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For Peer Review

Type I hyperprolinemia: genotype/phenotype correlations

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Abstract

Type I hyperprolinemia (HPI) is an autosomal recessive disorder associated with cognitive and psychiatric troubles, caused by alterations of the Proline Dehydrogenase gene (*PRODH*) at 22q11. HPI results from *PRODH* deletion and/or missense mutations reducing proline oxidase (POX) activity. The goals of this study were first to measure in controls the frequency of *PRODH* variations described in HPI patients, second to assess the functional effect of *PRODH* mutations on POX activity and finally to establish genotype/enzymatic activity correlations in a new series of HPI patients. 8/14 variants occurred at polymorphic frequency in 114 controls. POX activity was determined for 6 novel mutations and 2 haplotypes. The c.1331G>A, p.G444D allele has a drastic effect whereas the c.23C>T, p.P8L allele and the c.[56C>A; 172G>A], p.[Q19P; A58T] haplotype result in a moderate decrease in activity. Among the 19 HPI patients, 10 had a predicted residual activity <50%. 8 out of 9 subjects with a predicted residual activity \geq 50% bore at least one c.824C>A, p.T275N allele, which has no detrimental effect on activity but whose frequency in controls is only 3%. Our results suggest that *PRODH* mutations lead to a decreased POX activity or affect other biological parameters causing hyperprolinemia.

Introduction

Type I hyperprolinemia (MIM# 239500) is characterized by an increase in plasma proline level in absence of urinary pyrroline-5-carboxylate (P5C) (typical of type II hyperprolinemia).

It was initially described in 1962 as a nephropathy associated with cerebral dysfunction [Efron, 1965; Schafer et al., 1962]. The association with renal disease was fortuitous and was due to a recruitment bias since patients with nephropathy were preferentially screened by amino acid chromatography at this time. As a result of the analysis of new families with HPI ten years later, it was considered that hyperprolinemia was a benign condition without mental retardation or renal dysfunction [Mollica et al., 1976]. The gene associated with this condition, named *PRODH* (MIM# 606810), was mapped in 1997 on 22q11 [Campbell et al., 1997] and encodes for proline oxidase (POX), the first enzyme involved in proline catabolism. Its expression is limited to liver, brain and kidney. In 1999, a mouse model deficient for POX was described. These mice present behavioral problems, especially a sensory motor gating impairment, which is an endophenotype of schizophrenia [Gogos et al., 1999]. A few years later, [Humbertclaude et al., 2001] described a case with severe neurologic impairment and raised the question whether HPI really was a benign trait in humans, which opened up again the discussion.

In 2003, we described [Jacquet et al., 2003] a recurrent 350kb deletion encompassing the *PRODH* and *DGCR6* (MIM# 601279) genes, mediated by low copy repeats surrounding this region, which was subsequently found to be present in 1/250 individuals in the general population [Guilmatre et al., 2009; Ohtsuki et al., 2004]. Numerous missense mutations in the *PRODH* gene have also been described, most of them appearing recurrently because of a gene conversion between *PRODH* and a non-processed pseudogene located 1.4 Mb telomeric on chromosome 22 [Liu et al., 2002]. A functional characterization of POX enzymatic activity

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3 was established demonstrating that some of these mutations had a severe impact (> 70%
4 reduction) on its activity whereas others had a mild or moderate effect [Bender et al., 2005].
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6 Further genetic studies in 3 patients with mental retardation, epilepsy and very high plasma
7 proline level allowed us to establish that the severe form of HPI was a recessive disorder
8 resulting from homozygous *PRODH/DGCR6* deletion or mutations with a drastic effect on
9 enzymatic activity [Jacquet et al., 2003]. In accordance with the mouse model phenotype,
10 hyperprolinemia was then shown to be a risk factor for schizoaffective disorder [Jacquet et al.,
11 2005]. The link between hyperprolinemia and cognitive or psychiatric troubles was further
12 strengthened by the study of patients with velo cardio facial syndrome (VCFS) that results
13 from a 1.5-3Mb deletion on chromosome 22q11. Although all these patients are hemizygous
14 for the *PRODH* gene, only 30-50% have elevated plasma proline level [Goodman et al., 2000;
15 Raux et al., 2007], while 50% have mental retardation and 30% psychosis [Murphy et al.,
16 1999]. We have shown that in VCFS increased plasma proline level (in association with the
17 *COMT* (MIM# 116790) genotype) was a risk factor for psychosis and that prolinemia was
18 inversely correlated to IQ [Raux et al., 2007]. Based on the functional predictions, this study
19 also showed that a reduction of at least 50% of the enzymatic activity was required to produce
20 mild to moderate hyperprolinemia while severe hyperprolinemia was generally associated
21 with a near complete loss of enzymatic activity. In addition to these observations,
22 *PRODH/DGCR6* deletion and mild hyperprolinemia were recently found at a high frequency
23 in autistic subjects [Guilmatre et al., 2009].
24
25 Despite this large body of data, several points remain to be clarified. In particular, correlations
26 between *PRODH* genotype and various levels of hyperprolinemia, ranging from mild to
27 severe, remain poorly understood. In the present report, three issues were examined: first we
28 measured in controls the allelic frequency of *PRODH* variations found in HPI patients, second
29 we assessed the functional effect of previously uncharacterized mutations or haplotypes on
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3 enzymatic activity, and third we attempted to establish correlations between genotype and
4 enzymatic activity in a new series of HPI patients.
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10 **Material and methods**

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12 This study was approved by the Ethics Committee of Rouen University Hospital.
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15 **Controls**

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17 To establish the allelic frequency of *PRODH* (NM_016335) variants, we genotyped 114
18 healthy caucasian subjects originating from France (46 men, 68 females) previously included
19 in a study aimed to establish the normal values of plasma proline in the adult population
20 [Jacquet et al., 2005]. Nucleotide numbering reflects cDNA numbering with +1 corresponding
21 to the A of the ATG translation initiation codon in the reference sequence, according to
22 journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.
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34 **Patients with HPI**

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36 Blood samples from patients with HPI were sent to our laboratory from centers expert in the
37 diagnosis of Autism or mental retardation in order to analyze the *PRODH* gene. All patients
38 were examined by experienced clinicians and psychiatric diagnoses were made according to
39 DSM IV criteria. Biological investigations included MRI imaging, karyotype, Fragile X
40 testing and amino-acid chromatography. Plasma proline levels were determined by ion
41 exchange chromatography after overnight fasting. After informed consent was obtained, DNA
42 was extracted from peripheral lymphocytes. Patients as well as their parents were genotyped
43 in order to obtain the phase of the mutations.
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54 ***PRODH* sequencing and QMPSF**

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57 *PRODH* exons were sequenced using PCR conditions and primers previously described
58 [Jacquet et al., 2005; Raux et al., 2007]. In some subjects in which *PRODH* exon 7 could not
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3 be amplified , due to indels in both flanking introns, the presence of the c.824C>A, p.T275N
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5 variation was assessed using the forward primer 5'TGCAGTTCTCAGAGGTGCTG 3' in
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7 combination with the previously described reverse primer.
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11 *PRODH* deletions were characterized by quantitative multiplex analysis of short fluorescent
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13 fragments (QMPSF), as previously described [Jacquet et al., 2002].
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15 **Array CGH**

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17 DNA samples were analyzed using Human High-Resolution Discovery Microarray Kit
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19 4x180K (Agilent Technologies, Santa Clara, California, USA), using standard recommended
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21 protocols. Non commercial genomic DNA pool of 10 control individuals was used as
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23 reference. Hybridization results were extracted with Feature extraction software and analyzed
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25 using Agilent's DNA-analytics software. The data were processed using ADM-2 algorithm,
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27 with the threshold set at 6.0 SD.
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31 **Functional assay for POX activity**

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33 We purchased restriction enzymes and buffers from New England Biolabs: Pfu-Turbo DNA
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35 polymerase from Stratagene and chemicals from Sigma. ¹⁴C-proline was obtained from New
36
37 England Nuclear and purified prior to use by ion-exchange chromatography on Dowex AG
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39 50w-8x hydrogen form (BioRad) [Phang et al., 2001]. To assay green fluorescent protein
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41 (GFP) fluorescence, we used a Zeiss LSM 510 Meta confocal laser-scanning microscope.
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43 Functional test was realized as previously described [Bender et al., 2005]. Briefly, the
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45 *PRODH* ORF divided into three fragments previously cloned in the pBluescript KS vector
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47 (Stratagene) was used to perform the mutagenesis and the wild-type *PRODH* cDNA
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49 previously cloned into pTracer (Invitrogen) was used for expression analysis. We
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51 mutagenized the *ClaI/BstEII*-modified *PRODH* cDNA in pBluescript KS by PCR using the
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53 QuikChange Mutagenesis Kit (Stratagene). Dependent on the position of the desired
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55 mutation, we subcloned either the mutagenized *KpnI/ClaI* or mutagenized *ClaI/BstEII*
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3 fragment into the pTracer expression construct and resequenced the construct to verify the
4 presence of the mutation and the integrity of the ligations. For all expression studies, we
5 utilized a subclone (CHO-K1-C9) of CHO-K1 cells that lack endogenous POX activity [Valle
6 et al., 1973]. For electroporation, we used 30 μg of the indicated plasmid DNA and 350 volts/
7 400 Ω /960 μF in 300 μl of growth medium containing 1.25% DMSO and $5\text{--}7 \times 10^6$ cells.
8 After 48 h, we harvested the cells by washing the monolayer with PBS and scraping them into
9 cold PBS. The cells were collected by centrifugation at 480 x g and were resuspended in 0.1
10 M KPO_4 (pH 7.2). The cells and their organelles were disrupted by sonication for 1 min at a
11 setting of 25% (Branson Sonifier 450 [Branson Ultrasonics]). Total protein was determined
12 with the Pierce BCA protein assay (Pierce). POX-specific activity (nmol/prod/mg/hr) was
13 assayed radioisotopically, as described elsewhere [Phang et al., 1975]. To account for
14 variation in transfection efficiency, we expressed the data as a percentage of wild type
15 (specific activity of mutant allele/transfection efficiency)/(specific activity of wild-type
16 allele/transfection efficiency). For alleles with severe reduction in POX activity, we repeated
17 the assay with the addition of 1 mM FAD. All samples were assayed in triplicate in
18 independent transient transfection experiments.

41 **Ex vivo splicing assay using a splicing reporter minigene**

42 This assay was used to test the effect of the p.T275N variant in *PRODH* exon 7 splicing. This
43 assay was realized in both HeLa and ShSY5Y cells using the same protocol [Tournier et al.,
44 2008], with DMRIE-C (Invitrogen) as the transfection reagent for the SH-SY5Y cells. Primer
45 sequences are available upon request.

55 **Results**

59 **Frequency of *PRODH* variations in controls**

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3 The genotyping of 114 controls without psychiatric or neurological disorders (four of them
4 having plasma proline level above the upper threshold, range 389-482 $\mu\text{mol/l}$) allowed us to
5 determine in a control population the frequency of missense mutations that were present in
6 HPI patients [Raux et al., 2007] (and this study) (Table 1). For each mutation, the distribution
7 of genotypes followed the Hardy-Weinberg equilibrium. Among the 14 variations we
8 analyzed, 8 occurred at a polymorphic frequency (> 0.01), two of them (c.56C>A, p.Q19P
9 and c.553T>C, p.R185W) being very common with a minor allele frequency of 0.48 and 0.36,
10 respectively.
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25 **Functional consequences of *PRODH* missense mutations**

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27 In addition to data already reported, that are summarized in Supp Table S1 [Bender et al.,
28 2005], we describe here the results of functional analyses for a new series of *PRODH*
29 missense mutations (c.23C>T, p.P8L ; c.32G>C, p.R11P ; c.88C>T, p.P30S ; c.172G>A,
30 p.A58T ; p.T275N and c.1331G>A, p.G444D) and haplotypes (c.[56C>A; 172G>A],
31 p.[Q19P; A58T] and c.[56C>A; 88C>T], p.[Q19P; P30S]) (Figure 1). We show that the
32 p.G444D substitution has the most drastic effect resulting in only 27% of residual enzymatic
33 activity. p.P8L and the haplotype p.[Q19P; A58T] have both moderate effect. However the
34 other mutations have no effect on enzymatic activity or even a tendency to increase it. As
35 previously described for other haplotypes [Bender et al., 2005] the POX activity encoded by
36 the p.[Q19P; A58T] allele was dictated by the most severe missense mutation with no
37 evidence of intra allelic complementation. By contrast, analysis of the p.[Q19P; P30S]
38 haplotype showed that the p.P30S variation fully complemented the deleterious effect of the
39 p.Q19P mutation.
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Genotype/phenotype correlations in patients with type I hyperprolinemia

Clinical and demographic characteristics of a novel series of HPI patients whose *PRODH* genotype and plasma proline level have been determined are displayed in Table 2a. Most of these patients present with psychiatric and cognitive troubles and epilepsy. MRI findings were normal except for two patients who displayed agenesis of the corpus callosum, symmetrical temporal lobe hypoplasia, thalamus fusion and right opercular dysplasia, respectively.

Dysmorphism was noted in 4 patients who were all negatively screened for DNA rearrangement by aCGH, as was patient 109 who had an abnormal MRI but without dysmorphism.

In this series of patients, abnormal plasma proline levels ranged from 1667 to 327 $\mu\text{mol/l}$ with, for several subjects, considerable variation between two measures obtained at different time (Table 2b). Based on the results of functional assay, four subjects had a genotype predicted to result in a drastic loss of enzymatic activity. In accordance with these predictions, they all present with severe hyperprolinemia. Overall, only 10/19 patients had a predicted residual activity $<50\%$ which, according to our working hypothesis [Raux et al., 2007], is required to produce hyperprolinemia. Strikingly, 8 out of 9 subjects with a predicted residual enzymatic activity $\geq 50\%$ bore at least one p.T275N allele, which has no effect on enzymatic activity but whose frequency in the control population is only 3%. Using an ex vivo splicing assay to further analyse the effect of this variant, we observed no effect on the splicing pattern of *PRODH* exon 7 (data not shown).

Discussion

All patients included in this report present cognitive impairment and/or behavioral problems. Epilepsy was also frequent. It should however be stressed that this study was not designed to

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3 formally assess the clinical spectrum of HPI. Thus, although consistent with the view that HPI
4 is associated with psychiatric and neurologic disabilities, our data may just reflect a
5 recruitment bias since subjects presenting such conditions are more likely to be screened for
6 inborn error of amino acid metabolism than normal subjects.
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12 Nonspecific dysmorphic features were present in a subset of patients raising the question of a
13 putative link between HPI and syndromic MR. In these patients, array CGH analysis failed to
14 document the co-occurrence of another genetic abnormality that can explain these physical
15 features. Thus, it remains unclear whether dysmorphism may be a component of the HPI
16 syndrome or whether another as yet undetected genetic defect is present in this subset of
17 patients.
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29 Concerning the relationship between plasma proline and the predicted POX activity, a rather
30 poor correlation was observed. However, as previously noted [Bender et al., 2005] our
31 functional assay circumvent any effect these mutations might have on RNA splicing or
32 protein stability. It is thus conceivable that several variations may be wrongly classified as
33 benign on the basis of this sole test. In a previous report concerning VCFS patients we had
34 already noted the unexplained enrichment of the c.1292G>A, p.R431H mutation in patients
35 with high proline levels [Raux et al., 2007]. In the present report, the increased frequency of
36 the functionally neutral p.T275N mutation in HPI patients is noteworthy, pointing out that it is
37 indeed a probable pathologic variant. Since this variation does not modify the splicing pattern
38 of exon 7, its biological effect remains elusive and it is most likely in linkage disequilibrium
39 with another functional variant. Indeed, DNA variations in the regulatory regions or in introns
40 of *PRODH* may influence transcription or splicing of the gene. A polymorphism located in
41 the 5' region of *PRODH* and resulting in a 1.5-fold increase in promoter activity has been
42 described [Hoogendoorn et al., 2004]. However, genotyping this polymorphism in our
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3 patients did not reveal any relationship between the low transcriptional activity allele and HPI
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5 (data not shown). Characterization of further variations in these regulatory regions is thus
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7 required.
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10 As previously noted, plasma proline is subject to fluctuations over time in most patients, thus
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12 requiring, as a rule, repeated determinations. However, several patients bearing a potentially
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14 deleterious genotype had only mild hyperprolinemia on repeated measures. Since it seems
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16 unlikely that such a slight increase in proline level is sufficient to produce a detrimental
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18 effect, this result questions the value of plasma proline level determination as an estimate of
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20 CNS proline value. Because peripheral proline cannot gain access to the central nervous
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22 system (CNS) across the blood- brain barrier [Hawkins et al., 2006], blood and brain proline
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24 form two largely independent pools. Brain proline, the concentration of which in the cerebro
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26 spinal fluid (CSF) is low (4.2 $\mu\text{mol/l}$), is not subject to nutritional fluctuations. Its level
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28 depends on local synthesis from ornithine and glutamate and from degradation of collagen of
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30 the extra cellular matrix. In severe HPI [Efron, 1965; Humbertclaude et al., 2001] as well as
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32 in *PRODH* *-/-* mice [Gogos et al., 1999], CNS concentration of proline is elevated.
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34 Unfortunately, CSF data are not available for our patients.
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41 Another point to be kept in mind when interpreting slight increases in plasma proline is that
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43 normative values of plasma proline are lower in children as compared with adults.
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46 Accordingly, in mice *PRODH* transcripts are expressed at higher level in the young brain as
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48 compared with the mature brain [Maynard et al., 2003]. Physiologically, the fine tuning of
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50 *PRODH* expression facilitates proline catabolism during periods in which most refinement
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52 and stabilization of synaptic connections occur. Therefore, it can be hypothesized that any
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54 genetic defect causing even a modest increase in proline levels during critical periods of
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56 development may have a dramatic effect on CNS functioning.
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For Peer Review

References

- Bender H, Almashanu S, Steel G, Hu C, Lin W, Willis A, Pulver A, Valle D. 2005. Functional consequences of proth missense mutations. *Am J Hum Genet* 76:409-420.
- Campbell HD, Webb GC, Young IG. 1997. A human homologue of the drosophila melanogaster sluggish-a (proline oxidase) gene maps to 22q11.2, and is a candidate gene for type-i hyperprolinaemia. *Hum Genet* 101:69-74.
- Efron ML. 1965. Familial hyperprolinemia. report of a second case, associated with congenital renal malformations, hereditary hematuria and mild mental retardation, with demonstration of an enzyme defect. *N Engl J Med* 272:1243-1254.
- Gogos JA, Santha M, Takacs Z, Beck KD, Luine V, Lucas LR, Nadler JV, Karayiorgou M. 1999. The gene encoding proline dehydrogenase modulates sensorimotor gating in mice. *Nat Genet* 21:434-439.
- Goodman BK, Rutberg J, Lin WW, Pulver AE, Thomas GH. 2000. Hyperprolinaemia in patients with deletion (22)(q11.2) syndrome. *J Inherit Metab Dis* 23:847-848.
- Guilmatre A, Dubourg C, Mosca A, Legallic S, Goldenberg A, Drouin-Garraud V, Layet V, Rosier A, Briault S, Bonnet-Brilhault F, Laumonnier F, Odent S, Le Vacon G, Joly-Helas G, David V, Bendavid C, Pinoit J, Henry C, Impallomeni C, Germano E, Tortorella G, Di Rosa G, Barthelemy C, Andres C, Faivre L, Frebourg T, Saugier Veber P, Campion D. 2009. Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism, and mental retardation. *Arch Gen Psychiatry* 66:947-956.
- Hawkins RA, O'Kane RL, Simpson IA, Vina JR. 2006. Structure of the blood-brain barrier and its role in the transport of amino acids. *J Nutr* 136:218S-26S.

- 1
2
3 Hoogendoorn B, Coleman SL, Guy CA, Smith SK, O'Donovan MC, Buckland PR. 2004.
4
5 Functional analysis of polymorphisms in the promoter regions of genes on 22q11. *Hum Mutat*
6
7 24:35-42.
8
9
10 Humbertclaude V, Rivier F, Roubertie A, Echenne B, Bellet H, Vallat C, Morin D. 2001. Is
11
12 hyperprolinemia type i actually a benign trait? report of a case with severe neurologic
13
14 involvement and vigabatrin intolerance. *J Child Neurol* 16:622-623.
15
16
17 Jacquet H, Berthelot J, Bonnemains C, Simard G, Saugier-Veber P, Raux G, Campion D,
18
19 Bonneau D, Frebourg T. 2003. The severe form of type i hyperprolinaemia results from
20
21 homozygous inactivation of the proth gene. *J Med Genet* 40:e7.
22
23
24 Jacquet H, Demily C, Houy E, Hecketsweiler B, Bou J, Raux G, Lerond J, Allio G, Haouzir
25
26 S, Tillaux A, Bellegou C, Fouldrin G, Delamillieure P, Menard JF, Dollfus S, D'Amato T,
27
28 Petit M, Thibaut F, Frebourg T, Campion D. 2005. Hyperprolinemia is a risk factor for
29
30 schizoaffective disorder. *Mol Psychiatry* 10:479-485.
31
32
33 Jacquet H, Raux G, Thibaut F, Hecketsweiler B, Houy E, Demilly C, Haouzir S, Allio G,
34
35 Fouldrin G, Drouin V, Bou J, Petit M, Campion D, Frebourg T. 2002. Proth mutations and
36
37 hyperprolinemia in a subset of schizophrenic patients. *Hum Mol Genet* 11:2243-2249.
38
39
40 Liu H, Abecasis GR, Heath SC, Knowles A, Demars S, Chen Y, Roos JL, Rapoport JL,
41
42 Gogos JA, Karayiorgou M. 2002. Genetic variation in the 22q11 locus and susceptibility to
43
44 schizophrenia. *Proc Natl Acad Sci U S A* 99:16859-16864.
45
46
47 Maynard TM, Haskell GT, Peters AZ, Sikich L, Lieberman JA, LaMantia A. 2003. A
48
49 comprehensive analysis of 22q11 gene expression in the developing and adult brain. *Proc Natl*
50
51 *Acad Sci U S A* 100:14433-14438.
52
53
54 Mollica F, Pavone L. 1976. Hyperprolinaemia: a disease which does not need treatment?.
55
56
57 *Acta Paediatr Scand* 65:206-208.
58
59
60

1
2
3 Murphy KC, Jones LA, Owen MJ. 1999. High rates of schizophrenia in adults with velo-
4 cardio-facial syndrome. *Arch Gen Psychiatry* 56:940-945.

7 Ohtsuki T, Tanaka S, Ishiguro H, Noguchi E, Arinami T, Tanabe E, Yara K, Okubo T,
8 Takahashi S, Matsuura M, Sakai T, Muto M, Kojima T, Matsushima E, Toru M, Inada T.
9
10
11
12 2004. Failure to find association between proth deletion and schizophrenia. *Schizophr Res*
13 67:111-113.

16 Phang JM, Downing SJ, Valle DL, Kowaloff EM. 1975. A radioisotopic assay for proline
17 oxidase activity. *J Lab Clin Med* 85:312-317.

21 Phang JM, Hu C, Valle D. 2001. Disorders of proline and hydroxyproline metabolism. In:
22 Scriver CR, Beaudet AL, Sly, WS VD, editors. *The metabolic and molecular bases of*
23 *inherited disease*. McGraw Hill, New York. 1821-1838.

28 Raux G, Bumsel E, Hecketsweiler B, van Amelsvoort T, Zinkstok J, Manouvrier-Hanu S,
29 Fantini C, Breviere GM, Di Rosa G, Pustorino G, Vogels A, Swillen A, Legallic S, Bou J,
30 Opolczynski G, Drouin-Garraud V, Lemarchand M, Philip N, Gerard-Desplanches A, Carlier
31 M, Philippe A, Nolen MC, Heron D, Sarda P, Lacombe D, Coizet C, Alembik Y, Layet V,
32 Afenjar A, Hannequin D, Demily C, Petit M, Thibaut F, Frebourg T, Champion D. 2007.
33
34
35
36
37
38
39
40
41 Involvement of hyperprolinemia in cognitive and psychiatric features of the 22q11 deletion
42 syndrome. *Hum Mol Genet* 16:83-91.

45 Schafer IA, Scriver CR, Efron ML. 1962. Familial hyperprolinemia, cerebral dysfunction and
46 renal anomalies occurring in a family with hereditary nephropathy and deafness. *N Engl J Med*
47 267:51-60.

52 Tournier I, Vezain M, Martins A, Charbonnier F, Baert-Desurmont S, Olschwang S, Wang
53 Q, Buisine MP, Soret J, Tazi J, Frebourg T, Tosi M. 2008. A large fraction of unclassified
54
55
56
57
58
59
60 variants of the mismatch repair genes *mlh1* and *msh2* is associated with splicing defects. *Hum*
Mutat 29:1412-1424.

1
2
3 Valle D, Downing SJ, Phang JM. 1973. Proline inhibition of pyrroline-5-carboxylate
4
5 reductase: differences in enzymes obtained from animal and tissue culture sources. *Biochem*
6
7
8 *Biophys Res Commun* 54:1418-1424.
9
10
11
12
13
14
15
16
17
18
19
20
21
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23
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3 **Figure and table legends**
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8 **Table 1: Frequency of missense mutations in controls**
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10 *PRODH* (NM_016335)
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12 Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG
13 translation initiation codon in the reference sequence, according to journal guidelines
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15 (www.hgvs.org/mutnomen). The initiation codon is codon 1.
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22 **Table 2: Phenotypic and genotypic features of patients with Type I hyperprolinemia**
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24 **a: clinical features**
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26 F: female; M: male; age (years); #: born from consanguineous parents; * corpus callosum
27 agenesis, symmetrical temporal lobe hypoplasia, thalamus fusion; ** right opercular dysplasia
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29 MRI: Magnetic Resonance Imaging; aCGH: array Comparative Genomic Hybridization
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31 AF: autistic features; AP: atypical psychosis; At: ataxia; Au: autism; D: dyslexia; E: epilepsy;
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33 LD: learning disorder; MR: mental retardation; PE: pharmaco-resistant epilepsy; PMD:
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35 Psychomotor delay.
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40 + hypertelorism, telecanthus, high palate, ligamentar hyperlaxity; ++ frontal bossing, thin lips,
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42 syndactyly 2rd-3rd toes; +++ high forehead, frontal bossing, micrognathism, thin upper lip,
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44 short nose, short stature, microcephaly -2.5 SD; ++++ short nose with anteverted nostrils,
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46 prominent ridging of the metopic suture, long and flat philtrum and thin upper lip, small chin,
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55 **b: molecular features**
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57 *PRODH* (NM_016335); WT: wild type; del: deletion
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59 Fasting abnormal plasma proline values: age >18 years: males > 377 $\mu\text{mol/l}$, females > 316
60 $\mu\text{mol/l}$; 5 years > age < 18 years: > 270 $\mu\text{mol/l}$; age < 5 years: > 235 $\mu\text{mol/l}$.

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3 PREA: predicted residual enzymatic activity/normal value.
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6 Drastic mutations are in bold, mutations with moderate effect are in normal character and
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8 mutations without effect are in italics. The c.824C>A, p.T275N mutation is underlined.
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12 **Figure 1: proline oxidase activity of *PRODH* alleles**
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15 *PRODH* (NM_016335)
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17 The thin vertical lines indicate standard deviation.
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19 WT: wild type
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24 **Supp Table S1: proline oxidase activity of previously tested *PRODH* alleles** [Bender et al.,
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26 2005] *PRODH* (NM_016335)
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Table 1: Frequency of missense mutations in controls*PRODH* (NM_016335)

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG

translation initiation codon in the reference sequence, according to journal guidelines

(www.hgvs.org/mutnomen). The initiation codon is codon 1.

Variant	Exon	Genotypes (%)			Alleles (%)	
c.23C>T, p.P8L	2	PP (100)	PL (0)	LL (0)	P (100)	L (0)
c.32G>C, p.R11P	2	RR (100)	RP (0)	PP (0)	R (100)	P (0)
c.56C>A, p.Q19P, rs2008720	2	QQ (26)	QP (51,5)	PP (22,5)	Q (52)	P (48)
c.88C>T, p.P30S, rs3815655	2	PP (82)	PS (18)	SS (0)	P (91)	S (9)
c.172G>A, p.A58T	2	AA (95)	AT (4)	TT (1)	A (97)	T (3)
c.553T>C, p.R185W, rs4819756	5	RR (43)	RW (41)	WW (16)	R (63,5)	W (36,5)
c.824C>A, p.T275N, rs5747933	7	TT (93)	TN (7)	NN (0)	T (97)	N (3)
c.1217C>T, p.P406L, rs3970555	11	PP (97)	PL (3)	LL (0)	P (98,5)	L (1,5)
c.1279G>A, p.V427M, rs2238731	12	VV (100)	VM (0)	MM (0)	V (100)	M (0)
c.1292G>A, p.R431H, rs2904552	12	RR (78)	RH (18)	HH (4)	R (87)	H (13)
c.1322T>C, p.L441P, rs2904551	12	LL (98)	LP (2)	PP (0)	L (99)	P (1)
c.1331G>A, p.G444D	12	GG (100)	GD (0)	DD (0)	G (100)	D (0)
c.1357C>T, p.R453C, rs3970559	12	RR (96)	RC (4)	CC (0)	R (98)	C (2)
c.1397C>T, p.T466M, rs2870984	12	TT (99)	TM (1)	MM (0)	T (99,5)	M (0,5)

Table 2: Phenotypic and genotypic features of patients with Type I hyperprolinemia

a: clinical features

F: female; M: male; age (years); #: born from consanguineous parents; * corpus callosum agenesis, symmetrical temporal lobe hypoplasia, thalamus fusion; ** right opercular dysplasia
 MRI: Magnetic Resonance Imaging; aCGH: array Comparative Genomic Hybridization
 AF: autistic features; AP: atypical psychosis; At: ataxia; Au: autism; D: dyslexia; E: epilepsy;
 LD: learning disorder; MR: mental retardation; PE: pharmaco-resistant epilepsy; PMD: Psychomotor delay.
 + hypertelorism, telecanthus, high palate, ligamentar hyperlaxity; ++ frontal bossing, thin lips, syndactyly 2rd-3rd toes; +++ high forehead, frontal bossing, micrognathism, thin upper lip, short nose, short stature, microcephaly -2.5 SD; ++++ short nose with anteverted nostrils, prominent ridging of the metopic suture, long and flat philtrum and thin upper lip, small chin, hypertelorism, strabismus.

b: molecular features

PRODH (NM_016335); WT: wild type; del: deletion

Fasting abnormal plasma proline values: age >18 years: males > 377 μmol/l, females > 316 μmol/l; 5 years > age < 18 years: > 270 μmol/l; age < 5 years: > 235 μmol/l.

PREA: predicted residual enzymatic activity/normal value.

Drastic mutations are in bold, mutations with moderate effect are in normal character and mutations without effect are in italics. The c.824C>A, p.T275N mutation is underlined.

Table 2a

Subject	Sex	Age	Diagnosis	MRI	Dysmorphism	a-CGH
101	F	13	MR, Au, PE, At	normal	no	
102	M	3	MR, E	normal	yes +	normal
103	M	4	MR, PE, At	normal	no	
104	F	2	PMD, PE, At	normal	yes ++	normal
105	M	7	MR, Au	normal	no	
106	M	4	PE	normal	no	
107	F	14	LD	normal	no	
108	F	4	MR, E	normal	yes +++	normal
109	M	3	MR, E	abnormal*	no	normal
110	M	9	MR, E, , At	normal	no	
111	M	7	AF, E	normal	no	
112	M	9	LD, D	normal	no	
113	M	13	E	normal	no	
114	M	12	MR, E	abnormal **	yes ++++	normal
115	F	10	MR, E	normal	no	
116	M	31	AF, E	normal	no	
117	F	11	Au	normal	no	
116	M	14	MR, AP	normal	no	
119	F	9	LD	normal	no	

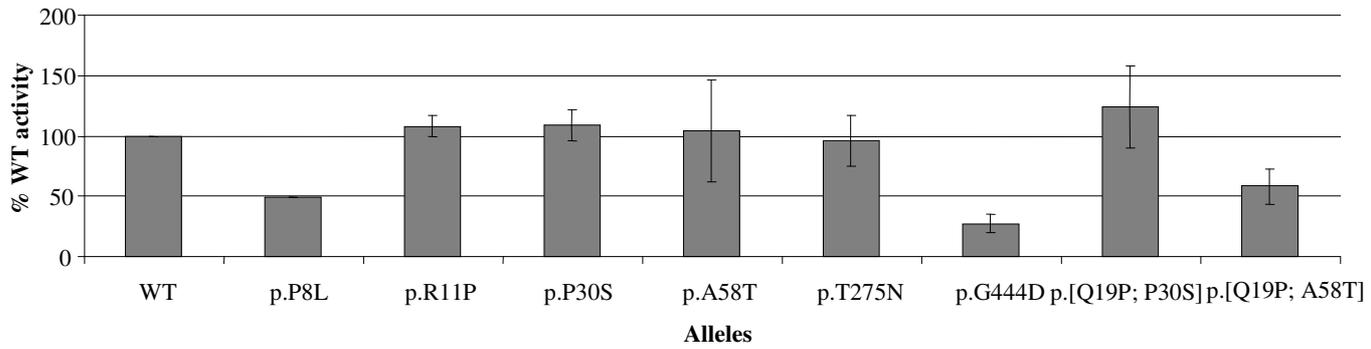
Table 2b

	Plasma proline		PREA
Subject	($\mu\text{mol/l}$)	Genotype	(%)
101	637-1667	p.R453C/p.T466M +p.R431H	15
102	1200	p.T466M+p.R453C/p.T466M+p.R453C	2
103	1084	p.L441P/p.L441P +p.R185W+p.Q19P	0
104	600-1000	p.R453C +p.V427M+p.R431H+ <u>p.T275N/p.T275N</u>	55
105	599-899	del/p.T275N	50
106	879	p.R453C/p.Q19P + <u>p.T275N</u>	35
107	692-878	del/del	0
108	826-862	p.L441P/p.Q19P +p.R185W	30
109	414-804	p.T466M+p.R453C/p.Q19P	30
110	781	p.R431H+ <u>p.T275N/p.T275N</u>	80
111	450-700	p.L441P + <u>p.T275N/WT</u>	50
112	679	p.T466M+p.R453C +p.R185W+p.Q19P+p.P30S/p.R431H	30
113	605	p.T466M +p.Q19P+p.R185W/p.R431H	40
114	574-847	p.L441P+p.R453C +p.R431H+p.R185W/ <u>p.T275N</u>	50
115	395-534	p.Q19P+ <u>p.T275N/p.V427M+p.T275N</u>	50
116	345-500	p.R431H/ <u>p.T275N</u>	80
117	488	del/p.V427M + <u>p.T275N</u>	25
118	400	p.Q19P+p.P30S/p.R431H	60
119	243-327	p.R453C/p.T275N	55

Figure 1: proline oxidase activity of *PRODH* alleles*PRODH* (NM_016335)

The thin vertical lines indicate standard deviation.

WT: wild type



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Supp Table S1: proline oxidase activity of previously tested *PRODH* alleles [Bender et al., 2005] *PRODH* (NM_016335)

Alleles	% WT activity
p.Q19P	65
p.A167V	65
p.R185W	55
p.R185Q	100
p.L289M	85
p.P406L	12
p.D426N	40
p.V427M	45
p.R431H	65
p.L441P	0
p.R453C	8
p.A455S	90
p.T466M	18
p.A472T	72
p.Q521E	25
p.Q521R	122
p.[L441P; R453C]	1
p.[V427M; R431H; L441P]	8
p.[R453C; T466M; A472T]	3