Investigation of the Relationship between MBP Gene Polymorphisms and Delayed Encephalopathy after Acute Carbon Monoxide Poisoning

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Research Article

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Abstract

Background Increasing evidence reveals that delayed encephalopathy after acute carbon monoxide poisoning (DEACMP) results from the combined effects of environmental and genetic factors. The main pathological feature of DEACMP was generalized demyelination of cerebral white matter. Myelin basic protein (MBP) levels in cerebrospinal fluid (CSF) and serum samples from DEACMP patients were elevated.

Objectives This study investigated the association of MBP single nucleotide polymorphisms (SNPs) (rs470555, rs470724, rs4890785, rs595997, rs76452994, and rs921336) with DEACMP.

Methods We genotyped 416 DEACMP patients and 785 age, educational level, and sex-matched ACMP patients for rs470555, rs470724, rs4890785, rs595997, rs76452994, and rs921336 SNPs using the Agena MassArray.

Results There were no significant differences in the allele frequency distribution, four genetic models, and genotype distributions between the DEACMP and ACMP groups for rs470555, rs470724, rs4890785, and rs595997. However, significant differences were observed for rs76452994 and rs921336.

Conclusions This study revealed that the MBP polymorphisms, rs470555, rs470724, rs4890785, and rs595997, were not associated with DEACMP. Based on the codominant, dominant, and overdominant genetic inheritability patterns, the MBP rs76452994 and rs921336 polymorphisms were associated with DEACMP. Furthermore, the G allele of rs76452994 and T allele of rs921336 could lead to higher DEACMP risk.

Introduction

Carbon monoxide (CO), referred to as a “silent killer,” is a colorless, tasteless, odorless, nonirritating, and highly toxic gas produced by the incomplete combustion of fossil fuels. As the specific gravity of CO is 0.97, it is slightly lighter than air^[1]. Acute carbon monoxide poisoning (ACMP) occurs after CO inhalation, which reduces the blood's ability to carry oxygen, leaving the body's organs and cells starved of oxygen. ACMP is increasingly recognized as a hazardous and relatively common cause of intoxication^[2]. In the United States, CO is estimated to poison approximately 50,000 people per year, with the mortality ranging between 1,000 to 2,000 individuals per year^[3]. Similar observations were reported between 1980 to 2008 by the World Health Organization (WHO), in which an average of 342 CO-related hospital admissions per year occurred with a total of approximately 140 to 490 CO-related deaths, resulting in an average annual death rate of 2.24 per 100,000 population^[4]. The primary causes of ACMP in China were improperly maintained heating systems, coal gas leakage, and vehicle exhaust, all of which are common in the winter and spring in northern Chinese cities^[5-6].

Delayed encephalopathy after acute carbon monoxide poisoning (DEACMP) is one of the most severe complications associated with ACMP, and patients exhibit recurring neuropsychiatric symptoms following an interval of apparent recovery (usually two to 60 days) from ACMP^[7]. Most patients who survive CO poisoning recover completely after inhalation of oxygen or exposure to hyperbaric oxygenation. Among all ACMP patients, the incidence of DEACMP ranges from 10% to 30% in China and 0.8% to 43% in other countries^[8-9]. Moreover, DEACMP primarily manifests as mental deterioration, behavioral disorders, autonomic dysfunction, parkinsonism, and severe dementia, resulting in clinical neurological complications^[10-11].

Considerable evidence shows that DEACMP results from the comprehensive effects of environmental and genetic factors. We found in this investigation of DEACMP patients that ACMP occurred simultaneously in husbands and wives. While DEACMP did not occur in severely intoxicated patients, it did occur in mildly intoxicated patients. Other studies have reported that after ACMP, patients with severe symptoms did not develop DEACMP and patients with mild symptoms did^[12]. Therefore, individual differences in genes and their polymorphisms might influence the occurrence of DEACMP. Our research group previously completed a genome-wide association study (GWAS) of 277 ACMP patients and 175 DEACMP patients in China and identified 842 SNP sites with differences greater than 0.5 that were related to DEACMP^[13]. We tested and verified several positive loci and discovered four single nucleotide polymorphisms, rs17068697/A, rs1539177/A, rs2236592/C, and rs9534475/C of the leucine-rich repeats and calponin homology domain containing 1 (LRCH1) that correlated with DEACMP^[14]. Two SNPs of the neurexin 3 gene, rs11845632 and rs2196447, also were identified as risk genes for DEACMP^[13]. Two neuron-specific enolase (NSE) SNPs, rs2071419 and rs3213434, were susceptibility sites for DEACMP. The NSE T allele of rs3213434, C allele of rs2071419, and the haplotypes, CCTTTC and GGTTTC, might be risk factors for DEACMP^[15]. However, we did not confirm a genetic correlation between the two gene SNPs (rs3790088 and rs4247109) of the WW domain that contains the E3 ubiquitin protein ligase 2 (*WWP2*) gene and the incidence of DEACMP^[16].

DEACMP is an acquired demyelinating disease and represents one uncommon subtype of acquired leukoencephalopathy. The main pathological features of DEACMP include generalized demyelination of cerebral white matter, symmetrical bilateral softening of the globus pallidus, and generalized or focal degeneration and necrosis of the cerebral cortex. Lesions also may involve the hippocampus and cerebellum^[17].

The myelin sheath is a tubular outer membrane wrapped around axons composed of protein and sphingomyelin^[18]. The primary function of myelin is to insulate the axon and allow increased nerve conduction velocities^[18]. MBP is a strong basic membrane protein, and MBP content in cerebrospinal fluid (CSF) is normally exceedingly low^[19]. Therefore, a significant increase in MBP levels in CSF is an indirect indication that a demyelination injury of white matter has occurred in the central nervous system (CNS)^[19]. One study found that the presence of chemically modified MBP was related to delayed CO-mediated neuropathology^[20]. Additional studies have demonstrated elevated MBP levels in DEACMP CSF and serum samples^[21, 22]. Previous studies have proposed that changes in serum MBP concentrations in patients with schizophrenia were related to gene polymorphisms in the single nucleotide polymorphism site, rs2000811^[23]. Therefore, based on the previous investigation of GWAS by our research group and analysis of relevant literature, we identified six SNP loci on the DEACMP-related *MBP* gene as test targets (rs470555, rs470724, rs4890785, rs595997, rs76452994, and rs921336).

Materials And Methods

Subjects

The study participants were Han Chinese older than 40 years of age and recruited in the northern Henan province from November 2006 to April 2019. All enrolled subjects met the national diagnostic criteria for occupational acute carbon monoxide poisoning (ACMP) (GBZ23-2002)^[24]. The ACMP patients were followed for more than 90 days. The subjects were divided into two groups based on the presence of DEACMP. If DEACMP occurred, the subject was assigned to the DEACMP group. The remaining subjects were assigned to the ACMP group. All DEACMP patients met the DEACMP diagnostic criteria based on the diagnoses of Zhao Xiangzhi and others^[25] and the Eighth Edition of "Internal Medicine"^[26]. There were 416 cases in the DEACMP group and 785 cases in the ACMP group. Patients exhibiting the following conditions were excluded: (1) recent treatment with hormone or immunosuppressive therapy; (2) vaccinated within the past six months; (3) a history of infection within the previous 15 days; (4) a history of allergies; (5) diagnosed with another CNS disease, serious diabetes, an immune system disease, severe heart illness, liver or kidney disease, alcohol dependence, malnutrition, or other mental disorders; and (6) pregnant or lactating.

A 3ml peripheral venous blood sample was obtained from each participant, placed in an EDTA anticoagulation tube, and stored in a -80°C freezer. The blood samples from the ACMP patients were collected within 24 hours after they achieved full consciousness following successful resuscitation. In DEACMP cases, the blood samples were collected between 6AM to 8AM on the second day of hospitalization.

Genotyping Tag-SNPs

Genomic DNA from the peripheral blood samples from each patient was extracted using a TIANGEN Blood DNA Midi Kit (DP304, Beijing TIANGEN Biotechnology Co., Ltd., Beijing, China). The sequences of the six MBP gene SNPs (rs470555, rs470724, rs4890785, rs595997, rs76452994, and rs921336) were obtained using online tools Assay design suite V2.0, located on the Agena website. Primer design and synthesis were carried out using Agena MassARRAY Assay Design Software (version 3.1, Agena Bioscience, San Diego, CA, USA). The genotyping was performed using the Agena MassArray following the platform directions. Finally, data management was carried out using Agena Typer Software (version 4.0, Agena Bioscience).

Statistical analysis

An independent-sample *t*-test was used for age comparison between the two groups. The Karl Pearson chi-squared test was applied to the 2x2 data tables with one degree of freedom to compare the patients' educational level and gender. The distribution of genotypes in conformity with the Hardy-Weinberg law was analyzed using the goodness-of-fit chi-square test. An association analysis between groups was performed using the binary logistic regression test. Statistical analyses were performed using SPSS version 19.0 statistical software (SPSS Inc., Chicago, IL, USA). Statistically significant differences were defined as $P < 0.05$.

Results

3.1 Clinical Characteristics.

The demographic data were matched between the two groups for rs470555 (average age: $P = 0.520$; gender distribution: $P = 0.077$; education level: $P = 0.109$), rs470724 (average age: $P = 0.487$; gender distribution: $P = 0.056$; education level: $P = 0.109$), rs4890785 (average age: $P = 0.476$; gender distribution: $P = 0.063$; education level: $P = 0.098$), rs595997 (average age: $P = 0.428$; gender distribution: $P = 0.065$; education level: $P = 0.092$), rs76452994 (average age: $P = 0.482$; gender distribution: $P = 0.066$; education level: $P = 0.092$), and rs921336 (average age: $P = 0.504$; gender distribution: $P = 0.063$; education level: $P = 0.098$) (Table 1). It is worth noting that the genotype distribution of these six SNPs conformed to the Hardy-Weinberg equilibrium law between the two groups ($P > 0.05$ for all cases; Table 2).

3.2 Analyses of the associations between the six SNP polymorphisms and DEACMP.

The analyses of the associations between the rs470555, rs470724, rs4890785, rs595997, rs76452994, and rs921336 SNP polymorphisms and DEACMP and the associated increased risk under different genetic models are shown in Table 3. For rs470555, rs470724, rs4890785, and rs595997, the correlation between genotype and DEACMP incidence was analyzed under each of the four genetic models (dominant inheritance, codominant inheritance, overdominant inheritance, and recessive inheritance). No differences were statistically significant ($P > 0.05$). The allele frequency distribution was analyzed for rs76452994 and rs921336, and the difference between the DEACMP and ACMP groups was statistically significant ($P < 0.05$). The G allele of rs76452994 and the T allele of rs921336 were correlated with the risk of DEACMP. Under the dominant, codominant (CG vs. CC), and overdominant genetic models of inheritance, the correlation analysis for rs76452994 and DEACMP incidence exhibited statistically significant differences ($P < 0.05$). Under the codominant (GG vs. CC) and recessive genetic models, the correlation analysis revealed no statistically significant differences ($P > 0.05$). Under dominant, codominant (GT vs. GG), and overdominant genetic models, the correlation analysis for rs921336 and DEACMP incidence did show statistically significant differences ($P < 0.05$). Under codominant (TT vs. GG) and recessive genetic models, the correlation analysis showed no statistically significant differences ($P > 0.05$).

For rs470555, rs470724, rs4890785, and rs595997, there were no significant differences in the allele frequency or genotype distributions between the DEACMP and ACMP groups ($P > 0.05$), suggesting that the genotype and allele frequency distributions of these four loci were not associated with DEACMP. On the other hand, rs7642994 and rs921336 did display statistically significant differences in the allele frequency and genotype distributions ($P < 0.05$), suggesting they were correlated with the incidence of DEACMP. No significant differences were observed in the allele frequency and genotype distributions of rs470555, rs470724, rs4890785, rs595997, or rs7642994 in males from the DEACMP and ACMP groups ($P > 0.05$), indicating that the genotype and allele

frequency distributions of these five loci were not associated with the specific incidence of DEACMP in men. However, it was noted that for rs921336, the allele frequency distribution between the two groups was significantly different ($P < 0.05$), suggesting that the allele frequency distribution for this locus was associated with DEACMP incidence in males.

There were no statistically significant differences observed in the allele frequency and genotype distributions between the DEACMP and ACMP groups for female patients for rs470555, rs470724, rs4890785, and rs595997 ($P > 0.05$), suggesting that the genotype and allele frequency distributions of these four loci were not associated with the incidence of DEACMP in women. However, the comparison of results from the rs76452994 and rs921336 allele frequency and genotype distributions in women did reveal statistically significant differences ($P < 0.05$), suggesting that the genotype and allele frequency distributions of these two loci were correlated with the incidence of DEACMP in women (Table 4).

Discussion

We analyzed six MBP polymorphisms (rs470555, rs470724, rs4890785, rs595997, rs76452994, and rs921336) in the ACMP and DEACMP groups. We also analyzed their associations with DEACMP in four different genetic models of inheritance. The results showed that the rs76452994 and rs921336 MBP polymorphisms were related to increased risk of DEACMP in the codominant (CG vs. CC, GT vs. GG), dominant, and overdominant genetic models. Also, the G allele of the rs76452994 polymorphism and the T allele of the rs921336 polymorphism might increase DEACMP risk.

In the previous study of gene polymorphisms, the allelic gene distribution frequency was not significantly different when the analysis did not include gender stratification. However, some parts were significantly different after the data were stratified by gender. In contrast, some statistically significant differences were observed without stratification by gender, and in other cases, no statistical significance was observed after stratification by gender. Our study found that when rs470555, rs470724, rs4890785, and rs595997 were analyzed based on gender in the DEACMP and ACMP groups, no significant differences were observed in the genotype distribution and allele frequency for males or females ($P > 0.05$). However, when rs76452994 was analyzed based on gender in the DEACMP and ACMP groups, no significant differences were observed for the genotype distribution, and allele frequency in males ($P > 0.05$), but a statistically significant difference was observed for females ($P < 0.05$). Furthermore, the analysis for rs921336 based on gender revealed that for the DEACMP and ACMP groups, a statistically significant difference in the genotype distribution and allele frequency existed for both males and females ($P < 0.05$).

The primary pathological changes associated with DEACMP included symmetrical lesions associated with softening of the bilateral globus pallidus and extensive demyelination in cerebral white matter. MBP plays an essential role in maintaining myelin structural stability and function in the CNS^[27]. Previous studies have shown that MBP concentrations in serum and CSF samples obtained from DEACMP patients are increased, indicating that MBP is closely related to DEACMP^[22,28]. Thus, the presence of elevated levels of MBP in the CSF could serve as a sensitive predictor for the development and outcome of DEACMP^[29].

The MBP gene, containing seven exons, is located in 18Q22-Q23 of human chromosome 18. The MBP gene spans 32 to 34Kb, and studies have documented that this gene is highly conserved. The other major CNS myelin and lipoprotein genes are located on the X chromosome and are not linked to the MBP gene. However, MBP synthesis is closely associated with lipoprotein expression in oligodendrocytes, which are the myelin-forming cells in the CNS^[23,30]. Numerous studies have described 18Q23 as a critical locus for bipolar disorder and suggest that this region contains genes associated with multiple psychiatric disorders and cognitive impairment^[31].

All eight genotyped tag-SNPs (rs9676113, rs3794832, rs7232502, rs12959006, rs61742988, rs3900176, rs11150997, and rs7233242) with $r^2 < 1$ in the MBP gene (chr18: 74690789–74844774) were included in our analysis. Zhou et al. discovered that one variant of the MBP gene, rs12959006, predicted worse outcomes with respect to the clinical course of multiple sclerosis (MS). The risk genotype (CT+TT) was significantly associated with relapse occurrence and with an increased yearly progression of disability^[32]. Napier et al. reported that the SNPs loci rs4890785/T, rs8096433/A, and rs17660901/G were associated with the incidence of MS^[33]. The C allele of the MBP SNP locus, rs12458282, exhibits considerable significance for mental disorders, including schizophrenia^[34]. DEACMP presents as a neuropsychiatric disorder including severe organic psychosis, which might be associated with the MBP gene. Our previous study found an association between the MBP 5'-side TGGA gene polymorphism and DEACMP and that the allele L increased the risk of occurrence in male patients with DEACMP^[35].

The pathogenesis of DEACMP has been associated with inflammation and immune damage, the ischemia hypoxia hypothesis, free radical damage, apoptosis, excitatory neurotransmitters, and others^[36]. However, the exact pathogenesis of DEACMP is still not clear. Therefore, a detailed study of the pathogenesis of DEACMP is needed. We discussed the pathogenesis of DEACMP based on molecular genetics as described in previous studies. We determined that the MBP gene might be a susceptibility gene associated with DEACMP. Two SNPs at MBP gene loci (rs76452994/G and rs921336/T) were associated with the onset of DEACMP under autosomal dominant inheritance, codominant inheritance, and super dominant inheritance. DEACMP is an acquired demyelinating disease of the CNS. MBP is a unique protein component of CNS myelin. The MBP concentrations in CSF and serum are elevated in DEACMP patients. Furthermore, the MBP gene investigated in our study was related to the genetic susceptibility for DEACMP, which provided a new theoretical basis to explain the pathogenesis of DEACMP. These findings also provided a new theoretical basis for the prediction, prevention, diagnosis, and treatment of DEACMP.

Conclusion

Based on the models of codominant, dominant, and overdominant inheritance, the rs921336/T polymorphism in the MBP gene was associated with an increased risk for DEACMP. Based on the models of codominant, dominant, and overdominant inheritance, the rs76452994/G polymorphism in the MBP

gene was associated with an increased risk of DEACMP in females. Finally, based on all four genetic models, four MBP gene SNP loci, rs470555, rs470724, rs4890785, and rs595997, were not associated with DEACMP.

Abbreviations

DEACMP: delayed encephalopathy after acute carbon monoxide poisoning; ACMP: acute carbon monoxide poisoning; MBP: Myelin basic protein; CSF: cerebrospinal fluid; CO: carbon monoxide; SNPs: single nucleotide polymorphisms; GWAS: genome-wide association study; NSE: neuron-specific enolase; CNS: central nervous system; MS: multiple sclerosis; LRCH1: leucine-rich repeats and calponin homology domain containing 1; *WWP2*: WW domain that contains the E3 ubiquitin protein ligase 2; EDTA: Ethylenediaminetetraacetic acid.

Declarations

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Fan Zhang coordinated and integrated genetic and neurological studies and wrote the manuscript. Jiao Zeng, Xiaoli Zhang, Jiapeng Gu, Yongkai Han, and Ping Zhang coordinated the clinical characterization of the patient. Wenqiang Li and Renjun Gu designed the study concept, interpreted the results, and critically revised the final version of the article. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Written informed consent from the participants prior to carrying out any study-related procedures was a prerequisite for inclusion in the study. The study was performed according to the principles of the Declaration of Helsinki. The research plan was approved by the Ethics Committee of the Second Affiliated Hospital of Xinxiang Medical University and the Ethics Committees of all participating hospitals and research institutes.

Consent for publication

Written informed consent for publication was obtained from the participant's legal guardian/next of kin.

Competing interests

The authors declare they have no competing interests.

References

- [1] Kinoshita H, Türkan H, Vucinic S, et al. Carbon monoxide poisoning[J]. Toxicol Rep, 2020, 7 :169-173.
- [2] Maffi L, Paganini M, Vezzani G, et al. Hyperbaric oxygen treatment for carbon monoxide poisoning in Italy: retrospective validation of a data collection tool for the Italian registry of carbon monoxide poisonings (IRCOP)[J]. Int J Environ Res Public Health, 2020, 17(2):574.
- [3] Hampson NB. U.S. mortality due to carbon monoxide poisoning, 1999–2014. Accidental and intentional deaths. Ann Am Thorac Soc 2016; 13(10): 1768–1774.
- [4] Braubach, M.; Algoet, A.; Beaton, M.; Lauriou, S.; Héroux, M.E.; Krzyzanowski, M. Mortality associated with exposure to carbon monoxide in WHO European Member States. Indoor Air 2013, 23, 115–125.
- [5] Ye Shanshan, Hu Zhuojun, Ruan Hailin, et al. Analysis of epidemiological characteristics of non-occupational acute carbon monoxide poisoning patients in Liuzhou from 2014 to 2017[J]. Chinese Journal of Disaster Medicine, 2019, 7(7):374-379.
- [6] Lin MS, Lin CC, Yang CC, et al. Myocardial injury was associated with neurological sequelae of acute carbon monoxide poisoning in Taiwan[J]. J Chin Med Assoc, 2018, 81(8) :682-690.
- [7] Huang YQ, Peng ZR, Huang FL, et al. Mechanism of delayed encephalopathy after acute carbon monoxide poisoning[J]. Neural Regen Res, 2020, 15(12) :2286-2295.
- [8] Qu PengPei, Yang Bing, Yang XiaoLei, et al. Progress on the Pathogenesis of Delayed Encephalopathy after Acute Carbon Monoxide Poisoning[J]. Occup and health. 2011, 27(19): 2251-2254.

- [9] Xu XM,Luo H,Rong BB,et al. Management of delayed encephalopathy after CO poisoning: an evidence-based narrative review[J]. *Medicine (Baltimore)*, 2019, 98(49): e18199.
- [10]Zhang P,Dai Y, Xiong J, et al. iTRAQ-based differential proteomic analysis of the brains in a rat model of delayed carbon monoxide encephalopathy[J]. *Brain Res Bull*, 2018,137:329-337.
- [11] Kumarihamy P, Kularatne SAM, Pathirage M, et al. A case of delayed neurological manifestation following carbon monoxide poisoning in Sri Lanka: epidemiology of exposure and literature review[J]. *BMC Pharmacol Toxicol*, 2019,20(1) :17.
- [12] Yanagiha K, Ishii K, Tamaoka A. A cetylcholinesterase inhibitor treatment alleviated cognitive impairment caused by delayed encephalopathy due to carbon monoxide poisoning: Two case reports and a review of the literature[J]. *Medicine (Baltimore)*, 2017,96(8):e6125.
- [13] Li WQ,Zhang YX,Gu RJ,et al.DNA pooling base genome-wide association study identifies variants at NRXN3 associated with delayed encephalopathy after acute carbon monoxide poisoning[J].*PLoS ONE*,2013,8(11):e79159(1-6).
- [14]Gu J, Zeng J, Wang X, et al.LRCH1 polymorphisms linked to delayed encephalopathy after acute carbon monoxide poisoning identified by GWAS analysis followed by Sequenom MassARRAY® validation[J].*BMC Med Genet*. 2019 12 16, 20(1):197.
- [15] Xu L, Liu X, Zhao J,et al. Association between Neuron-Specific Enolase Gene Polymorphism and Delayed Encephalopathy after Acute Carbon Monoxide Poisoning[J].*Behav Neurol*.2020 ;2020 :8819210. DOI:10.1155/2020/8819210]
- [16] Zhang F, Zeng J, Li WW,et al.The association study of WWP2 gene polymorphisms with delayed encephalopathy after acute carbon monoxide poisoning[J].*Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*.2020 Jul 20 ;38(7):485-489.
- [17] Geraldo AF, Silva C, Neutel D, et al.Delayed leukoencephalopathy after acute carbon monoxide intoxication[J]. *J Radiol Case Rep*,2014,8(5) :1-8.
- [18]Wen SY,Li AM,Mi KQ, et al.In vitro neuroprotective effects of ciliary neurotrophic factor on dorsal root ganglion neurons with glutamate-induced neurotoxicity[J].*Neural Regen Res*, 2017,12(10):1716-1723.
- [19] Takanashi J, Hayashi M, Yuasa S, et al. Hypomyelination in I-cell disease: MRI, MR spectroscopy and neuropathological correlation[J]. *Brain Dev*, 2012, 34(9):780-783.
- [20]Thom SR, Bhopale VM, Fisher D, Zhang J, Gimotty P. Delayed neuropathology after carbon monoxide poisoning is immune-mediated. *Proc Natl Acad Sci U S A*. 2004;101(37):13660–13665.
- [21] Ide T, Kamijo Y. Myelin basic protein in cerebrospinal fluid: a predictive marker of delayed encephalopathy from carbon monoxide poisoning[J]. *Am J Emerg Med*,2008,26(8):908-912.
- [22] Gu Renjun, Chen Wei, Zhang Xiuming,et al.Determination of myelin basic protein and neuron-specific enolase in elderly patients with delayed encephalopathy after acute carbon monoxide poisoning[J].*Chin J Geriatr*.2002,21(1):60-61.
- [23]Li Guohua,Xiong Peng,Wu Qiuxia,et al.Research progress of MBP and GFAP and their gene polymorphisms in schizophrenia[J].*Journal of Psychiatry*,2013,26(1):75-76.
- [24] Ministry of Health of the People's Republic of China.GBZ23-2002 Diagnostic criteria for occupational acute carbon monoxide poisoning[S]. Chinese Peking: Standards press of China,2002.
- [25]Zhao Xiangzhi, Zhao Xueding, Cheng Ziqiang. Delayed encephalopathy after acute carbon monoxide poisoning: report of 67 cases[J].*Chinese Journal of Neuropsychiatry*,1984,17(1):36-38.
- [26]Ge Junbo,Xu Yongjian. Internal medicine[M],The 8th edition,Chinese Peking:People's Medical Publishing House,2013:906-908.
- [27]Tzakos AG, Troganis A, Theodorou V, et al. Structure and function of the myelin proteins: current status and perspectives in relation to multiple sclerosis. *Curr Med Chem*. 2005;12(13):1569–1587.
- [28]TIAN Ying-hai, CHEN Qiu-xia, DENG Xiao-ying, et al. Value of myelin basic protein and neuron specific enolase to the prediction of delayed encephalopathy after acute carbon monoxide poisoning[J]. *J Chin Pract Diagn Ther*. 2017, 31(12): 1220-1221.
- [29]Hiroshi Kuroda, Kazuo Fujihara,Shigeki Kushimoto,et al. Novel clinical grading of delayed neurologic sequelae after carbon monoxide poisoning and factors associated with outcome. *Neurotoxicology*. 2015 May,48:35-43.
- [30] Kamholz J,Spielman R,Goglin K,et al. O'Brien S & Lazzarini R: the human myelin-basic-protein gene: chromosomal localization and RFLP analysis[J]. *Am J Hum Genet*, 1987, 40(4):365-373.
- [31] Hampson RM, Malloy MP, Mors O, et al. Mapping studies on a pericentric inversion (18) (p11.31 q21.1) in a family with both schizophrenia and learning disability[J]. *Psychiatric Genetics*, 1999,9(3):161-163.

[32]Zhou Y, Simpson S, Charlesworth JC, *et al.* Variation within MBP gene predicts disease course in multiple sclerosis[J]. Brain Behav, 2017, 7(4) :e00670.

[33] Napier MD, Poole C, Satten GA, *et al.* Heavy metals, organic solvents, and multiple sclerosis: An exploratory look at gene-environment interactions[J]. Arch Environ Occup Health, 2016, 71(1) :26-34.

[34]Baruch K, Silberberg G, Aviv A, *et al.*: Association between Golli-MBP and schizophrenia in the Jewish Ashkenazi population: are regulatory regions involved?[J]. Int J Neuropsychopharmacol,2009,12(7):885-94.

[35]SG Li, WQ Li, JK Wang , *et al.*Association of the genes for tumor necrosis factor- α and myelin basic protein with delayed encephalopathy after acute carbon monoxide poisoning[J].Genetics and Molecular Research, 2012, 11 (4): 4479-4486.

[36]Wang Wenlan, Zhang Yu, Li Ya, *et al.* Progress in the relationship between acute carbon monoxide poisoning and delayed neuropathological sequelae[J]. Chinese Journal of Critical Care Medicine, 2012,32(11):1041-1045.

Tables

Table 1 Demographic variables of DEACMP and ACMP patients genotyped for the rs470555, rs470724, rs4890785, rs595997, rs76452994 and rs921336 polymorphisms.

SNP	Characteristic	ACMP	DEACMP	tatistics	P value
rs470555	Age	64.34±12.33	64.81±11.71	t=0.643	0.520
	Gender:Male/Female	415/365	243/172	$\chi^2=3.132$	0.077
	Educational leve:1/2/3※	198/291/291	129/142/144	$\chi^2=4.437$	0.109
rs470724	Age	64.29±12.37	64.80±11.69	t=0.696	0.487
	Gender:Male/Female	412/367	244/172	$\chi^2=3.641$	0.056
	Educational leve:1/2/3※	289/291/200	130/142/144	$\chi^2=4.425$	0.109
rs4890785	Age	64.29±12.36	64.81±11.71	t=0.713	0.476
	Gender:Male/Female	415/369	243/172	$\chi^2=3.462$	0.063
	Educational leve:1/2/3※	200/293/291	130/141/144	$\chi^2=4.636$	0.098
rs595997	Age	64.28±12.36	64.86±11.69	t=0.793	0.428
	Gender:Male/Female	413/368	424/172	$\chi^2=3.393$	0.065
	Educational leve:1/2/3※	199/291/291	130/141/143	$\chi^2=4.763$	0.092
rs76452994	Age	64.26±12.35	64.78±11.70	t=0.703	0.482
	Gender:Male/Female	415/368	243/172	$\chi^2=3.378$	0.066
	Educational leve:1/2/3※	199/292/292	130/141/144	$\chi^2=4.777$	0.092
rs921336	Age	64.29±12.36	64.78±11.70	t=0.669	0.504
	Gender:Male/Female	415/369	243/172	$\chi^2=3.462$	0.063
	Educational leve:1/2/3※	200/293/291	130/141/144	$\chi^2=4.636$	0.098

※ 1=uneducated2=Primary school3=Middle school or above

Table 2 Results of Hardy-Weinberg equilibrium test for genotype distributions of MBP.

SNPs	Genotypes	Risk allele	Risk allele frequency ACMP/DEACMP	Actual value	Test value	P value
rs470555	AA	T	0.37/0.37	316/168	$\chi^2=0.784/1.149$	$P=0.676/0.563$
	AT			351/184		
	TT			113/63		
rs470724	CC	T	0.35/0.36	328/177	$\chi^2=0.253/1.444$	$P=0.881/0.486$
	CT			350/180		
	TT			101/59		
rs4890785	CC	T	0.22/0.24	464/239	$\chi^2=3.474/0.053$	$P=0.176/0.974$
	CT			290/153		
	TT			30/23		
rs595997	GG	G	0.47/0.48	177/91	$\chi^2=1.117/1.022$	$P=0.572/0.600$
	GA			374/217		
	AA			230/106		
rs76452994	GG	G	0.12/0.16	17/11	$\chi^2=4.256/0.000$	$P=0.119/1.000$
	GC			150/113		
	CC			616/291		
rs921336	GG	T	0.31/0.35	379/169	$\chi^2=0.020/1.333$	$P=0.990/0.513$
	GT			331/200		
	TT			74/46		

Table 3 Correlation analysis of MBP polymorphisms under different genetic models and DEACMP risk.

SNPs	Genetic models	Genotypes	ACMP	DEACMP	χ^2 value	P value	OR (95% CI)
rs470555	Allele	A/T	983/577	520/310	0.030	0.861	1.016(0.853,1.209)
	Codominant	AA/AT/TT	316/351/113	168/184/63	0.011/0.067	0.915/0.796	0.986(0.761,1.277)/1.049(0.731,1.504)
	Dominant	AA/AT+TT	316/464	168/247	0.000	0.992	1.001(0.786,1.276)
	Recessive	AA+AT/TT	667/113	352/63	0.104	0.747	1.056(0.756,1.476)
	Overdominant	AA+TT/AT	429/351	231/184	0.048	0.826	0.974(0.766,1.237)
rs470724	Allele	C/T	1006/552	534/298	0.035	0.851	1.017(0.853,1.212)
	Codominant	CC/CT/TT	328/350/101	177/180/59	0.135/0.177	0.713/0.674	0.953(0.737,1.232)/1.084(0.748,1.566)
	Dominant	CC/CT+TT	328/451	177/239	0.022	0.883	0.982(0.772,1.249)
	Recessive	CC+TT/TT	678/101	357/59	0.347	0.556	1.109(0.785,1.568)
	Overdominant	CC+TT/CT	429/350	236/180	0.303	0.582	0.935(0.735,1.188)
rs4890785	Allele	C/T	1218/350	631/199	0.842	0.359	1.097(0.900,1.339)
	Codominant	CC/CT/TT	464/290/30	239/153/23	0.035/1.923	0.851/0.166	1.024(0.797,1.316)/1.488(0.846,2.619)
	Dominant	CC/CT+TT	464/320	239/176	0.284	0.594	1.068(0.839,1.359)
	Recessive	CC+TT/TT	754/30	392/23	1.890	0.169	1.475(0.845,2.573)
	Overdominant	CC+TT/CT	494/290	262/153	0.002	0.967	0.995(0.777,1.273)
rs595997	Allele	A/G	834/728	429/399	0.543	0.461	1.065(0.900,1.261)
	Codominant	AA/AG/GG	230/374/177	106/217/91	2.522/0.393	0.112/0.531	1.259(0.947,1.673)/1.116(0.793,1.570)
	Dominant	AA/AG+GG	230/551	106/308	1.980	0.159	1.213(0.927,1.587)
	Recessive	AA+AG/GG	604/177	323/91	0.072	0.788	0.961(0.722,1.281)
	Overdominant	AA+GG/AG	407/374	197/217	2.219	0.136	1.199(0.944,1.522)
rs76452994	Allele	C/G	1376/184	695/135	9.360	0.002	1.453(1.143,1.847)
	Codominant	CC/CG/GG	613/150/17	291/113/11	10.451/0.624	0.001/0.429	1.587(1.198,2.102)/1.363(0.630,2.947)
	Dominant	CC/CG+GG	613/167	291/124	10.547	0.001	1.564(1.193,2.051)
	Recessive	CC+CG/GG	763/17	404/11	0.263	0.608	1.222(0.567,2.634)
	Overdominant	CC+GG/CG	630/150	302/113	10.095	0.001	1.572(1.188,2.079)
rs921336	Allele	G/T	1089/479	538/292	5.338	0.021	1.234(1.032,1.475)
	Codominant	GG/GT/TT	379/331/74	169/200/46	5.583/2.533	0.018/0.111	1.355(1.053,1.744)/1.394(0.925,2.101)
	Dominant	GG/GT+TT	379/405	169/246	6.348	0.012	1.362(1.071,1.733)
	Recessive	GG+GT/TT	710/74	369/46	0.816	0.366	1.196(0.811,1.765)
	Overdominant	GG+TT/TT	453/331	215/200	3.924	0.048	1.273(1.002,1.617)

Table 4 Genotype and allele analysis of six SNPs loci of MBP gene in DEACMP group and ACMP group.

SNP	sex	Genotypes	DEACMP	ACMP	Tatistics χ^2/P	Allele	DEACMP	ACMP	Tatistics χ^2/P
rs470555	sum	AA/AT/TT	168/184/63	316/351/113	0.024/0.876	A/T	520/310	983/577	0.030/0.861
	male	AA/AT/TT	99/106/38	156/196/63	0.229/0.633	A/T	304/182	508/322	0.235/0.628
	female	AA/AT/TT	69/78/25	160/155/50	0.501/0.479	A/T	216/128	475/255	0.529/0.467
rs470724	sum	CC/CT/TT	177/180/59	328/350/101	0.034/0.853	C/T	534/298	1006/552	0.035/0.851
	male	CC/CT/TT	106/101/37	162/195/55	0.171/0.679	C/T	313/175	519/305	0.176/0.675
	female	CC/CT/TT	71/79/22	166/155/46	0.444/0.505	C/T	221/123	487/247	0.460/0.498
rs4890785	sum	CC/CT/TT	239/153/23	464/290/30	0.883 /0.347	C/T	631/169	1218/350	0.842/0.359
	male	CC/CT/TT	142/88/13	232/163/20	0.176/0.675	C/T	372/114	627/203	0.168/0.682
	female	CC/CT/TT	97/65/10	232/127/10	3.377/0.066	C/T	259/85	591/147	3.197/0.074
rs595997	sum	AA/GA/GG	106/217/91	230/374/177	0.539 /0.463	A/G	429/399	834/728	0.543 /0.461
	male	AA/GA/GG	61/127/54	114/182/117	0.378/0.537	A/G	249/235	410/416	0.400/0.527
	female	AA/GA/GG	45/90/37	116/192/60	2.822/0.093	A/G	180/164	424/312	2.655/0.103
rs76452994	sum	CC/CG/GG	291/113/11	613/150/17	8.922/0.003	C/G	695/135	1382/184	9.348/0.002
	male	CC/CG/GG	173/66/4	313/90/12	0.540/0.463	C/G	412/74	716/114	0.557/0.456
	female	CC/CG/GG	69/118/47	219/303/60	13.889/0.000	C/G	283/61	666/70	14.868/0.000
rs921336	sum	GG/GT/TT	169/200/46	379/331/74	5.420 /0.020	G/T	538/292	1089/479	5.338 /0.021
	male	GG/GT/TT	100/120/23	160/181/74	3.828/0.050	G/T	320/166	501/329	3.926/0.048
	female	GG/GT/TT	69/80/23	219/150/0	37.238/0.000	G/T	218/126	588/150	32.818/0.000