Genetic epidemiology of BRCA1 mutations in Norway

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Abstract

Familial breast-ovarian cancer has been demonstrated to be frequent but unevenly distributed in Norway. This was assumed to be caused by the reduced population size created by the medieval Bubonic plagues 25 generations ago, and by the following rapid expansion. We have previously reported that four mutations account for 68% of the BRCA1 mutation carriers. Subsequent analysis has resulted in a total of 100 separate families carrying one of these founder mutations. The four mutations occurred on one specific BRCA1 haplotype each. The 1675delA, 816delGT and 3347delAG families originated from the South-West coast of Norway with a few families in the north, while the traceable ancestors of the 1135insA families clustered along the historical inland road from the South-East to mid-Norway. The carriers of each of the four mutations today are descendants of one or a few individuals surviving the plagues. We may identify the majority of BRCA1 mutation carriers in Norway by screening for local founder mutations.

Keywords: BRCA1; Inherited; Breast cancer; Ovarian cancer; Epidemiology; Founder mutation

1. Introduction

Of all breast cancers 5–10% are considered to be dominantly inherited [1,2]. The first breast cancer susceptibility gene (BRCA1) was located in 1990 and identified in 1994 [3,4], and the second (BRCA2) was identified shortly thereafter [5–7]. Following a nationwide survey of familial cancer in Norway during the early 1990s, our family cancer clinic uncovered a high incidence and uneven geographical distribution of familial breast–ovarian cancer. Based on the topographical conditions and historical events, we assumed the presence of local frequent BRCA1 mutations as the underlying cause. At that time, frequent mutations in some ethnic groups had been reported [8–10].

More than 10 000 years (400 generations) ago, all of Norway was covered with ice. As the inland ice began to melt, the coastal areas with deep fjords and valleys gradually became populated and prospered during the Viking age 40 generations ago. In the medieval ages, when the inhabitants numbered approximately 500 000, our country was struck by the Bubonic plagues. When brought to an end, 25 generations ago, the population had been reduced to approximately 150 000 [11]. A rapid population expansion, without any substantial immigration, followed. Today the population of Norway is approximately 4.5 million, and close to one million have emigrated to the USA over the last few generations. Thus, following a deep bottleneck, the population size has increased more than 30-fold over 25 generations. The small clusters of population surviving the plagues were scattered over a geographical distance.
equal to the distance from north to south across the European mainland. Until modern means of transportation appeared, population migration was limited due to the mountainous terrain and stormy waters.

Dominantly-inherited disease causing early death, such as the Li–Fraumeni and Cowden syndromes, will be selected against. Their prevalence will be low and maintained by new mutations [12,13]. However, since BRCA1-associated cancer most often occurs after childbearing age, fitness may not be reduced and negative selection may not take place. Assuming a prevalence of 0.09% BRCA1 mutation carriers in the general population [14], the Norwegian population surviving the Bubonic plagues would include approximately 135 mutation carriers. In a stable population and in the absence of positive or negative selection, it has been calculated that the probability of any new mutation to survive 25 generations is less than 10% (Fig. 1) [15]. It follows that if the Norwegian population had remained stable after the Bubonic plagues, without acquired or immigrated mutations added to it, we would expect less than 13 different BRCA1 mutations to be present today. The overall expectation is that the combined prevalence of mutations is maintained, but with loss of diversity. The distribution of mutations causing phenylketonuria shows the lack of diversity in Norway [16].

Because the number of mutation carriers is not expected to exceed the number of generations since its first occurrence [15], we would not expect any person surviving the plagues to have more than 25 mutation-carrying descendants today. However, the population has expanded and, in average, each survivor of the plagues has more descendants today than expected in a stable population. Such an expansion will also increase the probability that a single mutation survives.

A limited population may experience drifting prevalence of mutations due to random fluctuations in small numbers. The typical background for founder mutations is a limited and rapidly expanding population. Consequently, the estimated 4500000* 0.0009 = 4050 BRCA1 mutation carriers of today’s Norway, would be expected to carry approximately 13 old mutations. Drifting prevalence after the plagues, may have caused one or a few of these mutations to become more frequent than others. Obviously, the above estimates should be considered with great caution, but they do indicate that any frequent and dominantly-inherited disorder in Norway may be caused by founder mutations. Our intention with the given figures, is to indicate the low number of expected old mutations. The reference used [14] is not considered representative for our population. The example is to show how to estimate mutation numbers, all reasonable estimates of a priori mutation prevalences will give a low number of expected surviving mutations.

We have previously reported that our first series of breast cancer families from the Eastern part of Norway included multiple cases of BRCA1 1135insA mutations [9]. We have demonstrated that 15% of breast–ovarian cancer families from the South-West coast had BRCA1 1675delA mutations [10]. We have reported that 3% of incident ovarian cancer patients in the Southern part of the country had either 1135insA or 1675delA mutations [17]. We have described in a series of prospectively diagnosed inherited breast or ovarian cancers, that 68% of the mutation carriers had BRCA1 1135insA, 1675delA, 816delGT or 3347delAG. In the same series, 12 private mutations were described in one family each. How this series was selected, and the results, are discussed in detail in a previous report in Ref. [18]. In this report, we describe how we confirmed that the four mutations were more frequent than the twelve other mutations, how we located additional families with these four mutations, how we determined their geographical distribution and origin, and we compare the findings with historical information on the population.

2. Patients and methods

Initially, we had determined criteria to select kindreds with various inherited cancer syndromes [19]. Following media attention, thousands of individuals from all over the country referred themselves to benefit from the established follow-up examinations aiming at early diagnosis and treatment. The majority had familial breast cancer. This was unexpected, as familial colorectal cancer, familial ovarian cancer and familial prostate cancer were also invited to the clinic. Site-specific familial ovarian cancer was, besides a few small kindreds, not seen. Familial breast–ovarian cancer was a subgroup within the breast cancer kindreds and included most of the families with multiple cases of ovarian cancer. Initially conducted by the Unit of Medical
Breast–ovarian cancer cases in \textit{BRCA1}-mutated families will share alleles and haplotypes for intragenic markers (D17S855, D17S1322, D17S1323) or close (D17S1325) to \textit{BRCA1}, as will families with a common ancestor. In 1996, we identified 197 available families with a suitable structure and with breast or breast–ovarian cancers and established their \textit{BRCA1} haplotypes. The families were from all over the country. These 197 families were used as reference families.

We haplotyped all patients with prospectively demonstrated cancers at follow-up and with demonstrated \textit{BRCA1} mutations [18]. Reference families carrying the same haplotype as a prospectively-detected \textit{BRCA1} mutation-carrying patient were examined for that mutation.

The youngest available affected from each family in our nationwide series of breast cancer families was examined for the mutations found more than once in the combined series of prospective cancers and reference families.

Consecutive series of unselected breast or ovarian cancers cases from mid-Norway (Trondelag) were examined for presence of the 1675delA, 1135insA and 816delGT mutations.

All families with identified \textit{BRCA1} mutations were expanded by all possible means to obtain a complete family structure. Since the acceptance of clinical genetic services is high in Norway [20], most families could be expanded to include first- to third-degree cousins. All diagnoses were, whenever possible, confirmed in hospital medical files and/or The National Cancer Registry. All available affecteds were examined for the presence of the mutation, and all healthy adult family members were offered predictive testing. Informed consent was obtained in writing following genetic counselling for healthy family members according to our legislation on predictive genetic testing. The earliest known affected (typically three to five generations back) was identified from each family by a compilation of information from all members, and their birthplaces were established as postal zip codes.

Data was stored and analysed in computerised medical files in dBASE format and were exported to Cyrillic for graphic display of pedigree structures, and linked to a digital map of Norway with the ArcView software. The digital map included postal zip-codes and relevant demographic figures for the Norwegian population.

### 3. Results

As of October 2000, our medical files of familial cancer in Norway contained 15802 index patients, including 7065 cases with breast or ovarian cancer. To complete the structure for some of the kindreds, 16803 anonymous records of relatives to the patients were added, including 2802 cases with breast or ovarian cancer. Blood samples were obtained from 3123 patients with breast or ovarian cancer, of whom 1345 belonged to kindreds with suspected inherited cancer. In addition, 4185 young adult women at risk for breast cancer were followed-up. By December 1999, 82 initially healthy women had contracted breast and/or ovarian cancer. 40

#### Table 1

Norwegian \textit{BRCA1} founder mutations, their haplotypes, and number of mutation-carrying reference families, number of kindreds with each mutation, and number of demonstrated mutation carriers; see text for detailed explanations

<table>
<thead>
<tr>
<th>\textit{BRCA1} mutation</th>
<th>Haplotype(^a)</th>
<th>No. families identified in prospective cases series(^b)</th>
<th>No. (%) reference families with mutation(^c)</th>
<th>No. families identified in incident cases series(^d)</th>
<th>Total no. families with mutation</th>
<th>Total no. mutation carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1675delA</td>
<td>196-151-121-154(^e)</td>
<td>11</td>
<td>6 (3.0%)</td>
<td>13</td>
<td>37</td>
<td>137</td>
</tr>
<tr>
<td>1135insA</td>
<td>219'-145-124-162</td>
<td>6</td>
<td>5 (2.5%)</td>
<td>9</td>
<td>30</td>
<td>88</td>
</tr>
<tr>
<td>816delGT</td>
<td>198-143-121-154</td>
<td>5</td>
<td>3 (1.5%)</td>
<td>0</td>
<td>16</td>
<td>104</td>
</tr>
<tr>
<td>3347delAG</td>
<td>190-143-127-162</td>
<td>5</td>
<td>3 (1.5%)</td>
<td>–</td>
<td>17</td>
<td>65</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27</td>
<td>17 (8.6%)</td>
<td>22</td>
<td>100</td>
<td>394</td>
</tr>
</tbody>
</table>

\(^a\) Markers: D17S1325–D17S855–D17S1322–D17S1323. Marker D17S1325 is 600kb flanking, the others are intragenic.

\(^b\) This is the results previously published, see Ref. [18] for details.

\(^c\) The 197 reference families with assumed inherited breast cancer.

\(^d\) Previous [17] and present series combined.

\(^e\) Previously [10], the same haplotype was given as 195-xx-119-152, which is a calibration problem between two laboratories determining the same markers with different technology.

\(^f\) One family had a variant D17S1325 marker indicating a haplotype break.
(49%) of these prospectively-detected cases were identified as BRCA1 mutation carriers, whereas none had BRCA2 mutation [18]. Twelve of the 16 different BRCA1 mutations were identified in one kindred each, whereas four mutations were identified in more than one family.

By matching the BRCA1 haplotypes of each of the mutation carriers identified above, with the previously established haplotypes from the 197 breast cancer reference families, we identified 17 additional families with the four founder mutations (Table 1), but no additional families carrying the 12 private mutations previously described in Ref. [18] were found. Thus, this series confirmed that these four mutations were frequent, and the other 12 mutations were infrequent. It was outside our resources to screen all patients for unknown mutations. Subsequent screening of the breast cancer families and series of incident cancer patients was limited to the four founder mutations.

In the series of incident cancers taken from the Trondelag county in mid-Norway, we found that 2/60 (3%) ovarian cancer cases and 2/116 (2%) of breast cancer cases carried the 1135insA mutation, while 1675delA and 816delGT were tested for but not found.

Thirty-four families referred to our outpatient clinics were demonstrated to carry one of the four mutations. By all the different strategies together, the total number of 100 families and 394 individuals carrying either of the four founder mutations were identified (Table 1).

In addition to the five families previously haplotyped and demonstrated to carry a mutation [10], 8 prospectively-detected cases and 17 reference family cases with mutations were haplotyped in this study. All of these 30 families carried identical haplotypes for each mutation, except for one single flanking recombination between D17S855 and D17S1325 (600kb telomeric of BRCA1) in an 1135insA family. This further strengthens the common origin of the founder mutations.

The distribution of all living mutation carriers today paralleled the distribution of the general population. To elucidate the geographical origin of the four founder mutations, we traced the birthplace of the oldest affected females compatible with the recent cross-Atlantic emigration of about one million Norwegians, of whom a large number came from the South-West, and with the relative occurrence of the mutations in Norway and in North America. The 816delGT was found in 16 Norwegian and three US families, 3347delAG in 17 Norwegian and three US families and 1675delA in 37 Norwegian and 11 US families (as well as two Swedish families).

The findings of this study met our hypothesis that the uneven regional distribution of breast–ovarian cancer kindreds in the Norwegian population is due to the presence of founder mutations. We conclude that the great majority of today’s estimated 4000 BRCA1 mutation carriers are descendants of a small number of (possibly as few as four) survivors of the medieval Bubonic plagues 600 years ago. We have made no attempts to estimate the ages of these mutations. They may have been present before the plagues, since the combined probability that four new mutations have occurred and expanded in the limited population shortly after the plagues seems low.

We can only speculate that these founder mutations are of Norwegian origin. The 1135insA mutation has been reported a handful number of times from continental Europe, and around 10 times from the US [22], compared with 30 identified Norwegian 1135insA families. This mutation may have migrated from Northern to Central Europe during medieval or more recent inland trading journeys or, conversely, been introduced by early settlers and enriched in the agricultural areas of Norway. These figures are also compatible with the recent cross-Atlantic emigration of around one million Norwegians, of whom a large number came from the South-West, and with the relative occurrence of the mutations in Norway and in North America. The 816delGT was found in 16 Norwegian and three US families, 3347delAG in 17 Norwegian and three US families and 1675delA in 37 Norwegian and 11 US families (as well as two Swedish families).
Fig. 2. Birthplaces of oldest traceable affected or obligate carrier in each family, according to mutation. Birthplaces were mapped by postal zip-codes and located on a digital map of Norway with the ArcView® software. Numbers of families with each mutation are given in Table 1. All 100 families given in Table 1 are mapped, but numbers of families marked on the maps are less because some persons had the same postal zip-codes resulting in overlapping marks on the map. The concentration on certain areas was so high that giving each family a visible mark by dragging overlaps apart would have given a wrong impression of the distributions. (a) BRCA1 1135insA, (b) BRCA1 1675delA, (c) BRCA1 816delGT, (d) BRCA1 3347delAG.
The population-based sample of only 82 prospectively demonstrated cancers included 40 BRCA1 mutation carriers [18]. While there is no doubt that we have uncovered the major founder mutations, the low number of analysed cases and the selection procedures used to identify them, implies that additional frequent mutations may have been overlooked. Indeed, we have identified six families with BRCA1 G3297T, and four with BRCA1 3203del11, both mutations being restricted to families in Norway, Sweden and the US [22]. Since these mutations were revealed by other strategies than those described here, we did not include them in the present investigation and their prevalences remain to be described. The series also included BRCA1 mutations (such as 185delAG and 4184del4) observed in single families, but recognised as global founder alterations [22]. As previously reported in Ref. [18], we did find BRCA1 mutations in 21 out of 22 prospectively-detected epithelial ovarian cancers in breast–ovarian cancer families, suggesting that the mutation detection techniques used in this study are appropriate and that only a very few mutations are likely to have escaped detection.

We have no population-based figures for the prevalences of BRCA1 and BRCA2 mutations in the Nordic countries, but the relative prevalences within the group of inherited breast cancers seems to differ: we have reported 49% BRCA1 and 0% BRCA2 carriers [18]. In Sweden and Denmark, the proportions of BRCA1 and BRCA2 families were found to be 23 and 11%, respectively [23], whereas the corresponding figures in the Finnish population are even lower (10 and 11%, respectively) [24]. Iceland is an exception in this respect due to the strong impact of a single BRCA2 founder mutation, being responsible for approximately 8% of all breast and ovarian cancer cases and one third of all breast cancer families in this small population [25,26]. Norway, Sweden, Denmark and Iceland may be considered one ethnic group. The reported differences are parallel to the conclusions in this report, that subfractions of the populations have different prevalences due to genetic drift.

Unexpectedly, the prospective series did not include any BRCA2 mutation carriers. BRCA2 haplotype analysis of the reference families indicated intrafamilial associations with breast cancer, even if no extensive haplotype sharing between families was observed (data not shown). Although we cannot exclude the possibility that one or a few BRCA2 mutations have escaped detection due to technical reasons, the data suggested that other susceptibility genes may play a significant role in inherited breast cancer in Norway. Interestingly, such a putative gene was recently mapped within coupling distance of BRCA2 in families of Nordic origin [27]. If such a gene is demonstrated, we may be able to uncover any frequent mutations with this established material and with the same procedures as described in this study.

While this study was conducted, a governmental committee suggested that all new incident cancers may be examined for the presence of causative mutations [11]. This would pick up all families with founder mutations rapidly, give more precise prevalence estimates for each region, and provide population-based series for the determination of penetrances and expressions of the mutations. Cost-benefit calculations have indicated that this could be an efficient approach to improved healthcare [28]. The feasibility of this strategy was demonstrated in the study of the smaller series of incident cancers from Trondelag as reported here. Full mutation screening in all individuals with a possible predisposition for a dominantly-inherited disorder is presently unavailable or extremely expensive. The approach we have used to demonstrate the frequent BRCA1 mutations may be applied to any frequent dominantly-inherited disorder in populations like ours. Nordic regional laboratories that specialise in screening for locally frequent mutations should collaborate, and the mutation search in any patient could begin with a coordinated effect to demonstrate/exclude the most likely Nordic mutations, according to the birthplace and ethnic origin. Whenever a mutation carrier is demonstrated, the family may be expanded. Such a strategy may identify the majority of the mutation carriers within a short time and at a low cost.

In conclusion, we have shown that inherited breast cancer in Norway is characterised by the dominance of a low number of BRCA1 founder mutations. We suggest that this phenomenon is likely to be caused by past fluctuations in the population size, with severe bottlenecks followed by rapid expansion. We assume that similar founder effects may be present for other inherited diseases as well, including inherited breast cancer susceptibility genes that are not yet cloned, and that the Norwegian population offers an excellent opportunity to identify these genetic components.

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Appendix

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