

## ARTICLE

# A Genetic Association Study of Chromosome 11q22-24 in Two Different Samples Implicates the *FXYP6* Gene, Encoding Phosphohippolin, in Susceptibility to Schizophrenia

Khalid Choudhury, Andrew McQuillin, Vinay Puri, Jonathan Pimm, Susmita Datta, Srinivasa Thirumalai, Robert Krasucki, Jacob Lawrence, Nicholas J. Bass, Digby Quedsted, Caroline Crombie, Gillian Fraser, Nicholas Walker, Haitham Nadeem, Sophie Johnson, David Curtis, David St. Clair, and Hugh M. D. Gurling

Previous linkage analyses of families with multiple cases of schizophrenia by us and others have confirmed the involvement of the chromosome 11q22-24 region in the etiology of schizophrenia, with LOD scores of 3.4 and 3.1. We now report fine mapping of a susceptibility gene in the 11q22-24 region, determined on the basis of a University College London (UCL) sample of 496 cases and 488 supernormal controls. Confirmation was then performed by the study of an Aberdeen sample consisting of 858 cases and 591 controls (for a total of 2,433 individuals: 1,354 with schizophrenia and 1,079 controls). Seven microsatellite or single-nucleotide polymorphism (SNP) markers localized within or near the *FXYP6* gene showed empirically significant allelic associations with schizophrenia in the UCL sample (for *D11S1998*,  $P = .021$ ; for *rs3168238*,  $P = .009$ ; for *TTTC20.2*,  $P = .048$ ; for *rs1815774*,  $P = .049$ ; for *rs4938445*,  $P = .010$ ; for *rs4938446*,  $P = .025$ ; for *rs497768*,  $P = .023$ ). Several haplotypes were also found to be associated with schizophrenia; for example, haplotype Hap-F21 comprising markers *rs10790212-rs4938445-rs497768* was found to be associated with schizophrenia, by a global permutation test ( $P = .002$ ). Positive markers in the UCL sample were then genotyped in the Aberdeen sample. Two of these SNPs were found to be associated with schizophrenia in the Scottish sample (for *rs4938445*,  $P = .044$ ; for *rs497768*,  $P = .037$ ). The Hap-F21 haplotype also showed significant association with schizophrenia in the Aberdeen sample, with the same alleles being associated ( $P = .013$ ). The *FXYP6* gene encodes a protein called "phosphohippolin" that is highly expressed in regions of the brain thought to be involved in schizophrenia. The protein functions by modulating the kinetic properties of Na,K-ATPase to the specific physiological requirements of the tissue. Etiological base-pair changes in *FXYP6* or in associated promoter/control regions are likely to cause abnormal function or expression of phosphohippolin and to increase genetic susceptibility to schizophrenia.

Schizophrenia (SCZD [MIM #181500]) is a disease characterized by self-neglect, thought disorder, auditory hallucinations, delusions, and bizarre behavior.<sup>1</sup> It has a lifetime prevalence of 0.85% in the United Kingdom. Family, twin, and adoption studies have confirmed that the disorder is highly heritable. More than 20 whole-genome linkage scans of SCZD have demonstrated that there is heterogeneity of linkage, involving multiple loci on different chromosomal regions.<sup>2</sup> It is not yet known whether these loci interact. The linkage studies are interpretable only if it is accepted that at least some of these loci act independently in different families.

Chromosome 11q22 was first implicated in SCZD in a linkage study of two Japanese pedigrees in which positive LOD scores between 1.0 and 1.5 were reported with marker *D11S35*.<sup>3</sup> This was confirmed in a separate study of a large

Canadian pedigree, in which a maximum LOD score of 3.4 was obtained using the same genetic marker, *D11S35*.<sup>4</sup> Later, we published further evidence of linkage to SCZD at 11q23.3 in a genomewide scan of very large multiply affected families.<sup>5</sup> Overall, we found a 3-point LOD score of 3.1 with markers *D11S925* and *D11S934*. A LOD score of 3.2 was found in a single large Icelandic pedigree, again supporting the existence of locus heterogeneity. The chromosome 11q22-24 region has been shown to be one of the most well-established linkages to SCZD by a meta-analysis of 20 genome scans that employed nonparametric rank-order statistics to show that evidence of linkage at this region was nonrandom.<sup>2</sup> Chromosome 11q22.3-q24.1 was ranked third of all regions, and, when the different sample sizes of the 20 SCZD genome scans were taken into account, it was ranked fourth. This provided considerable

From the Molecular Psychiatry Laboratory, Department of Mental Health Sciences, University College London Medical School, Windeyer Institute of Medical Sciences (K.C.; A.M.; V.P.; J.P.; S.D.; R.K.; J.L.; N.J.B.; H.M.D.G.), West London Mental Health Trust, Hammersmith and Fulham Mental Health Unit and St Bernard's Hospital (D.Q.), and Queen Mary College, University of London and East London and City Mental Health Trust, Royal London Hospital (D.C.), London; West Berkshire National Health Service Trust, Reading, United Kingdom (S.T.); Department of Psychiatry, Warneford Hospital, University of Oxford, Oxford, United Kingdom (D.Q.; S.J.); Department of Mental Health, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom (C.C.; G.F.; D.S.C.); Ravenscraig Hospital, Greenock, United Kingdom (N.W.); and Buckinghamshire Mental Health National Health Service Trust, Aylesbury, United Kingdom (H.N.)

Received November 24, 2006; accepted for publication January 22, 2007; electronically published March 1, 2007.

Address for correspondence and reprints: Dr. Hugh M. D. Gurling, Molecular Psychiatry Laboratory, Department of Mental Health Sciences, University College London Medical School, Windeyer Institute of Medical Sciences, 46 Cleveland Street, London W1T 4JF, United Kingdom. E-mail: [h.gurling@ucl.ac.uk](mailto:h.gurling@ucl.ac.uk)

*Am. J. Hum. Genet.* 2007;80:664-672. © 2007 by The American Society of Human Genetics. All rights reserved. 0002-9297/2007/8004-0008\$15.00  
DOI: 10.1086/513475

**Table 1. Allelic Association Tests with SCZD at the *FXD6* Locus in the UCL Sample**

Marker and Sample	Marker Location (bp)	Previous Marker Distance (bp)	$\chi^2$	$P^a$	No. of Observed Alleles for Allelic Base			
					A	C	G	T
<i>rs11999</i> : Control Case	117196128		.91	.340	542 521	300 261		
<i>rs869789</i> : Control Case	117196596	468	2.75	.097	107 138		753 772	
<i>rs4579962</i> : Control Case	117197544	948	.03	.860	274 266		688 680	
<i>rs529623</i> : Control Case	117198465	921	.60	.440		473 475		395 427
<i>rs678776</i> : Control Case	117201954	3,489	.00	.975	285 282		671 666	
<i>rs479991</i> : Control Case	117202168	214	1.17	.280	665 715		197 187	
<i>D11S1998</i> <i>rs11216567</i> : Control Case	117202941 117203693	773 752	12.60 3.25	.021 <sup>b</sup> .072	880 829		70 89	
<i>rs10892181</i> : Control Case	117204796	1,103	.49	.484			351 320	609 594
<i>rs2019655</i> : Control Case	117205088	292	.60	.437	208 230		664 674	
<i>rs10790212</i> : Control Case	117207900	2,812	.47	.493		640 666		240 232
<i>rs3087563</i> : Control Case	117213147	5,247	.09	.763		415 422		451 472
<i>rs3168238</i> : Control Case	117214855	1,708	6.80	.009 <sup>c</sup>			76 47	874 887
<i>rs564989</i> : Control Case	117214964	109	.30	.584		222 226		742 712
<i>rs12363888</i> : Control Case	117218397	3,433	.04	.843		907 909		55 53
<i>rs11608153</i> : Control Case	117221873	3,476	.48	.489	821 805		129 115	
<i>rs512481</i> : Control Case	117224090	2,217	.90	.344	452 425		494 507	
<i>rs476130</i> : Control Case	117233093	9,003	.64	.423		890 853		70 77
<i>TTTC20.2</i> <i>rs7121573</i> : Control Case	117234550 117235040	1,457 490	17.54 3.15	.048 <sup>d</sup> .076	382 331		564 579	
<i>rs1815774</i> : Control Case	117236649	1,609	3.87	.049 <sup>e</sup>		342 311		528 583
<i>rs876798</i> : Control Case	117242650	6,001	.45	.503		125 123		739 797
<i>rs876797</i> : Control Case	117242957	307	2.94	.086	207 186		651 712	
<i>rs873713</i> : Control Case	117245126	2,169	2.86	.091	230 197			710 733

(continued)

**Table 1. (continued)**

Marker and Sample	Marker Location (bp)	Previous Marker Distance (bp)	$\chi^2$	$P^a$	No. of Observed Alleles for Allelic Base			
					A	C	G	T
<i>rs10790218</i> : Control Case	117247205	2,079	2.73	.099			374 334	576 602
<i>rs4938445</i> : Control Case	117250213	3,008	6.68	.010 <sup>f</sup>			299 250	579 632
<i>rs4938446</i> : Control Case	117250259	46	5.00	.025 <sup>g</sup>			296 271	
<i>rs11216598</i> : Control Case	117253662	3,403	.07	.798			296 271	570 655
<i>rs631898</i> : Control Case	117253688	26	2.31	.128			727 771	149 153
<i>rs11605223</i> : Control Case	117254054	366	1.41	.235				490 462
<i>rs3809043</i> : Control Case	117254325	271	.57	.452			490 462	470 494
<i>rs3809042</i> : Control Case	117254330	5	.21	.646			929 898	29 34
<i>rs497768</i> : Control Case	117255950	1,620	5.21	.023 <sup>h</sup>			27 23	925 899
							416 371	500 553

<sup>a</sup> Two-tailed significance from  $2 \times 2 \chi^2$ , with 1 df.

<sup>b</sup>  $P$  from CLUMP Monte Carlo substest T2.

<sup>c</sup> OR 1.64.

<sup>d</sup>  $P$  from CLUMP Monte Carlo substest T4.

<sup>e</sup> OR 1.21.

<sup>f</sup> OR 1.31.

<sup>g</sup> OR 1.26.

<sup>h</sup> OR 1.24.

confidence that an SCZD susceptibility locus was likely to exist in the 11q22-24 region.

## Material and Methods

Allelic association studies were performed to fine map an 11q22-24 SCZD susceptibility gene by detecting linkage disequilibrium (LD). The University College London (UCL) case-control sample consisted of 496 unrelated cases and 488 ancestrally matched controls. Research subjects were selected only if both parents were English, Scottish, or Welsh, with at least three grandparents having the same origins. Subjects were also included if the fourth grandparent was of another white European origin but were excluded if one grandparent was of Jewish or non-European Union (EU) (before the enlargement of the EU in 2004) ancestry. These data were recorded in an ancestry questionnaire, with confirmation from family histories noted on medical records. U.K. National Health Service (NHS) multicenter and local research ethics-committee approval was obtained, and all participating subjects signed an approved consent form after reading an information sheet. Each schizophrenic research subject had received a diagnosis and assessment by NHS psychiatrists as part of routine clinical diagnosis and treatment. Those with short-term drug-induced psychoses, psychoses with either learning disability or head injury, and other symptomatic psychoses were excluded. Schizophrenic subjects were recruited on the basis of having an International Classification of Diseases version 10 (ICD10) diagnosis

**Table 2. Fragment Sizes of Microsatellite Markers at the *FXD6* Locus in the UCL Sample**

Marker and Fragment Size (bp)	No. of Observed Alleles by Population	
	Control	Case
<i>D11S1998</i> :		
150	60	86
158	1	0
162	2	0
166	38	27
170	405	428
174	275	239
178	75	60
182	7	6
186	1	0
<i>TTTC20.2</i> :		
232	8	4
236	3	14
240	22	16
242	3	5
244	130	130
246	11	10
248	97	137
250	54	47
252	113	104
254	65	77
256	75	69
258	60	62
260	31	51
262	60	57
264	12	14
266	46	32
268	3	4
270	17	23
274	9	5
278	2	1
282	1	0

of SCZD recorded in medical case-history notes after clinical interview by NHS psychiatrists. The diagnoses were confirmed by a senior psychiatrist, usually within 1 wk. Schedule for Affective Disorders and Schizophrenia—Lifetime Version (SADS-L) interview was completed for all cases and controls by a research psychiatrist.<sup>6</sup> Schizophrenic subjects were then chosen on the basis of having received a diagnosis at the “probable level” of the Research Diagnostic Criteria (RDC).<sup>7</sup> Patients with schizoaffective bipolar disorder or schizomania were not included. All screened control individuals were also interviewed by a psychiatrist, specifically for the study, and were selected for having no family history of SCZD, alcoholism, or bipolar disorder, according to self-report by the research subject. They were then interviewed with the SADS-L schedule and were included only if they were found to have no present or lifetime history of any RDC-defined mental disorder. The UCL case-control sample has been used in studies of other chromosomal regions and has implicated the chromosome 5q33 *epsin 4* (*CLINT1*) gene encoding enthoproten, the chromosome 8p22 *PCMI* gene, and the chromosome 1q23 *UHMK1* gene as the cause of SCZD.<sup>8–10</sup> The sample has also been used to exclude association between SCZD and markers at the chromosome 1q23 *RGS4* and *CAPON* (*NOS1AP*) genes.<sup>11,12</sup>

Genomic DNA for the UCL case-control sample was extracted from whole-blood samples by use of standard cell lysis, proteinase K digestion, and phenol/chloroform ethanol precipitation method, as described elsewhere.<sup>8</sup> All DNA samples were quantified with Picogreen (Molecular Probes) by use of fluorimetry. Fifteen reference microsatellite markers at chromosomal loci not thought to be involved in SCZD were genotyped in a subset of samples (200 cases and 300 controls). This was done to detect genetic heterogeneity between the cases and controls and to confirm that the samples were genetically well matched. In addition, a statistical test with use of CHECKHET was employed to detect subjects with an atypical genetic background.<sup>13</sup> No evidence of genetic heterogeneity between cases and controls was found with use of the reference markers. The CHECKHET test detected two schizophrenic subjects with abnormal genotypes. These were excluded from the sample before any chromosome 11 markers were genotyped.

The Aberdeen sample consisted of 858 subjects with SCZD and 591 controls. The cases were recruited through Scottish psychiatric hospitals and met DSM-III-R or DSM-IV criteria of SCZD, with use of an operational criteria checklist (OPCRIT). Diagnosis of SCZD was confirmed through agreement by two independent senior psychiatrists on the basis of structured clinical interviews for DSM-III-R/DSM-IV (SCID) and inspection of psychiatric case notes. All controls were volunteers recruited through general practices from the same region of Scotland and were ethnically matched. The control samples were screened for absence of psychiatric illness. Informed consent was obtained from all patients and control individuals. A proportion of the Aberdeen sample was employed elsewhere in tests for genetic association between *NRG1* and SCZD.<sup>14</sup>

Random markers on chromosome 11q22–24, close to the maximum LOD score position in the UCL SCZD family-linkage study, were initially selected for fine mapping.<sup>5</sup> Five of these random microsatellite markers were genotyped before positive association with *D11S1998* was found. Further nonrandom microsatellite and SNP markers close to *D11S1998* were then chosen for genotyping. Microsatellite marker *TTTC20.2* was identified from a simple repeat found in the UCSC Genome Browser database May 2004 assembly. The forward (5'-AGGACCACACTCAGCCTCAC-3') and reverse (5'-GGGAAGGAAGGAGAGAGAGAG-3') primers were designed using Primer3.<sup>15</sup> The primer sequences for *D11S29*, *D11S4127*, *D11S1998*, *D11S939*, *D11S976*, *D11S4195*, *D11S925*, *D11S964*, *D11S836*, and *D11S934* were obtained from the GDB Human Genome Database and Ensembl. Microsatellite markers were genotyped in an initial sample of 443 controls and 480 schizophrenic patients in the UCL sample. PCR amplification of these markers was performed using an M13-tailed primer and a second nontailed primer. A third universal M13 sequence primer labeled with infrared dye IRD700 or IRD800 was used to hybridize against the M13-tailed locus-specific primer. Microsatellite-marker fragment sizes were separated and visualized with either of the two infrared dyes on LiCor 4200L sequencers. Genotyping was accomplished using SAGA-GT genotyping software (LiCor). The genotypes were then checked by eye, with all allele calls verified by a second independent person. Any discrepant genotypes were repeated. SNPs *rs11999*, *rs869789*, *rs529623*, *rs479991*, *rs2019655*, *rs10790212*, *rs3087563*, *rs1815774*, *rs876798*, *rs876797*, *rs14938445*, *rs4938446*, *rs11216598*, and *rs631898* were chosen from Ensembl. Further SNPs—*rs4579962*, *rs678776*, *rs11216567*, *rs10892181*, *rs3168238*, *rs564989*, *rs12363888*, *rs11608153*, *rs512481*, *rs476130*, *rs7121573*, *rs873713*, *rs10790218*, *rs11605223*,

**Table 3. Haplotypic Association Tests with SCZD at the *FXD6* Locus in the UCL Sample**

No. of Markers and Haplotype Reference	Haplotype	Global Empirical <i>P</i> <sup>a</sup>	Alleles Increasing in Cases	Estimated Haplotype Frequency (%)	
				Controls	Cases
2:					
HAP-F1	<i>rs3168238-rs497768</i>	.0028	T-G	47.9	56.0
3:					
HAP-F2	<i>rs564989-rs476130-rs876797</i>	.0043	C-C-C	11.4	14.9
HAP-F3	<i>rs564989-rs476130-rs497768</i>	.0048	T-C-G	41.6	48.7
HAP-F4	<i>rs564989-rs876797-rs873713</i>	.0010	C-C-T	16.9	23.8
HAP-F5	<i>rs476130-rs11605223-rs497768</i>	.0027	C-G-G	11.3	17.1
HAP-F6	<i>rs1815774-rs4938445-rs4938446</i>	.0066	G-G-T	58.1	64.0
HAP-F7	<i>rs11216598-rs11605223-rs497768</i>	.0013	A-G-G	11.4	16.8
HAP-F8	<i>rs11605223-rs3809043-rs497768</i>	.0021	G-C-G	11.5	17.3
4:					
HAP-F9	<i>rs3168239-rs564989-rs876797-rs873713</i>	.0013	T-C-C-T	17.9	23.1
HAP-F10	<i>rs3168238-rs12363888-rs11605223-rs497768</i>	.0057	T-C-G-G	10.8	17.2
HAP-F11	<i>rs3168238-rs476130-rs11605223-rs497768</i>	.0026	T-C-G-G	10.7	16.9
HAP-F12	<i>rs3168238-rs11605223-rs3809043-rs497768</i>	.0016	T-G-C-G	10.8	17.3
HAP-F13	<i>rs3168238-rs11605223-rs3809042-rs497768</i>	.0035	T-G-G-G	10.9	17.2
HAP-F14	<i>rs1815774-rs10790218-rs4938445-rs11605223</i>	.0055	G-G-G-G	40.5	46.7
HAP-F15	<i>rs1815774-rs4938445-rs4938446-rs11605223</i>	.0159	G-G-T-G	40.6	47.2
HAP-F16	<i>rs10790218-rs4938445-rs4938446-rs11605223</i>	.0066	G-G-T-G	40.8	46.3
HAP-F17	<i>rs11216598-rs11605223-rs3809043-rs497768</i>	.0019	A-G-C-G	11.4	16.8

<sup>a</sup> Haplotype-permutation test empirical *P*, based on 9,999 permutations.

*rs3809043*, *rs3809042*, and *rs497768*—were then selected from the International HapMap Project with use of the Haploview tagger function.<sup>16,17</sup> All SNP markers were determined using KASPar (KBioscience), a modified Amplifluor SNP-genotyping method. Of the samples, 17% from each microtitre plate were reduplicated to detect possible errors and to confirm the reproducibility of genotypes. The data were then analyzed to confirm Hardy-Weinberg equilibrium (HWE). Any markers lacking HWE were repeated using an alternative Taqman method. Before any association analysis, the genotype data were assessed using the SCANGROUP program in GENECOUNTING.<sup>18–20</sup> This program highlights any potential genotyping errors by identifying significant differences in haplotypic frequencies between any single 96-well microtitre plate and haplotypic frequencies in all other plates combined. It is argued that genotyping errors can show up as rare haplotypes occurring on just a single plate, whereas true haplotypic associations with disease will appear as differences in haplotype frequencies between cases and controls spread across a number of plates.

Allelic association analysis with SCZD was performed using a simple  $\chi^2$  test for biallelic SNPs and by using CLUMP for microsatellite markers. CLUMP employs an empirical Monte Carlo test of significance that does not require further correction for multiple alleles.<sup>21</sup> Subtests of the CLUMP program include T1, Pearson's  $\chi^2$  statistic of the "raw" contingency table; T2, the  $\chi^2$  statistic of a table with rare alleles grouped together to prevent small expected cell counts; T3, the largest of the  $\chi^2$  statistics of  $2 \times 2$  tables, each of which compares one allele against the rest grouped together; and T4, the largest of the  $\chi^2$  statistics of all possible  $2 \times 2$  tables, which compares any combination of alleles against the rest. To select regions of interest for further investigation, we took account of the output of the most significant of the four statistics by use of CLUMP. Likewise, we considered the pointwise *P* values for markers individually rather than attempting to derive

an overall *P* value for the region on the basis of combining results from multiple markers. The multilocus genotypes were then analyzed for haplotypic association with SCZD by use of GENECOUNTING, which computes maximum-likelihood estimates of haplotype frequencies from phase-unknown case-control data. The significance of any overall haplotypic association with SCZD was then obtained with a permutation test.<sup>18–20</sup> GENECOUNTING was also used to calculate pairwise LD between all markers with use of the LDPAIRS program; this was visualized using LocusView 2.0.

## Results

One of the five initial randomly selected microsatellites in the 11q22-24 region, *D11S1998*, showed allelic association with SCZD (*P* = .021; CLUMP T2) (tables 1 and 2). After this, other markers to be genotyped were selected nonrandomly by virtue of being very likely to be in LD with marker *D11S1998*. From these nonrandom microsatellite markers, *TTTC20.2* was also found to be associated with SCZD (*P* = .048; CLUMP T4). None of the other genotyped microsatellite markers localized to chromosome 11q22-24 showed association with SCZD (*P* values are for CLUMP T1 statistic: for *D11S29*, *P* = .899; for *D11S4127*, *P* = .521; for *D11S939*, *P* = .460; for *D11S976*, *P* = .393; for *D11S4195*, *P* = .287; for *D11S925*, *P* = .379; for *D11S964*, *P* = .132; for *D11S836*, *P* = .423; for *D11S934*, *P* = .561). After microsatellite analysis, SNP markers localized near positive markers *D11S1998* and *TTTC20.2* were selected for genotyping. SNPs *rs3168238* (*P* = .009; odds ratio [OR] 1.64), *rs1815774* (*P* = .049; OR 1.21), *rs4938445* (*P* = .010; OR 1.31), *rs4938446* (*P* = .025; OR 1.26), and



*rs497768* ( $P = .023$ ; OR 1.24) were all found to be associated with SCZD, with six further SNPs (for *rs869789*,  $P = .097$ ; for *rs11216567*,  $P = .072$ ; for *rs7121573*,  $P = .076$ ; for *rs876797*,  $P = .086$ ; for *rs873713*,  $P = .091$ ; and for *rs10790218*,  $P = .099$ ) showing a trend toward association.

Tests of three-marker haplotypic association with use of *rs11216598*, *rs11605223*, and *rs497768* (Hap-F7) provided significant evidence of association with SCZD, with a global permutation significance of  $P = .0013$ . Several other two-, three-, and four-marker tests of haplotypic association were also significantly positive, as shown in table 3. Nearly all the genotyped markers that show significant association with SCZD or a positive trend are localized within the *FXYP* domain—containing ion transport regulator 6 (*FXYP6* [MIM \*606683]) gene. The exceptions to these are markers *rs869789*, *D11S1998*, and *rs11216567*, situated on *FXYP2*. These three markers show significant LD with several of the markers in *FXYP6*. Results of pairwise LD statistics between all markers genotyped and their respective locations are shown in figure 1.

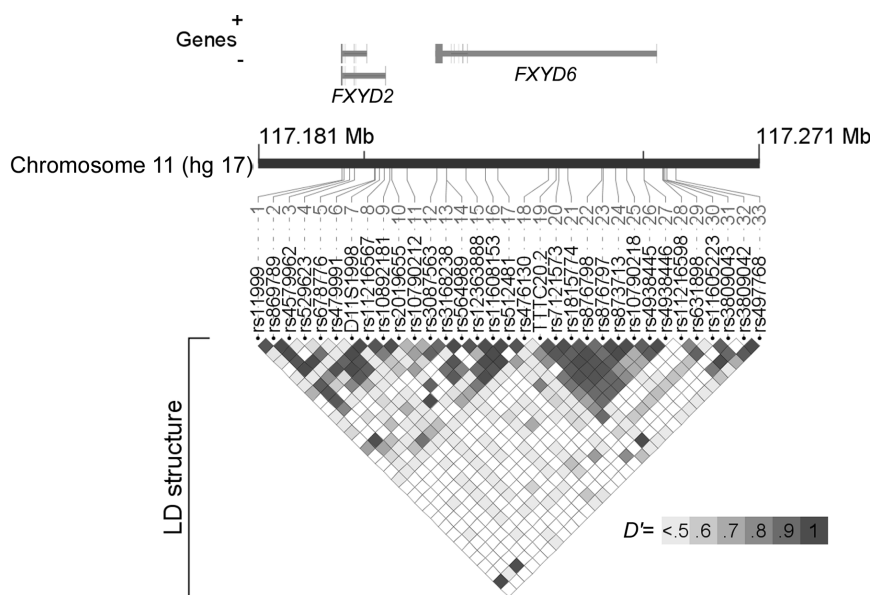
To verify the significance of the positive allelic and haplotypic association found in the UCL sample, replication was attempted in an Aberdeen case-control sample with use of SNPs *rs869789*, *rs11216567*, *rs10790212*, *rs3168238*, *rs876797*, *rs4938445*, and *rs497768*. With a one-tailed test of significance, two SNPs were found to show association with SCZD in the Aberdeen sample (for *rs4938445*,  $P = .044$ ; for *rs497768*,  $P = .037$ ) (table 4). The allele-frequency increase for SNP *rs3168238* ( $P = .074$ ), although not significantly associated with SCZD, displayed a trend

in the direction opposite to that found in the UCL sample. When the data from the UCL and the Aberdeen samples were combined, three SNP markers showed significant allelic association (for *rs869789*,  $P = .023$ ; for *rs4938445*,  $P = .002$ ; and for *rs497768*,  $P = .005$ ), with two further SNPs displaying a trend toward association (for *rs11216567*,  $P = .058$ , and for *rs876797*,  $P = .063$ ) (table 4).

The three-marker haplotype *rs10790212-rs4938445-rs497768* (Hap-F21), which produced a global empirical association in the UCL sample (empirical  $P = .002$ ), was also found to be replicated in the Aberdeen sample (empirical  $P = .013$ ). When data from both samples were combined, haplotype Hap-F21 exhibited strengthened permutation significance for haplotypic association with SCZD (empirical  $P = .0005$ ) (table 5). Other significantly associated haplotypes in the UCL sample were also found to be replicated in the Aberdeen sample and when both samples were combined.

## Discussion

The observation of allelic and haplotypic association in the UCL sample with use of nominal empirically derived  $P$  values and the fact that the same alleles and haplotypes were associated in the second Aberdeen sample suggest that it is unlikely that the association between *FXYP6* and SCZD is a false-positive result. The strongest allelic and haplotypic associations were found with markers within *FXYP6* and not in neighboring genes, which leads us to believe that this gene was the most likely to be involved



**Figure 1.** LD between markers genotyped in the *FXYP6* gene locus in the UCL sample. LD structure ( $D'$ ) between marker pairs is indicated by the shaded matrices. Marker positions are given in table 1, and their locations are shown relative to both *FXYP* genes displayed above the chromosome bar. The gene structures contain vertical lines representing exons. Genomic positions are in accordance with the National Center for Biotechnology Information build 35 hg 17 of the human genome assembly. The figure was generated using LocusView 2.0.

**Table 4. Allelic Association Tests with SCZD at the *FXVD6* Locus in the UCL, Aberdeen, and Combined Samples**

Marker and Sample	Marker Location (bp)	UCL <sup>a</sup>										Aberdeen										Combined (UCL and Aberdeen)									
		No. (%) of Observed Alleles for Allelic Base					$\chi^2$	<i>P</i> <sup>b</sup>	No. (%) of Observed Alleles for Allelic Base					$\chi^2$	<i>P</i> <sup>b</sup>	No. (%) of Observed Alleles for Allelic Base					$\chi^2$	<i>P</i> <sup>b</sup>									
		A	C	G	T	A			C	G	T	A	C			G	T	A	C	G			T								
<i>rs869789</i> :	117196596	107 (.12)	640 (.73)	753 (.88)	240 (.27)	2.75	.097	173 (.15)	860 (.74)	985 (.85)	296 (.26)	1.996	.079	280 (.14)	1500 (.74)	1,738 (.86)	536 (.26)	5.170	.023												
Control		138 (.15)	666 (.74)	772 (.85)	232 (.26)			282 (.17)	1242 (.75)	1384 (.83)	420 (.25)			420 (.16)	1908 (.75)	2156 (.84)	652 (.25)														
<i>rs11216567</i> :	117203693	880 (.93)		70 (.07)		3.25	.072	1040 (.92)		108 (.08)		2.477	.058	1920 (.92)		178 (.08)		3.585	.058												
Control		829 (.90)		89 (.10)				1528 (.92)		128 (.08)				2357 (.92)		217 (.08)															
<i>rs10790212</i> :	117207900		640 (.73)		240 (.27)	.47	.493		860 (.74)		296 (.26)	.040	.420		1500 (.74)		536 (.26)	.435	.510												
Control			666 (.74)		232 (.26)				1242 (.75)		420 (.25)				1908 (.75)		652 (.25)														
<i>rs3168238</i> :	117214855			76 (.08)	874 (.92)	6.80	.009			47 (.04)	1103 (.96)	3.184	.074 <sup>b</sup>					.501	.479												
Control				47 (.05)	887 (.95)					92 (.06)	1558 (.94)				123 (.06)		1977 (.94)														
Case						2.94	.086					.823	.182		139 (.05)		2445 (.95)														
<i>rs876797</i> :	117242957	207 (.24)	651 (.76)					250 (.22)	882 (.78)					457 (.23)	1533 (.77)			3.449	.063												
Control		186 (.21)	712 (.79)					337 (.21)	1295 (.79)					523 (.21)	2007 (.79)																
Case						6.68	.010											9.610	.002												
<i>rs4938445</i> :	117250213	299 (.34)		579 (.66)				376 (.33)		770 (.67)		2.936	.043	675 (.33)		1,349 (.67)															
Control		250 (.28)		632 (.72)				485 (.30)		1145 (.70)				735 (.29)		1,777 (.71)															
Case						5.21	.023											7.866	.005												
<i>rs497768</i> :	117255950	416 (.45)	500 (.55)					521 (.45)	633 (.55)					937 (.45)	1,133 (.55)																
Control		371 (.40)	553 (.60)					676 (.42)	944 (.58)					1,047 (.41)	1,497 (.59)																
Case																															

<sup>a</sup> Data as shown in tables 1.

<sup>b</sup> Two-tailed significance (*P*) from 2 × 2  $\chi^2$ , with 1 df.

<sup>c</sup> One-tailed significance (*P*) from 2 × 2  $\chi^2$ , with 1 df.

**Table 5. Tests of Haplotypic Association with SCZD at the *FXYP6* Locus for Comparison of UCL, Aberdeen, and Combined Samples**

No. of Markers, Haplotype Reference (Haplotype), and Sample	Global Empirical <i>P</i> <sup>a</sup>	Alleles Increasing in Cases	Estimated Haplotype Frequency (%)	
			Controls	Cases
2:				
HAP-F18 ( <i>rs10790212-rs4938445</i> ):				
UCL	.0183	C-G	44.7	48.9
Aberdeen	.0266	C-G	44.6	49.7
Combined	.0093	C-G	44.7	49.4
HAP-F1 ( <i>rs3168238-rs497768</i> ):				
UCL	.0028	T-G	47.9	56.0
Aberdeen	.1173	T-G	52.0	54.2
Combined	.0157	T-G	50.1	54.9
3:				
HAP-F19 ( <i>rs11216567-rs10790212-rs4938445</i> ):				
UCL	.0038	A-C-G	40.2	44.4
Aberdeen	.0453	A-C-G	38.6	44.2
Combined	.0687	A-C-G	39.1	44.1
HAP-F20 ( <i>rs11216567-rs10790212-rs497768</i> ):				
UCL	.0442	A-C-G	35.2	40.3
Aberdeen	.0104	A-C-G	39.3	40.0
Combined	.0348	A-C-G	37.5	40.0
HAP-F21 ( <i>rs10790212-rs4938445-rs497768</i> ):				
UCL	.0024	C-G-G	23.4	27.9
Aberdeen	.0127	C-G-G	25.5	27.6
Combined	.0005	C-G-G	24.7	27.7

<sup>a</sup> Haplotype permutation test empirical *P*, based on 9,999 permutations.

in the etiology of SCZD. One microsatellite marker in *FXYP2* was found to be associated with SCZD, but none of the SNPs within *FXYP2* were significantly associated in either the UCL or the Aberdeen samples. We found consistency as to which alleles and haplotypes were associated with SCZD in the UCL and Aberdeen samples. Even though an allele at marker *rs3168238* showed a trend to be associated with SCZD with an allele different from the one that showed significant association in the UCL sample, it was nevertheless found that the same allele was in the haplotype showing significant association with SCZD in the Aberdeen sample.

*FXYP6* encodes the protein phosphohippolin and is part of a family of seven *FXYP* genes.<sup>22</sup> The *FXYP* proteins share homology for a single common transmembrane domain.<sup>23</sup> Each *FXYP* protein is expressed in a tissue-specific manner and functions by altering the kinetic activity of Na,K-ATPase.<sup>24</sup> This involves changes in the active Na<sup>+</sup> and K<sup>+</sup> transports that are based on the specific requirements of different cells. The effects of *FXYP* proteins on kinetic parameters such as K<sub>1/2</sub> Na<sup>+</sup>, K<sub>1/2</sub> K<sup>+</sup>, and V<sub>max</sub> are usually approximately twofold.<sup>25-27</sup> However, these moderate effects are likely to have long-term physiological importance in maintaining cation homeostasis.<sup>28</sup> Regulation of Na,K-ATPase activity in a tissue- and isoform-specific manner is essential for tissue functions such as muscle contraction and neuronal excitability, because of the modification of its transport properties under changing physiological conditions.<sup>29</sup>

Phosphohippolin in rats has been found to be expressed in the neuronal fibers of the lateral habenula nucleus, hypothalamus, amygdaloid body, hippocampus, thalamus, stria terminalis, cingulum, olfactory bulb, cerebral cortex, and cerebellum.<sup>22,30</sup> The distribution in the cerebellum is unique, with a predominant expression pattern in the granule layer of the posterior lobe. Expression studies of the brain during development show the greatest amount of phosphohippolin in postnatal 3-wk-old rat brain, with substantial capacity of phosphohippolin still existing in the adult brain. This suggests that phosphohippolin may play an important role in neuronal excitability of the CNS during postnatal development and in the adult brain.<sup>22</sup> The Novartis gene-expression-atlas database (GNF SymAtlas) shows that the expression of *FXYP6* in humans is primarily in the brain, with the highest level of expression found in the prefrontal cortex, amygdala, hypothalamus, and occipital lobe. The prominent levels of expression in regions of the brain thought to be involved in SCZD, as identified by brain-imaging abnormalities, provide strong support that the *FXYP6* gene increases genetic susceptibility to SCZD.

Confirmation of genetic association with SCZD requires attention to the statistical power of future case-control replication studies. The critical issue is the proven presence of locus heterogeneity for SCZD. The linkage studies of SCZD have shown strong evidence of heterogeneity, with different subgroups of families containing different susceptibility loci. Therefore, any one susceptibility gene

will increase susceptibility to SCZD in only a minority of cases. The UCL SCZD sample has the power to detect allelic association of 0.99 at  $P < .05$  and of 0.91 at  $P < .001$ , with the assumption of complete LD between markers and disease alleles when the minor marker-allele frequency is  $< 10\%$  and with a difference of 5% in allele frequencies between cases and controls. If the allele frequency is as high as 50%, the power is 0.90 at  $P = .05$ , with a 10% allele-frequency difference. The power of the sample is only 0.41 at  $P = .05$  for a 5% allele-frequency difference. Further attempts at replication should aim to have 0.8 power for a significance of .05; this may require 1,300 cases and 1,300 controls, even with the assumption of complete LD between markers and disease mutations.

The *FXVD6* gene should now be sequenced in schizophrenic subjects from our sample who have inherited the alleles and haplotypes contributing to the positive association that has been found. This could identify etiological base-pair changes or mutations affecting the expression or function of *FXVD6* that are involved in the molecular pathology of SCZD. These may be "private" mutations not yet identified or genetic variants that are already in databases.

### Acknowledgments

This research was funded by the Neuroscience Research Charitable Trust. The sample collection in Scotland was funded by the University of Aberdeen and GlaxoSmithKline. We have no commercial conflict of interest. We thank the numerous NHS doctors who helped collect the samples.

### Web Resources

The URLs for data presented herein are as follows:

Ensembl, <http://www.ensembl.org/>  
 GDB Human Genome Database, <http://www.gdb.org/>  
 GNF SymAtlas, <http://symatlas.gnf.org/SymAtlas/>  
 International Haplotype Project, <http://www.hapmap.org/>  
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for SCZD and *FXVD6*)  
 UCSC Genome Browser, <http://genome.ucsc.edu/>

### References

- Andreasen NC (1995) Symptoms, signs, and diagnosis of schizophrenia. *Lancet* 346:477–481
- Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, Williams NM, Schwab SG, Pulver AE, Faraone SV, et al (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am J Hum Genet* 73:34–48
- Nanko S, Gill M, Owen M, Takazawa N, Moridaira J, Kazamatsuri H (1992) Linkage study of schizophrenia with markers on chromosome 11 in two Japanese pedigrees. *Jpn J Psychiatry Neurol* 46:155–159
- Maziade M, Raymond V, Cliche D, Fournier JP, Caron C, Gagneau Y, Nicole L, Marcotte P, Couture C, Simard C, et al (1995) Linkage results on 11Q21-22 in eastern Quebec pedigrees densely affected by schizophrenia. *Am J Med Genet* 60:522–528
- Gurling HMD, Kalsi G, Brynjolfsson J, Sigmundsson T, Sherrington R, Mankoo BS, Read T, Murphy P, Blaveri E, McQuillin A, et al (2001) Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. *Am J Hum Genet* 68:661–673
- Spitzer RL, Endicott J (1977) *The Schedule for Affective Disorders and Schizophrenia, Lifetime Version*, 3rd ed. New York State Psychiatric Institute, New York
- Spitzer RL, Endicott J, Robins E (1978) *Research Diagnostic Criteria for a selected group of functional disorders*, 3rd ed. New York State Psychiatric Institute, New York
- Pimm J, McQuillin A, Thirumalai S, Lawrence J, Queded D, Bass N, Lamb G, Moorey H, Datta SR, Kalsi G, et al (2005) The *Epsin 4* gene on chromosome 5q, which encodes the clathrin-associated protein enthoprotin, is involved in the genetic susceptibility to schizophrenia. *Am J Hum Genet* 76:902–907
- Gurling HM, Critchley H, Datta SR, McQuillin A, Blaveri E, Thirumalai S, Pimm J, Krasucki R, Kalsi G, Queded D, et al (2006) Genetic association and brain morphology studies and the chromosome 8p22 pericentriolar material 1 (PCM1) gene in susceptibility to schizophrenia. *Arch Gen Psychiatry* 63:844–854
- Puri V, McQuillin A, Choudhury K, Datta S, Pimm J, Thirumalai S, Krasucki R, Lawrence J, Queded D, Bass N, et al (2006) Fine mapping by genetic association implicates the chromosome 1q23.3 gene UHMK1, encoding a serine/threonine protein kinase, as a novel schizophrenia susceptibility gene. *Biol Psychiatry* (electronically published September 13, 2006; accessed February 19, 2007) (<http://www.journals.elsevierhealth.com/periodicals/bps/article/PIIS0006322306008067/abstract>)
- Puri V, McQuillin A, Thirumalai S, Lawrence J, Krasucki R, Choudhury K, Datta S, Kerwin S, Queded D, Bass N, et al (2006) Failure to confirm allelic association between markers at the *CAPON* gene locus and schizophrenia in a British sample. *Biol Psychiatry* 59:195–197
- Rizig MA, McQuillin A, Puri V, Choudhury K, Datta S, Thirumalai S, Lawrence J, Queded D, Pimm J, Bass N, et al (2006) Failure to confirm genetic association between schizophrenia and markers on chromosome 1q23.3 in the region of the gene encoding the regulator of G-protein signaling 4 protein (RGS4). *Am J Med Genet B Neuropsychiatr Genet* 141:296–300
- Curtis D, North BV, Gurling HM, Blaveri E, Sham PC (2002) A quick and simple method for detecting subjects with abnormal genetic background in case-control samples. *Ann Hum Genet* 66:235–244
- Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E, Gunnarsdottir S, Walker N, Petursson H, Crombie C, et al (2003) Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am J Hum Genet* 72:83–87
- Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 132:365–386
- Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–265



17. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D (2005) Efficiency and power in genetic association studies. *Nat Genet* 37:1217–1223
18. Zhao JH, Curtis D, Sham PC (2000) Model-free analysis and permutation tests for allelic associations. *Hum Hered* 50:133–139
19. Zhao JH, Lissarrague S, Essioux L, Sham PC (2002) GENE-COUNTING: haplotype analysis with missing genotypes. *Bioinformatics* 18:1694–1695
20. Curtis D, Knight J, Sham PC (2006) Program report: GENE-COUNTING support programs. *Ann Hum Genet* 70:277–279
21. Sham PC, Curtis D (1995) Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet* 59:97–105
22. Kadowaki K, Sugimoto K, Yamaguchi F, Song T, Watanabe Y, Singh K, Tokuda M (2004) Phosphohippolin expression in the rat central nervous system. *Brain Res Mol Brain Res* 125:105–112
23. Sweadner KJ, Rael E (2000) The FXYD gene family of small ion transport regulators or channels: cDNA sequence, protein signature sequence, and expression. *Genomics* 68:41–56
24. Crambert G, Geering K (2003) FXYD proteins: new tissue-specific regulators of the ubiquitous Na,K-ATPase. *Sci STKE* 2003:RE1
25. Beguin P, Crambert G, Monnet-Tschudi F, Uldry M, Horisberger JD, Garty H, Geering K (2002) FXYD7 is a brain-specific regulator of Na,K-ATPase  $\alpha$ 1- $\beta$  isozymes. *EMBO J* 21:3264–3273
26. Crambert G, Fuzesi M, Garty H, Karlsh S, Geering K (2002) Phospholemman (FXYD1) associates with Na,K-ATPase and regulates its transport properties. *Proc Natl Acad Sci USA* 99:11476–11481
27. Crambert G, Li C, Claeys D, Geering K (2005) FXYD3 (Mat-8), a new regulator of Na,K-ATPase. *Mol Biol Cell* 16:2363–2371
28. Garty H, Karlsh SJ (2006) Role of FXYD proteins in ion transport. *Annu Rev Physiol* 68:431–459
29. Geering K (2006) FXYD proteins: new regulators of Na-K-ATPase. *Am J Physiol Renal Physiol* 290:F241–F250
30. Yamaguchi F, Yamaguchi K, Tai Y, Sugimoto K, Tokuda M (2001) Molecular cloning and characterization of a novel phospholemman-like protein from rat hippocampus. *Brain Res Mol Brain Res* 86:189–192