Assignment¹ of the proteolipid protein 1 gene (*PLP1*) to tammar wallaby chromosome Xq by fluorescence in situ hybridization with a BAC clone

E. Koina J.A.M. Graves

The ARC Centre for Kangaroo Genomics, Comparative Genomics Group, Research School of Biological Science, The Australian National University, Canberra (Australia)

¹ To our knowledge this is the first time this gene has been mapped in tammar wallaby.

Manuscript received 4 November 2005; accepted in revised form for publication by M. Schmid, 16 December 2005.

Rationale and significance

Proteolipid protein 1 is the primary constituent of myelin, accounting for about half the protein content of the human central nervous system (Yool et al., 2000). The gene contains seven exons and spans approximately 17 kb. *PLP1* gene duplication and deletions or mutations in the coding portion of the gene (Mimault et al., 1999, Cailloux et al., 2000) cause disorders of the central nervous system including a range of phenotypes from Pelizaeus-Merzbacher disease (PMD) to spastic paraplegia 2 (SPG2). In humans *PLP1* maps to Xq22. We have isolated and mapped a BAC clone containing marsupial *PLP1*; adding to the comparative map between eutherians and marsupials (divergence 180 mya). Comparative mapping is a valuable tool for investigating the evolution of the X chromosome (Graves et al., 1998; Alsop et al., 2005).

Materials and methods

A *Macropus eugenii* (tammar wallaby) male BAC library (ME_KBa, commercially available through the Arizona Genomics Institute) was screened with an overgo probe as described by McPherson et al. (2001). The *PLP1* overgo was designed from the most conserved region of the gene (exon 3) with the overgo maker tool available through the Washington University Genome Sequencing Centre. (<http://genomeold.wustl.edu/tools/?overgo=1>). The overgo sequence was GTTCCAGAGGC-CAACATCAAGCTCATTCTTTGGAGCGGGT. BAC clones isolated from this screen were tested for the presence of the *PLP1* gene by PCR amplification, with the forward primer 5' CCACCCCTTGTTATCC-CAC 3' and reverse primer 5' GAAGGGGAGGGGTTCCAG 3'. Cycling parameters were: an initial cycle at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR product obtained (131 bp), from BAC clone ME_KBa 480K21, was 95% homologous to the human *PLP1* gene (GenBank accession number AJ00697). ME_KBa 480K21 was localized to male metaphase chromosomes, prepared from male fibroblasts, by DNA fluorescent in situ hybridization as described previously (Alsop et al., 2005).

Probe name: ME_KBa 480K21

Probe type: genomic DNA

Insert size: approximately 220 kb

Vector: pCUGIBAC1

Proof of authenticity: sequencing

Results

Mapping data

Most precise location: Xq3

No. of cells examined: 20

Number of male cells with specific signal: 1 (0), 2 (20) chromatids per male cell

We acknowledge support from the Australian Research Council.

Request reprints from Dr. Edda Koina

The ARC Centre for Kangaroo Genomics
Research School of Biological Sciences, The Australian National University
Building 46, Sullivans Creek Road, Canberra, ACT, 2602 (Australia)
telephone: +61 2 61252101; fax: +61 2 61254891
e-mail: Koina@rsbs.anu.edu.au

Fig. 1. DNA FISH mapping of ME_KBa 480K21, containing the marsupial *PLP1* gene, to the long arm of the tammar wallaby X chromosome (male metaphase).

References

- Alsop AE, Miethke P, Rofe R, Koina E, Sankovic N, Deakin JE, Haines H, Rapkins RW, Graves JAM: Characterizing the chromosomes of the Australian model marsupial *Macropus eugenii* (tammar wallaby). *Chromosome Res* 13: 627–636 (2005).
- Cailloux F, Gauthier-Barichard F, Mimault C, Isabelle V, Courtois V, Giraud G, Dastugue B, Boespflug-Tanguy O: Clinical European Network on Brain Dysmyelinating Disease: Genotype-phenotype correlation in inherited brain myelination defects due to proteolipid protein gene mutations. *Eur J Hum Genet* 8:837–845 (2000).
- Graves JA, Disteche CM, Toder R: Gene dosage in the evolution and function of mammalian sex chromosomes. *Cytogenet Cell Genet* 80:94–103 (1998).
- McPherson JD, Marra M, Hillier L, Waterston RH, Chinwalla A, Wallis J, Sekhon M, Wylie K, Mardis ER, Wilson RK, et al: A physical map of the human genome. The International Human Mapping Consortium. *Nature* 409:934–941 (2001).
- Mimault C, Giraud G, Courtois V, Cailloux F, Boire JY, Dastugue B, Boespflug-Tanguy O: Clinical European Network on Brain Dysmyelinating Disease: Proteolipoprotein gene analysis in 82 patients with sporadic Pelizaeus-Merzbacher disease: duplications, the major cause of the disease, originate more frequently in male germ cells, but point mutations do not. *Am J Hum Genet* 65:360–369 (1999).
- Yool DA, Edgar JM, Montague P, Malcolm S: The proteolipid protein gene and myelin disorders in man and animal models. *Hum Mol Genet* 9:987–992 (2000).