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Support for a Chromosome 18p Locus Conferring Susceptibility to Functional Psychoses in Families with Schizophrenia, by Association and Linkage Analysis

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Summary

The action of antipsychotic drugs on dopamine receptors suggests that dopaminergic signal transmission may play a role in the development of schizophrenia. We tested eight candidate genes (coding for dopamine receptors, the dopamine transporter, and G-proteins) in 59 families from Germany and Israel, for association. A *P* value of .00055 (.0044 when corrected for the no. of markers tested) was obtained for the intronic CA-repeat marker G-olf$_\alpha$ on chromosome 18p. The value decreased to .000088 (.0007) when nine sibs with recurrent unipolar depressive disorder were included. Linkage analysis using SSLP markers densely spaced around G-olf$_\alpha$ yielded a maximum two-point LOD score of 3.1 for a marker 0.5 cM distal to G-olf$_\alpha$. Multipoint analysis under the assumption of heterogeneity supported this linkage—whether the affected pheotype was defined narrowly or broadly—as did nonparametric linkage (NPL). In 12 families with exclusively maternal transmission of the disease, the NPL value also supported linkage to this marker. In order to test for association/linkage disequilibrium in the presence of linkage, the sample was restricted to independent offspring. When this sample was combined with 65 additional simplex families (each of them comprising one schizophrenic offspring and his or her parents), the 124-bp allele of G-olf$_\alpha$ was transmitted 47 times and was not transmitted 21 times ($P = .009$). These results suggest the existence, on chromosome 18p, of a potential susceptibility locus for functional psychoses.

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Introduction

Segregation analysis and epidemiological studies in schizophrenia (Risch and Baron 1984; Baron 1986) as well as in affective disorder (O'Rourke et al. 1983; Rice et al. 1987) suggest that oligo- or polygenic inheritance may be the most likely underlying mode of transmission for these disorders. The pattern of recurrence risk as reported in the literature is compatible with a small number of possibly interacting genes conferring susceptibility for schizophrenia and/or affective disorder (Risch 1990). Given the familial overlap between both syndromes, it is conceivable that some of these genes may be common to both types of functional psychoses and thus may contribute to vulnerabilty in general whereas others may specify the type.

On the basis of the pharmacological activity of antipsychotic and antidepressant drugs, schizophrenia has been considered to be associated with altered dopaminergic transmission and affective disorders with a disturbance of adrenergic or serotonergic transmission. In the past, investigations of functional changes in these neurotransmitter systems in the brain have dominated the search for the molecular causes of psychiatric disorders. Altered function of these neurotransmitters has been demonstrated, by a variety of techniques, in some but not all of the patients. However, the causes have not been unraveled for either of these disorders. Using association analysis and linkage analysis to investigate candidate genes and regions, which are selected on the basis of a possible involvement in the pathophysiology of the disease, should be one way to shed light on the etiology of schizophrenia and/or affective disorders.

We have studied genes that are involved in the dopaminergic pathway and that can be considered as candidate genes for the development of schizophrenia. The dopamine receptors D1 (Cichon et al. 1996) D2 (Hallmayer et al. 1994; Nöthen et al. 1994), D3 (Nöthen et

al. 1993), and D4 (Maier et al. 1994), the dopamine transporter (Maier et al. 1996), and, in addition, the genes for G-proteins, which may be coupled to these receptors—namely, the G-protein subunit Gs α (gene GNAS 1) and G-olf$_\alpha$ (gene GNAL)—have been tested by association analysis in 59 families, each of which was ascertained through an index patient with schizophrenia and had at least one affected sib pair. A statistically significant transmission/disequilibrium test (TDT) was obtained for the short sequence length polymorphism (SSLP) marker G-olf$_\alpha$, which maps to chromosome 18p (Ala-Kokko et al. 1995). It has been shown that G-olf$_\alpha$, which was first identified in the olfactory epithelium, is also highly expressed in caudate putamen and nucleus and appears to be coupled to the dopamine D1 receptor (Herve et al. 1993, 1995; Sakagami et al. 1995).

The TDT has been developed by Spielman et al. (1993) to detect linkage in the presence of association/linkage disequilibrium. Therefore, the initial finding was evaluated also by testing for linkage of G-olf$_\alpha$ and a series of SSLP markers in its neighborhood, by conventional linkage analyses using parametric and nonparametric methods. We obtained additional evidence for linkage when both diagnoses—schizophrenia and affective disorder—were included in the analysis. This increase in LOD-score value by addition of family members with affective disorders is of particular interest, since evidence for a susceptibility locus for bipolar disorder has been reported, for the same region, by Berrettini et al. (1994), a result partly replicated by Stine et al. (1995). Our results provide evidence for the existence of genetic factors on chromosome 18p that confer susceptibility to functional psychoses in families with schizophrenia.

Since other parts of chromosome 18 have been shown to be possibly linked to affective disorders (Stine et al. 1995; De bruyn et al. 1996; Freimer et al. 1996), the entirety of chromosome 18 was analyzed for evidence of linkage. No evidence for additional susceptibility loci was obtained in our sample of families with schizophrenic index patient.

Subjects, Material, and Methods

Family Sample

A total of 59 families were collected, consisting of 30 nuclear families from Haar (Germany), 13 nuclear and 5 extended families from Mainz (Germany), and 7 nuclear and 4 extended families from Israel. Of the families from Israel, nine were Jewish non-Ashkenazic, one was Ashkenazic, and one was of Arabic origin. The families recruited in Haar and Mainz were of German heritage. Further details of the pedigree sample are listed in table 1. There were 124 individuals affected with schizophrenia, and there were 32 individuals affected with schizoaffective disorder (on the basis of Research Diagnostic Criteria [RDC]) constituting the core diagnosis. These diagnoses were included in calculations using the narrowly defined disease ("narrow" disease) model. In addition, the sample contained 23 individuals with recurrent unipolar depression, 1 individual with bipolar disorder I, and 1 individual with bipolar disorder II. These individuals were considered as affected, in analyses considering a broadly defined disease ("broad" disease) model. The sample for linkage analysis also included 18 individuals with minor depression, 4 individuals with personality disorder, and 11 individuals with alcoholism. Since there is no epidemiological evidence for cosegregation with schizophrenia in families, these phenotypes were not included as affected, in the disease models used in this analysis.

For investigation of sex-dependent effects in linkage analysis, the families were subdivided according to the pattern of transmission. Only families with unilineal inheritance were considered. The affection status was schizophrenia, chronic schizoaffective disorder (RDC), bipolar disorder, and recurrent unipolar disorder. Four families in which inheritance was bilineal were excluded. In 30 families the affection status of parents or ancestors was not known to us, so that we could not determine either maternal or paternal inheritance unambiguously. The rest of the families were classified as being either only maternally transmitted (12 families) or maternally *and* paternally transmitted (13 families), according to the suggestions of Gershon et al. (1996). For sex-dependent transmission in linkage-disequilibrium analysis of the marker alleles of G-olf$_\alpha$, the TDT was performed only in families in which the parents differed in genotype, in order to determine unambiguously whether an allele was transmitted from either the father or the mother.

The sample of 65 simplex families (nuclear families with one affected sib [i.e., triads]) with family history was ascertained in the areas of Haar and Mainz, in order to increase the sample size for association/linkage-disequilibrium studies in the presence of linkage.

Cell Lines and Genomic DNA

Permanent cell lines were established from all individuals of all ascertained families by transformation of lymphocytes by means of Epstein-Barr virus, by standard techniques. Genomic DNA was prepared from either whole blood or cell lines by means of a Quiagen blood- and cell-culture DNA kit. The DNA was dissolved to a concentration of 10 μg/ml, which was used as stock solution.

Table 1

Characteristics of the Family Sample

Category	No. (No. with Affective Disorders) in			
	Jerusalem	Haar	Mainz	Total
All families:	11	30	18	59
Individuals	123	142	151	416
Affected	34 (40)	66 (78)	56 (66)	156 (184)
Affected/family	3.1 (3.6)	2.2 (2.6)	3.1 (3.6)	2.6 (3.1)
Nuclear families:				
Both parents	6	28	12	46
One parent	2	1	1	4
Individuals	83	124	60	277
Affected	22 (25)	63 (74)	30 (31)	115 (130)
Affected/family	2.75 (3.1)	2.1 (2.5)	2.3 (2.4)	2.3 (2.6)
Extended families:				
Two[a]/three generations	0/3	1/0	1/4	2/7
Individuals	40	8	91	139
Affected	12 (15)	3 (4)	26 (35)	41 (54)
Affected/family	4.0 (5.0)	3.0 (4.0)	5.2 (7.0)	4.6 (5.7)
Siblings:				
Pairs	7 (4)	28 (26)	17[b] (20)	52 (50)
Triplets	1 (4)	2 (4)	2 (2)	5 (10)
Quadruplets	2 (2)	0 (0)	0 (0)	2 (2)
Quintuplets	1 (0)	0 (0)	0 (0)	1 (0)
Sextuplets	0 (1)	0 (0)	0 (0)	0 (1)
Sib-pair totals:				
$(k-1)$ pairs	19 (23)	32 (34)	21 (24)	72 (81)
$k(k-1)\frac{1}{2}$ pairs	32 (43)	34 (38)	23 (26)	89 (107)

[a] Two generations include second-degree relatives.
[b] Two affected sib pairs are in different branches.

Markers

The following markers were from the Généthon map (Dib et al. 1996): D18S464, D18S1158, D18S1116, D18S53, D18S73, D18S453, D18S71, D18S1114, D18S478, D18S469, D18S57, D18S64, D18S61, D18S56, D18S59, and D18S68. Other markers included G-olf$_\alpha$ (Ala-Kokko; also see Genome Database [accession number GDB 196597]); D18S37 and D18S40 (Straub et al. 1993); and D18S852, D18S975, and D18S866 (Cooperative Human Linkage Center).

The order of the map for multipoint linkage was based on data form Giacalone et al. (1996), Esterling et al. (1997), and the Genetic Location Database map. Distances were taken from the literature as described in the Genome Database and, in the case of the family sample in the present study, also were calculated by GENEHUNTER. Marker-allele frequencies and heterozygosity were determined on the basis of genotypes of 110 unrelated individuals taken from the family sample.

Family Ascertainment

Families with index-patient with either schizophrenia or chronic schizoaffective disorder (mainly schizophrenic, on the basis of RDC; Spitzer et al. 1978) were ascertained consecutively at the State Mental Hospital at Haar (which is located in the area of Munich), under the supervision of M.A., and at the Psychiatric Hospital of the University of Mainz, under the supervision of W.M. Families from Israel were referred, from several Psychiatric Hospitals in Israel, to the Hadassah Medical School of the University of Jerusalem and were ascertained under the supervision of B.L.

The ascertainment plan for multiplex families was designed mainly for affected-sib-pair analysis using the core phenotype schizophrenia/chronic schizoaffective disorder. Therefore, families with aggregation of bipolar disorder—that is, families with either sibs with bipolar disorder, in addition to the schizophrenic individual, or with more than one relative with bipolar disorder—were excluded. No ascertainment restriction with regard to the unipolar depressive phenotype (recurrent) was applied.

The following types of families were ascertained according to the aforementioned procedure:

Nuclear families with two and more affected sibs: The minimum requirement for inclusion of a family within the sample for linkage studies was the availability of two affected sibs both affected with either schizophrenia or chronic schizoaffective disorder, together with both parents, independent of the latters' psychiatric diagnosis (for

identity-by-descent analysis). Additional, unaffected sibs were recruited for genotyping when only one parent was available; this was the case for four families.

Extended families: A nuclear family was extended to other branches when first- and second-degree relatives affected with either schizophrenia or chronic schizoaffective disorder were available for interview, diagnosis, and blood sampling.

Simplex families (*i.e., triads*)*:* Families with one individual affected with schizophrenia or schizoaffective disorder and with both parents available for both evaluation and blood sampling were ascertained when family history revealed the presence of at least one additional family member affected with either schizophrenia or bipolar or recurrent unipolar disorder.

Clinical Evaluation

The study has been approved by the local ethics committees, and written informed consent was obtained from all participants after they had received a description and explanation of the study. Probands and relatives were interviewed by means of the Schedule for Affective Disorders and Schizophrenia–Lifetime version (Fyer et al. 1985) and the Structured Clinical Interview for DSM-III Diagnoses–Personality Disorders (Spitzer and Williams 1985). In addition, the relatives of the proband were asked about the presence of psychiatric symptoms in the family, by means of the family-history research criteria. Lifetime diagnoses based on RDC (Spitzer et al. 1978) were obtained by the best-estimate diagnosis procedure (Leckman et al. 1982). Case records were evaluated by means of the Operational Criteria Checklist (McGuffin et al. 1991). The completed interview form, the family-history information, and the medical records of an individual were reviewed by an experienced psychiatrist, who was blinded both to the individual's familial relationship to the index case and to morbidity in the family. When a definite diagnosis could not be made, additional information was collected by reinterviewing of either the proband or other family members.

Genotypings

Amplification of marker loci was done by PCR either with ^{32}P–end-labeled oligonucleotides, for detection of radioactivity, or with fluorescence-labeled primers, for fragment analysis by means of an ABI sequencer type 377. PCR was performed in a 10-μl volume containing 50 ng of genomic DNA; 200 μM each of dATP, dGTP, dCTP, and dTTP; 2 pmol of each primer (for detection of radioactivity, the reverse primer had been 5′–end labeled by T4 polynucleotide kinase and γ-[^{32}P-]ATP); 50 mM KCl; 10 mM Tris-HCl (pH 8.3); 1.5 mM $MgCl_2$; and 1.25 U of *Taq* polymerase (Pharmacia). The samples were processed either in a Perkin-Elmer Thermo Cycler 9600 or in an MJ Research PTC-200 Peltier thermal cycler, by means of microtiter plates. An initial denaturation period of 4 min at 95°C was followed by 30 cycles each of 20 s at 95°C, 20 s at 55°C, and 30 s at 72°C. A final synthesis step, consisting of 10 min at 72°C, was added. In the case of the radioactively labeled product, 20 μl formamide tracking dye was added, the samples were heated for 5 min at 95°C, and 4 μl of each sample was loaded onto a sequencing gel containing 6% acrylamide and 8 M urea. Labeled products were detected by autoradiography overnight, by means of Kodak X-omat XAR. Autoradiograms were scored by two investigators independently, who were blinded to phenotype, and the alleles were assigned to the pedigree members. Fluorescence-based technology was used for markers D18S59, D18S56, D18S57, D18S64, D18S68, D18S61, and D18S469. After completion of PCR reactions, samples containing amplification products labeled with either 4,7,2′,7′-tetrachloro-6-carboxyfluorescein (TET), 6-carboxyfluorescein (6-FAM), or 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein (HEX), distilled water was added, to give a dilution of either 1:10, for HEX-labeled products, 1:20, for 6-FAM–labeled products, or 1:40, for TET-labeled products. To 1.5 μl of this dilution, 0.2 μl of GS 500 Tamra (ABI), 0.2 μl of loading buffer (ABI), and 1.6 μl of formamide were added. The samples were denatured, and 2 μl of each was loaded on a 6% denaturing polyacrylamide gel in the electrophoresis unit of the sequencer. Genotyping software GeneScan 2.0.2 and Genotyper 1.1 (ABI) were used for data collection and evaluation. Allele sizes were determined on the basis of analysis of representative samples by fluorescence technology and the molecular-weight marker GeneScan-500-TAMRA directly supplied by ABI.

Statistical Analysis

The following two definitions of the affected phenotype were used for calculations:

1. *Narrow disease model:* schizophrenia and schizoffective disorder, mainly schizophrenic;
2. *Broad disease model:* in addition to schizophrenia and schizoaffective disorder, as in the narrow disease model, recurrent unipolar and bipolar disorders I and II.

In both models, the index patient and at least one sib had a diagnosis of either schizophrenia or schizoaffective disorder (mainly schizophrenia).

Two-point LOD-score values were calculated by FASTLINK (Cottingham et al. 1993; Schäffer et al. 1994) version 3 of MLINK of the program package LINKAGE (Lathrop and Lalouel 1984). This analysis was performed, with markers D18S464, D18S1158, D18S53, D18S1116, D18S71, D18S453, D18S37,

D18S852, D18S1114, D18S40, D18S866, D18S478, and D18S975, for the whole family set, which included, in addition to the sibs, all other available relatives. A dominant model was used for linkage analysis. A penetrance of .55, a phenocopy rate of .001, and a disease-allele frequency of .02 were used with the narrow disease model. Two-point LOD-score values for the broad disease model were calculated with a penetrance of .77, a phenocopy rate of .05, and a disease-allele frequency of .06. These values are adopted from Su et al. (1993).

Multipoint analysis was performed by GENEHUNTER (Kruglyak et al. 1996). Because of time and memory constraints in the performance of multipoint analysis, the larger families were divided into smaller subfamilies. Sib-pair analysis was performed on the basis of identity by descent (Penrose 1953; Suarez and Van Eerdewegh 1984). The alleles transmitted from one informative parent to a pair of affected sibs were scored and counted as either shared or not shared. The mean test was used for calculation of *P* values (Knapp et al. 1994*a,* 1994*b*).

The TDTLIKE - Alpha Test Version for computation of TDT-like likelihood-ratio statistics, based on an algorithm of Terwilliger (1995), was used for calculation of transmissions of G-olf$_\alpha$ marker alleles to affected sibs, by TDT (Spielman et al. 1993). However, meioses are not independent among affected sibs if linkage has been detected (Spielman and Ewens 1996). Therefore, in the testing for linkage disequilibrium, a heterozygous parent in a family with two affected sibs was either discarded from the analysis, if this parent transmitted different alleles to both sibs, or was counted as a single observation, if this parent transmitted the same allele to both sibs (Martin et al. 1997). In a family with more than two affected sibs, this procedure was applied with two randomly chosen affected sibs from the family. With these data, a TDT statistic was calculated for each G-olf$_\alpha$ allele, by consideration of all other alleles as a single allele. The maximum of these TDT statistics was used in the testing for linkage disequilibrium. A randomization technique (with $R = 20{,}000$ permutations) as described by Morris et al. (1997) was employed to obtain *P* values corresponding to the observed maximum of TDT statistics.

Results

Evidence for Transmission Disequilibrium of G-olf$_\alpha$ Marker Alleles

While testing eight candidate genes for psychiatric disorders (i.e., the dopamine receptors D1–D5, the dopamine transporter, and the G-proteins Gs$_\alpha$ and G-olf$_\alpha$), we obtained, in a sample of 59 families with affected sib pairs, $P = .00055$ for the intronic dinucleotide-repeat marker G-olf$_\alpha$, by the TDT (see table 2). The *P* value remained significant when Bonferroni correction for the number of used markers was applied ($P = .0044$). When the disease definition was extended to include unipolar and bipolar disorder, the value decreased to .000088 (corrected $P = .0007$). The 124-bp allele of the CA repeat was transmitted more often than would be expected by chance (narrow disease definition, $P = .0007$; broad disease definition, $P = .00012$). Since the TDT simultaneously tests for association and linkage, we evaluated the finding further by applying parametric and nonparametric LOD-score methods to test for linkage.

Table 2

Transmission of G-olf$_\alpha$-Associated Marker Alleles to All Affected Sibs

Allele Size [in bp] (Frequency) and Model	No.		TDT (*P*)[a]
	Transmitted	Nontransmitted	
106 (.07):			
Narrow	15	15	.00
Broad	15	16	.032
112 (.10):			
Narrow	29	21	1.28
Broad	30	24	.9
114 (.155):			
Narrow	28	44	3.55
Broad	29	47	4.26
116 (.40):			
Narrow	48	77	6.73
Broad	52	79	5.56
118 (.015):			
Narrow	5	3	.5
Broad	6	3	1.09
120 (.015):			
Narrow	11	4	3.26
Broad	11	5	2.25
122 (.03):			
Narrow	4	6	.4
Broad	4	6	.49
124 (.175):			
Narrow	41	13	14.52 (.0007)
Broad	45	13	17.65 (.00012)

[a] Data are TDT-like likelihood-ratio statistics based on an algorithm of Terwilliger (1995); *P* values are multiple-test corrected. For the narrow model, the maximum-likelihood estimate of TDT is 10.654 ($P = .00055$); for the broad model, the maximum-likelihood estimate of TDT is 14.077 ($P = .000088$).

Evidence for Linkage to Chromosome 18p, by Parametric and Nonparametric Methods

Classic two-point LOD-score analysis was performed for the marker G-olf$_\alpha$ and revealed LOD-score values of 2.07 (recombination fraction [θ] .15), for the narrow disease definition, and 1.76 ($\theta = .2$), for the broad disease definition, in the total family sample (table 3). Subsequently, the region around the locus for G-olf$_\alpha$ was saturated with 13 additional SSLP markers (fig. 1).

For these analyses, we included the more distantly

Table 3

Marker Characteristics, Two-Point LOD Scores, and Allele Sharing

	Distance (cM)			Two-Point Z_{max} (θ)[b]		Allele Sharing[c]					
						Narrow			Broad		
Marker	Previously Published	Present Study[d]	Heterozygosity[a]	Narrow	Broad	Shared	Not Shared	P	Shared	Not Shared	P
D18S59 (A)	34.3	36	.831	.08 (.35)	.18 (.3)	51	54		51	54	
D18S464 (B)	5.7	6	.540	…	…	23	24		26	29	
D18S1158 (C)	.6	1	.650	.33 (.25)	.32 (.3)	53	44		59	48	
D18S1116 (D)	.5	0	.630	1.98 (.1)	1.98 (.15)	60	40	(.045)	72	39	(.0017)
D18S53 (E)	.5	0	.796	1.76 (.15)	3.1 (.15)	88	59	(.017)	103	64	(.0025)
GOLF (F)	.8	1	.764	2.07 (.15)	1.76 (.2)	80	56	(.04)	94	61	(.008)
D18S71 (G)	.6	1	.850	1.23 (.2)	2.16 (.2)	88	65		96	67	(.023)
D18S37 (H)	.6	1	.590	1.3 (.15)	1.64 (.2)	58	45		73	52	(.06)
D18S40 (I)	.2	1	.840	.81 (.25)	2.07 (.2)	85	73		100	80	
D18S852 (J)	.5	2	.450	.27 (.2)	.52 (.2)	31	26		35	29	
D18S114 (K)	.4	0	.780	.74 (.25)	1.73 (.25)	72	64		83	69	
D18S453 (L)	5.6	5	.825	.48 (.25)	.86 (.25)	71	67		84	72	
D18S975 (M)	.7	3	.684	.81 (.25)	2.17 (.2)	61	57		74	60	
D18S866 (N)	1.7	3	.652	…	.11 (.25)	52	48		64	54	
D18S478 (O)	2.9	4	.600	…	.75 (.25)	44	32		49	43	
D18S56 (P)	4.1	7	.759	…	…	51	53		51	53	
D18S57 (Q)	24.6	23	.832	.23 (.25)	.09 (.3)	42	38		42	38	
D18S64 (R)	12.7	14	.760	.25 (.25)	…	67	59		67	59	
D18S68 (S)	6.9	8	.821	…	…	54	57		54	57	
D18S61 (T)	6.4	7	.853	.84 (.25)	.04 (.35)	73	56		73	59	
D18S469 (U)			.681	…	…	59	47		59	47	

[a] Calculated in 110 individuals.
[b] An ellipsis (…) denotes that there were negative two-point LOD scores for all values of θ tested.
[c] Identical by descent, for all possible sib-pair combinations.
[d] Distances were calculated by GENEHUNTER.

related relatives suffering from schizophrenia and schizoaffective disorder and, for the broad disease model, also the relatives with affective disorders (table 1). Several markers revealed positive LOD-score values (table 3). A maximum LOD score (Z_{max}) of 3.1 was obtained with marker D18S53, at $\theta = .15$, for the broad disease model. D18S53 is 0.5 cM distal to G-olf$_{\alpha}$ (table 3). There was also evidence for linkage by nonparametric sib-pair analysis (table 3). The highest value, with 72 shared versus 39 nonshared ($P = .0017$), was observed for marker D18S1116, which is 0.5 cM distal to D18S53 and ~1 cM distal to G-olf$_{\alpha}$.

Multipoint Analysis

Recent findings in bipolar disorder (Berrettini et al. 1994; Stine et al. 1995; Freimer et al. 1996; McMahon et al. 1997) suggest, in addition, that susceptibility loci for bipolar disorders are on the long arm of chromosome 18. Therefore, additional markers, spanning the entire chromosome, were included for multipoint analysis (fig. 1). Multipoint linkage analysis was performed by the program GENEHUNTER (Kruglyak et al. 1996). The marker distances used in this analysis were taken from

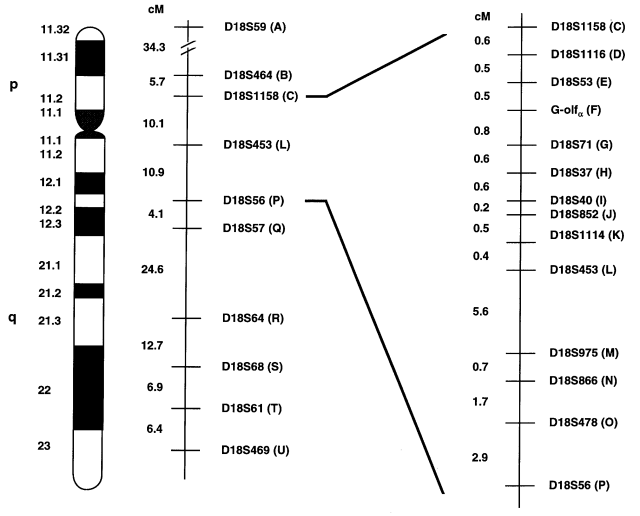

Figure 1 Diagram of chromosome 18 with markers used in linkage studies (ideogram is from Bray-Ward et al. 1996). Distances are from Genome Database.

the literature. In the family sample used in the present study, we also calculated marker distances by GENEHUNTER. In some cases, the distances were larger, probably because of the composition of our family sample, which differs from that of the CEPH families used for construction of the published maps. Since these differences could be due to typing errors, we formed haplotypes by using all markers and checked all recombinants by retyping. LOD-score analysis assuming heterogeneity (HLOD) reached a maximum value around marker D18S53—1.6 (37% of the families linked) for the narrow disease model and 2.92 (36% of the families linked) for the broad disease model. No additional peak was seen with markers on 18q (fig. 2).

Nonparametric analysis with GENEHUNTER (fig. 3) revealed a maximum NPL (NPL_{max}) around marker D18S53—2.41 ($P = .008$) for the narrow disease model and 2.9 ($P = .0023$) for the broad disease model. There was a small additional peak toward the end of the long arm (around marker D18S61), but this peak did not reach a statistically significant value (NPL = 1.3, $P = .097$).

Parent-of-Origin Effect

A parent-of-origin effect has been described in transmission of bipolar disorders (Stine et al. 1995; Gershon et al. 1996). To test for the effect in our sample with families with schizophrenia, we divided the sample into two categories—families transmitting the disease exclusively maternally (12 families) and families transmitting the disease paternally *and* maternally (13 families), as described above in the Family Sample subsection. NPL was calculated by GENEHUNTER. In contrast to the results from study of bipolar disorder, no evidence for linkage in paternally *and* maternally transmitting families was detectable in our sample (fig. 4). However, as shown in figure 4, an NPL_{max} of 2.83 ($P = .0022$) around the locus for G-olf$_\alpha$ was obtained when the 12 exclusively maternally transmitting families were analyzed.

The TDT as a Test for Association/Linkage Disequilibrium in the Presence of Linkage

As discussed by Spielman and Ewens (1996), the TDT is not valid as a test of association in multiplex families when linkage has been detected. If linkage is present, meioses cannot be considered as independent in affected sibs.

Therefore, the family sample was restricted to those heterozygous parents, of affected sib pairs, who transmitted the same allele to both sibs (Martin et al. 1997). The frequency difference between transmission and nontransmission of the 124-bp allele was statistically significant ($P = .0043$; see table 4), but the number of observations after application of this procedure was low. Therefore, we expanded the sample by including 65 simplex families (triads) that had been ascertained, for family-based association studies, in Haar and Mainz. The 124-bp G-olf$_\alpha$ marker allele, which, in the general

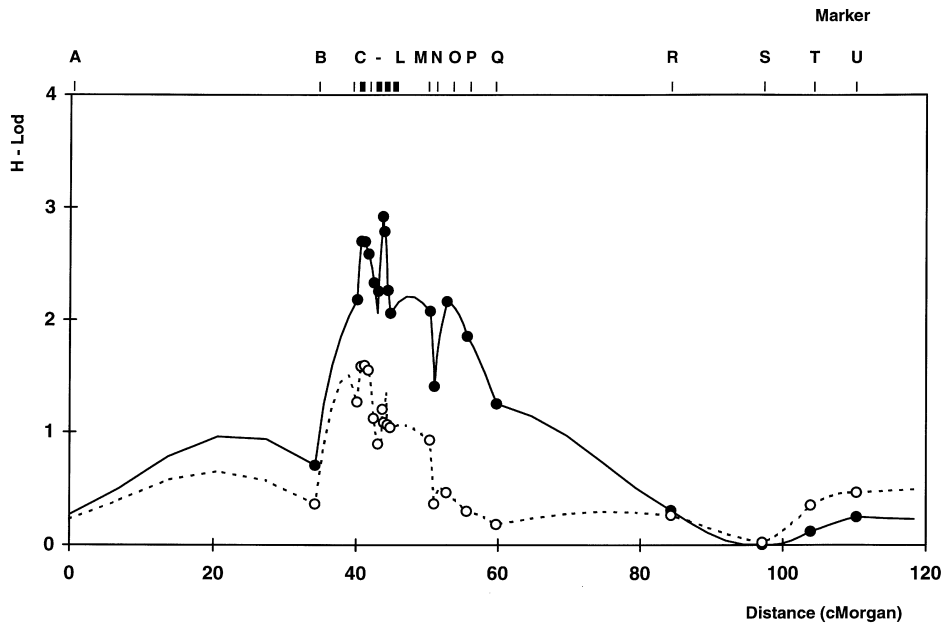

Figure 2 HLOD values (marker designations and distances are as in fig. 1). For the broad disease definition (*upper curve, with blackened circles*), $Z_{max} = 2.92$, $\alpha = .36$; for the narrow disease definition (*lower curve, with unblackened circles*), $Z_{max} = 1.59$, $\alpha = .37$.

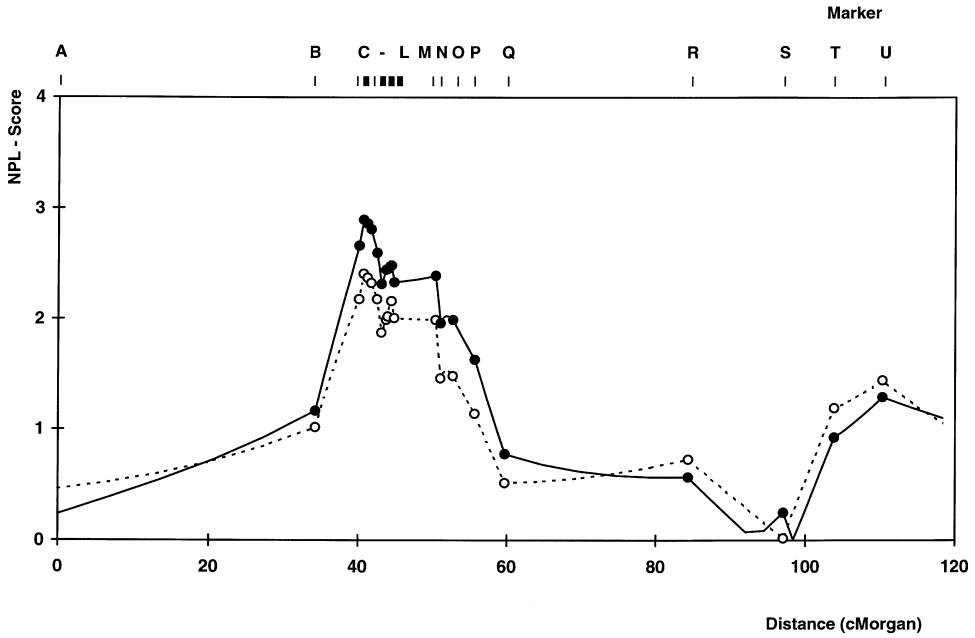

Figure 3 Nonparametric LOD values (marker designations and distances are as in fig. 1). For the broad disease definition (*upper curve, with blackened circles*), $NPL_{max} = 2.9$, $P = .0023$; for the narrow disease definition (*lower curve, with unblackened circles*), $NPL_{max} = 2.40$, $P = .0083$.

population, has a frequency of 17% (Ala-Kokko et al. 1995), was transmitted 33 times and was not transmitted 19 but failed to reach statistical significance (table 4). When both samples were analyzed together, the 124-bp allele was transmitted 47 times and was not transmitted 21 times. When the method of Morris et al. (1997) was used for correction, this result was statistically significant ($P = .0091$; table 4). The 124-bp allele was observed to be more frequently transmitted from the mother than from the father (table 4). Maternal transmission was statistical significant in the expanded family sample ($P = .0192$; table 4).

Discussion

The present study provides suggestive evidence, from both association/linkage disequilibrium studies and linkage studies, for a locus, on chromosome 18p, that may confer susceptibility to functional psychoses in families with schizophrenic index patients who have been ascertained for linkage studies in schizophrenia. Functional psychoses were first differentiated at the beginning of the century, by Emil Kraepelin (1899), into manic-depressive insanity (bipolar disorder) and dementia precox (schizophrenia). This classification was based on both the outcome of the disease and the presence of prominent mood symptoms in manic-depressive insanity. The concept has been very helpful in the development of diagnosis and therapy for these disorders but has been questioned in studies on symptomatology and genetic transmission (Taylor 1992; Maier et al. 1993):

1. Intermediate forms such as schizoaffective disorders occur in families with both disorders (Kendell and Gourlay 1970; Taylor and Amir 1994).
2. Family studies have revealed co-occurence of schizophrenia and affective disorder in some families (Gershon et al. 1988; Taylor 1992; Maier et al. 1993) but not in others. An increased risk for affective disorders among first-degree relatives of schizophrenia has been described (Gershon et al. 1988).
3. Presence of affective disorders predisposes to schizophrenia in later generations (Coryell et al. 1985).

These observations raise the possibility of familial or genetic factors that may be shared by both disorders. Consequently, a continuum of liability has been suggested for the functional psychoses (Crow 1986).

Evidence for a locus that may predispose to the development of functional psychoses is based on the following observations:

1. A preferential transmission of the 124-bp G-olf_{α} marker allele to sibs affected with schizophrenia and schizoaffective disorders is detected by TDT (table 2), suggesting association/linkage disequilibrium. The value increases when sibs with unipolar and bipolar disorder are included.
2. LOD-score analyses using parametric and nonpar-

ametric methods provide support for linkage, in the chromosomal region of 18p, centered around the markers D18S1116/D18S53/G-olf$_\alpha$ (table 3 and figs. 2–4).

3. LOD scores increase when a broad disease model, which includes recurrent unipolar (23 individuals) and bipolar (2 individuals) illness, is used for analysis (table 3 and figs. 2 and 3) An increase of LOD scores in our pedigree sample by a broadening of the phenotype has not been observed for the potential susceptibility loci, on chromosome 6 (Schwab et al. 1995) and 5 (Schwab et al. 1997), recently published by our group.

4. Evidence of linkage of bipolar disorder to chromosome 18 has been reported by several groups (Berrettini et al. 1994, 1997; Stine et al. 1995; Coon et al. 1996; De bruyn et al. 1996; Freimer et al. 1996; McMahon et al. 1997).

5. Allele sharing for marker D18S53 ($P = .02$) in sib pairs affected with schizophrenia has been reported by DeLisi et al. (1995).

The hypothesis for study of marker G-olf$_\alpha$ on chromosome 18p was based on the possible involvement of G-proteins in the etiology of schizophrenia. Our study was, a priori, not designed to test the previously reported linkage, for bipolar disorders, in the pericentromeric region of chromosome 18 in families with schizophrenia, nor was its purpose to test the nosological distinction between bipolar disorder and schizophrenia. However, when we obtained, by TDT, evidence of association of the SSLP marker G-olf$_\alpha$, we took into consideration the findings in bipolar disorders and scanned the entirety of chromosome 18, for the possible existence of additional susceptibility loci, by multipoint linkage analysis.

Recent findings in linkage studies of bipolar disorders support the view that we have obtained evidence for a susceptibility gene, which may be common to both disorders. Berrettini et al. (1994) have reported evidence, in the pericentromeric region of chromosome 18, of a gene for manic-depressive illness. With 11 markers and

Table 4

Transmission of 124-bp G-olf$_\alpha$ Marker Allele in Families, in a Sample of 65 Simplex Families (Triads), and in Combined Samples

Sample and Transmission	No.		P[a]
	Transmitted	Nontransmitted	
Families:			
Paternal	5	1	...
Maternal	7	1	.0523
Combined	14	2	.0043
Triads:			
Paternal	8	10	...
Maternal	17	7	...
Combined	33	19	.2531
All:			
Paternal	13	11	...
Maternal	24	8	.0193
Combined	47	21	.0091

Note.—Paternal/maternal transmission was determined only in parents with different genotypes.

[a] Corrected by the randomization technique of Morris et al. (1997).

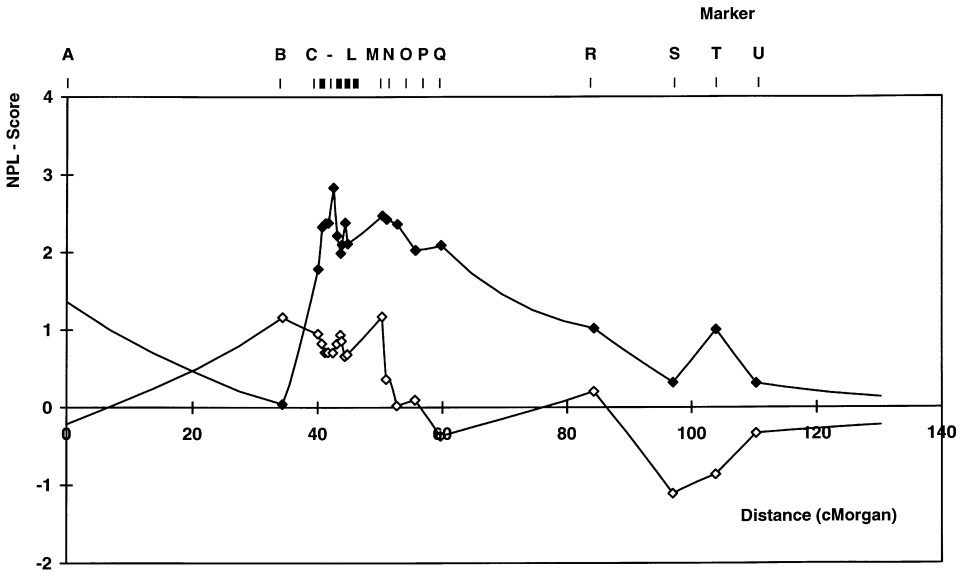

Figure 4 Nonparametric LOD values for parent-of-origin families (marker designations and distances are as in fig. 1). For the upper curve, which represents data for 12 maternal families, $NPL_{max} = 2.83$, $P = .0022$; for the lower curve, which represents data for 13 paternal/maternal families, $NPL_{max} = 1.1$, $P = .13$.

evaluation by parametric and nonparametric methods, two positive areas were detected, one around D18S53 on 18p, an area identical to the region with the Z_{max} in our sample, and another one around D18S56 on 18q. Stine et al. (1995) have studied 28 nuclear families transmitting the bipolar-affective-disorder phenotype, for linkage with markers on chromosome 18. Affected-sib-pair analysis revealed the greatest allele sharing for marker D18S37, which is 1.9 cM proximal to D18S53 and which also shows excess allele sharing in our sample. Stine et al. obtained additional evidence for a parent-of-origin effect. The strongest evidence for linkage was observed for two loci—one around D18S35 on 18p and another around D18S41 on 18q—when pedigrees with paternal transmission were identified and analyzed. Further support for linkage to 18q, but not to other regions on chromosome 18, was obtained in a sample of 30 additional pedigrees studied by McMahon et al. (1997), supporting linkage to 18q, linkage that previously had been reported by Stine et al. (1995). Supportive evidence for a locus on 18q has been also published by Coon et al. (1996) and De bruyn et al. (1996).

It also should be mentioned that several groups have failed to replicate linkage between bipolar disorders and chromosome 18 markers (Kelsoe et al. 1995; Maier et al. 1995; Pauls et al. 1995; Smyth et al. 1995; LaBuda et al. 1996; Detera-Wadleigh et al. 1997). Failure of replication in complex disorders may be due to sample size, which may be too small to have adequate power for replication (Suarez 1994), but it also may be due to other factors confounding linkage analyses in complex disorder—for example, heterogeneity, small relative risk, high allele frequencies, etc. In contrast to Berrettini et al. (1994), Stine et al. (1995), and McInnes et al. (1996), we observed, in our pedigrees with schizophrenic index patients, positive LOD scores only for markers in one area (D18S53/D18S37).

Another possible chromosome 18 susceptibility locus for bipolar disorders has been reported by Freimer et al. (1996), who analyzed markers in the telomeric region 18q22-qter, in a study of linkage disequilibrium in families of Costa Rican origin. We have included the markers—D18S64, D18S68, D18S61, and D18S469—from the region that was positive in these Costa Rican families, in scanning the long arm of chromosome 18. There was no evidence for an additional locus in this region, but the data are not sufficient to exclude an additional locus in our sample. There is also no support for this locus in the bipolar-disorder–pedigree samples reported by Berrettini et al. (1994, 1997).

If there is more than one locus on chromosome 18, as data from studies on bipolar-disorder families suggest, our results, which have been obtained in families with schizophrenia, favor a locus around D18S53/G-olf$_\alpha$ as contributing to the development of the illness. To our knowledge, there is only one report that has been published on linkage analysis of chromosome 18 markers in schizophrenic families (DeLisi et al. 1995). That study reported a failure to find chromosome 18 pericentric linkage in 32 families. However, the meaning of the reported *P* value of .023 for marker D18S53, a marker for which we obtained a two-point LOD score of 3.1 (table 3), may have to be reconsidered in the context of our findings.

In the present study, a positive TDT was obtained for marker G-olf$_\alpha$ in the analysis of a sample of families with affected sibs. The TDT (Spielman et al. 1993) examines transmission of alleles of a polymorphic locus from heterozygous parents to an affected offspring, but it can be applied to more than one affected sib if parents are available for genotyping. This has been done in the present study. However, in this case, the TDT is not valid as a test for linkage disequilibrium when linkage is present (Spielman and Ewens 1996), since, in the case of linkage, the sibs cannot be considered as independent, which is a requirement for application of the χ^2 test. One method that could be used to overcome this problem would be to reduce the size of the sibships, to a simplex family with only one affected offspring, by selecting one sib randomly from each sibship. A more powerful method has been proposed recently by Martin et al. (1997), which has been applied in the present study.

The TDT results in the family sample were further analyzed by investigation of an additional sample of 65 simplex families (i.e., triads). The failure to reach statistical significance may be due to the smaller sample size, heterogeneity, differences in etiology, or other factors confounding genetic analysis of complex disorders. However, a similar tendency was noticed, in that the 124-bp allele was transmitted more often to the affected offspring than to the unaffected offspring (33 cases vs. 19 cases).

Besides supporting linkage results, linkage disequilibrium may be useful in the definition of the region for physical mapping and gene identification and characterization. This should be particularly helpful in the case of polygenic disorders, in which a large number of families would be needed in order to narrow the region (Lander and Schork 1994).

Linkage disequilibrium is usually detected in isolates with founder populations, but, since it may persist for as many as 100 generations (Jorde 1995), it also may exist in populations in which the disease mutation cannot be traced to a common founder. Most of the families transmitting the 124-bp G-olf$_\alpha$ marker allele were from the southern part of Germany (the areas of Mainz and Munich), but there also were three families from Israel (one Ashkenazic, one non-Ashkenazic from Iraq, and one non-Ashkenazic from Morocco) that transmitted the allele. As Copeman et al. (1995) have shown for type 1

diabetes mellitus and markers on chromosome 2q, linkage disequilibrium may also be detected in mixed populations.

Our TDT results are also supported by the results of Tsiouris et al. (1996). While studying linkage disequilibrium of G-olf$_\alpha$ in families that were of northern European ancestry and had bipolar disorders, these authors observed a transmitted:nontransmitted ratio of 22:14 for the 124-bp allele. Although the *P* value is not significant, the 124-bp allele revealed the lowest *P* value in the likelihood analysis.

Several groups have reported, for chromosome 18, a parent-of-origin effect in bipolar disorder, resulting in linkage in families with either paternal- (Stine et al. 1995) or paternal *and* maternal (Gershon et al. 1996) transmission of the disease alleles. This was not seen in our sample with schizophrenic index patients when it was divided into maternally transmitting and paternally *and* maternally transmitting families. In contrast to the previous studies, we observed evidence for linkage in the 12 exclusively maternally transmitting families (fig. 4). However, we cannot rule out the possibility that a chance enrichment of linked families occurred when the sample size was reduced. On the other hand, as shown in table 4, TDT analysis provides some further evidence for preferential maternal transmission. In the family sample as well as in the expanded sample, we observed a preferential maternal transmission of the 124-bp marker allele of G-olf$_\alpha$, which reached statistical significance in the combined sample (table 4).

Gender differences in prevalence, age at onset, symptoms, course, and outcome of schizophrenia and in psychopathology are well known (Castle et al. 1995). These differences may not necessarily be caused by genetic factors; rather, they may be caused by gene × environment interactions and confounding factors such as ascertainment bias, artifacts in diagnosis, and reduced reproductive fitness, which may be different between affected males and females. On the other hand, maternal transmission and imprinting have been suggested, for schizophrenia, by Yaw et al. (1996), although they have not been detected by others (Asherson et al. 1994). A preferential maternal transmission in families with a schizophrenic index patient would suggest that a paternally imprinted locus in 18p is responsible for expression of the phenotype in schizophrenia, whereas the reverse may be seen in bipolar disorder. Thus, in analogy with the Prader-Willi/Angelman syndromes, it may be speculated that a differentially imprinted locus on chromosome 18 is responsible for the development of either schizophrenia or bipolar illness.

G-proteins are involved in transmembrane signaling and are coupled to neurotransmitter receptors, which may be involved in the etiology of schizophrenia. Therefore, G-olf$_\alpha$, encoding the stimulatory subunit of a G-protein can be considered as a candidate for a gene conferring susceptibility to schizophrenia. A preliminary examination of G-olf$_\alpha$ exons by SSCP analysis did not reveal mutations that might affect structure or function of the protein. Other candidate genes in the region include IMP (Yoshikawa et al. 1997) and a gene for an adrenocorticotropic-hormone receptor (Detera-Wadleigh et al. 1995).

If the positive TDT for G-olf$_\alpha$ indicates linkage disequilibrium with a susceptibility gene, the linked region can be narrowed to an area of 1 cM. This should be a good starting point for identification of additional candidate genes, which can be screened for disease-causing mutations.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cooperative Human Linkage Center, http://www.chlc.org/data/IntegratedMaps (for D18S852, D18S975, and D18S866

Généthon, http://www.genthon.fr/ (for D18S464, D18S1158, D18S1116, D18S53, D18S73, D18S453, D18S71, D18S1114, D18S478, D18S469, D18S57, D18S64, D18S61, D18S56, D18S59, and D18S68)

Genetic Location Database, http://cedar.genetics.soton.ac.uk/public_html/index.html

Genome Database, http://www.gdb.org (for G-olf$_\alpha$ [GDB 196597])

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