ONLINE MUTATION REPORT

# The severe form of type I hyperprolinaemia results from homozygous inactivation of the *PRODH* gene

**H Jacquet, J Berthelot, C Bonnemains, G Simard, P Saugier-Veber, G Raux, D Campion, D Bonneau, T Frebourg**

*J Med Genet* 2003;**40**:e7(http://www.jmedgenet.com/cgi/content/full/40/1/e7)

Since the original reports of hyperprolinaemia by Scriver *et al*[1] and Shafer *et al*,[2] two types of this rare metabolic disorder have been biochemically characterised[3]: type I (MIM 239500) results from the defect of the enzyme proline dehydrogenase (oxidase), which ensures the conversion of proline into Δ-1-pyrroline-5-carboxylate (P5C), the first step in the conversion from proline to glutamate,[4] and type II (MIM 239510) is the result of a defect of the P5C dehydrogenase/aldehyde dehydrogenase 4 enzyme and P5C is excreted in the urine.[5]

The phenotype of type II hyperprolinaemia is characterised by neurological manifestations including seizures and mental retardation.[3 6 7] Although type I hyperprolinaemia was originally described in a kindred with a familial nephropathy,[1 2] the renal disease was subsequently shown to be coincidental and type I hyperprolinaemia has been considered to be a benign disorder which can be asymptomatic.[3] Nevertheless, two studies have reported severe neurological manifestations (mental retardation, epilepsy) in two male children with type I hyperprolinaemia.[8 9] While mutations of the *ALDH4A1* gene, located on chromosome 1p36, have been identified in families with type II hyperprolinaemia,[10] the molecular basis of type I hyperprolinaemia has not been characterised until recently. We have recently identified in schizophrenic patients a heterozygous deletion and mutations of the *PRODH* gene, located on 22q11, which were associated with moderate hyperprolinaemia. We also found in two unrelated type I hyperprolinaemia children the same homozygous *PRODH* missense mutation.[11] We now report the identification of a complete homozygous *PRODH* deletion in a child with type I hyperprolinaemia with severe neurological manifestations.

**Key points**

- Type I hyperprolinaemia (MIM 239500) is a rare metabolic disorder which is biochemically characterised by a defect of the proline dehydrogenase (oxidase) enzyme involved in the conversion from proline to glutamate. Although type I hyperprolinaemia has been considered to be a benign disorder, severe neurological manifestations (mental retardation, epilepsy) have been reported in several affected subjects.
- We identified, in a child with a severe form of type I hyperprolinaemia with severe psychomotor delay and status epilepticus associated with a very high level of plasma proline level (2246 µmol/l), a complete homozygous deletion of the *PRODH* gene located on chromosome 22q11. This 22q11 deletion, also removing the *DGCR6*, *LOC200301*, and *DGS-A* loci, was estimated to be approximately 350 kb.
- The present study shows unambiguously that the severe form of type I hyperprolinaemia, characterised by neurological manifestations, results from homozygous inactivating alterations of the *PRODH* gene.

## CASE REPORT

The patient, a male, was the first child of healthy, consanguineous parents of Egyptian origin. At 4 years, he was referred for severe psychomotor delay, permanent hyperactivity, sleep disturbance with bruxism, and status epilepticus. Weight was 13 kg (−3 SD), length 95 cm (−2.5 SD), and head circumference 46 cm (−4 SD). There was no dysmorphism. Cerebral magnetic resonance imaging (MRI), performed at 4 years of age, showed normal myelination and no white matter abnormalities. Metabolic screening showed a very high level of plasma proline level (2246 µmol/l, n=133-227 µmol/l). Proline levels were also raised in urine (631 µmol/mmol creatinine, n<10 µmol/mmol creatinine) and cerebrospinal fluid (21 µmol/l,

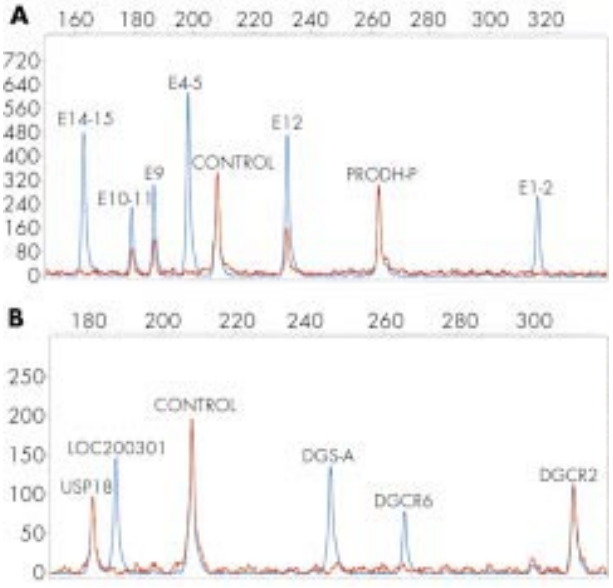


**Figure 1** Detection and characterisation of the *PRODH* homozygous deletion using QMPSF. In each panel, the electropherogram of the patient (red) was superimposed on that of a control (blue) by adjusting the peaks obtained for the control amplicon (*MSH2* exon 3) to the same level. The Y axis displays fluorescence in arbitrary units and the X axis indicates the size in bp. (A) QMPSF covering 10 exons (E) of the *PRODH* gene. Exons separated by short introns were covered by a single QMPSF amplicon. An exonic fragment specific for the *PRODH* pseudogene exon 8 (PRODH-P) was included. Exons 9-12 are present both in the *PRODH* gene and in the pseudogene. The *PRODH* homozygous deletion observed results, therefore, in a 50% decrease in the fluorescence of the corresponding peaks. (B) QMPSF covering five genes surrounding the *PRODH* locus.

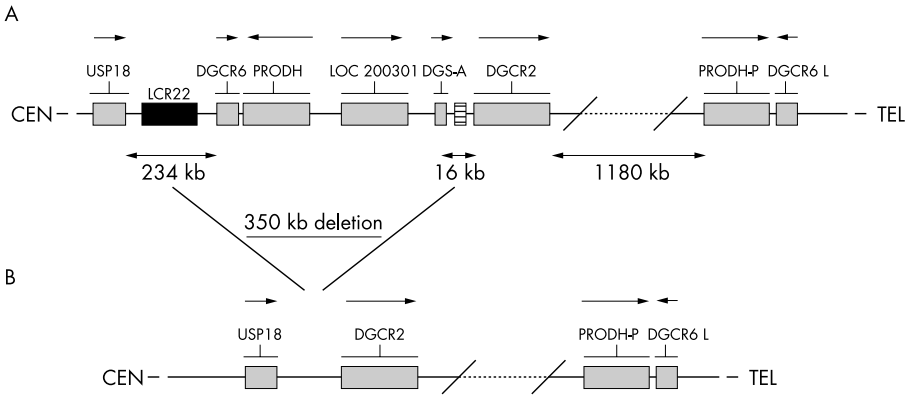


**Figure 2** Schematic representation of the *PRODH* deletion. (A) Organisation of the chromosome 22q11 region. The *USP18*, *DGCR6*, *PRODH*, *LOC200301*, *DGS-A*, and *DGCR2* genes, the *PRODH* pseudogene (PRODH-P), and the functional copy of *DGCR6* (DGCR6L[18]) are indicated by grey boxes. For each gene, the arrow above the box indicates the transcriptional orientation. CEN and TEL indicate the centromeric and telomeric sides. The centromeric LCR22, located within the USP18-DGCR6 intergenic sequence and involved in the 22q11DS,[18] is shown (black box). Analysis of the (DGS-A)-DGCR2 intergenic sequence, using the RepeatMasker program (http://repeatmasker.genome.washington.edu) and the Blast program from the National Center for Biotechnology Information showed that this region contains 21% of Alu and LINE repeated sequences sharing 80 to 90% of homology with the centromeric LCR22. (B) Rearranged 22q11 region.

n<4 μmol/l). Absence of P5C in urine led to the diagnosis of type I hyperprolinaemia.

While our previous observation of a homozygous L441P *PRODH* mutation in two unrelated children suffering from severe type I hyperprolinemia[11] suggested that type I hyperprolinaemia resulted from *PRODH* alterations, the functional consequence of this missense mutation had not been assessed. The present study shows unambiguously that the severe form of type I hyperprolinaemia, characterised by neurological manifestations, results from homozygous inactivating alterations of the *PRODH* gene and can indeed be considered as an autosomal recessive disease, although heterozygotes may have a moderate increase of prolinaemia.[3 11]

## DISCUSSION

We examined this patient for a genomic rearrangement of the *PRODH* gene, as previously described,[11] using quantitative multiplex PCR of short fluorescent fragments (QMPSF), a method based on the simultaneous amplification of multiple short exonic sequences using dye labelled primers under quantitative conditions.[12–15] The exploration of the *PRODH* locus is complicated by the presence on 22q11 of a non-functional *PRODH*-like pseudogene,[16] which shares exons 8-13 with *PRODH*. Because *PRODH* specific primers cannot be designed for these exons, detection of heterozygous and also of homozygous rearrangements of the *PRODH* gene requires quantitative conditions. QMPSF analysis showed a complete homozygous deletion of *PRODH* in this patient (fig 1A). Additional QMPSFs, exploring the centromeric *USP18* and *DGCR6* genes and the telomeric *LOC200301*, *DGS-A*, and *DGCR2* genes, surrounding *PRODH*, showed that the homozygous 22q11 deletion also removed the *DGCR6, LOC200301*, and *DGS-A* loci (fig 1B). This 22q11 deletion (fig 2), estimated to be approximately 350 kb, is similar to that previously identified, in a heterozygous state, in two related, white, schizophrenic patients[11] and has the same centromeric boundary as the 3 Mb and 1.5 Mb deletions[17] associated with the 22q11 deletion syndrome (22q11DS, MIM 192430). The boundaries of the deletion suggest that this recurrent *PRODH* deletion resulted from a recombination event between the centromeric low copy repeat (LCR 22) and repeated sequences within the *(DGS-A)-DGCR2* intergenic region (fig 2).

## ACKNOWLEDGEMENTS

We are grateful to Mario Tosi for critical review of the manuscript. HJ was supported by a grant from the Ministère de la Recherche.

### Authors' affiliations

**H Jacquet, P Saugier-Veber, G Raux, D Campion, T Frebourg,** INSERM EMI 9906 - IFRMP, Faculty of Medicine, Rouen, France
**J Berthelot, C Bonnemains, D Bonneau,** Department of Genetics, CHU d'Angers, France
**G Simard,** Department of Biochemistry, CHU d'Angers, France
**P Saugier-Veber, T Frebourg,** Department of Genetics, CHU de Rouen, France

Correspondence to: Dr T Frebourg, INSERM EMI 9906 - IFRMP, Faculty of Medicine, Rouen, France; Frebourg@chu-rouen.fr

## REFERENCES

1 **Scriver CR**, Schafer IA, Efron ML. New renal tubular amino-acid transport system and a new hereditary disorder of amino-acid metabolism. *Nature* 1961;**192**:672-3.
2 **Schafer IA**, Scriver CR, Efron ML. Familial hyperprolinemia, cerebral dysfunction and renal anomalies occurring in a family with hereditary nephropathy and deafness. *N Engl J Med* 1962;**267**:51-60.
3 **Phang JM**, Chien-an AH, Valle D. Disorders of proline and hydroxyproline metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*. Vol II. 8th ed. New York: McGraw-Hill, 2001:1820-38.
4 **Efron ML**. Familial hyperprolinemia: report of second case, associated with congenital renal malformation, hereditary hematuria and mild mental retardation, with demonstration of enzyme defect. *N Engl J Med* 1965;**272**:1243-54.
5 **Goodman SI**, Mace JW, Miles BS, Teng CC, Brown SB. Defective hydroxyproline metabolism in type II hyperprolinemia. *Biochem Med* 1974;**10**:329-36.
6 **Emery FA**, Goldie L, Stern J. Hyperprolinaemia type 2. *J Ment Defic Res* 1968;**12**:187-95.
7 **Flynn MP**, Martin MC, Moore PT, Stafford JA, Fleming GA, Phang JM. Type II hyperprolinaemia in a pedigree of Irish travellers (nomads). *Arch Dis Child* 1989;**64**:1699-707.
8 **Steinlin M**, Boltshauser E, Steinmann B, Wichmann W, Niemeyer G. Type I hyperprolinemia and white matter disease : coincidence or causal relationship? *Eur J Pediatr* 1989;**149**:40-2.
9 **Humbertclaude V**, Rivier F, Roubertie A, Echenne B, Bellet H, Vallat C, Morin D. Is type I hyperprolinemia actually a benign trait? Report of a case with severe neurologic involvement and vigabatrin intolerance. *J Child Neurol* 2001;**16**:622-3.
10 **Geraghty MT**, Vaughn D, Nicholson AJ, Lin W, Jimenez-Sanchez G, Obie C, Flynn MP, Valle D, Hu CA. Mutations in the $\Delta^1$-pyrroline 5-carboxylate dehydrogenase gene cause type II hyperprolinemia. *Hum Mol Genet* 1998;**7**:1411-15.
11 **Jacquet H**, Raux G, Thibaut F, Hecketsweiler B, Houy E, Demilly C, Haouzir S, Allio G, Fouldrin G, Drouin V, Bou J, Petit M, Campion D, Frebourg T. *PRODH* mutations and hyperprolinemia in a subset of schizophrenic patients. *Hum Mol Genet* 2002;**11**:2243-9.
12 **Charbonnier F**, Raux G, Wang Q, Drouot N, Cordier F, Limacher JM, Saurin JC, Puisieux A, Olschwang S, Frebourg T. Detection of exon deletions and duplications of the mismatch repair genes in hereditary nonpolyposis colorectal cancer families using multiplex polymerase chain reaction of short fluorescent fragments. *Cancer Res* 2000;**60**:2760-3.

13 **Saugier-Veber P**, Drouot N, Lefebvre S, Charbonnier F, Vial E, Munnich A, Frebourg T. Detection of heterozygous *SMN1* deletions in SMA families using a simple fluorescent multiplex PCR method. *J Med Genet* 2001;**38**:240-3.
14 **Charbonnier F**, Olschwang S, Wang Q, Boisson C, Martin C, Buisine MP, Puisieux P, Frebourg T. *MSH2* in contrast to *MLH1* and *MSH6* is frequently inactivated by genomic rearrangements in hereditary non polyposis colorectal cancer. *Cancer Res* 2002;**62**:848-53.
15 **Federica Casilli F**, Di Rocco ZC, Gad S, Tournier I, Stoppa-Lyonnet D, Frebourg T, Tosi M. Rapid detection of new BRCA1 rearrangements in high-risk breast-ovarian cancer families using multiplex PCR of short fluorescent fragments. *Hum Mutat* 2002;**20**:218-26.
16 **Liu H**, Heath SC, Sobin C, Roos JL, Galke BL, Blundell ML, Lenane M, Robertson B, Wijsman EM, Rapoport JL, Gogos JA, Karayiorgou M. Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. *Proc Natl Acad Sci USA* 2002;**99**:3717-22.
17 **Maynard TM**, Haskell GT, Lieberman JA, LaMantia A. 22q11DS : genomic mechanisms and gene function in DiGeorge/velocardiofacial syndrome. *Int J Dev Neurosci* 2002;**764**:1-13.
18 **Edelmann L**, Stankiewicz P, Spiteri E, Pandita RK, Shaffer L, Lupski J, Morrow BE. Two functional copies of the *DGCR6* gene are present on human chromosome 22q11 due to a duplication of an ancestral locus. *Genome Res* 2001;**11**:208-17.