Letter to the Editor

HTR and *GRIN2B* Variant Associated with Cognition Dysfunction in Electric Workers*

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The frequency of an extremely low frequency electromagnetic field (ELF-EMF) ranges from 3 to 3,000 Hz. Workers who are exposed to ELF-EMF include electric power installers and repairers, power plant operators, electricians, electrical fitters, other such professionals. The main work categories in an electrical company or for utility work can be defined according to the five main stages of electricity production and distribution. Studies on the central nervous system (CNS) reported depressed activity in the different neurotransmitter systems and decreased protein content in the brains of animals exposed to ELF-EMF. Some studies reported differences with respect to change in brain function after electromagnetic radiation among individuals[1]. A possible non-psychological basis for electromagnetic hypersensitivity was proven by the occurrence of field-onset and field-offset brain potential and the induction of steady-state changes in brain electrical activity; however, the mechanism is still unclear.

The serotonin system (5-HT) within the brain is associated with various behaviors and with the pathology of neuropsychiatric disorders. Catechol-O-methyltransferase (COMT) plays an important role in the catabolism of brain dopamine and norepinephrine. A single nucleotide polymorphism (SNP) in the serotonin receptor (5-HTR) and COMT gene has been associated with physiologic as well as behavioral effects[2]. N-methyl-d-aspartate receptors (NMDARs) are involved in several forms of synaptic plasticity, especially the NMDAR2B (NR2B) subunits that have been found to be associated with cognitive functions. Studies reported that polymorphisms in the NMDAR subunit 2B gene (*GRIN2B*) were associated with cognitive disorders, such as schizophrenia, Alzheimer's disease, Parkinson's disease, obsessive-compulsive disorder, bipolar disorder, and other such diseases[3]. Brain derived neurotrophic factor (BDNF) is involved in neuronal plasticity, neurogenesis, and response to antidepressant treatment. The Val66Met (rs6265) polymorphism, a functional BDNF SNP, impairs episodic memory and hippocampal function, which was implicated in neuroticism and in the pathogenesis of anxiety and depression[4].

However, the relationship between the SNPs in *HTR*, *GRIN2B*, *COMT* and *BDNF*, and the change in brain function of electric workers is still unknown. Therefore, this study evaluated the safety of electric company workers and studied the association of the SNP sites of HTR, *COMT*, *GRIN2B*, and *BDNF* with abnormal brain functions induced by ELF-EMF exposure.

All aspects of the study including epidemiological survey, protocols detailing procedures, recruitment materials, and consent forms were reviewed and approved by the Ethical Committee of Academy of Military medical Science. All participants from China were informed of the procedures and potential risks, and written consent of their participation was obtained.

The epidemiological survey composed of all workers from nine transformer substations of an electric company. Considering the confounding factors (age, physical condition, and working duration), the inclusive criteria were adjusted for

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workers aged 20-50 years and working duration of more than 2 years. The control group included 30 workers (16 males, 14 females) with an average age of 38 years; there were 80 workers (34 males, 46 females) in the operating group with an average age of 26 years, while the repairing group comprised 46 workers (all males) with an average age of 38 years. All participants signed the informed consent form for the study.

The participants of the control, operating, and repairing groups were selected and the intensity of ELF-EMF was detected. The control group comprised of office-related workers having no contact with electric work. The operating group individuals faced lower exposure (electric field intensity < 50 V/m, magnetic field strength < 10 μT) and worked in the master-control and switching room. The repairing group individuals faced higher exposure (electric field intensity ≥ 50 V/m, magnetic field strength ≥ 10 μT) as they were in charge of high-voltage wire repair and maintenance. Five radiation detection points were decided according to the size of operating room and the position of electrical transmission line.

The questionnaire contained eight parts: general condition, occupational status, family life and environment, lifestyle and habits, health history, women's menstrual and reproductive history, self-rating depression scale (SDS), and self-rating anxiety scale (SAS). The electromagnetic radiation-related content included occupational and residential exposure, where the former consisted of work type, work duration, safeguarding procedures, and the latter consisted of exposure to household appliances and venue-related intensities.

The physical examination indicators including peripheral blood cells, biochemical parameter, radioimmunity indices, electrocardiogram (ECG), and P300 event-related brain potential (ERP) were analyzed in the hospital. Eight SNPs of *HTR1A, HTR2A, HTR2A*, *GRIN2B*, *BDNF*, and *COMT* reported in the SNP database were examined, including rs6295, rs6311, rs6313, rs6314, rs6318, ENS10557853, rs6265, and rs4680 by polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing. In the samples, there were three genotypes each of *HTR2A* rs6311 (AA/AG/GG) and rs6313 (TT/TC/CC), two genotypes of *HTR1A* rs6295 (GG/CG), *GRIN2B* ENS10557853 (TT/CC), *BDNF* rs6265 (GA/AA), and *COMT* rs4680 (AA/GG), and one genotype of *HTR2C* rs6314 (CT), and rs6318 (GG).

The data were analyzed using the SAS 9.1 statistic software. The significance was defined as $P < 0.05$. Statistical analysis of hypomnesis, irritability, and insomnia of self-conscious symptoms and ECG parameter comparisons were carried out with logistic regression. Symptoms score, SDS score, SAS score, peripheral blood cell count, biochemical parameters, radioimmunity indices, and P300 parameters were compared using generalized linear regression. The effects of gender, age, working duration, and radiation dosage on the workers and the association with different genotypes were considered in the study.

In the results of questionnaires, the work-time (< 5 years or ≥ 5 years) had no influence on the mental symptoms. There were no differences with respect to health history, such as neurological and cardiovascular diseases, etc., between the control, operating, and repairing groups. Routine blood physical examination included peripheral blood cells and biochemical parameters, and there were statistical differences between different exposure dosages. The counts of red blood cells (RBC) in the operating group decreased compared to those in the control group, and the level of creatine kinase (CK) in the repairing group increased compared to that in the control and operating groups. However, the values of RBC and CK levels were within the normal human range for all groups.

ECG, P300, and radioimmunity indices in blood were used to evaluate the cardiac and cerebral functions. There were some abnormal characterizations of ECG in the operating and repairing groups as compared to the control group ($P < 0.05$), in the form of arrhythmia, bradycardia, T-wave abnormalities, etc., but there were no differences between the operating and repairing groups. The N2 latency of P300 in the repairing group was prolonged compared to that in the control and operating groups ($P < 0.05$), without any difference in P3 latency and amplitude. The brain natriuretic peptide (BNP) levels in the operating group increased compared to those in the control group ($P < 0.05$), whereas the leucine enkephalin (LENK) in the repairing group increased compared to that in the control and operating groups ($P < 0.05$).

The electric and magnetic field intensity in the electric company of this study was lower than the international and national standards. The ELF-EMF could interfere with the neuropsychological processes responsible for this short-term learning

effect supported by the brain synaptic plasticity. Clinical electroencephalogram (EEG) measurements during ELF-EMF were reported to show desynchronization of the alpha rhythm and other abnormalities in EEG activity. A promising neurophysiological approach to the assessment of disorders involving cognitive function was the application of the P300 ERP. Neuronal activity indexed by P300 latency is associated with the stimulus classification speed during memory updating and P300 amplitude is a measure of attention resource allocation when memory updating is engaged[5]. Thus, the observed P300 latency prolongation in the repairing group might reflect a slowing speed of cognitive performance.

BNP causes natriuresis, diuresis, and plasma shift to increase the oxygen transport in healthy humans to counteract hypoxic conditions. The natriuretic peptides are synthesized and released in response to the oxygen gradient among cardiocytes[6]. LENK is one of the opioid peptides that is mainly distributed in the CNS and physiologically serves as the chemical messenger in transformation, regulating metabolism, and coordinating organ function[7]. Hence, the BNP measurements play an important role in the diagnosis of the cardio-cerebral vascular disease, while LENK measurements play a role in cerebropathy. The increase of BNP in the operating group and LENK in the repairing group was still within the normal levels, which indicated a latent risk in electric workers.

Six SNP sites of *HTR1A, HTR2A*, *GRIN2B*, *BDNF*, and *COMT*, which were associated with cardiopathy and cerebropathy, of 150 workers in the electric company were genotyped. In 150 workers, there were 118 GG and 32 CG of *HTR1A* rs6295, in which the G-allele frequency was 89.3% and C-allele frequency was 10.7%. The *HTR2A* rs6311 in 150 workers included 44 AA, 74 AG, and 32 GG, wherein the A-allele frequency was 54.0% and G-allele frequency was 46.0%. The *HTR2A* rs6313 included 48 TT, 70 TC, and 32 CC, wherein T-allele frequency was 55.3% and C-allele frequency was 44.7%. The *GRIN2B* ENS10557853 contained 90 CC and 60 TT, wherein the C-allele frequency was 60.0% and T-allele frequency was 40.0%. Lastly, *BDNF* rs6265 had 38 AA and 112 GA, wherein the A-allele frequency was 62.7% and G-allele frequency was 37.3%, while *COMT* rs4680 had 138 AA and 12 GG, wherein the A-allele frequency was 92.0% and G-allele frequency was 8.0%.

The differences between the different genotypes of rs6311 and rs6313 with respect to the content of total protein (TP) and rs6295 in the content of Urea nitrogen were significant ($P < 0.05$). There were statistical differences in the interaction of rs6311 and exposure dosages with respect to the lymphocyte counts, the content of TP and CK, and between the rs6313 and exposure dosages with respect to the content of CK (Table 1). The counts of lymphocytes in AA genotype of rs6311 decreased with the increase of exposure dosage compared to AG and GG genotypes ($P < 0.05$). The TP content of rs6311 decreased, while that of CK in the AG genotype increased with the increased exposure dosage compared to that in the AA and GG genotypes ($P < 0.05$). The CK content of rs6313 in the TC genotype increased with the increasing exposure dosage compared to that in the TT genotype ($P < 0.05$).

There was no difference in the interaction of SNP sites with exposure dosages in ECG. There were statistical differences between rs6313, rs6295, ENS10557853, and exposure dosages in the latency of N2 (Table 2). The latency of N2 in TT genotype of rs6313, GG genotype of rs6295, and CC genotype of ENS10557853 was prolonged with the increase of exposure dosage as compared to CC, CG, and TT genotypes, respectively ($P < 0.05$). The P3 latency of rs6313 between the two genotypes showed a difference, while that in TT genotype of rs6313 was prolonged with the increase of exposure dosage as compared to CC genotype ($P < 0.05$).

Different genotypes of rs6295 and rs6265 varied with respect to the content of LENK and neuropeptide Y, respectively. The content of LENK increased with an increase in exposure dosage in the GG genotype of rs6295 compared to that in the CG genotype (Table 3).

A genetic variation of the 5-HT_{1A}R was reported to be associated with anxiety, depression, and suicidal behavior. Carriers of the *HTR1A* (rs6295) G allele presented more number of neurotic traits than those of C allele, and they wereassociated with changes of ligand binding to 5-HT_{1A}R, increased 5-HT_{1A}R activity, and the most unsatisfactory response to treatment with antidepressants[8]. In this study, the prolonged of N2 latency and the increase of LENK content were observed with an increase in exposure dosage in the GG genotype of rs6295 as compared to the CG genotype that showed an increased 5-HT_{1A}R activity in rs6295 G allele and

participated in the cognition retardation in electric workers.

A large number of studies have investigated the association of SNPs of *HTR2A* with a wide range of phenotypes, such as schizophrenia, mood, eating and anxiety disorders, psychiatric disorders, drug response and personality traits, attention-deficit hyperactivity disorders, suicide, and Alzheimer's disease[9]. The present study observed that prolonged N2 and P3 latency associated with increase in exposure dosage in the TT genotype of rs6313, as compared to the CC genotype, indicated that rs6313 T-allele might participate in the cognition retardation by influencing the expression

Table 1. Association of Polymorphisms with Serum Biochemicalparameters

Factors	*F* Value *P*										
	TP	ALB	TBIL	Ure	UA	Cr	ALT	AST	LDH	CK	CK-MB
Radiation dosage	0.39 0.5336	1.50 0.2255	0.04 0.8479	1.31 0.2566	1.86 0.1769	4.60 **0.0355***	0.06 0.8005	0.17 0.6835	0.03 0.8535	2.86 0.0956	0.08 0.7718
rs6311(AA/AG/GG)	3.57 **0.0335***	0.90 0.4105	0.51 0.5998	0.60 0.5542	61.07 0.3493	0.62 0.5432	0.32 0.7243	0.45 0.6371	0.18 0.8361	1.60 0.2085	0.02 0.9847
Radiation dosage×rs6311	3.33 **0.0415***	1.38 0.2586	0.42 0.6600	1.03 0.3614	71.59 0.2108	0.59 0.5565	0.38 0.6873	0.42 0.6576	0.19 0.8299	3.43 **0.0381***	0.40 0.6711
Radiation dosage	0.53 0.4672	1.89 0.1735	0.32 0.5720	1.06 0.3068	90.92 0.3420	3.72 0.0577	0.02 0.8780	0.12 0.7272	0.00 0.9723	1.23 0.2703	0.02 0.8944
rs6313 (TT/TC/CC)	3.40 **0.0391***	1.85 0.1646	0.93 0.3994	0.41 0.6632	61.21 0.3039	0.32 0.7304	0.55 0.5776	0.36 0.7011	0.28 0.7587	2.84 0.0651	1.46 0.2403
Radiation dosage×rs6313	2.88 0.0630	2.54 0.0858	1.75 0.1804	0.52 0.5985	10.57 0.5692	0.18 0.8327	0.18 0.8340	0.96 0.3897	0.36 0.7007	4.68 **0.0124***	1.96 0.1486
Radiation dosage	0.07 0.7879	1.09 0.3004	0.05 0.8288	2.98 0.0888	00.34 0.5621	0.97 0.3278	0.00 0.9910	0.37 0.5476	0.61 0.4367	4.22 **0.0438***	1.35 0.2494
rs6295(GG/CG)	0.07 0.7922	0.96 0.3294	0.06 0.8080	4.21 **0.0438***	0.32 0.5744	0.60 0.4429	0.33 0.5699	1.56 0.2152	1.22 0.2725	-	2.17 0.1447
Radiation dosage×rs6295	0.04 0.8336	0.62 0.4338	0.04 0.8497	2.35 0.1300	0.02 0.9023	0.06 0.8113	0.00 0.9492	0.61 0.4390	0.61 0.4358	-	1.72 0.1936
Radiation dosage	0.02 0.8907	1.45 0.2328	0.07 0.7892	0.58 0.4493	1.71 0.1956	4.88 0.0303	0.20 0.6586	0.30 0.5832	0.10 0.7540	3.64 0.0605	0.00 0.9710
ENS10557853 (TT/CC)	0.26 0.6109	0.27 0.6018	0.70 0.4040	0.22 0.6423	0.29 0.5949	0.01 0.9135	0.04 0.8395	0.01 0.9154	0.63 0.4313	0.43 0.5145	0.46 0.4988
Radiation dosage×ENS10557853	0.16 0.6911	0.77 0.3826	0.02 0.8792	0.23 0.6348	0.44 0.5116	0.18 0.6768	0.84 0.3636	0.34 0.5635	0.42 0.5206	0.01 0.9336	0.92 0.3418
Radiation dosage	0.02 0.8883	0.39 0.5352	0.00 0.9790	1.52 0.2211	2.44 0.1224	6.49 0.0130	0.20 0.6549	0.00 0.9694	0.00 0.9584	1.70 0.1960	0.00 0.9809
rs6265 (GA/AA)	0.64 0.4246	0.28 0.5974	0.09 0.7704	0.00 0.9683	0.51 0.4769	1.41 0.2388	2.70 0.1047	0.55 0.4615	0.00 0.9781	0.78 0.3794	0.13 0.7245
Radiation dosage×rs6265	0.81 0.3712	0.41 0.5240	0.02 0.9020	0.47 0.4936	0.31 0.5773	0.98 0.3267	2.57 0.1134	0.55 0.4621	0.13 0.7181	1.43 0.2364	0.21 0.6484
Radiation dosage	0.27 0.6055	0.78 0.3796	0.05 0.8279	1.73 0.1930	2.59 0.1116	5.06 **0.0276***	0.14 0.7130	0.02 0.8817	0.02 0.8831	1.60 0.2100	0.10 0.7579
rs4680 (AA/GG)	0.19 0.6608	0.04 0.8426	0.07 0.7985	0.40 0.5293	0.15 0.7012	0.27 0.6041	0.87 0.3534	0.12 0.7348	0.05 0.8236	0.03 0.8745	0.13 0.7162
Radiation dosage×rs4680	0.22 0.6428	0.19 0.6623	0.14 0.7058	1.06 0.3056	0.99 0.3226	1.25 0.2679	0.02 0.8766	0.44 0.5090	0.00 0.9977	0.00 0.9971	0.02 0.8776

Note. Statistical analysis was carried out with generalized linear regression. The influence of gender, age, work time and radiation dosage on the workers and the association with the different genotypes were considered. $^{*}P < 0.05$; TP: total protein; ALB: albumin; TBIL: total bilirubin; Ure: Urea nitrogen; UA: uric acid; Cr: creatinine; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactic dehydrogenase; CK: creatine kinase; CK-MB: creatine kinase isoenzyme MB.

Table 2. Association of Polymorphisms with the Latencies of N2 and P3 Wave and the Amplitude of P3

Factors	Latency of N2		Latency of P3		Amplitude of P3	
	F Value	*P*	*F* Value	*P*	*F* Value	*P*
Radiation dosage	8.95	**0.0039****	3.98	0.0502	0.18	0.6739
rs6311 (AA/AG/GG)	1.69	0.1934	1.25	0.2940	0.93	0.3990
Radiation dosage×rs6311	0.03	0.9717	0.17	0.8450	1.16	0.3197
Radiation Dosage	3.78	0.0563	0.51	0.4769	0.28	0.5956
rs6313 (TT/TC/CC)	2.51	0.0893	6.06	**0.0039****	0.47	0.6256
Radiation dosage×rs6313	5.65	**0.0055****	8.29	**0.0006****	0.79	0.4582
Radiation dosage	1.60	0.211	0.00	0.9472	0.92	0.3411
rs6295 (GG/CG)	3.50	0.0658	0.45	0.5064	0.46	0.4992
Radiation dosage×rs6295	5.07	**0.0276***	0.44	0.5104	0.74	0.3932
Radiation dosage	4.05	0.0483	1.77	0.188	0.73	0.3960
ENS10557853 (TT/CC)	1.37	0.2457	0.00	0.9579	1.20	0.2780
Radiation dosage×ENS10557853	4.29	**0.0423***	0.59	0.4462	3.03	0.0866
Radiation dosage	5.93	**0.0176***	4.23	**0.0437***	0.88	0.3527
rs6265 (GA/AA)	0.42	0.5207	0.38	0.5378	0.24	0.6283
Radiation dosage×rs6265	0.18	0.6758	1.23	0.2705	1.24	0.2702
Radiation dosage	0.60	0.4407	0.19	0.6651	0.08	0.7801
rs4680 (AA/GG)	0.61	0.4365	0.18	0.6737	0.39	0.5326
Radiation dosage×rs4680	1.18	0.2815	0.73	0.3959	0.44	0.5106

Note. Statistical analysis was carried out with generalized linear regression. The influence of gender, age, work time and radiation dosage on the workers and the association with the different genotypes were considered. $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 3. Association of Polymorphisms with BNP, LENK, and NPY

Factors	BNP		LENK		NPY	
	F Value	*P*	*F* Value	*P*	*F* Value	*P*
Radiation dosage	0.37	0.5454	3.12	0.0816	0.01	0.9298
rs6311 (AA/AG/GG)	0.32	0.7245	0.16	0.8555	0.22	0.8049
Radiation dosage×rs6311	0.51	0.6019	1.00	0.3744	0.10	0.9011
Radiation dosage	0.25	0.6159	2.72	0.1034	0.00	0.9944
rs6313 (TT/TC/CC)	0.12	0.8857	0.31	0.7328	0.24	0.7892
Radiation dosage×rs6313	0.02	0.9838	0.36	0.6957	0.04	0.9580
Radiation Dosage	0.13	0.7239	6.18	**0.0153***	0.11	0.7389
rs6295 (GG/CG)	0.62	0.4325	5.86	**0.0180***	0.61	0.4359
Radiation dosage×rs6295	0.28	0.5987	4.54	**0.0365***	0.17	0.6853
Radiation dosage	0.37	0.5438	2.41	0.1246	0.04	0.8519
ENS10557853 (TT/CC)	0.00	0.9906	0.91	0.3427	0.38	0.5409
Radiation dosage×ENS10557853	0.31	0.5824	0.41	0.5229	0.00	0.9475
Radiation dosage	0.23	0.6359	2.65	0.1079	0.00	0.9831
rs6265 (GA/AA)	5.81	**0.0185***	0.34	0.5615	0.22	0.6373
Radiation dosage×rs6265	1.09	0.3003	0.51	0.4755	0.00	0.9491
Radiation dosage	0.00	0.9576	1.03	0.3138	0.02	0.8861
rs4680 (AA/GG)	1.53	0.2195	0.73	0.3944	0.02	0.8803
Radiation dosage×rs4680	0.01	0.9218	0.21	0.6487	0.01	0.9310

Note. Statistical analysis was carried out with generalized linear regression. The influence of gender, age, work time and radiation dosage on the workers and the association with the different genotypes were considered. $^{*}P < 0.05$; BNP: brain natriuretic peptide; LENK: leucine enkephalin; NPY: Neuropeptide Y.

of 5-HT_{2A}R in electric workers. Although there were statistical differences in the interaction of *HTR2A* rs6311 and exposure dosages in the lymphocyte counts, the content of TP and CK, and between rs6313 and exposure dosages in the content of CK, the values of TP and CK in every group were within the normal range of humans. Thus, there was no significant change in TP and CK content, although it could indicate a potential caution for the workers.

Polymorphisms in GRIN2B are associated with cognitive disorders such as schizophrenia, Alzheimer's disease, Parkinson's disease, obsessive-compulsive disorder, and bipolar disorder. Most of them were concentrated on the exons or 3'-UTR of GRIN2B, and some reports about GRIN2B promoter variation with Alzheimer's disease found that −200T/G polymorphism in Caucasians and −1447T/C (ENS10557853) polymorphism in North Han Chinese population had no significant association, while −421C alleles within the North Han Chinese population increased the risk for sporadic Alzheimer's disease[10]. In the present study, the latency of N2 in CC genotype of ENS10557853 was prolonged with the increase in exposure dosage as compared to TT genotypes, which indicated that ENS10557853 C-allele was conducive to the cognition retardation in electric workers. However, the function of ENS10557853 C-allele is still unclear.

In this study, only 150 workers of the electric company were investigated, and some genotypes were lacking, such as CC in *HTR1A* rs6295, CT in *GRIN2B* ENS10557853, GG in *BDNF* rs6265, and AG in *COMT* rs4680 because of the small sample size. Hence, a negative finding about the association of *BDNF* and *COMT* polymorphisms for risk of cognition dysfunction in electric workers was possible, and further analyses may be worthwhile.

In conclusion, there were some abnormal characterizations of the brain and cardiac function in workers engaged in the electric company for a long time. The *HTR1A* in rs6295 G-allele, *GRIN2B* in ENS10557853 C-allele, and *HTR2A* in rs6313 T-allele could induce cognition retardation in the electric workers.

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