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The Association of Schizophrenia Risk D-Amino Acid Oxidase Polymorphisms With Sensorimotor Gating, Working Memory and Personality in Healthy Males

Panos Roussos*[,1,2], Stella G Giakoumaki[1,3], Eva Adamaki[1], Georgakopoulos Anastasios[2], Robakis K Nikos[2] and Panos Bitsios[1]

[1]Department of Psychiatry and Behavioral Sciences, Faculty of Medicine, University of Crete, Heraklion, Crete, Greece; [2]Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA; [3]Department of Psychology, Faculty of Social Sciences, University of Crete, Rethymno, Crete, Greece

There is evidence supporting a role for the D-amino acid oxidase (*DAO*) locus in schizophrenia. This study aimed to determine the relationship of five single-nucleotide polymorphisms (SNPs) within the *DAO* gene identified as promising schizophrenia risk genes (rs4623951, rs2111902, rs3918346, rs3741775, and rs3825251) to acoustic startle, prepulse inhibition (PPI), working memory, and personality dimensions. A highly homogeneous study entry cohort ($n = 530$) of healthy, young male army conscripts ($n = 703$) originating from the Greek LOGOS project (Learning On Genetics Of Schizophrenia Spectrum) underwent PPI of the acoustic startle reflex, working memory, and personality assessment. The QTPHASE from the UNPHASED package was used for the association analysis of each SNP or haplotype data, with *p*-values corrected for multiple testing by running 10 000 permutations of the data. The rs4623951_T-rs3741775_G and rs4623951_T-rs2111902_T diplotypes were associated with reduced PPI and worse performance in working memory tasks and a personality pattern characterized by attenuated anxiety. Median stratification analysis of the risk diplotype group (ie, those individuals homozygous for the T and G alleles (TG +)) showed reduced PPI and working memory performance only in TG + individuals with high trait anxiety. The rs4623951_T allele, which is the *DAO* polymorphism most strongly associated with schizophrenia, might tag a haplotype that affects PPI, cognition, and personality traits in general population. Our findings suggest an influence of the gene in the neural substrate mediating sensorimotor gating and working memory, especially when combined with high anxiety and further validate *DAO* as a candidate gene for schizophrenia and spectrum disorders.
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INTRODUCTION

Recent progress in psychiatric genetics has revealed several promising genetic susceptibility factors for schizophrenia, including D-amino acid oxidase (*DAO*), a gene located in chromosome 12q24 (for review see Verrall *et al*, 2010). On the basis of association, interaction, and functional analyses, Chumakov *et al* (2002) were the first to suggest that the brain-expressed gene *DAO* exerts an influence on the susceptibility to schizophrenia. Conflicting association results have since been reported by a number of independent studies providing the usual mixture of positive (Corvin *et al*, 2007; Liu *et al*, 2004; Ohnuma *et al*, 2009; Schumacher *et al*, 2004; Wood *et al*, 2007) and negative (Fallin *et al*, 2005; Jonsson *et al*, 2009; Liu *et al*, 2006; Shinkai *et al*, 2007; Vilella *et al*, 2008; Yamada *et al*, 2005) findings.

This inconsistency may be partly because of the clinical and genetic heterogeneity of this disorder. One approach to overcome this issue is by applying an endophenotypic approach, which involves a quantitative, heritable, trait-related, laboratory-assessed intermediate phenotype that is identified in patients and to a lesser degree in their unaffected relatives or other high risk individuals (Gottesman and Gould, 2003). A wide range of schizophrenia endophenotypes including neuropsychological, neurophysiological, structural and functional brain abnormalities have been evaluated up to date, and their relationship with various candidate genes has been assessed. One of the most promising schizophrenia endophenotype is the prepulse inhibition (PPI) of the acoustic startle reflex.

*Correspondence: Dr P Roussos, Department of Psychiatry and Behavioral Sciences, Faculty of Medicine, University of Crete, PO Box 2208, Heraklion 71003, Crete, Greece, Tel: + 30 2810 39 4610, Fax: + 30 2810 39 4610; E-mail: roussosp@edu.med.uoc.gr
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PPI is thought to reflect sensorimotor gating, a form of central nervous system inhibition wherein irrelevant sensory information is filtered out during the early stages of processing so that attention can be focused on more salient features of the environment (Braff *et al*, 1978). PPI in rodents is modulated by activity in a well-defined cortico–striatopallido–pontine circuitry (Swerdlow *et al*, 2001), which has been confirmed with neuroimaging studies in human subjects (Campbell *et al*, 2007; Kumari *et al*, 2003, 2005a, 2007a; Postma *et al*, 2006). Consistent with these neuroimaging findings and the notion that sensorimotor gating is important in human cognition (Geyer *et al*, 1990), neuropsychological studies show that higher PPI levels predict superior executive function (Bitsios and Giakoumaki, 2005; Bitsios *et al*, 2006; Csomor *et al*, 2008; Giakoumaki *et al*, 2006), whereas deficient PPI is well documented in conditions with frontostriatal pathology and deficient executive function such as schizophrenia (Braff *et al*, 2001a; Kumari *et al*, 2007b; Swerdlow *et al*, 2006).

DAO is now of interest in psychiatry (see Verrall *et al*, 2010 for review) because its major substrate in the brain is D-serine, a co-agonist of the *N*-methyl D-aspartate type of ionotropic glutamate receptor (NMDAR). Deficiency of D-serine signaling might contribute to NMDAR hypofunction, as there is evidence (1) for decreased D-serine level in both serum and cerebrospinal fluid in schizophrenia (Bendikov *et al*, 2007; Hashimoto *et al*, 2003, 2005; Yamada *et al*, 2005); (2) that addition of D-cycloserine to antipsychotic medication is beneficial for negative and probably cognitive symptoms (Coyle *et al*, 2002; Javitt, 2006; Shim *et al*, 2008; Tsai *et al*, 2006; Yang and Svensson, 2008); (3) D-serine produces behavioral and neurochemical alterations consistent with these clinical effects in animal models (Andersen and Pouzet, 2004; Karasawa *et al*, 2008; Lipina *et al*, 2005; Nilsson *et al*, 1997; Olsen *et al*, 2006; Tanii *et al*, 1994). More specifically, exogenous *DAO* reduces NMDAR function *in vivo* and *in vitro* (Gustafson *et al*, 2007; Hama *et al*, 2006; Katsuki *et al*, 2004; Mothet *et al*, 2000; Ren *et al*, 2006; Stevens *et al*, 2003; Yang *et al*, 2005), mutant mouse strain ddY/*DAO*−, which lacks *DAO* activity shows increased cerebellar NMDAR function (Almond *et al*, 2006) and enhanced hippocampal NMDAR-dependent long-term potentiation (Maekawa *et al*, 2005), and finally, systemically administered *DAO* inhibitors produce effects consistent with enhanced NMDAR function. Thus, *DAO* has the capability to regulate the function of NMDAR through D-serine breakdown and might contribute to NMDAR hypofunction in schizophrenia, or be relevant to its remediation.

Five *DAO* gene single-nucleotide polymorphisms (SNPs) have been associated with schizophrenia and subjected to meta-analysis in the SZGene Database (http://www.schizophreniaforum.org/res/sczgene/default.asp; Allen *et al*, 2008). Our aim was to investigate whether these risk *DAO* variants were related to variations in PPI levels, performance in cognitive tasks and personality traits in the LOGOS (Learning On Genetics Of Schizophrenia Spectrum) cohort. This would provide more evidence for the role of *DAO* as a schizophrenia candidate gene and would further our understanding of its functional mechanisms within the human brain.

MATERIALS AND METHODS

Subjects

Subjects were recruited from the first wave of the LOGOS study. This is a demographically and genetically highly homogeneous cohort of young male conscripts of the Greek Army with an age range 18–29 years (mean age 22.1 ± 3), which has been described in detail previously (Roussos *et al*, 2010). Briefly here, following presentation of the study and written informed consent, 703 randomly selected young male conscripts were recruited between June 2008 and July 2009. This study was approved by the Ethics Committee of the University of Crete, the Executive Army Bureau and the Bureau for the Protection of Personal and Sensitive Data of the Greek State. All subjects were thoroughly screened for past or current physical and mental health status by the army medical authorities, the study nurse and a trained research psychologist. Inclusion criteria were recent (last 2 months) conscript status in the camp and written informed consent. Exclusion criteria were left-handedness; personal history of head trauma, medical and neurological conditions, current use of prescribed drugs or a positive recreational drug screen; personal history of DSM-IV axis I disorders; and hearing threshold greater than 40 dB at 1 kHz.

Quantitative Trait Testing

Acoustic startle and PPI. 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. Pulses consisted of 40-ms, 115-dB white noise bursts, and prepulses consisted of 20-ms, 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. As variation of the lead interval may tap different aspects of early information processing from preattentional (eg, 30 ms) to attentional (eg, 120 ms) stimulus processing, three lead intervals (onset to onset) were used (30, 60, and 120 ms). 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 s (average 15 s). The entire test session lasted $\sim$15 min. Only subjects with high quality startle and PPI data (no >1 (out of 6) missing trial per trial type and/or no >2 (out of 12) missing pulse-alone trials) were included for further analyses ($n = 445$).

Neurocognitive assessment. We examined the performance in two different tasks that both recruit working memory process. We used one subtest of Cambridge Neuropsychological Test Automated Battery Sahakian and Owen, 1992) namely, that spatial working memory (SWM) test, which is a non-verbal test administered with the aid of a high-resolution touch-sensitive screen (Advantech). Visual working memory was assessed with the N-Back Sequential Letter Task (Fletcher and Henson, 2001).

Personality questionnaires. All subjects were administered the Eysenck Personality Questionnaire (EPQ; Eysenck *et al*, 1985), Cloninger's Temperament and Character Inventory (Cloninger *et al*, 1993), Spielberger's State-Trait Anxiety Inventory—Trait Scale (STAI-T; Spielberger, 1983), the Carver and White's Behavioral Inhibition/Behavioral Activation System (BIS/BAS) questionnaire (Carver and White, 1994), and the Schizotypal Traits Questionnaire Kelley and Coursey, 1992).

Genotyping

DNA was extracted from blood or cheek swab samples, using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The following five *DAO* SNPs were genotyped: rs4623951, rs2111902, rs3918346, rs3741775, and rs3825251, because they have been associated with schizophrenia in previous studies. Genotyping was performed blind to phenotype measures by K-Biosciences (Herts, United Kingdom; http://www.kbioscience.co.uk/) with a competitive allele-specific PCR system. Genotyping quality control was performed in 10% of the samples by duplicate checking (rate of concordance in duplicates $>99\%$). Call rate was $>96\%$ for all polymorphisms.

Statistical Analysis

Comparison of the genotype groups for each SNP across demographic variables and baseline startle was performed using separate one-way analyses of variances (ANOVAs) or the nonparametric Kruskal–Wallis test as appropriate, based on deviation from normality. For the sake of data reduction and variables classification we submitted performance scores on all personality questionnaires to principal component analysis (PCA). Hardy–Weinberg equilibrium (HWE) for five markers was checked using Haploview version 4.0 (Barrett *et al*, 2005). QTPHASE (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/) from the UNPHASED package version 3.1.4 was used for the analysis of genotype associations (Dudbridge, 2003). QTPHASE uses a generalized linear model for quantitative traits assuming normal distribution of the trait. The trait mean given an individual's genotype data are based on an additive model of haplotypes. Haplotypes with frequencies $<1\%$ in the whole sample were excluded. We used a two-step procedure to correct for multiple testing. The *p*-values for the trait differences were corrected for multiple testing by running 10 000 permutations of the data. In each permutation, the quantitative scores are randomly re-assigned among subjects and the minimum *p*-value is compared with the minimum *p*-value over all the analyses in the original data. This allows for multiple testing corrections over all tests performed in a run. Next, as an additional method we set α to 0.01 level of significance. Results with a $p<0.05$ are shown only as 'suggested findings' for future replication attempts. For each of those variants we performed separate mixed-model $3\times2\times3$ (genotype by prepulse by interval) ANOVAs of %PPI and latency data and assessment of cognitive performance and personality traits using ANOVA or the non-parametric Kruskal–Wallis test as appropriate based on the deviation from normality. Pearson product–moment correlation coefficients were used to assess the relations between PPI and personality traits. On the basis of our sample size, we were able to detect a small to medium effect size, which for 80% power and α set to 0.01, was Cohen's $d=0.33$.

RESULTS

Table 1 summarizes the genotype distribution and minor allele frequency of the *DAO* polymorphisms in our sample. Genotype frequencies were distributed in accordance with HWE. There were no differences in demographic variables between the *DAO* genotypes for each SNP (Supplementary Table 1). The rs3918346, rs3741775, and rs3825251 were in strong linkage disequilibrium (Supplementary Table 2, Supplementary Figure 1).

Single-Marker Association Analysis

Table 2 shows the *p*-values of the association of *DAO* SNPs with startle and PPI as revealed by the QTPHASE after correction with permutation test. A pattern of association can be seen whereby the T allele of the rs4623951 variant was significantly associated with lower PPI levels at $p<0.01$ and $p<0.05$ in 85 db_60 ms and 85 db_30 ms trials, respectively. A mixed-model ANOVA of PPI with rs4623951 genotype as the grouping factor (three levels) and prepulse and interval as the within-subject factors revealed a genotype main effect at trend level ($F(2,428)=3$, $p=0.051$, partial $\eta^2=0.016$). We repeated the UNPHASED analysis and the follow up ANOVAs described above, taking baseline startle and/or smoking and/or IQ as the covariates; following this procedure the results did not change or the *p*-values slightly improved.

Haplotype Analysis

We performed association tests for the haplotype analysis using two-marker combinations (Table 3). Overall, we found that the diplotypes rs4623951_T-rs2111902_T and rs4623951_T-rs3741775_G were associated with PPI at $p<0.01$ in at least one trial type, and this did not change when the UNPHASED analysis was repeated with baseline startle and/or smoking and/or IQ as covariates. For rs4623951-rs2111902 and rs4623951-rs3741775 diplotypes we divided our population into risk and no-risk groups

Table 1 Genotype, Allele and MAFs of the *DAO* Polymorphisms

Marker (%)	Genotype			Allele		MAF	HWE *p*-value
rs4623951 (97)	T/T 224	T/C 219	C/C 70	T 667	C 359	0.35	0.19
rs2111902 (98.5)	T/T 204	T/G 250	G/G 67	T 658	G 384	0.37	0.56
rs3918346 (96.2)	C/C 245	C/T 213	T/T 51	C 703	T 315	0.31	0.69
rs3741775 (98.5)	T/T 180	T/G 256	G/G 86	T 616	G 428	0.41	0.82
rs3825251 (98.1)	T/T 335	T/C 166	C/C 19	T 836	C 204	0.2	0.78

Abbreviations: *DAO*, D-amino acid oxidase; HWE, Hardy–Weinberg expectation; MAF, minor allele frequency.
The allele distributions are consistent with HWE. The percentage in the marker column refers to call rate for each polymorphism.

Table 2 Adjusted *p*-values from Permutation Test for Association of Startle and PPI for *DAO* Polymorphisms that Reached Significance at $p<0.05$

	rs4623951	rs2111902	rs3918346	rs3741775	rs3825251
Baseline startle	0.8	0.3	0.7	0.4	0.9
PPI 75_30	0.9	0.9	0.9	0.3	0.8
PPI 75_60	0.07	0.09	0.3	0.3	0.9
PPI 75_120	0.23	0.6	0.9	0.8	0.7
PPI 85_30	***0.015 (−0.008)***	0.8	0.9	0.6	0.4
PPI 85_60	**0.004 (−0.01)**	0.23	0.4	0.3	0.9
PPI 85_120	0.2	0.2	0.4	0.2	0.7
Pooled PPI					
PPI 30 ms	0.13	0.9	1	0.8	0.5
PPI 60 ms	**0.004 (−0.012)**	0.8	0.9	0.2	1
PPI 120 ms	0.16	0.3	0.7	0.6	0.7

Abbreviations: *DAO*, D-amino acid oxidase; PPI, prepulse inhibition.
Numbers represent the overall *p*-value for each single-nucleotide polymorphism after correction with permutation test. Numbers in brackets represent the estimated additive genetic value for the risk allele, relative to the protective common allele; plus or minus signs indicate increases or reductions in PPI respectively. The *p*-values <0.05 are in bold and italicized; *p*-values <0.01 are in bold. PPI data pooled across prepulse intensity are also shown for comparison.

based on homozygosity for both risk SNPs. TG+ represents subjects that were homozygous for the rs4623951_T and rs3741775_G diplotype and TG− are the non-homozygous individuals. TT+ represents subjects that were homozygous for the rs4623951_T and rs2111902_T diplotype and TT− are the non-homozygous individuals. There were no differences in demographic variables between the *DAO* diplotypic groups (Supplementary Table 3). Following this grouping, we performed separate mixed-model ANOVAs of PPI data with diplotype as the grouping factor (risk, no-risk) and prepulse and interval as the within-subject factors. These ANOVAs revealed significant main effects of genotype (rs4623951-rs2111902: $F(1,429)=5$, $p=0.026$, partial $\eta^2=0.013$; rs4623951-rs3741775: $F(1,428)=9.8$, $p=0.002$, partial $\eta^2=0.025$), with risk (TT+ and TG+) individuals presenting with PPI reductions (Figure 1). These results did not change when covarying with baseline startle and/or smoking and/or IQ. There were no significant findings for latency data following identical analyses.

Cognitive and Personality Variables

Table 4 shows the association of *DAO* rs4623951-rs3741775 and rs4623951-rs2111902 diplotype groups with our cognitive and personality phenotypic measures. Homozygosity (TG+ status) for the rs4623951_T and rs3741775_G diplotype was associated with statistically significant ($p<0.001$) lower number of correct responses in the three-back working memory test. In addition, TG+ individuals presented with lower anxious/negative mood as evidenced by higher EPQ extraversion ($p<0.002$) and lower STAI-T trait anxiety ($p<0.01$), TCI harm avoidance ($p<0.02$) and EPQ neuroticism ($p<0.004$).

Homozygosity for the rs4623951_T and rs2111902_T diplotype (TT+ status) was associated with statistically significant higher number of 'within' errors in the eight-box condition ($p<0.006$) and poorer strategy development as evidenced by higher scores in strategy ($p<0.001$) in the SWM task and lower number of correct responses in the three-back working memory test ($p<0.03$). In addition, TT+ individuals presented with less negative mood as evidenced by higher EPQ extraversion scores ($p<0.02$). These results did not change after covarying for age, IQ, and smoking status.

Factor loadings from the rotated component matrix are shown in the Supplementary Table 4. The Kaiser–Meyer–Olkin measure of sampling adequacy (0.83) and Bartlett's test of sphericity ($\chi^2=3246.1$, d.f. $=136$, $p<0.0001$) indicated that the data were appropriate for factor analysis. Five factors emerged with Eigenvalues >1, suggesting a multidimensional structure, and this five-factor solution accounted for 71% of the total variance (see Supplementary Table 4). Homozygosity for the risk rs4623951_T and rs3741775_G diplotype was associated with statistically significant lower score in the anxiety factor (mean ± SD: TG−, 0.04 ± 1; TG+, -0.3 ± 0.9; $p=0.019$; Cohen's d 0.33), but not in the other factors (all $p>0.3$). Similarly, TT+ individuals presented with lower score only in the anxiety factor (mean ± SD: TT−, 0.04 ± 1; TT+, -0.25 ± 0.9; $p=0.034$; Cohen's d 0.3), but no significant differences were found in any other factor (all $p>0.3$). Results did not change after covarying for age, IQ, and smoking status.

Trait Anxiety, PPI and Cognition

On the basis of weak association of rs4623951_T-rs2111902_T diplotype with personality ($p=0.02$) and the strong association of the rs4623951_T-rs3741775_G diplotype with personality traits, we further explored the association of the STAI-T trait anxiety, TCI harm avoidance and EPQ extraversion, and neuroticism with PPI trials within the TG+ and TG− groups. The only correlation within the TG+ group that passed the significance level

Table 3 Individual diplotype test for the *DAO* groups

	Baseline startle	PPI 75_30	PPI 75_60	PPI 75_120	PPI 85_30	PPI 85_60	PPI 85_120	Pooled 30 ms	Pooled 60 ms	Pooled 120 ms
rs4623951, rs2111902	0.6	0.7	***0.033, T-T: 0.005 (−0.012)***	0.15	0.2	**0.005, C-T: 0.0006 (0.015)**	***0.026, T-T: 0.01 (−0.01)***	*0.4*	***0.007, T-T: 0.007 (−0.003) C-T: 0.003 (0.02)***	***0.027, T-T: 0.016 (−0.01)***
rs4623951, rs3918346	0.8	0.9	0.1	0.3	0.07	***0.012, C-C: 0.001 (0.015)***	0.07	*0.3*	***0.011, C-C: 0.003 (0.019)***	*0.09*
rs4623951, rs3741775	0.4	***0.036, C-G: 0.016 (0.019)***	**0.0008, T-G: 0.008 (−0.013), C-G: 0.002 (0.023)**	***0.036, C-G: 0.012 (0.021)***	**0.006, T-G: 0.06 (−0.003), C-G: 0.002 (0.023)**	**0.001, T-G: 0.02 (−0.005), C-G: 0.0009 (0.025)**	**0.009, T-G: 0.04 (−0.009), C-G: 0.02 (0.017)**	***0.006, C-G: 0.0009 (0.03)***	***0.00006, T-G: 0.003 (−0.013), C-G: 0.0001 (0.033)***	***0.005, C-G: 0.007 (0.025)***
rs4623951, rs3825251	1	0.9	0.3	0.5	0.1	***0.03, C-T: 0.004 (0.01)***	0.4	*0.4*	***0.03, C-T: 0.006 (0.01)***	*0.6*
rs2111902, rs3918346	0.6	1	0.2	0.6	0.7	0.7	0.2	*0.9*	*0.9*	*0.3*
rs2111902, rs3741775	0.6	0.6	0.2	0.2	0.8	0.2	0.2	*1*	*0.3*	*0.1*
rs2111902, rs3825251	0.4	0.2	***0.03***	0.3	0.2	0.5	0.3	*0.07*	*0.8*	*0.3*
rs3918346, rs3741775	0.7	0.6	0.5	1	0.6	0.2	0.3	*1*	*0.3*	*0.7*
rs3918346, rs3825251	0.6	0.2	0.06	***0.019***	0.1	0.7	0.4	*0.05*	*0.6*	*0.1*
rs3741775, rs3825251	0.7	0.5	0.5	0.4	0.7	0.6	0.7	*0.8*	*0.6*	*0.7*

Abbreviations: *DAO*, D-amino acid oxidase; PPI, prepulse inhibition.
The number represents the overall *p*-value for diplotype. When the overall *p*-value was <0.05 we present *p*-value for specific alleles. The numbers in bracket represent the estimated additive genetic value between the specific diplotype and all other diplotypes pooled together. The *p*-values <0.05 are in bold and italicized; *p*-values <0.01 are in bold. PPI data pooled across prepulse intensity are also shown for comparison.

after false discovery rate correction and α set to 0.01 was between STAI-T trait anxiety and PPI 85 db_120 ms ($r = -0.334$, d.f. $= 68$; $p < 0.005$). There were no significant correlations within the TG− group or the entire cohort or any correlations between STAI-T score and startle amplitude (all *p*-values > 0.1). Median stratification of the TG+ group for STAI-T score, into low and high trait anxiety subgroups allowed groupwise comparison for PPI levels, with a mixed-model ANOVA with diplotype/anxiety as the grouping factor (TG−, TG+/low anxiety, TG+/high anxiety) and prepulse and interval as the within-subject factors. This analysis revealed a significant main effect of genotype ($F(2, 441) = 6.1$, $p < 0.002$), which was not altered when startle amplitude was taken as the covariate ($F(2, 440) = 6.1$, $p < 0.003$). *Post hoc* Bonferroni comparisons showed that PPI of the TG− group was greater than PPI of the highly anxious TG+ ($p < 0.003$), but not the non-anxious TG+ ($p > 0.4$) individuals (Figure 2). The three groups did not differ in terms of startle amplitude ($F < 1$). Figure 3 and Supplementary Table 5 show the effect size (Cohen's *d*) among our diplotypic groups. The subgroup of TG+ subjects combined with high trait anxiety revealed the most significant PPI deficits. We applied similar analyses for working memory measures (two- and three-back, and SWM eight-box within/between error and strategy data) between the three groups (TG−, TG+/low, and TG+/high) and we found a significant effect for three-back working memory test (Kruskal–Wallis $\chi^2 = 7.54$, d.f. $= 2$, $p = 0.023$). Follow-up pairwise group comparisons revealed that the TG− individuals had significantly greater number of correct responses in three-back compared with TG+/high (Mann–Whitney test $p = 0.025$) but not TG+/low (Mann–Whitney test $p = 0.081$) groups.

DISCUSSION

This is the first study to examine the effect of multiple schizophrenia risk *DAO* genetic variants on PPI, working memory and personality traits. We provide strong evidence that two *DAO* diplotypes, were associated with reduced PPI and working memory performance and a personality pattern characterized by attenuated anxiety/negative mood; this pattern was confirmed when personality questionnaires were grouped according to PCA. Moreover, median stratification analysis of the risk TG+ diplotype group showed reduced PPI and working memory performance only in those TG+ individuals with high trait anxiety, with notable increases in the effect size of the PPI data (see Figure 3). It is also notable that while SNP analyses provided marginally significant results, highly significant findings were revealed after haplotype analyses. Importantly, the LOGOS cohort, is a demographically, ethnically and genetically highly homogeneous sample of healthy young males.

Both PPI and working memory are important schizophrenia endophenotypes. Given the central role of the PFC in working memory and the reported association between PPI and working memory possibly via a PFC link (Giakoumaki *et al*, 2008; Roussos *et al*, 2008b, 2009b), it is important although perhaps not too surprising that the risk *DAO* diplotypes affected both PPI and working

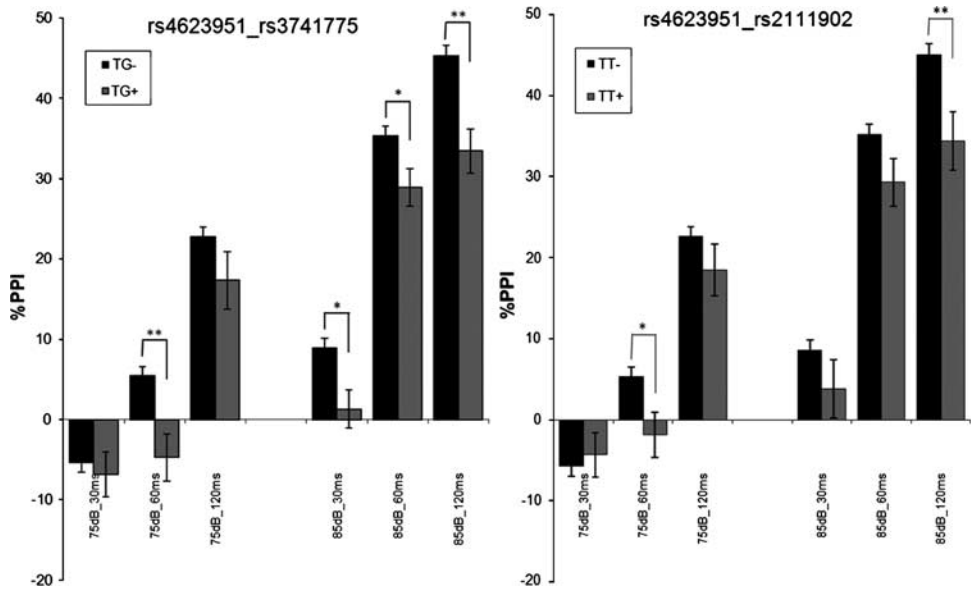

Figure 1 Percent prepulse inhibition (%PPI) for rs4623951_rs3741775 TG− and TG+ groups (left) and rs4623951_rs2111902 TT− and TT+ groups (right). Bars represent SEM. TG+ and TT+ subjects had significantly lower PPI levels compared with the TG− and TT− individuals, respectively. A 2 × 2 × 3 (TG or TT status × prepulse × interval) mixed model ANOVA of PPI data revealed significant main effects of TG ($F(1,428) = 9.8$, $p = 0.002$) and TT status ($F(1,429) = 5$, $p = 0.026$) status. Identical results were revealed with PPI data pooled across prepulse intensity $^{*}p < 0.05$, $^{**}p < 0.005$.

Table 4 Comparison of Cognitive and Personality Variables for the *DAO* Diplotype Groups

	rs4623951–rs3741775			rs4623951–rs2111902		
	TG− (*n* = 456)	**TG+ (*n* = 68)**	***p*-value**	**TT− (*n* = 437)**	**TT+ (*n* = 76)**	***p*-value**
SWM (errors eight-box condition)						
Within errors	1.5 ± 2.5	1.9 ± 3.2	0.4	1.5 ± 2.4	2.4 ± 3.6	**0.006**
Between errors	12.3 ± 10.5	12.2 ± 10.6	0.9	12.1 ± 10.7	13.8 ± 9.6	0.2
Strategy	32.2 ± 4	32.8 ± 3.7	0.2	32.1 ± 4.1	33.5 ± 3.1	**0.001**
N-back (correct responses)						
Two-back	2.5 ± 0.8	2.6 ± 0.8	0.7	2.5 ± 0.8	2.4 ± 0.8	0.5
Three-back	2.1 ± 1	1.7 ± 1.2	**0.001**	2.1 ± 1	1.8 ± 1.2	***0.03***
STAI-T						
STAI-T	36.4 ± 8.3	33.8 ± 7.3	**0.01**	36.4 ± 8.3	34.2 ± 7.2	0.2
EPQ						
Psychoticism	8.6 ± 3.2	8.6 ± 3.6	0.9	8.6 ± 3.2	8.6 ± 3.4	0.9
Extraversion	16.2 ± 4.3	17.7 ± 3.1	**0.002**	16.2 ± 4.3	17.2 ± 3.2	***0.02***
Neuroticism	10.4 ± 5	8.6 ± 4.3	**0.004**	10.2 ± 5	9.6 ± 4.8	0.3
Lie	10.4 ± 4	10.3 ± 4.7	0.9	10.4 ± 4	10.7 ± 4.3	0.6

Abbreviations: *DAO*, D-amino acid oxidase; EPQ, Eysenck Personality Questionnaire; STAI-T; Spielberger's State-Trait Anxiety Inventory—Trait Scale; SWM, spatial working memory.
TG+ represents subjects homozygous for the rs4623951_T and rs3741775_G diplotype and TG− are the non-homozygous individuals. TT+ represents subjects homozygous for the rs4623951_T and rs2111902_T diplotype and TT− are the non-homozygous individuals. Numbers are group means ± SD. The *p*-values < 0.05 are in bold and italicized; *p*-values < 0.01 are in bold. The *p*-values surviving correction for multiple testing (seven tests: corrected $p = 0.05/7 = p < 0.0071$) are bold and underlined. Results from the TCI, Schizotypal Traits Questionnaire and Behavioral Inhibition/Behavioral Activation System did not reveal any significant associations (all $p > 0.1$) and they are not shown.

memory, two functions characteristically deficient in schizophrenia. This finding confirms the role of *DAO* gene in the aetiopathogenesis of the disorder, and informs us on the potential routes by which these *DAO* variants increase risk for schizophrenia; it is indeed a possibility that the PPI and working memory attenuations seen in our risk

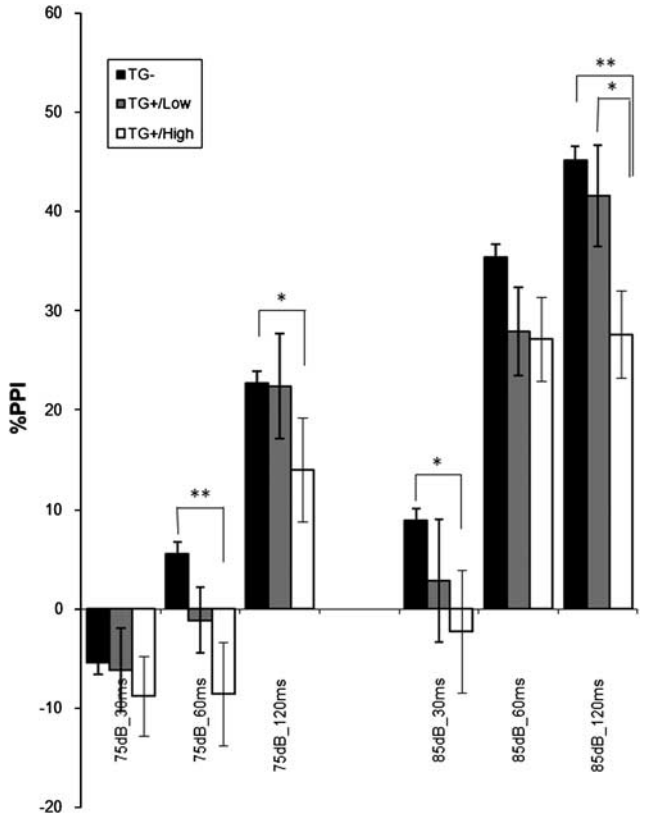

Figure 2 Percent prepulse inhibition (%PPI) for the TG− ($n = 456$), the non-anxious risk TG+ (TG+/low; $n = 34$) and the anxious risk TG+ (TG+/high; $n = 34$) groups as revealed following median split in the TG+ trait anxiety score. Bars represent SEM. A $3 \times 2 \times 3$ (TG status × prepulse × interval) mixed model ANOVA of PPI data revealed significant main effects of TG status ($F(2, 441) = 6.1$, $p < 0.002$). *Post hoc* Bonferroni comparisons revealed that PPI of the TG− group was greater than PPI of the high anxious TG+ only ($p < 0.003$). *$p < 0.05$, **$p < 0.005$.

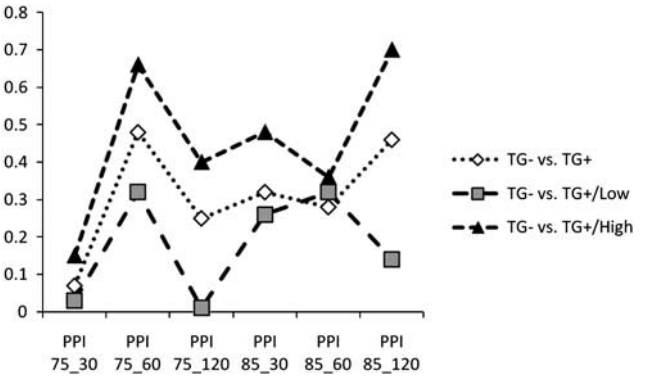

Figure 3 Cohen's d effect size of the *DAO* diplotype groups. TG+ represents subjects homozygous for the rs4623951_T and rs3741775_G diplotype and TG− are the non-homozygous individuals. TG+/low and TG+/high groups represent subjects homozygous for the risk rs4623951_T and rs3741775_G diplotype, with low and high anxiety, respectively, following a median split of their trait anxiety score.

diplotype groups (TG+ and TT+), reflect abnormalities in working memory and PPI overlapping circuitry, mediated via a *DAO* mechanism. It is important to emphasize that our subjects were normal functioning individuals and a 'ceiling effect' on performance is therefore built into our study, making the positive effects even more remarkable. It has to be mentioned however, that reduced or deficient PPI is a feature of a family of conditions with frontal–striatal pathology such as Tourette and Fragile × syndromes, Huntington's disease or OCD (Geyer, 2006), whereas deficient working memory may be a common feature at least in Fragile × syndrome (Hashimoto *et al*, 2011) and OCD (Purcell *et al*, 1998). Future research therefore, should explore the involvement of these *DAO* diplotypes in the spectrum of these syndromes, rather than merely narrowly defined schizophrenia.

In view of the high comorbidity of anxiety to schizophrenia and spectrum disorders (Huppert and Smith, 2005; Lewandowski *et al*, 2006) and its implicit role in the transition from prodromal states into psychosis (Hazlett *et al*, 1997; Yung *et al*, 2003), it is surprising that the same risk diplotypes are associated with lower anxiety levels. Although this was an unexpected finding, given that our risk diplotypes are theoretically associated with higher *DAO* activity (see below), our results are in keeping with animal data showing that inhibition/abolishment of *DAO* activity, improves gating, and cognition but is anxiogenic (Almond *et al*, 2006; Basu *et al*, 2009; Labrie *et al*, 2009a, b; Maekawa *et al*, 2005). Both diplotypes are highly prevalent in the general population (haplotype frequency: TG 36.2% and TT 38.2%) and it is possible that the risk for psychosis conferred, is compensated by an increase in emotional resilience against anxiety and negative mood. Similar conclusions have been reached for the COMT Val158Met (Enoch *et al*, 2003) and rs4818C/G polymorphisms (Roussos *et al*, 2010; Roussos *et al*, 2009b). It is important that the effect of the TG+ genetic variant on gating and working memory becomes more prominent in high anxiety individuals. Interestingly, patients with panic disorder present with a similar pattern of enduring gating deficits, ie, reduced PPI compared with controls which was more profound in patient subgroups with the highest trait (and state) anxiety (Ludewig *et al*, 2002). It would thus be interesting to re-examine gating in these patient samples stratified for at least the TG− and TG+ *DAO* variants. On the basis of these findings, we can further speculate that this risk *DAO* diplotype increases risk for schizophrenia and spectrum disorders when combined with other risk factors, such as genetic, epigenetic, or environmental ones that predispose to reduced resilience against stress.

As per SzGene database, the *DAO* SNP rs4623951 showed significant ($p < 0.026$) association across all ethnicities, with a protective effect of the C allele (OR = 0.88, 95% CI: 0.79–0.98). In a meta-analysis using only case–control data Sun *et al* (2008) showed that of the 75 genes that met a nominal $p < 0.05$ overall significance, *DAO* was eighth in the list, with a combined OR of 1.31, $p = 1.1 \times 10^{-6}$. Finally, Shi *et al* (2008) combined case–control and family-based studies and reported *DAO* as one of the 12 'top' genes. Conclusively, the three meta-analyses provide a moderate degree of support for an association between *DAO* and schizophrenia, specifically for rs4623951. However, neither have haplotype analyses been conducted, nor has a biological mechanism been identified. Nevertheless, *DAO* may be considered to be in the category of schizophrenia susceptibility genes for which there are reasonable grounds to defend, and continue

to investigate its candidacy. The only previous study on the relationship between *DAO* and cognitive schizophrenia endophenotypes found no association of three *DAO* SNPs with performance on a broad range of cognitive tasks; importantly however, the rs4623951 was not analyzed in that study (Goldberg *et al*, 2006). Our findings strongly suggest that this allele when combined with the G and T alleles of the rs3741775 and rs2111902 variants, respectively, is an important determinant of PPI and working memory in healthy subjects. Given the increasing prominence of PPI as a strong schizophrenia endophenotype, our study encourages further exploration of these variants in the pathophysiology of schizophrenia.

The mechanism underlying any genetic association of *DAO* with schizophrenia remains unclear. As the associated SNPs in the *DAO* gene are either non-coding or synonymous, any pathophysiological functionality is likely exerted through an effect on *DAO* expression. In turn, the altered *DAO* expression could affect D-serine or other *DAO* substrate levels as recent findings support that *DAO* expression and activity are increased in schizophrenia (Bendikov *et al*, 2007; Burnet *et al*, 2008; Kapoor *et al*, 2006; Madeira *et al*, 2008; Verrall *et al*, 2007). However, there was no genotype effect (including rs4623951) on *DAO* expression/activity or serum D-serine levels (Burnet *et al*, 2008; Yamada *et al*, 2005). Thus, there is not yet evidence to support the proposed molecular basis for the association of *DAO* with schizophrenia, although these negative studies are not definitive in terms of either SNP coverage, haplotypic assessment, or sample size.

As described in the introduction, *DAO* has the capability to regulate the function of NMDAR through D-serine breakdown and might contribute to NMDAR hypofunction in schizophrenia, or be relevant to its remediation. Complementing these data, *DAO* inhibition can correct NMDAR antagonist-induced deficits in PPI (Adage *et al*, 2008; Hashimoto *et al*, 2009; Horio *et al*, 2009). Moreover, serine racemase genetically modified mice with a $\sim$90% D-serine depletion have impaired spatial memory (Basu *et al*, 2009) and reduced PPI (Labrie *et al*, 2009b), indirectly supporting the possibility that restoring D-serine levels may be therapeutic against such deficits in schizophrenia. Importantly, ddY/*DAO*− mice exhibit increased anxiety (Labrie *et al*, 2009a), suggesting a possible anxiogenic side effect of *DAO* inhibition. Although the exact mechanism underlying the genetic association of *DAO* with schizophrenia-related endophenotypes remains unknown, based on the preclinical data and the results of the present human study we can speculate that the risk diplotypes might predict augmented expression activity of the *DAO* enzyme.

Because *DAO* activity seems to be increased in schizophrenia leading to NMDAR hypofunction, *DAO* inhibition is receiving attention as a potential alternative therapeutic mean (Verrall *et al*, 2010). It is intriguing that chlorpromazine was shown to be a *DAO* inhibitor *in vitro* $>$50 years ago (Yagi *et al*, 1956), a finding which was confirmed recently(Iwana *et al*, 2008) and was also extended to risperidone (Abou El-Magd *et al*, 2010); as clinical trials with *DAO* inhibitors in schizophrenia are lacking, it is unclear whether these observations are clinically relevant, however, they do provide a precedent for the potential therapeutic benefits of selective *DAO* inhibitors. Although findings from preclinical studies remain preliminary, they show that *DAO* inactivation, either in ddY/*DAO*− mice or after pharmacological *DAO* inhibition in rats and mice, produces behavioral, electrophysiological, and neurochemical effects suggestive of a pro-cognitive profile. Thus, studying the behavioral and cognitive impact of high-risk polymorphisms might provide fruitful results for applying pharmacogenomic approaches that will enhance the development of personalized treatment.

Sensorimotor-gating deficits are consistently observed in schizophrenia patients (Braff *et al*, 2001b; Kumari *et al*, 2007b; Kumari *et al*, 2000; Ludewig *et al*, 2003; Swerdlow *et al*, 2006), their first-degree relatives (Cadenhead *et al*, 2000; Kumari *et al*, 2005b) and individuals with schizophrenia spectrum disorder (Cadenhead *et al*, 2000; Quednow *et al*, 2008a), although they are not specific (Geyer, 2006). Twin (Anokhin *et al*, 2003) and family (Aukes *et al*, 2008; Greenwood *et al*, 2007) studies demonstrate that PPI is heritable and a growing number of recent genetic association studies have begun to elucidate its genetic architecture (Giakoumaki *et al*, 2008; Hong *et al*, 2008; Petrovsky *et al*, 2010; Quednow *et al*, 2008b, 2009, 2010; Roussos *et al*, 2010; Roussos *et al*, 2008a, b, 2009a, b). Interestingly, PPI showed a simple mode of transmission which is useful for successful application in molecular genetic research, whereas other endophenotypes, such as verbal fluency and SWM, demonstrated a polygenic, multifactorial model (Aukes *et al*, 2008), suggesting that PPI can be a superior endophenotype for identification of genetic variants in schizophrenia spectrum disorders. Conclusively, PPI has emerged as an important and validated endophenotypic marker, cross-fertilizing genetic studies of schizophrenia.

The LOGOS cohort provides a comprehensive endophenotypic assessment of schizophrenia-related intermediate phenotypes in a demographically and genetically homogeneous population of healthy, young, Greek males. This sample homogeneity coupled with high reliability of PPI recording (Abel *et al*, 1998; Flaten, 2002) and our stringent scoring criteria, increase multiplicatively the power of this cohort to detect genetic variants, thus obviating type I and II errors (Gottesman and Gould, 2003). Importantly, the healthy male volunteer model of studying functional mechanisms of genes is devoid of confounds which strongly impact the study and interpretation of PPI deficits in patient populations, such as gender and medication (Kumari *et al*, 2004; Swerdlow *et al*, 2006), presence of symptoms (Braff *et al*, 1999, 2001a) and the brain effects of psychiatric illness episodes. Last but not least, automatic sensorimotor gating as measured by the uninstructed PPI paradigm is uniquely independent of subjects' motivation, engagement, and social desirability biases. All the above taken together, increase confidence in the conclusions reached, about the functional mechanisms of the *DAO* gene. In the same cohort, we recently demonstrated a significant association of *NRG1* SNPs with PPI (Roussos *et al* 2010). Because *NRG1* and its erbB4 receptor control glutamatergic synapse maturation, modulation and plasticity (Li *et al*, 2007; Bjarnadottir *et al*, 2007), it is possible that genetic variants of both *NRG1* and *DAO* gene might converge on glutamatergic hypofunction. Interestingly, both our *NRG1* and the present *DAO* findings explain a small amount of PPI

variance ($\sim$1–3%), compared with the 10–20% of explained variance in previous studies when dopaminergic genes were examined (Roussos *et al*, 2008a, b; Quednow *et al*, 2009, 2010). The reason for this may be that the effects of dopamine genes are more proximal to the PPI endophenotype, as dopaminergic neurotransmission is directly relevant to PPI physiology, potently regulating PPI within its well identified neural circuitry (Geyer *et al*, 2001, Swerdlow *et al*, 2001). Other plausible reasons include overestimation of the effects of the dopaminergic gene variants as these were studied in independent smaller ($n \sim 100$) samples compared with the much larger LOGOS cohort.

In conclusion, we provide the first phenotypic configuration in a large and demographically/genetically highly homogeneous cohort of young healthy males carrying the *DAO* risk alleles. Pending replication, our findings suggest an influence of the gene in the neural substrate mediating sensorimotor gating and working memory, especially when combined with high anxiety and further validate *DAO* as a candidate gene for the schizophrenia and spectrum disorders.

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DISCLOSURE

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)