

Assignment¹ of the protein L-isoaspartate (D-aspartate) O-methyltransferase gene (PCMT1) to human chromosome bands 6q24→q25 with radiation hybrid mapping

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¹ This is a more precise localization of a gene previously mapped to 6q22.3→q24 by MacLaren et al. (1992).

Rationale and significance

The protein L-isoaspartyl (D-aspartyl) O-methyltransferase can initiate the repair of age-damaged proteins containing isomerized derivatives of aspartate and asparagine residues (Clarke, 1999). This pathway has been shown to be important for the maintenance of the structure and function of a number of cytosolic proteins (Brennan et al., 1994; Johnson et al., 1987), and mice deficient in this methyltransferase die of seizures between three and nine weeks of age (Kim et al., 1997). The human PCMT1 gene encoding this enzyme has been mapped to a 10-cM region of 6q22.3→q24 (MacLaren et al., 1992) and its genomic structure characterized (DeVry and Clarke, 1996). The mapping of the gene for a human progressive myoclonus epilepsy of the Lafora type to a 17-cM region in chromosome 6q23→q25 (Serratosa et al., 1995) suggested that the methyltransferase might be a candidate for the Lafora disease gene. Radiation hybrid mapping was carried out to more accurately determine the location of the PCMT1 gene with respect to the markers used in the linkage analysis of the Lafora study.

Materials and methods

Radiation hybrid panel mapping

The chromosome position of the PCMT1 gene was determined using the Stanford G3 Radiation Hybrid panel (Research Genetics Inc.). This method is useful for ordering markers in the region of interest as well as establishing the distance between these markers at an average resolution of 500 kb. DNA from these clones was tested for the presence of the PCMT1 gene as determined by PCR amplification of a 270-bp fragment including exon 2 using the flanking intron primers, 2INF5' (5'-CAGTCTCTATAGCACAGCTGGTGTATA-3') and 2INF3' (5'-GTGTCCCAGGCTTTTCT-3'), specific for human DNA. The 50 µl reaction contained 1 × buffer (Promega), 3.5 mM MgCl₂ provided by a HotWax™ bead (Invitrogen), 200 µM of each dNTP (Gibco BRL), 40 pmol of the primers 2INF5' and 2INF3', 50 ng of the RH clone DNA (Research Genetics Inc.), and 2 U of Taq DNA polymerase (Promega). The thermocycler parameters consisted of 95 °C for 2 min followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. The 83 reactions were run on 1 % agarose gels stained with ethidium bromide to identify the PCR results for each clone. Software for analysis of the results obtained from the panel was at <http://www.shgc.stanford.edu/index.html>.

Results

We have narrowed the location of the human PCMT1 gene to a more telomeric position in the q24→q25 region of chromosome 6 between the nearest flanking microsatellite markers D6S1564 and D6S1687 (Fig. 1). The SHGC marker which best links with the PCMT1 gene is SHGC-34285, a novel EST distinct in sequence from PCMT1. The two point maximum likelihood analysis results gave a LOD score of 1000 for this match. The resolution of this panel was able to position the PCMT1 gene approximately 0.6 Mb centromeric of D6S1687 and 1.1

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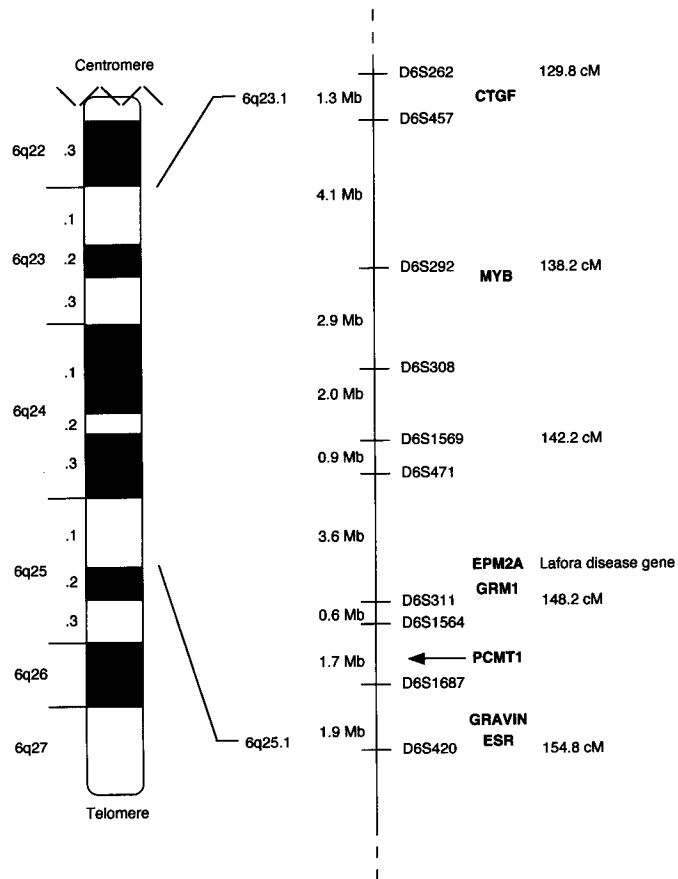


Fig. 1. Ideogram of human chromosome 6 depicting the updated mapping location of the PCMT1 gene relative to regional markers. The genetic map of the polymorphic markers and the location of PCMT1 is based on data from the Stanford Human Genome Center (<http://shgc-www.stanford.edu>) Map Version 2.0 and the recently released GeneMap '98 of the human genome (<http://www.ncbi.nlm.nih.gov/genemap>). The position of EPM2A denotes the newly established location of the Lafora disease gene relative to the D6S311 marker, according to Minassian et al. (1998). PCMT1 is located approximately 1.7 Mb telomeric of the D6S311 marker and 0.6 Mb centromeric of the D6S1687 marker. The Mb distances between markers was determined using the conversion factor of 26 kb per centirad (cR) as established by the Stanford Human Genome Center for the G3 RH mapping panel. The positions of CTGF, MYB, GRM1, GRAVIN, and ESR were taken from GeneMap '98 of the human genome with the G3 RH panel; the linkage between the chromosome ideogram at left and the map at the right is based on a 6q23.1 position for CTGF and a 6q25.1 position for ESR.

Mb telomeric of D6S1564. The PCMT1 gene is approximately 1.7 Mb telomeric of the D6S311 marker, which had been identified as the telomeric border of the 2.7-cM Lafora disease region (Maddox et al., 1997; Sainz et al., 1997), thus eliminating the repair methyltransferase as a candidate gene for Lafora disease. Moreover, the gene that causes the progressive myoclonus epilepsy of the Lafora type has recently been cloned (Minassian et al., 1998), and has been positioned approximately 1 Mb from PCMT1. Interestingly, the distance between markers reported by Minassian et al. (1998) is considerably less than we find in our analysis, although the relative positions of the markers and genes are the same.

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