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ORIGINAL PAPER

No Association Between NRG1 and ErbB4 Genes and Psychopathological Symptoms of Schizophrenia

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Abstract Neuregulin 1 (*NRG1*) and v-erb-a erythroblastic leukemia viral oncogene homolog 4 (*ErbB4*) have been extensively studied in schizophrenia susceptibility because of their pivotal role in key neurodevelopmental processes. One of the reasons for the inconsistencies in results could be the fact that the phenotype investigated has mostly the diagnosis of schizophrenia per se, which is widely heterogeneous, both clinically and biologically. In the present study we tested, in a large cohort of 461 schizophrenia patients recruited in Scotland, whether several SNPs in *NRG1* and/or *ErbB4* are associated with schizophrenia symptom dimensions as evaluated by the Positive and Negative Syndrome Scale (PANSS). We then followed up nominally significant results in a second cohort of 439 schizophrenia subjects recruited in Germany. Using linear regression, we observed two different groups of polymorphisms in NRG1 gene: one showing a nominal association with higher scores of the PANSS positive dimension and the other one with higher scores of the PANSS negative dimension. Regarding *ErbB4*, a small cluster located in the 5′ end of the gene was detected, showing nominal association mainly with negative, general and total dimensions of the PANSS. These findings suggest that some regions of *NRG1* and *ErbB4* are functionally involved in biological processes that underlie some of the phenotypic manifestations of schizophrenia. Because of the lack of significant association after correction for multiple testing, our analyses should be considered as exploratory and hypothesis generating for future studies.

Keywords NRG1 · ErbB4 · PANSS · Psychopathology · Schizophrenia

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Introduction

The NRG1 (Neuregulin 1) gene was initially associated with schizophrenia in the Icelandic population (Stefansson et al. 2002), and this association was subsequently replicated in a Scottish case–control study (Stefansson et al. 2003). Since then, a large number of studies in populations of various ethnic backgrounds have reported an association between schizophrenia and *NRG1* single nucleotide polymorphisms (SNPs) or haplotypes (Gong et al. 2009; Munafo et al. 2008; Tosato et al. 2005). The most consistent and strong association has been detected for a core region including five SNPs and two microsatellites located at the 5′ end of the NRG1 gene (Stefansson et al. 2002). Overall, dozens of SNPs of *NRG1* have been associated with schizophrenia, both at 5′ (Kukshal et al. 2013; Benzel et al. 2007; Corvin et al. 2004; Hall et al. 2004; Petryshen et al. 2005; Stefansson et al. 2002; Zhao et al. 2004) and 3′

regions (Bakker et al. 2004; Benzel et al. 2007; Lachman et al. 2006; Li et al. 2004; Petryshen et al. 2005). Recently, genome-wide association studies (GWAS) for schizophrenia have also reported a nominal association between several SNPs in NRG1 gene and schizophrenia, with p values spanning from 1.59×10^{-5} for rs4316112 to 1.38×10^{-3} for rs10095694 (Athanasiu et al. 2010; Shi et al. 2009). However, in none of these GWAS, the association survived correction for multiple testing at the threshold proposed for genome-wide significance ($p < 5 \times 10^{-8}$). Moreover, some meta-analyses have identified several SNPs in the NRG1 gene with overall odds ratios spanning from 0.85 for rs2439272 to 1.14 for rs2954041 (SZGene database http://www.szgene.org/geneoverview.asp?geneid=311).

This association between *NRG1* and schizophrenia thus suggests a role for *NRG1* as a genetic risk factor for schizophrenia. However, the allelic and population heterogeneity reported across data sets, together with the fact that multiple SNPs and haplotypes have been analyzed, makes it difficult to define *NRG1* as an unequivocal risk factor for schizophrenia. It is also possible that none of the previously analyzed SNPs represents a suitable proxy for a putative functional polymorphism. In fact, some studies have recently detected no association between schizophrenia and NRG1 gene variants (Crisafulli et al. 2012; Kim et al. 2011; Moon et al. 2011). In order to capture all the existing variability in *NRG1*, the association between *NRG1* and schizophrenia can be studied by looking for different tagSNPs or by analyzing hundreds of polymorphisms within the gene.

The multiple reports of an association between schizophrenia and v-erb-a erythroblastic leukemia viral oncogene homolog 4 (*ErbB4*) (Norton et al. 2006; Silberberg et al. 2006; Benzel et al. 2007), a gene that codes for NRG1 receptor, provide indirect support for the role of NRG1 gene in schizophrenia. In a GWAS, common variants in *ErbB4* and its ligand *NRG1* were found to be associated with schizophrenia, mostly in African-American subjects (Shi et al. 2009). Some recent meta-analyses suggest the involvement of *ErbB4* rs839523 in the susceptibility to schizophrenia in both Caucasian and Asian samples, with an overall odds ratio of 1.01 (SZGene database http://www.szgene.org/geneoverview.asp?geneid=273).

To date, most of the SNPs associated with schizophrenia are noncoding intronic variants, suggesting that the risk *ErbB4* SNPs may be functional through splicing regulation or may monitor functional elements within the gene itself (Law et al. 2007). It has therefore been suggested that NRG1 may mediate its effects on schizophrenia susceptibility through functional interactions with ErbB4. It is also possible that a genetic interaction between variants at the two loci increases the genetic risk for schizophrenia (Norton et al. 2006; Pitcher et al. 2011; Nicodemus et al. 2010). At the same time, however, a recent study has contradicted these results by showing no association between ErbB4 gene and schizophrenia, neither at the SNP nor at the haplotypic levels (Chen et al. 2011).

Overall, the inconsistent findings on the association between *NRG1*, *ErbB4* and schizophrenia could be due to the fact that the phenotype investigated has been the diagnosis of schizophrenia per se, which is widely heterogeneous, both clinically and biologically (Tosato and Lasalvia 2009; Davidson and McGlashan 1997; Hegarty et al. 1994). Some researchers have suggested that clinical phenotypes would be more closely related than diagnosis to susceptive genes (Li et al. 2013; DeRosse et al. 2012). It has also been suggested that quantitative traits, such as psychopathological dimensions, may lead to a better understanding of the genotype–phenotype relationship (Tao et al. 2006; Wilcox et al. 2002). Unfortunately, only few studies have investigated whether either NRG1 or ErbB4 genes are associated with schizophrenia symptom dimensions, often with contradictory results (Bakker et al. 2004, 2007; Rethelyi et al. 2010; Middle et al. 2010).

In the present study, we tested, in a large cohort of schizophrenia patients recruited in Aberdeen (Scotland), whether tagging SNPs spanning *NRG1* and/or *ErbB4* were associated with symptom dimensions as evaluated by PANSS. Our aim was to investigate whether genetic variants in NRG1 and ErbB4 genes can influence the severity of clinical symptoms. Moreover, recognizing the importance of replication studies in genetics, we followed up nominally significant results in a second cohort of schizophrenia patients recruited in Munich (Germany).

Materials and Methods

Sample

The discovery cohort comprised 461 schizophrenia patients (age 24.4 ± 8.1), all self-identifying as of Scottish or North European ancestry, recruited in Aberdeen, Scotland (Need et al. 2009). The confirmation cohort comprised 439 schizophrenia patients (age 39.2 ± 10.4), all self-identifying as of German or Central European ancestry, recruited in Munich, Germany (Van den Oord et al. 2006).

Patients were recruited using a consistent clinical protocol. To be enrolled as cases, participants had to fulfill the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV, American Psychiatric Association 1994) criteria for schizophrenia. The diagnosis was validated by Structured Clinical Interview for DSM-IV, SCID I and SCID II (First et al. 1997). All participants were outpatients or clinically stabilized in-patients. Detailed

medical and psychiatric histories were collected. According to the inclusion criteria, patients were excluded if they: (a) had a history of head injury and/or neurological disease; (b) had a diagnosis of schizoaffective disorder, or a mood disorder unrelated to schizophrenia; (c) were monozygotic twins of cases already included in the study; (d) had been diagnosed as intravenous drug users with dependency during the last 6 months. Further details of the cohorts and protocols are available in previous reports (Van den Oord et al. 2006; Need et al. 2009). After explanation of the procedures and before inclusion in the study, written informed consent was obtained from each subject enrolled in this study. The study was approved by the relevant local ethical committees.

Psychopathology Assessment

Patients who met inclusion criteria were assessed as soon as possible after they reached a remission of positive psychotic symptoms (i.e., clinical stabilization). Most of the patients were under treatment with antipsychotic medications. Psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS, Kay et al. 1987) completed with data and observations obtained during the SCID-I interview. The PANSS items yield four scores, regarding: positive symptoms, negative symptoms, general psychopathology and a total score, which is the sum of the first three.

Genotyping

Venous blood samples (15 ml) were collected in EDTA-containing tubes. DNA was extracted from blood leukocytes. Two hundreds and sixteen SNPs at the NRG1 locus and 177 SNPs at the ErbB4 locus were selected from the SNPs genotyped through Illumina HumanHap550 Genotyping BeadChip as previously described (Need et al. 2009). A series of quality control (QC) checks and tests of cryptic relatedness was carried out, ultimately excluding a total of 28 participants. A "one percent rule" was also employed, in order to discard from the analysis any SNP for which more 1 % of samples could not be reliably scored. This approach reduced the scope for spurious association while the average success rate of genotyping reached 98.4 % and the concordance rate for duplicate genotyping reached 99.997 % (Need et al. 2009).

Statistical Analysis

The association between *NRG1* and *ErbB4* SNPs and psychopathology was tested with a linear regression model using the PLINK software (Purcell et al. 2007) with no covariates. An additive genetic model was used. The null hypothesis H0 was the lack of association between the observed PANSS scores, fitted to a linear equation, and the genotype counts of the minor allele (0, 1, 2) of each SNP. The p values were corrected for the overall number of analyses conducted to test the PANSS negative, positive and general psychopathology and total scores.

Results

In the Aberdeen and Munich cohorts, schizophrenia DSM-IV subtypes were observed in the following proportions: paranoid 86.2 and 77.6 %, disorganized 7.5 and 15.6 %, catatonic 2.1 and 2.2 %, undifferentiated 4.2 and 4.6 %, respectively. PANSS scores were available for 451 subjects in the Aberdeen cohort and for 307 subjects in the Munich sample. PANSS total, positive, negative and general psychopathology scores were 43.46 (±12.48) and 50.31 (±15.78), 11.56 (±5.24) and 13.55 (±6.05), 10.50 (±3.98) and 11.61 (±4.68), 21.40 (±5.72) and 25.15 (±7.36), respectively. The Munich sample comprised significantly more severe cases than Aberdeen sample, and this could be due to different ascertainment methods at the two sites. While in Munich the participants were chronic outpatients, in Aberdeen they were significantly younger.

Using linear regression and correction for multiple testing, we found that none of the tested SNPs showed a significant association with PANSS psychopathological dimensions, for both *NRG1* and *ErbB4*, in the Aberdeen cohort. Among the initial 216 SNPs investigated in NRG1 locus, 35 SNPs showed a nominal association (before correction for multiple testing) with different psychopathological dimensions in the Aberdeen sample (Table 1).

All these SNPs are located in the region Chr8:31,201,548..33,230,933 (HapMap Data Rel 27 Phase II+III, on NCBI B36 assembly, dbSNP b126). This region comprises the full NRG1 gene and encompasses nearly 400-kbp upstream at its 5′ end and nearly 500-kbp downstream from its 3′ end.

Among the SNPs that showed a nominal association with the positive dimension, the majority was localized between position 31,392,134 and position 31,825,136 at the *NRG1* locus (Fig. 1). Among the SNPs that showed a nominal association with the negative dimension, the majority was localized at a different location, between position 32,008,036 and position 32,261,074 (Fig. 1). No such clusters of nominally associated polymorphisms were apparent for either general or total psychopathology dimensions.

Using Haploview (Haploview Software, version 4.2) with data downloaded from HapMap (Version 3, Release 27, reference population CEU, Chr8: from 31,200 to 33,231 kbp), linkage disequilibrium (LD) patterns for the

Table 1 All *NRG1* SNPs that produced a nominal significant *p* value (<0.05, before correction for multiple testing) with at least one PANSS item scores in the Aberdeen cohort

SNP	Allele	PANSS psychopathological dimensions			
		Positive	Negative	General	Total
rs2681600	2	**.0252**	.6592	.1686	.2466
rs2681599	2	**.0383**	.8186	.1012	.1884
rs2716960	1	**.0347**	.9248	.1132	.1504
rs2716959	1	**.0050**	.3693	.3383	.3398
rs7813593	2	.5055	.0712	**.0341**	.0521
rs7828595	2	**.0412**	.4991	.1329	.2914
rs1476540	1	.0548	**.0420**	.4544	.9184
rs1557800*	1	**.0049**	.1423	.2332	.4093
rs10489349	1	**.0028**	.5253	**.0104**	**.0167**
rs986110	1	**.0055**	.6656	.2582	.1131
rs4281084	1	**.0004**	.2112	.0512	**.0106**
rs10113797	2	**.0473**	.1412	.4065	.1027
rs7463426	2	**.0406**	**.0186**	.5986	.0597
rs4733094	1	**.0383**	**.0274**	.7702	.0853
rs1503486	1	.2207	**.0492**	.7784	.1785
rs1354334*	1	**.0284**	.0880	.9750	.1529
rs1566778	1	**.0318**	.0824	.9526	.1496
rs7827456	1	.5570	**.0233**	.1687	.1630
rs10954821	1	.6128	**.0122**	.1135	.1062
rs13249578	1	.5632	**.0103**	.1374	.1157
rs11776959	1	**.0241**	.3837	.1561	.0825
rs9297186	1	**.0113**	.8268	.3345	.2472
rs17620153	1	.1510	.1080	**.0197**	.0275
rs1481763	2	.0594	**.0072**	**.0081**	**.0032**
rs4733306	1	.4400	**.0118**	.4060	.0921
rs10097263	1	**.0485**	**.0396**	.1463	**.0306**
rs6468090	1	.3179	**.0072**	.2710	.0510
rs2200047	2	.3179	**.0072**	.2710	.0510
rs10503901	1	**.0359**	.3801	**.0119**	**.0284**
rs11782156	1	.0686	.2347	**.0349**	**.0405**
rs16879088	1	.9200	**.0098**	.5390	.1844
rs4733117	2	**.0435**	.1345	.1354	**.0497**
rs10503902	2	.9430	**.0364**	.9299	.3470
rs716144	2	.1532	.0951	**.0243**	**.0284**
rs4236723	2	.0515	.6770	**.0396**	.0819

Significant *p* values (<0.05, before correction for multiple testing) are highlighted in bold

* These SNPs have already been found as significantly associated by Petryshen et al. (2005)

investigated SNPs were checked in terms of r^2. We observed that the two clusters of SNPs mentioned above were not in strong LD (Fig. 2).

Even if our study is essentially a negative study, we followed up our results in the Munich cohort finding that in only a few instances there was a nominal association detected in both of the cohorts. These associations were between one psychopathological dimension and the same polymorphism, with the majority of SNPs showing nominal association only in the Aberdeen cohort but not in the Munich one. In detail, we found that only two SNPs have nominal association with both cohorts. In fact, rs2681599 was associated with the positive PANSS dimension in the Aberdeen sample (uncorrected $p = 0.0383$), but with the positive and total PANSS score in the Munich sample (uncorrected $p = 0.0297$ and 0.0479, respectively). rs7813593 was associated with the general psychopathology PANSS dimension in the Aberdeen sample (uncorrected $p = 0.0341$), but with the positive PANSS dimension in the Munich cohort (uncorrected $p = 0.0087$). There was therefore no notable overlap between significant SNPs in the two cohorts.

Regarding the ErbB4 gene, we found that 21 out of the initially 117 tested SNPs had a nominal association (before correction for multiple testing) with at least one of the four PANSS psychopathological dimensions before correction for multiple testing (Table 2).

All these SNPs were located in the region Chr2: 211,965,043..213,109,527 (HapMap Data Rel 27 Phase II+III, on NCBI B36 assembly, dbSNP b126), which comprised the ErbB4 gene and approximately 50-kbp downstream of its 3′ end and nearly 100 kbp upstream of its 3′ end. The nominally associated SNPs were distributed throughout the region of interest, but notably a small cluster was detected at the 5′ end of the gene (approximately between bp positions 211,847,782 and 211,934,422). In this region, the SNPs showed nominal associations mainly with negative, general and total dimensions of the PANSS (Fig. 3). Similarly to what was detected for *NRG1*, using Haploview (Haploview Software, version 4.2) with data downloaded from HapMap (Version 3, Release 27, reference population CEU, Chr2: from 211,800 to 213,110 kbp), we observed that the SNPs of interest were located in a low LD region (Fig. 4).

rs6728697 was the only SNP showing a nominal significant association with all four dimensions of the PANSS in the Aberdeen cohort (uncorrected $p = 0.016$ for positive, 0.009 for negative, 0.005 for general and 0.001 for total scores, respectively).

As for *NRG1*, we followed up our results in the Munich cohort. We found that rs6728697 was the only SNP showing an association (uncorrected *p* value = 0.016 in the Aberdeen sample, 0.021 in the Munich sample) with the positive PANSS dimension in both cohorts, before correction for multiple testing. In contrast, all other SNPs showed nominal significant *p* values <0.05 in the Aberdeen but not in the Munich sample.

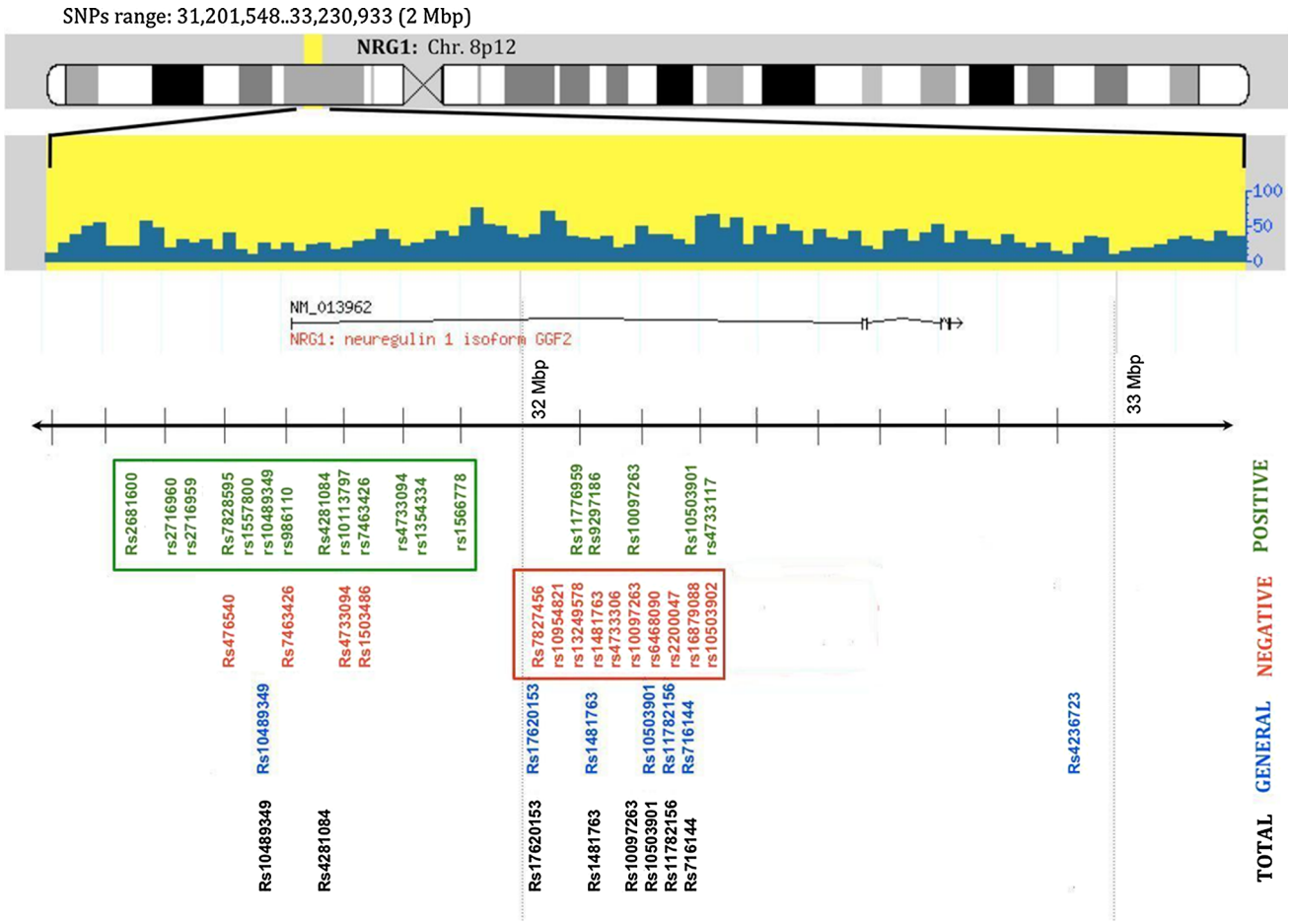

Fig. 1 *NRG1* locus along chr 8 and schematic representation of the SNPs that showed a positive correlation with psychopathological symptoms categories (*green* positive dimension, *red* negative dimension, *blue* general dimension, *black* total dimension) (Color figure online)

Discussion

To our knowledge, this is the first study testing the relationship between genetic variants in both NRG1 and ErbB4 genes and clinical features in two large cohorts of patients with schizophrenia. The main limitation of this study is the number of variables tested. We performed a substantial number of tests, which means that any Bonferroni correction for multiple testing would have to be large. Consequently, any significant *p* value should be regarded as nominal.

We also assessed the PANSS cross-sectionally in medicated subjects during the chronic stage of schizophrenia. This could raise the question as to whether the PANSS is a suitable instrument for the identification of real sub-types or categories of schizophrenia (or perhaps if this instrument can capture the intrinsic features of psychosis). Even with this conceptual limitation, the PANSS subscales are commonly used in association studies investigating the potential relationship between polymorphisms and psychopathological profiles (Li et al. 2013; Zhai et al. 2013; Bousman et al. 2013). In our study, chronic schizophrenia subjects were assessed as soon as possible after achieving remission of positive psychotic symptoms (i.e., clinical stabilization). Thus, it is reasonable to think that the psychopathological profile detected captures at least some intrinsic clinical features of psychosis.

Moreover, since patients were medically treated and stable at time of recruitment, it is also possible that our results reflect, at least in part, an effect of pharmacological treatment, partially covering the real impact of genetic variants on schizophrenia symptoms. Our analyses should therefore be considered as exploratory and hypothesis-generating.

In the present study, some putative "hot-spot" regions in both *NRG1* and *ErbB4* were identified, in which close SNPs, almost as a cluster, might be those associated with clinical psychopathological dimensions of schizophrenia. Interestingly, we observed that NRG1 SNPs showing a nominal association with schizophrenia had a tendency to group in two distinct regions along the gene length. The first region spans nearly 700 kb and covers the whole 5′

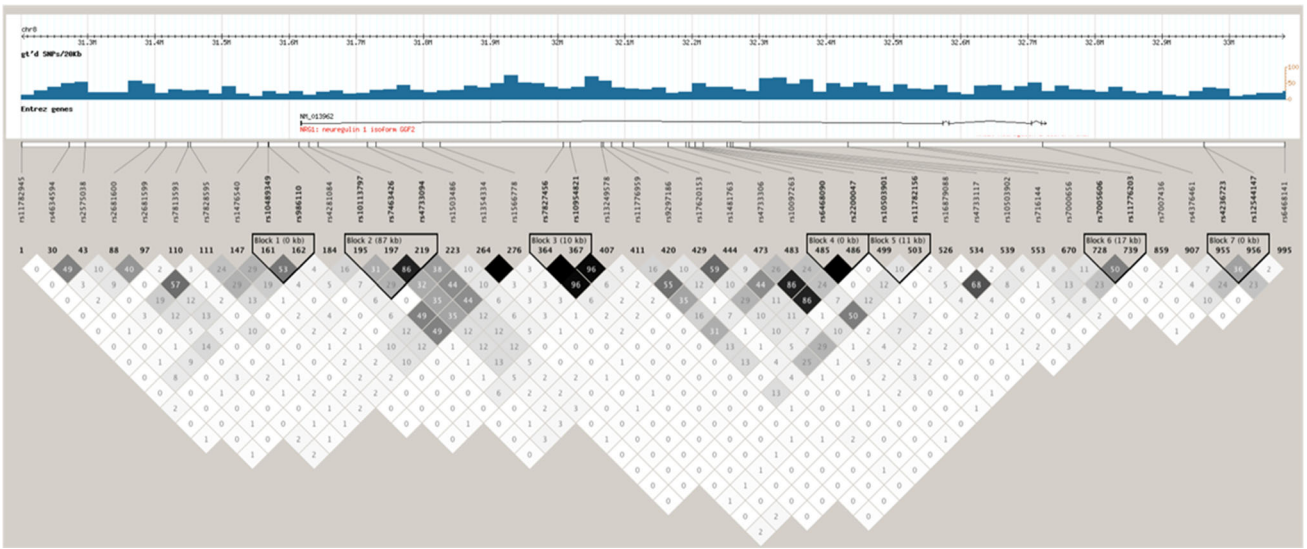

Fig. 2 LD patterns of NRG1 gene (Chr8:31,201,548..33,230,933) (Haploview 4.2, data from HapMap version 3, rel #27, reference population CEU). *Squares* indicate r^2 values: $r^2 = 0$: *white square*; $0 < r^2 < 1$: *shades* of *gray squares*; $r^2 = 1$: *black squares*

end and more upstream regions of the gene. We speculate that variants clustering in this area could be correlated with positive symptoms. The second region contains SNPs with a nominal association with negative symptoms, clustering in a nearly 600-kb long area and covering the gene length from its half toward its 3′ end. These findings might suggest that different portions of *NRG1* could be differentially implicated in the clinical presentation of schizophrenia or in the course of the illness. However, only a very limited number of studies used the symptom dimension approach with respect to *NRG1* (Wilcox et al. 2002; Kendler et al. 2000), making comparison with our findings difficult. It is therefore difficult to establish whether the clustering of SNPs we found is unusual or unexpected. Unfortunately, none of the markers chosen as tagSNPs for this study matched any of the Icelandic core haplotype polymorphisms (Stefansson et al. 2002). This is due to the fact that our genotyping analyses derive from a previous comprehensive hypothesis-free genome-wide association study carried out through Illumina HumanHap genotyping BeadChips (Need et al. 2009). Our current analysis comprises the most strongly associated polymorphisms derived from the above-mentioned GWAS, and it is conceivable to assume that, for the considered loci, "the Illumina SNP sets did not include the best-associated variants from previous studies" (Need et al. 2009).

Table 2 All *ErbB4* SNPs that produced a nominal significant *p* value (<0.05, before correction for multiple testing) with at least one PANSS item scores in the Aberdeen cohort

SNP	Allele	PANSS psychopathological dimensions			
		Positive	Negative	General	Total
rs4442936	2	**.0021**	.4262	**.0235**	**.0185**
rs7557116	2	**.0051**	.1474	**.0082**	**.0066**
rs6728697	2	**.0160**	**.0090**	**.0054**	**.0017**
rs1836729	1	.0649	**.0268**	.1812	**.0327**
rs1992027	1	.9029	**.0186**	.8481	.3487
rs1595068	2	.0575	.1465	**.0138**	**.0190**
rs1595067	2	.0725	**.0150**	**.0143**	**.0065**
rs6712652	1	.0718	**.0445**	**.0206**	**.0139**
rs953956	1	**.0223**	.3684	.0955	.0612
rs12619171	1	.4981	.2385	**.0346**	.0929
rs4130782	2	.9952	**.0112**	.3832	.1441
rs6722322	1	.8286	**.0423**	.2590	.1501
rs4672626	1	**.0295**	.6887	.0838	.0979
rs714393	1	.1161	**.0402**	.6796	.1206
rs1159709	1	.5837	**.0461**	.8601	.2746
rs9653337	2	**.0199**	.1789	.1964	.0575
rs1473636	1	.7123	**.0402**	.0506	.0607
rs7564926	2	**.0230**	.0524	**.0466**	**.0140**
rs1402766	1	**.0391**	.0666	.1778	**.0405**
rs1402769	1	**.0073**	.1554	.3198	.0563
rs10932428	1	**.0073**	.1554	.3198	.0563

Significant *p* values (<0.05, before correction for multiple testing) are highlighted in bold

For the ErbB4 gene, we observed no difference in the distribution of nominally significant SNPs among psychopathological dimensions. Nevertheless, we found that a small cluster of variants spanning approximately 100 kb and covering the 3′ region of the gene had a tendency toward an association with negative and general psychopathological dimensions and the total score of symptoms. It would be interesting to establish if this particular site of *ErbB4* is functionally involved in biological processes underling the phenotypic manifestations of schizophrenia, especially the

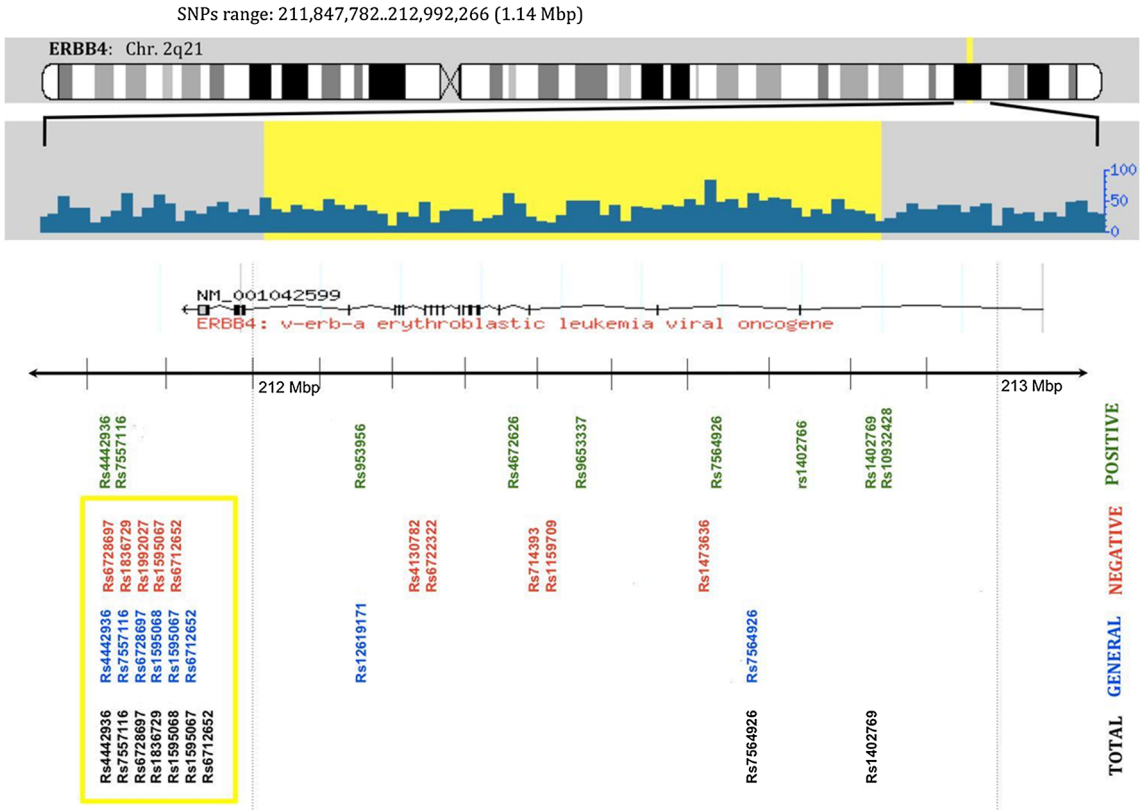

Fig. 3 *ErbB4* locus along chr 2 and schematic representation of the SNPs that showed a positive nominal correlation with psychopathological symptoms categories (*green* positive dimension, *red* negative dimension, *blue* general dimension, *black* total dimension) (Color figure online)

negative and/or general psychopathology dimensions. The latter is just a speculative interpretation of the results, and in order to obtain a clearer picture of these neurobiological processes, further studies would be needed.

From the present study, it is also clear that some degree of LD exists among the SNPs tested, especially between adjacent variants. In general, both these genes are large and span multiple regions of LD (D′ measures in LD blocks in Figs. 2, 4). Hence, the clusters of SNPs we observed could point to a role for distinct genetic regions in the underlying biology of the symptoms investigated, suggesting that these variants might actually represent good proxies to real causative markers.

A recent and comprehensive review (Buonanno 2010) highlighted a major possible role for the NRG1/ErbB4 signaling pathway in the pathogenesis of schizophrenia. In fact, the ErbB4 is a post-synaptic receptor with a high expression in GABAergic neurons, and NRG1 is also involved in the regulation of GABAergic and dopaminergic pathways, as well as in the synaptic plasticity processes mediated by glutamate release (Buonanno 2010). It has been proposed that impairments in the latter pathways might be involved in the development of the cognitive, positive, and negative symptoms observed in schizophrenia (Buonanno 2010). Within this hypothesis, only the cumulative impact of all the susceptibility variants investigated in this work would lead to the onset of psychotic symptoms, while considering each genetic polymorphism individually might lead to a loss of strength in association findings.

Finally, our hypothesis was first tested in the Aberdeen cohort and then we followed up the results in a second cohort recruited in Munich. Most of the SNPs clustering in the implicated regions were associated with schizophrenia in the Aberdeen sample, but no confirmation came from the Munich sample. This discrepancy might be due to either differences in clinical parameters, attributable to a different ascertainment at the two sites (Lelliott et al. 2010; Sederer 2010) or to real genetic differences between populations coming from different ancestries. Genetic differences between the Aberdeen and Munich populations were first assessed using the EIGENSTRAT software (Price et al. 2006). As a confirmation, the hidden population structure appeared to be low in the two populations. In fact, we calculated the Devlin and Roeder inflation factor λ (Devlin and Roeder 1999), adjusting for the known Munich/Aberdeen split. We obtained a value of 1.013, indicating that stratification was very slight and did not significantly affect

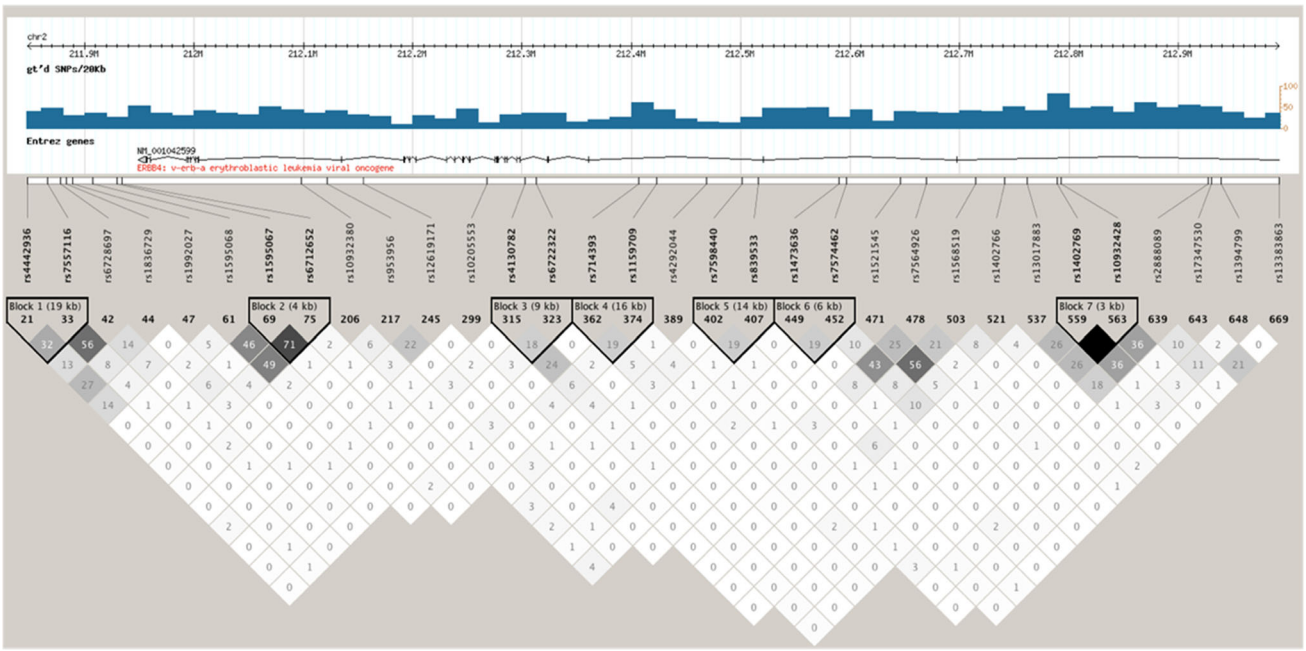

Fig. 4 LD patterns of *ErbB4* gene (Chr2:211,847,782..212,992,266) (Haploview 4.2, data from HapMap version 3, rel #27, reference population CEU). *Squares* indicate r^2 values: $r^2 = 0$: *white square*; $0 < r^2 < 1$: *shades* of *gray squares*; $r^2 = 1$: *black squares*

the detected genetic associations (Need et al. 2009). To our knowledge, the two populations are not phylogenetically different either. People from Scotland and Germany should share some common ancestors, tracing back to Anglo-Saxon invaders (Capelli et al. 2003). Moreover, neither Aberdeen nor Munich are known as recognizable geographical or genetic isolates. According to Novembre et al. (2008), geographically adjacent populations are typically very close from a genetic point of view, and they show a clear proximity between the Scotland and the German populations. An additional possible explanation for the lack of consistency in the nominal association signals between the Aberdeen and the Munich cohorts could be that these nominal associations have been detected by mere chance. For this reason, future confirmation studies are desirable in order to unravel any doubt about the validity of our results.

In conclusion, the difficulty in obtaining a consistent and clear-cut picture of the genetics of schizophrenia mirrors the marked clinical and neurobiological heterogeneity of the disorder. A comprehensive global model to capture the clinical heterogeneity in schizophrenia is still lacking. As long as we are not able to disentangle the question of heterogeneity at the clinical level, it is not likely that heterogeneity at the etiological and pathophysiological levels will be solved. Consequently, deconstructing schizophrenia into phenotypes based on symptoms, and then focusing on genes according to their biological function and putative involvement in this disorder, might be the best approach to overcome the lack of compelling genetic associations in schizophrenia genetic studies to date.

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