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Genetic Study Identifies CBLN4 as a Novel Susceptibility Gene for Accident Proneness

Abstract Frequent traffic accidents constitute a major danger to human beings. The accident-prone driver who has the stable physiological, psychological, and behavioral characteristics is one of the most prominent causes of traffic accidents. The internal link between the individual characteristics and the accident proneness has been a difficult point in the accident prevention research. The authors selected accident-prone drivers as cases and safe drivers as controls (case-control group) from 18,360 drivers who were enrolled from three public transportation incorporations of China using area stratified sampling method. The case-control groups were 1:1 matched. The authors performed genome-wide association study (GWAS) by 179 cases and 179 controls using the U.S. Affymetrix Genome-Wide Human Mapping SNP 6.0 Array. The authors observed that the gene frequencies of 34 single-nucleotide polymorphisms (SNPs) in three regions of cases were higher than those in the control ($P < 10^{-4}$). The authors then tested two independent replication sets for strong association 6 SNPs in 349 pairs of case-control drivers using the U.S. ABI 3730 sequencing method. The results indicated that SNP rs6069499 within linked CBLN4 gene are strongly associated with accident proneness ($P_{combined} = 6.37 \times 10^{-10}$). According to CBLN4 gene mainly involved in adrenal development and the regulation of secretion, the authors performed 12 biochemical parameters of the blood using radioimmunoassay. The levels of dopamine (DA) and adrenocorticotropic (ACTH) hormone showed significant differences between accident-prone drivers and safe drivers ($P_{DA} = 0.03$, $P_{ACTH} = 0.01$). It is suggested that the accident-prone drivers may have the idiosyncrasy of susceptibility.

Keywords: accident proneness, genome-wide association study (GWAS), dopamine (DA), ACTH, susceptibility gene, traffic accident epidemiology, accident prevention, traffic safety, three-dimensional model

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1 Introduction

Road traffic accidents are a major global public health crisis due to its injuries more than any other natural disasters (Jin, 1994; Mckenna, 1983), over 70% of accidents caused by drivers. Accident-prone drivers are one of the most prominent causes of traffic accidents, being responsible for 30%–40% of the total number of accidents in China (Jin, 1994). Accident proneness is an internal characteristic of individuals, leading them to more readily have accidents than others due to their physiological and psychological features within a certain period of time (Mckenna, 1983). The previous studies of accident proneness have been focused on the individual characteristics such as behavior, physical abilities, cognitive abilities, extroversion, nervous temperament, error reaction, low performance, etc. (Jin, 1994; Jin, Araki, Wu, Zhang, & Yokoyama, 1991; Norris, Matthews, & Riad, 2000; Pen, He, Chen, & Xiao, 2000; Petridou & Moustaki, 2000; Shen & He, 1994; Sun, Das, & He, 2014). However, the internal causes of recidivism accidents of accident proneness are still unknown. Studies on childhood injury proneness indicate that there is familial aggregation in child injuries, and the potential genetic factor plays an important role in the difference of individuals (Phillips & Matheny, 1995). If researches on the behavioral characteristics of the accident-prone drivers and their possible genetic clues of accident proneness make a break, it is possible to make a reasonable forecast and protective factor for the accident. For example, American scholars at the University of Virginia have studied the

young people who have been suffering from attention deficit syndrome, using sustained-release capsules to improve the attention of driving (Cox, Humphrey, Merkel, Penberthy, & Kovatchev, 2004). In 2010, the British scholars (Michie, van Stralen, & West, 2011) launched the "behavior changing wheel (BCW)" framework of pilot study. In the paper, the authors try to explore the genetic clues of the accident proneness, which may provide a new way for the selection of safe driver, targeted training of safe defect, behavior modification, and has important scientific value and social significance for individualized guidance to accident prevention.

2 Method subjects

According to the definition of the implementation of the international epidemiology, the authors chose bus drivers as target population using cluster sampling method from three public transportation incorporations in China. The three representative cities of Hangzhou (East China), Qiqihar (Northeast China), and Xi'an (Northwest China) were sampled by the geographical distribution. The authors selected accident-prone drivers as cases and safe drivers as controls who were 1:1 matched. Firstly, the authors performed genome-wide association study by 179 cases and 179 controls from 6,060 bus drivers in Hangzhou Public Transportation Incorporation of China using the U. S. Affymetrix Genome-Wide Human Mapping SNP 6.0 Array, and data analysis were executed by Chinese National Engineering Center for Biochip in Shanghai. Another two independent samples used in the replication study were from the Public Transportation Incorporation of Xi'an city and Qiqihar city in China, respectively. The first one included 158 cases and 158 controls, the second included 191 cases and 191 controls. All the cases and controls are healthy drivers, excluding the drivers who have driving contraindications. Informed consent was obtained from all the participants. The study was approved by each institutional ethical committee and conducted according to Declaration of Helsinki principles.

2.1 Selecting case and control

All participants were made a diagnostic test of accident proneness using the Chinese national standard (GB18463-2001) (National Standard of the People's Republic of China, 2001). The test indexes include speed anticipation, discrimination reaction judgment (error reaction), attention distribution and duration (error action), depth perception, dynamic vision, and night vision (Table 1). The drivers whose test indexes were all abnormal became candidates of the case group, and those whose test indexes were all normal were candidates of the control group.

Table 1

Diagnostic Test Standards for the Candidates of Cases and Controls (From Chinese National Standard GB18463-2001)

Index	Unit	Abnormal	Normal
Speed anticipation	ms	< 800 or > 2500	800–2500
Discrimination reaction judgment (error reaction)	time	> 5	$\leqslant 5$
Attention distribution and duration (error action)	time	> 110	$\leqslant 110$
Depth perception	mm	< -22 or > 22	± 22
Dynamic vision		< 0.2	$\geqslant 0.2$
Night vision	s	> 35	$\leqslant 35$

2.2 Confirming cases and controls

According to historical operating definition of accident-prone driver, cases are confirmed by those drivers who had three or more accidents at the level of more than equal-responsibility within 5 consecutive years (2005–2009) among case candidates. While the drivers who did not have liability for accidents over the same years (2005–2009) are confirmed as the controls among control candidates. To eliminate the bias of confounding factors, the matching conditions for case and control include: Similar age and driving duration (difference less than 2 years), same nationality, gender, residence, education and marital status, similar driving route and driving training, etc. (Table 2). Driver's responsibility for the accident is defined by the recognized five grades issued by the Chinese government, namely full responsibility, main responsibility, equal responsibility, secondary responsibility, and no responsibility (People's Republic of China, 2003). Accident frequency was accumulated from the original traffic accidents registered record by the safety department at the company.

Table 2

Matching Conditions of the Case Drivers and Control Drivers

Case group	Matching-control group
Age	Similar (difference less than 2 years old)
Gender	Same
Nationality	Same
Residence	Same
Education	Same
Driving duration	Similar (difference less than 2 years)
Driving training	Same (the same driving training school)
Driving route	Same (such as bus No. 2)
Marital status	Same

Two independent samples were used in the replication study. The first one was selected from 5,100 drivers recruited at the Public Transportation Incorporation of Qiqihar in China. Finally, there were 158 pairs of cases and controls selected into the study. The second one was selected from 7,200 drivers recruited at the Public Transportation Incorporation of Xi'an in China, and there were 191 pairs of cases and controls into the study.

2.3 Diagnostic test methods

(1) Speed anticipation. LJ9101 speed anticipation tester was used. In this testing instrument, a cursor moves at a constant speed in a slot. When the cursor moves behind a damper (blind area), the patient is asked to assume that the cursor still move at the same speed and to press the reply button while the cursor reaches the baffle on the other side at the same speed. Finally, the instrument displays the estimated time. Each patient is tested 5 times, and the average values are taken as the speed anticipation.

(2) Discrimination reaction judgment. LJ9102 discrimination reaction judgment tester was used. This instrument has one red, one yellow, and one green lamp. After the patient presses all the buttons, he is asked to operate according to the following instructions: While the red light is on, release the right foot button for the red light; while the green light is on, release the right hand button; and while the yellow light is on, release the left hand button; when the buzzer is ringing at the same time, or a certain lights turn on, or only the buzzer is ringing, does nothing. Each patient is tested 16 times, and the error reaction times and average reaction time are recorded.

(3) Attention distribution and duration. LJ9103 action judgment tester was used. The wheel of the equipment has 16 arrow identifiers and rotates at a constant speed. The patient attempts to manipulate the steering wheel so that the left pointer and right pointer can only pass behind the arrow identifier and cannot touch the turntable around the red line. If the pointer passes in front of the identifier or touches the identifier and the red line, the buzzer rings, and the counter records the error action times of left and right pointers during 3 min 30 s.

(4) Depth perception. LJ9204 depth perception tester was used. This instrument consists of a test box equipped with three poles on the same horizontal line. The middle pole can move back and forth in the mobile slot, and the others are fixed on the two sides of the slot. The patient is seated 2.5 m from the test box, looking at the center hole horizontally. When the moving pole reaches a horizontal line between both sides of the stem, the patient is asked to press the reply button. The instrument displays the deviation of the distances of the three poles. Each patient is tested 5 times, and the average value is recorded.

(5) Dynamic vision. LJ9205 dynamic vision tester was used. The patient sits in front of the dynamic vision tester. There is a dynamic vision target marked "C" in the instrument through the optical system. The dynamic vision target moves in front of the subject's eyes at a simulation speed of 30 km/h. As soon as the patient discerns the direction of the "C", he presses the reply button and answers the direction of the "C", and at the same time, the dynamic visual target stops moving. The dynamic vision value is shown on the side of the instrument automatically. Dynamic vision is tested 3 times, and the maximum value is taken.

(6) Night vision. LJ9206 night vision tester is used. An "E" is shown in the night vision instrument at the beginning of the test under a strong light. The patient stares at the mark "E" for 30 s. Then, the light source is shut off, and in the dark environment, if the patient can distinguish the direction of the mark "E", he will press the reply button. The night vision value, namely the dark adaptation time, is the recorded time from shutting of the light to pressing the button.

2.4 Genotype and quality control procedures for genome-wide association study (GWAS)

In all cases and controls, 2 mL of venous blood was collected into tubes with EDTA as an anticoagulant, and then stored in a –80 °C refrigerator, and DNA was extracted after defrosting the whole blood. Genome-wide scanning of SNPs was conducted using the U.S. Affymetrix SNP 6.0 chip and an experimental biochip at the Chinese National Engineering Research Center in Shanghai. All SNPs on the X, Y, and mitochondrial chromosomes (chr) as well as copy number variation-related SNPs were excluded. Additionally, SNPs were excluded if they showed a call rate lower than 90% in cases or controls, a minor allele frequency of $< 1\%$ in the population or significant deviation from Hardy-Weinberg equilibrium (HWE) in the controls ($P < 10^{-3}$). The authors then examined potential genetic relationships based on pair wise identity by state for all of the successfully genotyped samples using P-LINK 1.07 software and employed the Cochran-Armitage trend algorithm using P-LINK 1.07 software to calculate the allele frequencies of the case group and the control group as well as the P value, odds ratio (OR) and 95% confidence interval (95%CI). Principal component analysis (PCA) provided minimal evidence of population stratification (*Figure 1*). According to the GWAS data, the authors selected the most promising 6 SNPs among the 34 SNPs for replication in two independent samples using the U.S. ABI 3730 sequencing method.

2.4.1 Biochemical detection

Samples of 2 mL of venous blood were collected from the 179 case-control pairs of drivers. Then, the serum was separated, and 12 biochemical indexes of the blood were determined via radioimmunoassay including triiodothyr-

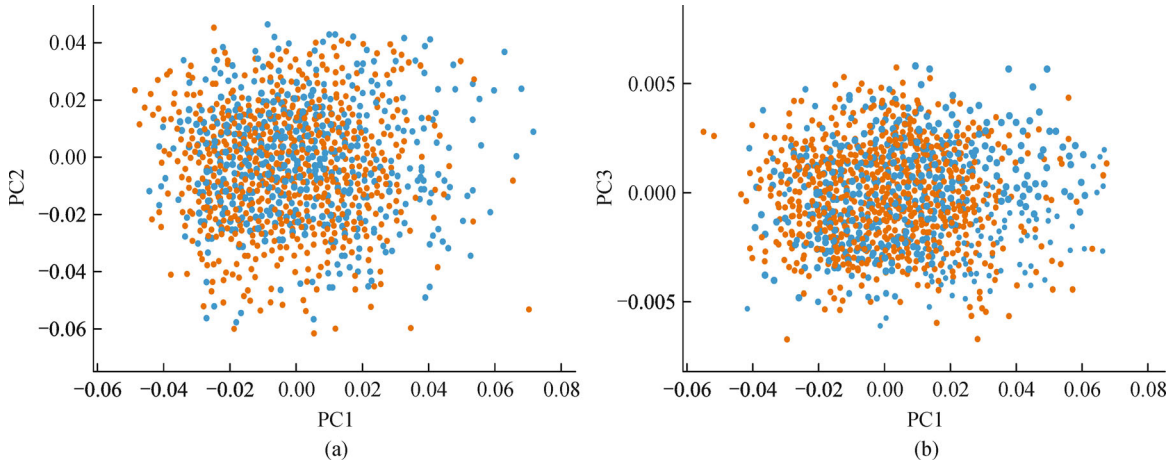

Figure 1. PCA plot of 1056 participants from 3 samples (528 cases and 528 controls). The orange points are cases, and the blue points are controls. (a) PCA plot of the GWAS and replication 1 samples; (b) PCA plot of the GWAS and replication 2 samples.

onine (T3), tetraiodothyronine (T4), testosterone (T), estradiol (E2), corticotropin (ACTH), cortisol (Cor), norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT), γ-aminobutyric acid (GABA), angiotensinII (ANG-II), dopamine-β-hydroxylase (DβH). Radioimmunoassay kits were purchased from the Beijing North Biotechnology Institute (batch number: 100920).

Statistical analysis:

(1) Genome-wide SNP correlation analysis, assessment of the minimum allele frequency (MAF) and Hardy-Weinberg equilibrium inspection were carried out using GeneSpring software for each SNP. Genotype-phenotype correlations were analyzed using the Cochran-Armitage trend test, Pearson's χ^2 test and Fisher's exact test.

(2) A control sample copy of the database was created using the control samples in GeneSpring software. Copy number analysis between the cases and the previous samples was performed to obtain the copy number in each sample. Then, determination of the copy number changes in common regions in the experimental sample, SNP chip testing and data analysis were executed by Chinese National Engineering Center for Biochip in Shanghai.

2.4.2 Results

Genome-wide association study: The GWAS dataset was comprised of 690,600 SNPs. Following the quality control procedures, the authors used the genotype data from 608,199 autosomal SNPs from 179 cases and 179 controls for association testing. Population structure analysis indicated minimal overall inflation of the genome-wide statistical results because of population stratification (λ_{GC}= 1.049). The authors carried out the Cochran-Armitage trend test to assess genotype-phenotype associations and observed 34 SNPs showing suggestive evidence of meeting the $10^{-6} < P < 10^{-3}$ criterion (*Figure 2*, Table 3).

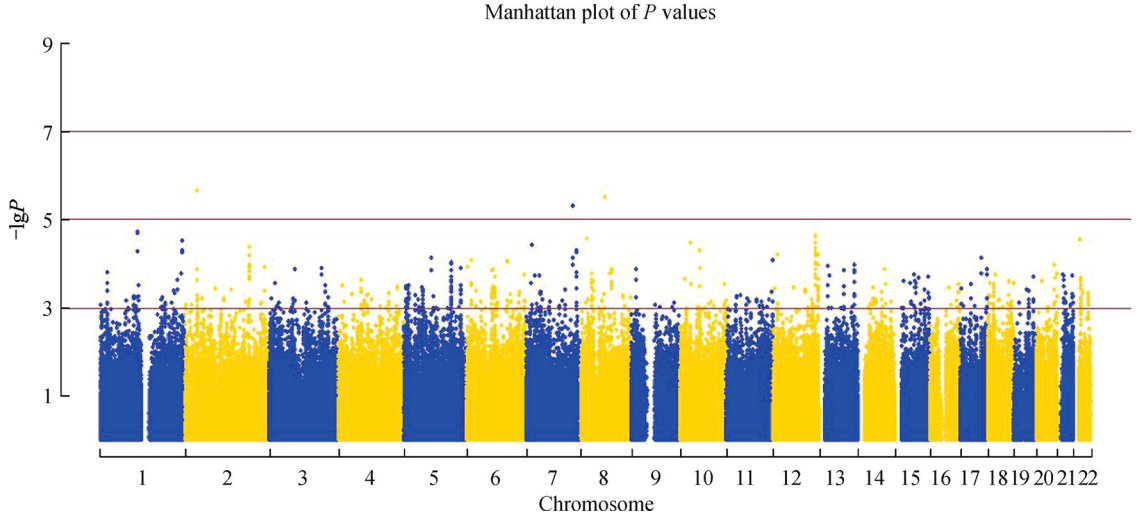

Figure 2. P value of all SNPs loci distribution on each chromosome.

Table 3

Information on SNP Loci at Associated Genes (n = 358)

dbSNP RS ID	Chr	Physical Position	Allele A/B	Association gene	*P*	*OR*	*OR* (95%CL)
rs2033581	8	69264902	A/G	DEPDC2	2.10×10^{-6}	0.4629	0.3365–0.6368
rs6978138	7	16856893	A/G	AGR2	4.70×10^{-5}	0.5443	0.4011–0.7385
rs1019621	7	135000000	A/G	CNOT4///STRA8	1.12×10^{-5}	0.4485	0.3047–0.6603
rs292661	7	135000000	C/T	CNOT4///STRA8	9.01×10^{-5}	0.4972	0.3317–0.7452
rs2972106	7	148000000	C/T	CNTNAP2	9.31×10^{-5}	0.5272	0.3748–0.7414
rs2717741	8	18490786	C/G	PSD3	1.80×10^{-5}	3.2696	1.7794–6.0078
rs2634449	8	18488730	A/G	PSD3	1.80×10^{-5}	0.2772	0.1489–0.5161
rs6069499	20	53973735	A/G	CBLN4	9.80×10^{-5}	0.2857	0.1478–0.5521
rs6035200	20	18970894	A/T	C20orf79///SLC24A3	9.66×10^{-5}	0.5884	0.4395–0.7879
rs6064290	20	53890201	C/T	Cerebellin-4 precursor	1.56×10^{-4}	1.5401	1.1458–2.0773
rs4811635	20	53864343	A/G	Cerebellin-4 precursor	4.02×10^{-4}	1.5202	1.1369–2.0292
rs1370271	20	53974426	A/G	Envelope protein	3.85×10^{-4}	0.3295	0.1767–0.6144
rs7732110	5	135547908	C/T	TRPC7	6.07×10^{-4}	0.6058	0.4505–0.8148
rs11242316	5	135547747	C/G	TRPC7	5.07×10^{-4}	1.6866	1.2560–2.2648
rs3734125	5	135551643	C/T	TRPC7	3.94×10^{-4}	1.7300	1.2873–2.3250
rs10045073	5	135557803	C/T	TRPC7	6.34×10^{-4}	1.7120	1.2740–2.3006
rs10041689	5	135565676	A/G	TRPC7	1.61×10^{-4}	1.7676	1.3141–2.3776
rs171101	5	135646103	C/G	TRPC7	3.35×10^{-4}	0.5696	0.4155–0.7809
rs7701815	5	135649997	A/C	TRPC7	4.07×10^{-4}	1.7555	1.2805–2.4067
rs1392170	5	135659201	A/C	TRPC7	4.01×10^{-4}	0.5806	0.4229–0.7970
rs950715	5	135708138	C/T	TRPC7	1.63×10^{-4}	0.5317	0.3784–0.7471
rs3777150	5	135705746	A/C	TRPC7	3.48×10^{-4}	0.5240	0.3655–0.7513
rs12515628	5	135712924	C/T	TRPC7	1.49×10^{-4}	1.8919	1.3443–2.6624
rs346644	5	135746033	A/G	TRPC7	3.48×10^{-4}	0.5538	0.3955–0.7755
rs2548979	5	135481392	C/T	SMAD5	5.24×10^{-4}	0.5924	0.4408–0.7962
rs2906830	5	135481829	C/T	SMAD5	8.77×10^{-4}	1.6487	1.2284–2.2128
rs2548978	5	135492030	C/T	SMAD5	3.86×10^{-4}	1.7065	1.2701–2.2928
rs9327743	5	135495208	C/G	SMAD5OS	3.21×10^{-4}	0.5714	0.4243–0.7695
rs13187638	5	135500093	C/G	SMAD5	9.59×10^{-4}	1.6324	1.2146–2.1940
rs6596288	5	135512462	C/T	SMAD5	3.82×10^{-4}	1.7060	1.2702–2.2915
rs10056474	5	135519295	C/G	SMAD5	3.82×10^{-4}	1.7061	1.2702–2.2915
rs10064147	5	135533740	A/G	SMAD5	1.67×10^{-4}	1.7823	1.3249–2.3976
rs6886699	5	135543637	C/T	SMAD5	2.03×10^{-4}	1.7546	1.3037–2.3614
rs7719008	5	164000000	A/G		9.67×10^{-5}	0.5480	0.4086–0.7348

Genome-wide association results from the initial GWAS analysis. The genome-wide *P* values of the Cochran-Armitage trend test from 179 pairs of case-control. The chromosomal distribution of all the *P* values (–lg*P*) is shown.

The authors selected the most promising 6 SNPs among the 34 SNPs for replication in two independent samples using the U.S. ABI 3730 sequencing method. The authors observed the MAF of the cases was higher than that of the controls ($P < 10^{-4}$). The replication analysis revealed the SNP rs6069499 within the CBLN4 gene that showed a consistent significant association with accident proneness ($P_{R1} = 4.01\times10^{-4}$, $OR_{R1} = 0.37$; $P_{R2} = 5.67\times10^{-5}$, $OR_{R2} = 0.23$) and presented a highly significant association in the joint analysis combining GWAS analysis with two replication samples ($P_{\text{combined}} = 6.37\times10^{-10}$, $OR_{\text{combined}} = 0.29$) (Table 4, *Figure 3*).

The authors plotted the *P* values of SNPs (shown as –lg*P*

Table 4

Association of SNP rs6069499 within the CBLN4 Gene with Accident Proneness in the GWAS and the Replication Studies

	MAF		*OR*	*P*
	Case	Control		
GWAS (358)	0.11 (179)	0.03 (179)	0.29	9.80×10^{-5}
Replication 1 (R1) (316)	0.10 (158)	0.04 (158)	0.37	4.01×10^{-4}
Replication 2 (R2) (382)	0.10 (191)	0.03 (191)	0.23	5.67×10^{-5}
Combined (1056)	0.10 (528)	0.03 (528)	0.29	6.37×10^{-10}

values on the y axis from the genome-wide single-marker association analysis using the Cochran-Armitage trend test) against their map positions (*x* axis). The color of each SNP spot reflects its r^2 value with the top SNP (large red square) within the association locus, ranging from red to blue. Estimated recombination rates (based on the combined CHB samples from the HapMap project) are plotted in light blue. Gene annotations were adapted from the UCSC Genome Browser (see URLs).

Association genomics functional Studies: Bioinformatics analysis showed that the functions of CBLN gene are mainly involved in the adrenal development and its endocrine regulation. When neurotransmitter and hormone levels were determined in the blood of drivers via radioimmunoassay, it was revealed that the level of DA in the case group was significantly lower than that in the control group, while the level of ACTH in the case group was higher than that in the control group ($P_{DA}= 0.03$ and $P_{ACTH}= 0.01$, respectively) (Table 5).

3 Discussions

Accident proneness can be objectively observed in various occupational populations (Visser, Pijl, Stolk, Neeleman, & Rosmalen, 2007), and its dangers are especially prominent in relation to human health and safety. Studies have shown that accident proneness is associated with certain typical physiological and psychological characteristics, such as insufficient motion perception, error reaction judgment, attention deficits, depth perception bias, and poor dynamic vision (Bergomi, Vivoli, Rovesti, Bussetti, Ferrari, & Vivoli, 2010; Gulliver & Begg, 2007; Jin, 2013; Nabi, Consoli, Chastang, Chiron, Lafont, & Lagarde, 2005; Parker, West, Stradling, & Manstead, 1995; Zhang, 2000). Previous studies have analyzed the relationships between the personality characteristics of accident-prone individuals and accidents, including anxiety, drunk-driving, provocation, violations, and other bad-driving behaviors (Moore & Dahlen, 2008; Özkan, Lajunen, Parker, Sümer, & Summala, 2010; Shahar, 2009). The evidence shows that these individual defects induce more driving errors in driving task experiments, resulting in repeated accidents (Uc & Rizzo, 2008). It has been found that accident-prone drivers may have individual idiosyncrasies related to fallible driving behaviors. The complex phenotype consisting of such internal idiosyncrasies may have a corresponding genetic basis (Plomin, Owen, & McGuffin, 1994). This study found that the SNP rs6069499 of CBLN4 gene variation was highly associated with accident proneness ($P < 10^{-10}$), which suggested that the accident-prone drivers may have the characteristics of susceptibility. Although the sample size in our study is small, the results are reliable by twice strict screenings of the samples to

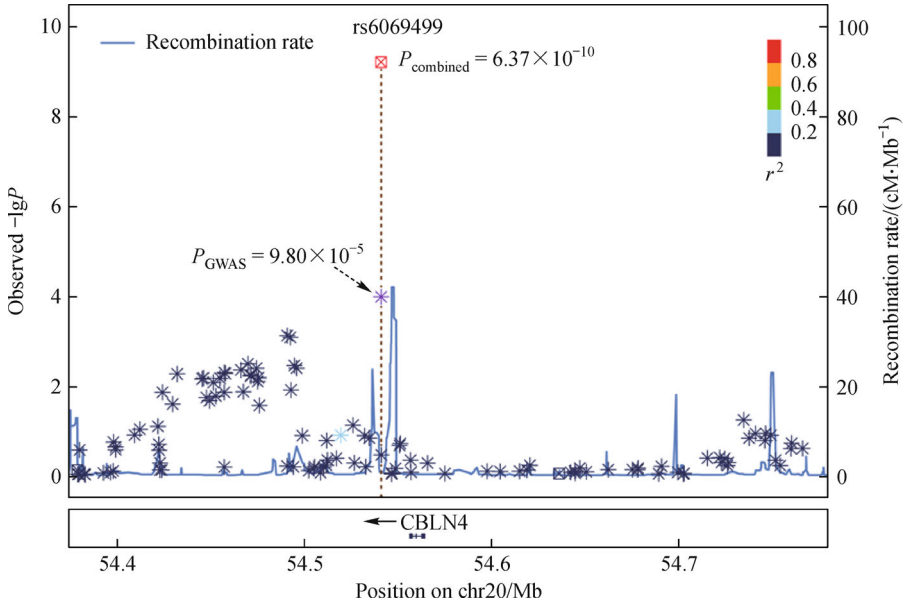

Figure 3. Association scatter plot for the accident proneness susceptibility locus.

Table 5

Comparison of Neurotransmitter and Hormone Levels in the Blood between Cases and Controls (n = 358)

Neurotransmitters and hormones	Unit	Case group (179)	Control group (179)	*P*
Triiodothyronine (T3)	ng/mL	1.06±0.14	1.11±0.20	0.38
Tetraiodothyronine (T4)	ng/mL	82.63±23.02	87.01±23.87	0.50
testosterone (T)	ng/mL	3.65±1.57	4.33±2.19	0.10
estradiol (E2)	pg/mL	42.53±15.75	43.11±12.86	0.89
Corticotropin (ACTH)	pg/mL	14.23±8.52	10.01±3.76	0.01**
Cortisol (Cor)	ng/mL	147.48±31.95	155.91±24.60	0.38
Norepinephrine (NE)	pmol/L	4424.85±2437.21	4927.11±1957.83	0.48
Dopamine (DA)	ng/L	250.15±122.81	329.94±153.96	0.03**
5-hydroxytryptamine (5-HT)	ng/L	2519.83±706.24	2042.47±857.90	0.57
γ-aminobutyric acid (GABA)	μmol/L	0.44±0.24	0.43±0.06	0.80
AngiotensinII (ANG-II)	pg/mL	1064.77±220.37	1116.88±196.25	0.39
Dopamine-β-hydroxylase (DβH)	ng/L	537.47±219.02	485.08±246.05	0.41

Note. **: $P < 0.05$

ensure the purity of the cases. Further researches will be carried out by expanding the samples.

CBLN4 gene is mainly involved in adrenal development and the regulation of secretion, and plays important roles in the regulation of the main hormones and neurotransmitters involved in the stress system (Rucinski & Malendowicz, 2009; Rucinski, Ziolkowska, Szyszka, & Malendowicz, 2009). Monoamine neurotransmitters are the most active among neurotransmitters in the body's stress system. When a source of stress (such as driving) stimulates the stress system and the hypothalamic-pituitary-adrenal (HPA) axis is overactive, this axis releases corticotrophin-releasing factor (CRF), which causes adrenocorticotropic hormone (ACTH) to be released, and cortisol levels will be increased (Mukherjee, Knisely, & Jacobson, 2004). Previous studies have indicated that accident proneness may be associated with monoamine neurotransmitter systems (e.g., 5-HA, DA) (Plomin, Owen, & McGuffin, 1994). Biochemical tests in the neurological system have shown that the movement coordination ability and learning and memory abilities are also associated with the dopamine system, and a low level of DA may result in anomalous emotions (Previc, 1999). High level of ACTH has positive relation with attention defect index (Lei & Yang, 2010). This study found that there is low level of dopamine (DA) and high level of adrenocorticotropic (ACTH) in accident prone drivers ($P_{DA} = 0.03$ and $P_{ACTH} = 0.01$). Low levels of DA may induce negative emotions leading to bad driving behaviors, and high levels of ACTH may induce distraction causing accidents in driving.

Animal experiments have shown that the bad driving behaviors (aggression) are related to the concentrations of serum testosterone (T) (Soma, 2006), and the levels of norepinephrine, 5-hydroxytryptamine and other neurotransmitters in cerebrospinal fluid are also associated with the animal and human stress response (Jin, Zhang, & Pan, 1999; Sluiter, van der Beek, & Frings-Dresen, 1998). However, the experimental data from the present study revealed no obvious differences in the serum levels of testosterone, norepinephrine, 5-hydroxytryptamine or other neurotransmitters and hormones. The above different results may be caused by the differences between animal behavior and human behavior parameters.

Accident proneness presents physiological, psychological and behavioral features, and the SNP rs6069499 of CBLN4 gene variation associated with accident proneness may determine these features of accident proneness; these features are both potential and stable. However, traffic accidents caused by accident proneness are also influenced by environment, which are in line with the quantitative trait genetic law of multi-gene effects associated with the impact of environment and other factors (Plomin, DeFries, McClearn, & McGuffin, 2007). When such multi-gene effects reach a certain threshold value (γ, *Figure 4*), they will trigger these internal features and induce accident proneness to mutate from incubation to dominance. In certain space and period, when environment factors reach certain threshold value (α, β, *Figure 4*), traffic accidents will occur. The discovery of the susceptibility SNP conferring accident proneness reasonably explains the theory of the three-dimensional "feature-period-environment" model of accident proneness (Jin, 1994).

The study showed that the rs6069499 mutation frequency of CBLN4 gene which reaches a certain level may increase the risk index of the accident-prone drivers. It has personalized instructing significance for drivers' self-risk prediction and safety defect target training. It also suggested that the accident-prone drivers may have the

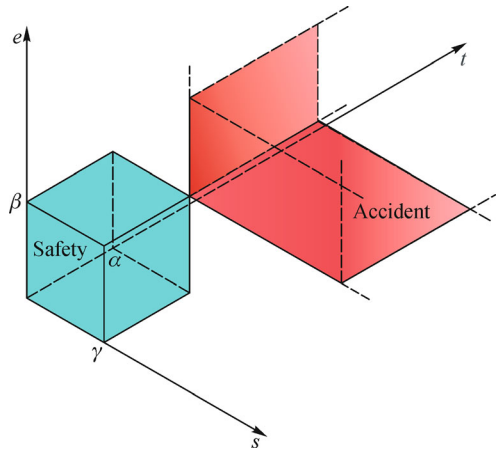

Figure 4. Three-dimensional threshold model of accident proneness.
s—Feature variables; *e*—Environmental variables; *t*—Period variables; *P*—Accident probability; α,β,γ—Threshold of three variables (period, environment, and feature)

susceptibility idiosyncrasy. Our findings related to the SNP rs6069499 in the CBLN4 gene not only contribute to our understanding of the genetic basis of accident proneness but also provide insights for future research on accident proneness. Moreover, the study sheds light on new mechanisms underlying the causes of accidents and pinpoints a potential new mode of accident prevention.

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Authors' contributions Hui-qing Jin conceived and designed this study and obtained financial support. Shu-lin Zhang and Liang-dan Sun analyzed the data and performed the epidemiological investigation. Yang Song undertook related data analysis and performed experiments. Wan-sheng Yu managed recruitment and obtained biological samples. All of the authors contributed to the final paper, and Hui-qing Jin played key roles. The authors declare no competing financial interests.

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