Schizophrenia susceptibility and chromosome 6p24-22

Sir — Diehl and co-workers[1] reported a lod score of 3.0 for linkage of schizophrenia to a chromosome 6 marker closely linked to the *SCA1* locus, with one of several models tested in a sample of pedigrees from Ireland[2], but failed to replicate this finding in 56 U.S pedigrees[1]. An analysis of the entire Irish cohort of 265 pedigrees[3] (published in this issue, but initially a personal communication by Kendler, Straub and MacLean to investigators in the field) resulted in a maximum lod score under the admixture test for linkage with heterogeneity of 3.4, with 15% of pedigrees linked, 2 centimorgans (cM) from *D6S296*, when using the 'PEN' model, a co-dominant model with incomplete penetrance, and a broadly defined disease phenotype[2]. In another analysis of the Irish sample (186 pedigrees), Wang *et al.*[4] obtained a maximum multipoint lod score of 3.9, assuming 50% of families linked, when the *F13A* locus and *D6S260* were analysed using the PEN model. Multilocus affected pedigree member analyses[5] involving *D6S260* and markers to either side of it (*D6S259* and *D6S285*) supported this finding.

We report here the analysis of the 6p24-22 markers *D6S296*, *D6S470*, *D6S259* and *D6S285* in 45 pedigrees multiply affected with schizophrenia and related disorders using our two (dominant, recessive) weighted screening models, and the PEN model. The 45 pedigrees were ascertained in Brisbane ($n = 13$) and Perth ($n = 7$), Australia, and in Philadelphia ($n = 14$), Iowa ($n = 8$) and New York ($n = 3$). They are ethnically diverse, including predominantly Caucasian-European ($n = 33$) and African–American families ($n = 9$), but also one Asian, one Hispanic and one Australian Aborigine/Micronesian family. Each pedigree was ascertained through a proband with chronic schizophrenia and extended through affected individuals with schizophrenia-related disorders. Subjects were diagnosed by DSM-III-R[6] criteria on the basis of psychiatric records and direct structured interviews: SADS[7] or CASH[8] for assessment of psychotic and affective disorders, and SSP/SIB[9,10] or SIDP[11] for schizotypal and paranoid personality disorders. Diagnoses were made by consensus procedures including review outside of the original site. Three categories of diagnoses were studied: Narrow (schizophrenia and chronic schizoaffective disorder), Intermediate (other non-affective psychoses including probable schizophrenia, delusional disorder, schizophreniform disorder, non-chronic schizoaffective disorder, psychosis not otherwise specified), and Broad (schizotypal or paranoid personality disorder)[12]. There were 111 subjects with Narrow, 20 with Intermediate, and 9 with Broad diagnoses, for a mean of 3.11 affected individuals per pedigree. Genotypes were obtained for 264 individuals including 124 unaffected individuals (available parents and grandparents of affecteds, and siblings if one or both parents were unavailable) using a standard radioactive STRP protocol, similar to that used by Straub *et al.*[3] . All individuals without definite consensus diagnoses were considered 'diagnosis unknown' in linkage analyses[13].

The four markers *D6S296*, *D6S470*, *D6S259* and *D6S285*, have respective sex-averaged inter-marker distances of 2.6, 7.5 and 5.9 in the large Irish dataset[3], and 4, 9 and 7 in the Généthon map[14]. *D6S260*, the marker most postive within the study of Wang *et al.*[4] , maps very closely to *D6S259* (ref. 3). For each marker, Table 1 shows cumulative lod scores (under homogeneity) for our families at various recombination fractions (θ) for each of the three models, including the maximum lod score (Z_{max}) and its corresponding θ, as well as the maximum lod score under the assumption of heterogeneity (Z_{het}) and the θ and α (proportion of linked families) at which Z_{het} occurred. Assuming homogeneity, no marker gave significant linkage to schizophrenia using any model. Both the dominant and the PEN models showed significant exclusion for a distance of at least 5 cM around each marker. The only slightly positive lod scores occurred with the recessive model near *D6S259* ($Z_{max} = 0.34$ at $\theta = 0.25$) and *D6S285* ($Z_{max} = 0.23$ at $\theta = 0.26$). None of the tests for hetero-

Table 1 Two-point lod scores for chromosome 6p24-22

	θ											
Locus	0.0	0.01	0.05	0.1	0.2	0.3	0.4	Z_{max}	at θ	Z_{het}	at θ	α
						DOM-W						
D6S296	−10.91	−8.99	−4.88	−2.50	−0.56	−0.03	0.03	0.035	0.38	0.035	0.38	1.0
D6S470	−12.70	−10.56	−5.87	−3.10	−0.78	−0.09	0.03	0.025	0.4	0.025	0.4	1.0
D6S259	−9.78	−8.12	−4.48	−2.35	−0.62	−0.12	−0.01	0.0	0.5	0.0	0.5	n.a.
D6S285	−8.12	−6.85	−3.92	−2.12	−0.60	−0.12	−0.01	0.0	0.5	0.018	0.0	0.05
						REC-W						
D6S296	−7.05	−6.31	−4.04	−2.25	−0.52	−0.01	0.03	0.038	0.37	0.064	0.02	0.1
D6S470	−5.75	−5.15	−3.28	−1.78	−0.34	0.05	0.05	0.069	0.34	0.069	0.34	1.0
D6S259	−3.45	−2.97	−1.55	−0.51	0.28	0.29	0.10	0.341	0.25	0.348	0.16	0.55
D6S285	−3.56	−3.08	−1.68	−0.66	0.14	0.21	0.08	0.228	0.26	0.366	0.0	0.25
						PEN						
D6S296	−6.48	−5.64	−3.39	−1.84	−0.46	−0.06	0.0	0.001	0.42	0.023	0.07	0.1
D6S470	−7.59	−6.69	−4.18	−2.35	−0.65	−0.10	0.0	0.003	0.43	0.003	0.43	1.0
D6S259	−5.51	−4.83	−2.96	−1.62	−0.42	−0.06	0.0	0.0	0.5	0.001	0.32	0.1
D6S285	−3.74	−3.30	−2.04	−1.09	−0.21	0.02	0.02	0.029	0.35	0.096	0.0	0.15

Dominant and recessive genetic models were selected on the basis of epidemiological data[15,16] and estimates of penetrance from a subset of our families after correction for ascertainment[17]. DOM-W and REC-W screening models were created by reducing the penetrance ratios for the intermediate and broad cases to down-weight their contribution[13,18], based on simulation analyses. The PEN co-dominant model is from Su *et al.*[2] and was used in the Irish study[3]. Disease allele frequency was set at 0.01 (DOM-W), 0.14 (REC-W) and 0.032 (PEN). Predicted population prevalence was 0.006-0.027 (depending on diagnostic model) for DOM-W and REC-W and 0.03 for PEN. Penetrances for normal phenotype, disease heterozygote and disease homozygote were 0.002, 0.2, 0.2 (DOM-W); 0.003, 0.003, 0.3 (REC-W); 0.0064, 0.375, 0.75 (PEN). For DOM-W, these values were 0.07, 0.35, 0.35 for intermediate and 0.15, 0.45, 0.45 for broad cases; for REC-W, 0.1, 0.1, 0.5 for intermediate and 0.185, 0.185, 0.55 for broad cases. Unaffected individuals were assigned unknown diagnosis (penetrance ratio 1:1) in all analyses. Two-point lod scores were computed with the MLINK program from LINKAGE 5.0[19,20] using marker allele frequencies calculated by the method of Boehnke[21].

geneity carried out with the HOMOG program[13] were statistically significant.

Our results do not provide independent evidence of linkage of schizophrenia to markers in the 6p24-22 region, but as the mode of transmission is unclear, they do not definitely exclude linkage either. For the PEN model, which yielded support for linkage in the Irish cohort, our data demonstrate clearly negative results. A multicenter collaborative analysis using parametric and non-parametric methods will help clarify the importance of the 6p finding.

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1. Diehl, S.R. *et al. Am. J. hum.Genet. suppl.* **55**, 867 (1994).
2. Su, Y. *et al. Arch. gen. Psychiat.* **50**, 205-211 (1993).
3. Straub, R. *et al. Nature Genet.* 287–293.
4. Wang, S. *et al. Nature Genet.* **10**, 41-46 (1995).
5. Weeks, D.E. & Lange, K. *Am. J. hum. Genet.* **50**, 859-868 (1992).
6. American Psychiatric Association. *DSM-III-R: Diagnostic and Statistical Manual of Mental Disorders. (3rd ed., revised).* (The American Psychiatric Association, Washington, 1987).
7. Endicott, J. & Spitzer, R.L *Arch. gen. Psychiat.* **35**, 837-844 (1978).
8. Andreason, N.C. *Comprehensive Assessment of Symptoms and History.* (University of Iowa, Iowa City, 1987).
9. Baron, M., Asnis, L. & Gruen, R. *Psychiat. Res.* **4**, 213-228 (1981).
10. Baron, M. & Gruen, R. *The Schedule for Interviewing Borderlines (SIB).* (New York State Psychiatric Institute, New York, 1980).
11. Stangl, D., Pfohl, B. & Zimmerman, M. *Arch. gen. Psychiat.* **42**, 591-596 (1985).
12. Levinson, D.F. & Mowry, B.J. *Schizophr. Bull.* **17**, 491-514 (1991).
13. Ott, J. *Analysis of Human Genetic Linkage (rev.ed.).* (Johns Hopkins University Press, Baltimore, 1991).
14. Gyapay, G. *et al. Nature Genet.* **7**, 246-339 (1994).
15. Gottesman, I.I. & Shields, J. *Schizophrenia: The Epigenetic Puzzle.* (Cambridge University Press. Cambridge, 1982).
16. McGue, M., Gottesman, I.I. & Rao, D.C *Am. J. hum. Genet.* **35**, 1161-1178 (1983).
17. Levinson, D. *et al. Genet. Epidemiol.* (in the press)
18. Terwilliger, J.T. & Ott, J. *Handbook of Human Genetic Linkage.* (Johns Hopkins University Press, Baltimore 1994).
19. Lathrop, G.M. *et al. Proc. natn. Acad. Sci U.S.A.* **81**, 3443-3446 (1984).
20. Lathrop, G.M. *et al. Am. J. hum. Genet.* **37**, 482-498 (1985).
21. Boehnke, M. *Am. J. hum. Genet.* **48**, 22-25 (1991).

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Sir — In 1994 we received a personal communication from Straub, Kendler and colleagues that two markers on chromosome 6p22-25, *D6S296* and *D6S285*, gave positive lod scores above 2.00 in schizophrenia pedigrees from Ireland. Results obtained with these and other nearby markers have now been published[1,2]. Based on this information, we investigated markers at *D6S296* and *D6S285* in our own sample of 12 British and 11 Icelandic pedigrees multiply affected with schizophrenia. Diagnoses were made based on information from an interview by a psychiatrist using the Lifetime Version of the Schizophrenia and Affective Disorders Schedule (SADS-L), a rating for schizoid personality and schizotypal disorder using DSM-III-R criteria and all other available sources of clinical information, and were made by consensus between two psychiatrists who were blind to genotyping according to Research Diagnostic Criteria (RDC). Pedigrees were included on the basis of containing multiple cases of schizophrenia but no cases of bipolar affective disorder and of appearing to demonstrate unilineal inheritance. Two affection classes were used for the linkage analyses: a narrow category, denoted DOMS, consisting of schizophrenia, schizoaffective disorder and unspecified functional psychosis, and a broader category, denoted DOMSS, consisting additionally of schizoid and schizotypal personality disorder according to DSM-III-R criteria and schizotypal features according to the RDC. Of the 377 individuals in the 23 pedigrees, 95 fell into the DOMS category and an additional 18 fell into the DOMSS category. Dinucleotide repeat polymorphisms at *D6S296* and *D6S285* (ref. 3) were genotyped using standard techniques[4] and the genotypes were assigned by consensus between two raters (HG and GK) blind to diagnostic data. Allele frequencies were calculated from unrelated founders in the pedigrees. Linkage analyses were carried out using FASTLINK[5–7] assuming dominant and recessive transmission and allowing for locus heterogeneity, using the transmission models detailed in Table 1.

Lod scores totalled across pedigrees were strongly negative for both markers, for both affection models and for dominant and recessive transmission, and most individual families produced negative lod scores at most recombination fractions. The maximum lod2 scores obtained under the assumption of locus heterogeneity[8] with values of α (proportion of families linked) ranging from 0.05 to 1.0 are shown in Table 1. They fall well within chance expectation. If α was