Supplementary Methods

Human cortical brain sample collection

Tissues were obtained with full informed consent from the legal next of kin at autopsy from the Washington, DC, and Northern Virginia medical examiners' offices. 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). Toxicological analysis was performed on every case for the presence of ethanol, illicit drugs, nicotine and cotinine as well as prescribed drugs such as anti-depressants and antipsychotics. All non-psychiatric control subjects were free of a history of psychiatric illness, and were excluded if any illicit substance or a significant level of ethanol intoxication was detected.

Statistical analysis

Effects of developmental stage on NRG3 expression were conducted on each subclass separately using analysis of covariance with developmental stage, race and sex as fixed variables and RNA Integrity Number (RIN) and Post mortem interval (PMI) as covariates. Diagnostic effects on NRG3 subclass expression were explored using analysis of covariance between age matched unaffected non-psychiatric controls and individuals diagnosed with either BPD or MDD, with diagnosis, sex and race as fixed variables and RIN, PMI and pH as covariates. To examine the potential effects of medication status on gene expression the effects of antipsychotic and antidepressant medication status in BPD and MDD patients on NRG3 gene expression was explored using analysis of covariance, with antipsychotic status and antidepressant status as fixed variables. To assess the effect of nicotine exposure on NRG3 subclass expression univariate analysis was carried out in unaffected non-psychiatric controls, BPD and MDD individuals with diagnosis and nicotine status at time of death (positive or negative) as fixed variables to determine any main effects of nicotine status or an interaction between diagnosis and nicotine status. Effects of genetic variation at rs10748842 and rs6584400 on NRG3 gene expression levels in individuals with BPD or MDD were examined using ANCOVA with genotype and diagnosis as fixed factors and pH, PMI and RIN as covariates. Sex and Race were included as fixed factors where a main effect of these variables was identified. Post Hoc comparisons between allele groups were performed within diagnostic categories.

Supplementary Results

Effects of medication, suicide status and smoking status on NRG3 subclass expression

To rule out effects of antidepressant and antipsychotic medication use as a confound in interpreting expression changes in affective disorders, we investigated the effects of antidepressant and antipsychotic medication use (positive or negative) on expression levels of NRG3 Class I-IV in the DLPFC. As this was an exclusion criteria, all 179 unaffected non-psychiatric controls were negative for both antidepressant and antipsychotic use. Eighteen patients diagnosed with BPD were positive for antidepressant use and 9 were positive for antipsychotic use (antipsychotic status was unavailable for 2 individuals), whereas in patients diagnosed with MDD disorder, 37 individuals were positive for antidepressant use and 6 positive for antipsychotic use (medication status was unavailable for 1 individual). There was no main effect of antipsychotic status on the expression levels of NRG3 Class I-IV (all $p>0.05$). Similarly, antidepressant medication status did not significantly affect expression levels of NRG3 Class I-IV (all $p>0.05$).

We also assessed the association between suicide as a cause of death in the affective disorder diagnostic groups on the expression levels of NRG3 Class I-IV. 23 individuals diagnosed with BPD, and 47 individuals diagnosed with MDD cause of death was by suicide. There was no main effect of cause of death by suicide (compared to death by non-suicide, or cause of death undetermined) on the expression levels of NRG3 Class I-IV in the affective disorder groups (all $p>0.05$).

Additionally, since smoking status has been previously shown to regulate other molecules in the Neuregulin pathway (48), we investigated the effect of nicotine status at time of death (positive or negative) on expression levels of NRG3 Class I-IV in the DLPFC. As expected we identified an increase in the percentage of smokers in affective disorders compared to non-psychiatric control individuals (26.8% of individuals positive for significant nicotine levels at time of death in non-psychiatric controls compared to 55.9% and 50.7% in individuals diagnosed with BPD or MDD, respectively). Nicotine status had no effect on the expression of NRG3 Class I, II or IV (all $p>0.05$). NRG3 Class III expression, however, was significantly affected by nicotine status ($F(1,265)=11.055$, $p<0.001$), whereby positive nicotine status was commensurate with decreased NRG3 Class III expression. This effect was observed across all 3 diagnostic groups, with no significant interaction between diagnosis and nicotine status ($p>0.05$).

Table S1. Demographic information of lifespan cohort.

Developmental Stage	Age (Years)	number	% Female	Race (AA:CAU:AS:HIS)	pH (mean±SD)	PMI (mean±SD)	RIN (mean±SD)
Fetus	GW 14-39	39	51.3	34:5:0:0	N/A	2.56±2.21	8.68±1.32
Neonate	0-0.5	19	21.1	11:8:0:0	6.39± 0.24	32.90±14.52	8.11±0.97
Infant	1-3	7	57.1	2:5:0:0	6.41±0.35	27.43±14.41	7.91±0.88
Child	4-6	7	14.3	1:6:0:0	6.51±0.31	21.14±7.10	7.69±1.45
Adolescent	12-18	45	30.4	16:30:0:0	6.46±0.39	20.37±12.22	8.64±0.63
Young Adult	19-25	21	22.7	16:5:0:1	6.60±0.31	27.79±11.60	8.25±1.45
Adult	26-55	110	35.5	67:39:2:2	6.54±0.28	32.20±15.01	8.23±0.69
Aging	56-85	38	39.5	21:15:1:1	6.45±0.25	36.72±14.67	7.99±0.78

Abbreviations: GW= Gestational week; AA= African American; CAU= Caucasian; AS= Asian; HIS= Hispanic; PMI= Post mortem interval; RIN: RNA Integrity number

Table S2. Demographic information of diagnostic cohort.

Diagnosis	Age (Years)	number	% Female	Race (AA:CAU:AS:HIS)	pH (mean±SD)	PMI (mean±SD)	RIN (mean±SD)	Antipsychotic use	Antidepressant use	Smokers
Unaffected	18-85	179	50.4	108:64:3:4	6.53±0.28	32.1±15.0	8.23±0.71	N/A	N/A	48/179 (26.8%)
Bipolar Disorder	20-79	34	38.2	5:28:1:0	6.23±0.19	31.33±15.4	7.98±0.73	9/34 (26.4%)	18/34 (52.9%)	19/34 (55.9%)
Major Depressive Disorder	18-83	69	43.7	14:51:2:2	6.22±0.22	34.21±22.8	8.11±0.73	6/69 (8.7%)	37/69 (53.6%)	35/69 (50.7%)

Abbreviations: AA= African American; CAU= Caucasian; AS= Asian; HIS= Hispanic; PMI= Post mortem interval; RIN: RNA Integrity number

Figure S1. Exon structure.