Association and interaction analyses of *NRG1* and *ERBB4* genes with schizophrenia in a Japanese population

Sae Shiota · Mamoru Tochigi · Hiroko Shimada ·
Jun Ohashi · Kiyoto Kasai · Nobumasa Kato ·
Katsushi Tokunaga · Tsukasa Sasaki

Received: 29 May 2008 / Accepted: 25 July 2008 / Published online: 14 August 2008
© The Japan Society of Human Genetics and Springer 2008

Abstract Neuregulin 1 (*NRG1*) is one of the most promising candidate genes for schizophrenia. A number of replication studies have been conducted, although the results were inconsistent and no susceptible variant has yet been identified. The inconsistency might be attributed to the ethnic difference in allele and haplotype frequencies. However, it is equally possible that one or more genes interacting with *NRG1* may also be implicated in schizophrenia and attribute to the inconsistency. To test the hypothesis, we conducted an interaction analysis between *NRG1* and one of its receptor's (*ERBB4*) polymorphisms as well as the association analysis of the two genes associated with schizophrenia in Japanese. We observed no significant difference between patients and controls in allele frequencies or genotypic distributions of the 18 polymorphisms of the genes. The permutation test showed no significant differences in estimated haplotype frequencies between patients and controls, including the haplotype

HAP_{ICE}. In the interaction analysis, significant interaction was observed between rs2919381 in *NRG1* and rs7560730 in *ERBB4* ($P = 0.047$, corrected). Thus, our results suggest the possibility that interaction between variants in *NRG1* and *ERBB4* might contribute to susceptibility for schizophrenia in a Japanese population. Further investigation may be necessary to confirm our results.

Keywords Neuregulin 1 (*NRG1*) · *ERBB4* · Schizophrenia · Epistasis · Chromosome 8

Introduction

Chromosome 8p has been implicated as a locus for schizophrenia in several linkage studies, which has been confirmed by meta-analyses (Badner and Gershon 2002; Lewis et al. 2003). Stefansson et al. (2002) conducted an extensive fine mapping of the locus using Icelandic pedigrees, which identified an associated haplotype (HAP_{ICE}) in the 5' region of the neuregulin 1 (*NRG1*) gene. *NRG1* is a plausible candidate gene for schizophrenia considering its involvement in neurodevelopment, regulation of glutamate and other neurotransmitter receptor expression, and synaptic plasticity (Falls 2003). The association was soon replicated in a Scottish sample by the same group (Stefansson et al. 2003). However, results of subsequent replication studies were inconsistent, and no susceptible variant has yet been identified (Tosato et al. 2005; Li et al. 2006; Munafo et al. 2008). Some studies observed the association of variants located in the 3' end region of *NRG1*, mainly from exon 3 to intron 7 (Petryshen et al. 2005; Yang et al. 2003).

The inconsistency may be explicable by the ethnic difference in allele and haplotype frequencies among the

Electronic supplementary material The online version of this article (doi:10.1007/s10038-008-0332-9) contains supplementary material, which is available to authorized users.

S. Shiota · J. Ohashi · K. Tokunaga
Department of Human Genetics,
Graduate School of Medicine, University of Tokyo,
7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan

M. Tochigi · H. Shimada · K. Kasai · N. Kato · T. Sasaki
Department of Neuropsychiatry,
Graduate School of Medicine, University of Tokyo,
7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan

T. Sasaki (✉)
Health Service Center, University of Tokyo,
7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan
e-mail: psytokyo@yahoo.co.jp

studies. However, it is equally possible that one or more genes interacting with *NRG1* may also be implicated in schizophrenia and attribute to the inconsistency. The mechanism of signaling by *NRG1* involves binding to the extracellular domain of the tyrosine kinase receptors ERBB3 or ERBB4, which leads to the formation of ERBB homo- or heterodimers, often including ERBB2 (Falls 2003). In the study by Stefansson et al. (2002), mutant mice heterozygous for either *Nrg1* or *ErbB4* showed a behavioral phenotype that overlaps with mouse models for schizophrenia. Thus, *NRG1*–*ERBB* signaling pathway may be a candidate for genetic network involved in the pathogenesis of schizophrenia (Corfas et al. 2004). Two studies have investigated the association between *ERBB3* and schizophrenia using a Japanese sample, although no association was observed (Kanazawa et al. 2007; Watanabe et al. 2007). Conversely, four studies provided evidence for an association between *ERBB4* and schizophrenia (Nicodemus et al. 2006; Norton et al. 2006; Silberberg et al. 2006; Benzel et al. 2007), two of which observed that interaction between *NRG1* and *ERBB4* increased susceptibility for schizophrenia (Norton et al. 2006; Benzel et al. 2007). However, no other study to date has examined the interaction between the two genes. In addition, no study has investigated the association between *ERBB4* and schizophrenia in Asian populations. In this study, we conducted association analysis of the two genes, *NRG1* and *ERBB4*, in Japanese schizophrenics. With respect to *NRG1*, polymorphisms in the 3' end region were investigated in addition to the HAP_{ICE} region. The interaction analysis was also conducted between *NRG1* and *ERBB4* polymorphisms in the same sample.

Subjects and methods

Subjects comprised 416 unrelated Japanese patients with schizophrenia [225 men and 191 women; age, 45.0 ± 14.7 years, mean $\pm$ standard deviation (SD)] recruited from several psychiatric hospitals around Tokyo. All patients met the *Diagnostic and Statistical Manual for Mental Disorders*, Fourth Edition (DSM-IV) criteria for schizophrenia. Patient diagnosis was confirmed by at least two experienced psychiatrists independently through repeated unstructured interviews and clinical chart review. Controls comprised 520 gender-matched unrelated healthy volunteers (281 men and 239 women; age: 37.5 ± 11.3 years) residing in the same area of Japan. All patients and controls were ethnically Japanese. The objective of the study was clearly explained, and written informed consent was obtained from all subjects. The study was approved by the Ethical Committee of the Faculty of Medicine, the University of Tokyo.

Genomic DNA was extracted from leukocytes using the standard phenol–chloroform method. We genotyped 13 polymorphisms in *NRG1* and five in *ERBB4*, as listed in Table 1. Markers that form HAP_{ICE} were all genotyped, except for two single nucleotide polymorphisms (SNPs), SNP8NRG221132 and SNP8NRG433E1006, whose minor allele frequencies were extremely low in our Japanese population [0 and 1.1%, respectively, in Iwata et al. (2004)]. We also genotyped rs1081062, located in the HAP_{ICE} region, which was observed to be significantly associated with schizophrenia in the previous Japanese study (Fukui et al. 2006). To investigate the association of the 3' end region, we genotyped seven SNPs, five of which showed a significant association with schizophrenia in the previous two studies (Yang et al. 2003; Petryshen et al. 2005). With respect to *ERBB4*, we covered SNPs that showed a significant association with schizophrenia in the previous two studies (Norton et al. 2006; Silberberg et al. 2006), except for two SNPs, IVS12 – 15C>T and IVS2 + 67C>G, which showed no polymorphism in a Japanese population in the National Center for Biotechnology Information (NCBI) dbSNP database. To cover IVS2 + 67C>G, the nearest tag SNP, rs7560730, was genotyped.

All SNPs were analyzed using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, CA, USA). Two microsatellites were analyzed using the ABI 3130xl Genetic Analyzer (Applied Biosystems) following polymerase chain reaction (PCR) amplification using the same primer sets as Stefansson et al. (2002). Deviation from the Hardy–Weinberg equilibrium was assessed using the χ^2 test comparing observed genotype frequencies with those expected from allele frequencies. χ^2 was used to compare SNP frequencies between patients and controls. For comparison of microsatellite allele frequencies, Fisher's exact test was used. Lewontin's D' was used to analyze pairwise linkage disequilibrium (LD) (Lewontin 1964). Haplotype block analysis was conducted with the Gabriel method and the four-gamete method (Gabriel et al. 2002; Wang et al. 2002). Haplotypes of the polymorphisms and their frequencies were estimated by the maximum-likelihood method, with an expectation–maximization algorithm (Excoffier and Slatkin 1995). Permutation P values were calculated in comparison with haplotype frequencies between patients and controls (Fallin et al. 2001). The SNPalyze 5.1 Standard (DYNACOM, Japan) and Haploview 3.32 (Barrett et al. 2005) were used to conduct LD, haplotype block, and haplotype analyses. Statistical power was calculated using a Web-based statistical program, Genetic Power Calculator (Purcell et al. 2003). To test interactions between two polymorphisms, standard logistic regression analyses were performed using the model by Cordell (2002):

Table 1 Allele frequencies of polymorphisms in the *NRG1* and *ERBB4* genes

Locus	Polymorphism	Alleles ^a	Allele frequency ^b		
			Schizophrenia	Control	<i>P</i> value ^c
<i>NRG1</i> HAP _{ICE}	SNP8NRG221533	C/T	0.464	0.469	0.83
	SNP8NRG241930	G/T	0.117	0.111	0.68
	SNP8NRG243177	T/C	0.478	0.470	0.73
	rs1081062	T/C	0.120	0.122	0.90
	478B14-848	12	0	0.001	0.04
		13	0.013	0.008	
		14	0.178	0.221	
		15	0.005	0.005	
		16	0.024	0.010	
		17	0.719	0.690	
		18	0.047	0.050	
		19	0.014	0.014	
		20	0	0.001	
		11	0.002	0.001	
	420M9-1395	12	0.776	0.743	0.11
		13	0.193	0.231	
		14	0.005	0.004	
		15	0.002	0.001	
		16	0	0.002	
		17	0.016	0.010	
		18	0.006	0.008	
		19	0.006	0.008	
<i>NRG1</i> 3' end	rs3924999	A/G	0.210	0.227	0.40
	rs2439272	G/A	0.203	0.193	0.60
	rs2466058	C/T	0.106	0.130	0.13
	rs2954041	G/T	0.359	0.316	0.06
	rs7823899	T/A	0.513	0.474	0.09
	rs6988339	A/G	0.516	0.471	0.06
	rs2919381	G/A	0.442	0.439	0.87
	rs7424835	G/A	0.237	0.261	0.23
<i>ERBB4</i>	rs7598440	T/C	0.464	0.469	0.82
	rs839523	C/T	0.396	0.399	0.91
	rs707284	C/T	0.388	0.396	0.72
	rs7560730	C/T	0.222	0.238	0.42

^a Major/minor alleles were described in case of single nucleotide polymorphisms (SNPs); the number of AC repeats in case of microsatellites

^b Minor allele frequencies were described in case of SNPs

^c The chi-square and Fisher's exact tests were used to compare the SNP and microsatellite frequencies, respectively, between patients and controls

$$\log(p/(1-p)) = \mu + a_1x_1 + d_1z_1 + a_2x_2 + d_2z_2 + i_{aa}x_1x_2 + i_{ad}x_1z_2 + i_{da}z_1x_2 + i_{dd}z_1z_2$$

where x_i and z_i are dummy variables related to the underlying genotype at locus i . We set x_i as a proportional component to the amount of major (or minor) alleles, and z_i as a component corresponding to dominance, i.e., $x_i = 1$ and $z_i = -0.5$ for major homozygote, $x_i = 0$ and $z_i = 0.5$ for heterozygote, and $x_i = -1$ and $z_i = -0.5$ for minor homozygote,

respectively. The coefficients μ , a_1 , a_2 , d_1 , d_2 , i_{aa} , i_{ad} , i_{da} , and i_{dd} are parameters to be estimated. Lack of interaction in this model implies that all interaction coefficients (i_{aa} , i_{ad} , i_{da} , and i_{dd}) are zero. If there is no interaction, the resulting model is

$$\log(p/(1-p)) = \mu + a_1x_1 + d_1z_1 + a_2x_2 + d_2z_2.$$

Statistical significance was assessed by a likelihood ratio test, comparing the full model with the reduced model. When significant interaction was observed, odds ratio (OR)

was calculated for each genotype combination. The χ^2 test or Fisher's exact test was used to test the statistical significance of the OR. The statistical package SPSS for Windows (SPSS Inc. 1999) was used for interaction analyses.

Results

Polymorphism allele frequencies and genotypic distributions compared between patients and controls are shown in Tables 1 and 2, respectively. All polymorphism distributions followed Hardy–Weinberg equilibrium in both patients and controls. A nominally significant difference was observed in microsatellite 478B14-848 allele frequencies between patients and controls (Fisher's exact test after unifying the alleles in which frequencies <2%, $P = 0.042$) (Table 1), although the statistical level became insignificant after correction for multiple testing. No significant difference was observed in allele frequencies of other polymorphisms or genotypic distributions of all SNPs between patients and controls.

The strength of LD denoted as D' between pairs of polymorphisms is shown in Fig. 1 (values of r^2 are shown in

supplementary material Fig. 1). The LD pattern was broadly similar in the patient and control groups. In haplotype block analysis, a block consisting of SNP8NRG241930 and SNP8NRG243177 was suggested in *NRG1* HAP_{ICE} region; two blocks, rs2466058–rs2954041 and rs7823899–rs6988339, in *NRG1* 3' end region; and a block rs7598440–rs707284 in *ERBB4* (Fig. 1). We then compared frequencies of the suggested four haplotypes (SNP8NRG241930–SNP8NRG243177, rs2466058–rs2954041, rs7823899–rs6988339, and rs7598440–rs707284) between patients and controls in addition to three haplotypes consisting of all polymorphisms in each region (i.e., SNP8NRG221533–420M9-1395, rs3924999–rs2919381, and rs7424835–rs7560730). No significant difference was observed in the distributions of haplotypes between patients and controls (data not shown). The three-marker haplotype consisting of SNP8NRG221533 and the two microsatellites did not show significant difference.

Interaction analyses were performed between two polymorphisms, one in the *NRG1* and another in the *ERBB4* gene (Table 3). As a result, significant interactions were observed between rs2954041 in *NRG1* and rs7424835 in *ERBB4* and between rs2919381 in *NRG1* and rs7560730 in *ERBB4* [2-log (likelihood ratio) = 12.6

Table 2 Genotypic distributions of polymorphisms in the *NRG1* and *ERBB4* genes

Locus	Polymorphism	Genotypic distribution ^a								<i>P</i> value ^b
		Schizophrenia				Control				
		N	AA	Aa	aa	N	AA	Aa	aa	
<i>NRG1</i> HAP _{ICE}	SNP8NRG221533	412	0.291	0.491	0.218	511	0.301	0.460	0.239	0.63
	SNP8NRG241930	414	0.781	0.205	0.014	518	0.794	0.191	0.015	0.86
	SNP8NRG243177	413	0.288	0.467	0.245	517	0.292	0.476	0.232	0.91
	rs1081062	413	0.770	0.220	0.010	509	0.766	0.224	0.010	0.99
	478B14-848	414	0.533	0.370	0.097	518	0.476	0.427	0.097	0.18
	420M9-1395	412	0.605	0.342	0.053	516	0.554	0.382	0.064	0.30
<i>NRG1</i> 3' end	rs3924999	416	0.611	0.358	0.031	514	0.602	0.344	0.054	0.23
	rs2439272	409	0.648	0.298	0.054	505	0.655	0.303	0.042	0.69
	rs2466058	404	0.800	0.188	0.012	501	0.750	0.240	0.010	0.17
	rs2954041	396	0.416	0.450	0.134	491	0.487	0.395	0.118	0.11
	rs7823899	415	0.231	0.511	0.258	520	0.287	0.478	0.235	0.16
	rs6988339	416	0.226	0.517	0.257	520	0.287	0.485	0.229	0.10
<i>ERBB4</i>	rs2919381	415	0.333	0.450	0.217	520	0.319	0.485	0.196	0.56
	rs7424835	414	0.580	0.367	0.053	515	0.532	0.414	0.054	0.33
	rs7598440	416	0.289	0.495	0.216	518	0.282	0.498	0.220	0.97
	rs839523	415	0.357	0.494	0.149	515	0.344	0.514	0.142	0.82
	rs707284	414	0.370	0.485	0.145	504	0.351	0.506	0.143	0.65
	rs7560730	415	0.607	0.342	0.051	501	0.589	0.347	0.064	0.66

^a A and a represent major and minor alleles at each single nucleotide polymorphism site, respectively. In case of microsatellites, minor allele was defined as allele other than major allele

^b The χ^2 test was used to compare genotypic distributions between patients and controls

Fig. 1 The strength of linkage disequilibrium (LD) between pairs of polymorphisms in patients and controls. The values of D' for patients are shown in the *upper portion* and those for controls in the *lower portion* (the *empty box* means D' value of 1.0). The *heavy-line frames* show suggested haplotype blocks. **a** *NRG1* HAP_{ICE} region. Two microsatellites were treated as binary polymorphisms consisting of major allele and others. **b** *NRG1* 3' end region. **c** *ERBB4*

Table 3 Interaction analyses between two polymorphisms of *NRG1* and *ERBB4* genes

Locus	Polymorphism	<i>ERBB4</i>				
		rs7424835	rs7598440	rs839523	rs707284	rs7560730
<i>NRG1</i> HAP _{ICE}	SNP8NRG221533	0.12 (7.33)	0.38 (4.21)	0.35 (4.45)	0.38 (4.20)	0.30 (4.86)
	SNP8NRG241930	0.50 (3.37)	0.53 (3.17)	0.41 (4.00)	0.54 (3.11)	0.76 (1.88)
	SNP8NRG243177	0.074 (8.52)	0.50 (3.35)	0.32 (4.68)	0.59 (2.80)	0.53 (3.19)
	rs1081062	0.28 (5.12)	0.22 (5.76)	0.19 (6.10)	0.29 (4.98)	0.21 (5.86)
	478B14-848	0.23 (5.56)	0.48 (3.46)	0.28 (5.11)	0.40 (4.05)	0.12 (7.23)
	420M9-1395	0.12 (7.24)	0.86 (1.31)	0.57 (2.99)	0.56 (2.99)	0.38 (4.24)
<i>NRG1</i> 3' end	rs3924999	0.52 (3.25)	0.98 (0.37)	0.83 (1.50)	0.78 (1.76)	0.49 (3.42)
	rs2439272	0.98 (0.46)	0.56 (2.98)	0.86 (1.28)	0.80 (1.65)	0.49 (3.41)
	rs2466058	0.60 (2.77)	0.50 (3.35)	0.44 (3.78)	0.58 (2.86)	0.73 (2.05)
	rs2954041	0.013 (12.6)	0.59 (2.79)	0.46 (3.59)	0.14 (6.93)	0.19 (6.17)
	rs7823899	0.09 (7.99)	0.99 (0.35)	0.91 (0.98)	0.99 (0.27)	0.71 (2.16)
	rs6988339	0.12 (7.36)	0.99 (0.24)	0.89 (1.14)	0.92 (0.93)	0.84 (1.41)
	rs2919381	0.66 (2.44)	0.61 (2.69)	0.60 (2.76)	0.085 (8.20)	0.00073 (19.2)

The results were denoted as P value [2-log (likelihood ratio)]

and 19.2, $P = 0.013$ and 0.00073, respectively]. The statistical level of interaction between rs2919381 in *NRG1* and rs7560730 in *ERBB4* remained significant after Bonferroni correction ($P = 0.047$, corrected for the 65 interaction analyses). Table 4 shows distribution and OR for each genotype combination between *NRG1* rs2919381 and *ERBB4* rs7560730. Increased risk for schizophrenia was observed in subjects with genotype combinations of GA–CT, GG–TT, and AA–TT for *NRG1*–*ERBB4* (OR = 2.70, 3.56, and 7.47 compared with AA–CC combination, $P = 0.049$, 0.016, and 0.018, respectively) (Table 4). No significant interaction was observed between the other two pairs of polymorphisms in the *NRG1* and *ERBB4* genes.

Discussion

Three Japanese studies have investigated the association between the HAP_{ICE} region of *NRG1* and schizophrenia (Iwata et al. 2004; Fukui et al. 2006; Ikeda et al. 2008). The first study observed no significant association (Iwata et al. 2004), whereas the second observed the association of rs1081062 located in intron 1 of *NRG1* (Fukui et al. 2006). Thus, the results contradicted each other. However, the third study, which is a recently published two-stage association study with 60 SNPs along the whole gene and large sample sets (1,126 cases and 1,022 controls for screening, and 1,262 cases, 1,022 controls, and 166 trios for confirmation), did not observe a significant association between

Table 4 Distribution and odds ratio for each genotype combination between *NRG1* rs2919381 and *ERBB4* rs7560730

<i>NRG1</i> rs2919381	<i>ERBB4</i> rs7560730		
	CC	CT	TT
GG			
Schizophrenia	91	99	61
Control	99	148	48
Odds ratio (<i>P</i> value) ^a	2.57 (0.058)	1.87 (0.17)	3.56 (0.016)
GA			
Schizophrenia	42	79	21
Control	47	82	45
Odds ratio (<i>P</i> value) ^a	2.50 (0.077)	2.70 (0.049)	1.31 (0.44)
AA			
Schizophrenia	5	8	8
Control	14	15	3
Odds ratio (<i>P</i> value) ^a	1 (ND)	1.49 (0.40)	7.47 (0.018)

^a Odds ratio was calculated for each genotype combination compared with the combination of AA–CC for *NRG1*–*ERBB4*. χ^2 or Fisher's exact test was used to test the statistical significance of the odds ratio

SNPs and schizophrenia (Ikeda et al. 2008). Our results did not observe the association between *NRG1* itself and schizophrenia, which correlates with the results of Ikeda et al. (2008).

The interaction analysis in our study, however, suggested the possibility that *NRG1* may be implicated in schizophrenia through epistatic interaction with *ERBB4*. In a Japanese population, *ERBB4* may be required as an additional factor for *NRG1* to be a susceptibility locus for schizophrenia. It is unclear at this point how the two SNPs interact with each other and contribute to the pathogenesis of schizophrenia from the biological point of view. Both SNPs, rs2919381 in *NRG1* and rs7560730 in *ERBB4*, are located in the intron of each gene. There is a possibility that these SNPs may not themselves be the etiological variants but, rather, in LD with the true susceptible variants. However, to our knowledge, no coding mutation has been identified in the *ERBB4* gene to date. A portion of intronic variants could be related to altered splice-variant expression, as shown in other intronic SNPs in *ERBB4*, such as rs4673628, rs7598440, rs707284, and rs839523 (Law et al. 2007).

There are differences in details in the results between our study and others (Norton et al. 2006; Benzel et al. 2007), although all studies observed interaction between *NRG1* and *ERBB4*. Norton et al. (2006) observed a significant interaction between HAP_{ICE} in *NRG1* and IVS12 – 15C>T in *ERBB4* in a Caucasian sample. On the other hand, no evidence for interaction was found for IVS2 + 67C>G in their study, the nearest tag SNP of which is rs7560730, and we observed a significant interaction between this SNP and *NRG1* rs2919381. This might be partly attributable to the difference in ethnicity. The IVS12 – 15C>T showed no

polymorphism in a Japanese population, and differences in allele and haplotype frequencies of *NRG1* are remarkable between the populations (Gardner et al. 2006). The difference in ethnicity or selection of SNPs may account for the difference between our result and that in Benzel et al. (2007), which suggested 45 pair-wise SNP interactions between *NRG1* and *ERBB4* in Caucasian.

Several limitations may be acknowledged in our study. One is the possibility of type II error regarding the relationship between schizophrenia and each gene. Power analysis showed that the statistical power was more than 80% when genotype relative risk was set at 1.75–2.7 for SNPs used in the study (minor allele frequency = 0.111–0.474) under a dominant inheritance model ($\alpha = 0.05$). This may not be enough to detect susceptibility loci of modest effects for schizophrenia. Second, we did not investigate the entire region of each gene. We selected markers that showed a significant association with schizophrenia in previous studies (Stefansson et al. 2002; Stefansson et al. 2003; Yang et al. 2003; Iwata et al. 2004; Petryshen et al. 2005; Fukui et al. 2006; Norton et al. 2006; Silberberg et al. 2006). Larger sample size and a gene-based approach using tag SNPs may be needed to obtain the conclusion, especially for the relationship between *ERBB4* itself and schizophrenia in the Japanese population. Third is subject attributes. Patient diagnosis was not assessed using the standard structured interview; therefore, it may be possible that not all subjects completely satisfied the DSM-IV criteria for schizophrenia. Also, controls were not age-matched, which could also affect the results.

In conclusion, our results suggest that interaction between variants in *NRG1* and *ERBB4* might contribute to susceptibility for schizophrenia in the Japanese population. However, larger sample size and a systematic LD-based marker selection may be necessary to confirm our results and clarify the role of *ERBB4* in the Japanese population.

References

- Badner JA, Gershon ES (2002) Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 7:405–411
- Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–265
- Benzel I, Bansal A, Browning BL, Galwey NW, Maycox PR, McGinnis R, Smart D, St. Clair D, Yates P, Purvis I (2007) Interactions among genes in the ErbB-Neuregulin signaling network are associated with increased susceptibility to schizophrenia. *Behav Brain Funct* 3:31
- Cordell HJ (2002) Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. *Hum Mol Genet* 11:2463–2468
- Corfas G, Roy K, Buxbaum JD (2004) Neuregulin 1–erbB signaling and the molecular/cellular basis of schizophrenia. *Nat Neurosci* 7:575–580

- Excoffier L, Slatkin M (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 12:921–927
- Fallin D, Cohen A, Essioux L, Chumakov I, Blumenfeld M, Cohen D, Schork NJ (2001) Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res* 11:143–151
- Falls DL (2003) Neuregulins: functions, forms, and signaling strategies. *Exp Cell Res* 284:14–30
- Fukui N, Muratake T, Kaneko N, Amagane H, Someya T (2006) Supportive evidence for neuregulin 1 as a susceptibility gene for schizophrenia in a Japanese population. *Neurosci Lett* 396:117–120
- Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. *Science* 296:2225–2229
- Gardner M, Gonzalez-Neira A, Lao O, Calafell F, Bertranpetit J, Comas D (2006) Extreme population differences across neuregulin 1 gene, with implications for association studies. *Mol Psychiatry* 11:66–75
- Ikeda M, Takahashi N, Saito S, Aleksic B, Watanabe Y, Nunokawa A, Yamanouchi Y, Kitajima T, Kinoshita Y, Kishi T, Kawashima K, Hashimoto R, Ujike H, Inada T, Someya T, Takeda M, Ozaki N, Iwata N (2008) Failure to replicate the association between *NRG1* and schizophrenia using Japanese large sample. *Schizophr Res* 101:1–8
- Iwata N, Suzuki T, Ikeda M, Kitajima T, Yamanouchi Y, Inada T, Ozaki N (2004) No association with the neuregulin 1 haplotype to Japanese schizophrenia. *Mol Psychiatry* 9:126–127
- Kanazawa T, Glatt SJ, Tsutsumi A, Kikuyama H, Koh J, Yoneda H, Tsuang MT (2007) Schizophrenia is not associated with the functional candidate gene *ERBB3*: results from a case-control study. *Am J Med Genet B Neuropsychiatr Genet* 144:113–116
- Law AJ, Kleinman JE, Weinberger DR, Weickert CS (2007) Disease-associated intronic variants in the *ErbB4* gene are related to altered *ErbB4* splice-variant expression in the brain in schizophrenia. *Hum Mol Genet* 16:129–141
- Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, Williams NM, Schwab SG, Pulver AE, Faraone SV, Brzustowicz LM, Kaufmann CA, Garver DL, Gurling HM, Lindholm E, Coon H, Moises HW, Byerley W, Shaw SH, Mesen A, Sherrington R, O'Neill FA, Walsh D, Kendler KS, Ekelund J, Paunio T, Lonnqvist J, Peltonen L, O'Donovan MC, Owen MJ, Wildenauer DB, Maier W, Nestadt G, Blouin JL, Antonarakis SE, Mowry BJ, Silverman JM, Crowe RR, Cloninger CR, Tsuang MT, Malaspina D, Harkavy-Friedman JM, Svrakic DM, Bassett AS, Holcomb J, Kalsi G, McQuillin A, Brynjolfsson J, Sigmundsson T, Petursson H, Jazin E, Zoega T, Helgason T (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73:34–48
- Lewontin RC (1964) The interaction of selection and linkage.I. General considerations; heterotic models. *Genetics* 120:849–852
- Li D, Collier DA, He L (2006) Meta-analysis shows strong positive association of the neuregulin 1 (*NRG1*) gene with schizophrenia. *Hum Mol Genet* 15:1995–2002
- Munafo MR, Attwood AS, Flint J (2008) Neuregulin 1 genotype and schizophrenia. *Schizophr Bull* 34:9–12
- Nicodemus KK, Luna A, Vakkalanka R, Goldberg T, Egan M, Straub RE, Weinberger DR (2006) Further evidence for association between *ERBB4* and schizophrenia and influence on cognitive intermediate phenotypes in healthy controls. *Mol Psychiatry* 11:1062–1069
- Norton N, Moskvina V, Morris DW, Bray NJ, Zammit S, Williams NM, Williams HJ, Preece AC, Dwyer S, Wilkinson JC, Spurlock G, Kirov G, Buckland P, Waddington JL, Gill M, Corvin AP, Owen MJ, O'Donovan MC (2006) Evidence that interaction between neuregulin 1 and its receptor *ERBB4* increases susceptibility to schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 141:96–101
- Petryshen TL, Middleton FA, Kirby A, Aldinger KA, Purcell S, Tahl AR, Morley CP, McGann L, Gentile KL, Rockwell GN, Medeiros HM, Carvalho C, Macedo A, Dourado A, Valente J, Ferreira CP, Patterson NJ, Azevedo MH, Daly MJ, Pato CN, Pato MT, Sklar P (2005) Support for involvement of neuregulin 1 in schizophrenia pathophysiology. *Mol Psychiatry* 10:366–374
- Purcell S, Cherny SS, Sham PC (2003) Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 19:149–150
- Silberberg G, Darvasi A, Pinkas-Kramarski R, Navon R (2006) The involvement of *ERBB4* with schizophrenia: association and expression studies. *Am J Med Genet B Neuropsychiatr Genet* 141:142–148
- SPSS Inc. (1999) SPSS base system: syntax reference guide. Release 10.0. SPSS Inc., Chicago
- Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, Hjaltason O, Birgisdottir B, Jonsson H, Gudnadottir VG, Gudmundsdottir E, Bjornsson A, Ingvarsson B, Ingason A, Sigfusson S, Hardardottir H, Harvey RP, Lai D, Zhou M, Brunner D, Mutel V, Gonzalo A, Lemke G, Sainz J, Johannesson G, Andresson T, Gudbjartsson D, Manolescu A, Frigge ML, Gurney ME, Kong A, Gulcher JR, Petursson H, Stefansson K (2002) Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 71:877–892
- Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E, Gunnarsdottir S, Walker N, Petursson H, Crombie C, Ingason A, Gulcher JR, Stefansson K, St Clair D (2003) Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am J Hum Genet* 72:83–87
- Tosato S, Dazzan P, Collier D (2005) Association between the neuregulin 1 gene and schizophrenia: a systemic review. *Schizophr Bull* 31:613–617
- Wang N, Akey JM, Zhang K, Chakraborty R, Jin L (2002) Distribution of recombination crossovers and the origin of haplotype blocks: the interplay of population history, recombination, and mutation. *Am J Hum Genet* 71:1227–1234
- Watanabe Y, Fukui N, Nunokawa A, Muratake T, Kaneko N, Kitamura H, Someya T (2007) No association between the *ERBB3* gene and schizophrenia in a Japanese population. *Neurosci Res* 57:574–578
- Yang JZ, Si TM, Ruan Y, Ling YS, Han YH, Wang XL, Zhou M, Zhang HY, Kong QM, Liu C, Zhang DR, Yu YQ, Liu SZ, Ju GZ, Shu L, Ma DL, Zhang D (2003) Association study of neuregulin 1 gene with schizophrenia. *Mol Psychiatry* 8:706–709