

# Evidence for Common Ancestral Origin of a Recurring *BRCA1* Genomic Rearrangement Identified in High-Risk Hispanic Families

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## Abstract

**Background:** Large rearrangements account for 8% to 15% of deleterious *BRCA* mutations, although none have been characterized previously in individuals of Mexican ancestry. **Methods:** DNA from 106 Hispanic patients without an identifiable *BRCA* mutation by exonic sequence analysis was subjected to multiplexed quantitative differential PCR. One case of Native American and African American ancestry was identified via multiplex ligation-dependent probe amplification. Long-range PCR was used to confirm deletion events and to clone and sequence genomic breakpoints. Splicing patterns were derived by sequencing cDNA from reverse transcription-PCR of lymphoblastoid cell line RNA. Haplotype analysis was conducted for recurrent mutations.

**Results:** The same deletion of *BRCA1* exons 9 through 12 was identified in five unrelated families. Long-range PCR and

sequencing indicated a deletion event of 14.7 kb. A 3-primer PCR assay was designed based on the deletion breakpoints, identified within an *AluSp* element in intron 8 and an *AluSx* element in intron 12. Haplotype analysis confirmed common ancestry. Analysis of cDNA showed direct splicing of exons 8 to 13, resulting in a frameshift mutation and predicted truncation of the *BRCA1* protein.

**Conclusions:** We identified and characterized a novel large *BRCA1* deletion in five unrelated families—four of Mexican ancestry and one of African and Native American ancestry, suggesting the possibility of founder effect of Amerindian or Mestizo origin. This *BRCA1* rearrangement was detected in 3.8% (4 of 106) of *BRCA* sequence-negative Hispanic families. An assay for this mutation should be considered for sequence-negative high-risk Hispanic patients. (Cancer Epidemiol Biomarkers Prev 2007;16(8):1615–20)

## Introduction

Large genomic rearrangement mutations (deletions or duplications of one or more exons) are not detectable by sequence-based genotyping and are estimated to account for 8% to 15% of all deleterious mutations in the *BRCA1* and *BRCA2* genes (1, 2). Most *BRCA* rearrangements are unique; however, some *BRCA1* rearrangements show apparent founder effect in European and American populations, five of which are included in the commercial assay in the United States (3–6).

The majority of *BRCA1* rearrangements are believed to result from recombination of *Alu* repeats, which make up ~41.5% of the *BRCA1* gene (3, 7, 8).

Most genetic epidemiology studies of the *BRCA* genes have been conducted in predominantly Caucasian cohorts (9–11). The few studies involving other ethnic groups have focused primarily on African Americans (12–14). We recently reported

on a study of the prevalence of *BRCA* mutations in a Hispanic high-risk clinic population residing in an urban center in the United States, with deleterious *BRCA* mutations detected in 31% of 110 unrelated families (15).

To our knowledge, there are no published studies on the prevalence of *BRCA* rearrangements among individuals of Hispanic<sup>4</sup> descent residing in the United States. The purpose of this study was to identify rearrangements in the *BRCA* genes in a cohort of high-risk patients of predominantly Mexican ancestry.

## Materials and Methods

**Sample.** Individuals of Hispanic descent with a personal or family history of breast and/or ovarian cancer were enrolled in an Institutional Review Board–approved prospective Hereditary Cancer Registry and underwent genetic cancer risk assessment and *BRCA* testing between 1998 and 2006. Information about family history for the individuals in this study was obtained by an experienced genetic counselor in a 2-h face to face interview. Histories were often confirmed or expanded by contact with select relatives. Demographic data, including country of origin and a three- to five-generation pedigree, were obtained. Individuals of Hispanic descent included those of Spanish, Mexican, Central and South American, Cuban, or Puerto Rican origin. Participants with

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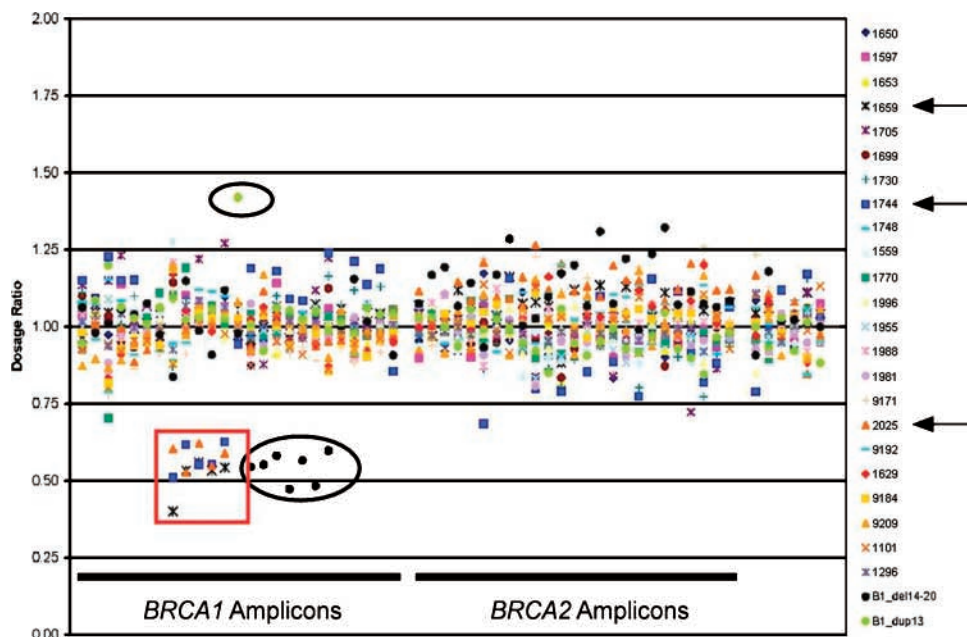
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<sup>4</sup> Although we use “Hispanic,” the most common census term for individuals of Mexican, Central and South American, Cuban, or Puerto Rican descent, referring to “ethnicity,” is “Latino.” Latino is generally considered a more ethnically/culturally based term for individuals of the aforementioned groups.



**Figure 1.** Gene copy number analysis by multiplexed quantitative differential PCR for *BRCA1* and *BRCA2* for 23 DNA specimens obtained from patients of Hispanic ancestry. Three samples (1659, 1744, 2025) revealed a deletion of exons 9 to 12 in *BRCA1* (boxed in the graph, arrows in the graph legend). A dosage ratio of 0.8 to 1.25 indicates normal copy number (two alleles); a value above 1.3 indicates gain of one allele; and a value less than 0.7 indicates a loss of one allele. Each gene has been arranged 5' to 3'. Control DNAs are encircled (*BRCA1* duplication exon 13; *BRCA1* deletion exons 14-20). The elevated ratios of the *BRCA2* exons for the del 14-20 control and positive patient samples is a normalization echo created by using the two genes as dosage controls for each other in the multiplexes.

Hispanic ancestry only on one parental side were eligible if that side was significant for a history of breast or ovarian cancer.

Probabilities of carrying a mutation in the *BRCA1* or *BRCA2* genes were estimated using the Couch model, the Myriad model, and BRCAPRO (16-18). The Couch model estimate, which calculates the probability of a *BRCA1* mutation, was modified by a factor of 1.33 to account for *BRCA2* (19).

DNA from 106 patients—76 without an identifiable mutation in the original cohort of 110 unrelated high-risk Hispanic families (15), and an additional 30 sequence-negative high-risk Hispanic families recruited in 2006—was screened for large *BRCA* rearrangements (see below). Genomic DNA was isolated from peripheral blood leukocytes using the FlexiGene DNA kit (Qiagen).

**Large Rearrangement Screening.** Multiplexed quantitative differential PCR was used to assess gene dosage of all *BRCA1* and *BRCA2* exons, and regions immediately 5', including the promoter, and 3' of the genes through Myriad Genetic Laboratories Clinical Development Division (20). Based on the technology of fluorescent quantitative multiplexed PCR (21, 22), a number of design improvements were implemented to achieve high levels of sensitivity and specificity (23). A total of 11 PCR multiplexes are used to assess both *BRCA1* and *BRCA2* copy number. Each region is represented by a minimum of two unique amplicons. Each of these amplicons and those for adjacent exons are amplified in different multiplexes. This approach allows mutations to be observed using multiple data points from multiple reactions, reducing false-positive events that are commonly seen in single reaction quantitative assays. G/C-specific PCR chemistry was used for the G/C-rich sequence at the 5' region of the genes to achieve improved amplification reproducibility. All multiplexes were designed as a combination of *BRCA1* and *BRCA2* and each gene acts as a dosage control for the other. To control for complete loss of either *BRCA1* or *BRCA2* in a specimen, amplicons from highly conserved genes found in regions that are haplolethal or triplolethal (24) were included in each multiplex. Finally, wherever possible, coding region primers for this assay reside within the sequencing amplicons for the commercial BRACAnalysis test, allowing for easy resolution of false positives caused by primer binding site polymorphisms. Samples were assayed in

sets of 30 with two positive controls (one duplication and one deletion mutation).

Dosage quotient values for each region were calculated for each sample. This was accomplished by converting the amplitude of each peak to a ratio of itself against the average peak height of the *BRCA2* amplicons when analyzing *BRCA1*, and the average of the *BRCA1* amplicons when analyzing *BRCA2*. Next, the average ratio for each amplicon across the sample set was calculated and used to normalize the values from the first step. These data were used to generate a scatterplot where the value 1.0 represents normal gene copy number, 0.5 represents deletions (loss of one allele), and 1.5 represents duplication mutations (gain of one allele; Fig. 1). Deletion events were called for data points below 0.7; specimens were considered to have normal copy number at 0.8 to 1.25; and duplications were called for data points over 1.3. All mutation-positive samples and specimens with data points outside the call thresholds were repeated, and positive results were confirmed by using long-range PCR.

**Rearrangement Breakpoint Characterization.** After a deletion of *BRCA1* exons 9 to 12 [*BRCA1* del (ex 9-12)] was identified by multiplexed quantitative differential PCR in three unrelated families, primers were designed closely flanking the rearrangement, and ~50 to 100 ng of genomic DNA from each case were PCR amplified using long-range conditions and a touchdown program to generate a 2.7-kb fragment, including the breakpoint for sequencing. The PCR products were then extracted and purified using Qiagen QIAquick Gel Extraction kit (Qiagen) protocol, sequenced by cycle sequencing using Applied Biosystems BigDye Terminator v3.1 sequencing kit (Applied Biosystems) and electrophoresis on an ABI 3730 sequencer (Applied Biosystems), and analyzed with GeneMapper software (Version 4.0, Applied Biosystems). Comparison with published genomic *BRCA1* sequences [National Center for Biotechnology Information (NCBI) accession no. L78833] was done to determine the breakpoint region. RepeatMasker v3.1.X (A.F.A. Smit, R. Hubley, and P. Green),<sup>5</sup> a program that screens DNA sequences for interspersed repeats, was used to identify *Alu* elements at the breakpoints.

<sup>5</sup> <http://repeatmasker.org>

A 3-primer PCR assay was designed, based on the intronic deletion breakpoints, and results in coamplification of the mutant allele 742 bp breakpoint fusion product and a 1,145 bp wild-type allele product (Fig. 2). Briefly, ~20 bp amplimers with similar predicted  $T_m$  values were selected and tested under varying PCR conditions until a protocol was derived that reliably generated wild-type and breakpoint mutant products with a 10-fold range of input DNA. This assay was subsequently used to confirm the rearrangement positive cases, including the mother of patient 2025 (she was also used to establish phase in the haplotyping experiments, below).

**Characterization of RNA Splicing and Lost Functional Domains.** RNA was extracted with TRIzol reagent (Invitrogen) from a lymphoblastoid cell line (created from rearrangement positive case 1659) subjected to reverse transcription-PCR with combinations of primers flanking exons 2 to 15 and the resulting cDNA products were sequenced. A sequence file representing the observed predominant RNA species, direct splicing of exons 8 to 13, was generated and subjected to open reading frame analysis using MacVector (v6.5.3, Oxford Molecular Group 1999). The literature was reviewed for putative *BRCA1* functional domains to assess the effect of a deletion of exons 9 through 12.

**Haplotype Analysis for Recurrent Mutations.** Seven short tandem repeat markers were genotyped across the *BRCA1* locus for all four patients that were found to be carriers of the *BRCA1* del (ex 9-12) mutation, the mother of patient 2025 (to establish phase), and for a fifth case identified by multiplex ligation-dependent probe amplification (25) from a separate high-risk cohort study. The approach generally followed that of Neuhausen et al. (26, 27). Standard PCR protocols were used. Primer sequences for chromosome 17 (D17S1320, D17S1321, D17S1322, D17S1323, D17S1327, and D17S1325) microsatellite markers were obtained from the Genome

Database.<sup>6</sup> For map pair D17S855 (*BRCA1*), novel primers were designed (F: 5'-TGCCATTTCTTTTCACTCTGG/R: 5'-GCAAATAACTTGGGTATCACTTAAAA) to facilitate multiplex analysis of samples. All synthesis and labeling of primers with fluorescein derivatives (5'-6-FAM, 5'-TET, or 5'-HEX) was provided by Integrated DNA Technologies. PCR amplification of 50 ng of genomic DNA in 1× reaction buffer, with 200 μmol/L deoxynucleotide triphosphates, 0.4 μmol/L primers, 1.5 mmol/L MgCl<sub>2</sub>, and 0.625 unit of Taq DNA Polymerase (Qiagen) was used to amplify microsatellite markers (D17S855, D17S1320, D17S1323, D17S1325, and D17S1327) according to standard cycling protocols. Amplification of D17S1321 and D17S1322 was done using 50 ng of genomic DNA in 1× reaction buffer, with 200 μmol/L deoxynucleotide triphosphates, 0.3 μmol/L primers, 1.5 to 3.5 mmol/L MgCl<sub>2</sub>, and 1.25 units of AmpliTaq Gold under a modified cycling program (95°C 10 min, 35 cycles of 94°C 30 s, 59°C or 62°C 45 s, 72°C 30 s, followed by 72°C 60 min). Following amplification, all PCR products were size separated on a 3130 xl capillary Genetic Analyzer (Applied Biosystems) using a 3130 POP-7 polymer matrix (Applied Biosystems). ROX-labeled GeneScan 500 (Applied Biosystems) was used as a size standard in each lane. Data output was analyzed with GeneMapper software (Version 4.0, Applied Biosystems) to determine fragment lengths.

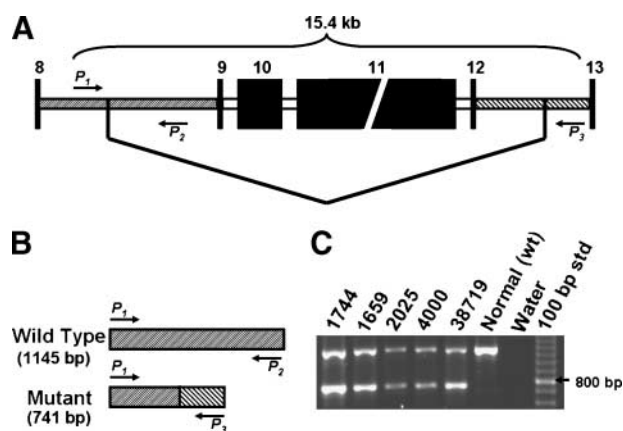
**Data Analysis.** Statistical analyses of demographic and clinical data were conducted using the Statistical Package for the Social Sciences software, version 11.0 (copyright 2001 by SPSS, Inc.). Means and SDs for continuous data or proportions for categorical data were generated.

## Results

The majority of subjects were female (99%) and had a history of invasive cancer: 91 (85.8%) had breast cancer only, 3 (2.8%) had both breast and ovarian cancer, and 4 (3.8%) had ovarian cancer only (Table 1). Three (2.8%) subjects had a history of ductal carcinoma *in situ*. Average age of first breast cancer diagnosis was 37.8 years (SD ± 7.8, range 23-66 years). Reported ancestry for both parental lineages included 70 (66.0%) Mexican, 14 (13.2%) Central American, 3 (2.8%) South American, 2 (1.9%) Spanish, and 17 (16.0%) of mixed country of origin (Table 1).

Multiplexed quantitative differential PCR analysis identified a *BRCA1* del (ex 9-12), indicated by a 50% loss of signal in those exons, in 4 of 106 (3.8%) unrelated families, and a fifth case was identified by multiplex ligation-dependent probe amplification in a separate high-risk cohort study. Long-range PCR resulted in the generation of a 2.7-kb product in these samples, consistent with a deletion event of 14.7 kb. Subsequent sequence analysis of the 2.7-kb product identified the breakpoints within an *AluSp* element in intron 8 and an *AluSx* element in intron 12 (Fig. 3). The rearrangement breakpoints were determined to be located between nucleotides 29,624 and 29,702 in intron 8 and nucleotides 44,280 and 44,358 in intron 12 (National Center for Biotechnology Information [NCBI] accession no. L78833). All five unrelated families were found to have the same rearrangement.

Sequenced cDNA showed that the predominant mRNA species was a direct splice of exons 8 to 13, and is predicted to result in an in-frame stop codon (Fig. 4). No significant alternate splicing patterns were evident. Consequently, the *BRCA1* del (ex 9-12) mutation results in both the loss of multiple functional domains in the deleted segment and premature truncation, thus strongly suggesting that this is a deleterious mutation.



**Figure 2.** Diagram of the 3-primer *BRCA1* del (ex 9-12) assay. **A**, genomic structure of breakpoint region with numbered exons. *Solid blocks*, *BRCA1* exons 8 to 13; *close crosshatch bar*, intron 8; *distinct crosshatch patterns*, introns involved in rearrangement; *open bars*, introns 9 to 11. *Bracket below the diagram*, positions of the respective breakpoints. Primer positions for the specific rearrangement assay ( $P_1$ ,  $P_2$ ,  $P_3$ ) are indicated along with respective orientation. Although not detectable in assay, predicted product for  $P_1$  and  $P_3$  (15.4 kb) wild-type allele is indicated by parentheses above diagram. **B**, depiction of the wild-type ( $P_1$  and  $P_2$ ) and rearrangement mutation-specific ( $P_1$  and  $P_3$ ) PCR products. **C**, photograph of ethidium bromide-stained agarose gel with cases (*first five lanes*) and controls (*wt*, *water*). Size standard indicated on right. Clean bands of 1,145 and 742 bp represent the wild-type and mutant alleles in positive cases (*lanes 1-5*), while the wild-type control (*lane 6*) shows only the wild-type allele.

<sup>6</sup> <http://www.gdb.org>

**Table 1. Demographics and clinical characteristics (N = 106)**

Variable	BRCA1 del (ex 9-12) mutation status	
	Carrier	Noncarrier
n (%)	4 (3.8%)	102 (96.2%)
Gender		
Male	0	1 (0.1%)
Female	4 (100%)	101 (99.0%)
Cancer status		
Unaffected (%)	0	5 (4.9%)
Affected with BC and/or OC (%)	4 (100%)	97 (95.1%)
BC (%)	3 (75.0%)	77 (79.4%)
Bilateral BC	1 (25.0%)	10 (10.3%)
OC (%)	0	4 (4.1%)
BC and OC (%)	0	3 (3.1%)
DCIS (%)	0	3 (3.1%)
Average age at first BC diagnosis ± SD	37.0 ± 3.6	37.9 ± 8.0
Probands' parental country(ies) of origin*		
No. Mexican (%)	3 (75.0%)	66 (64.7%)
No. Central American/Caribbean (%)	0	14 (13.7%)
No. South American (%)	0	3 (2.9%)
No. Spanish (%)	0	2 (2.0%)
No. mixed† (%)	1 (25.0%)	17 (16.7%)

Abbreviations: BC, breast cancer; OC, ovarian cancer; DCIS, ductal carcinoma *in situ*.

\*Applies to both parental lineages; except those with mixed ancestry indicated in the last category.

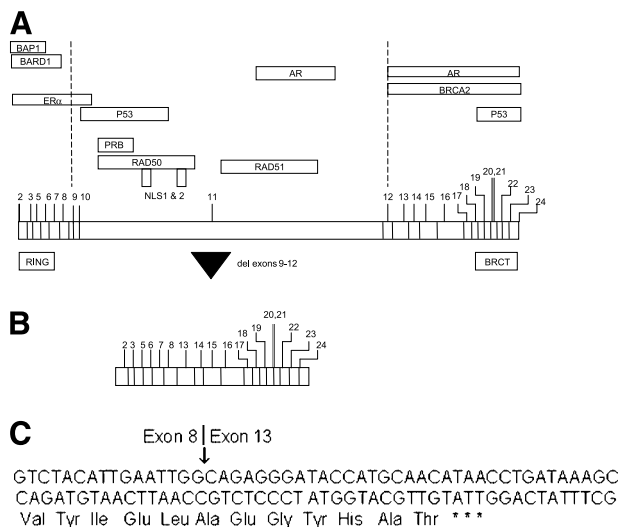
† "Mixed" means a combination of above or combination of Hispanic and non-Hispanic ancestry.

The *BRCA1* del (ex 9-12) haplotype was determined from the mother-daughter pair (samples 2025-14 and 2025-1, respectively) and all six (at \*6 markers from D17S1320 through the *BRCA1* locus, to D17S1325; Fig. 5) individuals carrying the mutation carried the alleles associated with the haplotype\*, suggesting common ancestry. Marker D17S1323 is deleted in the mutant allele.

All four *BRCA1* del (ex 9-12) cases detected by multiplexed quantitative differential PCR were affected with breast cancer, with an average age at first breast cancer diagnosis of 37.0 years (SD ± 3.6, range 33-40 years). Multigenerational pedigrees are depicted in Fig. 6. The mean pretest *BRCA* mutation probability for all three models combined was 25.2% (SD ± 15.4%, range 9.9-38.9%) for these four cases.

**INTRON 8** *AluSp*  
 GAAGCCTTTAAGAAGAGCTGAATTGCCAGGCGCAGTGGCTCACGCCTGT  
AATCCACGACTTTGGGAGGCCGAGGCGGGCGGATCACCTGAGGTCGGGA  
GTTCAGACCGCTGACAACATGGTGAACCCCGTCTCTACTAAAAAT  
ACAAAAATTAGGCCAGGTGTGGTGGCTCACGCCTG  
*AluSx* **INTRON 12**

**Figure 3.** *BRCA1* deletion exon 9 to 12 breakpoint localized within *AluSp* sequence in intron 8 and *AluSx* sequence in intron 12. The sequence of the 2.7-kb product spanning the breakpoint was determined and mapped against *BRCA1* genomic sequence [National Center for Biotechnology Information (NCBI) accession no. L78833] by RepeatMasker v3.1.X software (A.F.A. Smit, R. Hubley and P. Green RepeatMasker at <http://repeatmasker.org>). The breakpoint is depicted as a consensus block of identity; thus, the precise point of recombination and which respective *Alu* element contributed to that cannot be determined. The intronic breakpoint region is depicted, with unique intron 8 sequence leading to the specific *AluSp* (single underline) element, adjacent to the consensus block (boxed), and the 3' *AluSx* element (double underline) in intron 12 sequence.

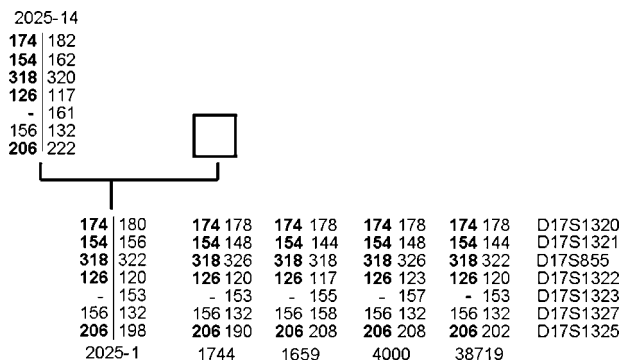


**Figure 4.** *BRCA1* protein functional domains and predicted frameshift and premature truncation. **A**, adapted from Welch et al., 2000 (42): Translated *BRCA1* exons and distribution of known/putative functional domains for *BRCA1* protein. **B**, depiction of truncated coding sequence resulting from deletion of exons 9 to 12. **C**, predicted frameshift and premature stop codon that would result from direct splicing of the remaining exons (8 joined to 13), presuming no use of cryptic or alternate splice sites.

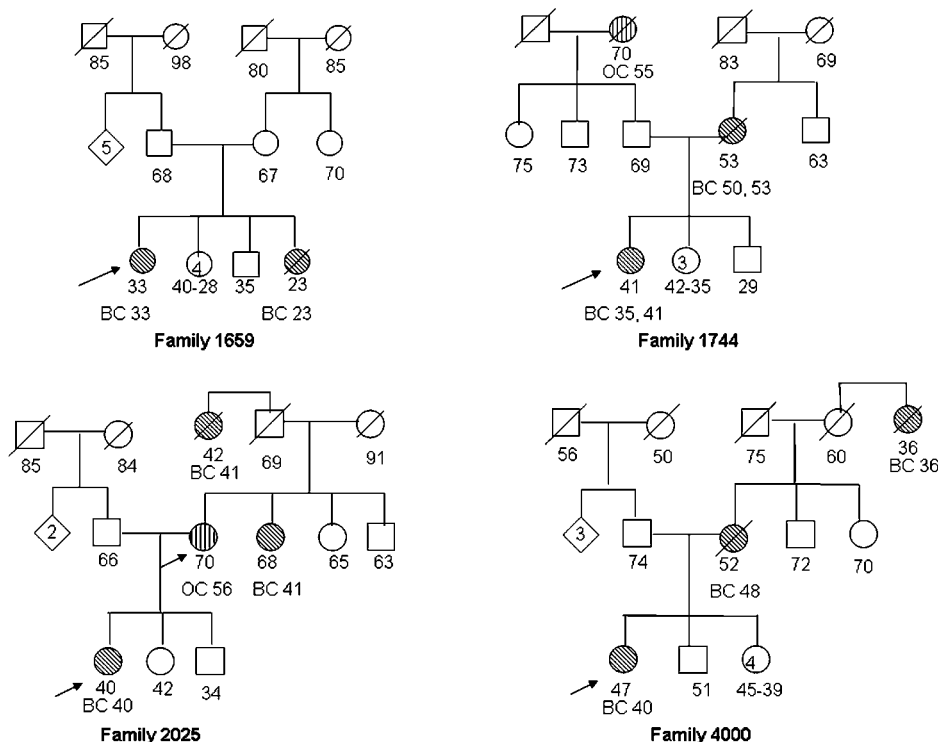
## Discussion

The same *BRCA1* del (ex 9-12) mutation was observed in 3.8% (4 of 106) of unrelated Hispanic families in a high-risk clinic cohort. We previously reported on 34 sequence-detected *BRCA* mutations in a cohort of 110 high-risk Hispanic families (15). Adding the three *BRCA1* del (ex 9-12) mutations found among the sequence negative cases brings the overall prevalence of *BRCA* mutations in the cohort to 34% (37 of 110)—the large rearrangement representing 8.8% (3 of 37) of all *BRCA* carriers. This proportion of rearrangements among *BRCA1*-positive individuals is consistent with previous studies in European and American populations (3, 4, 6, 28-31).

A recent study of 384 Spanish families with breast and ovarian cancer cases, residing in Spain, found that *BRCA1* large rearrangements accounted for 8.2% of the *BRCA1* mutations



**Figure 5.** *BRCA1* del (ex 9-12) haplotype. The haplotype was determined from the mother (2025-14) and daughter (2025-1) pair (*in bold type*). No determination was made for marker D17S1327 as the genotype is equivalent in both mother and daughter. *Dash*, deleted marker D17S1323 in the mutant allele. For the other four cases, a genotype was established. All the individuals carrying the mutation carried the alleles associated with the haplotype, suggesting that this is a founder mutation.



**Figure 6.** Pedigrees for four families with the *BRCA1* del (ex 9-12) mutation. Squares, males; circles, females. Age at cancer risk assessment or death is provided below each individual; deceased individuals are indicated by a hatch mark and age at death. The arrow by the filled circle indicates the tested individual; age at breast cancer (BC) or ovarian cancer (OC) onset is shown.

identified (6). Five of eight *BRCA1* rearrangements (five deletions, two duplications, and one amplification) identified in this cohort were novel; one has been reported to be a French founder mutation (deletion of exons 8-13; ref. 32) and two others (deletion of exons 11-15 and duplication of exon 20) were also reported in Portuguese (31) and Italian cohorts (28). However, the *BRCA1* del (9-12) mutation has not been reported in any other study population to date. Although Walsh et al. (4) detected 36 large *BRCA* rearrangements among 300 sequence-negative high-risk families, there were only 15 Hispanics in the cohort and none were found to carry a *BRCA* rearrangement.

Data from several studies has led to the hypothesis that the majority of rearrangements within the *BRCA* genes are due to *Alu* repeats that are susceptible to recombination (3, 7). The identification of the breakpoints within *Alu* elements Sp and Sx supports the hypothesis that the *BRCA1* del (ex 9-12) mutation is due to an *Alu* repeat-mediated recombination event.

Based on our analyses, the *BRCA1* del (ex 9-12) mutation is likely deleterious, resulting in loss of critical functional domains as well as premature truncation of the *BRCA1* protein (Fig. 4). The rearrangement results in the complete loss of the p53, pRB, Rad50, Rad51, NLS1, and NLS2 interacting domains and partial loss of the ER $\alpha$  domain.

For the four *BRCA1* del (ex 9-12) mutation families reporting Mexican ancestry, all were unrelated through at least a three- to five-generation pedigree and shared the microsatellite alleles associated with the haplotype, suggesting the possibility of founder effect (Fig. 6). Marker D17S1320 is located ~500 kbp centromeric to *BRCA1*, and D17S1325 is located ~350 kbp telomeric to *BRCA1*—the entire segment is within the 17q21 region. The extent of identity beyond these markers (traditionally used in most *BRCA* haplotyping studies) is unknown. The region of origin within Mexico was known for two of the four rearrangement families; family 1653 originated in Zacatecas, Mexico, whereas family 1744 originated in Chihuahua, Mexico. Interestingly, the *BRCA1* del (ex 9-12) case identified via multiplex ligation-dependent probe amplification in a separate cohort (from Texas) shares the same genotype but is of African American and Native American ancestry. In addition, the *BRCA1* del (ex 9-12)

mutation was recently seen in a Mexican-American family residing in Laredo, Texas.<sup>7</sup> Thus, the families appeared distinct for at least four generations and were also somewhat geographically dispersed. Although we determined that the recurrent *BRCA1* 185delAG mutations in our Hispanic cohort occurred on the Jewish haplotype, and are observed primarily in colonial Hispanics (15, 33), based on the family histories, haplotype data and finding the mutation in an African American/Native American woman, we speculate that *BRCA1* del (ex 9-12) may have originated in a Mestizo or Amerindian population.

The population of Mexico is 60% Mestizo, 30% Amerindian, 9% White, and 1% other (34). Mexican Mestizos are the result of admixture between Spanish, Amerindian, and African populations, whereas those of Amerindian ancestry are descendants of indigenous populations, believed to have come from Asia between 12,000 and 30,000 years ago, that inhabited Mexico before Spanish colonization (35, 36). African ancestry in the Mexican population is believed to have derived from African slavery in Mexico during the 16th to 18th centuries (37).

Studies of the genetic admixture in Mexico have found that various regions have different compositions. In Zacatecas, Spanish genes comprise the greatest proportion (60%) of the population, whereas Amerindian and African genes make up 37% and 3% of the gene pool, respectively (38). Conversely, the composition in Chihuahua was found to be 91% Native American, 5% African, and 3% Spanish (37). The latter study was of mitochondrial DNA haplotypes and the lower frequency of European haplotypes was thought to have been because mitochondrial DNA is maternally inherited and historically more Spanish males than Spanish females colonized Mexico. Our observation of the *BRCA1* del (ex 9-12) mutation in five families of Mexican ancestry and an African American/Native American woman, and its absence in a recent Spanish *BRCA1* rearrangement study (6), support the hypothesis that this mutation may be of Mestizo or Amerindian origin, although additional studies in native Mexican populations would be helpful to define the prevalence and origin of the mutation. The

<sup>7</sup> G.W. Unzeitig, personal communication.

origins of this rearrangement may also be clarified by studying ancestral informative markers (39, 40).

Commercial *BRCA* rearrangement testing using the multiplexed quantitative differential PCR method (BRACAnalysis Rearrangement Testing; Myriad Genetics Laboratory, Salt Lake City, UT) became available in August of 2006, and the assay is included in the cost of BRACAnalysis if the family meets certain high-risk criteria (generally approximating a 30% *a priori* mutation probability). Only two of the five patients with a rearrangement in this study would have met the selection criteria for BRACAnalysis Rearrangement Testing.

The overall prevalence of deleterious *BRCA* mutations was 33.6% (37 of 110) in our well-characterized [sequencing (15) followed by rearrangement analysis in all uninformative cases (current study)] cohort of 110 Hispanic high-risk clinic families. Although the incidence of breast cancer in Hispanic women is less than that for non-Hispanic White women (41), our results suggest that *BRCA* mutations may account for a higher proportion of the breast cancers in young Hispanic women, analogous to the situation for Ashkenazim. Studies are ongoing to more precisely determine the prevalence of this mutation in population-based Hispanic cohorts and in additional clinic based cohorts of Hispanic women with breast cancer. However, given the apparent founder effect and relatively high prevalence we observed in our high-risk clinic population to date, it would be worthwhile to consider an assay that detects the *BRCA1* del (ex 9-12) mutation for sequence-negative Hispanic patients.

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## Evidence for Common Ancestral Origin of a Recurring *BRCA1* Genomic Rearrangement Identified in High-Risk Hispanic Families

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