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A high frequent BRCA1 founder mutation identified in the Greenlandic population

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Abstract Approximately 10% of all breast and ovarian cancers are dominantly inherited and mutations are mainly found in the BRCA 1 and 2 genes. The penetrance of BRCA1 mutations is reported to be between 68 and 92% and confers a 36–92% life time risk of breast cancer. Most mutations in BRCA1 are uniquely occurring mutations, but founder mutations have been described. In this study we describe a founder mutation with wide spread presence in the Inuit population. We have screened 2,869 persons from Greenland for the presence of a BRCA1 mutation (p.Cys39Gly) only found in the Inuit population. The overall carrier frequency was 1.6% in the general population, but the frequency differs geographically from 0.6% on the West coast to 9.7% in the previously isolated population of the East coast. This is to our knowledge the highest population frequency of a BRCA1 mutation ever to be described. To determine the clinical relevance of the mutation, we have examined ten breast cancer patients and nine ovarian cancer patients from Greenland for the presence of the p.Cys39Gly mutation. We found three ovarian cancer patients (33%) and one breast cancer patient (10%) carrying the mutation. The high number of women carrying a BRCA1 mutation known to trigger the development of potentially lethal diseases leads us to recommend an offer of genetic counselling and test for the mutation to all females of Inuit origin, thereby hopefully preventing a number of breast and ovarian cancer deaths.

Keywords Breast cancer · BRCA1 · Founder mutation · Inuit

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Introduction

Breast cancer is the leading form of cancer affecting women worldwide today. The prognosis is generally good with an estimated survival rate of 73% in developed countries [1]. Ovarian cancer is a less frequent form of cancer and it accounts for 4% of all cancer cases affecting women. Due to the often late time of diagnosis and the propagation to the abdominal cavity, it is the seventh most common cause of death due to cancer in women [1]. Approximately 10% of all breast and ovarian cancers can be ascribed to a dominantly inherited disposition [2, 3]. Germline mutations in BRCA1 and BRCA2 are responsible for a large proportion of these hereditary breast and ovarian cancers and confer a 36–92% lifetime risk of breast cancer [4–8] and a 16–63% lifetime risk of ovarian cancer [5, 7, 9] in women. The major part of reported mutations in BRCA1 are changes only found in a few from unrelated families [10]. The remaining changes are founder mutations occurring in different ethnic groups and populations. One of the most studied populations are the Ashkenazi Jews in whom just three mutations account for approximately 85%

of the mutations found in the BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) genes [11].

To evaluate the significance and geographic distribution of a specific BRCA1 mutation, c.234T > G (p.Cys39Gly), localised in the RING domain we screened a representative proportion (5%) of the Greenlandic population.

Materials and methods

Subjects

DNA from 1,071 inhabitants from the Municipality of Ammassalik, East Greenland was screened for the p.Cys39Gly mutation. The samples cover 36.8% of the Ammassalik population. Of these samples, 18 large families (a total of 358 subjects) with a varying number of members (1–40) were identified.

In addition, 1,798 blood samples collected in 2006 and 2007 from pregnant females in Greenland were examined for the presence of the p.Cys39Gly BRCA1 mutation.

The first sample set mentioned above has been collected between 1989 and 2004 in a population based investigation of carrier status for two autosomal recessive diseases [Cholestasis Familiaris Groenlandica (CFG) and Propionic Acidemia (PA)] [12, 13]. The second sample set consists of pregnant females from Greenland participating in a population screening for CFG and PA covering more than 90% of all pregnant. All samples have been anonymised.

DNA extraction from bloodspots

All the above mentioned samples were spotted onto filter paper test cards and stored at −20°C until use. A fragment of 1 mm in circumference was cut out of the blood spot on the test cards and DNA was extracted from the filter paper fragment by the Chelex 100 method [14].

Patients

Patients of Greenlandic Inuit origin, who were treated for either breast or ovarian cancer, were identified at the tumour bank at the Department of Pathology, Rigshospitalet. We received paraffin embedded tissue from nine ovarian cancer samples and ten breast cancer samples. In the case of five of the ovarian samples and all of the breast cancer samples, we also received normal, respectively ovarian and breast tissue. All samples have been anonymised.

DNA extraction from paraffin embedded tissue

Sections of 10 μm cut from paraffin embedded breast or ovarian cancer tumour tissue were treated with Ultra Clear (JT Baker) to deparaffinate the tissue. The tissues were treated with a proteinase K/extraction buffer solution (10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.45% TWEEN 20, 0.45% TRITON × 100, BSA : 0.1 g/L and 6 U/mL proteinase K) for 48 h. The samples were incubated at 100°C for 10 min and then cooled on ice. The DNA is ready to use after cooling.

Computer analysis of the mutation

The mutation was analysed using the program Align-GVGD which is an extension of the original Grantham difference [15]. The program predicts whether a missense mutation is deleterious or neutral [16]. The Align-GVGD calculates a value for the input amino acid changes. Values for strongly dissimilar pairs have scores above 100. The highest score obtainable is for a Cys to Thr change, with a value of 215. The mutation was also analysed with the PolyPhen [17] and the SIFT [18] algorithms.

Mutation analysis

The *BRCA1* p.Cys39Gly mutation genotyping was performed using allele-specific PCR amplification with mutation-specific forward primer BRCA1-39syg-F: 5′-AG GAACCTGTCTCCACAAACG-3′and reverse primer BR CA1-39-R: 5′-TCCTGGGTTATGAAGGACAAA-3′. All reactions were performed in the presence of an additional primer set either specific for the exon 5 of the SLC30A9 gene (F: 5′-AGCCATTCACATCAATATTTC-3′and R: 5′-ATTTGGTTTGCACTTTTATTC-3′) located on chromosome 4. The extra primer set served as internal control of the reaction.

PCR amplifications were carried out in a 16 μL reaction mixture containing 6 μL DNA, 1× Amplicon standard buffer with 15 mM $MgCl_2$ (Bie & Berntsen, Copenhagen, Denmark), 2.5 mM of each dNTP, 10 pmol of each primer and 1 unit of Amplicon Taq DNA Polymerase (Bie & Berntsen, Copenhagen, Denmark). PCR conditions consisted of an initial melting step for 5 min at 95°C followed by 35 cycles of 95°C for 30 s, 54°C for 30 s and 72°C for 30 s, and a final extension step at 72°C for 7 min. PCR products were separated on a 2.5% agarose gel containing ethidium bromide. All reactions were performed in the presence of a positive and negative external control.

Restriction enzyme digest

As a further control of the validity of the PCR test, all positive samples were subjected to a new round of PCR amplification with the following primers: BRCA1C39GF: 5′-GCTCAC TGAAGGTAAGGATCG-3′ and BRCA1C39GR: 5′-GAG AAAGAATGAAATGGAGTTGG-3′.

PCR amplifications were carried out in a 16 μL reaction mixture containing 4 μL DNA, 1× Amplicon standard buffer with 15 mM $MgCl_2$ (Bie & Berntsen, Copenhagen, Denmark), 2.5 mM of each dNTP, 10 pmol of each primer and 1 unit of Amplicon Taq DNA Polymerase (Bie & Berntsen, Copenhagen, Denmark). The PCR conditions were as follows: an initial melting step for 5 min at 95°C followed by 35 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 30 s, and a final extension step at 72°C for 7 min. The fragments were incubated with the *Bst*E II enzyme 2 h at 60°C. *Bst*E II digests the following sequence 5′-GGT-NACC-3′ only present in the mutant fragment generating two bands of 140 and 260 base pairs. Digested PCR products were separated on a 2% agarose gel containing ethidium bromide.

DNA sequencing

To validate the specificity of the analysis, one of the generated PCR fragments was cloned using the TOPO TA cloning kit according to manufactures protocol (Invitrogen), transformed into TOP10 cells (Invitrogen). Plasmid DNA was extracted by mini preparations according to manufactures protocol (Qiagen). A total of six plasmids were sequenced using standard M13 forward and reverse primers.

Statistical methods

Fisher's Exact test was used for examining the p.Cys39Gly mutation distribution between cancer patients and population samples. $P < 0.05$ was considered statistically significant.

Results

A total number of 2,869 samples (5% of the population) of Greenlandic Inuit origin have been screened for a novel BRCA1 mutation localised within the RING domain. The mutation changes a conserved cysteine to a glycine at amino acid position 39. The GD value obtained from the Align-GVGD analysis for the BRCA1 39 Cys to Gly amino acid change is 158.23. Further analyses with both the PolyPhen and SIFT algorithms predicted the Cys to Gly change to be damaging to the function of the protein. Therefore it is highly likely that the mutation will have a deleterious effect on the protein function of BRCA1.

The p.Cys39Gly mutation was found in 30 individuals from 18 families (Table 1) in whom a total of 358 subjects were tested. All families were from the municipality of Ammassalik on the east coast of Greenland. Furthermore 713 inhabitants of the municipality of Ammassalik, not determined to belong to any of the aforementioned large families, were tested and 74 were found to carry the mutation. In 2000 the municipality of Ammassalik had 2,911 inhabitants (statgreen.gl). We have hereby screened 36.8% of the population of Ammassalik, which is the heaviest populated municipality on the east coast of Greenland and found a carrier frequency of 9.7%.

Table 1 Frequency of the BRCA1 C39G mutations in the population of Greenland and in the control samples

	N	Heterozygous	% Positive	Homozygous
Samples from pregnant	1,798	29	1.6	None
Ammassalik samples	1,071	104	9.7	None
Ovarian cancer patients	9	3	33	2, in cancer tissue
Breast cancer patients	10	1	10	1, in cancer tissue

Moreover, samples from pregnant females in 2006 and 2007 were screened for the p.Cys39Gly mutation. A total of 1,798 samples were screened and we found 29 positives, which corresponds to a carrier frequency of 1.6%. This frequency varies geographically (Fig. 1).

A total of nine patients with ovarian cancer and ten patients with breast cancer were genotyped, both groups were from Greenland. Of the nine patients diagnosed with ovarian cancer we found three (33%) carrying the p.Cys39Gly mutation. In the group of breast cancer patients we found one (10%) carrier out of a total number of ten tested. Two of the p.Cys39Gly positive ovarian cancer samples and the positive breast cancer sample showed homozygosity for the mutation in the tumour sample.

Fisher's Exact test showed a statistically significant higher frequency of the mutation in the cancer patients compared to the background population of pregnant women ($P < 0.001$).

Discussion

Pathogenesis of the mutation

The p.Cys39Gly mutation in BRCA1 was first identified in a 43 years old Inuit ovarian cancer patient who belonged to a Ittoqqortoormiit family and has subsequently been reported to occur in the Inuit population [19, 20], and in one study without information about ethnicity [21].

The mutation is a missense mutation, a T to G transversion at amino acid position 39. Codon 39 is localised

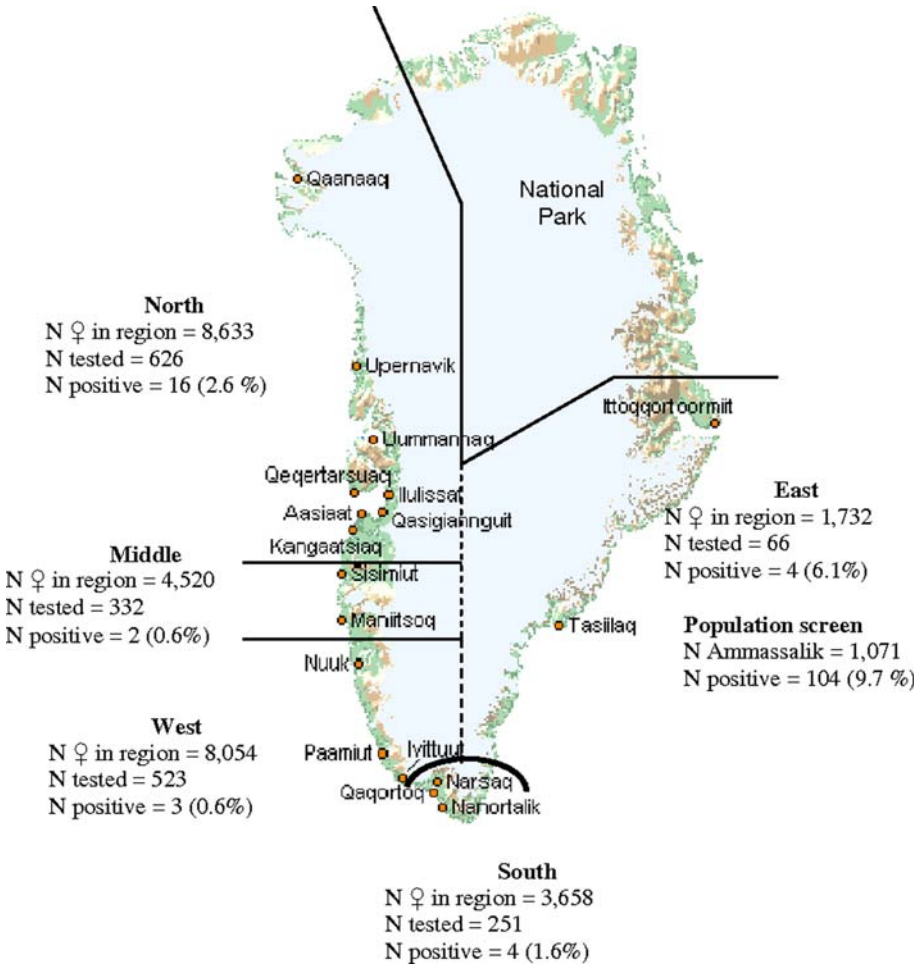

Fig. 1 Number of tested individuals and their location. Greenland is divided into four municipalities: North, Middle, South and East-West, which have been further divided into East and West by the *dotted line* for our purpose. Map modified from http://www.statgreen.gl/dk/kort.html

within the RING domain which spans amino acids 11–98 [22]. This domain is highly conserved and the cysteine residue is conserved from *Tetraodon* through to humans. The RING domain is also known to be important for protein-protein interactions of the BRCA1 protein [23]. The Cys to Gly amino acid change in codon 39 has now been reported to the Breast Cancer Information Core data base (BIC, http://research.nhgri.nih.gov/bic/). Other amino acid changes at this position have previously been reported and been judged to be deleterious for the function of the protein [24]. When the p.Cys39Gly mutation is analysed by the Align-GVGD, PolyPhen and SIFT algorithms, it is unanimously predicted to be deleterious.

The pathogenity of the mutation is further verified by the finding of loss of heterozygosity (LOH) in three out of four cancers from mutation carriers, where the LOH serves as the second hit in accordance with Knudson's two hit theory for tumour suppressor genes [25], especially when considering that somatic BRCA1 mutations are rare in sporadic breast and ovarian cancer [26, 27]. Not surprisingly we found the mutations to occur statistically significant more often in breast and ovarian cancer patients compared to the background population ($P < 0.001$).

Cancer in Greenland

Few studies have examined the breast and ovarian cancer incidence in Greenland let alone the spectrum of BRCA1 or BRCA2 mutations in the population. A study examining the development in cancer incidence in Greenland in the period of 1973–1997 found an increase in the incidence of lung (23%), stomach (24%), cervix uteri (10%) and breast cancer (14%). There was no significant increase in ovarian cancer with the incidence of ovarian cancer being comparable to the incidence in Denmark [28]. The authors suggest that the increasing Westernised life style in Greenland together with the increase in average life expectancy is part of the explanation for the increase in breast cancer incidence. Furthermore the diagnostic possibilities have also increased and more women now undergo examination for breast or ovarian cancer. This may also contribute to the increase in incidence of breast

cancer. The breast cancer risk in Greenlandic Inuit migrating to Denmark doubles, which may be explained partly by change in environmental factors and changes in the fertility pattern [29]. Further factors influencing the onset of breast cancer is parity, onset of menarche and menopause [30]. All of which are factors that have changed in Greenland within the last few decades. The penetrance of p.Cys39Gly mutation is currently not known, but it can be speculated that the mutation has been a low penetrance mutation until recently. After the changes in life style in Greenland, the mutation may now have changed into a high penetrance mutation, which may in part explain the increase in breast cancer incidence.

From our results we can conclude that the p.Cys39Gly mutation is an unknown variant outside the Inuit population, and is most likely an Inuit founder. Most of our material consists of people with Inuit background though it cannot be ruled out that there may be a number of samples of a different ethnic origin, mainly Danish.

BRCA1 mutation spectrum

The prevalence of BRCA1 mutation carriers has been estimated to range between 0.125 and 0.25% in different populations (Peto J 1999, Ford D 1995, Antoniou AC 2002, Antoniou AC 2000, McClain MR 2005). Among the populations most frequently studied for founder mutations are the Askenazi Jews. Familial breast and ovarian cancer is seen in this population, and the BRCA-1 and -2 spectrums of the Askenazi Jews have therefore been thoroughly described. It has been found that two mutations in the BRCA1 gene (185delAG and 5382insC) and one mutation in BRCA2 gene (6174delT) are the major mutations responsible for hereditary breast and ovarian cancer in this population [11]. In the general population of Askenazi Jews it has been showed that the two BRCA1 mutations are present in approximately 1% and 0.13% respectively [31, 32]. Another well-studied population is the population of Iceland. Here, one mutation in BRCA2 (999del5) is the only high-frequency founder mutation. It has been found in 0.4–0.6% of unaffected Icelanders, in 7.7–8.5% of breast cancer cases that had not been selected on the basis of family history or age of onset, and in 6–7.9% of ovarian cancer cases [33–35]. These carrier frequencies are somewhat lower than the 1.6% our study has observed in the general population of Greenland, and the Inuit population seems to have the highest carrier frequency of a BRCA1 mutation found in any population so far. Hansen et al. have studied the frequency of the p.Cys39Gly mutation in a smaller area on the West coast of Greenland. They find a frequency of almost 1% which is in concordance with our results from the same area (Fig. 1).

Origin of Inuit in Greenland

The geographic difference in carrier frequency is most likely due to a high degree of isolation and small number of inhabitants as well as the possibly different ancestry of the east Greenlanders [36]. The ancestors (the Thule culture) of the current population of Greenland are thought to have migrated into Greenland from the Canadian islands about year 900 [37]. It is likely that they brought the mutation with them, as the highest frequencies of the mutation is found in the east coast and the northern part of the west coast, and the frequency decreases towards the south in accordance with the increased distance from the passage from Canada.

According to mitochondrial DNA studies the Thule culture interbred with the existing Dorset culture creating a mixed population on the west and south coast of Greenland [36].

Conclusion

The p.Cys39Gly mutation in BRCA1 mutation is especially prevalent in the population of Greenland and in patients diagnosed with breast or ovarian cancer. The observed carrier frequency of 1.6% is higher than the prevalence of many genetic diseases for which routine screenings are offered, such as PKU and congenital hypothyroidisme (Andrews LB). Also it is almost double the frequency of BRCA1 mutations found in Askenazi population. Furthermore, collaboration with the national health authorities of the US and Canada (NIH and Health Canada) would be of interest to assess the occurrence of the p.Cys39Gly mutation in the North American Inuit population. We recommend that the female population of Greenland, along with the approximately 4.600 women of Greenlandic origin living in Denmark, should be offered genetic counselling and subsequent screening for the p.Cys39Gly mutation.

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