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Double Heterozygotes for the Ashkenazi Founder Mutations in BRCA1 and BRCA2 Genes

To the Editor:

Three Jewish founder mutations, 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 genes, have been identified in breast cancer (BC) and ovarian cancer (OC) Ashkenazi patients. In the Ashkenazi general population, the carrier frequencies of these founder mutations are 1% for 185delAG (Struewing et al. 1996), 0.13% for 5382insC, and 1.35% for 6174delT (Roa et al. 1996; Oddoux et al. 1996). Given these high population frequencies, one would expect to find individuals homozygous for the mutations 185delAG/185delAG, 6174delT/6174delT, and 5382insC/5382insC, compound heterozygous for 185delAG/5382insC, or double heterozygous for 185delAG/6174delT or 5382insC/ 6174delT, provided the individuals are viable. The effect of two mutations in a single individual is important both for an understanding of the mode of action and interaction between the BRCA1 and BRCA2 genes and for appropriate genetic counseling. To date, two double heterozygous patients (185delAG/6174delT; Ramus et al. 1997; Gershoni-Baruch et al. 1997) and one patient homozygous for a mutation in exon 11 of the BRCA1 gene (Boyd et al. 1995) have been reported.

By pooling results from four cancer/genetics centers in Israel, we have analyzed ~1,500 BC/OC Ashkenazi patients. All subjects received genetic counseling and signed informed consent forms in compliance with institutional ethics committees (institutional review boards). Each patient was tested for the three Ashkenazi founder mutations: in BRCA1, the mutations 185delAG and 5382insC, and in BRCA2, the mutation 6174delT (Abeliovich et al. 1997; Levy-Lahad et al. 1997; Bruchim Bar-Sade et al. 1998). Four patients were found to be double heterozygotes. Summaries of their clinical status and pedigrees are presented in table 1 and figure 1.

Patient 1 is an Ashkenazi mother of two children who was diagnosed with unilateral breast cancer at the age of 38 years. Her family history was positive for both OC, with which her mother was diagnosed at the age of 50 years, and breast cancer, with which her paternal aunt was diagnosed at the age of 60 years and her daughter at the age of 35 years. Her paternal grandfather had lung cancer at the age of 45 years. A test for 185delAG/ 6174delT in her father revealed neither mutation; DNA could not be retrieved from the paraffin block of her mother. Analysis of the polymorphic markers D17S855, D17S1322, D17S1323, D9S55, and D11S1337 in the father and in Patient 1 confirmed paternity. It was thus

Table 1

Genotypes and Clinical Status of the Patients

Individual	Genotype	Clinical Status	Age at Diagnosis (years)
Patient 1	185delAG/6174delT	BC	38
Mother of Patient 1	185delAG/6174delT[a]	OC	50
Patient 2	185delAG/6174delT	OC	57
Patient 3	185delAG/6174delT	Healthy	50
Patient 4	5382insC/6174delT	BC	45

[a] Inferred genotype.

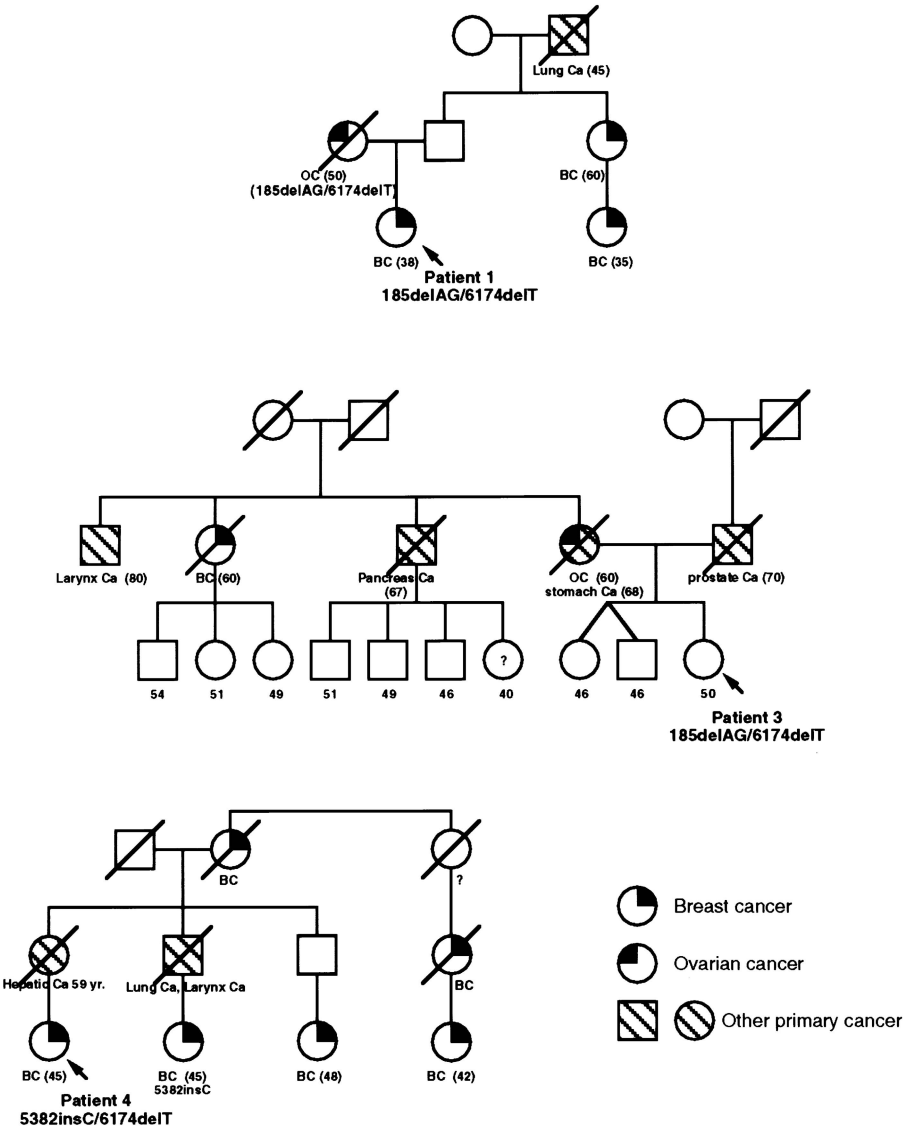

Figure 1 Pedigrees of Patients 1, 3, and 4. In parentheses is the inferred genotype and the ages at diagnosis.

assumed that she had inherited both mutations from her double heterozygous mother.

Patient 2 is a 57-year-old Ashkenazi woman who presented with stage IV OC. The patient is alive with no evidence of disease 5 years after treatment. Her family history includes breast cancer in her mother (age at diagnosis unknown). No further information was available. Patient 2 had irregular menses and primary sterility, which were treated with low doses of steroids.

Patient 3 is a 50-year-old asymptomatic Ashkenazi woman who was referred for evaluation of her breast cancer risk before starting hormonal replacement therapy for increasing loss in bone density. The maternal family history was positive for ovarian, breast, pancreas, stomach, and laryngeal cancers. Her father had prostate cancer. The patient had idiopathic premature menopause at the age of 37 years after bearing three children.

Patient 4 is a 46-year-old Ashkenazi woman who was diagnosed with breast-infiltrating ductal carcinoma. The family history was positive for cancer: hepatic carcinoma at the age of 59 years in her mother and breast cancer in her maternal grandmother. Two of her maternal cousins and two more distant relatives had breast cancer at the ages of 45, 48, and 42 years (the age at diagnosis of one of the relatives is unknown). One of them is a carrier of the mutation 5382insC. The others were not available for mutation analysis.

As compared with carriers of single mutations, the four double heterozygotes we observed did not have a particularly severe phenotype, based on the tumor types and age at diagnosis: one was unaffected at the age of 50 years; two were affected with unilateral breast cancer, one at the age of 38 years and one at the age of 46 years; and one was diagnosed with OC at the age of 57 years. An inferred double heterozygote (the mother of Patient 1) had OC at the age of 50 years. None had more than one primary tumor, and tumor histology and clinical course were unremarkable. Two other 185delAG/6174delT carriers were reported: one had BC and OC diagnosed at the ages of 48 and 50 years, respectively (Ramus et al. 1997); the other had bilateral BC at the ages of 41 and 50 years, respectively (Gersoni-Baruch et al. 1997).

Although the small number of cases precludes definite conclusions, our results suggest that the phenotypic effects of double heterozygosity for BRCA1 and BRCA2 germ-line mutations are not cumulative. This is in agreement with the observation that the phenotype of mice that were homozygote knockouts for the BRCA1 and BRCA2 genes was similar to that of mice that were BRCA1 knockouts. This suggests that the BRCA1 mutation is epistatic over the BRCA2 mutation (Ludwig et al. 1997).

Interestingly, two of the double heterozygotes described have had reproductive problems: one (Patient 2) had primary sterility and irregular menses, and another (Patient 3) had premature menopause at the age of 37 years. This latter patient was asymptomatic at the age of 50 years. These preliminary observations raise the possibility of hormonal effects in double heterozygotes, including the possibility that the lack of estrogen may have a protective effect.

At the population level, given the known heterozygote frequencies in Ashkenazi Jews, the expected frequencies of double heterozygotes would be the multiplication of the heterozygote frequencies 185delAG/6174delT (1.35×10^{-4}) and 5382insC/6174delT (1.75×10^{-5}). The expected frequencies of BRCA1 and BRCA2 homozygotes will be the multiplication of the mutation frequencies (approximately one-half of the heterozygote frequency), which are 2.5×10^{-5} for 185delAG homozygotes and 4.6×10^{-5} for 6174delT homozygotes. Therefore the ratio of 185delAG/6174delT double heterozygotes and 6174delT and 185delGA homozygotes is 3:1:0.5, respectively. Namely, the double heterozygotes should be about three to six times more common than the homozygotes 185delAG or 6174delT. In this respect, we might have expected to observe 185delAG or 6174delT homozygotes. The fact that we did not observe these or any other homozygotes may be due to chance, and more patients should be tested before a homozygous patient is found or, alternatively, before homozygosity for 185delAG or 6174delT decreases viability or causes different phenotypic consequences.

The clinical implication of this study is that mutation analysis in Ashkenazi Jews should include all known founder mutations. Identification of additional carriers of more than one mutation will increase our understanding of the interaction between various mutations and will improve genetic counseling.

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Partial Triplication of mtDNA in Maternally Transmitted Diabetes Mellitus and Deafness

To the Editor:

Maternally inherited diabetes and deafness (MIDD) is a recently recognized subtype of diabetes mellitus (DM) that is associated with mtDNA mutations (Maassen et al. 1997). The first mtDNA defect described for MIDD was a deletion associated with a duplication of the mtDNA in a family presenting DM and deafness over three generations (Ballinger et al. 1992, 1994). Subsequent to this observation, a mutation in nucleotide (nt) 3243 was reported in several pedigrees presenting DM and deafness (Reardon et al. 1992; van den Ouweland et al. 1992; Kadowaki et al. 1993). We report a partial tandem triplication of 9.2 kb in one member of a family presenting MIDD associated with a tandem duplication of 4.6 kb.

In 1966, a 44-year-old man (II-7) of Italian origin was hospitalized for insulin-dependent DM and hearing loss. In 1973, his nephew (III-2), who was born in 1932, was hospitalized for non–insulin-dependent DM and deafness. At that time, the morbid association led to a study of the pedigree (fig. 1), which showed transmission of DM and deafness over four generations, with a total of 13 affected individuals (Kressmann 1976). Seven individuals from the pedigree (III-3, III-4, IV-1, IV-2, IV-3, IV-4, and IV-5) were examined by clinicians. The clinical history was the same for all affected patients: the first manifestation was deafness, beginning at 20–30 years of age, with a rapid and severe increase in bilateral sensory hearing loss. DM developed later in the 3d decade, and insulin was required either immediately or at a later date. At that time, the individuals from the fourth generation, who were <20 years of age, presented no deafness or DM. No pedigree member had ptosis, ophthalmoplegia, or muscle weakness. Recently, the maternal inheritance pattern of DM and deafness in this family

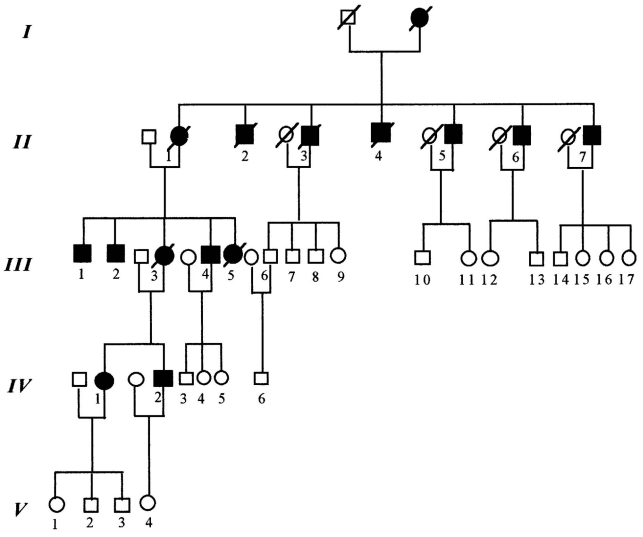

Figure 1 Pedigree of family analyzed in this study. Unblackened symbols indicate unaffected individuals, and blackened symbols indicate affected individuals. Nine family members (II-7, III-2, III-3, III-4, IV-1, IV-2, IV-3, IV-4, and IV-5) were examined in 1973.