Assignment¹ of the protocadherin 20 (PCDH20) gene to tammar wallaby chromosome 6q by fluorescence in situ hybridisation

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¹ To our knowledge, this is the first time this gene has been mapped in the tammar wallaby or any other marsupial.

Rationale and significance

Protocadherin genes are members of the cadherin superfamily and are thought to be involved in cell-cell recognition in the central nervous system. Cadherin genes are divided into two groups, classical and non-classical. Protocadherins are the major non-classical cadherins (Suzuki, 1996). In humans, PCDH20 maps to chromosome 13q21 (Yagi and Takeichi, 2000). PCDH20 is also known to map to mouse chromosome 18, rat chromosome 18p12→p11, and Chinese hamster chromosome 2q17 (Hirano et al., 1999, Ono et al., 2000).

Marsupials are of particular value in comparative genetics because of their early divergence (about 130 million years ago) from eutherian mammals. In this study we cloned and mapped the genomic homologue of PCDH20 by fluorescence in situ hybridisation to the long arm of chromosome 6 in the model marsupial *Macropus eugenii* (tammar wallaby). PCDH20 is the first gene to be mapped to tammar wallaby chromosome 6 and may represent a newly defined region of homology between human chromosome 13 and wallaby chromosome 6. Secondary signals were also observed on the proximal region of the long arm of the X chromosome that could represent a related protocadherin gene in this region.

Materials and methods

A tammar wallaby lambda genomic male DNA library was screened with a 700-bp PCDHX PCR product amplified from human male genomic DNA with the primers PCDHX-2020f (5' GAA TAA CTC TCC TGG CAT CC 3') and PCDHX-2735r (5' TCT CTT GTG TTT CCT CCT AC 3'). The cycling parameters used to amplify the expected product were 30 cycles of (94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min) following 2 min denaturation at 94 °C. Eight positive clones were isolated, and sequencing revealed that three contained the wallaby PCDH20 gene. Two of the PCDH20 clones were biotin-14-dCTP labelled by nick translation and hybridised to tammar wallaby metaphase chromosomes. Both gave the same location. Hybridisation signals were detected with anti-biotin raised in goat and anti-goat coupled with FITC and counterstained with DAPI.

Probe names: MeugPCDHX-1.3 and MeugPCDHX-1.4

Probe type: genomic DNA

Insert size: approximately 20 kb

Vector: lambda EMBL3

Proof of authenticity: sequencing

Results

Mapping data

Location: 6q (Fig. 1)

Number of cells examined: MeugPCDHX-1.3, 15; MeugPCDHX-1.4, 10

Number of cells with specific signals:

MeugPCDHX-1.3; 1 (0), 2 (3), 3 (3), 4 (5) chromatids per cell

MeugPCDHX-1.4; 1 (1), 2 (2), 3 (1), 4 (3) chromatids per cell

Most precise assignment: central 6q

Location of background signals (sites with > 2 signals): proximal Xq

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Fig. 1. FISH mapping of MeugPCDHX-1.3 (**a**) and MeugPCDHX-1.4 (**b**). Both clones had hybridisation signals on the long arm of chromosome 6. Secondary signals were also detected in the proximal region of Xq.

References

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