

Original Article

Association of two polymorphisms in *MSH2* and *XRCC1* genes with multiple sclerosis in Iranian population

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Abstract

Introduction: To protect genomes of all organisms from internal and external damages and maintain the genome integrity and the continuity of life, repair system has been developed in all living cells. Defects in repair system are responsible for various kinds of disease including cancers and neurodegenerative diseases such as Multiple sclerosis (MS). The relationship between various components of the repair system and MS has been confirmed by investigations on separate cohorts in independent research. The main aim of this study was to discover the genetic association of two functional polymorphisms of rs1799782 in *XRCC1* and rs2303425 in *MSH2* genes as the key players in DNA repair system; with MS.

Methods: The genotypes of 105 MS patients and 102 age and sex matched healthy controls for these polymorphisms were determined by a PCR-RFLP technique.

Results: Genotype and allele frequencies of rs1799782 in patients with MS compared to the control group demonstrated a significant difference and a possible role for this polymorphism in MS pathogenesis (P value (0.02) and OR (3.4)). The rs2303425 polymorphism showed no significant correlation (P value = 0.41 and OR=1.5) with the risk of MS in Iranian population.

Conclusion: Our results suggest a possible role for repair system genes and their significance in the pathogenesis of multiple sclerosis.

Keywords:

DNA repair;
Multiple Sclerosis;
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Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease with autoimmune and neurodegenerative hallmarks which involves inflammation, demyelination, and axonal damage of central nervous system (CNS) (Owens, 2003; Frischer et al., 2009). The inflammatory niche in the CNS causes lesions leading to inappropriate signal transduction between

different parts of the brain and causes lifelong disabilities (Gregory et al., 2012) in up to 2.5 million peoples over the worldwide (Westerlind et al., 2014). There are four disease courses in MS disease including clinically isolated syndrome (CIS), relapsing-remitting (RR), primary progressive (PP), and secondary progressive (SP) that are categorized based on the relapse rate and disease progression. RR MS is the most common subtype of the disease and usually end up in SP MS (Polman et al., 2011).

Several genetic and environmental factors like mutation and gene deregulation (Naghavi Gargari et al., 2015a; Naghavi Gargari et al., 2015b), smoking (Wingerchuk, 2012), EBV infection (Levin et al., 2010), and deficiency in vitamin D (Cantorna, 2006), seems to be involved in MS pathogenesis.

Mutations happen in genome and nucleotide pool and lead to numerous clinical conditions like several types of cancers and neurodegenerative disease (Gon et al., 2011). Damages with internal and external sources, like slippages in DNA polymerase replication, radiation and reactive oxygen species, can cause mutation and damage to the genetic information.

All of living cells are equipped with the DNA repair system to maintain genome integrity (Jeggo et al., 2016). DNA repair system consists of three main mechanisms of mismatch repair, nucleotide or base excision repair and homologous recombination, and all of them act on DNA level (Kunkel and Erie, 2015).

X-Ray Repair Cross-Complementing Protein 1 (XRCC1) is a vital component of base excision repair system which takes part in efficient repair of single-strand breaks caused by ionizing radiations and chemical compounds like alkylating agents in collaboration with DNA ligase III, polymerase beta, and poly (ADP-ribose) polymerase (Breslin et al., 2015; Ying et al., 2016). MSH2 is one of the main components of mismatch repair (MMR) system and can detect mismatched regions in DNA strands (Kunkel and Erie, 2005; Jiricny, 2006). MSH2 protein plays its important role as an element of a complex with MSH3 or MSH6 to identify mismatched bases or mismatched slipped repeats, respectively (Owen et al., 2005; Wilson et al., 2005). After detection of the mutated site, downstream factors will be recruited and repair the error (Jiricny, 2006).

Polymorphisms in the different part of the genome are characterized as the predisposing elements to various kinds of pathologies and are the main basis of personalized medicine (Sachidanandam et al., 2001). Several polymorphisms in the *XRCC1* and *MSH2* genes have been found to be associated with cancers including lung, breast, nonmelanoma, skin, and gastric (Palli et al., 2010).

The association of some repair genes' polymorphisms with MS (Ebers et al., 1996) and also other neurodegenerative diseases have been verified, but the possible association of two functional

polymorphisms of rs1799782 in *XRCC1* and rs2303425 in *MSH2* genes with MS is not examined yet (Simon-Sanchez et al., 2009). In ALS, a neurodegenerative disease, the decreased function of repair system in the patients' brains has been reported. Also in 2010, Briggs et al. confirmed the association of several polymorphisms in the repair genes, including *GTF2H4*, with MS (Briggs et al., 2010).

Based on previous information about the possible association of DNA repair system and the onset of MS, the aim of the present study was to investigate the frequencies of polymorphic rs2303425 located in the promoter of *MSH2* and rs1799782 in the coding region of *XRCC1* in a population of Iranian MS patients.

Materials and methods

Patients and controls

Case samples from approved MS patients, based on McDonald criteria (Poser et al., 1983; Polman et al., 2005) and based on Brain Magnetic Resonance Imaging (MRI), were collected from Sina Hospital (Tehran, Iran). Control samples were collected from volunteers with no neurological history, family history of autoimmune disease or multiple sclerosis at the time of sampling with coincident sexuality, age, and race. In this study, 105 cases and 102 controls with listed demographic features were included (Table 1). This study was approved by the ethical committee of Tarbiat Modares University and all the participants signed a written informed consent prior to the blood sampling.

Genomic DNA extraction

Two ml peripheral blood was collected from each participant and stored in -80 °C in tubes contain 3 mg EDTA till DNA extraction. Peripheral Blood Mononuclear cells (PBMCs)' DNA was extracted by using DNG PLUS™ kit (CinnaGen, Iran) according to manufacturer's instruction. The extracted DNA was washed and desalted by 70% ethanol and dissolved in TE buffer and stored in -20 °C till molecular analysis. The quality and quantity of extracted DNA were visualized by and measured by electrophoresis in 1% agarose gel and spectrophotometry, respectively.

Table 1: Demographic features of the control group and MS patients

	Male			Female			Types of MS frequencies		
	Number	Age (years)	Mean age onset	Number	Age (years)	Mean age onset	Relapse-remitting (RR)	Secondary progressive (SP)	Primary progressive (PP)
Patients	32	23-50	29.7	73	17-53	29.7	69.7%	25.3%	5%
Controls	32	21-52		70	16-60	-	-	-	-

SNP Genotyping

For genotyping rs1799782 and rs2303425 polymorphisms, 100- 150 ng of genomic DNA were used as template for PCR-RFLP techniques. Specific primers for Arg194Trp (rs1799782) with followed sequences of 5'-ACGCCGGAACGTCCCTAATTC-3' and 5'-AGAGGCGCGACTGGGCTTG-3' that amplified a 427 bp fragment, were designed by primer 3 software. For amplification of MSH2 polymorphism (rs2303425), a 350 bp region was amplified by designing specific primers, 5'-CGGAAACAGCTTAGTGGGT-3' and 5'-TCTCTGAGGCGGAAAGGAG-3'.

The amplification was performed using master mix PCR (Solis BioDyne, Estonia) through following instruction: an initial pre-denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C (30 s), annealing at 60°C (30 s) and extension at 72°C (45 s) followed by 5 minutes final extension at 72°C. The PCR-amplified fragments were digested with restriction endonucleases (Thermo Scientific) Pvu II for cutting C allele at rs1799782 and BstN I (Fermentas) for T allele at rs2303425. The digestion reaction was performed in a total volume of 20 μ l followed by overnight incubation at 37°C and the genotypes of participants were observed by running the digested products on 2% agarose gel. To verify the designed genotyping procedures, the DNA sequences of some randomly selected samples for each genotype was determined by an ABI automated DNA sequencer (Macrogen, Korea).

Statistical analysis and bioinformatics analysis

Statistical calculations were performed by SPSS software package (Version.19.0). The Chi-square test

was used to analyze the association of two SNPs with MS using frequency of alleles and genotypes between the studied groups. In this study, P-value \leq 0.05 was considered as significant.

The bioinformatics analysis was performed using consite (<http://consite.genereg.net>), JASPAR (jaspar.genereg.net) and micro-PIR (www4a.biotech.or.th/micropir) for identifying transcription and microRNA binding sites overlap with these two SNPs, respectively.

Results

In this study, we analyzed the genotypes and allele frequencies of 105 MS patients and 102 healthy controls for two polymorphisms in *XRCC1* and *MSH2* genes using the PCR-RFLP technique. Calculated genotype frequencies for both SNPs in patients and healthy control groups were in Hardy-Weinberg equilibrium.

The result of enzyme's digestion for rs1799782 polymorphism on the agarose gel (2%) is shown in Figure 1. The allelic frequencies (C, T) in case population were 93.33% and 6.67% vs. 98% and 2% in controls, respectively; while the genotype frequencies (CC, CT) in patients were 87% and 13% vs. 96% and 4% in healthy controls, respectively. To evaluate the relationship between the risk of MS and the CT genotype, χ^2 test declared significant differences between patients and healthy volunteers (P-value= 0.024 and OR = 3.400 (1.158, 9.985)). The χ^2 test for calculating the association between T allele and the risk of MS onset reached the significance level (P-value= 0.028, OR= 3.400 (1.138 10.157)). Gender stratification confirmed a significant association between CT genotype and MS risk in female (P-value= 0.05, OR= 4.253(0.952, 18.994))

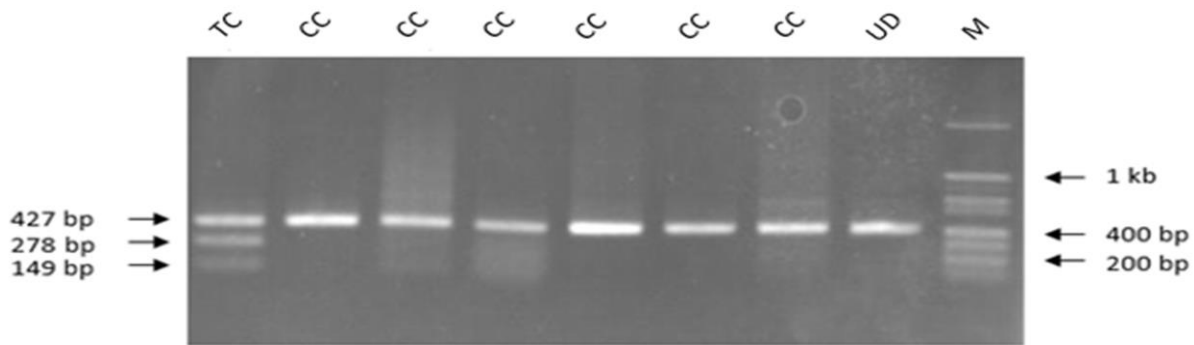


Fig.1. A PCR-RFLP technique used for genotyping of rs1799782 SNP (Arg 194 Trp) by Pvu II as a restriction endonuclease enzyme. The presence of T allele in SNP position can be recognized by this method. Digestion process produces 278 and 149 bp fragments. The genotype of each sample is shown on the top of the gel. Undigested control and ladder are shown by UD and M, respectively.

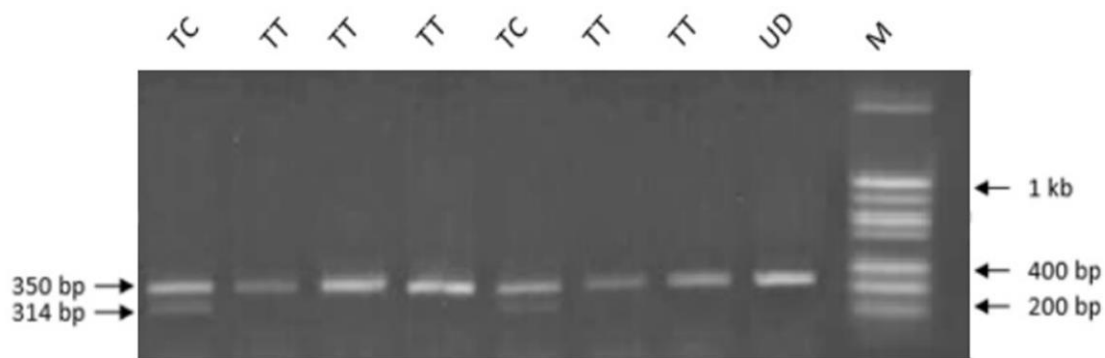


Fig.2. To genotype patients and control individuals for SNP rs2303425 in MSH2 gene, a PCR –RLFP used. In the presence of C allele in SNP position, restriction endonuclease BstNI can cut the amplicon of 350 bp to two fragments with 314 and 36 bp. The products of restriction enzyme digestion were separated by 2% agarose gel electrophoresis.

but not in male patients and, also no significant association observed between allelic frequencies and MS risk in both genders.

The allele frequencies for rs2303425 (T, C) in the case population were calculated as 93.8% and 6.2%, while in the control group were 91.66% and 8.34%, respectively. The genotype frequencies (TT, CT) in patients were measured 88% and 12% vs. 91.67% and 8.33% in controls. The X^2 test between the case and control cohorts, did not validate the association of CT genotype and the C allele with MS risk with P-value of 0.451 and 0.433, respectively. After gender stratification, statistical analysis showed no significant differences in genders for the association of C allele and CT genotype with the risk of MS (Table 3).

For further investigation, bioinformatics analysis using Consite and Jaspas software indicated that rs2303425 polymorphism, located in *MSH2* promoter,

is a recognition site for a forkhead transcription factor called FREAC-3.

Discussion

Multiple sclerosis is one of the most prominent neurodegenerative disorders in the world, primes autoimmune reactions resulting in an inflammatory niche in the CNS, myelin degradation, axonal loss, and irreversible disability (Owens, 2003; Frischer et al., 2009). Various investigations have been done all over the world to clarify the association between different components of the DNA repair systems and the risk of MS incidence. In previous studies, the relationship between polymorphisms of repair system genes of *XRCC4*, *XAB2*, *RAD23A*, and *RPA3* and MS has been confirmed (Ebers et al., 1996). In addition to polymorphisms' role in the pathogenesis

Table 2: Genotype and allele frequencies of rs1799782 among case and control population

cohort	Maj. Allele%	Min Allele%	P-Value	OR (CI 50%)	CC genotype%	CT genotype%	P value	OR (CI 50%)
Total MS	93.33	6.67	.028	3.400 (1.138, 10.157)	87	13	0.024	3.400 (1.158, 9.985)
Male MS	92.19	7.81	0.27	2.578 (0.519, 12.813)	84.38	15.62	0.25	2.578 (0.538, 12.344)
Female MS	93.83	6.16	0.061	4.253 (0.935, 19.339)	87.68	12.32	0.05	4.253 (0.952, 18.994)
Control	98	2			0.96	0.04		
Male control	97	3			94	6		
Female control	98.56	1.44			97.1	2.9		

Table 3: Genotype and allele frequencies of rs2303425 among case and control population

cohort	Maj. Allele%	Min Allele%	P-Value	OR (CI 50%)	TT genotype%	CT genotype%	P value	OR (CI 50%)
Total MS	93.8	6.2	0.451	0.743 (0.370, 1.490)	88	12	0.433	0.743 (0.381, 1.450)
Male MS	92.19	7.81	1.000	1.031 (0.313, 3.393)	84.38	15.62	1.000	1.031 (0.330, 3.225)
Female MS	94.52	5.48	0.356	0.630 (0.266, 1.495)	89.05	10.95	0.337	0.630 (0.274, 1.448)
Control	91.66	8.34			91.67	8.33		
Male control	92.43	7.57			84.85	15.15		
Female control	91.3	8.7			82.6	17.4		

of MS, alternation in the repair system genes' expression level such as PARP1, OGG1, UNG, and RPA1, has been detected in the lymphocytes of MS patients (Briggs, 2010).

XRCC1 gene is located on chromosome 17q and its protein works as a scaffold in the base excision and single-strand break repair systems. Base excision repair is a primary mechanism for responding to the DNA damage and can be activated by production of reactive oxygen species (ROS) from intracellular hydroxylation (Ying et al., 2016). This protein causes recruitment of proteins involved in the repair system such as polynucleotide kinase, human AP endonuclease (APE1), DNA ligase III, poly (ADP-ribose) polymerases (PARP) and DNA polymerase β (Breslin et al., 2015; Ying et al., 2016). In the last few

years, many molecular studies have investigated the possible associations between *XRCC1* polymorphisms and cancer risk. The presence of certain polymorphisms seem to be associated with increased or decreased cancer susceptibility, depending on the cancer type and the levels of environmental exposure to DNA damaging agents. Arg194Trp polymorphism is a missense variation located in the coding region of *XRCC1* causing a substitution in the interaction surface with PARP and DNA polymerase β . This polymorphism might alter the *XRCC1* function and susceptibility to cancer and neurodegenerative disease occurrence (Tae et al., 2004).

Several Genetic variations in *XRCC1* gene are associated with increased risk of breast, stomach,

and prostate cancers, heart diseases and autoimmune diseases such as rheumatoid arthritis and lupus erythematosus. In this study, we found that the genotype and allele frequencies in rs1799782 of *XRCC1* possess a significant difference between MS patients and control group, indicating a possible role of this genetic variation in MS pathogenesis (Langsenlehner et al., 2011). rs1799782 at codon 194 occurs near the N-terminal domain and results in an arginine (R) to tryptophan (W) substitution. This polymorphism may alter RNA stability and degradation so it can affect protein functions.

The output of the PolyPhen-2 software prediction showed that a query substitution is predicted to be benign with high confidence. The query substitution is predicted to be damaging, but with low confidence.

The null mice for *MSH2* gene located on 2p chromosome, showed an increased sensitivity to UV light, skin and stomach cancers (Owen et al., 2005; Wilson et al., 2005). The association between *MSH2* polymorphisms and various types of cancers has been confirmed (Hsieh et al., 2016); however, we could not find any significant association between rs2303425 in *MSH2* gene and multiple sclerosis in both genders.

ConSite and Jasper software predict that rs2303425 polymorphism located in *MSH2* promoter region could be a recognition site for FREAC-3 forkhead transcription factor (Sandelin et al., 2004a; Sandelin et al., 2004b). Hence, it's supposed that in the presence of the mutant allele (C), the efficacy of FREAC-3 recognition and triggering the expression would be decreased. Based on our finding, rs2303425 polymorphism is not involved in the incidence rate of MS and so, FREAC-3 transcription factor recognition is not influenced by this promoter variation. The frequencies of polymorphic allele and its association with the clinical conditions is influenced by the selected population; so the lack of association between rs2303425 and MS risk could be the result of indigenous dissimilarities between Iranian population and the rest of the world or inaccuracy in bioinformatics prediction. Therefore, performing of this study in other populations seems to be essential for understanding the actual role of this SNP in MS pathogenesis.

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Conflict of interest

The authors have declared no conflict of interest.

References

- Breslin C, Hornyak P, Ridley A, Rulten SL, Hanzlikova H, Oliver AW, et al. The XRCC1 phosphate-binding pocket binds poly (ADP-ribose) and is required for XRCC1 function. *Nucleic acids res* 2015; 43: 6934-6944.
- Briggs FB, Goldstein BA, McCauley JL, Zuvich RL, De Jager PL, Rioux JD, et al. Variation within DNA repair pathway genes and risk of multiple sclerosis. *Am J Epidemiol* 2010; 172: 217-224.
- Briggs FBS. Characterization of complex genetic component contributing to the susceptibility for multiple sclerosis and rheumatoid arthritis. 2010.
- Cantorna MT. Vitamin D and its role in immunology: Multiple sclerosis, and inflammatory bowel disease. *Prog Biophys Mol Biol* 2006; 92: 60-64.
- Ebers GC, Kukay K, Bulman DE, Sadovnick AD, Rice G, Anderson C, et al. A full genome search in multiple sclerosis. *Nat Genet* 1996; 13: 472-476.
- Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 2009; 132: 1175-1189.
- Gon S, Napolitano R, Rocha W, Coulon S, Fuchs RP. Increase in dNTP pool size during the DNA damage response plays a key role in spontaneous and induced-mutagenesis in *escherichia coli*. *Proc Natl Acad Sci U S A* 2011; 108: 19311-19316.
- Gregory AP, Dendrou CA, Atfield KE, Haghikia A, Xifara DK, Butter F, et al. TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis. *Nature* 2012; 488: 508-511.
- Hsieh YC, Cho EC, Tu SH, Wu CH, Hung CS, Hsieh MC, et al. Msh2 rs2303425 polymorphism is associated with early-onset breast cancer in taiwan. *Ann Surg Oncol* 2016.
- Jeggo PA, Pearl LH, Carr AM. DNA repair, genome stability and cancer: A historical perspective. *Nat Rev Cancer* 2016; 16: 35-42.
- Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* 2006; 7: 335-346.
- Kunkel TA, Erie DA. DNA mismatch repair. *Annu Rev Biochem* 2005; 74: 681-710.
- Kunkel TA, Erie DA. Eukaryotic mismatch repair in relation to DNA replication. *Annu Rev Genet* 2015; 49: 291-313.
- Langsenlehner T, Renner W, Gerger A, Hofmann G,

- Turner EM, Kapp KS, et al. Association between single nucleotide polymorphisms in the gene for *xrcc1* and radiation-induced late toxicity in prostate cancer patients. *Radiother Oncol* 2011; 98: 387-393.
- Levin LI, Munger KL, O'Reilly EJ, Falk KI, Ascherio A. Primary infection with the epstein-barr virus and risk of multiple sclerosis. *Ann Neurol* 2010; 67: 824-830.
- Naghavi Gargari B, Behmanesh M, Sahraian MA. Effect of vitamin D treatment on interleukin-2 and interleukin-4 genes expression in multiple sclerosis. *Physiology and Pharmacology* 2015a; 19: 14-21.
- Naghavi Gargari B, Behmanesh M, Shirvani Farsani Z, Pahlevan Kakhki M, Azimi AR. Vitamin D supplementation up-regulates IL-6 and IL-17A gene expression in multiple sclerosis patients. *Int Immunopharmacol* 2015b; 28: 414-9.
- Owen BA, Yang Z, Lai M, Gajec M, Badger JD, Hayes JJ, et al. (CAG) (n)-hairpin DNA binds to Msh2-Msh3 and changes properties of mismatch recognition. *Nat Struct Mol Biol* 2005; 12: 663-670.
- Owens T. The enigma of multiple sclerosis: Inflammation and neurodegeneration cause heterogeneous dysfunction and damage. *Curr Opin Neurol* 2003; 16: 259-265.
- Palli D, Polidoro S, D'Errico M, Saieva C, Guarrera S, Calcagnile AS, et al. Polymorphic DNA repair and metabolic genes: A multigenic study on gastric cancer. *Mutagenesis* 2010; 25: 569-575.
- Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald Criteria. *Ann Neurol* 2011; 69: 292-302.
- Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol* 2005; 58: 840-846.
- Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, et al. New diagnostic criteria for multiple sclerosis: Guidelines for research protocols. *Ann Neurol* 1983; 13: 227-231.
- Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001; 409: 928-933.
- Sandelin A, Alkema W, Engstrom P, Wasserman WW, Lenhard B. Jaspar: An open-access database for eukaryotic transcription factor binding profiles. *Nucleic Acids Res* 2004a; 32: D91-4.
- Sandelin A, Wasserman WW, Lenhard B. ConSite: Web-based prediction of regulatory elements using cross-species comparison. *Nucleic Acids Res* 2004b; 32: W249-252.
- Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet* 2009; 41: 1308-1312.
- Tae K, Lee HS, Park BJ, Park CW, Kim KR, Cho HY, et al. Association of DNA repair gene *xrcc1* polymorphisms with head and neck cancer in korean population. *Int J Cancer* 2004; 111: 805-808.
- Westerlind H, Ramanujam R, Uvehag D, Kuja-Halkola R, Boman M, Bottai M, et al. Modest familial risks for multiple sclerosis: A registry-based study of the population of sweden. *Brain* 2014; 137: 770-778.
- Wilson TM, Vaisman A, Martomo SA, Sullivan P, Lan L, Hanaoka F, et al. MSH2-MSH6 stimulates DNA polymerase η , suggesting a role for a: T mutations in antibody genes. *J Exp Med* 2005; 201: 637-645.
- Wingerchuk DM. Smoking: Effects on multiple sclerosis susceptibility and disease progression. *Ther Adv Neurol Disord* 2012; 5: 13-22.
- Ying S, Chen Z, Medhurst AL, Neal JA, Bao Z, Mortusewicz O, et al. DNA-pkcs and *parp1* bind to unresected stalled DNA replication forks where they recruit *xrcc1* to mediate repair. *Cancer Res* 2016, 76:1078-1088.