(GSK3-α), was the site of phosphorylation in each phosphopeptide, both *in vitro* (Fig. 4*b*) and *in vivo* (not shown). The ^{32}P-labelling of other (more acidic) tryptic phosphopeptides was not increased by insulin (Fig. 4*d*). These peptides have been noted previously in GSK3 from A431 cells and shown to contain phosphoserine and phosphotyrosine[11].

PKC-δ, ε and ζ are reported to be activated by mitogens, and PKC-ζ activity is stimulated *in vitro* by several inositol phospholipids, including PI(3,4,5)P_3, the product of the PI 3-kinase reaction[26]. However, purified PKC-ε[27], PKC-δ and PKC-ζ (data not shown) all failed to inhibit GSK3-α or GSK3-β *in vitro*. Moreover, although PKC-α, β1 and γ inhibit GSK3-β *in vitro*[27], GSK3-α is unaffected, while their downregulation in L6 myotubes by prolonged incubation with phorbol esters abolishes the activation of MAPKAP kinase-1 in response to subsequent challenge with phorbol esters, but has no effect on the inhibition of GSK3 by insulin (not shown).

Taken together, our results identify GSK3 as the first physiologically relevant substrate for PKB. The stimulation of glycogen synthesis by insulin in skeletal muscle involves the dephosphorylation of Ser residues in glycogen synthase that are phosphorylated by GSK3 *in vitro*[2]. Hence the 40–50% inhibition of GSK3 by insulin, coupled with a similar activation of the relevant glycogen synthase phosphatase[28], can account for the stimulation of glycogen synthase by insulin in skeletal muscle[2] or L6 myotubes[29]. The activation of glycogen synthase and the resulting stimulation of glycogen synthesis by insulin in L6 myotubes is blocked by wortmannin, but not by PD 98059 (ref. 29), just like the activation of PKB and inhibition of GSK3. However, GSK3 is unlikely to be the only substrate of PKB *in vivo*, and identifying other physiologically relevant substrates will be important because PKB-β is amplified and overexpressed in many ovarian neoplasms[23]. □

Received 4 August; accepted 14 November 1995.

1. Embi, N., Rylatt, D. B. & Cohen P. *Eur. J. Biochem.* **107,** 519–527 (1980).
2. Parker, P. J. J., Caudwell, F. B. & Cohen, P. *Eur. J. Biochem.* **130,** 227–234 (1983).
3. Welsh, G. I. & Proud, C. G. *Biochem. J.* **294,** 625–629 (1993).
4. Nikolakaki, E., Coffer, P., Hemelsoet, R., Woodgett, J. & Defize, L. *Oncogene* **8,** 833–840 (1993).
5. de Groot, R., Auwerx, J., Bourouis, M. & Sassone-Corsi, P. *Oncogene* **8,** 841–847 (1993).
6. Fol, C. *et al. J. biol. Chem.* **269,** 32187–32193 (1994).
7. Siegfried, E., Chou, T.-B. & Perrimon, N. *Cell* **71,** 1167–1179 (1992).
8. He, X., Saint-Jeannet, J.-P., Woodgett, J. R., Varmus, H. E. & Dawid, I. B. *Nature* **374,** 617–622 (1995).
9. Hughes, K., Ramakrishna, S., Benjamin, W. B. & Woodgett, J. R. *Biochem. J.* **288,** 309–314 (1992).
10. Cross, D. A. E. *et al. Biochem. J.* **303,** 21–26 (1994).
11. Saito, Y., Vandenheede, J. R. & Cohen, P. *Biochem. J.* **303,** 27–31 (1994).
12. Sutherland, C., Leighton, I. A. & Cohen, P. *Biochem. J.* **296,** 15–19 (1993).
13. Sutherland, C. & Cohen, P. *FEBS Lett.* **338,** 37–42 (1994).
14. Welsh, G. I., Foulstone, E. J., Young, S. W., Tavare, J. M. & Proud, C. G. *Biochem. J.* **303,** 15–20 (1994).
15. Alessi, D. R., Cuenda, A., Cohen, P., Dudley, D. & Saltiel, A. L. *J. biol. Chem.* **270,** 27489–27494 (1995).
16. Kuo, C. J. *et al. Nature* **358,** 70–73 (1992).
17. Stambolic, V. & Woodgett, J. R. *Biochem. J.* **303,** 701–704 (1994).
18. Eldar-Finkelman, H., Seger, R., Vandenheede, J. R. & Krebs, E. G. *J. biol. Chem.* **270,** 987–990 (1995).
19. Vlahos, C. J., Matter, W. F., Hui, K. Y. & Brown, R. F. *J. biol. Chem.* **269,** 5241–5248 (1994).
20. Coffer, P. J. & Woodgett, J. R. *Eur. J. Biochem.* **201,** 475–481 (1991).
21. Jones, P. F., Jakubowicz, T., Pitossi, F. J., Maurer, F. & Hemmings, B. A. *Proc. natn. Acad. Sci. U.S.A.* **88,** 4171–4175 (1991).
22. Ahmed, N. N. *et al. Molec. cell. Biol.* **15,** 2304–2310 (1995).
23. Cheng, J. Q. *et al. Proc. natn. Acad. Sci. U.S.A.* **89,** 9267–9271 (1992).
24. Franke, T. F. *et al. Cell* **81,** 727–736 (1995).
25. Burgering, B. M. Th. & Coffer, P. J. *Nature* **376,** 599–602 (1995).
26. Palmer, R. H. *et al. J. biol. Chem.* **270,** 22412–22416 (1995).
27. Goode, N., Hughes, K., Woodgett, J. R. & Parker, P. J. J. *J. biol. Chem.* **267,** 16878–16882 (1992).
28. Dent, P. *et al. Nature* **348,** 302–308 (1990).
29. Lazar, D. F. *et al. J. biol. Chem.* **270,** 20801–20807 (1995).

ACKNOWLEDGEMENTS. We thank A. Klip for providing L6 cells, and H. McDowell for advice and help in growing them; A. Saltiel for PD 98059; J. Vandenheede for anti-GSK3 antibodies; C. Marshall for anti-p42 MAP kinase antibodies; P. Parker for PKC-δ and ζ; N. Morrice for providing GSK3; B. Caudwell for peptide sequencing; M. Frech for the PH domain antibodies; and J. Thorner for critical reading of the manuscript. D.A.E.C. is the recipient of a CASE studentship from the U.K. Biotechnology and Biological Sciences Research Council and SmithKline Beecham Pharmaceuticals. This work was supported by the British Diabetic Association, the U.K. Medical Research Council, and The Royal Society.

Identification of the breast cancer susceptibility gene *BRCA2*

Richard Wooster*, Graham Bignell*, Jonathan Lancaster‡, Sally Swift†, Sheila Seal*, Jonathan Mangion*, Nadine Collins*, Simon Gregory§, Curtis Gumbs‖, Gos Micklem§, Rita Barfoot*, Rifat Hamoudi*, Sandeep Patel*, Catherine Rice§, Patrick Biggs*, Yasmin Hashim*, Amanda Smith†, Frances Connor†, Adalgeir Arason¶, Julius Gudmundsson¶, David Ficenec¶* David Kelsell#, Deborah Ford☆, Patricia Tonin**, D. Timothy Bishop††, Nigel K. Spurr#, Bruce A. J. Ponder‡‡, Rosalind Eeles*, Julian Peto☆, Peter Devilee§§, Cees Cornelisse§§, Henry Lynch‖‖, Steven Narod** ☆☆, Gilbert Lenoir¶¶, Valdgardur Egilsson¶, Rosa Bjork Barkadottir¶, Douglas F. Easton##, David R. Bentley§, P. Andrew Futreal‖, Alan Ashworth† & Michael R. Stratton***

Sections of * Molecular Carcinogenesis and ☆ Epidemiology, and † CRC Centre for Cell and Molecular Biology, Institute of Cancer Research, Haddow Laboratories, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK, and Chester Beatty Laboratories, Fulham Road, London SW3 6JB, UK
‡ Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709, USA
§ The Sanger Centre, Hinxton Hall, Hinxton, Cambridgeshire CB10 1RQ, UK
‖ Duke University Medical Centre, Departments of Surgery and Genetics, and Division of Gynaecologic Oncology, Research Drive, Medical Sciences Research Building, Room 363, Durham, North Carolina 27710, USA
¶ Laboratory of Cell Biology, University Hospital of Iceland, P.O. Box 1465, IS-121 Reykjavik, Iceland
# ICRF Clare Hall Laboratories, Blanche Lane, South Mimms, Potters Bar EN6 3LD, UK
** Division of Medical Genetics and Division of Human Genetics, Dept of Medicine, McGill University, 1650 Cedar Avenue, Montreal, H3G 1A4, Canada
†† ICRF Genetic Epidemiology Laboratory, 3K Springfield House, Hyde Terrace, Leeds LS2 9LU, UK
‡‡ CRC Human Cancer Genetics Research Group, Level 3, Laboratories Block, Box 238, Addenbrookes Hospital, Hills Road, Cambridge CB2 2QQ, UK
§§ Department of Human Genetics and Pathology, Leiden University, Wassenaarseweg 72, P. O. Box 9503, 2300 RA, Leiden, The Netherlands
‖‖ Department of Preventive Medicine and Public Health, Creighton University School of Medicine, Omaha, Nebraska 68178, USA
¶¶ International Agency for Research on Cancer, 150 Cours Albert-Thomas, 69372 Lyon Cedex 08, France
CRC Genetic Epidemiology Group, Department of Community Medicine, Institute of Public Health, University of Cambridge, University Forvie Site, Robinson Way, Cambridge CB2 2SR, UK
☆☆ Women's College Hospital, Toronto, Ontario, Canada
*** Genome Sequencing Centre, Washington University in St Louis, School of Medicine, St Louis, MO, USA

In Western Europe and the United States approximately 1 in 12 women develop breast cancer. A small proportion of breast cancer cases, in particular those arising at a young age, are attributable to a highly penetrant, autosomal dominant predisposition to the disease. The breast cancer susceptibility gene, *BRCA2*, was recently localized to chromosome 13q12-q13. Here we report the identification of a gene in which we have detected six different germline mutations in breast cancer families that are likely to be due to *BRCA2*. Each mutation causes serious disruption to the open reading frame of the transcriptional unit. The results indicate that this is the *BRCA2* gene.

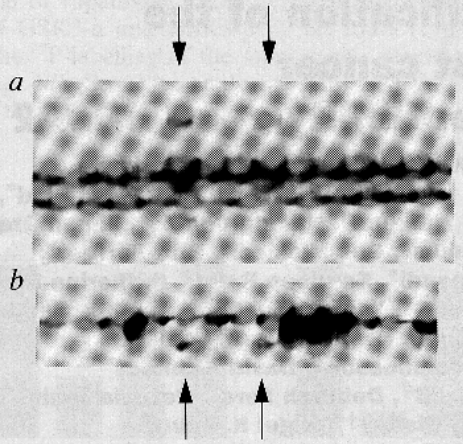

FIG. 1 Detection of the *BRCA2* gene mutation in family IARC 2932. Mutation screening by migration shift assays. The arrows indicate abnormally migrating bands in two early onset breast cancer cases from IARC 2932.
METHODS. A ^{32}P-labelled, 271-bp genomic fragment was amplified from lymphocyte DNAs from affected individuals in 46 breast cancer families. The PCR product was denatured in 50% formamide and electrophoresed through *a*, 4.5% non-denaturing polyacrylamide gels and *b*, 6% denaturing polyacrylamide gels.

Abnormalities of several genes are known to confer susceptibility to breast cancer. The *BRCA1* gene accounts for the large majority of families with both breast and ovarian cancer cases, but only half of families with site-specific breast cancer[1]. Using families with multiple cases of early-onset breast cancer showing evidence against linkage to *BRCA1* we recently demonstrated the existence of a second major breast cancer susceptibility locus, *BRCA2*, on chromosome 13q12-q13 (ref. 2). Preliminary studies indicate that mutations in *BRCA2* confer a similar risk of female breast cancer to *BRCA1*. However, the risk of ovarian cancer appears to be lower and the risk of male breast cancer substantially higher. Risks of other cancers, including prostate and laryngeal cancer, may also be elevated in carriers of *BRCA2* mutations (unpublished data).

BRCA2 was originally positioned within a 6-cM region between *D13S289* and *D13S267* that was defined on the basis of meiotic recombinants in early-onset breast cancer cases within clearly linked families[2]. (The genetic map in this region is centromere–*D13S289*–3cM–*D13S260*–1cM–*D13S171*–2cM–*D13S267*–telomere[3].) We further mapped the centromeric boundary of the interval within which the gene lies to *D13S260* using a set of Icelandic families (unpublished data). Subsequently, using recombinants in other families and additional microsatellite markers isolated from the region, we established that *BRCA2* is likely to be located in a 600-kb interval centred around *D13S171*. An unexpected contribution to the fine localization of *BRCA2* was provided by the detection of a homozygous somatic deletion in a single pancreatic cancer[4]. The centromeric boundary of this deletion is approximately 300 kb centromeric to *D13S171* and the telomeric boundary close to, but still centromeric of, *D13S171* (ref. 5). Despite the ambiguity of the relationship between this deletion and *BRCA2*, we combined the genetic recombinant information from families and the physical localization from the homozygous deletion, and prioritized analysis of the 300-kb region immediately centromeric to *D13S171*.

Yeast artificial chromosome (YAC)[6] and P1 artificial chromosome (PAC)[7] contigs extending approximately 700 kb centromeric and 300 kb telomeric to *D13S171* were constructed and a minimally overlapping set of 14 PACs was identified. Transcribed sequences located on these genomic contigs were identified using two methods: exon amplification (exon trapping) from subcloned PAC DNA[8], and direct selection by solution hybridization of complementary DNA to PAC genomic DNA[9]. To identify *BRCA2*, genomic DNA fragments of less than 300 bp containing putative coding sequences were screened for mutations. At least one affected member of 46 breast cancer families was examined. Each family included in this set either shows evidence of linkage to *BRCA2*, and/or shows evidence against linkage to *BRCA1*, and/or has not been found to carry a *BRCA1* mutation, and/or includes a case of male breast cancer. Most, but probably not all, of these families would be expected to have cases caused by *BRCA2* mutations.

Disease-associated mutations in most known cancer susceptibility genes usually result in truncation of the encoded protein and inactivation of critical functions. In the course of the mutational screen of candidate coding sequences from the *BRCA2* region, the first detected sequence variant that was predicted to disrupt translation of an encoded protein was observed in IARC 2932 (Fig. 1). This family is clearly linked to *BRCA2* with a multipoint LOD score of 3.01 using *D13S260* and *D13S267*. A deletion of 6 bp removes the last five bases of the exon examined (exon S66), deletes the conserved G of the 5′ splice site of the intron, and directly converts the codon TTT for phenylalanine to the termination codon TAA. By sequencing, this mutation has been detected in lymphocyte DNA from two other early-onset breast cancer cases in this family. The individuals examined share only the disease-associated haplotype. The mutation is absent in more than 500 chromosomes from normal individuals and in the remaining families and cancers. This finding therefore identified a strong candidate for the *BRCA2* gene.

TABLE 1 *BRCA2* mutations in breast cancer families

	FBCs	FBCs < 50	OvCs	MBCs	LOD score at *BRCA1*	LOD score at *BRCA2*	*BRCA2* mutation
IARC 2932	15	10	0	0	−2.38	3.01	CCC.TTT.CGgtaa
IARC 3594	6	5	0	0	nd	nd	CAT.AAC.TCT.CTA
CRC B211	5	3	4	0	−0.48	0.49	AGT.CTT.CAC
CRC B196	17	12	0	0	−2.21	0.92	AAA.ACT.GAA.ACT
Montreal 681	3	2	0	1	nd	nd	GCA.AGT.GGA
Montreal 440	2	2	0	2	nd	nd	GAT.AAA.CAA.GCA

LOD scores at *BRCA1* were calculated using the markers *D17S250* and *D17S579*; those at *BRCA2* were calculated using the markers *D13S26* and *D13S267*. Exon sequence is denoted by upper case, intron sequence by lower case; Codons are indicated by stops. The underlined letter indicate the deleted bases in each family. Abbreviations: FBCs, female breast cancers; OvCs, ovarian cancers, MBCs, male breast cancers.

```
HIGKSMPNVLEDEVYETVVDTSEEDSFSLCFSKCRTKNLQKVRTSKTRKKIFHEANADEC   60
EKSKNQVKEKYSFVSEVEPNDTDPLDSNVANQKPFESGSDKISKEVVPSLACEWSQLTLS  120
GLNGAQMEKIPLLHISSCDQNISEKDLLDTENKRKKDFLTSENSLPRISSLPKSEKPLNE  180
ETVVNKRDEEQHLESHTDCILAVKQAISGTSPVASSFQGIKKSIFRIRESPKETFNASFS  240
GHMTDPNFKKETEASESGLEIHTVCSQKEDSLCPNLIDNGSWPATTTQNSVALKNAGLIS  300
TLKKKTNKFIYAIHDETSYKGKKIPKDQKSELINCSAQFEANAFEAPLTFANADSGLLHS  360
SVKRSCSQNDSEEPTLSLTSSFGTILRKCSRNETCSNNTVISQDLDYKEAKCNKEKLQLF  420
ITPEADSLSCLQEGQCENDPKSKKVSDIKEEVLAAACHPVQHSKVEYSDTDFQSQKSLLY  480
DHENASTLILTPTSKDVLSNLVMISRGKESYKMSDKLKGNNYESDVELTKNIPMEKNQDV  540
CALNENYKNVELLPPEKYMRVASPSRKVQFNQNTNLRVIQKNQEETTSISKITVNPDSEE  600
LFSDNENNFVFQVANERNNLALGNTKELHETDLTCVNEPIFKNSTMVLYGDTGDKQATQV  660
SIKKDLVYVLAEENKNSVKQHIKMTLGQDLKSDISLNIDKIPEKNNDYMNKWAGLLGPIS  720
NHSFGGSFRTASNKEIKLSEHNIKKSKMFFKDIEEQYPTSLACVEIVNTLALDNQKKLSK  780
PQSINTVSAHLQSSVVVSDCKNSHITPQMLFSKQDFNSNHNLTPSQKEQITELSTILEDS  840
GSQFEFTQFRKPSYILQKSTFEVPENQMTILKTTSEECRDADLHVIMNAPSIGQVDSSKQ  900
FEGTVEIKRKFAGLLKNDCNKSASGYLTDENEVGFRGFYSAHGTKLNVSTEALQKAVKLF  960
SDIENISEETSAEVHPISLSSSKCHDSVVSMFKIENHNDKTVSEKNNKCQLILQNNIEMT 1020
TGTFVEEITENYKRNTENEDNKYTAASRNSHNLEFDGSDSSKNDTVCIHKDETDLLFTDQ 1080
HNICLKLSGQFMKEGNTQIKEDLSDLTFLEVAKAQEACHGNTSNKEQLTATKTEQNIKDF 1140
ETSDTFFQTASGKNISVAKESFNKIVNFFDQKPEELHNFSLNSELHSDIRKNKMDILSYE 1200
ETDIVKHKILKESVPVGTGNQLVTFQGQPERDEKIKEPTLLGFHTASGKKVKIAKESLDK 1260
VKNLFDEKEQGTSEITSFSHQWAKTLKYREACKDLELACETIEITAAPKCKEMQNSLNND 1320
KNLVSIETVVPPKLLSDNLCRQTENLKTSKSIFLKVKVHENVEKETAKSPATCYTNQSPY 1380
SVIENSALAFYTSCSRKTSVSQTSLLEAKKWLREGIFDGQPERINTADYVGNYLYENNSN 1440
STIAENDKNHLSEKQDTYLSNSSMSNSYSYHSDEVYNDSGYLSKNKLDSGIEPVLKNVED 1500
QKNTSFSKVISNVKDANAYPQTVNEDICVEELVTSSSPCKNKNAAIKLSISNSNNFEVGP 1560
PAFRIASGKIVCVSHETIKKVKDIFTDSFSKVIKENNENKSKICQTKIMAGCYEALDDSE 1620
DILHNSLDNDECSTHSHKVFADIQSEEILQHNQNMSGLEKVSKISPCDVSLETSDICKCS 1680
IGKLHKSVSSANTCGIFSTASGKSVQVSDASLQNARQVFSEIEDSTKQVFSKVLFKSNEH 1740
SDQLTREENTAIRTPEHLISQKGFSYNVVNSSAFSGFSTASGKQVSILESSLHKVKGVLE 1800
EFDLIRTEHSLHYSPTSRQNVSKILPRVDKRNPEHCVNSEMEKTCSKEFKLSNNLNVEGG 1860
SSENNHSIKVSPYLSQFQQDKQQLVLGTKVSLVENIHVLGKEQASPKNVKMEIGKTETFS 1920
DVPVKTNIEVCSTYSKDSENYFETEAVEIAKAFMEDDELTDSKLPSHATHSLFTCPENEE 1980
MVLSNSRIGKRRGEPLILVGEPSIKRNLLNEFDRIIENQEKSLKASKSTPDGTIKDRRLF 2040
VHHVSLEPITCVPFRTTKERQEIQNPNFTAPGQEFLSKSHLYEHLTLEKSSSNLAVSGHP 2100
FYQVSGNKNGKMRKLITTGRPTKVFVPPFKTKSHFHRVEQCVRNINLEGNRQKQNIDGHG 2160
SDDSKNKINDNEIHQFNKNNSNQAAAVTFTKCEEEPLDLITSLQNARDIQDMRIKKKQRQ 2220
RVFPQPGSLYLAKTSTLPRISLKAAVGGQVPSACSHKQLYTYGVSKHCIKINSKNAESFQ 2280
FHTEDYFGKESLWTGKGIQLADGGWLIPSNDGKAGKEEFYRALCDVKAT             2329
```

FIG. 2 Predicted amino acid sequence of the *BRCA2* gene. The positions of the frameshift mutations indicated in Table 1 are boxed, and the positions of intron–exon boundaries are arrowed above the amino acid sequence.

METHODS. Exon S66 and others that had been trapped in association with it were used to isolate segments of the candidate cDNA by hybridization to normal human fetal brain, placental, monocyte and breast cancer cDNA libraries. Additional fragments were isolated by PCR amplification from known exon sequences to vector ends. In the course of these analyses, other previously trapped exons and cDNAs selected by solution hybridization were incorporated into an extended cDNA sequence. In addition, the exon prediction program Genemark was used to define the location of adjacent candidate transcribed sequences from the genomic sequence. Putative intron–exon boundaries were confirmed by amplification from cDNA and direct sequencing of amplification products. Northern analysis indicates that the transcript from the *BRCA2* gene is large (approximately 10–12 kb), and hence the N terminus of the *BRCA2* protein may well be missing from the above sequence.

To characterize this gene further, exon S66 was used to isolate a series of cDNA clones which represented segments of the *BRCA2* candidate (see Fig. 2 legend). At this stage the initial shotgun sequence data from a 900-kb region thought to contain *BRCA2* was completed at the Sanger Centre and Washington University and became available to us through the public release of the assembled sequence (at ftp://ftp.sanger.ac.uk/pub/human/sequences/13q and ftp://genome.wustl.edu/pub/gsc1/brca2 from 23 November 1995). From alignment of the cDNA and genomic sequence data, the candidate *BRCA2* gene was found to lie in three sequence contigs which also contained other previously isolated transcribed sequences. The exon and open reading frame prediction program Genemark was used to define putative additional 5′ exons of the gene. Contiguity of the transcription unit was confirmed by reverse-transcription–polymerase chain reaction (RT–PCR) on cDNA and sequence analysis. The availability of extensive sequence information at the cDNA and genomic level allowed mutational analysis of further coding regions of the putative *BRCA2* gene in samples from breast cancer families.

A TG deletion and a TT deletion were detected in families CRC B196 and CRC B211 respectively (Table 1). In both families the mutation has been detected by sequencing other individuals with early onset breast cancer who share only the haplotype of 13q microsatellite markers that segregates with the disease. Therefore, the mutations are on the disease-associated chromosomes. A CT deletion was detected in family IARC 3594. This mutation has arisen within a short repetitive sequence (CTCTCT), a feature that is characteristic of deletion/insertion mutations in many genes, and which is presumed to be due to slippage during DNA synthesis. Finally, a T deletion and an AAAC deletion have been found in Montreal 681 and 440, respectively. Both these families include a male breast cancer case, and previous analyses have indicated that the large majority of such families will have *BRCA2* mutations[10]. All these mutations are predicted to generate frameshifts leading to premature termination codons. None of the mutations have been found in over 500 chromosomes from healthy women and are therefore unlikely to be polymorphisms. The identification of several different germline mutations that truncate the encoded protein in breast cancer families that are highly likely to be due to *BRCA2* strongly suggests that we have identified the *BRCA2* gene.

Northern analysis has demonstrated that *BRCA2* is encoded by a transcript of 10–12 kb (data not shown), which is present

in normal breast epithelial cells, placenta and the breast cancer cell line MCF7. This suggests that our present contig of cDNAs covering approximately 7.3 kb (including 300 bp of 3′ untranslated sequence) may not include the whole *BRCA2* coding sequence. The known sequence of 2,329 amino acids encoded by the *BRCA2* gene does not show strong homology to sequences in the publicly available DNA or protein databases, and therefore we have no clues to its functions. However, some weak matches were detected including, intriguingly, a very weak similarity to the *BRCA1* protein over a restricted region (amino acids 1394–1474 in *BRCA1*, and 1783–1863 in the portion of *BRCA2* shown in Fig. 2). The significance of this is unclear.

Loss of heterozygosity on chromosome 13q has been observed in sporadic breast and other cancers, suggesting that there is a somatically mutated tumour suppressor gene in this region[11,13]. *BRCA2* is a strong candidate for this gene, and the analysis of a large series of cancers is underway to investigate if *BRCA2* is somatically mutated during oncogenesis.

The identification of *BRCA2* should now allow more comprehensive evaluation of families at high risk of developing breast cancer. However, the roles of environmental, lifestyle or genetic factors in modifying the risks of cancer in gene carriers are unknown, and further studies will be required before routine diagnosis of carrier status can be considered. □

Received 5 December; accepted 7 December 1995.

1. Easton, D. F., Bishop, D. T., Ford, D. & Crockford, G. P. *Am. J. hum. Genet.* **52,** 678–701 (1993).
2. Wooster, R. *et al. Science* **265,** 2088–2090 (1994).
3. Gyapay, G. *et al. Nature Genet.* **7,** 246–339 (1994).
4. Schutte, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **92,** 5950–5954 (1995).
5. Schutte, M. *et al. Cancer Res.* **55,** 4570–4574 (1995).
6. Cohen, D., Chumakov, I. & Weissenbach, J. *Nature* **366,** 698–701 (1993).
7. Ioannou, P. A. *et al. Nature Genet.* **6,** 84–89 (1994).
8. Nehls, M., Pfeifer, D. & Boehm, T. *Oncogene* **9,** 2169–2175 (1994).
9. Tagle, D. A., Swaroop, M., Lovett, M. & Collins, F. S. *Nature* **361,** 751–753 (1993).
10. Stratton, M. R. *et al. Nature Genet.* **7,** 103–107 (1994).
11. Devilee, P. & Cornelisse, C. J. *Biochim. biophys. Acta* **1198,** 113–130 (1994).
12. Lundberg, C., Skoog, L., Cavenee, W. K. & Nordenskjold, M. *Proc. natn. Acad. Sci. U.S.A.* **84,** 2372–2376 (1987).
13. Cleton-Jansen, A.-M. *et al. Br. J. Cancer* **72,** 1241–1244 (1995).

ACKNOWLEDGEMENTS. We thank the families for their continuing help and encouragement in this work and the many clinicians who have referred families; F. Dion, R. Carter, K. Anderson, M. Ponder, H. Vasen, J. Feuntein, O. Serova, R. Gwilliam, S. Humphray, M. Leversha, Y. Ramsey, H. King, D. Cain, D. Averill, P. Mitchell, M. Crompton, C. Cochran, J. Marks, D. Iglehart, R. Wiseman, J. Lancaster and the members of the Human Sequencing teams at the Sanger Centre and the Genome Sequencing Centre at Washington University for making their data publicly available; M. Schutte and S. Kern for discussions; and C. Marshall, C. Cooper and P. Garland for encouragement. This work was supported by the Cancer Research Campaign, BREAKTHROUGH Breast Cancer Charity and the Jean Rook Appeal, the Institute of Cancer Research, the Wellcome Trust, the Medical Research Council, the Imperial Cancer Research Fund, the U.S. Army, Duke University SPORE in Breast Cancer, the National Cancer Institute, the Icelandic Cancer Research Fund, the Nordic Cancer Union, the Ligue National contre le Cancer, the Dutch Cancer Society, the Cancer Research Society, the Canadian Breast Cancer Foundation, the Fonds de la recherche en Sante du Quebec, and the Canadian Genetic Diseases Network. Access to BRCA2 sequences can be obtained from rich_w@icr.ac.uk.

RETRACTION

Cloning and functional expression of a rat heart K_{ATP} channel

M. L. J. Ashford, C. T. Bond, T. A. Blair & J. P. Adelman

Nature **370,** 456–459 (1994)

IN this letter we described the cloning and expression of an inward rectifier potassium-channel subunit from rat heart (Kir 3.4) which, when transfected into HEK293 and BHK21 cells, endowed them with ATP-sensitive potassium channels. Since this paper appeared, we have not been able regularly to reproduce those findings. In addition, the data presented by Krapivinsky *et al.*[1] presents a compelling argument that Kir 3.4 is an intrinsic component of the channel underlying I_{KACh} in atrium, and that it does not contribute to the channel underlying cardiac I_{KATP}. Therefore, we cannot support our previous statement that Kir 3.4 represents a subunit of cardiac K_{ATP} channels. □

1. Krapivinsky, G. *et al. Nature* **374,** 135–141 (1995).