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Research paper

Decreased plasma levels of neureglin-1 in drug naïve patients and chronic patients with schizophrenia

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HIGHLIGHTS

- A specific decrease of NRG1β1 levels was found in patients with schizophrenia.
- Antipsychotics did not affect expression of NRG1β1 levels.
- NRG1 may serves as a specific disease marker for schizophrenia.

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ABSTRACT

Although the neuregulin-1 (NRG1) gene is one of the susceptibility genes for schizophrenia and various other psychiatric diseases, it remains unclear how individual psychiatric diseases affect the expression of the NRG1 protein in patients. A previous study reported a schizophrenia-linked decrease in serum NRG1 levels. The present study aimed to replicate this initial finding and to assess its disease specificity for schizophrenia. We collected plasma samples from drug-naïve patients with first-episode schizophrenia ($n = 80$), patients with chronic schizophrenia ($n = 86$), patients with bipolar I disorder ($n = 60$), patients with bipolar II disorder ($n = 60$) and patients with major depressive disorder ($n = 60$), we measured the plasma levels of NRG1β1 and compared the levels with those of age- and sex-matched healthy volunteers ($n = 82$). One-way ANOVA and post hoc analyses detected specific NRG1β1 decreases in the participants with first-episode and chronic schizophrenia but not in those with bipolar I disorder, bipolar II disorder or major depressive disorder. The mean plasma levels of NRG1β1 immunoreactivity were 4.27 ± 0.71 ng/mL in the participants with first-episode schizophrenia, 4.08 ± 0.64 ng/mL in the participants with chronic schizophrenia and 7.21 ± 0.91 ng/mL in the healthy controls. Although we analyzed the pathological correlations of NRG1β1 immunoreactivity in terms of the clinical parameters of the sample, we observed only weak positive correlations with the age of the participants with chronic schizophrenia and the disease onset times of the participants with bipolar II disorder. We failed to identify correlations between other clinical parameters and plasma NRG1β1 immunoreactivity among all patient subjects. These findings suggest that NRG1 may serve as a relatively specific disease marker for schizophrenia. However, the pathological role of this decrease must be explored further.

1. Introduction

Schizophrenia, bipolar disorder and major depressive disorder are three of the most common and severe psychiatric disorders [1] and are complex illnesses that are induced by both genes and the environment [2]. These psychiatric disorders have similar clinical phenotypes and genetic risk factors [1,3–5]. Numerous genes are associated with these conditions [6–8]. Among these genes, neuregulin-1 (NRG1) was first to be identified as a risk factor for schizophrenia [9]. Previously studies have also reported that

Abbreviations: NRG, neuregulin; ELISA, enzyme-linked immunosorbent assay; FESZ, drug-naïve patients with first-episode schizophrenia; CSZ, chronic schizophrenia; BPI, bipolar I disorder; BPII, bipolar II disorder; MDD, major depressive disorder; ANOVA, analysis of variance; ANCOVA, analysis of covariance.

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NRG1 is linked to bipolar disorder and major depressive disorder in different countries and populations, although this evidence is far from convincing [10–12].

NRG1 is thought to encode over 30 proteins via interactions with ErbB receptors, and these proteins have many functions in the central and peripheral nervous systems (CNS and PNS, respectively) [13]. NRG1 mRNA levels are altered in patients with schizophrenia; specifically, the mRNAs that encode type I NRG1 splice variants are up-regulated in the hippocampus and prefrontal cortex [14,15]. Certain studies have found significant correlations between NRG1 mRNA levels and single nucleotide polymorphisms (SNPs; [15]; however, mRNA analyses that use postmortem samples are controversial [16–19]. Measurements of the expressions of mRNA and protein levels in the peripheral blood are usually used to diagnose psychiatric diseases [16,20,21]. Among the molecules examined in the CNS and PNS, cytokines have been found to exhibit prominent alterations. NRG1 mRNA expression levels exhibit marked reductions in the peripheral lymphocytes of patients with schizophrenia [21]. However, few studies have detected NRG1 protein levels in the peripheral blood of bipolar disorder or major depressive disorder patients.

A previous study found a schizophrenia-linked decrease in serum NRG1 levels. The present study used an enzyme-linked immunosorbent assay (ELISA) to measure the levels of plasma NRG1β1 in drug-naïve participants with first-episode schizophrenia, participants with chronic schizophrenia, participants with bipolar I disorder, participants with bipolar II disorder and participants with major depressive disorder. We sought to replicate our previous findings and to determine the disease specificity of the plasma NRG1 level as a disease marker for schizophrenia.

2. Methods and materials

2.1. Participants and samples

The participants included 86 patients with chronic schizophrenia between the ages of 18 and 40 years. 82 healthy controls, 80 drug-naïve patients with first-episode schizophrenia, 60 patients with bipolar I disorder, 60 patients with bipolar II disorder and 60 patients with major depressive disorder who matched the chronic schizophrenia group as well as possible in terms of age and sex were also included. The patients who met the *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition (DSM-V) criteria for schizophrenia, bipolar I disorder, bipolar II disorder and major depressive disorder were recruited from among the in and out patients of the psychiatric department of the First Hospital of Hebei Medical University. A total of 82 healthy volunteers were recruited from the staff or students of the same hospital as controls. All control participants were assessed via a structured psychiatric interview (SCID). Healthy controls and patient with malignant tumors, autoimmune disorders, family histories, nerve–muscle coupling disorders and pregnancy were excluded.

Blood samples were collected in vacuum collection tubes with EDTA (Insepack, ST750EK, Sekisui, Osaka, Japan) between 7 and 8 a.m. and centrifuged within 0.5 h of collection. The plasma samples from all participants were collected and frozen at −80 °C until the measurements. This study was approved by the Ethics Committee of the First Hospital of Hebei Medical University. All participants or their families (when the patients were unable to sign for themselves) provided written informed consent.

2.2. ELISA

In brief, ELISA black plates were coated with 70 ng of mouse anti-human NRG1β1 isoform antibody (R&D Systems, Minneapolis, MN) at 4 °C overnight, and 100 μL of plasma or standard NRG1β1 (R&D Systems, Minneapolis, MN; 10–1000 pg) was added into the wells. After washing, 15 ng of biotinylated goat anti-human NRG1β1 antibody (R&D Systems, Minneapolis, MN) was added into each well. Biotinylated antibodies were then added to each well with 100 μL of avidin-β-galactosidase (1:10,000; Merck Millipore, Darmstadt, Germany) followed by the addition of 200 μM of the fluorogenic substrate MUG (Sigma Chemicals, St. Louis, MO, USA). The fluorescence was measured using a Varioskan Flash microplate reader (Thermo Scientific; Waltham, MA) with excitation and emission wavelengths of 365 and 450 nm [22]. The lowest detectable concentration was 125 pg/mL, and the coefficients of variation were <10% in this ELISA system. To test the cross-reactivity of NRG1β1 via our ELISA system, we used the five following recombinant human proteins: the human NRG1 core epidermal growth factor β1(EGFβ1) domain, EGF, the EGF-like growth factor (HB-EGF), transforming growth factor-α (TGF-α), and betacellulin.

2.3. Statistical analyses

The ELISA results were initially analyzed using ANOVA with gender, disease, and/or diagnosis type as between-subject factors. The relationships between the plasma NRG1β1 levels and each potential confounding factor (including age, disease onset, illness duration and antipsychotic medication dosage) were evaluated using Pearson's correlation analyses. Moreover, we also used ANCOVA to examine each potential confounding factor as a covariate separately and in combination to confirm the conclusions of the ANOVAs. Post hoc analyses were conducted with Fisher's least significant difference (LSD) tests, and p-values < 0.05 were considered significant. All of the data were analyzed using software SPSS version 22.0.

3. Results

3.1. Measurements of the plasma NRG1β1 immunoreactivities in the control participants and participants with first-episode schizophrenia, chronic schizophrenia, bipolar I disorder, bipolar II disorder or major depressive disorder

We obtained plasma from the patients and healthy controls (Table 1). ELISA was used to determine the linearity of the NRG1β1 detection with concentrations that ranged from 10 to 1000 pg/ml of recombinant human NRG1β1 (Fig. 1)

The mean NRG1β1 level of the 80 healthy controls was 7.21 ± 1.11 ng/mL. The mean concentration in the 43 males was 6.36 ± 1.09 ng/mL and that of the 39 females was 8.29 ± 1.42 ng/mL. We failed to identify a significant difference between the genders (p = 0.204). The NRG1β1 concentrations of the first-episode schizophrenia patients (5.19 ± 0.58 ng/mL) were compared with those of the chronic schizophrenia participants (4.08 ± 0.64 ng/mL), and we did not identify any significant difference between these groups (p = 0.08). The concentrations of NRG1β1 in the participants with first-episode schizophrenia and chronic schizophrenia were significantly lower than that of the control participants (control vs. first-episode schizophrenia, p = 0.017; control vs. chronic schizophrenia, p = 0.001). No significant differences in the levels NRG1β1 were detected between the healthy controls (7.21 ± 1.11 ng/mL) and the participants with bipolar I disorder (7.19 ± 0.91 ng/mL; n = 60, p = 0.98), bipolar II disorder (6.41 ± 1.26 ng/mL; n = 60, p = 0.358) or major depressive disorder (6.69 ± 1.43 ng/mL; n = 60, p = 0.955) (Fig. 2).

To test the cross-reactivity of NRG1 via our ELISA system, we used recombinant proteins of the human NRG1 core EGFβ1