86. Mulle JG, Chowdari KV, Nimgaonkar V, Chakravarti A. No evidence for association to the G72/G30 locus in an independent sample of schizophrenia families. *Mol. Psychiatry* 2005; **10**: 431–433.

87. Chen YS, Akula N, Detera-Wadleigh SD *et al.* Findings in an independent sample support an association between bipolar affective disorder and the G72/G30 locus on chromosome 13q33. *Mol. Psychiatry* 2004; **9**: 87–92; image 5.

88. Hattori E, Liu C, Badner JA *et al.* Polymorphisms at the G72/G30 gene locus, on 13q33, are associated with bipolar disorder in two independent pedigree series. *Am. J. Hum. Genet.* 2003; **72**: 1131–1140.

89. Zhang F, St Clair D, Liu X *et al.* Association analysis of the RGS4 gene in Han Chinese and Scottish populations with schizophrenia. *Genes Brain Behav.* 2005; **4**: 444–448.

90. Williams NM, Preece A, Spurlock G *et al.* Support for RGS4 as a susceptibility gene for schizophrenia. *Biol. Psychiatry* 2004; **55**: 192–195.

91. Morris DW, Rodgers A, McGhee KA *et al.* Confirming RGS4 as a susceptibility gene for schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2004; **125**: 50–53.

92. Chen X, Dunham C, Kendler S *et al.* Regulator of G-protein signaling 4 (RGS4) gene is associated with schizophrenia in Irish high density families. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2004; **129**: 23–26.

93. Chowdari KV, Mirnics K, Semwal P *et al.* Association and linkage analyses of RGS4 polymorphisms in schizophrenia. *Hum. Mol. Genet.* 2002; **11**: 1373–1380.

94. Cordeiro Q, Talkowski ME, Chowdari KV, Wood J, Nimgaonkar V, Vallada H. Association and linkage analysis of RGS4 polymorphisms with schizophrenia and bipolar disorder in Brazil. *Genes Brain Behav.* 2005; **4**: 45–50.

95. Sobell JL, Richard C, Wirshing DA, Heston LL. Failure to confirm association between RGS4 haplotypes and schizophrenia in Caucasians. *Am. J Med. Genet. B Neuropsychiatr. Genet.* 2005; **139**: 23–27.

96. Grillet N, Pattyn A, Contet C, Kieffer BL, Goridis C, Brunet JF. Generation and characterization of Rgs4 mutant mice. *Mol. Cell. Biol.* 2005; **25**: 4221–4228.

97. Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat. Genet.* 2004; **36**: 131–137.

98. Schwab SG, Hoefgen B, Hanses C *et al.* Further evidence for association of variants in the AKT1 Gene with schizophrenia in a sample of European Sib-Pair families. *Biol. Psychiatry* 2005; **58**: 446–456.

99. Ikeda M, Iwata N, Suzuki T *et al.* Association of AKT1 with schizophrenia confirmed in a Japanese population. *Biol. Psychiatry* 2004; **56**: 698–700.

100. Ohtsuki T, Inada T, Arinami T. Failure to confirm association between AKT1 haplotype and schizophrenia in a Japanese case-control population. *Mol. Psychiatry* 2004; **9**: 981–983.

101. Brazil DP, Park J, Hemmings BA. PKB binding proteins. Getting in on the Akt. *Cell* 2002; **111**: 293–303.

102. Rodgers EE, Theibert AB. Functions of PI 3-kinase in development of the nervous system. *Int. J. Dev. Neurosci.* 2002; **20**: 187–197.

103. Brunet A, Datta SR, Greenberg ME. Transcription-dependent and – independent control of neuronal survival by the PI3K-Akt signaling pathway. *Curr. Opin. Neurobiol.* 2001; **11**: 297–305.

Lithium response and Val66Met polymorphism of the brain-derived neurotrophic factor gene in Japanese patients with bipolar disorder

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Lithium is a first-line agent for the treatment of bipolar disorder. A significant association between the Val66Met polymorphism of the brain-derived neurotrophic factor gene and bipolar disorder has been reported. We investigated whether this polymorphism is associated with the response to lithium treatment in Japanese patients with bipolar disorder. Patients had been treated with lithium carbonate for more than 1 year, and the response was retrospectively evaluated. No significant differences were found in the genotype distribution or allele frequency between responders and non-responders. Our results suggested that the brain-derived neurotrophic factor Val66Met polymorphism might not greatly contribute to the efficacy of lithium in bipolar disorder. *Psychiatr Genet* 16:49–50 © 2006 Lippincott Williams & Wilkins.

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Lithium is a first-line agent for the treatment of bipolar disorder (BPD). Although its therapeutic mechanisms remain poorly understood, recent studies suggested a potential role of the brain-derived neurotrophic factor (BDNF) (Hashimoto *et al.*, 2004). A significant association between the Val66Met single-nucleotide polymorphism (SNP) of the BDNF gene and BPD has been reported (Neves-Pereira *et al.*, 2002). This SNP affects activity-dependent secretion of BDNF in cultured neurons, and human memory and hippocampal function. Therefore, we investigated whether the Val66Met SNP of the BDNF gene is associated with the response to lithium treatment in Japanese patients with BPD.

Study participants were 161 patients with BPD [83 bipolar I disorders (BPI) and 78 bipolar II disorders (BPII)]. Consensus diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition criteria. They were composed of 76 male and 85 female patients, with age of 48.2 ± 12.8 (mean ± SD) years and a mean age at onset of 34.1 ± 11.7 years. All the participants were biologically unrelated Japanese. Patients had been treated with lithium carbonate and its serum concentration was maintained between 0.4–1.2 mEq/l at least for 1 year. After a complete description of the study, written, informed consent was obtained from every participant. The study protocol was approved by institutional ethics committees.

Response to lithium treatment was retrospectively determined according to the criteria described previously (Masui *et al.*, in press). Briefly, lithium responders were defined as those patients with less frequent and/or severe relapse, including no relapse, during the maintenance period of lithium treatment compared with the period before the initiation of lithium treatment. During the maintenance period, administration of antidepressants or antipsychotics was regarded as a relapse. The genotyping of the Val66Met SNP (rs6265) of the BDNF gene was determined by TaqMan 5′-exonuclease allelic discrimination assay.

Among 161 patients, 110 were determined as responders and 51 patients as non-responders. The genotype distribution for responders (Val/Val = 41, Val/Met = 55, Met/Met = 14) and non-responders (Val/Val = 16, Val/Met = 27, Met/Met = 8) was in Hardy–Weinberg equilibrium ($P = 0.50$ and $P = 0.54$, respectively, χ^2 test).

No significant difference was found in the genotype distribution or allele frequency between the responders and non-responders ($P = 0.73$ and $P = 0.45$, respectively, χ^2 test). When a subtype of BPD (BPI or BPII) or sex was examined separately, there were no differences in genotype distributions or allele frequencies between the responders and non-responders.

Our results suggest that the Val66Met SNP of the BDNF gene is unlikely to be associated with lithium prophylaxis in Japanese patients with BPD. It is noteworthy that the significant association between this SNP and BPD has been demonstrated in Caucasian populations (Neves-Pereira et al., 2002), although the subsequent studies in Asian populations failed to replicate it (Kunugi et al., 2004). Therefore, the effects of this SNP might be different between ethnicities. The association between lithium prophylaxis and this SNP should be further tested in other ethnicities.

References

Hashimoto K, Shimizu E, Iyo M (2004). Critical role of brain-derived neurotrophic factor in mood disorders. Brain Res Brain Res Rev 45:104–114.

Kunugi H, Iijima Y, Tatsumi M, Yoshida M, Hashimoto R, Kato T, et al. (2004). No association between the Val66Met polymorphism of the brain-derived neurotrophic factor gene and bipolar disorder in a Japanese population: a multicenter study. Biol Psychiatry 56:376–378.

Masui T, Hashimoto R, Kusumi I, Suzuki K, Tanaka T, Nakagawa S, et al. (in press). A possible association between the -116C/G single nucleotide polymorphism of the XBP1 gene and lithium prophylaxis in bipolar disorder. Int J Neuropsychopharmacol [published online June 1–6].

Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F, Kennedy JL (2002). The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. Am J Hum Genet 71:651–655.

Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5′ SNPs associated with the disease

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Genetic variation in neuregulin 1 (NRG1) is associated with schizophrenia. The disease-associated SNPs are noncoding, and their functional implications remain unknown. We hypothesized that differential expression of the NRG1 gene explains its association to the disease. We examined four of the disease-associated SNPs that make up the original risk haplotype in the 5′ upstream region of the gene for their effects on mRNA abundance of NRG1 types I–IV in human postmortem hippocampus. Diagnostic comparisons revealed a 34% increase in type I mRNA in schizophrenia and an interaction of diagnosis and genotype (SNP8NRG221132) on this transcript. Of potentially greater interest, a single SNP within the risk haplotype (SNP8NRG243177) and a 22-kb block of this core haplotype are associated with mRNA expression for the novel type IV isoform in patients and controls. Bioinformatic promoter analyses indicate that both SNPs lead to a gain/loss of putative binding sites for three transcription factors, serum response factor, myelin transcription factor-1, and High Mobility Group Box Protein-1. These data implicate variation in isoform expression as a molecular mechanism for the genetic association of NRG1 with schizophrenia.

genetics | mRNA | human postmortem brain | hippocampus | ErbB

S chizophrenia is a complex, heritable psychiatric disorder. Recently, several putative schizophrenia susceptibility genes have been identified (1). Genomewide linkage studies and metaanalyses of linkage scans have highlighted chromosome 8p as a susceptibility locus (2–8). Extensive fine-mapping of the 8p locus, haplotype-association analysis, and linkage disequilibrium (LD) tests subsequently implicated neuregulin 1 (NRG1) (6), a gene with pleiotropic roles in neurodevelopment and plasticity (9).

The NRG1 gene spans 1.2 Mb (6) and gives rise to many structurally and functionally distinct isoforms, through alternative promoter usage. These isoforms are divided into three classic groups (9): type I (previously known as acetylcholine receptor inducing activity, heregulin, or neu differentiation factor), type II (glia growth factor) and type III (cysteine-rich domain containing), which are based on distinct amino termini. All isoforms have a bioactive EGF-like domain that is responsible for activation of ErbB receptor tyrosine kinases (ErbB2–ErbB4). Additional NRG1 5′ exons have recently been identified, giving rise putatively to novel NRG1 types IV–VI in the human brain (10). No biological information is available presently for these novel isoforms.

In the original report of association with schizophrenia in an Icelandic population, Stefansson and colleagues (6) identified a "core at-risk haplotype" consisting of five SNPs (SNP8NRG221132, SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, and SNP8NRG433E1006) and two microsatellites covering the 5′ end of the NRG1 gene and extending into the second intron (hereafter referred to as the "deCODE haplotype"). Separate follow-up studies in Scottish, Irish, mixed United Kingdom, and Dutch populations confirmed the genetic association between schizophrenia and NRG1 by using markers within the

same core haplotype (11–14) or with overlapping markers in the 5′ region (15, 16). Studies in four Asian populations also showed a strong association between schizophrenia and NRG1 polymorphisms at the 5′ (17–20) and 3′ end of the gene (19). Together these results, not withstanding two negative studies (21, 22), provide strong evidence that NRG1 is a schizophrenia-susceptibility gene. Additional support for NRG1's role in schizophrenia comes from the phenotype of NRG1 and ErbB4 mutant mice (6, 23–25), which exhibit behaviors similar to those of established rodent models of schizophrenia (26).

Exactly how genetic variation in NRG1 impacts on disease susceptibility remains uncertain because the SNPs associated with schizophrenia are all noncoding, being either intronic, synonymous exonic substitutions, or upstream of the transcription start site. It is possible that an as-yet unknown (rare) coding mutation(s) exists, but it is more probable that the noncoding SNPs themselves, or other SNPs with which they are in LD, are functionally associated with the disease. One plausible explanation is that the NRG1 SNPs are regulatory and affect disease susceptibility by altering expression (via altering transcriptional activity, alternative splicing, or stability of the RNA) and, thereby, the amount or distribution of the protein and ultimately its function. Support for this hypothesis comes from data demonstrating increased NRG1 type I mRNA in the prefrontal cortex in schizophrenia (27) and from altered gene expression and splicing being associated with polymorphic variation in other brain diseases (1, 28, 29).

The aim of this study was to address whether disease-associated polymorphic loci in the 5′ upstream region of NRG1 modulate NRG1 mRNA expression and contribute to its association with schizophrenia. We performed a series of hypothesis-driven experiments aimed at confirming previously published positive and negative expression data in schizophrenia (i.e., elevated type I, NRG1, and no change in type II or III (27) and to test specifically the hypothesis that disease-associated SNPs in the original risk haplotype influence expression of the novel type IV isoform based on their physical proximity to its 5′ regulatory region.

To test for differential expression of NRG1 in schizophrenia, we examined mRNA abundance for NRG1 types I–IV in the human hippocampus, a region prominently implicated in the pathogenesis of schizophrenia (30) and in the neurobiology of NRG1 (31). Examination of the effects of genetic variation on NRG1 expression included 4 SNPs from the deCODE core haplotype (6), with each SNP being tested individually for associations with NRG1 mRNA levels in patients and controls. LD between SNPs was examined, and the deCODE at-risk haplotype region was tested for association
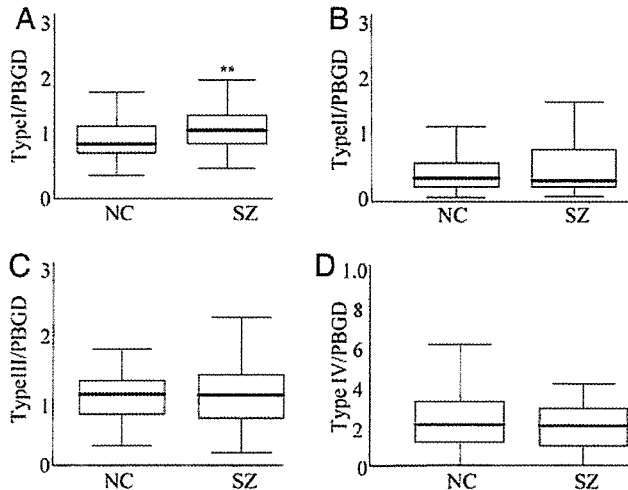

Fig. 1. *NRG1* types I–IV mRNA expression in the hippocampus of schizophrenic patients and control individuals. Quantitative RT-PCR analysis of *NRG1* types I–IV normalized to PBGD. (*A*–*C*) *n* = 53 normal control subjects (NC) and 38 patients with schizophrenia (SZ). (*D*) *n* = 46 normal controls subjects and 28 patients with schizophrenia. Box represents the proportion of the distribution falling between the 25th and 75th percentiles. Bars outside the box represent the SD. Bar inside represents the mean. Significant differences were found between controls and patients for type I *NRG1* expression. **, significant differences (*P* < 0.01)
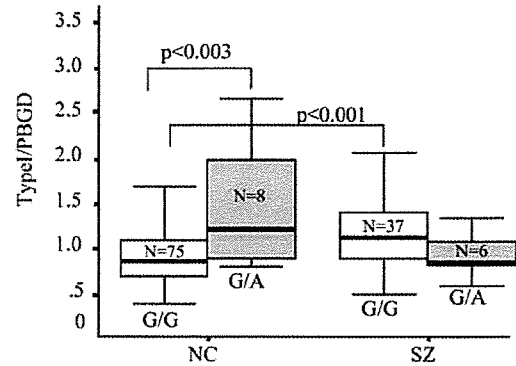

Fig. 2. Association between SNP8NRG122132 and type I *NRG1* mRNA in normal controls (NC) and schizophrenics (SZ). A significant interaction of genotype and diagnosis was observed on normalized type I mRNA expression (*P* < 0.003). Post hoc *t* tests revealed a main effect of genotype on type I *NRG1* expression in controls, with A allele carriers having increased levels compared with homozygous G individuals (*P* < 0.003). Schizophrenic patients homozygous for the G allele had higher levels of type I mRNA compared with controls with the same genotype (*P* < 0.001). Two individuals were excluded from analysis because of genotyping failure. Box represents the proportion of the distribution falling between the 25th and 75th percentiles. Bars outside the box represent the SD. The bar inside represents the mean.

with *NRG1* mRNA abundance. Our results support our primary hypotheses and indicate that the region of the gene implicated by the core at-risk haplotype impacts on specific *NRG1* isoforms and interacts with their expression in schizophrenia.

Results

NRG1 Isoform mRNA Expression in Schizophrenia. Normalized hippocampal *NRG1* type I mRNA expression levels were increased by 34% in schizophrenic patients compared with control subjects (Fig. 1; *F* (1, 85) = 8.65; *P* = 0.004). Similar significant findings were observed in the less well matched full cohort (84 controls vs. 44 schizophrenics; data not shown). No significant differences were observed between groups for the other *NRG1* isoforms {Fig. 1; type II [*F* (1, 85) = 0.10; *P* = 0.74]; type III, [*F* (1, 85) = 0.36; *P* = 0.54]; type IV, [*F* (1, 68) = 1.6; *P* = 0.20]; 17 individuals were not available for the type IV study because of a shortage of RNA]. Expression ratios for all *NRG1* isoforms were calculated to investigate relative expression abnormalities, given previous reports of altered isoform ratios in the dorsolateral prefrontal cortex in schizophrenia (27). Type I *NRG1* mRNA expression was increased relative to all other isoforms in schizophrenia: type I/II [*F* (1, 85) = 5.20; *P* = 0.02], I/III [*F* (1, 85) 5.0; *P* = 0.02], and I/IV [*F* (1, 68) = 10.11; *P* = 0.002]. No changes in any other isoform ratio were seen. No significant differences were observed between diagnostic groups for any of the housekeeping genes *PBGD*, [*F* (1, 85) = 0.27; *P* = 0.6]; TBP, [*F* (1, 85) = 0.16; *P* = 0.21], and *GUSB*, [*F* (1, 68) = 0.001; *P* = 0.98]. Covariation for pH, postmortem interval (PMI), and age was used in all analyses (see *Supporting Text*, which is published as supporting information on the PNAS web site).

Effects of 5' SNPs on NRG1 Expression. The effect of SNPs on *NRG1* type I-III isoform expression were examined in the whole cohort (*n* = 84 controls and *n* = 44 schizophrenics). For type IV analysis, 24 individuals were not available for analysis because of a shortage of RNA (leaving *n* = 74 controls and *n* = 30 schizophrenics). None of the SNPs examined showed any effect on expression of type II or type III isoforms.

Genetic Variation and Type I NRG1 mRNA. A genotype × diagnosis interaction [*F* (3, 122) = 9.21; *P* = 0.003] was found for SNP8NRG221132 and type I *NRG1* mRNA. There was no main effect of genotype. Post hoc comparisons showed that the genotype was significant in the controls alone (post hoc *t* test; *P* = 0.003; Fig. 2) with individuals carrying the rare A (2) allele expressing higher levels than individuals homozygous for G (1/1 genotype). The G allele constitutes the risk allele in the deCODE haplotype (6, 11). However, this pattern was not seen in schizophrenic patients who tended in the opposite direction (Fig. 2). Schizophrenic patients homozygous for the risk allele (G) had increased expression of type I *NRG1* mRNA compared with control subjects homozygous for the same allele (*t* test; *P* = 0.001). To confirm these findings, we genotyped SNP8NRG221132 in brain tissue from an earlier study of a separate cohort, in whom *NRG1* mRNA expression for types I–III had been measured by identical quantitative RT-PCR methods (controls *n* = 13, schizophrenics *n* = 16; *n* = 22 African Americans and *n* = 7 Caucasians) (27), and *NRG1* type I expression was found to be increased in the dorsolateral prefrontal cortex of these patients with schizophrenia. In this cohort we observed a main effect of SNP8NRG221132 genotype in the whole sample [*F* (3, 25) = 15.17; *P* = 0.0005] on prefrontal type I *NRG1* mRNA, with subjects carrying the A allele (*n* = 4) again showing higher type I *NRG1* levels compared with homozygous G cases (*n* = 25, data not shown). A genotype × diagnosis interaction also was found in this cohort [*F* (3, 25) = 16.26; *P* = 0.0005], with schizophrenic patients homozygous for the G allele (*n* = 14) having greater expression of type I *NRG1* mRNA than control subjects (*n* = 11) with the same genotype (*P* = 0.02). No other SNPs examined in the study were associated with type I *NRG1* expression.

Genetic Variation and Type IV NRG1 mRNA. Because >10% of individuals in the cohort were homozygous for the rare risk allele (T) at SNP8NRG243177, a complete analysis was conducted based on the three genotype groups (C/C, C/T, and T/T). We found a main effect of genotype for SNP8NRG243177 on type IV *NRG1* mRNA abundance in the whole sample [*F* (5, 98) = 3.15; *P* = 0.04; Fig. 3*A*]. The data suggest an allele dose effect with individuals heterozygous for the (T) risk allele (6, 11) having 21% more type IV *NRG1* mRNA than homozygous C/C individuals and individuals homozygous for the risk allele having 49% more type IV *NRG1*
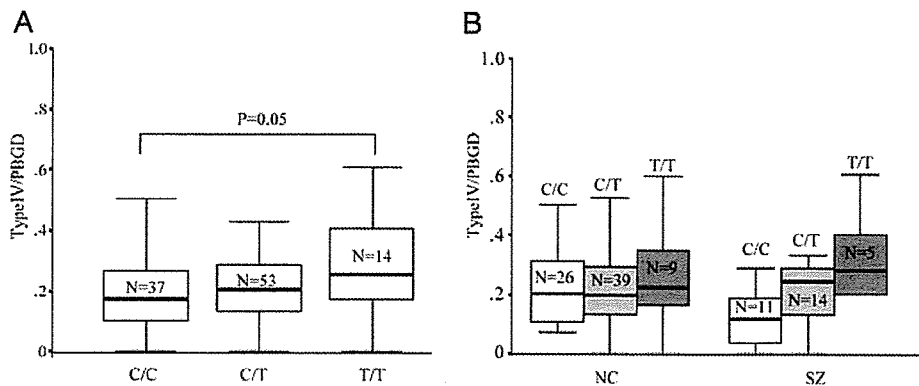
223

A

P=0.05

B

mRNA expression than homozygous C/C individuals. However, standard post hoc contrasts between the three groups revealed only the two homozygote groups to be significantly different in terms of type IV abundance (post hoc *t* test; *P* = 0.05; Fig. 3*A*). This effect appeared more pronounced in the schizophrenia group alone (Fig. 3*B*), although no hint of a diagnosis × genotype interaction was observed. Of note, normal control individuals homozygous for the T allele also showed the relatively greatest type IV *NRG1* mRNA expression (Fig. 3*B*).

Significant diagnosis × genotype interactions were found between five of the six haplotype-tagging SNPs (htSNPs) and type IV mRNA abundance (rs4268090, rs4298458, rs4452759, rs4733263, and rs4476964) [range *F* (3, 100) = 3.35–5.82; *P* = 0.033–0.018]. A trend for a main effect of genotype was observed for the htSNP, rs4268090 [*F* (3, 100) = 3.55; *P* = 0.06]. Carrying the T (2) allele at this SNP was associated with higher levels of type IV mRNA compared with subjects homozygous for the C (1/1) allele.

Haplotype Analysis. Results of LD tests between pairs of all 10 5' SNPs in African American and Caucasian individuals can be found in Tables 1 and 2, which are published as supporting material on the PNAS web site. The four markers chosen from the deCODE haplotype were in significant, but moderate, LD in both groups (see *Supporting Text*). The frequencies for the four common haplotypes comprised of the four deCODE SNPs are shown in Table 3, which is published as supporting information on the PNAS web site). Hap 4 contains the specific alleles that form part of the deCODE haplotype.

To test whether this four SNP risk haplotype (hap4) was associated with *NRG1* mRNA levels, we used SNPHAP (www-gene.cimr.cam.ac.uk/clayton/software) to assign a diplotype (haplotype pair) to each individual. We then compared hap4 carriers

(diplotypes hap1/hap4, hap2/hap4, hap3/hap4, and hap4/hap4) to non-hap4 individuals (diplotypes hap1/hap1, hap1/hap2, hap1/hap3, hap2/hap2, hap3/hap3, and hap2/hap3), testing for an effect of carrying the risk haplotype on *NRG1* mRNA abundance. ANOVA revealed a main effect of hap4 on type IV mRNA abundance in the entire sample [*F* (3, 89) = 3.38; *P* = 0.04]. Hap4 carriers had 27% more type IV mRNA compared with non-hap4 individuals (Fig. 4*A*). This effect appeared more pronounced in the schizophrenic patients, where a 53% increase in type IV *NRG1* mRNA was seen in hap4 carriers compared to noncarriers; in controls, only a 14% increase was observed (Fig. 4*B*). However, no diagnosis by genotype interaction was found. Race was not included as a factor in the analysis because of the small number of African American individuals carrying hap4. Hap4 showed no effect on the expression of any of the other *NRG1* isoforms.

Promoter Analysis Based on Transcription Factor Binding Sites. As a further exploration of the functional relevance of disease-associated SNPs in the *NRG1* gene, we performed an analysis of putative transcription factor binding sites by using MATINSPECTOR software (Genomatix, Munich), a computational suite for promoter informatics. Two SNPs in the haplotype were indicated to be contained within transcriptional regulatory elements; notably, these elements were the two SNPs that, as described above, were found to impact upon expression of *NRG1* isoforms, SNP8NRG221132, which is associated with type I *NRG1*, and SNP8NRG243177, which is associated with type IV *NRG1*. SNP8NRG221132 is within a predicted transcription factor binding domain for serum response factor (SRF), with the risk allele (G) abolishing SRF binding. SNP8NRG243177 is also within a putative binding site for SRF and for myelin transcription factor 1. Carrying the risk allele (T) results in a predicted loss of binding to both of these transcription factors
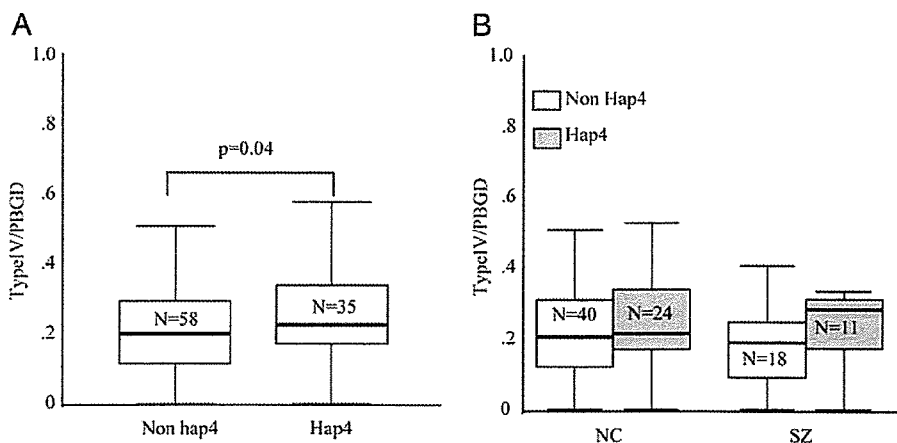
A

p=0.04

B

and the acquisition of the transcription factor binding site for High Mobility Group Box Protein-1. None of the other 8 SNPs genotyped in the study mapped to transcription factor binding domains.

Analysis of Negative SNP Controls. The two negative control SNPs (rs10954867 and rs7005288) showed no association with any *NRG1* isoform in either controls or schizophrenic patients (all *P* > 0.2).

Regional Distribution of Hippocampal NRG1 mRNA in Schizophrenia. Because no information is available regarding the distribution of *NRG1* in the human hippocampus, and this data was not provided from the quantitative RT-PCR experiments, we examined *NRG1* mRNA in the hippocampus in schizophrenia by using *in situ* hybridization with a "*pan*" *NRG1* probe (*Supporting Text*; see also Fig. 5, which is published as supporting information on the PNAS web site). No differences were seen in the distribution of *pan* NRG1 mRNA between subfields or its overall abundance in schizophrenia.

Discussion

We have investigated the expression of *NRG1* type I–IV mRNA in the human hippocampus and examined the effects of schizophrenia and disease-associated polymorphisms in the 5' upstream region on expression of these transcripts. We hypothesized that the genetic association of *NRG1* with schizophrenia is mediated by altered expression of the gene based on the location and noncoding nature of the disease-associated polymorphisms and the fact that extensive sequencing of *NRG1* has failed to identify pathogenic coding mutations (6). We report three principal findings: (*i*) up-regulation of type I expression in the hippocampus in schizophrenia, (*ii*) association of type I expression with a single SNP residing in the original deCODE risk haplotype, and (*iii*) association of type IV expression with a single SNP and a four-marker haplotype representing the 5' upstream region of the original at-risk haplotype associated with schizophrenia. We provide evidence of association between disease linked-variation in *NRG1* and altered *NRG1* isoform expression in the brain, and we propose that altered transcript regulation is a potential molecular mechanism behind the genetic association of *NRG1* with schizophrenia.

Our finding of increased type I mRNA *NRG1* expression in the hippocampus in schizophrenia replicates the finding in the dorsolateral prefrontal cortex of a smaller and separate brain series (27). These findings suggest that enhanced type I expression is robust and found in two separate brain regions in schizophrenia. In addition, we also replicate the finding that type II and type III isoform expression is unaltered in schizophrenia, suggesting that these isoforms may not be directly relevant to the pathophysiology of the disease. However, we did observe increases in the relative abundance of type I to type II–IV, suggesting that the contribution of these latter isoforms to *NRG1* signaling in the hippocampus may be indirectly compromised in patients with schizophrenia. At present, it is unclear whether type I up-regulation in schizophrenia is primary or secondary to other abnormalities in *NRG1* isoform regulation or to other molecular changes associated with the disease

When the four individual SNPs representing the 5' region of the deCODE at-risk haplotype were tested for association with type I *NRG1* mRNA, no main effects of genotype were seen for any of the SNPs. A diagnosis × genotype interaction was observed at a single SNP, SNP8NRG221132, and post hoc tests showed an effect of genotype only in control subjects on type I mRNA abundance. In a second independent cohort of brains in which increased type I mRNA expression previously had been reported in the schizophrenia samples, we found a main effect of a genotype at this SNP in the entire sample and again a genotype by diagnosis interaction. This main effect of the genotype was not seen in the first cohort; however, we note that the main effect in the entire sample is driven primarily by the controls. The observations that the main effect of

the genotype was primarily in the controls, and that the four-marker risk haplotype had no effect on type I *NRG1* expression, raise the possibility that SNP8NRG221132 influences type I expression independent of its contribution to risk for schizophrenia. Additional support for the functional relevance of SNP8NRG221132 comes from the bioinformatic promoter analysis that predicts the risk allele (G) leads to a loss of binding for SRF. SRF is a transcription factor that regulates the expression of genes encoding cytoskeletal proteins, such as cofilin and actin (32), both of which have been linked directly to *NRG1*s role in actin dynamics (33). The loss of SRF binding in controls homozygous for the risk allele, therefore, may be related to lower levels of type I mRNA transcription, as reported here. The direct functional consequences of this SNP for type I *NRG1* transcriptional control remain difficult to predict because the SNP resides 1 Mb upstream from the transcriptional start site of type I. However, SNP8NRG221132 could conceivably reside in a regulatory element of the gene, as is seen in other key developmental genes where genomic regions harboring cis-regulatory elements can be located as far as 1 Mb from the transcription unit (34).

The interrelationship between SNP8NRG221132, type I *NRG1* expression, and schizophrenia is somewhat more difficult to interpret, because in contrast to the effect seen in controls, we did not see a similar genotype effect in the patients. This issue is discussed in *Supporting Text, Discussion: Genetic Association and Type I Expression in Schizophrenia*).

In contrast to the type I finding, which is not manifestly related to genetic variation in *NRG1* associated with schizophrenia, the association between both SNP8NRG243177 and the four-marker at-risk haplotype with expression of a novel isoform of *NRG1*, type IV, suggests that we may have identified a genetic mechanism and a molecular phenotype underlying the involvement of *NRG1* in susceptibility for schizophrenia. The risk allele of SNP8NRG243177 and the deCODE haplotype predicted higher levels of type IV *NRG1* expression in our entire sample. Analysis of the three genotype groups for SNP8NRG243177 revealed that individuals homozygous for the risk allele had the highest levels of type IV expression, with evidence of an allele dose-dependant effect. This observation appeared more pronounced in the patients, although trends in the same direction were found in the normal controls and no diagnosis × genotype interaction was observed. SNP8NRG243177 is the most 3' of the SNPs in the four-marker deCODE haplotype and is located ≈1.2 kb upstream of the transcriptional start of type IV. Because none of the other single SNPs in this haplotype were associated with type IV *NRG1* expression, our results suggest that SNP8NRG243177 is a functional polymorphic variant that regulates type IV *NRG1* mRNA levels or is in strong LD with a nearby functional mutation. Additional support for the functional relevance of SNP8NRG243177 for gene regulation comes from the bioinformatic prediction that this SNP determines a putative transcription factor binding domain for SRF, myelin transcription factor 1, and High Mobility Group Box Protein-1. Of note, SRF and myelin transcription factor 1 play critical roles in neuronal migration, synaptic plasticity, and oligodendrocyte proliferation and survival, respectively, providing a striking molecular convergence with current hypotheses regarding the neurobiology of schizophrenia and the potential role of *NRG1* (35). However, we do not know which, if any, of these changes in transcription factor binding sites might mediate the association between SNP8NRG243177 and type IV expression and schizophrenia. Of potential interest is High Mobility Group Box Protein-1, an abundant chromatin-binding protein, which acts as an architectural facilitator in transcription (36). In our sample, acquisition of two High Mobility Group Box Protein-1-binding motifs (i.e., homozygosity for the risk allele) was associated with significantly elevated type IV *NRG1* expression, whereas acquisition of one (i.e., heterozygosity for the risk allele) was not. This observation suggests (*i*) that this binding site may potentiate

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type IV *NRG1* transcription (and that SRF binding is necessary for optimal levels of type IV transcription) and 2) that this effect may be recessive.

The absence of overall changes in type IV *NRG1* gene expression levels in schizophrenia suggest that altered type IV, unlike type I, is not a general characteristic of the disease state, *per se*. Indeed, if altered *NRG1* type IV expression is part of the genetic architecture of susceptibility for schizophrenia, it would not be expected to show an effect at the general population level, assuming that the at-risk haplotype is relevant for, at most, 10% of cases. Furthermore, our finding that the deCODE risk haplotype is associated specifically with type IV *NRG1* expression argues that the clinical association with *NRG1* is based on this molecular effect.

We further report association of type IV *NRG1* mRNA in schizophrenia with five additional htSNPs, which span a 17-kb gap between the four SNPs from the deCODE haplotype. To our knowledge, these SNPs have not been tested for association with schizophrenia in the same clinical samples in which the deCODE SNPs were positive. We genotyped these SNPs to address the possibility that the deCODE haplotype might not provide sufficient information regarding genetic diversity in our sample. None of these SNPs showed main effects, and their association with *NRG1* type IV expression in schizophrenia is likely via LD with SNP8NRG243177.

In our sample, the deCODE risk haplotype, which we termed hap4, was present in both Caucasian and African American populations but more common in the Caucasian sample. The significant degree of LD across this region of the gene suggests that, at least in Caucasians, it has undergone very little recombination (37). Furthermore, the region is highly conserved between species, including chimpanzee, dog, mouse, and rat, suggesting that this region of the gene is functional, probably involved in transcriptional regulation of *NRG1* (38). We found no evidence to suggest that the frequency of the deCODE haplotype was higher in our patient population compared with controls, but our sample is too small to meaningfully test for association with clinical phenotype. Of note, we observed that the frequency of hap2 was somewhat greater in the African American patients (34%) compared with African American controls (25%), suggesting that in different ethnic groups, different haplotypes in the same region of the gene may be associated with schizophrenia. However, because of the small sample size involved, conclusions are limited. Interestingly, hap2 in the African American sample contains the same allele at SNP8NRG243177 as hap4.

In the original report by Stefansson *et al.* (6), association in Icelandic families was mapped to a seven-marker haplotype spanning a 270-kb LD block starting at SNP8NRG221132 and ending with a synonymous SNP in exon two (SNP8NRG433E1006) and two microsatellites in the second intron (478N14-848 and 420M9-13950). Evidence of association to this region of the gene in other samples has been primarily to SNPs at the 5′ end of this haplotype, encompassing the SNPs typed in this study. Thus, although we cannot exclude the possibility that the causative mutation(s) accounting for our association with type IV lies downstream from our typed SNPs, we tend to doubt this possibility for three reasons: (*i*) the exon 2 SNP and the microsatellites typed in the deCODE haplotype have not shown single point (pairwise) association with schizophrenia in any study (6, 18, 19, 39), in contrast to the four SNPs tested here, (*ii*) the physical location of SNP8NRG243177 (i.e., ≈1,200 bases upstream from the exon 1 start site) makes it a far better candidate for being located in a transcriptional regulatory region for type IV, and (*ii*) this SNP is in a putative functional transcription factor binding domain.

The known biological functions of *NRG1* (9) fit well with current hypotheses regarding the neurobiology of schizophrenia (35), including regulation of synaptogenesis, *in vivo* synaptic transmission, long-term potentiation, activity-dependent synaptic plasticity, and neuronal migration as well as neurotransmitter function (NMDA,

GABA, α-7, and dopamine) and oligodendrocyte biology, all of which are proposed to interact or be altered in schizophrenia (30, 40). Of particular relevance is the recent finding that *NRG1* down-regulates NMDA-receptor currents in prefrontal cortical pyramidal neurons and slices (41). These data suggest that increased expression of *NRG1* type I or IV would translate into decreased NMDA receptor-mediated signaling, one of the principal neurotransmitter hypotheses of schizophrenia.

Finally, it should be noted that we have performed a number of tests in this study, and correction for multiple testing was not performed. Correction for random effects, such as Bonferroni correction, would be an excessively conservative approach, particularly given that we have restricted our primary analyses to planned comparisons (based on strong prior clinical association and physical location of the SNPs) of four SNPs and a single haplotype comprised of these SNPs. Because the SNPs are in moderate LD, the degree of independence between markers is low and, therefore, correcting for multiple testing would result in a high type II error rate. The prior probability and the predictable association between the deCODE haplotype and expression of NRG1 isoforms (especially type IV, which is its immediate physical neighbor) combined with the LD between SNPs in this haplotype makes statistical correction for these comparisons inappropriate. Nevertheless, our finding regarding type IV expression and the deCODE haplotype and SNP8NRG243177 requires independent replication.

In summary, we provide evidence of splice variant-specific alterations of *NRG1* gene expression in schizophrenia and demonstrate that disease-associated polymorphisms in a 5′ regulatory region of *NRG1* are associated with differential *NRG1* isoform expression. We suggest that the mechanism behind the clinical association of *NRG1* with schizophrenia is altered transcriptional regulation, which modifies, probably to a small degree and in an isoform-limited fashion, the efficiency of *NRG1* signaling effects on neural development and plasticity. Such alterations may compromise cortical and hippocampal function through one or more of the roles of *NRG1* and reflect, at least partly, the contribution of *NRG1* to the genetic risk architecture for the disease.

Materials and Methods

Human Postmortem Tissue. Postmortem hippocampal tissue was collected at the Clinical Brain Disorders Branch, National Institute of Mental Health, from 84 normal controls (22 females/62 males, 53 African American/25 American Caucasian/5 Hispanic/1 Asian, mean age 40.5 ± (SD) 15.4 years, PMI 30.7 ± 13.9 h, pH 6.59 ± 0.32) and 44 schizophrenic patients (15 females/29 males, 24 African American/20 Caucasians, mean age 49.7 ± 17.2 years, PMI 36.3 ± 17.7 h, pH 6.48 ± 0.28). This whole cohort was used for the analysis of effects of genetic variation on *NRG1* isoform expression. The different genotype groups in this cohort did not differ on any of the potential variables that affect gene expression in the human postmortem brain (i.e., age, PMI, pH, and age). Because the diagnostic groups in the whole cohort were not perfectly matched for these variables, we selected a subcohort of 53 controls (17 females/36 males, 31 African Americans/17 Caucasians/5 Hispanic individuals, mean age 44 ± 14.2 years, PMI 33.3 ± 13.7, pH 6.53 ± 0.24) and 38 schizophrenic individuals (12 females/26 males, 18 African Americans/19 Caucasians/1 Hispanic individuals, mean age 49.3 ± 19.3 years, PMI 38.1 ± 18.8, pH 6.40 ± 0.26), matched for these potential confounding variables. This subcohort was used for diagnostic comparisons of *NRG1* expression levels. Details of brain collection, neuroleptic medication history, and RNA extraction are described in *Supporting Text*.

Oligonucleotide and Primer Design. Primer and probe designs for *NRG1* types I–III were as described in ref. 27. Details of type IV and type I–III design can be found in *Supporting Text* (see also Table 4, which is published as supporting information on PNAS web site).

Quantitative Real-Time RT-PCR. *NRG1* mRNA expression levels were measured by quantitative RT-PCR by using an ABI Prism 7900 sequence detection system with a 384-well format (Applied Biosystems) as described (see *Supporting Text*). Our primary data analysis is based on normalization of *NRG1* mRNAs to an endogenous control gene, because it accounts for variability in the initial concentration and quality of total RNA and in the conversion efficiency of the reverse transcription reaction (42). Optimal normalization of a target mRNA to an endogenous control gene requires that the two transcripts have similar expression levels and that the control gene expression levels do not differ between the comparison groups. Based on this finding, PBGD was considered the most reliable for normalization here. Similar results were, however, obtained with normalization to TBP or GUSB (data not shown).

NRG1 Genotype Determination. DNA was extracted from cerebellar tissue by using a standard protocol supplied by PUREGENE (Gentra Systems). We genotyped four SNPS from the deCODE core haplotype (SNP8NRG221132, SNP8NRG221533, SNP8NRG241930, and SNP8NRG243177) and selected six additional SNPs (rs10096573, rs4268090, rs4298458, rs4452759, rs4733263, and rs4476964; Table 5 and Fig. 6, which are published as supporting material on the PNAS web site) from HAPMAP (www.hapmap.org) based on designation of these as htSNPS by using HAPLOVIEW (www.broad.mit.edu/mpg/haploview). The additional markers define the common haplotypes in an LD block containing a 22-kb region upstream of the first exon in type IV *NRG1*, which includes the four most 5′ SNPs of the deCODE core risk haplotype. These six htSNPs were chosen to maximize genetic coverage because they are highly informative tags for the common haplotypes in this region of the gene.

Two SNPs at the 3′ end of *NRG1* were selected from the dbSNP database as negative control genotypes (rs10954867 and rs7005288). These SNPs previously have not been associated with schizophrenia, are not in known regulatory domains, and were included in the analysis as a control for random statistical effects.

Genotyping was performed by using the Taqman 5′ exonuclease allelic discrimination assay (details available upon request). Genotype reproducibility was routinely assessed by regenotyping all samples for selected SNPs and was generally >99%. LD between 5′ SNPs was determined by using the program LDMAX/GOLD (43). The program SNPHAP written by David Clayton (version 1.0) was used to calculate haplotype frequencies and to assign diplotypes to individuals.

Statistical Analyses. Correlations of mRNA levels with demographic variables were performed for all subjects by using Spearman's correlations. Correlations of mRNA levels with neuroleptic medication (lifetime neuroleptic exposure, daily dose, and final neuroleptic dose) were investigated in the schizophrenic cohort. Primary planned comparisons between diagnostic groups were made by using univariate ANCOVA for each mRNA with diagnosis as the independent variable and age, pH, and PMI as covariates. Effects of genetic variation on *NRG1* mRNA expression were examined by using ANOVA with genotype and diagnosis as independent factors. Primary comparisons examined the effects of four SNPs and the core haplotype on type I–IV expression in patients and controls. Secondary, post hoc analyses included examination of the 6 htSNPS, where warranted. Where there was a significant genotype × diagnosis interaction, individual group post hoc tests were examined as part of the standard ANOVA readout. Analysis of the effects of race was restricted to African American and Caucasian individuals because of the small sample size in other ethnic groups. The genotype groups did not differ on any of the demographic variables, and no correlations were seen between *NRG1* isoform expression, age, pH, or PMI in the different genotype groups; therefore straight ANOVAs are reported for the effects of genotype, but the statistical results were not changed when covariates were included. To increase power for statistical analyses of SNPs with minor allele frequencies <10%, we grouped individuals heterozygous and homozygous for the rare allele. Examination of all three genotype groups was conducted when the minor allele frequency was >10%. All experiments were conducted blind to diagnosis.

1. Harrison, P. J. & Weinberger, D. R. (2005) *Mol. Psychiatry* 10, 40–68.
2. Pulver, A. E., Lasseter, V. K., Kasch, L., Wolyniec, P., Nestadt, G., Blouin, J. L., Kimberland, M., Babb, R., Vourlis, S., Chen, H., *et al.* (1995) *Am. J. Med. Genet.* 60, 252–260.
3. Kendler, K. S., MacLean, C. J., O'Neill, F. A., Burke, J., Murphy, B., Duke, F., Shinkwin, R., Easter, S. M., Webb, B. T., Zhang, J., *et al.* (1996) *Am. J. Psychiatry* 153, 1534–1540.
4. Kendler, K. S., Myers, J. M., O'Neill, F. A., Martin, R., Murphy, B., MacLean, C. J., Walsh, D. & Straub, R. E. (2000) *Am. J. Psychiatry* 157, 402–408.
5. Blouin, J. L., Dombroski, B. A., Nath, S. K., Lasseter, V. K., Wolyniec, P. S., Nestadt, G., Thornquist, M., Ullrich, G., McGrath, J., Kasch, L., *et al.* (1998) *Nat. Genet.* 20, 70–73.
6. Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., Brynjolfsson, J., Gunnarsdottir, S., Ivarsson, O., Chou, T. T., *et al.* (2002) *Am. J. Hum. Genet.* 71, 877–892.
7. Badner J. A. & Gershon E. S. (2002) *Mol. Psychiatry* 7, 405–411.
8. Lewis, C. M., Levinson, D. F., Wise, L. H., DeLisi, L. E., Straub, R. E., Hovatta, I., Williams, N. M., Schwab, S. G., Pulver, A. E., Faraone, S. V., *et al.* (2003) *Am. J. Hum. Genet.* 73, 34–48.
9. Falls, D. L. (2003) *J. Neurocytol.* 32, 619–647.
10. Steinthorsdottir, V., Stefansson, H., Ghosh, S., Birgisdottir, B., Bjornsdottir, S., Fasquel, A. C., Olafsson, O., Stefansson, K. & Gulcher, J. R. (2004) *Gene* 342, 97–105.
11. Stefansson, H., Sarginson, J., Kong, A., Yates, P., Steinthorsdottir, V., Gudfinnsson, E., Gunnarsdottir, S., Walker, N., Petursson, H., Crombie, C., *et al.* (2003) *Am. J. Hum. Genet.* 72, 83–87.
12. Williams, N. M., Preece, A., Spurlock, G., Norton, N., Williams, H. J., Zammit, S., O'Donovan, M. C. & Owen, M. J. (2003) *Mol. Psychiatry* 8, 485–487.
13. Bakker, S. C., Hoogendoorn, M. L., Selten, J. P., Verduijn, W., Pearson, P. L., Sinke, R. J. & Kahn, R. S. (2004) *Mol. Psychiatry* 9, 1061–1063.
14. Hall, D., Gogos, J. A., Karayiorgou, M. (2004) *Genes Brain Behav.* 3, 240–248.
15. Corvin, A. P., Morris, D. W., McGhee, K., Schwaiger, S., Scully, P., Quinn, J., Meagher, D., Clair, D. S., Waddington, J. L. & Gill, M. (2004) *Mol. Psychiatry* 9, 208–213.
16. Petryshen, T. L., Middleton, F. A., Kirby, A., Aldinger, K. A., Purcell, S., Tahl, A. R., Morley, C. P., McGann, L., Gentile, K. L., Rockwell, G. N., *et al.* (2005) *Mol. Psychiatry* 10, 366–374.
17. Yang, J. Z., Si, T. M., Ruan, Y., Ling, Y. S., Han, Y. H., Wang, X. L., Zhou, M., Zhang, H. Y., Kong, Q. M. & Liu, C. (2003) *Mol. Psychiatry* 8, 706–709.
18. Tang, J. X., Chen, W. Y., He, G., Zhou, J., Gu, N. F., Feng, G. Y. & He, L. (2004) *Mol. Psychiatry* 1, 11–20.
19. Li, T., Stefansson, H., Gudfinnsson, E., Cai, G., Liu, X., Murray, R. M., Steinthorsdottir, V., Januel, D., Gudnadottir, V. G., Petursson, H., Ingason, A., *et al.* (2004) *Mol. Psychiatry* 9, 698–704.
20. Zhao, X., Shi, Y., Tang, J., Tang, R., Yu, L., Gu, N., Feng, G., Zhu, S., Liu, H., Xing, Y., *et al.* (2004) *J. Med. Genet.* 41, 31–34.
21. Iwata, N., Suzuki, T., Ikeda, M., Kitajima, T., Yamanouchi, Y., Inada, T. & Ozaki, N. (2004) *Mol. Psychiatry* 9, 126–127.
22. Thiselton, D. L., Webb, B. T., Neale, B. M., Ribble, R. C., O'Neill, F. A., Walsh, D., Riley, B. P. & Kendler, K. S. (2004) *Mol. Psychiatry* 9, 777–783.
23. Gerlai, R., Pisacane, P. & Erickson, S. (2000) *Behav. Brain Res.* 109, 219–227.
24. Bao, J., Wolpowitz, D., Role, L. W. & Talmage, D. A. (2003) *J. Cell Biol.* 161, 1133–1141.
25. Rimer, M., Barrett, D. W., Maldonado, M. A., Vock, V. M. & Gonzalez-Lima, F. (2005) *NeuroReport* 16, 271–275.
26. Lipska, B. K. (2004) *J. Psychiatry Neurosci.* 29, 282–286.
27. Hashimoto, R., Straub, R. E., Weickert, C. S., Hyde, T. M., Kleinman, J. E. & Weinberger, D. R. (2004) *Mol. Psychiatry* 9, 299–307.
28. Farrer, M., Chan, P., Chen, R., Tan, L., Lincoln, S., Hernandez, D., Forno, L., Gwinn-Hardy, K., Petrucelli, L., Hussey, J., *et al.* (2001) *Ann. Neurol.* 50, 293–300.
29. Bertram, L., Hiltunen, M., Parkinson, M., Ingelsson, M., Lange, C., Ramasamy, K., Mullin, K., Menon, R., Sampson, A. J., Hsiao, M. Y., *et al.* (2005) *N. Engl. J. Med.* 352, 884–894.
30. Harrison, P. J. (2004) *Psychopharmacology* 174, 151–162.
31. Law, A. J., Shannon, W. C., Hyde, T. M., Kleinman, J. E. & Harrison, P. J. (2004) *Neuroscience* 127, 125–136.
32. Alberti, S., Krause, S. M., Kretz, O., Philippar, U., Lemberger, T., Casanova, E., Wiebel, F. F., Schwarz, H., Frotscher, M., Schutz, G., *et al.* (2005) *Proc. Natl. Acad. Sci. USA* 102, 6148–6153.
33. Nagata-Ohashi, K., Ohta, Y., Goto, K., Chiba, S., Mori, R., Nishita, M., Ohashi, K., Kousaka, K., Iwamatsu, A., Niwa, R., *et al.* (2004) *J. Cell Biol.* 165, 465–471.
34. Kleinjan, D. A. & van Heyningen, V. (2005) *Am. J. Hum. Genet.* 76, 8–32.
35. Corfas, G., Roy, K. & Buxbaum, J. D. (2004) *Nat. Neurosci.* 7, 575–580.
36. Thomas, J. O. & Travers, A. A. (2001) *Trends Biochem. Sci.* 26, 167–174.
37. Cardon, L. R. & Abecasis, G. R. (2003) *Trends Genet.* 19, 135–140.
38. Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Kent, W. J., Mattick, J. S. & Haussler, D. (2004) *Science* 304, 1321–1325.
39. Hong, C. J., Huo, S. J., Liao, D. L., Lee, K., Wu, J. Y. & Tsai, S. J. (2004) *Neurosci. Lett.* 366, 158–161.
40. Harrison, P. J. (1999). *Brain* 122, 593–624.
41. Gu, Z., Jiang, Q., Fu, A. K., Ip, N. Y. & Yan, Z. (2005) *J. Neurosci.* 25, 4974–4984.
42. Burbach, G. J., Dehn, D., Del Turco, D. & Deller, T. (2003) *J. Neurosci. Methods* 131, 83–91.
43. Abecasis, G. R. & Cookson, W. O. (2000) *Bioinformatics* 16, 182–183.

Antipsychotic medication and cognitive function in schizophrenia

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Abstract

Antipsychotic polypharmacy and excessive dosing still prevail worldwide in the treatment of schizophrenia, while their possible association with cognitive function has not well been examined. We examined whether the "non-standard" use of antipsychotics (defined as antipsychotic polypharmacy or dosage >1000 mg/day of chlorpromazine equivalents) is associated with cognitive function. Furthermore, we compared cognitive function between patients taking only atypical antipsychotics and those taking only conventionals. Neurocognitive functions were assessed in 67 patients with chronic schizophrenia and 92 controls using the Wechsler Memory Scale-Revised (WMS-R), the Wechsler Adult Intelligence Scale-Revised (WAIS-R), the Wisconsin Card Sorting Test (WCST), and the Advanced Trail Making Test (ATMT). Patients showed markedly poorer performance than controls on all these tests. Patients on non-standard antipsychotic medication demonstrated poorer performance than those on standard medication on visual memory, delayed recall, performance IQ, and executive function. Patients taking atypical antipsychotics showed better performance than those taking conventionals on visual memory, delayed recall, and executive function. Clinical characteristics such as duration of medication, number of hospitalizations, and concomitant antiparkinsonian drugs were different between the treatment groups (both dichotomies of standard/non-standard and conventional/atypical). These results provide evidence for an association between antipsychotic medication and cognitive function. This association between antipsychotic medication and cognitive function may be due to differential illness severity (e.g., non-standard treatment for severely ill patients who have severe cognitive impairment). Alternatively, poorer cognitive function may be due in part to polypharmacy or excessive dosing. Further investigations are required to draw any conclusions.
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Keywords: Schizophrenia; Cognitive function; Atypical antipsychotics; Conventional antipsychotics; Polypharmacy

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1. Introduction

Schizophrenia is associated with wide-ranging deficits in neurocognitive function, including memory, attention, executive function, and working memory (Bozikas et al., 2006; Fioravanti et al., 2005; Gold et al., 1992; Keefe et al., 2005; Reed et al., 2002; Silver et al., 2003; Suwa et al., 2004), and these deficits are considered to be core to the pathophysiology of the illness. Reports of cognitive impairments in schizophrenia date back to the pioneering efforts of Kraepelin (1919) and Bleuler (1950) and, more recently, the characteristics of these deficits have been clarified with increasing sophistication and precision. School children who will later develop schizophrenia are more likely than their classmates to under-perform in school (Erlenmeyer-Kimling et al., 2000; Fuller et al., 2002; Kremen et al., 1998; Reichenberg et al., 2005), and cognitive deficits become widely present at the onset of psychosis (Bilder et al., 1992; Hoff et al., 1992). Growing evidence has suggested that cognitive deficits in schizophrenia are not byproducts of positive symptoms (Addington et al., 1991; Davidson et al., 1995) or negative symptoms (Bell and Mishara, 2006; Harvey et al., 1996; Harvey et al., 2005).

As impaired performance on measures of neurocognition is more closely linked to functional outcome than symptoms (Green, 1996; Green et al., 2000), enhancement of cognitive functioning is considered an important component of treatment for schizophrenia (Green et al., 2005; Hofer et al., 2005). Investigators have focused on the cognitive pathology of schizophrenia and have sought to assess the effects of treatment on this dimension. A large number of treatment studies have demonstrated that therapeutic effects of conventional drugs are limited to the positive symptoms of the illness and they have substantially less impact on cognitive impairments (Medalia et al., 1988; Spohn and Strauss, 1989), whereas atypical antipsychotics may ameliorate cognitive deficits (Bender et al., 2006; Bilder et al., 2002; Harvey et al., 2006; Keefe et al., 1999, 2006; Kern et al., 1999; Meltzer and McGurk, 1999; Muller et al., 2005; Purdon et al., 2000; Rossi et al., 1997; Sumiyoshi et al., 2005; Thornton et al., 2006).

In spite of extensive research and recommendations as to the optimal prescription of antipsychotics, antipsychotic polypharmacy and excessive dosing are still highly prevalent worldwide, especially in Japan (Bitter et al., 2003; Chong et al., 2004; Faries et al., 2005; Ganguly et al., 2004; Procyshyn et al., 2001; Sim et al., 2004a; Weissman, 2002). This may be due in part to the scarcity of evidence for the possible association between antipsychotic medication in terms of dosage or type and cognitive function in these countries. In this context, the present study was aimed (1) to examine whether the "non-standard" use of antipsychotics (defined as antipsychotic polypharmacy or dosage >1000 mg/day of chlorpromazine equivalents) is associated with cognitive functions and (2) to compare cognitive deficits between patients treated with atypical antipsychotics and those with conventional drugs, using a comprehensive set of neurocognitive tests and by examining extensive clinical characteristics of patients.

2. Methods

2.1. Subjects

Patients with schizophrenia ($n = 67$) who were under treatment at the National Center of Neurology and Psychiatry Musashi Hospital, Tokyo, Japan were recruited. All patients met the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia. Consensus diagnosis was made by treating and research clinicians who were all senior psychiatrists, based on clinical interviews, observations, and case notes. Patients were chronic schizophrenia and were prescribed a stable dose of antipsychotic medication for at least 3 months prior to neuropsychological test sessions. Schizophrenic symptoms were rated by using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Healthy volunteers ($n = 92$) who had no history of current or past contact to psychiatric services were recruited from the hospital staffs and their associates through fliers and by word of mouth. Those individuals who had a history of regular use of psychotropic agents were not enrolled in the control group. Participants were excluded from both the patient and control groups if they had prior medical histories of central nervous system disease or severe head injury, or if they met criteria for alcohol/drug dependence or mental retardation. All subjects were biologically unrelated Japanese who resided in the same geographical area (Western part of Tokyo Metropolitan). Written informed consent was obtained from all subjects and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Neuropsychological test measures

A comprehensive battery of neurocognitive tests was administered to all subjects in a random order that took at least 4 h to complete. The battery included the Wechsler Memory Scale-Revised (WMS-R, Sugishita,

2001; Wechsler, 1987), the Wechsler Adult Intelligence Scale-Revised (WAIS-R, Shinagawa et al., 1990; Wechsler, 1981), the Wisconsin Card Sorting Test (WCST, Heaton, 1981; Kashima et al., 1987), and the Advanced Trail Making Test (ATMT, Nakahachi et al., 2006; Takahashi et al., 2005).

2.2.1. Wechsler Memory Scale-Revised

A full version of the WMS-R (Wechsler, 1987) was administered. The average score and standard deviation (S.D.) of WMS-R in the general population are 100 and 15, respectively. This test mainly measures memory functions, while it can also assess attention. Its four main outcome measures were verbal memory, visual memory, attention, and delayed recall.

2.2.2. Wechsler Adult Intelligence Scale-Revised

A full version of the WAIS-R (Wechsler, 1981) was administered, yielding scores of verbal IQ, performance IQ, and full-scale IQ.

2.2.3. Wisconsin Card Sorting Test

The WCST (Heaton, 1981) mainly assesses executive function including cognitive flexibility in response to feedback. We used a modified and computerized version of the test (Kashima et al., 1987; Kobayashi, 1999). Outcome measures were numbers of categories achieved, total errors, and perseverative errors of Milner and Nelson types.

2.2.4. Advanced Trail Making Test

The ATMT (Takahashi et al., 2005) is a computerized task modified from the original Trail Making Test (Reitan and Wolfson, 1993), and is considered to measure subjects' abilities of spatial working memory and psychomotor speed. In the present study, only spatial working memory was rated in all subjects.

2.3. Grouping procedures

Daily doses of antipsychotics, including depot antipsychotics, were converted to approximate chlorpromazine equivalents (CPZeq) using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999). The patient group was subdivided into two different types of subgroups by medication patterns. One grouping criterion was a "standard" or "non-standard" use of antipsychotics. The "standard" was defined as receiving antipsychotic monotherapy with a CPZeq dose of 1000 mg/day or less, and "non-standard" as polypharmacy (the use of more than one antipsychotic) or a CPZeq dose of more than 1000 mg/day. This classification was

according to several precedent studies (Diaz and De Leon, 2002; Edlinger et al., 2005; Ito et al., 2005; Lehman and Steinwachs, 1998; Sim et al., 2004b; Waddington et al., 1998; Weissman, 2002). The other grouping criterion was whether patients were treated only with conventional or only with atypical antipsychotics, and those who were treated with both types of antipsychotics were excluded from this grouping criterion.

2.4. Statistical analyses

Averages are reported as means±S.D. Demographic characteristics and test results were compared between groups. We used the t-test to compare mean scores. Categorical variables were compared with χ^2 test or Fisher's exact test where appropriate. The analysis of covariance (ANCOVA) was used to compare neuropsychological test results of patients and those of controls, controlling for a confounding variable. All comparisons were made between two groups, namely between patients and controls, the "standard" and "non-standard" groups, or conventional and atypical groups. Statistical significance was set at two-tailed $p < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

3.1. Sample characteristics

Demographic and clinical characteristics are presented in Table 1. There were no differences between patients and controls in sex, age, or handedness. Patients with schizophrenia demonstrated significantly shorter education years and a higher rate of cigarette smoking compared to controls. The "non-standard" group, as expected, showed significantly greater CPZeq and more frequent use of conventional antipsychotics and antiparkinsonian drugs than the "standard" group. The number of hospitalizations was significantly larger in "non-standard" than in "standard" group. The conventional group showed significantly longer duration of medication, more frequent use of antiparkinsonian drugs, and larger number of hospitalizations than the atypical group.

3.2. Neuropsychological test scores in patients vs. controls

Patients with schizophrenia showed significantly poorer performance than healthy controls on all the neuropsychological tests (Table 2). Although control

Table 1
Demographic and clinical characteristics of schizophrenia patients and controls (including comparisons of "standard" vs. "non-standard" and conventional vs. atypical group)

Variable	Schizophrenia patients ($N=67$)	Healthy controls ($N=92$)	p value (patients vs. controls)	Standard group ($N=26$)	Non-standard group ($N=41$)	p value (standard vs. non-standard)	Conventional group ($N=23$)	Atypical group ($N=22$)	p value (conventional vs. atypical)
Sex (male/female)	40/27	54/38	0.90	14/12	26/15	0.44	16/7	11/11	0.18
Age (years)	42.7±11.9	43.0±14.5	0.90	42.4±14.5	42.9±10.1	0.87	46.2±11.7	39.8±13.6	0.095
Education (years)	13.4±2.7	16.4±3.1	<0.001	13.8±2.1	13.0±3.0	0.24	13.5±3.3	13.6±2.2	0.93
Handedness (right/left)	64/3	84/8	0.50	26/0	38/3	0.28	22/1	22/0	1.0
Smoking (yes/no)	32/35	23/69	0.0029	12/14	20/21	0.83	12/11	10/12	0.65
Family history of schizophrenia (yes/no)	15/52			7/19	8/33	0.48	17/6	17/5	0.79
Age at onset (years)	25.0±8.3			26.2±9.4	24.2±7.5	0.33	24.2±7.2	26.7±10.1	0.34
Duration of medication (years)	13.3±10.4			10.6±12.8	15.1±8.3	0.090	17.9±11.5	8.2±9.9	0.0045
CPZeq of total antipsychotic drugs (mg/day)	877±749			473±268	1134±840	<0.001	804±634	521±322	0.065
Antipsychotics (conventional/both/atypical)	23/22/22			5/0/21	18/22/1	<0.001	–	–	
Antiparkinsonian drug use (yes/no)	48/19			14/12	34/7	0.010	2/21	12/10	0.0012
Age at first hospitalization (years)	29.1±12.2			29.0±15.2	29.1±10.7	0.98	30.3±10.7	27.5±14.9	0.52
Number of hospitalizations	2.1±2.1			1.2±1.7	2.7±2.1	0.0043	2.4±2.1	1.2±1.7	0.042
Duration of total hospitalizations (months)	35.0±78.4			27.0±68.8	40.1±84.4	0.51	46.3±107.2	20.0±53.1	0.31
Outpatients/inpatients	45/22			19/7	26/15	0.41	17/6	16/6	0.93
PANSS total score	63.1±17.4			59.7±17.9	65.1±17.1	0.36	63.3±17.0	57.6±16.4	0.39

Underlined figures represent significant differences.

Table 2
Test results of patients with schizophrenia and control subjects (including comparisons of standard vs. non-standard and conventional vs. atypical group)

Variable	Patients with schizophrenia (N=67)	Control subjects (N=92)	p value (patients vs. controls)	Standard group (N=26)	Non-standard group (N=41)	p value (standard vs. non-standard)	Conventional group (N=23)	Atypical group (N=22)	p value (conventional vs. atypical)
WMS-R									
Verbal memory	81.9±18.3	112.2±14.2	<0.001	86.0±19.9	79.2±16.8	0.14	85.3±16.0	83.6±20.1	0.75
Visual memory	83.8±21.0	110.1±10.4	<0.001	92.2±20.1	78.4±20.0	0.008	79.6±21.0	94.7±19.1	0.016
Attention	90.8±14.0	107.9±14.5	<0.001	92.3±14.6	89.8±13.7	0.46	92.0±12.5	92.5±14.6	0.89
Delayed recall	80.4±19.8	113.2±12.8	<0.001	88.8±20.0	75.1±17.8	0.005	78.2±14.7	90.8±20.6	0.023
WAIS-R									
Information	8.7±3.7	11.3±3.0	<0.001	9.4±3.2	8.2±3.9	0.16	9.5±3.5	8.6±3.5	0.38
Digit span	8.5±2.8	11.4±3.1	<0.001	8.7±2.5	8.4±3.0	0.74	8.5±3.3	8.8±2.0	0.72
Vocabulary	8.3±3.3	12.1±2.9	<0.001	9.5±2.9	7.6±3.3	0.015	8.4±3.4	9.2±3.2	0.40
Arithmetic	8.0±3.2	12.6±3.0	<0.001	8.3±2.6	7.8±3.5	0.47	8.7±3.4	8.3±3.0	0.66
Comprehension	7.6±3.3	11.7±2.8	<0.001	8.4±3.3	7.2±3.3	0.15	7.1±3.0	8.3±3.4	0.22
Similarities	9.6±3.3	12.7±2.0	<0.001	10.2±3.4	9.2±3.2	0.22	9.5±3.3	10.2±3.5	0.49
Picture completion	8.5±3.0	10.6±2.4	<0.001	9.3±2.3	8.0±3.3	0.056	8.8±3.1	9.1±2.2	0.70
Picture arrangement	8.1±3.3	11.7±2.4	<0.001	8.3±3.0	7.9±3.4	0.59	7.9±2.8	8.5±3.2	0.48
Block design	9.3±3.5	13.1±2.6	<0.001	10.6±3.3	8.4±3.4	0.012	9.1±3.4	10.6±3.0	0.12
Object assembly	8.3±3.4	11.5±3.0	<0.001	9.4±3.0	7.7±3.5	0.036	7.6±3.3	9.4±3.0	0.062
Digit symbol	7.0±2.8	12.6±2.7	<0.001	7.2±2.6	6.8±3.0	0.58	7.6±2.1	7.1±2.8	0.52
Verbal IQ	90.2±16.7	112.7±13.7	<0.001	94.4±14.2	87.6±17.8	0.090	91.3±16.2	93.0±15.3	0.72
Performance IQ	86.8±16.6	112.1±11.5	<0.001	92.1±13.9	83.4±17.4	0.036	86.4±14.0	91.7±14.2	0.21
Full-scale IQ	87.7±17.0	113.5±12.4	<0.001	92.7±13.9	84.4±18.0	0.0502	88.3±15.0	91.7±14.6	0.44
WCST									
Categories achieved	2.0±2.0	3.7±2.0	<0.001	2.6±2.1	1.7±1.8	0.080	1.6±1.9	2.4±2.2	0.18
Total errors	26.1±10.3	18.4±8.5	<0.001	22.1±9.8	28.7±9.9	0.010	30.6±11.3	21.9±10.0	0.0089
Perseverative errors of Milner	7.5±9.2	2.7±3.7	<0.001	5.8±8.2	8.5±9.7	0.26	11.3±12.5	5.3±7.1	0.051
Perseverative errors of Nelson	9.9±8.9	4.9±5.2	<0.001	7.2±8.1	11.5±9.1	0.055	13.5±11.3	6.0±7.0	0.0098
ATMT (spatial working memory)	27.8±8.9	35.8±8.6	<0.001	30.3±8.7	26.3±8.8	0.068	27.3±8.4	30.4±9.1	0.25

Underlined figures represent significant differences.

subjects in the present study performed rather better than general population on the standardized WMS-R and WAIS-R, performance on all indices of the two tests in patients were poorer than that in general population. Since the difference in education years between the two diagnostic groups had a possibility of confounding the difference in the test results, we performed ANCOVA, controlling for education years. It revealed that all the performance on the tests were significantly poorer in patients than in controls (all $p < 0.01$).

3.3. Test scores in standard vs. non-standard group

As presented in Table 2, mean scores on all indices of the cognitive tests were better in patients treated with the standard use of antipsychotics than in those treated with the non-standard use; seven measures reached statistical significance, i.e., visual memory, delayed recall (WMS-R), vocabulary, block design, object assembly, performance IQ (WAIS-R), and number of total errors (WCST).

3.4. Test scores in conventional vs. atypical antipsychotics group

Test results in patients treated with conventional drugs and those with atypical drugs are presented in Table 2. All mean scores except verbal memory (WMS-R), information, arithmetic, and digit symbol (WAIS-R) were favorable to the atypical antipsychotic group. The atypical group performed significantly better than the conventional group on visual memory, delayed recall, WCST total errors, and perseverative errors of Nelson.

4. Discussion

Our results confirmed that a wide range of cognitive functions including memory, attention, working memory, executive function, and general intellectual function are substantially impaired in patients with chronic schizophrenia, which is consistent with an abundance of studies (Bozikas et al., 2006; Fioravanti et al., 2005; Gold et al., 1992; Joyce and Huddy, 2004; Keefe et al., 2005; Reed et al., 2002; Silver et al., 2003; Suwa et al., 2004).

4.1. Standard vs. non-standard medication

Congruent with recent reports (Bitter et al., 2003; Chong et al., 2004; Sim et al., 2004a), non-standard use of antipsychotics (i.e., excessive use of antipsychotics or polypharmacy) was frequent in our Japanese patients with schizophrenia (standard 39% vs. non-standard

61%). Patients in non-standard group showed significantly poorer performance than those in standard group on visual memory, delayed recall, performance IQ, and executive function. Since the symptom severity (assessed with PANSS) of the two groups was similar, the difference in cognitive performance cannot be ascribed to difference in symptom severity at the time of neurocognitive tests. However, other clinical characteristics such as number of hospitalizations (with statistical significance) and duration of medication (with statistical trend), from which the original illness severity would be presumed, were different between the two treatment groups. In this situation, the illness of the non-standard group might be severer than that of the standard group at the outset, thus requiring the additional medication to reach the same level of improvement. Moreover, Joyce et al. (2005) reported that cognitive heterogeneity was present in patients with schizophrenia at illness onset. In this context, primary explanation for the association between differences of antipsychotic medication (standard/non-standard) and of cognitive function could be that both of them are due to the same cause, namely the difference of original illness severity. Alternatively, the other plausible explanation for the difference of cognitive performance between the two medication groups might be that polypharmacy and excessive dosing of antipsychotics have detrimental effects on brain and cause poorer cognitive function. This raises the possibility that cognitive deficits could be reduced by changing non-standard to standard prescription if symptoms of patients permit. Since the non-standard treatment group was more likely to be on concomitant antiparkinsonian medication, such drugs could also play a causal role in the poorer cognitive function, which was in line with prior reports (McGurk et al., 2004; Minzenberg et al., 2004; Strauss et al., 1990). To draw any conclusion, longitudinal studies that investigate from illness onset to chronic phase are necessary.

4.2. Conventional vs. atypical

When patients were divided into conventional and atypical antipsychotic groups, the latter demonstrated significantly better performance than the former on visual memory, delayed recall, and executive function. In our subjects, most patients in the atypical group were medicated with either risperidone or olanzapine. Indeed, these drugs have been reported to be superior to conventional drugs or even to other atypical antipsychotics (Bilder et al., 2002; Cuesta et al., 2001; Kern et al., 1999; McGurk et al., 2005; Mori et al., 2004;

Thornton et al., 2006). Although findings to date on specific effects of these two agents on cognition have been somewhat inconsistent, beneficial effects of risperidone on several cognitive domains including memory (Bilder et al., 2002; Keefe et al., 2006; Kern et al., 1999), executive function (Keefe et al., 2006; Meltzer and McGurk, 1999; Rossi et al., 1997), and working memory (Keefe et al., 2006; Meltzer and McGurk, 1999; Mori et al., 2004), and olanzapine on memory (Keefe et al., 2006; Meltzer and McGurk, 1999; Mori et al., 2004; Sumiyoshi et al., 2005; Thornton et al., 2006) and executive function (Bender et al., 2006; Bilder et al., 2002; Keefe et al., 2006; Meltzer and McGurk, 1999) have been reported. Precedent studies have demonstrated that atypical antipsychotics had favorable effects especially on verbal memory out of memory function (Meltzer and McGurk, 1999), which was not in harmony with our results that suggested favorable effects of atypical agents on visual memory instead of verbal memory. In general, our results might be consistent with prior studies reporting superiority of atypical to conventional antipsychotics in terms of cognitive function. In the present study, however, clinical characteristics such as duration of medication, number of hospitalizations, and antiparkinsonian drug use were significantly different between the two medication groups. Moreover, CPZeq reached nearly significant difference between the two groups. In this study, therefore, causal relationship between medication type (conventional/atypical) and cognitive function is quite difficult to argue due to these confounders.

4.3. Limitations

There were several limitations to the current study. The cross-sectional nature of the study did not allow drawing any definite conclusions regarding causality between antipsychotic medication and its correlates. Since control subjects in the present study performed better than the general population on the standardized WMS-R and WAIS-R, it was possible that the differences between patients and controls were exaggerated. As the patients involved in this study suffered from chronic schizophrenia, the findings can not be generalized to recent-onset schizophrenia. The sample size was not very large, which may have resulted in type II errors.

4.4. Conclusion

In conclusion, this study confirms that patients with chronic schizophrenia have wide-ranging cognitive impairments and provides evidence for an association between antipsychotic medication (both standard/non-standard and conventional/atypical) and cognitive function. The differences of medication and of cognitive function are associated but both could be due to original illness severity. Alternatively, cognitive deficits in schizophrenia could be reduced in part by specific medication pattern, especially by atypical antipsychotic monotherapy at ordinary dosage.
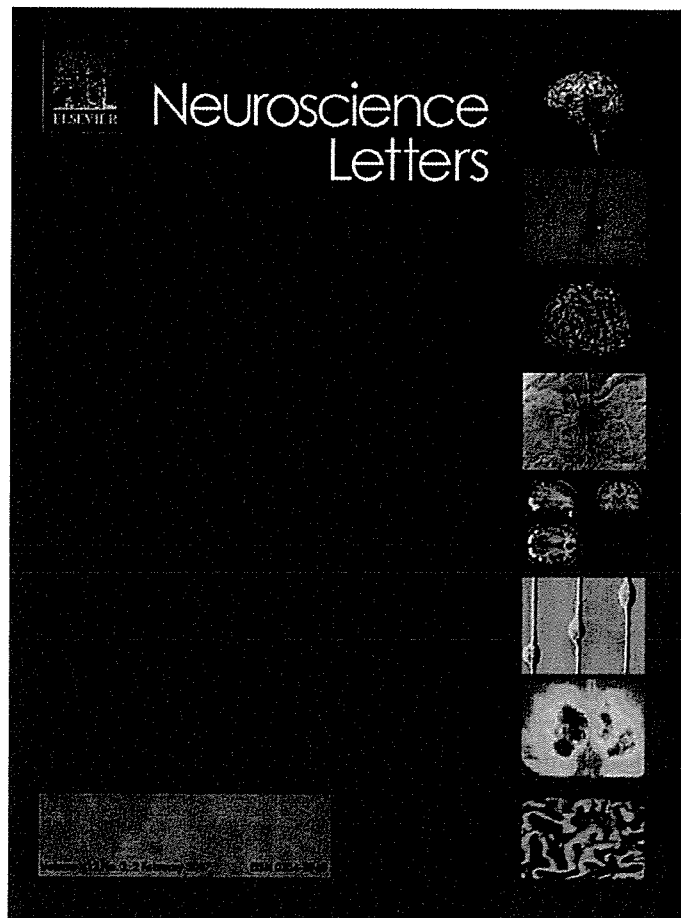
References

Addington, J., Addington, D., Maticka-Tyndale, E., 1991. Cognitive functioning and positive and negative symptoms in schizophrenia. Schizophr. Res. 5, 123–134.

American Psychiatric Association, 1994. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Association, Washington, DC.

American Psychiatric Association, 1997. Practice Guidelines for the Treatment of Patients with Schizophrenia. American Psychiatric Press, Washington, DC.

Bell, M.D., Mishara, A.L., 2006. Does negative symptom change relate to neurocognitive change in schizophrenia? Implications for targeted treatments. Schizophr. Res. 81, 17–27.

Bender, S., Dittmann-Balcar, A., Schall, U., Wolstein, J., Klimke, A., Riedel, M., Vorbach, E.U., Kuhn, K.U., Lambert, M., Dittmann, R.W., Naber, D., 2006. Influence of atypical neuroleptics on executive functioning in patients with schizophrenia: a randomized, double-blind comparison of olanzapine vs. clozapine. Int. J. Neuropsychopharmacol. 9, 135–145.

Bilder, R.M., Lipschutz-Broch, L., Reiter, G., Geisler, S.H., Mayerhoff, D.I., Lieberman, J.A., 1992. Intellectual deficits in first-episode schizophrenia: evidence for progressive deterioration. Schizophr. Bull. 18, 437–448.

Bilder, R.M., Goldman, R.S., Volavka, J., Czobor, P., Hoptman, M., Sheitman, B., Lindenmayer, J.P., Citrome, L., McEvoy, J., Kunz, M., Chakos, M., Cooper, T.B., Horowitz, T.L., Lieberman, J.A., 2002. Neurocognitive effects of clozapine, olanzapine, risperidone, and haloperidol in patients with chronic schizophrenia or schizoaffective disorder. Am. J. Psychiatry 159, 1018–1028.

Bitter, I., Chou, J.C., Ungvari, G.S., Tang, W.K., Xiang, Z., Iwanami, A., Gaszner, P., 2003. Prescribing for inpatients with schizophrenia: an international multi-center comparative study. Pharmacopsychiatry 36, 143–149.

Bleuler, E., 1950. In: Zinkin, J. (Ed.), Dementia Praecox or the Group of Schizophrenias. International Universities Press, New York.

Bozikas, V.P., Kosmidis, M.H., Kiosseoglou, G., Karavatos, A., 2006. Neuropsychological profile of cognitively impaired patients with schizophrenia. Compr. Psychiatry 47, 136–143.

Chong, M.Y., Tan, C.H., Fujii, S., Yang, S.Y., Ungvari, G.S., Si, T., Chung, E.K., Sim, K., Tsang, H.Y., Shinfuku, N., 2004. Antipsychotic drug prescription for schizophrenia in East Asia: rationale for change. Psychiatry Clin. Neurosci. 58, 61–67.

Cuesta, M.J., Peralta, V., Zarzuela, A., 2001. Effects of olanzapine and other antipsychotics on cognitive function in chronic schizophrenia: a longitudinal study. Schizophr. Res. 48, 17–28.

Davidson, M., Harvey, P.D., Powchik, P., Parrella, M., White, L., Knobler, H.Y., Losonczy, M.F., Keefe, R.S., Katz, S., Frecska, E., 1995. Severity of symptoms in chronically institutionalized geriatric schizophrenic patients. Am. J. Psychiatry 152, 197–207.

Diaz, F.J., De Leon, J., 2002. Excessive antipsychotic dosing in 2 U.S. state hospitals. J. Clin. Psychiatry 63, 998–1003.

Edlinger, M., Hausmann, A., Kemmler, G., Kurz, M., Kurzthaler, I., Walch, T., Walpoth, M., Fleischhacker, W.W., 2005. Trends in the pharmacological treatment of patients with schizophrenia over a 12 year observation period. Schizophr. Res. 77, 25–34.

Erlenmeyer-Kimling, L., Rock, D., Roberts, S.A., Janal, M., Kestenbaum, C., Cornblatt, B., Adamo, U.H., Gottesman II, 2000. Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: the New York High-Risk Project. Am. J. Psychiatry 157, 1416–1422.

Faries, D., Ascher-Svanum, H., Zhu, B., Correll, C., Kane, J., 2005. Antipsychotic monotherapy and polypharmacy in the naturalistic treatment of schizophrenia with atypical antipsychotics. BMC Psychiatry 5 (26).

Fioravanti, M., Carlone, O., Vitale, B., Cinti, M.E., Clare, L., 2005. A meta-analysis of cognitive deficits in adults with a diagnosis of schizophrenia. Neuropsychol. Rev. 15, 73–95.

Fuller, R., Nopoulos, P., Arndt, S., O'Leary, D., Ho, B.C., Andreasen, N.C., 2002. Longitudinal assessment of premorbid cognitive functioning in patients with schizophrenia through examination of standardized scholastic test performance. Am. J. Psychiatry 159, 1183–1189.

Ganguly, R., Kotzan, J.A., Miller, L.S., Kennedy, K., Martin, B.C., 2004. Prevalence, trends, and factors associated with antipsychotic polypharmacy among Medicaid-eligible schizophrenia patients, 1998–2000. J. Clin. Psychiatry 65, 1377–1388.

Gold, J.M., Randolph, C., Carpenter, C.J., Goldberg, T.E., Weinberger, D.R., 1992. Forms of memory failure in schizophrenia. J. Abnorm. Psychology 101, 487–494.

Green, M.F., 1996. What are the functional consequences of neurocognitive deficits in schizophrenia? Am. J. Psychiatry 153, 321–330.

Green, M.F., Kern, R.S., Braff, D.L., Mintz, J., 2000. Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"? Schizophr. Bull. 26, 119–136.

Green, M.F., Barnes, T.R., Danion, J.M., Gallhofer, B., Meltzer, H.Y., Pantelis, C., 2005. The FOCIS international survey on psychiatrists' opinions on cognition in schizophrenia. Schizophr. Res. 74, 253–261.

Harvey, P.D., Lombardi, J., Leibman, M., White, L., Parrella, M., Powchik, P., Davidson, M., 1996. Cognitive impairment and negative symptoms in geriatric chronic schizophrenic patients: a follow-up study. Schizophr. Res. 22, 223–231.

Harvey, P.D., Koren, D., Reichenberg, A., Bowie, C.R., 2005. Negative symptoms and cognitive deficits: what is the nature of their relationship? Schizophr. Bull. 32, 250–258.

Harvey, P.D., Bowie, C.R., Loebel, A., 2006. Neuropsychological normalization with long-term atypical antipsychotic treatment:

results of a six-month randomized, double-blind comparison of ziprasidone vs. olanzapine. J. Neuropsychiatry Clin. Neurosci. 18, 54–63.

Heaton, R.K., 1981. The Wisconsin Card Sorting Test (Manual). Psychological Assessment Resources, Odessa, FL.

Hofer, A., Baumgartner, S., Bodner, T., Edlinger, M., Hummer, M., Kemmler, G., Rettenbacher, M.A., Fleischhacker, W.W., 2005. Patient outcomes in schizophrenia: II. The impact of cognition. Eur. Psychiatr. 20, 395–402.

Hoff, A.L., Riordan, H., O'Donnell, D.W., Morris, L., DeLisi, L.E., 1992. Neuropsychological functioning of first-episode schizophreniform patients. Am. J. Psychiatry 149, 898–903.

Inagaki, A., Inada, T., Fujii, Y., Yagi, G., 1999. Equivalent Dose of Psychotropics. Seiwa Shoten, Tokyo. in Japanese.

Ito, H., Koyama, A., Higuchi, T., 2005. Polypharmacy and excessive dosing: psychiatrists' perceptions of antipsychotic drug prescription. Br. J. Psychiatry 187, 243–247.

Joyce, E., Huddy, V., 2004. Defining the cognitive impairment in schizophrenia. Psychol. Med. 34, 1151–1155.

Joyce, E.M., Hutton, S.B., Mutsatsa, S.H., Barnes, T.R., 2005. Cognitive heterogeneity in first-episode schizophrenia. Br. J. Psychiatry 187, 516–522.

Kashima, H., Handa, T., Kato, M., Sakura, K., Yokoyama, N., Murakami, M., Shigemori, K., Muramatsu, T., Saito, H., Ooe, Y., Mimura, M., Asai, M., Hosaki, H., 1987. Neuropsychological investigation on chronic schizophrenia—aspects of its frontal functions. In: Takahashi, R., Flor-Henry, P., Gruzelier, J., Niwa, S. (Eds.), Cerebral Dynamics, Laterality and Psychopathology. Elsevier, Amsterdam, pp. 337–345.

Kay, S.R., Opler, L.A., Fiszbein, A., 1987. Positive and Negative Syndrome Scale (PANSS) manual. Schizophr. Bull. 13, 261–276.

Keefe, R.S., Silva, S.G., Perkins, D.O., Lieberman, J.A., 1999. The effects of atypical antipsychotic drugs on neurocognitive impairment in schizophrenia: a review and meta-analysis. Schizophr. Bull. 25, 201–222.

Keefe, R.S., Eesley, C.E., Poe, M.P., 2005. Defining a cognitive function decrement in schizophrenia. Biol. Psychiatry 57, 688–691.

Keefe, R.S., Young, C.A., Rock, S.L., Purdon, S.E., Gold, J.M., Breier, A., HGGN Study Group, 2006. One-year double-blind study of the neurocognitive efficacy of olanzapine, risperidone, and haloperidol in schizophrenia. Schizophr. Res. 81, 1–15.

Kern, R.S., Green, M.F., Marshall Jr., B.D., Wirshing, W.C., Wirshing, D., McGurk, S.R., Marder, S.R., Mintz, J., 1999. Risperidone versus haloperidol on secondary memory: can newer medications aid learning? Schizophr. Bull. 25, 223–232.

Kobayashi, S., 1999. Wisconsin Card Sorting Test Program Keio-F-S-version (Web site, in Japanese). Available at: http://cvddb.shimane-med.ac.jp/user/wisconsin.htm. Accessed May 14, 2003.

Kraepelin, E., 1919. In: Barclay, R.M., Roberston, G.M. (Eds.), Dementia Praecox and Paraphrenia. E 7 S Livingstone, Edinborough.

Kremen, W.S., Buka, S.L., Seidman, L.J., Goldstein, J.M., Koren, D., Tsuang, M.T., 1998. IQ decline during childhood and adult psychotic symptoms in a community sample: a 19-year longitudinal study. Am. J. Psychiatry 155, 672–677.

Lehman, A.F., Steinwachs, D.M., 1998. Translating research into practice: the Schizophrenia Patient Outcomes Research Team (PORT) treatment recommendations. Schizophr. Bull. 24, 1–10.

McGurk, S.R., Green, M.F., Wirshing, W.C., Wirshing, D.A., Marder, S.R., Mintz, J., Kern, R., 2004. Antipsychotic and anticholinergic effects on two types of spatial memory in schizophrenia. Schizophr. Res. 68, 225–233.

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McGurk, S.R., Carter, C., Goldman, R., Green, M.F., Marder, S.R., Xie, H., Schooler, N.R., Kane, J.M., 2005. The effects of clozapine and risperidone on spatial working memory in schizophrenia. Am. J. Psychiatry 162, 1013–1016.

Medalia, A., Gold, J., Merriam, A., 1988. The effects of neuroleptics on neuropsychological test results of schizophrenics. Arch. Clin. Neuropsychol. 3, 249–271.

Meltzer, H.Y., McGurk, S.R., 1999. The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. Schizophr. Bull. 25, 233–255.

Minzenberg, M.J., Poole, J.H., Benton, C., Vinogradov, S., 2004. Association of anticholinergic load with impairment of complex attention and memory in schizophrenia. Am. J. Psychiatry 161, 116–124.

Mori, K., Nagao, M., Yamashita, H., Morinobu, S., Yamawaki, S., 2004. Effect of switching to atypical antipsychotics on memory in patients with chronic schizophrenia. Prog. Neuro-psychopharmacol. Biol. Psychiatry 28, 659–665.

Muller, U., Werheid, K., Hammerstein, E., Jungmann, S., Becker, T., 2005. Prefrontal cognitive deficits in patients with schizophrenia treated with atypical or conventional antipsychotics. Eur. Psychiatr. 20, 70–73.

Nakahachi, T., Iwase, M., Takahashi, H., Honaga, E., Sekiyama, R., Ukai, S., Ishii, R., Ishigami, R., Kajimoto, O., Yamashita, K., Hashimoto, R., Shimizu, A., Takeda, M., 2006. Discrepancy of performance among working memory related tasks in autism spectrum disorders was caused by task characteristics except working memory which could interfere with task execution. Psychiatry Clin. Neurosci. 60, 312–318.

Procyshyn, R.M., Kennedy, N.B., Tse, G., Thompson, B., 2001. Antipsychotic polypharmacy: a survey of discharge prescriptions from a tertiary care psychiatric institution. Can. J. Psychiatry 46, 334–339.

Purdon, S.E., Jones, B.D., Stip, E., Labelle, A., Addington, D., David, S.R., Breier, A., Tollefson, G.D., 2000. Neuropsychological change in early phase schizophrenia during 12 months of treatment with olanzapine, risperidone, or haloperidol. The Canadian Collaborative Group for Research in Schizophrenia. Arch. Gen. Psychiatry 57, 249–258.

Reed, R.A., Harrow, M., Herbener, E.S., Martin, E.M., 2002. Executive function in schizophrenia: is it linked to psychosis and poor life functioning? J. Nerv. Ment. Dis. 190, 725–732.

Reichenberg, A., Weiser, M., Rapp, M.A., Rabinowitz, J., Caspi, A., Schmeidler, J., Knobler, H.Y., Lubin, G., Nahon, D., Harvey, P.D., Davidson, M., 2005. Elaboration on premorbid intellectual performance in schizophrenia: premorbid intellectual decline and risk for schizophrenia. Arch. Gen. Psychiatry 62, 1297–1304.

Reitan, R.M., Wolfson, D., 1993. The Halstead Reitan Neuropsychological Test Battery: Theory and Clinical Interpretation. Neuropsychology Press, Tucson, AZ.

Rossi, A., Mancini, F., Stratta, P., Mattei, P., Gismondi, R., Pozzi, F., Casacchia, M., 1997. Risperidone, negative symptoms and cognitive deficit in schizophrenia: an open study. Acta Psychiatr. Scand. 95, 40–43.

Silver, H., Feldman, P., Bilker, W., Gur, R.C., 2003. Working memory deficit as a core neuropsychological dysfunction in schizophrenia. Am. J. Psychiatry 160, 1809–1816.

Sim, K., Su, A., Fujii, S., Yang, S.Y., Chong, M.Y., Ungvari, G.S., Si, T., Chung, E.K., Tsang, H.Y., Chan, Y.H., Heckers, S., Shinfuku, N., Tan, C.H., 2004a. Antipsychotic polypharmacy in patients with schizophrenia: a multicentre comparative study in East Asia. Br. J. Clin. Pharmacol. 58, 178–183.

Sim, K., Su, A., Leong, J.Y., Yip, K., Chong, M.Y., Fujii, S., Yang, S., Ungvari, G.S., Si, T., Chung, E.K., Tsang, H.Y., Shinfuku, N., Kua, E.H., Tan, C.H., 2004b. High dose antipsychotic use in schizophrenia: findings of the REAP (Research on East Asia Psychotropic Prescriptions) study. Pharmacopsychiatry 37, 175–179.

Shinagawa, F., Kobayashi, S., Fujita, K., Maekawa, H., 1990. Japanese Wechsler Adult Intelligence Scale-Revised. Nihonbunkakagakusha, Tokyo. in Japanese.

Spohn, H.E., Strauss, M.E., 1989. Relation of neuroleptic and anticholinergic medication to cognitive functions in schizophrenia. J. Abnorm. Psychology 98, 367–380.

Strauss, M.E., Reynolds, K.S., Jayaram, G., Tune, L.E., 1990. Effects of anticholinergic medication on memory in schizophrenia. Schizophr. Res. 3, 127–129.

Sugishita, M., 2001. Japanese Wechsler Memory Scale-Revised. Nihonbunkakagakusha, Tokyo. in Japanese.

Sumiyoshi, C., Sumiyoshi, T., Roy, A., Jayathilake, K., Meltzer, H.Y., 2005. Atypical antipsychotic drugs and organization of long-term semantic memory: multidimensional scaling and cluster analyses of category fluency performance in schizophrenia. Int. J. Neuropsychopharmacol. 29, 1–7.

Suwa, H., Matsushima, E., Ohta, K., Mori, K., 2004. Attention disorders in schizophrenia. Psychiatry Clin. Neurosci. 58, 249–256.

Takahashi, H., Iwase, M., Nakahachi, T., Sekiyama, R., Tabushi, K., Kajimoto, O., Shimizu, A., Takeda, M., 2005. Spatial working memory deficit correlates with disorganization symptoms and social functioning in schizophrenia. Psychiatry Clin. Neurosci. 59, 453–460.

Thornton, A.E., Van Snellenberg, J.X., Sepehry, A.A., Honer, W., 2006. The impact of atypical antipsychotic medications on long-term memory dysfunction in schizophrenia spectrum disorder: a quantitative review. J. Psychopharmacol. 20, 335–346.

Waddington, J.L., Youssef, H.A., Kinsella, A., 1998. Mortality in schizophrenia. Antipsychotic polypharmacy and absence of adjunctive anticholinergics over the course of a 10-year prospective study. Br. J. Psychiatry 173, 325–329.

Wechsler, D., 1981. Wechsler Adult Intelligence Scale, Revised. Psychological Corporation, New York.

Wechsler, D., 1987. Wechsler Memory Scale Manual, Revised. Psychological Corporation, San Antonio.

Weissman, E.M., 2002. Antipsychotic prescribing practices in the Veterans Healthcare Administration—New York Metropolitan Region. Schizophr. Bull. 28, 31–42.

Neuroscience Letters

A new gain-of-function allele in chimpanzee tryptophan hydroxylase 2 and the comparison of its enzyme activity with that in humans and rats

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Abstract

Tryptophan hydroxylase 2 (TPH2) is a rate-limiting enzyme of neuronal serotonin biosynthesis. Recently, two single nucleotide polymorphisms (SNPs) at the exon 11 coding region that resulted in amino acid substitutions in the C-terminal domain have been reported to affect enzyme activity in humans and mice. We determined 175 base-pair sequences of the exon 11 region in nine primate species from all recognized lineages. All nucleotide sequence substitutions were synonymous, with the exception of one adenine (A) to guanine (G) substitution at the 1404th position in the open reading frame (ORF). This substitution leads to a glutamine (Q) to arginine (R) amino acid substitution at the 468th position within chimpanzee sequences. The frequency of the G allele was 0.24 among 66 chimpanzees. Therefore, it is a novel SNP observed in chimpanzees, and we have named these two alleles as *ch468Q* and *ch468R*, respectively. When expressed in HeLa cells, *ch468R* caused an approximate 20% increase in enzyme function during L-5-hydroxytryptophan (5HTP) production ($P < 0.001$). We also surveyed the interspecies difference in enzyme activity among human, chimpanzee, and rat. Although the rat showed an identical amino acid sequence at the C-terminal region as those of human and *ch468Q*, the rat enzyme was more active than those of human or chimpanzee ($P < 0.001$), indicating the importance of substitutions in other regions. Our findings on the chimpanzee SNP will be a useful genetic marker in understanding the individual difference in the serotonin-related behavior.
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Serotonin synthesis is mediated by the rate-limiting enzyme tryptophan hydroxylase (TPH), which catalyzes the pterin-dependent hydroxylation of L-tryptophan to L-5-hydroxytryptophan (5HTP). Additionally, this reaction is the first step in the synthesis of melatonin, which is involved in the regulation of mammalian reproduction and circadian rhythms [27]. The TPH belongs to the family of tetrahydrobiopterin-dependent aromatic amino acid hydroxylases, which includes phenylalanine hydroxylase (PAH) and tyrosine hydroxylase (TH) [14]. These enzymes

share similar protein organization composed of a regulatory N-terminal domain, a catalytic domain, and a short C-terminal oligomerization domain (Fig. 1a) [6–8]. Although the precise manner is often unique for each hydroxylase, they can be activated by similar mechanisms [4,9,10].

Recently, TPH isoform 2 was discovered to be preferentially located in brain dorsal raphe [23,28] and in the peripheral myenteric neurons in the gut [4]. The discovery of TPH2 has renewed great interest in studying the role of this enzyme in the neurochemical function of serotonin. To date, over 500 single nucleotide polymorphisms (SNPs) have been identified in the TPH2 gene in humans and mice, but coding non-synonymous SNPs are only six [3,33]. A number of recent studies have