

Localisation of the Breast-Ovarian Cancer Susceptibility Gene (*BRCA1*) on 17q12-21 to an Interval of ≤ 1 cM

Simon A. Smith, Richard A. DiCioccio, Jeffery P. Struewing, Douglas F. Easton, Holly H. Gallion, Hans Albertsen, Sylvie Mazoyer, Bertil Johansson, Elisabeth Steichen-Gersdorf, Mike Stratton, Debbie Ford, Gill Marshall, Raymond L. White, M. Steven Piver, and Bruce A. J. Ponder

CRC Human Cancer Genetics Research Group, Department of Pathology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QP, UK (S.A.S., H.H.G., S.M., B.J., E.S.-G., B.A.J.P.); Roswell Park Cancer Institute, Department of Gynecologic Oncology, Buffalo, New York 14263, USA (R.A.D., M.S.P.); National Institutes of Health, Genetic Epidemiology Branch, Bethesda, Maryland 20892, USA (J.P.S.); Sections of Epidemiology and Molecular Carcinogenesis, Institute of Cancer Research, Belmont, Sutton, Surrey SM2 5NG, UK (D.F.E., M.S., D.F., G.M.); Eccles Institute of Human Genetics, Howard Hughes Medical Institute & University of Utah, Salt Lake City, Utah 84112, USA (H.A., R.L.W.)

A breast-ovarian cancer susceptibility gene, *BRCA1*, which is responsible for disease in approximately 45% of breast cancer families and most families that contain breast and ovarian cancer, has been assigned by genetic linkage to 17q12-21. Here, we report the analysis of three marker-disease recombinants in families that contain breast and ovarian cancer, two of which strongly suggest a location for *BRCA1* telomeric to D17S702, a microsatellite polymorphism, and a third which suggests a location centromeric to *EDH17B*, the gene encoding estradiol-17 β dehydrogenase. If the interpretation of these recombinants is correct, the results localise *BRCA1* to an interval of ≤ 1 cM. *Genes Chrom Cancer* 10:71-76 (1994).

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A susceptibility locus for breast cancer has been mapped by genetic linkage to the long arm of chromosome 17, in the interval 17q12-21 (Hall et al., 1990). Predisposition at this locus, named *BRCA1*, is responsible for the disease in approximately 45% of families that contain multiple cases of breast cancer only and most families that contain breast cancer and at least one case of epithelial ovarian cancer (Easton et al., 1993). The cloning of *BRCA1* will help identify those individuals who are at risk of cancer through the inheritance of predisposing mutations and is the first step towards elucidating the role of this gene in the pathogenesis of breast and ovarian cancer. The *BRCA1* gene has already been mapped to a region of approximately 2cM defined proximally by D17S857 and distally by D17S78 (Anderson et al., 1993; Kelsell et al., 1993; Simard et al., 1993). Here, we report the further genetic mapping of *BRCA1*, based upon recombination events in three breast-ovarian cancer families.

Fifteen microsatellite polymorphisms (Table 1) spanning 16cM were typed by PCR using standard techniques (Smith et al., 1993). The genetic order of the markers is as described by Anderson et al. (1993). In addition, D17S702 has been mapped on a YAC contig of the region to lie between D17S800 and D17S846 (S. Mazoyer et al., unpublished results). Lod scores were calculated assuming an autosomal dominant disease gene with a risk to carriers of 0.8 by age 80 years and a risk of 0.08 in

non-carriers as previously described (Easton et al., 1993).

The families and the most likely haplotypes constructed by typing markers in the region D17S250-*NME1* are shown in Figures 1-3. In family 8101 (Figure 1), five of the six affected individuals for whom we have typings share a common haplotype. The sixth case, who was diagnosed with ovarian cancer aged 41 years, appears to inherit a recombinant chromosome from her affected mother that contains the "linked" haplotype distal to D17S702, suggesting that the disease locus, *BRCA1*, is located telomeric to D17S702. A recombinant in another ovarian cancer case, diagnosed at 51 years in a second family (family 801, Figure 2), also suggests that *BRCA1* is located telomeric to D17S702. The third family, family 64 (Figure 3), contains a recombination in an individual diagnosed with breast cancer at age 34 years, which suggests that *BRCA1* is located centromeric to *EDH17B*, the gene encoding estradiol-17 β dehydrogenase. The polymorphism in *EDH17B* which is recombinant in family 64, HSD-DEL, occurs in the 5' untrans-

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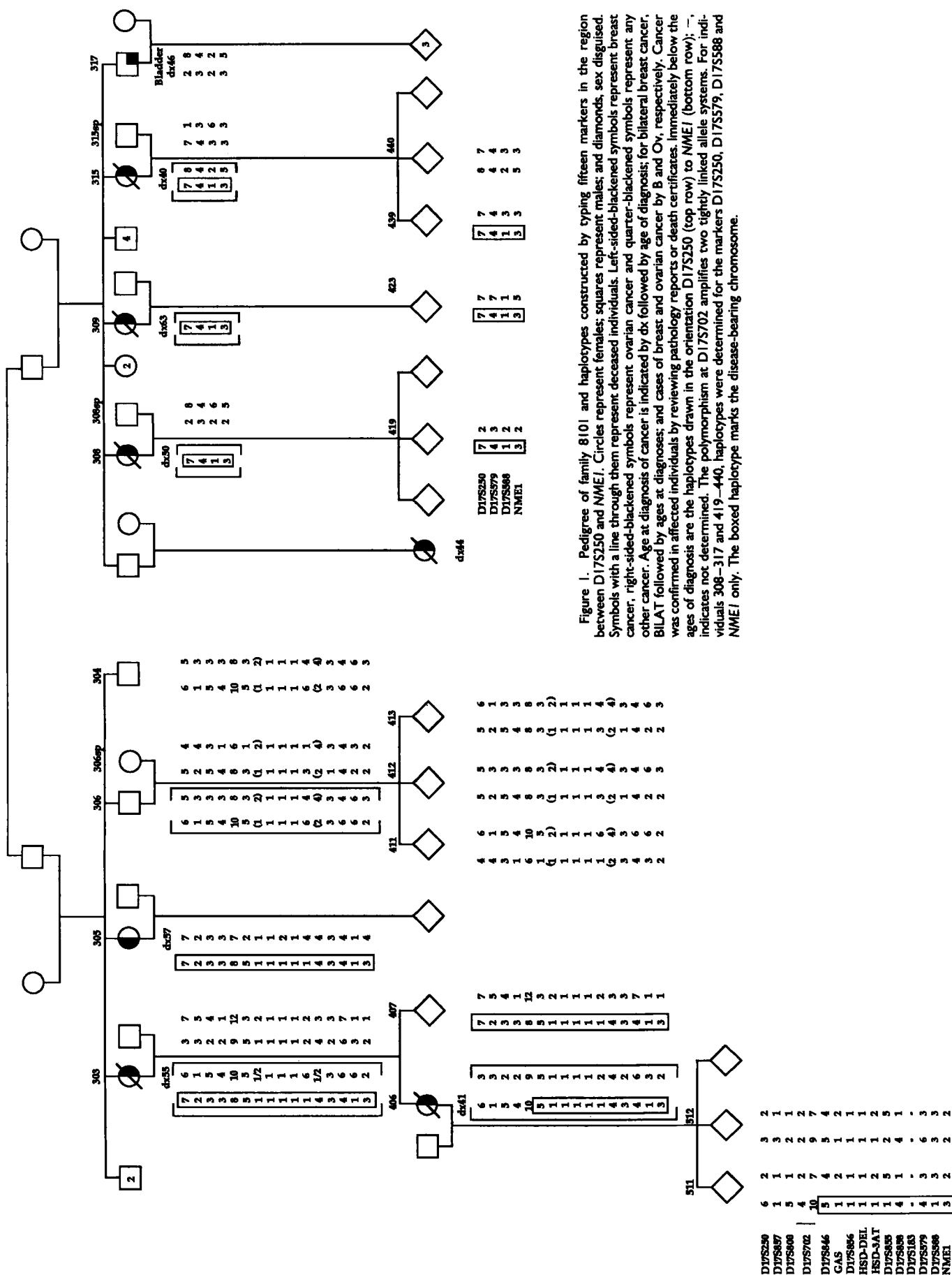
Address reprints to, Dr. S. A. Smith, CRC Human Cancer Genetics Research Group, Department of Pathology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QP, UK.

Present addresses: Department of Obstetrics and Gynecology, University of Kentucky, Lexington, Kentucky 40536, USA (H.H.G.); Univ. Kinderklinik, Anichstrasse 35, A-6020 Innsbruck, Austria (E.S.-G.).

Table 1. Polymorphic Genetic Markers on 17q12-21*

Locus	Name	Primer sequence	TEMP	References
D17S250	Mfd 15	GGA AGA ATC AAA TAG ACA AT GCT GGC CAT ATA TAT ATT TAA ACC	55°C	Weber et al., 1990
D17S857	OF1	TTT GTC TGC AAA CAT GGA GG TCA GCT GAA GAG AAA AAT GGC	55°C	Anderson et al., 1993
D17S800	200zf4	GGT CTC ATC CAT CAG GTT TT ATA GAC TGT GTA CTG GGC ATT GA	55°C	Weissenbach et al., 1992
D17S702	UT394	AGC AAC ACA TAT CAG GGG C TGT AGG TTG ACC TTA AGG C	52°C	Albertsen et al., in press
D17S846	UM8	TGC ATA CCT GTA CTA CTT CAG TCC TTT GTT GCA GAT TTC TTC	58°C	Fleijter et al., 1993
GAS		CAGAGAGGATGGGGGAGAAG (GAS1) GATGTACATAAAGCGCCCTG (GAS2)	65°C	Epstein et al., 1990
		TGA GC(C/T)(G/A)(A/T)GAT(C/T)(G/A) (C/T)(G/A)CCA(C/T)TGCACTCCAGCCTG GG (ALU-CON)	55°C	
D17S856	OF2	AAG GCA AGA CTT CGT CGA GA CAT TCC CTG GTC CTG TGC	55°C	Anderson et al., 1993
EDH17B	HSD-DEL	GTG ACC CAC GAA ACA CAG G CAG AAG GTGAAG AAC TCA TCC A	55°C	Friedman et al., 1993
EDH17B	HSD-A3T	CAG TAC TAA AGG CCC TAT TAT CAA A AGG CTG CAGTGA GTC CAG AT	55°C	Friedman et al., 1993
D17S855	248yg9	GGA TGG CCT TTT AGA AAG TGG ACA CAG ACT TGT CCT ACT GCC	56°C	Weissenbach et al., 1992
D17S858	OF3	GCA CTC TGA CTA GAA TCT GGG G TCC AAG TGG GAA TGA GTG C	55°C	Anderson et al., 1993
D17S183	SCG43	ACA AAC TGA TGT GGG CTC TAG GTA CAT AGC ATG GGT GCA GCT	55°C	Black et al., 1993
D17S579	Mfd 188	AGT CCT GTA GAC AAA ACC TG CAG TTT CAT ACC AAG TTC CT	55°C	Hall et al., 1992
D17S588	42D6	CCT GGT CTA GGA AGA GTG TCA GTG TAA GCA TCT GTG TAT ACT AC	55°C	Easton et al., 1993
NME1	Nm 23	TTG ACC GGG GTA GAG AAC TC TCT CAG TAC TTC CCG TGA CC	55°C	Easton et al., 1993

*The polymorphisms are ordered as they appear on the chromosome from D17S250 (centromeric) to NME1 (telomeric). TEMP indicates the optimum annealing temperature of the primers during PCR amplification



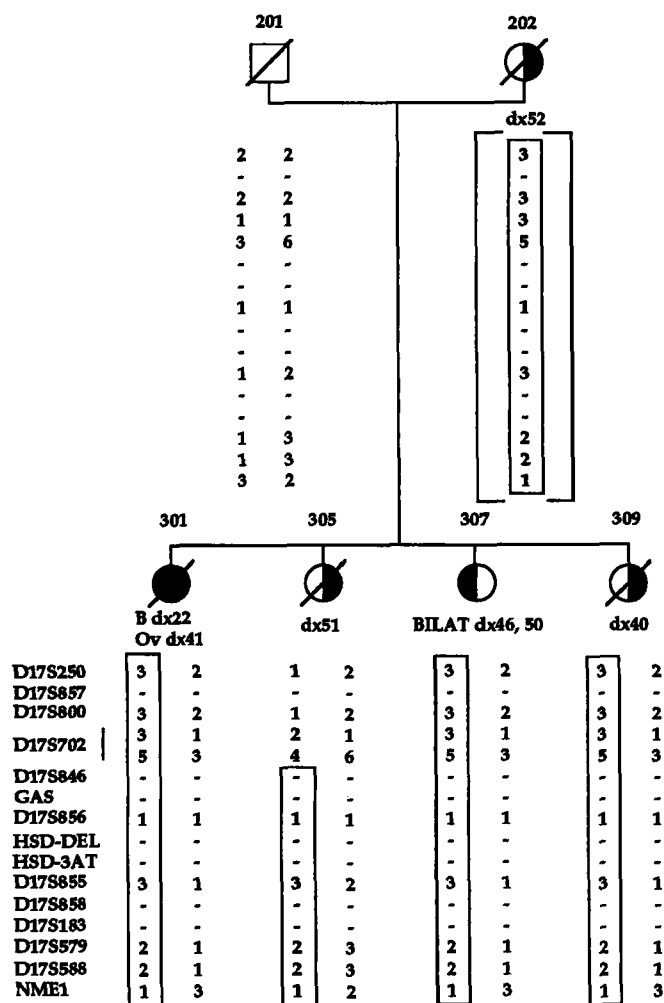


Figure 2. Pedigree of family 801. Symbols and legend as for Figure 1, except that -, indicates alleles not amplified by PCR from archival pathology samples.

lated region of the gene (Friedman et al., 1993). However, since the orientation of *EDH17B* on the chromosome is not known, *EDH17B* cannot be excluded with certainty as the *BRCA1* gene.

Thus, we report two observations, both in breast-ovarian cancer families, which identify D17S702 as the closest proximal marker which flanks *BRCA1*. Since each of these two families contain ovarian as well as breast cancer, they have a high prior probability of being linked to *BRCA1* (Easton et al., 1993), and the recombinant individuals, being ovarian cancer cases, are unlikely to be phenocopies. The closest previously reported proximal marker is stated to be D17S857 (Kelsell et al., 1993), based upon an observation in a breast cancer case in which both D17S857 and D17S800 were recombinant with *BRCA1*. However, the relative order of the two markers D17S857 and

D17S800 has been unresolved (Anderson et al., 1993). A recombination on a non-disease chromosome in individual 413 of family 8101 (Figure 1), indicates that D17S857 maps centromeric to D17S800. Having mapped D17S800 and D17S702 on a YAC contig of the region (S. Mazoyer et al., unpublished results) we have determined the order of these markers to be cen-D17S857-D17S800-D17S702-qter, which, in conjunction with the recombinants in families 801 and 8101, defines D17S702 as the new proximal boundary for the region containing *BRCA1*.

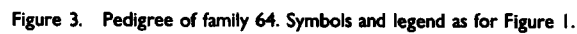
The recombinant in family 64 suggests a new distal boundary for the *BRCA1* region, defined by the locus for *EDH17B*. The interpretation of this result depends upon the evidence firstly, that family 64 is linked to *BRCA1* and secondly, that the recombinant individual is not a sporadic case. The formal evidence for linkage to 17q from the lod scores is not very strong (multipoint lod score 0.12). However, the presence in this family of an ovarian cancer case indicates a prior probability of linkage of at least 80% (Easton et al., 1993) and the posterior probability of linkage is thus at least 84%. An allele loss for the marker D17S250, in the tumour of individual 500, provides further evidence that the family is linked to *BRCA1*, because it affects the wild-type chromosome (Figure 4). In previous studies of tumours from 17q-linked families, each of sixteen allele losses that were detected affected the wild-type chromosome, consistent with *BRCA1* being a tumour suppressor gene (Smith et al., 1992; Kelsell et al., 1993). Finally, the young age of breast cancer diagnosis in the recombinant individual in family 64, at 34 years, argues against it being a phenocopy. Thus, although not conclusive, the evidence favours the interpretation that *BRCA1* lies proximal to *EDH17B* and distal to D17S702. This is an interval of 1cM or less, and is a significant narrowing of the region over that reported previously (Bowcock et al., 1993; Simard et al., 1993; Kelsell et al., 1993).

NOTE ADDED IN PROOF

Subsequent analysis of the offspring of individual 309 in family 64 has indicated that the ovarian cancer case did not inherit the putative linked haplotype. This suggests that either the ovarian cancer is a sporadic case or that the family is not linked to 17q12-21.

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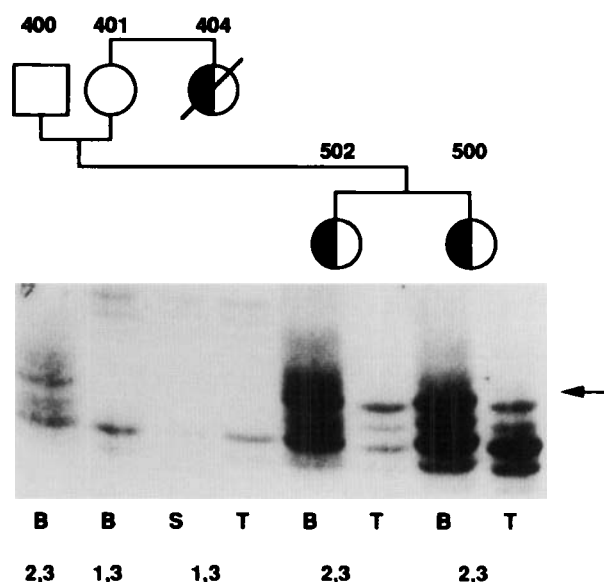


Figure 4. Analysis of loss of heterozygosity in the breast tumours of family 64 using the marker D17S250. Alleles were amplified from constitutional DNA either from blood (B) or normal breast stroma (S), and from the tumour (T) for the affected individuals. Allelotypes are indicated at the bottom of the figure and the arrow indicates the loss of allele 2 in the tumour of individual 500.

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