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Large regional differences in the frequency of distinct *BRCA1/BRCA2* mutations in 517 Dutch breast and/or ovarian cancer families

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Abstract

In 517 Dutch families at a family cancer clinic, we screened for *BRCA1/2* alterations using the Protein Truncation Test (PTT) covering approximately 60% of the coding sequences of both genes and direct testing for a number of previously identified Dutch recurrent mutations. In 119 (23%) of the 517 families, we detected a mutation in *BRCA1* ($n=98$; 19%) or *BRCA2* ($n=21$; 4%). *BRCA1/2* mutations were found in 72 (52%) of 138 families with breast and ovarian cancer (HBOC), in 43 (13%) of the 339 families with breast cancer only (HBC), in 4 (36%) of 11 families with ovarian cancer only (HOC), and in nine of 29 families with one single young case (<40 years) of breast cancer. Between the different subgroups of families (subdivided by the number of patients, cancer phenotype and age of onset) the proportion of *BRCA1/2* mutations detected, varied between 6 and 82%. Eight different mutations, each encountered in at least six distinct families, represented as much as 61% (73/119 families) of all mutations found. The original birthplaces of the ancestors of carriers of these eight recurrent mutations were traced. To estimate the relative contribution of two important regional recurrent mutations (*BRCA1* founder mutation IVS12-1643del3835 and *BRCA2* founder mutation 5579insA) to the overall occurrence of breast cancer, we performed a population-based study in two specific small regions. The two region-specific *BRCA1* and *BRCA2* founder mutations were detected in 2.8% (3/106) and 3.2% (3/93) of the unselected breast tumours, respectively. Of tumours diagnosed before the age of 50 years, 6.9% (3/43) and 6.6% (2/30) carried the region-specific founder mutation. Thus, large regional differences exist in the prevalence of certain specific *BRCA1/BRCA2* founder mutations, even in very small areas concerning populations of approximately 200 000 inhabitants.

Keywords: Breast cancer; *BRCA1*; *BRCA2*; Mutation detection rate; Founder mutations; Population-based studies; Family cancer clinic

1. Introduction

Since the identification of the breast cancer susceptibility genes *BRCA1* [1] and *BRCA2* [2] in 1994 and 1995, respectively, a growing number of members from families with clustering of breast and/or ovarian cancer have sought genetic counselling [3]. In general, genetic testing of individuals at risk can only be offered when a specific mutation that segregates with the disease has been identified within the family. Both *BRCA1* and *BRCA2* are large genes and germline mutations in these genes are scattered throughout the coding sequences.

Both for practical and cost-effectiveness reasons, the probability that an individual with breast or ovarian cancer may have a mutation in *BRCA1/BRCA2* is an important consideration in genetic testing. Therefore models have been developed, based on characteristics such as age at diagnosis of breast cancer and the number of breast and/or ovarian cancer patients in a family, to predict mutation carrier status before testing [4–7].

The ethnic background of a patient can strongly influence these probability models. For example, Ash-

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kenazi Jewish breast cancer patients have significantly higher probabilities for carrying a *BRCA1* mutation [4]. This is explained by the fact that 3 *BRCA1/2* founder mutations (*BRCA1* 185delAG, 5382insC and *BRCA2* 6174delT) are encountered at frequencies of 1, 0.1 and 1.5%, respectively, in the Ashkenazi Jewish population [8,9]. Similar effects were observed in breast cancer patients from the Icelandic population, in which the *BRCA2* 999del5 founder mutation is prevalent (population frequency of 0.6%) [10,11].

In other countries, including The Netherlands, several recurrent mutations in the *BRCA1* and *BRCA2* genes have been described [12–21]. Thus far, haplotype analysis of Dutch recurrent mutations was consistent with a single origin of these mutations, indicating that they are founder mutations. In particular, the recurrent mutations IVS12-1643del3835 in *BRCA1* and 5579insA in *BRCA2*, highlighted in the present study, were also shown to be founder mutations [15,18].

By the end of 1998, 517 families with either clustering of breast and/or ovarian cancer or a single case of early onset breast cancer were registered at the Family Cancer Clinic of the Daniel den Hoed Cancer Center and/or the Department of Clinical Genetics of the Erasmus University Rotterdam. We determined family characteristics in terms of the age at onset of breast cancer, the presence of ovarian cancer and the number of affected individuals in the pedigree in relation to the percentage of mutations identified with a routinely applied set of mutation-detection methods.

Frequencies of *BRCA1* and *BRCA2* founder mutations detected in the Southwestern part of The Netherlands differed from those reported elsewhere in The Netherlands [16] (see the BIC database). Therefore, we looked more closely into the geographical origin of the families with an identified mutation and investigated the prevalence of certain founder mutations in population-based series of breast cancer patients from specific regions within the South-western part of The Netherlands.

2. Patients and methods

2.1. Families and geographical distribution of families with a mutation

A series of families with clustering of breast and/or ovarian cancer was referred for onco-genetic and medical counselling to our departments between 1 January 1994 and 1 January 1999; this closing date was chosen because the routinely applied mutation-detection methods at that time took 6 to 12 months. Eligible for the present study were all families out of these series in which *BRCA1/BRCA2* mutation analysis was performed ($n = 517$), according to a protocol approved by the Medical Ethical Committees of our institutes. In general, a family was eligible for screening for mutations in *BRCA1/BRCA2* when it met one of the criteria listed in Table 1. The number of first and second-degree relatives with breast and/or ovarian cancer was determined by the relationship of an affected relative to the nearest affected individual in the pedigree. Considering the high penetrance of *BRCA1/BRCA2* mutations in women, as well as the heterogenetic origin of breast cancer, we excluded second-degree affected relatives who were daughters of unaffected women, whereas second-degree affected relatives who were daughters of men were included.

For each family, a detailed pedigree encompassing at least four generations was constructed. Whenever possible, hospital records and pathology reports were collected from individuals with malignancies to confirm the diagnosis. Age at onset of breast cancer was registered in three categories: the number of relatives diagnosed before the age of 40 years, the number of relatives diagnosed from 40 to 49 years and the number of relatives diagnosed with breast cancer from the age of 50 years and over. Pedigree data were used to identify the ancestors most likely to have transmitted the genetic predisposition in each of the families. On average, such an ancestor was born around 1890. The place of birth of that ancestor was taken as the place of origin of a family. Occasionally, it was possible to link separate families of which the probands were not aware they were related; these families were then considered as one family.

2.2. Population-based breast cancer patients

In view of the results with respect to the geographical origin of the two founder mutations IVS12-1643del3835 (*BRCA1*) and 5579insA (*BRCA2*), we performed a population-based study for the prevalence of these two mutations (Fig. 1).

From previously isolated DNA of 1052 stored breast tumour samples which were sent to our regional central laboratory for routine steroid receptor assays [22], two

Table 1

Minimal criteria for *BRCA1/BRCA2* mutation analysis
- A single woman affected by breast cancer before the age of 40
- A single woman affected by both breast and ovarian cancer
- Two first- or second-degree[a] relatives affected by breast cancer, one of them diagnosed before the age of 45 years
- Two first- or second-degree[a] relatives, one of them affected by ovarian cancer and the other affected by breast cancer before the age of 50 years
- Two first- or second-degree[a] relatives affected by ovarian cancer
- Three first- or second-degree[a] relatives affected by either breast cancer or ovarian cancer

[a] Only second-degree relatives who were paternally related to another affected relative, and not maternally, were taken into account.

groups of breast cancer patients were selected on the basis of their region of residence: (a) patients ($n=106$) who at time of diagnosis were living in the region (West-Brabant) of clustering of the IVS12-1643del3835 *BRCA1* founder mutation; and (b) patients ($n=93$) from the region (Zuid-Beveland) of clustering of the 5579insA *BRCA2* founder mutation (see also Fig. 1). In both groups, no selection was made for age at diagnosis or family history.

These 199 DNA samples were irreversibly made anonymous, with only the geographical region of where the patient lived and the age at diagnosis recorded. The region was defined and registered as the zip code area. The Netherlands (population of approximately 16 million inhabitants) is divided into 90 zip code areas. All samples were tested for both mutations, and for 2804delAA which is one of the most frequently detected *BRCA1* mutation throughout the whole Dutch population.

2.3. DNA analysis

In 517 separate families, DNA analysis was performed using genomic DNA, preferably of all living affected relatives with breast and/or ovarian cancer. On average, 1.9 patients per family were tested. As screening of the entire coding sequences of both genes is costly and was not accessible in our clinical setting on a routine basis, we applied in all these families a set of mutation-detection assays covering at least 60% of the coding sequences of both genes. This set consisted of a Protein Truncation Test (PTT) of exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* that was performed as previously described [23] with minor modifications. In addition, single strand conformation polymorphism (SSCP) analysis of exon 2 of *BRCA1* (which included detection of the mutations 185insA and 185delAG); allele specific oligonucleotide hybridisation (ASO) analysis of the founder mutations 5382insC and IVS20+1G>A was performed. Finally, the founder mutations IVS12-1643del3835 and IVS21-36del510 were tested by a polymerase chain reaction (PCR) analysis specific for these large genomic deletions [15].

In a subset of 106 families, SSCP analysis of the remaining coding exons of the *BRCA1* gene was performed and in subset of 23 families, PTT analysis of the complete *BRCA2* gene from reverse transcriptase (RT)-PCR obtained products was undertaken. The *BRCA1/2* mutations identified by these additional analyses were not taken into account with regard to the proportion of mutations identified in relation to family characteristics (Table 2) to make comparisons between the subgroups possible. For the population-based study, DNA from tumour samples of breast cancer patients was tested with an ASO analysis for the *BRCA1* mutation 2804delAA and the *BRCA2* mutation 5579insA. Deletion-specific PCR analysis was used to detect the *BRCA1* mutation IVS12-1643del3835.

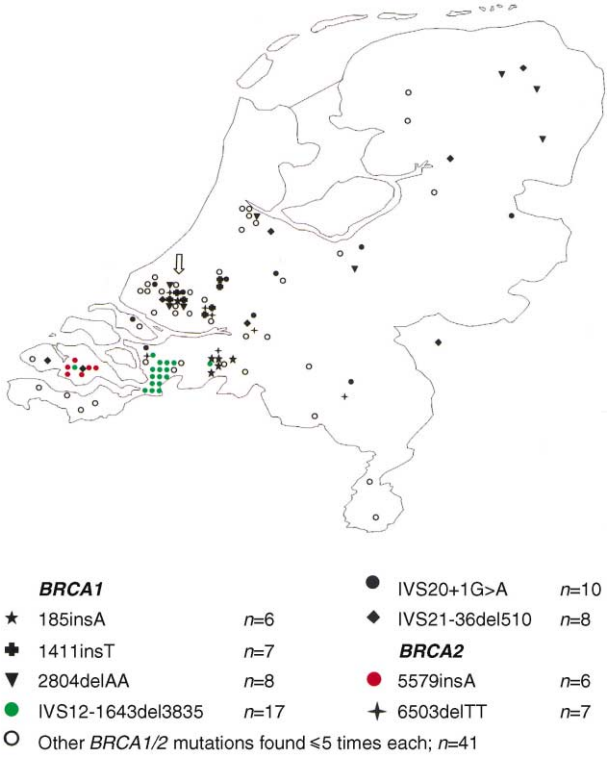

Fig. 1. Geographical distribution of the places from which each family with one of the eight recurrent *BRCA1*/*BRCA2* mutations originate; the arrow indicates Rotterdam.

Table 2
Frequency of *BRCA1* and *BRCA2* mutations, depending on the number, age at diagnosis and site of origin of the cancers in the family[a]

	0 BC below 40 years	1 BC below 40 years	⩾2 BC below 40 years	Total
HBC				
Total	172	118	49	339
Mutations				
BRCA1	8	14	9	31
BRCA2	3	4	4	11
Either gene	11 (6%)	18 (15%)	13 (27%)	42 (12%)
HBOC with 1 OC				
Total	47	30	17[b]	94
Mutations				
BRCA1	7	12	12	31
BRCA2	1	3	0	4
Either gene	8 (17%)	15 (50%)	12 (71%)	35 (37%)
H(B)OC with ⩾2 OC				
Total	27	12[b]	16[b]	55
Mutations				
BRCA1	12	7	8	27
BRCA2	2	1	1	4
Either gene	14 (52%)	8 (67%)	9 (56%)	31 (56%)

BC, breast cancer; OC, ovarian cancer; HBC, hereditary breast cancer; HBOC, hereditary breast and ovarian cancer; HOC, hereditary ovarian cancer.
[a] Only mutations detected with the routinely applied set of mutation-detection methods are shown.
[b] Taking these 3 subgroups together, the proportion of identified *BRCA1*/*BRCA2* mutations rose from 64% (29/45) to 78% (35/45) when results of the more extensive mutation analyses were included.

2.4. Statistical analysis

P values were calculated using the two-sided Fisher's Exact test. All analyses were performed using STATA 6.0 software.

3. Results

3.1. Family characteristics and mutation spectrum

Overall, in the 517 families 119 (23%) mutations in total were detected in *BRCA1* ($n=98$; 19%) and in *BRCA2* ($n=21$; 4%). Table 3 lists the general clinical characteristics of the families in which genetic analysis was performed and the number of mutations found per gene. In 52% ($n=72$) of 138 families with both breast and ovarian cancer (HBOC), a mutation was identified: in *BRCA1* in 46% ($n=64$) and in *BRCA2* in 6% ($n=8$). In families with breast cancer only (HBC), in 13% ($n=43$) of the 339 families a mutation was detected: in *BRCA1* in 9% ($n=31$) and in *BRCA2* in 4% ($n=12$); and in families with ovarian cancer only (HOC) in 36% ($n=4$) of 11 families a mutation was detected: in *BRCA1* in 27% ($n=3$) and in *BRCA2* in 9% ($n=1$).

Table 3
Frequency of *BRCA1* and *BRCA2* mutations in relation to the presence of breast cancer and ovarian cancer

Family characteristics	No of families	BRCA1 (%)	BRCA2 (%)	Either gene (%)
HBC				
1 patient with BC below 40 years	29	0	0	0
2 patients with BC	131	11 (8)	4 (3)	15 (11)
⩾3 patients with BC	208	20 (10)	8 (4)	28 (13)
HOC				
⩾2 patients with OC	11	3 (27)	1 (9)	4 (36)
HBOC				
1 patient with both BC and OC and ⩾1 patients with BC	27	14 (52)	2 (7)	16 (59)
1 patient with OC and ⩾1 patients with BC	67	22 (33)	3 (4)	25 (37)
2 patients with OC and ⩾1 patients with BC	27	15 (56)	2 (7)	17 (63)
⩾3 patients with OC and ⩾1 patients with BC	17	13 (76)	1 (6)	14 (82)
Total	517	98 (19)	21 (4)	119 (23)

BC, breast cancer; OC, ovarian cancer; HBC, hereditary breast cancer; HOC, hereditary ovarian cancer; HBOC, hereditary breast and ovarian cancer.

The majority (68%; 67 out of 98 families) of *BRCA1* mutations were found in the HBOC/HOC families, whereas less than half of *BRCA2* mutations were detected in the HBOC/HOC families (43%; 9 out of 21 families); this difference was statistically significant ($P = 0.04$).

Table 4 lists all 38 distinct mutations identified and the number of families in which each mutation was found. In addition, the total number of breast and ovarian cancer cases and relative percentages per mutation are shown. Fig. 2a and b show for each family the position of the mutation in the gene and the relative contribution of the number of breast cancer cases and ovarian cancer cases to the clinical phenotype. By far the most frequent mutation was the large 3.8 kb genomic deletion IVS12-1643del3835 encompassing exon 13 in *BRCA1*, which was found in 20 families with a total of 109 breast and/or ovarian cancer cases. Six of the *BRCA1* and two of the *BRCA2* recurrent mutations were encountered six times or more, together being responsible for 61% (73/119) of the families with a detected mutation.

Table 2 shows the probability of finding a *BRCA1* and *BRCA2* mutation with the routinely applied mutation screen, in relation to the cancer phenotype in the family. Initially, the number of affected relatives diag-

Table 4
Number of families for each mutation, and frequency of cases of breast cancer (BC) and ovarian cancer (OC) per mutation

BRCA1	Exon	No. of families	No. of BC/OC[a]	No. of BC (%)	No. of OC (%)
185insA	2	6	26	21 (78)	6 (22)
185delAG	2	3	9	8 (89)	1 (11)
W372X	11	1	2	1 (50)	1 (50)
1411insT	11	7	35	30 (86)	5 (14)
S510X	11	1	3	2 (67)	1 (33)
2312del5	11	3	8	7 (78)	2 (22)
Q780X	11	3	17	14 (82)	3 (18)
2524delTG	11	1	3	1 (33)	2 (67)
2765delTGC	11	1	6	4 (67)	2 (33)
2804delAA	11	8	27	17 (63)	10 (37)
E908X	11	4	19	12 (57)	9 (43)
2846del4	11	1	7	5 (71)	2 (29)
3604delA	11	1	2	2 (100)	0
3668delAGinsT	11	1	5	2 (40)	3 (60)
E1214X	11	1	5	5 (100)	0
3875del4	11	1	3	3 (100)	0
3889delAG	11	2	8	4 (44)	5 (56)
4284delAG	12	2	12	11 (79)	3 (21)
IVS12-1643del3835	13	20	109	82 (71)	33 (29)
R1443X	13	2	7	6 (75)	2 (25)
5149del4	17	1	3	3 (75)	1 (25)
5256delG	18	1	4	4 (80)	1 (20)
IVS18-1G > A	19	1	8	6 (67)	3 (33)
5382insC	20	5	19	16 (80)	4 (20)
IVS20 + 1G > A	20	10	30	26 (81)	6 (19)
5448insC	22	1	5	4 (67)	2 (33)
IVS21-36del510	22	9	35	30 (75)	10 (25)
IVS22 + 5G > A	22	1	14	11 (79)	3 (21)
Total		98	431	337	120
BRCA2	Exon	No of families	No of BC/OC[a]	No of BC (%)	No of OC (%)
862delAG	8	1	4	3 (75)	1 (25)
4682del4	11	1	3	3 (100)	0
4708insA	11	1	8	7 (64)	4 (36)
5578delAA	11	1	2	2 (67)	1 (33)
5579insA	11	6	22	13 (59)	9 (41)
S1882X	11	1	3	3 (100)	0
Y1894X	11	1	4	4 (100)	0
6503delTT	11	7	25	25 (93)	2 (7)
6872del4	11	1	4	3 (60)	2 (40)
9900insA	27	1	3	3 (100)	0
total		21	78	66	19

[a] Figures of breast and ovarian cancer do not add up because of cases with both breast and ovarian cancer.

nosed with breast cancer at ages 40–49 years and at ages ≥ 50 years were also taken into account for each of the subgroups of Table 2. However, these 2 parameters did not play a role in the probability of detecting *BRCA1*/*BRCA2* mutations once the classification according to the number of early onset breast cancer and number of ovarian cancers was made. The only exception was a minor influence of the number of breast cancer patients diagnosed between 40 and 49 years in families with HBC (data not shown).

The proportion of *BRCA1*/*BRCA2* mutations that was detected varied from 6% (11/172) in the HBC families without breast cancer patients diagnosed before the age of 40 years (Table 2), to 82% (14/17) in the HBOC families with 3 or more ovarian cancer patients and one or more breast cancer patients (Table 3). In our series, we recorded in total 13 male breast cancer patients in 12 different families. In 5 (42%) out of these 12 families, a mutation was detected: four *BRCA1* mutations (in three HBOC and one HBC families) and one *BRCA2* mutation (in a HBOC family).

3.2. Geographical distribution of families with a mutation

The geographical origin of the families with the eight most frequently occurring recurrent *BRCA1* and *BRCA2* mutations is shown in Fig. 1. Five pairs of

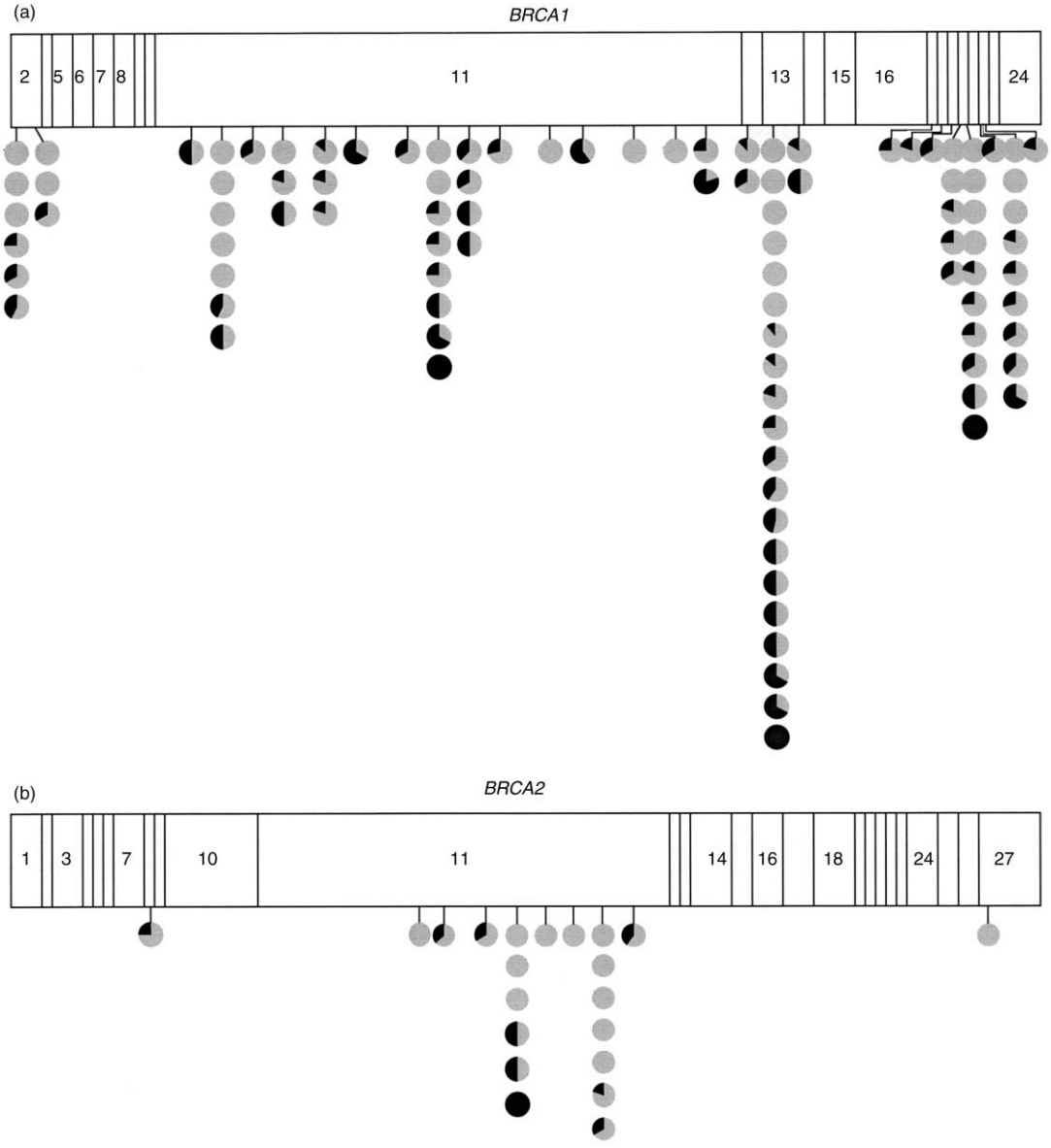

Fig. 2. (a) Position of germline mutations in *BRCA1* in 98 families; the ratio of cases of ovarian cancer (black) to cases of breast cancer in each separate family is represented within one circle. (b) Position of germline mutations in *BRCA2* in 21 families; the ratio of cases of ovarian cancer (black) to cases of breast cancer in each separate family is represented within one circle.

families, of which the probands were not aware that they were related, appeared to be linked; each of those pairs was mapped out as a single family.

The arrow in Fig. 1 refers to the urban area of Rotterdam, in which at present approximately 750 000 inhabitants are living. Geographical clustering was seen for a number of recurrent mutations, particularly the *BRCA1* mutations 185insA, 1411insT, IVS12-1643del3835, and the *BRCA2* mutation 5579insA. Most striking is the situation for the *BRCA1* IVS12-1643del3835 mutation and for the *BRCA2* 5579insA mutation: these cluster in two distinct, geographically adjacent regions of a number of small towns and villages that until now were independent rural districts with current populations of approximately 250 000 and 150 000 inhabitants, respectively.

3.3. Mutation analysis in population-based tumour samples

Of the 199 tumours selected for testing for either the *BRCA1* mutation IVS12-1643del3835 ($n=106$) or the *BRCA2* 5579insA mutation ($n=93$), the mean age at diagnosis was 57 years (range 24–85 years). In both regions, the 'region-specific' mutation was found in 3/106 (2.8%; breast cancers diagnosed at ages 34, 43 and 48 years) and 3/93 (3.2%; diagnosed at ages 42, 47 and 53 years), respectively, of the unselected breast tumours. In the '*BRCA1*-founder' region, the *BRCA2* founder mutation 5579insA and the other Dutch founder *BRCA1* mutation (2804delAA), were detected once (both 1/106; 0.9% age at diagnosis, 42 and 39 years, respectively). In the '*BRCA2*-founder region' none of the other two founder mutations were detected. Of the eight tumours with one of the three germline mutations, seven were diagnosed before the age of 50 years. If only breast tumours diagnosed below the age of 50 years were considered, the prevalence of these founder mutations in the regions of clustering was 6.9% (3/43) for the *BRCA1* mutation and 6.6% (2/30) for the *BRCA2* mutation. Regarding all tumours from both regions diagnosed before the age of 50 years, in 10% (7/73) one of the three *BRCA1*/*BRCA2* founder mutations was detected.

4. Discussion

In this report, we describe the results of a *BRCA1*/*BRCA2* germline mutation analysis in a large series of 517 families visiting our Family Cancer Clinic. Overall, we detected a *BRCA1* mutation in 19% of the families, while in 4% a *BRCA2* mutation was identified. In accordance with others, we found that the presence of ovarian cancer, early onset of breast cancer (<40 years), and increasing numbers of young affected women in a family, greatly enhanced the probability of finding a mutation [4–7,24]. In addition, our data confirm that apart from *BRCA2*, BRCA1 mutations are also involved in male breast cancer [2,24] and that both *BRCA1* and *BRCA2* analysis is warranted in HBC/HBOC families with a case of male breast cancer.

We detected no *BRCA1*/*BRCA2* mutations in 29 families with a single case of breast cancer before the age of 40 years. This seems to be in contrast with breast cancer population studies, where *BRCA1* or *BRCA2* mutations were identified in 5.9–9.4% of the patients diagnosed at ages below 35/36 years [25,26]. However, our 29 patients in fact were strongly selected for not having a positive family history for the disease since for each family an extended pedigree encompassing at least 4 generations was constructed. In contrast, in population-based studies cases with a positive family history for the disease will inevitably be included. Therefore, detailed pedigree analysis is an important tool in determining the probability of finding a mutation in *BRCA1*/*BRCA2*.

Currently, only a few studies describe a complete analysis of the coding sequences of the *BRCA1* and *BRCA2* genes in a series of families visiting a family cancer clinic [7,16,27]. With the set of mutation-detection methods completed in all 517 families, we analysed approximately 60% of the coding sequences of the *BRCA1* and *BRCA2* genes, and therefore will have missed an unknown number of mutations. Despite this limitation, our overall *BRCA1*/*BRCA2* mutation-detection rate in 138 HBOC families (52%) was similar to the *BRCA1*/*BRCA2* mutation-detection rate (50%) found by Frank and colleagues [7] in another large series of clinically ascertained HBOC families ($n=117$). This could indicate that we have detected the majority of identifiable mutations in these families. Moreover, our results appear to be nearly identical to those of two recently presented smaller studies involving 100 HBOC [27] and 268 HB(O)C families [28], respectively, analysing the complete coding sequence of *BRCA1* and/or *BRCA2*.

The two Dutch founder mutations in *BRCA1* (IVS12-1643del3835) and *BRCA2* (5579insA) were mainly detected in families originating from small, confined regions in the South-western part of The Netherlands. The cause of the geographical differences in the prevalence of founder mutations on such a small map-scale may be specific demographic or geographical conditions. In the 16th century, the region of clustering of the *BRCA1* founder mutation (West-Brabant) was nearly de-populated due to a religious war (Roman-Catholics against protestants); afterwards the region was re-populated by large scale reproduction of a limited number of people. Our findings may be explained by a founder mutation carried by one of these ancestors. Interestingly, one village in the *BRCA1* founder-region has already been shown to be a genetic isolate for other

inherited diseases [29]. In the past, religious preferences contributed also significantly to the isolation of communities in our country. We found that all ancestors of the families with the *BRCA1* founder mutations were Roman Catholics, while all ancestors of the families with the *BRCA2* founder mutation were protestant. Furthermore, the region of clustering of the *BRCA2* founder mutation (Zuid-Beveland) was a rather isolated island until the nineteenth century. Apart from migration-characteristics of a population, the time period of origin of the mutation is an important factor with respect to geographical clustering of founder mutations. In this respect, it is interesting to note that families with the Dutch *BRCA1* founder mutation 2804delAA, which was estimated to have originated about 32 generations ago, have places of origin more scattered across The Netherlands [14] (Fig. 1).

In order to estimate the clinical impact of these two specific founder mutations on breast cancer incidence in the two geographical regions, we performed a population-based study of breast tumours from these regions. First of all, it is noteworthy that there are no significant regional differences in the age-adjusted mortality rates from either breast or ovarian cancer in The Netherlands [30]. As much as 7% of breast tumours selected for age at diagnosis below 50 years, but unselected for family history, were due to the region-specific founder mutations only. In a British population-based study, 6.1% of patients with breast cancer at ages below 50 years were estimated to be carriers of any *BRCA1* or *BRCA2* mutation [25]. Since we tested for only three *BRCA1/2* mutations in the population study, all other mutations remained undetected. Thus, already a relatively large proportion of breast cancer below the age of 50 years from these two regions was due to the *BRCA1/BRCA2* founder mutations (10%; 7/73).

By further comparison, at least one of the three founder mutations in the Ashkenazi Jewish population and the single founder mutation in the Icelandic population are found in 14 and 7.7%, respectively, of women with breast cancer below the age of 50 years that were unselected for family history [11,31]. Finally, the percentage of mutations we detected in our population-based study was comparable to the prevalence of the total of *BRCA1* mutations identified in a hospital-based study of 642 breast cancer patients from the Western part of The Netherlands [32].

Mapping out the origin of the ancestors of the HBC/HBOC/HOC families may facilitate the search for as yet undetectable *BRCA1/BRCA2* mutations in families from the same geographical region by reconstructing haplotypes [19]. In well-defined populations, it may even be possible to map unknown breast cancer susceptibility genes using haplotype-sharing.

In conclusion, even in a small and densely populated industrial country such as The Netherlands, large regional differences may exist in the prevalence of a *BRCA1* and a *BRCA2* founder mutation. In addition to the familial cancer history (early onset breast cancer as well as ovarian cancer), knowledge about the presence and prevalence of founder mutations in specific populations is of importance for selecting families eligible for *BRCA1/BRCA2* analysis and will greatly facilitate the detection of mutations.

5. 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); Dutch database: http://ruly70.-medfac.leidenuniv.nl/~devilee/Lab/b1nl5.htm; http://ruly70.medfac.leidenuniv.nl/~devilee/Lab/b2nl5.htm; (for *BRCA1* and *BRCA2* mutations in The Netherlands); 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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