Identification of a large rearrangement of the *BRCA1* gene using colour bar code on combed DNA in an American breast/ovarian cancer family previously studied by direct sequencing

Sophie Gad, Maren T Scheuner, Sabine Pages-Berhouet, Virginie Caux-Moncoutier, Aaron Bensimon, Alain Aurias, Mark Pinto, Dominique Stoppa-Lyonnet

J Med Genet
2001;**38**:388–392

Service de Génétique Oncologique, Institut Curie, Section Médicale, 26 rue d'Ulm, F-75248 Paris Cedex 05, France
S Gad
A Pages-Berhouet
V Caux-Moncoutier
D Stoppa-Lyonnet

GenRISK Program, Division of Medical Genetics, Cedars-Sinai Medical Center, UCLA School of Medicine, California, USA
M T Scheuner
M Pinto

Laboratoire de Biophysique de l'ADN, Institut Pasteur, Paris, France
A Bensimon

INSERM U509, Pathologie Moléculaire des Cancers, Institut Curie, Paris, France
A Aurias

Correspondence to
Dr Stoppa-Lyonnet
dominique.lyonnet@curie.net

EDITOR—Linkage data suggest that *BRCA1* and *BRCA2* gene alterations account for the majority of hereditary breast/ovarian cancer cases.[1] Though comprehensive screening of the *BRCA1* gene has been attempted on a number of occasions, only two thirds of expected mutations have been detected so far.[1–3] Most efforts have relied on standard PCR techniques, including direct sequencing, single strand conformation polymorphism (SSCP) analysis, heteroduplex analysis (HDA), denaturing gradient gel electrophoresis (DGGE), and protein truncation testing (PTT), with a focus on point and small mutations.[2] The relatively low rate of detection of *BRCA1* gene mutations may be because of the existence of large rearrangements, which are not detected by such approaches. Supporting this hypothesis, a number of large rearrangements, ranging from 0.5 to 23.8 kb and spanning the entire *BRCA1* gene, have recently been detected by Southern blotting, analysis of *BRCA1* lymphocyte transcripts, and long range PCR.[4–14] In most cases, the characterised rearrangements are the result of unequal recombination events between Alu sequences, which cover 41.5% of *BRCA1* introns.[15]

Here we report the identification, using colour bar coding on combed DNA, of a previously undescribed large rearrangement of the *BRCA1* gene in an American breast/ovarian cancer family with ancestors from France and Germany (fig 1).

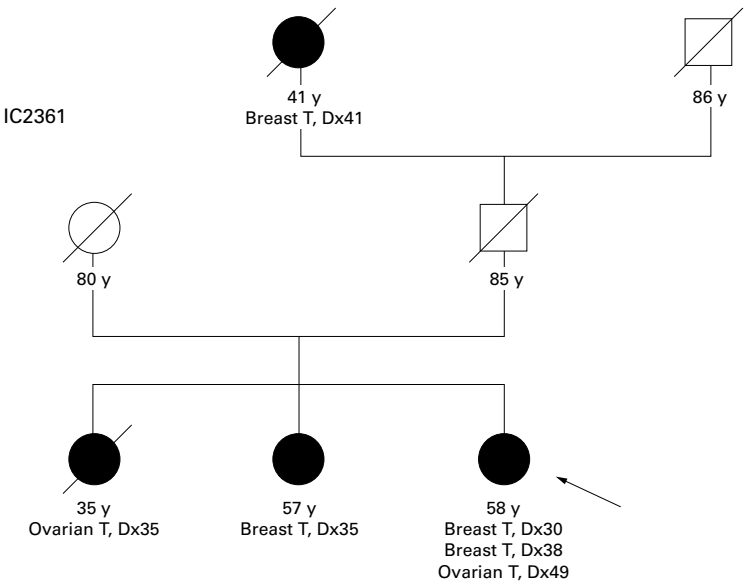

Figure 1 Pedigree of American family IC2361. Current ages or ages at death are indicated below each symbol. Tumour locations (T) and ages at diagnosis (Dx) are reported. The arrow indicates the patient studied.

Material and methods

The index case was diagnosed with breast cancer at the age of 30 and ovarian cancer at the age of 49. She had one sister with breast cancer diagnosed at the age of 35, another sister with ovarian cancer diagnosed at the age of 35, and a paternal grandmother with breast cancer diagnosed at the age of 41. The index case was referred to Cedars-Sinai Medical Center (Los Angeles, USA) for a genetic consultation. She elected to participate in *BRCA* gene testing, as she hoped to characterise her apparent genetic susceptibility so that her daughter could know her own status with greater certainty. No *BRCA1* or *BRCA2* gene mutation was identified by direct DNA sequencing (BRACAnalysis™, Myriad Genetic Laboratories Inc, Salt Lake City, USA). Because the a priori likelihood of carrying a *BRCA* gene mutation was high, the case was referred to our laboratory to search for large rearrangements in the *BRCA1* gene (family quoted IC2361).

The strategy for the detection of large rearrangements developed in our laboratory is based on a full four colour bar code of the *BRCA1* region on combed DNA.[16] Combing relies on homogeneous stretching of DNA molecules at a constant rate of 2 kb/μm.[17] Fluorescence in situ hybridisation (FISH) is then performed on combed DNA.[18] The probes used include a PAC covering the whole *BRCA1* region and long range (LR) PCR products (6.5 to 10 kb long) covering a number of exons, therefore bar coding the PAC. We have optimised the *BRCA1* bar code reported by Gad *et al*[16] by adding new LR products to allow for the detection of rearrangements as small as 2 kb. Finally, in addition to the PAC, a complex bar code of the *BRCA1* region was designed with seven probes (fig 2). This approach allows for a panoramic view of the *BRCA1* gene and its flanking regions.

Germline DNA was extracted from a lymphoblastoid cell line, with a step in agarose blocks in order to preserve its integrity. After combing of the DNA on silanised surfaces[17 18] and FISH with the set of probes, microscope screening was then performed.

Results

With a few fields of microscopic view, a number of full signals without LR13-15 probes were

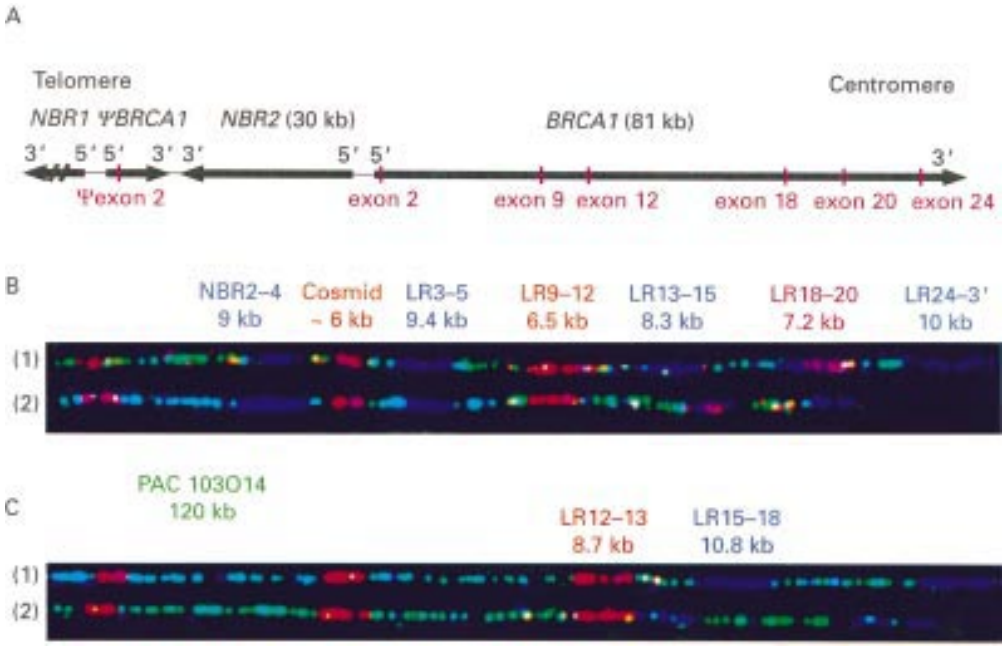

Figure 2 BRCA1 bar code of IC2361. (A) The BRCA1 region in 17q21. The BRCA1 gene is spread over 81 kb and has a common promoter with the NBR2 gene (Next to BRCA1 gene 2), which covers approximately 30 kb.[15 26] The BRCA1 pseudogene (ΨBRCA1) lies next to NBR2 and corresponds to a partial duplication of BRCA1, from the promoter region to intron 2.[27 28] The NBR1 gene (Next to BRCA1 gene 1) is located 5' to ΨBRCA1 and may be phylogenetically at the origin of NBR2.[26 29] The figure is not drawn to scale and is adapted from Xu et al[26] and Brown et al.[28] (B) Analysis of the BRCA1 region with our standard bar code. (1) This full signal corresponds to the normal allele. The PAC 103O14 insert covers the region between the first exons of NBR1 and the 3' BRCA1 UTR (exon 24), which is approximately 120 kb long.[15 26 28 30] The cosmid ICRFc105D06121 clone used in our experiment is rearranged, with an insert which is approximately 6 kb long and covers the region between NBR2 intron 1 and BRCA1 intron 2.[16 26 28] The other probes are long range (LR) PCR products, covering the regions between the exons quoted, except LR24-3', which covers BRCA1 exon 24 to 10 kb downstream (primers used are available on http://www.curie.net/genetique). (2) This full signal does not include the LR13-15 probe, which represents a first indication of a deletion of at least exons 13 to 15 in the BRCA1 gene for this patient. (C) Adaptation of the BRCA1 bar code to the detected deletion. The hybridisation of two additional long range products covering exons 12 to 13 and exons 15 to 18 on the normal allele (1) and the deleted allele (2) shows that the deletion comprises only exons 13 to 15.

observed, identifying the existence of a large deletion of the *BRCA1* gene (fig 2B). In order to characterise the deleted exons better, the bar code was adapted by using two additional probes, covering exons 12-13 and exons 15-18. Taking the sizes of *BRCA1* exons and introns into account,[15] as shown in fig 2C, the deleted region was expected to comprise exons 13, 14, and 15.

To characterise the mutant mRNA, we performed RT-PCR using primers located at the 3' end of exon 11 and in exon 16. A normal product was detected both in control and patient DNA corresponding to the 824 bp expected product (fig 3A). In the patient, a mutant product was also observed, corresponding to the 334 bp product expected in the absence of exons 13 to 15 (fig 3A). Sequencing of this shorter product showed that exons 12 and 16 were adjacent (fig 3B), leading to a premature stop codon at position 1437 and truncation of the BRCA1 protein. Thus, the deletion of exons 13 to 15 appears to be the origin of the cancer predisposition in this family.

In order to define the boundaries of the deletion, long range PCR was performed on genomic DNA using primers located in exons 12 and 16. From the patient's DNA, an abnormal product was observed at 8.5 kb, whereas the 20.2 kb expected product was not observed in control DNA (fig 3A). The 8.5 kb product was gel extracted. Its restriction map was determined (data not shown) and compared to the restriction map of the normal region of exons 12 and 16 (accession number Genbank L78833[15]). From this comparison and by taking the location of the Alu sequences in this region into account, we hypothesised that a recombination event had occurred between two Alu sequences, in the 3' end of intron 12 and the 5' end of intron 15, respectively. Primers were designed to amplify a DNA fragment comprising the putative breakpoints. From the patient's DNA, a 550 bp fragment was obtained, gel extracted, and sequenced (fig 3A). It showed that unequal recombination had occurred between an Alu Sx in intron 12 and an Alu Sp in intron 15. These two Alu sequences share 86% homology (data not shown). Recombination breakpoints were located between nucleotides 44 377 and 44 397 in Alu Sx and between nucleotides 55 980 and 56 000 in Alu Sp (accession number Genbank L78833[15]), resulting in a 11 604 bp deletion (fig 3C).

In order to examine the frequency of this rearrangement among breast/ovarian cancer families, we screened a series of 90 women affected with breast or ovarian cancer and ascertained at the cancer genetics clinic of the Institut Curie (Paris, France) according to family criteria previously reported.[3] Most of patients had French ancestors. These women, screened negative for *BRCA1* and *BRCA2* point mutations, were then tested for the presence of the 550 bp PCR product by using

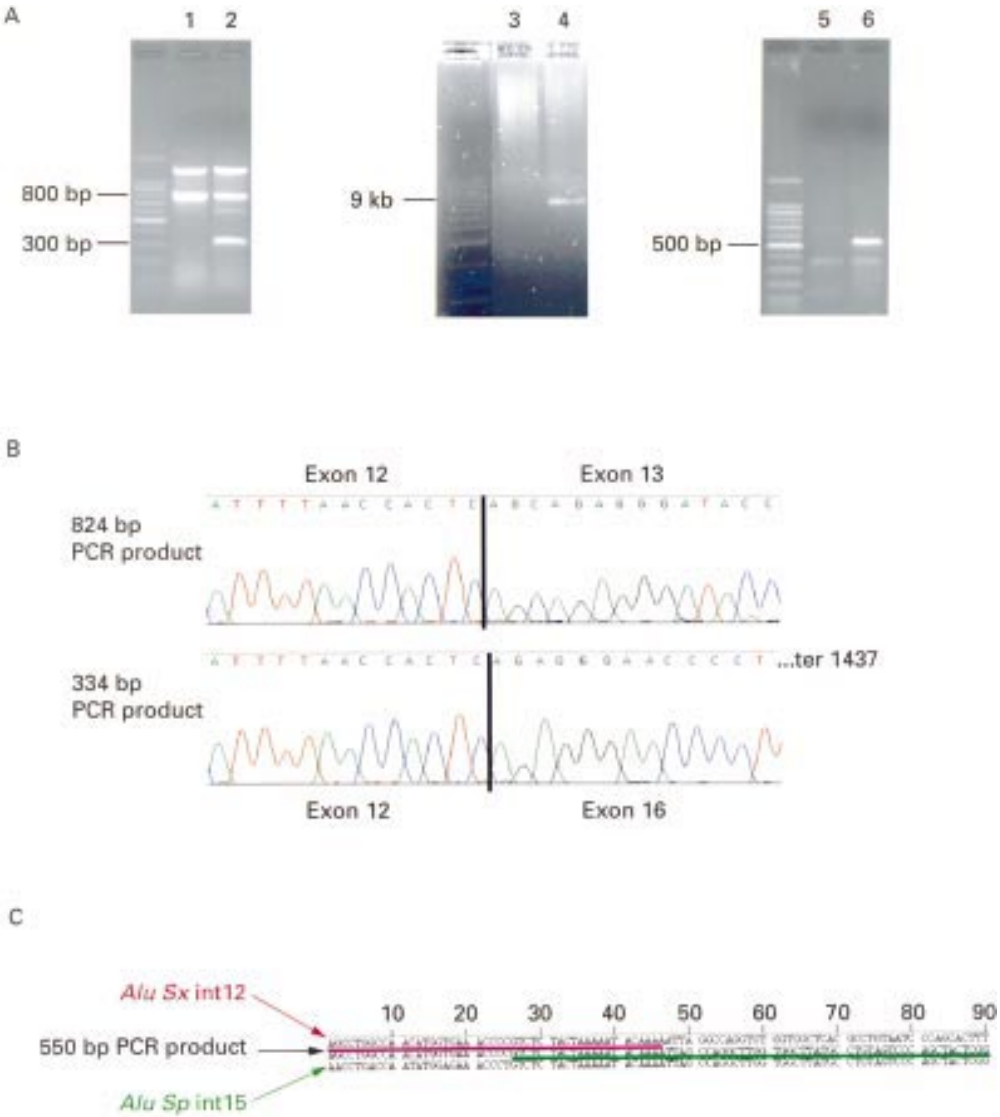

Figure 3 Characterisation of the IC2361BRCA1 rearrangement. (A) RT-PCR products with primers "exon 11 forward" and "exon 16 reverse" on (lane 1) control RNA (824 bp expected) and on (lane 2) IC2361 RNA: a product between 300 and 400 bp is observed and corresponds to the deletion of exons 13 to 15 (334 bp). RNA extraction was performed according to the TRIzol protocol (Gibco, Gaithersburg, MD, USA). Long range PCR products with primers "ex12 forward" and "ex16 reverse" on (lane 3) genomic control DNA showing, as expected, no product and on (lane 4) IC2361 genomic DNA showing the 8.5 kb abnormal product comprising the breakpoints. PCR products with primers "Alu intron 12 forward" and "Alu intron 15 reverse" on (lane 5) genomic control DNA showing as expected no product and on (lane 6) IC2361 genomic DNA showing the 550 bp narrowing the breakpoint region. (B) Sequences of IC2361 RT-PCR products. The 824 and 334 bp PCR products were gel extracted and sequenced with primers "exon 11 forward" and "exon 16 reverse". Sequence of the 824 bp product shows normal transition between exons 12 and 13, whereas sequence of the 334 bp product shows a junction of exons 12 to 16. (C) Box in which recombination has occurred, leading to a 11.6 kb deletion. The 550 bp was gel extracted and sequenced with primers "Alu intron 12 forward" and "Alu intron 15 reverse". Alignment of this sequence with Alu Sx in intron 12 and Alu Sp in intron 15 shows the recombination box located between nucleotides 44 377 and 44 397 in intron 12 and between nucleotides 55 980 and 56 000 in intron 15 (accession number Genbank L78833[15]), thus resulting in a 11 604 bp deletion.

primers "Alu intron 12 forward" and "Alu intron 15 reverse". No 550 bp fragment was observed, suggesting that this rearrangement is not frequent in the population studied (data not shown).

Discussion

Our report of a previously undescribed 11.6 kb deletion encompassing exons 13 to 15 of the *BRCA1* gene illustrates the diversity of large rearrangements and their contribution to the molecular pathology of the *BRCA1* gene. Few series of breast/ovarian cancer families have been systematically screened for large rearrangements of the *BRCA1* gene. The reported frequencies of *BRCA1* rearrangements range between 12% and 36%.[4 6 7 14] Even with a conservative estimate of 10%, it would be advisable to include a search for large rearrangements in *BRCA1* when analysing high risk breast/ovarian cancer families. The family reported here serves as a prime example of a case in which additional testing was warranted in the absence of a detectable point mutation with standard PCR methods. The prior probability of the index case being a *BRCA1/2* mutation carrier has been estimated at 95%. This value was obtained by using the MLINK program of the LINKAGE package, with the parameters of

the Claus segregation model modified by Easton and the estimated contributions of *BRCA1* and *BRCA2* mutations to breast/ovarian cancer predisposition.[1 19–21] In the absence of an identifiable mutation, closely related family members would have to be considered to be at high risk and would have to make decisions regarding cancer prevention on the basis of empirical data. With the identification of the familial *BRCA1* deletion, at risk family members can now consider testing for the identified familial mutation and can learn their mutation status with certainty.

The broad diversity of rearrangements, ranging from 0.5 to 23.8 kb and spread over the 81 kb of the *BRCA1* region, requires methods that allow for complete analysis of the gene. In this respect, colour bar coding on combed DNA appears useful. It allows for a panoramic view of the *BRCA1* region and for the detection of a rearrangement of about 6 kb (the size of a probe deleted or duplicated) at a glance. In addition, deletions and duplications as small as 2 kb can be detected with measurement of the probe signals.[16] Finally, more complex rearrangements involving inversions can also be detected. We think that software allowing for the automatic capture and analysis of signals would streamline the approach and, therefore, favour the use of colour bar coding on combed DNA. Searching for large gene rearrangements is a recurrent challenge for molecular geneticists. In addition to Southern blotting, other promising PCR based methods have recently been reported, including a long range PCR strategy and quantitative PCR.[11 22–24] Haploid conversion of human lymphocytes via a cell fusion strategy may be another alternative to these methods, as it allows for suppression of the normal allele, facilitating the detection of large rearrangements by standard PCR.[25] Comparative analysis of the different methods listed above, taking both sensitivity and cost into consideration, are now needed to improve genetic testing for breast and ovarian cancer predisposition.

We are indebted to the family for participating in this study. This work was supported by the Institut Curie "Programme Incitatif et Coopératif: Génétique et Biologie des Cancers du Sein". SG is supported by a fellowship from the MENRT. AA thanks the Ligue Nationale Contre le Cancer.

1 Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BAJ, Gayther SA, Birch JM, Lindblom A, Stoppa-Lyonnet D, Bignon Y, Borg A, Hamann U, Haites N, Scott RJ, Maugard CM, Vasen H, Seitz S, Cannon-Albright LA, Schofield A, Zelada-Hedman M, the Breast Cancer Linkage Consortium. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. *Am J Hum Genet* 1998;**62**:676-89.

2 Gayther SA, Ponder BA. Mutations of the *BRCA1* and *BRCA2* genes and the possibilities for predictive testing. *Mol Med Today* 1997;**3**:168-74.

3 Stoppa-Lyonnet D, Puig PL, Essioux L, Pages S, Ithier G, Ligot L, Fourquet A, Salmon RJ, Clough KB, Pouillard P, the Institut Curie Breast Cancer Group, Bonaiti-Pellie C, Thomas G. *BRCA1* sequence variations in 160 individuals referred to a breast/ovarian family cancer clinic. *Am J Hum Genet* 1997;**60**:1021-30.

4 Petrij-Bosch A, Peelen T, van Vliet M, van Eijk R, Olmer R, Drüsedau M, Hogervost FBL, Hageman S, Arts PJW, Ligtenberg MJL, Meijers-Heijboer H, Klijn JGM, Vasen HFA, Cornelisse CJ, van't Veer LJ, Bakker E, van Ommen GJB, Devilee P. *BRCA1* genomic deletions are major founder mutations in Dutch breast cancer patients. *Nat Genet* 1997;**17**:341-5.

5 Puget N, Torchard D, Serova-Sinilnikova O, Lynch HT, Feunteun J, Lenoir GM, Mazoyer S. A 1 kb Alu-mediated germ-line deletion removing *BRCA1* exon 17. *Cancer Res* 1997;**57**:828-31.

6 Puget N, Stoppa-Lyonnet D, Sinilnikova OM, Pages S, Lynch HT, Lenoir GM, Mazoyer S. Screening for germ-line rearrangements and regulatory mutations in *BRCA1* led to the identification of four new deletions. *Cancer Res* 1999;**59**:455-61.

7 Puget N, Sinilnikova OM, Stoppa-Lyonnet D, Audoynaud C, Pages S, Lynch HT, Goldgar D, Lenoir GM, Mazoyer S. An Alu-mediated 6 kb duplication in the *BRCA1* gene: a new founder mutation? *Am J Hum Genet* 1999;**64**:300-2.

8 Swensen J, Hoffman M, Skolnick MH, Neuhausen SL. Identification of a 14 kb deletion involving the promoter region of *BRCA1* in a breast cancer family. *Hum Mol Genet* 1997;**6**:1513-17.

9 Carson N, Gilpin C, Hunter A, Allanson J, Aubry H. An in-frame deletion of *BRCA1* exon 20 in a family with early-onset breast cancer and ovarian cancer. *Am J Hum Genet* 1999;**65**:A1610.

10 Montagna M, Santancatterina M, Torri A, Menin C, Zullato D, Chieco-Bianchi L, D'Andrea E. Identification of a 3 kb Alu-mediated *BRCA1* gene rearrangement in two breast/ovarian cancer families. *Oncogene* 1999;**18**:4160-5.

11 Payne SR, Newman B, King MC. Complex germline rearrangement of *BRCA1* associated with breast and ovarian cancer. *Genes Chrom Cancer* 2000;**29**:58-62.

12 Rohlfs EM, Yang Q, Skrzynia C, Graham ML, Silverman LM. Complex rearrangement in *BRCA1* results in the in-frame loss of exons in the BRCT domain. *Am J Hum Genet* 1999;**65**:A1792.

13 Rohlfs EM, Puget N, Graham ML, Weber BL, Garber JE, Skrzynia C, Halperin JL, Lenoir GM, Silverman LM, Mazoyer S. An Alu-mediated 7.1 kb deletion of *BRCA1* exons 8 and 9 in breast and ovarian cancer families that results in alternative splicing in exon 10. *Genes Chrom Cancer* 2000;**28**:300-7.

14 Unger MA, Nathanson KL, Calzone K, Antin-Ozerkis D, Shih HA, Martin AM, Lenoir GM, Mazoyer S, Weber BL. Screening for genomic rearrangements in families with breast and ovarian cancer identifies *BRCA1* mutations previously missed by conformation-sensitive gel eletrophoresis or sequencing. *Am J Hum Genet* 2000;**67**:841-50.

15 Smith TM, Lee MK, Szabo CI, Jerome N, McEuen M, Taylor M, Hood L, King MC. Complete genomic sequence and analysis of 117 kb of human DNA containing the gene *BRCA1*. *Genome Res* 1996;**6**:1029-49.

16 Gad S, Aurias A, Puget N, Mairal A, Schurra C, Montagna M, Pages S, Caux V, Mazoyer S, Bensimon A, Stoppa-Lyonnet D. Colour bar coding the *BRCA1* gene on combed DNA: a useful strategy for detecting large gene rearrangements. *Genes Chrom Cancer* (in press).

17 Bensimon A, Simon A, Chiffaudel A, Croquette V, Heslot F, Bensimon D. Alignment and sensitive detection of DNA by a moving interface. *Science* 1994;**265**:2096-8.

18 Michalet X, Ekong R, Fougerousse F, Rousseaux S, Schurra C, Hornigold N, van Slegtenhorst M, Wolfe J, Povey S, Beckmann JS, Bensimon A. Dynamic molecular combing: stretching the whole human genome for high-resolution studies. *Science* 1997;**277**:1518-23.

19 Easton DF, Bishop DT, Ford D, Crockford GP, Breast Cancer Linkage Consortium. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. *Am J Hum Genet* 1993;**52**:678-701.

20 Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. *Am J Hum Genet* 1991;**48**:232-42.

21 Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 1984;**36**:460-5.

22 Laurendeau I, Bahuau M, Vodovar N, Larramendy C, Olivi M, Bieche Y, Vidaud M, Vidaud D. TaqMan PCR-based gene dosage assay for predictive testing in individuals from a cancer family with INK4 locus haploinsufficiency. *Clin Chem* 1999;**45**:982-6.

23 Charbonnier F, Raux G, Wang Q, Drouot N, Cordier F, Limacher JM, Saurin JC, Puisieux A, Olschwang S, Frebourg T. Detection of exon deletions and duplications of the mismatch repair genes in hereditary nonpolyposis colorectal cancer families using multiplex polymerase chain reaction of short fluorescent fragments. *Cancer Res* 2000;**60**:2760-3.

24 Robinson MD, Chu CE, Turner G, Bishop DT, Taylor GR. Exon deletions and duplications in *BRCA1* detected by semiquantitative PCR. *Genet Test* 2000;**4**:49-54.

25 Yan H, Papadopoulos N, Marra G, Perrera C, Jiricny J, Boland CR, Lynch HT, Chadwick RB, de la Chapelle A, Berg K, Eshleman JR, Yuan W, Markovitz S, Laken SJ, Lengauer C, Kinzler KW, Vogelstein B. Conversion of diploidy to haploidy. *Nature* 2000;**403**:723-4.

26 Xu CF, Brown MA, Nicolai H, Chambers JA, Griffiths B, Solomon E. Isolation and characterization of the *NBR2* gene which lies head to head with the human *BRCA1* gene. *Hum Mol Genet* 1997;**6**:1057-62.

27 Barker DF, Liu X, Almeida ERA. The *BRCA1* and *1A1.3B* promoters are parallel elements of a genomic duplication at 17q21. *Genomics* 1996;**38**:215-22.

28 Brown MA, Xu CF, Nicolai H, Griffiths B, Chambers JA, Black D, Solomon E. The 5' end of the *BRCA1* gene lies within a duplicated region of human chromosome 17q21. *Oncogene* 1996;**12**:2507-13.

29 Campbell IG, Nicolai HM , Foulkes WD, Senger G, Stamp GW, Allan G, Boyer C, Jones K, Bast RC Jr, Solomon E, Trowsdale J, Black DM. A novel gene encoding a B-box protein within the *BRCA1* region at 17q21.1. *Hum Mol Genet* 1994;**3**:589-94.

30 Brown MA, Jones KA , Nicolai H, Bonjardim M, Black D, McFarlane R, De Jong P, Quirk JP, Lehrach H, Solomon E. Physical mapping, cloning, and identification of genes within a 500 kb region containing *BRCA1*. *Proc Natl Acad Sci USA* 1995;**92**:4362-6.

A unique form of autosomal dominant cataract explained by gene conversion between β-crystallin B2 and its pseudogene

Vanita, Virinder Sarhadi, André Reis, Martin Jung, Daljit Singh, Karl Sperling, Jai Rup Singh, Joachim Bürger

J Med Genet 2001;**38**:392–396

Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar, India
Vanita
V Sarhadi
J R Singh

Gene Mapping Centre, Max-Delbrück-Centre, Berlin, Germany
A Reis
M Jung

Institute of Human Genetics, Charité, Humboldt-Universität zu Berlin, Germany
A Reis
K Sperling
J Bürger

Dr Daljit Singh Eye Hospital, Amritsar, India
D Singh

Correspondence to:
Dr Bürger, Institut für Humangenetik, Charité, Augustenburger Platz 1, 13353 Berlin, Germany, joachim.buerger@charite.de or Prof Dr Singh, Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar 143005, India, jairup@vsnl.com jairup@hotmail.com

EDITOR—Using linkage analysis, a large Indian family with autosomal dominant sutural cataract and cerulean opacities was mapped to chromosome 22 and two cosegregating sequence changes (475C→T and 483C→T) were identified in the *CRYBB2* gene. The first was previously described in two genetically unrelated families with other inherited forms of cataract. The two sequence alterations are identical to the sequence of the *CRYBP1* pseudogene that is 228 kb apart. Furthermore, the pseudogene-like fragment within the *CRYBB2* gene is flanked by chromosomal junction sequences. Therefore, we conclude that gene conversion is the most likely mechanism leading to this mutation. Alternatively, dual point mutation would explain our findings. In addition, since the three families with Q155X mutations all show different types of cataract, we conclude that mutant *CRYBB2* causes cataract formation but other modifying factors determine the type of cataract.

Autosomal dominant congenital cataract (ADCC) is a clinically and genetically heterogeneous group of disorders that cause blindness. More than 13 independent loci have been mapped, and 10 different genes identified so far. Five of them are crystallin genes that are categorised into the α, β, γ, μ, and ζ subgroups. The crystallins constitute the main lens proteins, whereby β-crystallin B2 is the only abundant protein in the adult lens fibre in man.[1 2] Causative mutations have been recognised in the α-crystallin A gene (zonular central nuclear cataract),[3] the β-crystallin A3/A1 gene (zonular cataracts with sutural opacities),[4] the γ-crystallin C gene (Coppock-like cataract),[5] and the γ-crystallin D gene (progressive juvenile onset punctate cataract).[6] These and all other ADCC mutations identified so far are private mutations, with one exception. Litt *et al*[7] described a nonsense mutation, Q155X, in the β-crystallin B2 gene leading to cerulean cataract. Exactly the same mutation was identified by Gill *et al*[8] in familial Coppock-like cataract. Here, we report the identical mutation in a large Indian family exhibiting sutural cataract with punctate and cerulean opacities. In addition, we present evidence that this mutation in the β-crystallin B2 gene is an independent event and most likely the result of gene conversion.

Identification and characterisation of the mutation

After obtaining informed consent, we performed linkage analysis in an Indian five generation family with 33 affected members, based on semi-automated genotyping with microsatellite markers from the Généthon linkage map; 48 members of this family, 25 of them affected, were selected for mapping (fig 1A). Assuming autosomal dominant inheritance with full penetrance and equal allele frequencies for each marker and using the LINKAGE program package, we calculated two point lod scores. After having excluded the autosomal dominant cataract loci on chromosomes 1, 2, 12, 13, 14, 16, 17, and 19, we detected linkage in our family to marker D22S315, with a lod score of Zmax = +8.500 at θmax = 0.05.

This region on chromosome 22 harbours four β-crystallin genes, *CRYBA4*, *CRYBB1*, *CRYBB2*, *CRYBB3*, and the pseudogene *CRYBP1*.[9] We amplified the translated exons 2-6 of the *CRYBB2* gene by PCR as described previously.[7] In addition, we designed primer sets for maximum discrimination between gene and pseudogene sequences: CRYBLg (5'-TGACCTTGTAGCTGGGCTTG-3'), CRYBLpsg (5'-TGACTTTGCAGCCAGGCTTG-3'), 596rg (5'-CACTGCATGTCGCGGATACG-3'), 596rpsg (5'-CCCTGCATGTCGTGGATGCA-3'). PCR products were purified with a Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced directly using the Big-Dye-Terminator Cycle Sequencing Kit (PE Biosystems, Weiterstadt, Germany). Sequencing reactions were purified with a Dye-Ex Kit (Qiagen, Hilden, Germany) and run and analysed on ABI 310 and 377 sequencers (PE Biosystems, Weiterstadt, Germany).

Sequencing of exon 6 showed a C→T mutation at nucleotide position 475 (Q155X). This stop mutation truncates the protein by 51 residues and has previously been described.[7 8] However, our sequencing of exon 6 showed an additional variant, a C→T substitution at nucleotide position 483. This silent polymorphism was found exclusively in patients. Since