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Original Article

BDNF gene polymorphism and serum level correlate with liver function in patients with hepatitis B-induced cirrhosis

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Abstract: We investigate the correlation of serum brain-derived neurotrophic factor (BDNF) level and its gene polymorphism with liver function classification in patients with hepatitis B virus (HBV) induced liver cirrhosis. A total of 182 patients with HBV induced liver cirrhosis were collected as a case group, and 186 healthy subjects in the same period were used as the control group. ELISA measured serum BDNF levels. Polymerase chain reaction-restriction fragment length polymorphism was used to detect rs6265 (A/G) and rs10835210 (A/C) in the *BDNF* gene. The serum BDNF level was significantly lower in the case group than in the control group. With the elevation of Child-Pugh classification in patients with HBV induced liver cirrhosis, the decrease trend of serum BDNF level was even lower. The difference in frequency distribution between the case group and the control group was statistically significant regarding GG, GA, and AA genotypes, as well as G and A alleles in rs6265 (all $P < 0.05$). The frequency distribution of genotypes and alleles of rs6265 was statistically different in HBV induced liver cirrhosis patients with different liver function grades ($P < 0.05$). In patients with HBV induced liver cirrhosis, the AA genotype of BDNF gene rs6265 had the lowest level of serum BDNF. Our study suggests that serum BDNF plays an important role in the grading and early diagnosis of liver function in patients with HBV-induced liver cirrhosis, and AA genotype at rs6265 of BDNF gene is a negative factor for liver cirrhosis. Moreover, the polymorphism of this locus could affect the serum BDNF level.

Keywords: Brain-derived neurotrophic factor, gene polymorphism, cirrhosis, Child-Pugh classification, liver function, frequency, haplotype, risk

Introduction

Liver cirrhosis is induced by the repeated occurrence of diffuse degeneration and necrosis of the liver cells, hyperplasia of fibrous tissue, and nodular regeneration of cells, which results in the degeneration and hardening of the liver [1, 2]. Liver cirrhosis is mainly manifested by hepatic dysfunction and portal hypertension, and associated with complications such as digestive tract hemorrhage and hepatic encephalopathy [3-5]. It has been demonstrated that cirrhotic livers often have hepatitis B virus (HBV) or hepatitis C virus (HCV) [6]. The compensatory status of liver function plays an important role in the development, clinical treatment, and prognosis of patients with liver cirrhosis [7]. Therefore, a correct assessment of liver function in patients with liver cirrhosis is essential to the formulation of a reasonable treatment plan [8, 9]. At present, Child-Pugh classification is widely used as an indicator of liver function reserve, prognosis, and survival period in patients with liver cirrhosis [10-12].

Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophic factor family, mainly expressed in the hippocampus and cerebral cortex [13]. Previous studies have found that BDNF is associated with the growth, differentiation, and repair of neurons [14, 15]. In recent years, BDNF has been found to modulate synaptic plasticity and enhance long-term potentiation in hippocampus, which is closely related to cognitive processes such as learning and memory [16, 17]. In cognitive functional

tests such as water maze and passive avoidance, BDNF inhibition can cause a decrease in long-term potentiation and impairment of memory function [18]. In addition to the influence of BDNF on learning and memory, a large number of animal experiments confirm that BDNF can also participate in the regulation of blood glucose and energy metabolism [19], and has a significant hypoglycemic effect. Furthermore, BDNF gene is suggested to be located at 11p13 chromosome, consisting of more than 5 exons, and each exon has its own promoter to adjust its mRNA splicing [20]. Multiple previous studies have shown that the expression of BDNF and its genetic polymorphism are associated with several diseases, such as cognitive dysfunction, depression, stroke, epilepsy, and several tumors [21-26], among which rs6265 (A/G) and rs10835210 (A/C) of this gene have aroused great concern in the field of genetic polymorphism study [27, 28]. A previous study has suggested that BDNF is crucial for tumor angiogenesis and growth, and it may represent a potential target for antiangiogenic therapy in hepatocellular carcinoma (HCC) [29]. Also, it has been demonstrated that higher serum BDNF level is related to a more advanced tumor status in HCC patients, and serum BDNF might play an important role in tumor progression of HCC [30]. In addition, in view of the current status of liver cirrhosis, as mentioned above, several studies have paid attention to investigate the molecular mechanism of this type of disease, but there are few studies focused on the exploration of BDNF in liver cirrhosis. Accordingly, the present study was carried out to explore the correlation of serum BDNF level and its gene polymorphism with liver function classification in patients with HBV-induced liver cirrhosis.

Materials and methods

Ethics statement

This study was approved by the institutional ethics review board of Shangrao People's Hospital. This study applied the voluntary principle for the consent of the patients and their families. At the same time, patients were fully aware of the study, had the ability to complete all the treatment plans, and signed the relevant informed consent.

Study subjects

A total of 182 first visit patients with HBV-induced liver cirrhosis were collected as a case group in Shangrao People's Hospital from January 2017 to November 2017. Among them, there were 150 males and 32 females. The age of the patients was 24~74 years in the case group, and the average age was 45.52 ± 11.58 years old. Child-Pugh classification: 56 cases of grade A, 69 cases grade B, and 57 cases grade C. 186 healthy subjects who underwent physical examination in the medical center of Jiangxi Institute of Gastroenterology & Hepatology, The First Affiliated Hospital of Nanchang University were used as the control group in the same period. In this group, there were 142 males and 44 females, and the average age was 43.68 ± 10.54 years old (ranging from 22~70 years old). The control group was selected according to the principle of matching the age and gender of the case group. Inclusion criteria of the case group: HBV-infected subjects, positive HBsAg test result, HBV-DNA $\geq 10^5$ copy/mL; Computed tomography scan showed fibrosis and cirrhosis of the liver; serum liver fibrosis index exceeded the standard [Hyaluronic acid (HA) > 110 mg/L, laminin (LN) > 110 μg/mL, type IV collagen (PC IV) > 75 μg/L and proline peptidase (PLD) > 1126 ug/L]; serum ALT concentration > 40 U/L; classification of liver fibrosis > S0. Exclusion criteria: Non-HBV infection of liver cirrhosis; patients who have been treated with hepatitis or liver cancer; patients who had a genetic history of hepatitis and HCC; and acute hepatitis B patients. Inclusion and exclusion criteria of the control group: Negative imaging, HBsAg, HBV-DNA and AFP test, normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and being without history of cancer and other major disease.

Specimen collection

5 mL fastingvenous blood was extracted from each subject in the case group on the next day at 8:00 am after admission, and 5 mL fasting venous blood was extracted from each subject in the control group at 8:00 am on the day of physical examination. The extracted venous blood was then placed into EDTA anticoagulant and non-anticoagulant tube, respectively, followed by centrifugation (604 g) (3-16 L high-speed centrifuge, Sigma-Aldrich (St. Louis, MO,

USA)) at 4°C for 10 min to separate serum and blood cells. Samples were saved in cryogenic refrigerator at -80°C for further usage.

Measurement of liver function indexes

The liver function indexes (alanine aminotransferase [ALT], aspertate Aminotransferase [AST], total bilirubin [TBIL], alkaline phosphatase [ALP], cholinesterase [CHE], albumin [ALB] and total bile acid [TBA]) were measured by Beckman Coulter DxC 800 automatic biochemical analyzer (Brea, California, USA) and the original automatic biochemistry instrument reagent (Synchron CX/LX/UniCel DxC automatic clinical biochemical instrument reagent catalogue is found in http://www.beckmancoulter.cn/diagnostics/chemistry/dxc/test-menu.html). ALT, AST, ALP and CHE were measured by performance rate method with ALT, AST, ALP and CHE kits (Beckman Instruments Inc, Brea, USA). TBIL was measured by diazotization analytical method with TBIL kit (Beckman Instruments Inc, Brea, USA). ALB was measured by bromocresol green method with ALB kit (Beckman Instruments Inc, Brea, USA). TBA was measured by enzyme cycle method with TBA kit (Beckman Instruments Inc, Brea, USA). After testing, two groups of people recorded the test results of their liver function test items, and compared the two groups of data. The data after recording were compiled and sorted. At the same time, the relevant data of two groups of inspected personnel were sorted into corresponding data.

Genomic DNA extraction

A 6 mL erythrocyte lysate (Beijing Solarbio Technology Co., Ltd., Beijing, China) was added into a 10 mL centrifuge tube. The 3 mL anticoagulant which was completely thawing was fully mixed and acted at room temperature for 20 min, with 4~5 times of mixing during the process. Centrifugation at 2415 g (3-16 L high-speed centrifuge, Sigma-Aldrich (St. Louis, MO, USA)) was then performed for 15 min. After discarding the supernatant, samples were then oscillated fully, and the precipitate was re-suspended (If the color was still red, the above operation could be repeated again). With the addition of 3 mL erythrocyte lysis buffer + 50 uL protease K (10 mg/mL) + 60 uL 10% SDS (Beijing Solarbio Technology Co., Ltd., Beijing, China), a water bath was performed at 65°C for 20-30 min, with a number of mixings during the period. Then, 750 uL supersaturated sodium acetate was added, followed by light flick or oscillation to mix well, and centrifugation at 1358 g (3-16 L high-speed centrifuge, Sigma-Aldrich (St. Louis, MO, USA)) for 15 min. Afterwards, the new tube was added with 3.75 mL isopropanol, and the supernatant after centrifugation with the addition of supersaturated sodium acetate was poured into the isopropanol and was gently reversed and mixed. It was visible that the white flocculent DNA was clustered and twisted into a mass. DNA was then aspirated into the 1.5 mL Eppendorf tube, and centrifuged at 11920 g (3-16 L high-speed centrifuge, Sigma-Aldrich (St. Louis, MO, USA)) for 2 min. After discarding the supernatant, 500 μL 70% ethanol was added and gently reversed for many times, and then centrifuged at 11920 g (3-16 L high-speed centrifuge, Sigma-Aldrich (St. Louis, MO, USA)) for 3 min. After discarding the supernatant, repeated washing was performed with ethanol for another two times. EP centrifuge tube was tilted and put on clean absorbent paper to dry. With the addition of 300 μL DNA heavy solution, another water bath was carried out at 65°C for 1 h (or overnight at 4°C) for dissolution.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) used to detect SNP and genotyping of BDNF gene

Human genome databases (http://www.ncbi.nlm.nih.gov, http://www.ncbi.nlm.nih.gov/SNP and http://snp.cshl.org/) were used to determine rs6265 (A/G) and rs10835210 (A/C) in *BDNF* gene. PCR-RFLP was utilized to evaluate the gene polymorphisms of rs6265 and rs10835210. Primers were designed with Primer Premier 5 software, and the primer sequence was synthesized by Shanghai Sangon Biotech Company (Shanghai, China). BDNF-rs6265 primer sequence: forward: 5'-CAAACATCCGAGGACAAGGT-3', reverse: 5'-AGAAGAGGAGGCTCCAAAGG-3', BDNF-rs10835210 primer sequence: forward: 5'-AAACATGTGTGGACCTGCAA-3', reverse: 5'-CCACATGGCTCATTATGCAC-3'. PCR reaction system was 20 μL, including DNA 100 ng, 2.4 μL 10 × PCR buffer solution containing $MgCl_2$ 15 nmol/L, 1.0 UTaq DNA polymerase, 200 μmol/L dNTPs, 1 μL forward and reverse primers, respectively. PCR reaction condition (Bio-Rad PCR instrument, Hercules, California, USA) was as follows: 94°C predenaturation for 5 min, 94°C for 30 s, 58°C for 45 s, and 72°C for 45 s, with a total of 30 cycles, followed by extension at 72°C for 10 min. The PCR products were treated by specific restric-

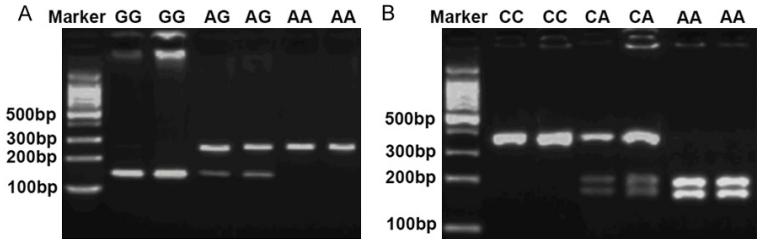

Figure 1. Enzyme electrophoresis of rs6265 (A) and rs10835210 (B) in BDNF gene.

tion endonuclease and were verified by 3% agarose gel electrophoresis (Bio-Rad PCR instrument, Hercules, California, USA). Genotypes were identified by enzyme digestion, namely cut homozygosity, cut heterozygosity, and uncut homozygosity. The rs6265 locus of the *BDNF* gene was a A/G dimorphism, which consisted of three different genotypes of G/G, A/G and A/A in the population. After enzyme digestion of PCR products by Pml I, two 250 bp fragments were produced, indicating that the two alleles were not cut apart, and the genotype of the individual was AA; the length of fragments after enzyme digestion by Pml I was 125 bp and 250 bp, respectively, indicating that only one allele was cut apart, and the genotype of the individual was AG; when the length of fragments after enzyme digestion by Pml I was 125 bp, the two alleles were all cut apart, and the genotype of the individual was GG. Detailed information is shown in **Figure 1A**. The rs10835210 locus was a A/C dimorphism, which consisted of three different genotypes of A/A, A/C and C/C in the population. The length of the fragment of the PCR product was 338 bp after the Bcl I enzyme digestion, indicating that both the two alleles were uncut, and the genotype of the individual was CC; when the length of the fragment was 338 bp, 181 bp and 157 bp after Bcl I enzyme digestion, only one allele was cut apart, and the genotype of the individual was CA. Furthermore, when the length of the fragment was 181 bp and 157 bp after Bcl I enzyme digestion, both the two alleles were suggested to be uncut, and the genotype of the individual was AA, which is shown in **Figure 1B**.

Determination of serum BDNF level

Enzyme linked immunosorbent assay (ELISA) was used to determine the concentration of BDNF in the serum. The ELISA kit was purchased from R&D Biology Inc. (Total BDNF Quantikine ELISA Kit from R&D Systems, Minneapolis, MN, Catalog # DBNT00) and the test was operated in strict accordance with instructions. Concrete steps were showed as follows: The reagents, samples and standard products were prepared. The prepared samples and standard products were added to react for 120 min at 37°C. The plate was washed three times and added with antibody working solution, and reacted for 60 min at 37°C. The plate was washed three times and added with horseradish peroxidase (HRP), and reacted for 30 min at 37°C. The plate was washed three times and added with substrate working solution, and reacted for 5-10 min in dark. After adding stop buffer, BioTek ELx800 full-automatic enzyme labeling system (Vermont, USA) was used to detect optical density (OD) at 450 nm wavelength within 30 min.

Statistical analysis

SPSS21.0 (SPSS, Inc, Chicago, IL, USA) software was involved in all data analysis. Hardy-Weinberg equilibrium was used to test the population representation of genotypic distribution. Relative risk of genotypes was expressed by the odds ratio (OR) and 95% confidence interval (CI). χ^2 test was used to compare allele and genotype frequency. Categorical data were expressed by rate, percentage, or composition ratio, and statistical analysis was carried out by chi-square test. Measurement data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used in multiple groups. Fisher's least significant difference t test (LSD-t) was used for the pairwise comparison after ANOVA analysis. t test was used in the two groups. Haplotype analysis and linkage disequilibrium of *BDNF* gene was achieved by Shesis software. The correlation analysis was carried out by Pearson correlation. $P < 0.05$ meant that a statistical difference was significant.

Results

General data of the case group and control group and the level of serum BDNF

There was no statistically significant difference in age and gender between the two groups

Table 1. Basic information and serum BDNF of the case group and the control group

	Case group (n = 182)	Control group (n = 186)	*P*
Age (Years)	45.52 ± 11.58	43.68 ± 10.54	0.112
Gender (Male/Female)	150/32	142/44	0.159
Serum BDNF (μg/L)	15.68 ± 3.21	23.54 ± 3.58	< 0.001
Alanine aminotransferase (U/L)	128.0 ± 15.8	15.1 ± 2.6	< 0.001
Aspartate aminotransferase (U/L)	77.4 ± 20.1	15.4 ± 2.6	< 0.001
Total bilirubin (μmol/L)	86.4 ± 8.6	11.1 ± 4.1	< 0.001
Alkaline phosphatase (U/L)	181.1 ± 23.4	75.0 ± 10.5	< 0.001
Serum cholinesterase (U/L)	2396 ± 829	8625 ± 899	< 0.001
Albumin (g/L)	31.2 ± 4.4	48.4 ± 4.1	< 0.001
Total bile acid (μmol/L)	47.4 ± 24.9	5.9 ± 2.0	< 0.001

Note: Enumeration data were expressed by rate or percentage or constituent ratio, and chi-square test was used for statistical analysis. The measurement data were mean ± standard deviation and t test was used for comparison between the two groups. BDNF, brain-derived neurotrophic factor.

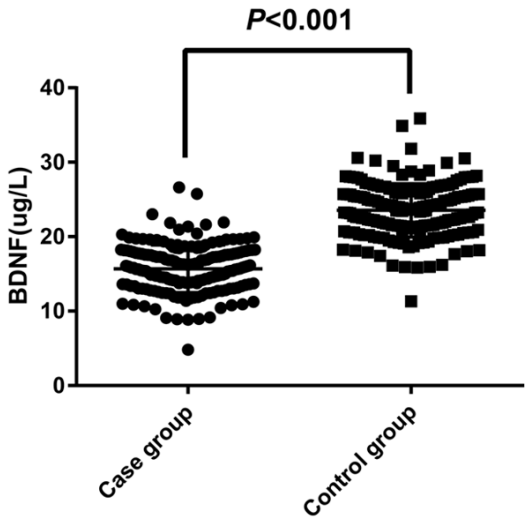

Figure 2. Serum BDNF levels in the case group (n = 182) and the control group (n = 186). Note: The measurement data were mean ± standard deviation, and t test was used for comparison between the two groups. BDNF, brain-derived neurotrophic factor.

(both $P < 0.05$). After comparison, the serum BDNF level of the patients in the case group was significantly lower than that of the control group ($P < 0.05$). In the detection of serum biochemical indexes in patients, the serum cholinesterase activity, albumin, and serum cholesterol were significantly lower in the case group than in the control group (all $P < 0.05$). The alanine aminotransferase, total bilirubin, aspartate aminotransferase, alkaline phosphatase and total bile acid level were significantly higher than those in the control group (all $P < 0.05$), as shown in **Table 1** and **Figure 2** (**Figure 2** shows serum BDNF levels in the case group and the control group only).

Comparison of liver function indexes and serum BDNF in different grades of HBV-related liver cirrhosis

The liver function index and serum BDNF in different grades of HBV-induced liver cirrhosis was analyzed. Compared with patients with grade A cirrhosis, serum cholinesterase activity, albumin, serum cholesterol and serum BDNF were all decreased, and total bile acid level was increased in patients with grade B and C cirrhosis (all $P < 0.05$). Furthermore, compared with patients with grade B cirrhosis, the serum cholinesterase activity, albumin, serum cholesterol, and serum BDNF were all reduced; alanine aminotransferase, total bilirubin, aspartate aminotransferase, alkaline phosphatase, and total bile acid level were increased in patients with C grade cirrhosis (all $P < 0.05$; **Table 2** and **Figure 3**, **Figure 3** shows only serum BDNF levels in patients with different grades of HBV-induced liver cirrhosis). Corresponding results showed that with the elevation of Child-Pugh classification in patients with HBV induced liver cirrhosis, the serum cholinesterase activity, albumin and serum cholesterol were reduced accordingly; meanwhile, the decreased trend of serum BDNF level was even lower, and the increase of alanine aminotransferase, total bilirubin, aspartate aminotransferase, alkaline phosphatase, and total bile acid level was even higher ($r < 0$, $P < 0.05$).

Correlation analysis between serum BDNF level and liver function indexes

To analyze the correlation between the serum BDNF level and the liver function index, the Pearson correlation analysis was used. Corresponding results (**Figure 4**) revealed that in patients with liver cirrhosis, the level of serum BDNF was negatively correlated with alanine

Table 2. Comparison of liver function indexes and serum BDNF in different grades of HBV-induced liver cirrhosis

	Grade A (n = 56)	Grade B (n = 69)	Grade C (n = 57)	P^*	$P^\#$	$P^\&$
Serum BDNF (μg/L)	26.13 ± 3.04	16.87 ± 2.36	7.26 ± 1.63	< 0.001	< 0.001	< 0.001
Alanine aminotransferase (U/L)	119.38 ± 15.37	126.53 ± 11.08	142.19 ± 11.71	0.003	< 0.001	< 0.001
Aspartate aminotransferase (U/L)	68.82 ± 18.16	77.55 ± 21.65	85.64 ± 16.42	0.018	< 0.001	0.022
Total bilirubin (μmol/L)	82.56 ± 7.44	86.05 ± 9.44	90.60 ± 6.62	0.026	< 0.001	0.003
Alkaline phosphatase (U/L)	170.60 ± 19.20	180.91 ± 25.53	191.65 ± 19.83	0.014	< 0.001	0.011
Serum cholinesterase (U/L)	3145 ± 1021	1833 ± 462	1346 ± 363	< 0.001	< 0.001	< 0.001
Albumin (g/L)	42.7 ± 3.3	34.5 ± 2.2	21.2 ± 3.5	< 0.001	< 0.001	< 0.001
Serum cholesterol (mmol/L)	3.9 ± 2.0	3.3 ± 1.4	2.3 ± 0.8	0.044	< 0.001	< 0.001
Total bile acid (μmol/L)	22.5 ± 6.5	41.4 ± 20.9	80.6 ± 40.1	< 0.001	< 0.001	< 0.001

Note: P^* refers to the comparison between grade B and A; $P^\#$ refers to the comparison between grade C and A; $P^\&$ refers to the comparison between grade C and B. The measurement data were mean ± standard deviation and one-way ANOVA was used for comparison among multiple groups. Fisher's least significant difference t test (LSD-t) was used for the pairwise comparison after ANOVA analysis. BDNF, brain-derived neurotrophic factor; HBV, hepatitis B virus; ANOVA, analysis of variance.

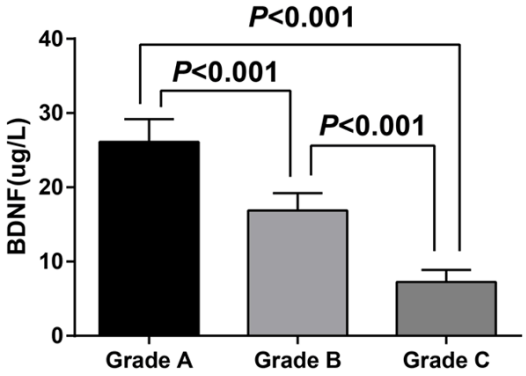

Figure 3. Comparison of serum BDNF in different grades of HBV induced liver cirrhosis. Note: Grade A (n = 56), grade B (n = 69) and grade C (n = 57). The measurement data were mean ± standard deviation and one-way ANOVA was used for comparison among multiple groups. Fisher's least significant difference t test (LSD-t) was used for the pairwise comparison after ANOVA analysis. BDNF, brain-derived neurotrophic factor; HBV, hepatitis B virus; ANOVA, analysis of variance.

aminotransferase (r = -0.610, $P < 0.001$), aspartate aminotransferase (r = -0.716, $P < 0.001$), total bilirubin (r = -0.470, $P < 0.001$), alkaline phosphatase (r = -0.485, $P < 0.001$), and total bile acid (r = -0.565, $P < 0.001$). Meanwhile, there were positive correlations of serum BDNF level with cholinesterase (r = 0.754, $P < 0.001$), albumin (r = 0.558, $P < 0.001$), and cholesterol (r = 0.509, $P < 0.001$).

Polymorphism distribution of BDNF gene loci

Hardy-Weinberg equilibrium was detected using goodness of fit test. The results revealed that the genotype distribution of two loci in the *BDNF* gene was in accordance with Hardy-Weinberg equilibrium ($P > 0.05$), indicating that the sample of this study was representative.

The distribution of genotype frequencies and allele frequencies of *BDNF* gene are shown in **Table 3**. The frequency distribution of GG, GA, and AA genotypes in the rs6265 loci was statistically different in the case group and the control group ($P = 0.003$). Compared with patients carrying GG genotype, the distribution of GA, AA and GA + AA genotypes was higher in the case group than that in the control group (all $P < 0.05$). The risk of HBV-induced liver cirrhosis in patients with GA genotype was about 1.9 times as high as that of the GG genotype carriers (OR = 1.939, 95% CI = 1.176-3.198). The risk of HBV induced liver cirrhosis in patients with AA genotype was about 2.5 times as high as that of the GG genotype carriers (OR = 2.532, 95% CI = 1.431-4.480). Furthermore, the risk of HBV-induced liver cirrhosis in patients with GA + AA genotype was about 2.6 times as high as that of the GG genotype carriers (OR = 2.565, 95% CI = 1.598-4.117). The frequency distribution of G and A alleles was statistically different in the case group and the control group ($P = 0.001$). The A allele might increase the risk of HBV-related cirrhosis (OR = 1.652, 95% CI = 1.234-2.211). However, there was no obvious difference in the comparison of CC, CA, and AA genotypes in the rs10835210 between the case group and the control group ($P = 0.300$). No statistical difference was also found in the C and A alleles between the case group and the control group ($P = 0.359$).

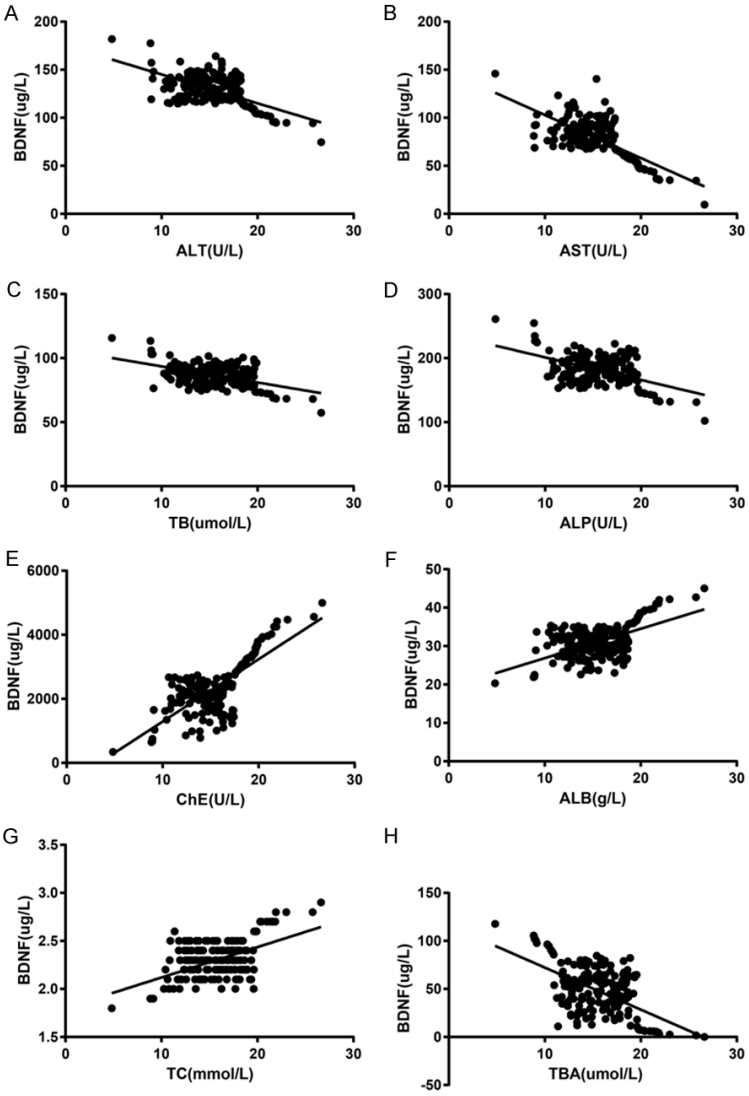

Figure 4. Correlation analysis between serum BDNF level and liver function indexes. Note: A-H referred to the correlation analysis of serum BDNF level with alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, cholinesterase, albumin, cholesterol, and total bile acid. Correlation analysis was carried out by Pearson analysis. ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; ALP, alkaline phosphatase; ChE, cholinesterase; ALB, albumin; TC, cholesterol; TBA, total bile acid.

Haplotype analysis

The linkage disequilibrium (LD) level between rs6265 and rs10835210 of BDNF gene was analyzed by SHEsis software. D is the basic unit of LD, measuring the observed deviation between the haplotype frequency and the expected frequency in the equilibrium state. D' = 0 means that the two loci are completely independent. D' = 1 means the two loci are not recombined and are in complete linkage disequilibrium. The other measure of LD is r^2, which represents the statistical correlation between the two loci. r^2 = 0 means that the two loci are completely independent. If the value of D' and r^2 is 0, the linkage equilibrium is complete, and if the value of D' and r^2 is 1, the linkage is in complete disequilibrium. The results in this study showed that the D' = 0.999 and r^2 = 0.453 between rs6265 and rs10835210 showed strong linkage disequilibrium, indicating that the two SNPs are commonly inherited together, i.e. in linkage disequilibrium with each other.

Using SNPs at rs6265 and rs10835210 of BDNF gene, a haplotype system consisting of four haplotypes was constructed. The distribution of each haplotype in the case group and the control group was analyzed. The haplotype SHEsis software with frequency less than 0.03 was automatically filtered. As shown in **Table 4**, the difference of AC and GC haplotype had statistical difference between the case group and the control group (P < 0.05). Among them, AC haplotype might increase the risk of HBV induced liver cirrhosis (OR = 1.875, 95% CI = 1.281-2.743). GC haplotype might be a protective haplotype of HBV induced liver cirrhosis (OR = 0.605, 95% CI = 0.452-0.810). However, AA haplotype was not associated with the risk of HBV-induced liver cirrhosis (P > 0.05).

Relationship between polymorphism of BDNF gene locus and liver function classification of HBV-induced liver cirrhosis

Table 5 reveals the results of the distribution of rs6265 and rs10835210 in HBV-induced liver cirrhosis patients with different liver function classification. Differences of genotype frequen-

Table 3. Distribution of genotype frequencies and allele frequencies of rs6265 and rs10835210

Genotypes	Control group (n = 186)	Case group (n = 182)	χ^2	OR (95% CI)	*P*
rs6265					
GG	67 (36.02%)	38 (20.88%)		Ref	
GA	80 (43.01%)	88 (48.35%)	6.815	1.939 (1.176-3.198)	0.009
AA	39 (20.97%)	56 (30.77%)	10.37	2.532 (1.431-4.480)	0.001
GA + AA	119 (63.98%)	144 (79.12%)	15.64	2.565 (1.598-4.117)	< 0.001
G	214 (57.52%)	164 (45.05%)			
A	158 (42.48%)	200 (54.95%)	11.46	1.652 (1.234-2.211)	0.001
rs10835210					
CC	95 (51.08%)	80 (43.96%)			
CA	76 (40.86%)	89 (48.90%)	2.298	1.391 (0.9074-2.131)	0.129
AA	15 (8.06%)	13 (7.14%)	0.005	1.029 (0.4623-2.291)	0.944
CA + AA	91 (48.92%)	102 (56.04%)	1.869	1.331 (0.883-2.006)	0.172
C	266 (71.50%)	249 (68.41%)			
A	106 (28.50%)	115 (31.59%)	0.841	1.159 (0.845-1.589)	0.359

Note: Ref, reference. The enumeration data are expressed by rate or percentage or constituent ratio, and chi-square test was used for statistical analysis. Relative risk of genotypes was expressed by odds ratio (OR) and 95% confidence interval (CI).

Table 4. Haplotype analysis of rs6265 and rs10835210 of BDNF gene

	Case group (freq)	Control group (freq)	χ^2	*P*	OR (95% CI)
A A	115.00 (0.316)	106.00 (0.285)	0.841	0.359	1.159 (0.845-1.589)
A C	85.00 (0.234)	52.00 (0.140)	10.671	0.001	1.875 (1.281-2.743)
G C	164.00 (0.451)	214.00 (0.575)	11.456	0.001	0.605 (0.452-0.810)

Note: OR, Odds Ratio; CI, Confidence interval; BDNF, brain-derived neurotrophic factor.

cy distribution of GG, GA, and AA in rs6265 were statistically significant in grade A and B, grade A and C as well as grade B and C liver cirrhosis patients (all $P < 0.05$). The number of grade B and C liver cirrhosis patients with the AG + AA genotype was significantly increased in comparison with the patients with grade A cirrhosis ($P < 0.05$). Meanwhile, there was statistical difference in the comparison of allele frequency distribution of rs6265 G and A alleles in grade A and B, grade A and C as well as grade B and C liver cirrhosis patients (all $P < 0.05$). Grade B and C liver cirrhosis patients with an A allele were significantly more than those with grade A cirrhosis ($P < 0.05$). However, there was no statistical differences in the comparison of genotype and allele frequency distribution of rs10835210 in HBV-induced liver cirrhosis patients with different liver function classification ($P > 0.05$).

Comparison of serum BDNF levels between different genotypes

For the investigation of the relationship between serum BDNF level and genetic polymorphism, we analyzed the serum BDNF levels of BDNF genotype rs6265 and rs10835210 in different cirrhotic patients. The results (**Table 6**) show that there was a significant difference in the level of serum BDNF among GG, AG, and AA genotypes of rs6265 in patients with HBV-induced liver cirrhosis (all $P < 0.05$), and the serum BDNF level of patients with AA genotype was the lowest ($P < 0.05$). The level of serum BDNF in patients with G allele was higher than that of A alleles ($P < 0.05$). Furthermore, no significant difference was found in the level of serum BDNF between patients with CC, CA, and AA genotype at rs10835210 (all $P > 0.05$). In addition, there was no significant difference in the level of serum BDNF in patients with C allele and A allele ($P > 0.05$). The above results indicate that in patients with HBV-induced liver cirrhosis, the BDNF gene rs6265 and rs10835210 polymorphisms were associated with the serum BDNF concentration.

Discussion

Liver cirrhosis is a common disease and main cause of death in China [31]. Liver cirrhosis is the result of abnormal over-deposition of the extracellular matrix of the liver, and its process is extremely complex and is affected by multi-

Table 5. Frequency distribution of gene loci in different grades of HBV-related liver cirrhosis

Genotypes	Grade A (n = 56)	Grade B (n = 69)	Grade C (n = 57)	P^*	$P^\#$	$P^\&$
rs6265						
GG	22 (39.28%)	13 (18.84%)	3 (5.27%)	0.011	< 0.001	0.003
AG	28 (50.00%)	37 (53.62%)	23 (40.35%)			
AA	6 (10.72%)	19 (27.54%)	31 (54.38%)			
AG + AA	34 (60.72%)	56 (81.16%)	54 (94.73%)	0.016	< 0.001	0.023
G	72 (64.28%)	63 (45.65%)	29 (25.44%)	0.004	< 0.001	0.001
A	40 (35.72%)	75 (54.35%)	85 (74.56%)			
rs10835210						
CC	24 (42.86%)	30 (43.48%)	26 (45.61%)	0.929	0.14	0.236
CA	26 (46.43%)	33 (47.83%)	30 (52.63%)			
AA	6 (10.71%)	6 (8.69%)	1 (1.76%)			
CA + AA	32 (57.14%)	39 (56.52%)	31 (54.39%)	0.944	0.85	0.858
C	74 (66.07%)	93 (67.39%)	82 (71.93%)	0.893	0.389	0.493
A	38 (33.93%)	45 (32.61%)	32 (28.07%)			

Note: P^* refers to the comparison between grades B and A; $P^\#$ refers to the comparison between grades C and A; $P^\&$ refers to the comparison between grades C and B. The enumeration data were expressed by rate or percentage or constituent ratio, and chi-square test was used for statistical analysis. HBV, hepatitis B virus.

Table 6. Comparison of serum BDNF levels between different genotypes

Genotypes	Cases	Serum BDNF level	*P*
rs6265			
GG	38 (20.88%)	17.23 ± 2.73	< 0.001
AG	88 (48.35%)	15.94 ± 2.56	
AA	56 (30.77%)	14.85 ± 2.35	
G	164 (45.05%)	16.05 ± 3.02	0.009
A	200 (54.95%)	15.24 ± 2.82	
rs10835210			
CC	80 (43.96%)	16.15 ± 3.27	0.152
CA	89 (48.90%)	15.21 ± 3.15	
AA	13 (7.14%)	15.36 ± 2.63	
C	249 (68.41%)	15.81 ± 3.26	0.114
A	115 (31.59%)	15.24 ± 3.02	

Note: The measurement data are mean ± standard deviation, and one-way ANOVA was used for comparison among multiple groups. Fisher's least significant difference t test (LSD-t) was used for the pairwise comparison after ANOVA analysis, and t test was used in the two groups. BDNF, brain-derived neurotrophic factor; ANOVA, analysis of variance.

ple extrinsic and intrinsic factors [32]. The assessment of liver function in patients with HBV-induced liver cirrhosis, as well as the analysis of the severity of cirrhosis to achieve a clear and reasonable treatment plan has important clinical significance for the prognosis of patients. At present, there are many evaluation indexes and methods of the degree of liver damage and liver reserve function in liver cirrhotic patients, biochemical indexes such as alanine aminotransferase, aspartate aminotransferase, total bilirubin, albumin, cholinesterase, serum lipid, also including Child-Pugh classification [33], the model for end-stage liver disease (MELD) score [34] and MELD-Na score [35].

In the study, for the purpose of exploring the correlation of serum BDNF level with liver function classification in patients with HBV-induced liver cirrhosis, first, ELISA was used to detect serum BDNF levels, combined with the evaluation of other indexes related to the reflection of liver functions. It was found that serum cholinesterase activity, albumin, serum cholesterol, and serum BDNF level were significantly lower in the case group than in the control group, but the alanine aminotransferase, total bilirubin, aspartate aminotransferase, alkaline phosphatase and total bile acid level were higher than those of the latter group. A possible reason for the above results might be as follows. First, the liver plays an important role in the metabolic process of lipid synthesis and transportation [36]. In cirrhosis patients, liver cells undergo degeneration and necrosis, and many enzymes and proteins that liver cells synthesize to regulate lipid metabolism can lead to a decrease of blood lipid level. Secondly, patients with liver cirrhosis may develop malnutrition and hypoproteinemia [37] due to gastrointestinal congestion, malabsorption, and loss of appetite. Thirdly, in patients with liver cirrhosis, liver cell damage, hormone synthesis and decomposition, as well as glucose metabolism are disordered [38]. At the same time, liver cirrhosis cell degeneration, necrosis, and fibrosis result in destroyed normal structure, leading to the restriction of blood supply, and liver func-

tion is further impaired. With the elevation of Child-Pugh classification [39] in patients with HBV-induced liver cirrhosis, the serum cholinesterase activity, albumin, and serum cholesterol were reduced accordingly. The decreased trend of serum BDNF level was even lower, and the increase of alanine aminotransferase, total bilirubin, aspartate aminotransferase, alkaline phosphatase, and total bile acid level was even higher. It was considered that with the increased Child-Pugh score, the injury of liver cells gradually increased, as did the area of hepatic fibrosis.I This induced severe damage of liver cell mitochondria, endoplasmic reticulum, and lysosome organelles, significantly reduced enzyme synthesis and metabolism of blood lipids, and disordered cell synthesis and lipid metabolism [40, 41]. Further correlation analysis suggested that serum BDNF level was negatively correlated with alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, and total bile acid, whereas serum BDNF level was positively correlated with cholinesterase, albumin, and cholesterol.

The relationship between *BDNF* rs6265 and rs10835210 gene polymorphism and different liver function classification in HBV-induced liver cirrhosis has not been reported. Furthermore, in this study, a Chinese population was involved as the patients of the study, and PCR-RFLP [42, 43] was applied to detect the distribution of genotypes and alleles of SNP loci at *BDNF* in the Chinese Han population for the analysis of allele and genotype polymorphism of *BDNF* SNPs. Results of one-site model showed that the frequency distribution of GG, GA, and AA genotypes in the rs6265 loci was statistically different in the case group and the control group. Furthermore, the distribution of GA, AA and GA + AA genotypes was higher in the case group than that in the control group when compared to those carrying GG genotype. The risk of HBV-induced liver cirrhosis in patients with GA and AA genotype was about 1.9 times and 2.5 times as high as that of the GG genotype carriers, respectively. Furthermore, the risk of HBV-induced liver cirrhosis in patients with GA + AA genotype was about 2.6 times as high as that of the GG genotype carriers. It was suggested that this region might be closely linked to the susceptibility genes of liver cirrhosis. The frequency distribution of G and A alleles was statisticallydifferent in the case group and the control group. The A allele might increase the risk of HBV-induced liver cirrhosis. However, there was no obvious difference in the comparison of CC, CA and AA genotypes in the rs10835210 between the case group and the control group. This was speculated to be associated with two major causes. One was the relatively smaller frequency of genotype and alleles at rs10835210 locus, which affected the statistical effectiveness. Secondly, it might be related to the limited sample size of the present study, which remains to be further investigated in relatively larger population.

In the study of susceptibility genes of diseases, SNP is a genetic marker of dimorphism, and its heterozygosity and polymorphic information are relatively low in the population. For liver cirrhosis, a complex disease caused by the interaction between genes and other genes, as well as genes and environment [44-46], the simple analysis of polymorphism of a single locus in a gene cannot accurately draw a true relationship between genes and diseases. In order to make more effective use of genetic information to explore the genetic mechanism of disease, it is necessary to conduct multi-site analysis besides one-site analysis, namely, a haplotype analysis [47, 48].

Linkage disequilibrium test is the basis of haplotype analysis to investigate of the correlation between haplotype and disease incidence in linkage disequilibrium block [49, 50]. To be specific, it is significant to locate the susceptible gene in a linkage disequilibrium block, or to explore the correlation between haplotype and disease incidence among adjacent linkage disequilibrium block [51]. In this study, the Shesis software was used to analyze the degree of linkage disequilibrium between every two SNPs of *BDNF*. In the linkage disequilibrium analysis of rs6265 and rs10835210 in *BDNF* gene, it was found that there was relatively stronger linkage disequilibrium, suggesting the possibility of the performance of haplotype analysis. Therefore, haplotype analysis of rs6265 and rs10835210 in *BDNF* gene was achieved by Shesis software. In the overall samples, there were statistically significant differences of genotype frequency distribution of GG, GA and AA in rs6265 in grade A and B, grade A and C as well as grade B and C liver cirrhosis patients. Also, the number of grade B and C liver cirrhosis patients with the AG + AA genotype was sig-

nificantly increased in comparison with the patients with a grade A cirrhosis; Grade B and C liver cirrhosis patients with A allele were significantly more than those with grade A cirrhosis. However, there was no statistical difference in the comparison of genotype and allele frequency distribution of rs10835210 in HBV-induced liver cirrhosis patients with different liver function classification. In addition, in haplotype analysis, the difference of AC and GC haplotype had statistical significance between the case group and the control group. Among them, AC haplotype might increase the risk of HBV induced liver cirrhosis. GC haplotype might be a protective haplotype in HBV-induced liver cirrhosis.

In sum, the level of serum BDNF and the rs6265 of *BDNF* gene are associated with Child-Pugh classification of liver function in patients with HBV-induced cirrhosis. In addition, serum BDNF plays an important role in the grading and early diagnosis of liver function in patients with liver cirrhosis associated with HBV, and AA genotype at rs6265 locus of BDNF gene is a negative factor for liver cirrhosis. However, the results also need to be tested in different populations to further confirm the relationship with the Child-Pugh classification of liver function in patients with liver cirrhosis. In addition, in view of the relationship between BDNF gene polymorphism and liver function grading in patients with HBV induced liver cirrhosis, it is necessary to further expand the sample size in order to improve the quality of the study. Furthermore, we also need to use gene expression analysis and western blot analysis in order to further explore the relationship between the BDNF gene and liver cirrhosis.

Disclosure of conflict of interest

None.

Abbreviations

BDNF, Brain-derived neurotrophic factor; HBV, hepatitis B virus; PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; ELISA, Enzyme linked immunosorbent assay; HA, Hyaluronic acid; LN, laminin; PLD, proline peptidase.

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References

[1] Gonçalves-Macedo L, Lopes EP, Domingues ALC, Markman B Filho, Mota VG, Luna CF. Schistosomiasis and hepatopulmonary syndrome: the role of concomitant liver cirrhosis. Mem Inst Oswaldo Cruz 2017; 112: 469-473.

[2] Deutsch M, Manolakopoulos S, Andreadis I, Giannaris M, Kontos G, Kranidioti H, Pirounaki M, Koskinas J. Bacterial infections in patients with liver cirrhosis: clinical characteristics and the role of C-reactive protein. Ann Gastroenterol 2018; 31: 77-83.

[3] Paternostro R, Wagner D, Reiberger T, Mandorfer M, Schwarzer R, Ferlitsch M, Trauner M, Peck-Radosavljevic M, Ferlitsch A. Low 25-OH-vitamin D levels reflect hepatic dysfunction and are associated with mortality in patients with liver cirrhosis. Wien Klin Wochenschr 2017; 129: 8-15.

[4] Fialla AD, Thiesson HC, Bie P, Schaffalitzky de Muckadell OB, Krag A. Internal dysregulation of the renin system in patients with stable liver cirrhosis. Scand J Clin Lab Invest 2017; 77: 298-309.

[5] Piotrowski D, Boroń-Kaczmarska A. Boron-Kaczmarska, Bacterial infections and hepatic encephalopathy in liver cirrhosis-prophylaxis and treatment. Adv Med Sci 2017; 62: 345-356.

[6] Liu XE, Desmyter L, Gao CF, Laroy W, Dewaele S, Vanhooren V, Wang L, Zhuang H, Callewaert N, Libert C, Contreras R, Chen CY. N-glycomic changes in hepatocellular carcinoma patients with liver cirrhosis induced by hepatitis B virus. Zhonghua Gan Zang Bing Za Zhi 2008; 16: 74-5.

[7] Krautbauer S, Wiest R, Liebisch G, Buechler C. Associations of systemic sphingolipids with measures of hepatic function in liver cirrhosis are related to cholesterol. Prostaglandins Other Lipid Mediat 2017; 131: 25-32.

[8] Yamada S, Morine Y, Imura S, Ikemoto T, Arakawa Y, Iwahashi S, Saito Y, Yoshikawa M, Teraoku H, Shimada M. Liver regeneration after splenectomy in patients with liver cirrhosis. Hepatol Res 2016; 46: 443-9.

[9] Krautbauer S, Eisinger K, Wiest R, Liebisch G, Buechler C. Systemic saturated lysophosphatidylcholine is associated with hepatic function

in patients with liver cirrhosis. Prostaglandins Other Lipid Mediat 2016; 124: 27-33.
[10] Abbasi A, Bhutto AR, Alam MT, Aurangzaib M, Masroor M. Frequency of hepatic hydrothorax and its association with child pugh class in liver cirrhosis patients. J Coll Physicians Surg Pak 2016; 26: 566-9.
[11] Jia JB, Zhang D, Ludwig JM, Kim HS. Radiofrequency ablation versus resection for hepatocellular carcinoma in patients with Child-Pugh A liver cirrhosis: a meta-analysis. Clin Radiol 2017; 72: 1066-1075.
[12] Genda T, Ichida T, Sakisaka S, Tanaka E, Mochida S, Ueno Y, Inui A, Egawa H, Umeshita K, Furukawa H, Kawasaki S, Inomata Y; Assessment Committee of Indication for Transplantation. Survival in patients with Child-Pugh class C cirrhosis: analysis of the liver transplant registry in Japan. Hepatol Res 2017; 47: 1155-1164.
[13] Popova NK, Ilchibaeva TV, Naumenko VS. Neurotrophic factors (BDNF and GDNF) and the serotonergic system of the brain. Biochemistry (Mosc) 2017; 82: 308-317.
[14] Li XT, Liang Z, Wang TT, Yang JW, Ma W, Deng SK, Wang XB, Dai YF, Guo JH, Li LY. Brain-derived neurotrophic factor promotes growth of neurons and neural stem cells possibly by triggering the phosphoinositide 3-kinase/AKT/glycogen synthase kinase-3beta/beta-catenin pathway. CNS Neurol Disord Drug Targets 2017; 16: 828-836.
[15] Chung CY, Lin MH, Lee IN, Lee TH, Lee MH, Yang JT. Brain-derived neurotrophic factor loaded PS80 PBCA nanocarrier for in vitro neural differentiation of mouse induced pluripotent stem cells. Int J Mol Sci 2017; 18.
[16] Parrini M, Ghezzi D, Deidda G, Medrihan L, Castroflorio E, Alberti M, Baldelli P, Cancedda L, Contestabile A. Aerobic exercise and a BDNF-mimetic therapy rescue learning and memory in a mouse model of Down syndrome. Sci Rep 2017; 7: 16825.
[17] Tan L, Yang H, Pang W, Li H, Liu W, Sun S, Song N, Zhang W, Jiang Y. Investigation on the role of BDNF in the benefits of blueberry extracts for the improvement of learning and memory in Alzheimer's disease mouse model. J Alzheimers Dis 2017; 56: 629-640.
[18] Yang Y, Jing XP, Zhang SP, Gu RX, Tang FX, Wang XL, Xiong Y, Qiu M, Sun XY, Ke D, Wang JZ, Liu R. High dose zinc supplementation induces hippocampal zinc deficiency and memory impairment with inhibition of BDNF signaling. PLoS One 2013; 8: e55384.
[19] Lin CC, Hung YY, Tsai MC, Huang TL. Increased serum brain-derived neurotrophic factor in male schizophrenic patients with metabolic syndrome. Medicine (Baltimore) 2017; 96: e7089.
[20] Rosier MF, Goguel AF, Martin A, Le Paslier D, Couillin P, Houlgatte R, Bernheim A, Auffray C, Devignes MD. A 1.7-Mb YAC contig around the human BDNF gene (11p13): integration of the physical, genetic, and cytogenetic maps in relation to WAGR syndrome. Genomics 1994; 24: 69-77.
[21] Callaghan CK, Rouine J, O'Mara SM. Exercise prevents IFN-alpha-induced mood and cognitive dysfunction and increases BDNF expression in the rat. Physiol Behav 2017; 179: 377-383.
[22] Jiang H, Chen S, Li C, Lu N, Yue Y, Yin Y, Zhang Y, Zhi X, Zhang D, Yuan Y. The serum protein levels of the tPA-BDNF pathway are implicated in depression and antidepressant treatment. Transl Psychiatry 2017; 7: e1079.
[23] Jiang C, Zuo F, Wang Y, Lu H, Yang Q, Wang J. Progesterone changes VEGF and BDNF expression and promotes neurogenesis after ischemic stroke. Mol Neurobiol 2016; [Epub ahead of print]
[24] de Almeida AA, Gomes da Silva S, Lopim GM, Vannucci Campos D, Fernandes J, Cabral FR, Arida RM. Physical exercise alters the activation of downstream proteins related to BDNF-TrkB signaling in male Wistar rats with epilepsy. J Neurosci Res 2018; 96: 911-920.
[25] Xia H, Li Y, Lv X. MicroRNA-107 inhibits tumor growth and metastasis by targeting the BDNF-mediated PI3K/AKT pathway in human non-small lung cancer. Int J Oncol 2016; 49: 1325-33.
[26] Dooley LN, Ganz PA, Cole SW, Crespi CM, Bower JE. Val66Met BDNF polymorphism as a vulnerability factor for inflammation-associated depressive symptoms in women with breast cancer. J Affect Disord 2016; 197: 43-50.
[27] Li W, Zhou N, Yu Q, Li X, Yu Y, Sun S, Kou C, Chen DC, Xiu MH, Kosten TR, Zhang XY. Association of BDNF gene polymorphisms with schizophrenia and clinical symptoms in a Chinese population. Am J Med Genet B Neuropsychiatr Genet 2013; 162B: 538-45.
[28] Cai X, Shi X, Zhang X, Zhang A, Zheng M, Fang Y. The association between brain-derived neurotrophic factor gene polymorphism and migraine: a meta-analysis. J Headache Pain 2017; 18: 13.
[29] Lam CT, Yang ZF, Lau CK, Tam KH, Fan ST, Poon RT. Brain-derived neurotrophic factor promotes tumorigenesis via induction of neovascularization: implication in hepatocellular carcinoma. Clin Cancer Res 2011; 17: 3123-33.
[30] Yang ZF, Ho DW, Lau CK, Tam KH, Lam CT, Yu WC, Poon RT, Fan ST. Significance of the serum brain-derived neurotrophic factor and platelets in hepatocellular carcinoma. Oncol Rep 2006; 16: 1237-43.

[31] Wang SB, Wang JH, Chen J, Giri RK, Chen MH. Natural history of liver cirrhosis in south China based on a large cohort study in one center: a follow-up study for up to 5 years in 920 patients. Chin Med J (Engl) 2012; 125: 2157-62.
[32] Prystupa A, Szpetnar M, Boguszewska-Czubara A, Grzybowski A, Sak J, Załuska W. Activity of MMP1 and MMP13 and amino acid metabolism in patients with alcoholic liver cirrhosis. Med Sci Monit 2015; 21: 1008-14.
[33] Sebastian A, Varghese SM. Fuzzy logic for Child-Pugh classification of patients with cirrhosis of liver. International Conference on Information Science 2017; IEEE: 168-171.
[34] Zhou XD, Chen QF, Sun DQ, Zheng CF, Liang DJ, Zhou J, Wang SJ, Liu WY, Van Poucke S, Wang XD, Shi KQ, Huang WJ, Zheng MH. Remodeling the model for end-stage liver disease for predicting mortality risk in critically ill patients with cirrhosis and acute kidney injury. Hepatol Commun 2017; 1: 748-756.
[35] Li HY. Clinical value of end-stage liver disease model combined with MELD-Na in prevention short-term prognosis in patients with decompensated liver cirrhosis. China Modern Medicine 2016.
[36] Zhang X, Zhang P, Gao J, Huang Q. Autophagy dysregulation caused by ApoM deficiency plays an important role in liver lipid metabolic disorder. Biochem Biophys Res Commun 2018; 495: 2643-2648.
[37] Nishikawa H, Enomoto H, Ishii A, Iwata Y, Miyamoto Y, Ishii N, Yuri Y, Takata R, Hasegawa K, Nakano C, Nishimura T, Yoh K, Aizawa N, Sakai Y, Ikeda N, Takashima T, Iijima H, Nishiguchi S. Prognostic significance of low skeletal muscle mass compared with protein-energy malnutrition in liver cirrhosis. Hepatol Res 2017; 47: 1042-1052.
[38] Nishida T. Diagnosis and clinical implications of diabetes in liver cirrhosis: a focus on the oral glucose tolerance test. J Endocr Soc 2017; 1: 886-896.
[39] Albers I, Hartmann H, Bircher J, Creutzfeldt W. Superiority of the Child-Pugh classification to quantitative liver function tests for assessing prognosis of liver cirrhosis. Scand J Gastroenterol 1989; 24: 269-76.
[40] Kantarcıoğlu M, Demirci H, Avcu F, Karslıoğlu Y, Babayiğit MA, Karaman B, Öztürk K, Gürel H, Akdoğan Kayhan M, Kaçar S, Kubar A, Öksüzoğlu G, Ural AU, Bağcı S. Efficacy of autologous mesenchymal stem cell transplantation in patients with liver cirrhosis. Turk J Gastroenterol 2015; 26: 244-50.
[41] Balakrishnan CH, Rajeev H. Correlation of serum prolactin level to child pugh scoring system in cirrhosis of liver. J Clin Diagn Res 2017; 11: 0C30-0C33.
[42] Sun YL, Lin CS. Establishment and application of a fluorescent polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for identifying porcine, caprine, and bovine meats. J Agric Food Chem 2003; 51: 1771-6.
[43] Suzuki A, Matsumoto Y, Shibuya N, Ryoichi S, Kamata M, Enokido M, Goto K, Otani K. Interaction effect between the BDNF Val66Met polymorphism and parental rearing for interpersonal sensitivity in healthy subjects. Psychiatry Res 2012; 200: 945-8.
[44] Ramos-Lopez O, Martinez-Lopez E, Roman S, Fierro NA, Panduro A. Genetic, metabolic and environmental factors involved in the development of liver cirrhosis in Mexico. World J Gastroenterol 2015; 21: 11552-66.
[45] Ehsani Ardakani MJ, Safaei A, Arefi Oskouie A, Haghparast H, Haghazali M, Mohaghegh Shalmani H, Peyvandi H, Naderi N, Zali MR. Evaluation of liver cirrhosis and hepatocellular carcinoma using Protein-Protein Interaction Networks. Gastroenterol Hepatol Bed Bench 2016; 9 Suppl 1: S14-S22.
[46] Li YL, Zheng MX, Wang G. A personalized approach identifies disturbed pathways and key genes in hepatitis C virus-cirrhosis with hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2016; 20: 4266-4273.
[47] Stram DO. Multi-SNP haplotype analysis methods for association analysis. Methods Mol Biol 2017; 1666: 485-504.
[48] Su ZG, Zhang SZ, Xiao CY, Tong Y. A method of haplotype analysis for multiple single-nucleotide polymorphisms. Yi Chuan Xue Bao 2005; 32: 243-7.
[49] McCaskie PA, Carter KW, McCaskie SR, Palmer LJ. The effect of missing data on linkage disequilibrium mapping and haplotype association analysis in the GAW14 simulated datasets. BMC Genet 2005; 6 Suppl 1: S151.
[50] Pittman AM, Myers AJ, Abou-Sleiman P, Fung HC, Kaleem M, Marlowe L, Duckworth J, Leung D, Williams D, Kilford L, Thomas N, Morris CM, Dickson D, Wood NW, Hardy J, Lees AJ, de Silva R. Linkage disequilibrium fine mapping and haplotype association analysis of the tau gene in progressive supranuclear palsy and corticobasal degeneration. J Med Genet 2005; 42: 837-46.
[51] Mitra N, Ye TZ, Smith A, Chuai S, Kirchhoff T, Peterlongo P, Nafa K, Phillips MS, Offit K, Ellis NA. Localization of cancer susceptibility genes by genome-wide single-nucleotide polymorphism linkage-disequilibrium mapping. Cancer Res 2004; 64: 8116-25.