A Risk *PRODH* Haplotype Affects Sensorimotor Gating, Memory, Schizotypy, and Anxiety in Healthy Male Subjects

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Background: Significant associations have been shown for haplotypes comprising three *PRODH* single nucleotide polymorphisms (SNPs; 1945T/C, 1766A/G, 1852G/A) located in the 3′ region of the gene, suggesting a role of these variants in the etiopathogenesis of schizophrenia. We assessed the relationship between these high-risk *PRODH* polymorphisms and schizophrenia-related endophenotypes in a large and highly homogeneous cohort of healthy males.

Methods: Participants (*n* = 217) were tested in prepulse inhibition (PPI), verbal and working memory, trait anxiety and schizotypy. The QTPHASE from the UNPHASED package was used for the association analysis of each SNP or haplotype data. This procedure revealed significant phenotypic impact of the risk CGA haplotype. Subjects were then divided in two groups; levels of PPI, anxiety, and schizotypy, verbal and working memory were compared with analysis of variance.

Results: CGA carriers (*n* = 32) exhibited attenuated PPI (*p* < .001) and verbal memory (*p* < .001) and higher anxiety (*p* < .004) and schizotypy (*p* < .008) compared with the noncarriers (*n* = 185). There were no differences in baseline startle, demographics, and working memory. The main significant correlations were schizotypy × PPI [85-dB, 120-msec trials] in the carriers and schizotypy × anxiety in the entire group and the noncarriers but not the carriers group.

Conclusions: Our results strongly support PPI as a valid schizophrenia endophenotype and highlight the importance of examining the role of risk haplotypes on multiple endophenotypes and have implications for understanding the continuum from normality to psychosis, transitional states, and the genetics of schizophrenia-related traits.

The 22q11.2 deletion syndrome (22q11.2DS), also known as velocardiofacial syndrome, is a hemizygous microdeletion on 22q11.2 of typically 3 Mb, encompassing approximately 30 genes. The most prevalent symptoms include cognitive dysfunction with mild mental retardation, behavioral difficulties, and a 30-fold increased risk of schizophrenia (1). Schizophrenia patients have higher-than expected frequency of 22q11.2 microdeletions (1–3), and linkage analysis and linkage disequilibrium studies point to a schizophrenia susceptibility locus at chromosome 22q11.2.

The proline dehydrogenase (oxidase 1) (*PRODH)* gene (MIM: 606810) is located on chromosome 22q11.2, within the narrower 1.5-Mb "psychosis critical region" of the 22q11.2DS, and is widely expressed in the brain and other tissues (4). Proline oxidase is localized within the mitochondria where it catalyses the conversion of proline to D-1-pyrroline-5-carboxylate (P5C). P5C then converts to glutamate or γ-aminobutyric acid, two neurotransmitters critically implicated in the pathophysiology of schizophrenia (5). Recent studies suggest a possible role of the *PRODH* gene variations, located in the 3′ region of the gene, in the etiopathogenesis of schizophrenia (6–8). More specifically, significant associations have been shown for haplotypes consisting of three single nucleotide polymorphisms (SNPs): rs372055

(1945T>C), rs450046 (1766A>G), and rs385440 (1852G>A); the alleles 1945C, 1766G, and 1852A have been shown to be overtransmitted in schizophrenia patients (7,8). Moreover, the *PRODH* 1945C–1852A haplotype was an important determinant of executive functions in schizophrenia patients (9). However, several other studies have failed to replicate these associations (10–12).

Mice lacking the *PRODH* gene have deficient prepulse inhibition (PPI) (4), learning, and memory (13), all characteristic traits of schizophrenia (14,15). Also, children with the 22q11.2DS have deficient PPI (16). PPI is a cross-species operational measure of sensorimotor gating, through which prepulses reduce the effect of subsequent sensory stimuli. It is reliably deficient in schizophrenia, in which reduced gating is thought to lead to sensory overload, which then gives rise to attentional deficits, cognitive and behavioral fragmentation, and some of the complex symptoms of this disorder (14). PPI is emerging as an important endophenotype for schizophrenia (17) because of its high heritability (18) and the presence of PPI deficits in high-risk subjects (19) and in healthy carriers of alleles conferring increased risk for the disorder (20–22).

In this study, we were interested in the relationship between PPI and the high-risk *PRODH* polymorphisms 1945T/C, 1766A/G, and 1852G/A. Given the associations of these *PRODH* variants with schizophrenia, we hypothesized that healthy carriers of the risk alleles would show attenuated PPI. In an attempt to better characterize the phenotypic impact of these *PRODH* variants, we also measured other well-known schizophrenia-related endophenotypes such as verbal and working memory (15,23). Finally, we measured schizotypy as a quantitative trait of liability to psychosis (24) and trait anxiety because it correlates with schizotypy (25) and is thought to increase susceptibility for psychosis (26). A more detailed understanding of the specific role of these risk *PRODH* gene variants in phenotype shaping may help clarify

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important aspects of the pathophysiology of schizophrenia and spectrum disorders.

Methods and Materials

Subjects

The study was approved by the Ethics Committee of the University of Crete. Two hundred and sixty unrelated, right-handed Greek/Caucasian healthy men aged 18–35 years were recruited from the pooled volunteer list of the University staff and students. All subjects were of southeast European ancestry on the basis of self-report and further confirmed by STRUCTURE (27) analysis using 52 ancestry informative unlinked markers selected for maximal informativeness; none of the subjects deviated from a single population, which makes genetic inhomogeneity of the tested population unlikely. Exclusion criteria were personal history of head trauma, medical and neurological conditions, use of prescribed and recreational drugs, personal or family history of DSM-IV Axis I disorders, and hearing threshold lower than 40 dB at 1 kHz. Following written informed consent, all subjects underwent IQ testing with the Raven's progressive matrices, psychiatric assessment using the Mini-International Neuropsychiatric Interview (28), and physical assessment including urine toxicology and a hearing test. Family history of psychiatric disorders was assessed using the Family Interview for Genetic Studies (29), supplemented by medical notes as necessary. Six subjects were excluded because of a psychiatric condition or a family history of psychiatric illness (or both), 22 subjects were startle nonresponders (mean startle amplitude $< 10~\mu V$), and 15 had a positive drug screen. Two hundred and seventeen subjects (mean age $\pm$ SD, 26.1 $\pm$ 4.4) entered and completed the study.

Genotyping

Blood samples were obtained and DNA was extracted using the Flexigene DNA kit (Qiagen, Hilden, Germany). The *PRODH* genotypes were determined by restriction fragment length polymorphism after polymerase chain reaction (PCR) amplification and digestion with restriction enzymes (New England Biolabs, Frankfurt/Main, Germany). See Supplement 1 (Genotyping) for details.

Measurement of the Startle Response

Twelve pulse-alone (40-msec, 115-dB) and 36 prepulse (20-msec, 75- and 85-dB) pulse trials with three lead intervals were used (30, 60, 120 msec). For each interval, there were six trials with 75-dB prepulse and six with 85-dB prepulse. See Supplement 2 (Startle Measurement) for details.

Verbal and Working Memory

We used the Word Lists subtest of the Weschler Memory Scale (WMS-III) from the WAIS-R (30), to assess verbal learning and memory. A list of 12 words was read, and subjects were asked to recall the words in any order (immediate recall); this procedure was repeated four times. After Trial 4, an interference trial with a new list occurred, and subjects were subsequently asked to recall as many words as possible from the first list (short-delay recall). Thirty minutes later, subjects were asked to recall the words from the first list again (long-delay recall). The test finished with a recognition memory trial: a list of words was read, and subjects were asked to identify the words included in the first list (recognition). Outcome variables were the number of correct responses per recall condition (immediate four trials, short delay, long delay) and intrusion errors (words identified that were not included in the list). Working memory was assessed with the

widely used N-Back Sequential Letter Task (31). Outcome variables were the number of correct responses and reaction time.

Questionnaires

All subjects completed the Schizotypal Traits Questionnaire (STQ) (32) and the State-Trait Anxiety Inventory—Trait Scale (STAI-T) (33). The STQ scale is a 37-item self-report questionnaire derived from the criteria for Schizotypal Personality Disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM) and is thought to provide the best measure of the underlying schizotypy dimension (34). Trait anxiety refers to relatively stable individual differences in proneness to anxiety. The STAI-T is a 20-item scale with high internal consistency, high stability, and adequate validity (33). Scores on personality measures are also given for sample characterization purposes and comparison with future studies (Supplement 3) (20).

Statistical Analysis

Comparison of the genotype groups for each SNP (1945T/C: three groups; 1766A/G: 2 groups and 1852G/A: three 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. Hardy-Weinberg equilibrium for *PRODH* markers was checked using Haploview version 4.0 (35). QTPHASE (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/) from the UNPHASED package was used for the association analysis of haplotype data (36). 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 less than 1% in the whole sample were excluded. We used a two-step procedure to correct for multiple testing. First, we used the permutation test option as provided in the QTPHASE to avoid spurious results and correct for multiple testing. The most significant *p* value in the haplotype analysis was corrected for multiple testing by running 1000 permutations of the data. In each permutation, the trait values are randomly shuffled between subjects, and the best *p* value is stored to provide an empirical frequency distribution, followed by comparison of the minimum *p* value to 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, we used false discovery rate (FDR) as an additional multiple testing correction (37), separately for SNPs and haplotypes analyses for consistency with previous studies (38). The FDR correction controls the proportion of false positives among the significant results. Both Permutation and FDR procedures correct for multiple testing and may serve as a better approach for complex disease traits such as most psychiatric diseases, in which multiple genes with modest contribution are involved; they are less conservative than a Bonferroni correction, which is appropriate for independent tests such as unlinked markers. We set the FDR at .05. On the basis of our population size, we were able to detect an anticipated effect (F^2) of .051 (or R^2 = .049) with 80% power and α set to .05.

Results

Table 1 summarizes the *PRODH* genotype and allele frequencies in our sample. Linkage disequilibrium (LD) was strongest between 1766 and 1852 (r^2 = .880) and weak between 1945 and 1766 or between 1945 and 1852 (r^2 = .216 and .245, respectively; all D' values = 1; (Supplement 4). There were no differences in

Table 1. Genotype, Allele, and Minor Allele Frequencies (MAFs) of the *PRODH* Groups

Marker	Genotype			Allele		MAF	HWE p Value
1945	TT	CT	CC	T	C	.27	.19
(rs372055)	120	77	20	317	117		
1766	AA	AG	GG	A	G	.074	.58
(rs450046)	185	32	0	402	32		
1852	GG	AG	AA	G	A	.083	.33
(rs385440)	184	30	3	398	36		

The allele distributions are consistent with Hardy-Weinberg expectations (HWE).

demographic and startle variables between the *PRODH* genotypes for each SNP (Table 2).

Table 3 shows the association of *PRODH* markers with our phenotypic measures as revealed by the QTPHASE. A pattern of association can be seen in which the C, G, and A alleles of the 1945, 1766, and 1852 variants, respectively, were associated with lower PPI levels, lower number of correct words recalled at short and long delays, and increased STQ and STAI scores. Table 4 shows the individual haplotype test for %PPI, verbal and working memory, and personality traits for the three *PRODH* haplotype groups (TAG [73%], CAG [18.7%] and CGA [7.4%]) revealed by the QTPHASE. Overall, the TAG haplotype was associated with higher PPI levels, better memory performance, and lower STQ and STAI scores, whereas the psychosis risk CGA haplotype showed attenuated PPI levels and verbal memory performance and higher STQ and STAI scores.

We divided our sample into carriers (CGA+, n = 32) and noncarriers (CGA−, $n = 185$) of the psychosis risk CGA haplotype (Table 5; Supplement 5). CGA+ individuals scored 40.0 ± 9.1 in the STAI-T and 11.2 ± 7.3 in the STQ, whereas CGA− individuals scored 35.2 ± 7.4 and 8.1 ± 5.6, respectively. One-way ANOVA comparisons revealed higher STQ [$F(1,215) = 7.3, p = .008$; Cohen's $d = .5$] and STAI-T [$F(1,215) = 8.3, p = .004$; Cohen's $d = .6$] scores in CGA+ individuals. Median stratification analysis showed a greater proportion of CGA+ with elevated schizotypy (23/32 = 72%) and trait anxiety (20/32 = 62.5%) compared with CGA− [84/185 = 45.7% and 86/185 = 46.7% respectively, with $\chi^2_{schiz}(1) = 7.22, p = .007$ and $\chi^2_{anxiety}(1) = 4.04, p = .045$]. STAI-T scores correlated with STQ in the entire group ($r = .375, p < .001$) and the CGA− ($r = .4, p < .001$), but not the CGA+ group ($r = .21, p > .26$). STQ was negatively correlated with PPI at the 85-dB/120-msec trial type only (entire group: $r = −.266, df = 216, p < .001$; CGA+ r = −.496, df = 31,

Table 2. Demographic and Startle Comparisons of the *PRODH* Genotype Groups

	1945	1766	1852
Age (years)[a]	1.0 (0.5)	1.4 (.2)	1.5 (.4)
Education (years)[a]	.6 (.7)	< .1 (.7)	.5 (.7)
Estimated IQ	.4 (.6)	.7 (.3)	.6 (.5)
Smokers/Nonsmokers[b]	< .1 (.9)	.6 (.4)	2.1 (.3)
Cigarettes/Day[a]	.2 (.8)	1.9 (.1)	2.8 (.2)
Baseline Startle (μV)[a]	1.5 (.4)	.7 (.3)	1.0 (.6)
Onset Latency (msec)	.6 (.5)	1.2 (.2)	.6 (.5)
Peak Latency (msec)	< .1 (.9)	.7 (.3)	.4 (.6)

[a]For this measures, the nonparametric Kruskal-Wallis procedure was applied.
[b]Chi-square comparison. Numbers represent F or χ^2 score; numbers in brackets represent p values.

Table 3. Association of Percent Prepulse Inhibition (PPI), Memory Measures, and Personality Traits for the Three *PRODH* Groups

	1945	1766	1852
PPI 75_30	.91	.059	**.043**
PPI 75_60	**.034**	**_.003_**	**_.001_**
PPI 75_120	**.019**	**.001**	**_.0002_**
PPI 85_30	.07	**_.002_**	**_.002_**
PPI 85_60	**.04**	.09	.08
PPI 85_120	**.006**	**.01**	**_.003_**
STQ	**_.002_**	**.01**	**.01**
STAI-T	**.006**	**.007**	**.006**
WL Total Correct Recalls (Immediate)	.45	**_.0009_**	**_.0001_**
WL Total Correct Recalls (Short Delay)	.24	**.03**	**.01**
WL Total Correct Recalls (Long Delay)	.46	**_.003_**	**_.001_**
WL Total Intrusions (Immediate)	.17	.51	.37
WL Total Intrusions (Short Delay)	.71	.63	.74
WL Total Intrusions (Long Delay)	.16	.32	.19
N-Back Total Correct	.27	.58	.66
N-Back Reaction Time	.25	.75	.67

p values < .05 are in bold. p values that are both < .05 and survived correction for multiple testing using the false discovery rate approach are bold and underlined.
PPI, prepulse inhibition; STAI-T, State-Trait Anxiety Inventory; STQ, Schizotypal Traits Questionnaire; WL, word lists.

$p = .005$ and CGA− $r = −.146, df = 185, p = .047$), and these correlations remained significant when the effects of anxiety were partialled out (r values: −.268, −.512, and −.178, respectively). Fisher's Z test showed a significant difference between the CGA+ and the CGA− groups in the correlation between STQ and PPI at 85 dB/120 msec ($Z = 1.985; p < .05$). All other correlations were not significant.

Figure 1 shows that the CGA+ subjects had significantly lower PPI levels compared with the CGA− individuals. A 2 × 2 × 3 (CGA status × prepulse × interval) mixed model ANOVA of PPI data revealed significant main effects of CGA status [$F(1,214) = 15.18, p < .001$] and the expected main effects of prepulse and interval [$p < .001$] but no interactions.

Figure 2 (left) shows that more words were recalled with repeated trials (Trials 1–4), but the CGA+ subjects recalled fewer words correctly compared with the CGA− individuals. A 2 × 4 (CGA status × trial) mixed-model ANOVA revealed significant main effects of trial [$F(1,215) = 221.1, p < .001$] and CGA status [$F(1,215) = 11.18, p < .001$] but no interaction ($F < 1$). Figure 2 (right) also shows that, compared with the CGA- individuals, CGA+ carriers had fewer total correct recalls at the short and long delays [One-way ANOVAs: $F(1,215) = 5.3, p < .023$ and $F(1,215) = 10.1, p < .002$, respectively]. There was no effect of CGA status on word intrusions at immediate or at short and long delay recalls (all Fs < 1).

All effects remained significant at $p < .001$ when smoking status was entered as an additional between-subject factor or when anxiety and age were taken as covariates. There was no group difference in accuracy or reaction time (total correct responses) of the N-Back Task (Mann-Whitney $U = 2815.5; p = .9$ and $F < 1$ respectively).

Discussion

This is the first attempt to characterize the phenotype of the CGA *PRODH* haplotype that confers increased risk for schizophrenia (7,8). We found that this haplotype was associated with attenuated PPI, according to prediction, in a demographically,

Table 4. Individual Haplotype Test for the Three *PRODH* Groups

	Global P	TAG[a] (n = 317)	CAG[a] (n = 81)	CGA[a] (n = 32)
PPI 75_30	.07	.91	.12	.06 (−1.4%)
PPI 75_60	.009	**.03**	.84	**.004** (−2.8%)
PPI 75_120	.003	**.02**	.9	**.001** (−2.9%)
PPI 85_30	.01	.07	.92	**.002** (−2.2%)
PPI 85_60	.09	**.04**	.27	.09 (−1.4%)
PPI 85_120	.01	**.006**	.33	**.01** (−1.8%)
STQ	.004	**.001**	.06 (5.0%)	**.01** (8.2%)
STAI-T	.007	**.005**	.24	**.006** (2.2%)
WL Total Correct Recalls (Immediate)	.0008	.45	**.04** (5.5%)	**.0009** (−14.8%)
WL Total Correct Recalls (Short Delay)	.09	.23	.64	**.03** (−26.7%)
WL Total Correct Recalls (Long Delay)	.005	.45	.1	**.003** (−28.5%)
WL Total Intrusions (Immediate)	.45	.19	.36	.53
WL Total Intrusions (Short Delay)	.87	.70	.84	.61
WL total intrusions (long delay)	.42	.15	.54	.28
N-Back Total Correct	.26	.28	.13	.57
N-Back Reaction Time	.53	.26	.32	.75

PPI, prepulse inhibition; STAI-T, State-Trait Anxiety Inventory—Trait Scale; STQ, Schizotypal Traits Questionnaire; WL, word lists.

[a]Difference in risk between a haplotype and all the others pooled together. *p* values < .05 are in bold. *p* values that are both < .05 and survived correction for multiple testing using the false discovery rate approach are underlined and bold. Numbers in brackets represent the estimated additive genetic value for this haplotype relative to TAG.

ethnically, and genetically highly homogeneous sample of healthy male student volunteers. Although the CGA+ subjects had attenuated PPI at all trial types, differences were more prominent in intermediate trials of the dynamic range used here, possibly because of the operation of floor and ceiling effects at the 75-dB/30-msec and 85-dB/120-msec trials, respectively, because the startle response to a pulse is known to be inhibited least efficiently with the former and most efficiently with the latter trial type (Figure 1). Verbal memory is thought to be a trait marker of schizophrenia because it is impaired in ultra-high-risk cohorts (39). The CGA+ subjects had fewer words recalled at immediate, short, and long delays, suggesting a learning/memory difficulty. Although trait anxiety and schizotypy fell short of survival for multiple testing in the QTPHASE analysis, they were both significantly higher in the CGA+ group with moderate effect sizes. Anxiety correlated with schizotypy in the entire group and in the CGA– subgroup, in agreement with previous studies reporting correlations between trait anxiety and schizotypal traits (25). However, this relationship was not present in the

CGA+ group, suggesting that different mechanisms may operate to increase anxiety and schizotypy in this group. The lack of relationship between anxiety and other measures, and the ab-

Table 5. Demographic and Startle Comparisons of the Two CGA Haplotype Groups

	CGA+ (n = 32)	CGA− (n = 185)	p Value
Age (years)[a]	26.8 ± 4.1	26.1 ± 4.5	> .3
Education (years)[a]	17.6 ± 3.5	17.0 ± 2.5	> .7
Estimated IQ	112.0 ± 13.5	113.3 ± 12.4	> .5
Smokers/Nonsmokers[b]	18/14	84/101	> .2
Cigarettes/Day[a]	19.5 ± 9.5	17.2 ± 8.7	> .2
Baseline startle (μV)[b]	165.6 ± 101.4	150.7 ± 95.1	> .4
Onset Latency (msec)	42.1 ± 6.3	43.6 ± 7.3	> .2
Peak Latency (msec)	60.5 ± 4.5	59.8 ± 4.1	> .3

Numbers are group means ± SD.

[a]For these measures, the nonparametric Mann-Whitney procedure was applied.

[b]Chi-square comparison. Age distribution was identical between groups (Levene's test $F = .0, p > .99$).
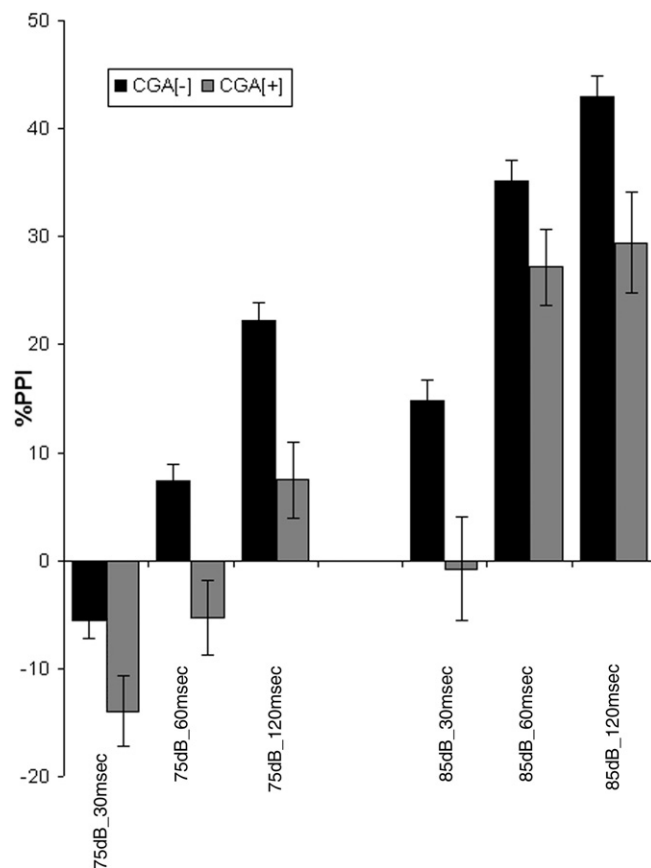
Figure 1. Percent prepulse inhibition (%PPI) for the CGA+ and the CGA− groups. Bars represent SEM.
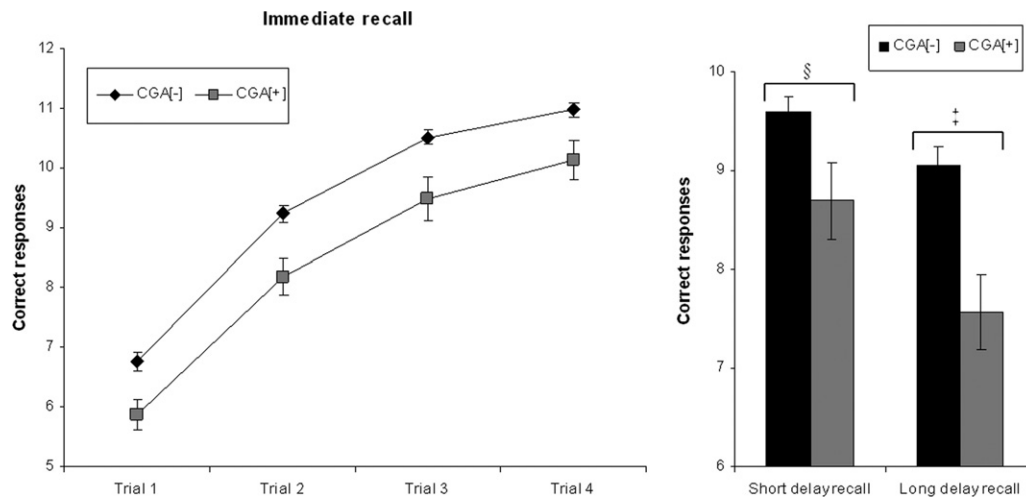
Figure 2. Verbal memory for the CGA+ and the CGA− groups. Bars represent SEM. §$p < .05$; ‡$p < .002$.

sence of intercorrelations suggests that the risk CGA haplotype affects the endophenotypic measures tested by altering the functional outcome of possibly dissociable and nonredundant circuits.

Schizotypy is conceptualized as a nonclinical manifestation of the same underlying biological factors that give rise to schizophrenia and schizophrenia spectrum disorders (40), and it is presumed to result from neural dysmaturation processes (41). Anxiety is highly comorbid in schizophrenia and spectrum disorders (25,42), florid psychotic episodes are often preceded by anxious hyperarousal (43), and the presence of anxiety in prodromal schizotypy appears to increase the risk of transition into psychosis (26), suggesting an interaction between anxiety and schizotypy.

In light of these links between schizotypy, anxiety, and schizophrenia, the association of the risk CGA *PRODH* haplotype with higher anxiety and schizotypy in healthy carriers brings face validity to the finding of reduced PPI in this group and has substantial theoretical implications. Gating ability, as measured by PPI levels, predicts the level of cognitive function in healthy samples (44–46), and deficient gating of exteroceptive stimuli may lead to cognitive dysfunction (14), whereas deficient gating of interoceptive stimuli may lead to increased awareness of "preconscious" material (14), increasing the risk for hallucinations and delusions (47,48). It is tempting to think that at least one path to psychosis in CGA+ individuals is exacerbation of their gating deficiency beyond a certain threshold, following vicious-cyclic interactions between anxiety and schizotypy. Indeed, anxiety induces attentional bias toward negative, threatening stimuli contexts (49), which independently cause attentional interference (50) and reasoning errors (51) in positive schizotypy. It must be noted, however, that deficient PPI is a feature of a family of conditions (52) in which anxiety and schizotypy are common clinical manifestations (Tourette and Fragile X syndromes, Huntington's disease) (53–55) or there are substantial overlaps with schizophrenia (OCD) (56–59) or the severity of symptomatology is a function of schizotypy (PTSD) (60–62). Future research should therefore explore the involvement of this PRODH haplotype in the spectrum of syndromes marked with high anxiety and schizotypy, rather than merely narrowly defined schizophrenia.

The existence of PPI differences in "normal" populations, schizotypal personality disorder, and schizophrenia implies not

only a range of gating abilities but also that different levels of abnormality arise in the same circuit. This abnormality is likely to include genetic components, and it is interesting in this respect that the inverse correlation between schizotypy and PPI at the long 120-msec intervals was much stronger in the CGA+ group, which was more biased toward the high end of the schizotypy score spectrum. This relationship is interesting because PPI at long 120-msec intervals is thought to be less preattentive and more "frontally" mediated (63), and it is consistent with recent findings (64). Previous literature shows PPI deficiencies in normal volunteers scoring high on different psychometric measures of proneness to psychosis (65–68), with some notable negative findings (69–71). These were unstratified samples for CGA status, and our results suggest that such stratification of normal population samples might reveal stronger and reliable, CGA+-dependent relationship between PPI and measures of thought disorder. This relationship strengthens the notion that gating is pivotal to the structure and cohesiveness of thought (72–76) and may reflect a causal link between gating and cognitive and thought processes or common underlying circuitry. The prefrontal cortex (PFC) and the hippocampus-amygdala complex are the most obvious candidate areas of overlap among PPI, verbal memory, thought processes, and anxiety. These major brain regions are shared components of the forebrain networks subserving PPI (7), verbal memory, and anxiety, and they are highly relevant to the etiopathogenesis of schizophrenia (77,78) in which deficiencies in gating, learning and memory, and thought disorders constitute core and interlinked characteristics. Given the central role of the PFC in working memory and the reported association between PPI and working memory through a PFC link (21,31), it is surprising that the CGA *PRODH* haplotype did not affect working memory, a function characteristically deficient in schizophrenia. It may be that behavioral performance in the N-Back is not a sensitive measure of the effect of this *PRODH* haplotype on cognitive function, at least in healthy subjects. It is also possible that unaffected working memory may reflect the operation of a compensatory protective or "resilience" mechanism in our carefully selected, highly functioning CGA+ individuals. Other possibilities include that working memory impairment requires the additional effect of other risk haplotypes or environmental stressors (e.g., alcohol/drug abuse, chronic stress, mood episodes) and may not be evident until prodromal symptom formation or some nonspecific decline in function appears.

Certainly, more detailed analysis of the impact of this haplotype on cognitive function is required, but 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 is a tentative but plausible possibility that the PPI and learning and memory attenuations and the higher schizotypy and anxiety of our CGA+ subjects reflect abnormalities in PFC-hippocampal circuitry mediated through a *PRODH* mechanism. Given that the CGA haplotype might be associated with higher glutamate (Glu) levels (discussed later), our results agree with animal data showing that perturbations of Glu neurotransmission in neonatal rats cause anxiety, as well as PPI and learning/memory, but not working memory, deficits later in life (79).

The CGA haplotype is associated with POX/PRODH hyperactivity. Indeed, the 1766 polymorphism is the only missense variation within the CGA haplotype and leads to a glutamine to arginine substitution in position 521 (Q521R) of the coding protein, encoding for a POX/PRODH enzyme with an approximately 20% higher activity than that of the common glutamine amino acid (80). POX/PRODH is the first enzyme of proline catabolism, converting proline to D-1-pyrroline-5-carboxylate (P5C) (81). Proline functions as an inhibitory neurotransmitter-neuromodulator, as a metabolic precursor of Glu in subpopulations of Glutamatergic neurons (81), or both. Therefore, the Q521R substitution and the subsequent POX/PRODH hyperactivity would correlate with reduced proline levels and increased P5C/Glu availability in the central nervous system (81). Our results could be a consequence of POX/PRODH hyperactivity, reduced proline, increased P5C/Glu availability, or all of these factors. However, we cannot entirely exclude a contribution to the observed alterations in our endophenotypes by the silent SNPs 1945 and 1852; indeed, there is increasing evidence supporting the notion that synonymous SNPs are capable of alterations in protein amounts, structures, or function (82).

The role of increased Glu in excitotoxicity, aberrant neurodevelopment, apoptosis, and sublethal synaptic apoptosis and its contribution to increased schizophrenia risk are well documented (83–88). Thus, the intriguing possibility emerges that the observed PPI and learning/memory attenuation in our CGA+ subjects were the result of increased Glu/glutamine levels. Although speculative, this interpretation agrees with the available evidence, because induction of Glu release in mice is associated with PPI disruption and increased apoptotic cell death in the PFC (89–91) and hippocampus (92). Moreover, mice strains with low PPI present with upregulated pro-apoptotic genes (93). Also, ketamine-induced *N*-methyl-D-aspartate receptor blocking is associated with deficits in immediate and delayed verbal recall episodic memory in humans (94) and memory impairments in the Morris water maze in adult rats (95), which may be related to apoptotic cell death (89). It would thus be interesting for future studies on the CGA phenotype to examine these subjects' Glu levels with magnetic resonance spectroscopy. The high trait anxiety in CGA+ subjects is also interesting in the context of 1) the proposed role of proline as an inhibitory neurotransmitter (81), which is presumably reduced in CGA+ subjects with hyperactive POX/PRODH enzyme; 2) recently established links between anxiety and the oxidative stress metabolism pathway in mice (96); and 3) converging evidence on the causal role of high Glu levels in human anxiety (97), and social anxiety in particular (98), which is a central feature of schizotypy. Finally, given its documented role in apoptosis and oxidative stress (81), the possibility remains that our findings

could be a direct consequence of POX/PRODH hyperactivity. Clearly our results remain phenomenologic in nature and need replication in future studies with direct measurements of PRODH activity, glutamate levels, and apoptosis.

The *COMT* and the *ZDHHC8* genes are located within the same 1.5-Mb "psychosis-critical region" of the 22q11DS as the *PRODH* gene, and both have been associated with schizophrenia (1). We therefore checked for COMT and ZDHHC8 allele distribution between our PRODH groups and, importantly, found no difference. Also, analysis of our data did not reveal a relationship between PPI and the rs175174 polymorphism of the *ZDHHC8* gene (data not shown). Age and its distribution, which could affect gene-function relationship (99), were identical between the PRODH haplotypes, and potential confounding effects were controlled with ANCOVA models. Positive genetic association findings have been previously reported by our group, using subsamples of the current population (21,22); thus, it is possible that our results may not be entirely immune to Type I error. Clearly these results need replication in larger samples of healthy and high-risk subjects. Favoring our findings, however, is the fact that a recent study revealed that the same allele as that in our risk haplotype (1766A/G) was more strongly associated with schizophrenia in families and predicted poorer prefrontal efficiency during performance in a working memory test (100).

In summary, a PRODH haplotype associated with schizophrenia was associated with attenuated gating and verbal memory and higher anxiety and schizotypy in healthy male subjects. These findings support the concept of a continuum of liability to psychosis in the general population and highlight the importance of examining the role of risk haplotypes rather than single SNPs on multiple endophenotypes in the context of a multimodal phenotypic assessment. This strategy applied in the general population and high- and ultra-high-risk groups may advance understanding of the etiology of and transition to psychosis by allowing a dimensional approach to symptomatology. It would be important to examine the phenotypic modulation of CGA+ subjects by stress or psychotropic medication. College graduates scoring higher in schizotypy are at heightened risk for developing psychotic and schizophrenia-spectrum disorders (101), and attempts to identify high-risk and prodromal individuals are receiving increasing attention. These findings suggest that early identification and prophylactic treatment strategies in high-risk groups should include genotyping for these *PRODH* variants because the CGA haplotype is associated with schizophrenia-related endophenotypes, such as reduced gating and memory and high schizotypy and trait anxiety, which may all be negative indicators of decompensation.

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