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**BRIEF REPORT**

# Case–control study of association between the functional candidate gene *ERBB3* and schizophrenia in Caucasian population*

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**Abstract**

Schizophrenia is a common psychiatric disorder with a complex genetic aetiology. Evidence shows that the oligodendrocyte and myelin-related genes including *ERBB3* are closely related to schizophrenia. Two recent studies (Kanazawa et al. (Am J Med Genet B Neuropsychiatr Genet 2007;144:113)) and Watanabe et al. (Neurosci Res 2007;57:574) reported there was no association between *ERBB3* and schizophrenia in Japanese population. We investigated the *ERBB3* gene given the putative functional nature of the gene and population heterogeneity between Asian and Caucasian. Scottish case and control samples were sequenced with four SNPs (rs705708 at intron 15, rs2271189, rs773123, rs2271188 at exon 27). We detected rs773123, which is a nonsynonymous Ser/Cys polymorphism located seven bases downstream of rs2271189, with $P=0.034$. The subgroups of male patients and patients with age at onset $<45$ showed evidence of significant association with $P$ values of 0.0046 and 0.0055, respectively. To our knowledge, this is the first association study between *ERBB3* and schizophrenia in the Caucasian population. Further investigation with large sample size should be helpful to clarify the nature of the gene.

**Key words:** *association, case-control, ERBB3, myelin, schizophrenia*

## Introduction

Schizophrenia is a severe, often chronic and common complex debilitating mental illness with a large genetic component (Faraone et al. 2002; Rujescu 2008). The oligodendrocyte and myelin-related genes including *ERBB3* (*HER3*) have been found to be highly related to schizophrenia (Hakak et al. 2001; Tkachev et al. 2003). The *ERBB3* gene maps to human chromosome 12q13 covering 22,702 base pairs. The region 12q13 has been reported to be involved in schizophrenia (Deb-Rinker et al. 2002). *ERBB3* expresses a 6.2-kb transcript in a variety of normal tissues of epithelial origin (Kraus et al. 1989). The neuregulin receptor ERBB3 is a member of the epidermal growth factor receptor family, which plays fundamental roles in the regulation of cell survival, proliferation, differentiation, and cellular transformation in response to various specific growth factors (Holbro et al. 2003; Walters et al. 2003).

In a microarray study, Tkachev et al. (2003) found that the amount of the *ERBB3* mRNA was reduced by 2.29-fold in patients with schizophrenia. Moreover, quantitative polymerase chain reaction (PCR) analysis on brains have confirmed significant down-regulation of *ERBB3* by 1.97 ($P=0.0005$) and 2.62 ($P=0.001$) in schizophrenia and bipolar disorder, respectively (Tkachev et al. 2003). DNA microarray has also shown the ERBB3 receptor to be down-regulated in schizophrenics (Hakak et al. 2001). The high degree of correlation between the expression changes in schizophrenia and bipolar disorder provide compelling evidence for common pathophysiological pathways that may govern the disease phenotypes of schizophrenia (Hakak et al. 2001; Deb-Rinker et al. 2002; Tkachev et al. 2003). Moreover, it has been found that loss of *ERBB3*

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results in embryonic lethality in mice with brain defects (Cho and Leahy 2002; Niendorf et al. 2007). In addition, the neuregulin 1 gene (*NRG1*), which codes for the neuron-derived ligand for the ERBB3 receptor, has been identified as a promising susceptibility gene for schizophrenia by our group (Tang et al. 2004; Zhao et al. 2004; Li et al. 2006). There was evidence showing co-expression and functional inter-activity between *ERBB3* and *NRG1* (Britsch 2007; Parlapani et al. 2008), such as the entry of *NRG1* beta1 to the parenchyma of the brain and spinal cord (Kastin et al. 2004). Two recent studies (Kanazawa et al. 2007; Watanabe et al. 2007) reported there was no association between *ERBB3* and schizophrenia in the Japanese population. We studied the Scottish population by sequencing four single nucleotide polymorphisms (SNPs) (rs705708 at intron 15, rs2271189, rs773123, rs2271188 at exon 27).

## Methods

One hundred and ninety cases and 196 controls of Caucasian origin were genotyped using sequencing analysis. The cases contained 82 (71.9%) male and 32 (28.1%) female with a mean age at onset of 26.39 years, SD = 11.29 and the controls comprised 105 (59.3%) male and 72 (40.7%) female with a mean age at onset of 38.52 years, SD = 11.07 (based on the samples genotyped successfully). All the patients were interviewed and diagnosed by two independent psychiatrists according to American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders – IV (DSM-IV). A standard informed consent for the genetic analysis, which was reviewed and approved by the local psychiatry research ethical committee in Scotland, was given by all subjects after the nature of study had been fully explained. Peripheral blood samples were taken from the subjects, and genomic DNA was then extracted from the whole blood using the modified phenol-chloroform method (Gao et al. 2001).

The SNPs were genotyped using the Nested PCR-based sequencing analysis. The primers were shown in supplementary Table I. The PCR products were purified, and then subjected to heat inactivation. The products were then sequenced using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3100 DNA sequencer (Applied Biosystems). The protocols used were as described in one of our previous study (Liu et al. 2005).

The chi-square test was used to check if genotype distributions of the samples were in Hardy–Weinberg equilibrium. The statistical significance of the differences in the allele frequency distributions was estimated using the program Clump 2.2 (Sham and Curtis 1995). The *P* values were corrected as they were assessed using the Monte Carlo approach rather than the $\times 2$ distribution (Sham and Curtis 1995). Each computation was performed with at least 100,000 simulations. The odds ratio and relative risk (95% CI) were calculated using EPI (Ver. 5). The frequencies of multiple marker haplotypes were estimated using both the EHPLUS program (Zhao et al. 2000) and PHASE 2.02 (Stephens et al. 2001). The standardized measure of linkage disequilibrium (LD) for each pair of markers, denoted as D′, was estimated by 2LD software (Zapata et al. 2001). Linkage disequilibrium (LD) plots were generated by Haploview (Barrett et al. 2005). All tests were two tailed and significance was accepted at $P < 0.05$. We also combined our data with the data from Kanazawa et al. (2007) and Watanabe et al. (2007) using meta-analysis. The method was described in our previous studies (Li et al. 2006). The SNP rs2271188, which was investigated in Kanazawa's study, was excluded from the analysis because of low heterozygosity in our samples.

## Results

Genotype distributions of the SNPs were in Hardy–Weinberg equilibrium except rs2271189 in cases ($P = 0.0006$) (Supplementary Table II). The results showed that there was no evidence of significant association for rs705708 and rs2271189. However, we found rs773123 with low heterozygosity of 83% "TT" genotype and 17% "AT" genotype. It is located seven bases downstream of the synonymous SNP (rs2271189, Arg) in the 27th exon. Kanazawa et al. 2007 reported rs773123 was not associated with schizophrenia in Japanese population. However, there was a big difference on allele frequency at rs773123 between Asian and Caucasian populations (such as the T [Cys] allele is 69% in Kanazawa et al.'s study and 92% in our study). We detected evidence of statistically significant difference in the allele frequency distributions between patients and controls with *P* value 0.034 after Monte Carlo simulation (OR 0.41, 95% C.I. 0.18–0.96) (Table I). The subgroups of male patients and patients with age at onset $< 45$ showed evidence of significant association with schizophrenia with *P* values of 0.0046 and 0.0055, respectively (Table II). The *P* values from genotypic analysis were even slightly smaller than the *P* values from allelic analysis (Tables I and II). This nonsynonymous SNP can lead to the change of coding protein residue from AGC[Ser] to TGC[Cys]. Strong linkage disequilibrium was observed between rs2271189 and two other SNPs

Table I. Results of case-control analyses for each SNP.

| | | | | Patients | | Controls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SNPs[a] | Alleles | Distance[b] | Function | Allele1[c] | Allele2[d] | Allele1[c] | Allele2[d] | *P* value | Odds ratio (95% CI) | Relative risk (95% CI) |
| rs705708 | A/G | 0 | intron | 74 (48.68%) | 78 (51.32%) | 132 (42.86%) | 176 (57.14%) | 0.24 | 1.26 <0.84–1.90 > | 1.14 <0.92–1.40 > |
| rs2271189 | C/T | 6.078 | synonymous [Arg] | 119 (61.98%) | 73 (38.02%) | 208 (62.65%) | 124 (37.35%) | 0.88 | 0.97 <0.66–1.43 > | 0.99 <0.86–1.14 > |
| rs773123 | A/T | 6.085 | nonsynonymous, [Ser] to [Cys] | 7 (3.65%) | 185 (96.35%) | 28 (8.43%) | 304 (91.57%) | **0.034** | 0.41 <0.18–0.96 > | 0.43 <0.19–0.97 > |
| | | | | | | | | **0.028**[e] | | |

[a]NCBI SNP Cluster ID.
[b]Positions of SNPs are shown as distances (kb) from rs705708.
[c]Number of the first polymorphism alleles in alphabetical order for each SNP.
[d]Number of the second polymorphism alleles in alphabetical order for each SNP.
[e]*P* value from the genotypic analysis of AT vs. TT (OR = 0.39 <0.16, 0.93 >). *P* value <0.05 is in boldface.
The experimental work and statistical analyses were carried out during 2003–2004. Eight SNPs were selected for genotyping on a small set of samples, and only the SNPs with evidence of association were replicated in the total Caucasian subjects.

Table II. Results of different sex and age at onset groups.

| SNPs/Sex/Age at onset | *P* values | Odds ratio (95% CI) |
|---|---|---|
| rs705708 | | |
| Male | 0.46 | 1.19 (0.74,1.93) |
| Female | 0.47 | 1.33 (0.61,2.93) |
| Early age at onset | 0.62 | 1.12 (0.72,1.73) |
| Late age at onset | 0.43 | 1.76 (0.41,7.51) |
| rs2271189 | | |
| Male | 0.56 | 0.87 (0.54,1.41) |
| Female | 0.92 | 0.97 (0.49,1.91) |
| Early age at onset | 0.87 | 0.97 (0.63,1.48) |
| Late age at onset | 0.93 | 1.06 (0.26,4.39) |
| rs773123 | | |
| Male | **0.0046** | 0.09 (0.01,0.71) |
| | **0.0034**[a] | |
| Female | 0.44 | 0.55 (0.12,2.59) |
| Early age at onset | **0.0055** | 0.21 (0.06,0.7) |
| | **0.0039**[a] | |
| Late age at onset | 0.41 | 1.5 (0.16,13.75) |

[a]*P* value from the genotypic analysis of AT vs. TT. Early age at onset group was defined as individuals with age at onset <45.

($D' > 0.97$); however, LD between rs705708 and rs773123 was very weak (Supplementary Table III and Supplementary Figure). Meta-analysis of each SNP showed no evidence of significance (Supplementary Table IV). There was no evidence of significance for either overall or individual haplotypic analysis (overall $P = 0.068$) for the haplotypes with frequencies >5% in both cases and controls. However, the haplotype (GCT) showed a *P* value of 0.047 with frequency of 0.01 in cases and frequency of 0.054 in controls (Table III).

## Conclusion

To conclude, our study showed evidence of association between the nonsynonymous SNP (rs773123) with schizophrenia in the Caucasian population, in particular in male patients and patients of early age

Table III. Results of multi-marker haplotypes of the case-control study in the Caucasian population.

| Haplotype[a] | Frequency in cases | Frequency in controls | *P* value[b] |
|---|---|---|---|
| 111 | 0.0801 | 0.0401 | 0.10 |
| 121 | 0.4298 | 0.3609 | 0.19 |
| 211 | 0.455 | 0.5122 | 0.30 |
| 212 | 0.01 | 0.0536 | **0.047** |
| Overall *P* value, on 3 degrees of freedom = 0.068 (chi-square = 7.1291) | | | |

[a]For each haplotype, alleles are concatenated according to the order rs705708, rs2271189 and rs773123. 1 = the first polymorphism allele; 2 = the second polymorphism allele, as shown in Table I.
[b]*P* values <0.05 are in boldface italic.
The haplotypes with frequencies <0.05 in both cases and control were not shown.

at onset. To our knowledge, this is the first association study between *ERBB3* and schizophrenia in Caucasian population. Further study with large sample size should be carried out. The involvement of the epidermal growth factor receptor family genes including *ERBB3* in the pathogenesis of schizophrenia deserves further investigation.

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## Statement of interest

None.

## Electronic-database information

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ for *ERBB3*;

Online dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ for *ERBB3*;

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ for *ERBB3*.

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## Supplements

Supplementary Table I. The primers used for the PCR-based sequencing analysis.

| Markers | Primer sequence |
|---|---|
| rs705708 | L (+): AGCGAAGGATTGGAGAAAGG |
| | R (–): TCAGCACCAGTGTTTGTCCT |
| rs2271189[a] | L (+): AGGCAGTGAACAACCCAATA |
| rs773123[a] | R (–): ACCGTTGACATCCTCTTCCT |

[a]Same primer was used for the two SNPs.

Supplementary Table II. Calculation of deviation from Hardy–Weinberg equilibrium.

| SNPs | Ethnicity | Cases | Controls |
|---|---|---|---|
| rs705708 | Caucasian | 0.16 | 0.68 |
| rs2271189 | Caucasian | **0.0006** | 0.98 |
| rs773123 | Caucasian | 0.71 | 0.39 |

Supplementary Table III. Pairwise LD of the case-control study in the Caucasian population.

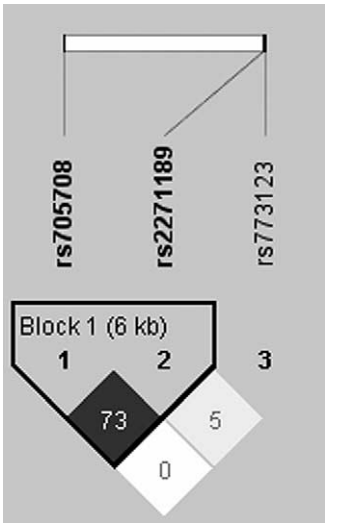
| SNPs[a] | rs705708 | rs773123 | rs2271189 |
|---|---|---|---|
| rs705708 | – | | |
| rs773123 | 0.12 | – | |
| rs2271189 | **0.97** | **0.99** | – |

[a]For each pair of SNPs, the standardized D′ coefficient is shown. D′ values >0.7 are in boldface.

Supplementary Table IV. Results of meta-analysis for each SNP.

| SNPs[a] | Overall OR (95% CI) | *P*(*Z*) values | *P*(*Q*) values |
|---|---|---|---|
| rs705708[a] | 1.03 (0.86,1.24) | 0.74 | 0.24 |
| rs2271189[a] | 0.97 (0.81,1.15) | 0.70 | 0.97 |
| rs773123[b] | 0.94 (0.68,1.29) | 0.68 | 0.04 |
| rs2271189 & rs773123[c] | 0.96 (0.82,1.12) | 0.59 | 0.23 |

[a]Our study with Kanazawa et al. (2007).
[b]Our study with Watanabe et al. (2007).
[c]rs773123 and rs2271189 were combined.
*P*(*Q*) Cochran's Chi-square-based *Q* statistic test was used to assess the heterogeneity.




Supplementary Figure. Pairwise Linkage disequilibrium (LD) plot of three *ERBB3* SNPs based on healthy controls. *The degree of shade of gray is corresponding with the $r^2$ score ($0 < r^2 < 1$).