Assignment\(^1\) of BReast Cancer Associated 1 (BRCA1) to tammar wallaby (Macropus eugenii) chromosome 2q3 by in situ hybridization

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

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**Rationale and significance**

BRCA1, located at 17q21.31 in humans, is a significant gene in familial breast and ovarian cancer. BRCA1 functions as an important regulator of pathways governing DNA repair, cell-cycle progression, ubiquitylation and transcriptional regulation in mammals. Orthologs are not found in the yeast, fly or worm genomes (Venkitaraman, 2002; Narod and Foulkes, 2004). Previously SOX9, located at 17q24.3 in humans, was mapped to the long arm of chromosome 2 in the tammar wallaby (Pask et al., 2002). Therefore mapping of the tammar wallaby BRCA1 homologue to chromosome 2q3 has extended the region of synteny with human chromosome 17q by 29 megabases, identifying a large segment of a chromosome block (C14) that is conserved in marsupial evolution (Rens et al., 2003).

**Materials and methods**

A 1.4-kb tammar wallaby (MEU) probe spanning exon 11 of BRCA1 was amplified from male MEU genomic DNA with the forward primer 5'-ATTGCCCAACACAGACAGAATG-3' and reverse primer 5'-GAAACAA-TGAGGTACTGTCYTGAG-3'. Cycling parameters included a denaturation step of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 90 s. This probe was labeled with 32P and used to screen a male MEU genomic BAC library (Arizona Genomics Institute). PCR and sequencing of the 1.4-kb product was performed on DNA extracted from positive clones. A single significant tblastx hit to human chromosome 17 (NC_000017.9 at base 38499118, E-value 2e-32) confirmed that the clone Me-KBa-354-P-19 contained the MEU BRCA1 homologue. This clone was labeled with digoxigenin-11-dUTP by nick translation, pre-annealed with 1 μg tammar wallaby C0t-1 DNA at 37 °C for 30 min and hybridized overnight at 37 °C to male MEU metaphase chromosomes that had been denatured for 75 s at 70 °C in 70% denaonismed formamide:2× SSC. Hybridization signals were detected with Cy3 conjugated to anti-digoxigenin antibodies; chromosomes were counterstained with DAPI.

**Probe name:** Me-AGI-354-P-19  
**Probe type:** genomic BAC clone  
**Vector:** pCUGI-BAC1  
**Proof of authenticity:** Partial sequencing

**Fig. 1.** Localisation of BRCA1 to tammar wallaby chromosome 2q3. A male Macropus eugenii metaphase spread showing the BAC clone Me-KBa-354-P-19, which contains sequences homologous to human BRCA1, hybridized to the long arm of chromosome 2.
Results

Mapping data:
Most precise location: 2q3
No. of cells examined: 30
Number of cells with specific signal: 1 (0), 2 (4), 3 (19), 4 (7) chromatids per cell

References