The Dopamine D_3 Receptor Ser9Gly Polymorphism Modulates Prepulse Inhibition of the Acoustic Startle Reflex

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Background: The dopamine D_3 receptor (DRD_3) is suspected to modulate prepulse inhibition (PPI) in animals and humans, but definite conclusions cannot be drawn due to lack of selective DRD_3 ligands. The Ser9Gly polymorphism is a common variant of the DRD_3 gene and determines the gain of function of the D_3 receptor. This is the first study to examine the influence of the DRD_3 Ser9Gly polymorphism on human PPI.

Methods: Prepulse inhibition was measured in 101 healthy male subjects presented with 75-dB and 85-dB prepulses at 30-, 60-, and 120-msec prepulse-pulse intervals. Subjects were grouped according to their DRD_3 status into a Gly/Gly, a Ser/Gly, and a Ser/Ser group.

Results: Analyses of variance showed that at all prepulse and interval conditions, Gly/Gly individuals had the lowest PPI and the greatest onset latency facilitation and Ser/Ser individuals had the highest PPI and the lowest onset latency facilitation, while Ser/Gly individuals were intermediate.

Conclusions: These results suggest that PPI is modulated by the D_3 receptor and its levels depend on the Ser9Gly polymorphism.

Key Words: Dopamine, DRD_3, healthy males, sensorimotor gating, Ser9Gly polymorphism, startle

Prepulse inhibition (PPI) of the acoustic startle response refers to a reliable reduction in the magnitude of the blink reflex component of the startle response to a strong auditory stimulus (the pulse) if this is preceded 30 msec to 500 msec by a weak stimulus (the prepulse). Prepulse inhibition is demonstrable across species from mice to humans (1) and it is considered a measure of "sensorimotor gating," whereby prepulses reduce the effect of subsequent sensory stimuli to protect the brain from sensory overload (2). Prepulse inhibition deficit is a reliable feature of neuropsychiatric disorders such as schizophrenia, where reduced gating is thought to be one possible neurobiological mechanism underlying some basic cognitive abnormalities associated with this disorder (3). The PPI deficits observed in schizophrenia can be mimicked in animals by the administration of dopamine (DA) agonists and reversed by antipsychotic drugs (4,5). Also, typical and atypical antipsychotic drugs reverse the PPI deficits observed in schizophrenic patients (6).

The D_2 receptor family is involved in the regulation of PPI in rats (7) with the dopamine D_2 receptor (DRD_2) subtype being essential (8,9). Evidence from agonist-antagonist studies in rats suggests a role for the dopamine D_3 receptor (DRD_3) as well (10–14), but progress in this area is hurdled by the lack of selective DRD_3 ligands. Thus, the involvement of DRD_3 in PPI modulation remains an open issue, and it was questioned recently since preferential D_3 antagonists had either no effect or were required at higher doses to reverse the strain-specific PPI deficit in the DBA/2J mice (15). In the human brain, the highest expression of the D_3 receptor is in the ventral and the association striatum (16–19). Gurevich and Joyce (19) have reported an overlap in the expression of D_3 and D_2 receptors, as D_3 messenger RNA (mRNA) is expressed in at least 30% of the neurons of the ventral striatum and D_2 mRNA is expressed in over 75% of the neurons. Consistent with DRD_3 involvement in PPI modulation, we have recently shown that ropinirole disrupts PPI in healthy male subjects (20) but the issue of selectivity continues to prohibit firm conclusions. Ropinirole is a preferential $D_{3/2}$ agonist (21) with a twentyfold selectivity for D_3 over D_2 receptors (22) and has also affinity for the dopamine D_4 receptor (DRD_4) subtype (23). The DRD_4 may also play a role in PPI modulation (24–26). Therefore, a contribution from agonistic activity at the D_2 and D_4 receptors cannot be entirely excluded.

In view of these difficulties with agonist-antagonist studies, an alternative strategy to test the involvement of DRD_3 in PPI modulation may be to study PPI in human subjects characterized for the DRD_3 Ser9Gly polymorphism. This polymorphism is a common variant of the DRD_3 gene, localized on 3q13.3, and determines the gain of function of the D_3 receptor. Indeed, the DRD_3 receptor of Gly-9 homozygous individuals shows a greater than fourfold increased dopamine affinity and signaling responses such as dopamine-mediated cyclic adenosine monophosphate (cAMP) response and mitogen-associated protein kinase (MAPK) signal compared with the Ser-9 variant (27). There is growing evidence supporting a role for the DRD_3 receptors in the pathophysiology and treatment of schizophrenia (28,29). A detailed understanding of the specific role of the DRD_3 receptor subtype in the modulation of human PPI may help clarify important aspects of the pathophysiology of neuropsychiatric disorders characterized by deficient sensorimotor gating. In the present study, we were interested in the relationship of the DRD_3 Ser9Gly polymorphism and PPI. We hypothesized that subjects carrying the Gly allele (high gain of D_3 function) would show reduced PPI amplitudes compared with homozygous subjects for the Ser allele (low gain of D_3 function). We used a range of stimulus parameters designed to explore potential stimulus-dependent effects of the DRD_3 Ser9Gly polymorphism.

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Methods and Materials

Subjects

The study was approved by the Ethics Committee of the University of Crete. All participants gave written informed consent before screening. We restricted the sample to men to avoid PPI variability related to gender (30,31) and menstrual cycle (32). Inclusion criteria included right-handedness; absence of personal history of head trauma, medical and neurological conditions, or use of prescribed and recreational drugs; absence of personal or family (up to second-degree relatives) history of DSM-IV Axis I disorders; and hearing threshold greater than 40 dB at 1 kHz.

One hundred twenty-one unrelated healthy male subjects of Greek/central European descent were randomly recruited by advertisement from university students and the general population of Crete, Greece. All underwent psychiatric assessment using the Mini-International Neuropsychiatric Interview (33) and physical assessment including a urine toxicology screening and a hearing test. Seven subjects were excluded because of a psychiatric condition and/or a family history of psychiatric illness, 11 subjects were startle nonresponders (mean startle amplitude $<$ 10 μV), and 2 subjects had a positive drug screen. One hundred one Greek/Caucasian healthy male subjects aged 18 to 35 years (mean $\pm$ SD, 26.0 $\pm$ 4.1) entered and completed the study. Participants were seen and assessed on a single occasion.

Genotyping

Genomic DNA was extracted from venous blood samples using the Flexigene DNA kit (Qiagen, Hilden, Gemrany). The DRD_3 Ser9Gly genotype was determined by restriction fragment length polymorphism after polymerase chain reaction (PCR) amplification and digestion with HaeIII restriction enzyme (New England Biolabs, Frankfurt/Main, Germany), similar to a previously described methodology by Lannfelt *et al.* (34).

Measurement of the Startle Response

A commercially available electromyographic (EMG) startle system (EMG SR-LAB; San Diego Instruments, San Diego, California) was used to examine the eye-blink component of the acoustic startle response from the right orbicularis oculi muscle. Equipment descriptions, setup, and scoring criteria have been previously described in detail (35). Pulses consisted of 40-msec, 115-dB white noise bursts, and prepulses consisted of 20-msec, 75-dB and 85-dB white noise bursts over 70-dB background noise. Recording began with 3 min of acclimation when only background noise was present. The recording period comprised 12 pulse-alone trials and 36 prepulse-pulse trials. Three lead intervals (onset to onset) were used (30, 60, 120 msec). For each interval, there were six trials with 75-dB prepulse and six trials with 85-dB prepulse. All trials were presented in pseudorandom order with the constraint that no two identical trials occurred in succession. The intertrial interval varied between 9 and 23 sec (average 15 sec). The entire test session lasted approximately 15 min.

Statistical Analysis

The maximum amplitudes of the raw EMG responses from each trial were averaged across all trials of the same type, and the percentage PPI (%PPI) was calculated using the formula [(AmplitudePulse-alone-AmplitudePrepulse-pulse)/AmplitudePulse-alone] $\times$ 100. Comparison of the genotype groups across demographic variables and baseline startle was performed using separate one-way analyses of variance (ANOVAs) or the nonparametric Kruskal-Wallis test as appropriate based on the deviation from normality. Separate mixed-model ANOVAs with genotype as the grouping factor and prepulse and interval as the within-subject factors were used to analyze %PPI and latency data. Significant findings were followed up with Bonferroni corrections.

Results

Forty-three subjects were homozygous for the Ser allele, 43 were heterozygous for Ser/Gly, and 15 were homozygous for Gly/Gly, a distribution consistent with Hardy-Weinberg expectations (χ^2 = .61, df = 2, p = .74). There were no differences in demographic and startle variables between the three genotype groups (Table 1). As an additional control to rule out gross stratification effects, genotyping was also performed for six unrelated gene polymorphisms. A contingency table approach (36) was used to test for differences in the allelic distributions of these additional markers for DRD_3 Ser/Ser and Gly/Gly subjects. This analysis revealed no significant differences in allele frequencies for each locus (with significance set at $p < .05$) (Table 2). This finding makes genetic inhomogeneity of the tested population unlikely.

Figure 1 shows the %PPI of the three groups. A mixed-model ANOVA of PPI with genotype as the grouping factor (three levels) and prepulse and interval as the within-subject factors revealed significant main effects of genotype [$F(2,98) = 5.13$, $p < .008$, $\eta^2 = .10$], prepulse [$F(1,98) = 108.2$, $p < .001$, $\eta^2 = .51$], and interval [$F(2,196) = 63.6$, $p < .001$, $\eta^2 = .39$] but no significant interactions. Post hoc comparisons with the Bonfer-

Table 1. Demographic and Startle Characteristics for DRD_3 Genotype Groups

	Ser/Ser	Ser/Gly	Gly/Gly	F (or x^2)	p
Sample Size	43	43	15		
Age (years)[a]	25.8 $\pm$ 4.4	26.5 $\pm$ 4.1	25.7 $\pm$ 2.9	<1	>.6
Education (years)[a]	17.1 $\pm$ 2.9	17.1 $\pm$ 2.4	16.8 $\pm$ 2.1	<1	>.8
Smokers/Nonsmokers[b]	16/27	20/23	8/7	1.4	>.4
Cigarettes/Day	6.5 $\pm$ 10.3	7.9 $\pm$ 10.0	7.5 $\pm$ 8.2	<1	>.6
Baseline Startle (μV)	319.4 $\pm$ 220.0	280.9 $\pm$ 191.9	389.1 $\pm$ 247.3	1.4	>.2
Onset Latency, msec	45.5 $\pm$ 6.4	44.9 $\pm$ 8.3	41.3 $\pm$ 8.6	1.7	>.1
Peak Latency, msec	60.4 $\pm$ 4.3	59.3 $\pm$ 3.9	61.2 $\pm$ 3.8	1.5	>.2

Mean $\pm$ SD.

DRD_3, dopamine D_3 receptor.

[a]For this measure, the overall distribution of the score differed from normality, and the equivalent nonparametric Kruskal-Wallis procedure was applied.

[b]Chi-square comparison.

Table 2. Test for Significant Group Differences in Allele Frequencies for Each Locus When Dividing Samples by DRD_3 Ser9Gly in Ser9 and Gly9 Homozygote Groups

Locus	Chromosome	*X2 (df = 1)*	*p*
DRD2A1 rs1800497	11q23	.00	1
DAT1 rs28363170	5p15	.02	.88
PRODH rs372055	22q11	.02	.88
DRD1 rs4532	5q35	.06	.80
COMT rs4680	22q11	.06	.80
ZDHHC8 rs175174	22q11	.01	.92

COMT rs4680, catechol-O-methyltransferase Val158Met polymorphism; DAT1 rs28363170; a 40-base pair (bp) tandem repeat polymorphism in the 3′ region of the SLC6A3 gene; DRD_1 rs4532, dopamine receptor D_1 A-48G polymorphism in the 5′ untranslated region of DRD_1 gene; DRD2A1 rs1800497, dopamine receptor D_2 TaqIA restriction fragment length polymorphism; DRD_3, dopamine D_3 receptor; PRODH rs372055, proline dehydrogenase T1945C polymorphism; ZDHHC8 rs175174, zinc finger DHHC domain-containing protein 8 A/G polymorphism.

roni correction test revealed that PPI of the Ser/Ser group was greater than PPI of the Gly/Gly group ($p < .01$) and the Ser/Gly group (trend $p = .09$).

Figure 2 shows the onset latency in pulse-alone trials and onset latency facilitation by the 75-dB (left) and the 85-dB prepulse (right) across the three intervals for the three genotype groups. A 3 × 4 (genotype × trial type) ANOVA for the 75-dB prepulse intensity revealed significant main effects of genotype [$F(2,98) = 3.2$, $p < .05$, $\eta^2 = .06$] and trial type [$F(3,294) = 15.4$, $p < .001$, $\eta^2 = .14$] but no significant interaction [$F < 1$]. Bonferroni post hoc tests showed significant differences in onset latency facilitation between the Ser/Ser and the Gly/Gly groups ($p < .044$). An identical ANOVA for the 85-dB prepulse intensity revealed significant main effects of genotype [$F(2,98) = 4.6$, $p < .012$, $\eta^2 = .09$] and trial type [$F(3,294) = 20.7$, $p < .001$, $\eta^2 = .17$] but no significant interaction [$F = 1.2$; $p > .3$]. Bonferroni post hoc tests showed significant differences in onset latency facilitation between the Ser/Ser and the Gly/Gly groups ($p < .012$). Figure 2 shows a different amount of latency facilitation between the two prepulses at the 30-msec interval. Indeed, this impression was confirmed by an overall 3 × 2 × 3 (genotype × prepulse × interval) ANOVA that revealed a significant prepulse × interval interaction [$F(2,196) = 11.9$, $p < .001$, $\eta^2 = .11$]. Peak latency data are shown in Table 3. Analyses of variance with identical factorial design as above revealed no significant genotype main effects for this measure ($p > .05$). It can be seen in Table 3 that while peak latencies of the Ser/Ser and the Ser/Gly groups followed the same pattern as that seen in onset latency, peak latencies of the Gly/Gly group were at Ser/Ser levels. Given that the Gly/Gly group had the lowest PPI and by extension the greatest startle amplitude at all trial types, we controlled for the possibility that greater time-to-peak in this group was the result of greater startle. Indeed, controlling for the effect of startle amplitude with analyses of covariance (ANCOVAs), the genotype effects on peak latency became significant. Table 4 shows the Cohen's *d* values for the two homozygote groups (Ser/Ser vs. Gly/Gly) along PPI and onset latency facilitation at all trial types used.

Discussion

We found that in healthy male subjects the DRD_3 Ser9Gly polymorphism is associated with PPI levels. More specifically, we observed a linear relationship between PPI levels and Gly allele load; Gly9 homozygotes had the lowest PPI and Ser9 homozygotes the highest PPI, while heterozygotes were intermediate for both prepulse intensities and all three lead interval conditions. A 10% of total PPI variance was attributable to DRD_3 genotype. These findings strongly suggest that the D_3 receptor is involved in PPI modulation in humans. We also found an effect of the Gly allele on prepulse latency facilitation; Gly9 homozygous individuals had greater onset latency facilitation than Ser homozygotes, while heterozygotes were intermediate. These results taken together suggest that Gly-allele load is associated with faster prepulse detection but poorer prepulse processing. Importantly, our findings were obtained in a homogeneous cohort of healthy male subjects and cannot be attributed to differences in demographic characteristics or genetic inhomogeneity, since the genotype groups did not differ in that respect (Tables 1 and 2).

Although the present results strongly suggest that the D_3 receptors are indeed involved in PPI modulation, it cannot ascertain the likely central site(s) responsible for this effect. The

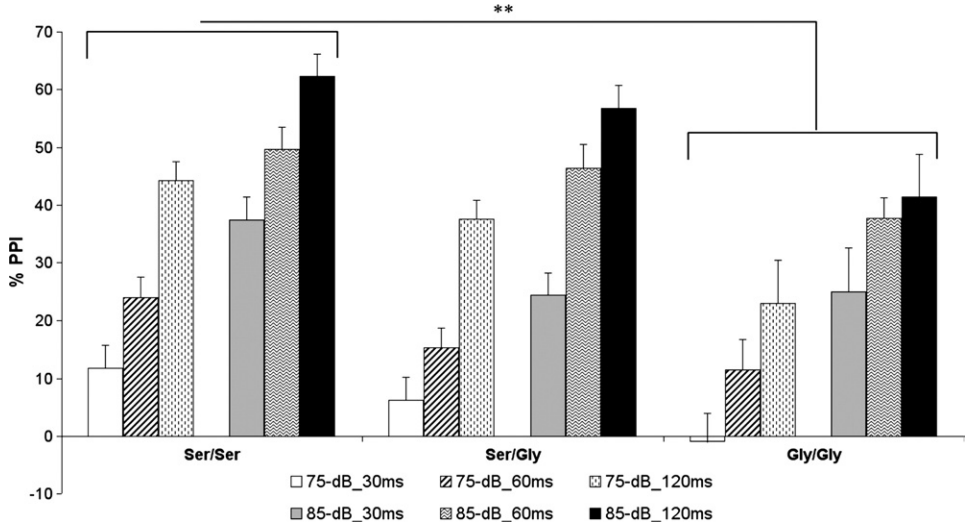

Figure 1. Group means and SEM for %PPI for the three genotype groups with 75-dB and 85-dB prepulses at 30-, 60-, and 120-msec prepulse-pulse intervals. $^{**}p < .01$. PPI, prepulse inhibition.

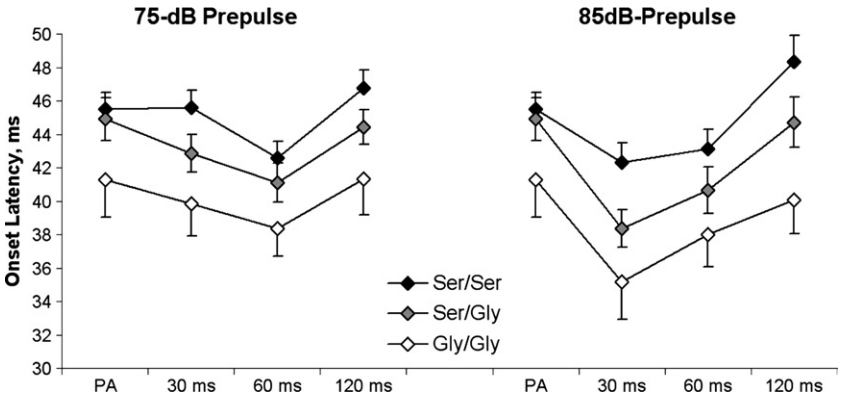

Figure 2. Group means and SEM for prepulse onset latency facilitation for the three genotype groups with 75-dB (left panel) and 85-dB prepulses (right panel) at 30-, 60-, and 120-msec prepulse-pulse intervals. PA, pulse-alone trials.

distribution of the D_3 receptor suggests that its functions are related to the mesolimbic rather than the nigrostriatal DA system (37). The D_3 receptors are expressed preferentially in subcortical basal ganglia and limbic structures (37–39), which overlap with the neural circuitry regulating PPI (40,41). They are also functionally associated with the modulation of prefrontal cortex (PFC) functions (42), due to their dopaminergic projections to the PFC (43). The PFC is also a critical node in the neural circuitry regulating PPI in animals (44–51), and its role in human PPI is supported by positron-emission tomography (PET) (52) and structural (53) and functional magnetic resonance imaging (MRI) studies (54,55). One possibility is that Gly allele-loading subjects had reduced PPI due to the high function of subcortical presynaptic D_3 autoreceptors. These normally inhibit ventral tegmental dopaminergic neurons, thus reducing the DA levels in the PFC (56,57). It has been shown recently that reduced PFC DA levels in healthy male subjects may be associated with reduced PPI levels (58). Alternatively, reduced PPI in the Gly allele-loading subjects could also be due to high function of D_3 receptors at extrastriatal sites such as the CA1 area of the dorsal hippocampus. Indeed, there is evidence that D_3 receptors in the hippocampus are involved in PPI modulation, since local hippocampal application of the D_3 agonist quinpirole reduces PPI in the rat (59). Finally, the possibility remains that compensatory changes in other dopamine receptors closely linked to DRD_3, such as the D_2 receptors, participate in the observed genotype differences in PPI.

The effect of the Ser9Gly polymorphism on prepulse latency facilitation was unexpected and we do not have a definite explanation for it. Longer prepulse-pulse intervals result in greater startle inhibition (PPI) but smaller amount of onset latency facilitation (1,60–62), and this pattern was seen in all three genotype groups in the present study. Therefore, longer onset latencies are associated with greater PPI for a given prepulse, and, interestingly, the three genotype groups complied perfectly with this principle. The Ser/Ser group with the greatest PPI had the longest onset latencies (smallest prepulse latency facilitation) and the opposite was true for the Gly/Gly group, while the Ser/Gly group was intermediate. Thus, low function of the DRD_3 (Ser/Ser group) is associated with prolonged onset latencies and better PPI. This is surprisingly homologous to findings from D_3 mutant mice, which have prolonged response latencies coupled with improved accuracy in attentional tasks (63). These authors concluded that one role of D_3 receptors may be to speed up response selection, perhaps at the expense of response accuracy. It is tempting to speculate that the D_3 receptors speed up prepulse stimulus detection (increased onset latency facilitation) at the expense of prepulse stimulus processing (low PPI). That onset latency facilitation is indeed a measure of prepulse stimulus detection finds unexpected support by the finding of earlier latency facilitation (at 30 msec) with the easy to detect 85-dB prepulse compared with the harder to detect 75-dB prepulse (Figure 2). It is notable that the impact of the Gly allele load was greater for onset latency facilitation than %PPI, especially at the "preattentive" 30-msec interval for both prepulses (Table 4). This raises the issue of whether the PPI deficits associated with Gly allele load observed in this study are partly attributable to fast stimulus detection already at very early stages of information processing. It is indeed conceivable that fast stimulus detection in the constant streamline of incoming information in real life may lead to sensory stimulus overload. This could compromise later stage stimulus processing by obstructing attentional prioritization of salient stimuli from less important ones that ought to be filtered out. A significant genotype main effect on peak latency was revealed only after we controlled for the effects of greater startle amplitude in the Gly/Gly group. It appears that although the Gly9 homozygotes startle faster than the Ser9 carriers at all trial types, it may take them the same time to peak because of their greater startle amplitudes. Clearly, more

Table 3. Peak Latency Data at Each Trial Type for the Three Genotype Groups

	Ser/Ser	Ser/Gly	Gly/Gly
75 dB			
30 msec	60.8 ± 3.5	58.9 ± 4.2	61.2 ± 3.5
60 msec	57.6 ± 4.7	57.1 ± 5.3	57.9 ± 3.7
120 msec	60.5 ± 4.2	59.0 ± 4.4	61.6 ± 3.9
85 dB			
30 msec	56.2 ± 4.3	53.8 ± 5.3	56.2 ± 5.8
60 msec	55.3 ± 4.9	53.8 ± 6.0	55.9 ± 6.0
120 msec	59.2 ± 5.5	57.7 ± 4.6	58.1 ± 6.2

Mean ± SD.

Table 4. Effect Size (Cohen's *d* Values) for Differences in %PPI and Onset Latency Facilitation Between the Two Ser9Gly Homozygote Groups (Ser/Ser vs. Gly/Gly) at Each Trial Type

Prepulse	Interval	%PPI	Onset Latency
75 dB	30 msec	.36	.80
	60 msec	.30	.64
	120 msec	1.01	.70
85 dB	30 msec	.54	.87
	60 msec	.45	.66
	120 msec	1.06	.90

PPI, prepulse inhibition.

research is required to clarify the role of DRD_3 in onset latency facilitation, peak latency, and shape of the EMG waveform, and the meaning of these findings and the mechanisms involved.

Prepulse inhibition is considered a candidate endophenotype for schizophrenia (64,65) because of its high heritability (66) and the presence of PPI deficits in the unaffected first-degree relatives of probands (67,68). Therefore, any gene polymorphisms associated with PPI attenuation ought to at least be considered as potentially increasing the risk for schizophrenia. In this context, it is relevant to our findings that a significant association between DRD_3 Gly9 homozygosity and schizophrenia was revealed in a recent meta-analysis (69). It is an intriguing possibility that gating deficits may be at least one way by which Gly9 homozygosity increases the risk for psychosis. Our findings link with those of Mulert *et al*. (70) who studied the effects of the DRD_3 Gly9Ser polymorphism in relation to the P300 amplitude, another candidate endophenotype for schizophrenia (71). They found that Gly9 homozygotes had diminished parietal and increased frontal P300 amplitudes compared with Ser9 carriers, a pattern seen in patients with schizophrenia (72,73).

To our knowledge, this is the first demonstration in humans of the influence of DRD_3 Ser9Gly polymorphism on PPI levels. The present study contributes to the understanding of individual differences and the genetic architecture of human PPI. It also buttresses the status of PPI as an endophenotype for schizophrenia. Future studies examining the effect of the DRD_3 Ser9Gly polymorphism on pharmacological manipulations of the PPI following administration of preferential D_3 agonists or antagonists could further enhance our understanding of the relationship between DRD_3 and PPI.

PR and SGG contributed equally to this article.

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