Molecular Psychiatry (2004) 9, 784–795

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Multicenter linkage study of schizophrenia loci on chromosome 22q

BJ Mowry[*1,2], PA Holmans[3], AE Pulver[4], PV Gejman[5], B Riley[6], NM Williams[7], C Laurent[8], SG Schwab[9], DB Wildenauer[9], S Bauché[8], MJ Owen[7], B Wormley[6], AR Sanders[5], G Nestadt[4], KY Liang[10], J Duan[5], R Ribble[6], N Norton[7], S Soubigou[11], W Maier[9], KR Ewen-White[12], N deMarchi[13], B Carpenter[5], D Walsh[14], H Williams[7], M Jay[8], M Albus[15], DA Nertney[1], G Papadimitriou[16], A O'Neill[17], MC O'Donovan[7], J-F Deleuze[11], FB Lerer[18], D Dikeos[16], KS Kendler[6], J Mallet[8], JM Silverman[19], RR Crowe[20] and DF Levinson[21]

[1]*Queensland Centre for Mental Health Research, The Park – Centre for Mental Health, Wacol, Queensland, Australia;* [2]*Department of Psychiatry, University of Queensland, Brisbane, Queensland, Australia;* [3]*MRC Biostatistics Unit, Cambridge, UK;* [4]*Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [5]*Northwestern University, ENH Research Institute, Evanston, IL, USA;* [6]*Department of Psychiatry, Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA, USA;* [7]*Department of Psychological Medicine, University of Wales College of Medicine, Cardiff, UK;* [8]*Laboratoire de Génétique Moléculaire de la Neurotransmission et des Processus Neurodégénératifs, Centre National de la Recherche Scientifique, Hôpital de la Pitié Salpêtrière, Paris, France;* [9]*Department of Psychiatry, University of Bonn, Bonn, Germany;* [10]*Department of Biostatistics, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA;* [11]*Aventis Pharma SA, Evry, France;* [12]*Australian Genome Research Facility, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia;* [13]*Clinica Psichiatrica, Seconda Universita degli Studi di Napoli, Naples, Italy;* [14]*The Health Research Board, Dublin, Ireland;* [15]*State Mental Hospital, Haar, Germany;* [16]*Department of Psychiatry, University of Athens Medical School, Athens, Greece;* [17]*Department of Psychiatry, Queens University, Belfast, Northern Ireland;* [18]*Department of Psychiatry, Hadassah-Hebrew University Medical Center, Jerusalem, Israel;* [19]*Department of Psychiatry, Mt Sinai School of Medicine, New York, NY, USA;* [20]*Mental Health Clinical Research Center and Department of Psychiatry, The University of Iowa College of Medicine, Iowa City, IW, USA;* [21]*Department of Psychiatry, University of Pennsylvania, Philadelphia, PA, USA*

The hypothesis of the existence of one or more schizophrenia susceptibility loci on chromosome 22q is supported by reports of genetic linkage and association, meta-analyses of linkage, and the observation of elevated risk for psychosis in people with velocardiofacial syndrome, caused by 22q11 microdeletions. We tested this hypothesis by evaluating 10 microsatellite markers spanning 22q in a multicenter sample of 779 pedigrees. We also incorporated age at onset and sex into the analysis as covariates. No significant evidence for linkage to schizophrenia or for linkage associated with earlier age at onset, gender, or heterogeneity across sites was observed. We interpret these findings to mean that the population-wide effects of putative 22q schizophrenia susceptibility loci are too weak to detect with linkage analysis even in large samples.
Molecular Psychiatry (2004) **9,** 784–795. doi:10.1038/sj.mp.4001481
Published online 4 March 2004

Keywords: linkage (genetics); chromosomes, human, pair 22; schizophrenia; genetics, molecular; genetics, medical

Introduction

Schizophrenia is a severe, debilitating disorder characterized by delusional beliefs, hallucinations, disordered speech, and deficits in emotional and social behavior. It is strongly familial, but the pattern of inheritance is complex, with most data favoring the interaction of multiple genes, each exerting a small to moderate effect on overall disease risk. There are now a number of candidate genomic regions supported by converging evidence from independent studies.[1,2] One of these is a broad region of chromosome 22q.

Pulver *et al*[3] reported a maximum LOD score (MLS) of 1.54 at IL2RB in 39 multiplex European–American pedigrees. There have been other positive reports, including some in partially or completely overlapping multicenter samples, and these are summarized in Table 1. Most of these findings have focused on two regions: 22q12, in the vicinity of D22S278, and 22q11, closer to the microdeletion responsible for the

*Correspondence: BJ Mowry, Queensland Centre for Mental Health Research, The Park – Centre for Mental Health, Wolston Park Road, Wacol, Queensland 4076, Australia.
E-mail: bryan_mowry@qcsr.uq.edu.au
Received 29 August 2003; revised 28 November 2003; accepted 08 December 2003

Table 1 Schizophrenia linkage findings on chromosome 22q

First author, year	*#Peds*	*#Aff*	*Phenotype; diagnostic criteria*	*Ethnicity*	*Cytogenetic location*	*Physical location (Mb)*	*Genetic location (cM)*	*Most positive result (test)*
Studies of chromosome 22 markers[a]								
Pulver, 1994[3]	39		SZ, SA (RDC)	Eur, Afr-Am	q13.1	35.76	44.75	LOD = 2.82 (dom)
Lasseter, 1995[4]	57		SZ, SA (RDC)	Eur; Afr-Am	q11.1–q13	23.94	29	LOD = 1.71 (dom)
Vallada, 1995[5]	23	84	SZ/SA	UK, Japan	q12	34.67	40.73	LOD = 1.51 (rec)
Schwab, 1995[6,b]	30	84	SZ/SA-S (RDC)	Israel, (Seph), Ger	q12	33.69	42.1	LOD = 0.612 (dom)
Kalsi, 1995[7,c]	23		SZ, UFP (RDC)	UK, Iceland	q12	35.02–43.54	47–56.47	NS (par and nonpar)
Riley, 1996[8]	20	58	SZ	Bantu	p11.1–q12	15.54–35.02	0–47	NS (par and nonpar)
Parsian, 1997[9]	23	46	SZ; spectrum	US; Eur	q12–q13	28.88–40.63	34.7–50.81	NS (par and nonpar)
Genome scans reporting evidence for chromosome 22 linkage								
Coon, 1994[10]	9	35	SA, SA-S (RDC)	Eur	q12–q13	40.25	49.28	LOD = 2.09
Blouin, 1998[11,d]	54	146	SZ/SA, (D4)	Eur; Afr-Am	q12	33.71	39.06	NPL = 2.42 ($P = 0.009$)
Stober, 2001[12]	12	57	Periodic catatonia	German	q13	47.62	68.82	NPL = 1.85 ($P = 0.0018$)
DeLisi, 2002[13,e]	294	669	SZ/SA (D3R)	US, UK, Italian, Chilean, Belgian	q12	35.02	47	MLS = 2.0 (and $P < 0.00005$ for maternal allele sharing, ASPEX)
Combined or meta-analyses[f]								
Pulver, 1994[14]	256		SZ, SA (RDC)	Diverse, predom Eur	q12–q13.1	28.88–40.63	34.7	NS (dom and nonpar)
Gill, 1996[15]	620		SZ/SA (D3R/ RDC)	Diverse, predom Eur	q12	34.67	40.73	$P = 0.004$ (ASP possible triangle method)
Badner, 2002[1,g]	681	1978	D3R, D4, RDC	Diverse; predom Eur	pter-q12.3	32.63	38.55	$P < 9 \times 10^{-5}$ (Multiple Scan Probability meta-analysis method, 'best' analysis)
Lewis, 2003[2,h]	1208	2945	D3R, D4, RDC	Diverse; predom Eur	pter-q12.3	0–34.02	0–39.85	Eighth highest rank of $120 \approx 30$ cM bins in rank-based Genome Scan Meta-Analysis (see text)

#Peds = number of pedigrees.
#Aff = number of genotyped affected individuals (not given in all papers).
Phenotype: SZ = schizophrenia; SA = schizoaffective disorder (-S = mainly schizophrenic); UFP = unspecified functional psychosis.
Diagnostic criteria: D3R = DSM-III-R; D4 = DSM-IV; RDC = Research Diagnostic Criteria.
Ethnicity: Eur = European ancestry; Af-Am = African-American; Seph = Sephardic; Ger = German; Swe = Swedish; Fin = Finnish.
Physical locations are in distances from pter, and are mostly taken from NCBI Map View Build 33, assembly of human genome sequence data available on April 10, 2003, supplemented where necessary by the deCODE map.[16] Genetic locations are taken from the deCODE map. Test: par = parametric; nonpar = nonparametric; dom = dominant; rec = recessive; ASP = affected sibpair analysis; MLS = maximum LOD score; NPL = nonparametric linkage score (Zall); LOD = lod score; NS = nonsignificant.
[a]Shown are nonoverlapping studies reporting specifically on chromosome linkage results. Preliminary reports of positive genome scan results are not shown (see footnotes for individual scans below).
[b]Preliminary report of genome scan.[17]
[c]Preliminary report of genome scan.[18]
[d]Final report of a genome scan whose preliminary data produced the first report of possible chromosome 22 linkage[3] and a follow-up report.[4]
[e]Final report of a genome scan; two preliminary reports described chromosome 22 data.[19,20]
[f]Clinical samples considered by these analyses are partially overlapping. For the two meta-analyses, Lewis *et al*[2] describe the degree of overlap with Badner and Gershon[1].
[g]Badner and Gershon[1] locate the 'regional' *P*-value at 32 cM (Marshfield); this is approximated here by D22S1162 (32.63 Mb; 31.84 cM Marshfield map).
[h]Distal boundary of the highly ranked chr22 bin occurs at D22S424: physical location: 34.02 Mb; genetic location: 33.8 cM (Marshfield).

velocardiofacial syndrome (VCFS). No study has produced genome-wide statistically significant results in these regions, and the most positive linkage report in a single sample (max LOD = 3.8) is in 22q13 for bipolar disorder.[21]

Badner and Gershon[1] carried out a meta-analysis of published schizophrenia and bipolar genome scan data using Multiple Scan Probability, which combines *P*-values from linkage regions with clusters of positive results. At 32 cM on the Marshfield map, approximately 2 Mb centromeric to D22S278, they reported evidence for linkage to schizophrenia ($P = 0.0002$), and stronger evidence for linkage to bipolar disorder ($P = 0.00003$). Lewis *et al*[2] reported on a rank-based Genome Scan Meta-Analysis method,[22] which considers all available (published and unpublished) genome scan data for the schizophrenia phenotype (approximately 1200 pedigrees), including most although not all of the samples shown in the table. Chromosome 22 did not produce one of the five most positive linkage results in any of the 20 scans, yet many scans produced small positive scores on proximal 22q, and the 30 cM proximal 22q region, which includes the VCFS region, produced the eighth-best rank in the genome. This was one of 12×30 cM 'bins' in 10 chromosomal regions that met aggregate criteria for significance, more often than expected by chance (ie clusters of nominally significant *P*-values that did not occur in 1000 replicates of the entire unlinked data set). A companion analysis of all available bipolar disorder data did not produce a significant result.[23] The authors concluded that many or all of these regions, including chromosome 22q, were likely to contain weakly linked schizophrenia susceptibility loci.

Chromosome 22q has been of particular interest because schizophrenia or schizoaffective disorder is observed in over 20% of patients with VCFS,[14,24] which is caused by 1.5–3 Mb microdeletions in 22q11. VCFS is characterized by cleft palate, cardiac defects, learning disabilities and a typical facial appearance.[25] Psychosis is associated with a variable deletion size (1.5–3.0 Mb),[26] and may occur in the absence of cardiac defects and cleft palate.[27,28] VCFS microdeletions have been found in 0.6%[29] to 2%[30] of adult patients with schizophrenia and 6% of those in whom onset was below the age of 16 years.[31] Thus, it appears highly likely that deletion of one or more 22q11 genes can produce a schizophrenia-like phenotype. It is less clear whether sequence variations in any of these genes can also increase risk for schizophrenia in individuals without microdeletions. It is possible that one or more genes on 22q11 will be associated with an early-onset phenotype, given the higher 22q11 microdeletion rate in children with schizophrenia, and the multiple premorbid cognitive and social impairments and distinguishing MRI features of children with VCFS and schizophrenia.[31]

Evidence for association with schizophrenia has recently been reported for several 22q loci as shown in Table 2. In the VCFS deletion region, these include PRODH (proline dehydrogenase, 22q11);[32] COMT (catechol-*O*-methyltransferase, 22q11.21);[33] ZNF74 (zinc-finger protein 74, 22q11.21);[34] PCQAP (PC2 (positive cofactor 2, multiprotein complex) glutamine/Q-rich-associated protein, 22q11.21) to age at onset but not to schizophrenia;[35] UFD1L (ubiquitin fusion degradation 1 like, 22q11.21);[36] and SNAP29 (synaptosomal-associated protein, 29 kDa, 22q11.21).[37] Two groups have reported evidence for association between a microsatellite marker in the VCFS deletion region, D22S944, and schizophrenia.[38,39] Analyses of small samples of parent–proband trios[40] or cases and controls[41] showed no linkage disequilibrium between D22S278 and schizophrenia after correction for multiple tests. The strongest findings on 22q thus far are those for PRODH and COMT, although the PRODH finding was not confirmed in a recent study.[42] Distal to the microdeletion region, weak association to schizophrenia was reported for a VNTR in the 5′-untranslated region of YWHAH (tyrosine-3-monooxygenase, 14-3-3 protein eta gene, 22q12.3).[43] Also, in a microarray study of 300 candidate genes in three independent brain sample cohorts, significant upregulation of gene expression in schizophrenia was reported for APOL1 (apolipoprotein L1, 22q13.1), APOL2 (apolipoprotein L2, 22q12) and APOL4 (apolipoprotein L4, 22q13.1).[44]

Owing to these reports of linkage, and association between schizophrenia and diverse loci on chromosome 22q and of expression of genes on 22q in the brains of people with schizophrenia, we carried out a linkage study of 10 microsatellite markers covering all of 22q in a large multicenter schizophrenia pedigree sample, and incorporated both age at onset (AAO) and sex into the analysis as covariates. As noted in Table 1, the clinical sample overlapped partially with several previous multicenter analyses; however, these were limited to a few markers near IL2RB or D22S278. Linkage has not been previously analyzed in a large multicenter sample using markers spanning all of chromosome 22q.

Materials and methods

Clinical sample

The sample is summarized in Table 3 and has been described previously.[45] Briefly, clinical samples collected by eight research groups (the seven participating collaborators plus the NIMH Human Genetics Initiative sample) have been combined into a sample of 779 informative pedigrees (679 of them of predominantly European ancestry) containing 838 independent affected sib pairs and 1918 genotyped individuals with schizophrenia or schizoaffective disorder. Each sample was collected with appropriate human subjects approvals including informed consent. Subjects were interviewed by research clinicians using standardized diagnostic instruments, and best-estimate diagnoses were carried out based on interviews, medical records, and family informant reports. A case was considered affected if the DSM-IIIR diagnosis was schizophrenia or schizoaffective disorder. Predominant ethnic origins across the sample

Table 2 Schizophrenia association findings on chromosome 22q

Study	*Sample*	*Ethnicity*	*Markers*	*Evidence*
COMT	22q11.21		18.30–18.33 Mb	
Li *et al*, 1996[46]	178 trios	Han; Caucasian		TDT: Val-108 pref. transm. ($P=0.005$);
Kunugi *et al*, 1997[47]	22 peds	Japanese	$Val^{108/158}Met$	Both studies combined ($P=0.0015$)
Chen *et al*, 1996[48]	177 SZ, 99 controls	Chinese (Taiwan)	Four SNPs, one ins/del	No differences in genotype/haplotype frequencies; no support
Daniels *et al*, 1996[49]	78 SZ, 78 controls	UK	NlaIII polymorphism	NS
Ohmori *et al*, 1998[50]	150 SZ, 150 controls	Japanese	$Val^{108/108}Met$	Met-108 allele
Li *et al*, 2001[38]	198 trios	Chinese (Sichuan)	Five SNPs in COMT (incl $Val^{108/158}Met$)	$P=0.04$ (186T/C); $P=0.01$ (Val158/Met); $P=0.0006$ (five marker haplotype)
Liou *et al*, 2001[51]	198 SZ, 188 controls	Chinese	Val $^{158/158}$ Met	NS; $P=0.005$: age at onset
Takase *et al*, 2001[34]	(a) 299 SZ, 363 controls	Japanese	Five STRPs, $Val^{108/158}Met$, 4 SNPs in ZNF74 (19.07Mb)	(a) $P=0.04$ (D22S264, SZ); $P=0.0001$ (ZNF74*1150T/C; age at onset)
	(b) 169 additional SZ *vs* controls	Japanese		(b) $P=0.0001$ (ZNF74*1150T/C; age at onset)
Egan *et al*, 2001[52]	(a) 175 SZ, 219 unaff sibs, 55 controls	Eur-Am	$Val^{108/158}Met$	(a) $P=0.001$ (Met allele; better cognitive performance)
	(b) 104 trios			(b) TDT: Val allele pref transm ($P=0.03$; OR = 1.5)
Joober *et al*, 2002[53]	104 SZ, 96 controls	Quebec	$Val^{108/158}Met$	NS
Shifman *et al*, 2002[33]	720SZ, 2970 controls	Ashkenazi	12 SNPs incl $Val^{108/158}Met$	$P=9.5\times10^{-8}$ (rs737865–rs165688–rs165599 G-G-G haplotype)
WKL1(MLC1)	22q13.33		48.67–48.70 Mb	
Meyer *et al*, 2001[54]	One pedigree	German	WKL1	Exon11: 1120C/A: Leu309Met mutation
Devaney *et al*, 2002 [55]	(a) 28 + 15 SZ	Eur-Am; Ger	WKL1: 15 SNP (noncoding), three SNP (3′-UTR), four SNP (syn), two (nonsyn)	NS
Jorgensen *et al*, 2002[56]	10 SZ, 44 controls	Faroe Is	D22S279–276	Differential haplotype freq ($P=0.0075$);
			WKL1: exon11: 1120C/A	NS
McQuillin *et al*, 2002[57]	174 SZ, incl 22 cat sz	UK	WKL1: exon11: 1120C/A	NS
PRODH	22q11.21		17.27–17.29 Mb	
Liu *et al*, 2002[32]	(a) adult SZ: 107 trios	U.S. (NIMH-GI), diverse	10 SNPs (after screening 18 SNPs in nine genes across microdeletion region)	(a) $P=0.04$ (PRODH 1945 allele 3); $P=0.003$ (PRODH 1766/19452-2 haplotype); OR = 4.6 (with early childhood deviance) or OR = 3.3 (onset before 19) for PRODH 1766/1945/2026 2-2-1 haplotype
	(b) COS: 29 trios	U.S. (diverse)		
	(c) 109cases/75 controls	SA Afrikaner		(b) $P=0.001$, PRODH 1766/1945 2-2 haplotype; $P=0.003$, PRODH 1945/2026 2-1 haplotypes (c) $P=0.01$, OR = 2.6, PRODH 1766/1945/2026 2-2-1 haplotype, (onset < 19)
Williams *et al*, 2003[42]	(a) 677 SZ, 679 controls	UK	SNPs 1945, 2026	NS
	(b) 55 COS trios	Bulgaria		
OTHER GENES				
Saleem *et al*, 2001[58]	103BP, 103 SZ, 120 controls	Indian	38.91 Mb *(GAN: 9295519); CAG repeat-containing locus	22CH3: eight-repeat allele and 8/8 genotype: higher freq. in cases ($P<0.02$)
De Luca *et al*, 2001[36]	(a) 88 SZ, 92 controls	Italian	UFD1L (22q11.21; 17.81–17.84 Mb)—SNP in promoter	(a) $P=0.009$
	(b) 38 trios	Canadian		(b) $P=0.03$
Li *et al*, 2000[38]	198 trios	Chinese (Sichuan)	ARVCF (22q11.21; 18.33–18.37 Mb)—two SNPs, D22S264	$P=0.0006$ (ARVCF930C)
De Luca *et al*, 2003[35]	189 SZ/222 controls	Caucasian: Italian	PCQAP (22q11.21; 19.18–19.26 Mb)—exon 7 CAG repeat	$P=0.0051$ (CAG repeat size)

Table 2 Schizophrenia association findings on chromosome 22q

Study	*Sample*	*Ethnicity*	*Markers*	*Evidence*
Saito *et al*, 2001[37]	105 SZ/107 controls	Eur-Am	SNAP29 (22q11.21; 19.53–19.56 Mb)—four SNPs	$P = 0.018$ (allele) and $P = 0.009$ (genotype), promoter SNP
Toyooka *et al*, 1999[43]	118 SZ/118 controls	Japanese	YWHAH (14-3-3 protein η chain gene; 22q12.3; 30.66–30.67 Mb)—5′ tandem repeat	$P = 0.05$ (two-repeat allele) $P = 0.02$ in early-onset cases)
Mimmack *et al*, 2002[44]	10 SZ, 10 controls (and BP, MDD cases)	Eur-Am	APOL1, APOL2, APOL4 (22q11.2–13.2; 34.85–34.93 Mb). Prefrontal cortex	Upregulation of these three genes by microarray expression studies

Sample: All cases were DSM-IIIR or DSM-IV schizophrenia, with schizoaffective disorder included in De Luca *et al*,[36] Egan *et al*[52] and Liu *et al*,[32] except that Meyer *et al*[54] studied a pedigree with periodic catatonia, and Toyooka *et al*[43] restricted their sample to early onset (before age 22 years); SZ = schizophrenia (with or without schizoaffective disorder). NIMH-GI = NIMH Human Genetics Initiative schizophrenia collection.
Markers: COMT = catechol-*O*-methyltransferase; ARVCF = armadillo repeat gene deletes in velocardiofacial syndrome; ZNF74 = zinc-finger protein 74 (Cos52); WKL1 = megalencephalic leukoencephalopathy with subcortical cysts 1; SNAP29 = synaptosomal-associated protein, 29 kDa; UFD1L = ubiquitin fusion degradation 1 like; PRODH = proline dehydrogenase (oxidase); PCQAP = PC2 (positive cofactor 2, multiprotein complex) glutamine/Q-rich-associated protein; APOL1, APOL2, APOL3 = apolipoprotein L1, L2, L3; NS-nonsignificant. Physical locations were taken from 'Build 33', NCBI's assembly of human genomic sequence data available on April 10, 2003.
*Constructed from NCBI blast; the 22 CH3 repeats are part of a predicted gene encoding 1203 AA's.
Evidence: LOD = Lod score.

were as follows: Bonn: German, Israeli (predominantly non-Ashkenazi); Chicago, AUS/US, JHU, NIMH: European, African-American; CNRS: French, French/African/Indian mixtures (Reunion Island); and VCU/Ireland: Irish; Cardiff: English, Welsh.

Markers and genotyping

The 10 microsatellite markers are listed in Table 4. Eight are from the Applied Biosystems (Foster City, CA, USA) HD5 map, and two additional markers are within the VCFS microdeletion region (D22S1638, D22S941), and had been previously successfully typed by the Cardiff group. Fluoresceinated primers were synthesized by ABI and distributed to each lab. Each group genotyped its own samples except that the AUS/US, JHU, and NIMH samples were genotyped by the Australian Genome Research Facility. The standard semiautomated genotyping methods used by each group have been described in the references listed in Table 1. The markers covered 60.8 cM of sex-averaged genetic distance, with an average marker spacing of 6.76 cM and average marker heterozygosity of 0.76. Marker allele frequencies were determined for each data set, and each analysis was set up so that the allele frequencies assumed for each pedigree were those computed for its data set.

Statistical analyses

Planned primary analyses were multipoint affected sibling pair (ASP) analysis using the possible triangle algorithm and yielding MLS,[59] and multipoint nonparametric linkage (NPL) scores (Z_{ALL}).[60] In addition, logistic regression analysis[61] was used to test for intersample heterogeneity of ASP sharing, for overall significance of linkage allowing for this heterogeneity, and for linkage while accounting for covariates as described below. Unless otherwise noted, *P*-values are regional values determined empirically by simulating 5000 replicates of the sample with no linkage present.

Additional multipoint analyses of identity-by-descent (IBD) sharing in ASPs were carried out to test the effect of age at onset (of psychosis) and sex as covariates. The IBD sharing probability for each ASP was modeled as a logistic regression on the observed covariates, as suggested by Rice,[62] enabling likelihood-ratio tests of the effect of each covariate on the IBD sharing probability to be tested. Note that these analyses produced slightly different results than the possible triangle algorithm discussed above, even without covariates, in part because ASPs were omitted if AAO was not known for either sib, and in part because a multiplicative model for IBD is applied, where the probabilities of an ASP sharing 0, 1, or 2 alleles IBD, respectively, are assumed to be $(1-p)^2$, $2p(1-p)$, and p^2, and the likelihood is then maximized with respect to p such that $P \geqslant 0.5$.

The following covariates were used in the analyses: (1) the mean age-at-onset of the two sibs (AAO-Mean); (2) the absolute value of the difference in ages at onset

Table 3 Description of the sample

Sample	*Informative pedigrees*	*Individuals typed*	*Affected cases typed*	*Informative sibships*	*ASPs*	*Indep ASPs*
Aus/US[a]	67	358	170	55	72	63
Bonn[b]	65	275	150	65	91	76
Cardiff[c]	145	429	311	145	179	159
Chicago[d]	62	311	146	62	92	73
CNRS[e]	43	274	136	44	89	64
JHU[f]	95	589	234	80	115	96
NIMH[g]	109	469	269	99	145	120
VCU/IRE[h]	193	1304	502	158	220	187
Total	779	4009	1918	708	1003	838

References to clinical data collection and genotyping methods are as follows:
[a]Levinson *et al*,[63] Ewen *et al*.[64]
[b]Schwab *et al*.[17]
[c]Williams *et al*.[65]
[d]Cao *et al*.[66]
[e]Campion *et al*[67] and Bonnet-Brilhault *et al*.[68]
[f]Blouin *et al*.[11]
[g]Cloninger *et al*.[69]
[h]Kendler *et al*.[70]

Table 4 Chromosome 22q marker map

Marker	LOC
D22S420	0
D22S1638[a]	3.5
D22S941[a]	4
D22S539	10
D22S315	16.6
D22S1163	23.2
D22S277	31.9
D22S423	40.6
D22S274	49.1
D22S1169	60.8

[a]Markers within the minimal VCFS deletion region.

(AAO-Diff); and (3) sex (coded as a factor with three levels corresponding to M–M, M–F, or F–F pairs). The effects of AAO-Mean and AAO-Diff were then evaluated while allowing for sex effects, by fitting both variables simultaneously (Mean + Sex, Diff + Sex). Models containing main effects and interactions between AAO and Sex were also fitted. Region-wide *P*-values, the probability of observing a 'LOD score anywhere in the region being tested' greater than the observed LOD score, were estimated by randomly permuting covariates among the affected sib pairs.

Note that all of the MLS results for logistic regression with covariates are associated with large numbers of degrees of freedom, so that thresholds for genome-wide significance are quite high and are not approached here, even where scores are above 4 or even 5.

Results

Multipoint ASP and NPL analyses

Table 5 and Figures 1 and 2 show results for multipoint ASP and NPL analyses for each sample and for the entire data set. For the entire data set, there were no statistically significant linkage findings for either analysis, with a peak MLS = 0.97 (60.8 cM, $P = 0.19$) and a peak NPL = 1.39 (60.8 cM, $P = 0.066$). Although results varied across the samples, there was no statistically significant evidence for intersample heterogeneity. The overall MLS for IBD sharing in ASPs allowing for heterogeneity was 3.28 (60.8 cM, $P = 0.057$). Nominally significant MLS results were observed in the Chicago and JHU samples, on distal and proximal 22q, respectively, and nominally significant NPL results were observed in the AUS/US and NIMH samples, with a trend ($P = 0.06$) in the JHU sample, at three different locations. In the logistic regression analysis without covariates, nominally significant results were observed in the Chicago, JHU, and NIMH samples, with a trend ($P = 0.051$) in the Cardiff sample.

AAO and sex effects

Table 6 shows peak MLS results and their locations for each sample and the combined sample. As a whole, the sample showed no AAO or gender effect. All observed relationships between AAO and IBD were in the opposite direction to the predicted one, that is, *older* AAO predicted increased IBD sharing in the AUS/US and Chicago samples. In two samples (but not the entire sample), there were interactions between sex and increased AAO-Mean in predicting IBD. In one of these (NIMH), this interaction was observed in very proximal 22q (2.1 cM) near the VCFS region.

Table 5 MLS and NPL analyses (no covariates)

Sample	*MLS*	*IBD*	*CM*	*P-value*	*NPL*	*cM*	*P-value*
AU/US	0.29	0.56	51.4		1.89	16.6	0.035
Bonn	0.27	0.54	14.0		0.96	23.2	
Cardiff	0.69	0.54	2.1		0.93	0.0	
Chicago	2.06	0.71	60.8	0.013	1.08	40.6	
CNRS	0.13	0.53	60.8		−0.24	60.8	
JHU	1.68	0.59	26.7	0.009	1.73	31.9	(0.06)
NIMH	1.00	0.57	45.7		2.02	49.1	0.043
VCU/IRE	0.22	0.53	3.5		0.68	60.8	
ALL	0.97	0.52	60.8	0.19	1.39	49.1	(0.066)
LOD (intersample heterogeneity)	3.40		60.8	0.20			
LOD (linkage accounting for intersample heterogeneity)	3.28		60.8	0.057			

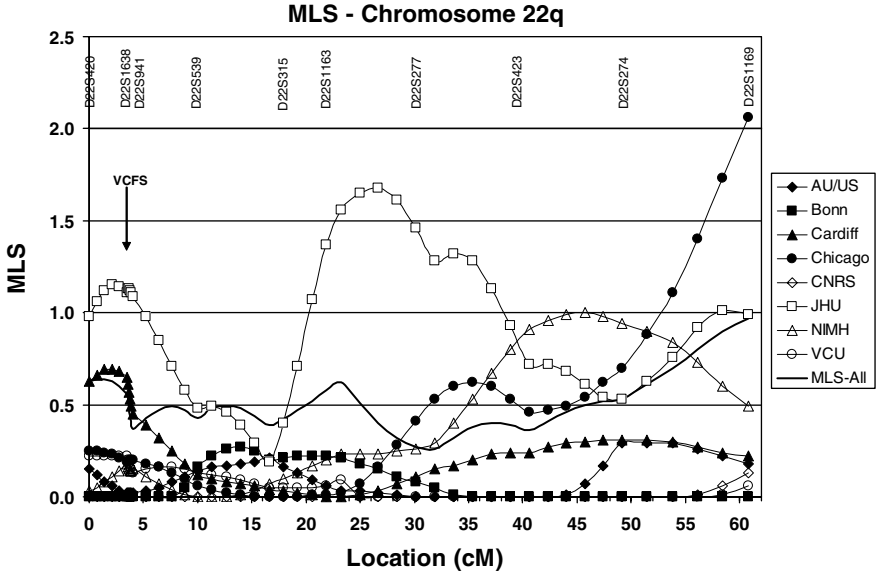

Figure 1 MLS results. Shown are multipoint maximum LOD scores for each sample and for the entire sample combined.

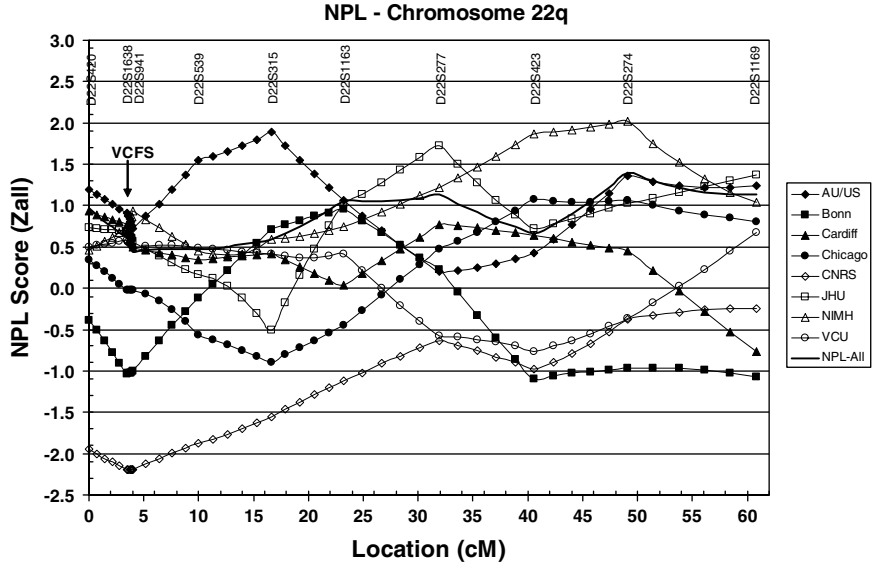

Figure 2 NPL results. Shown are multipoint NPL (Z_{ALL}) scores for each sample and for the entire sample combined.

Table 6 Logistic regression analyses with and without covariates

Sample	*N pairs*	*No covar*		*AAO-Mean*		*AAO-Diff*		*Sex*		*Mean + Sex*		*Diff + Sex*	
		MLS	*cM*	*MLS*	*cM*	*MLS*	*cM*	*MLS*	*cM*	*MLS*	*cM*	*MLS*	*cM*
Aus/US	68	0.16	0.0	**1.59**[a]	17.9	0.58	49.1	0.93	16.6	**3.27**[b,c]	16.6	0.94	49.1
Bonn	76	0.22	21.9	1.27	44.0	0.33	3.5	1.59	10.0	2.02	10.0	1.61	10.0
Card	160	0.58	0.0	0.87	0.0	0.58	0.0	1.11	49.1	1.17	51.4	1.11	49.1
Chicago	88	**2.12**[‡]	60.8	**3.08**[d]	60.8	2.12	60.8	2.87	60.8	**4.05**[e]	60.8	2.87	60.8
CNRS	70	0.00		0.17	0.0	1.75	3.5	0.58	38.9	0.58	38.9	1.91	3.5
JHU	110	**1.44**[†]	23.2	2.03	30.2	1.72	60.8	1.82	60.8	2.28	30.2	2.59	60.8
NIMH	122	**1.14***	45.7	1.21	45.7	1.28	47.4	1.37	45.7	2.17[f]	2.1	1.50	45.7
VCU/IRE	213	0.26	0.0	0.57	16.6	0.83	23.2	0.39	23.2	0.88	16.6	1.39	24.9
ALL	907	3.59	60.8	3.78	60.8	4.14	60.8	4.07	60.8	4.23	60.8	4.44	60.8

MLS = maximum LOD score for IBD sharing among affected sibling pairs in logistic regression analysis (considering only pairs where age at onset was known for both cases).
cM = location of the peak score shown, in cM, ABI map; No covar = results with no covariates; AAO-Mean = results for IBD sharing after introducing the mean age at onset (AAO) of the two affected siblings as a covariate; AAO-Diff = as for AAO-Mean, but using the difference in AAO for each pair; Sex = results after introducing sex of the pair (M–M, M–F, F–F) as a covariate; Mean + Sex = results with both AAO-Mean and Sex as covariates; Diff + Sex = results with both AAO-Diff and Sex as covariates.
Results associated with $P<0.05$ are bolded. *P*-values are empirical region-wide values unless noted otherwise.
[‡] $P=0.0009$.
[†] $P=0.005$.
* $P=0.011$.
[a] $P=0.05$ for increase in MLS for AAO-Mean *vs* no covar (greater AAO predicting increased IBD sharing).
[b] $P<0.001$ for increase in MLS for Mean + Sex *vs* Sex (ie adding effect of mean AAO after allowing for sex effects) (greater AAO predicting increased IBD; increased IBD for F/F pairs).
[c] $P=0.011$ for increase in MLS for Mean + Sex *vs* AAO mean (ie adding the effect of sex after allowing for Mean-AAO effects). There was also a significant interaction between increasing AAO and Sex effects ($P=0.014$, by χ^2 approximation with 2 df).
[d] $P=0.043$ for increase in MLS for AAO-Mean *vs* no covar (greater AAO predicting increased IBD sharing).
[e] $P=0.017$ for increase in MLS for Mean + Sex *vs* Sex.
[f] $P=0.011$ for interaction between increasing AAO-Mean and Sex effects (by χ^2 approximation with 2 df).

Parent-of-origin effects
To follow up DeLisi *et al*'s[13] report of excess (paternal/maternal) sharing, we tested for differences between the IBD probabilities of paternally and maternally inherited alleles in our affected sib pairs. This was done by modeling the IBD probabilities of the paternal and maternal alleles as logistic regressions with the same intercept, but including a parameter in the regression of maternal IBD to reflect a difference in the IBD probabilities (ie a parent-of-origin effect). The significance of the parent-of-origin effect was assessed by randomly permuting parental genotypes within sibships, maximizing the LOD score allowing for a parent-of-origin effect, and comparing the maximized LOD score to that obtained from the actual data. No significant results were obtained either when the parent-of-origin effect was assumed constant across the combined sample or when it was allowed to differ between groups. Groups were also analyzed individually, but none exhibited a significant parent-of-origin effect.

Discussion

In our large collaborative sample, we found no statistically significant linkage findings across our entire data set, for MLS, NPL, or logistic regression analyses. Nominally significant results were observed in some samples, but these were in diverse locations. Therefore, even with this large multicenter sample, we have been unable to use linkage analysis to clarify whether and where there are genes on chromosome 22q that influence susceptibility to schizophrenia in individuals without VCFS microdeletions. Age at onset and sex were taken into account in these analyses, because of reports that these factors mediated association to schizophrenia of two genes in the VCFS region, PRODH (AAO) and COMT (sex). These covariates did not have a significant effect on this multicenter sample, and nominally significant effects were observed in only a few samples at diverse locations, with all AAO effects in the direction opposite to that predicted (increased rather than decreased AAO predicting increased IBD).

Weakly significant P-values
It may be helpful to discuss briefly the interpretation of weakly significant *P*-values. In this field, there is a need to prevent false discoveries through the application of sufficiently stringent criteria for significance,[71,72] while at the same time avoiding the risk

that true effects are missed; the latter is an important consideration particularly when the effect of each gene might be expected to be small and thus the power of linkage studies even of this scale is limited; this is probably particularly true for follow-up (replication) studies such as ours, which are likely to yield smaller (and truer) effect sizes than the original report.[73] Some investigators have recently encouraged optimizing procedures for controlling and eliminating false discoveries, thereby achieving an acceptable ratio of true and false discoveries.[74] One interpretation of our weakly significant *P*-values is that these results most likely reflect considerable disease heterogeneity in our sample and in these populations more generally.

The phenotype used in these analyses, DSM-IIIR schizophrenia and schizoaffective disorder, is consistent with other current schizophrenia linkage and association studies, but it is possible that a different definition of the phenotype would be more strongly linked to one or more chromosome 22q loci. The ethnicity of the samples could also be an issue, in that about 13% of the pedigrees were not of European ancestry. Evidence for linkage or association to schizophrenia on chromosome 22q has been previously observed in some ethnically diverse samples.[13,32–45,59–62]

The most parsimonious explanations of these results would be either that there are no schizophrenia susceptibility loci on chromosome 22q or that their population-wide effects are sufficiently weak that they cannot be reliably detected by linkage methods with our sample size. We would tentatively favor the second hypothesis, for two reasons:

(1) Evidence for schizophrenia linkage on proximal 22q was observed in two meta-analyses of schizophrenia genome scans, as discussed in the introduction. The larger of these analyses considered about 1200 pedigrees, of which about 500 were included here (parts of the AUS/US, Bonn, Cardiff, JHU, NIMH and VCU/IRE samples). The rank-based method used in this analysis can detect 'aggregate' evidence for linkage (nominally significant results by two empirical criteria in more bins than expected by chance) even when the actual combined linkage scores do not reach genome-wide significance, that is, it is more powerful than a combined linkage analysis but localizes the result only to broad bins.[75] It is not known how either meta-analysis method is affected by multiple weakly linked loci in the same region (perhaps affecting different overlapping sets of families). Given the meta-analysis results, it is quite possible that one or more susceptibility loci exist on 22q but cannot be detected by linkage methods in our sample. The sporadic evidence for linkage in individual samples could reflect the variable locations of weak linkage peaks that can be observed in samples with inadequate power to detect them reliably.[76,77]

(2) Both the relationship between VCFS and schizophrenia, and the evidence for association with schizophrenia reported for loci such as COMT, suggest that there are one or more susceptibility loci on 22q that underlie the meta-analysis linkage result. If this is the case, then it is likely that their population-wide effects are too weak to be reliably detected by linkage methods even in rather large samples.

Limitations

Although greater power is achieved by collaborative pooling of data sets, there are a number of limitations associated with this type of study:

(a) There may be undetected differences across samples, which obscures linkage. For example, there could be undetected intersite differences in sampling frames and diagnostic approaches despite the fact that each group uses internationally accepted diagnostic criteria.
(b) The ethnicity of the study population across diverse samples has a greater potential for variation, which could result in locus heterogeneity at certain susceptibility loci (although the vast majority of families studied here are of predominantly European ancestry). Indeed, a recent study by Lung *et al*[78] found ethnic heterogeneity in allele variation in the DRD4 gene in schizophrenia.
(c) Moreover, this type of heterogeneity compounds the well-known difficulty in genetically complex diseases, namely, that the magnitude of the genetic effect of any one locus is unknown.
(d) Differences in genotyping methods may be present across laboratories.
(e) More generally, linkage analyses of complex traits can never rule out false-negative findings; indeed, we assume that there are one or more genes on chromosome 22q that can influence psychosis vulnerability, given the prevalence of psychosis in VCFS patients, but we and others have not been able to detect these loci using genetic linkage methods.

Suggestions

A number of suggestions with regard to multicenter follow-up studies may be helpful:

(a) In the composition of the collaborative group, it is important to avoid preferential selection bias for groups with positive findings. For example, we formed our multicenter sample 5 years ago and have kept it constant regardless of the region being tested (except for permitting individual groups to add newly available families for whom linkage results in new regions of interest were not known).
(b) In the case of findings that clearly originated in one or two of the samples that are included in the multicenter analysis, it can be useful to agree in advance upon a differentiation between the 'new'

families and the 'old' families associated with the original positive evidence for linkage. However, for some findings, the pattern of previous results will not permit such a clear differentiation, as was the case here where none of the samples had produced previous suggestive or significant evidence for linkage on chromosome 22q.

(c) A common set of markers should be genotyped in all samples, with adequate coverage of the broad region that produced evidence for linkage in other studies, and with as dense a map as is feasible to maximize linkage information. The markers that produced evidence for linkage in the original study should generally be included, although sometimes there is subsequent evidence that other nearby markers perform better and are more informative.

(d) It may be useful to carry out linkage analyses such that linkage scores for each sample are computed using its own marker allele frequencies (as was done here). On the other hand, we have not observed significant allele frequency differences among our samples. If a somewhat larger and more ethnically heterogeneous multicenter study were attempted, it would be possible to use raw allele sizes (whether from one central genotyping lab, or using a set of common controls to standardize sizes across labs) to study population substructure directly, and then either to subdivide the sample for linkage analysis or to enter the structured groupings into an analysis (such as logistic regression analysis) as covariates.

(e) Analyses are needed to model and systematically test differences between centers; for example, in this and previous studies, we have used logistic regression of IBD allele sharing of ASPs using site as a covariate[62] to evaluate: (i) the significance of difference in sharing proportions among sites (ie test for intersite heterogeneity) and (ii) the overall significance of linkage while testing for intersite heterogeneity.

(f) We make the assumption, based on our many experiences with crosssite collaboration, that there are undetected but systematic differences in the ways that standard criteria for the diagnosis of schizophrenia are applied across research groups – for example, each group is likely to develop a degree of internal consistency in determining when to exclude a subject for excessive substance abuse or when to consider affective features to be predominant. It might be therefore useful to introduce a more dimensional approach to the rating of symptoms and symptom clusters[79–81] in order to describe phenotypic subgroups for linkage analysis. We are planning to attempt this type of crosssample dimensional strategy in future studies.

Conclusion

Linkage analysis of 10 chromosome 22q microsatellite markers in a large multicenter sample failed to produce significant evidence for linkage to schizophrenia or for linkage associated with earlier age at onset, the sex of the ASPs, or heterogeneity across sites. Slightly positive evidence for linkage was observed on distal 22q for the entire sample, with $P = 0.057$ in a logistic regression analysis of IBD sharing in ASPs while taking intersite heterogeneity into account, and $P = 0.066$ in an NPL analysis. While it is possible that there are no schizophrenia susceptibility loci on 22q, evidence from meta-analyses of schizophrenia genome scan data and several reports of association to specific genes all suggest that there may, indeed, be such loci, but that their population-wide effects are too weak to detect with linkage analysis even in a rather large sample.

Acknowledgements

We gratefully acknowledge participation of family members. Supported by NIMH Grants KO2-01207 and K24-MH64197 (DFL); the Australian NHMRC Grants 33505 and 35016, and Queensland Department of Health (BJM); NIMH Grant MH61602 (DFL, CL, BR, AEP, PVG, DBW, MJO); NIMH Grants MH 41953, 52537, and 45390 (BR and KSK); the UK Medical Research Council (MJO, MO'D); Deutsche Forschungsgemeinschaft Grant SFB 400 (DBW, WM); the German–Israeli Foundation for Scientific Research (BL, DBW); the NIMH Intramural Program and the Brain Research Foundation, University of Chicago (PVG); NIMH Grant RO1-MH57314 (AEP); and CNRS and Aventis Pharma SA (JM, CL). Specimens from the NIMH Schizophrenia Genetics Initiative (NIMH SGI) were used in this study. Data and biomaterials were collected in three projects that participated in the NIMH SGI. From 1991 to 1997, the principal investigators and coinvestigators were Harvard University (Grant U01 MH46318) (MT Tsuang, S Faraone, and J Pepple); Washington University, St Louis (Grant U01 MH46276) (CR Cloninger, T Reich, and D Svrakic); and Columbia University (Grant U01 MH46289) (C Kaufmann, D Malaspina, and J Harkavy Friedman).

Electronic-Database Information

'Build 33', NCBI's assembly of human genomic sequence data available on April 10, 2003, http://www.ncbi.nlm.nih.gov/genome/guide/human/HsStats.html (for physical locations of markers).

References

1 Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 2002; **7**: 405–411.

2 Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I *et al*. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am J Hum Genet* 2003; **73**: 34–48.

3 Pulver AE, Karayiorgou M, Wolyniec PS, Lasseter VK, Kasch L, Nestadt G *et al*. Sequential strategy to identify a susceptibility gene

for schizophrenia: report of potential linkage on chromosome 22q12–q13.1: part 1. *Am J Med Genet* 1994; **54**: 36–43.
4 Lasseter VK, Pulver AE, Wolyniec PS, Nestadt G, Meyers D, Karayiorgou M *et al.* Follow-up report of potential linkage for schizophrenia on chromosome 22q: part 3. *Am J Med Genet* 1995; **60**: 172–173.
5 Vallada HP, Gill M, Sham P, Lim LC, Nanko S, Asherson P *et al.* Linkage studies on chromosome 22 in familial schizophrenia. *Am J Med Genet* 1995; **60**: 139–146.
6 Schwab SG, Lerer B, Albus M, Maier W, Hallmayer J, Fimmers R *et al.* Potential linkage for schizophrenia on chromosome 22q12–q13: a replication study. *Am J Med Genet* 1995; **60**: 436–443.
7 Kalsi G, Brynjolfsson J, Butler R, Sherrington R, Curtis D, Sigmundsson T *et al.* Linkage analysis of chromosome 22q12–13 in a United Kingdom/Icelandic sample of 23 multiplex schizophrenia families. *Am J Med Genet* 1995; **60**: 298–301.
8 Riley B, Mogudi Carter M, Jenkins T, Williamson R. No evidence for linkage of chromosome 22 markers to schizophrenia in southern African Bantu-speaking families. *Am J Med Genet* 1996; **67**: 515–522.
9 Parsian A, Suarez BK, Isenberg K, Hampe CL, Fisher L, Chakraverty S *et al.* No evidence for a schizophrenia susceptibility gene in the vicinity of IL2RB on chromosome 22. *Am J Med Genet* 1997; **74**: 361–364.
10 Coon H, Holik J, Hoff M, Reimherr F, Wender P, Myles Worsley M *et al.* Analysis of chromosome 22 markers in nine schizophrenia pedigrees. *Am J Med Genet* 1994; **15**: 72–79.
11 Blouin JL, Dombroski BA, Nath SK, Lasseter VK, Wolyniec PS, Nestadt G *et al.* Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. *Nat Genet* 1998; **20**: 70–73.
12 Stober G, Pfuhlmann B, Nurnberg G, Schmidtke A, Reis A, Franzek E *et al.* Towards the genetic basis of periodic catatonia: pedigree sample for genome scan I and II. *Eur Arch Psychiatry Clin Neurosci* 2001; **251**: 125–130.
13 DeLisi LE, Shaw SH, Crow TJ, Shields G, Smith AB, Larach VW *et al.* A genome-wide scan for linkage to chromosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. *Am J Psychiatry* 2002; **159**: 803–812.
14 Pulver AE, Nestadt G, Goldberg R, Shprintzen RJ, Lamacz M, Wolyniec PS *et al.* Psychotic illness in patients diagnosed with velo-cardio-facial syndrome and their relatives. *J Nerv Ment Dis* 1994; **182**: 476–478.
15 Gill M, Vallada H, Collier D, Sham P, Holmans P, Murray R *et al.* A combined analysis of D22S278 marker alleles in affected sib-pairs: support for a susceptibility locus for schizophrenia at chromosome 22q12. Schizophrenia Collaborative Linkage Group (Chromosome 22). *Am J Med Genet* 1996; **67**: 40–45.
16 Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B *et al.* A high-resolution recombination map of the human genome. *Nat Genet* 2002; **31**: 241–247.
17 Schwab SG, Hallmayer J, Albus M, Lerer B, Eckstein GN, Borrmann M *et al.* A genome-wide autosomal screen for schizophrenia susceptibility loci in 71 families with affected siblings: support for loci on chromosome 10p and 6. *Mol Psychiatry* 2000; **5**: 638–649.
18 Gurling HM, Kalsi G, Brynjolfson J, Sigmundsson T, Sherrington R, Mankoo BS *et al.* 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* 2001; **68**: 661–673.
19 Polymeropoulos MH, Coon H, Byerley W, Gershon ES, Goldin L, Crow TJ *et al.* Search for a schizophrenia susceptibility locus on human chromosome 22. [Special Issue: Search for a schizophrenia susceptibility locus on human chromosome 22]. *Am J Med Genet* 1994; **15**: 93–99.
20 Shaw SH, Kelly M, Smith AB, Shields G, Hopkins PJ, Loftus J *et al.* A genome-wide search for schizophrenia susceptibility genes. *Am J Med Genet* 1998; **81**: 364–376.
21 Kelsoe JR, Spence MA, Loetscher E, Foguet M, Sadovnick AD, Remick RA *et al.* A genome survey indicates a possible susceptibility locus for bipolar disorder on chromosome 22. *Proc Natl Acad Sci USA* 2001; **98**: 585–590.
22 Wise LH, Lanchbury JS, Lewis CM. Meta-analysis of genome searches. *Ann Hum Genet* 1999; **63**: 263–272.
23 Segurado R, Detera-Wadleigh SD, Levinson DF, Lewis CM, Gill M, Nurnberger Jr JI *et al.* Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: bipolar disorder. *Am J Hum Genet* 2003; **73**: 49–62.
24 Murphy KC, Jones LA, Owen MJ. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry* 1999; **56**: 940–945.
25 Shprintzen RJ. Velo-cardio-facial syndrome: a distinctive behavioral phenotype. *Ment Retard Dev Disabil Res Rev* 2000; **6**: 142–147.
26 Karayiorgou M, Morris MA, Morrow B, Shprintzen RJ, Goldberg R, Borrow J *et al.* Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. *Proc Natl Acad Sci USA* 1995; **92**: 7612–7616.
27 Sugama S, Namihira T, Matsuoka R, Taira N, Eto Y, Maekawa K. Psychiatric inpatients and chromosome deletions within 22q11.2. *J Neurol Neurosurg Psychiatry* 1999; **67**: 803–806.
28 Arinami T, Ohtsuki T, Takase K, Shimizu H, Yoshikawa T, Horigome H *et al.* Screening for 22q11 deletions in a schizophrenia population. *Schizophr Res* 2001; **52**: 167–170.
29 Ivanov D, Kirov G, Norton N, Williams HJ, Williams NM, Nikolov I *et al.* Chromosome 22q11 deletions, velo-cardio-facial syndrome and early-onset psychosis. Molecular genetic study. *Br J Psychiatry* 2003; **183**: 409–413.
30 Lindsay EA, Morris MA, Gos A, Nestadt G, Wolyniec PS, Lasseter VK *et al.* Schizophrenia and chromosomal deletions within 22q11.2. *Am J Hum Genet* 1995; **56**: 1502–1503.
31 Usiskin SI, Nicolson R, Krasnewich DM, Yan W, Lenane M, Wudarsky M *et al.* Velocardiofacial syndrome in childhood-onset schizophrenia. *J Am Acad Child Adolesc Psychiatry* 1999; **38**: 1536–1543.
32 Liu H, Heath SC, Sobin C, Roos JL, Galke BL, Blundell ML *et al.* Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. *Proc Natl Acad Sci USA* 2002; **99**: 3717–3722.
33 Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weizman A *et al.* A highly significant association between a COMT haplotype and schizophrenia. *Am J Hum Genet* 2002; **71**: 1296–1302.
34 Takase K, Ohtsuki T, Migita O, Toru M, Inada T, Yamakawa-Kobayashi K *et al.* Association of ZNF74 gene genotypes with age-at-onset of schizophrenia. *Schizophr Res* 2001; **52**: 161–165.
35 De Luca A, Conti E, Grifone N, Amati F, Spalletta G, Caltagirone C *et al.* Association study between CAG trinucleotide repeats in the PCQAP gene (PC2 glutamine/Q-rich-associated protein) and schizophrenia. *Am J Med Genet* 2003; **116**(Suppl): 32–35.
36 De Luca A, Pasini A, Amati F, Botta A, Spalletta G, Alimenti S *et al.* Association study of a promoter polymorphism of UFD1L gene with schizophrenia. *Am J Med Genet* 2001; **105**: 529–533.
37 Saito T, Guan F, Papolos DF, Rajouria N, Fann CS, Lachman HM. Polymorphism in SNAP29 gene promoter region associated with schizophrenia. *Mol Psychiatry* 2001; **6**: 193–201.
38 Li T, Ball D, Zhao J, Murray RM, Liu X, Sham PC *et al.* Family-based linkage disequilibrium mapping using SNP marker haplotypes: application to a potential locus for schizophrenia at chromosome 22q11. *Mol Psychiatry* 2000; **5**: 77–84.
39 Williams NM, Spurlock G, Norton N, Williams HJ, Hamshere ML, Krawczak M *et al.* Mutation screening and LD mapping in the VCFS deleted region of chromosome 22q11 in schizophrenia using a novel DNA pooling approach. *Mol Psychiatry* 2002; **7**: 1092–1100.
40 Moises HW, Yang L, Kristbjarnarson H, Wiese C, Byerley W, Macciardi F *et al.* An international two-stage genome-wide search for schizophrenia susceptibility genes. *Nat Genet* 1995; **11**: 321–324.
41 Williams NM, Jones LA, Murphy KC, Cardno AG, Asherson P, Williams J *et al.* No evidence for an allelic association between schizophrenia and markers D22S278 and D22S283. *Am J Med Genet* 1997; **74**: 37–39.
42 Williams N, Spurlock G, Norton N, Ivanov D, McCreadie RG, Preece A *et al.* Association between PRODH and schizophrenia is not confirmed. *Mol Psychiatry* 2003; **8**: 644–645.

43 Toyooka K, Muratake T, Tanaka T, Igarashi S, Watanabe H, Takeuchi H *et al.* 14-3-3 protein eta chain gene (YWHAH) polymorphism and its genetic association with schizophrenia. *Am J Med Genet* 1999; **88**: 164–167.
44 Mimmack ML, Ryan M, Baba H, Navarro-Ruiz J, Iritani S, Faull RL *et al.* Gene expression analysis in schizophrenia: reproducible up-regulation of several members of the apolipoprotein L family located in a high-susceptibility locus for schizophrenia on chromosome 22. *Proc Natl Acad Sci USA* 2002; **99**: 4680–4685.
45 Levinson DF, Holmans PA, Laurent C, Riley B, Pulver AE, Gejman PV *et al.* No major schizophrenia locus detected on chromosome 1q in a large multicenter sample. *Science* 2002; **296**: 739–741.
46 Li T, Sham PC, Vallada H, Xie T, Tang X, Murray RM *et al.* Preferential transmission of the high activity allele of COMT in schizophrenia. *Psychiatr Genet* 1996; **6**: 131–133.
47 Kunugi H, Vallada HP, Sham PC, Hoda F, Arranz MJ, Li T *et al.* Catechol-*O*-methyltransferase polymorphisms and schizophrenia: a transmission disequilibrium study in multiply affected families. *Psychiatr Genet* 1997; **7**: 97–101.
48 Chen CH, Lee YR, Liu MY, Wei FC, Koong FJ, Hwu HG *et al.* Identification of a BglI polymorphism of catechol-*O*-methyltransferase (COMT) gene, and association study with schizophrenia. *Am J Med Genet* 1996; **67**: 556–559.
49 Daniels JK, Williams NM, Williams J, Jones LA, Cardno AG, Murphy KC *et al.* No evidence for allelic association between schizophrenia and a polymorphism determining high or low catechol *O*-methyltransferase activity. *Am J Psychiatry* 1996; **153**: 268–270.
50 Ohmori O, Shinkai T, Kojima H, Terao T, Suzuki T, Mita T *et al.* Association study of a functional catechol-*O*-methyltransferase gene polymorphism in Japanese schizophrenics. *Neurosci Lett* 1998; **243**: 109–112.
51 Liou YJ, Tsai SJ, Hong CJ, Wang YC, Lai IC. Association analysis of a functional catechol-*O*-methyltransferase gene polymorphism in schizophrenic patients in Taiwan. *Neuropsychobiology* 2001; **43**: 11–14.
52 Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE *et al.* Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA* 2001; **98**: 6917–6922.
53 Joober R, Gauthier J, Lal S, Bloom D, Lalonde P, Rouleau G *et al.* Catechol-*O*-methyltransferase Val-108/158-Met gene variants associated with performance on the Wisconsin Card Sorting Test. *Arch Gen Psychiatry* 2002; **59**: 662–663.
54 Meyer J, Huberth A, Ortega G, Syagailo YV, Jatzke S, Mossner R *et al.* A missense mutation in a novel gene encoding a putative cation channel is associated with catatonic schizophrenia in a large pedigree. *Mol Psychiatry* 2001; **6**: 302–306.
55 Devaney JM, Donarum EA, Brown KM, Meyer J, Stober G, Lesch KP *et al.* No missense mutation of WKL1 in a subgroup of probands with schizophrenia. *Mol Psychiatry* 2002; **7**: 419–423.
56 Jorgensen TH, Borglum AD, Mors O, Wang AG, Pinaud M, Flint TJ *et al.* Search for common haplotypes on chromosome 22q in patients with schizophrenia or bipolar disorder from the Faroe Islands. *Am J Med Genet* 2002; **114**: 245–252.
57 McQuillin A, Kalsi G, Moorey H, Lamb G, Mayet S, Quested D *et al.* A novel polymorphism in exon 11 of the WKL1 gene, shows no association with schizophrenia. *Eur J Hum Genet* 2002; **10**: 491–494.
58 Saleem Q, Dash D, Gandhi C, Kishore A, Benegal V, Sherrin T *et al.* Association of CAG repeat loci on chromosome 22 with schizophrenia and bipolar disorder. *Mol Psychiatry* 2001; **6**: 694–700.
59 Holmans P. Asymptotic properties of affected-sib-pair linkage analysis. *Am J Hum Genet* 1993; **52**: 362–374.
60 Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 1996; **58**: 1347–1363.
61 Dorr DA, Rice JP, Armstrong C, Reich T, Blehar M. A meta-analysis of chromosome 18 linkage data for bipolar illness. *Genet Epidemiol* 1997; **14**: 617–622.
62 Rice JP. The role of meta-analysis in linkage studies of complex traits. *Am J Med Genet* 1997; **74**: 112–114.
63 Levinson DF, Mahtani MM, Nancarrow DJ, Brown DM, Kruglyak L, Kirby A *et al.* Genome scan of schizophrenia. *Am J Psychiatry* 1998; **155**: 741–750.
64 Ewen KR, Bahlo M, Treloar SA, Levinson DF, Mowry B, Barlow JW *et al.* Identification and analysis of error types in high-throughput genotyping. *Am J Hum Genet* 2000; **67**: 727–736.
65 Williams NM, Rees MI, Holmans P, Norton N, Cardno AG, Jones LA *et al.* A two-stage genome scan for schizophrenia susceptibility genes in 196 affected sibling pairs. *Hum Mol Genet* 1999; **8**: 1729–1739.
66 Cao Q, Martinez M, Zhang J, Sanders AR, Badner JA, Cravchik A *et al.* Suggestive evidence for a schizophrenia susceptibility locus on chromosome 6q and a confirmation in an independent series of pedigrees. *Genomics* 1997; **43**: 1–8.
67 Campion D, d'Amato T, Bastard C, Laurent C, Guedj F, Jay M *et al.* Genetic study of dopamine D1, D2, and D4 receptors in schizophrenia. *Psychiatry Res.* 1994; **51**: 215–230.
68 Bonnet-Brilhault F, Laurent C, Campion D, Thibaut F, Lafargue C, Charbonnier F *et al.* No evidence for involvement of KCNN3 (hSKCa3) potassium channel gene in familial and isolated cases of schizophrenia. *Eur J Hum Genet* 1999; **7**: 247–250.
69 Cloninger CR, Kaufmann CA, Faraone SV, Malaspina D, Svrakic DM, Harkavy-Friedman J *et al.* Genome-wide search for schizophrenia susceptibility loci: the NIMH Genetics Initiative and Millennium Consortium. *Am J Med Genet* 1998; **81**: 275–281.
70 Kendler KS, O'Neill FA, Burke J, Murphy B, Duke F, Straub RE *et al.* Irish study on high-density schizophrenia families: field methods and power to detect linkage. *Am J Med Genet* 1996; **67**: 179–190.
71 Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995; **11**: 241–247.
72 Thomson G. Identifying complex disease genes: progress and paradigms. *Nat Genet* 1994; **8**: 108–110.
73 Goring HH, Terwilliger JD, Blangero J. Large upward bias in estimation of locus-specific effects from genomewide scans. *Am J Hum Genet* 2001; **69**: 1357–1369.
74 van den Oord EJ, Sullivan PF. False discoveries and models for gene discovery. *Trends Genet* 2003; **19**: 537–542.
75 Levinson DF, Levinson MD, Segurado R, Lewis CM. Genome scan meta-analysis of schizophrenia and bipolar disorder, part I: Methods and power analysis. *Am J Hum Genet* 2003; **73**: 17–33.
76 Hauser ER, Boehnke M. Confirmation of linkage results in affected-sib-pair linkage analysis for complex genetic traits. *Am J Hum Genet Suppl* 1997; **61**: A:278.
77 Barr CL, Wigg K, Malone M, Schachar R, Tannock R, Roberts W *et al.* Linkage study of catechol-*O*-methyltransferase and attention-deficit hyperactivity disorder. *Am J Med Genet* 1999; **88**: 710–713.
78 Lung FW, Tzeng DS, Shu BC. Ethnic heterogeneity in allele variation in the DRD4 gene in schizophrenia. *Schizophr Res* 2002; **57**: 239–245.
79 Kendler KS, Karkowski LM, Walsh D. The structure of psychosis: latent class analysis of probands from the Roscommon Family Study. *Arch Gen Psychiatry* 1998; **55**: 492–499.
80 Levinson DF, Mowry BJ, Escamilla MA, Faraone SV. The Lifetime Dimensions of Psychosis Scale (LDPS): description and interrater reliability. *Schizophr Bull* 2002; **28**: 683–695.
81 McGuffin P, Farmer A, Harvey I. A polydiagnostic application of operational criteria in studies of psychotic illness. Development and reliability of the OPCRIT system. *Arch Gen Psychiatry* 1991; **48**: 764–770.