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Frequently Occurring Germ-Line Mutations of the *BRCA1* Gene in Ovarian Cancer Families from Russia

To the Editor:

Germ-line mutations of the *BRCA1* gene are thought to be responsible for the cancer in 80%–90% of families containing multiple cases of both breast and ovarian cancer (Easton et al. 1993). In addition, *BRCA1* is predicted to be responsible for the cancer in the majority of site-specific ovarian cancer families containing three or more cases of epithelial ovarian cancer and no cases of early-onset breast cancer (Steichen-Gersdorf et al. 1994). Since the isolation of *BRCA1* (Miki et al. 1994), >130 distinct germ-line mutations have been reported, primarily in multiple-case breast/ovarian cancer families (Couch et al. 1996). The majority of these mutations

are predicted to result in truncation of the BRCA1 protein, as a result of frameshift, nonsense, or splice-site alterations. Two mutations occur with a relatively high frequency: 185delAG and 5382insC account for ~22% of all mutations reported. The 185delAG mutation is particularly common in Ashkenazi Jews; it has a frequency of ~1% in this population and is present in almost 20% of Ashkenazi Jews with early-onset breast cancer (Streuwing et al. 1995; Fitzgerald et al. 1996; Roa et al. 1996). The 5382insC mutation also appears to be relatively common in the Ashkenazi Jewish population, with a frequency of 0.13% in a large series of unselected Ashkenazi Jews (Roa et al. 1996).

Epidemiological data suggest that the risk of ovarian cancer varies between different families linked to the *BRCA1* locus; in the majority of these families, there appears to be a high risk of breast cancer but a relatively low risk of ovarian cancer, whereas in a minority of families there appears to be an equally high risk of both breast cancer and ovarian cancer (Easton et al. 1995). In support of this, two recent studies have indicated that the incidence of breast cancer and ovarian cancer in families is correlated with the location of the *BRCA1* mutation (Gayther et al. 1995; Holt et al. 1996). When mutations that result in a truncated BRCA1 protein occur in the first two-thirds of the gene, the risk of ovarian cancer relative to breast cancer in the family is significantly higher than when truncating mutations occur in the last one-third of the gene.

We have analyzed a series of predominantly ovarian cancer families, collected in Russia, for germ-line mutations of the *BRCA1* gene. Nineteen families containing at least two first-degree relatives with epithelial ovarian cancer (borderline ovarian cancer was not included) were identified through the Russian Academy of Medical Sciences in Moscow. For the analysis, genomic DNA was available from one affected individual of each family. In total, these families contained 47 cases of ovarian cancer, diagnosed at any age, and 3 cases of breast cancer, diagnosed at $\leq$60 years of age (table 1).

Mutation analysis was performed by use of a combination of single-strand conformation analysis and heteroduplex analysis (SSCA/HA) and the protein-truncation test (PTT), as described in previous studies (Gayther et al. 1996; Friedman et al. 1997). Variants identified by SSCA/HA and/or PTT were characterized further by fluorescent sequencing using the model 373A semiautomated sequencing system (Applied Biosystems). In addition, genotyping with four microsatellite polymorphisms (D17S855, D17S1322, D17S1323, and D17S1327) located within or flanking *BRCA1* was performed for each individual (Neuhausen et al. 1996).

The results of this analysis are presented in table 1. Four different truncating mutations were identified in 14 (74%) apparently distinct families. Two of these mutations, 2073delA and 4153delA, have not been reported in any population outside this series of ovarian cancer families (Couch et al. 1996; Breast Cancer Information Core 1997). A novel missense variant, Met1628Thr, also was identified in one family (SDK 262). Although this alteration has not been reported previously, the possibility remains that it is a rare variant without disease association. The amino acid change is relatively conservative, and cross-species comparison indicates that, although the amino acid at this position is similar between human *BRCA1* and murine *Brca1*, it is not identical (Abel et al. 1995; Sharan et al. 1995). A population-based analysis of this variant in breast cancer and ovarian cancer cases, as compared with controls from Russia, will be required for further assessment of its disease association. In addition, the Met1628Thr variant is located within a region of the protein that demonstrates transcriptional transactivation activity (Chapman and Verma 1996); it therefore may be possible to test directly the functional significance of this alteration.

The 5382insC mutation was detected in nine apparently distinct families. This mutation has been shown in previous studies to be associated with a rare haplotype, suggesting that it is derived from a common ancestor (Neuhausen et al. 1996). In this study, only one affected individual was available from each family with the 5382insC mutation, and, therefore, phase discrimination of genotypes was not possible. The genotype established for four microsatellite polymorphisms in or near the *BRCA1* gene is consistent with the fact that all nine individuals with the 5382insC mutation share the same rare haplotype, which has been shown in previous studies to be associated with this mutation (data not shown). Allelotyping also was consistent with the fact that the three families with the 4153delA mutation share a common haplotype.

The prevalence of germ-line *BRCA1* mutations in these families is much greater than would have been predicted from previous epidemiological studies, considering the histories of cancer in the families (Easton et al. 1993; Steichen-Gersdorf et al. 1994). Truncating mutations were detected in all 7 families containing three or more cases of ovarian cancer and/or breast cancer and in 7 (58%) of 12 families containing two cases of ovarian cancer only. In a study of families, ascertained in the United Kingdom, containing two or more first-degree relatives with ovarian cancer, *BRCA1* mutations were detected in 52% of families containing three or more cases of ovarian cancer and/or breast cancer and in 17% of families containing two cases of ovarian cancer only (S. A. Gayther and B. A. J. Ponder, unpublished data).

Perhaps the most interesting finding of this study is the suggestion that mutations in *BRCA1* may be responsible

Table 1

Germ-line Mutations of the BRCA1 Gene Identified in Ovarian Cancer and Ovarian/Breast Cancer Families Ascertained in Russia

Family	No. of Reported Cases of Cancer		Mutation[a]			Origin of Proband	
	Ovarian	Breast[b]	Exon	Codon	Alteration	Birthplace	Stated Nationality or Ethnicity
EAL 3/92	5	...	20	1756	5382insC	Moscow	Jewish
EAW 5/92	3	1	20	1756	5382insC	Moscow	Russian
JNK 4/92	2	1	20	1756	5382insC	Moscow region	Russian
KFS 1/92	4	...	11	1252	3875del4	Moscow region	Russian
LCB 13/92	2	...	...	...	...	Moscow region	Russian
LIK 12/92	2	1	20	1756	5382insC	Ukraine	Russian
MNN 14/92	2	...	20	1756	5382insC	Azerbaijan	Tatarvian
NIV 6/92	2	...	...	...	...	Moldova	Moldovian
NNS 23/93	2	...	20	1756	5382insC	Belarus	Belarussian
OMI 8/92	2	...	20	1756	5382insC	Moscow	Russian
PVM 9/92	2	...	...	...	...	Moscow	Russian
SAD 7/92	2	...	20	1756	5382insC	Moscow	Russian
TIR 11/92	3	...	20	1756	5382insC	Siberia	Jewish
SDK 262	2	...	16	1628	M1628T	Irkutsk, Siberia	Russian
VIK 18/92	2	...	11	652	2073delA	Moscow	Russian
VKA 19/92	2	...	11	1345	4153delA	Moscow	Russian
VMB 15/92	2	...	11	1345	4153delA	Moscow	Russian
VVN 21/93	2	...	...	...	...	Udmurt Republic	Russian
YPT 2/92	4	...	11	1345	4153delA	Moscow region	Russian

[a] A portion of the data presented here has been published previously (Gayther et al. 1996).
[b] Diagnosed at ≤60 years of age.

for almost 75% of familial ovarian cancer in Russia. Furthermore, two apparent founder mutations, 5382insC and 4153delA, account for 86% of all mutations. Until a larger sample set that is more clearly representative of the ethnic diversity of the Russian population can be analyzed, these findings must be interpreted with caution. A bias could have occurred if the method of ascertainment led us unknowingly to sample several closely related branches of the same family, although this appears unlikely because of the apparently diverse geographic and ethnic spread reported for the probands of these families, particularly for the 5382insC mutation (table 1).

It is perhaps surprising, in the light of previous findings, that the 5382insC mutation is so frequent in this series of predominantly ovarian cancer families. This mutation is located in exon 20 of *BRCA1,* in a region that is more commonly associated with a low risk of ovarian cancer and/or with an increased risk of breast cancer (Gayther et al. 1995; Holt et al. 1996). Similarly, a recent study suggests that the 5382insC mutation occurs frequently in predominantly breast cancer families from Hungary (Ramus et al. 1997 [in this issue]). The most likely explanation for the apparent discrepancy is that the results reported here were obtained on a set of samples that were ascertained specifically for ovarian cancer. However, it may be that there is real variation, between different populations, in the breast cancer and/or ovarian cancer risks associated with the 5382insC mutation and that these risks are modified by genetic background (Phelan et al. 1996) or by environmental factors. Analysis of breast cancer families and of population-based series of breast cancer and ovarian cancer cases from Russia is needed to resolve this issue.

If population-based studies indeed confirm that the 5382insC and 4153delA mutations are common and account for a significant proportion of breast cancers and ovarian cancers, screening may be possible along the same lines as has been suggested for the Ashkenazi Jewish population. However, as in the case of the Ashkenazi Jews, it will be necessary to know the risks associated with each mutation and to have the resources and the ability to act on the information.

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Analysis of BRCA1 and BRCA2 Mutations in Hungarian Families with Breast or Breast-Ovarian Cancer

To the Editor:

Breast cancer is the most common malignancy among women; and the strongest epidemiological risk factor for the disease is a positive family history. A breast-ovarian cancer–susceptibility gene, BRCA1, on chromosome 17q21 has been isolated by positional cloning (Futreal et al. 1994; Miki et al. 1994). More than 130 different mutations have been described in the BRCA1 gene (Couch et al. 1996*b*). A second breast cancer–susceptibility gene, BRCA2, has been identified on chromosome 13q12-13 (Wooster et al. 1994, 1995; Tavtigian et al. 1996). Founder mutations have been described in several populations, for both BRCA1 and BRCA2 (Struewing et al. 1995; Tonin et al. 1995; Couch et al. 1996*a;* Neuhausen et al. 1996*a;* Thorlacius et al. 1996).

Hungarian individuals with a family history of breast or breast-ovarian cancer were studied to identify the number and frequency of BRCA1 and BRCA2 mutations. The minimum criterion for inclusion was that the family contain at least two first-degree relatives with breast or ovarian cancer diagnosed at any age. Cases with a strong family history of breast or ovarian cancer were selected preferentially. Seventy-five percent of the families selected for this study had three or more cases of breast or ovarian cancer. Genomic DNA samples were available from affected individuals in 32 families with breast or breast-ovarian cancer. One affected individual