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Neurexin 1 (NRXN1) Splice Isoform Expression During Human Neocortical Development and Aging

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Abstract

Neurexin 1 (NRXN1), a presynaptic adhesion molecule, is implicated in several neurodevelopmental disorders characterized by synaptic dysfunction including, autism, intellectual disability, and schizophrenia. To gain insight into NRXN1's involvement in human cortical development we used quantitative real time PCR to examine the expression trajectories of NRXN1, and its predominant isoforms NRXN1-α and NRXN1-β in prefrontal cortex from fetal stages to aging. Additionally, we investigated whether prefrontal cortical expression levels of NRXN1 transcripts are altered in schizophrenia or bipolar disorder in comparison to non-psychiatric control subjects. We observed that all three NRXN1 transcripts were highly expressed during human fetal cortical development, dramatically increasing with gestational age. In the postnatal DLPFC, expression levels were negatively correlated with age, peaking at birth until approximately 3 years of age, after which levels declined dramatically to be stable across the lifespan. NRXN1-β expression was modestly but significantly elevated in the brains of patients with schizophrenia compared to non-psychiatric controls, whereas NRXN1-α expression was increased in bipolar disorder. These data provide novel evidence that NRXN1 expression is highest in human prefrontal cortex during critical developmental windows relevant to the onset and diagnosis of a range of neurodevelopmental disorders, and that NRXN1 expression may be differentially altered in neuropsychiatric disorders.

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Conflict of Interest

The authors declare no conflict of interest.

Introduction

Genetic studies have identified neurexin-1 (NRXN1; 2p16.3) as a risk gene for several neurodevelopmental disorders, including autism spectrum disorder, schizophrenia, developmental delay and mental retardation,[1–7] implicating a pleotropic role for NRXN1 in human cortical development. In schizophrenia, copy number variations (CNVs) disrupting the NRXN1 promoter and first exon have been observed,[8–15] and meta-analyses reveal that the presence of deletions within the gene confer a substantial increase in risk of the disorder (OR 4.78), which increases further when restricted to functional deletions >100kb (OR 7.44).[8] Common genetic variation in NRXN1 has also been shown to impact clinical responsiveness to antipsychotic[16–18] and antidepressant treatments,[19] as well as nicotine dependence.[20,21]

The NRXN1 gene spans 1.12 Mb and contains 23 exons, making it one of the largest within the human genome.[22] Two major isoforms, NRXN1-α and -β, are each transcribed from alternative promoters.[23] The NRXN1-α promoter is located at the proximal end of the gene and is most frequently eliminated by microdeletions, whilst the -β promoter is located further downstream of exon 17 and usually is unaffected. In addition, the gene contains five canonical splice sites, which could give rise to potentially more than 1,000 unique isoforms.[23,24]

NRXN1 is a member of the larger neurexin family of proteins (NRXN1–3), which function in the vertebrate nervous system as presynaptic cell adhesion molecules and receptors that play critical roles in synaptic development.[25] NRXN1 is a critical mediator of the assembly and maturation of synapses, with expression of NRXN1 sufficient to induce synapse formation in cultured non-neuronal cells.[26] Presynaptic neurexin proteins form trans-synaptic adhesion complexes with postsynaptic neuroligin proteins. These neuroligin/neurexin adhesion complexes are responsible for the development of glutamatergic and GABAergic synapses, governing the balance of excitatory and inhibitory synapse formation and function in the central nervous system.[27–30] Additionally, several members of the neuroligin family have also been implicated in genetic risk for neurodevelopmental and neuropsychiatric disorders including schizophrenia and autism spectrum disorder.[31,32]

Despite strong genetic candidacy for involvement in neurodevelopmental disorders, and NRXN1 being a fundamental regulator of synaptic function, the quantitative expression profiles of NRXN1 and its major isoforms during human brain development and maturation remain unknown. In this study we sought to characterize the expression of NRXN1 and its two major isoforms throughout normal human brain development as well as in a cohort of patients with schizophrenia or bipolar disorder. Together, our findings support the hypothesis that NRXN1, both alpha and beta isoforms, are highly expressed during critical periods of synapse development being enriched in the prefrontal cortex during the late gestational period and early postnatal life. Additionally, we identify that NRXN1 expression levels are altered in a transcript specific manner in the context of neuropsychiatric disorders, with the expression of NRXN1-α and NRXN1-β being subtly but significantly upregulated in the prefrontal cortex of patients with bipolar disorder and schizophrenia, respectively. This data

provides the first evidence for a divergence in the involvement of individual NRXN1 isoforms in distinct neuropsychiatric disorders.

Materials and Methods

Human Postmortem Brain Samples

Postmortem human brains from the Clinical Brain Disorders Branch were obtained at autopsy primarily from the Washington, D.C. and Northern Virginia medical examiners' offices, all with informed consent from the legal next of kin (protocol 90-M-0142 approved by the NIMH/National Institutes of Health Institutional Review Board). Additional postmortem fetal, infant, child, and adolescent brain tissue samples were provided by the National Institute of Child Health and Human Development Brain and Tissue Bank for Developmental Disorders (www.btbank.org) under contracts NO1-HD-4–3368 and NO1-HD-4–3383. The institutional review board of the University of Maryland at Baltimore and the State of Maryland approved the protocol, and the tissue was donated to the NIMH under the terms of a Material Transfer Agreement. Clinical characterization, diagnoses, and macro- and microscopic neuropathological examinations were performed on all CBDB cases using a standardized paradigm. Cases at the University of Maryland at Baltimore were handled similarly (http://medschool.umaryland.edu/BTBank/ProtocolMethods.html). Toxicological analysis was performed on every case. All non-psychiatric control subjects were free of a history of psychiatric illness or significant alcohol or substance abuse.

A total of 245 non-psychiatric control individuals ranging in age from 0–85 years [mean age 33.3 y; 82 female, 163 male; 133 African American, 105 Caucasian, 4 Hispanic, 3 Asian; Post Mortem Interval (PMI) 29.92 (SD=14.86 h); pH 6.49 (SD= 0.30); RNA Integrity Number (RIN) 8.2 (SD= 0.86)], 110 individuals with schizophrenia ranging in age from 18–85 years were available for this study [mean age, 52.2y; 42 female, 68 male; 67 African American, 39 Caucasian; 3 Hispanic, 1 Asian; PMI, 38.68 (SD=14.83 h); pH 6.34 (SD=0.25); RIN 7.98 (SD=0.95)], and 34 individuals with bipolar disorder ranging in age from 20–79 years were available for this study [mean age, 42.26y; 13 female, 21 male; 5 African American, 28 Caucasian; 1 Asian; PMI, 31.34 (SD=15.4 h); pH 6.23 (SD=0.19); RIN 7.98 (SD=0.73)]. Fetal frontal cortex samples were derived from 43 subjects from elective termination. 5 subjects were removed from analysis due to identification of maternal substance abuse, therefore the remaining 39 normal subjects are reported in the current study (age range, gestational weeks 14–39, 20 female, 18 male, 1 unknown gender; 34 African American, 5 Caucasian; PMI 2.48 (SD=2.1h); RIN 8.72 (SD=1.27)). Diagnoses were determined using the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) criteria. Toxicological screening was performed on all cases for ethanol, cocaine and metabolites, opiates (including prescription narcotics, methadone, and heroin), benzodiazepines, and phencyclidine (substance abuse definition). Additional supplemental, toxicology testing was done through National Medical Services (NMS Labs, Inc., www.nmslabs.com, Willow Grove, PA), with screening for cannabinoids and metabolites, nicotine and cotinine and prescribed medications such as antidepressants, antipsychotics in psychiatric cases. Ethanol intoxication above 0.06 mg/dL or the presence of any illicit drug were exclusion criteria for non-psychiatric controls. Toxicology was routinely performed on

blood or cerebellar tissue, but some testing was performed in bile or urine depending on the availability of tissues. Full demographic information is also provided in Supplemental Table 1.

Sample preparation and Real-Time Quantitative PCR

Total RNA extraction, RNA quantitation and qualification as well as first strand cDNA synthesis was carried out as previously described.[33] Expression levels of human NRXN1 and its predominant splice isoforms NRXN1-α and NRXN1-β, were measured using Taqman Gene Expression Assays (Applied Biosystems, CA, USA): Hs00985129_m1, Hs00373346_m1, and Hs00985123_m1, respectively, by real time RT-PCR using an ABI Prism 7900 sequence detection system with 384-well format (Applied Biosystems) and quantified via the standard curve method, as described previously.[33] The expression of NRXN1 transcripts in the human brain were highly abundant and the selected TaqMan Gene Expression Assays efficiently detected each transcript with the Ct values for NRXN1 transcripts of 19–25 (pan NRXN), 20–26 (NRXN1-α), 20–28 (NRXN1-β). Our primary data analysis was based on normalization of NRXN1 mRNA expression to the geometric mean of the quantity of three internal control genes: β-actin, GADPH, and PBGD as described previously.[34]

Statistical Analyses

Statistical analyses were performed using IBM Statistic SPSS, version 21. Effects of gestational age on NRXN1 isoform expression were analyzed in fetal brain samples (14–39 weeks) using Linear Regression analysis, controlling for sex. The effect of age on NRXN1 expression was assessed in non-psychiatric control individuals (0–85 years) by Linear Regression analysis controlling for sex, race, pH, RIN and PMI. The effect of diagnosis on NRXN1 expression was explored in age matched samples (18–85 years) using ANCOVA with diagnosis, sex and race as the fixed variables and RIN, pH, PMI as covariates. To examine potential effects of neuroleptic treatment on gene expression, correlations were explored between lifetime chlorpromazine equivalents and NRXN1 isoform expression in schizophrenia subjects using Spearman's correlation analysis. Additionally, the effects of antipsychotic or antidepressant medication in bipolar patients on NRXN1 gene expression were explored using ANCOVA with antipsychotic status and antidepressant status as fixed variables. Effects of substance abuse (positive or negative) on NRXN1-α, or NRXN1-β gene expression in subjects diagnosed with bipolar disorder or schizophrenia, respectively, were assessed by ANCOVA. To assess effects of nicotine exposure on NRXN1 gene expression, univariate ANOVA was used to explore main effects and the interaction between diagnosis and positive identification of nicotine. All experiments were conducted blind to diagnosis and age. All available samples were included in final analyses, with the exception of 5 fetal subjects (described above) who were excluded based on positive toxicology.

Results

NRXN1-α and NRXN1-β expression increases during human fetal cortical development

We assessed quantitative expression of NRXN1 isoforms in human fetal prefrontal cortex derived from 39 fetal samples during the second trimester of gestation (gestational age 14–

39 weeks). Expression levels of NRXN1, NRXN1-α and NRXN1-β were highly significantly correlated with gestational age (NRXN1: β=0.785, F(2,36)=31.509, $p=1.23E^{-8}$; NRXN1-α: β=0.383, F=(2,36)=5.024, p=0.012; NRXN1-β: β=0.666, F(2,36)=15.513, $p=1.38E^{-5}$). Expression of the transcripts increased significantly throughout fetal brain development, reaching peak expression levels at 39 weeks gestation (Figure 1a–c).

Significant effects of age on NRXN1-α and β expression during the normal postnatal human lifespan

We next explored the temporal dynamics of NRXN1 isoform expression across postnatal development in DLPFC tissue derived from a large cohort of non-psychiatric controls ranging from 0–85 years of age. Analysis of quantitative expression trajectories of NRXN1, NRXN1-α and NRXN1-β revealed a strong negative correlation between expression levels and postnatal age (NRXN1: β=−0.615, F(6,240)=27.123, $p=1.3E^{-24}$; NRXN1-α: β=−0.577, F=(6,240)=19.379, $p=2.1E^{-18}$; NRXN1-β: β=−0.499, F(6,240)=15.043, $p=6.28E^{-15}$) (Figure 2a, b, c). Similar to the findings in fetal brain development, expression levels of all three transcripts showed highly overlapping expression profiles during postnatal cortical development, with NRXN1, NRXN1-α and NRXN1-β expression levels being highest at birth, then steadily decreasing until approximately 3 years of age, after which expression remained steady throughout childhood, adolescence and aging. These patterns of expression trajectories across late prenatal development, neonatal life, peri-adolescence and into aging are shown as interpolation plots (Figure 3a–c).

NRXN1 isoform expression is differentially increased in the DLPFC of patients with schizophrenia or bipolar disorder

Given the genetic association of variants in NRXN1 and increased risk for schizophrenia and other neurodevelopmental disorders, we compared quantitative expression of NRXN1, NRXN1-α and NRXN1-β in the DLPFC of a large cohort of individuals diagnosed with either schizophrenia (n=110) or bipolar disorder (n=34) compared to group age matched non-psychiatric controls (n=178). Expression levels of NRXN1-α were significantly elevated in patients with bipolar disorder compared to the non-psychiatric controls (F(1,198)=6.706, p=0.01) (Supplemental Fig S1a). Of the 34 individuals with bipolar disorder, 9 were positive for antipsychotic medication (status not available for 2 subjects) and 18 positive for antidepressants at time of death, as assessed by toxicological examination. There was no main effect of antipsychotic medication status (F(1,188)=0.319, p>0.05) or antidepressant status (F(1,188)=0.589, p>0.05) on NRXN1-α gene expression levels.

In schizophrenia, levels of NRXN1-β were significantly elevated in the DLPFC of patients compared to the non-psychiatric controls (F(1,273)=6.816, p=0.01) (Supplemental Fig S1b). Correlation analysis in a subset of schizophrenia patients (data available for n=32) failed to demonstrate association of lifetime chlorpromazine equivalents with NRXN1-α expression (r= −0.087, p>0.05). Regarding substance abuse, 28 of the 34 patients diagnosed with bipolar disorder and 38 of the 110 patients with schizophrenia had a positive toxicology at time of death. Since this was an exclusion criterion, no non-psychiatric control subjects were positive for substance abuse. ANCOVA analysis showed no main effect of substance abuse

on NRXN1-α expression in bipolar patients ($F(1,196)=0.217$, $p>0.05$) or on NRXN1-β expression in schizophrenia patients (($F1,267)=2.839$, $p>0.05$). Nicotine status, as assessed at time of death, was available for a subset of each diagnostic group (n=176 non-psychiatric controls, n=109 schizophrenia patients and n=33 bipolar disorder patients). Univariate ANOVA revealed no main effect of nicotine ($p>0.05$) or interaction between diagnosis and nicotine status ($F(1,203)=0.418$, $p>0.05$) on NRXN1-α expression in bipolar disorder patients and non-psychiatric controls. In addition, no main effect of nicotine ($p>0.05$) or interaction between diagnosis and nicotine status ($F(1,279)=0.362$, $p>0.05$) was observed on NRXN1-β expression in schizophrenia patients and non-psychiatric controls.

Discussion

Here we provide quantitative insight into the developmental trajectory of NRXN1 isoform expression in human brain across the prenatal and postnatal lifespan. In support of recent genetic studies that have implicated the disruption of NRXN1 in the pathogenesis of several neurodevelopmental disorders[1–7], we demonstrate that NRXN1 expression is highest during critical periods of human brain development, specifically during the 2nd trimester to approximately 3 years of postnatal age. In addition, we show that divergent isoform-specific expression changes exist in patients with schizophrenia or bipolar disorder. Together, these data provide insight into the relevance of NRXN1 in normal brain development and function and demonstrate how alterations in NRXN1 expression and modulation of NRXN1 splicing may be pathophysiologically relevant to neurodevelopmental and neuropsychiatric disorders.

Our findings of peak expression levels of NRXN1 in the human cortex during the late embryonic period and early postnatal life strengthen the link between NRXN1 and neurodevelopmental disorders.[1–7] In accordance with previous findings that NRXN1 is essential for synaptogenesis, we found that NRXN1 expression peaks during critical periods for synapse construction, maturation, refinement and modulation.[35,36] Remarkably, the expression trajectory of NRXN1-α and β in the human cortex parallels the synaptic density profile in the postnatal human cortex.[37] In addition, previous studies have demonstrated that in the murine and chick brain NRXN1 expression is detectable from embryonic day 10 and increases with development.[38–40] To our knowledge the current study is the first to demonstrate the developmental expression trajectories of NRXN1 and its splice isoforms in the human brain.

Alternative splicing of NRXN1 is a dynamic process that is dependent upon neuronal activity.[41–43] Splicing and alternative promoter usage in the NRXN1 gene is a highly regulated process that determines the interactions between NRXN1 variants and their preferential postsynaptic partners. Studies examining the alternative regulation of NRXN1 isoforms suggest that these differential trans-synaptic protein combinations confer a molecular code specifies the type of synapse that will eventually form in the developing brain.[26,29,44–47] For example, NRXN1-α isoforms have been implicated in the development of GABAergic synapses,[26,29,44] whereas NRXN1-β variants are more selective in binding to glutamatergic postsynaptic partners such as Neuroligin-1 and LRRTM1.[29,45–47]

Interestingly, we identified subtle, but significant, disorder-specific increases in NRXN1-α and β expression in bipolar disorder and schizophrenia, respectively; changes which did not correlate with potential confounds, including neuroleptic medication, nicotine exposure or substance abuse. Although the pathophysiological relevance of the changes is unknown, our data suggest an immature pattern of NRXN1 expression in these disorders, which may contribute to diagnostic-specific disruptions in the excitatory/inhibitory balance of the brain. Although these findings are somewhat unexpected in the context of observations that the majority of rare CNVs identified in NRXN1 in psychiatric disorders, are hemizygous exonic microdeletions[8–15] - which are predicted to decrease gene expression, they are not totally surprising. To our knowledge, empirical data on NRXN1-α or β gene expression in brain (or the balance between the two isoforms) is absent in patients carrying microdeletions, making it unclear exactly what impact the mutations have on gene expression. Furthermore, our findings in conjunction with recent data demonstrating that increased NRXN1 expression in adult rodent cortex is associated with impaired learning and memory[48] lend support to the notion that elevated NRXN1 expression may be relevant to neuropsychiatric illness. We also note, that given the low rates of CNVs reported in schizophrenia (0.12–1.07%[8–15]) and bipolar disorder (0.2%[49]) it is unlikely that rare CNVs contribute to the expression changes observed in our study. Finally, despite the proposed genetic overlap in schizophrenia and bipolar disorder,[50] we report diagnostic-specific effects on NRXN1 isoform expression. Although the mechanistic basis of these molecular differences is unknown, they may reflect differential effects of illness state, disease course or disorder-specific genetic risk factors which directly or indirectly affect NRXN1 expression. Further studies are required to address these questions.

In summary, our results demonstrate that NRXN1 alternative isoform expression is temporally regulated during critical periods of human neocortical development and identify potential differential molecular contributions of NRXN1-α and NRXN1-β to schizophrenia and bipolar disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, et al. Autism Genome Project C. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat Genet. 2007; 39(3):319–328. [PubMed: 17322880]
2. Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, et al. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet. 2008; 82(2):477–488. [PubMed: 18252227]

3. Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, et al. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature. 2009; 459(7246):569–573. [PubMed: 19404257]

4. Bena F, Bruno DL, Eriksson M, van Ravenswaaij-Arts C, Stark Z, Dijkhuizen T, et al. Molecular and clinical characterization of 25 individuals with exonic deletions of NRXN1 and comprehensive review of the literature. Am J Med Genet. 2013; 162B(4):388–403. [PubMed: 23533028]

5. Ching MS, Shen Y, Tan WH, Jeste SS, Morrow EM, Chen X, et al. Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders. Am J Med Genet. 2010; 153B(4):937–947. [PubMed: 20468056]

6. Gregor A, Albrecht B, Bader I, Bijlsma EK, Ekici AB, Engels H, et al. Expanding the clinical spectrum associated with defects in CNTNAP2 and NRXN1. BMC Med Genet. 2011; 12:106. [PubMed: 21827697]

7. Tucker T, Zahir FR, Griffith M, Delaney A, Chai D, Tsang E, et al. Single exon-resolution targeted chromosomal microarray analysis of known and candidate intellectual disability genes. Eur J Hum Genet. 2014; 22(6):792–800. [PubMed: 24253858]

8. Kirov G, Gumus D, Chen W, Norton N, Georgieva L, Sari M, et al. Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. Hum Mol Genet. 2008; 17(3):458–465. [PubMed: 17989066]

9. Ikeda M, Aleksic B, Kirov G, Kinoshita Y, Yamanouchi Y, Kitajima T, et al. Copy number variation in schizophrenia in the Japanese population. Biol Pysch. 2010; 67(3):283–286.

10. International Schizophrenia C. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature. 2008; 455(7210):237–241. [PubMed: 18668038]

11. Need AC, Keefe RS, Ge D, Grossman I, Dickson S, McEvoy JP, et al. Pharmacogenetics of antipsychotic response in the CATIE trial: a candidate gene analysis. Eur J Hum Genet. 2009; 17(7):946–957. [PubMed: 19156168]

12. Rujescu D, Ingason A, Cichon S, Pietilainen OP, Barnes MR, Toulopoulou T, et al. Disruption of the neurexin 1 gene is associated with schizophrenia. Hum Mol Genet. 2009; 18(5):988–996. [PubMed: 18945720]

13. Vrijenhoek T, Buizer-Voskamp JE, van der Stelt I, Strengman E, Genetic R, et al. Outcome in Psychosis C. Recurrent CNVs disrupt three candidate genes in schizophrenia patients. Am J Hum Genet. 2008; 83(4):504–510. [PubMed: 18940311]

14. Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science. 2008; 320(5875):539–543. [PubMed: 18369103]

15. Todarello G, Feng N, Kolachana BS, Li C, Vakkalanka R, Bertolino A, et al. Incomplete penetrance of NRXN1 deletions in families with schizophrenia. Schizo Res. 2014; 155(1–3):1–7.

16. Souza RP, Meltzer HY, Lieberman JA, Le Foll B, Kennedy JL. Influence of neurexin 1 (NRXN1) polymorphisms in clozapine response. Hum Psychopharm. 2010; 25(7–8):582–585.

17. Lett TA, Tiwari AK, Meltzer HY, Lieberman JA, Potkin SG, Voineskos AN, et al. The putative functional rs1045881 marker of neurexin-1 in schizophrenia and clozapine response. Schiz Res. 2011; 132(2–3):121–124.

18. Jenkins A, Apud JA, Zhang F, Decot H, Weinberger DR, Law AJ. Identification of candidate single-nucleotide polymorphisms in NRXN1 related to antipsychotic treatment response in patients with schizophrenia. Neuropsychopharm. 2014; 39(9):2170–2178.

19. Tansey KE, Rucker JJ, Kavanagh DH, Guipponi M, Perroud N, Bondolfi G, et al. Copy number variants and therapeutic response to antidepressant medication in major depressive disorder. Pharmacogenomics J. 2014; 14(4):395–399. [PubMed: 24445990]

20. Nussbaum J, Xu Q, Payne TJ, Ma JZ, Huang W, Gelernter J, et al. Significant association of the neurexin-1 gene (NRXN1) with nicotine dependence in European- and African-American smokers. Hum Mol Genet. 2008; 17(11):1569–1577. [PubMed: 18270208]

21. Yang J, Wang S, Yang Z, Hodgkinson CA, Iarikova P, Ma JZ, et al. The contribution of rare and common variants in 30 genes to risk nicotine dependence. Mol Psych. 2014

22. Rowen L, Young J, Birditt B, Kaur A, Madan A, Philipps DL, et al. Analysis of the human neurexin genes: alternative splicing and the generation of protein diversity. Genomics. 2002; 79(4): 587–597. [PubMed: 11944992]
23. Missler M, Sudhof TC. Neurexins: three genes and 1001 products. TIG. 1998; 14(1):20–26. [PubMed: 9448462]
24. Tabuchi K, Sudhof TC. Structure and evolution of neurexin genes: insight into the mechanism of alternative splicing. Genomics. 2002; 79(6):849–859. [PubMed: 12036300]
25. Sudhof TC. Neuroligins and neurexins link synaptic function to cognitive disease. Nature. 2008; 455(7215):903–911. [PubMed: 18923512]
26. Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. Cell. 2004; 119(7):1013–1026. [PubMed: 15620359]
27. Song JY, Ichtchenko K, Sudhof TC, Brose N. Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. PNAS. 1999; 96(3):1100–1105. [PubMed: 9927700]
28. Varoqueaux F, Jamain S, Brose N. Neuroligin 2 is exclusively localized to inhibitory synapses. Eur J Cell Bio. 2004; 83(9):449–456. [PubMed: 15540461]
29. Boucard AA, Chubykin AA, Comoletti D, Taylor P, Sudhof TC. A splice code for trans-synaptic cell adhesion mediated by binding of neuroligin 1 to alpha- and beta-neurexins. Neuron. 2005; 48(2):229–236. [PubMed: 16242404]
30. Zhang C, Atasoy D, Arac D, Yang X, Fucillo MV, Robison AJ, et al. Neurexins physically and functionally interact with GABA(A) receptors. Neuron. 2010; 66(3):403–416. [PubMed: 20471353]
31. Sun C, Cheng MC, Qin R, Liao DL, Chen TT, Koong FJ, et al. Identification and functional characterization of rare mutations of the neuroligin-2 gene (NLGN2) associated with schizophrenia. Hum Mol Genet. 2011; 20(15):3042–3051. [PubMed: 21551456]
32. Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, et al. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat Genet. 2003; 34(1):27–29. [PubMed: 12669065]
33. 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. PNAS. 2006; 103(17):6747–6752. [PubMed: 16618933]
34. Kao WT, Wang Y, Kleinman JE, Lipska BK, Hyde TM, Weinberger DR, et al. Common genetic variation in Neuregulin 3 (NRG3) influences risk for schizophrenia and impacts NRG3 expression in human brain. PNAS. 2010; 107(35):15619–15624. [PubMed: 20713722]
35. Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? Neuroscience Biobehav Rev. 2003; 27(1–2):3–18.
36. Tau GZ, Peterson BS. Normal development of brain circuits. Neuropsychopharmacology. 2010; 35(1):147–168. [PubMed: 19794405]
37. Huttenlocher PR. Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Research. 1979; 163(2):195–205. [PubMed: 427544]
38. Puschel AW, Betz H. Neurexins are differentially expressed in the embryonic nervous system of mice. J Neurosci. 1995; 15(4):2849–2856. [PubMed: 7722633]
39. Paraoanu LE, Becker-Roeck M, Christ E, Layer PG. Expression patterns of neurexin-1 and neuroligins in brain and retina of the chick embryo: Neuroligin-3 is absent in retina. Neurosci Lett. 2006; 395(2):114–117. [PubMed: 16300891]
40. Brown SM, Clapcote SJ, Millar JK, Torrance HS, Anderson SM, Walker R, et al. Synaptic modulators Nrxn1 and Nrxn3 are disregulated in a Disc1 mouse model of schizophrenia. Mol Psych. 2011; 16(6):585–587.
41. Rozic-Kotliroff G, Zisapel N. Ca2+ -dependent splicing of neurexin IIalpha. Biochem Biophys Res Comm. 2007; 352(1):226–230. [PubMed: 17107668]
42. Iijima T, Wu K, Witte H, Hanno-Iijima Y, Glatter T, Richard S, et al. SAM68 regulates neuronal activity-dependent alternative splicing of neurexin-1. Cell. 2011; 147(7):1601–1614. [PubMed: 22196734]

43. Rozic G, Lupowitz Z, Piontkewitz Y, Zisapel N. Dynamic changes in neurexins' alternative splicing: role of Rho-associated protein kinases and relevance to memory formation. PloS One. 2011; 6(4):e18579. [PubMed: 21533271]
44. Kang Y, Zhang X, Dobie F, Wu H, Craig AM. Induction of GABAergic postsynaptic differentiation by alpha-neurexins. J Bio Chem. 2008; 283(4):2323–2334. [PubMed: 18006501]
45. Chih B, Gollan L, Scheiffele P. Alternative splicing controls selective trans-synaptic interactions of the neuroligin-neurexin complex. Neuron. 2006; 51(2):171–178. [PubMed: 16846852]
46. Graf ER, Kang Y, Hauner AM, Craig AM. Structure function and splice site analysis of the synaptogenic activity of the neurexin-1 beta LNS domain. J Neurosci. 2006; 26(16):4256–4265. [PubMed: 16624946]
47. Siddiqui TJ, Pancaroglu R, Kang Y, Rooyakkers A, Craig AM. LRRTMs and neuroligins bind neurexins with a differential code to cooperate in glutamate synapse development. J Neurosci. 2010; 30(22):7495–7506. [PubMed: 20519524]
48. Kumar D, Thakur MK. Perinatal exposure to bisphenol-A impairs spatial memory through upregulation of neurexin1 and neuroligin3 expression in male mouse brain. PloS One. 2014; 9(10):e110482. [PubMed: 25330104]
49. Noor A, Lionel AC, Cohen-Woods S, Moghimi N, Rucker J, Fennell A, et al. Copy number variant study of bipolar disorder in Canadian and UK populations implicates synaptic genes. Am J Med Genet. 2014; 165B(4):303–313. [PubMed: 24700553]
50. International Schizophrenia C, Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature. 2009; 460(7256):748–752. [PubMed: 19571811]

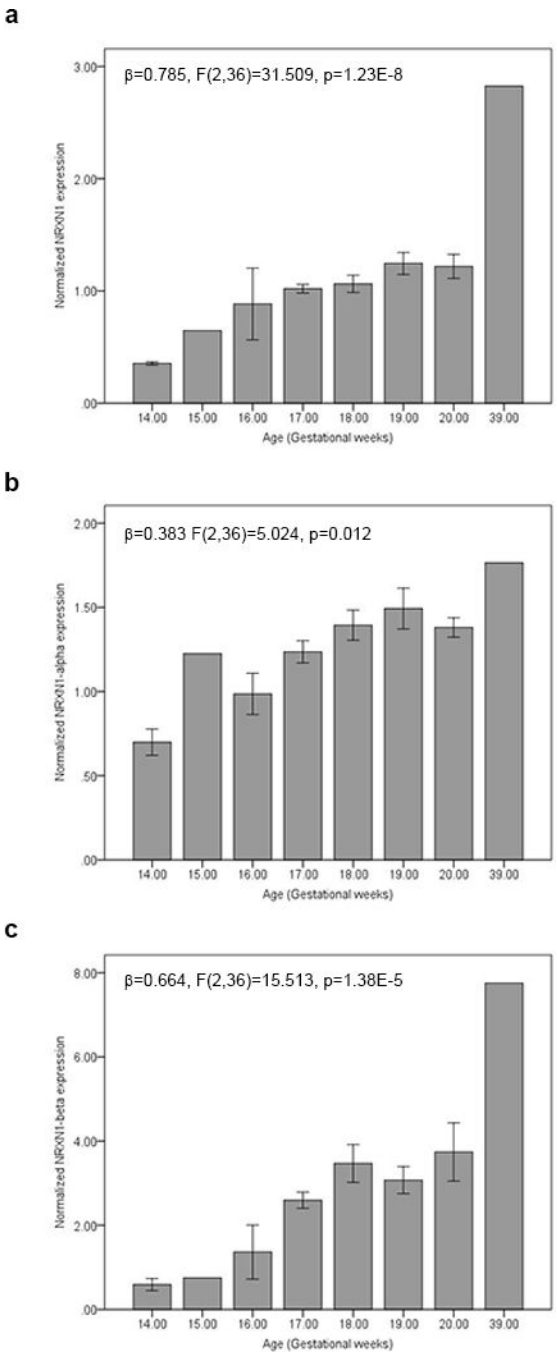

Figure 1. Expression trajectories of NRXN1 and its major splice isoforms NRXN1-α and NRXN1-β in the human prefrontal cortex during fetal brain development

Quantitative PCR analysis of NRXN1 (a), NRXN1-α (b) and NRXN1-β (c) mRNA expression in human prefrontal cortex from gestational weeks 14–39. (Gestational age (weeks) 14, n=4; 15, n=1; 16, n=2; 17, n=5; 18, n=11; 19, n=13; 20, n=2; 39, n=1). Data represent the mean ± S.E.M. Data normalized to the geometric mean of three housekeeping genes (β-actin, GAPDH, PBGD).

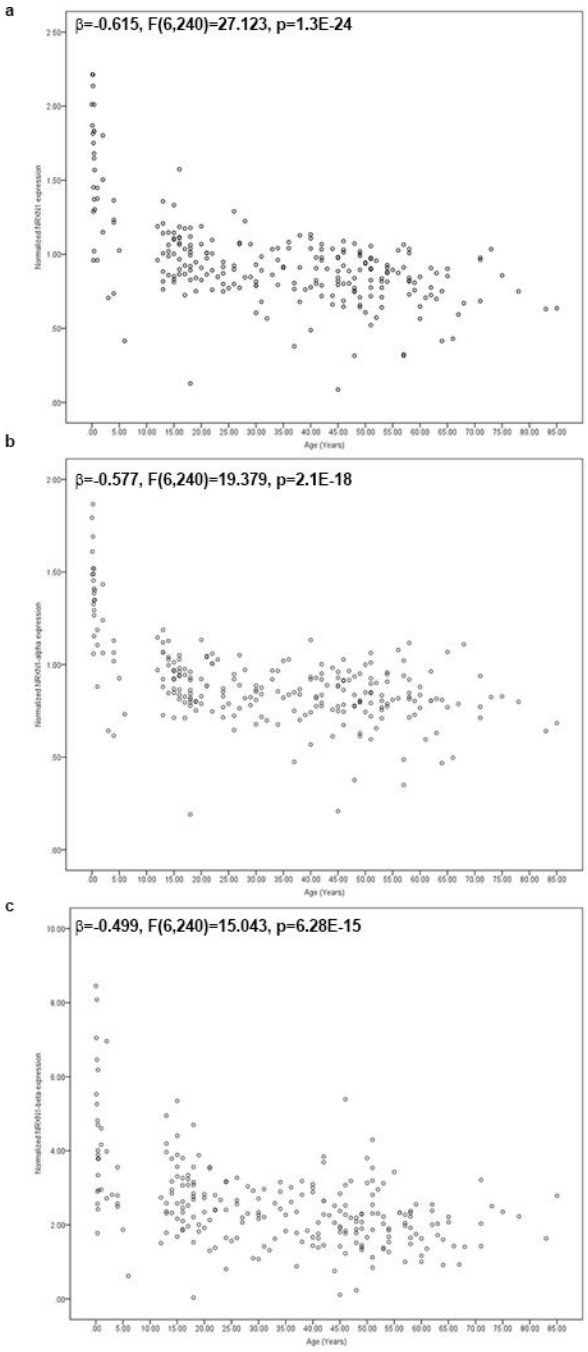

Figure 2. Expression of NRXN1 transcripts in the human dorsolateral prefrontal cortex across the postnatal lifespan

Quantitative PCR analysis of NRXN1 (a), NRXN1-α (b) and NRXN1-β (c) mRNA expression in human dorsolateral prefrontal cortex from birth, throughout aging (age range 0–85 years) (n=245). Each sample is represented by an individual data point; data represent mRNA expression normalized to the geometric mean of three housekeeping genes (β-actin, GAPDH, PBGD).

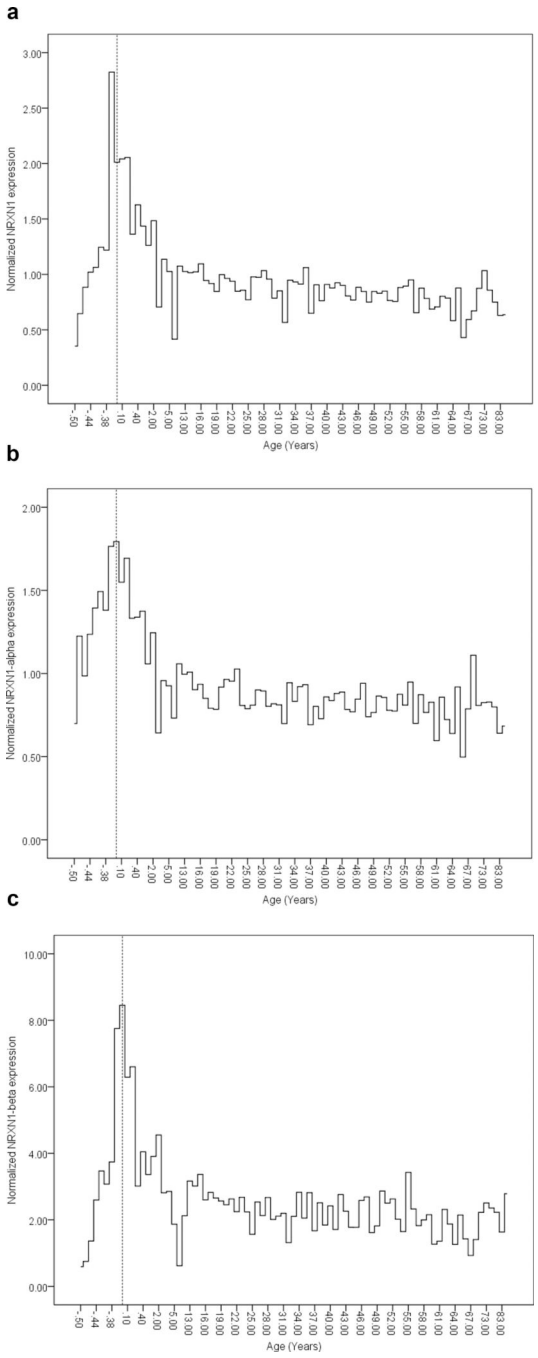

Figure 3. Expression trajectories of NRXN1 transcripts in the human dorsolateral prefrontal cortex throughout development and aging

Interpolation plots of NRXN1 (A), NRXN1-α (b) and NRXN1-β (c) mRNA expression in the human prefrontal cortex throughout fetal development, early life and aging (age range gestational week 16–85 years) (n=284). Data represent mRNA expression normalized to the geometric mean of three housekeeping genes (β-actin, GAPDH, PBGD).