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RESEARCH

Does Lrig1 expression have a relationship with ERBB1 and ERBB2 expression in schizophrenia?

Lrig1 ekspresyonunun şizofrenide ERBB1 ve ERBB2 ekspresyonuyla ilişkisi var mıdır?

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Abstract

Purpose: This study aims to investigate Lrig1 expression and to reveal the possible relationship between Lrig1, ERBB1 and ERBB2 in schizophrenia.

Materials and Methods: In this study, peripheral blood samples of 70 schizophrenia patients and 60 healthy controls were used. Real Time PCR was applied for Lrig1, ERBB1 and ERBB2 gene expression analysis.

Results: The level of Lrig1 mRNA was lower in the patients when compared to healthy controls. Expression levels of ERBB1 and ERBB2 were decreased in the patients versus in the healthy controls. According to the receiver operating characteristic curve analysis, the three genes had the power to discriminate the patients from the healthy controls (Lrig1 AUC: 0.66, ERBB1 AUC: 0.64, ERBB2 AUC: 0.79). There was a weak, positive correlation between the Lrig1 and ERBB1 expressions in the schizophrenia patients. No significant correlation was detected between the Lrig1 and ERBB2 expressions or the ERBB1 and ERBB2 expressions in the patients.

Conclusion: The results revealed that the Lrig1 and ERBB relationship changes in schizophrenia. These genes may have the potential to be a biomarker that can be used in schizophrenia.

Keywords: Schizophrenia, Lrig1, ERBB1, ERBB2, EGFR

Öz

Amaç: Bu çalışmanın amacı şizofrenide Lrig1 ekspresyonunu araştırmak ve şizofrenide Lrig1, ERBB1 ve ERBB2 arasındaki olası ilişkiyi ortaya koymaktır.

Gereç ve Yöntem: Çalışmaya 70 şizofreni hastası ve 60 sağlıklı bireye ait periferik kan örneği dahil edildi. Lrig1, ERBB1 ve ERBB2 gen ekspresyon analizi için Real Time PCR kullanıldı.

Bulgular: Lrig1 mRNA düzeyi hastalarda sağlıklı kontrollere nispeten düşüktü. ERBB1 ve ERBB2'nin hastalarda sağlıklı kontrollerden daha düşük ifade düzeylerine sahip olduğu görüldü. Alıcı işletim karakteristiği eğrisi analizine göre, üç genin hastaları sağlıklı kontrollerden ayırt etme gücü vardı (Lrig1 AUC: 0,66, p = 0,006, ERBB1 AUC: 0,64 ERBB2 AUC: 0,79). Şizofreni hastalarında Lrig1 ve ERBB1 ifadeleri arasında zayıf, pozitif bir korelasyon vardı. Hastaların gen ifadelerine bakıldığında Lrig1 ile ERBB2 arasında ve ERBB1 ile ERBB2 arasında anlamlı bir korelasyon olmadığı görülmüştür.

Sonuç: Sonuçlar Lrig1 ve ERBB ilişkisinin şizofrenide değiştiğini ortaya koydu. Bu genlerin şizofrenide kullanılabilecek bir biyobelirteç olma potansiyeline sahip olduğu düşünülmektedir.

Anahtar kelimeler: Şizofreni, Lrig1, ERBB1, ERBB2, EGFR

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INTRODUCTION

Schizophrenia is a psychiatric disease that affects a significant portion of society[1], has positive and negative symptoms, and causes cognitive impairment[2]. Current medical treatment for it, which has many side effects, does not give patients the desired results[1]. The discovery of biomolecules in this area is important in supporting both diagnosis and treatment.

Leucine-rich repeat and immunoglobulin domain-containing protein 1 (Lrig1) is a family member of transmembrane leucine-rich repeats and is involved in growth and neutrophilic factor signaling[3,4]. Lrig1 expression in healthy tissues is seen commonly and its expression may be altered in several disorders[5,6]. It is associated with receptor tyrosine kinases (RTKs) and regulates their signaling by regulating receptor ubiquitylation and degradation[7]. Studies have shown that Lrig1 ubiquitylates the epidermal growth factor receptor (ERBB) family, including ERBB1, causing its lysosomal degradation independently of ERBB1 ligands[3,7]. An animal model study demonstrated the role of Lrig1 in hippocampus dendrite development and its effect on learning and memory[8].

RTKs are cell surface receptors that bind to a large number of ligands, including hormones, growth factors, cytokines stimulating cell signaling that lead to cell growth, development, differentiation, and migration[9,10]. RTKs are important molecules due to their roles in neuroprotection, synaptic plasticity, and cell survival. Maintaining neuronal health depends on the regulated activation of RTKs, including epidermal growth factor receptors (EGFRs), insulin receptors, and tropomyosin-related kinase receptors[11]. The dysregulation of RTKs is associated with neurodegeneration due to their roles in amyloid-beta (Aβ) aggregation, oxidative stress, and neuroinflammation[12]. Impaired insulin signaling in the brain exacerbates Aβ pathology and neuroinflammation[11]. RTKs comprise the ERBB subfamily, which consists of four essential members [ERBB1 (EGFRs, human epidermal growth factor receptor 1 (HER1)), ERBB2, ERBB3, and ERBB4 (HER2, HER3, and HER4)][13]. The expression of ERBB family members occurs in different regions of the brain (including dopaminergic neurons) and has an effect on adhesion, cell growth, migration, and proliferation[9,10]. Receptors belonging to the ERBB family can mediate messages from more than one EGF ligand by forming homodimers or heterodimers[10]. The expression and activity of different ERBB family members have been seen in neural stem cells (NSCs) and postnatal neuronal cell functions. The activation of EGFRs, and RTKs, leads to the stimulation of proliferation and motility of NSCs and their derivatives[14]. The contribution of ERBB1 to the postnatal activation of dopaminergic neurons has been previously demonstrated[15]. ERBB2 expression was shown in proliferating NSCs and precursors[9]. Neocortex ERBB2:ERBB3 complex signaling has been revealed to regulate the expression of disrupted schizophrenia 1 (DISC1), which is a genetic schizophrenia gene[16].

In schizophrenia, which exhibits both neurodevelopmental and neurodegenerative characteristics, the underlying gene expressions and networks remain unclear. Studies have shown that irregularities in the development of the nervous system, in which many growth factors play crucial role, may cause the development of schizophrenia[16]. Understanding the molecular changes behind schizophrenia is crucial for uncovering the nature of the disease and developing effective treatments. ERBB1 and ERBB2 have important roles in both neural development and the pathology of schizophrenia. Similarly, Lrig1 has important roles in nervous system development in both prenatal and postnatal life. Lrig1 is also a negative regulator of ERBB signaling, as it causes the destruction of ERBB receptors. For this reason, the gene expressions of ERBB1 and ERBB2, and their negative regulator, Lrig1, were examined herein.

According to literature, several studies have revealed the role of ERBB family members in schizophrenia. However, the results are still contradictory and the function of the ERBB family in neuropsychiatric diseases needs to be investigated further. Lrig1 is an important protein that regulates the quiescence of stem cells. Decreased adult NSC proliferation has been shown in psychiatric disorders[17].

Changes in the Lrig1 expression level are expected to differ between healthy individuals and those with psychiatric disorders. It is also believed that the receptors targeted for degradation by Lrig1 may play a role in this process. In addition, the Lrig1 gene remains an unexplored topic in schizophrenia patients. The Lrig1 mRNA expression level and relationship between the Lrig1 and ERBB1 and ERBB2 expression levels were explored for the first time in schizophrenia patients in this research.

This study hypothesized that Lrig1 expression is also reduced in schizophrenia patients, reflecting the reported decrease in neural stem cells (NSCs) associated with the disorder[17]. Another hypothesis was that an inverse relationship between the Lrig1 and ERBB1 and ERBB2 expression levels would be observed in schizophrenia patients, based on the knowledge that Lrig1 is a negative regulator of ERBB family members[7].

MATERIALS AND METHODS

Sample

The study population included 70 schizophrenia patients and 60 age-matched healthy controls. G*Power 3.1.9.2[18] analysis software was utilized in calculating the sample of the study. It was determined that at least 116 individuals (58 patient and 58 healthy controls) were needed to reach α err prob < 0.05 and 1- β err prob of 0.95. The research was conducted at the Atatürk University Mental Health and Diseases Department after receiving approval from the Atatürk University Non-Interventional Clinical Research Ethics Committee (Decision No.: 3-39, 02.05.2023). Patients were diagnosed with schizophrenia using the Diagnostic and Statistical Manual of Mental Disorder-5th Edition (DSM-5)[19]. All the patients were admitted to a psychiatric clinic and received hospital treatment for a certain period of time. The patients were selected in order from inward schizophrenic patients during their hospitalization between February15th and December 31st, 2023. Written informed consent was given by all the participants. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki[20]. Selection, scale applications, and obtaining the clinical data of the patients were performed by Prof. Dr. Halil Özcan at Atatürk University, Faculty of Medicine. Laboratory studies were conducted at Atatürk University, Department of Medical Pharmacology Laboratory and Bayburt University, Medical Biology Laboratory by Assoc. Prof. Dr. Sevgi Karabulut Uzunçakmak and Assoc. Prof. Dr. Pelin Aydın. Writing, revision, and continuation of the process of the article were conducted by Prof. Dr. Zekai Halıcı and Assoc. Prof. Dr. Sevgi Karabulut Uzunçakmak, Prof. Dr. Halil Özcan, and Assoc. Prof. Dr. Pelin Aydın. Patients with a diagnosis of cancer, chronic physical diseases such as hypertension, diabetes, etc., those under the age of 18, and those with different neuropsychiatric diseases and comorbid mental disorders [mental retardation, neurodegenerative disorders, alcohol and substance use disorders, mood disorders except mild depressive symptoms but not clinically major depression diagnosis (differentiating depressive symptoms with negative symptoms of schizophrenia is a very problematic area in schizophrenic symptoms)] were not included in the study. Any healthy controls with any past or present psychiatric disorder mentioned above, a relative with a major psychiatric disorder, a diagnosis of cancer or any chronic physical diseases were also excluded. Based on the inclusion and exclusion criteria, 70 patients were included and 42 were excluded. For the healthy controls, 60 were included and 46 were excluded. Clinical and demographic information were obtained from the patients and their hospital records.

Psychiatric evaluation

The patients' sociodemographic data and scale scores were obtained (some from the patients during hospitalization and some from their hospital records after discharge). The Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression Scale (CGI), Global Assessment of Functioning Scale (GAF), and Brief Psychiatric Rating Scale (BPRS) were used for data collection. All the scales were filled out by an experienced psychiatrist two times based on the examination and history of the patients at the time of hospitalization and before discharge. Some sociodemographic data and scales scores were unavailable due to the lack of some data in their hospital records. All the patients were evaluated by a psychiatrist on admission to the hospital and before discharge. Symptom severity was evaluated using the PANSS, CGI, GAF, and BPRS.

The PANSS, developed by Kay et al.,[21] is a 30-item rating scale that evaluates the extent and severity of schizophrenia symptoms. The scale includes a positive symptoms subscale, negative symptoms subscale, and general psychopathology subscales. The positive and negative symptoms subscales include 7 items each, which are scored from 1 to 7 points. The general psychopathology subscale includes 16 items, also scored from 1 to 7 points. Obtaining a higher score indicates more severe schizophrenia symptoms.

Developed by Guy[22], the CGI evaluates the course of all psychiatric disorders including schizophrenia, regardless of age. It has 3 subscales, including disease

severity, improvement of symptoms, and side effects. Only the disease severity subscale was used, which is scored between 1 and 7 points, 1 indicating a normal participant and 7 indicating severe illness.

The GAF is an assessment instrument used worldwide that rates the functioning level of participants in life and evaluates loss of functioning directly (scoring is between 0 and 100 points, where a higher score indicates better functioning) and a lower score shows the severity of illness indirectly[23].

The BPRS is one of the most widely used scales to measure psychiatric symptoms including depression, anxiety, and hallucinations[24]. It is generally used in patients with psychotic disorders such as schizophrenia to evaluate psychotic and other possible psychiatric symptoms. It has 18 items, scored between 0 and 6 points, where 0 indicates no symptoms and 6 indicates severe symptoms. A higher score indicates increased psychiatric symptoms. All the patients were under antipsychotic treatment, most of them used atypic antipsychotics, and some used typic and atypic antipsychotics (paliperidone, olanzapine, clozapine, quetiapine, aripiprazole, amisulpiride, haloperidol, etc.). Moreover, some were taking benzodiazepines (alprazolam, diazepam, clonazepam) and antidepressant agents (selective serotonin reuptake inhibitors, such as sertraline, escitalopram, venlafaxine, etc.).

RNA isolation and cDNA synthesis

Peripheral blood samples, drawn into anticoagulant tubes, were taken from all the participants during their hospitalization. Total RNA isolation was conducted using an EcoPURE Total RNA Kit (EcoTech Biotechnology, Erzurum, Türkiye) according to the manufacturer's instructions. RNA samples were examined using an Epoch Spectrophotometer System and Take3 Plate (BioTek Instruments Inc., Winooski, VT, USA). An iScript cDNA Synthesis Kit (Bio-Rad Laboratories GmbH, Feldkirchen, Germany) was used to synthesize cDNA from the total RNA. The samples were stored at –20 °C until the experiments.

Gene expression analysis

Determination of the gene expressions was performed using iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories GmbH) with an ABI StepOne Plus Real-Time Polymerase Chain Reaction (RT-PCR) System (Applied Biosystems, Waltham, Massachusetts, USA). β-actin was determined as the internal control gene. The PCR mix included 10 μL of supermix, 2 μL of primers (forward and reverse), 4 μL of cDNA, and 4 μL of nuclease-free water. The RT-PCR reactions were performed as follows: polymerase activation and DNA denaturation (at 95 °C for 30 s), denaturation (at 95 °C for 15 s), and annealing/extension and plate reading (at 60 °C for 60 s) for 40 cycles. All the reactions were run in triplicate. The ERBB1, ERBB2, and Lrig1 gene expressions were determined using the delta-delta cycle threshold method. The primers for Lrig1[25] were: forward 5'-CTGGACGCGGAGCCTAAAC-3', reverse 5'-TGTAGGTTCGGCAAGTCCTCA-3'. The primers for ERBB1[26] were: forward 5'-AGGCACGAGTAACAAGCTCAC-3', reverse 5'-ATGAGGACATAACCAGCCACC-3'. The primers for ERBB2[27] were: forward 5'-TGGCCTGTGCCCACTATAAG-3', reverse 5'-AGGAGAGGTCAGGTTTCACAC-3'. The primers for β-actin[27] were: forward 5'-CCAACCGCGAGAAGATGA-3', reverse 5'-CCAGAGGCGTACAGGGATAG-3'.

Statistical analysis

GraphPad Prism 5.0 (San Diego, CA, USA) was used to analyze the results statistically. The Kolmogorov–Smirnov/Shapiro–Wilk tests were used to test the distribution of the results. The results were expressed as the mean ± standard deviation or median (min and max) values. The Mann–Whitney U test was used to compare the Lrig1, ERBB1 and ERBB2 gene expressions of patients and healthy controls. While evaluating the clinical data, the Student's t test was used for the PANSS and the Mann–Whitney U test was used for the CGI, GAF, and BPRS. The power of the Lrig1, ERBB1, and ERBB2 expression levels to distinguish the patients from the healthy controls was analyzed using receiver operating characteristic (ROC) curve analysis. The relationship between the Lrig1 and ERBB1 and ERBB2 gene expression levels were examined using Spearman's correlation analysis. $p < 0.05$ was accepted as statistically significant.

RESULTS

The patients comprised 40 (57%) males and 30 (43%) females, with a mean age of 41 ± 11.3 years. Moreover, 21% were married, 47% were single, and the marital status of the others was unknown. The mean duration of illness was 15 ± 8.7 years. Of the

patients, 45.7% were smokers, 31.4% were nonsmokers, and the smoking status of 22.8% was unknown. While 11% of the patients were university graduates, 48% had a lower level of education, and the education level of 41% was unknown. Moreover, 9% of the patients were employed, 60% were unemployed, and the employment status information of the remaining 31% could not be obtained.

The results of the PANSS, CGI, GAF, and BPRS, performed after the patients were hospitalized and just before they were discharged, are given in the Table 1. When the differences between the test scores during hospitalization and at discharge were examined, a statistically significant difference was observed, and the applied treatment improved the test scores (PANSS: $p < 0.0001$, CGI: $p = 0.0389$, GAF: $p < 0.0001$, and BPRS: $p < 0.0001$).

Table 1. PANSS, CGI, GAF, BPRS scores of patients

Time with patients' status	**PANSS**	**CGI**	**GAF**	**BPRS**
	(mean±SD)	Median(Min- Max)	Median(Min- Max)	Median(Min- Max)
Hospitalized	94.18±2.53	6 (5-10)	35(25-55)	44(5-71)
Discharge	59.7±2.28	4.5 (1-8)	55(25-85)	21.5(3-42)
p	<0.0001	0.0389	<0.0001	<0.0001

PANSS; Positive and Negative Syndrome Scale CGI; Clinical Global Impression GAF; Global Assessment of Functioning BPRS; Brief Psychiatric Rating Scale. Bold numbers represent statistically significant results.

This study investigated whether the genes were differentially expressed between the diagnostic groups. Therefore, Lrig1 and ERBB family gene expression analysis was performed for the schizophrenia patients and healthy controls. The results showed that the Lrig1 mRNA expression level decreased in the schizophrenia patients compared to the healthy controls ($p = 0.0068$) (Figure 1A) Similarly, the ERBB1 and ERBB2 expression levels were lower in the schizophrenia patients than in the healthy controls ($p = 0.0109$ and $p < 0.0001$, respectively) (Figures 1B and 1C).

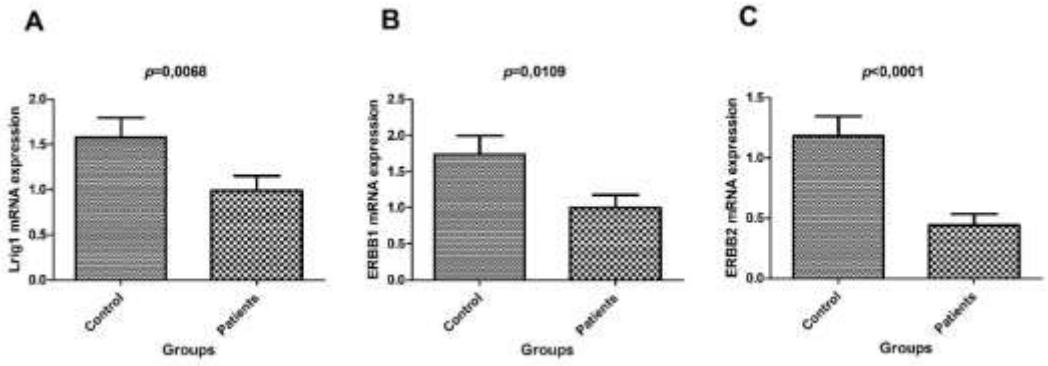

Figure1. mRNA expression of Lrig1, ERBB1 and ERBB2 in patients and healthy controls.

The power of the genes to distinguish the patients from the healthy controls was investigated using ROC curve analysis. The area under the ROC curve of Lrig1 was 0.66 (Figure 2A), that of ERBB1 was 0.64 (Figure 2B), and that of ERBB2 was 0.79 (Figure 2C). The sensitivity and specificity of Lrig1 were 65.31% and 56% ($p = 0.006$), those of ERBB1 were 61.54% and 52.08% ($p = 0.01$), and those of ERBB2 were 64.29% and 91.67% ($p < 0.0001$). The gene with the highest discrimination power was ERBB2.

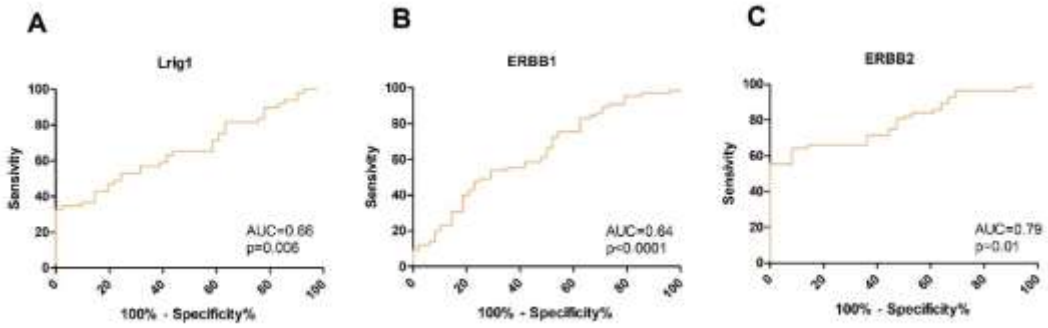

Figure 2. ROC Curve analysis of A Lrig1 mRNA expression B ERBB1 mRNA expression C ERBB2 mRNA expression.

According to the expression analysis results, the expressions of all three genes were low in the schizophrenia group. Based on this, the existence of a possible relationship between the genes was investigated. The results showed a weak positive correlation between the Lrig1 and ERBB1 expressions ($r = 0.355$, $p = 0.0152$). No correlation was found between the Lrig1 and ERBB2 expression ($r = 0.11$, $p = 0.472$). Similarly, there was no significant correlation between the ERBB1 and ERBB2 expression ($r = –0.05$, $p = 0.708$).

DISCUSSION

Schizophrenia, a disease with a molecular basis that has not yet been elucidated, affects the work and private lives of individuals and is accompanied by various cognitive, affective, and behavioral disorders. When the numerous studies conducted on schizophrenia were investigated, it was seen that molecular studies that will facilitate the direct diagnosis of schizophrenia or prove the effectiveness of treatment for it are ongoing. Therefore, ERBB family member (ERBB1, ERBB2) expressions and their regulator Lrig1 expression in schizophrenia patients were investigated herein.

Some psychiatric disorders have been associated with dendritic overgrowth and thus disordered social behavior[28,29]. In the study of Alsina et al.,[4] Lrig1 was shown to restrict primary dendrite formation and dendrite branching in hippocampal neurons. Their Lrig1-deficient mouse model revealed differences in social behavior that may be related to abnormal dendrites in the hippocampus. They suggested that Lrig1 abnormalities may be related to brain disorders. A study conducted by Vincenti et al.[30] revealed increased pyramidal glutamatergic neurons in the postnatal neocortex of Lrig1-knockout mice. Alterations in dendrite morphology and connections may be predictive of psychiatric disorders[31]. No studies investigating the Lrig1 expression in schizophrenia patients could be found in the literature. However, in previous studies, changes such as social withdrawal, social discrimination, spatial memory deficit, and obsessive-compulsive disorder-like behaviors were observed in animal models as a result of genetically modifying leucine-rich repeat (LRR) proteins[29,32,33]. Lrig1 is a transmembrane protein containing 15 LRRs[34].

The current study is the first to investigate Lrig1 expression in schizophrenia patients, and the results revealed lower Lrig1 mRNA levels in the schizophrenia patients compared to the healthy controls. Although there were no studies to compare these results with, when considering the studies summarized above, it can be seen that Lrig1 has a very important role in neural development. The ROC curve analysis results herein also showed that Lrig1 is important in schizophrenia and has the power to distinguish patients from healthy controls. The decrease in the hippocampal volume in psychiatric disorders has also been shown in the lower hippocampal areas of schizophrenia patients[35]. This decreased volume may be the result of decreased neurogenesis. Lrig1 is an important molecule in maintaining stem cell quiescence[36]. Therefore, high Lrig1 expression is expected in schizophrenia patients. However, the patients participating in the

study were in the treatment process, and studies have shown that antidepressants aim to and are successful at increasing the hippocampal volume[37]. Therefore, it can be thought that decreased Lrig1 expression may be a positive result of the drugs used in the treatment of the disease. Even decreased Lrig1 expression creates a difference that can be used to distinguish between patients and healthy individuals. Therefore, Lrig1 has the potential to be an important gene to be considered in schizophrenia. More extensive and detailed studies on Lrig1 expression in schizophrenia patients are needed to better understand its implications.

The ERBB family is an important receptor group that mediates the signals of EGF family ligands and supports neural development[14,38,39]. ERBB1 is involved in cell proliferation and the migration of NSCs and their descendents[14,39]. Furthermore, at postnatal stages, some differentiated neurons also express ERBB1[9]. In addition to the ERBB1 expression, ERBB2 is highly expressed in NSCs or their precursors during proliferation[9]. According to some studies, the results obtained from postmortem brain tissue and serum may differ from each other. Increased EGF signaling in the brain decreases the ERBB1 expression level, leading to signal attenuation[38]. When the ERBB1 subunit binds to its ligand, it initiates homodimerization or heterodimerization with other EGF receptor subunits, such as ERBB2[40]. Futamura et al.[38] investigated EGF family members in the prefrontal cortex of the brain in postmortem schizophrenia patients. They found that EGFR (ERBB1) immunoreactivity was higher in the schizophrenia patients than in the controls. They observed that the EGF levels in the prefrontal cortex and striatum and serum EGF levels were lower in the schizophrenia patients compared to the controls. There was no significant correlation between the ERBB family and their ligands in the prefrontal cortex. However, they observed no interaction between the EGF level and EGFR (ERBB1) reactivity. Mostaid et al.[41] conducted a study with treatment-resistant schizophrenia (TRS) patients. Their results showed that NRG-ERBB signaling was upregulated in the whole blood of the TRS patients. According to their analysis, the ERBB2 expression level was higher in the TRS patients than in the controls; however, it was not statically significant. Keri et al.[42] investigated the ERBB expression in schizophrenia patients via the flow cytometry method. Their results demonstrated unchanged ERBB2/3 but decreased ERBB4 expression levels in the schizophrenia patients compared with the controls. According to the studies summarized above, the expression levels obtained from different tissues are not consistent with each other. It is also difficult to say that there is a direct relationship between EGF and its receptors. The current study examined the expression levels of ERBB1 and ERBB2. According to the results, the ERBB1 and ERBB2 mRNA expression levels were lower in the schizophrenia patients than in the healthy controls. In addition, the ROC curve analysis revealed that both the ERBB1 and ERBB2 expression levels had the power to distinguish the patients from the healthy controls. In the present study, the whole blood of the patients was used. Although the study structure herein was similar to that of Mostaid et al.,[41] different results were obtained. This difference may have been due to the patient population. While they worked with a group of patients resistant to treatment, the current study employed a group of patients who had been treated for years and whose treatment resistance had not been reported. In addition, their patient group consisted of patients who were hospitalized and not hospitalized, while the present patient group consisted only of patients who were hospitalized. Moreover, the current patient population comprised those who were not only hospitalized, but also actively receiving treatment. Differences in the tissues, experimental methods, and treatment protocols used, and taking samples from patients at different times may also have caused differences in the study results. There are also many cytokines and neutrophic factors that play a role in prenatal and postnatal neurogenesis, and the ERBB family is just one of them. In addition to the existence of many molecular changes, the present study also revealed differences in the expression levels of ERBB family members ERBB1 and ERBB2 in the schizophrenia patients compared to the healthy controls.

The negative regulatory effect of Lrig1 on RTKs suggests that it affects multiple cellular signaling pathways[3,36]. Lrig1 ubiquitinates ERBB family receptors and causes their degradation, which weakens ERBB signaling in the cells they are found together with[3]. In the developing brain, Jeong et al.[43] reported an increased expression of Lrig1 when radial glial progenitors (RGPs) transformed to adult NSCs. They also showed that Lrig1 deficiency leads to increased RGP proliferation through increased

EGFR activation. In addition, recent studies have revealed the effect of Lrig1 on pathways other than the ERBB family[30,36]. In other words, Lrig1 plays a role in various functions in cells, independent of the ERBB family, and this is mediated by different signaling pathways. Vincenti et al.[30] observed an increase in postnatal pyramidal glutamatergic neurons of the neocortex in mice, in which the Lrig1 gene was knocked out. They suggested that Lrig1 regulates neural progenitor proliferation via the EGF-independent pathway. Ouzikov et al.[36] studied adult NSCs and revealed that the loss of Lrig1 expression resulted in increased cell proliferation, independent of EGFR (ERBB1) signaling. They showed that there was no difference in pEGFR immunostaining and immunoblotting in the adult ventricular-subventricular zone with or without Lrig1 expression[36]. This may suggest that prenatal and postnatal Lrig1 function via different signaling pathways. In the present study, correlation analysis was performed to reveal a possible relationship between the Lrig1 and ERBB1 and ERBB2 expression levels. Lrig1 is a negative regulator of the ERBB family and there is an expected negative correlation between them. However, in this study, the Lrig1 expression levels were high in the patient group due to the patient profile. It is known that ERRBB expression levels are irregular in diseases such as schizophrenia. Therefore, although a positive correlation was seen between the Lrig1 and ERBB1 expression levels in this study, it was not a strong correlation. No significant correlation was observed between the Lrig1 and ERBB2 expression levels. This supports the possibility that Lrig1 may not be acting through the ERBB family in schizophrenia. Lrig1 may show its effects via other pathways or molecules, including mitogen-activated protein kinase and bone morphogenetic protein[44,45]. Therefore, this suggests that other targets of Lrig1 should be examined together with Lrig1 in schizophrenia. Validation of this study with a larger patient population will strengthen the results.

One limitation of the study was the small number of patients. Another limitation was that the protein levels of the genes were not measured. More comprehensive studies with larger patient populations will be effective in revealing the roles of the genes active in schizophrenia.

This study demonstrated the Lrig1 mRNA expression in the blood of schizophrenia patients for the first time. In addition, the relationship between the Lrig1 expression and ERBB1 and ERBB2 expressions in schizophrenia was investigated for the first time. Conflicting studies in the literature have indicated that ERBB expression and its functions in schizophrenia need to be studied further. Herein, it was shown that the Lrig1, ERBB1, and ERBB2 expression levels were relatively low in the schizophrenia patients compared to the healthy controls. These genes also had the power to distinguish the patients from the healthy controls. This revealed that the relationship between the Lrig1 and ERBB expressions changes in schizophrenia. We believe that these genes have the potential to be a biomarker for the disease. Therefore, it should not be overlooked that these genes also play roles in important functions that should be considered in the clinic.

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