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Replicated genetic evidence supports a role for HOMER2 in schizophrenia

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ABSTRACT

Schizophrenia is a heritable mental disorder with a complex genetic aetiology potentially implicating glutamatergic dysfunction. Following a search for functionally relevant genes with evidence of linkage to schizophrenia, we selected HOMER2 for as a candidate gene for investigation using a multi-stage association design. Twenty-six tagging SNPs were genotyped in 401 cases and 812 controls and associated SNPs were analysed in an independent sample of 408 cases and 804 controls, all from Ireland. Secondary replication analysis was undertaken using the International Schizophrenia Consortium (ISC) European sample of 1287 cases and 1128 controls. Significant associations were found at five SNPs in the first Irish sample ($p < 0.05$), but were not replicated in the second Irish sample. SNP rs2306428 was significantly associated when the two samples were combined ($p = 0.008$, OR = 0.73) and also by proxy in the ISC sample (rs17158184, $r^2 = 1.0$, $p = 0.019$, OR = 0.75). The protective allele at rs2306428 removes a predicted splice-enhancer binding site where Homer2 is naturally truncated. We did not detect an allelic effect of rs2306428 on neuropsychological function nor on HOMER2 splicing. This study supports a role for HOMER2 gene in schizophrenia susceptibility. Further work is required to confirm and elucidate the role of HOMER2 and interacting genes in schizophrenia aetiology.

Schizophrenia (OMIM 181500) is a substantially heritable ($h^2 \approx 0.8$) mental disorder of complex genetic aetiology characterized by deficits in perception, cognition, motivation and social interaction [4]. Recent studies are starting to elucidate the involvement of rare copy number variants (CNVs) [10] and common risk variants in schizophrenia aetiology [11,16,21] but may be underpowered to explain much of the genetic variance.

Knowledge of gene function and positional cloning evidence remain important investigative tools in complex disease genetics. A substantial body of evidence implicates N-methyl-D-aspartic acid (NMDA)-type glutamate receptors in schizophrenia pathogenesis [8,25,30]. Several genes implicated in schizophrenia to date have important functions at the synapse of glutamatergic neurons e.g. DISC-1, Neuregulin-1 and DTNBP1. To identify novel candidate genes for schizophrenia we cross-referenced the results of three searches: (i) involvement in glutamate processing; (ii) products localizing to the synapse [18] and (iii) genes located in loci linked to schizophrenia [13]. Of the fourteen genes which fulfilled these criteria (CAMK2A, GABBR1, GAD2, GLUL, GRIA1, GRIA4, GRIK2, GRIK4, GRM4, GPR158, HOMER2, SIAH1, SYNGAP1 and TUBA8), we selected HOMER2 for comprehensive evaluation as most of the other genes had been investigated previously.

There are three HOMER genes, all highly expressed in the brain [27]. The Homer proteins form a physical scaffold and signal transduction system for glutamate signalling at the post-synaptic density [3]. They bind many post-synaptic proteins, notably metabotropic and NMDA-glutamate receptors [22,31]. Mice lacking either the Homer1 or Homer2 gene have an exacerbated locomotor response to NMDA receptor antagonists and abnormalities in neuronal glutamate processing [29]. Of three human HOMER genes, only HOMER2 (OMIM 604799) maps to a region suggestive of linkage to schizophrenia (15q21.3–q26.1) [13]. This gene has only been investigated in one small previous study with partial gene coverage [14].

We report a comprehensive investigation of common variation at HOMER2 using three independent case–control schizophrenia samples. In Stage 1 (discovery) we investigated tag SNPs and potentially functional SNPs across the locus in Irish Sample 1. We characterized significant findings at the locus using additional re-sequencing and genotyping. Identified associations were then investigated for replication in an independent Irish case–control sample (Irish Sample 2) and a larger dataset from the International Schizophrenia Consortium (ISC). We investigated phenotypic and functional effects of risk variants detected by analysing clinical and

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Table 1
Association results from Irish Sample 1.

#	SNP ID	Phase	Position[a]	Alleles[b]	Case MAF	Control MAF	χ^b	P	OR	95% CI
1	rs1256430	1	81,316,526	A/G	0.165	0.162	0.02	*0.878*	1.02	0.80–1.29
2	rs12442119	1	81,319,799	T/C	0.269	0.272	0.02	*0.883*	0.99	0.81–1.20
3	rs1256426	2	81,320,422	G/C	0.473	0.454	0.71	*0.400*	1.08	0.90–1.29
4	**rs2306428**	**2**	**81,320,441**	**T/C**	**0.054**	**0.085**	**7.43**	***0.006***	**0.61**	**0.43–0.87**
5	rs1256444	1	81,337,307	C/T	0.462	0.443	0.71	*0.398*	1.08	0.90–1.29
6	rs1256455	1	81,341,576	T/C	0.251	0.226	1.73	*0.189*	1.15	0.93–1.41
7	rs2621226	1	81,342,479	A/G	0.441	0.429	0.27	*0.602*	1.05	0.88–1.26
8	rs17359494	1	81,346,341	C/T	0.224	0.243	1.08	*0.300*	0.89	0.73–1.10
9	rs2621227	2	81,349,516	A/G	0.493	0.498	0.04	*0.843*	0.98	0.82–1.17
10	SNP36	2	81,352,332	A/T	0.010	0.014	0.71	*0.399*	0.69	0.29–1.63
11	**rs7174726**	**1**	**81,354,763**	**C/T**	**0.066**	**0.095**	**4.97**	***0.026***	**0.68**	**0.48–0.96**
12	rs7496832	2	81,355,797	C/A	0.109	0.138	3.71	*0.054*	0.76	0.58–1.01
13	SNP19	2	81,366,190	A/T	0.026	0.017	2.14	*0.144*	1.55	0.86–2.81
14	**SNP20**	**2**	**81,366,498**	**T/C**	**0.112**	**0.142**	**4.18**	***0.041***	**0.76**	**0.59–0.99**
15	**rs12913501**	**1**	**81,372,191**	**T/C**	**0.109**	**0.143**	**4.84**	***0.028***	**0.74**	**0.56–0.97**
16	**rs869498**	**1**	**81,376,406**	**T/C**	**0.133**	**0.100**	**6.09**	***0.014***	**1.39**	**1.07–1.81**
17	rs12148275	1	81,379,899	G/A	0.486	0.484	0.01	*0.918*	1.01	0.85–1.21
18	rs12050725	1	81,381,349	A/T	0.201	0.186	0.71	*0.401*	1.10	0.88–1.37
19	rs12443081	2	81,394,567	A/G	0.101	0.099	0.01	*0.930*	1.01	0.76–1.36
20	rs2061947	1	81,397,236	T/C	0.467	0.448	0.71	*0.400*	1.08	0.90–1.29
21	rs7170046	2	81,402,099	T/C	0.102	0.113	0.63	*0.429*	0.89	0.67–1.19
22	rs1010820	2	81,405,958	A/T	0.194	0.219	1.84	*0.175*	0.86	0.69–1.07
23	rs11857990	2	81,414,427	G/A	0.295	0.328	2.52	*0.112*	0.86	0.71–1.04
24	rs4842928	2	81,417,560	A/T	0.172	0.170	0.01	*0.918*	1.01	0.80–1.28
25	rs1871658	2	81,428,253	G/C	0.433	0.396	2.99	*0.084*	1.17	0.98–1.39
26	rs6603038	2	81,437,335	T/C	0.510	0.478	2.03	*0.155*	1.14	0.95–1.36

[a] Chromosome 15, build 36.
[b] The minor allele is stated first. All alleles are called on the forward strand.

neuropsychological indices of schizophrenia and by examining for effects of detected variants on gene expression.

This study utilized data from three different schizophrenia case–control association samples, termed Irish Sample 1, Irish Sample 2 and the International Schizophrenia Consortium sample. The Irish case sample was 66% male, with mean age at onset of 25 years (SD 8.9) and duration of illness at interview of 19.3 years (SD 12.4). The mean medication dosage was 457 Chlorpromazine equivalents (SD 416). There were no significant demographic differences between Samples 1 and 2.

This sample consisted of 401 cases and 812 controls from the Republic of Ireland. Ethics Committee approval was obtained from all participating hospitals and centres. Case individuals provided written informed consent. Diagnosis was made by the consensus lifetime best estimate method using all available information (Structured Clinical Interview for DSM (SCID-P), family or staff report and chart review). Cases met criteria for DSM-IV schizophrenia ($n = 312$) or schizoaffective disorder ($n = 89$), all were over 18 years of age and of Irish origin (self reported Irish grandparents). The control sample, drawn from anonymized Irish blood donors, was 64.3% male.

This sample consisted of 408 cases and 840 controls. Patients were clinically stable with a diagnosis of DSM-IV schizophrenia ($n = 338$) or schizoaffective disorder ($n = 70$), gave written informed consent and were recruited from five sites across the Republic of Ireland and Northern Ireland using the same inclusion/exclusion criteria as for Sample 1. This case sample was 66.3% male. The control sample, which was 29.6% male, was collected by the Irish Blood Transfusion Service.

The ISC sample has been described in detail elsewhere [11]. Irish cases (from Sample 1) which contributed to the ISC were excluded from this analysis. We excluded samples which were genotyped on the Affymetrix 5.0 array as there was not sufficient coverage of the HOMER2 for replication analysis. The ISC sample used in this study consisted of 1287 cases and 1128 controls from Sweden, Bulgaria and Scotland.

The Caucasian Europeans from Utah (CEU) trio sample from HapMap was used as a reference panel for selecting SNPs at HOMER2 for association analysis. To maximise capture of potential functional variation, eight SNPs in the coding exons and 3′UTR of HOMER2 of unknown minor allele frequency (MAF; rs11629943, rs8041066, rs1051936, rs11541402, rs28550214, rs1802494, 34287296, rs34273427), were tested for heterozygosity in a small discovery panel of fifteen schizophrenia cases. This gave 95% power to detect a SNP with a MAF > 0.1. Those found to be polymorphic were genotyped in the HapMap CEU sample. This data was combined with available online data to give a refined HOMER2 HapMap that was analysed to select tag SNPs that capture the majority of common SNPs across the gene. Tag SNPs (MAF > 0.1) for genotyping were chosen from HapMap CEU data using Tagger as implemented in Haploview 4.1 with $r^2 > 0.8$ [2,6].

The SNaPshot® primer extension method was used to genotype the eight exonic SNPs in 15 schizophrenia cases on an ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems, Warrington, UK). Genotyping of SNPs in the case–control samples was either out-sourced to KBiosciences (www.kbioscience.co.uk/; Hoddesdon, UK), who use a Kaspar assay or genotyped in-house by TaqMan® assay on an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems). DNA samples from the HapMap CEU sample were randomly distributed in our case–control DNA plates for the purposes of quality control. Direct nucleotide sequencing was used to search for novel SNPs in predicted functional sites of HOMER2 in 15 schizophrenia cases using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI PRISM® 3130xl Genetic Analyzer. Details of all genotyping and sequencing assays are available on request. Full details of the genotyping and quality control measures used for the ISC samples are detailed elsewhere [10].

The UCSC Genome Browser [12] was used for determination of intron–exon boundaries and relative position of SNPs. The conservation and DNase H1 hypersensitive site tracks (April 2005 assembly) were used for prediction of functional non-coding sites. The 'Cis-ter' (http://zlab.bu.edu/~mfrith/cister.shtml) *cis*-element cluster finder [7] was queried with the genomic sequence of the HOMER2 gene including a substantial 5′ extragenic region (chr15: 81,300,000–81,450,000, reverse strand). Splice-