European Journal of Human Genetics (2001) 9, 773–779
© 2001 Nature Publishing Group All rights reserved 1018-4813/01 $15.00

www.nature.com/ejhg

ARTICLE

Haplotype analysis in Icelandic and Finnish *BRCA2* 999del5 breast cancer families

Rosa B Barkardottir*[,1], Laura Sarantaus[2], Adalgeir Arason[1], Paula Vehmanen[2], Pär-Ola Bendahl[3], Tommi Kainu[4], Kirsi Syrjäkoski[4], Ralf Krahe[5], Pia Huusko[6], Seppo Pyrhönen[7], Kaija Holli[8], Olli-P Kallioniemi[4], Valgardur Egilsson[1], Juha Kere[5,9] and Heli Nevanlinna[2]

[1]Department of Pathology, University Hospital of Iceland, Iceland; [2]Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, Finland; [3]Department of Oncology, University Hospital of Lund, Sweden; [4]Laboratory of Cancer Genetics, Institute of Medical Technology, Tampere University Hospital, Finland; [5]Department of Medical Genetics, University of Helsinki, Finland; [6]Department of Clinical Genetics, University of Oulu and Oulu University Hospital, Finland; [7]Department of Radiotherapy and Oncology, Turku University Hospital, Finland; [8]Department of Oncology, Tampere University Hospital, Finland; [9]Finnish Genome Centre, University of Helsinki, Finland

The 999del5 mutation is the single, strong *BRCA2* founder mutation in Iceland and the most common *BRCA1/2* founder mutation in Finland. To evaluate the origin and time since spreading of the 999del5 mutation in Iceland and in Finland, we constructed haplotypes with polymorphic markers within and flanking the *BRCA2* gene in a set of 18 Icelandic and 10 Finnish 999del5 breast cancer families. All Icelandic families analysed shared a common core haplotype of about 1.7 cM. The common ancestors for the Icelandic families studied were estimated to trace back to 340–1000 years, not excluding the possibility that the mutation was brought to Iceland during the settlement of the country. Analysis of the Finnish families revealed two distinct haplotypes. A rare one, found in three families in the old settlement region in southwestern Finland, shared a four-marker (0.5 cM) core haplotype with the Icelandic 999del5 haplotype. A distinct ~6 cM haplotype was shared by seven 999del5 Finnish families estimated to have a common ancestry 140–300 years ago. These families cluster in two geographical regions in Finland, in the very same area as those with the rare haplotype and also in the most eastern, late settlement region of Finland. The results may indicate a common ancient origin for the 999del5 mutation in Iceland and in Finland, but distinct mutational events cannot be ruled out. The surprising finding of the same mutation in two completely different haplotypes in a sparsely populated area in Finland may suggest gene conversion. *European Journal of Human Genetics* (2001) **9,** 773–779.

Keywords: Founder mutation; *BRCA2*; hereditary breast cancer; mutation age estimation

Introduction

The two major high-risk breast cancer susceptibility genes, *BRCA1* and *BRCA2*,[1–3] have been widely studied in different populations, and at the Breast Cancer Information Core database, more than 1700 different mutations have been reported. Most of the mutations have been reported only once (about 1100) but several population-specific founder mutations, as well as founder mutations found in geogra-

*Correspondence: Rosa B Barkardottir, Laboratory of Cell Biology, Department of Pathology, University and National Hospital of Iceland, House 14, v/Eiriksgötu, Reykjavik, Iceland. Tel: +354 5601906; Fax: +354 5601943; E-mail: rosa@landspitali.is
Received 22 March 2001; revised 6 August 2001; accepted 15 August 2001

phically diverse populations have been described.[4] The proportion of non-recurrent *versus* founder *BRCA1* and *BRCA2* mutations varies widely among populations, reflecting historical influences of migration, population structure, and geographic or cultural isolation.

Only one mutation has been identified for each of the *BRCA1* and *BRCA2* genes in Icelandic breast cancer families. In *BRCA1*, a splice site mutation in exon 17, 5173 G>A, has been identified in three families sharing a common haplotype.[5] In *BRCA2*, a 5 bp deletion in exon 9[3] has been found in the majority (2/3) of high-risk Icelandic breast cancer families[6,7] and to a lesser extent (1/3) in randomly selected pairs of sisters diagnosed with breast cancer at the age of 60 years or younger.[8] The population prevalence of this mutation in Iceland is 0.4%, and among breast cancer or ovarian cancer patients, 8.5 and 7.9% respectively.[9] The 999del5 mutation is also found in 40% of Icelandic male breast cancer cases.[10]

So far, more than 20 distinct *BRCA1* and *BRCA2* mutations have been found in Finnish families, accounting for about 20% of Finnish breast and breast-ovarian cancer families.[11–13] Fourteen of the mutations are founder mutations and account for the vast majority of all *BRCA1/2* families as well as 1.8% of breast[14] and 5.0% of ovarian cancer cases in Finland.[15] Of all *BRCA1/2* mutations in Finland, the 999del5 appears to be the most common founder mutation, accounting for 17% of all *BRCA1/2* families and one third of *BRCA2* families.[12] In addition to Iceland and Finland, the *BRCA2* 999del5 mutation (reported as 995del5) has also been reported in one family of Puerto Rican origin[16] but, as yet, it has not been reported in the other Nordic countries.

In this study we constructed haplotypes in sets of Icelandic and Finnish breast and breast-ovarian cancer families with markers within and flanking the *BRCA2* gene to study the origin and the time since spreading of the 999del5 mutation i.e. the number of generations since the common ancestors of the families studied.

Subjects and methods

Kindreds

Eighteen Icelandic and ten Finnish breast and breast-ovarian ovarian cancer families were included in this study. The Icelandic breast cancer families were all ascertained at the University Hospital of Iceland. Pedigree data was obtained from the Icelandic Genetic Council at the University Hospital of Iceland and verification of cancer diagnoses from the Department of Pathology, University Hospital of Iceland, and at the Icelandic Cancer Registry. Five of the families (2F, 4, 5A/B, 6, and 7A/C) were large high risk families and 13 were pairs of sisters (2, 10, 13, 15, 16, 21, 23, 24, 32, 51, 57, 59, and 67) diagnosed with breast cancer at the age of 60 years or younger. The Finnish 999del5 families were ascertained by four different research groups at the Helsinki, Tampere and Oulu University Hospitals by mutation screening of breast and breast-ovarian cancer families. For most of the Finnish kindreds pedigree data and the birthplaces of parents or grandparents of the probands were traced using genealogy registries and church parish records as described.[17] For families 97, 102 and 113 only the birthplace of the proband was available. Cancer diagnoses were verified through hospital records and the Finnish Cancer Registry when possible.

Genotyping

The haplotypes segregating within the five large Icelandic families were mapped extensively with 28 markers covering 29 cM and sister pairs were typed with 5 of these 28 markers (Figure 1). Twenty-four markers spanning 36 cM were used in genotyping of the Finnish families (Figure 3). Primer sequences published in Couch *et al*.[18] were used for markers D13S1694, D13S1695, D13S1696, D13S1697, D13S1698, D13S1699, D13S1701, and those for markers SLS320, SLS385, SLS165, SLS234, SLS163, SLS312, SLS321, SLS329, SLS886 were kindly provided by Dr Michael Stratton. Primers were designed for the intron 11 polymorphism (EX11) at nucleotide 7069 (+78) and are available on request. All markers except for EX11 are microsatellite markers. Primer sequences and genetic distances for the other markers were obtained from Généthon.[19]

Marker order and physical distances for markers D13S260 through D13S267 were determined by using the genomic sequence for this region. Genomic sequences were obtained from The Sanger Centre and marker primer sequences positioned using Sequencher v3.0 (Gene Codes Corporation, Ann Arbor, MI, USA). Physical distances were converted to cM assuming 1 cM=0.5 Mb, which was the average observed ratio at this region. In a study of recurrent *BRCA2* mutations, Neuhausen *et al*.[20] used between markers D13S290 and D13S267 an assumption of 1 cM=0.67 Mb, which is similar to the ratio used in our study.

PCR amplification and genotype scoring of the Icelandic material was as previously described elsewhere[21] Genotyping of the Finnish families and selected 999del5 mutation carriers from the Icelandic families for direct comparison of the allele sizes was carried out with fluorescent technique using ABI 377 model sequencer (Applied Biosystems, Foster City, CA, USA). CEPH individual 1347-02 (Coriell Cell Repositories and Applied Biosystems) was used to ensure consistent allele calling between individual gels. Genotype data were analysed using GeneScan v3.1 and Genotyper v2.0 software (Applied Biosystems). For estimating the population prevalence of the alleles, 96 Finnish population controls and 50 Icelandic controls were genotyped. For estimating the population prevalence of the core haplotype 14 Icelandic parents-child control pairs were genotyped.

Haplotype construction and estimation of the time since the common ancestors of the families

Haplotypes within the families were constructed using Genehunter (that gives the pattern of inheritance at each

marker and likely positions of recombinants). The haplotypes were proofread and some reconstructed by hand due to Genehunter's limited capability to assign haplotypes in pedigrees of complex structure. The history of recombinations in the 999del5-carrying chromosomes between the families was reconstructed by assuming minimum diversity of the haplotypes. Starting from the site of the mutation and moving outward to both directions, historical recombinations were noted as the branching of the haplotype when two or more different alleles were observed for a marker. The branched haplotype reconstruction of the Icelandic and the Finnish families is shown in Figures 1 and 3, respectively.

The number of generations (g) since the common ancestors of the families studied, denoted as the time since the spreading of the mutation, was estimated using the Luria-Delbrück calculation[12,22,23] of $p_{excess}=\alpha(1-\theta)^g$, where $\alpha=1$ (all chromosomes carry the same mutation), and θ refers to the recombination fraction between the mutation and marker locus. The definition of p_{excess} was based on the haplotype reconstructions (Figures 1 and 3). Two modifications of the calculation[12] were used; in one population allele frequencies were taken into consideration and p_{excess} was defined as $p_{excess}=(p_{affected}-p_{normal})/(1-p_{normal})$ (modification 1) whereas in the other p_{excess} was assumed equal to $p_{affected}$ (modification 2). To achieve the minimum and maximum estimates for the time since the common ancestors, the $p_{affected}$ value at each marker was calculated either as the fraction of haplotypes carrying the allele that was present in the most common haplotype (minimum estimate) or as one of the alleles observed in different haplotypes (maximum estimate). Modification 2 was applied in calculating both the minimum and maximum estimates, whereas modification 1 was applied only in calculating the minimum estimate and there p_{normal} was the frequency of the allele that was present in the most common mutation haplotype in normal population chromosomes. The markers for which the genetic distance from the mutation was known were used for age estimations (Figures 1 and 3). The average of values obtained at different markers was considered as the most likely time estimate.

Results and discussion

This article reports the results of haplotype and genealogical studies on a large number of breast and breast-ovarian cancer families in Iceland and in Finland. The results of the haplotype analysis show that all the Icelandic families share a common core *BRCA2* 999del5 haplotype whereas in Finland two distinct haplotypes are associated with this mutation.

Haplotype and genealogy studies of Icelandic *BRCA2* 999del5 families

One common core haplotype was found to segregate within all the Icelandic families analysed, indicating identity by descent of the mutation carrier families. The shared haplotype covered the chromosome region of markers SLS312 through SLS886 (Figure 1) which is about 0.85 Mb or approximately 1.7 cM according to the physical distance of the markers and observed recombination fraction in this region. The modification 1 of the Luria-Delbrück equation yielded an estimation of 25 generations or 500–625 years (assuming 20–25 years per generation) since the common ancestor of the families, while the modification 2 used in estimating both the minimum and maximum time since the common ancestor gave the range of 16–40 generations or 320–1000 years.

It is believed that Iceland was settled mainly by Vikings from western Scandinavia and the British Isles between the years 860–1060.[24–27] This is supported by studies on mitochondria DNA and the Y chromosome indicating that the settlers of Iceland were mainly people of Scandinavian and Celtic origin.[28–30] The finding of only two *BRCA1/2* mutations in Iceland and both shown to be founder mutations probably reflects the demographic history of subsequent drastic and repeated population bottlenecks, which is likely to have reduced the genetic diversity introduced by the original settlers.

Nine variants of the haplotype could be identified in 16 of the families as shown in Figure 1, where the 999del5 families have been given different symbols based on sharing of the haplotype between markers D13S220 and D13S1246. In two families the haplotype variant was uncertain due to lack of information on markers centromeric to the 999del5 mutation. Locating the families on the map of Iceland according to the birthplaces of the grandparents shows that the different haplotype variants tend to cluster in different regions of the country (Figure 2), despite the rather even distribution of Icelandic inhabitants on the coastal regions around the country at the end of the 19th century and beginning of the 20th century. Genetic drift is likely to be responsible for the difference in the frequency of the 999del5 haplotype variants and the clustering of these variants in different parts of the country.

Haplotype and genealogy studies of Finnish *BRCA2* 999del5 families

In Finland, two different haplotypes were observed in the 999del5 families (Figure 3). A rarer haplotype (denoted as haplotype A) was present in three families, and a more common haplotype (denoted as haplotype B) was present in seven families. The number of families sharing the rare haplotype A is too low for time estimation. All three families share a haplotype covering 16 markers from marker D13S260 telomeric to marker D13S267 (approximately 3 cM) with the exception of marker D13S1698. At this marker allele 1 is found to segregate with the mutation in family 158 and allele 2 in families 6003 and 102. This difference in allele sizes in the middle of an otherwise shared haplotype is probably due to a mutation[31] or a null allele.[32]

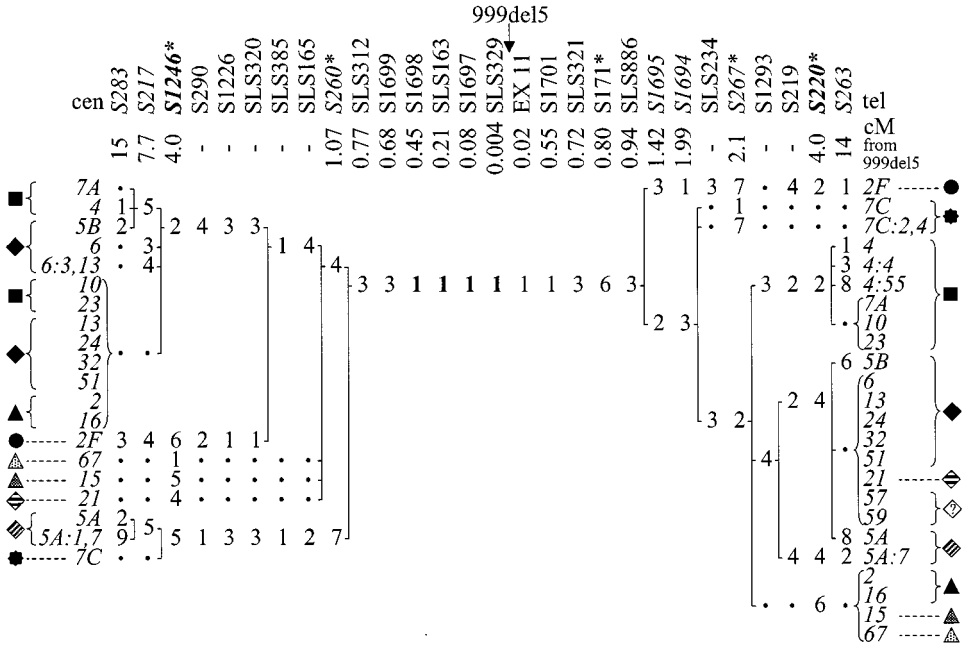

Figure 1 The branched haplotype reconstruction of the Icelandic families, revealing the minimum number of historical recombinations. The order of markers and their relative distances from the 999del5 mutation in cM are shown on the top of the figure. The five markers that were used in genotyping sister pairs are indicated with *. The markers that were used for age estimations span 29 cM around the *BRCA2* gene and are marked with italics. Family members are indicated after a colon in case of recombination within families. The haplotype variants found to segregate in the families have been marked with different symbols according to the extent of sharing a common haplotype between the markers D13S220 and D13S1246 (marked with bold). A four-marker haplotype 1-1-1-1 (indicated with bold) at markers D13S1698-SLS163-D13S1697-SLS329 is present in the Icelandic core haplotype and in the Finnish haplotype A. ●=unknown allele.

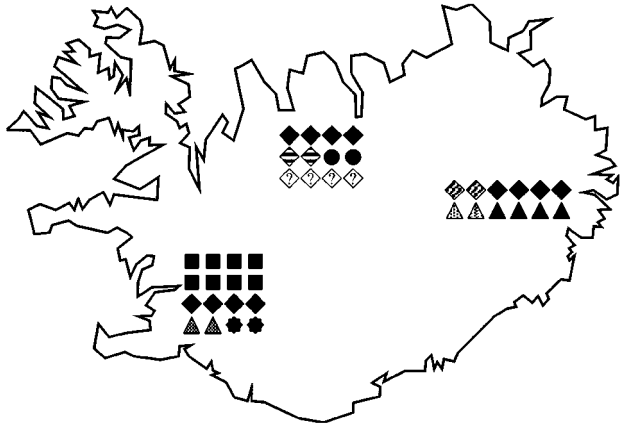

Figure 2 Schematic drawing of Iceland showing geographical distribution of the birthplaces of the grandparents of the proband of each of the 18 Icelandic families analysed (two symbols for each family). The nine haplotype variants are represented by distinct symbols (see the text for Figure 1).

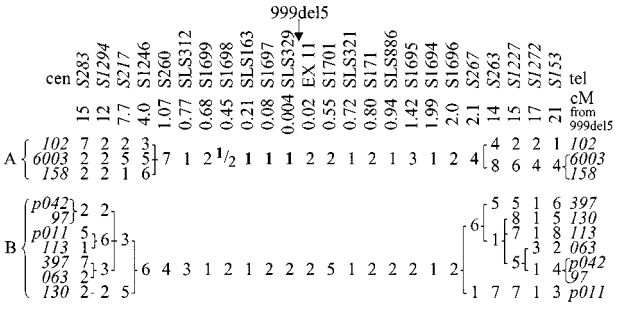

Figure 3 Haplotype reconstruction A of the three Finnish families and B of the seven Finnish families. The order of the markers studied and their relative distances in cM are shown on the top of the figure. The markers that were used for age estimations span 36 cM around the *BRCA2* gene and are marked with Italics. A four-marker haplotype 1-1-1-1 (indicated with bold) at markers D13S1698-SLS163-D13S1697-SLS329 is shared between the Icelandic families and the Finnish families with haplotype A.

Haplotype A shares allele sizes also with the Icelandic haplotype at three markers (SLS329, D13S1697, D13S163) in two families and in one family also at the fourth marker (D13S1698) adjacent to the mutation spanning about 200 kb or approximately 0.5 cM. The estimated frequency of this haplotype in normal chromosomes is about 1.3%

$(0.82 \times 0.18 \times 0.37 \times 0.24)$ in the Finnish population and 2.2% $(0.82 \times 0.22 \times 0.53 \times 0.23)$ in the Icelandic population, assuming equilibrium between markers. The actual frequency of a two-marker haplotype available (allele 1 at D13S1698 and allele 1 at D13S1697, estimated frequency 4.3%)[12] was 2% in 102 normal chromosomes in Finland and for a three marker haplotype (allele 1 at D13S1698, allele 1 at SLS163 and allele 1 at SLS329, estimated frequency 10.0%) in Icelandic controls (28 chromosomes) it was 3.6%. Thus, a coincidence of similar short haplotypes in the Icelandic and Finnish families cannot be ruled out. On the other hand, if these haplotypes are identical by descent and there is only one mutational event then the common origin must be significantly older than the Icelandic haplotype expansion.

The seven Finnish families segregating haplotype B all share a common region covering markers D13S1246 through D13S1696 and spanning approximately 6 cM (Figure 3). Time estimation since the common ancestry of these families using the modification 1 of the Luria-Delbrück equation was eight generations or 160–200 years whereas using the modification 2 it was 7–12 generations or 140–300 years.

Tracing the origin of the grandparents of the 10 Finnish 999del5 families revealed that the families form two clusters in geographically distinct regions in Finland. The families carrying haplotype A have their origins in the old agricultural area in Pirkanmaa, belonging to the early settlement region in the southwest of Finland (Figure 4). The families segregating haplotype B originate from two distinct geographical regions: from the very same small region in Pirkanmaa as the families segregating haplotype A, and from the new settlement region in the most eastern part of the country (Figure 4). Finland is likely to have been inhabited continuously since the last glacial period about 9000 years ago by small groups mainly from the south and east, but also from the west.[33] For centuries, only the coastal regions of the south, southwest, and southeast (old settlement region) were permanently inhabited. An internal migration towards the central and northern parts of the country (new settlement region) began only in the 16th century and resulted in rural subisolates whose major population expansion began in the 17th century.[34]

The presence of two distinct 999del5 haplotypes in the Finnish population is quite surprising, and even more so as families carrying both haplotypes cluster in the very same small area in the old settlement region. It is possible that the 999del5 region of the *BRCA2* gene represents a mutational hotspot and the two distinct haplotypes in Finland represent different mutational events as well as the one in Iceland and the 999del5 mutation in a family of Puerto Rican Hispanic ancestry. It has been proposed that short symmetric elements predispose DNA sequences to meiotic microdeletion[35] and indeed, partially overlapping and closely flanking the 999del5 mutation three symmetric elements can be identified.

However, another possible explanation for two haplotypes in Finland is a gene conversion. Both haplotypes A and B are present in a very small geographical region, in adjacent communities in an old sparsely populated region and haplotype B also in a more recently inhabited region. Interestingly, a similar situation has been observed in autosomal recessive congenital ichtyosis: an identical transglutaminase 1 gene (*TGM1*) mutation is present in two haplotypes in the Savo region in southeastern Finland, and one of the haplotypes is also present in central Finland[36] that was inhabited in the 16th century mainly by population movement from South Savo.[37]

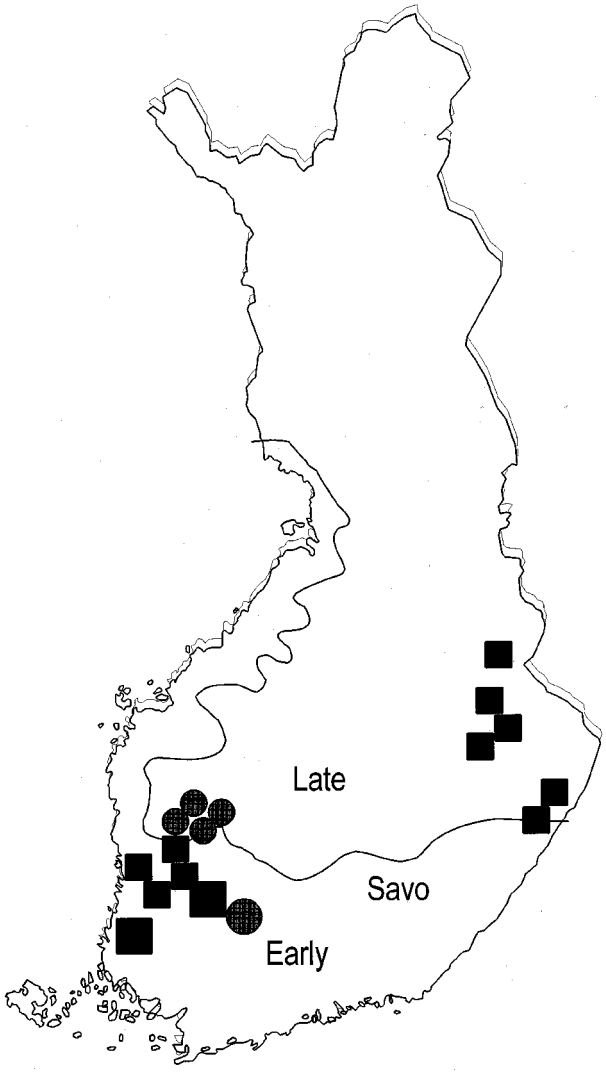

Figure 4 Map of Finland showing the geographical distribution of the ancestry of the 999del5 families. The circles indicate origins of the families carrying the haplotype A and the squares indicate origins of the families carrying the haplotype B. The small symbols represent birthplaces of the earliest known cancer generation (two symbols for each family) and the large symbols birthplaces of the proband, if information of the previous generations was not available.

Thus, an alternative theory for a mutational hotspot and independent mutational events could be an ancient common origin for the Icelandic and Finnish 999del5 mutations. The ancient 999del5 mutation could have been introduced to both Iceland and Finland during different time periods. The short common core haplotype seen in the present day families in Iceland and in Finland, as well as the absence of the mutation in other populations studied, may reflect extinction of ancient branches in populations with repeated bottlenecks. In Finland, the haplotype A in the old settlement region may be the original one from which haplotype B may have diverged by gene conversion. Individuals carrying the new haplotype may have migrated to the east of Finland during the more recent settlement of this part of the country, forming a regional cluster there. Although this theory remains unproven, it would be in agreement with the population historical records.

Electronic-database information

Accession numbers and URLs for data in this article are as follows: Information on markers: Genethon, ftp://ftp.genethon.fr/pub/Gmap/Nature-1995/Haplotype construction: Genehunter, http://waldo.wi.mit.edu/ftp/distribution/software/genehunter/gh2/Information on genomic sequences: The Sanger Centre, ftp://ftp.sanger.ac.uk/pub/human/sequences/Chr_13/; *BRCA1/2* mutation database: Breast Cancer Information Core database (BIC), http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/.

Acknowledgements

We wish to thank the families for participating in this study by donating blood samples and Dr Mike Stratton at the Institute of Cancer Research, Sutton, Surrey, UK, for kindly providing information for the SLS markers used and Dr Hannaleena Eerola and Ms Minna Merikivi, R.N. at the Department of Oncology, Helsinki University Hospital, for their kind help. Professor Jonas Hallgrimsson and the staff at the Department of Pathology, Iceland is gratefully acknowledged for access to tissue samples, Anna G Hafsteinsdottir, Oddny Vilhjalmsdottir at the Genetic Committee, University of Iceland for help with the pedigree data, the Red Cross in Tampere, Finland, and the Blood Bank of Iceland for providing the blood samples for allele frequency estimations. We also warmly thank Elizabeth Gillanders and Diana Freas-Lutz at NHGRI for technical advice and support, Ella Kristin Geirsdottir, Gudrun Johannesdottir and Omar Kristinsson at Department of Pathology, Iceland, for technical assistance and Sigrun Kristjansdottir and Kristrun Olafsdottir at the Department of Pathology, Iceland , for the help with tissue processing. This work was supported by the Nordic Cancer Union, Finnish Cancer Society, the Sigrid Juselius Foundation and the Clinical Research Fund of Helsinki University Central Hospital.

References

1 Miki Y, Swensen J, Shattuck-Eidens D *et al*: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994; **266**: 66–71.
2 Wooster R, Bignell G, Lancaster J *et al*: Identification of the breast cancer susceptibility gene BRCA2 [see comments] [published erratum appears in Nature 1996 Feb 22;379(6567):749]. *Nature* 1995; **378**: 789–792.
3 Tavtigian SV, Simard J, Rommens J *et al*: The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds [see comments]. *Nat Genet* 1996; **12**: 333–337.
4 Szabo CI, King MC: Population genetics of BRCA1 and BRCA2 [editorial; comment]. *Am J Hum Genet* 1997; **60**: 1013–1020.
5 Bergthorsson JT, Jonasdottir A, Johannesdottir G *et al*: Identification of a novel splice-site mutation of the BRCA1 gene in two breast cancer families: screening reveals low frequency in Icelandic breast cancer patients. *Hum Mutat* 1998; Suppl 1: S195–S197.
6 Gudmundsson J, Johannesdottir G, Arason A *et al*: Frequent occurrence of BRCA2 linkage in Icelandic breast cancer families and segregation of a common BRCA2 haplotype. *Am J Hum Genet* 1996; **58**: 749–756.
7 Thorlacius S, Olafsdottir G, Tryggvadottir L *et al*: A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes [see comments]. *Nat Genet* 1996; **13**: 117–119.
8 Arason A, Jonasdottir A, Barkardottir RB *et al*: A population study of mutations and LOH at breast cancer gene loci in tumours from sister pairs: two recurrent mutations seem to account for all BRCA1/BRCA2 linked breast cancer in Iceland. *J Med Genet* 1998; **35**: 446–449.
9 Johannesdottir G, Gudmundsson J, Bergthorsson JT *et al*: High prevalence of the 999del5 mutation in icelandic breast and ovarian cancer patients. *Cancer Res* 1996; **56**: 3663–3665.
10 Thorlacius S, Sigurdsson S, Bjarnadottir H *et al*: Study of a single BRCA2 mutation with high carrier frequency in a small population [see comments]. *Am J Hum Genet* 1997; **60**: 1079–1084.
11 Vehmanen P, Friedman LS, Eerola H *et al*: Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes. *Hum Mol Genet* 1997; **6**: 2309–2315.
12 Sarantaus L, Huusko P, Eerola H *et al*: Multiple founder effects and geographical clustering of BRCA1 and BRCA2 families in Finland. *Eur J Hum Genet* 2000; **8**: 757–763.
13 Vahteristo P, Eerola H, Tamminen A, Blomqvist C, Nevanlinna H: A probability model for predicting BRCA1 and BRCA2 mutations in breast and breast-ovarian cancer families. *Br J Cancer* 2001; **84**: 704–708.
14 Syrjakoski K, Vahteristo P, Eerola H *et al*: Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients. *J Natl Cancer Inst* 2000; **92**: 1529–1531.
15 Sarantaus L, Vahteristo P, Bloom E *et al*: BRCA1 and BRCA2 mutations among 233 unselected Finnish ovarian carcinoma patients. *Eur J Hum Genet* 2001; **9**: 424–430.
16 Schubert EL, Lee MK, Mefford HC *et al*: BRCA2 in American families with four or more cases of breast or ovarian cancer: recurrent and novel mutations, variable expression, penetrance, and the possibility of families whose cancer is not attributable to BRCA1 or BRCA2 [see comments]. *Am J Hum Genet* 1997; **60**: 1031–1040.
17 Eerola H, Blomqvist C, Pukkala E, Pyrhonen S, Nevanlinna H: Familial breast cancer in southern Finland: how prevalent are breast cancer families and can we trust the family history reported by patients? *Eur J Cancer* 2000; **36**: 1143–1148.
18 Couch FJ, Rommens JM, Neuhausen SL *et al*: Generation of an integrated transcription map of the BRCA2 region on chromosome 13q12-q13. *Genomics* 1996; **36**: 86–99.
19 Dib C, Faure S, Fizames C *et al*: A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996; **380**: 152–154.

20 Neuhausen SL, Godwin AK, Gershoni-Baruch R *et al*: Haplotype and phenotype analysis of nine recurrent BRCA2 mutations in 111 families: results of an international study. *Am J Hum Genet* 1998; **62**: 1381 – 1388.
21 Barkardottir RB, Arason A, Egilsson V, Gudmundsson J, Jonasdottir A, Johannesdottir G: Chromosome 17q-linkage seems to be infrequent in Icelandic families at risk of breast cancer. *Acta Oncol* 1995; **34**: 657 – 662.
22 Lehesjoki AE, Koskiniemi M, Norio R *et al*: Localization of the EPM1 gene for progressive myoclonus epilepsy on chromosome 21: linkage disequilibrium allows high resolution mapping. *Hum Mol Genet* 1993; **2**: 1229 – 1234.
23 Hoglund P, Haila S, Scherer SW *et al*: Positional candidate genes for congenital chloride diarrhea suggested by high-resolution physical mapping in chromosome region 7q31. *Genome Res* 1996; **6**: 202 – 210.
24 Berry RJ, Muir VML: The natural history of man in Shetland. *J Biosoc Sci* 1975; **7**: 319 – 344.
25 Magnusson SA. *Northern Sphinx*. Montreal: McGill University Press, 1977.
26 Davies N. *The isles: a history.* London: Macmillan, 1999.
27 Rafnson S. The Atlantic islands. In: Sawyer P, ed. *The Oxford illustrated history of the Vikings*. Oxford: Oxford University Press, 1999.
28 Helgason A, Sigurdandottin S, Gulcher JR, Ward R, Stefansson K: mtDNA and the origin of the Icelanders: deciphering signals of recent population history. *Am J Hum Genet* 2000; **66**: 999 – 1016.
29 Helgason A, Sigurdandottin S, Nicholson J *et al*: Estimating scandinavian and gaelic ancestry in the male settlers of Iceland. *Am J Hum Genet* 2000; **67**: 697 – 717.
30 Helgason A, Hickey E, Goodacre S *et al*: mtDNA and the islands of the North Atlantic: estimating the proportions of Norse and Gaelic ancestry. *Am J Hum Genet* 2001; **68**: 723 – 737.
31 Weber JL, Wong C: Mutation of human short tandem repeats. *Hum Mol Genet* 1993; **2**: 1123 – 1128.
32 Callen DF, Thompson AD, Shen Y *et al*: Incidence and origin of 'null' alleles in the (AC)n microsatellite markers. *Am J Hum Genet* 1993; **52**: 922 – 927.
33 Jutikkala E, Pirinen K: *A history of Finland*. Juva: WSOY, 1996.
34 Nevanlinna HR: The Finnish population structure. A genetic and genealogical study. *Hereditas* 1972; **71**: 195 – 236.
35 Schmucker B, Krawczak M: Meiotic microdeletion breakpoints in the BRCA1 gene are significantly associated with symmetric DNA-sequence elements [letter]. *Am J Hum Genet* 1997; **61**: 1454 – 1456.
36 Laiho E, Ignatius J, Mikkola H *et al*: Transglutaminase 1 mutations in autosomal recessive congenital ichthyosis: private and recurrent mutations in an isolated population. *Am J Hum Genet* 1997; **61**: 529 – 538.
37 de la Chapelle A: Disease gene mapping in isolated human populations: the example of Finland. *J Med Genet* 1993; **30**: 857 – 865.