A Longitudinal Study of the Association between the LEPR Polymorphism and Treatment Response in Patients with Bipolar Disorder

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Abstract: Patients with bipolar disorder (BD) exhibit individual variability in the treatment outcome, and genetic background could contribute to BD itself and the treatment outcome. Leptin levels significantly change in BD patients treated with valproate (VPA), but whether LEPR polymorphisms are associated with treatment response is still unknown. This longitudinal study aimed to investigate the associations between LEPR polymorphisms and VPA treatment response in BD patients who were drug naïve at their first diagnosis of BD. The single-nucleotide polymorphisms (SNPs) of LEPR (rs1137101, rs1137100, rs8179183, and rs12145690) were assayed, and the LEPR polymorphism frequencies of alleles and genotypes were not significantly different between the controls (n = 77) and BD patients (n = 130). In addition, after the 12-week course of VPA treatment in BD patients, the LEPR polymorphisms showed significant effects on changes in disease severity. Moreover, considering the effect of the LEPR haplotype, the frequency of the CAGG haplotype in BD patients was higher than that in the controls (9.3 vs. 2.9%, p = 0.016), and the LEPR CAGG haplotype was associated with a better treatment response than the other haplotypes in BD patients receiving VPA treatment. Therefore, LEPR polymorphisms might serve as mediators involved in the therapeutic action of VPA treatment.

Keywords: bipolar disorder; LEPR; leptin receptor; valproate; single-nucleotide polymorphisms; pharmacogenetics

1. Introduction

Bipolar disorder (BD) is a mental disorder that typically starts in adolescence or early adulthood [1]. The disorder is characterized by the Diagnostic and Statistical Manual of Mental Disorders (DSM) as severe bouts of recurring mania and depression. Mania is defined by a decreased need for sleep, increased hyperactivity and irritability. During depressive episodes, patients face feelings of guilt, hopelessness and suicidal thoughts. The symptom of switching between depression and mania or hypomania is a specific feature of BD [2]. The natural course of BD tends to worsen without treatment, while people with BD can lead healthy and productive lives when the illness is effectively treated. In addition, the life expectancy of individuals with BD is significantly reduced, and poor psychosocial...
functions, such as impaired executive functions and verbal memory function, have been found in BD [3].

Due to the individual variability in the treatment outcome of BD, more mood swings and hospitalizations result in cognitive impairment. Genetic background could contribute to mood disorders and treatment outcomes. Pharmacogenomics/pharmacogenetics is the study of the stratification of the pharmacological response to a drug by a population based on the genetic variation of that population, and applications of pharmacogenomics/pharmacogenetics include accurately predicting the response to treatment or medication-related adverse effects [4]. It can also provide some new insights into the mechanisms of drugs and the development of new therapeutic agents [5]. With construction for the entire human genome based on high-throughput single-nucleotide polymorphisms (SNPs), the detection of specific DNA sequences affecting responses to drugs can now be made possible [4,6]. In addition, C. Li reported that if a drug matches one of the following characteristics, it can be applied in pharmacogenomics/pharmacogenetics: (1) variation in drug response, (2) narrow therapeutic indices, (3) severe idiosyncratic adverse reactions, and (4) increased incidence of treatment-related complications [7]. Valproate (VPA) users in BD apparently match the first criterion. Important components of the individual variability in treatment outcome, as well as treatment outcome among BD patients treated with mood stabilizers, such as VPA, may have a basis in common genetic variation that can be revealed directly through genetic association methods [8–10]. Thus, we expect to determine the genetic factors implicated in mechanisms of therapeutic action and predict the treatment outcome in BD patients receiving VPA through a pharmacogenetics approach. Among mood stabilizers, VPA is usually widely used in treating BD at different stages [11] and the most prescribed drug among patients newly diagnosed with BD in Taiwan [12]. In addition, VPA demonstrates neuroprotective effects [13–15]. Thus, we focused on the treatment response of VPA in the current study. As a result, pharmacogenetics studies in BD patients receiving VPA are warranted.

Leptin is an essential pleiotropic hormone released by white adipose tissue, and the leptin–leptin receptor responds to changes in energy intake and regulates energy homeostasis [16,17]. Not only does leptin have a role in energy intake and expenditure, but leptin–leptin receptors also play critical roles in many other neurocognitive processes and interact with various other hormones and neurotransmitters in the CNS [18–20], especially in BD patients [21]. Interestingly, our previous studies, similar to other reports, have demonstrated that leptin levels were associated with BD and significantly increased after mood stabilizers treatment [22–25]. Leptin levels were significantly higher in BD patients treated with VPA than in healthy controls or in remitted BD patients. Leptin signaling depends on leptin receptor function, and variation in the leptin receptor (LEPR) gene directly influences receptor function. In addition, a recent study indicated an association of LEPR gene polymorphisms with weight gain in epilepsy patients receiving VPA [25]. However, whether LEPR polymorphisms correspond to treatment response in BD patients treated with VPA is still unknown. To clarify the role of LEPR polymorphisms in BD patients, we aimed to investigate the associations of LEPR polymorphisms between BD patients and controls. Furthermore, we aimed to investigate whether the LEPR polymorphism has an effect on treatment response in BD patients receiving VPA through this 12-week follow-up longitudinal study. We also examined whether the haplotype frequencies of LEPR were associated with BD itself and VPA treatment response.

2. Results

We recruited 130 BD patients and 77 controls, and the demographic data are shown in Table 1. There were no significant differences between BD patients and controls, except for the HAMD and YMRS scores. The mean concentration of VPA was 80.6 ± 18.0 mg/L in BD patients. In addition, the LEPR polymorphism frequencies of alleles and genotypes were not significantly different between the controls and BD patients (Supplementary Table S1). The genotype frequencies of LEPR polymorphisms in the current study did not depart
significantly from the Hardy–Weinberg equilibrium in the patients with BD or in the controls. In investigating the demographic characteristics of LEPR polymorphisms on HAMD and YMRS scores, we found no significant differences between LEPR polymorphisms and disease severity in BD patients, except for LEPR rs1137100 (Table 2). BD patients with the AA + AG genotype of LEPR rs1137100 had higher YMRS scores than those with the GG genotype (10.4 ± 4.1 vs. 8.8 ± 3.9, p = 0.030).

Table 1. Demographic characteristics and measurements between controls and BD patients.

|                          | Controls (n = 77) | BD Patients (n = 130) | BD Patients after Treatment | 95% CI a | t a/χ² | p Value a | 95% CI b | t b | p Value b |
|--------------------------|------------------|-----------------------|-----------------------------|---------|--------|-----------|---------|-----|-----------|
| Age (y) (Range, min–max) |                  | 31.0 ± 10.7 (19–59)   | 32.1 ± 11.6 (18–64)         |         | −2.4  | 0.069    | 0.491   | −   | −         |
| Gender, female (%)       |                  | 43 (55.8)             | 66 (50.8)                   | 0.1–0.2 | 0.564  | 0.453    |        | −   | −         |
| HAMD scores              |                  | 3.2 ± 1.6             | 19.4 ± 5.5                  |         | −      | −         | −       | −   | −         |
| YMRS scores              |                  | 4.1 ± 0.4             | 9.2 ± 4.0                   |         | −      | −         | −       | −   | −         |

Abbreviations: YMRS, 11-item Young Mania Rating Scale; HAMD, 17-item Hamilton Depression Rating Scale.

After the 12-week course of VPA treatment, all of the BD patients exhibited significant reductions in the YMRS and HDRS scores (Table 1). Figure 1 shows the effects of LEPR polymorphisms on changes in disease severity in BD patients during the 12-week treatment course. For LEPR rs1137100, there were no effects on HAMD scores, while BD patients with the AA + AG genotype had a higher change in YMRS scores during the treatment course (p = 0.010). For LEPR rs1137101, there were no effects on the HAMD and YMRS scores. For LEPR rs12145690, there were no effects on the HAMD scores, while BD patients with the CC genotype had a higher change in YMRS scores during the treatment course.
For LEPR rs8179183, BD patients with the GG genotype had a higher change in HAMD scores during the treatment course \((p = 0.037)\), while there were no effects on the YMRS scores.

**Figure 1.** Effects of LEPR polymorphisms on changes in disease severity in BD patients during the 12-week VPA treatment course. Plots show the effect of LEPR polymorphisms (a) LEPR rs1137100, (b) LEPR rs1137101, and (c) LEPR rs12145690 and LEPR rs8179183 on the changes in disease severity (I) \(\Delta\)HDRS scores and (II) \(\Delta\)YMRS scores. (a) (I) The change in HAMD scores significantly decreased over time \((p = 0.004)\), but no effects of rs1137100 on the HAMD scores were found. (a) (II) The change in YMRS scores decreased significantly over time \((p = 0.011)\), and allele carriers of LEPR rs1137100 had a higher change in YMRS scores during the treatment course \((p = 0.010)\). (b) (I) The change in HAMD scores decreased significantly over time \((p = 0.004)\), but no effects of rs1137101 on the HAMD scores were found. (b) (II) The change in YMRS scores decreased significantly over time \((p = 0.011)\), but no effects of rs1137101 on the YMRS scores were found. (c) (I) The change in HAMD scores decreased significantly over time \((p = 0.003)\), but no effects of rs12145690 on the HAMD scores were found. (c) (II) The change in YMRS scores was not different between different genotypes during the treatment course. Patients with the CC genotype had a higher change in YMRS scores during the treatment course \((p = 0.030)\). (d) (I) The change in HAMD scores decreased over time; BD patients with the GG genotype of LEPR rs8179183 had a higher change in HAMD scores during the treatment course \((p = 0.037)\). (d) (II) The change in YMRS scores decreased significantly over time, but no effects of rs8179183 on the YMRS scores were found. Abbreviations: YMRS, 11−item Young Mania Rating Scale; HAMD, 17−item Hamilton Depression Rating Scale; BD, bipolar disorder.

To determine the effect of LEPR polymorphisms on BD and its treatment response, we further performed a haplotype analysis. Table 3 shows that the haplotype frequencies of the four polymorphisms in the LEPR gene between BD patients and controls and the frequency of the LEPR CAGG haplotype (order: rs12145690, rs1137100, rs1137101, and rs8179183) were significantly different between BD and controls \((9.3\% \text{ vs. } 2.9\%, p = 0.016)\). Furthermore, we found that the LEPR CAGG haplotype could not only differentiate remitters from nonremitters using HAMD scores but also responders and nonresponders using YMRS scores (Tables 4 and 5). BD patients with the LEPR CAGG haplotype had more remitters \((13.9\% \text{ vs. } 7.3\%, p = 0.038)\) and responders \((16.6\% \text{ vs. } 6.7\%, p = 0.013)\) than those with other haplotypes.
Table 3. Haplotype frequencies of the 4 polymorphisms in the LEPR gene in BD patients and healthy controls.

| Haplotype * | BD Patients | Healthy Controls | $X^2$ | $p$  | Odds Ratio | 95% CI |
|-------------|-------------|------------------|-------|------|------------|--------|
| 1           | CGGG        | 69.9             | 68.1  | >0.001 | 0.995      | 1.0    | 0.6–1.6 |
| 2           | AAGG        | 7.4              | 11.2  | 1.964  | 0.161      | 0.6    | 0.3–1.2 |
| 3           | CAGG        | 9.3              | 2.9   | 5.756  | 0.016 *    | 3.3    | 1.2–9.2 |
| 4           | CAAG        | 6.1              | 7.6   | 0.429  | 0.512      | 0.8    | 0.4–1.7 |
| 5           | CGAG        | 2.5              | 1.5   | 0.378  | 0.539      | 1.6    | 0.4–7.2 |
| 6           | AAAC        | 1.4              | 1.8   | 0.129  | 0.720      | 0.8    | 0.2–3.6 |
| 7           | AAGG        | 0.9              | 1.8   | 0.688  | 0.407      | 0.5    | 0.1–2.7 |
| 8           | CGGC        | 1.3              | 1.2   | >0.001 | 0.985      | 1.0    | 0.2–6.3 |

The SNP order in each haplotype: 1: rs12145690 (A/C); 2: rs1137100 (A/G); 3: rs1137101 (A/G); 4: rs8179183 (C/G). The comparison of frequencies between BD patients and controls was performed using a chi-square test. * Frequency threshold was set to >1%. * $p < 0.05.$

Table 4. Haplotype frequencies of the 4 polymorphisms in the LEPR gene in HAMD remitters and nonremitters.

| Haplotype * | HAMD Remitter (%) | HAMD Nonremitter (%) | $X^2$ | $p$  | Odds Ratio | 95% CI |
|-------------|-------------------|----------------------|-------|------|------------|--------|
| 1           | CGGG              | 69.6                 | 69.4  | 0.009 | 0.923      | 1.0    | 0.6–1.7 |
| 2           | CAGG              | 13.9                 | 7.3   | 2.912 | 0.038 *    | 2.0    | 1.9–4.6 |
| 3           | AAGG              | 7.1                  | 8.1   | 0.103 | 0.748      | 0.9    | 0.3–2.2 |
| 4           | CAAG              | 5.2                  | 6.3   | 0.163 | 0.687      | 0.8    | 0.3–2.4 |
| 5           | CGAG              | 2.2                  | 2.8   | 0.093 | 0.761      | 0.8    | 0.2–3.9 |
| 6           | AAAC              | 0                    | 2.5   | 2.536 | 0.111      | 0      | –       |
| 7           | CGGC              | 1                    | 1.5   | 0.130 | 0.718      | 0.7    | 0.1–6.8 |

The SNP order in each haplotype: 1: rs12145690 (A/C); 2: rs1137100 (A/G); 3: rs1137101 (A/G); 4: rs8179183 (C/G). The comparison of frequencies between remitters and nonremitters was performed using a chi-square test. * Frequency threshold: >1%. * $p < 0.05.$

Table 5. Haplotype frequencies of the 4 polymorphisms in the LEPR gene in YMRS responders and nonresponders.

| Haplotype * | YMRS Responder (%) | YMRS Nonresponder (%) | $X^2$ | $p$  | Odds Ratio | 95% CI |
|-------------|---------------------|-----------------------|-------|------|------------|--------|
| 1           | CGGG                | 63.3                  | 72.3  | 2.283 | 0.131      | 0.6    | 0.4–1.1 |
| 2           | CAGG                | 16.6                  | 6.7   | 6.108 | 0.013 *    | 2.8    | 1.2–6.3 |
| 3           | AAGG                | 7.1                   | 8.0   | 0.074 | 0.786      | 0.9    | 0.3–2.4 |
| 4           | CAAG                | 6.4                   | 5.7   | 0.044 | 0.834      | 1.1    | 0.4–3.4 |
| 5           | CGAG                | 2.7                   | 2.5   | 0.008 | 0.929      | 1.1    | 0.2–5.5 |
| 6           | AAAC                | 1.2                   | 1.7   | 0.085 | 0.771      | 0.7    | 0.1–7.1 |
| 7           | CGGC                | 1.2                   | 1.3   | 0.006 | 0.937      | 0.9    | 0.1–9.5 |

The SNP order in each haplotype: 1: rs12145690 (A/C); 2: rs1137100 (A/G); 3: rs1137101 (A/G); 4: rs8179183 (C/G). The comparison of frequencies between responders and nonresponders was performed using a chi-square test. * Frequency threshold was set to >1%. * $p < 0.05.$

3. Discussion

To our knowledge, this is the first longitudinal study to report associations between LEPR polymorphisms and VPA treatment response in BD patients who were drug naïve at their first diagnosis of BD. In the current study, the LEPR frequencies of alleles and genotypes were not significantly different between the controls and BD patients, and LEPR polymorphisms and disease severity were not different in BD patients when they were diagnosed with BD at baseline, except LEPR rs1137100. During the 12-week VPA treatment period, LEPR polymorphisms showed significant effects on changes in disease severity in BD patients. In addition, considering the effect of the LEPR haplotype on BD and its treatment response, we found that the frequency of the CAGG haplotype in BD patients was higher than that in the controls. Moreover, the LEPR CAGG haplotype was associated with a better treatment response than the other haplotypes in BD patients receiving VPA.
treatment. Therefore, the results of the current study suggested that the polymorphisms of LEPR, especially the CAGG haplotype, might serve as mediators involved in the therapeutic action of VPA treatment.

Leptin is an appetite-regulating hormone produced in white adipose tissue that acts on the hypothalamus to suppress food intake and increase energy expenditure, and it comprises 167 amino acids [17,26]. Leptin influences glucose homeostasis by direct action on the liver and muscle and pancreas β cells [27]. Leptin decreases glucose and insulin levels by enhancing peripheral glucose uptake and insulin sensitivity [28]. In addition, studies in humans have provided evidence about the role of leptin in mood disorders [29,30]. Lower plasma leptin levels were observed in patients with bipolar disorder [31]. However, our previous study found that bipolar patients who received valproate had increased peripheral leptin levels [22], and VPA has been confirmed to stimulate appetite and decrease thermogenesis in the hypothalamus, resulting in weight gain and metabolic syndrome [32]. Overall, leptin was found to be another common mediator regulating mood disorder and metabolic homeostasis [29]. In our study, although we did not investigate whether leptin levels were associated with BD itself or with metabolic indices in BD patients, we found that LEPR polymorphisms influence the individual treatment response. The relevant mechanisms of leptin and LEPR in BD need to be clarified.

Leptin exerts its function by binding to leptin receptors [17]. The leptin receptor is a member of the cytokine receptor superfamily, it is a single transmembrane protein, and its different isoforms are distributed in different tissues. For example, Ob-Rb, which is a biologically active isoform of the leptin receptor, is primarily expressed in the hypothalamus and is also found in tissues and cells that regulate glucose homeostasis, such as pancreatic β cells [33]. Leptin receptors are encoded by the LEPR gene [27]. LEPR is located on chromosome 1p31, and it includes more than 70 thousand base pairs and consists of 20 exons. According to previous studies, several polymorphisms of the LEPR gene, such as Arg109Lys (rs1137100), Gln223Arg (rs1137101) and Asn656Lys (rs8179183), were reported to be associated with type 2 diabetes [34]. On the other hand, rs1137101 was associated with weight gain in epilepsy patients receiving VPA [25]. Furthermore, a study reported that the polymorphism of the LEPR gene was associated with resistance to antidepressant treatment in MDD patients [35]. Therefore, we selected four LEPR SNPs (rs1137100, rs1137101, rs8179183, and rs12145690) and investigated the associations between the polymorphisms and treatment outcome in BD patients during the 12-week valproate treatment course. Our results indicated that polymorphisms of the LEPR gene were associated with treatment response in patients receiving VPA, and we would like to further investigate the possible therapeutic mechanism of the LEPR gene as leptin and leptin receptors might be involved in the pathophysiology of BD. Moreover, histone deacetylase (HDAC) is known to be a direct target of VPA, and recent studies have suggested that the modulation of G-protein signaling by VPA might occur through its effects on HDAC and gene transcription [36–38]. HDAC inhibitors have been noted to play roles in glucose homeostasis and metabolic regulation [39,40]. Therefore, it is important to know whether the therapeutic mechanism of VPA is correlated with the effect on leptin regulation through HDAC in BD.

In addition to various individual differences in treatment response in BD, previous studies have shown that patients with BD are prone to metabolic syndrome, which may increase the risk of cardiovascular disease and diabetes mellitus. The comorbidity of BD and metabolic syndrome has been associated with a worse disease course [41]. Previously, a review summarized the prevalence of MS in BD patients from different countries, and the prevalence rate ranged from 16% to 60% [22,42]. Therefore, investigating the causal relationship between BD and metabolic syndrome is an important issue. Previous studies have noted that some factors may increase the risk of bipolar patients developing metabolic syndrome. These factors include increased proinflammatory cytokine production [43] and psychiatric medications, such as mood stabilizers, valproate (VPA), and antipsychotics [23,44]. Genetic effects [45,46], such as GNB3, and environmental factors, such as smoking and education level, may contribute to the risk for metabolic syndrome.
Another common mediator of energy balance and mood symptoms might be leptin, as leptin and leptin receptors play roles in obesity [26]. Here, we demonstrated that polymorphisms of LEPR play roles in changes in disease severity during treatment response. Furthermore, it is interesting to investigate whether genetic biomarkers could predict treatment-related metabolic syndrome in BD patients and provide intervention early. Whether LEPR polymorphisms are also associated with metabolic syndrome in BD patients receiving VPA remains to be further investigated.

Although the data were presented carefully, this study has certain limitations. The first limitation is the relatively small sample size of BD patients and controls from a single site. Although there was a small sample size, the frequencies of the LEPR polymorphisms in controls were consistent with those presented in the Ensembl project for participants of Asian ethnicity (Supplementary Table S1). The second limitation was that the study period was short, 12 weeks of observation after the initiation of VPA treatment. The third limitation was the lack of controlling for some confounding factors, such as diet, smoking, drinking, exercise, socioeconomic status and general health status, which might influence treatment response in BD. The fourth limitation was that all BD patients were drug naïve and at their first diagnosis of BD, criteria aimed at decreasing the bias and confounding factors. Thus, the current results might not be extrapolated to other BD patients. Further study is needed to clarify whether LEPR polymorphisms could be a useful clinical indicator for monitoring treatment response and even metabolic disturbance in BD patients.

In conclusion, the present study showed that BD patients exhibited significant reductions in the YMRS and HDRS scores, while LEPR polymorphisms showed significant effects on changes in disease severity in BD patients during the VPA treatment course. Regarding the LEPR haplotype, the CAGG haplotype could be associated with BD itself and with VPA treatment response. This finding might help to develop tailored individual therapies for BD patients to optimize VPA treatment, as BD is a severe chronic psychiatric disease that requires maintenance therapy with mood stabilizers. Previously, most pharmacogenetic studies of BD have focused on the response to lithium, and few studies have focused on VPA, especially in BD patients who are drug naïve [47,48]. Therefore, our study demonstrated that the pharmacogenetics approach can enable clinical utility. Our long-term goal is to establish personalized treatment programs for patients with BD by leveraging a pharmacogenetics approach.

4. Materials and Methods

4.1. Subjects

The Institutional Review Board for the Protection of Human Subjects at National Cheng Kung University Hospital (NCKUH) approved the research protocol (IRB No. A-ER-104-031). All participants (18 to 65 years old) recruited consecutively (from March 2015 to August 2019) from outpatient settings at the National Cheng Kung University Hospital and provided written informed consent regarding their willingness to participate in the research. In addition, to avoid possible confounding factors, we also recruited controls from the community after individuals with mental illnesses were excluded by a senior psychiatrist using the Chinese version of the Mini International Neuropsychiatry Interview. All of the BD patients were initially evaluated in an interview by an attending psychiatrist using the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia–Life Time, which has good interrater reliability, to determine the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition diagnoses (DSM-5). The same rater conducted the initial and subsequent ratings for each patient and was blinded to the subject’s genotype. The patients also met the following inclusion criteria: (i) diagnosis of BD II using the 2-day minimum for hypomania [49,50], (ii) major depressive status at the time of study entry, with a 17-item Hamilton Depression Rating Scale (HDRS) score > 15, and (iii) drug-naïve with a diagnosis of BD for the first time, with no history of mood stabilizer treatment. The exclusion criteria included (i) the presence of other major psychiatric illnesses (such as schizophrenia, BD I), (ii) a history of substance or alcohol abuse or dependence, (iii) severe...
physical illness, (iv) previous or ongoing treatment for metabolic disturbance, and (v) a history of psychotropic agent use. To minimize the effect of ethnic bias, we recruited only Taiwanese participants who were unrelated.

After enrollment in this study, the patients received VPA 500 to 1500 mg orally per day for 12 weeks. Serum trough concentration of VPA was assessed by the homogeneous enzyme immunoassay method at the laboratory of the Pathology Research Center of NCKUH [51]. Concomitant fluoxetine (20 mg/day) was permitted to treat depressive symptoms, and lorazepam (<8 mg) was used for night-time sedation and to treat agitation and insomnia during the study, the dosage of which was adjusted according to the clinical manifestation and the patient’s tolerance. To avoid valproate use in pregnant women, subjects meeting the following criteria are not prescribed to receive valproate treatment according to the guidance Taiwan Food and Drug Administration: (i) during pregnancy for the treatment of bipolar disorder, and (ii) women of childbearing potential unless they have birth control (contraception). The mood of each patient was evaluated using the HDRS and the 11-item Young Mania Rating Scale (YMRS) at baseline and 12 weeks after the initiation of treatment. Predefined cutoffs for remission were a HAMD or YMRS score ≤ 7 [52,53], and those for response were the percentage change in HAMD score (defined as ΔHAMD score %) or percentage change in YMRS score (defined as ΔYMRS score %) ≤ −50%.

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\begin{align*}
\Delta \text{HAMD score} % &= \frac{(\text{HAMD score at 12 weeks}) - (\text{HAMD score at baseline})}{\text{HAMD score at baseline}} \\
\Delta \text{YMRS score} % &= \frac{(\text{YMRS score at 12 weeks}) - (\text{YMRS score at baseline})}{\text{YMRS score at baseline}}
\end{align*}
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4.2. Genotyping

Ten milliliters of whole blood were withdrawn from the antecubital vein of each participant using a heparin tube. A buffy coat was isolated from plasma and red blood cells after centrifugation at 3000 × g for 15 min at 4 °C and immediately stored at −80 °C. Genomic DNA was extracted from each buffy coat sample using a QIAamp DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The quality of the extracted genomic DNA was checked by agarose gel electrophoresis analysis. The DNA was stored at −80 °C until use. The SNPs of the LEPR gene (rs1137101, rs1137100, rs8179183, and rs12145690) were chosen based on literature reports [25,54–56] and were analyzed using commercially available TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. Amplification and dissociation were conducted using an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The PCR system automatically calculated the negative derivative of the change in fluorescence. The SNP genotype of each tested sample was determined by a software program and confirmed manually. In case of disagreement, the analysis was repeated.

4.3. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 19.0 (SPSS Inc., Chicago, IL, USA). Categorical variables are expressed as numbers and percentages, and continuous variables are expressed as the means ± standard deviations (SDs) unless otherwise specified. Categorical variables were assessed using chi-square tests, and continuous variables were assessed using t tests. In addition, intent-to-treat analyses using the mixed-effects model for repeated measures were used to analyze the baseline disease severity as the primary outcome at baseline and after VPA treatment. Data were analyzed using the last observation carried forward (LOCF) method, in which the last observation was entered for missing visits [57]. We further investigated the effects of the polymorphisms of LEPR on the changes in disease severity in BD patients. The observed and expected genotype frequencies were compared using a chi-square goodness-of-fit test to ensure that the loci did not depart from the Hardy–Weinberg equilibrium. Genotype and allele frequencies in different groups were compared through chi-square tests. Dominant models were used to compare the differences in disease severity, which were calculated...
by using Student’s t test. In addition, we investigated whether the LEPR polymorphism had an effect on treatment outcomes during the 12-week VPA treatment course. We used repeated-measures ANOVA with the four visit values of HAMD and YMRS as dependent variables, while the intervention and the LEPR polymorphism × intervention interaction were independent variables. Haplotype frequencies and linkage disequilibrium coefficients (D’) were assessed by the expectation-maximization (EM) algorithm using SNP chi-square tests. The odds ratio and its 95% confidence interval between groups were calculated. The level of significance was set at 0.05 for two-sided tests.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23179635/s1.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board for the Protection of Human Subjects at National Cheng Kung University Hospital (IRB No. A-ER-104-031 and date of approval 7 April 2015).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data that support the findings of this study are available from National Cheng Kung University. Restrictions apply to the availability of these data, which were used under license for this study. The data presented in this study are available on request from the corresponding author with the permission of National Cheng Kung University.

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