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Identifying Bipolar Disorder Susceptibility Loci in a Densely Affected Pedigree

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Bipolar disorder (BIP) is a highly heritable disorder with complex patterns of genetic inheritance, and recent genetic findings highlight the role of numerous common variants each with subtle effects.[1] The existence of Mendelian subtypes of BIP (rare variants of very strong effects) has been postulated, particularly as such variants could prove to be more tractable for subsequent biological investigation.[2] One way to evaluate this hypothesis is via the study of pedigrees densely affected with BIP in which a genetic variant of strong effect inherited from a common ancestor may be more likely. With the advent of high-throughput technologies, we can now search densely-affected pedigrees for specific variants that may contribute to risk for BIP.

We therefore evaluated a Spanish multi-generational pedigree with an exceptional prevalence of BIP using multiple complementary genomic techniques (Table S1). This pedigree contains 18 cases with BIP (including a sibship with six of 11 affected) and seven individuals with recurrent major depressive disorder (Figure 1a). The lifetime prevalence of mood disorders in this large pedigree (6 generations; 120 individuals, 30 with known mood disorders, 42 with DNA) makes it a strong candidate for identifying genetic risk factors of near-Mendelian effects. Our search strategy is depicted in Figure 1b. All protocols were IRB approved and all subjects provided written informed consent.

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Conflicts of Interest

Dr. Sullivan was on the SAB of Expression Analysis (Durham, NC, USA). The other authors report no conflicts.

First, we used genome-wide linkage analysis and an affecteds-only approach to identify genomic regions sharing identity-by-descent. Linkage analysis was performed using microsatellite data from 13 individuals.[3] We used data from Illumina HumanOmni Quad genotyping arrays to identify shared segments using Beagle,[4] Germline,[5] PedIBD,[6] and runs of identity-by-state.[7] Regions identified by linkage or with two sharing methods were considered candidate regions (Table S2, six regions totaling 93.7Mb).

Second, we hypothesized that one of these regions contained a novel, rare functional single nucleotide variant (SNV) of high penetrance. We attempted to identify novel SNVs in these six genomic regions shared by BIP cases using exome sequencing (five BIP cases) and whole genome sequencing (three BIP cases). Whole genome sequence data was screened to identify high-quality homozygous or heterozygous SNVs that were within the candidate regions, novel, of predicted functional consequence, and present in all three BIP cases. This procedure identified 26 SNVs in three olfactory receptors (*OR4C3*, *OR9G9*, and *OR4C45*). Because multiple variants were present in each gene, given the existence of other highly similar members of the large olfactory gene family, and manual review of alignment patterns, we believed all to be due to misalignment. We repeated this process for the exome sequencing data, and identified 21 SNVs (17 were also identified by whole genome sequencing), and all were in olfactory genes (*OR4C3*, *OR9G9*, and *OR8U8/OR8U1*), and likely due to incorrect alignment. We next evaluated CNVs in these regions. Using PennCNV[8] calls from Illumina arrays, no novel CNVs were identified in these candidate regions that were >1kb, present in ≥8 of 11 BIP cases, and confirmed via whole genome sequencing. Therefore, we were unable to identify any potential SNVs or CNVs within these candidate regions that were promising for follow-up.

Third, we then extended our analysis genome-wide. From the whole genome sequencing of three individuals, we identified novel SNVs of predicted functionality that were present in all three BIP cases (Table S3). We excluded SNVs in olfactory receptors, with questionable alignments, or that were not confirmed in exome sequencing of five BIP cases. This resulted in eight SNVs that we sequenced in 11 affected individuals using Sanger sequencing. Five SNVs verified and had plausible inheritance patterns, and were then genotyped in 42 individuals in the pedigree.

Two SNVs were potentially interesting (chr12:52452495 C>T in *NR4A1* and chr18:47793974 G>C in *MBD1*). Neither exhibited unequivocal Mendelian inheritance (Figures S2–S3). The *NR4A1* (nerve growth factor IB, NGFIB or Nur77) SNV is novel but common in this pedigree (homozygous or heterozygous in 30/34 individuals descended from the founders, excluding married-in individuals) and did not clearly segregate in BIP cases as the SNV was found in 92% of BIP cases and 86% of subjects without a mood disorder (although the number of unaffecteds is small). The *MBD1* (methyl-CpG-binding domain protein 1) SNV tracked with BIP (66%), other mood disorders (88%), and less so in unaffecteds (21%). However, this SNV was recently identified by the 1000 Genomes Project in subjects from the United Kingdom. Therefore, this variant is not completely novel, but has an interesting pattern of segregation. Both variants warrant additional follow-up in a larger case-control samples although neither appears to be a strong novel Mendelian variant.

Genome-wide expansion of the CNV search identified no novel CNVs that were present in ≥8 BIP cases, >1kb in size, overlapped an exon, and verified by whole genome sequencing.

Fourth, given the absence of compelling results in support of a near-Mendelian variant, we evaluated the contribution of common variation in this pedigree. The presence of many common variant risk alleles in a pedigree is a potential explanation for a dense pedigree.[9] This can be due to or exacerbated by assortative mating (in which mental illness in the

family of a spouse is more likely to be tolerated if one has it in one's own family) and result in an accumulation of common risk alleles. We therefore calculated risk profile scores, which are the weighted number of BIP risk alleles in each subject. Based on results from the Psychiatric GWAS Consortium[10] (7,481 BIP cases and 9,250 controls), risk profile scores were computed for the GAIN BIP cases (N=1080) and controls (N=1058),[11] and the 11 BIP cases in the densely-affected pedigree (Figure 1c).

As anticipated, the GAIN BIP cases had significantly higher risk profile scores than the GAIN controls ($p < 0.001$). Risk profile scores for 11 BIP cases from the Spanish pedigree were also significantly greater than controls ($p < 0.001$) but not significantly different from GAIN BIP cases ($p = 0.19$). It is particularly notable that the BIP pedigree cases did not have markedly lower risk profile scores (e.g., low common variant profiles might be consistent with the present of a strong mutation). Rather, the BIP pedigree cases appeared to have common variant risk profile scores similar to European-American BIP cases ascertained clinically without regard to family history.

In conclusion, we systematically assessed this large pedigree dense with BIP for genetic variants of strong effect. This comprehensive analysis did not conclusively identify any SNVs or CNVs of near-Mendelian effect. However, we cannot exclude the presence of risk variants with more complex inheritance patterns, variants with more cryptic functional effects, or variants missed due to coverage or individuals sequenced. However, the common variant risk profiles of BIP cases in this pedigree are similar to those of BIP cases ascertained without regard to family history. Therefore, it is possible that the etiology of BIP in this pedigree is more related to multiple common risk variants rather than one or a few variants of extremely strong effect.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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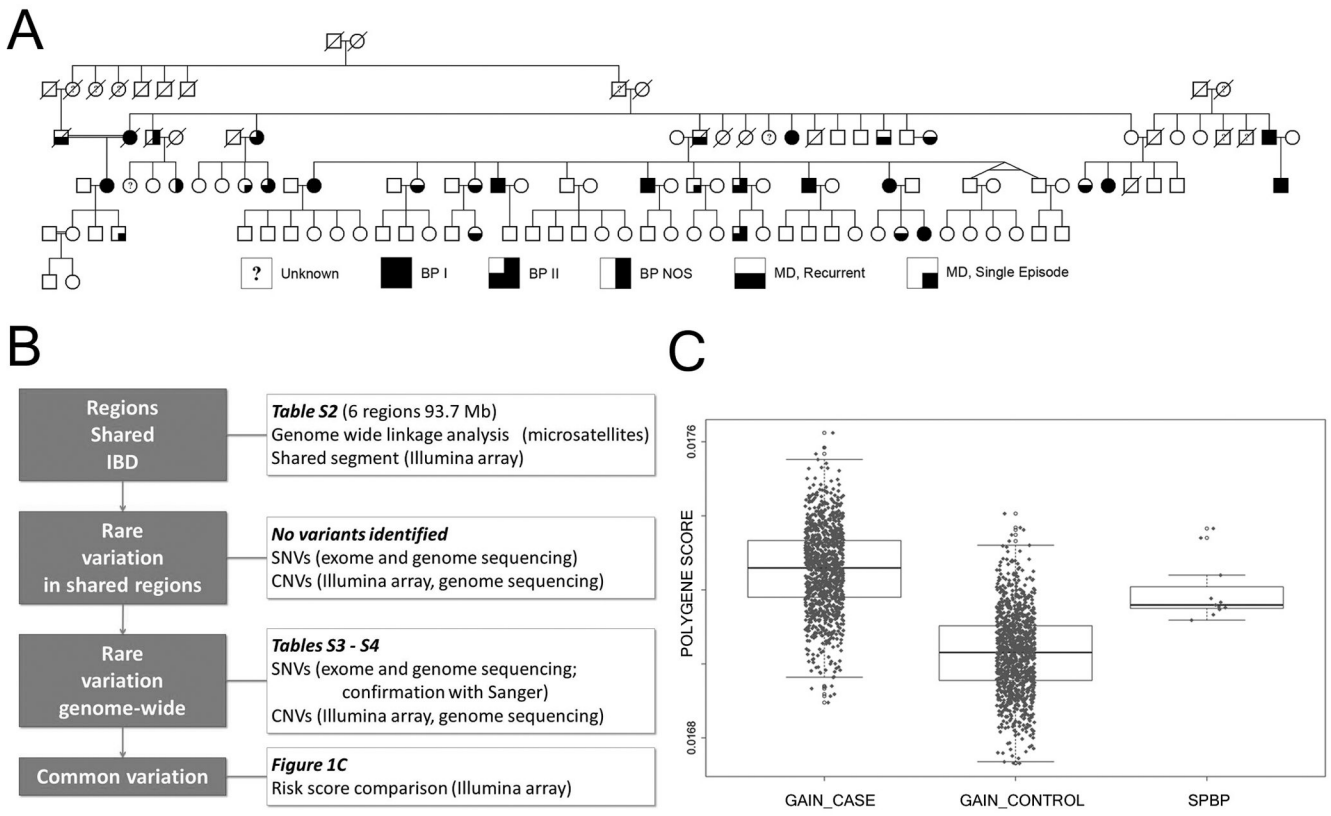

Figure 1.

(a) The pedigree was ascertained in Spain, and has a high prevalence of mood disorders, particularly bipolar disorder (type 1) and recurrent major depressive disorder.

(b) Summary of experimental flowchart and results. IBD=identity-by-descent, SNV=single nucleotide variant, CNV=copy number variation.

(c) Risk profile scores in GAIN BIP cases, GAIN controls, and 11 BIP cases from the pedigree in Figure 1a. Profile scores were based on the PGC BIP mega-analysis.