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A High Proportion of Novel Mutations in BRCA1 with Strong Founder Effects among Dutch and Belgian Hereditary Breast and Ovarian Cancer Families

T. Peelen,[1] M. van Vliet,[1] A. Petrij-Bosch,[1] R. Mieremet,[1] C. Szabo,[5] A. M. W. van den Ouweland,[6] F. Hogervorst,[8] R. Brohet,[9] M. J. L. Ligtenberg,[11,12] E. Teugels,[13] R. van der Luijt,[14] A. H. van der Hout,[15] J. J. P. Gille,[10] G. Pals,[10] I. Jedema,[1] R. Olmer,[3] I. van Leeuwen,[1,4] B. Newman,[16] M. Plandsoen,[6] M. van der Est,[6] G. Brink,[8] S. Hageman,[8] P. J. W. Arts,[11] M. M. Bakker,[11] H. W. Willems,[11] E. van der Looij,[11] B. Neyns,[13] M. Bonduelle,[13] R. Jansen,[14] J. C. Oosterwijk,[3,15] R. Sijmons,[15] H. J. M. Smeets,[17] C. J. van Asperen,[3] H. Meijers-Heijboer,[6] J. G. M. Klijn,[7] J. de Greve,[13] M.-C. King,[5] F. H. Menko,[10] H. G. Brunner,[11] D. Halley,[6] G.-J. B. van Ommen,[1] H. F. A. Vasen,[4] C. J. Cornelisse,[2] L. J. van 't Veer,[8] P. de Knijff,[1] E. Bakker,[1,3] and P. Devilee[1,2]

Departments of [1]Human Genetics, [2]Pathology, and [3]Clinical Genetics, Leiden University Medical Center, and[4]Foundation for the Detection of Hereditary Tumors, Leiden; [5]Department of Medicine, Division of Medical Genetics, University of Washington, Seattle; [6]Department of Clinical Genetics, Erasmus University and University Hospital, and [7]Daniel den Hoed Cancer Clinic, Rotterdam; Departments of [8]Pathology and [9]Epidemiology, The Netherlands Cancer Institute, and [10]Department of Clinical Genetics, Free University of Amsterdam, Amsterdam; Departments of [11]Human Genetics and [12]Pathology, University Hospital, Nijmegen; [13]Laboratory of Molecular Oncology, Department of Medical Genetics and Oncology, Free University Academic Hospital, Brussels; [14]Department of Human Genetics, University of Utrecht, Utrecht; [15]Department of Medical Genetics, University of Groningen, Groningen; [16]University of North Carolina, Chapel Hill; and [17]Department of Clinical Genetics, Limburg, Maastricht, The Netherlands

Summary

We have identified 79 mutations in BRCA1 in a set of 643 Dutch and 23 Belgian hereditary breast and ovarian cancer families collected either for research or for clinical diagnostic purposes. Twenty-eight distinct mutations have been observed, 18 of them not previously reported and 12 of them occurring more than once. Most conspicuously, a 2804delAA mutation has been found 19 times and has never been reported outside the Netherlands. A common haplotype spanning ⩾375 kb could be identified for each of the nine examined recurrent mutations, indicating the presence of multiple BRCA1 founder mutations in the Dutch population. The 2804delAA mutation has been estimated to have originated ∼32 generations ago. No specific breast or ovarian cancer phenotype could be assigned to any of the common mutations, and the ovarian cancer incidence among 18 families with the 2804delAA mutation was heterogeneous.

Introduction

BRCA1 is one of several genes that predisposes strongly to the development of breast and ovarian cancer (Ford and Easton 1995). The gene has been mapped to 17q12-q21 (Hall et al. 1990; Miki et al. 1994), and germ-line mutations have been identified in >300 families. They are nearly ubiquitously distributed over the coding region, and >85% are frameshift or nonsense mutations leading to premature termination of protein translation (Shattuck-Eidens et al. 1995; Couch et al. 1996). A number of mutations have been found repeatedly, reducing the number of distinct mutations to ∼130. Two of these, the 185delAG mutation and the 5382insC mutation, each represent ∼11% of all mutations thus far reported (Couch et al. 1996).

The incidence of specific mutations seems to be strongly dependent on the population from which the breast cancer families were ascertained. Thus the 185delAG mutation was found mainly in families of Ashkenazi Jewish origin (Tonin et al. 1995). Subsequently, a carrier frequency of ∼1% was estimated for the 185delAG mutation among Ashkenazi Jews unselected for the presence of either breast cancer or a positive family history (Struewing et al. 1995*a;* Roa et al. 1996), eight times the incidence of all mutations together in the general population (Ford et al. 1995). Specific mutations also have been recurrently detected in breast cancer families of Swedish, British, and Austrian origin (Gayther et al. 1995; Shattuck-Eidens et al. 1995; Johannsson et al. 1996; Wagner et al. 1996). Reconstruction of the haplotypes bearing some of these common mutations has provided evidence for strong founder effects (Simard et al. 1994; Johannsson et al. 1996; Neuhausen et al. 1996), although the

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Address for correspondence and reprints: Dr. Peter Devilee, Department of Human Genetics, University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands. E-mail: devilee@ruly46.medfac.leidenuniv.nl

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age at origin has been estimated for only a few of them (Neuhausen et al. 1996).

To further explore the existence of population-specific BRCA1 mutations, we have screened >650 families of Dutch and Belgian origin for the presence of mutations. Our results are in agreement with the concept that the incidence of specific BRCA1 mutations can be strongly influenced by the geographic origin of the study population and indicate that this is due to the presence of one or more founder mutations.

Subjects and Methods

Family Ascertainment

Families were accrued essentially through two routes: (1) as "research" families, which were ascertained by the Netherlands Foundation for the Detection of Hereditary Tumors and from which blood samples were taken by the Department of Human Genetics in the Leiden University Medical Center, and (2) as "counseling" families, which were recruited by self- or physician referral to a family cancer clinic or clinical genetics department in either Amsterdam, Brussels, Groningen, Leiden, Maastricht, Nijmegen, Rotterdam, or Utrecht. Research families were sampled when they contained at least three first-degree relatives with either breast or ovarian cancer, at least one of whom had been diagnosed at <50 years of age. Of the 176 families thus collected, there were 48 with at least four cases of breast cancer diagnosed at <60 years of age and with any number of ovarian cancers. Approximately one-third of the research families thus would meet current cancer-incidence eligibility criteria established by the Breast Cancer Linkage Consortium (Easton et al. 1995). Intake criteria for counseling families, as applied by the various centers, were heterogeneous both in terms of numbers and ages at onset of breast and ovarian cancers and in terms of the degree of kinship in the family. In general, families were offered DNA testing if their breast and/or ovarian cancer incidence suggested a ⩾10% prior probability of being due to BRCA1 (Shattuck-Eidens et al. 1995). In addition, some centers forwarded isolated unilateral breast cancer cases, diagnosed at either <40 years of age (Rotterdam) or <35 years of age (Leiden and Amsterdam), or bilateral breast cancer cases in which the first primary cancer had occurred at <40 years of age (Leiden). The center in Brussels used, as an additional criterion, at least two first-degree relatives with breast cancer diagnosed at <60 years of age. Counseling families were often less extensively sampled then research families. For all families, cancer diagnoses were confirmed by retrieval of pathology and/or medical records, wherever possible. Additionally, two DNA samples from Northern American families were included in the haplotype analysis. Both had the 2457C→T mutation also found in the Dutch series, and one derived from the population-based Carolina Breast Cancer Study.

Blood samples from a consecutive series of 662 breast cancer patients were collected during the years 1986–94. These patients were diagnosed in two hospitals in the Leiden area and were not selected for either family history or age at onset. DNA samples were irreversibly anonymized before being subjected to BRCA1-mutation screening.

Genetic Studies

Human genomic DNA was isolated from either 20 ml EDTA or heparinized blood samples (Miller et al. 1988). Total RNA was isolated from either peripheral blood lymphocytes by use of either Histopaque 1077 (Sigma) or lysed erythrocytes, followed by RNAzol B extraction as described by the manufacturer (Cinna; Biotecx Laboratories). Eight polymorphic microsatellite markers located on chromosome 17q and spanning a 5.7-cM interval including BRCA1 were used in a radioactive PCR under conditions described elsewhere (Cornelis et al. 1995). From centromere to telomere, the order reported in the Genome Database (GDB) is as follows: D17S250, THRA1, D17S1321, D17S855, D17S1322, D17S1323, D17S1327, D17S579. Primer sequences to amplify these markers can be retrieved online from the GDB. A physical map for a number of these markers has been resolved (Neuhausen et al. 1996), and these distances (in kb) are as follows: D17S1321–(225 kb)–D17S855–(5 kb)–D17S1322–(45 kb)–D17S1323–(100 kb)–D17S1327. The sex-average genetic distance between THRA1 and D17S579 is 3.2 cM (Easton et al. 1993). CEPH1347 was used as a reference to size alleles in terms of base pairs.

Screening for Mutations

A variety of methods were applied to detect the presence of a BRCA1 mutation in a total of 666 families (table 1). Chain-terminating mutations were screened for by the protein-truncation test (PTT) as described elsewhere (Hogervorst et al. 1995), with a nonradioactive modification performed in some of the participating centers. Exon 11 was examined completely from genomic DNA in virtually all of the families. When RNA was available, exons 2–10 also were screened by the PTT. One center used allele-specific oligonucleotide (ASO) hybridization, according to standard procedures (Saiki et al. 1986), to screen for known exon 11 mutations. Direct screening for deletions and insertions (DSDI) was performed with a multiplex PCR targeted at the mutations 185delAG (exon 2), 2804delAA (exon 11), 4184del4 (exon 11), and 5382insC (exon 20). Four primer pairs were designed, producing PCR products of different sizes—113, 130, 139, and 93 bp for the wild type of the four target sequences, respectively. Four microliters of ^{32}P-dCTP–labeled PCR product were electrophoresed on a 6% polyacrylamide gel (7 M urea, 1 × TBE) and were then fixed, dried, and exposed to a

Table 1

Number of Families Screened, by Various Techniques, in Seven Dutch Centers and One Belgian Center

	No. of Families									No. of Mutations Found
Technique	Leiden	Amsterdam	Rotterdam	Nijmegen	Utrecht	Groningen	Maastricht	Brussels	Total	
PTT, exon 11[a]	106	123	52	59	36	35	56	23	490	44
PTT, exon 11[b]	176								176	18
PTT, exons 2–10	25	57		26		3			111	0
PTT exons 12–24	10	57							67	0
DSDI[c]	138						56		194	5
DSDI[d]				50					50	1
ASO hybridization[e]			131						131	6
T-tracking, exon 2		52							52	0
Sequencing exon 2		57							57	3
Sequencing exons 16–24		28							28	0
SSCP[f]								23	23	2

[a] Families participated via family cancer clinics; all centers reported, on average, the same mutation rate.
[b] Families were collected for research purposes.
[c] For mutations in exon 2, exon 11 (2804delAA and 4184del4), and exon 20.
[d] For exons 2, 3, 5, 6, 20, 23, and 24.
[e] For mutations 185delAG, 1411insT, 1438delT, 2312del5, 2457C→T, 2804delAA, 2841G→T, 2846del4, 3604delA, 3668AG→T, 3759G→T, 3939insG, and 3960C→T.
[f] For exons 2, 5, and 20.

Konica AX X-ray film. One center used DSDI for the screening of exons 2, 3, 5, 6, 20, 23, and 24, using fluorescently labeled primers and analysis on the Perkin Elmer ABI 373A automated sequencer in the GeneScan mode. One center screened exon 2, using the T-reaction only in standard sequencing methodology. The Belgian center used SSCP analysis to screen for mutations in exons 2, 5, and 20. Primer sequences to obtain PCR products and to detect the mutation of interest are either retrievable electronically from the Breast Cancer Information Core (BIC) (1996) Web site (http://www.nhgri.nih.gov/Intramural research/Lab transfer/Bic) or available on request, for all applied methodologies. All mutations detected by the various screening methods were verified by direct sequencing using either a Pharmacia ALF or Perkin Elmer ABI 373A or 377 automated sequencer and laser detection of fluorescently labeled primers or nucleotides, according to the suppliers' protocols. Mutations were classified according to the nomenclature recently proposed elsewhere (Beutler et al. 1996), except for the 185delAG and 5382insC, to avoid confusion with other publications concerning these common mutations.

Allele Frequencies in the Dutch Population

The allele frequencies at THRA1, D17S855, and D17S579 were determined by typing a panel of 168 unrelated Dutch control individuals. Obtained allele frequencies were essentially comparable to those reported in the GDB, except for some alleles of THRA1. The 167-bp allele, reported in 13% of the Caucasian individuals, was found in 32% of the Dutch control population. The 165-bp allele was not found among controls, whereas the GDB reports a frequency of 13% in Caucasians. A discordancy in allele sizes was noted for THRA1, for which we determined allele lengths to be an odd number of bases whereas the GDB reports even-numbered allele lengths.

Statistical Analysis

For dating the origin of the 2804delAA mutation, two algorithms were used—one developed to compute the age of the idiopathic torsion dystonia mutation in Ashkenazi Jews (Risch et al. 1995) and one developed to derive the age of six BRCA1 mutations (Neuhausen et al. 1996). Both give an estimate of the number of generations (G) since the origin of the mutation. G depends on the progenitor marker-allele frequency in the normal population, the recombination fraction between the marker allele and the disease locus, and Q, the observed frequency of disease chromosomes not carrying the progenitor marker allele. The analysis was performed under the assumption that the risk alleles for the flanking and intragenic microsatellite markers were as follows: 167 bp (THRA1), 145 bp (D17S1321), 155 bp (D17S855), 133 bp (D17S1327), and 119 bp (D17S579). The age was determined by use of either the physical distances (under the assumption that 100 kb corresponds to a genetic distance of 0.1 cM) (Neuhausen et al. 1996) or the sex-average recombination fractions (Easton et al. 1993). The sex-average distance be-

tween THRA1 and D17S579 is 3.2 cM, and the disease locus, coinciding with D17S855, was assumed to lie halfway between (Anderson et al. 1993).

Results

A total of 666 families were screened for the presence of a BRCA1 mutation, by use of a variety of methodologies, in seven different centers in the Netherlands and one center in Belgium. Exons 2 and 11 were most consistently investigated (table 1). This revealed 79 families with a mutation (table 2), 8 of which have been reported elsewhere (Hogervorst et al. 1995). Twenty-two families were found among the 176 collected for research purposes, and the remaining 57 were among the 490 families that are part of genetic-counseling programs, for which different intake criteria apply (see Subjects and Methods). For each family, at least one individual with the highest probability of being a gene carrier, generally an early-onset breast or ovarian cancer patient, was selected for mutation testing.

Mutation Spectrum

The 79 mutations (table 2) comprise 28 different alterations, 18 of which had not been reported in the BIC database (Couch et al. 1996) by investigators other than those participating in this study at the time that the manuscript of this paper was submitted. The 12 recurrent mutations accounted for 63 (80%) of the 79 families in which a BRCA1 mutation was detected. Most conspicuous was the occurrence of the 2804delAA mutation, which was observed in 19 families and has never been reported elsewhere. Six Dutch centers reported families with this mutation, and so they do not seem to cluster in a highly specific geographic area of the Netherlands. Similarly, the 1411insT and 2312del5 mutations, found in seven and eight families, respectively, are novel and were found in different centers. These three mutations together thus comprise 34 (43%) of 79 families in which a mutation was detected. The known frequent mutations, 185delAG and 5382insC, were found in eight and two families, respectively. The Dutch BRCA1-mutation spectrum thus consists of a very high proportion of novel changes, relative to the spectrum currently available (Couch et al. 1996). Since the

Table 2

Dutch and Belgian BRCA1 Mutations

Mutation	Effect on Protein	No. of Families	Reported outside Netherlands/Belgium?[a]
185delAG	Asn 23 end 39	8	Y
Unknown	Cys 64 end 310 (RNA)	1	N
IVS5+3A→G	del exon 5	1	N
1135delA	Val 339 end 340	1	N
1135insA	Val 339 end 345	1	Y
1240delC	Thr 374 end 376	1	Y
1406insA	Glu 430 end 434	2	N
1411insT	Leu 431 end 434	7	N
1438delT	Leu 440 end 439	2	N
1623del5	Leu 502 end 505	1	Y
2140delC	Leu 674 end 699	1	N
2312del5	Glu 732 end 736	8	N
2331del4	Val 738 end 751	1	N
2457C→T	Gln 780 end 779	3	Y
2804delAA	Gln 895 end 901	19	N
2841G→T	Glu 908 end 908	3	Y
2846del4	Asn 909 end 998	1	N
3109insAA	Asn 998 end 1001	2	N
3604delA	Asp 1162 end 1209	4	Y
3668AG→T	Lys 1138 end 1209	1	N
3759G→T	Glu 1214 end 1214	1	N
3780G→T	Glu 1221 end 1221	1	N
3867G→T	Glu 1250 end 1250	1	Y
3875del4	Leu 1252 end 1262	1	Y
3939insG	Val 1274 end 1286	3	N
3960C→T	Gln 1281 end 1281	1	Y
5382insC	Gln 1756 end 1892	2	Y
5389del7	Asp 1796 end 1800	1	N

[a] As in the BIC database, as of December 15, 1996 (Breast Cancer Information Core 1996). Y = yes; and N = no.

2804delAA mutation alone comprises 24% of all mutations found, we investigated its incidence by DSDI among a series of 662 breast cancer patients, consecutively collected and unselected for either family history or age at onset. One patient, who had breast cancer at 41–45 years of age, was found to carry the mutation in lymphocyte DNA. The 185delAG, 4184del4, and 5282insC mutations were not found.

Haplotype Analysis

Disease-associated haplotypes of 9 of the 12 recurrent mutations were reconstructed by typing three intragenic markers (D17S855, D17S1322, and D17S1323) and five flanking markers (table 3). For the 2804delAA mutation, this analysis was completed in 18 of 19 families, and unambiguous phasing of disease haplotypes at the three intragenic markers was possible in 8 of them. These families were completely concordant for the 155-122-151 haplotype. In at least three families, haplotype conservation extended proximally to D17S250, but D17S579 at the distal end was recombinant in all three. The 156-, 122-, and 151-bp alleles at D17S855, D17S1322, and D17S1323, respectively, were observed in all 18 families with the 2804delAA mutation, and the same was true for both the 145-bp allele at D17S1321 and the 133-bp allele at D17S1327. This indicates that a segment is conserved among 8 families—and, possibly, among all 18 families—and spans a region between 375 kb (defined by D17S1321 and D17S1327) and 3.2 cM (defined by THRA1 and D17S579).

The shared haplotypes among five families with the 185delAG mutation cover the same region as that shared among the 2804delAA-mutation carriers (table 3). It is noteworthy that the linked alleles at the intragenic markers D17S1321, D17S855, and D17S1327 are identical to the previously reported disease-associated alleles for Ashkenazi Jewish families carrying the 185delAG mutation (Simard et al. 1994). For families with the 2312del5 and 1411insT mutations, the shared haplotype also might extend from THRA1 to D17S579. However, among seven families with the 2312del5 mutation, D17S855 differs, with one dinucleotide (CA) repeat unit from the most commonly shared allele, in one family (RUL57). Likewise, among five families with the 1411insT mutation, a three-(CA)-unit difference was noted at D17S1323 in family B7489. Given the allele lengths at flanking markers in family RUL57, a mutation in the repeat block is the most likely explanation for this difference (Weber and Wong 1993; Neuhausen et al. 1996); in B7489 a recombination event cannot be fully excluded. Finally, the haplotypes associated with any of the other mutations that were found more than once also suggested that they each have a common origin (table 3). The 2457C→T mutation, which was found three times in the Netherlands, was also reported in two Northern American families (Friedman et al. 1995; B. Newman, unpublished data). Analysis of the disease-carrying haplotype nonetheless supported a common origin. The index case of one of these families reported a Dutch ancestry; the other is of unknown but supposedly western European ethnic background.

Age at Origin of the 2804delAA Mutation

The allele frequencies of THRA1, D17S855, and D17S579 were determined in a control population of 168 individuals (data not shown). The frequency of the 155-bp allele at D17S855 was 7%. Since the 2804delAA mutation is associated with this allele in at least eight kindreds, this makes it extremely unlikely that the mutation occurred twice independently on the same haplotype.

By use of THRA1 and the disease locus (coincident with D17S855) at a genetic distance of 1.6 cM, the origin of the mutation was estimated to have been ~15 generations ago. However, when D17S579 was used (also at 1.6 cM), the estimated age was 49 generations. The average number of generations when data from both markers were used was 32. When the algorithm developed by Goldgar and coworkers (Neuhausen et al. 1996) was used on the same data set, 35 generations were derived (1-LOD-unit support interval = 14–59), with .97 being the estimated proportion of families due to the mutation occurring on the presumed ancestral haplotype.

Genotype-Phenotype Correlation

Table 4 lists the incidence of breast and ovarian cancer in the families with any one of the five most frequently found mutations. Among 18 families carrying the 2804delAA mutation, a heterogeneous phenotype was observed. Some families had only breast cancer, with or without bilateral incidence; others contained either ovarian cancer cases only or both breast and ovarian cancer cases. A remarkable paucity of ovarian cancer relative to breast cancer was observed in the families with the 2457C→T mutation. Also, the families with either the 185delAG, 2312del5, or 1411insT mutation did not show a consistent phenotype, in terms of breast or ovarian cancer incidence.

Discussion

We have screened >650 Dutch and Belgian breast and ovarian cancer families for the presence of gene alterations in BRCA1 and have found that, of the 28 distinct mutations detected in 79 families, 18 have not been reported elsewhere. In addition, 12 mutations were found more than once in a total of 63 families (80%). Although difficult to compare, the proportion of mutations accounted for by the 185delAG mutation and 5382insC was not significantly different from that reported in other studies (Shattuck-Eidens et al. 1995;

Table 3

Haplotyping of 47 Dutch and Belgian BRCA1 Families

Mutation and Family	Haplotype at Marker							
	D17S250	THRA1	D17S1321	D17S855	D17S1322	D17S1323	D17S1327	D17S579
2804delAA:								
RUL60	164	167	145	155	122	151	133	119
MOC52	164	167	145	155	122	151	133	111
PFT8	164	167	145	155	122	151	133	127
N3172	156–166	177–179	145	155	122	151	133	119–113
MOC440	164–162	167	145–155	155	122	151	133	127
MOC493	164–152	167–169	145	155	122	151	133	119–113
MOC506	164–156	167	145–155	155	122	151	133	111–129
EUR13	164	167–173	145–155	155	122	151	133	119–129
MOC66	164–152	167–169	145–153	155–147	122–128	151	133–157	125–127
RUL82	154	171	145	155	122–125	151	133	119
B5513	164	167	145	155	122	151–157	133	119
MOC559	164–162	167	145	155–145	122–125	151–159	133–167	119–127
B7178	152–154	171	145–155	155–147	122–125	151–157	133–159	111–123
PFT7	164–158	167	145	155–145	122–125	151–157	133–161	111
B7533	152–164	167–171	145–149	155–145	122–125	151–157	133–157	109–113
G394	162–168	167–175	145–155	155–151	122	151	133	119–131
U644	—	171–159	145–153	155–151	122	151	133	—
U484	—	167	145–155	155–143	122–125	151–157	133–165	121–123
185delAG:								
RUL85	156	177	155	145	128	157	163	113–121
RUL26	158	167	155	145	128–122	157–151	163	123
RUL101	150	167	155	145	128	157	163	113
N3538	152–154	171–173	155–157	145–151	128–122	157–151	163–133	113–115
B7219	160	167	155–145	145	128	157	163	123
2312del5:								
RUL21	150	181	145	149	122	151	133	125
RUL57	164–154	171	145	147	122	151	133	125
MOC580	164	171	145–163	149	122	151	133	121
N3507	152–156	171–175	145	149–151	122–119	151	133	121–125
BEL030	164–152	171	145–147	149	122–128	151–157	133	125
BEL049	—	171	145	149	122–125	151	133	125–113
PFT135	—	181–175	145–147	149–143	122–125	151	133	—
1411insT:								
EUR17	150	175	153	145	122–125	151–159	161	111
EUR33	150	175	153	145	125	159	161	123
MOC549	150	175	153	145	125	159–157	161	123
M51013	150–160	175–171	153–151	145–151	125–122	159–151	161–133	123–121
B7489	154	171	153	145	125	153	161	111–125
2457C→T:								
RUL47	—	171	149	145	131	157	167	125
RUL49	—	171	149	145	131	157	167	117
FAM7	—	171	149	145	131	157	167	117
930654	—	171	149	145	128–122	157	167	115–123
1438delT:								
MOC46	—	169	157	153	122	151	133	125
PFT9	—	169–175	—	149–153	122–125	151	133	125–129
3604delA:								
MOC662	—	167	—	145	128	157	133	111–113
N3110	—	161–171	145	145–151	122–131	157–151	133–165	121–125
3939insG:								
MOC3	—	145	147–153	145–153	122	151	133	123–117
RUL77	—	145	143	145	122	151	133	123
5382insC:								
EUR5	—	167	153	151	122	151	129	121
BEL002	—	169–171	149–153	151–145	122–125	151–157	129–167	115–127

Table 4

Incidence of Breast and Ovarian Cancer in Families Carrying One of the More Frequent Founder Mutations

Mutation	No. of Cases[a]		No. of Families		
	Breast Cancer[b]	Ovarian Cancer	Breast Cancer Only	Ovarian Cancer Only	Breast and Ovarian Cancer
185delAG	24 (13)	8 (4)	4	0	4
1411insT	23 (20)	8 (8)	2	1	4
2312del5	9 (9)	7 (4)	3	1	2
2457C→T	24[c] (22)	6 (5)	1	0	3
2804delAA	36 (22)	19 (9)	7	5	6

[a] Numbers in parentheses are number of cases for which pathological or medical records were available.
[b] Bilateral breast cancer cases were counted as two separate primary breast tumors.
[c] Includes one case of male breast cancer.

Couch et al. 1996). These mutations are particularly frequent among Ashkenazi Jews (Roa et al. 1996). The families under study here have not been selected for ethnic background, although two families with the 185delAG mutation were reported to be of Jewish origin. The 4184del4 mutation, which frequently is found among breast-ovarian cancer families in the United Kingdom (Gayther et al. 1995) and which would be missed by our PTT, was not detected in any Dutch family by DSDI. Our results thus add further weight to the concept that the proportions of specific BRCA1 mutations are strongly dependent on the ethnic background of the study population. Specific and novel mutations in BRCA1 also have been detected in Swedish, Austrian, Norwegian, and Italian families (Andersen et al. 1996; Caligo et al. 1996; Johannsson et al. 1996; Wagner et al. 1996). This will have important consequences for the routine of genetic testing, for which assessing the ethnic origin of the index case may direct mutation screening to specific regions of the gene.

The Netherlands and Belgium are neighboring countries and occupy a relatively small geographic area of northwestern Europe. Until ~150 years ago, the Flemish part of Belgium and the Netherlands formed one nation. Their joint current population of ~20 million has remained fairly immobile and isolated until post–Second World War times. These conditions may have led to the fixation of quite a distinct spectrum of BRCA1 mutations. It is noteworthy that the 3604delA mutation reported here was submitted recently to the BIC database by a group from Düsseldorf, just across the Netherlands' border, but has not yet been reported outside this region. Although the Dutch population contains substantial ethnic minorities from Indonesia, Turkey, Morocco, and Surinam, the age at origin of BRCA1 mutations likely predates their recent immigrations, and families with these backgrounds are very rarely seen in family-cancer clinics. We are convinced therefore that the obtained mutation spectrum genuinely derives from a Dutch Caucasian White population.

It is difficult to estimate how representative this spectrum is of the general Dutch population. In none of the centers did the mutation-screening approach cover the complete coding region, and the possibility that other frequent but as yet unknown mutations exist among the breast cancer families studied here cannot be excluded. In particular, since PTT was the most broadly used screening module, we will have underestimated the incidence of missense mutations. Yet, for exon 11, we probably have obtained a representative mutation set, since 94% of all exon 11 mutations lead to premature chain termination during translation (Couch et al. 1996), and 90% of the 3.4-kb exon is screened reliably by PTT (Van der Luijt et al. 1994; Hogervorst et al. 1995). A selection for families whose cancer incidence has a high prior probability of being due to BRCA1, in the presence of a genotype-phenotype correlation, also might affect the mutation spectrum. The intake criteria applied for research families were different than those applied for families asking for genetic advice via genetic-counseling centers. Both criteria are expected to give rise to a mixture of high- to medium-risk families with considerable but partial overlap. For example, two sisters each diagnosed with breast cancer at <40 years of age would not be eligible under the research criteria but would be eligible under the counseling criteria. Intriguingly, the BRCA1 mutation–identification rate in both groups of families was approximately the same. This rate is lower than those reported by others, but those studies have selected families on the basis of linkage analysis and/or ovarian cancer incidence (Castilla et al. 1994; Friedman et al. 1994; Struewing et al. 1995*b*; Gayther et al. 1995; Serova et al. 1996), which will strongly increase the proportion due to BRCA1. A complete description of all families, in terms of breast and ovarian cancer incidence, in conjunction with a complete screening of the

entire BRCA1 coding region, would be required in order to resolve this.

Haplotype analysis of 9 of the 12 recurrent mutations indicated strong founder effects, in particular for those mutations that occurred more than five times. At the intragenic markers, the obtained haplotypes for the 185delAG mutation were identical to those reported elsewhere (Simard et al. 1994; Friedman et al. 1995). Except for two families, we were unable to retrieve evidence that the families with the 185delAG are of Ashkenazi Jewish origin, but this mutation has been estimated to have arisen ~38 generations, or ~760 years, ago (Neuhausen et al. 1996). Several waves of eastern European immigrants during several centuries therefore render it conceivable that this mutation also migrated to the Netherlands. Intriguingly, the 2804delAA mutation appears to have arisen at approximately the same time. It is striking that the haplotypes carrying any one of the nine recurrent mutations investigated here are all in agreement with the possibility that they each have descended from a common ancestor. This is in contrast with haplotype data obtained on the 185delAG and 4184del4 mutations, which provide evidence for multiple, independent mutational events (Berman et al. 1996; Neuhausen et al. 1996). Our data nonetheless suggest that a considerable number of the mutations known to date, including the ones that occur less frequently but particularly those that are unique to certain geographic areas, might have arisen once. It should be noted that no de novo BRCA1 mutation has been reported yet, and our data and those of others (Neuhausen et al. 1996) suggest that these mutations will be extremely rare.

The 2804delAA mutation, although accounting for 24% of all mutations in the families studied here, was found only once among 662 breast cancer cases consecutively collected in the Leiden area and unselected for either age at diagnosis or family history. If the mutation distribution found in these families also applies to unselected cases, and if we correct for the fact that an estimated 60% of the current mutation spectrum in the BIC would have been identified by our screening protocols, then the estimated population gene frequency of BRCA1 (Ford et al. 1995) predicts 1.6 carriers among 662 cases (1.7% × 24% × 60% × 662). This suggests that the frequency of BRCA1 in the Dutch population is not higher than that estimated by linkage (Ford et al. 1995) and that the high proportion of the 2804delAA mutation is due mainly to the founder effect. Although the mutation was identified by six centers located in all corners of the Netherlands, this area is still small relative to the whole of Europe, which would be in agreement with the low migration rates of the Dutch.

No consistent phenotype, as defined by occurrence of breast and ovarian cancer (table 4), could be discerned among 18 families with the 2804delAA mutation. Some families contained breast cancer cases only, and others contained only ovarian cancer cases, although the number of cases per family was small because many were ascertained through family-cancer clinics. For the five most frequently encountered mutations, the breast:ovarian cancer-incidence ratio was 2.5 (table 4), in agreement with our observations elsewhere (Peelen et al. 1996). Friedman et al. (1995) also found that the expressivity of the 185delAG mutation varies, from early-onset breast cancer and ovarian cancer to late-onset breast cancer without ovarian cancer. Linkage analysis has indicated that ovarian cancer risk in BRCA1-linked families is heterogeneous (Ford et al. 1994). Subsequently, mutations occurring before codon 1435 in exon 13 were found to confer a significantly higher ovarian cancer risk than was found in those occurring after this point (Gayther et al. 1995). We cannot confirm this observation here, mainly because almost all mutations occur before codon 1435. Yet, we found 13 families in which only breast cancer was ascertained that are not in agreement with it, most conspicuously the families with the 2457C→T mutation. Alternatively, a genetic modifier of ovarian cancer risk might exist that is closely linked to BRCA1. It therefore will be of interest to more accurately determine the extent and overlap of the shared haplotype among families with an identical mutation but with distinct phenotypes or different ethnic backgrounds.

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