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Serum NR1 and NR2 concentrations in first-episode schizophrenia and clinical high-risk for psychosis

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Abstract

Background This study evaluated the utility of serum NR1 and NR2 concentrations in identifying individuals with first-episode schizophrenia (FES) and clinical high risk (CHR) as well as their correlations with clinical symptoms and cognitive domains.

Methods This cross-sectional study compared peripheral blood NR1 and NR2 concentrations among the FES, CHR, and healthy control (HC) groups and examined their association with cognitive function. Serum concentrations of NR1 and NR2 subunits were measured using ELISA, and cognitive function was assessed using the MATRICS Consensus Cognitive Battery. Concentrations were compared among groups using the analysis of covariance or non-parametric tests and ROC curve analysis, and correlation was determined using the Pearson or Spearman method.

Results A total of 41 FES cases, 34 CHR cases, and 41 HC were included in the study. Serum NR1 concentrations significantly varied among the three groups ($Z = 16.19$, $P < 0.001$) and were significantly different between the FES group and the CHR ($Z = -4.04$, $P < 0.001$) and HC groups ($Z = -2.49$, $P = 0.01$). Additionally, serum NR2 concentration was significantly different between the CHR and HC groups ($F = 5.37$, $P = 0.02$). In the FES group, serum NR1 concentration was negatively correlated with speed of processing ($r = -0.41$, $P = 0.02$), whereas serum NR2 concentration was negatively correlated with verbal learning ($r = -0.40$, $P = 0.02$). In the CHR group, serum NR1 concentration was positively correlated with the total MCCB score ($r = 0.40$, $P = 0.04$). ROC curve analysis showed that NR2 level was better for discriminating FES (AUC: 69%; sensitivity: 56%; specificity: 85%; optimal cutoff value: 32.80 ng/mL) and CHR (AUC: 74%; sensitivity: 62%; specificity: 85%; optimal cutoff value: 32.77 ng/mL).

Conclusions Serum NR1 and NR2 concentrations show potential for early identification of individuals with psychosis, but further validation is needed, and they are also correlated with cognition. Furthermore, serum NR2 concentration is more stable and serves as a promising objective biomarker for quantitative assessment.

Keywords NR1, NR2, First-episode schizophrenia, Clinical high risk

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Background

Schizophrenia is a neurodevelopmental disorder characterized by positive symptoms, negative symptoms, and cognitive deficits, with clinical onset in late adolescence to early adulthood. Although the pathogenesis of schizophrenia is not yet fully understood, genetic, autopsy, and neuroimaging studies all demonstrated that dysfunction of glutamate neurotransmitters, particularly the hypofunction of the N-methyl-D-aspartate receptor (NMDA-R), plays a crucial role in driving the onset of schizophrenia [1–3]. As early as 30 years ago, researchers found that patients with schizophrenia have reduced glutamate levels in their cerebrospinal fluid [4]. Subsequent studies have shown that administering NMDA-R antagonists, such as phencyclidine and ketamine, to healthy controls (HC) can induce positive, negative, and cognitive symptoms similar to those observed in schizophrenia [5]. Mice with 5% of normal NR1 expression can survive to adulthood but exhibit behavioral abnormalities, including increased motor activity, stereotyped behaviors, and deficits in social and sexual interactions. These behavioral changes are similar to those observed in drug-induced animal models of schizophrenia and can be alleviated by treatment with haloperidol or clozapine [6].

NMDA-R is a heterotetramer composed of different subunits, including NR1, NR2, and NR3 [7]. There are eight different isoforms of NR1 (NR1 1a/b-4a/b), which are derived from the same gene and produced by alternative splicing at different sites [8, 9]. NR2 has four different isoforms (NR2 A to NR2D) and NR3 has two isoforms (NR3 A and NR3B) [7]. An active NMDA-R must be composed of at least one NR1 and one NR2 subunit, and must be co-activated by both glutamate and glycine [10–12]. NR1 is the functional subunit, and dysregulation of its gene expression can lead to a loss of receptor function. NR2 is the regulatory subunit of the receptor complex [13]. NMDA-R is involved in various processes, including learning, memory, and synaptic plasticity. However, excessive activation can lead to seizures and excitotoxicity [14, 15].

NMDA-R is primarily expressed on neurons. The NR1 subunit is ubiquitously expressed throughout the brain, whereas the expression of the NR2 subunit is region-specific [16]. The NR2 A and NR2B isoforms are primarily found in specific forebrain regions of the normal brain, such as the neocortex and hippocampus. In contrast, the NR2 C and NR2D isoforms are primarily expressed in the healthy cerebellum, as well as in the midbrain and hindbrain of humans and animals [7, 16]. NMDA-R may be involved in the pathogenesis of schizophrenia through various mechanisms across different neural circuits and cell types [17]. NMDA-R expression and activity have been found in non-neuronal peripheral tissues, such as glial cells, lungs, osteocytes, and the heart [18, 19]. This suggests that NMDA-R plays a variety of physiological roles. Activity in the central nervous system (CNS), particularly in neurons and glial cells, can influence NMDA-R levels in the peripheral blood. This occurs through complex signaling pathways, including neurotransmitters, cytokines, and neuromodulators. These signals are transmitted through the blood–brain barrier, cerebrospinal fluid, or the meningeal lymphatic system. They ultimately trigger responses that may affect the peripheral blood levels of NMDA-R [20–23]. Loureiro et al. found a decrease in the plasma concentrations of NR1 and NR2 in individuals with schizophrenia spectrum disorders, suggesting that NR2 may serve as a plasma biomarker for psychosis [24]. This preliminary finding indicates that peripheral NR1 and NR2 levels may reflect those in the CNS.

Despite advancements in psychiatry, there has been no significant breakthrough in the prevention and control of schizophrenia, and its onset and outcomes remain largely unchanged. In fact, many patients already exhibit some non-specific clinical symptoms before disease onset, such as perceptual abnormalities, bizarre thoughts, memory impairments, difficulty concentrating, anxiety, and depression, which are collectively referred to as the clinical high-risk (CHR) state for psychosis [25]. Approximately one-third of CHR individuals will transition to severe psychotic disorders, primarily schizophrenia, within the next 2 to 3 years [26, 27]. Cognitive deficits are a core feature of schizophrenia [28]. Several studies have found that individuals at risk for psychotic disorders exhibit mild to moderate widespread neurocognitive abnormalities prior to the onset of the illness, including deficits in attention, executive function, and working memory [29, 30]. Research suggests that abnormalities in NMDA-R function may be related to these cognitive deficits, although the conclusions are not yet fully conclusive [31–33]. Therefore, evaluating the relationship between serum NR1 and NR2 concentrations and cognitive function may help to better understand the role of NMDA-R in the cognitive deficits associated with schizophrenia.

The plasma concentrations of NMDA-R subunits in psychosis have not been well characterized, with only preliminary evidence provided by Loureiro et al. [24]. Based on the hypothesis of NMDA-R dysfunction in schizophrenia, they suggested that changes in NMDA-R subunit concentrations in the peripheral blood may indirectly reflect functional abnormalities in the brain. However, current studies are limited by small sample sizes and primarily focus on populations with schizophrenia spectrum disorders. Additionally, they have not differentiated patients based on risk levels, nor have their findings been validated across diverse ethnic groups. Therefore,

building on previous work, this study measured the serum concentrations of NR1 and NR2 in first-episode schizophrenia (FES) patients and CHR individuals to evaluate their potential utility in the early identification of psychosis, as well as their correlation with clinical symptoms and cognitive function.

Methods

Participants

The participants were help-seeking outpatients and inpatients at Beijing Anding Hospital of Capital Medical University between January 2015 and December 2018. They were aged 14–50 years, included both males and females, and had at least a primary school education. The participants were divided into three groups: (1) FES: Participants were first diagnosed with schizophrenia based on the Structured Clinical Interview for DSM-IV (SCID-I/P). They had a total illness duration of less than 5 years, had used antipsychotic medication for less than 2 weeks in the past year, and had a cumulative total duration of antipsychotic medication use of less than 6 weeks [34]. (2) CHR: Participants were screened using the Structured Interview for Psychosis Risk Syndromes (SIPS) and had one or more of the following conditions: brief intermitted psychotic symptoms syndrome (BIPS), attenuated psychotic symptoms syndrome (APSS), or genetic risk and deterioration syndrome (GDR) [35]. (3) HC: Healthy individuals with no family history of mental disorders were recruited through advertisements. They were matched with the above two groups in terms of age, gender, and education level. Screening with the aforementioned assessment tools showed no evidence of mental disorders. Exclusion criteria: Presence of organic brain diseases or serious unstable physical illnesses, history of alcohol or drug abuse or dependence, risk of suicide or violence, having received modified electroconvulsive therapy within the past 6 months, or individuals with comorbidities of other psychiatric disorders.

The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee (approval number: 2015127 FS-2). All participants and legal guardians were fully informed of and understood the study details and voluntarily signed the informed consent form.

Clinical evaluations

The severity of FES was assessed using the Positive and Negative Syndrome Scale (PANSS). The scale includes 3 subscales, namely the Positive Scale (7 items), the Negative Scale (7 items), and the General Psychopathology Scale (16 items) [36]. Psychiatric examinations were conducted by psychiatrists trained in the use of the scale, based on all information from the week prior to the assessment. Each item is rated on a 7-point scale: 1—Absent; 2—Minimal; 3—Mild; 4—Moderate; 5—Moderately severe; 6—Severe; 7—Extreme.

The severity of positive, negative, disorganized, and general symptoms in the CHR and HC groups from the past few months was assessed using the Scale of Prodromal Symptoms (SOPS) from the SIPS tool. The symptoms were rated on a scale from 0 (never, absent) to 6 (severe, psychotic) based on the intensity, frequency, and duration of symptoms [35].

The participants' neurocognitive function was evaluated using the Chinese version of the MATRICS Consensus Cognitive Battery (MCCB), which includes seven cognitive domains: speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem-solving, and social cognition [37, 38].

Analysis of plasma NR1 and NR2 levels

A total of 5 mL of fasting venous blood was collected in a clot activator tube, let sit at room temperature for 30 min, and centrifuged at 3000 rpm for 15 min. Serum was extracted and stored at −80 °C until analysis. Serum NR1 and NR2 levels were measured using the NMDA-R ELISA kit according to the manufacturer's instructions (My BioSource, San Diego, CA, USA). Optical density (OD) was measured at 450 nm using a microplate reader. The intensity of the color is inversely proportional to the NMDA-R concentration. A standard curve was generated by plotting the OD against the concentrations of the standards. The NMDA-R concentration in each sample was determined using the standard curve.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics 23.0 for Windows (SPSS, Inc., Chicago, IL, USA). Demographic and clinical data were compared using the *t*-test or one-way analysis of variance (ANOVA). Categorical data were compared using the chi-square test. Cognitive domain data were compared among groups using multivariate analysis of variance (MANOVA), with age, gender, and education level as covariates. Serum NR2 concentrations were compared among groups using the analysis of covariance (ANCOVA), with gender and age as covariates. Post-hoc pairwise comparisons were performed using Bonferroni correction. Due to the non-normal distribution of the data and unequal variances, serum NR1 concentrations were compared among the three groups using a non-parametric test. The effect size was represented by Cohen's d, which quantifies the standardized magnitude of the mean difference between two groups. A Cohen's d value of 0.2–0.5 indicates a small effect size, 0.5–0.8 indicates a medium effect size, and values above 0.8 indicate a large effect size. The correlation of serum

NR1 and NR2 concentrations with clinical symptoms and MCCB domains was evaluated using Pearson correlation or Spearman rank correlation analysis. The discriminatory power of serum NR1 and NR2 concentrations in early psychosis detection was assessed by ROC curve analysis, and the optimal cut-off values were determined. A $P < 0.05$ was considered statistically significant.

Results

Demographics and clinical characteristics

This study included 41 FES cases, 34 CHR cases, and 41 HC who met the eligibility criteria. Demographic and clinical data analysis are detailed in Table 1. Age significantly varied across the three groups (F = 7.29, $P = 0.001$, ANOVA). In particular, age was significantly lower in the CHR group compared to the HC group (t = −3.80, $P < 0.001$, independent sample *t*-test) but comparable between the FES group and the CHR and HC groups. Gender ratio was not significantly different among the three groups ($\chi^2 = 5.59$, $P = 0.06$, chi-square test). However, symptom severity significant differed between the CHR and HC groups ($P < 0.001$). 70.73% of participants in the FES group reported a brief history of antipsychotic medication use.

Cognitive function

Total MCCB scores were significantly different among the three groups (F = 12.88, $P < 0.001$, ANCOVA). Cognitive impairment was most severe in the FES group, followed by the CHR and HC groups (Table 2). Pairwise comparisons with Bonferroni correction revealed that, except for social cognition, the FES group showed worse cognitive function in the other six domains compared to the HC group, with medium to large effect sizes distinguishing the two groups. Specifically, the FES group performed worse than the CHR group in speed of processing, attention/vigilance, visual learning, reasoning, and problem-solving. However, there was no difference between the CHR and HC groups.

Plasma NR1 and NR2 concentrations

Serum NR1 concentrations were highest in the FES group, followed by the HC and CHR groups. Serum NR2 concentrations were the highest in the HC group, followed by the FES and CHR groups (Table 3). Serum

Table 1 Demographic and clinical characteristics of study participants

	FES	CHR	HC	F/t	*P*
Subjects, n	41	34	41	—	—
Age, y	24.29 ± 5.87	21.41 ± 5.51	26.66 ± 6.30	7.29	0.001
Education, y	14.27 ± 4.56	12.97 ± 2.63	12.51 ± 3.33	2.53	0.08
Duration of illness, mo	27.86 ± 27.45	—	—	—	—
Age at onset	22.22 ± 5.71	—	—	—	—
SIPS-Positive	—	9.00 ± 3.44	0.34 ± 1.30	14.83	< 0.001
SIPS-Negative	—	7.84 ± 5.16	0.17 ± 0.70	9.43	< 0.001
SIPS-Disorganization	—	4.23 ± 2.63	0.07 ± 0.35	10.02	< 0.001
SIPS-General	—	5.39 ± 2.88	0.12 ± 0.51	11.48	< 0.001
SIPS-Total	—	26.45 ± 10.56	0.71 ± 2.77	14.97	< 0.001
PANSS-Positive	24.25 ± 5.44	—	—	—	—
PANSS-Negative	20.03 ± 8.05	—	—	—	—
PANSS-general psychopathology	43.16 ± 6.03	—	—	—	—
PANSS-Total	87.87 ± 13.52	—	—	—	—
				χ^2	*P*
Men, n (%)	17 (41.46)	21 (61.76)	27 (65.85)	5.59	0.06
Married, n (%)	8 (19.51)	4 (11.76)	15 (36.59)	6.91	0.03
Family history, n (%)	8 (19.51)	11 (32.35)	—	1.62	0.20
Smoking, n (%)	3 (7.32)	2 (5.88)	6 (14.63)	2.00	0.37
Medication, n (%)	33 (80.49)	19 (55.88)	—	—	—
Unmedicated	8 (19.51)	15 (44.12)	—	—	—
AP	29 (70.73)	11 (32.35)	—	—	—
AD	0	2 (5.88)	—	—	—
AD + AP	0	4 (11.77)	—	—	—
Unspecified	4 (9.76)	2 (5.88)	—	—	—

AD Antidepressant, *AP* Antipsychotic

Table 2 Comparison of MCCB domain scores among the FES, CHR and HC groups

MCCB Domains	FES	CHR	HC	Statistic[a]		Pairwise comparison[b]		
				F	*P*		*P*	*Effect size*[c]
Speed of Processing	32.14 ± 8.80	40.34 ± 9.22	44.54 ± 8.23	15.55	< 0.001	FES < CHR	0.001	0.91
						FES < HC	< 0.001	1.46
Attention/Vigilance	31.00 ± 10.07	42.28 ± 11.48	43.82 ± 9.21	14.32	< 0.001	FES < CHR	< 0.001	1.05
						FES < HC	< 0.001	1.33
Working memory	36.88 ± 9.81	35.61 ± 17.37	44.74 ± 7.92	2.64	0.08	FES < HC	0.04	0.89
Verbal learning	38.63 ± 9.89	43.62 ± 11.03	47.95 ± 10.37	4.15	0.02	FES < HC	0.006	0.92
Visual learning	37.31 ± 15.52	44.73 ± 11.34	43.87 ± 10.81	5.83	0.004	FES < CHR	0.002	0.55
						FES < HC	0.008	0.50
Reasoning/problem solving	32.57 ± 9.70	40.12 ± 12.92	40.21 ± 9.80	8.76	< 0.001	FES < CHR	< 0.001	0.67
						FES < HC	< 0.001	0.78
Social recognition	34.59 ± 11.23	37.39 ± 9.43	36.41 ± 11.53	0.13	0.88	—	—	—
Total	35.15 ± 5.82	41.46 ± 9.26	43.08 ± 6.05	12.88	< 0.001	FES < CHR	0.001	0.84
						FES < HC	< 0.001	1.34

[a] Multivariate analysis of covariance

[b] Post-hoc Bonferroni correction

[c] Effect size was calculated using Cohen's d for significant pairwise comparisons

NR1 concentrations significantly differed among the three groups (Z = 16.19, $P < 0.001$, non-parametric test) and was significantly different between the FES group and the CHR group (Z = −4.04, $P < 0.001$, Cohen's d = 1.03), as well as between the FES group and the HC group (Z = −2.49, $P = 0.01$, Cohen's d = 0.59). Furthermore, ANCOVA using age and gender as covariates showed a significant difference in serum NR2 concentrations between the CHR and HC groups (F = 5.37, $P = 0.02$, Cohen's d = 0.82). The serum NR2 concentration in FES group is lower than in HC group, but the difference between the two groups is not statistically significant (F = 2.61, $P = 0.11$, Cohen's d = 0.48).

Correlation of serum NR1 and NR2 concentrations with demographics, clinical symptoms and cognition

Linear correlation analysis of the FES group showed that serum NR1 concentration was negatively correlated with speed of processing ($r = -0.41$, $P = 0.02$), while serum NR2 concentration was negatively correlated with verbal learning ($r = -0.40$, $P = 0.02$) (Fig. 1). In the CHR group, serum NR1 concentration was positively correlated with the total MCCB score ($r = 0.40$, $P = 0.04$; Table 4). However, serum NR1 and NR2 concentrations were not correlated with demographics or clinical symptoms in both the FES and CHR groups.

ROC curve analysis

The areas under the ROC curves for serum NR1 and NR2 concentrations were greater than 50% for discriminating FES and CHR (Fig. 2). Additionally, NR2 level was better for discriminating both FES (AUC: 69%; sensitivity: 56%; specificity: 85%; optimal cutoff value: 32.80 ng/mL) and CHR (AUC: 74%; sensitivity: 62%; specificity: 85%; optimal cutoff value: 32.77 ng/mL).

Discussion

This is the first study to evaluate serum NR1 and NR2 concentrations and their associated factors in FES and CHR. We found that: (1) Serum NR1 and NR2

Table 3 Serum NR1 and NR2 concentrations among the three study groups

Serum concentrations	FES	CHR	HC	Statistic		Pairwise comparison		
				F/Z	*P*		*P*	*Effect size*[c]
NR1, ng/ml[a]	11.73 ± 5.46	7.12 ± 3.49	8.74 ± 4.76	16.19	< 0.001	CHR < FES	< 0.001	1.03
						HC < FES	0.01	0.59
NR2, ng/ml[b]	36.19 ± 12.53	32.85 ± 11.02	41.82 ± 10.94	3.05	0.05	CHR < HC	0.02	0.82

[a] Non-parametric test

[b] Analysis of Covariance

[c] Effect size was calculated using Cohen's d

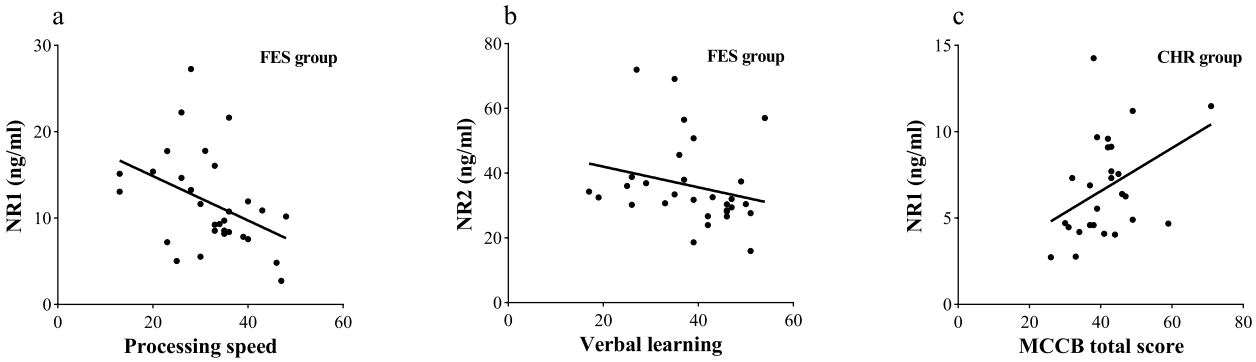


Fig. 1 Scatter plot of the relationship between serum NMDA-R subunit concentrations and cognitive domains

Table 4 The correlation between serum NR1 and NR2 concentrations and cognition (r)

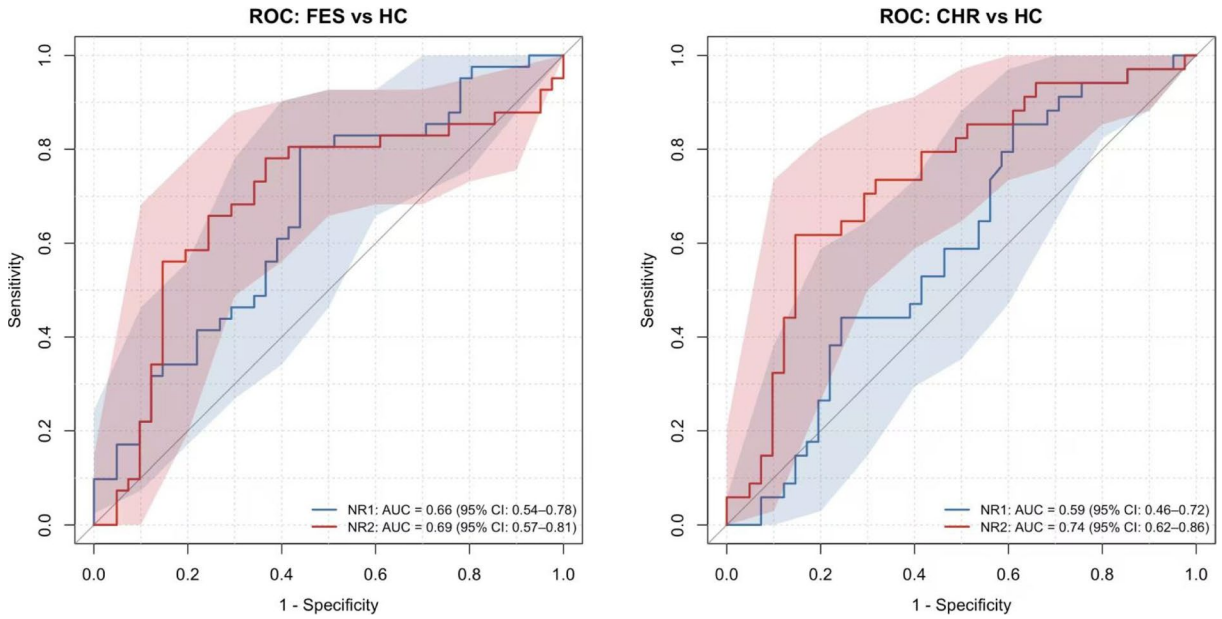
MCCB Domains	FES		CHR	
	NR1	NR2	NR1	NR2
Speed of Processing	−0.41*	−0.06	0.10	0.08
Attention/Vigilance	−0.24	0.06	0.04	0.12
Working memory	−0.08	0.02	0.04	−0.11
Verbal learning	−0.14	−0.40*	0.15	0.04
Visual learning	0.16	0.24	0.25	0.06
Reasoning/problem solving	−0.11	−0.05	0.03	−0.29
Social recognition	0.18	0.07	0.24	0.15
Total	−0.13	0.02	0.40*	0.14

* $P < 0.05$

concentrations were lower in CHR individuals than in HC, with a significant difference in NR2. In FES patients, NR1 was higher, but NR2 was lower than in HC, though not significantly. (2) In FES patients, serum NR1 correlated negatively with speed of processing and NR2 with verbal learning. In CHR individuals, serum NR1 correlated positively with total MCCB score. (3) Serum NR2 concentration showed better potential than NR1 for early identification of FES and CHR.

Compared to HC, FES patients exhibited lower serum NR2 concentrations, while CHR individuals showed reduced serum NR1 and NR2 levels. These data align with the hypothesis of NMDA-R dysfunction in schizophrenia and are consistent with previous findings in brain autopsies, magnetic resonance imaging, and serum/plasma NMDA-R subunit concentrations [1, 2, 24], further supporting the value of the serum concentrations of NMDA-R subunits in early psychosis detection. In this study, the concentrations of serum NR1 and NR2 exhibit a"decrease followed by an increase"trend. Specifically, in CHR individuals, glutamate function is reduced, resulting in a decrease in NR1 and NR2 concentrations, which aligns with the disease's susceptibility state. However, once individuals enter the disease state (FES), NR1 and NR2 concentrations may increase due to compensatory mechanisms for glutamatergic dysfunction [39]. Autopsy findings revealed a significant increase in NR1 expression in the anterior cingulate cortex of individuals with schizophrenia. This upregulation was associated with changes in NMDA-R expression on the cell membrane in this region. However, no changes in NR2 subunit expression were observed [40]. Additionally, the gene expression patterns of NMDA-R and related molecules can vary across age and symptoms prior to disease onset [7]. These changes may also be related to the use of antipsychotic medications. Studies have shown that after taking antipsychotic medications, NR1 expression is increased in the parietal lobe, temporal lobe, sensory cortex, and hypothalamus. This upregulation may be related to the blockade of D2 receptors by antipsychotic drugs [41, 42].

NMDA-R is a key regulator of synaptic plasticity and long-term potentiation, influencing central learning and memory functions [43]. However, the role of each subunit in specific cognitive processes remains unclear [44]. Deletion of NR1 impaired the working memory, executive function, and the ability to explore novel stimuli in mice [33]. Moreover, deletion of NR2 in the forebrain or hippocampus impaired spatial working memory [31, 32], while its overexpression enhanced learning and memory in mice [45]. Previous studies have reported that patients with schizophrenia have downregulated NR1 and NR2 A expression in the dorsolateral prefrontal cortex [46, 47] and disrupted NR2B trafficking. Additionally, the absence of NR2B further impaired NMDA-R function [48]. Other studies have revealed upregulated NR2 A expression in the frontal and occipital cortices [49], as well as upregulated NR2B expression in the temporal cortex and dorsomedial prefrontal cortex [50, 51]. On the other hand, NR1 expression is reduced while NR2B expression is increased in the hippocampus [52]. Patients with schizophrenia



	FES group						CHR group					
	AUC	Sen	Spe	PPV	NPV	cut-off	AUC	Sen	Spe	PPV	NPV	cut-off
NR1	66%	80%	56%	64%	74%	7.52 ng/ml	59%	85%	39%	54%	76%	9.74 ng/ml
NR2	69%	56%	85%	79%	66%	32.80 ng/ml	74%	62%	85%	78%	73%	32.77 ng/ml

Fig. 2 Receiver operating characteristic (ROC) curves of serum NR1 and NR2 concentrations for discriminating FES and CHR

exhibit widespread cognitive impairments early in the course of the illness, with verbal learning and speed of processing closely linked to the function of the frontal and temporal lobes [53, 54]. This study found that serum NR1 and NR2 concentrations were negatively correlated with speed of processing and verbal learning in FES patients, respectively. These negative correlations may be attributed to the elevated serum NR1 and NR2 concentrations in the FES group. Consistent with the findings in animal studies, serum NR1 concentration was positively correlated with the total cognitive score in CHR individuals. These results indirectly support the correlation between NMDA-R subunit expression in the frontal and temporal lobes and cognitive function.

In agreement with Loureiro et al., ROC curve analysis in this study revealed that serum NR2 concentration exhibited higher specificity and accuracy than NR1 in discriminating FES, CHR, and HC [24]. Similarly, changes in plasma NR2 concentrations have been observed in Alzheimer's disease and after acute ischemic stroke, with plasma NR2 concentration positively correlating with the severity of neuronal damage [55, 56]. Although this study found no correlation between serum NR2 concentrations and clinical symptoms or psychosis severity, lower serum NR2 levels, despite the influence of various confounding factors, still effectively identified CHR individuals, suggesting its potential in early identification of individuals with psychosis, but further validation is needed.

This study also has certain limitations. Firstly, most FES patients and CHR individuals had a brief history of antipsychotic use; however, the existing data do not allow for a comparison between those with and without medication. Short-term use of medication (< 2 weeks) may have an improving effect on cognitive function, but its overall impact on the results of this study is minimal. Secondly, the three groups were not fully age-matched, with participants in the CHR group being younger. Animal studies have demonstrated that both internal and external environmental factors during growth and development can lead to dynamic changes in NMDA-R gene expression, with expression levels being age-dependent and exhibiting a certain degree of plasticity [7]. In this study, age

was included as a covariate in the statistical analysis to minimize its impact on the study results. Finally, due to the small sample size, there are certain limitations in the subgroup analysis. Additionally, future follow-up studies will be conducted to longitudinally validate the changes in NR1 and NR2 concentrations over time in these participants.

Conclusion

Serum concentrations of NR1 and NR2 are decreased prior to the onset of psychosis, with NR2 showing greater potential as a biomarker for early identification of psychosis, although further validation is needed. Serum NR1 and NR2 concentrations are associated with cognitive function but not with the demographic and clinical characteristics of FES patients and CHR individuals. Follow-up studies in CHR individuals are needed to evaluate the changes in the serum concentrations of NMDA-R subunits and their predictive value in the transition to psychosis.

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Authors' contributions
Data acquisition and collation: Feifei Wang, Xueqi Wang, Yongying Cheng, Weiwei Hou, Lu Wang, Xinke Shi, Wenpeng Hou. Yujie Wen and Yushen Ding managed the literature searches. Qijing Bo and Lige Ge undertook the statistical analysis. Zhen Mao and Feng Li wrote the complete first draft. Final review and editing by Fang Dong. All authors have read and approved the manuscript.

Authors' information
All researchers in the study were trained regarding the protocol and Good Clinical Practice guidelines.

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Data availability
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
All procedures of the present study were performed in accordance with the Declaration of Helsinki. The study protocols were approved by the clinical research ethics committees of Beijing Anding Hospital, Capital Medical University. All the individuals were aware of the purpose of the study and signed an informed consent form.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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