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Report

Founder Mutations in the BRCA1 Gene in Polish Families with Breast-Ovarian Cancer

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We have undertaken a hospital-based study, to identify possible BRCA1 and BRCA2 founder mutations in the Polish population. The study group consisted of 66 Polish families with cancer who have at least three related females affected with breast or ovarian cancer and who had cancer diagnosed, in at least one of the three affected females, at age <50 years. A total of 26 families had both breast and ovarian cancers, 4 families had ovarian cancers only, and 36 families had breast cancers only. Genomic DNA was prepared from the peripheral blood leukocytes of at least one affected woman from each family. The entire coding region of BRCA1 and BRCA2 was screened for the presence of germline mutations, by use of SSCP followed by direct sequencing of observed variants. Mutations were found in 35 (53%) of the 66 families studied. All but one of the mutations were detected within the BRCA1 gene. BRCA1 abnormalities were identified in all four families with ovarian cancer only, in 67% of 27 families with both breast and ovarian cancer, and in 34% of 35 families with breast cancer only. The single family with a BRCA2 mutation had the breast-ovarian cancer syndrome. Seven distinct mutations were identified; five of these occurred in two or more families. In total, recurrent mutations were found in 33 (94%) of the 35 families with detected mutations. Three BRCA1 abnormalities—5382insC, C61G, and 4153delA—accounted for 51%, 20%, and 11% of the identified mutations, respectively.

Mutations in BRCA1 (MIM 113705) and BRCA2 (MIM 600185) confer a high lifetime risk for both breast and ovarian cancer (Futreal et al. 1994; Miki et al. 1994; Wooster et al. 1994, 1995). Many different BRCA1 and BRCA2 mutations have been described in families with early-onset breast and ovarian cancer (Couch et al. 1996; Couch and Weber 1996). The presence of recurrent mutations in BRCA1 suggests the presence of founder effects; this was first confirmed in the Ashkenazi Jewish population (Tonin et al. 1995, 1996). Founder mutations in other populations have also been described. To identify possible founder mutations in individuals in Poland, we evaluated a panel of 66 Polish families with familial aggregation of breast or ovarian cancers. Knowledge of the nature and frequency of population-specific mutations in BRCA1 and BRCA2 is a critical step in the development of simple and inexpensive diagnostic approaches to DNA analysis in particular ethnic groups.

The study group consisted of 66 Polish families with breast-ovarian cancer who were drawn from the Oncology Centers in Szczecin (58 families), Wrocław (5 families) and Łódź (3 families), Poland. In each family, at least three related female relatives were affected by breast or ovarian cancer, and at least one cancer was diagnosed at a patient age <50 years. A total of 27 families had both breast and ovarian cancers, whereas 4 families had ovarian cancers only and 35 families had breast cancer only.

Blood samples for genetic analyses were taken from at least one affected woman from each family; generally, the samples were taken from the youngest of these

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women. Genomic DNA was prepared from peripheral blood leukocytes, by use of the nonenzymatic, rapid method described by Lahiri and Nurnberger (1991). The entire coding region of BRCA1 and BRCA2 was screened for the presence of germline mutations in BRCA1 and BRCA2, by use of SSCP followed by direct sequencing of variants. The primers used for PCR amplification of the BRCA1 gene have been described in the Breast Cancer Information Core (BIC) database, and those used for BRCA2 have been described by Friedman et al. (1997). Samples were sequenced with the use of fluorescently labeled dideoxy-chain terminators (Sanger et al. 1997) from the ABI Prism Kit (PE Biosystems), in a model 373 automated DNA sequencer (PE Biosystems). Members of families with detected mutations were interviewed to identify the geographic region where their ancestors lived before World War II.

Mutations were found in 35 (53%) of the 66 families studied. All but one of the mutations were detected within the BRCA1 gene. The mutation-detection rate was dependent on the number of ovarian cancers: BRCA1 abnormalities were identified in 100% of four families with ovarian cancers only, in 67% of 27 families with breast and ovarian cancers, and in 34% of 35 families with breast cancers only (table 1). The single family with a BRCA2 mutation presented with the breast-ovarian cancer syndrome.

Seven distinct mutations were identified, and five of these occurred in at least two different families (table 2). Recurrent alterations were found in 33 (94%) of the 35 families with detected mutations. Three BRCA1 mutations—5382insC, C61G, and 4153delA—accounted for 51%, 20%, and 11% of all identified mutations, respectively.

For individuals from families with mutations and for individuals from families without detected mutations, the average age at diagnosis of breast cancers was 47.8 and 48.2 years, respectively. For families with BRCA1 mutations, the average age at diagnosis of ovarian cancers was 44.4 years.

It was possible to establish the family area of origin before World War II, for 25 probands with BRCA1 mutations. This was done with reference to the branch of the family that was most likely to be transmitting the mutation. No particular geographic aggregation of mutations was observed (fig. 1).

The results of the present study suggest that Poland can now be included in the select group of countries in which a small number of founder mutations accounts for the majority of changes in families with BRCA1 and BRCA2 and a genetic predisposition to breast and ovarian cancers. Three BRCA1 abnormalities—5382insC, C61G, and 4153delA—were found in 82% of families with a strong aggregation of breast or ovarian cancers, and they were found in 94% of families with detected mutations.

Table 1

Frequency of BRCA1 and BRCA2 Mutations, Depending on the Site of Origin of Cancers in a Family

	Frequency of Mutation		
Cancer	BRCA1	BRCA2	Total
Breast and ovarian	18/27 (66.7%)	1/27 (3.7%)	19/27 (70.4%)
Ovarian only	4/4 (100%)	0/4 (0%)	4/4 (100%)
Breast only	12/35 (34.3%)	0/35 (0%)	12/35 (34.3%)
Total	34/66 (51.5%)	1/66 (1.5%)	35/66 (53%)

Other ethnic groups with high frequencies of founder mutations include Ashkenazi Jews, Icelanders, French Canadians, the Dutch, Norwegians, Swedes, and, possibly, other populations of central and eastern Europe (Streuwing et al. 1995; Szabo and King 1997; Dorum et al. 1999; table 3).

The most frequent mutation in the present series was the BRCA1 5382insC, which occured in approximately one-half of the families with mutations. The majority of families with this common mutation originate from eastern or central Europe. This mutation is also common in Ashkenazi Jews, and it constitutes ~25% of the mutations found in Jewish women with a high genetic risk of breast and ovarian cancers (Struewing et al. 1997). The findings from small case series suggest that the 5382insC mutation also occurs frequently in families from Hungary and Latvia who have breast-ovarian cancer (Gayther et al. 1997; Ramus et al. 1997; Csokay et al. 1999).

The individuals analyzed in this study were collected predominantly from northwest Poland. However, because most of the migration (from all regions of Poland) to this northwest region occured after World War II, we believe that these mutations are representative of mutations in individuals in the country at large (fig. 1). Furthermore, the results of independent studies have verified that BRCA1 5382insC is the dominant founder mutation in Silesia, which is a southern region of Poland (E.G., personal communication).

The second most commonly observed mutation in the present study was the BRCA1 C61G missense mutation in exon 5, which accounted for 20% of families with mutations. This mutation has previously been reported in various ethnic groups; however, to our knowledge, its incidence in Poland is higher than that in any population described to date, suggesting that this BRCA1 abnormality is characteristic of the Polish population.

The third recurrent mutation found in Polish patients, BRCA1 4153delA, was detected in 6% of Polish families. This mutation has previously been described and has been found in multiple families from Russia (Gayther et al. 1997). Thus, the 4153delA mutation appears to

Table 2

Germline Mutations Detected in BRCA1 and BRCA2 Genes in Families from Poland Who Have Breast and/or Ovarian Cancer

Gene and Family Number	Mutation			Site and No. of Cancers		
	Exon	Codon	Alteration	Breast	Ovaries	Other
BRCA1:						
4506	20	1756	5382insC	3	...	...
3311	20	1756	5382insC	3	...	...
4412	20	1756	5382insC	3	...	...
1633	20	1756	5382insC	3	...	Colon
4508	20	1756	5382insC	2	1	...
3319	20	1756	5382insC	2	1	...
3088	20	1756	5382insC	2	2	Lymphoma
3572	20	1756	5382insC	3	...	...
4545	20	1756	5382insC	3	...	Colon, stomach
1738	20	1756	5382insC	4	3	Colon
1582	20	1756	5382insC	3	...	Prostate
4478	20	1756	5382insC	3	...	...
1387	20	1756	5382insC	4	1	Colon
2863	20	1756	5382insC	4	2	...
4968	20	1756	5382insC	...	4	Stomach, cancer site unknown
5715	20	1756	5382insC	...	3	...
5726	20	1756	5382insC	1	2	...
4030	20	1756	5382insC	3	...	Lung, leukemia
1581	5	61	C61G	2	2	Cancer site unknown
1888	5	61	C61G	4	...	...
4859	5	61	C61G	3	...	...
3804	5	61	C61G	7	...	...
4858	5	61	C61G	4	...	...
5850	5	61	C61G	2	1	...
4854	5	61	C61G	3	...	Skin
2984	11	1345	4153delA	2	1	...
4278	11	1345	4153delA	...	4	...
3080	11	1345	4153delA	2	2	Colon
5939	11	1345	4153delA	...	4	Leukemia
1601	2	23	185delAG	3	...	...
703	2	23	185delAG	3	...	Lung
3910	11	1234	3819del5	3	...	...
5763	11	1234	3819del5	2	2	Colon, lung
5746	5	64	C64R	4	1	Lung, colon, leukemia
BRCA2:						
3874	27	3401	T3401M	2	1	Colon

be another BRCA1 mutation characteristic of individuals in eastern Europe.

The 185delAG mutation is the second BRCA1 abnormality that is common to both the Polish and the Ashkenazi populations. In combination, the 5382insC and 185delAG BRCA1 mutations account for ~60% of the total BRCA1 and BRCA2 mutations in Poland and in the Ashkenazi population. This similarity may reflect the common history of these two populations. In some instances, Polish families with these mutations may be descendants of individuals who converted from Judaism in recent generations.

The final recurrent mutation found in this study was the 3819del5 BRCA1 mutation. This mutation has not previously been reported, and it may be characteristic of the Polish population.

Other Polish families with BRCA1 or BRCA2 mutations have been reported (see the BIC database; Sobczak et al. 1997; Jakubowska et al. 1999). Mutations that have been reported in other studies of Polish families but that are not present in the population studied here include BRCA1 W1782X (Sobczak et al. 1997) and BRCA2 T3401M, 6886del5, 7883del4, and 9630delC (see the BIC database; Jakubowska et al. 1999). The 9630delC mutation was present in three families from Silesia (E.G., personal communication).

It is possible that we have not yet identified all founder mutations that occur in Poland. SSCP does not detect all mutations, and the technique used here is not sensitive to the detection of large deletions or duplications. However, the proportion of families with detected mutations was high; mutations were identified in 70% of families

with breast and ovarian cancers and in 34% of families with breast cancers only; therefore, it is unlikely that we have missed many mutations. It is possible that there are local founder mutations that are specific to subregions of Poland and that are not represented here. Furthermore, we have sequenced only a single individual per family, and it is possible that we have inadvertently chosen to sequence a sporadic case in a family in which a mutation is present in other affected individuals.

Of note is the preponderance of BRCA1 mutations over BRCA2 mutations in the present series. To our knowledge, this is the most extreme example of an excess of BRCA1 mutations over BRCA2 mutations in a particular population. The results of epidemiological studies in the United Kingdom, Canada, the United States, and Israel suggest that BRCA2 mutations account for ⩽40% of all mutations identified in families with breast-ovarian cancer and for the majority of mutations in families with site-specific breast cancer (Easton et al. 1995; Couch et al. 1996; Peto et al. 1999). Examples of populations in which there are predominantly BRCA2 mutations include Iceland (Thorlacius et al. 1997), Italy (Santarosa et al. 1998), and the Philippines (S.A.N., unpublished data). In the present study, BRCA2 mutations were found in only 1.5% of all families with an aggregation of breast or ovarian cancer. The reason for the preponderance of BRCA1 mutations in this population is not clear. The most likely explanation is that the founder effects were restricted to this gene. We do not believe that the families were selected for the study in a way that preferentially led to the inclusion of BRCA1-positive families. Although 45% of the families in the present series included one or more cases of ovarian cancer, the number of BRCA1 mutations far exceeded the number of BRCA2 mutations in families with site-specific breast-cancer syndrome. In fact, all 12 mutations in families with site-specific breast cancer were found in BRCA1 (table 1).

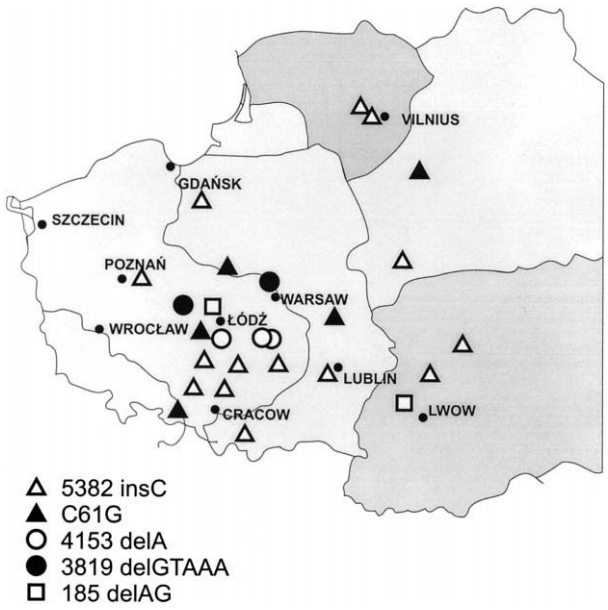

Figure 1 Map with localization of geographic areas where ancestors of BRCA1-mutation carriers were living before World War II.

The results of the present study will be of significance both for diagnostic testing and for epidemiological studies. BRCA1 and BRCA2 are both large genes, and complete analysis of the coding regions is expensive and time-consuming. The identification of five founder mutations allows for the rapid diagnosis of BRCA1-mutation carriers in Polish individuals with relatively high sensitivity. Furthermore, in countries with a large Polish population, such as the United States, DNA testing for founder mutations prior to complete gene analyses may be cost-effective. The simplicity of detection of the BRCA1 mutation in Poland creates a unique opportunity for rapid identification of hundreds and, possibly, thousands of Polish women who carry constitutional mutations. This group will be exceedingly valuable for use in the study of chemoprevention and treatment in high-risk women.

Table 3

Populations with a High Proportion of BRCA1 and BRCA2 Founder Mutations

Population	Mutation		Reference
	BRCA1	BRCA2	
Ashkenazi Jews	185delAG		Simard et al. (1994)
	5382insC		
		6174delT	Neuhausen et al. (1996)
British	4184del4		Gayther et al. (1995)
		6503delTT	Mazoyer et al. (1996)
Icelanders		999del5	Thorlacius et al. (1996)
Dutch	2804delAA		Peelan et al. (1997)
	2312del5		Petrij-Bosch et al. (1997)
	1411insT		
	C2457T		
Norwegians	1135insA		Andersen et al. (1996)
	1675delA		Dorum et al. (1997)
Swedes	2594delC		Johannsson et al. (1996)
	C1806T		Hakansson et al. (1997)
	3172ins5		
	1201del11		
	3829delT		
		4486delG	Hakansson et al. (1997)
		A3508T	
		4075delGT	
Russians	5382insC		Gayther et al. (1997)
	4153delA		

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

BIC, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/ (for BRCA1 and BRCA2 mutations)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for BRCA1 [MIM 113705] and BRCA2 [MIM 600185])

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