# The Human Insulin-like Growth Factor-Binding Protein 4 Gene Maps to Chromosome Region 17q12–q21.1 and Is Close to the Gene for Hereditary Breast–Ovarian Cancer

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**The gene for insulin-like growth factor-binding protein 4 (IGFBP4) codes for a serum protein that binds to the family of insulin-like growth factors and modulates their activity. It has been mapped by *in situ* hybridization to chromosome region 17q12–q21.1. We have developed a CA-repeat polymorphism from a cosmid clone containing IGFBP4. By linkage analysis, IGFBP4 maps to the chromosome 17q interval THRA1–D17S579. This interval also contains the gene for hereditary breast–ovarian cancer, BRCA1. Genetic recombination between IGFBP4 and BRCA1 places IGFBP4 centromeric to the cancer susceptibility gene and effectively excludes it as a candidate gene for BRCA1. IGFBP4 is, however, one of the closest known centromeric markers for BRCA1; the estimated recombination fraction is 0.015. IGFBP4 and D17S579 together define a 2.8-cM interval that contains BRCA1.**

Insulin-like growth factors (IGFs) play important roles in regulating normal breast development and are potent mitogens for breast cancer cells (6, 13, 14). IGF bioactivity in normal and neoplastic breast tissue is influenced not only by circulating IGF concentration and local IGF production, but also by the concentration of specific IGF-binding proteins (IGFBP) (15). So far, six IGFBP genes have been identified and four of these have been localized (1). IGFBP4 has been mapped to chromosome region 17q12-q21 by *in situ* hybridization (3). The gene for hereditary breast and ovarian cancer was assigned to this region (9, 11). We investigated IGFBP4 as a candidate gene for BRCA1 by linkage analysis in 22 hereditary breast–ovarian cancer families.

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A cosmid clone (cCLBP4-5) containing the entire IGFBP4 gene was isolated from a placental library using a PCR fragment corresponding to the published cDNA sequence (16). The clone was used for fluorescence *in situ* hybridization to assign IGFBP4 to chromosome region 17q12–q21.1. The insert of cCLBP4-5 was subcloned in pGEM-5Zf vectors and sequenced on an ALF DNA sequencer (Pharmacia) using fluorescence-labeled vector primers. The subclone 14D5 contained a CA-repeat domain located in intron 1 of IGFBP4. We con-

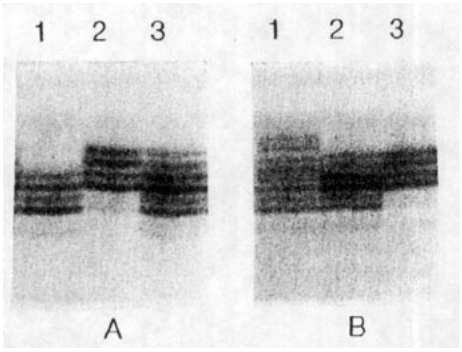


**FIG. 1.** The result of a PCR amplification of the CA-repeat polymorphism for IGFBP4 is shown. The primers are sense, 5'-CTTTC'T CAGCCACAAGATAGAGC-3', and antisense, 5'-GGCAGACCACAC-AGGAGG-3'. The 12.5-$\mu$l reaction volumes contained 100 ng of genomic DNA, 50 pmol of each primer, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 25 $\mu$*M* dATP, 200 $\mu$*M* each of dCTP, dGTP, dTTp, 1 $\mu$Ci[$^{35}$S]dATP, 10 m*M* Tris–HCl (pH 8.3), and 0.1% Triton. The DNA was denatured at 96°C for 5 min followed by the addition of 0.75 U *Taq* polymerase and then amplified for 35 cycles, each cycle consisting of 1 min at 96°C, 30 s at 56°C, and 1 min at 72°C. The amplified fragments were then electrophoresed on denaturing polyacrylamide gels (5%) and detected by autoradiography (Kodak X-Omat, AR-5). The PCR reaction detects three polymorphic alleles. Based on 70 unrelated individuals in the breast–ovarian cancer families, the allele frequencies for the IGFBP4 polymorphism were estimated to be 0.44 (**A**, lane **2**), 0.55 (**A**, lane **1**), and 0.007 (the rare allele was present on a single chromosome; **B**, lane **1**).

**TABLE 1**

**Two-Point Lod Scores for Linkage between IGFBP4 and Other Chromosome Region 17q12–q21 Loci**

| Locus | Recombination fraction | | | | Lod | $\hat{\theta}$ |
|---|---|---|---|---|---|---|
| | 0.01 | 0.05 | 0.10 | 0.20 | | |
| D17S250 | 31.46 | 30.76 | 27.34 | 19.11 | 31.81 | 0.02 |
| THRA1 | 16.80 | 18.57 | 17.12 | 12.27 | 18.61 | 0.04 |
| 17$\beta$-HSD II | 12.88 | 11.67 | 9.96 | 6.44 | 13.15 | 0.00 |
| D17S579 | 19.12 | 19.40 | 17.61 | 12.63 | 19.70 | 0.03 |
| D17S588 | 9.41 | 13.71 | 13.71 | 10.42 | 13.98 | 0.07 |
| D17S74 | −12.95 | 2.50 | 7.09 | 7.91 | 8.25 | 0.16 |

*Note.* Linkage was carried out using the LINKAGE programs (10). Polymorphisms have been previously described (4, 7, 12, 18). For computational efficiency, each polymorphism with six or more observed alleles was reduced in the linkage analysis to a system of six equally frequent alleles. The six previously mapped loci are in chromosome order from centromere to telomere. From our data, we estimate the two-point (sex-average) recombination fractions for the intervals to be 0.05, 0.01, 0.02, 0.08, and 0.10, respectively.

structed PCR primers flanking this repeat and identified a polymorphism with three alleles (Fig. 1). The IGFBP4 polymorphism and six other chromosome 17q markers were used in a linkage analysis of 22 breast and ovarian cancer families; the families have been previously described (8, 17). IGFBP4 maps to the region of chromosome 17q12–q21 by two-point linkage analysis (Table 1). No recombination was observed between IGFBP4 and 17$\beta$-HSD II (LOD = 13.15), but was observed between IGFBP4 and the other chromosome 17 markers. The results of three-point linkage analysis favor the location of IGFBP4 in the 6.4-cM interval between THRA1 and D17S579 with odds of greater than $10^5$:1 over a location centromeric to THRA1 and odds greater than 100:1 over a location telomeric to D17S579 (Fig. 2). This interval also contains BRCA1, retinoic acid receptor $\alpha$ (RARA), and 17$\beta$-HSD II (4, 5, 17).

Hereditary breast cancer is a genetically heterogeneous disease, and roughly 50% of all families studied to date show linkage to BRCA1 (7). Assuming genetic homogeneity of breast cancer susceptibility in the 22 families studied here, the maximum multipoint lod score for BRCA1 of 10.57 was observed at a position in the interval between THRA1 and D17S579 (17). Fourteen of the 22 families give positive lod scores for linkage of the breast–ovarian cancer trait to the interval, and in these linkage-positive families, the maximum lod score for linkage between BRCA1 and IGFBP4 was 2.68 at a recombination fraction of 0.015. A single unambiguous recombination event was observed between the cancer phenotype and IGFBP4. This crossover maps IGFBP4 to a position centromeric to BRCA1 (Fig. 3). Because there are two early-onset cases of breast cancer in family 1816 who carry the recombinant chromosome, the crossover is very informative (i.e., the sporadic occurrence of cancer in both of these individuals is unlikely). In conclusion, these results provide support for IGFBP4 as one of the closest markers centromeric to BRCA1 and that

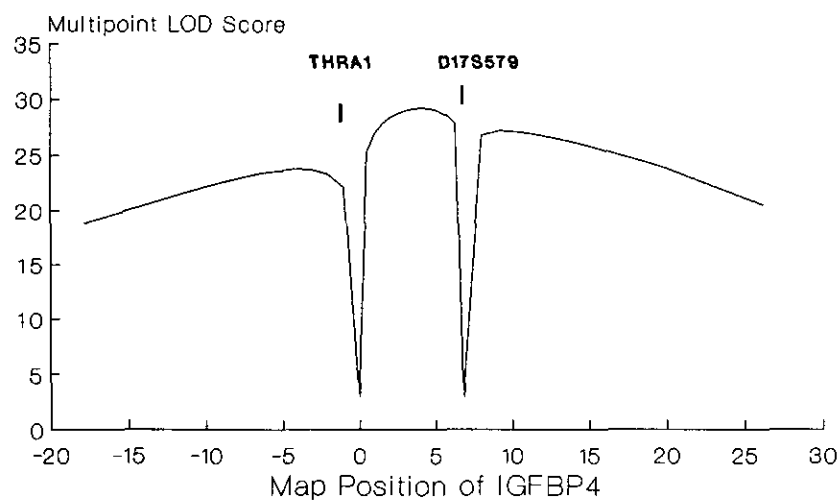


**FIG. 2.** Three-point mapping of IGFPB4 gene. Origin indicates the THRA1 locus. The analysis was based on 22 families. Multipoint linkage analysis was performed assuming no interference. The recombination rate between THRA1 and D17S579 was previously estimated to be 0.064 in these data (17).

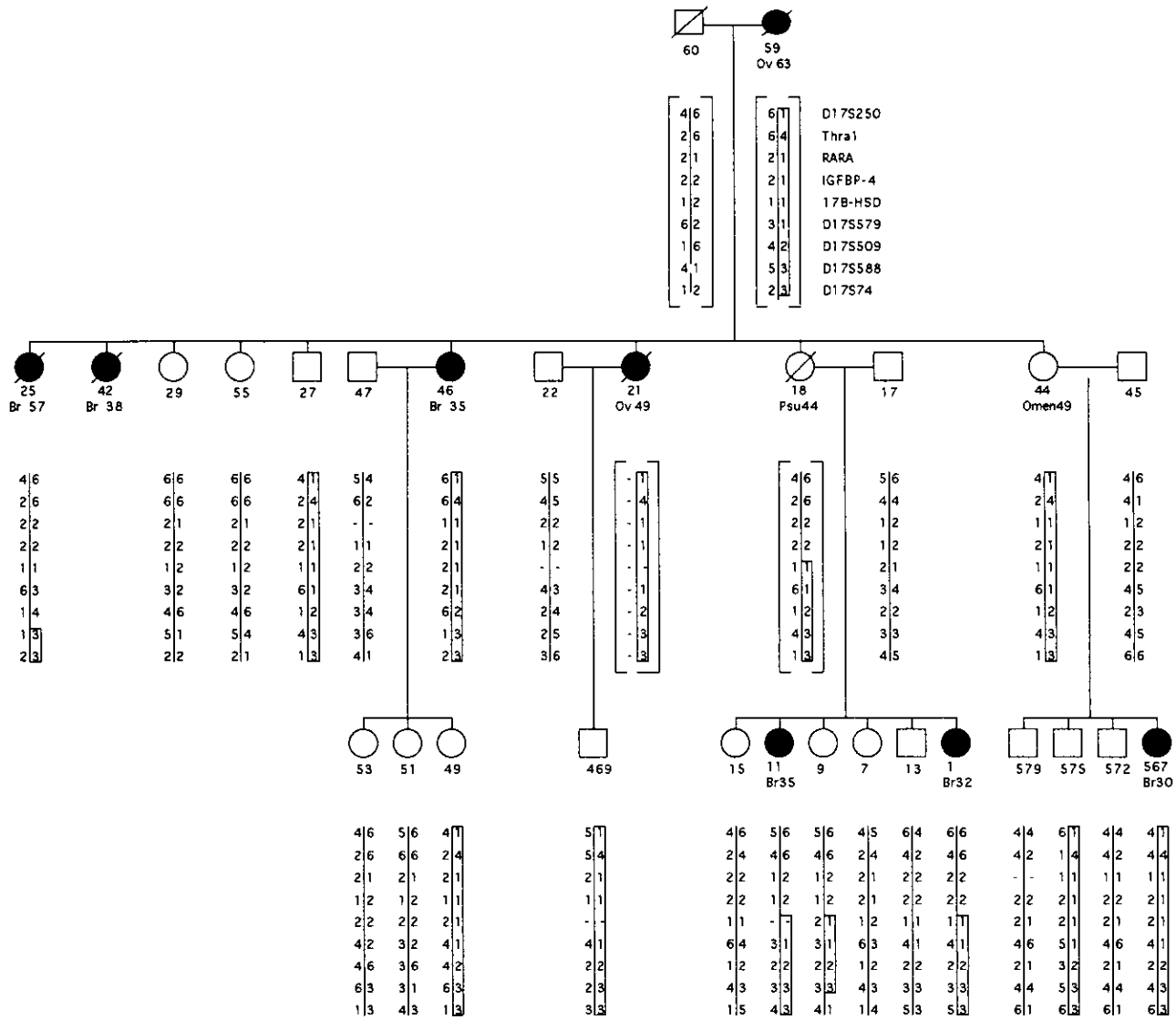


**FIG. 3.** Abridged pedigree of family 1816. Black circles represent affected women. Br, breast cancer; Ov, ovarian cancer; Psu, abdominal cancer, site unknown; Omen, omental cancer. Ages at cancer diagnosis are given. Diagonal slashes indicate deceased. Numbers arranged vertically refer to haplotypes of the markers indicated. Haplotypes in brackets are inferred. The haplotype indicated in the rectangle contains, by inference, the BRCA1 gene. A dash in the place of an allele number indicates that a typing is unavailable. Not all typed individuals appear. The multipoint lod score for linkage of the breast–ovarian cancer trait in this family to the interval THRA1 D17S579 is 3.62. The RARA data are based on the polymorphism of Arvelier *et al.* (2). Individual 25 is likely to have a sporadic case of breast cancer.

BRCA1 is in the 2.8-cM interval flanked by IGFBP4 and D17S579. Because of the observed genetic recombination, IGFBP4 is not likely to be the breast–ovarian cancer gene.

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