosum group C poly(AT) insertion/deletion polymorphism. Carcinogenesis 21:1821–1825
27. Willeford KT, Rapp J (2012) Smoking and age-related macular degeneration: biochemical mechanisms and patient support. Optom Vis Sci 89:1662–1666
28. Modenese A, Gobba F (2019) Macular degeneration and occupational risk factors: a systematic review. Int Arch Occup Environ Health 92:1–11
29. Shaw PX, Stiles T, Douglas C, Ho D, Fan W, Du H, Xiao X (2016) Oxidative stress, innate immunity, and age-related macular degeneration. AIMS Mol Sci 3:196–221
30. Rezaei Z, Saadat I, Saadat M (2012) Association between three genetic polymorphisms of glutathione S-transferase Z1 (*GSTZ1*) and susceptibility to bipolar disorder. Psychiatry Res 198:166–168
31. Mohammadynejad P, Saadat I, Ghanizadeh A, Saadat M (2011) Bipolar disorder and polymorphisms of glutathione S-transferases M1 (*GSTM1*) and T1 (*GSTT1*). Psychiatry Res 186:144–146
32. Kordestanian N, Saadat M (2017) A 50-bp Ins/Del polymorphism at the promoter region of the superoxide dismutase-1 and bipolar disorder type 1. Nord J Psychiatry 71:570–573
33. Cameron DJ, Yang Z, Gibbs D, Chen H, Kaminoh Y, Jorgensen A, Zeng J, Luo L, Brinton E, Brinton G, Brand JM, Bernstein PS, Zabriskie NA, Tang S, Constantine R, Tong Z, Zhang K (2007) *HTRA1* variant confers similar risks to geographic atrophy and neovascular age-related macular degeneration. Cell Cycle 6:1122–1125

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