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Dense SNP association study for bipolar I disorder on chromosome 18p11 suggests two loci with excess paternal transmission

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Parent-of-origin effects have been implicated as mediators of genetic susceptibility for a number of complex disease phenotypes, including bipolar disorder. Specifically, evidence for linkage on chromosome 18 is modified when allelic parent-of-origin is accommodated in the analysis. Our goal was to characterize the susceptibility locus for bipolar I disorder on chromosome 18p11 and investigate this parent-of-origin hypothesis in an association context. This was achieved by genotyping single nucleotide polymorphisms (SNPs) at a high density (1 SNP/5 kb) along 13.6 megabases of the linkage region. To increase our ability to detect a susceptibility locus, we restricted the phenotype definition to include only bipolar I probands. We also restricted our study population to Ashkenazi Jewish individuals; this population has characteristics of a genetic isolate and may therefore facilitate detection of variants for complex disease. Three hundred and forty-four pedigrees (363 parent/child trios) where probands were affected with bipolar 1 disorder were genotyped. Transmission disequilibrium test analysis revealed no statistically significant association to SNPs or haplotypes within this region in this sample. However, when parent-of-origin of transmitted SNPs was taken into account, suggestive association was revealed for two separate loci.
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Introduction

Bipolar I disorder is a severe mood disorder characterized by episodes of both mania and depression. Lifetime prevalence of bipolar I disorder is estimated at 0.4–1.6%; approximately 10% of bipolar I patients commit suicide (Diagnostic and Statistical Manual of Mental Disorder (DSM IV)). Annual costs for bipolar disorder are estimated at $24–45 billion dollars.[1] Despite this heavy burden of disease, the underlying pathology of bipolar disorder remains poorly understood. A greater understanding of disease etiology is imperative for the development of successful treatment and management options.

There is substantial evidence that genetic factors contribute to the development of bipolar disorders. Heritability is estimated between 47 and 89%,[2] and heritability of specifically bipolar 1 disorder has been estimated to be 93%.[3] Despite this high heritability, segregation analyses do not support Mendelian paradigms of inheritance. The complex inheritance of bipolar disorder, as well as phenotypic and etiologic heterogeneity, implies that multiple susceptibility loci may exist.

Numerous whole-genome linkage studies have been modestly successful in identifying regions of the genome that may harbor susceptibility loci, including six studies that find significant or suggestive evidence for linkage to chromosome 18p11.[4–9] A meta-analysis of 18 whole-genome linkage scans of bipolar disorder identified chromosome 18 as nominally significant;[10] another meta-analysis of 11 studies found the 18p region significant at $P<0.003$.[11] There is also evidence for a parent-of-origin effect on linkage evidence at the 18p11 locus.[5,9,12] It is important to note that many studies have failed to find linkage evidence to chromosome 18p11. The lack of agreement in findings from genetic studies may be attributable to etiologic heterogeneity of bipolar disorder; it may also be due to lack of consistency in ascertainment or diagnostic methods, or to low statistical power, as many linkage studies have a small number of pedigrees for analysis.

A number of candidate genes on chromosome 18p11 have been investigated for association with genetic markers, including *NAPG, PACAP, MC5R, IMPA2, NDUFV2, GNAL* and *CLUL1*[13–21] (Table 1).

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Table 1 Candidate genes studied for bipolar disorder on chromosome 18p11

Candidate gene	*Position, build 34*	*Study, year*	*Sample size*	*Parent-of-origin analyzed?*	*No. of markers tested*	*Evidence for association*
NAPG	10.5	Weller *et al.*[21]	460 BPI cases, 191 controls	Not possible	5	$P=0.027$, single SNP genotypic test, uncorrected
ADCYAP1 (PACAP)	0.9	Ishiguro *et al.*[15]	86 bipolar (I and II) cases, and 251 controls	Not possible	1	No association
GNAL	11.7	Tsiouris *et al.*[13]	224 trios from 106 families	Yes	1 (11 alleles)	No association; no parent-of-origin association
MC5R	13.8	Lohoff and Berrettini[20]	345 BPI cases, 275 controls	Not possible	3	No association
IMPA2	11.9	Sjoholt *et al.*[18]	44 Norwegian bipolar cases, 47 controls	Not possible	10	0.03, single SNP, uncorrected
			92 Arab BPI trios	No	10	0.002, single SNP, uncorrected
		Yoshikawa *et al.*[14]	22 bipolar cases; 96 bipolar cases, 59 controls	Not possible	3	0.051, uncorrected
NDUFV2	9.1	Washizuka *et al.*;[19] Washizuka *et al.*[17]	189 bipolar (I and II) Japanese cases, 222 controls	Not possible	6	0.0001 (4-SNP haplotype), corrected
			104 bipolar (I and II) case–parent trios	No	9	0.11 (2-SNP haplotype), corrected
CLUL1	0.6	McInnes *et al.*[16]	227 (nonindependent) BPI cases, 52 controls	No	30	0.01, uncorrected

Some of these studies show promising genetic-association evidence, and await replication and biology-based evidence for involvement in disease. The majority of candidate gene studies have utilized a case–control design; testing of a parent-of-origin hypothesis is not possible in these studies.

Lack of consistency among linkage and association findings for bipolar disorder is complicated by the definition of affection status. Many linkage studies do not distinguish between bipolar I, bipolar II, unipolar depression and schizoaffective disorder; often, all are considered affected. A reduction in heterogeneity may be achieved by restricting the investigated phenotype to include a single and more reliable diagnosis.

The investigation of disease in an isolated population may also reduce heterogeneity and facilitate identification of susceptibility variants. Because isolated populations arise from a limited number of founders, genetic variation is reduced and the probability that affected individuals share a common susceptibility locus is increased. The investigation of isolated populations for complex genetic disease has been previously successful for both linkage and association for complex diseases. For example, the *EDNRB* locus for Hirschsprung disease was discovered by linkage in Mennonite kindreds, and mutations in the gene were also first identified in the Mennonite population.[22,23] Subsequently, it has been shown that mutation in the *EDNRB* gene is responsible for 5% of Hirschsprung disease in outbred populations.[24] Recent studies in the Icelandic population have identified associated regions or specific variants for prostate cancer and type II diabetes; these associations have been replicated in non-Icelandic populations.[25,26] In the Ashkenazi Jewish population, variation in the genes *MSH2*, *BRCA1* and *BRCA2* contribute to the burden of prostate, breast and colon cancer in the Ashkenazim as well as outbred populations.[27–30] We have elected to focus on the Ashkenazi Jewish population, which has arisen from a limited number of founders and where endogamous mating is culturally reinforced.

We have previously investigated evidence for linkage in 41 Ashkenazi pedigrees multiplex for bipolar disorder (I and II). The results of this scan were suggestive for linkage to chromosome 18p11; this linkage evidence increased when analysis was restricted to a diagnosis of BPI only (22 pedigrees), and is due to excess sharing of *paternal* alleles among affected relatives in the 18p11 region.[9] Our goal in the present study was to fine map the linkage region with informative SNPs, and further investigate the parent-of-origin effect observed in our linkage study. To this end, we ascertained 344 pedigrees (363 BPI trios) all of Ashkenazi descent. This sample was genotyped for 2777 SNP markers in a 13.6 Mb region on chromosome 18p11. We find suggestive association evidence for paternally transmitted SNPs. Further investigation and close scrutiny of this region is warranted.

Materials and methods

Study subjects

Ashkenazi Jewish individuals affected with BPI were recruited nationally over a 6-year period through

advertisements in newspapers and Jewish newsletters, talks to community organizations, letters to leaders of the Jewish community and a study Web site (Johns Hopkins Epidemiology/Genetics Program in Psychiatry Home Page, www.hopkinsmedicine.org/epigen). Case–parent trios were eligible for inclusion in these analyses if: the proband met DSM-IV criteria for a BPI diagnosis, both parents were available for DNA collection, and all four grandparents were of Ashkenazi Jewish descent. This recruitment effort led to 344 eligible families for analysis (363 trios), including 24 families (39 trios) from our previous linkage study. Forty-eight percent of affected individuals in our study were male; mean age of onset for BPI was 18.9 years.

Probands were assessed for psychiatric illness according to established procedure, as follows: a trained clinical examiner (doctoral-level clinical psychologist) interviewed each study subject in person (usually in their home) using the Diagnostic Interview for Genetic Studies (DIGS), a standard, semi-structured instrument widely used for phenotypic classification of individuals for psychiatric genetic studies. These interviews were tape-recorded. For each study subject, medical records were also obtained, and collateral interviews with at least one additional informant were conducted. The examiner then completed a written diagnostic workup, including relevant clinical features and course of illness. All collected clinical information (tape-recorded interviews, DIGS interview booklets, medical records, collateral interview information and written diagnostic workup) was forwarded to two independent, trained clinicians, who reached a consensus diagnosis of bipolar I disorder before an individual was included for study. Complete details about clinical methods are available in Fallin *et al*.[9]

We assessed power in a *post hoc* manner by first accounting for the number of tests done while incorporating the LD structure of this region in our sample, as follows: all SNPs in an LD block (defined as $r^2 > 0.8$) were regarded as a single test per block (524 blocks, 1883 SNPs). All remaining 'orphan' SNPs were considered independent tests ($n = 894$). By these rules, 1418 independent single SNP tests were conducted in this experiment, requiring a Bonferroni-corrected *P*-value of 0.000034 for statistical significance. QUANTO[31,32] was then used to calculate power for varying relative risks and disease allele frequencies, given a sample size of 363 trios and an alpha level of 0.000034.

Genotyping strategy

On chromosome 18p11, 13.6 Mb of the bipolar linkage region was selected for dense SNP genotyping. SNPs were genotyped using the Illumina BeadArray genotyping technology,[33] which has been shown to have a high degree of assay conversion, a low rate of missing data and error rates of less than one error in 1000 genotypes (Altshuler *et al.*,[34] Supplemental Tables 2 and 3).

SNP selection. At the time SNPs were selected for this experiment, the HapMap Consortium genotyping project was at an early stage; 'tagging' methods had not been developed nor validated. We therefore developed our own criteria for SNP selection, balancing our desire to obtain maximal information with efficiency and cost. As a first step, all SNPs in dbSNP that mapped to the first 13.6 Mb of chromosome 18p11 were vetted using an algorithm proprietary to Illumina, which calculates a 'design score' for each SNP (ranging from 0 to 1); this is approximately the probability that an assay will successfully generate data in the Illumina system. SNPs with a design score above 0.6 were considered for assay development. SNPs with reported minor allele frequency below 0.05 were excluded. Of SNPs that met these criteria, those that were in the HapMap or had been deposited in dbSNP by Perlegen were preferentially selected for genotyping at a maximum density of one SNP every 1.8 kb, though there were many regions where large gaps existed. This scaffold was then supplemented from the remaining pool of SNPs at variable density depending on the partial LD information available from HapMap. In regions of low LD, we selected SNPs at a maximum density of one SNP every kb; in regions of high LD maximum selection density was one SNP every 2 kb. This resulted in 3072 SNPs chosen for assay development with an average spacing of one SNP every 4.4 kb. The largest gap between SNPs was 26.5 kb. Seventy-seven genes are cataloged in RefSeq in this region (Pruitt *et al.*,[35] http://www.ncbi.nlm.nih.gov/RefSeq/). One thousand two hundred and thirty-nine SNPs were selected for genotyping in these 77 transcripts. The average SNP density in transcribed sequence was one SNP every 4.2 kb.

Analysis

Data quality was assessed by (1) consistency of working assays, (2) genotype analysis of replicate samples and (3) the rate of Mendelian errors in families. For (2), genotype analyses of duplicate samples, four CEPH samples were used as controls. These four samples were genotyped on every 96-well plate (13 plates total). To assess the rate of Mendelian errors, family-based study samples were used. Mendelian errors underestimate the true genotyping error rate, because many genotyping errors are consistent with Mendelian inheritance. We therefore assumed that observed Mendelian errors represent one-third of the actual genotyping errors[36] and adjusted the estimated error rate in this study accordingly. Hardy–Weinberg equilibrium was also tested for each SNP in the parental population; SNPs out of HWE at $P < 0.001$ were analyzed separately.

Association was investigated in this study via the transmission disequilibrium test (TDT), where transmission of alleles from heterozygous parents to affected offspring are tested, and statistical significance is assessed using the TDT χ^2 test with 1 d.f.[37] Adjusted *P*-values were assessed by permutation of

transmitted alleles from heterozygous parents to affected offspring 10 000 times.

Haplotypes were inferred using the Stephens–Smith–Donnelly algorithm as modified by Lin *et al.*,[38,39] which accounts for missing data, can exploit inheritance information to generate accurate haplotypes between related individuals, can accurately phase between regions of high LD and can handle very large amounts of data.[38–40] This algorithm is accurate over long distances (100–300 kb), particularly for family-based samples. After haplotypes were generated, 'exhaustive allelic' testing was accomplished by investigating all haplotypes of every possible window size (linear combinations), using the TDT test (EA-TDT[38,39]). Statistical significance for haplotype-based tests was calculated unadjusted for multiple tests, and was also adjusted for multiple tests via permutation (>100 000 permutations).

A recent report by Lin *et al.*[41] suggested that evidence for linkage to chromosome 18p11 is enhanced in later-onset (age of onset $\geqslant$21) bipolar pedigrees. We explored whether late age at onset also led to detectable differences in association evidence by stratifying our sample and conducting association analysis on trios with probands <21 (64.8% of probands) and $\geqslant$21 (33.8% of probands) separately. Seven probands lacked information about age-at onset and were excluded from this analysis.

Parent-of-origin analyses were conducted in multiple ways. To assess whether allelic parent-of-origin was relevant to association results, informative transmissions/nontransmissions were stratified by source parent for all SNPs, and the TDT was recalculated. By this method, four SNPs showed excess paternal transmission at $P<0.0001$ (unadjusted), with no excess maternal transmission. Stratified TDT can sometimes give spurious results, particularly in the presence of (a) maternal effects or (b) skewed distribution of genotypes between parental classes. We therefore assessed these four SNPs for parent-of-origin association in two additional ways. First, we performed the TAT as suggested by Weinberg, which is similar to the TDT but excludes double heterozygote mating pairs.[42] Next, we formulated a conditional logistic regression matched by parental-child genotype combination, and included an interaction term for paternal origin of risk allele, such as was used by Le Stunff *et al.*:[43]

$$\ln\left(\frac{\mathrm{P}(t)}{1-\mathrm{P}(t)}\right) = \alpha \mathrm{A} + \beta \mathrm{A} * \mathrm{F}$$

where outcome is probability of transmission, $A=1$ if putative risk allele is transmitted for a given parent–child pair, 0 otherwise, and $F=1$ if the transmission was paternally informative. Parent-of-origin effects are assessed by a likelihood ratio test between models with and without the interaction term. This analysis has the added advantage of allowing for estimation of the odds ratio for paternal inheritance. Analysis was carried out using standard statistical software (STATA/SE 8.2 for windows).

Results

Three thousand and seventy-two SNP assays were attempted in this study; 183 (5.96%) failed to generate data. Of the remaining 2889 SNPs, 100 were monomorphic and 12 were out of Hardy–Weinberg equilibrium at $P<0.001$, leaving 2777 SNPs for analysis. The estimated error rate for these SNPs, as assessed by presence of Mendelian errors and replicate analysis, is 2.8 and 3.3 errors per 100 000 genotypes, respectively. This implies there are between 95 and 112 errors in 3.3 million genotypes in this study.

Genotyping was attempted on 1167 unique samples. Genotyping was unsuccessful on four individuals, as assessed by missing data rates and repeated poor clustering of genotype data. One pedigree had >10% of genotypes with Mendelian errors, and was subsequently removed from analysis due to suspected non-paternity or sample mislabeling (four individuals removed).

Analysis

We estimated the power of this study in a *post hoc* way to account for the number of independent tests conducted in this study, given 363 trios tested and the LD structure of our sample. For experiment-wide significance, a *P*-value of 0.000034 is required (1418 independent tests). This sample has 80% power to detect genetic relative risks $\geqslant$2.2 if disease allele frequency is 10%, given a log-additive genetic model. If disease allele frequency is 50%, this study is powered to detect genetic relative risks $\geqslant$1.8.

Transmissions of alleles from heterozygous parents to affected offspring were tabulated, and the TDT statistic was calculated for individual SNPs (Figure 1). The marker with the strongest evidence for association was rs541039, position 4265455 (185 transmitted vs 125 untransmitted, $P<0.0008$, permutation-adjusted $P<0.86$). No SNP in this analysis had an adjusted *P*-value <0.05. Details about the 10 SNPs with the highest evidence for association are provided (Table 2).

Haplotypes were generated, and transmission of all possible contiguous SNP haplotypes of all possible window sizes was assessed via the TDT. A 14 SNP haplotype of size 120 kb, midpoint at 6121080, was the most strongly associated (59 transmitted: 20 untransmitted, unadjusted $P<0.000013$; adjusted $P<0.38$).

No compelling evidence for differential association related to age at onset (>20 vs <21) of bipolar disorder was observed.

Parent-of-origin effects were first investigated by stratifying informative transmissions/nontransmissions by source parent for all SNPs. This method revealed four SNPs with paternal transmission distortion at $P<0.0001$ (unadjusted for multiple tests), and no excess maternal transmission. Three of these SNPs (rs1893157, rs494412, rs620504) map within 13 kb, and are correlated with one another (all $r^2>0.7$). For these SNPs, the minor allele is paternally overtransmitted. (These three SNPs are approximately

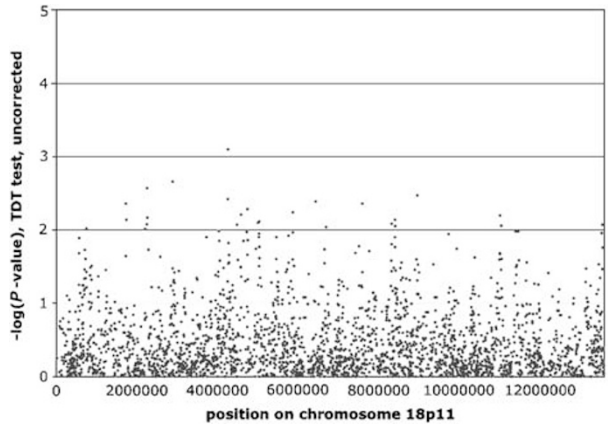

Figure 1 Distribution of association evidence, allelic TDT test. Uncorrected *P*-values are plotted by chromosomal position for each SNP tested. Highest evidence for association is at rs541039, position 4,265,455, 184 T vs 125 U, uncorrected *P*-value 0.0008, permuted *P*-value 0.86.

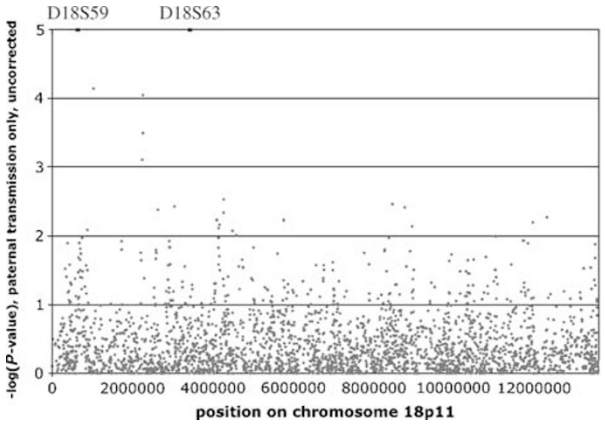

Figure 2 TDT results, paternal transmission only. Transmission from paternal heterozygotes only was tabulated for each SNP. Unadjusted *P*-values are plotted for each SNP tested. D18S59 and D18S63 are microsatellite markers that previously showed evidence for excess paternal allele sharing among sibs affected with BPI.[9]

Table 2 Ten most associated SNPs, allelic TDT

SNP	*Position*	*T:U*	χ^2	P-*value*
rs541039	4265455	184:125	11.265	0.0008
rs11080994	2887993	222:162	9.375	0.0022
rs1893157	2257456	81:47	9.031	0.0027
rs10853370	8960615	204:149	8.569	0.0034
rs551920	4261385	100:63	8.399	0.0038
rs6506386	6437482	177:127	8.224	0.0041
rs16952222	7598051	51:26	8.117	0.0044
rs12607596	1732207	193:141	8.096	0.0044
rs9635859	4747894	91:57	7.811	0.0052
rs1940594	5872643	125:85	7.619	0.0058

equally informative for a single putative susceptibility variant; to avoid redundancy we report detailed results for rs1893157 only.) The remaining SNP (rs789024) is 1.2 Mb telomeric to this locus, and is not correlated with the others (all $r^2 = 0$). We therefore regard rs789024 as an independent putative susceptibility locus. These two loci map within D18S59 and D18S63, two microsatellite markers that describe the peak linkage region of paternal allele sharing previously reported in this population[9] (Figure 2).

The transmission asymmetry test (TAT), as suggested by Weinberg, excludes mating pairs of two heterozygotes in order to avoid bias from these matings, which contribute twice to a stratified TDT.[42] After this exclusion, excess paternal transmission is still observed for both loci (Table 3a). Next, we formulated a conditional logistic regression matched by parental mating type, and included an interaction term for paternal origin of risk allele, such as was used by Le Stunff *et al*.[43] Likelihood ratio tests for the parent-of-origin interaction term were significant at $P < 0.001$ for both loci tested (Table 3b). For SNP rs789024, the estimated odds ratio for developing bipolar disorder when the risk allele is paternally transmitted is 4.27 (CI 2.00–9.10), relative to maternal transmission. For SNP rs1893157, estimated odds ratio for bipolar disorder given paternal transmission is 6.53 (CI 2.22–19.20) relative to maternal transmission of the risk allele.

SNP rs789024 is out of Hardy–Weinberg equilibrium; there are 11% too many heterozygotes compared to what is expected. Excess heterozygosity is consistent for both parent populations. To glean additional information about this SNP, we examined the genotypic distribution in the four HapMap populations. The trend of aberrant heterozygosity is also observed; there are 6.92% too many heterozygotes in the CEU population, and 8.66% too few heterozygotes in the CHB population. This trend is not observed for SNP rs1893157 (Figure 3).

Discussion

The aim of this study was to characterize the linkage region for bipolar disorder on chromosome 18p11, and investigate the paternal parent-of-origin hypothesis for susceptibility, as was suggested by an earlier linkage study in the Ashkenazi Jewish population.[9] In this study, our sample of 363 trios had 80% power at an alpha of 0.000034 to detect risk ratios of at least 1.8–2.2-fold, at a disease allele frequency of 50–10%, respectively and a log-additive model of genetic risk. Without taking parent-of-origin into account, our inability to detect a statistically significant association implies that a common allele that confers this magnitude of risk does not exist in the 18p11 region.

There is evidence for a parent-of-origin effect at the 18p11 locus from a linkage study in the Ashkenazi Jewish population,[9] as well as other populations.[5,12] Parent-of-origin effects imply an epigenetic mechanism of inheritance and may be a compelling hypothesis to explain complex genetic disorders such

Table 3a Transmission of alleles to bipolar I offspring, stratified by parent-of-origin of alleles

	rs789024					*rs1893157*			
	T	*U*	P-*value*	*Tau*		*T*	*U*	P-*value*	*Tau*
Stratified TDT									
Paternal	77	35	0.00007	0.69	Paternal	52	19	0.00009	0.73
Maternal	60	57	0.78	0.51	Maternal	24	23	0.88	0.51
Total[a]	137	92	0.003	0.60	Total[a]	76	42	0.002	0.64
TAT									
Paternal	45	24	0.01	0.65	Paternal	43	17	0.0008	0.72
Maternal	32	42	0.24	0.43	Maternal	21	19	0.75	0.53
Total	77	66	0.35	0.54	Total	64	36	0.005	0.64

[a]Excludes trios where parents and off spring are all heterozygous, as these are not informative.

Table 3b Test for parent-of-origin effects

	Model	*Estimates*				*−2LL*	χ^2	*LRT*χ^2	*d.f.*	P-*value*
		α	*(s.e.)*	β	*(s.e.)*					
rs789024	Allele effect (A)	0.79	(0.18)			589.32				
	Allele and PO (A + A*F)	0.10	(0.26)	1.45	(0.37)	574.90	PO	14.41	1	0.0001
rs1893157	Allele effect (A)	1.15	(0.26)			285.55				
	Allele and PO (A + A*F)	0.08	(0.41)	1.87	(0.55)	273.73	PO	11.82	1	0.0006

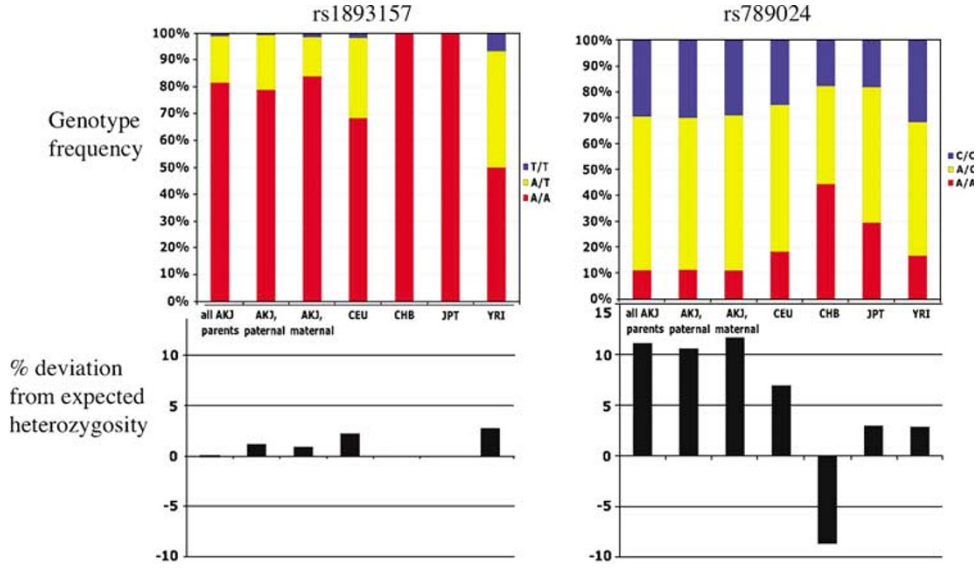

Figure 3 Genotype frequencies and deviation from expected heterozygosity for two paternally transmitted SNP are shown for Ashkenazi Jewish parents, Ashkenazi maternal and paternal populations separately, and Hapmap populations.

as bipolar disorder.[44–46] The paternal allele sharing in the Fallin *et al*.[9] linkage study is described by microsatellite markers D18S59 and D18S63; the two loci (four SNPs) with putative parent-of-origin effects in this study map between these two markers and is therefore consistent with previous results. These loci confer substantial risk for bipolar disorder (fourfold and sixfold respectively, relative to maternal transmission). They may represent two different regions that each control the same transcript, two different

regions that are related to two different susceptibility genes, or one or both may be false positives.

A note about parent-of-origin transmission tests for association: As these methods are only now emerging, the methods to apply empirical *P*-value estimation in this framework are lacking. The simple permutation of transmission status used for standard TDT analyses would not provide the appropriate null distribution for hypothesis of parent-of-origin. Therefore, we caution the reader to interpret the distribution-based *P*-values we provide with the understanding that they are based on a χ^2 assumption.

One of the SNPs with excess paternal transmission, rs789024, is out of Hardy–Weinberg equilibrium, with 11% too many heterozygotes observed. This may be due to chance, however, this trend is also observed in two of the four HapMap populations. This aberrant genotypic distribution may be a consequence of selection, implying that rs789024 or a linked variant is involved in susceptibility for bipolar disorder. It is also possible this excess heterozygosity represents genotyping error, perhaps owing to a yet undetected segmental duplication in the human genome. The other SNP with excess paternal transmission, rs1893157, is consistently in Hardy–Weinberg equilibrium, but has a wide spectrum of allele frequencies between populations. This too may represent the action of selection.

In this study, we have pursued a family-based study design. Family-based samples are not without drawbacks; they require more collection effort and increased genotyping costs compared to case–control designs. However, these drawbacks are balanced by two important advantages: first, it is possible to investigate parent-of-origin hypotheses. Second, family-based study designs are robust to interference from population stratification. It has been shown that population stratification can mask true positive findings, and cryptic substructure can create false positives.[47,48]

Parent-of-origin effects have been shown to be important for a number of genetic diseases, usually through an imprinting mechanism. At least eight syndromes have been shown to involve aberrant transmission or regulation of imprinted genes.[49] For example, Prader–Willi syndrome[50] involves either loss of the paternally transmitted region of chromosome 15q11–13, or maternal uniparental disomy (UPD) of this same region. Angelman's syndrome,[50] a distinct phenotype, results from loss of maternal 15q11–13 or paternal UPD. Several genes in the critical region on chromosome 15q11–13 including *UBE3A*, *SNRPN*, *PAR1*, *PAR5* and *PAR7*, have shown parent-of-origin-specific expression. Loss of parent-of-origin expression is principally responsible for disease. It is likely that for complex diseases such a bipolar disorder, a similar mechanism contributes to susceptibility.

There has not yet been reported evidence for an imprinted region on chromosome 18p11. However, a recent study finds evidence of parent-of-origin-dependent expression for an alternative transcript of *GNAL*, which encodes G_{olf}, the alpha subunit of a heretotrimeric stimulatory G-protein that may functionally associate with the dopamine D1 receptor.[51] *GNAL* has previously been investigated for parent-of-origin effects in bipolar disorder, and no association has been reported; however, this study tested a single marker in a small number of trios.[13] *GNAL* maps more than 10 Mb distal to the loci reported in this study. It is unlikely that the loci we report here are responsible for control of *GNAL* expression; 800 kb is the greatest distance yet reported between a regulatory element and its target gene.[52] The mitochondrial subunit gene *NDUVF2* has been reported to be associated with both bipolar disorder and schizophrenia,[17,19,53] as has *IMPA2*,[14,18] though these loci are also located 8–10 Mb distal to the loci reported herein. Parent-of-origin associations have not been investigated at these loci. Of candidate genes investigated, *CLUL* and *ADCYAP (PACAP)* are located within 1 Mb of the paternally transmitted SNPs we report herein. These loci have been investigated previously in a case–control study design, and evidence for association has been marginal (*CLUL1*[16]) or nonexistent (*PACAP*[15]). It is possible that accounting for parent-of-origin would strengthen association evidence. The results of the current study suggest a reexamination of these loci; of particular interest is whether parent-of-origin-specific expression of these genes can be detected.

The results of this study suggest that parent-of-origin of transmission plays a significant role in susceptibility to bipolar disorder for variants on chromosome 18p11. This statistical evidence must be supplemented with biological information about candidate loci near SNPs reported in this paper; further experimentation in this region is warranted.

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