The Gene for Pancreatic Polypeptide (PPY) and the Anonymous Marker D17S78 Are within 45 kb of Each Other on Chromosome 17q21

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A gene for early-onset breast and ovarian cancer (BRCA1) has been localized to a small region of chromosome 17q21 (2, 3, 13). A combination of genetic linkage studies, radiation-reduced hybrid analysis, and physical mapping by FISH has identified several genes/markers that lie in this interval (1, 2, 6). Among these are the gene encoding pancreatic polypeptide (PPY) and a polymorphic marker at locus D17S78. Our efforts to construct a physical map of this region by isolating a large number of yeast artificial chromosome (YAC) and cosmid clones demonstrate that PPY and D17S78 are present within the same cosmid clone, and therefore no farther than 45 kb apart. This observation takes on particular significance since it excludes a recently described BRCA1 candidate gene (4) from the interval defined by meiotic mapping.

The probe p131 recognizes the *MspI* polymorphism at the locus D17S78 (10). To isolate YAC and cosmid clones for the marker D17S78, an STS (sequence-tagged site) was developed by sequencing a part of the probe p131. The STS (5′-TCT AGA GGA TCA GAG GAG TG-3′ and 5′-GTG TGA GAG TGG TGT ATG TG-3′) generates a PCR product of 177 bp from a human DNA template under the cycling conditions of denaturation at 94°C for 1 min, annealing at 62°C for 1 min,

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and extension at 72°C for 1 min for 35 cycles. These primers were employed to screen a total human genomic YAC library, constructed at the Center for Molecular Medicine at Washington University, St. Louis. A YAC clone was identified (A260E11). This YAC clone, when tested by PCR, was found also to contain an STS for PPY. Similarly, among the three YAC clones (26D6, 237E9, and 251H5) isolated for PPY from the CEPH YAC library, two (26D6 and 251H5) contained the STS for p131, confirming the proximity of these two STSs. The PCR primers for PPY were 5′-CCT TTC TCT CCC ATG CAT TTG GCA-3′ and 5′-TAC ACT GCC ATG TTC TGC CCT GT-3′ (5). The PCR product (177 bp) generated by the p131 STS was radiolabeled and hybridized to an arrayed chromosome 17 cosmid library, prepared and supplied to us by Larry Deaven of Los Alamos National Laboratory. Ten cosmid clones (1C9, 3H6, 8D6, 10G5, 39B6, 78A11, 79B6, 115F11, 119E12, and 145C7) were isolated, and all 10 clones were tested for the presence of PPY by hybridization to radiolabeled PCR product of the PPY primers. Two clones, 10G5 and 78A11, were found to contain the PPY STS, showing that the STSs for PPY and p131 are no more than 45 kb apart from each other.

To confirm that the polymorphism identified by D17S78 and the PPY gene are present in the same cosmid, DNA from the cosmid clones (10G5 and 78A11) was digested with *Msp*I, transferred to membrane after electrophoresis, and hybridized sequentially to the probes for both D17S78 and PPY. Results of such an analysis for the cosmid 78A11 are shown in Fig. 1. The sizes of the two alleles detected by the probe p131 (D17S78) are 4.5 kb and a doublet consisting of 1.9-plus 2.1-kb fragments, in addition to a 2.7-kb common fragment (10). As shown in Fig. 1a, the smaller size allele, a doublet of 1.9 and 2.1 kb, along with the 2.7-kb common fragment, was present in the cosmid 78A11. Subsequent hybridization of the same blot to the labeled PCR product of the PPY STS as a probe detected a 2-kb fragment (Fig. 1b), showing that the polymorphic marker D17S78 and the STS for PPY are present in the same cosmid. Similar observations were made for the DNA from the cosmid clone 10G5 (data not shown).

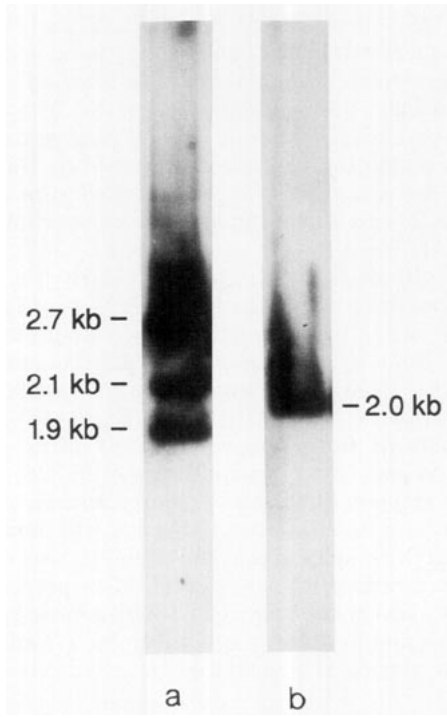

FIG. 1. DNA from the cosmid clone 78A11, after digestion with *Msp*I, was separated by electrophoresis and transferred to GeneScreen Plus. The 2.3-kb *Bam*HI fragment from plasmid p131 was labeled and used as a probe for D17S78 (**a**). After removal of the probe, the blot was hybridized to the probe for PPY, and the 258-bp PCR product was amplified from human total genomic DNA using an STS for PPY (**b**).

Although PPY and D17S78 were found to be no farther than 45 kb apart, identification of a smaller fragment that hybridizes to both probes would indicate that these two are much closer. Both cosmids, 10G5 and 78A11, were digested with individual or combinations of enzymes *Bam*HI, *Pst*I, and *Eco*RI, transferred to a membrane, and hybridized sequentially to labeled PCR products from STSs for PPY and p131. The fragments detected by each probe were distinct, however, and no common fragment was identified.

The probe p131 and the gene PPY were previously mapped to 17q21–q23 and to the proximal long arm of chromosome 17, respectively (10, 14). The demonstration of the close proximity of these markers should allow them to be treated as a single locus in terms of long-range genomic mapping of this region, and the genomic clones isolated should serve as useful resources for the identification of the BRCA1 gene. A recent report excludes the marker D17S78 from the BRCA1 region by means of a crossover observed in one affected member of a linked family (13), and therefore the D17S78/PPY locus serves as the distal boundary for the BRCA1 locus.

Analysis of a large number of familial and sporadic breast and ovarian cancers has identified frequent loss of heterozygosity near the BRCA1 locus. A recent report has suggested the responsible interval lies just telomeric to PPY, and a suggested candidate gene (MCD) for BRCA1 was found to be somatically rearranged in two of several hundred sporadic breast tumors (4). The identification of a convincing recombinant placing BRCA1 centromeric to D17S78 (13) and the data reported here showing that D17S78 and PPY lie in the same cosmid cast doubt on the identity of MCD as the heritable BRCA1 gene and are more compatible with a role for a somatic mutation of MCD in tumor progression. Such an observation is not without precedent. The MCC gene, for example, was originally suggested as a candidate for familial polyposis coli (8), but the actual responsible gene was later found nearby (7, 11). Prohibitin (12) and NME1 (9) are both located in 17q21–q22 and show somatic alterations in breast cancer, but have been previously excluded from the BRCA1 region by meiotic mapping of affected pedigrees. The value of closely interdigitated and robust physical and genetic maps is at once apparent in all these examples.

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