JAMA Psychiatry | Original Investigation

Association of Schizophrenia Risk With Disordered Niacin Metabolism in an Indian Genome-wide Association Study

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IMPORTANCE Genome-wide association studies (GWASs) in European populations have identified more than 100 schizophrenia-associated loci. A schizophrenia GWAS in a unique Indian population offers novel findings.

OBJECTIVE To discover and functionally evaluate genetic loci for schizophrenia in a GWAS of a unique Indian population.

DESIGN, SETTING, AND PARTICIPANTS This GWAS included a sample of affected individuals, family members, and unrelated cases and controls. Three thousand ninety-two individuals were recruited and diagnostically ascertained via medical records, hospitals, clinics, and clinical networks in Chennai and surrounding regions. Affected participants fulfilled *DSM-IV* diagnostic criteria for schizophrenia. Unrelated control participants had no personal or family history of psychotic disorder. Recruitment, genotyping, and analysis occurred in consecutive phases beginning January 1, 2001. Recruitment was completed on February 28, 2018, and genotyping and analysis are ongoing.

MAIN OUTCOMES AND MEASURES Associations of single-nucleotide polymorphisms and gene expression with schizophrenia.

RESULTS The study population included 1321 participants with schizophrenia, 885 family controls, and 886 unrelated controls. Among participants with schizophrenia, mean (SD) age was 39.1 (11.4) years, and 52.7% were male. This sample demonstrated uniform ethnicity, a degree of inbreeding, and negligible rates of substance abuse. A novel genome-wide significant association was observed between schizophrenia and a chromosome 8q24.3 locus (rs10866912, allele A; odds ratio [OR], 1.27 [95% CI, 1.17-1.38]; $P = 4.35 \times 10^{-8}$) that attracted support in the schizophrenia Psychiatric Genomics Consortium 2 data (rs10866912, allele A; OR, 1.04 [95% CI, 1.02-1.06]; $P = 7.56 \times 10^{-4}$). This locus has undergone natural selection, with the risk allele A declining in frequency from India (approximately 72%) to Europe (approximately 43%). rs10866912 directly modifies the abundance of the nicotinate phosphoribosyltransferase gene (*NAPRT1*) transcript in brain cortex (normalized effect size, 0.79; 95% CI, 0.6-1.0; $P = 5.8 \times 10^{-13}$). *NAPRT1* encodes a key enzyme for niacin metabolism. In Indian lymphoblastoid cell lines, (risk) allele A of rs10866912 was associated with *NAPRT1* downregulation (AA: 0.74, n = 21; CC: 1.56, n = 17; $P = .004$). Preliminary zebrafish data further suggest that partial loss of function of *NAPRT1* leads to abnormal brain development.

CONCLUSIONS AND RELEVANCE Bioinformatic analyses and cellular and zebrafish gene expression studies implicate *NAPRT1* as a novel susceptibility gene. Given this gene's role in niacin metabolism and the evidence for niacin deficiency provoking schizophrenialike symptoms in neuropsychiatric diseases such as pellagra and Hartnup disease, these results suggest that the rs10866912 genotype and niacin status may have implications for schizophrenia susceptibility and treatment.

JAMA Psychiatry. doi:10.1001/jamapsychiatry.2019.1335
Published online July 3, 2019.

Editorial
Supplemental content

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Schizophrenia is a severe mental illness characterized by delusional beliefs, hallucinations, disordered speech, and deficits in cognitive, emotional, and social behavior. This cross-cultural disorder has a lifetime prevalence of approximately 1%[1] and significant mortality. The disease imposes a substantial burden on individuals, families, and societies, ranking twelfth globally in years lived with disability.[2] Onset is typically in early adulthood with a frequently chronic trajectory. Schizophrenia's defining pathophysiology is poorly understood, and current treatments have limited efficacy. High heritability (approximately 0.8)[3] has driven the search for genetic variants, the identification of which will contribute to unravelling disease mechanisms and provide essential biological understanding needed for improved evidence-based therapeutics and personalized treatments.

Most schizophrenia genome-wide association studies (GWASs) have been conducted in Europeans, with a minority in African American and East Asian populations. Common genetic variants are ancient and shared across ethnicities, with evidence of shared common genetic variation for schizophrenia between major global populations.[4] The Psychiatric Genomics Consortium 2 (PGC2),[5] using predominantly European ancestry samples, has provided major insights into the genetic basis of the disorder by identifying 108 genome-wide significant loci from a study of 36 989 cases and 113 075 controls. These loci explain approximately 7% of the liability to disease, but whole-genome analysis methods[6] suggest that variants tagged by common single-nucleotide polymorphisms (SNPs) (minor allele frequency, > .01) on GWAS arrays collectively explain approximately 50% of the genetic liability.[7] The remaining heritability will likely be accounted for by additional common SNPs of small effect size identified as GWAS sample sizes increase[7] and by rare variants that are largely population specific[8] and poorly correlated with common SNPs in GWASs.[6] We report herein, to our knowledge, the first schizophrenia GWAS in an Indian population, recruited from ethnically homogeneous schizophrenia pedigrees and unrelated cases and controls.[9]

Key Points

Question Can new causes of schizophrenia be identified within the Indian population, given its unique genetic makeup and environment?

Findings In this genome-wide association study that included 3092 individuals from southern India, a genome-wide significant association with schizophrenia was observed on chromosome 8q24.3. Bioinformatic, cellular, and animal model evidence points to *NAPRT1*, a gene that encodes a key niacin metabolism enzyme, as the top gene within this locus.

Meaning These findings suggest that the genotype of the top association signal and niacin status may be relevant in schizophrenia susceptibility and treatment.

Methods

Study Participants

All participants gave written informed consent, and the study was approved by relevant institutional ethics committees at each participating institution. This study followed the Strengthening the Reporting of Genetic Association Studies (STREGA) reporting guideline. Our sample was recruited at The Schizophrenia Research Foundation, Chennai, India, and consists almost exclusively of individuals of Tamil ethnicity (>97%) (eTables 1-3 in the Supplement). Within Tamil ethnicity, we initially focused on Tamil Brahmin and other Brahmin castes to maximize sample homogeneity; we subsequently recruited other castes. We used standardized instruments (administered in Tamil where necessary), including the Diagnostic Interview for Genetic Studies,[10] the Family Interview for Genetic Studies,[11] *DSM-IV* diagnostic criteria, and the consensus diagnostic procedure (eMethods in the Supplement). The GWAS sample included (1) a family data set ascertained for multiple affected family members (of 1376 individuals, 505 with schizophrenia [36.7%]) and (2) a case-control data set (of 1716 individuals, 816 with schizophrenia [47.6%]). The total sample included 3092 individuals, 1321 (42.7%) of whom had schizophrenia. For details of genotyping and preimputation, imputation, and postimputation quality control, see the eMethods and eFigures 1-3 in the Supplement. Recruitment, genotyping, and analysis occurred in consecutive phases beginning January 1, 2001. Recruitment was completed on February 28, 2018, and genotyping and analysis are ongoing.

SNP Heritability and Genome-wide Association Analyses

To quantify the proportion of variance attributed to all genome-wide SNPs (SNP-based heritability) and to test for population stratification, we applied the GREML function in GCTA (genome-wide complex trait analysis), version 1.24.722[12] (eMethods and eFigure 4 in the Supplement). We used GCTA's mixed linear model-based function to conduct the analysis in the case-control and family data sets independently (eMethods in the Supplement). The final meta-analysis of both data sets did not show any inflation ($\lambda = 1.00$ [eFigure 5 in the Supplement] and $\lambda = 0.98$ [eFigure 6 in the Supplement]), confirming that our leading association signal is not a false-positive finding.

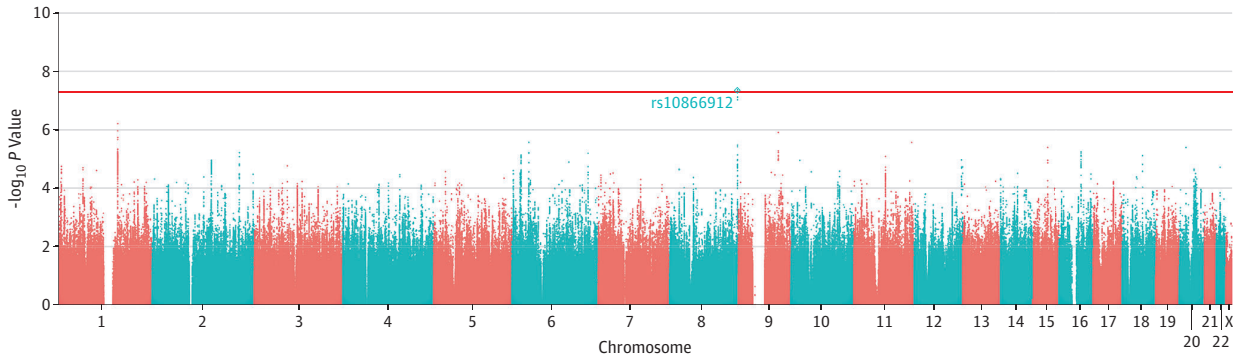
Transethnic Meta-analysis

Given the ancestral difference between our sample and the predominantly European PGC2, we conducted transethnic meta-analysis using RE2C, an extension of METASOFT,[13] which accounts for heterogeneity in effect sizes of SNPs across populations. This process evaluated whether the addition of Tamil Nadu samples to PGC2 resulted in novel and/or statistically stronger, already identified genome-wide significant loci (eMethods in the Supplement).

Post-GWAS Extended Bioinformatic Analyses

We conducted the following analyses: (1) genomic profile risk scoring to capture the contribution to disease of the many SNPs that do not reach genome-wide statistical significance[14]; (2) statistical fine mapping analysis of Indian

Figure 1. Manhattan Plot of Observed Schizophrenia Association Signals



Manhattan plot shows genome-wide schizophrenia associations in 3092 individuals (1321 cases and 1771 controls) from Tamil Nadu, India. The phase three 1000 Genome Project South Asian population was used to calculate linkage disequilibrium. The x-axis shows the chromosomal position, and the y-axis shows the significance of association ($-\log_{10}P$ value). The horizontal red line represents the level of genome-wide significance ($P = 5 \times 10^{-8}$). Our top genome-wide significant locus is situated on chromosome 8q24.3 (rs10866912, $P = 4.35 \times 10^{-8}$).

GWAS data with functional annotation data sets using PAINTOR, version 3.1[15,16] to determine which SNP at our top locus was associated with most functional annotations and reveal the SNP with the highest posterior probability of being causal and therefore the one to be prioritized for functional investigation; (3) expression quantitative trait loci (eQTL) analyses to investigate the regulatory effects of our top SNP on gene expression; (4) natural selection analyses to examine the global pattern of allele frequency distribution for the lead SNP to detect signatures of positive selection; (5) vegetarian diet analysis to test whether this environmental factor was associated with disease status in individuals homozygous for the risk allele of the lead SNP; (6) SMR (summary data-based mendelian randomization), version 0.712 analyses[17] to identify genes within the top locus whose expression levels were associated with schizophrenia; (7) pathway and network connectivity enrichment analyses to identify gene sets enriched for association with disease and risk genes enriched for association with tissue types, respectively; and (8) transethnic genetic correlation for schizophrenia between India and Europe to evaluate the similarity of genetic architecture of this disease. Details are provided in eMethods in the Supplement.

Post-GWAS Functional Analyses

To assess the effect of allele-specific expression, we measured expression of genes at the rs10866912 locus in lymphoblastoid cell lines established from 60 individuals (30 cases and 30 controls) within the study population (eMethods in the Supplement). We identified the presence of 4 distinct haplotypes across the rs10866912 locus, encompassing 3054 of 3092 of the study population (98.8%) (eTable 5 in the Supplement). For *naprt1* knockdown in zebrafish, we used a microRNA-mediated gene-silencing approach optimized for zebrafish as described previously[18] (eMethods and eTable 4 in the Supplement). We used the 2-sided *t* test with significance set at $P < .05$ to compare findings.

Results

Clinical Sample

Of the 1321 individuals with schizophrenia (mean [SD] age, 39.1 [11.4] years), 170 (13.8%) were from consanguineous families (uncle-niece, first cousin, or second cousin); all were living with their families; 696 (52.7%) were male and 625 (47.3%) were female; 433 (32.8%) had a tertiary level of educational attainment; and 368 (27.9%) had at least part-time employment (>30% time). The clinical phenotype was homogeneous, and negative symptoms were frequently observed (660 [50.0%]). The negligible to low rates of comorbid alcohol and substance abuse further enhanced the phenotypic homogeneity of this sample (eMethods and eTables 1-3 in the Supplement).

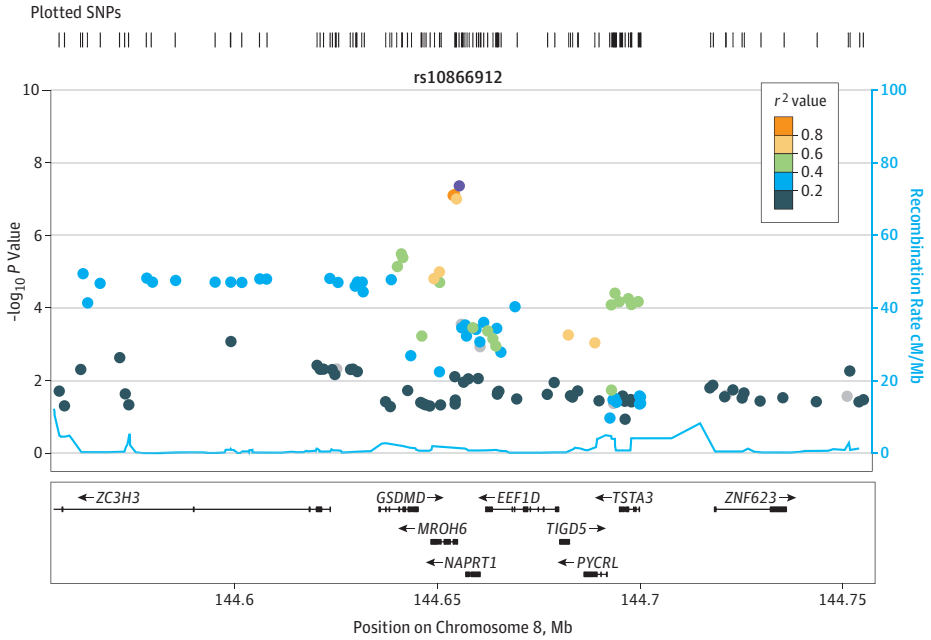
SNP Heritability

SNP heritability on the liability scale was estimated to be 0.287 (standard error, 0.073) (eFigure 4 in the Supplement), falling within the expected range. Additional heritability analyses confirmed the lack of population stratification in our data set (eMethods in the Supplement).

Genome-wide Association Analyses

The meta-analysis genome-wide association results are summarized in **Figure 1**. We observed a genome-wide significant locus on chromosome 8q24.3 (rs10866912, allele A; odds ratio [OR], 1.27 [95% CI, 1.17-1.38]; $P = 4.35 \times 10^{-8}$; chr8: 144655315; hg19). The index SNP is located approximately 7 kilobases from the 5′ end of *MROH6* [GenBank NM_001100878], lying within a linkage disequilibrium (LD) block defined by r^2>0.6. The 6 genes within this block are *GSDMD* (GenBank NM_024736), *MROH6*, *NAPRT1* (GenBank NM_145201), *EEF1D* (GenBank NM_032378), *TIGD5* (GenBank NM_032862), and *PYCRL* (GenBank NM_023078) (**Figure 2**). We found no signs of confounding due to caste status (666 Brahmins and 2426 non-Brahmins; rs10866912, allele C; β coeffi-

Figure 2. Regional Plot of Chromosome 8q24.3 Locus (100-Kilobase Window)



The index single-nucleotide polymorphism (SNP) rs10866912 is colored purple, with other SNPs colored according to the degree of linkage disequilibrium (measured by r^2 value) with the index SNP. SNPs with missing linkage disequilibrium information are shown in gray. The x-axis shows the SNP locus position on chromosome 8 (GRCh37/hg19 build). The y-axis shows the significance of association ($-\log_{10}$ *P* value) in our Indian population. Nine genes are located within the 100-kilobase window, with the direction of transcription (upstream/downstream) being annotated with arrows. cM indicates centimorgans; Mb, megabase.

cient, 0.007; standard error [SE], 0.005; $P = .22$) in our data set. The top locus was replicated in PGC2 (rs10866912, allele A; OR, 1.04 [95% CI, 1.02-1.06]; $P = 7.56 \times 10^{-4}$) (eFigure 7 in the Supplement), with the same direction of effect in the Indian (OR, 1.27) and PGC2 (OR, 1.04) data sets.

We observed no significant associations between chromosome X SNPs and disease status. Further verification based on imputation batch-based meta-analysis showed consistent results (eTable 6 in the Supplement).

Transethnic Meta-analysis

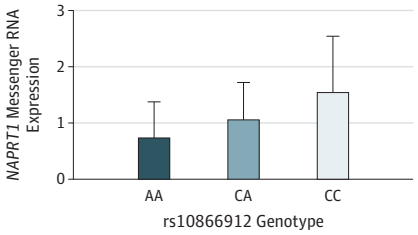
Transethnic meta-analysis revealed a stronger genome-wide significance for our top locus (rs10866912) after meta-analysis with PGC2, increasing from $P = 4.35 \times 10^{-8}$ for India (rs10866912, allele A; OR, 1.27; 95% CI, 1.17-1.38) to $P = 2.09 \times 10^{-9}$ for India plus PGC2 (β coefficient, 0.05; SE, 0) (eTable 7 and eFigures 8-10 in the Supplement). This more significant *P* value indicates that our top SNP is in strong LD with the causal variant in the PGC2 and Indian data sets. Moreover, of the 108 PGC2 genome-wide significant loci, 23 became more significantly associated after this meta-analysis (eTable 8 in the Supplement). In addition, at our top locus, we sought to observe the difference in LD block pattern in the 2 populations that may facilitate fine mapping of the true causal SNP(s) (eFigure 9 and eFigure 10 in the Supplement).

Post-GWAS Extended Bioinformatic Analyses

For genomic profile risk scoring, PGC2 SNPs below the *P* value threshold of .05 had the strongest association with schizophrenia in India (eResults, eTable 9, and eFigure 11 in the Supplement). Statistical fine mapping analysis revealed that, of all the SNPs in our top locus, the top SNP rs10866912 had the highest probability of being a causal SNP (*z* score, 5.476; posterior probability, 1.0) (eFigure 12 in the Supplement).

To investigate possible molecular mechanisms underlying the top SNP, we interrogated eQTL databases for *cis*-eQTL effects of this SNP on neighboring genes. Using the Genotype-Tissue Expression project (GTEx, version 7),[19] the top SNP had the strongest regulatory effect for *NAPRT1* expression in brain cortex (normalized effect size, 0.79; 95% CI, 0.6-1.0; $P = 5.8 \times 10^{-13}$ [eFigure 13 in the Supplement]). In the Brain eQTL almanac,[20] the index SNP was significantly associated with *NAPRT1* expression across all brain regions (meta-analysis $P = 2.9 \times 10^{-13}$) and in particular with frontal cortex (133 samples; AA expression [$\log_2$ scale], 8.7; CC expression, 9.1; genotype counts: 23 for AA, 67 for AC, and 43 for CC; $P = 4.7 \times 10^{-7}$) and temporal cortex (133 samples; AA expression [$\log_2$ scale], 8.7; CC expression, 8.9; genotype counts: 23 for AA, 67 for AC, and 43 for CC; $P = 8.7 \times 10^{-7}$) (eTable 10 and eFigure 14 in the Supplement); other strongly associated SNPs in the locus (rs10866911, rs4873803, and rs4873804) also showed association with *NAPRT1* expression. Importantly, the lead SNP and these other associated SNPs were not associated with expression of any other genes in the LD block ($r^2 > 0.6$) [eTable 10 in the Supplement]). Spatiotemporal analyses using the human brain transcriptome[21] indicated that the pattern of *NAPRT1* expression was relatively high in most brain regions (highest in the hippocampus and neocortex) during early prenatal developmental stages, with a gradual decline in expression during postnatal development. Of the 5 other genes in the LD block, *TIGD5* had a similar pattern of perinatal expression to *NAPRT1* (eFigure 15A-E in the Supplement). In addition, the most active transcription factor binding to the top locus, *POL2RA* (NM_000937), is highly expressed during the

Figure 3. Expression of *NAPRT1* in Indian Lymphoblastoid Cell Lines



Expression of *NAPRT1* was analyzed in lymphoblastoid cell lines from 20 individuals (10 cases and 10 controls) of each rs10866912 genotype, including AA homozygous for risk allele, CA heterozygous, and CC homozygous for the protective allele. *NAPRT1* expression shows a dose-response association with the rs10866912 genotype in these samples, with the A risk allele downregulating expression. P = .004 for AA vs CC.

early prenatal developmental stages in all brain regions (eFigure 15F in the Supplement), with decreasing expression during later prenatal and early postnatal stages.

For natural selection analyses, the worldwide pattern of allele frequency distribution for the top SNP revealed a declining frequency of the risk allele (A) from African (approximately 96%) to Indian (approximately 72%) to European (approximately 43%) populations (eFigure 16 in the Supplement). Natural selection analysis using 1000 Genome Project data suggested that the top locus has undergone higher selection in Europeans than other world populations (integrated haplotype score for the European population, −2.52 [P < .01]; for the South Asian population, −1.03 [P = .30]). Similarly, the cross-population extended haplotype homozygosity test revealed more suggestive evidence of natural selection in Europeans compared with other populations (−2.16 [P < .03] [eFigure 17D in the Supplement]). The results of other tests of natural selection, corroborating these results, are shown in eFigure 17A to C in the Supplement. We observed a higher proportion of vegetarian diet in cases compared with controls (161 of 754 [21.4%] vs 154 of 783 [19.7%]; P = .67). Detailed results of other post-GWAS extended analyses, including SMR, pathway and network connectivity enrichment, transethnic genetic correlation for schizophrenia between India and Europe, and PGC2 replication in our Indian data set, are found in eResults, eFigures 18 to 21, and eTables 11 and 12 in the Supplement.

Post-GWAS Functional Analyses

Messenger RNA Expression Associated With the rs10866912 Locus

We sought to measure the effects of the index SNP on gene expression at the top locus using lymphoblastoid cell lines established from study participants. Our top locus (defined by r^2>0.6) contains the following 6 genes: *GSDMD, MROH6, NAPRT1, EEF1D, TIGD5,* and *PYCRL*. All except *NAPRT1* showed negligible gene expression. By contrast, *NAPRT1* showed a dose-response association with the rs10866912 genotype, the A risk allele downregulating expression of *NAPRT1* for AA vs CC (AA: 0.74, n = 21; CC: 1.56, n = 17; P = .004 for AA vs CC) (**Figure 3**).

This result is consistent with the CommonMind Consortium[22] eQTL finding that the risk allele (A) of the index SNP, rs10866912, significantly downregulates *NAPRT1* in a large postmortem collection of human brains (allele A; β coefficient, −0.45; $P = 1.40\times10^{-28}$). This result is also consistent with Brain eQTL almanac results showing allele-specific expression for rs10866912 (AA > AC > CC) across all measured brain regions, with the most significant result occurring in the frontal cortex (133 samples; AA expression [$\log_2$ scale], 8.7; CC expression, 9.1; genotype counts: 23 for AA, 67 for AC, and 43 for CC; $P = 4.7\times10^{-7}$) and with AA genotypes showing the least expression (eFigure 14 in the Supplement).

Zebrafish In Vivo Analysis

We further investigated in vivo the pathogenicity of *NAPRT1* loss of function using the zebrafish model. Based on a 2016 microRNA-mediated gene-silencing technology developed for the zebrafish,[23] we generated a transgene presenting a ubiquitous promoter driving expression of the dsRED marker and 3 different synthetic microRNAs targeting *dre-naprt1* messenger RNA (**Figure 4**A). We injected this construct into the zebrafish along with transposase to promote its integration into the zebrafish genome for stable and heritable expression. Interestingly, we found that, in the injected F0 animals and in a stable F1 line (named UBI-NAPRT1-123), the expression of anti-*naprt1* microRNAs trigger the developmental defect holoprosencephaly (Figure 4B), the most common congenital forebrain malformation, in which the brain fails to fully divide into 2 hemispheres. Importantly, injection of wild-type *dre-naprt1* messenger RNA without microRNA target sequences into F1 UBI-NAPRT1-123 zebrafish was sufficient to fully rescue this developmental phenotype (Figure 4C), confirming both efficient endogenous *naprt1* knockdown and the specificity of this defect. Further study will be required to fully understand the role of *naprt1* in the development of the fish.

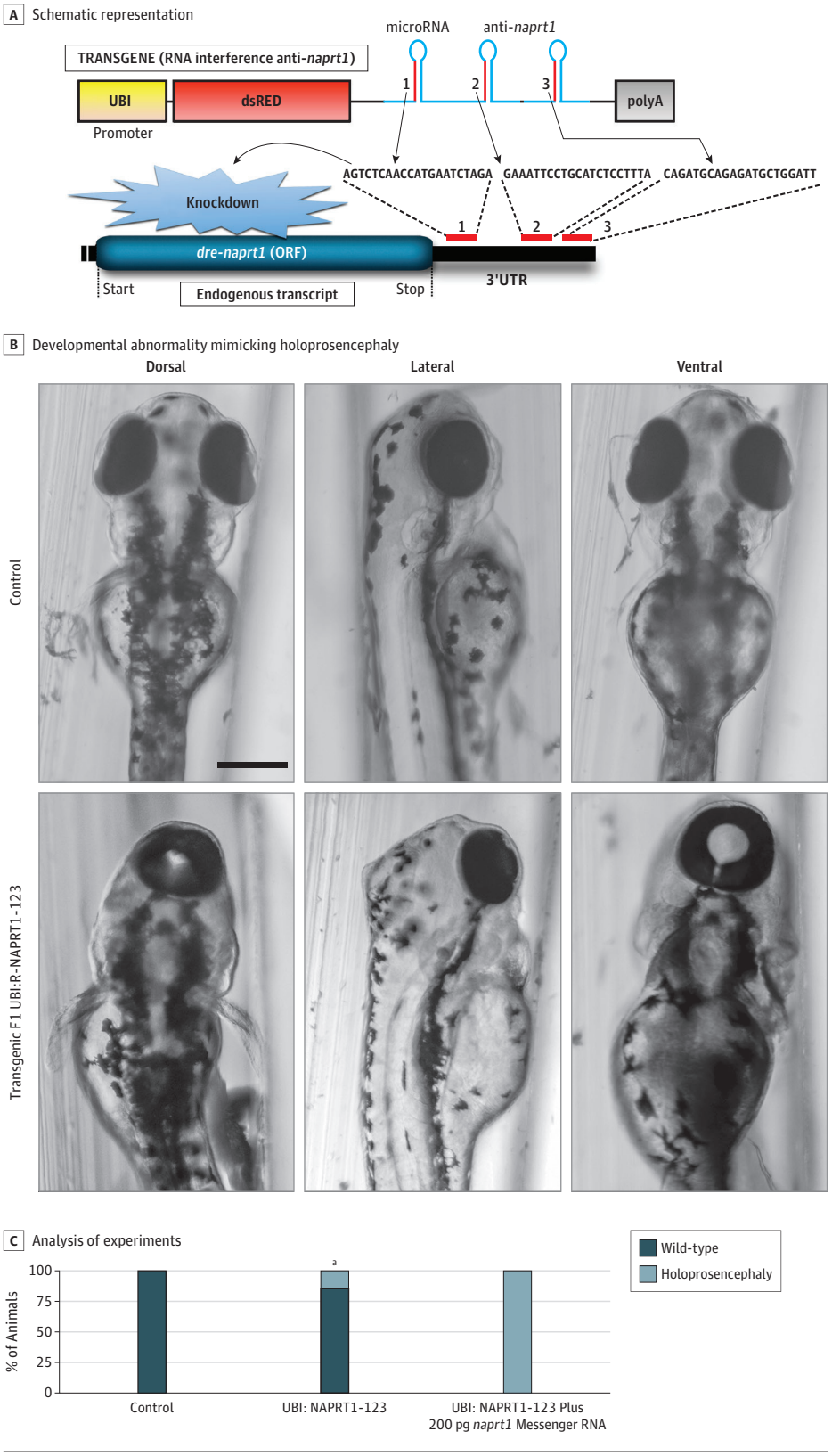
Discussion

Most schizophrenia GWASs have been conducted in Europeans, with a minority in Asian and African American populations. Our results provide further evidence for a cross-ethnic polygenic contribution to schizophrenia, although caution is required when estimating disease across disparate world ancestries given differences in genetic (LD patterns, allele frequencies, and genetic architecture)[24] and environmental factors.[25]

To our knowledge, this report describes the first Indian schizophrenia GWAS. Our sample (n = 3092) is characterized by a uniform ethnicity (>97% Tamil), a degree of inbreeding (typical F_HET coefficient range, −0.2 to 0.2), and a homogeneous schizophrenia phenotype with negligible rates of alcohol and illicit substance abuse, features that can be advantageous for genome-wide analyses.

We identified a schizophrenia locus in 8q24.3 that surpassed the threshold of genome-wide significance. It has not been possible to test replication of this finding in a separate Indian schizophrenia GWAS because, to our knowledge, none

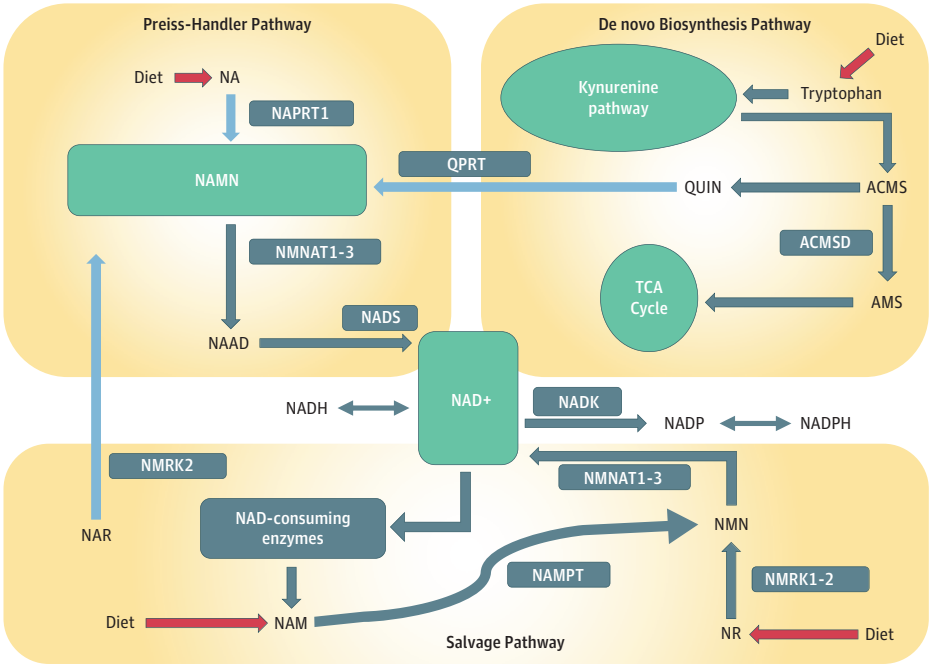
Figure 4. In Vivo Functional Analysis of Zebrafish *naprt1* Loss of Function



A, Schematic representation of the F1 line UBI:NAPRT1-123 transgene used to generate stable transgenic *naprt1* knockdown in zebrafish. B, Transgenic F1 UBI:NAPRT1-123 fish present a developmental abnormality mimicking holoprosencephaly. Black bar indicates 50 µm. C, Rescue experiments demonstrated that injection of *dre-naprt1* (with a custom 3'UTR not recognized by the anti-*naprt1* microRNAs) rescued the holoprosencephaly observed in the F1 UBI:NAPRT1-123 clutches. Analysis was performed 3 times with 50 animals per condition. ORF indicates open reading frame.

[a] $P \leq .02$ compared with control.

Figure 5. Nicotinamide Adenine Dinucleotide (NAD+) Biosynthetic Pathways



Nicotinic acid mononucleotide (NAMN) levels are maintained by 3 independent pathways (see light blue arrows). First, the Preiss-Handler pathway uses dietary nicotinic acid (NA) and the enzyme nicotinic acid phosphoribosyltransferase (NAPRT1) to generate NAMN, which is then transformed into nicotinic acid adenine dinucleotide (NAAD) by NAMN transferase (NMNAT) and finally into NAD^+ by NAD^+ synthase (NADS). Second, the de novo synthesis pathway of NAD^+ from tryptophan occurs through the kynurenine pathway to produce 2-amino-3-carboxymuconate semialdehyde (ACMS). This metabolite is converted nonenzymatically into quinolinc acid (QUIN), which is transformed into NAMN by quinolinate phosphoribosyltransferase (QPRT). Third, nicotinic acid riboside (NAR) is converted into NAMN by nicotinamide riboside kinase (NMRK2). The second and third pathways converge with the Preiss-Handler pathway via NAMN. The NAD salvage pathway recycles the nicotinamide generated as a byproduct of the enzymatic activities of NAD^+-consuming enzymes. Nicotinamide phosphoribosyltransferase (NAMPT) recycles nicotinamide into nicotinamide mononucleotide (NMN). NAD^+ is converted to nicotinamide adenine dinucleotide phosphate (NADP) by NAD^+ kinase (NADK). Pathways are described at https://reactome.org/content/detail/R-HSA-197264 and by Verdin et al.[28] ACMSD indicates aminocarboxymuconate semialdehyde decarboxylase; AMS, α-aminomuconate semialdehyde; NADH, reduced form of nicotinamide adenine dinucleotide; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NAM, nicotinamide; NR, nicotinamide riboside; and TCA, tricarboxylic acid cycle.

currently exist. However, our locus was replicated in the European ancestry schizophrenia PGC2 GWAS with similar direction and smaller magnitude of effect.[5] Analyses suggest that this locus has undergone natural selection in Europeans, with the derived allele (C) undergoing positive selection and the ancestral or risk allele (A) declining in frequency from approximately 72% in India to approximately 43% in Europe.[26] The high frequency of this Indian risk allele suggests it may have arisen recently, transforming from a neutral allele (ie, no effect on disease phenotype) to a risk allele, triggered perhaps by interaction with an environmental or lifestyle factor.[24]

Although the lead SNP is located closer to *MROH6* than to *NAPRT1* and other top SNPs lie within *MROH6*, *cis*-eQTL evidence and cellular expression studies indicate that these SNPs regulate the expression of *NAPRT1*. This result is consistent with analytic studies reporting that only a minority of associated genes are physically closest to the top GWAS SNP.[17] To our knowledge, no prior genome-wide significant evidence exists for association between *NAPRT1* and psychiatric traits, although 1 early study reports a nominal *P* value for a synonymous SNP, rs2290416, in exon 10 being associated with attention-deficit/hyperactivity disorder.[27]

NAPRT1 is the key rate-limiting enzyme involved in metabolizing nicotinic acid (NA), the major dietary source of niacin[28] (Preiss-Handler pathway, **Figure 5**). *NAPRT1* converts NA to nicotinic acid mononucleotide (NAMN), which is then converted to nicotinamide adenine dinucleotide (NAD^+), a ubiquitous coenzyme fundamental to all living cells and vital for cellular biochemistry, energy metabolism, and DNA synthesis. Levels of NAMN are maintained by 3 distinct pathways, but only the Preiss-Handler pathway operates in the brain under most conditions; thus, *NAPRT1* is crucial for contributing to the production of NAMN in the brain.[29] We suggest that *NAPRT1* underexpression could lead to NAD^+ deficiency, known to produce (1) neurodegenerative disorders such as pellagra and Hartnup disease, which can present with schizophrenialike symptoms such as auditory hallucinations, persecutory delusions, and delusional parasitosis[30]; and (2) oxidative stress via its negative effect on NADP levels, which have been implicated in schizophrenia.[31]

We further investigated whether a tryptophan-deficient (vegetarian) diet was potentially contributing to disease in the subset of 1627 samples homozygous for the rs10866912 risk allele (A). We observed a trend toward a higher proportion of

vegetarian diet in cases compared with controls (21.4% vs 19.7%; $P = .07$), which appears to be evidence of gene-environment interaction, although further metabolic studies in this cohort would be necessary to establish the association. In the absence of this evidence and antedating clinical trials, we speculate that vegetarian diet and homozygosity of the rs10866912 risk allele (A) may additively contribute to schizophrenia susceptibility in this Indian cohort. In this context, niacin supplementation has been used to prevent congenital malformations associated with NAD^+ deficiency.[32]

Based on post-GWAS bioinformatics analyses, cellular gene expression, and zebrafish knockdown data, *NAPRT1* is the prime candidate at our locus. Our in vivo experiments showed that partial loss of function of *NAPRT1* can cause abnormal brain development (holoprosencephaly) in early zebrafish (Figure 4). Holoprosencephaly is the most common human forebrain developmental defect, and many factors are known to be involved in pathogenesis.[33] Evidence for such defects occurring in schizophrenia include a recent report by the ENIGMA Schizophrenia DTI Working Group[34] of widespread microstructural deficits across the white matter skeleton in a large sample (1963 individuals with schizophrenia and 2359 healthy controls), with the corpus callosum being 1 of 2 regions showing greatest effects. Although only a modest effect was seen with common variants in *NAPRT1* on schizophrenia risk, the fact that *NAPRT1* plays an essential role in NAD^+ metabolism in the brain suggests the possibility of a global effect on brain development.

Strengths and Limitations

One limitation of our study is the sample size, because large sample sizes are needed for GWAS discovery in schizophrenia. We maximized the sample size over many years according to available resources. As a consequence of this strategy, another limitation is that genotyping was conducted in 3 batches over time and used 3 different genotyping arrays. Our careful quality control steps and statistical analyses have provided strategies to overcome potential confounding introduced through these technical batch effects, but given this limitation, further replication of our results is needed before strong conclusions can be drawn. We note that this study includes one of the few Indian schizophrenia research cohorts and, to our knowledge, the only one that has undergone a GWAS. Importantly, supported by our DNA analyses, this cohort is from a single region with uniform ethnicity. Another strength of our study is the comprehensive phenotyping using the consensus diagnostic procedure (see eMethods in the Supplement) that documented a "pure" schizophrenia phenotype and negligible substance abuse.

Conclusions

We have identified *NAPRT1* as a novel susceptibility gene for schizophrenia in an Indian population. Further studies in larger Indian samples are needed to replicate this novel genome-wide significant association.

ARTICLE INFORMATION

Accepted for Publication: April 7, 2019.

Published Online: July 3, 2019. doi:10.1001/jamapsychiatry.2019.1335

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Obtained funding: Nyholt, Mowry.
Administrative, technical, or material support: Periyasamy, John, Rajendran, Thirunavukkarasu, Nancarrow, Nertney, Smith, Filippich, Patel, Fowdar, McLean, Tirupati, Gundugurti, Selvaraj, Brown, Suetani, Giacomotto, Thara, Mowry.
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Conflict of Interest Disclosures: Dr Nancarrow reported receiving grants from the Australian National Health and Medical Research Council (NHMRC) during the conduct of the study. Dr Tirupati reported receiving grants from the Australian NHMRC during the conduct of the study. Dr Wray reported receiving grants from the Australian NHMRC during the conduct of the study. Dr Mowry reported receiving grants from the Australian NHMRC during the conduct of the study. No other disclosures were reported.

Funding/Support: This study was supported by grants 143027, 339454, 613602, and 1047956 from the Australian NHMRC (Drs Mowry and Thara); Career Development Fellowship grants 1103418 and 1127440 from the Australian NHMRC (Dr Gratten); grant 1165850 from the Australian NHMRC (Drs Mowry and Giacomotto); grants 1113400 and 1078901 from the Australian NHMRC (Dr Wray); grants GM59290, GM104390, and GM118335 from the National Institutes of Health (Dr Jorde); and a Senior Principal Research Fellowship from the Australian NHMRC (Dr Brown).

Role of the Funder/Sponsor: The sponsors had no role in the design and conduct of the study; collection, management, analysis, and

interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank the patients and family members who participated in this study and the staff of Sowmanasya Hospital, Tiruchirappalli, Athma Hospitals and Research Private Ltd, Tiruchirappalli, Asha Hospital, Hyderabad, Vazhikatti Mental Health Centre & Research Institute, Coimbatore, and Manthin Maiyam Hospital, Erode, for their support.

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