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Report

The Exon 13 Duplication in the *BRCA1* Gene Is a Founder Mutation Present in Geographically Diverse Populations

The *BRCA1* Exon 13 Duplication Screening Group*

Recently, a 6-kb duplication of exon 13, which creates a frameshift in the coding sequence of the *BRCA1* gene, has been described in three unrelated U.S. families of European ancestry and in one Portuguese family. Here, our goal was to estimate the frequency and geographic diversity of carriers of this duplication. To do this, a collaborative screening study was set up that involved 39 institutions from 19 countries and included 3,580 unrelated individuals with a family history of the disease and 934 early-onset breast and/or ovarian cancer cases. A total of 11 additional families carrying this mutation were identified in Australia (1), Belgium (1), Canada (1), Great Britain (6), and the United States (2). Haplotyping showed that they are likely to derive from a common ancestor, possibly of northern British origin. Our results demonstrate that it is strongly advisable, for laboratories carrying out screening either in English-speaking countries or in countries with historical links with Britain, to include within their *BRCA1* screening protocols the polymerase chain reaction–based assay described in this report.

Methods used to screen for mutations in the *BRCA1* gene (MIM 113705) focus mainly on genomic DNA, and, being PCR based, they do not enable the detection of large DNA rearrangements. This may explain why only 12 large germline insertions or deletions have been described (Petrij-Bosch et al. 1997; Puget et al. 1997, 1999*a*, 1999*b;* Swensen et al. 1997; Montagna et al. 1999; Rohlfs et al. 2000), compared with ~400 point mutations, small insertions, and deletions scattered across the whole coding sequence and over the splice junctions (Breast Cancer Information Core). However, in two independent studies performed on Dutch (Petrij-Bosch et al. 1997) and U.S. (Puget et al. 1999*a*) families with breast and/or ovarian cancer, rearrangements have been found to represent 36% and 15% of all mutations, respectively.

Recently, a 6-kb duplication of exon 13, ins6kbEx13, which creates a frameshift in the coding sequence, has been identified in the *BRCA1* gene (Puget et al. 1999*b*). It was initially found in three, apparently unrelated, U.S. families of European ancestry and in one Portuguese family. Haplotype data suggested a common founder for all these families.

To estimate the frequency and geographic diversity of carriers of this duplication, which will not be identified if not specifically sought, a collaborative study was set up. This involved 39 institutions in which the screening for ins6kbEx13 was done by PCR (fig. 1) using specific primers and a positive control. A total of 3,580 unrelated individuals with a family history of breast and/or ovarian cancer who were from 19 different countries (Australia, Austria, Belgium, Canada, Finland, France, Germany, Great Britain, Hungary, Ireland, Israel, Italy, the Netherlands, Norway, Spain, Sweden, Switzerland, Turkey, and the United States) were screened. To be enrolled, the family histories had to fulfill at least one of the following criteria: (1) at least three cases of female breast and/or ovarian cancer; (2) at least one case of female breast cancer and one case of male breast cancer; (3) one case of female breast cancer diagnosed at age <50 years and one case of ovarian or female breast cancer; (4) one case of female breast cancer and one case of ovarian cancer in first-degree relatives; (5) one case of female breast cancer and one case of female bilateral breast cancer; or (6) two cases of ovarian cancer. This heterogeneous sample is representative of the diversity of patients who attend clinics for individuals at high risk for cancer. At the time of the analysis, the whole coding sequence and the splice sites of the *BRCA1* and *BRCA2* genes had been screened for the presence of mutations

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* The complete list of group members can be found in the Appendix.

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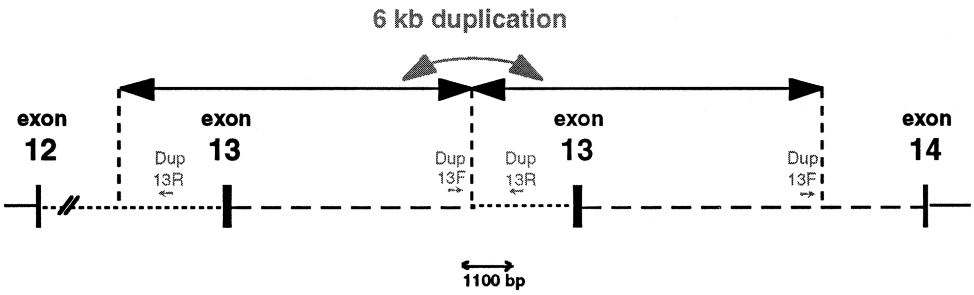

Figure 1 Schematic representation of *BRCA1* exon 13 duplication. The location and orientation of the dup13F (GAT TAT TTC CCC CCA GGC TA) and dup13R (AGA TCA TTA GCA AGG ACC TGT G) primers are represented, along with the position of the 6-kb duplicated region and of the 1.1-kb PCR fragment generated with dup13F/R. Blackened boxes denote exons; dotted lines denote intron 12; and broken lines denote intron 13.

in 61% and 37%, respectively, of the families; the screening had not been completed in 31% (*BRCA1*) and 43% of them (*BRCA2*); it had not been attempted in 8% (*BRCA1*) and 20% (*BRCA2*).

From this series of families, 1,831 were recruited and screened in English-speaking countries, and 10 apparently independent families were found to carry the duplication in Australia (1), Canada (1), Great Britain (6), and the United States (2) (table 1). No duplications were found in the 1,749 families who were recruited and screened in non–English-speaking countries (table 2). Ten of the institutions involved in this project also sought the presence of the duplication in 934 additional samples, mainly breast cancer cases selected for an early age at onset (table 3). One non–English-speaking duplication carrier was found in this second series: a Belgian woman with breast cancer diagnosed at age 34 years whose mother also developed breast cancer. If we also include the 4 families previously identified (Puget et al. 1999*a*), a total of 15 apparently unrelated families have been found to carry ins6kbEx13 (table 4). As seen with the other recurrent mutations (Tonin et al. 1996), these 15 families display a spectrum of different breast and ovarian cancer phenotypes.

Haplotype analysis was conducted with seven polymorphic short-tandem-repeat markers within or flank-

Table 1

***BRCA1* Exon 13–Duplication Screening in English-Speaking Countries**

Country and Town	No. of Positive Famliies	No. of Screened Families[a]			
		Total	Breast Cancer Only	Breast and Ovarian Cancer	Ovarian Cancer Only
Australia:					
Westmead	1	40	33	7	0
Canada:					
Montreal	0	5[b]	1	4	0
Toronto	1	504	319	174	11
Ireland:					
Dublin	0	73	15	50	8
United Kingdom:					
Cambridge	0	56	0	15	41
St Andrews	1	150	112	38	0
Leeds	4	44	0	44	0
London	1	278	210	68	0
Sutton	0	94	NC	NC	NC
United States:					
Chapel Hill	0	61	39	21	1
New York	0	92	64	27	1
Philadelphia	1	110	65	45	0
Rochester	1	257	206	51	0
Salt Lake City	0	67	43	24	0
Total	10	1,831	>1,107	>568	>62

[a] NC = not communicated.
[b] No French Canadian or Jewish Ashkenazi families were included.

Table 2

***BRCA1* Exon 13–Duplication Screening in Non–English-Speaking Countries**

Country and Town	No. of Positive Families	No. of Screened Families			
		Total	Breast Cancer Only	Breast and Ovarian Cancer	Ovarian Cancer Only
Austria:					
Vienna	0	238	177	52	9
Belgium:					
Gent	0	30	26	3	1
Canada:					
Quebec	0	45	40	5	0
Finland:					
Helsinki	0	161	126	35	0
France:					
Clermont-Ferrand	0	36	24	9	3
Lille	0	115	103	5	7
Nantes	0	48	39	8	1
Villejuif	0	70	46	24	0
Paris	0	51	44	7	0
St. Cloud	0	117	96	20	1
Germany:					
Dusseldorf	0	45	36	8	1
Heidelberg	0	75	52	22	1
Hungary:					
Budapest	0	25	24	1	0
Israel:					
Tel Hashomer	0	76	62	14	0
Italy:					
Milan	0	48	35	13	0
Padua	0	96	54	33	9
Pisa	0	17	3	14	0
Netherlands:					
Leiden	0	30	26	4	0
Utrecht	0	200	168	27	5
Norway:					
Oslo	0	19	1	13	5
Spain:					
Barcelona	0	85	58	27	0
Madrid	0	76	60	16	0
Sweden:					
Lund	0	29	0	20	9
Switzerland:					
Geneva	0	8	6	2	0
Turkey:					
Ankara	0	9	8	0	1
Total	0	1,749	1,314	382	53

ing the *BRCA1* locus and spanning ~1.9 Mb (from centromere to telomere, these markers are D17S1185, D17S1321, D17S1323, D17S1322, D17S855, D17S1326, and D17S1325; data not shown). In 8 of the 15 families, a single sample was available; thus, it was not possible to determine phase. Nevertheless, all exon 13–duplication carriers had genotypes compatible with their sharing the same haplotype. Because the particular alleles shared by duplication carriers at D17S1185 and D17S855 have population frequencies <15%, we can conclude that this duplication has most likely spread from a common ancestor. Unfortunately, because of an insufficient number of families in which haplotypes could be determined, it was not possible to accurately date this mutation.

We then tried to determine where the duplication originated. The origins of three of the families positive for the duplication could not be traced (table 4). However, the remaining ones are, in all but one case, compatible with the assumption that the ancestor for this mutation was British, because they are either of British descent or from countries—Ireland, Portugal, and Belgium—that have trading or other historical links with Britain. Five of the British families are from northern England, mainly Yorkshire, suggesting a northern British origin for the ancestor of this mutation. The exception is a U.S. family

Table 3

***BRCA1* Exon 13–Duplication Screening in Breast and/or Ovarian Cancer Cases**

Country and Town	No. of Positive Cases	No. (Age [Years]) of Screened Cases		
		Breast Cancer	Ovarian Cancer	Breast and Ovarian Cancer
Canada:				
Toronto	0	24 (<45)	…	5
Ireland:				
Dublin	0	14 (<50)	…	…
United Kingdom:				
London	0	59 (<35)	…	2
Sutton	0	617 (<45)	…	…
Austria:				
Vienna	0	14 (<35)	4 (<40)	…
Belgium:				
Gent	1	16 (<35)	1 (<35)	…
Germany:				
Dusseldorf	0	2 (<40)	…	…
Heidelberg	0	70 (<40)	…	…
Spain:				
Barcelona	0	90 (<35)	…	…
Turkey:				
Ankara	0	16 (<32)	…	…
Total	1	922	5	7

of Norwegian and Swedish ancestries. Another possibility is that the duplication could be of Viking origin, since all the countries mentioned above also had contact with Vikings. However, against this possibility, no duplication carrier has been identified in Sweden or in Norway; but the number of families screened was low in both countries (29 and 19, respectively; table 2). Alternatively, this U.S. carrier may not be fully aware of all her ancestors.

Eleven more families have been identified as carrying the exon 13 duplication, either in different series or after this study: two from Australia, one from New Zealand (E. Edkins, personal communication), five from Canada (S. Narod and C. Phelan, personal communication; N. Carson, personal communication), and three from Great Britain (A. Haworth, personal communication). In conclusion, the *BRCA1* exon 13 duplication is most likely a founder mutation distributed mainly in English-speaking countries or in countries with historical links with Britain. It would be strongly advisable, for laboratories

Table 4

Origin and Phenotype of the Families Carrying the *BRCA1* Exon 13 Duplication

Town Where Screening Was Done	Nationality	Origin	No. of Cases	
			Breast Cancer	Ovarian Cancer
Lyon[a] (France)	American	Irish/Dutch	1	4
Lyon[a](France)	American	English	7	3
Lyon[a](France)	American	Unknown	7	0
Lyon[a](France)	Portuguese	Portuguese	3	0
Gent (Belgium)	Belgian	Unknown	2	0
Perth (Australia)	Australian	Unknown	4	1
Toronto (Canada)	American	Irish/Scottish/English/German	4	0
St. Andrews (Great Britain)	British	English/Polish	3	0
Leeds (Great Britain)	British	English	3	3
Leeds (Great Britain)	British	English	2	3
Leeds (Great Britain)	British	French/English	4	1
Leeds (Great Britain)	British	English	5	1
London (Great Britain)	British	English	1	2
Philadelphia (United States)	American	Dutch/German/French/English	8	3
Rochester (United States)	American	Norwegian/Swedish	4	0
Total			58	21

[a] Families reported by Puget et al. (1999*a*).

carrying out screening for *BRCA1* mutations in these countries, to include within their protocols the PCR-based assay described here. This study emphasizes once more the necessity of screening not only for mutations in the coding sequence and splice sites but also for gene rearrangements, when one is analyzing for the presence of disease-causing mutations in the *BRCA1* gene.

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Appendix

The *BRCA1* Exon 13 Duplication Screening Group

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Breast Cancer Information Core (BIC), http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omin (for *BRCA1* [MIM 113705])

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