Schizophrenia Bulletin vol. 42 no. 2 pp. 288–300, 2016
doi:10.1093/schbul/sbv135
Advance Access publication September 24, 2015

Exome Sequence Data From Multigenerational Families Implicate AMPA Receptor Trafficking in Neurocognitive Impairment and Schizophrenia Risk

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Schizophrenia is a mental disorder characterized by impairments in behavior, thought, and neurocognitive performance. We searched for susceptibility loci at a quantitative trait locus (QTL) previously reported for abstraction and mental flexibility (ABF), a cognitive function often compromised in schizophrenia patients and their unaffected relatives. Exome sequences were determined for 134 samples in 8 European American families from the original linkage study, including 25 individuals with schizophrenia or schizoaffective disorder. At chromosome 5q32–35.3, we analyzed 407 protein-altering variants for association with ABF and schizophrenia status. For replication, significant, Bonferroni-corrected findings were tested against cognitive traits in Mexican American families (n = 959), as well as interrogated for schizophrenia risk using GWAS results from the Psychiatric Genomics Consortium (PGC). From the gene *SYNPO*, rs6579797 (MAF = 0.032) shows significant associations with ABF (P = .015) and schizophrenia (P = .040), as well as jointly (P = .0027). In the Mexican American pedigrees, rs6579797 exhibits significant associations with IQ (P = .011), indicating more global effects on neurocognition. From the PGC results, other *SYNPO* variants were identified with near significant effects on schizophrenia risk, with a local linkage disequilibrium block displaying signatures of positive selection. A second missense variant within the QTL, rs17551608 (MAF = 0.19) in the gene *WWC1*, also displays a significant effect on schizophrenia in our exome sequences (P = .038). Remarkably, the protein products of *SYNPO* and *WWC1* are interaction partners involved in AMPA receptor trafficking, a brain process implicated in synaptic plasticity. Our study reveals variants in these genes with significant effects on neurocognition and schizophrenia risk, identifying a potential pathogenic mechanism for schizophrenia spectrum disorders.

Key words: schizophrenia/cognition/*SYNPO*/*WWC1*/synaptic plasticity

Introduction

Schizophrenia is a complex, highly heritable brain disorder characterized by disturbances in behavior, thought, and emotion.[1] Although a number of genes and neurobiological pathways have been implicated in linkage and genome-wide association studies, much of the genetic liability of schizophrenia remains to be explained,[2,3] suggesting a polygenic architecture,[4] likely confounded by the clinical heterogeneity of the disorder.[5]

Deficits in cognitive functions have been commonly observed in schizophrenia patients, and in smaller magnitude in unaffected family members, especially for executive function, learning, and memory,[6–10] which may reflect innate, underlying differences that mediate the familial risk of schizophrenia. Analysis of such endophenotypes can delineate the psychiatric phenome and allow for identification of etiological mechanisms that are more proximate to gene action than disease endpoints.[11] Thus, genes that moderately influence the risk of schizophrenia may exhibit substantially stronger effects on cognition, making them easier to detect at genome-wide significance levels.

This approach was employed by Almasy et al[12] who conducted a genome-wide linkage screen of schizophrenia and cognitive performance in affected families,

discovering a quantitative trait locus (QTL) for abstraction and mental flexibility (ABF) on chromosome 5q (log of odds [LOD] = 3.43; P = .011), with effects on schizophrenia risk revealed through bivariate analysis. Although several linkage studies of schizophrenia have implicated chromosome 5q,[13–20] these findings provide insight into the potential neuropathology of the region, as the neural underpinnings for ABF have been localized primarily to the prefrontal brain circuitry,[21] including the dorsolateral and superior prefrontal cortices,[22] whose anatomical abnormalities and activity levels have been strongly associated with schizophrenia.[23–27]

The specific genetic variants contributing to this broad linkage region have yet to be determined. Previous endophenotype studies that have targeted schizophrenia candidate genes have been successful in identifying risk variants, including ones for cognitive traits.[28–38] In this article, we investigate the QTL at chromosome 5q, focusing on nonsynonymous variation from 238 local genes. Exome sequencing was conducted on 134 samples from 8 European American families drawn from the original linkage study, including 25 diagnosed with schizophrenia or schizoaffective disorder. Significant SNP associations for ABF and schizophrenia were followed up in independent Mexican American families for select neurocognitive measurements, as well as examined among the GWAS results from the Psychiatric Genomics Consortium (PGC). We identify a number of potential risk loci, with implications for the neurobiological basis of cognitive impairment as observed in schizophrenia patients.

Methods

Family Samples

The Multiplex-Multigenerational Genetic Investigation of Schizophrenia (MGI) has been described previously.[12,39] Families were recruited through a European American individual with schizophrenia, who had at least 1 first-degree relative with schizophrenia or schizoaffective disorder (SAD), depressed type. From the extended families, all the available first-, second- and third-degree relatives 15 years of age or older were invited to participate. MGI was approved by the Institutional Review Board of each of the 3 collaborating institutions, with all participants providing informed consent. In the case of minors under age 18 who provided assent, consent was obtained from a parent. Of the 43 MGI families (n = 676 participants), 8 of the largest, most densely affected ones were analyzed in this study. A total of 134 samples were exome sequenced, including 23 diagnosed with schizophrenia and 2 with SAD (see supplementary table S1 for pairwise familial relationships). Based on the original ABF linkage,[12] 5 of the families selected exhibit appreciable pedigree-specific LOD scores at the QTL, ranging from 0.16 to 0.33, representing 37.8% of the overall signal (LOD = 3.43; supplementary table S2).

Phenotyping

DSM-IV diagnoses were determined from: (1) the Diagnostic Interview for Genetics Studies, version 2.0[40]; (2) the Family Interview for Genetics Studies[41]; and (3) reviews of medical records. Lifetime best-estimate diagnoses were arrived at by 2 investigators, each blind to the familial relationships among participants (kappa > 0.8). In total, 106 individuals were diagnosed with schizophrenia or SAD, with 75% undergoing treatment at the time of assessment. Effects of medication on neurocognitive measures have been shown to be negligible or subtle.[42–44] In addition to schizophrenia, other psychiatric conditions identified included schizotypal personality disorder, psychotic disorder, and different forms of bipolar disorder.

Participants completed a computerized test battery[45,46] designed to evaluate 9 neurocognitive domains.[39] ABF was assessed using the Penn Conditional Exclusion Test (PCET),[47] for which participants are required to select one of 4 shapes for exclusion based on a sorting principle. An efficiency score was calculated as the average z score for performance accuracy and speed.

Exome Sequencing

We used the Illumina TruSeq platform (Illumina) for sample preparation, exome enrichment, and sequencing on the Illumina HiSeq 2000 instrument. In total, 62 Mb were sequenced, yielding uniform coverage of 201 121 exons from 20 794 genes. FASTQ files of demultiplexed paired sequencing reads of 100 bp were produced by CASAVA 1.8 suite and mapped to the UCSC human genome reference assembly 19 (hg19) using BWA (v. 0.6.1).[48] Mapped reads were analyzed with SAMtools (v. 0.1.12a)[49] and Picard (v. 1.56) (http://picard.sourceforge.net) to mark likely PCR duplicates and ensure consistency of the mapped data, with the output processed with the GATK (v. 1.6) package[50] (for more detail, see supplementary methods).

We called a total of 380 895 high-quality SNPs, with an average of 35 reads per variant, each functionally annotated with ANNOVAR.[51] At the QTL for ABF efficiency at 5q32–35.3, spanning approximately 35 Mb, 6518 SNPs were called, encompassing 366 different genes. To focus on sites of potential functional relevance, sequence data were filtered for variant quality LOD scores of 4.0 or greater, have no missing genotype data, and represent nonsynonymous mutations, leaving 407 SNPs available for association analysis. Mendelian consistency of these loci was confirmed with Sequential Oligogenic Linkage Analysis Routines (SOLAR).[52]

Replication Samples

For any significant, Bonferroni-corrected associations for ABF and/or schizophrenia risk, replications were sought in independent Mexican American families from the Genetics of Brain Structure and Function (GOBSF)

study for select neurocognitive measurements[53,54]: PCET accuracy (n = 519 subjects), Wechsler Adult Intelligence Scale II (WAIS-II; n = 430), Wechsler Test of Adult Reading (WTAR) (n = 264), California Verbal Learning Test (CVLT) total recall (n = 520), and CVLT delayed recall (n = 518). Unlike MGI, these families were not ascertained based on schizophrenia probands. Genotypes were obtained from whole genome sequences and imputed data (n = 959).[55]

For potential risk effects related to schizophrenia, we interrogated GWAS results from the PGC (available at http://www.med.unc.edu/pgc/downloads). Specifically, we examined 2 data sets: stage I, representing 17 population samples of European ancestry (n = 9394 cases and 12462 controls), with imputation based on HapMap3 reference panel[56]; and stage I plus additional Swedish cohorts (n = 5001 cases and 6243 controls), using 1000 Genomes phase 1 data for imputation.[57]

Statistical Analysis

All genetic analyses were performed in SOLAR, using a maximum likelihood (ML), variance decomposition approach. To evaluate ABF as an endophenotype to schizophrenia, both the genetic correlation and endophenotype ranking variable (ERV) were computed.[58] SNP association testing was performed using measured genotype analyses.[59] This single degree of freedom test assumes genetic additivity and compares a model saturated for both the random effects of kinship and the main effect of a SNP genotype to a null model with the SNP effect constrained to 0. Covariates include sex, age, age squared, and their interactions. P values were adjusted for multiple testing (n = 407 SNPs) via Bonferroni correction, corresponding to an alpha threshold of approximately 1.2×10^{-4}. Bivariate models of ABF and schizophrenia were also examined for any SNPs of interest. Tail area-based false discovery rate (FDR) q-values were computed in the R package fdrtool.[60] Multimarker, gene-based analyses were conducted using the sequence kernel association tests (SKAT) with the R script famSKAT.[61] Diversity and neutrality test statistics were computed for genomic regions of interest using PopGenome,[62] with coalescent simulations (1000 iterations) based on Hudson's MS algorithm[63] performed to evaluate significance of observed deviations from the neutral evolutionary model.

Results

Descriptive Statistics and Heritability Estimates

Measures of ABF efficiency were available for 113 of the 134 sequenced individuals, with a mean of -0.45 ± 0.11, ranging from −2.03 to 1.39, with no evidence of kurtosis (g_2 = −1.55). No significant differences are observed between the sexes. Age is negatively correlated with ABF (r = −.34; $P = 2.0\times10^{-4}$). For schizophrenia, affected individuals (n = 21) scored significantly worse for ABF efficiency ($\mu = -1.41\pm0.24$) than unaffected individuals ($\mu = -0.24\pm0.12$; $P = 1.3\times10^{-4}$). Both ABF and schizophrenia are significantly heritable, with respective estimates of 0.53 ± 0.19 ($P = 1.8\times10^{-3}$) and 0.84 ± 0.40 ($P = 8.6\times10^{-3}$). The genetic correlation between the traits is -0.19 ± 0.11 (P = .34; ERV = 0.13), with a more robust genetic correlation of -0.47 ± 0.15 (P = .021; ERV = 0.28) observed for the entire set of MGI families.

Genetic Associations at 5q32–35.3

In total, 407 nonsynonymous variants from the QTL at 5q32–35.3 were tested for association with ABF (λ = 1.09; supplementary table S3). After Bonferroni adjustment for multiple testing, 1 SNP, rs6579797 (MAF = 0.032) from the gene *SYNPO*, was significantly associated with ABF, with the minor allele showing poorer performance ($\beta_{ABF} = -2.01\pm0.48$; $P = 3.70\times10^{-5}$; corrected P = .015; q = 0.014). This SNP, along with 23 others that exhibit nominal association with ABF (ie, P < .05), were also tested for association with schizophrenia, of which 7 show evidence of risk (table 1 and figure 1A). Of these, 2 SNPs remained significant after Bonferroni correction: the top hit for ABF, rs6579797 ($\beta_{SCZ} = 1.84\pm0.63$; P = .0017; corrected P = .040) and the SNP rs17551608 (MAF = 0.19), located in *WWC1*, with its minor allele associated with improved ABF performance ($\beta_{ABF} = 0.42\pm0.21$; P = .041) and decreased liability for schizophrenia ($\beta = -1.09\pm0.39$; P = .0016; corrected P = .038). When considered jointly in a bivariate model, the 2 traits are significantly associated with both rs6579797 ($\beta_{ABF} = -1.95\pm0.47$; $\beta_{SCZ} = 1.75\pm0.55$; $P = 1.11\times10^{-4}$) and rs17551608 ($\beta_{ABF} = 0.45\pm0.19$; $\beta_{SCZ} = -1.00\pm0.22$; P = .0048). These 2 SNPs are in linkage equilibrium (r^2 = .019).

Based on SIFT[64] and PolyPhen2[65] algorithms, which predict the effects of amino acid substitutions on protein function, 2 SNPs from table 1 are considered potentially deleterious: rs17551608, and rs17660042 from the gene *SLC36A3* (P_{ABF} = .0018; P_{SCZ} = .010). Interestingly, in addition to rs17551608, 7 other missense variants were identified in *WWC1* (figure 1B), representing a high concentration of protein-altering variation (top 10th percentile for genes in the region, accounting size). Of these 7 variants, 4 are observed among the 25 schizophrenia cases, 3 of which are predicted to impact protein function. The most noteworthy of these are: rs145963282 (MAF = 0.0083), which is nominally associated with ABF ($\beta_{ABF} = -2.30\pm0.72$; P = .0029), and nearly so with schizophrenia risk ($\beta_{SCZ} = 1.44\pm0.82$; P = .070; bivariate association P = .026) and rs3822659 (MAF = 0.076), which shows increased risk of schizophrenia ($\beta_{SCZ} = 0.99\pm0.41$; P = .012). Interestingly, genetic interactions between rs17551608 and these other *WWC1* missense variants were detected, notably rs3822659 for both ABF (P = .054)

Table 1. Top Association Results for ABF and Schizophrenia at 5q32–35.3

				Function Prediction		ABF		Schizophrenia	
dbSNP 137	Position (bp)	Gene	MAF	SIFT	PolyPhen2	Beta (SE)	*P* Value	Beta (SE)	*P* Value
rs6579797[a]	149 998 128	*SYNPO*	0.032	Tolerated	Benign	−2.01 (0.48)	3.7×10^{-5}[c]	1.84 (0.63)	.0017[c]
rs17660042	150 666 946	*SLC36A3*	0.073	Deleterious	Probably damaging	−1.02 (0.32)	.0018	0.94 (1.00)	.010
rs2303063	147 480 027	*SPINK5*	0.47	Tolerated	Benign	0.41 (0.18)	.023	−0.52 (1.03)	.033
rs2303067	147 480 955	*SPINK5*	0.47	Tolerated	Benign	0.41 (0.18)	.023	−0.52 (1.03)	.033
rs2961944	159 835 658	*SLU7*	0.20	Tolerated	Benign	−0.40 (0.18)	.027	0.49 (0.24)	.037
rs61740602	150 646 888	*GM2A*	0.12	Tolerated	Benign	−0.56 (0.26)	.028	0.79 (0.29)	.0068
rs17551608[b]	167 835 539	*WWC1*	0.19	Deleterious	Possibly damaging	0.42 (0.21)	.041	−1.09 (0.39)	.0016[c]

Note: The table lists nonsynonymous variants from 5q32–35.3 with at least nominal evidence (P < .05) for association with ABF and schizophrenia. SNP rs numbers are based on dbSNP build 137. MAFs are based on maximum likelihood estimates that account for familial relationships. Predicted effects of amino acid changes on protein function are based on the SIFT and PolyPhen2 algorithms, which were obtained with the Ensembl online tool Variant Effect Predictor (VEP). For the association results, positive beta estimates (SE in parentheses) for schizophrenia correspond to increased risk. All 7 of the SNP variants presented here show the expected directions of effect for the 2 traits (ie, decrease in ABF performance corresponds with an increase in schizophrenia risk, and vice versa). ABF, Abstraction and Mental Flexibility; MAFs, Minor Allele Frequencies;
[a]G199A; aspartic acid substituted for asparagine, D67N.
[b]C798T; arginine substituted for cysteine, R250C.
[c]Significant after Bonferroni correction for multiple testing (α = .05): 407 tests for ABF; 24 tests for risk of schizophrenia.

and schizophrenia (P = .057), as well as rs61730019 (P_{ABF} = .039; P_{SCZ} = .0042; supplementary table S4), with no significant ablation of the main effects of rs17551608.

To assess the independence of these *WWC1* variants, haplotypes were phased using MERLIN v. 1.1.2.[66] With the exception of rs3822659, which is in perfect linkage disequilibrium (LD) with an adjacent SNP, rs3822660, each of the missense variants were phased to separate haplotypes. Collectively, the haplotypes account for 10.2% of the variation in ABF ($P = 5.95 \times 10^{-5}$) and 20.3% of the risk for schizophrenia (Kullback-Leibler R^2 value; $P = 4.40 \times 10^{-7}$) in our families. Based on multimarker SKAT analyses of ABF, *WWC1* yielded the fifth strongest association among genes tested from the QTL region (P = .11; supplementary table S5), with the lone, nominally significant result belonging to the gene *SLC36A3* (P = .029).

Replication of Neurocognitive Effects in GOBSF Families

The 2 SNPs showing significant effects on ABF and/or schizophrenia risk in our MGI families, rs6579797 (*SYNPO*) and rs17551608 (*WWC1*), were tested against neurocognitive measurements obtained in Mexican American pedigrees from GOBSF. Although PCET efficiency, representing a *z* score of performance accuracy and speed, was not assessed in these independent samples, accuracy scores were available (n = 519). However, we found no evidence of association with either rs6579797 (β = −0.041 ± 0.18; P = .82) or rs17551608 (β = −0.14 ± 0.12; P = .24).

Given the broad cognitive impairment typically observed in schizophrenia, which extends beyond executive functions, such as ABF, we examined a pair of IQ measures for general intelligence (table 2), revealing significant, detrimental effects of rs6579797 (WAIS-II: β = −6.28 ± 2.79, P = .025; WTAR: β = −7.54 ± 2.96, P = .011). Also, with previous research strongly implicating *WWC1* in verbal memory performance (see "Discussion" section), we examined CVLT scores, with the *WWC1* variant rs61730019 showing significant association with both delayed recall (P = .0031) and total recall (P = .0091).

PGC GWAS Results for Schizophrenia

To investigate the effects of *SYNPO* and *WWC1* on schizophrenia, we interrogated GWAS results from the PGC. For stage I analyses (n = 21 856), as reported by Ripke et al,[56] rs6579797 (*SYNPO*) was neither directly genotyped nor imputed from HapMap 3 data, however a near significant risk effect was observed for a nearby tagging variant (r^2 = 1.0), intronic SNP rs9324647 (OR = 4.90 ± 0.91; P = .080; MAF = 0.0046). More recently, the PGC sample collection has expanded to include large Swedish cohorts (total n = 32 143), for which Ripke et al[57] have reported updated association results (supplementary table S6). Based on 1000 Genomes phase 1 data, rs6579797 was successfully imputed but failed to show evidence of risk effects (OR = 1.04 ± 0.070; P = .61; MAF = 0.02). For other nearby *SYNPO* variants (±10 kb from rs6579797), a near significant association was identified for a rare intronic SNP, rs192542133 (OR = 0.79 ± 0.08; P = .0025; corrected P = .059; MAF = 0.015). As for *WWC1* variants, including rs17551608, no evidence was found.

Population Genetics and Signatures of Positive Selection

According to 1000 Genomes data, the putative risk allele of rs6579797 shows marked frequency differences between global populations (supplementary table S7).

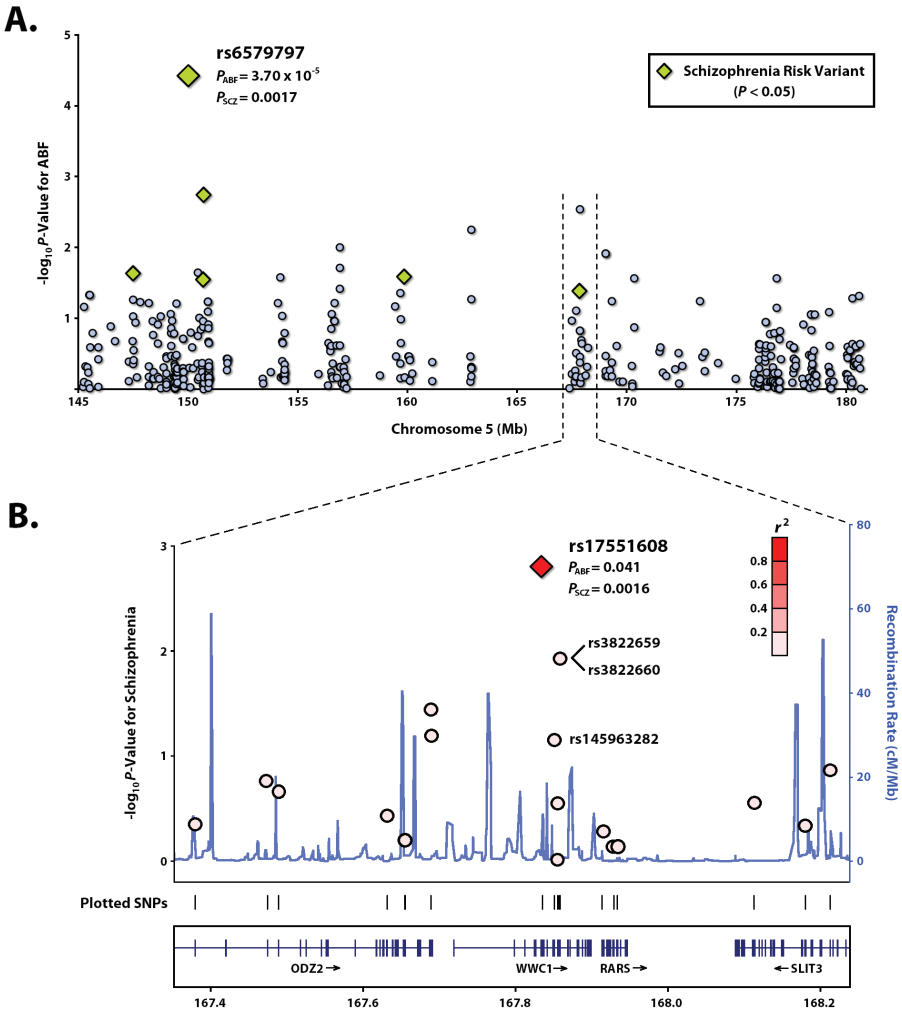

Fig. 1. (A) Plot of association *P* values for missense SNPs from chromosome 5q32–35.3 (n = 407) for ABF. Variants that are also nominally associated with schizophrenia risk ($P < .05$) are represented as diamonds. (B) Regional plot of association results for dbSNP 137 variants from *WWC1* and neighboring genes for schizophrenia. Recombination rate based on hg19 assembly for 1000 Genomes data (2012) for European populations. Plotted using LocusZoom.[149]

Table 2. Association Results for rs6579797 and *WWC1* Variants for Select Cognitive Measures in Mexican American Families

			IQ				CVLT			
T2D-GENES Families (n = 959)			WASI-II (n = 430)		WTAR VIQ (n = 264)		Total Recall (n = 520)		Delayed Recall (n = 518)	
dbSNP 137	Gene	MAF	Beta (SE)	*P* Value	Beta (SE)	*P* Value	Beta (SE)	*P* Value	Beta (SE)	*P* Value
rs6579797	*SYNPO*	0.022	−6.28 (2.79)	**.025**	−7.54 (2.96)	**.011**	−1.55 (1.96)	.43	−0.38 (0.56)	.50
rs17551608	*WWC1*	0.077	−2.86 (1.78)	.11	0.74 (1.53)	.63	−1.60 (1.26)	.20	−0.52 (0.36)	.15
rs61730019	*WWC1*	0.015	1.58 (3.71)	.67	5.24 (3.71)	.16	7.63 (2.57)	**.0031**	1.93 (0.74)	**.0091**

Note: The table presents association results between *SYNPO* and *WWC1* variants and measures of IQ and verbal memory in Mexican American families using whole genome sequences and imputed data from the T2D-GENES consortium. Two measures of IQ were tested for genetic association: WAIS II and WTAR verbal IQ. For verbal memory, total and delayed recalls for the CVLT were examined. MAFs are based on maximum likelihood estimates that account for familial relationships. Significant association *P* values (< .05) are highlighted in bold. CVLT, California Verbal Learning Test; IQ, Intelligent quoteint; MAFs, Minor allele frequencies; WAIS, Wechsler Adult Intelligence Scale; WTAR, Wechsler Test of Adult Reading.

Among Europeans (EUR) and admixed Americans (AMR), the respective frequencies are 0.015 and 0.039, whereas higher frequencies are observed for African (AFR) and Asian (ASN) populations, around 0.25, yielding substantial pairwise F_{ST} scores with the EUR groups (0.22 and 0.21, respectively). As for rs17551608,

population differentiation is also evident, with its minor allele ranging in frequency from 0.16 in EUR populations to its complete absence in the ASN samples.

Interestingly, the minor risk allele (A) of rs6579797 appears to be the ancestral state, as determined from phylogenetic sequence alignments of nonhuman primate species, a possible signature of selection. To investigate this further, we computed the local LD structure of rs6579797 for the 5 EUR populations from 1000 Genomes (figure 2), observing a cluster of *ancestral* minor alleles in strong LD ($r^2 > .9$), indicative of an evolutionary sweep. Diversity and neutrality test statistics support this, as the EUR groups exhibit low nucleotide diversity within the LD block ($\pi = 7.36$), with highly significant deviations from the neutral evolutionary model, including Tajima's D ($P = .010$) and Fu and Li's F ($P = .001$) (table 3 and supplementary table S8). Similar negative deviations from neutrality are observed in admixed American populations, a likely reflection of their European origins.

Discussion

Synaptopodin: Neurocognitive Implications for Schizophrenia Risk

From our analysis of exome sequence data for chromosome 5q32–35.3, a region encompassing a QTL for neurocognition,[12] we identified nonsynonymous variants from 2 genes, *SYNPO* and *WWC1*, which are significantly associated with ABF and/or schizophrenia risk. Of these, rs6579797 (*SYNPO*) is particularly compelling. The minor allele is carried by 6 heterozygotes in 3 MGI families, representing a modest enrichment relative to EUR populations. Four of the carriers are affected, corresponding to significantly decreased ABF performance (corrected $P = .015$) and heightened risk for schizophrenia (corrected $P = .040$). Based on the original linkage results for ABF, 2 of the 3 families harboring the rs6579797 risk allele have pedigree-specific LODs of 0.33 and 0.28. In the third family, a near zero LOD score was observed, with 2 heterozygote carriers, both unaffected for schizophrenia, although one diagnosed with severe major depression with symptoms of psychosis.

With genome-wide microsatellite data available for many of the MGI samples,[12] we estimated identity-by-descent sharing for chromosome 5, allowing us to impute ML genotypes (.95 probability threshold) for rs6579797 for an additional 82 individuals in our 8 study families (see supplementary methods). Combining the exome sequence data and imputed genotypes, the association signals at rs6579797 remained significant for both ABF (corrected $P = .0031$) and schizophrenia (corrected $P = .024$; supplementary table S9).

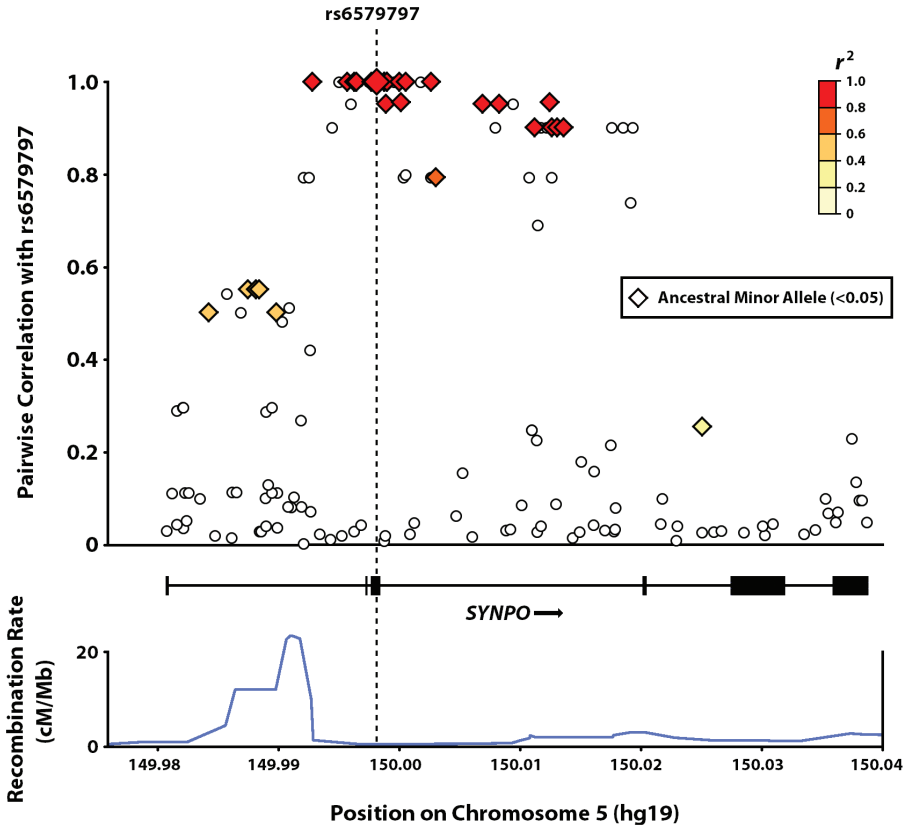

Fig. 2. Plot of pairwise LD correlations for rs6579797 with local *SYNPO* variants, based on population samples of European ancestry from 1000 Genomes data. Ancestral minor alleles (frequency < 0.05) are highlighted.

Table 3. Diversity and Neutrality Test Scores Based on 1000 Genomes Data for LD Block of *SYNPO* Variant rs6579797

1000 Genomes Populations[a]	*N*	*S*	π	Tajima's *D*	Fu and Li's *F*	Fu and Li's *D*	Fay and Wu's *H*	Zeng's *E*
African	246	211	34.36	−0.04	0.07	0.18	0.73	−0.66
Admixed American	181	156	10.91	−1.89**	−2.83**	−2.83**	0.43[c]	−2.06**
East Asian	286	107	26.59	1.66	1.53	0.90	−4.44	5.14[b]
European	379	116	7.36	−1.75**	−3.02**	−3.37**	0.44[c]	−1.90**
Total	1092	298	23.04	−1.19	−2.15**	−2.81**	0.69	−1.59*

Note: Diversity measurements (segregating sites [S], nucleotide diversity [π]), and neutrality test statistics, as determined by differences in unbiased estimators of θ = 4 Neμ, were computed for variants found within an observed LD block for rs6579797 in the gene SYNPO (pairwise $r2 > .90$, carrying ancestral minor alleles), corresponding to hg19 coordinates chr5: 149 992 784–150 013 606 (delineated by SNPs rs10074935 and rs61051686).

[a]1000 Genomes phase 1 version 3 data for 4 "super populations": African (AFR), representing Yoruba in Nigeria (YRI), Luhya in Kenya (LWK), and Americans of African ancestry from southwestern United States (ASW); Admixed American (AMR), representing Colombians from Medellin, Colombia (CLM), Puerto Ricans (PUR), and Americans of Mexican ancestry from Los Angeles (MXL); East Asian (ASN), representing Han Chinese from Beijing (CHB), Southern Han Chinese (CHS), and Japanese from Tokyo (JPT); and European (EUR), representing Utah residents with Northern and Western European ancestry (CEU), Toscani in Italy (TSI), British in England and Scotland (GBR), Iberian population in Spain (IBS), and Finnish in Finland (FIN).

[b]For the other tail of the distribution (ie, positive), this score is significant, with an empirical *P* value of .034.

[c]Positive deviation Fay and Wu's *H*, a statistic that utilizes information from the intermediate- and high-frequency parts of the frequency spectrum, coupled with the negative score for Zeng's *E* based on low- and high-frequency variant classes, suggests that the locus may be entering a recovery phase for these 2 populations.

*Empirical *P* value (1-tailed) <0.05, ** <0.01, based on an observed distribution for 1000 samples generated via coalescent simulation.

When examined in Mexican American pedigrees from GOBSF, the *SYNPO* variant showed significant association with IQ measures (smallest $P = .011$), indicating more generalized cognitive effects. This is consistent with the overlapping signals observed at the QTL, ranging in LODs from 1.05 to 1.70, for various neurocognitive traits: verbal memory, spatial processing, language and reasoning, and attention. Interestingly, when tested against these other measurements in the MGI families, rs6579797 showed significant detrimental effect on verbal memory accuracy ($\beta = -1.44 \pm 0.45$; $P = .031$). As for schizophrenia risk, we found suggestive evidence for *SYNPO* variants from GWAS results reported by the PGC, although rs6579797 displayed no association in the most current data.

Remarkably, *SYNPO* appears to be under selective pressure, further hinting at its potential relevance. The putative risk allele of rs6579797 represents the ancestral evolutionary state, yet is uncommon in EUR and AMR populations from 1000 Genomes. Pairwise correlations with rs6579797 reveal an LD block enriched with minor ancestral alleles, a potential footprint of an evolutionary sweep. Neutrality test statistics for EUR and AMR support this, revealing significant deviations from the neutral model. Notably, negative deviation was not observed for Fay and Wu's *H*, which, when coupled with the negative score for Zeng's *E*, suggests that the locus may be entering a recovery phase (ie, accumulation of neutral genetic variation).[67] This finding adds to a growing list of genes implicated in neurocognition and brain development that appear to have undergone selection over the course of human evolution.[68]

Although SIFT and PolyPhen2 yield low probabilities that rs6579797 is damaging, both algorithms have false negative rates >10%[69] and thus do not necessarily preclude it from having important functional consequences. The product of *SYNPO*, synaptopodin, is an actin-binding protein found in the dendritic spines of telencephalic neurons,[70] with the D67N substitution encoded by rs6579797 situated within a PEST motif, which may serve as a molecular signal for proteasomal degradation.[71] Interestingly, when we re-examined *SYNPO* variants excluded from our analyses (eg, those without SIFT and PolyPhen2 scores), we discovered a splice site variant, rs59962087, that is 469 bp upstream of rs6579797 and in perfect LD, with an ancestral minor allele, thus representing another functional candidate for the observed association signal.

What makes this result compelling for the pathology of schizophrenia is that within the dendritic spine, synaptopodin is believed to be an essential component of the spine apparatus (SA), influencing local calcium storage[72] and protein synthesis,[73] with synaptopodin-deficient mice exhibiting deficits in synaptic plasticity and spatial learning.[74,75]More specifically, synaptopodin directly regulates the release of calcium[76] and the accumulation of glutamate receptor 1 (GluR1) in the spine head, a subunit of the α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) receptor induced during long-term potentiation (LTP). This establishes a potential mechanistic link with synaptic plasticity,[77] a key neural process that underlies ABF and mediates synaptic dynamics in the prefrontal cortex (PFC),[78] a brain region strongly implicated in schizophrenia.[79–87]

Moreover, in a recent exome sequencing study by Timms et al,[88] protein-altering variants in genes involved in *N*-methyl-D-aspartate (NMDA) receptor hypofunction

were found to segregate in schizophrenia families, supporting the glutamatergic dysfunction hypothesis for schizophrenia,[89,90] which ties in well with our findings. NMDA and AMPA are the 2 primary types of receptors activated by glutamate in the mammalian brain, each playing a critical, interrelated role in calcium-induced potentiation. Antagonists of NMDA receptors can replicate schizophrenia symptomatology in healthy people, including deficits in mental flexibility,[91,92] whereas enhancers have been found to reduce negative features and improve cognition in patients.[93,94] Studies of knockout mice have revealed impairments in behavioral flexibility,[95–97] with effects on potentiation.[98,99] In postmortem brain tissue of schizophrenia patients, altered mRNA and protein levels of glutamate receptors have been observed,[100] including irregularities in AMPA receptor trafficking and localization, particularly in the PFC.[101–104] Association studies of schizophrenia have identified a number of SNPs and copy number variants (CNVs) in genes involved in glutamatergic neurotransmission,[105–109] including the synaptic adhesion molecule neurexin,[110,111] which has been found to regulate AMPA receptor endocytosis and control excitatory synaptic strength.[112]

WWC1: Another Regulator of AMPA Receptor Trafficking

The other gene implicated in our analysis, *WWC1*, shows an enrichment of protein-altering variation in our MGI families, including 3 independent SNPs with significant or suggestive associations with ABF and schizophrenia, maintained by the imputed data (supplementary table S9): rs17551608, rs145963282, and rs3822659. Collectively, the *WWC1* variants account for significant portions of the variability in ABF and schizophrenia risk, with evidence of genetic interaction effects. Interestingly, when affection status was broadened to include other schizophrenia spectrum diagnoses in these pedigrees, namely schizotypal personality disorder (n = 7) and psychosis disorder (n = 6), a stronger association was observed for rs17551608 ($P = 3.80 \times 10^{-4}$; $\beta = -1.07 \pm 0.08$). However, these findings were not independently replicated in the GOBSF families for PCET accuracy and IQ, as well as among the GWAS results for schizophrenia from the PGC, perhaps a reflection of the genetic loads carried by our multiplex families.

Nonetheless, *WWC1* remains intriguing. Its protein product, the WW domain containing protein 1 (WWC1), a postsynaptic scaffolding molecule expressed in the human brain, exhibits protein-protein interactions (PPIs) with other postsynaptic proteins, most notably dendrin and synaptopodin,[70] via its WW domains.[113,114] Other binding features include a C2-like motif that interacts with phospholipids[115] and a region that binds protein kinase C (PKC)ζ,[116] a molecule integral for neuronal plasticity[117] and known to affect long-term memory.[118] Based on the online database BioGRID (v. 3.2.102),[119] other PPIs have been detected for synaptopodin and WWC1, including proteins of genes implicated in schizophrenia risk (supplementary table S10).[120–127] However, no genetic interaction effects were detected between rs6579797 and *WWC1* missense variants for ABF or schizophrenia risk, as well as with other variants from these PPI networks (supplementary table S11).

The influence of *WWC1* on neurocognition, however, is well supported. In a seminal paper by Papassotiropoulos et al,[128] an intronic SNP within *WWC1*, rs17070145, was reported to be associated with human memory, with allelic differences in hippocampal activations during memory tasks. This finding has been replicated in multiple studies involving both healthy subjects and patients with mild cognitive impairment,[129–134] with differential effects on memory in psychotic individuals,[135] as well as a predisposition for late-onset Alzheimer's disease.[136] Remarkably, rs17070145 has also been linked to cognitive flexibility, with tobacco use possibly modulating this effect,[137] a notable interaction given its prevalence among schizophrenia patients,[138] although we found no such effects. However, the chromosome 5 QTL does show suggestive linkage for verbal memory accuracy in MGI, as measured by the Penn Word Memory Test (LOD = 1.50, P = .0043), with a near significant association ($\beta = -1.05 \pm 0.55$, P = .059) detected for the *WWC1* missense variant rs61730019 (MAF = 0.061), that also displays significant effects on CVLT total recall (P = .0031) and delayed recall (.0091) scores in GOBSF.

Despite the convincing case for its neurocognitive implications, how WWC1 controls higher brain function at the molecular level remains to be elucidated. Research has shown that it binds to PICK1,[139] a synaptic protein involved in AMPA receptor trafficking[140] and considered crucial for hippocampal synaptic plasticity,[141] as WWC1 knockdown accelerates the rate of AMPA receptor recycling, with knockout mice exhibiting profound learning and memory impairment. It has been hypothesized that WWC1 may serve as a docking station for AMPA receptors,[142] mediating linkage between endosomes containing phosphatidylinositol-3-phosphate, a key regulator of vesicular traffic in excitatory neurons,[143] and components of the postsynaptic cytoskeleton that include dendrin and synaptopodin.

Conclusion

From our analysis of exome sequence data from chromosome 5q32–35.3, a region linked to neurocognition in families impacted by schizophrenia, we identify missense variants in 2 genes involved in AMPA receptor trafficking and neuronal plasticity, *SYNPO* and *WWC1*, that are associated with ABF performance and schizophrenia susceptibility. When examined in Mexican American pedigrees, the *SYNPO* variant, rs6579797, shows deleterious effects on

general intelligence, with evidence of selection operating at this locus. Thus, these findings suggest that disruptions in AMPA receptor turnover in the postsynaptic cell have important pathological consequences on neurocognition, lending support to the glutamatergic dysfunction hypothesis. Recognizing that functional improvement in schizophrenia patients is likely to require treatment of cognitive capabilities,[144,145] as global impairment represents a core feature,[146] augmenting synaptic transmission and plasticity may have therapeutic potential, with a diverse class of allosteric agents available for modulating AMPA receptor activity.[147] Of course, schizophrenia is a highly complex disorder involving perhaps thousands of risk alleles[4] from genes involved in other neurotransmitter systems, with increasing evidence for the central importance of calcium channel signaling,[148] thus necessitating an integrated, neurobiological approach to developing effective treatment strategies for this devastating mental illness.

Supplementary Material

Supplementary material (references 150–152 are cited in the supplementary material) is available at http://schizophreniabulletin.oxfordjournals.org.

Funding

This work was supported by the National Institutes of Health (MH061622, MH042191, and MH063480 for the MGI study; MH0708143, MH078111, and MH083824 for the GOBSF study; MH059490 for the development of SOLAR; and C06 RR017515) and Texas Biomedical Research Institute Forum Grant (11–4311 to M.Z.K).

Acknowledgments

We thank the participants of the MGI and GOBSF studies, as well as our research staffs. We would also like to recognize the invaluable comments and suggestions we received by the anonymous reviewers of this article. The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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