Whole Genome Association Study Identifies Polymorphisms in the *NPAS3* Gene Associated With Enhanced Response to Iloperidone Treatment in Patients With Schizophrenia

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#1035/T

INTRODUCTION

Schizophrenia, a psychotic disorder affecting approximately 1% of the US population, is characterized by the presence of positive symptoms (eg, hallucinations), negative symptoms (eg, social withdrawal), and impaired cognitive functions.[1] Although many antipsychotic drugs are approved to treat this chronic illness, patient response to treatment remains highly variable,[2,3] and no specific, reliable markers predictive of response have been identified. Iloperidone is an investigational mixed D_2/5-HT_2 receptor antagonist antipsychotic that has demonstrated clinical efficacy in a broad range of schizophrenia symptoms and has a reduced potential for extrapyramidal side effects.[4-7] Through a whole genome association study (WGAS) conducted in a phase 3 clinical trial of iloperidone for the treatment of schizophrenia, we identified several single nucleotide polymorphisms (SNPs) strongly associated with iloperidone efficacy (see Poster #1036/T). We focus here on SNPs in the *NPAS3* gene that had previously been directly linked to schizophrenia.[8]

METHODS

The WGAS was conducted in a randomized, double-blind, placebo- and ziprasidone-controlled, multicenter, phase 3 clinical trial that evaluated the efficacy, safety, and tolerability of a 24-mg/d dosage of iloperidone (12 mg twice a day) for 28 days in patients with acute exacerbations of schizophrenia. The primary efficacy variable in this trial was change from baseline to the last scheduled observation in the Positive and Negative Syndrome Scale Total score (PANSS-T).[9]

Genotyping

DNA samples from 218 patients who received iloperidone and 105 who received placebo were genotyped for >500,000 SNPs using a microarray set (GeneChip Human Mapping 500K Array Set; Affymetrix, Santa Clara, California). Genotype calls were made using the dynamic model-based (DM) genotyping algorithm[10] and the latest Bayesian robust linear model with Mahalanobis distance classifier (BRLMM), with a confidence threshold of 0.5.[11] Only arrays with ≥93% call rates and with <30% heterozygote calls were retained. After filtering for minor allele frequency (≥10%) and quality of genotype calls, 334,563 autosomal SNPs were used in the analysis. To verify the validity of the microarray genotype calls of specific SNPs of interest, ≥15 DNA samples were sequenced, with a minimum of at least 5 samples for each genotype.

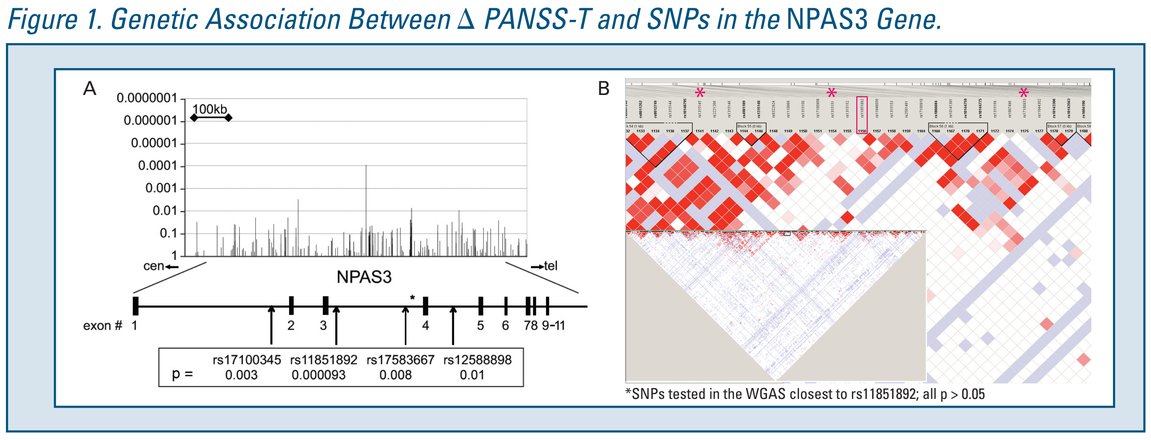
Statistical Analysis

Three different analyses were conducted. The first analysis used a 2-phase approach in which the DNA samples were equally and randomly split into the discovery and confirmatory groups. For the 50% of samples used in the discovery phase, each SNP was tested for potential association between genotype and change in PANSS-T for patients in the bottom and top 30% of the distribution of Day 28 last-observation-carried-forward (LOCF) data. The confirmatory phase used the other 50% of the samples as a holdout group and tested the top 100 SNPs from the discovery phase. Two single-phase analyses were performed on all the samples available using analysis of variance (ANOVA) on the LOCF data and mixed-model repeated-measures (MMRM) analysis, respectively. The Benjamini and Hochberg (BH)[12] procedure was used to control for the expected proportion of false discovery rate (FDR).

RESULTS AND DISCUSSION

The WGAS identified 6 SNPs associated with iloperidone efficacy, including rs11851892 located on chromosome 14q12-q13 within the *NPAS3* gene, raw p=0.000093, BH-adjusted p=0.59 (**Figure 1A**). Data for the other five SNPs are reported in Poster #1036/T. Three other SNPs within the *NPAS3* gene were shown to be significantly ($p < 0.01$) associated with changes in PANSS-T (**Figure 1A**). SNPs rs11851892 and rs17583667 are both located in intron 3, where a chromosomal breakpoint has been described by Kamnasaran et al[8] in a family affected with schizophrenia (**Figure 1A**). SNP rs11851892 was not contained in any haplotype block as defined by the HapMap CEPH samples (**Figure 1B**).[13-14]

Figure 1. Genetic Association Between Δ PANSS-T and SNPs in the NPAS3 *Gene.*



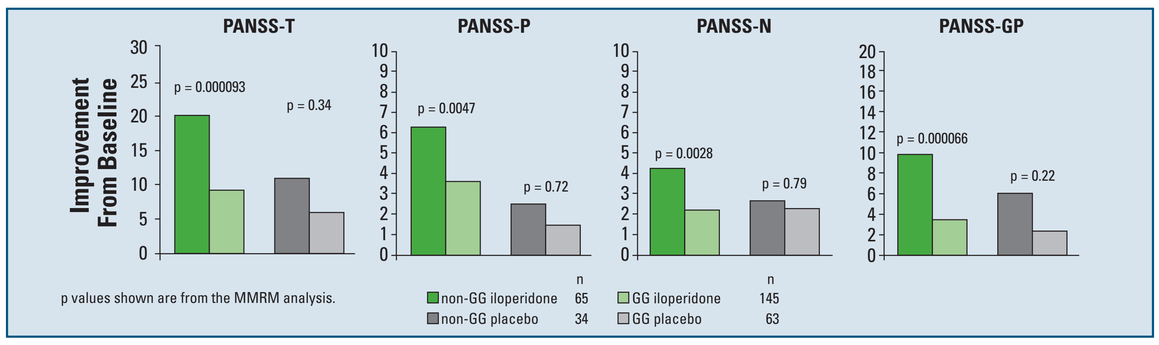
(A) p values (vertical axis) from the mixed-model repeated measures analysis are shown for the genomic region containing *NPAS3* in order of the SNPs' physical locations (horizontal axis). The direction of the map relative to the centromere (cen) and to the telomere (tel) is indicated by horizontal arrows. SNPs with $p \leq 0.01$ are boxed. *Approximate location in *NPAS3* of the breakpoint described by Kamnasaran et al[8] in a family affected with schizophrenia. (B) Linkage disequilibrium plot of *NPAS3* gene region. This map was generated using Haploview v4.0[13] on 30 CEPH trios from the International HapMap Project.[14] The standard Haploview color scheme is used: LOD > 2 and D′ = 1, red; LOD > 2 and D′ < 1, shades of pink/red; LOD < 2 and D′ = 1, blue; LOD < 2 and D′ < 1, white. The SNP of interest is boxed in red.

SNP rs11851892

The non-GG rs11851892 genotype, which occurred at a frequency of 31%, was present in a group of iloperidone-treated patients who averaged a >20-point improvement from baseline in PANSS-T, 68% greater than the mean response of all iloperidone-treated patients (**Figure 2**) (Cutler AJ et al, manuscript in preparation). Patients who carried the non-GG genotype were approximately 3 times more likely to experience a 20% improvement than patients who carried the GG genotype (odds ratio, 2.74; 95% confidence interval, 1.492-5.028; p = 0.0011). This association was consistent between men and women and across races. The non-GG genotype effect on PANSS-T was also seen for the PANSS positive (PANSS-P), negative (PANSS-N), and general psychopathology (PANSS-GP) scores (**Figure 2**).

In differentiating patients with superior response to iloperidone, SNP rs11851892 had a sensitivity of 42%, a specificity of 79%, a negative predictive value of 60%, and a positive predictive value of 65%. This SNP had the greatest specificity and positive predictive value of any of the 6 SNPs studied (see Poster #1036/T).

Figure 2. Treatment Response per Genotype.



NPAS3 and Schizophrenia

The NPAS3 protein belongs to a family of transcription factors that contains a basic helix-loop-helix (bHLH) motif and a PAS domain. NPAS3 is expressed in multiple regions of the brain, including the hippocampus, thalamus, and cortex.[8]

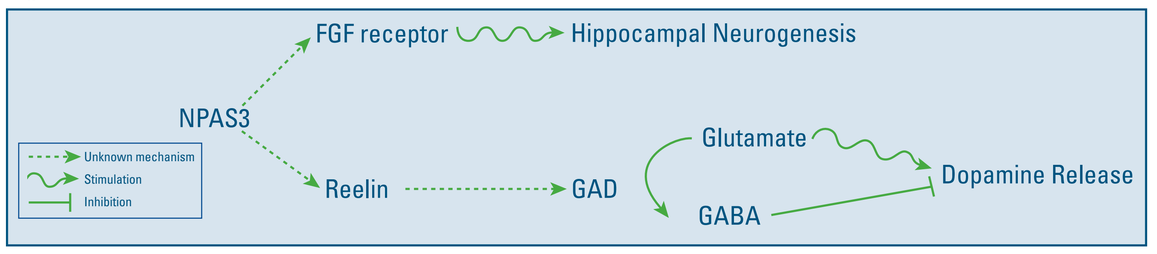
A study has described a mother and daughter with schizophrenia who carried a t(9;14)(q34;q13) chromosome, with a breakpoint in intron 3 of the *NPAS3* gene (**Figure 1A**). This breakpoint disrupted the bHLH and PAS domains involved in the DNA binding and dimerization functions of the protein.

Recently, studies of mice with disruptions in the *NPAS3* and *NPAS1* genes have shown that the encoded transcription factors may control regulatory pathways relevant to schizophrenia. *NPAS3* knockout mice are deficient in expression of fibroblast growth factor (FGF) receptor subtype 1 mRNA, have reduced hippocampal neurogenesis, and show behavioral and neuroanatomic abnormalities reminiscent of schizophrenia.[15] This suggests that impaired hippocampal neurogenesis may be related to schizophrenia-associated hippocampal pathology.[15,16]

In addition, *NPAS3* homozygous knockout adult mice have a marked reduction in reelin in the cortex, dentate gyrus, and amygdala and display schizophrenia-like behavioral disturbances.[17] Reelin protein, reelin mRNA, and GAD67 levels have also been shown to be significantly reduced in several brain areas of patients with undifferentiated or paranoid schizophrenia.[18,19] Reduced reelin may lead to reduced glutamate acid decarboxylase (GAD) and γ-aminobutyric acid (GABA), resulting in increased dopamine release (**Figure 3**). The behavioral disturbances seen in *NPAS3* knockout mice are also consistent with impairment in glutamatergic and dopaminergic systems involved in human psychopathology.[16]

NPAS3 may therefore be associated with schizophrenia through the involvement of different pathways: FGF-mediated adult hippocampal neurogenesis and reelin-mediated expression of the GAD enzyme, which is necessary for the production of GABA inhibitory neurotransmitter (**Figure 3**).

Figure 3. NPAS3 Pathway.



CONCLUSIONS

- This study provides the first evidence of a possible link between *NPAS3* and the efficacy of an antipsychotic treatment.
- Patients with the rs11851892 *NPAS3* non-GG genotype showed significantly greater improvement on PANSS-T and PANSS subscales than the GG genotype.
- Our data suggest that *NPAS3* may affect an individual patient's response to antipsychotic treatment with iloperidone.
- *NPAS3* should be further investigated as a candidate gene for schizophrenia because it has previously been shown to be involved in schizophrenia in one family,[8] has been implicated in the regulation of hippocampal neurogenesis and neurotransmitter pathways, and has now been associated with patient response to antipsychotic treatment.
- Additional studies specifically designed to confirm and elucidate the clinical value of this finding should provide new insight into response to iloperidone.
- This type of genetic data may soon guide clinicians in directing iloperidone therapy with optimal benefit-to-risk ratio.

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