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ORIGINAL RESEARCH ARTICLE

Hyperprolinemia is a risk factor for schizoaffective disorder

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DNA sequence variations within the 22q11 DiGeorge chromosomal region are likely to confer susceptibility to psychotic disorders. In a previous report, we identified several heterozygous alterations, including a complete deletion, of the proline dehydrogenase (*PRODH)* gene, which were associated with moderate hyperprolinemia in a subset of DSM III schizophrenic patients. Our objective was (i) to determine whether hyperprolinemia is associated with increased susceptibility for any of three psychiatric conditions (schizophrenia, schizoaffective disorder and bipolar disorder) and (ii) to establish a correlation between hyperprolinemia and *PRODH* genotypes. We have conducted a case–control study including 114 control subjects, 188 patients with schizophrenia, 63 with schizoaffective disorder and 69 with bipolar disorder. We report that, taking into account a confounding effect due to valproate treatment, hyperprolinemia is a risk factor for DSM IIIR schizoaffective disorder ($P = 0.02$, Odds ratio = 4.6, 95% confidence interval 1.3–16.3). We did not detect 22q11 interstitial deletions associated with the DiGeorge syndrome among the 320 patients of our sample and we found no association between common *PRODH* polymorphisms and any of the psychotic disorders. In contrast, we found that five rare *PRODH* alterations (including a complete *PRODH* deletion and four missense substitutions) were associated with hyperprolinemia. In several cases, two variations were present simultaneously, either in *cis* or *trans* in the same subject. A total of 11 from 30 hyperprolinemic subjects bore at least one genetic variation associated with hyperprolinemia. This study demonstrates that moderate hyperprolinemia is an intermediate phenotype associated with certain forms of psychosis.
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An increased frequency of several psychotic disorders, such as children-onset schizophrenia,[1] schizophrenia,[2] and bipolar disorder,[3] has been observed in patients with the 22q11 deletion syndromes (22q11DS),[4] which include the DiGeorge syndrome (MIM 188400) and the velo-cardio-facial syndrome (MIM 192430). This has led to the hypothesis that sequence variations of one or several genes located within the 3 Mb region commonly deleted in most of the 22q11DS patients[5] might confer susceptibility for psychoses in the general population. In a previous study, we had identified in two schizophrenic relatives (diagnosed according to DSM III criteria) a 350 kb heterozygous deletion within the DiGeorge critical region (DGCR) removing entirely the proline dehydrogenase (*PRODH*) gene. This deletion was associated in patients with moderate hyperprolinemia. In addition, two heterozygous *PRODH* missense mutations (L441P and L289M) were detected in three of 63 schizophrenic patients but not in 68 controls, and these mutations were also associated with moderate hyperprolinemia.[6] Interestingly, we subsequently found the same *PRODH* deletion and the L441P substitution at the homozygous state in children suffering from a severe form of type I hyperprolinemia, a condition characterized by high plasma proline levels, seizures and mental retardation.[7] To determine whether the association between hyperprolinemia and psychosis was circumstantial or whether mild to moderate hyperprolinemia is indeed a risk factor for psychotic illness, we undertook a large case–control study including 434 subjects (114

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controls, 188 patients with schizophrenia, 63 with schizoaffective disorder and 69 with bipolar disorder). We report here a significant association between DSM IIIR schizoaffective disorder and hyperprolinemia.

Materials and methods

Subjects

All subjects were unrelated Caucasian born in France. Subjects with alcohol abuse at the time of the study were excluded from the study. Control subjects ($n=114$, 40% males, mean age 38 ± 11 years) were mainly staff members. Subjects with a history of psychiatric or metabolic disorder or with first-degree relatives with history of psychiatric disorder were not included. All controls were drug-free (except oral contraceptives in women). Affected subjects were ascertained among in- or outpatients from four psychiatric hospitals. Consensus diagnoses for psychiatric cases were established by two raters according to DSM IIIR criteria from all available information following examination of case notes and in most cases direct interview of patients with the Positive and Negative Schizophrenia Scale (PANSS, Kay *et al*[8]) and the appropriate sections of the Schedule for Affective disorder and Schizophrenia.[9] The schizophrenic sample consisted of 188 subjects (73% males, mean age 37 ± 10 years; clinical subtypes: 35 undifferentiated, 90 paranoid, 47 disorganized, 15 residual and 1 catatonic), while the schizoaffective sample contained 63 subjects (60% males, mean age 40 ± 12 years) and the bipolar sample 69 subjects (52% males, mean age 48 ± 15 years). Clinical data were compiled into case vignettes and diagnoses were made blind of proline data. Informed consent was obtained from all participants and the Local Research Ethics Committee approved the study protocol.

Determination of plasma proline levels

Plasma proline levels were determined in the morning after overnight fasting. All samples were analyzed by the same laboratory using ion exchange chromatography on a BIOTRONIK LC 3000 system.

Statistical analyses

Data were expressed as mean $\pm$ SD. Sex and age effects were assessed by analysis of variance (ANOVA) or regression and included as covariates in subsequent analyses when significant. Comparisons for continuously distributed variables were performed using the Mann–Whitney nonparametric test. Statistical comparisons for categorical variables were performed using χ^2 test or Fisher's exact test when the cells size was small. All tests reported were two-tailed. Odds ratio (OR) are given with their 95% confidence interval (CI). Haplotypes frequency was estimated by the EH program.[10]

Quantitative multiplex PCR of short fluorescent fragments (QMPSF)

In all, 10 exons (1, 2, 4, 5, 9, 10, 11, 12, 14, 15) of the *PRODH* gene were analyzed by QMPSF in all subjects. When a rearrangement involving the *PRODH* gene was detected, four different QMPSF covering the entire DGCR region were performed as described previously.[6]

Table 1 Primers used for sequence analysis of the *PRODH* exons

Exon	*Sense primer*	*Antisense primer*
2[a]	5′-CAGCTGAAGTGCCAGGGTGC-3′	5′-CCCGTTTCAGGCCAGGAAG-3′
3[b]	5′-CATGGCTGGGGTGAAGTGT-3′	5′-ATACATCCCACCTGCTCCC-3′
4[c]	5′-GGGAACGTGCCTGTCACCT-3′	5′-CCACTTGTGCCCCAGCCTA-3′
5[d]	5′-ATGCTTGGAGCCCTCCTGA-3′	5′-GCCCCAGCTTAGTGTTCACC-3′
6[c]	5′-CCCTCCCTGTCGCACCCT-3′	5′-GCCTCTAAGGCATGGGCAC-3′
7[c]	5′-GTGCCCATGCCTTAGAGGC-3′	5′-GGGTGAGTTCCCAAAACCAC-3′
8[a]	5′-CCAATGGGTGGGGGGAAGG-3′	5′-CTTCAGCACTGTCATCCCAG-3′
9[a]	5′-CACAGGTGGGGGTGCC-3′	5′-GGTCTGTGGGGCCATAGTG-3′
10[c,e]	5′-TTCCCGCCCTTGCTGTG-3′	5′-CGGCATCCACCATCAGC-3′
11[a]	5′-GCAACAGTTGCTCCCACTCT-3′	5′-ACTCCCTCCTCCATCCCAT-3′
12[c]	5′-TGACAATGTGACCCTGGACG-3′	5′-CATGACATAAAAGCTGAGGAAATA-3′
13[c,e]	5′-CTGGAACTGGGCTCACAT-3′	5′-GCCTTTCCCCACAAATGCC-3′
14[c]	5′-CCCTCAGTTCCTGGCTTCAC-3′	5′-GGGCAGCACCTCCATCACGG-3′
15[b]	5′-GGGCCTCATGGTGTATCT-3′	5′-CCAGAGCTGACCCTGTGT-3′

[a]PCR conditions: after a 3 min denaturation at 94°C, 10 cycles of 10 s at 94°C, 15 s at 65°C (with a decrease of 1°C/cycle) and 15 s at 72°C, followed by 30 cycles of 10 s at 94°C, 15 s at 55°C, and 15 s at 72°C, and a final 5 min extension at 72°C.
[b]PCR conditions: after a 3 min denaturation at 94°C, 10 cycles of 20 s at 94°C, 20 s at 65°C (with a decrease of 1°C/cycle) and 50 s at 72°C, followed by 30 cycles of 20 s at 94°C, 20 s at 55°C, and 50 s at 72°C, and a final 5 min extension at 72°C.
[c]PCR conditions: after a 3 min denaturation at 94°C, 10 cycles of 10 s at 94°C, 15 s at 60°C (with a decrease of 1°C/cycle) and 15 s at 72°C, followed by 30 cycles of 10 s at 94°C, 15 s at 50°C, and 15 s at 72°C, and a final 5 min extension at 72°C.
[d]PCR conditions: after a 3 min denaturation at 94°C, 35 cycles of 10 s at 94°C, 15 s at 52°C, and 20 s at 72°C, and a final 5 min extension at 72°C.
[e]Indicated primers amplified both the *PRODH* gene and the *PRODH* pseudogene.

Sequence analysis
Exons 2–15 of *PRODH* were PCR amplified using primers listed in Table 1. PCR was performed in a final volume of 50 μl containing 100 ng of DNA, 1 μM of each primer, 200 μM of dNTP, 0.5 U of *Taq* polymerase (Abgene, Epsom, UK), 1.5 mM $MgCl_2$, 67 mM Tris-HCl (pH 8.8), 16 mM $(NH4)_2SO_4$ and 0.01% Tween 20. For exons 2, 4, 6, 8, 9, 10 and 11, we added 10% DMSO. After purification by low-melt agarose gel electrophoresis, PCR products were directly sequenced on both strands using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing kit (PE Applied Biosystems, Foster city, CA, USA) and an Applied Biosystems model 3100 automated sequencer (PE Applied Biosystems).

To determine the phase of the substitutions in patients bearing several mutations, PCR products were cloned using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA, USA) and sequenced.

Screening for specific mutations
The L289M substitution was detected by digestion of the PCR-amplified exon 8 using the restriction enzyme *Bsa*BI (New England Biolabs, Beverly, CA, USA) as described previously.[6] The P406L substitution, corresponding to the C1421T transition, was detected by digestion of the PCR-amplified exon 11 (330 bp) using the restriction enzyme *Bsr*BI (New England Biolabs). The C allele produces three 44, 128 and 158 bp sized fragments, whereas the T allele produces two fragments of 128 and 202 bp.

Results

Association study
We examined the inter-relationships between plasma proline levels and gender and age in the whole sample (ie all patients *vs* controls) with the use of ANOVA. This showed no diagnosis effect ($F = 1.19$, $P = 0.27$), a significant gender effect ($F = 14.5$, $P = 0.0002$) with no diagnosis × sex interaction. Age group effects and age × gender or diagnosis interactions were not significant ($F = 1.4$, $P = 0.23$, $F = 2.1$, $P = 0.14$ and $F = 0.0001$, $P = 0.98$, respectively). In controls, the mean plasma proline values were significantly higher in males (249 ± 63.8 μmol/l) than in females (196 ± 60 μmol/l) ($P < 0.0001$) as reported previously.[11] Subjects whose plasma proline level were two standard deviations above the controls mean values (ie 316 μmol/l in females and 377 μmol/l in males) were considered to be hyperprolinemic. Using these two thresholds in subsequent analyses, we found that among the 434 subjects included in this study, 30 (50% males) had moderate hyperprolinemia (mean plasma proline level $= 430 \pm 90$ μmol/l; range 317–726 μmol/l). As shown in Table 2, the distribution of hyperprolinemic subjects did not differ between control subjects and schizophrenic patients but was significantly different in schizoaffective and bipolar patients, as compared with controls ($P = 0.002$, OR = 5.4, 95% CI 1.7–17 and $P = 0.005$, OR = 4.8, 95% CI 1.5–15, respectively).

Confounding factors
Since most schizoaffective and bipolar patients ($n = 132$) were treated by mood-stabilizing drugs—either Lithium or valproate—we examined a possible effect of these treatments on plasma proline levels. While no effect of Lithium was detected (data not shown), the mean plasma proline level in 57 patients treated by valproate was significantly higher than in those not receiving this drug (281 ± 104 *vs* 236 ± 104 μmol/l, $P = 0.001$, Mann–Whitney nonparametric test). Two factors ANOVA (sex and treatment) failed to show any sex effect ($F(1,128) = 1.77$, $P = 0.19$) or any interaction between sex and treatment ($P = 0.45$) in these 132 schizoaffective or bipolar patients. In this subsample, 14 of the 57 valproate-positive subjects were hyperprolinemic as compared with eight of the 75 valproate-free subjects ($P = 0.06$, Fisher's exact test). Although this latter result did not reach statistical significance, a confounding effect of valproate treatment on plasma proline level is likely. We therefore decided to restrict the association study to valproate-free subjects. The association between hyperprolinemia and disease remained valid in schizoaffective patients ($n = 40$ patients, $P = 0.02$, OR = 4.6, 95% CI 1.3–16.3) but was no longer significant in bipolar patients ($n = 35$ patients, $P = 0.6$). Given the effect of valproate on proline

Table 2 Distribution of hyperprolinemia in controls and patients

	Control subjects (n = 114)	*Schizophrenic patients (n = 188)*[a]	*Schizoaffective patients (n = 63)*[b]	*Bipolar patients (n = 69)*[c]
Hyperprolinemic subjects ($n = 30$)	4 (3.5%)	4 (2.1%)	11 (17.5%)	11 (15.9 %)
Nonhyperprolinemic subjects ($n = 404$)	110 (96.5%)	184 (97.9%)	52 (82.5%)	58 (84.1 %)

[a] $P = 0.48$.
[b] $P = 0.002$, OR = 5.4, 95% CI 1.7–17.
[c] $P = 0.005$, OR = 4.8, 95% CI 1.5–15.

levels, we next checked the effect of any medication that might conceivably have a similar effect. Only five subjects were treated with other anticonvulsants and none of them was hyperprolinemic. Finally, to assess a potential effect of neuroleptic treatment on plasma proline level, we compared the mean plasma proline level in schizophrenic patients (all but three of them receiving classical neuroleptics) and controls (224 ± 54 and 217 ± 67 μmol/l, respectively). Controlling for the sex effect, these values did not differ between schizophrenic patients and controls. This argues against an effect of classical neuroleptic treatment on plasma proline levels.

Absence of association between frequent PRODH polymorphisms and hyperprolinemia or psychotic disorders

Several DNA pseudogene-like substitutions of the *PRODH* gene had previously been identified.[12] We focused on two frequent polymorphisms located in exon 12 (R431H and A472T), one of which (A472T) had been associated with schizophrenia in a previous report.[12] All tested samples were in Hardy–Weinberg equilibrium. No association between these polymorphisms and any psychotic disorder was detected. Moreover, no polymorphism was associated with hyperprolinemia (Table 3). No linkage disequilibrium was found between the two polymorphisms and haplotypes analysis failed to reveal any association.

Rare DNA variations and hyperprolinemia

In all hyperprolinemic subjects, we sequenced the coding sequence of *PRODH* and searched for heterozygous deletions, using QMPSF as described previously.[6] A complete deletion and five missense mutations, located in exons 8, 11 and 12 of the *PRODH* gene, were found, respectively, in two and 13 of the 30 hyperprolinemic subjects. The estimated size of the *PRODH* deletion, approximately 350 kb, was identical in the two unrelated patients and was similar to that previously identified, in the homozygous state, in a patient suffering from a severe form of type I hyperprolinemia.[7] The frequency of the detected *PRODH* variations was then assessed in the whole sample including patients and controls. As shown in Table 4, the allele frequencies, except in one case, significantly differed between hyperprolinemic and nonhyperprolinemic subjects. A detailed account of phenotypic and genotypic characteristics for each hyperprolinemic subject is given in Table 5. It is interesting to note that, in all patients and controls, QMPSF analysis of the *PRODH* gene allowed us to exclude the presence of the common interstitial deletions associated with the 22q11DS.

Discussion

When assessing a possible association between high plasma proline levels and three psychotic disorders, we reasoned that this risk factor might be present only in a small subset of affected subjects and we anticipated a small variation in the upper tail of the distribution rather than a global shift of the distribution in patients. As a result, a categorical analysis was carried out rather than a quantitative analysis. To define abnormal plasma proline level, we used a stringent threshold (2 SD above the sex-specific control mean) that, assuming a Gaussian distribution, predicts a specificity of 97.5%. Using this approach, the case–control study reveals an association between hyperprolinemia and two psychotic disorders (schizoaffective disorder and bipolar disorder). However, a confounding effect of valproate treatment is probable. Although no effect of valproate on proline metabolism has so far been described, the inhibitory potential of this drug on metabolic enzymes has been documented

Table 3 Distribution of R431H and A472T variations in 114 controls and 320 patients and comparison of genotypic and allelic frequencies

R431H	*RR*	*RH*	*HH*	*P-value (genotypic)*	*P-value (allelic)*
Controls ($n=114$)	89	20	5		
Schizophrenic patients ($n=188$)	151	32	5	0.68	0.54
Schizoaffective patients ($n=63$)	49	11	3	1	0.9
Bipolar patients ($n=69$)	52	15	2	0.75	0.9
Hyperprolinemic subjects ($n=30$)	20	9	1		
Nonhyperprolinemic subjects ($n=404$)	321	69	14	0.18	0.15
A472T	*AA*	*AT*	*TT*	*P-value (genotypic)*	*P-value (allelic)*
Controls ($n=114$)	101	10	3		
Schizophrenic patients ($n=188$)	167	18	3	0.89	0.9
Schizoaffective patients ($n=63$)	53	10	0	0.19	0.7
Bipolar patients ($n=69$)	54	13	2	0.12	0.06
Hyperprolinemic subjects ($n=30$)	24	6	0		
Nonhyperprolinemic subjects ($n=404$)	351	45	8	0.26	0.7

Table 4 Allelic distribution of rare *PRODH* variations in nonhyperprolinemic and hyperprolinemic subjects[a]

	Nonhyperprolinemic subjects (n = 808 chromosomes)	*Hyperprolinemic subjects (n = 60 chromosomes)*	*P-value*
L289M	5 (0.6%)	2 (3%)	0.08
P406L	3 (0.4%)	3 (5%)	0.005
L441P	2 (0.2%)	3 (5%)	0.003
R453C	7 (0.9%)	4 (7%)	0.005
T466M	1 (0.1%)	3 (5%)	0.001
Del[b]	0	2 (3%)	0.005

[a]Allele frequencies are indicated within parentheses.
[b]Del: 350 kb deletion removing entirely the *PRODH* locus.

Table 5 Phenotypic and genotypic characteristics of the 30 hyperprolinemic subjects

Subject	*Diagnosis*	*Sex*	*Proline (μmol/l)*	*Valproate*	*Genotype*[a]
T1	Control	F	396	No	**L441P**/R431H
T2	Control	M	482	No	**P406L** + R431H/R431H
T3	Control	M	446	No	Wt
T4	Control	M	389	No	**R453C** + R431H/**P406L**
S1	Schizophrenia	F	365	No	Wt
S2	Schizophrenia	F	340	No	Wt
S3	Schizophrenia	F	319	No	**L441P**/wt
S4	Schizophrenia	M	454	No	Wt
SA1	Schizoaffective disorder	F	429	Yes	L289M/wt
SA2	Schizoaffective disorder	F	322	Yes	Wt
SA3	Schizoaffective disorder	F	317	Yes	**R453C**/wt
SA4	Schizoaffective disorder	F	726	No	**L441P**/R431H
SA5	Schizoaffective disorder	F	503	No	Wt
SA6	Schizoaffective disorder	F	424	No	**del/R453C**
SA7	Schizoaffective disorder	F	317	No	**T466M**/wt
SA8	Schizoaffective disorder	M	612	Yes	Wt
SA9	Schizoaffective disorder	M	452	Yes	Wt
SA10	Schizoaffective disorder	M	508	No	Wt
SA11	Schizoaffective disorder	M	504	No	Wt
BP1	Bipolar disorder	F	470	Yes	**del/P406L**
BP2	Bipolar disorder	F	444	Yes	Wt
BP3	Bipolar disorder	F	401	Yes	Wt
BP4	Bipolar disorder	F	325	No	Wt
BP5	Bipolar disorder	M	488	Yes	Wt
BP6	Bipolar disorder	M	410	Yes	Wt
BP7	Bipolar disorder	M	402	Yes	Wt
BP8	Bipolar disorder	M	397	Yes	Wt
BP9	Bipolar disorder	M	394	Yes	**R453C** + **T466M**/wt
BP10	Bipolar disorder	M	377	Yes	L289 M/wt
BP11	Bipolar disorder	M	493	No	**T466M**/R431H

[a]Missense mutations whose effect on enzyme activity is drastic *in vitro* are in bold. The common substitution R431H, which reduces the enzyme activity from 35% and might act as a contributing factor in patients bearing a mutation, is also indicated. Two mutations linked by the sign + are located in *cis*. A slash indicates mutations located in *trans*. Wt: wild type. Whenever more than one plasma sample was obtained from the same subject, the mean plasma proline value was used. Sex indication: F = female; M = male.

in vivo and *in vitro*.[13] This confounding effect does not affect our conclusion that hyperprolinemia is associated with increased susceptibility to schizoaffective disorder but hampers any firm conclusion in the bipolar group. Constitution of a large sample of valproate-free bipolar patients will be required to resolve this issue. Since the description of schizoaffective disorder over 70 years ago by Kasanin,[14] this clinical entity has raised much debates. The DSM III classification provided no criteria to diagnose schizoaffective disorder. Specific criteria were introduced in the DSM IIIR and have been shown to define a

syndrome that differs meaningfully from both schizophrenia and affective disorder in symptoms, course, outcome and patterns of familial psychopathology.[15] The association described here with a biological marker adds an important criterion to confirm that DSM IIIR schizoaffective disorder is a valid construct. The question of whether schizoaffective disorder constitutes a separate disorder or shares a common etiology with either schizophrenia or bipolar disorder remains highly controversial.[16–18] While the present study does not support an involvement of hyperprolinemia in DSM IIIR schizophrenia, we cannot exclude at the present stage that this risk factor might be shared between schizoaffective disorder and bipolar disorder. Finally, it should be stressed that the evidence for an association is modest ($P = 0.02$) and has not been corrected for multiple testing (three clinical phenotypes). Therefore, this finding needs to be confirmed in a replication sample. In our previous study,[6] we reported high plasma proline levels in four DSM III schizophrenic patients with rare *PRODH* gene alterations (*PRODH* deletion or L441P missense mutation). This finding is not in conflict with the present data since three of these patients meet the DSM IIIR criteria for schizoaffective disorder.

Molecular analysis, in accordance with two recent reports[19,20] revealed no association between common *PRODH* polymorphisms and schizophrenia. The frequency of the *PRODH* region deletion was recently set to 1 in 250 subjects (95% CI, 1 in 115 to 1 in 547 subjects) in the Japanese population,[21] indicating that the prevalence of this deletion is increased by 10-fold relative to 22q11 interstitial deletions. Therefore, failure to detect the *PRODH* deletion in our controls probably results from our limited sample size. It should also be noted that no interstitial deletion of chromosome 22q11 was found in our sample of patients, thus supporting recent reports[22,23] indicating that 22q11DS might be less common among adult psychotic patients than initially thought.[24] Our study demonstrates that several rare *PRODH* mutations but not common DNA polymorphisms are associated with hyperprolinemia. Functional consequences of 16 *PRODH* mutations have recently been established (Bender *et al*, communication no. 73 at the American Society of Human Genetic meeting, 2003). Interestingly, the four missense mutations associated with hyperprolinemia in the present study (P406L, L441P, R453C and T466M) have indeed a drastic effect on enzyme activity with a residual activity of less than 20%. The L289M mutation, which shows a trend towards an association with hyperprolinemia ($P = 0.08$), has only a mild impact on enzyme activity (residual activity: 84%). Even if a deleterious effect of the L289M mutation on enzyme activity is ruled out, we cannot exclude that this substitution might be in linkage disequilibrium in our population with another DNA variation located within regulatory regions of the *PRODH* gene. Aside from the L289M mutation, 11 out of 30 hyperprolinemic subjects bore at least one genetic variation associated with hyperprolinemia (eight from 16 in valproate-free hyperprolinemic subjects). In several cases, two variations were simultaneously present, either in *cis* or *trans*. The loose correlation between hyperprolinemia and *PRODH* coding region genotypes indicates that other genetic factors located outside of the coding region are likely to influence plasma proline levels. This conclusion is supported by the fact that although approximately half of the DiGeorge patients are hyperprolinemic,[25] all 22q11 DS subjects sequenced so far by our group bear a wild-type *PRODH* coding sequence in the remaining allele (unpublished results).

Earlier reports[26,27] had implicated potentiation of glutamate excitotoxicity as a possible mechanism for neurological dysfunction in hyperprolinemic subjects. Recently, more insight into the deleterious effect of elevated proline level in brain has been gained with reports showing that proline decreases $Na+$, $K+$-ATPase and acetylcholinesterase activities and elicits oxidative stress in rat brains.[28–30]

The present study emphasizes the importance of intermediate phenotypes (ie plasma proline level) to reveal the determinism of genetically complex diseases such as psychotic disorders. Moreover, the finding of an association between hyperprolinemia and schizoaffective disorder but not with schizophrenia is potentially important to delineate the etiological boundaries of these clinical entities.

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