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Research paper

Neuregulin1 types mRNA level changes in autism spectrum disorder, and is associated with deficit in executive functions

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ABSTRACT

Background: Autism spectrum disorder (ASD) is a pediatric heterogeneous psychiatric and neurodevelopmental disorder with social and communication deficits, language impairment and ritualistic or repetitive behaviors. ASD has significant genetic bases but candidate genes and molecular mechanisms of disorder are not clarified. Neuregulin1 (*NRG1*) gene, located in 8p12 is involved in development of central nervous system and was indicated as candidate gene in schizophrenia.

Methods: mRNA level of types I, II and III of *NRG1* gene were studied in peripheral blood of 1540 ASD patients (IQ > 70) and 1490 control children by quantitative Real Time PCR. Also three domains of executive functions (working memory, response inhibition and vigilance) were examined in all subjects.

Findings: All three types were significantly down regulated in ASD patients. Significant deficiencies in executive functions (EF) were found in ASD patients. EF deficiencies mostly were associated with down expression of mRNA level of types I and III. Also correlations were found between *NRG1* expression with gender and severity of ASD symptoms.

Interpretations: Findings primarily have been suggested involvement of *NRG1* in etiology of ASD. Also correlation of *NRG1* mRNA level with EF deficiencies could shed lights on EF mechanisms and may suggest targeted treatments to improve particular executive functions.

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1. Introduction

Autism spectrum disorder (ASD) is a complex psychiatric disorder which characterized by social and communication deficits, language

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Research in context

Evidence before this study

Autism spectrum disorder is heterogenic developmental problem with strong and complicated genetic bases. Several shared genes have been found between schizophrenia and Autism. Based on pub-med, science direct and Scopus data bases genetic variants and gene expression alterations of Neureguline1 (*NRG1*) had been reported in schizophrenic patients. Several studies also reported single nucleotide polymorphisms in *NRG1* associated with autism. We started working on Neureguline1 (*NRG1*) in psychiatric disorder in 2013 by a case control study in gene expression of *NRG1* types in schizophrenic patients and possible correlation with clinical symptoms. Then we continued the same approach in bipolar disorder, attention deficit hyperactivity disorder and Autism until now.

Added value of this study

Findings of present study help to clarify the role of *NRG1* in etiology of Autism and also its role in executive functions not just in autistic children also in normal children as well. In the other hand our study brings new evidence about disruption in excitation/inhibition (E/I) balance in autism. In addition sex effect and correlation of each types of NRG1 with severity of symptoms in patients showed more detail about pathophisiology of autism.

Implications of all the available evidence

Present study may help to understand about the role of epidermal growth factors such as *NRG1* in etiology of autism. Also study of each types of *NRG1* could clarify the role of each molecular pathway in neurons and neuroglia in severity of autism symptoms and executive functions deficits. Future studies may consider the expression of *NRG1* expression in neuron made by induced pluripotent stem cells from children with autism. Also future study may consider drug therapy to increase the expression of *NRG1* that based on our findings may reduce the severity of autism symptoms and also improve the executive functions ability in children.

impairment and ritualistic or repetitive behaviors and can be diagnosed after age three [1]. ASD has different prevalence in different countries. Center for disease control and prevention (CDC) of United States has estimates 1 in 59 children have been identified with ASD in USA. Studies in Asia, Europe, and North America have identified 1% average prevalence of ASD [2]. Etiology of autism spectrum is not clear but high heritability and strong genetic bases of disorder including chromosomal abnormalities and point mutations were reported in number of studies. Genetic bases of ASD have elaborated due to interactions among multiple genes and epigenetic factors which influence gene expression [3].

Number of shared genes were identified which are involved in etiology of psychiatric disorders such as Schizophrenia(SCZ), bipolar disorder, attention deficit hyperactivity disorder(ADHD), major depressive disorder(MDD) and ASD [4].Previous gene expression studies in autism spectrum disorder had been revealed expression changes in several genes in different pathways. Expression alteration of genes that are involved in serotonin pathway like *5-HTT* [5], development and functions of nervous system like *CNTNAP2* and transcription factors such as *FOXP1* and *FOXP2* were reported in ASD patients [6].

Neuregulin1 (*NRG1*) gene, located in 8p12, plays critical role in development of central nervous system and activation of neurotransmitter receptors, including glutamate receptors [7]. Several genes in human chromosome 8 are candidate genes in neuropsychiatric disorders. *VMAT1/SLC18A1*, *NRG1*, *PPP3CC* and *DPYSL2* in 8p12–13 are clearly associated with Schizophrenia and probably bipolar disorder [8].

The *NRG1* gene has at least 31 isoforms in mammal, which can be grouped into six types of proteins with different structures and distinct functional characteristics. *NRG1* types are involved in modulation of neuronal migration, synaptogenesis, glycogenesis, dendritic growth, neuron-glia communication, myelination and neurotransmission. Types I, II and III of *NRG1* express in human peripheral blood and types IV and V are brain specific [9]. Type I is related to acetylcholine receptor inducing activity, type II is a Glial growth factor and type III is a sensory and motor neuron-derived factor [10], so expression changes in each type could affect specific function in brain independently or with other types. Expression of each *NRG1* isoforms is distinctly regulated by neuronal activity [11].

Several polymorphisms and rare mutations in *NRG1* were found associated with schizophrenia [12]. Over expression of type I [13]and down expression of type III in hippocampus of schizophrenic patients were reported in post mortem studies [14].Zhang et al. were showed low expression of *NRG1* in peripheral blood of schizophrenia and Shibuya et al. later were detected decrease in serum level of *NRG1* in patients with chronic schizophrenia [15]. Studies on transgenic animal models have been showed over expression of *NRG1* type I could affect hippocampal functions and circuitry, mediated via inhibitory interneuron which induces impairment in hippocampus-dependent spatial working memory [16].Several shared genetic variants were found between schizophrenia and autism spectrum disorders especially in genes involved in synaptic activity [17].

Previous study about NRG1 expression in ASD mouse model showed positive correlation between *NRG1* mRNA level in microglia and peripheral blood mononuclear cells (PBMCs) in mice. It may support *NRG1* as potential peripheral marker. In the other hand mRNA levels of *NRG1* type III in PBMCs were positively correlated with impairments in social interaction in children with ASD which might strength evidences about *NRG1* role in etiology of Autism as well as other psychiatric disorders [18]. Recent studies bring evidence about down expression of *NRG1* types I, II and III in peripheral blood of methamphetamine and heroin dependent individuals as well as schizophrenic patients which are correlated with severity of psychotic symptoms in these patients [19].

Present study aimed to examine the expression level of types I, II and III of *NRG1* gene in peripheral blood of ASD patients. Several executive functions (EF) were examined in ASD and non psychiatric children. Also correlation of *NRG1* mRNA level with executive functions abilities were assessed.

2. Material and methods

2.1.1. Subject selection

The study was included 1540 unrelated ASD patients (1020 male,520 female)aged 7 to 11 years diagnosed by two independent senior psychiatrists by unstructured or semi structured behavioral observations in patients and interviews with patients, their parents and teachers based on DSM-V criteria and have been confirmed by exceeding threshold of Autism Diagnostic Observation Schedule (ADOS) score [2].Higher score in ADOS is referring to high sever symptoms in ASD patients. Control group was included 1490 unrelated control children (970 male, 520 female) aged 7 to11 years. Patients with IQ score (Wechsler Abbreviated Intelligence Scale, 1999) lower than 70 were excluded from study. All of the ASD samples were early diagnosed and did not start treatment when participated in study. Patients have been recruited from the outpatient psychiatric clinics of 18 provinces (from 31 provinces) of Iran. The subjects of control group were recruited from children who received regular medical checkups for school enrolment in local medical centers. The control children were recruited with matched sex, age, race, socioeconomic situation and familial situation to patients group and had no history of any psychological or somatic problem.

Supplementary Table 1 showed matching statistical data. Statistical data of 24 patients with combined ASD and ADHD were excluded from study and finally 1540 patients included to the study. None of subjects or their parents had current or history of sever medical condition, neurological disorder, history of head trauma with loss of consciousness, any psycho-stimulant or opioid drug abuse and alcohol or nicotine dependence. All subjects' parents were given an explanation on the purpose of the study and next, written informed consent has been provided. Study was approved by central ethical committee of Islamic Azad University.

2.1.2. Neuropsychological tests

Four neuropsychological tests (as listed below) were performed to analyze response inhibition, vigilance and working memory as main executive functions (EF) which their deficiency had reported in ASD patients.

2.1.2.1. 1) Integrated visual and auditory (IVA) continuous performance test (CPT). The integrated visual and auditory (IVA) continuous performance test (CPT) was designed as screening and symptoms severity test of ADHD, but also using for several neurodevelopmental psychiatric disorders including ASD. The IVA associates vigilance and impulsivity in a balanced design by visual and auditory modalities. The visual response control quotient (VRCQ) and auditory response control quotient (ARCQ) are the primary dependent variables [20,21].

2.1.2.2. 2) IVA visual attention quotient (VAQ) and Auditory attention quotient (AAQ):. We used VAQ and AAQ as primary dependent variables in this measure to examine the vigilance. Tests were performed based on previous studies method [21,22]. The VRCQ, ARCQ, VAQ and AAQ are all part of IVA-CPT test.

2.1.2.3. 3) Spatial span (SSP) of CANTABexpedio. Cambridge neuropsychological test automated battery (CANTABexpedio) examine cognitive domains including attention, executive function, memory, processing speed, and visuo-spatial ability. Spatial Span (SSP) measures both forward and reverse spatial memory span. Test was performed based on previous studies method [21,22].

2.1.3. 4) Spatial working memory (SWM)

Spatial Working Memory (SWM) measures the ability to maintain spatial information and to subsequently manipulate the presented items in working memory. This test uses boxes and colors and is suitable for children. Method of performing test had followed based on previous researches [22]. The total spatial working memory between search errors (SWM Btwn Error) and strategy scores (SWM Strategy) were considered in results [22].

2.1.4. Blood sampling and quantitative real time PCR

Blood (5 ml) was collected from the cubital vein without tourniquet between 10.00 and 11.00 AM. Total RNA was extracted from peripheral blood samples immediately after samplings according to standard protocols using by RNA Purification kit (GeneJET™ RNA Purification Kit#K0732, Thermo scientific - Fermentas, Latvia). Total RNA was treated with DNase for the removal of contaminating genomic DNA using DNase Treatment & Removal Reagents (DNase I, RNase-free (#EN0521) Fermentas, Latvia), according to the manufacturers protocol. Quality of RNA was evaluated by Agarose gel electrophoresis and UV- spectroscopy. The cDNA was synthesized using a Transcription First Strand cDNA Synthesis Kit (RevertAid Premium First Strand cDNA Synthesis Kit #K1652, Thermo scientific -Fermentas, Latvia) according to manufacturer's protocol. After alignment the sequence of all three types of *NRG1* gene and finding types' specific sequences, primers designed by "oligo7" software and were blasted on NCBI website. The phosphoglycerate kinase 1 (PGK1) gene was used for normalization as an endogenous reference gene. Primers were provided in Table 1. Agarose gel electrophoresis was used to verify the predicted size of PCR amplicons of genes. Standard curves for each gene were prepared using serial dilutions (1: 4) of pooled cDNA from total RNA extracted from blood samples of 10 control subjects. In each experiment, the R^2 value of the standard curve was >0.99 and no-template control assays was resulted in no detectable signal. Quantitative RT-PCR was performed by using SYBR green (Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2×) #K0221, Thermo scientific - Fermentas, Latvia). CFX96 Touch Real-Time PCR Detection System (BIO-RAD, California, United States) used for triplicate method Quantitative Real Time-PCR. Pfafle formula used to calculate the ratio. Quantitative Real time PCR and analysis of expression data were conducted based on previous study [23].

Table 1
Sequences of primers used for quantitative Real time PCR.

Genes primer	primer sequence
*PGK1*forward primer	5′ GTGCCAAATGGAACACGGAG3′
*PGK1*reverse primer	5′ TGCCAAGTGGAGATGCAGAA3′
NRG1 type I forward primer	5′ AGCCTCAACTGAAGGAGCAT3′
NRG1 type I reverse primer	5′ ACTCCCCTCCATTCACACAG3′
NRG1 type II forward primer	5′ CCCTATCTGGTGAAGGTGCA3′
NRG1 type II reverse primer	5′ GTCGGGCTCCATGAAGAAGA3′
NRG1 type III forward primer	5′ ATCTGCATTGTCCCCATCCT3′
NRG1 type III reverse primer	5′ GCAGTTGCGTCCAGAGAAAT3′

2.2. Statistical analysis

Descriptive data are expressed as mean ± SD (range) and level of statistical significance was set at $P < 0.05$.Compliance with normal distribution for continuous variables was assessed via the Kolmogorov-Smirnov test. Statistical differences were calculated by one-way ANOVA followed by independent Student's *t*-test for multiple group comparisons. Pearson correlation analysis was conducted to determine the relationship between variables. Bonferroni correction was used for multiple comparisons corrections. Statistical analysis was conducted by using SPSS version23.

3. Results

Demographic and clinical data have been presented in Table 2.

3.1.1. Gene expression results

Significant down regulation of all three types of *NRG1* in patient's group in compare with control subjects were detected. No significant correlation was found between gene expression results and age, age of onset, duration of illness and intelligence quality score. Exact *p* values and ratio for each type have been presented in Table 3.

Table 2
Demographic and clinical variables (Means and Standard Deviations).

VARIABLE	ASD	Normal
Gender	1020 male,520 female	970 male, 520 female
Age	9.2 ± 1.6	9.4 ± 2.3
Age of onset	3.4 ± 1.2	–
illness time	5.3 ± 3.4	–
IQ score	91.32 ± 23.42	102.4 ± 14.48
VRCQ	57.21 ± 37.66	96.22 ± 17.28
ARCQ	66.29 ± 14.62	97.46 ± 24.36
VAQ	59.31 ± 17.7	95.29 ± 28.15
AAQ	66.56 ± 28.16	97.83 ± 20.49
SSP	3.32 ± 1.34	5.74 ± 0.89
SWM Btwn Errors	66.32 ± 16.11	42.39 ± 26.48
SWM Strategy	40.21 ± 1.15	31.38 ± 3.56

VRCQ = Visual Response Control Quotient, ARCQ = Auditory Response Control Quotient, VAQ = Visual Attention Quotient, AAQ = Auditory Attention Quotient, SSP = Spatial Span, SWM = Spatial Working Memory,

Table 3
Gene expression ratio (fold change) and *p* value for *NRG1* types between groups.

Gene	ASD vs. Normal
NRG1 type I	Ratio: 0.63 p value: 0.001
NRG1 type II	Ratio: 0.65 p value: 0.002
NRG1 type III	Ratio: 0.47 p value: 0.001

3.1.2. Neuropsychological results and correlations with gene expression

3.1.2.1. 1) IVA tests' results. Significant deficiency and lower performance in ASD group in compare with control group were showed in both VRCQ (*P* value = 0.0002) and ARCQ (P value = 0.001). Also significant lower performances were detected in ASD group in compare with control group in VAQ (*P* value = 0.001) and AAQ (P value = 0.0001). In ASD group, correlation analysis between *NRG1* types expression with IVA tests results have showed significant correlation between down expression of type I and type III with lower performance of all IVA tests (VRCQ, ARCQ, VAQ and AAQ). Also down expression in type II were significantly correlated with lower performance of VRCQ. Analysis of correlation between *NRG1* expression level and IVA tests in all subjects as combined group showed significant correlation between down expression of type III with lower performance of all IVA tests. Results presented in Tables 4 and 5.

3.1.3. 2) Working memory tests results

Difference of SSP (P value = 0.002), SWM Btwn Errors (P value = 0.001) and SWM strategy (P value = 0.001) were significant between ASD and control groups which indicate significant deficiency in working memory of ASD in compare with controls. Significant correlation was found between types of *NRG1* expression and working memory deficiency. In ASD group, significant correlations were detected between down expression of type I and III with increase of SWM Btwn Errors and SWM strategy score. Also significant correlations were detected between down expression of type I, type II and type III with decrease of SSP score. Correlations between *NRG1* expression level and working memory tests in all subjects as combined group showed significant correlation between down expression of type III with increase of SWM strategy score. Summary of all correlations have been provided in Tables 4 and 5.

3.1.4. Severity of symptoms in ASD and gene expression

Diagnosis of ASD patients, have been confirmed by ADOS tests. Also severity of symptoms and their relation with gene expression and executive functions patterns were assessed. As the patients were high function, age compatible module three had been used for all ASD patients. Findings revealed significant correlation between down expression of type II and higher score of ADOS (r: −0.66, *P* value = 0.003). In addition significant correlation was found between lower score of VRCQ and higher score of ADOS (r: −0.72, P value = 0.001).

3.1.5. Sex effect analysis in ASD group

Effects of gender in severity of symptoms, executive functions and *NRG1* expression had been assessed with in ASD group. Findings showed significant down expression of type III in boys in compare with girls (P value = 0.002). In the other hand significant increase of SWM strategy score (P value = 0.002) were found in males in compare with females. Also type III down expression was significantly correlated with increase of SWM strategy score in overall ASD group (P value = 0.001, $r = -0.77$) and in ASD girls (P value = 0.003, $r = -0.62$) and ASD boys (P value = 0.003, $r = -0.71$) as independent groups.

4. Discussion

NRG1 has been implicated as a susceptibility gene in schizophrenia and there are evidences that the pattern of expression of *NRG1* isoforms may alter in schizophrenia. Present study is one of the first studies of *NRG1* role in ASD patients and suggests *NRG1* as novel potential candidate gene in etiology of ASD. Unlike the previous post-mortem genome wide expression profiling study that showed slight down expression of *NRG1* in cortex tissue, strong down regulation of *NRG1* detected in present cohort study [24]. Neuropsychological results and correlations suggest potential *NRG1* role in executive functions specially response inhibition, vigilance and working memory which their deficits previously were reported in ASD patients. However these finding probably has been improved knowledge about *NRG1* role in neuropsychiatric disorder.

NRG1 type I has a major role in neuronal plasticity which shows activity-dependent regulation, and involvement in neurotransmitter receptor expression regulations. Number of deregulations in neuronal activity such as seizures and long-term potentiation, affect type I expression. *NRG1* promotes the switch from the immature form of NMDAR, which contains primarily NR2B subunits to one containing more NR2C subunits in the central nervous system. *NRG1* also potentiates α7 nicotinic acetylcholine receptor transmission in hippocampal neurons, and expression of the β2 subunit of the γ-amino butyric acid receptor in cerebellar granule cells. Thus, significant decrease in type I expression in ASD patients may alter neuronal signaling of NRG1 per se, or induces abnormalities in maturation of NMDA receptors and GABA receptor signaling or may be both of them eventually in brain of ASD patients [25].

NRG1 type II (GGF) expression is essential for development of cortical neurons, and promotes the transformation and differentiation of radial glial cells. Glial cells in turn are involved in cortical neuronal cell migration and differentiation. It may make it reasonable that down regulation of type II mRNA level could lead to putative abnormalities of oligodendroglial functions including those functions which is related to communication and abilities in ASD patients [25]. Also correlation between type II and ADOS score may relate to the role of low functioning

Table 4
Correlation of gene expression results with demographic and clinical characteristics in ASD patients.

Gene expression	Gender	Age	Age of onset	illness time	IQ score	VRCQ	ARCQ	VAQ	AAQ	SSP	SWM Btwn Errors	SWM Strategy
NRG1 type I	*p* value: 0.16 r: - 0.14	p value: 0.36 r: 0.07	p value: 0.25 r: 0.03	p value: 0.13 r: 0.08	p value: 0.19 r: 0.04	p value: 0.001 r: 0.83	p value: 0.001 r: 0.86	p value: 0.001 r: 0.78	p value: 0.002 r: 0.72	p value: 0.0001 r: 0.8	p value: 0.001 r: −0.73	p value: 0.007 r: - 0.63
NRG1 type II	p value: 0.08 r: 0.18	p value: 0.17 r: - 0.12	p value: 0.12 r: 0.1	p value: 0.17 r: 0.07	p value: 0.15 r: 0.05	p value: 0.001 r: 0.72	p value: 0.08 r: 0.37	p value: 0.16 r: 0.29	p value: 0.46 r: 0. 14	p value: 0.003 r: 0.64	p value: 0.17 r: - 0.26	p value: 0.34 r: - 0.18
NRG1 type III	p value: 0.3 r: 0.11	p value: 0.19 r: 0.08	p value: 0.09 r: 0.11	p value: 0.23 r: 0.14	p value: 0.09 r: 0.18	p value: 0.003 r: 0.92	p value: 0.001 r: 0.94	p value: 0.002 r: 0.88	p value: 0.003 r: 0.81	p value: 0.002 r: 0.84	p value: 0.002 r: - 0.89	p value: 0.001 r: - 0.77

VRCQ = Visual Response Control Quotient, ARCQ = Auditory Response Control Quotient, VAQ = Visual Attention Quotient, AAQ = Auditory Attention Quotient, SSP = Spatial Span, SWM = Spatial Working Memory.

Table 5
Correlation of gene expression results with demographic and clinical characteristics in all subjects.

Gene expression	Gender	Age	IQ score	VRCQ	ARCQ	VAQ	AAQ	SSP	SWM Btwn Errors	SWM Strategy
NRG1 type I	p value: 0.12 r: - 0.9	p value: 0.16 r: 0.09	p value: 0.23 r: 0.11	p value: 0.17 r: 0.15	p value: 0.44 r: 0.18	p value: 0.11 r: 0.23	p value: 0.14 r: 0.19	p value: 0.37 r: 0.04	p value: 0. 2 r: −0.17	p value: 0.23 r: −0.06
NRG1 type II	p value: 0.07 r: 0.11	p value: 0.9 r: 0.03	p value: 0.07 r: 0.16	p value: 0.14 r:0.27	p value: 0.07 r: 0.2	p value: 0.09 r: 0.34	p value: 0.08 r: 0.32	p value: 0.12 r: 0.29	p value: 0.07 r: - 0.3	p value: 0.14 r: −0.12
NRG1 type III	p value: 0.4 r: 0.13	p value: 0.1 r: 0.2	p value: 0.13 r: 0.2	p value: 0.002 r:0.62	p value: 0.001 r: 0.68	p value: 0.002 r: 0.73	p value: 0.003 r: 0.56	p value: 0.01 r: 0.27	p value: 0.03 r: - 0.41	p value: 0.002 r: −0.53

VRCQ = Visual Response Control Quotient, ARCQ = Auditory Response Control Quotient, VAQ = Visual Attention Quotient, AAQ = Auditory Attention Quotient, SSP = Spatial Span, SWM = Spatial Working Memory.

of glial cells in severity of symptoms and cognitive disabilities in ASD patients.

NRG1 type III (SMDF) is involved with early development and survival of the Schwann cells. Previous studies were confirmed the role of type III signaling in the maintenance of corticostriatal components and in the neural circuits involved in sensorimotor gating and working memory [26]. Nicodemus et al. were reported down expression of type III in hippocampus -which is the main region of memory in schizophrenic patients [14]. Down regulation of type III mRNA level in ASD patients could explains sensorimotor abnormalities such as language impairments (as sensorimotor related symptom) in these patients [27,28].

NRG1-ErbB4 signaling regulates the function of amygdale. Inhibition of NRG1-ErbB4 signaling which is natural consequence of *NRG1* down regulation, decrease GABAergic activity and disrupts the excitation/inhibition (E/I) balance. Altered E/I balance is a pathophysiological mechanism of many psychological diseases, such as autism and schizophrenia [29]. Different lines of evidence were showed reduced GABAergic signaling in the brains of autistics children. It may be related to down regulation of *NRG1* as an upper hand growth factor which is involved in signal transduction of GABAergic neurons. Significant down expression of type III in boys in compare with girls may strength the evidence about association of GABAergic signaling abnormalities and repetitive behaviors (which are most sever in boys) in ASD patients.

4.1. Executive functions deficits association with NRG1

Results of neuropsychological battery of EF measures across three domains (response inhibition, vigilance and working memory) were confirmed previous studies about EF deficit in ASD patients [19,30]. Associations between schizophrenia related risk allele of *NRG1* and *NRG3* with memory and attention deficits had been previously reported [31,32]. In addition impacts of type I over expression on working memory abnormalities was reported in transgenic mice [33]. Present findings were showed impacts of down regulation of *NRG1* types on working memory and attention deficits which may cause by abnormalities in neuronal plasticity, gelial cell functions and sensorimotor. Type III which is a sensory and motor neuron-derived factor has showed strongest correlation with all three domains.

NRG1 mRNA level increase by particular antipsychotic treatments in schizophrenic patients [34,35]. Correlation of *NRG1* down regulation with executive functions deficits that were detected in ASD patients may suggest that same antipsychotic treatments might increase *NRG1* mRNA level and improve executive functions such as memory and attention in ASD patients as well.

Neuroimaging assessments in addition to gene expression and genotyping examinations could provide more reliable results about role of neuregulin family and their receptors in ASD. Also evaluation of antipsychotic effects on *NRG1* mRNA level in ASD patients would provide interesting data for therapists.

5. Conclusion

We primarily suggest involvement of *NRG1* main types in etiology of autism spectrum disorders. Correlation of executive functions deficits with down regulation of *NRG1* types have showed important role of *NRG1* in orchestration of number of executive functions including response inhibition, vigilance and working memory in ASD patients.

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Declaration of interest

The authors declare that they have nothing to disclose.

Ethics committee approval

The project followed the Helsinki declaration obligations and had been approved by central ethical committee of Islamic Azad University.

Author Contributions

Samane Abbasy, Fazlollah Shahraki, Masoume Ghasemzadeh Qazvini, Sahel Towfigh Rafiei, Horie Rezvani asl, Atena alsadat Shiryazdi, Mehdi Farhadi, Elnaz Noshadirad and Rana Ghamari were involved in clinical and laboratory data collection and data analysis. They have also participated in editing the manuscript. Zeinab Tabrizi, Rashed Mehrfard, Fereshteh Esmaili kakroudi, Mahsima Azarnoosh, Seyede Mahsa Bagheri, Faeghe Younesi, Narges Parsamehr, Nooriyeh Garaei, Soroush Abyari, Melika Pourmehrabi, Pardis Zolfaghari, Maryam Gholami, Maede Salehi, Elham Rastegarimogaddam, Elnaz Nobakht, Elmira Nobakht and Rayan Partovi were involved in laboratory data collection and data analysis. Arvin Haghighatfard was the head of the research team. He was the study designer and was involved in clinical and laboratory data collection, analysis of data and writing the manuscript.

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Appendix A. Supplementary data

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References

[1] Gregg JP, Lit L, Baron CA, Hertz-Picciotto I, Walker W, Davis RA, et al. Gene expression changes in children with autism. Genomics 2008;91(1):22–9.
[2] Frieden TR, Jaffe HW, Cono J, Richards CL, Iademarco MF: Prevalence of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network,11 sites, United States, 2010. MMWR 2014, vol. 63(No. 2):1.
[3] Rapin I, Tuchman RF. Autism: definition, neurobiology, screening, diagnosis. Pediatr Clin North Am 2008;55(5):1129–46 [viii].
[4] Nothen MM, Nieratschker V, Cichon S, Rietschel M. New findings in the genetics of major psychoses. Dialogues Clin Neurosci 2010;12(1):85–93.
[5] Hu VW, Frank BC, Heine S, Lee NH, Quackenbush J. Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in severity of autism reveals differential regulation of neurologically relevant genes. BMC Genomics 2006;7:118.
[6] Chien WH, Gau SS, Chen CH, Tsai WC, Wu YY, Chen PH, et al. Increased gene expression of FOXP1 in patients with autism spectrum disorders. Mol Autism 2013;4(1):23.
[7] Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, et al. Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 2002;71(4):877–92.
[8] Tabares-Seisdedos R, Rubenstein JL. Chromosome 8p as a potential hub for developmental neuropsychiatric disorders: implications for schizophrenia, autism and cancer. Mol Psychiatry 2009;14(6):563–89.
[9] Deng C, Pan B, Engel M, Huang XF. Neuregulin-1 signalling and antipsychotic treatment: potential therapeutic targets in a schizophrenia candidate signalling pathway. Psychopharmacology (Berl) 2013;226(2):201–15.
[10] Mei L, Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nat Rev Neurosci 2008;9(6):437–52.
[11] Liu X, Bates R, Yin DM, Shen C, Wang F, Su N, et al. Specific regulation of NRG1 isoform expression by neuronal activity. J Neurosci 2011;31(23):8491–501.
[12] Kukshal P, Bhatia T, Bhagwat AM, Gur RE, Gur RC, Deshpande SN, et al. Association study of neuregulin-1 gene polymorphisms in a North Indian schizophrenia sample. Schizophr Res 2013;144(1–3):24–30.
[13] Law AJ, Lipska BK, Weickert CS, Hyde TM, Straub RE, Hashimoto R, et al. Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5' SNPs associated with the disease. Proc Natl Acad Sci U S A 2006;103(17):6747–52.
[14] Nicodemus KK, Law AJ, Luna A, Vakkalanka R, Straub RE, Kleinman JE, et al. A 5′ promoter region SNP in NRG1 is associated with schizophrenia risk and type III isoform expression. Mol Psychiatry 2009;14(8):741–3.
[15] Shibuya M, Komi E, Wang R, Kato T, Watanabe Y, Sakai M, et al. Measurement and comparison of serum neuregulin 1 immunoreactivity in control subjects and patients with schizophrenia: an influence of its genetic polymorphism. J Neural Transm (Vienna) 2010;117(7):887–95.
[16] Deakin IH, Nissen W, Law AJ, Lane T, Kanso R, Schwab MH, et al. Transgenic overexpression of the type I isoform of neuregulin 1 affects working memory and hippocampal oscillations but not long-term potentiation. Cereb Cortex 2012;22(7):1520–9.
[17] Kenny EM, Cormican P, Furlong S, Heron E, Kenny G, Fahey C, et al. Excess of rare novel loss-of-function variants in synaptic genes in schizophrenia and autism spectrum disorders. Mol Psychiatry 2014;19(8):872–9.
[18] Ikawa D, Makinodan M, Iwata K, Ohgidani M, Kato TA, Yamashita Y, et al. Microglia-derived neuregulin expression in psychiatric disorders. Brain Behav Immun 2017 Mar 1;61:375–85.
[19] Haghighatfard A, Hosseini Z, Mahmoodi A, Mahmoodi A, Qazvini MG. NRG1 Types down Regulate in Methamphetamine and Heroin Dependent patients. MOJ Addict Med Ther 2016;3(2) (00030).
[20] Sanford JA, Turner A. Manual for the integrated visual and auditory continuous performance test. Richmond, VA: Brain Train; 2004 (INC).
[21] Corbett BA, Constantine LJ, Hendren R, Rocke D, Ozonoff S. Examining executive functioning in children with autism spectrum disorder, attention deficit hyperactivity disorder and typical development. Psychiatry Res 2009;166(2–3):210–22.
[22] C C. CANTAB: Cambridge; England; 2002.
[23] Haghighatfard A, Andalib S, Amini Faskhodi M, Sadeghi S, Ghaderi AH, Moradkhani S, et al. Gene expression study of mitochondrial complex I in schizophrenia and paranoid personality disorder. World J Biol Psychiatry 2017;16:1–4.
[24] Parikshak NN, Swarup V, Belgard TG, Irimia M, Ramaswami G, Gandal MJ, et al. Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. Nature 2016;540(7633):423.
[25] Hashimoto R, Straub R, Weickert CS, Hyde T, Kleinman J, Weinberger D. Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. Mol Psychiatry 2004;9(3):299–307.
[26] Leimeroth R, Lobsiger C, Lüssi A, Taylor V, Suter U, Sommer L. Membrane-bound neuregulin1 type III actively promotes Schwann cell differentiation of multipotent progenitor cells. Dev Biol 2002;246(2):245–58.
[27] Chen Y-JJ, Johnson MA, Lieberman MD, Goodchild RE, Schobel S, Lewandowski N, et al. Type III neuregulin-1 is required for normal sensorimotor gating, memory-related behaviors, and corticostriatal circuit components. J Neurosci 2008;28(27):6872–83.
[28] Takarae Y, Luna B, Minshew NJ, Sweeney JA. Patterns of visual sensory and sensorimotor abnormalities in autism vary in relation to history of early language delay. J Int Neuropsychol Soc 2008;14(6):980.
[29] Lu Y, Sun X-D, Hou F-Q, Bi L-L, Yin D-M, Liu F, et al. Maintenance of GABAergic activity by neuregulin 1-ErbB4 in amygdala for fear memory. Neuron 2014;84(4):835–46.
[30] Goldberg MC, Mostofsky SH, Cutting LE, Mahone EM, Astor BC, Denckla MB, et al. Subtle executive impairment in children with autism and children with ADHD. J Autism Dev Disord 2005;35(3):279–93.
[31] Krug A, Markov V, Krach S, Jansen A, Zerres K, Eggermann T, et al. The effect of Neuregulin 1 on neural correlates of episodic memory encoding and retrieval. Neuroimage 2010;53(3):985–91.
[32] Meier S, Strohmaier J, Breuer R, Mattheisen M, Degenhardt F, Mühleisen TW, et al. Neuregulin 3 is associated with attention deficits in schizophrenia and bipolar disorder. Int J Neuropsychopharmacol 2013;16(3):549–56.
[33] Deakin IH, Nissen W, Law AJ, Lane T, Kanso R, Schwab MH, et al. Transgenic overexpression of the type I isoform of neuregulin 1 affects working memory and hippocampal oscillations but not long-term potentiation. Cereb Cortex 2012;22(7):1520–9.
[34] Deng C, Pan B, Engel M, Huang X-F. Neuregulin-1 signalling and antipsychotic treatment. Psychopharmacology (Berl) 2013;226(2):201–15.
[35] Zhang H-X, Zhao J-P, Lv L-X, Li W-Q, Xu L, Ouyang X, et al. Explorative study on the expression of neuregulin-1 gene in peripheral blood of schizophrenia. Neurosci Lett 2008;438(1):1–5.