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Dopamine Transporter Polymorphism Modulates Oculomotor Function and DAT1 mRNA Expression in Schizophrenia

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Abstract

Smooth pursuit eye movement (SPEM) deficit is an established schizophrenia endophenotype with a similar neurocognitive construct to working memory. Frontal eye field (FEF) neurons controlling SPEM maintain firing when visual sensory information is removed, and their firing rates directly correlate with SPEM velocity. We previously demonstrated a paradoxical association between a functional polymorphism of dopamine signaling (*COMT* gene) and SPEM. Recent evidence implicates the dopamine transporter gene (*DAT1*) in modulating cortical dopamine and associated neurocognitive functions. We hypothesized that *DAT1* 10/10 genotype, which reduces dopamine transporter expression and increases extracellular dopamine, would affect SPEM. We examined the effects of *DAT1* genotype on: Clinical diagnosis in the study sample (n=418; 190 with schizophrenia), SPEM measures in a subgroup with completed oculomotor measures (n=200; 87 schizophrenia), and *DAT1* gene expression in FEF tissue obtained from postmortem brain samples (n=32; 16 schizophrenia). *DAT1* genotype was not associated with schizophrenia. *DAT1* 10/10 genotype was associated with better SPEM in healthy controls, intermediate SPEM in unaffected first-degree relatives of schizophrenia subjects, and worse SPEM in schizophrenia subjects. In the gene expression study, *DAT1* 10/10 genotype was associated with significantly reduced *DAT1* mRNA transcript in FEF tissue from healthy control donors (p<0.05), but higher expression in schizophrenia donors. Findings suggest regulatory effects of another gene(s) or etiological factor in schizophrenia, which modulate *DAT1* gene function.

Keywords

DAT1 gene; expression; endophenotype; schizophrenia

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INTRODUCTION

The use of endophenotypes may help reduce phenotypic and genetic heterogeneity in schizophrenia. For genetic studies, useful endophenotypes should be heritable, frequently expressed in probands, and stable over time (Gottesman and Gould, 2003). Deficits in smooth pursuit eye movement (SPEM) are some of the most established markers in schizophrenia (Holzman, 2000; Hong et al., 2006b; Thaker et al., 1998; Calkins and Iacono, 2000). SPEM deficits are also present in unaffected relatives of schizophrenia patients (i.e., relatives without DSM-IV schizophrenia), suggesting that they may reflect underlying genetic liabilities for schizophrenia (Karoumi et al., 2001). The predictive component of the SPEM phenotype has an estimated broad sense heritability of 0.9 in families with schizophrenia vs. 0.27 for the traditional global measure of eyetracking (Hong et al., 2006a). Pursuit eye movements are initiated by the sensory signal of a target's image on the retina. Once the eyes achieve the velocity of the moving target, accurate pursuit is maintained by predicting target position based on internal representations of previous target velocity. FEF neurons controlling predictive pursuit maintain firing when the target sensory information is removed; and their firing rates directly correlate with predictive eye velocity (Tanaka and Fukushima, 1998). Dopamine signaling modulates overlapping components of the predictive pursuit circuitry (Krauzlis, 2004; Fukushima et al., 2003).

Evidence implicates dopamine dysregulation in schizophrenia-related prefrontal dysfunction. Catechol-*O*-methyltransferase (COMT) and dopamine transporter (DAT) are major regulators of dopamine signaling in the brain. Several studies note association between schizophrenia-related neurocognitive phenotypes and the functional Val¹⁵⁸Met single nucleotide polymorphism in *COMT* gene (Tunbridge et al., 2006). The dopamine transporter regulates the duration of extracellular dopamine activity (Tunbridge et al., 2006). Its gene (*DAT1*) maps to chromosome 5p15.3 and contains 15 exons spanning about 53kb (HGNC: 11049: [SLC6A3]). *DAT1* contains a 40-bp variable number of tandem repeats (VNTR) polymorphism in the 3'-untranslated region (3'-UTR), which repeats 3-13 times, with the 9- and 10- repeat alleles displaying the highest frequencies in human populations.

The VNTR has been associated with attention deficit hyperactivity disorder and bipolar disorder, with inconsistent findings in schizophrenia and Parkinson's disease (Cook, Jr. et al., 1995; Kelada et al., 2005; Gamma et al., 2005). Reports suggest a functional effect of the *DAT1* 10-repeat variant (10/10 genotype) in modulating cortical dopamine compared to non-10/10 genotypes. Compared to non-10/10 genotypes, the 10/10 genotype results in decreased DAT expression and putatively, increased synaptic dopamine tone (Jacobsen et al., 2000; van Dyck et al., 2005), but other reports have demonstrated opposite genotype effects (Kelada et al., 2005; VanNess et al., 2005; Brookes et al., 2007). Although *DAT1* VNTR is not in a coding region, it is known to regulate gene expression by affecting stability and translational efficiency of transcribed mRNA (Conne et al., 2000; Greenwood and Kelsoe, 2003).

We have previously demonstrated a paradoxical association between *COMT* Val¹⁵⁸Met and predictive pursuit gain (PPG), a refined component of SPEM (Thaker et al., 2004) (see Figure 1). Our preliminary findings with *COMT* gene suggest that in healthy subjects, increased dopamine (*COMT* Met/Met genotype) resulted in improved SPEM performance, but was detrimental to SPEM performance in schizophrenia patients (O'Driscoll et al., 2000). Based on our *COMT* findings, we hypothesized that *DAT1* 10/10 genotype, which reduces DAT expression and hence increases extracellular dopamine, would have a similar effect on SPEM function. Moreover, given the inconsistent reports on the functional effect of *DAT1* VNTR on DAT expression, we examined *DAT1* genotype effects on mRNA transcript expression in postmortem brain tissue from the frontal eye fields (FEF); a cortical area related to abnormal

SPEM in schizophrenia patients and their relatives (Hong et al., 2005; O'Driscoll et al., 1999; Berman et al., 1999).

MATERIALS AND METHODS

Subjects

Four hundred and eighteen individuals participated in this study. Schizophrenia subjects were recruited from outpatient programs at the Maryland Psychiatric Research Center (MPRC), Community Mental Health Centers, and by newspaper advertisements targeting the Baltimore Metropolitan area. Healthy controls were recruited in two ways, 1) Matched controls (matched to patients by age, ethnicity, sex and zip code) were recruited using motor vehicle administration records, and, 2) Unmatched controls who responded to newspaper advertisements. Consent to contact family members of schizophrenia subjects' was obtained to screen and recruit eligible first-degree relatives.

Our sample included 243 self-identified European-American and 175 self-identified African-American subjects (total n=418:190 schizophrenia, 68 unaffected first-degree relatives of schizophrenia patients, and 160 healthy controls). Oculomotor testing and scoring has been completed in 200 of the 418 subjects, consisting of 87 schizophrenia individuals, 40 relatives (9 with schizophrenia spectrum personality, and 31 without Axis II disorder), and 73 controls (Table 1). All subjects gave written informed consent in accordance with University of Maryland IRB guidelines. *The Structured Clinical Interview for DSM-IV* (SCID-IV) was administered to all subjects to obtain DSM-IV diagnoses. Patients were individuals with DSM-IV schizophrenia. Healthy controls and unaffected relatives were administered *The Structured Interview for DSM-IV Personality Diagnoses* (SIDP), and *The Structured Interview for the Assessment of Schizotypal Symptoms* (SIS). Healthy controls did not meet DSM-IV criteria for Axis I disorders and had no family history of schizophrenia. Nine unaffected relatives met the study criteria for schizophrenia spectrum personality (SSP) - the presence of 3 or more paranoid or schizoid traits, or 4 or more schizotypal traits. In schizophrenia subjects, evaluation of the capacity to sign consent was performed to assess their understanding of the study prior to signing consent.

Based on the known effects of age on smooth pursuit eye movements, only subjects within the age range of 16-58 years were included (Ross et al., 1999). We excluded subjects with general medical, ophthalmologic, or neurological conditions. Additionally, subjects were excluded if they reported substance dependence within 6 months prior to study enrollment or had current substance abuse. Specifically, no subject was taking stimulants, abusing alcohol, or on other substances known to affect expression of the dopamine transporter.

Human postmortem tissue

Human postmortem brain samples (n=32) were collected from the Maryland Brain Collection (MBC) at the MPRC from the FEF, a portion of Brodmann's area 6. FEF tissue was dissected as shown in Rosano et al (2003), i.e., from the caudal end of superior frontal sulcus (SFS) at the SFS-precentral sulcus junction, and stored at -80°C (Rosano et al., 2003). Imaging studies identify pursuit-related human FEF in precentral sulcus (PCS), posterior to the caudal end of the SFS (Berman et al., 1999; O'Driscoll et al., 2000; Rosano et al., 2002).

The samples were from 16 schizophrenia donors (6 on conventional antipsychotics, 5 atypicals, 3 not on medications for 6 months or more before death, and 2 of unknown medication status at time of death), and 16 normal controls with no psychiatric history. Samples were selected as case-control pairs and were tightly matched on sex, age, postmortem interval (PMI), pH, hemisphere, and freezer time (Table 2). Tissues were obtained with consent from the legal next

of kin. Postmortem clinical diagnosis was established according to the method of Roberts and colleagues (1998)(Roberts et al., 1998). Clinical information was obtained through family interviews with the next of kin, including the Structured Clinical Interview for DSM-IV (SCID-IV) (First et al., 1997). Past medical and psychiatric records were reviewed in a consensus diagnosis performed by senior psychiatrists (Dr. Robert Conley, and either Dr. William T. Carpenter, Jr. or, Dr. Carol Tamminga), using the Diagnostic Evaluation After Death (DEAD) (Zalcman and Endicott, 1983).

RNA extraction and reverse transcription

RNA extraction and reverse transcription was performed following the method of Lipska et al (2006) (Lipska et al., 2006). Briefly, tissue was pulverized and stored at -80°C. Total RNA was extracted using TRIZOL Reagent (Life Technologies Inc., Grand Island, NY, USA). Total RNA yield was determined by absorbance at 260 nm. RNA quality was confirmed by high-resolution capillary electrophoresis on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA). An RNA integrity number (RIN), obtained from the entire Agilent electrophoretic trace with RIN software algorithm, was used for the assessment of RNA quality (scale 1-10, with 1 being the lowest and 10 being the highest RNA quality)(Schroeder et al., 2006). RNA samples showing clearly defined, sharp, 18S and 28S ribosomal peaks, 28S/18S ratios >1.2 and RIN > 5.0 were included (all 32 samples met these criteria). Total RNA (1mg) was used for 20µl of reverse transcriptase reaction to synthesize cDNA, using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA).

Probes and primers

Commercial TaqMan FAM dye-labeled MGB probe/primer sets were used for *DAT1* (Gene Expression Assay ID: Hs00997371_m1, Applied Biosystems, Foster City, CA, USA), and for two endogenous control genes: glyceraldehyde-3-phosphate dehydrogenase (GAPDH: Hs99999905_m1) and β -actin, (ACTB: Hs99999903_m1).

Real-Time Quantitative PCR (RT-qPCR)

Expression levels of *DAT1* mRNA were measured with RT-qPCR using relative quantitation (RQ) by comparative C_T assay, in an ABI 7900HT Fast System with 96-well reaction plates (Applied Biosystems) normalized to two reference genes (GAPDH and ACTB) (Lipska et al, 2006). Each 10-20µl reaction contained 900 nM of primer, 250 nM of probe and TaqMan Universal PCR Mastermix (Applied Biosystems) containing Hot Goldstar DNA Polymerase, deoxynucleoside triphosphates (dNTP)s with deoxyuridine triphosphate (dUTP), uracil-N-glycosylase, passive reference, and cDNA template generated from 100-200 ng of RNA. The PCR cycle parameters were 50°C for 2 min, 95°C for 10min, 40 cycles of 95°C for 15sec, and 59°C or 60°C for 1 min. PCR data were acquired from the Sequence Detection Software (SDS version 2.2.2, Applied Biosystems) and quantified by RQ analysis. Amplification efficiency was quantified by a standard curve method with serial dilutions of pooled cDNA derived from RNA obtained from FEF of 6 normal control samples. All sample wells were run singleplex for target gene and endogenous control genes as triplicates. One sample was chosen as the reference for each plate and SDS calculated gene expression levels as an average of RQ values.

DAT1 VNTR genotyping

Genomic DNA was isolated from peripheral leukocytes in whole blood and from pulverized postmortem tissue using the QIAamp DNA Blood Maxi Kit (Qiagen) and DNeasy Tissue Kit (Qiagen), respectively. Genotyping was performed by PCR amplification of the 40-bp sequence of the VNTR polymorphic loci as described elsewhere (Szekeres et al., 2004).

Oculomotor measures

Oculomotor methods are described in detail elsewhere (Thaker et al., 2003). In the smooth pursuit task, a target starts from a center fixation and moves back and forth across the screen as the eyes “track” it. Subjects were instructed to follow the moving target (velocity of 18.7°/s; amplitude $\pm 12^\circ$) across the computer monitor with their eyes. After four to six half-cycles, the target was unpredictably masked (made invisible) for 500 ms. Of the 25 trials presented, 15 had the mask appearing variably sometime during a half-cycle, and in the rest of the trials at the change in ramp direction. Participants were instructed to follow the moving target even when it became briefly invisible. Pursuit gain measures from the mask period from these two types of trials were averaged to obtain a measure of predictive pursuit gain (PPG) (Figure 1). Maintenance pursuit gain was obtained by dividing average artifact-free eye velocity for the half cycle preceding the mask, by the target velocity. Scoring of eye movement data was performed blind to subject group or diagnosis and are described elsewhere (Thaker et al., 2003).

Statistical analyses

Genotype frequencies were compared between groups using chi-square tests. We considered the ‘at risk’ genotype to be 10/10 based on previous reports of a functional effect of 10/10 genotype on *DAT1* expression, compared to non-10/10 genotypes (van Dyck et al., 2005). The association of *DAT1* genotype with predictive pursuit gain (PPG) variance was examined using mixed model repeated measures ANOVA, with familial relatedness as a fixed factor, and genotype (10/10 vs. non-10/10) and diagnosis group (schizophrenia, unaffected relatives [with and without SSP], and controls) modeled as independent variables. In this sample there was no significant correlation between age and PPG ($r = -0.06$, NS). Racial distribution was even across groups and the initial analysis showed no effects of race and therefore race was not considered as a between subjects factor. Since the mRNA expression data were skewed (skewness of 4.1, SE 0.4), these data were first Log10 transformed (skewness 0.05, SE 0.4). There were no effects of age, gender, PMI and pH on the Log10 mRNA expression. Subsequent analyses used ANOVA with diagnosis and genotype (10/10 or non-10/10) to examine the effects of genotype on mRNA expression.

RESULTS

Seven alleles were identified, corresponding to base pair repeats of sizes 5, 6, 7, 8, 9, 10, and 11. *DAT1* genotype frequencies were 10/10: 70.6%; 10/9: 13.0%; 9/9: 9.9%. The others (5/5, 9/8, 10/6, 10/7, 10/8, 11/9, and 11/10) had a combined frequency of 6.5%. The frequencies of the 10/10 vs. non-10/10 genotypes did not differ significantly between European and African origin subjects ($n = 418$, $\chi^2 = 0.88$, $df = 1$, $p = 0.40$), and subjects were therefore combined across ethnicity for all analyses. The frequency of 10/10 genotypes did not differ significantly between cases and controls (72.9% vs. 64.7%, $n = 350$, $\chi^2 = 0.41$, $df = 1$, $p = 0.52$). Repeated measures logistic model comparison of 10/10-genotype frequency between cases, relatives and controls, taking into account familial correlations was not significant ($p > 0.50$).

For PPG analysis, we initially assessed the effects of *DAT1* genotype on PPG pooling all three-subject groups (schizophrenia, unaffected relatives and controls), but allowing for group by genotype interactions. These analyses revealed that the effects of genotype on PPG differed significantly by group [group by genotype interaction, $F(2, 189) = 6.33$, $p < 0.005$; Figure 2]. Post hoc comparisons were therefore carried out and revealed that, among healthy controls, those with the 10/10 genotype had a significantly higher PPG than those with non-10/10 genotypes ($p < 0.02$); however, among schizophrenia subjects, the opposite effect was observed, i.e., those with the 10/10 genotype had significantly lower PPG compared to those with non-10/10 genotypes ($p < 0.01$).

We hypothesized that the absence of a genotype effect on PPG in the relatives group might be attributed to the heterogeneous nature of this group, which includes individuals with and without schizophrenia liability (SSP and non-SSP relatives, respectively). Therefore, in exploratory analyses we divided the relatives who had completed oculomotor testing into SSP (marking schizophrenia liability; n=9) and non-SSP relatives (n=31). The repeat analyses again showed a significant group by genotype interaction [$F(3,187)=4.33$, $p<0.005$]. The non-SSP relatives with 10/10 genotype (n=22) had similar PPG as healthy controls, but significantly higher PPG than in the schizophrenia group ($p=0.05$). PPG in SSP relatives with 10/10 genotype (n=7) was not significantly different from PPG in schizophrenia patients ($p=0.14$; Figure 3). Our analysis of mRNA expression in the postmortem sample revealed a similar pattern of divergent genotype effect as observed for PPG. The 10/10 genotype was associated with higher mRNA transcripts in schizophrenia cases, but lower levels in healthy controls. As before, we observed a significant diagnosis by genotype interaction on mRNA expression through ANOVA analysis [$F(1,28)=8.1$, $p<0.01$; Figure 4]. In post-hoc analysis by group, healthy control postmortem tissue with the 10/10 genotype had significantly lower *DAT1* mRNA expression compared to tissue with non-10/10 genotypes ($p<0.05$). In contrast, analysis of postmortem tissue from schizophrenia subjects revealed the 10/10 genotype to be associated with higher mRNA expression, although not significantly so ($p<0.09$). The mRNA expression in the schizophrenia and healthy control 10/10 tissues was significantly different ($p<0.001$); there were no significant between-group differences for tissue with non-10/10 genotypes ($p>0.6$). Figure 4 shows data from two 10/10 and one non-10/10 tissue of schizophrenia donors who were not on medications (n=3); their values were similar to the mean values observed in the schizophrenia group. There was not effect of age or ethnicity on the *DAT1* mRNA levels ($p>0.5$).

DISCUSSION

The results from this study demonstrate an opposite effect of *DAT1* genotype on PPG in schizophrenia compared to control subjects. *DAT1* 10/10 genotype has been shown to reduce expression of DAT. Our postmortem mRNA expression data in a small sample suggest that this is the case in controls, but not in schizophrenia. Healthy controls with 10/10 genotype (i.e., with increased synaptic dopamine) showed superior predictive pursuit compared to controls without the 10/10 genotype. In contrast, schizophrenia patients with 10/10 genotype showed poorer predictive pursuit than patients without the 10/10 genotype. These findings are consistent with our previous findings with the COMT Val¹⁵⁸Met polymorphism - the Met/Met COMT genotype (increased dopamine tone) was associated with better predictive pursuit in healthy, but not schizophrenia subjects (Thaker et al, 2004). The similar findings across these two dopamine-regulating genes suggest that the observed associations of the two genes with PPG are not because these genes are in linkage disequilibrium with another gene(s). Rather, these data suggest that genes that increase frontal cortical-subcortical dopaminergic tone directly increase predictive pursuit function in healthy individuals but have opposite effects in schizophrenia patients.

The findings from the mRNA expression study in the FEF were informative regarding the opposite effects of *DAT1* 10/10 genotype on PPG in schizophrenia compared to healthy subjects. In the healthy control group, 10/10 genotype correlated with reduced mRNA expression (increased extracellular dopamine), which might explain the high PPG. In contrast, 10/10 genotype had opposite effects on mRNA expression in schizophrenia, increasing *DAT1* expression resulting in reduced synaptic dopamine, and poor PPG. The reasons for the opposite effects of *DAT1* variation on mRNA expression in the two groups are not clear. A schizophrenia susceptibility gene (s) that is in linkage disequilibrium with the VNTR might explain our results. In this context, the findings in the relatives group were interesting. One would expect that only a subgroup of relatives would inherit this modulating gene with similar

effects to that observed in schizophrenia. In the remaining relatives, the effects of *DAT1* should mimic the findings in healthy subjects. We indeed observed this pattern; non-SSP relatives were similar to healthy controls, while SSP relatives were similar to schizophrenia participants. Nonetheless, our observation in the SSP relatives group needs replication considering the small number in this group (n=9). Notably, SSP traits (and associated neurocognitive deficits) are thought to be under similar genetic control in biological relatives of schizophrenia patients (Siever and Davis, 2004). Thus, in the relatives as a group, *DAT1* effects would be intermediate to healthy subjects and patients, with SSP relatives more similar to schizophrenia patients. The findings in the current study are consistent with this prediction.

Reports are contradictory regarding which *DAT1* VNTR genotype (10/10 vs. 9/9) reduces *DAT1* expression ((Jacobsen et al., 2000; van Dyck et al., 2005; VanNess et al., 2005; Brookes et al., 2007; VanNess et al., 2005; Brookes et al., 2007). In our study sample, 10/10 genotype was associated with reduced gene expression in normal controls but not in schizophrenia subjects. A reasonable assumption is that in schizophrenia, additive contributions of genetic variation might act on *DAT1* 3'UTR and affect *DAT1* mRNA transcription efficiency, sub-cellular localization or stability (Conne et al., 2000; Greenwood et al., 2003). Our unexpected results could also be explained by variation upstream of *DAT1* gene (i.e., 5' promoter region), which we did not examine in this study. The 5' promoter region contains several binding domains for enhancing and silencing factors, including sites for nuclear receptor subfamily 4, group A, member 2 (NR4A2), an established transcriptional regulator of *DAT1* expression (see review by (Bannon et al., 2001; Greenwood et al., 2003). We did not examine variations in about 22 SNPs previously discovered in the 5' region (Kelada et al., 2005; Rubie et al., 2001; Greenwood et al., 2003). In their original work, Kelada and colleagues (2005), found no association between Parkinson's disease and 5' region haplotypes nor the *DAT1* VNTR; and Parkinson's disease. Furthermore, they demonstrated a significant difference in activity of *DAT1* 5' haplotypes using an *in vivo* reporter gene assay. Findings from our study using *ex vivo* human brain tissue from a region known to control SPEM were similar - no association with schizophrenia, and different effect of *DAT1*-10/10 genotype on gene expression in cases compared to controls. Further complexity to *DAT1* regulation is provided by animal data, which supports regulatory elements in a genomic region that is homologous to human chromosomal regions 9q21 and 11q12-13 (Janowsky et al., 2001); regions which have been implicated in schizophrenia. Comparable regulatory genes/proteins might make the 10/10 genotype detrimental in schizophrenia.

There was no association between schizophrenia and *DAT1* genotype in this sample, which is consistent with published studies (Gamma et al., 2005). Recent reports have demonstrated effects of *DAT1* variation on neurocognitive phenotypes that are impaired in schizophrenia. Demiralp and coworkers (2006) demonstrated an effect of *DAT1* and *DRD4* polymorphisms on evoked gamma band responses in healthy volunteers (Demiralp et al., 2006). Another study using functional imaging demonstrated additive effects of *COMT* and *DAT1* polymorphism on blood oxygen level-dependent (BOLD) signals in the working memory cortical network: subjects with *COMT* Met/Met and *DAT1* 10/10 genotype exhibited a more focused BOLD response during the N-back task (Bertolino et al., 2006). We were unable to examine the interactive effects of *COMT* and *DAT1* genotype on PPG because of very small size of the cells. Consistent with our finding of reduced *DAT1* mRNA expression in healthy control tissue, Jacobsen and colleagues (2000) demonstrated reduced dopamine transporter density in healthy subjects with 10/10 genotype compared to non-10/10 genotype (Jacobsen et al., 2000). More recently, Bellgrove and colleagues showed an effect of this functional VNTR on spatial attention in healthy children (Bellgrove et al., 2007).

To our knowledge, our report is the first demonstration of 1) a *DAT1* 10/10 effect on smooth pursuit eye movements, 2) a differential effect of *DAT1* variation on an endophenotype across

3 subject groups: schizophrenia, unaffected first-degree relatives, and healthy controls, and 3) differential *DAT1* mRNA expression in FEF between schizophrenia and controls. Previous evidence suggests segregated regulation of dopaminergic neurons in frontal cortical (FEF is located here) compared to mesocortical regions. For instance, Horvath and colleagues (2007) demonstrated significant downregulation of *DAT1* mRNA and protein in nigrostriatal, but not mesolimbic regions. Interestingly, there were complementary alterations in *DAT1* transcriptional regulators, α -synuclein (SNCA) and Nurr1 (alias, NR4A2) in these regions (Horvath et al., 2007). Notably, their brain tissue samples were from heroin abusers and were analyzed with immunohistochemistry. Furthermore, Akil and coworkers (1999) demonstrated altered dopaminergic innervation of prefrontal cortex Brodmann's area 9, which was neurotransmitter and laminar-specific, and not an artifact of antipsychotic medication treatment (Akil et al., 1999). In our study, tissue was dissected from Brodmann's area 6 of the pursuit-related human FEF.

Our results do not suggest that *DAT1* is a major schizophrenia liability gene. Important limitations of this study include the small sample size, and that little is known about the precise *in vivo* effect of the *DAT1* VNTR on dopamine signaling (Mill et al., 2005). Furthermore, we did not measure brain synaptic dopamine levels or dopamine transporter density in this sample. We measured *DAT1* mRNA transcript, a reasonable corollary of gene expression. The observed effect of mRNA expression in schizophrenia could also be explained by environmental factors and not a gene effect (e.g., effect of psychotropic medications or psychostimulant abuse). The records of schizophrenia donors that were on medications at the time of death suggest that they were on antipsychotic medications only. None of them was on a psychostimulant or antidepressant drug. Mateos and colleagues (2007) have demonstrated a null effect of antipsychotic medications on DAT density in antipsychotic-treated and naïve schizophrenia patients, even after a 4-week antipsychotic trial (Mateos et al., 2007). Their data suggest that, in schizophrenia, reduced DAT density is a trait rather than a state finding. Comparison between *DAT1* mRNA expression in postmortem tissue from patients who were not on medication at the time of death to those who were is consistent with this. However, our postmortem sample size is very small and replication in a powered sample is necessary. The small number of SSP relatives is another important limitation. Lastly, though no *DAT1* genotype differences were observed between ethnic groups in this sample, it is possible that the association findings may have been biased by cryptic population substratification. This could have been addressed by genotyping a panel of ancestral markers, chosen for having discordant allele frequencies in African and European ancestral populations, subpopulations assigned to individuals and used as covariates for the association analyses (Pritchard and Rosenberg, 1999). Our results support further studies directly examining the relationship between *DAT1* and SPEM by *in vivo* neuroimaging measurement of dopamine transporter density, and transcriptional factors that regulate the *in vivo* phenotype.

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FIGURE 1.

An example of maintenance and predictive smooth pursuit. Predictive pursuit was measured using a target mask manipulation. Dashed line indicates target traces; solid line, actual eye traces. In this trial, a mask of 500-millisecond duration occurs in the beginning of the cycle. The subject's eyes turn and pursue for approximately 250 milliseconds without immediate target motion information.

FIGURE 2.

Effect of having *DAT1* 10/10 genotype vs. non-10/10 genotype on predictive pursuit gain (mean $\pm$ S.E.) in schizophrenia subjects, their unaffected 1st degree relatives, and healthy controls. Schizophrenia subjects with 10/10 genotype had poor predictive pursuit compared to controls, with intermediate effect in relatives.

DAT1, dopamine transporter gene; *DAT1* 10/10, homozygous for 10-repeat alleles; *DAT1* non-10/10, not homozygous 10/10 genotype.

FIGURE 3.

Effect of *DAT1* genotype on predictive pursuit gain in unaffected first-degree relatives of schizophrenia subjects (Mean $\pm$ SE). In unaffected first-degree relatives, the effect of *DAT1* genotype on predictive pursuit gain in non-SSP relatives was similar to the genotype effect in healthy control individuals. Rel_SSP, Relatives with SSP; Rel_nSSP, Relatives without SSP.

FIGURE 4.

Effects of *DAT1* genotype on *DAT1* mRNA expression in postmortem brain tissue from the frontal eye fields, in schizophrenia and healthy control subjects (Mean $\pm$ SE). The figure also shows data from three schizophrenia subjects that were not on medications (two 10/10 and one non-10/10-genotype). The values of *DAT1* mRNA expression from these three samples were similar to the mean values observed in corresponding schizophrenia groups.

SZ, Schizophrenia; Medication-free SZ, Schizophrenia patients who were off psychotropics at the time of death; FEF, Frontal Eye Fields