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NRG1 Gene in Recurrent Major Depression: No Association in a Large-Scale Case–Control Association Study

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The *Neuregulin 1* (*NRG1*) gene was initially implicated in schizophrenia (SZ) and has recently been associated with bipolar disorder (BPD) in two studies. An association with major depressive disorder (MDD) has not yet been investigated but is warranted in view of the genetic overlap between MDD and BPD. We have performed a large-scale case–control study investigating the association between *NRG1* polymorphisms and MDD, genotyping a selection of 14 single nucleotide polymorphisms (SNPs) spanning the *NRG1* gene in a sample of 1,398 patients of White European ancestry with a diagnosis of MDD and 1,304 ethnically matched controls from three clinical sites in the UK. We found no single marker or haplotype associations that withstood correction for multiple testing. Our findings do not provide evidence that *NRG1* plays a role in MDD or that this gene explains part of the genetic overlap with BPD. © 2009 Wiley-Liss, Inc.

Key words: **NRG1; depression; association; affective disorders**

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INTRODUCTION

An increased incidence of unipolar major depressive disorder (MDD) in the relatives of bipolar disorder (BPD) probands compared to the general population [McGuffin and Katz, 1989; Jones et al., 2002], together with twin study evidence [McGuffin et al., 2003] points to a genetic overlap between MDD and BPD. Further evidence for shared genetic liability includes the evidence of an overlapping linkage region on 12q22-24 [Ewald et al., 2002; McGuffin et al., 2005; Shink et al., 2005]. There is also emerging evidence from twin [Carno et al., 2002] and candidate gene association studies of shared genetic factors between BPD and schizophrenia (SZ) [for review, see Craddock and Forty, 2006; Kato, 2007]. One such gene is *Neuregulin 1* (*NRG1*), which maps to chromosome 8p12 and is one of four neuregulin genes (*NRG1*, *NRG2*, *NRG3*, and *NRG4*). The neuregulins are a family of signaling proteins that mediate, via binding to the extracellular domain of their receptor tyrosine kinases ErbB-1 to ErbB-4, a variety of cell–cell interactions [Buonanno and Fischbach, 2001]. *NRG1* is known to mediate cell–cell interactions in the nervous system, heart, breast, muscle, and other organs, and has been implicated in the etiology of breast cancer, heart disease, multiple sclerosis, and SZ [for review, see Falls, 2003]. *NRG1* was initially implicated in SZ with a five marker "core" haplotype called HAP_{ICE}, encompassing the 5′-end of the gene and consisting of three single nucleotide polymorphisms (SNPs) and two microsatellites [Stefansson et al., 2002]. This initial finding has been replicated in multiple SZ

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samples [Yang et al., 2003; Hall et al., 2004; Li et al., 2004; Tang et al., 2004; Zhao et al., 2004], but not in all [Hall et al., 2004; Hong et al., 2004; Iwata et al., 2004] [for meta-analysis see Dawei et al., 2006]. These contradictory results may be attributed to population differences in allele and haplotype frequencies of the SNPs analyzed [Gardner et al., 2006]. Recently, two studies have associated *NRG1* with BPD [Green et al., 2005; Thomson et al., 2007] suggesting that it is one of the genes accounting for the overlap between SZ and BPD. However, a family-based collection failed to replicate an association of *NRG1* with BPD in the Irish population [Cassidy et al., 2006].

An association between *NRG1* and MDD has not yet been investigated; however, a recent study of postmortem brain tissue [Bertram et al., 2007] reported that the density of NRG-1α expressing neurons in the prefrontal gray matter was reduced in individuals with both SZ and MDD. This together with the evidence of genetic correlation in the liabilities to MDD and BPD merits an exploration of the role of *NRG1* in unipolar depression. To our knowledge, this is the first study testing for association of *NRG1* with MDD in a large-scale case–control association study.

MATERIALS AND METHODS

Samples

Our sample of 1,398 patients with a diagnosis of recurrent unipolar depression (MDD) (432 men, 964 women; mean age SD: 47.37 12.32) was recruited from three clinical sites: London (n = 495), Cardiff (n = 492), and Birmingham (n = 411), UK. Subjects were identified from psychiatric clinics, hospitals, general medical practices, and from volunteers responding to media advertisements. Subjects were included if they were over the age of 18, had experienced a minimum of two episodes of Diagnostic Statistical Manual 4th edition operational criteria [DSM-IV; American Psychiatric Association, 1994] and International Classification of Diseases 10th edition operational criteria [ICD-10; World Health Organisation, 1993] depression of at least moderate severity, separated by at least 2 months of remission as defined by the DSM-IV or the ICD-10. All subjects were White and of European parentage and were interviewed using the Schedules for Clinical Assessment in Neuropsychiatry [SCAN; Wing et al., 1990]. Subjects were excluded (1) if they ever fulfilled criteria for mania, hypomania, schizoaffective disorder or SZ, (2) if they experienced psychotic symptoms that were mood incongruent or present when there was no evidence of a mood disturbance, (3) intravenous drug use with a lifetime diagnosis of dependency, (4) depression occurring solely in relation to alcohol or substance abuse, or depression only secondary to medical illness or medication, (5) a clear diagnosis of BPD, SZ, schizoaffective disorder, or acute or transient psychotic disorders in first- or second-degree relatives, and (6) if they were related to an individual already included in the study. One thousand three hundred four control subjects (554 men, 747 women, mean age - SD: 41.70 13.10), screened for lifetime absence of psychiatric disorder using a modified version of the Past History Schedule [PHS; McGuffin et al., 1986] were recruited in London. They were either contacted via the MRC general practice research framework and screened using the Sham et al. [2000] composite index (G) of depressive and anxiety symptoms then telephone interviewed using the PHS, or were healthy volunteers who were staff or students of King's College London, again screened for mental health using the PHS. All controls subjects were White and of European parentage. Subjects were excluded if they or a first-degree relative ever fulfilled criteria for major depression, BPD, schizoaffective disorder, or SZ. Subjects were also excluded if they scored 10 or above on the Beck Depression Inventory [BDI; Beck and Steer, 1984], did not return consent, or failed to return cheek swabs (had to be returned by mail). All participants gave written informed consent and the study was approved by the Local Ethical Committees of the three centers.

Genotyping

Blood samples were obtained from all patients and either blood or buccal mucosa swabs obtained from controls. All samples were labeled with a unique ID bar code, pending DNA extraction. Genomic DNA was extracted by an in-house validated procedure from bloods and cheek swabs as described previously [Freeman et al., 1997, 2003]. A selection of 14 SNPs spanning the *NRG1* gene was investigated: rs35753505 (=SNP8NRG221533, 5′ near gene), rs62510682 (5′ near gene), rs10954811 (intron 1), rs1354334 (intron 1), rs553950 (intron 1), rs3924999 (exon 2), rs2439272 (intron 5), rs10095694 (intron 5), rs2466058 (intron 5), rs2954041 (intron 5), rs2919390 (intron 5), rs6988339 (intron 5), rs4262285 (intron 5), and rs3757930 (intron 6). Genotyping was performed applying SNPlex™ Genotyping System (Applied Biosystems, Foster City, CA) for SNPs rs1354334, rs3924999, rs2439272, rs2466058, rs2954041, rs6988339, and rs4262285. Genotyping of the remaining SNPs was performed using Sequenom MassARRAY® iPLEX Gold. SNPlex™ Genotyping System uses multiplex oligonucleotide ligation reactions (OLA), polymerase chain reaction (PCR), and capillary electrophoresis to analyze bi-allelic SNPs. Genotyping was performed blind with regard to all phenotypic information, including affection status, and 2,162 samples were successfully genotyped with SNPlex™ Genotyping System. Analysis of the raw data was performed using GeneMapper Software v3.7 and Microsoft Office Excel 2003. The following quality control criteria were applied: SNPs were omitted from analysis if poor genotype clustering prevented GeneMapper from making calls. Individual genotypes were omitted if their peak heights were <20% of the average for that genotypic group across the entire sample to avoid false heterozygosity assignment due to background noise in poor quality samples. As low call rates may indicate inaccurate genotyping, markers were omitted if the call rate after the previous exclusions was less than 80%. The Sequenom MassARRAY® iPLEX Gold assay uses PCR amplification and primer extension, resulting in an allele-specific difference in mass between extension products. This mass difference allows the data analysis software to differentiate between SNP alleles, and 2,475 samples were successfully genotyped with Sequenom MassARRAY® iPLEX Gold.

Statistics

To test for deviation from Hardy–Weinberg Equilibrium (HWE), the computer program FINETTI (http://ihg.gsf.de/cgi-bin/hw/

hwa1.pl) was used to perform exact statistics, and cases and controls were considered separately. The *P*-value for the exact test was calculated by generating the possible samples of genotype counts consistent with the observed allele counts, under a null hypothesis of HWE, calculating the appropriate Pearson (χ^2) test statistics for each sample. The exact *P*-value was calculated by comparing the value of the Pearson (χ^2) test statistics of the observed data with the distribution of the test statistics under the null hypothesis (http://www.meb.ki.se/genestat/tl/genass_ldmap/hwe_gterror/testing.htm). A standard chi-square (χ^2) statistic was calculated to test for genotypic association with each SNP using SPSS version 15.0. The computer program FINETTI (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to calculate the Cochran–Armitage trend statistic to test for allelic association. UNPHASED version 3.0.10 program [Dudbridge, 2003] was applied using two- and three-marker slide windows to analyze for haplotypic association. Individuals with incomplete genotyping data were excluded from haplotype analyses. UNPHASED uses the standard Expectation– Maximization (EM) algorithm in order to estimate haplotypes from genotypes. The rare haplotype frequency threshold was taken as 0.01 (both in cases and controls). UNPHASED uses unconditional logistic regression to perform likelihood ratio tests under a log-linear model of the probability that an allele or haplotype belongs to the case rather than control group. The global null hypothesis is that the odds ratios of all haplotypes are equal between cases and controls. Individual haplotypes were also tested for association by grouping the frequencies of all other haplotypes together. Haploview 4.0 program [Barrett et al., 2005] was used to perform linkage disequilibrium (LD) analysis of all SNPs in our sample. The measure of LD, denoted as D′ and r^2, was calculated from the haplotype frequency using the EM algorithm. To calculate the power of our case–control sample, we used the PS program [Dupont and Plummer, 1990]. In this program the method of Schlesselman [1982] is used for independent case and control groups, applying an uncorrected chi-squared test. When the case and control sample sizes are unequal, PS uses the generalization of Casagrande's method proposed by Fleiss [1981]. The alternative hypothesis is specified in terms of odds ratios. In this study, all *P*-values reported were two-tailed. Multiple testing corrections were performed by application of the false discovery rate [FDR, Benjamini et al., 2001] to both single-marker and haplotype analyses. In the case of single-marker analyses, it was assumed that 14 independent tests were performed in this study when testing 14 markers. Since two SNPs were excluded from haplotypic analyses, only 12 independent tests were assumed in the case of multi-marker analyses.

RESULTS

Genotyping results for each SNP were available for different number of samples and the percentage of missing data for each SNP are shown in Table I. The genotypic distributions of the 14 SNPs investigated were in HWE in both the control group and the case group as a whole, and when the genders were separated (data not shown). We performed statistical analyses in the overall group of MDD, and dividing cases and controls according to gender.

TABLE I. Single-Marker Analyses of the Investigated SNPs in *NRG1*

						Total χ^2 (*P*)		Male χ^2 (*P*)		Female χ^2 (*P*)	
SNP ID	**Position**	**Alleles**	**MD %**	**Cases**	**N (controls)**	**Genotypic**	**Allelic**	**Genotypic**	**Allelic**	**Genotypic**	**Allelic**
rs3753505	31593683	C/T	1.40	1,239	1,197	1.139 [0.566]	1.12 [0.289]	0.074 [0.964]	0.05 [0.831]	1.878 [0.391]	1.78 [0.183]
rs62510682	31613877	G/T	0.61	1,255	1,207	2.924 [0.232]	2.72 [0.099]	0.215 [0.898]	0.20 [0.657]	3.535 [0.171]	3.23 [0.072]
rs10954811	31705995	A/G	9.13	1,258	952	6.646 [0.036]	1.85 [0.174]	1.440 [0.487]	0.71 [0.401]	5.069 [0.079]	0.71 [0.400]
rs1354334	31799612	G/T	1.02	1,195	945	0.323 [0.851]	0.27 [0.604]	1.03 [0.597]	0.85 [0.357]	0.018 [0.991]	0.01 [0.931]
rs553950	31910904	G/T	1.36	1,233	1,205	1.161 [0.560]	0.81 [0.367]	4.205 [0.122]	3.54 [0.060]	0.108 [0.947]	0.08 [0.773]
rs3924999	32572900	C/T	0.23	1,200	957	**5.711 (0.058)**	**2.13 (0.143)**	**6.420 (0.04)**	**5.46 (0.0195)**	2.881 [0.237]	0.02 [0.878]
rs2439272	32612630	C/T	4.63	1,112	950	2.919 [0.232]	0.31 [0.575]	6.054 [0.048]	1.82 [0.178]	0.484 [0.785]	0.29 [0.589]
rs10095694	32619282	C/T	0.78	1256	1,199	1.211 [0.546]	1.16 [0.282]	1.615 [0.446]	0.21 [0.643]	0.855 [0.652]	0.39 [0.531]
rs2466058	32626691	A/G	1.48	1,187	943	0.165 [0.921]	0.16 [0.685]	4.839 [0.089]	**4.59 (0.032)**	1.582 [0.453]	1.55 [0.212]
rs2954041	32642168	G/T	7.72	1,107	888	1.438 [0.476]	1.43 [0.232]	0.897 [0.334]	0.90 [0.344]	1.567 [0.457]	0.49 [0.484]
rs2919390	32646497	G/T	0.03	1,266	1,208	0.005 [0.998]	0.00 [0.998]	1.650 [0.438]	0.74 [0.390]	1.28 [0.527]	0.63 [0.427]
rs6988339	32665458	A/G	0.42	1,195	958	2.487 [0.288]	0.98 [0.323]	1.628 [0.443]	0.34 [0.557]	0.766 [0.682]	0.57 [0.449]
rs422285	32702243	C/T	2.17	1,184	931	0.001 [0.979]	0.00 [0.979]	0.417 [0.519]	0.42 [0.557]	0.389 [0.528]	0.4 [0.528]
rs3757930	32708660	C/T	0.44	1,254	1,208	2.930 [0.231]	2.65 [0.104]	4.055 [0.132]	**3.65 (0.056)**	0.739 [0.691]	0.01 [0.914]

P-values are shown prior to correction for multiple testing [for corrected *P*-values see "Results" Section]. Position on chromosome 8 according to HapMap Genome Browser (B36), MD %, missing data in % (missing data refers to the number of samples successfully genotyped with each method); N, number of cases/controls for this SNP.
Bold values represent significant *P*-values.

Single-marker association was performed for all SNPs (Table I). In the overall group of MDD, neither significant genotypic nor allelic association was observed with any of the SNPs genotyped. Prior to correction for multiple testing, marker rs10954811 showed genotypic association ($P = 0.036$, FDR$P = 0.50$), however, not showing allelic association (uncorrected $P = 0.174$) in Cochran–Armitage's trend test. Performing gender-specific single-marker analyses, marker rs3924999 showed a difference in both genotypic ($P = 0.04$; FDR$P = 0.56$) and allelic ($P = 0.0195$; FDR$P = 0.27$) distribution between cases and controls in males, but not in females; however, this does not resist multiple testing correction. In males, the adjacent SNP rs2439272 showed a difference in genotypic distribution between cases and controls ($P = 0.048$; FDR$P = 0.34$), again only prior to correction for multiple testing. In short, all differences in genotypic and allelic distributions between cases and controls failed to withstand correction for multiple testing (see FDR*P*-values in brackets). LD between the analyzed loci is shown in Table II. The strongest LD was observed between SNPs rs35753505 and rs62510682, rs10954811 and rs1354334, rs2439272 and rs10095694, rs2439272 and rs2466058, rs10095694 and rs2466058, rs2466058 and rs2919390, rs2466058 and rs6988339, rs2919390 and rs4262285, rs6988339 and rs4262285, and rs4262285 and rs3757930. A two- and three-marker sliding window approach was applied using UNPHASED version 3.0.10. Haplotypes with a frequency below 1% were excluded from analyses, and markers with a minor allele frequency below 5% were excluded from haplotype analyses (rs2954041 and rs4262285). There was a different distribution between male cases and controls with two three-marker haplotypes in *NRG1* (rs2466858–rs2919390–rs6988339, uncorrected global $P = 0.023$ and rs2919390–rs6988339–rs3757930, uncorrected global $P = 0.026$), both containing the G-allele of rs2919390 and rs6988339. However, these differences did not withstand correction for multiple testing (FDR$P = 0.27$ in the first and FDR$P = 0.15$ in the latter case). Two two-marker haplotypes showed different distributions between male cases and controls (rs553950–rs3924999, uncorrected global $P = 0.05$ and rs2919390–rs6988339, uncorrected global $P = 0.042$), again not withstanding multiple testing correction. Haplotype-specific (''individual'') *P*-values showed a different distribution between male cases and controls for several two- and three-marker haplotypes (see Table III); however, these do not withstand multiple testing correction. The results of haplotypic analyses (two- and three-marker sliding approach) are shown in Table III.

TABLE II. Pair-Wise Comparisons (LD, Linkage Disequilibrium) of the SNPs Investigated in the NRG1 Gene, Performed Using Haploview 4.0 Program [Barrett et al., 2005]

	rs35733505	rs62510682	rs10954811	rs1354334	rs553950	rs3924999	rs2439272	rs10095694	rs2466058	rs2954041	rs2919390	rs6988339	rs4262285	rs3757930
rs35753505		0.908	0.530	0.274	0.329	0.001	0.01	0.004	0.048	0.253	0.032	0.081	0.129	0.005
rs62510682	0.261		0.369	0.103	0.274	0.012	0.009	0.03	0.067	0.256	0.085	0.067	0.42	0.008
rs10954811	0.062	0.096		0.923	0.714	0.012	0.043	0.013	0.053	0.351	0.001	0.035	0.354	0.011
rs1354334	0.068	0.004	0.215		0.739	0.036	0.023	0.012	0.106	0.671	0.016	0.015	0.203	0.057
rs553950	0.021	0.005	0.022	0.096		0.044	0.091	0.082	0.202	0.413	0.161	0.04	0.126	0.046
rs3924999	0.0	0.0	0.0	0.001	0.0		0.308	0.241	0.724	0.706	0.287	0.298	0.06	0.109
rs2439272	0.0	0.0	0.0	0.001	0.001	0.094		0.969	0.935	0.46	0.56	0.638	0.305	0.514
rs10095694	0.0	0.0	0.0	0.0	0.001	0.042	0.678		0.959	0.402	0.256	0.593	0.079	0.123
rs2466058	0.0	0.0	0.001	0.001	0.0	0.032	0.054	0.083		0.169	0.94	0.955	0.125	0.771
rs2954041	0.002	0.001	0.001	0.014	0.03	0.014	0.003	0.003	0.0		0.676	0.693	0.058	0.683
rs2919390	0.001	0.003	0.0	0.0	0.004	0.041	0.154	0.044	0.109	0.013		0.764	0.086	0.006
rs6988339	0.003	0.003	0.001	0.0	0.0	0.088	0.402	0.254	0.057	0.014	0.29		0.873	0.098
rs4262285	0.0	0.003	0.001	0.002	0.004	0.0	0.002	0.0	0.005	0.002	0.0	0.016		0.941
rs3757930	0.0	0.0	0.0	0.002	0.001	0.004	0.082	0.006	0.118	0.021	0.0	0.003	0.059	

D′ values are shown in the upper right triangle, r^2 values are shown in the lower triangle.

DISCUSSION

We have performed the first large-scale case–control study investigating the association between *NRG1* polymorphisms and recurrent unipolar depression, genotyping a selection of 14 SNPs spanning the *NRG1* gene in a sample of 1,398 patients with a diagnosis of recurrent unipolar depression (MDD) and 1,304 control subjects from three clinical sites in the UK (London, Cardiff, and Birmingham). We found no significant genotypic or allelic association that withstood correction for multiple testing with any of the SNPs genotyped, either in the overall group of MDD, or when the genders were separated. Multi-marker analyses were performed

TABLE III. UNPHASED Version 3.0.10 Program [Dudbridge, 2003] Was Applied Using (b) Two- and (a) Three-Marker Slide Windows to Analyze for Haplotypic Association in Males

SNP	MDD males haplotypes									
(a) Three-marker slide window										
rs35753505	C									
rs62510682	T	T								
rs10954811	G	G	G							
rs1354334		G	T	G						
rs553950			T	G	G					
rs3924999				C	C	T				
rs2439272					C	T	T			
rs10095694						T	C	T		
rs2466058							G	A	G	
rs2919390								G	G	G
rs6988339									G	G
rs3757930										T
Global *P*-value	0.376	0.425	0.253	0.142	0.070	0.347	0.094	0.278	**0.023**	**0.026**
Individual *P*-value	**0.019**	0.129	**0.029**	**0.033**	**0.032**	0.071	**0.006**	0.081	**0.036**	**0.040**
Frequency cases/controls	0.01/0.00	0.09/0.06	0.10/0.07	0.32/0.38	0.39/0.42	0.27/0.22	0.01/0.00	0.07/0.09	0.03/0.05	0.03/0.05

SNP	MDD males haplotypes										
(b) Two-marker slide windows											
rs35753505	C										
rs62510682	T	T									
rs10954811		G	A								
rs1354334			G	T							
rs553950				T	G						
rs3924999					C	C					
rs2439272						C	T				
rs10095694							C	T			
rs2466058								A	A		
rs2919390									G	G	
rs6988339										G	A
rs3757930											T
Global *P*-value	0.466	0.709	0.910	0.251	**0.05**	0.177	0.162	0.13	0.174	**0.042**	0.181
Individual *P*-value	0.097	0.310	0.539	0.047	**0.009**	**0.044**	**0.048**	0.073	0.067	**0.034**	**0.031**
Frequency cases/controls	0.02/0.01	0.20/0.17	0.26/0.28	0.10/0.07	0.51/0.57	0.42/0.47	0.01/0.00	0.07/0.10	0.07/0.09	0.02/0.05	0.17/0.22

Global and individual *P*-values of the significant haplotypes are shown, prior to correction for multiple testing (for corrected *P*-values see "Results" Section).
Bold values represent significant *P*-values.

applying a two- and three-marker sliding approach, with two SNPs excluded from haplotypic analyses due to minor allele frequencies below 5%. In males we report a different distribution between cases and controls with two three-marker haplotypes (rs2466058–rs2919390–rs6988339, uncorrected global $P = 0.023$ and rs2919390–rs6988339–rs3557930, uncorrected global $P = 0.026$), both containing the G-alleles of rs2919390 and rs6988339, and with two two-marker haplotypes (rs553950–rs3924999, uncorrected global $P = 0.05$ and rs2919390–rs6988339, rs6988339, uncorrected global $P = 0.042$), the latter again containing the G-alleles of rs2919390 and rs6988339. However, none of these differences withstood correction for multiple testing, and thus cannot be taken as evidence for association. Assuming a disease allele and/or genotype with a population frequency of 0.2, which confers risk of disease at an odds ratio of 1.4 or 1.3, we have 93.85% or 77.12% power of our MDD sample to detect association at $P = 0.05$. Limitations of the current study are that the controls are slightly younger in age (41.70 vs. 47.37), and they might possibly be at risk to develop MDD in the future. In addition, 43% of controls but only 31% of cases are male, which might have an impact on gender-specific analyses. A core risk haplotype (HAP_{ICE}) at the 5′ end of the *NRG1* gene was found to be associated with SZ in the Icelandic population and directly replicated in the Scottish population [Stefansson et al., 2002, 2003]. We have genotyped two SNPs in the 5′ region described in this original association report (rs35753505 = SNP8NRG221533 and rs62510682); however, we do not find an association with MDD. The microsatellites of the HAP_{ICE} haplotype have not been genotyped in the current study. In a large association study of *NRG1* in SZ [Petryshen et al., 2005], SNPs rs2439272 and rs2466058 were associated with the disease; however, in another study [Addington et al., 2007] the same markers failed to be associated with SZ. Association with marker rs3924999 was found in a rather small study performed in Chinese Han schizophrenic family trios [Yang et al., 2003]. Green et al. [2005] genotyped one SNP (SNP8NRG221533) and two microsatellites within the core risk haplotype (HAP_{ICE}) in BPD patients and controls from UK, and found an association with BPD. Another study investigating the association of polymorphisms in the *NRG1* gene with BPD [Thomson et al., 2007] found significant haplotypic association of SNP8NRG221533, rs10954811 (in a combined SZ and BPD sample), rs553950, rs2919390, and rs3757930, and significant single marker and haplotypic association of rs10095694 and rs6988339 with BPD. This is of interest due to the genetic overlap of BPD and MDD; however, in the current study we report no association of any of these markers with MDD. Other evidence implicating NRG1 and MDD has previously come from investigations on NRG1-α immunoreactivity in neurons in the prefrontal cortex in SZ and affective disorder (MDD and BPD) patients [Bertram et al., 2007]. They observed reduced immunoreactivity only in the SZ sample; however, the density of NRG1-α expressing neurons in the prefrontal gray matter was reduced in individuals with SZ and MDD. The implications of this small study in which only one NRG1 isoform was investigated in context of genetic variants of NRG1 remain to be elucidated.

In conclusion, our findings do not provide evidence that *NRG1* plays a role in MDD or that it explains part in the genetic overlap with BPD.

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