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CORRESPONDENCE

Family-based association of an *ANK3* haplotype with bipolar disorder in Latino populations

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Over the past several years, genome-wide association studies (GWAS) have successfully identified genetic loci strongly associated with bipolar disorder (BP).[1–3] Some of the most consistent findings have been in variants located in the *ANK3* (ankyrin-G) gene located on chromosome 10q. Schultz *et al.*[4] found that BP risk alleles in *ANK3*, rs9804190 and rs10994336, contributed independently to BP with no significant marker–marker interaction. Subsequent association studies support the *ANK3* association in other populations of primarily European or Asian ancestries.[5,6] To date, no studies have been reported that focus in Latino populations.

To evaluate the role of *ANK3* in the Latino population, we have designed a family-based association study to evaluate single-nucleotide polymorphisms (SNPs) spanning the *ANK3* gene, including GWAS-significant SNPs rs9804190 and rs10994336, in 215 Latino pedigrees with reported ancestry from Mexico or Central America. Inclusion criteria required a proband with a bipolar disorder type I (BPI) diagnosis with at least one sibling with a clinical diagnosis of BPI or schizoaffective bipolar disorder (SABP), and a minimum of two additional first-degree relatives willing to participate. Additional family members with a history of affective or psychotic disorders were included when possible. The sample consisted of 157 case–parent trios and 258 affected subjects with one parent genotyped. All study participants were diagnosed using Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria using a best-estimation consensus procedure as previously described.[7] Of the 929 individuals in this study, 466 were diagnosed with BPI and 16 were diagnosed with SABP.

DNA was isolated from lymphoblastoid cell lines. SNP selection covering the *ANK3* gene was based on a tagging SNP approach ($r^2 \geqslant 0.9$) using the SNPbrowser software version 4.0.1 (Life Technologies Corporation, Carlsbad, CA, USA). Known BP risk variants in *ANK3*, rs9804190 and rs10994336, were included in the panel as well as additional proximal SNPs to these known variants. A total of 38 SNPs were genotyped using a custom-designed Illumina GoldenGate SNP assay (Illumina, San Diego, CA, USA). After assessment of quality control, a total of 36 SNPs for 213 pedigrees (913 individuals) were retained for statistical analysis.

Analyses of individual SNPs and haplotypes were completed with the Family Based Association Test (FBAT) version 2.0.3,[8] using an additive genetic model (Table 1). We were unable to replicate associations of the BP risk variants rs9804190 and rs10994336 in our dataset. To thoroughly test the loci identified by the two individual SNPs previously reported to be associated with BP, Haploview version 4.2 (http://www.broadinstitute.org/haploview/haploview)[9] was used to visualize linkage disequilibrium (LD) relationships between all genotyped variants (36 SNPs) within and surrounding the *ANK3* region and to construct LD blocks following the D′ method described by Gabriel *et al.*[10] Five SNPs were found to be in high LD with rs10994336. SNPs

Table 1 Family-based association tests for *ANK3* variants and haplotypes under additive model

Marker	*Over-transmitted*	*Allele frequency*	*Z-score*	P-*value*	*Adjusted* P-*value*[a]
rs1380455	T	0.728	2.108	**0.035**	0.193
rs16914968	T	0.076	1.643	0.100	0.470
rs1551684	C	0.914	1.111	0.267	0.844
rs3808942	T	0.745	0.094	0.925	1.000
rs3808943	T	0.311	2.430	**0.015**	0.087
rs10994336	T	0.310	1.939	0.053	0.276
Haplotype	*Informative families*	*Haplotype frequency*	*Z-score*	P-*value*	*Permutation* P-*value*[b]
TCCTTT	103	0.315	2.266	**0.023**	**0.035***
GCCTCC	111	0.279	−1.572	0.116	0.126
TCCCCC	100	0.247	−0.565	0.572	0.552
TCTTCC	43	0.070	−0.778	0.436	0.396
TTCTCC	39	0.069	1.737	0.082	0.079

Abbreviation: *ANK3*, ankyrin-G.
[a]Bonferroni-adjusted *P*-values. [b]*P*-values derived from 10 000 permutations; *denotes statistical significance.
Non-adjusted *P*-values <0.05 are in bold.

rs1380455 and rs3808943 were nominally associated with BP; however, the associations did not meet the Bonferroni threshold of significance ($P = 0.008$). No other genotyped SNPs were in high LD with the rs9804190 risk allele and were therefore dropped from further analysis. Haplotype analyses were performed on the six-locus haploblock encompassing the rs10994336 BP risk variant. The TCCTTT haplotype showed an over-transmission in BP, which was statistically significant after permutation test with 10 000 simulations. Interestingly, the rs10994336 (T) BP risk allele was also present in the TCCTT**T** haplotype showing increased risk ($Z = 2.266$, permuted $P = 0.035$). The whole marker permutation test was also significant ($P = 0.022$).

In closing, we attempted to validate in nearly 1000 Latinos the significant *ANK3* variants associated with BP identified in populations of predominantly European ancestry. The associations between previous reported genetic variants of *ANK3* (rs9804190 and rs10994336) and risk of BP were not significant in individual SNP analysis on our population. However, a six-locus *ANK3* haplotype encompassing the rs10994336 risk allele was significantly associated with BP. This suggests that the rs10994336 risk allele associated with BP in European and Asian ancestry populations is also part of a more specific haplotype associated with BP in Latino populations. Targeted sequencing within these haploblock regions will be helpful in identifying the true functional variants in *ANK3* that underlie BP. These results provide additional evidence that *ANK3* is associated with BP and provide the first evidence that variations in this gene might have a role in the pathogenesis of this disorder in the Latino population.

Conflict of interest

The authors declare no conflict of interest.

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