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A common Greenlandic Inuit *BRCA1* RING domain founder mutation

Thomas v. O. Hansen · Bent Ejlersen · Anders Albrechtsen · Eva Bergsten · Peter Bjerregaard · Torben Hansen · Torben Myrhøj · Peter B. Nielsen · Vera Timmermans-Wielenga · Mette K. Andersen · Lars Jønson · Finn C. Nielsen

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Abstract Germ-line mutations in the tumour suppressor proteins *BRCA1* and *BRCA2* predispose to breast and ovarian cancer. We examined 32 breast and/or ovarian cancer patients from Greenland for mutations in *BRCA1* and *BRCA2*. Whereas no mutations were identified in 19 families, 13 families exhibited a *BRCA1* exon 3 nucleotide 234 T > G mutation, which has not previously been reported in the breast cancer information core (BIC) database. The mutation changes a conserved cysteine 39 to a glycine in the Zn²⁺ site II of the RING domain, which is essential for BRCA1 ubiquitin ligase activity. Eight of the families had members with ovarian cancer, suggesting that the RING domain may be an ovarian cancer hotspot. By SNP array analysis, we find that all 13 families share a 4.5 Mb genomic fragment containing the *BRCA1* gene, showing that the mutation originates from a founder. Finally, analysis of 1152 Inuit, representing almost ~2%

of the total Greenlandic Inuit population, showed that the frequency of the mutation was 1.0%. We conclude that the *BRCA1* nucleotide 234 T > G is a common Greenlandic Inuit founder mutation. The relative high frequency in the general population, together with the ease of screening and possibility to reduce mortality in gene carriers, may warrant screening of the Greenlandic Inuit population. Provided screening is efficient, about 5% of breast- and 13% of ovarian cancers, respectively, may be prevented.

Keywords *BRCA1* · RING domain · Cys39Gly · SNP array · Greenland · Inuit · Founder mutation

Introduction

Breast cancer is the leading cancer among women. Loss of BRCA1 (MIM 113705) and BRCA2 (MIM 600185) tumour suppressor function predispose to hereditary breast and ovarian cancer, conferring a lifetime risk of approximately 80% for breast cancer and 20–50% for ovarian

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cancer in female carriers [1]. BRCA1 and BRCA2 are involved in a variety of cellular processes, including maintenance of genome stability [2, 3], DNA repair via homologous recombination [4, 5], transcriptional regulation [6], cell cycle control [7], chromatin remodelling [8] and ubiquitylation [9].

BRCA1 exhibit a conserved amino terminus containing a ~110 residue RING (*Really Interesting New Gene*) domain [10] and a conserved acidic carboxyl terminus containing two ~90 residues BRCT (*BRCA1 C-terminal*) domains [11], which are involved in phosphor–protein interactions [12]. The RING domain is characterized by a conserved pattern of seven cysteines and one histidine residue forming two distinct Zn^{2+} binding sites, termed site I and II [13–15] (Fig. 1). The BRCA1 RING domain interacts with the RING domain of BARD1 in vivo [16, 17] and the two proteins co-localize in nuclear dots during S-phase of the cell cycle and in nuclear foci following DNA damage [18, 19]. Moreover, loss of BARD1, reduces the stability of BRCA1 [20]. The complex is involved in ubiquitylation [21–24] and recently the factors were shown to bind RAP80 via CDCC80 (Abraxas) and to target BRCA1 to ubiquitin structures at DNA damage sites [25–29]. Several BRCA1 ubiquitylation targets have been identified, including histones, γ -tubulin, the RNA polymerase II subunit RPB1, CtIP, ERalpha and

RPB8 [21, 30–35], suggesting that BRCA1 dependent ubiquitylation regulates many cellular activities. One of the most interesting targets is ERalpha, which could explain the tissue specific effects mediated by *BRCA1* mutations. Mutations in the Zn^{2+} binding loops of the RING domain are assumed to be cancer predisposing, since they disrupt the ubiquitin ligase activity of BRCA1 [36].

Here we report the identification of a common *BRCA1* nucleotide (nt) 234 T > G founder mutation, that is present in about 1% of the Greenlandic Inuit population. The mutation changes the conserved cysteine 39 in the Zn^{2+} site II of the RING domain to a glycine. The high frequency of the mutation in the general population indicates that screening of the Greenlandic population may be beneficial.

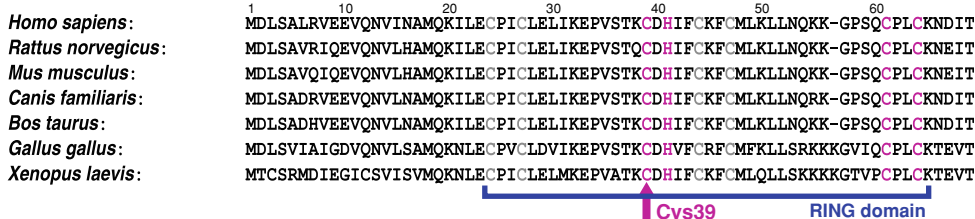
Patients and methods

Patients

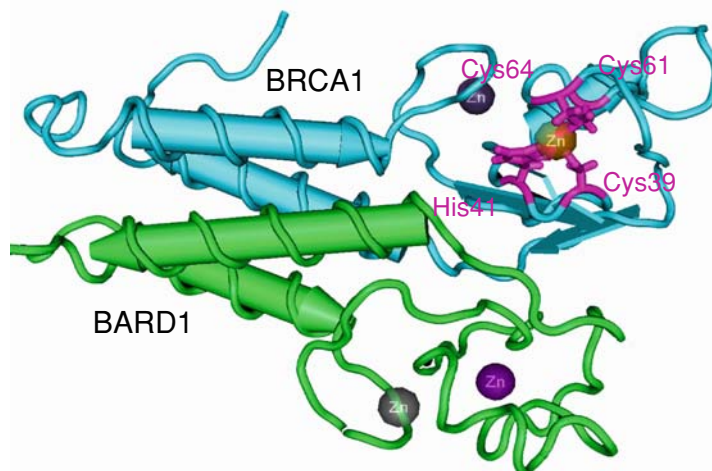
Following referral to oncogenetic counselling, patients from Greenlandic families with clustering of breast and/or ovarian cancer have since 1999 been offered *BRCA1* and *BRCA2* mutation screening according to the guidelines of the Danish Breast Cancer Cooperative Group (DBCG)

Fig. 1 (a) Primary structure of the BRCA1 RING domain in different species. The RING domain is underlined in blue and the conserved cysteines and histidines, that form the Zn^{2+} site I and site II are indicated in grey and pink, respectively. The position of the mutated cysteine 39 is indicated by the arrow. **(b)** Solution structure of the BRCA1/BARD1 RING-domain heterodimer. The BRCA1 subunit is shown in blue and the BARD1 subunit in green. The Cys 39, 61 and 64 and His 41 residues of the Zn^{2+} Site II in the RING domain, are shown in pink. Helices that flank the core RING motif of both BRCA1 and BARD1 form the dimerization interface. The illustration was generated with the Cn3D vers. 4.1 software (NCBI, Molecular Modelling Data Base (MMDB)) and based on data from [15]

A BRCA1 RING domain



B Structure of the BRCA1-BARD1 heterodimer



(www.dbcg.dk). Blood samples were obtained from breast and ovarian cancer patients after informed consent and the original consent encompassed additional screening in case of new methods or targets. The family history was verified using the registry of the DBCG, hospital medical records and pathology reports and genetic counselling was provided for every family member.

BRCA1 and *BRCA2* screening

Genomic DNA was purified from whole blood using the QIAamp DNA mini kit (Qiagen), and paraffin embedded tumour tissue was purified using the Chemagic DNA tissue kit (Chemagen) according to the manufacturer's instructions. *BRCA1* and *BRCA2* were amplified using intronic primer pairs flanking each exon. PCR products were pre-screened by dHPLC (denaturing high performance liquid chromatography) using the Wave system (Transgenomic) and sequenced using an ABI3730 DNA analyzer (Applied Biosystems). Sequence variations were verified in a new blood sample. Moreover, genomic DNA was examined by MLPA analysis as recommended by the manufacturer (MRC-Holland). *BRCA1* variants are numbered according to Genbank accession number U14680 in which the A in the AUG start codon has number 120, while the *BRCA2* variants are numbered according to Genbank accession number NM_000059 in which the A in the AUG start codon has number 229. Furthermore, the guidelines from the Human Genetic Variation Society (www.hgvs.org/mutnomen) were used.

SNP chip analysis

Genomic DNA was applied to 250 K *StyI* (~238,000 SNPs) SNP-microarray chips and processed according to the manufacturer's instructions (Affymetrix). Briefly, 250 ng of genomic DNA was digested with *StyI* and ligated to adapters. Adapter ligated DNA was amplified, purified, fragmented and labeled with biotin and hybridized to the arrays for 18 h. The Affymetrix 450 fluidics station and the Affymetrix 3000 G7 gene scanner were used to wash, stain and scan the arrays. The CEL files were analysed using the BRLMM algorithm from Affymetrix Genotyping analysis software (GTTYPE).

In order to assess whether the mutation happens once or several times, we investigate the local relatedness across the genome. The population allele frequencies for the Greenlandic population are difficult if not impossible to estimate because of the small population size, possible very large genomic variation between local communities (population stratification) and large variations in inbreeding coefficients. Because of the inbreeding, the problems obtaining an appropriate allele frequency and because of

the linkage disequilibrium between SNPs, we could not use standard method for estimating relatedness. Instead we looked at the length of possible shared haplotype across the genotype. We defined a possible shared haplotype by a region where all individuals share at least one allele. Region with no markers i.e. centromeric regions and SNPs with missing data, were excluded.

Population samples

Blood samples were collected between 1999 and 2001 from Greenlanders with Greenlandic ethnicity (Inuit), defined as Greenlanders or mixed Greenlandic/Danish aged 18 and above living in three areas of West Greenland, including Nuuk, Qasigiannnguit and four villages in the district of Uummannaq representing towns of varying size [37]. For all participants informed consent was obtained in writing and orally. The relevant ethical review committees approved the study. All together 1152 samples were analysed by TaqMan genotyping.

Genotyping

BRCA1 nt 234 T > G genotyping was performed using TaqMan assay (Applied Biosystems). The PCR reaction was performed as described by the manufacturer (Applied Biosystems). The *BRCA1*-234 primer and probe sequences were 5'-AGTCTGGAGTTGATCAAGGAACCT-3' (forward primer), 5'-GGAGCCACATAACACATTCAAACCT-3' (reverse primer), 5'-VIC-TCTCCACAAAGtGTGACC-MGB-3' (probe 1) and 5'-6-FAM-TCCACAAAGGTGACC-MGB-3' (probe 2). The allelic-specific fluorescence was measured using an ABI PRISM 7900HT Sequence Detector System (Applied Biosystems). Water control and previously genotyped samples were included in each plate to ensure accuracy of genotyping. Moreover all mutation positive samples as well as 35 random chosen wild-type samples were verified by sequencing.

Results

To identify disease-causing *BRCA1* and *BRCA2* mutations in 32 breast and/or ovarian cancer patients from Greenland, the entire coding region and the exon-intron boundaries of *BRCA1* and *BRCA2*, were examined by dHPLC and sequencing. Moreover, *BRCA1* and *BRCA2* were examined for large genomic rearrangements by MLPA analysis. Several known polymorphisms, as well as five unknown variants, including two undescribed intronic *BRCA2* variations—IVS8-14delG and IVS24-8 C > T—were recognized (Supplementary Table 1). No disease causing mutations were identified in 19 of the families. The

majority of these patients had weak or no family history of breast or ovarian cancer (Supplementary Table 2). In the remaining 13 families, in contrast, a *BRCA1* exon 3 nt 234 T > G mutation was observed (Fig. 2), which has not previously been described in the breast cancer information core (BIC) database (www.research.nhgri.nih.gov/bic/). The mutation changes the conserved cysteine 39 in the Zn²⁺ site II of the RING domain to a glycine. The mutation was verified in a second blood sample, as well as in paraffin embedded breast cancer tumour tissue from one patient, where we also found that the corresponding wild-type allele was reduced in agreement with the two-hit model of carcinogenesis [38].

All families exhibiting the *BRCA1* nt 234 T > G mutation had a history of breast and ovarian cancer (Table 1 and Supplementary Fig. 1). Mutation analysis was not possible in all affected members, due to lack of material or because the patients were deceased. The mutation was however present in all tested family members with breast and/or ovarian cancer, supporting that the

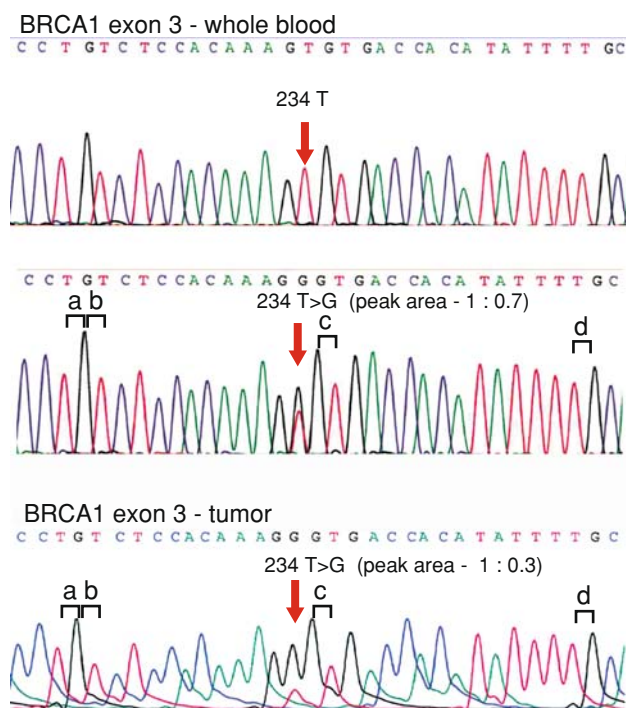


Fig. 2 Identification of the *BRCA1* nt 234 T > G mutation. DNA was purified from blood and tumour samples from patients and *BRCA1* exon 3 were amplified using intronic primer pairs flanking exon 3 and sequenced. DNA Sequences from blood (wild-type and mutation positive patient) and from tumour tissue are shown. The *BRCA1* nt 234 position is indicated by an arrow. The relative peak area for the nt 234 T and G from mutation positive blood and tumour samples were calculated to be approximately 1:0.7 and 1:0.3, suggesting loss of heterozygosity in the tumour tissue. To estimate the average peak variation, four GT dinucleotide pairs (a, b, c and d) from blood and tumour samples were compared and found to be 1:0.9 ± 0.2

mutation co-segregates with breast and/or ovarian cancer in the families. All together eight family members aged between 33 and 49 years were tested positive in the absence of any tumours. The average age of onset for all female mutation positive probands was 41.8 years, ranging from 28 to 69 years, while the average onset for all mutation negative breast/ovarian cancer (and one fallopian tube cancer) patients was slightly higher (43.7 years, ranging from 28 to 68 years).

Since the mutation has not been reported in other populations and we did not previously encounter the mutation in Danish (Caucasian) breast and/or ovarian cancer patients, a SNP array analysis was performed to determine if the mutation represented an Inuit founder mutation. The Greenlandic individuals showed large variation in inbreeding, with an inbreeding coefficient ranging from no inbreeding ($F = 0$) to being extremely inbred ($F = 0.21$). If the *BRCA1* mutation is a founder mutation, we would expect the carriers to share a common haplotype around the *BRCA1* gene. Fig. 3 shows the length of possible shared haplotypes between the 13 mutation carriers on chromosome 17. Around *BRCA1*, a 4.5 Mb shared haplotype consisting of 274 SNPs was noted. None of the individuals without mutation shared this haplotype or any haplotype longer than 1 Mb around the *BRCA1* gene. The shared haplotype between carriers is also the longest possible shared haplotype in the whole genome excluding centromeric regions without any SNPs (Supplementary Fig. 2). The possible shared haplotype with the second highest number of SNPs is 1.5 Mb long located on the right side of the *BRCA1* gene on chromosome 17 and consists of 87 SNPs. Accordingly, we infer that the *BRCA1* nt 234 T > G mutation is an Inuit founder mutation.

To estimate the frequency of the mutation in the Greenlandic Inuit population, we applied a simple and inexpensive TaqMan based *BRCA1* nt 234 T > G mutation analysis (Fig. 4). The assay also included known *BRCA1* nt 234 T > G mutation carriers as well as 100 Danish negative controls. Totally, 1152 Greenlandic Inuit were analysed and 11 *BRCA1* nt 234 T > G mutation carriers corresponding to a population frequency of ~1%, were identified. The 11 mutation positive carriers included 7 females (aged 27, 27, 33, 36, 40, 56, 60 years, respectively) and 4 males (aged 31, 41, 50, 55 years, respectively). Their cancer status is currently unknown. All mutation positive carriers identified in this assay, as well as 35 random chosen wild-types were sequenced to verify the genotype.

Discussion

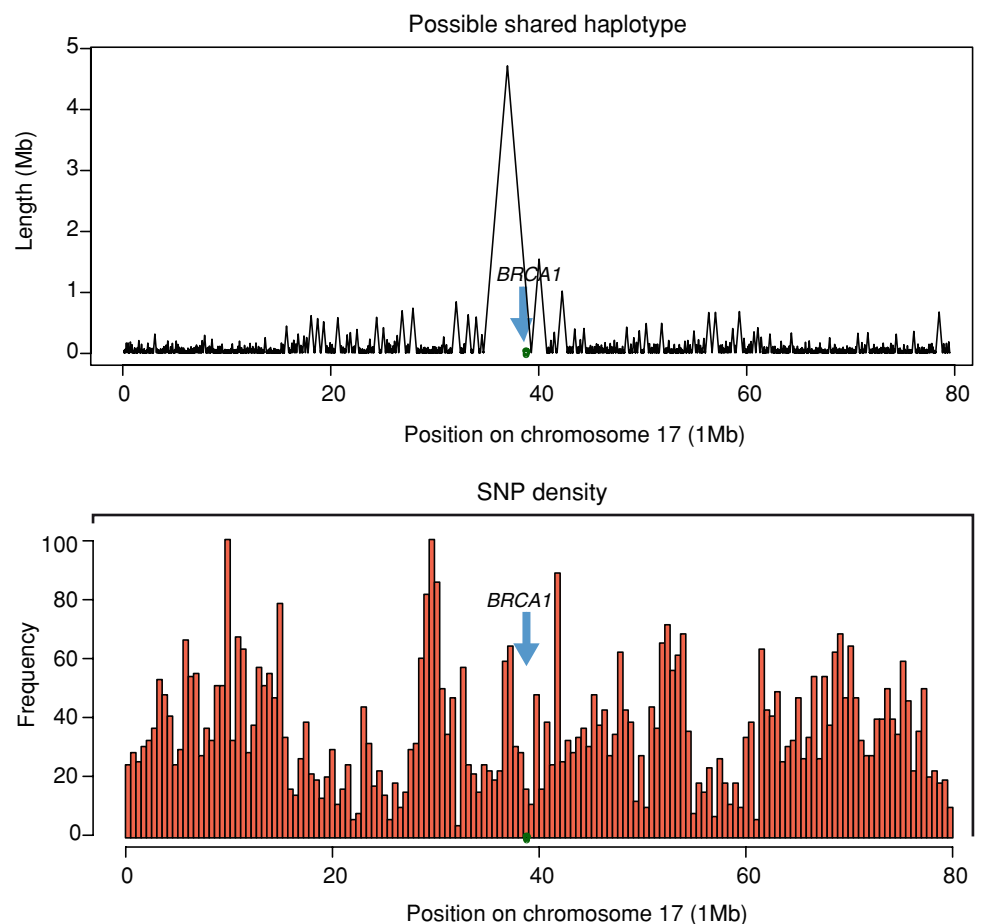
Despite Greenland is the world's largest island, the population is only ~56.000. About 88% of the population is of

Table 1 Greenlandic families with the *BRCA1* nt 234 T > G mutation

Family ID	Sex of proband	Phenotype and age at diagnosis	Family history of breast and ovarian cancers and age of diagnosis	Family history of other cancers
14829	Female	BC 43	M BC 49	None
16142	Female	BC 28	S OC 31	Unknown
21395	Female	BC 38 +41	M BC 47	None
56387	Female	BC 43 + 49, OC 55	N OC 27	Gyn?
58756	Female	OC 69	S BC 45, D BC 52, PC OC 57, PC BC 36, PC BC 51, PC BC 49, PCD, BC 34	Colon?, Rhinopharynx
60493	Female	OC 33	M BC 55	None
60949	Female	OC 45	MC BC 50 PC BC > 50	Rectal, Colon
61273	Female	BC 45	S OC 50	Abdominal
71572	Female	OC 39	M BC 40	Unknown
72316	Female	BC 39	BC 45	Rectal, Gyn, Lung
73428	Female	BC 30	M OC 40, MA OC	Gyn
73429	Female	BC 39	M BC 50	None
73430	Female	BC 52	S OC, M BC	Thyroid

BC—breast cancer, OC—ovarian cancer, Gyn—undefined gynecological cancer, M—mother, S—sister, N—Niece, D—daughter, PC—paternal cousin, MA—maternal aunt, MC—maternal cousin, PCD—paternal cousin's daughter

Fig. 3 SNP analysis of 13 Greenlandic Inuit families harbouring the *BRCA1* nt 234 T > G mutation. The lengths of the possible shared haplotypes are shown as lines in the top figure and the *BRCA1* gene is shown in green at position 38,449,840–38,530,994. The bottom picture is the SNP density on chromosome 17



Inuit origin, many of which live in relatively isolated communities, under specialized polar conditions. Compared to the urbanized world, the population is relatively

genetically homogenous, and the disease spectrum differs in many aspects from that found in other parts of the earth. Greenlandic Inuit exhibit a distinct pattern of cancer,

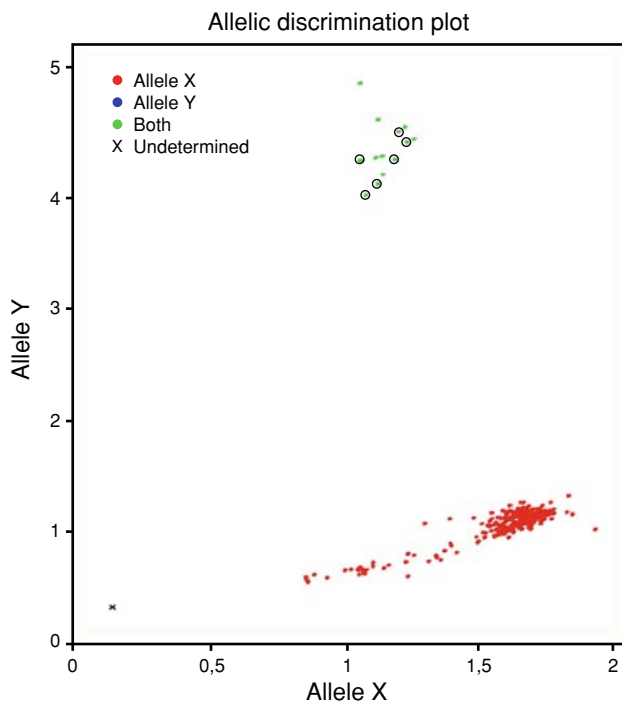


Fig. 4 Analysis of the frequency of the *BRCA1* nt 234 T > G mutation in the Greenlandic population. DNA from 1152 Greenlandic subjects were analysed by TaqMan genotyping. The Scatter diagram shows the end-point fluorescence detection from one 384-well plate using probes and primers detecting *BRCA1* nt 234 position. X is a water control, the cluster of red dots represents nt 234 T subjects (wild-types), while the cluster of green dots represents subjects having the nt 234 T > G mutation, and includes known mutation positive controls (circled)

characterized by a high frequency of e.g. Epstein-Barr Virus (EBV)-associated nasopharyngeal- and salivary gland carcinoma. Moreover Inuit exhibit a low frequency of tumours, that are common in Western countries, such as breast cancer, although the risk of breast cancer in Inuit women has increased markedly over the past 25–30 years [39].

During examination of breast and/or ovarian patients from Greenland for mutations in the *BRCA1* and *BRCA2* genes, we discovered a *BRCA1* nt 234 T > G mutation in exon 3 (Cys39Gly) in 13 out of 32 families. Mutations substituting the conserved cysteine and histidine residues in the Zn²⁺ site I or site II of the *BRCA1* RING domain have previously been reported as disease causing and are deleterious in functional and *in silico* assays [22, 24, 36, 40, 41]. Some mutations disrupt the ubiquitin ligase activity by interfering with the heterodimerization between *BRCA1* and *BARD1*, while others change the ubiquitin ligase activity by disturbing the binding of E2 proteins like UbcH5c to the *BRCA1/BARD1* complex.

The 13 Greenlandic families with the *BRCA1* nt 234 T > G mutation all exhibited several members with breast and/or ovarian cancer. In the families where more family members were examined, the mutation co-segregated with

the disease. In the mutation positive families several family members with the *BRCA1* nt 234 T > G mutation did not have any signs of cancer (the oldest being 49 years). This is not surprising, considering that the penetrance of *BRCA1* mutations is not 100% and that the age of onset normally is variable [1]. In 8 out of the 13 families, there was both breast and ovarian cancer. We have also seen this in Danish families, carrying Cys44Ser and Cys61Gly RING domain mutations. Four families out of 4 with the Cys44Ser mutation and two out of 4 families with the Cys61Gly mutation exhibited ovarian cancer (unpublished data). Previous reports on the genotype-phenotype correlations in hereditary *BRCA1* breast and ovarian cancer, have indicated that mutations towards the 3' end of the gene were associated with lower ovarian cancer risk [42, 43]. It was later proposed, that mutations in the central region of *BRCA1* had a higher risk of ovarian cancer compared with the C-terminal and N-terminal end of the gene, respectively [44]. Our findings may indicate, that mutations in the RING domain in the N-terminus of *BRCA1* could predispose to ovarian cancer in the same way as mutations in the ovarian cancer cluster region (OCCR) of *BRCA2* [45].

Founder mutations have previously been reported in *BRCA1* and *BRCA2*, including the Norwegian *BRCA1* nt 1135insA and nt 1675delA, the Swedish *BRCA1* nt 3171ins5, the Icelandic *BRCA2* nt 999del5, and the Ashkenazi Jews *BRCA1* nt 185delAG mutations [46–49]. In the Inuit families, the 13 probands shared a possible 4.5 Mb haplotype in the *BRCA1* region, indicating that the mutation in the *BRCA1* region happened once and segregated in the population to the 13 positive individuals as a founder mutation.

The frequency of the mutation in the Greenlandic population is close to 1.0%. The analysis was performed on individuals from Nuuk, Qasigiannuit and four villages in the district of Uummannaq at the west coast where 95% of the Greenlandic population lives. Mutation positive carriers were identified in Nuuk and Qasigiannuit similar to some of the probands (Fig. 5). Moreover, three of the mutation positive probands were from Ittoqqortoormiit and three were from Tasiilaq, on the east coast. Thus the mutation is widespread in Greenland and it is possible that it originated 800–1000 years ago before the arctic populations migrated into Greenland from Alaska [50]. Unfortunately, we did not have sufficient power to directly address the admixture among the probands. The population frequency is comparable to the population frequency found for the *BRCA2* nt 999del5 founder mutation in the Icelandic population (0.6%) [51] and for the *BRCA1* nt 185delAG founder mutation in Ashkenazi Jews (~1.0%) [52, 53]. Based on the population frequency about 280 Greenlandic Inuit women are estimated to carry the mutation. The employed TaqMan analysis has a high capacity per day and it is therefore useful for population screening. Due to the

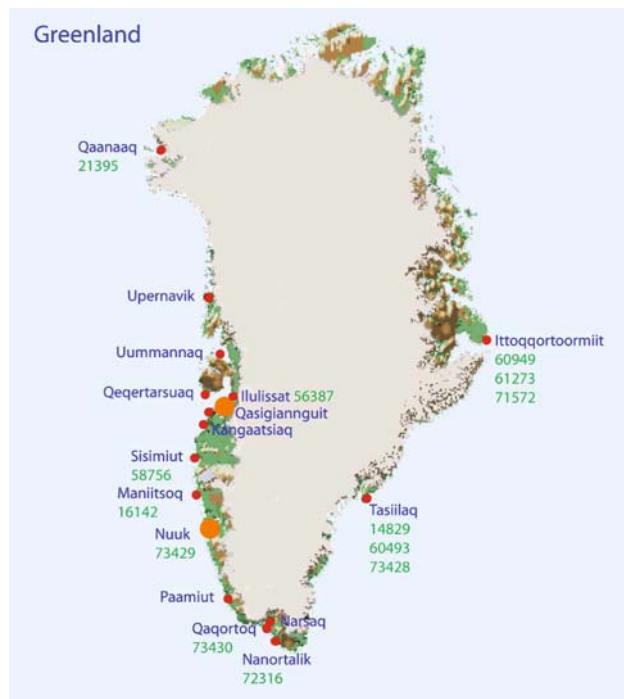


Fig. 5 Geographic localization of identified Greenlandic *BRCA1* nt 234 T > G mutation carriers. The localization of the 13 mutation positive carriers with breast and/or ovarian cancer was based on the birthplace of the proband. We have moreover indicated where Greenlandic *BRCA1* nt 234 T > G mutation carriers from the population screening were identified (orange). The figure is adapted from www.statgreen.gl/images/gmap.gif

relative small size of the Greenlandic population and potential preventive and therapeutic benefit of identifying gene carriers, population screening could be warranted. In Denmark gene carriers are offered a screening program and treatment as described in national guidelines from the Danish Breast Cancer Group (DBCG), and these could also apply to the Inuit population. The total number of breast- and ovarian cancer cases is 68 and 16 per year, respectively [39]. Assuming a penetrance of 80% and 50% for breast and ovarian cancer, respectively [1], we estimate, that the *BRCA1* nt 234 T > G founder mutation accounts for about 5% of all breast cancers and up to 13% of all ovarian cancers among the Greenlandic Inuit population.

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