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Original Paper

A *BRCA1* Founder Mutation, Identified with Haplotype Analysis, Allowing Genotype/Phenotype Determination and Predictive Testing

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We searched for a founder mutation in a population from one geographic region of Norway with prevalent breast/ovarian cancer families. We sampled 33 breast/ovarian cancer families and determined haplotypes of four markers linked to the *BRCA1* region. Of the affected 33 index women, 13 (39.4%) shared one haplotype. In five (15% of total), an identical mutation was indicated by an abnormal truncated protein test (PTT) of exon 11 and shown to represent a 1675delA mutation. In the other index women, PTT of exon 11 showed no abnormality. No other *BRCA1* founder mutation of this prevalence is likely because no other haplotype was more frequent in affecteds than in controls. All families with the 1675delA mutation in this geographic region may be considered as part of one large kindred. This allows a genotype–phenotype correlation to be precisely determined and used in genetic counselling for predictive testing within this kindred. Identification of identical haplotypes between unrelated affected individuals may be used to estimate the extent of founder effects for any mapped disease, without knowledge of the specific founder mutation. © **1997 Elsevier Science Ltd.**

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INTRODUCTION

LINKAGE AND segregation analyses indicate that some clustering of breast cancer is caused by rare, highly penetrant dominant genes [1, 2]. Recently, two genes *BRCA1* and *BRCA2* that harbour mutations with high penetrance for breast and ovarian cancer, have been identified [3, 4]. This has led to detection of mutations segregating with breast cancer. The possibility exists that additional and/or non-familially occurring mutations remain to be discovered [5–7]. Easton and associates [8] calculated that approximately 50% of highly penetrant dominantly inherited breast cancer and the majority of familial breast–ovarian cancer may be related to *BRCA1* mutations. Risks of female breast cancer are similar for *BRCA1* and *BRCA2*. Ovarian cancer is more prevalent in mutated *BRCA1* kindreds, whilst male breast cancer may be associated with *BRCA2* mutations [4, 9]. Distinct mutations within the same genes may give distinct phenotypes [10]. It is unclear whether all *BRCA1* mutations implicate risk for ovarian cancer or not. Thus, the predictive value of demonstrating a *BRCA1* mutation is not clear. Demonstration of one large kindred would make it possible to determine the phenotype–genotype correlation for one specific mutation, and may then be used for predictive testing and genetic counselling.

Because of the general occurrence of breast/ovarian cancer after childbearing age, there is probably little selection against mutation carriers, and genetic drift will have the effect that the majority of families carry a few prevalent mutations, while other mutations may be present with low prevalence. The most prevalent mutations are most likely also the oldest, because of the combined effect of genetic drift and population growth [11].

By use of a few closely linked markers at an interval of less than 1 cM, a highly informative haplotype can be observed

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that is unlikely to be broken by recombination, even if the founder mutation was present many (up to 100) generations ago. *BRCA1* mutations are expected to follow this kind of population genetics.

Determination of haplotypes can be done by typing affecteds and relatives to determine phase. The identification of a common haplotype in affected individuals could point to a common ancestral origin for a mutation. Only a few individuals within the families carrying this haplotype would subsequently have to be screened to find the associated mutation(s). Identification at the DNA level of such a specific mutation in affected individuals and their healthy relatives with the same haplotype by a direct method would require less laboratory effort.

Our hospital covered all of Norway, but an area on the south-west coast in Rogaland and Hordaland counties (a population of about 400 000) appeared to have an increased prevalence of breast/ovarian cancer families, and was therefore chosen as the study population. Due to the Bubonic Plague (the Black Death), the population examined had a substantial bottle-neck year 1349 (the population in all of Norway was at that time 300 000–400 000, and was reduced by approximately 60% and has been expanding since then).

MATERIALS AND METHODS

Affected index persons and relatives from 33 breast–ovarian cancer families from this geographical area were selected. We have previously established clinical criteria for identifying families with inherited breast–ovarian cancer and shown that the cancer incidence in these families remains high [12, 13]. The inclusion criteria are stated in Table 1. 31 families had two or more cases with ovarian cancer (25 families had pathological confirmed ovarian cancer diagnoses, and in 6 families only one of the ovarian cancers was possible to confirm by pathological reports). Blood samples and written informed consent from all 170 participants were obtained. Apart from the index women, relatives and spouses from two or three generations were included to establish haplotypes in the affected family members, in some families more than one affected. All families received genetic counselling. Pedigrees including first- and second-degree relatives were obtained. All reports of cancers relevant to the study were verified by pathological reports. No family members were immigrants.

Microsatellite analysis

Microsatellites within and flanking the *BRCA1* gene on chromosome 17q have been described [14]. The markers used were *D17S800*, *D17S1322*, *D17S855*, and *D17S1325*, all within an interval of less than 1 cM. *D17S855* and *D17S1322* are localised within *BRCA1*. The PCR products were obtained under standard conditions and resolved using an automated sequencer (ALF, Pharmacia, Uppsala, Sweden). Allele lengths were calibrated using internal length standards as well as external standards to insure comparability between gels. Haplotypes were constructed assuming a minimum number of recombinants within each family. Haplotypes shared by two or more affecteds in the families examined were initially subjected to mutation analyses.

Mutation analysis

Mutation scanning of exon 11 of *BRCA1* was carried out using a protein truncation test (PTT) and primers as described in Hagervorst and associates [15]. For direct sequencing we used primers from base pairs 1602–1621 (forward) and 1961–1942 (reverse). The PCR product was subjected to direct sequencing by an automated sequencer (ALF, Pharmacia).

Table 1. *Clinical criteria for inclusion*

Number of families	Description
31	One case with ovarian cancer who has one first-degree relative (or second-degree relative through male) with ovarian cancer
2	One case with both ovarian and breast cancer (breast cancer diagnosed $\leq$ 60 years)

RESULTS

The most prevalent haplotype was found in 13/33 (39.4%) of the index women: 152–119–195 for *D17S1322*, *D17S855*, and *D17S1325*. Of these women, 5/13 had additional affected relatives sharing the haplotype, 3/13 did not share the haplotype and 5/13 did not have affected relatives alive. PTT showed a truncated protein in exon 11 in 3/5 index women sharing haplotype, demonstrated to be 1675delA (the remaining 2 index women had one affected examined in her family). The same mutation was subsequently found in 2/5 index women who did not have affected relatives alive. Altogether, 5/13 (38.5%) index women carrying the most prevalent haplotype and 5/33 (15%) of all index women had this mutation. In the 5 families with this mutation, all affecteds examined carried both the haplotype and the mutation. These mutation carrying families had 16 breast cancers occurring at median/mean age of 39.5/43.7 and 12 ovarian cancers occurring at 49/50.9 years (Table 2). The mutation was searched for but not found in the remaining index women, and none of them tested abnormal for PTT of exon 11.

The prevalence of the haplotype in question was 8/56 (14.3%) in the index women without the mutation, versus 6/52 (11.5%) in controls (non-transmitted parental haplotypes and spouses). The second most prevalent haplotype in the index women had a prevalence of 4/66 (6%) versus 4/52 (7.7%) in controls.

DISCUSSION

We have demonstrated a large kindred with one disease-associated *BRCA1* mutation. This gives the possibility of determining risk of breast and ovarian cancer in young mutation carriers in this kindred (= predictive value of genetic testing). As noted above, there were theoretical reasons to assume the existence of founder effects in breast–ovarian cancer families. While this study was carried out, others have indeed reported a number of frequent mutations both in *BRCA1* and *BRCA2* [14, 16, 17]. Determining haplotypes may be used to find and characterise such mutations, even in

Table 2. *Number of breast and ovarian cancer phenotypes in five families with the* 1675delA *mutation*

	Breast cancer (n = 16)	Ovarian cancer (n = 12)
30–39 years	8	1
40–49 years	3	5
50–59 years	5	3
60–69 years	0	3
Median/mean age at diagnosis	39.5/43.7 years	49/50.9 years

the absence of known mutations or data that link patients genealogically. As described in this study, the same haplotype may carry both mutated and non-mutated alleles, indicating that the mutation once occurred in this frequent haplotype, and that both mutated and non-mutated haplotypes may be found. The probability of recombination within the four loci of the haplotype is small, but the haplotypes at which the mutation was found differ at the marker *D17S800*. This indicates that the mutation is possibly quite old. The same mutation has been found in a Swedish family [18] not known to be related to our families. The examined population had ancient connections to the populations surrounding the North Sea. In the 19th century, emigration to the Northern part of the U.S.A. took place. It is likely that the same mutation is present in descendants from these emigrants.

Among the families without the demonstrated mutations, the (152, 119, 195) haplotype is still the most prevalent one. If there is another founder mutation at the *BRCA1* locus, the same haplotype would still be the most probable candidate to carry this as well. However, the prevalence of this haplotype without mutation in affecteds did not exceed the prevalence in controls. It is therefore not likely that this haplotype carries additional highly penetrant founder mutations. The same argument goes for the second most prevalent haplotype found. This means that in the general population, demonstration of the risk haplotype cannot, at present, discriminate between the possibilities of having the 1675delA mutation, having no mutation, or having another yet unidentified mutation, although the latter possibility seems less likely.

Prevalences of haplotypes spanning a limited gene distance are subject to genetic drift in founder populations [19], which reduces their theoretical informativeness based on linkage equilibrium between markers. In this investigation, we found a fraction of the theoretically possible haplotypes. We expected to find founder haplotypes in the young, recently expanded population studied, and any random mutation to have occurred would most probably be located on the most prevalent haplotype [20]. The finding of a founder mutation in the most prevalent haplotype in the controls was not surprising.

For practical purposes, all breast–ovarian cancer families and their relatives in Norway may now be screened at the DNA level for the mutation to identify other branches of this ancient family. As mentioned, different mutations of the *BRCA1* gene may have distinct consequences for the carriers [9, 21]. The presence of the founder mutation indicates risk of early breast and ovarian cancer. As the families were selected on the bases of having ovarian cancer (Table 1), the early onset breast cancer in the families with the mutation may be a significant observation without substantial ascertainment error. Thus, empirical data for genetic counselling and predictive testing within this large kindred have been obtained. By extending the patient population, the genotype–phenotype correlation for this specific mutation may be further determined.

In the 5 pedigrees with the demonstrated mutation, 12 individuals had ovarian cancer, the median age/mean age (range) was 49/50.9 years (39–66 years) which is more than 10 years earlier than the onset of sporadic ovarian cancer as reported by Amos and associates [22]. Of the 16 individuals with breast cancer, the median age/mean age (range) was 39.5/43.7 years (35–58), which is in keeping with expected age of onset of *BRCA1*-related breast cancer [9, 23]. The results of follow-up of women at risk of breast–ovarian cancer are good enough to warrant the use of predictive testing within such families [12, 13] as to provide mutation carriers with the healthcare they need.

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