Peer reviewed article

BRCA1/2 mutations in Swiss patients with familial or early-onset breast and ovarian cancer[1]

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Summary

Questions under study: Germ-line alterations in BRCA1 and BRCA2 genes account for 30–50% of all forms of familial breast and ovarian cancer syndromes. Specific mutations in specific populations and ethnic groups have been identified in BRCA1 and BRCA2. However, it is not known whether such specific mutations prevail in the Swiss population.

Methods: We started to screen patients with primary breast and ovarian cancer and a strong family history of both cancers by sequencing the full-length coding regions of BRCA1 and BRCA2.

Results: With the selection criteria used in this study we identified 19 mutations in the first 38 patients screened (50%). These mutations were either defined as deleterious and resulted in a protein truncation (n = 10) or were defined as unclassified variants (n = 9). One novel truncating mutation was found in BRCA2 and two novel unclassified variants were detected in BRCA1. These three mutations are not described in the BIC and HGMD databanks.

Conclusions: We detected three unknown mutations among 38 patients in a Swiss study of BRCA1/2 mutation patterns. One of these novel mutations is clearly deleterious as it leads to protein truncation at nucleotide 133 of BRCA2.

Keywords: BRCA1; BRCA2; mutations; breast cancer; Swiss population

Introduction

One of the promising approaches to reducing the high incidence and mortality associated with breast and ovarian cancer lies in a better understanding of the risk factors for the disease and implementation of strategies to reduce these risk factors. It is currently estimated that 5–10% of all breast cancer cases are inherited in a dominant autosomal fashion through highly penetrant mutations in breast cancer susceptibility genes. Identification of two of these genes, BRCA1 and BRCA2, makes it possible to screen women with a strong family history of predisposition to breast and ovarian cancer [1–3]. Over the next few years it is likely that predictive testing for hereditary breast and ovarian cancer will become part of the medical management of women at high risk [4, 5].

The BRCA1 gene encodes a large protein of 1863 amino acids, and more than 300 mutations predisposing to disease have been identified throughout the gene. The BRCA2 gene encodes a protein of 3418 amino acids, also with a large array of different mutations identified throughout the coding region. However, a number of base substitutions do not alter the amino acid sequence or result in amino acid changes not associated with the disease (normal polymorphism) [6]. Hence the biggest challenge in interpreting the mutation analysis of BRCA1 and BRCA2 genes is to distinguish between normal polymorphisms and deleterious mutations associated with increased cancer risk. Only with a thorough knowledge of the impact of all mutations on cancer risk will it be possible to interpret genetic test results and to estimate individual familial risk of disease.

In addition, specific mutations in specific populations and ethnic groups have been identified in both genes. For example, specific BRCA1 and BRCA2 mutations were reported for Ashkenazi Jews [7]. BRCA1 germline screening in Italian families from Tuscany revealed a high frequency of unique mutations [8]. Other common BRCA1 mutations were found in Canadian, Belgian or Dutch breast cancer families [9–11]. These specific mutations indicate that there is a need to define the

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risks of breast and ovarian cancer in population-based studies, and that the type and frequency of the different mutations may be dependent on the different ethnic groups within each country.

Patients and methods

Patients

To increase the likelihood of BRCA1/2 mutations in the respective individuals, only breast and ovarian cancer patients entered our non-randomised study. The following additional selection criteria were used: (a) family history including one first-degree individual or two or more second-degree individuals on the same side of the family with breast or ovarian cancer or associated tumours such as prostate, colorectal and pancreas carcinomas at any age; (b) diagnosis of both breast and ovarian cancer at any age; (c) bilateral breast cancer; (d) breast or ovarian cancer before the age of 40; (e) male breast cancer patients; (f) Ashkenazi Jewish patients with early-onset breast or ovarian cancer or a family history of breast and ovarian cancers. Non-Caucasian patients were excluded from the study. From each patient a complete family history was taken in accordance with ethical and data-security guidelines and with the patient's express agreement. Patients were genetically tested only if pre- and post-test counselling was provided. The counselling relied on the standards proposed by the SIAK Network for Cancer Predisposition Testing and Counselling (Bern, Switzerland) [12]. The study required that the patients' informed consent be obtained prior to testing.

Sequencing

DNA was isolated from peripheral mononuclear blood cells by standard procedures. Coding sequences of both genes, including the intron-exon boundaries, were amplified from purified genomic DNA. The PCR products were purified and used as templates in a set of 160 cycle sequencing reactions using BigDye terminator chemistry (Applied Biosystems, Foster City, CA). Each gene was sequenced on both strands. Sequence alignment to a reference sequence was performed with the AutoAssembler (Applied Biosystems). The classification of the gene alterations was performed in accordance with the entries in the BIC (Breast Cancer Information Core, Bethesda, MD) and the HGMD (Human Gene Mutation Database, Institute of Medical Genetics, Cardiff, UK) databanks.

Results and discussion

38 patients were selected on the basis of the criteria described in "Patients and methods" and tested for BRCA1 and BRCA2 mutations. 50% of this patient collective showed no mutation in BRCA1 or BRCA2 when screened by sequencing the coding region of the two genes (Table 1). The changes found in the coding regions of the two genes of these patients were all clearly classified as polymorphisms. 15 of the patients showed a base substitution in one of the genes and 4 patients had a small deletion in one of the cancer predisposition genes. No insertions were found in any of the 38 patients. In six patients the base substitution resulted in an in-frame stop codon. Thus, of the 19 patients with mutations, 10 showed, according to the base sequence, a truncation of one of the resulting proteins (Table 2) most probably leading to a breast and ovarian cancer predisposition. Changes other than frame shifts or nonsense mutations were found in almost 50% of the patients with gene alterations. These mutations were all in accordance with the databanks' unclassified variants without a clear indication of predisposition. Thus, approximately 25% of all cases screened showed unclassified variants. One change could not be defined either as polymorphism or as an unclassified variant, since both classifications were found in the two databanks.

The types of mutation detected and the family members affected are listed in Table 3. Nine different mutations were detected in BRCA1 with 5 changes in exon 11. Eight different mutations were found in BRCA2 with only two changes in exon 11. Three out of 17 different mutations (~18%) were not listed in the BIC and HGMD databanks (G1487A and G2522A, both in exon 11 of BRCA1 and G133T in BRCA2). One mutation results in a protein truncation at nucleotide 133 of BRCA2 and is therefore classified as deleterious (patient #135). The other two mutations are unclassified (patients #105 and #153). Although the origin of these three mutations is unknown, they may be specific for the Swiss population. One of the unknown unclassified variants, the base substitution

Table 1

Type and frequency of mutations in 38 Swiss patients with primary breast and ovarian cancer.

Mutation	number of cases	% of total
None	19	50
Base substitution	15	39
Small deletion*	4	11

* One to ten bases were deleted in either of the two genes of these patients.

Table 2

Frequency of truncating mutations in 19 Swiss patients.

Mutation	number of cases	% of all changes	clinical consequence
Truncation*	10	53	predisposition
Other	8	42	unclassified
	1	5	unclassified/ polymorphism

Truncations were either caused by base substitution resulting in a stop codon (nonsense mutation) or a frameshift caused by small deletions.

Table 3

Definition of the individual 17 mutations found in 19 Swiss patients.

Patient ID	affected gene	exon	nucleotide change[a]	amino acid change	type[b]	age	affected family members[c]		
							breast cancer	ovarian cancer	other cancers
147	BRCA1	11	A1067G	Gln356Arg	M	36	mother, sister		
105		11	G1487A*	Arg496His	M bilateral	64	sister		grandfather (P) pancreas
124		11	C1687T	Gln563Stop	M	45	sister	sister	
153		11	G2522A*	Arg841Gln	M	36			father colon, grandfather (P) colon
146		11	3600-3610del	1160Stop	M	33	mother		
148		16	G4956A	Met1652Ile	M	46	mother, grandmother (M)		grandfather (P) prostate
121		16	G4956A	Met1652Ile	M	45	mother, grandmothr (M)		
151		18	G5080T	Glu1654Stop	O	44	mother, grandmother (P)		
102		18	G5199T	Glu1694Stop	M	36	grandmother (M), grandmother (P)		
116		18	5234delG	1707Stop	M	35	mother		
135	BRCA2	3	G133T*	Glu45Stop	M	28	mother, aunt (M)		
149		11	C5744T	Thr1915Met	M	46	sister, mother	sister	
134		11	C6100T	Arg2034Cys	M	54	mother, grandmother (M)		grandfather (P) prostate
100		14	7253delAA	2341Stop	M	30	Mother		
101		14	7253delAA	2341Stop	M bilateral	52			
150		20	T8503C	Ser2835Pro	M	45	mother, grandmother (P),		
125		22	G8850T	Lys2950Asn	M	41	mother, grandmother (M), aunt (P)		uncle (P) prostate
144		25	C9382T	Arg3128Stop	M	45	mother, grandmother (M), aunt (M)		
132		27	A9976T	Lys3326Stop	M bilateral	51 male			

[a] Nucleotide coordinates are relative to the A of the initiator ATG codon; [b] M: breast cancer; O: ovarian cancer; [c] P: paternal; M: maternal; * Mutations not found in the BIC and HGMD databanks

G2522A, was found in the BRCA1 gene of a woman with primary breast cancer (patient #153) and one other unclassified mutation in the same gene (A1067G). This patient had no first- or second-degree relative with breast or ovarian cancer but both the father and his father were diagnosed with colon carcinoma between the ages of 30 and 40. However, it is not known whether these two men were affected by the same BRCA1 mutations.

Two of the screened patients were males, both with primary breast cancer. One showed a mutation in BRCA2 (patient # 132, nonsense mutation, A3326T) in exon 27, which leads to a truncation of BRCA2. Since the truncating mutation is at the very end of the protein, it is possible that the protein's functions are not affected. Most of the few entries in databanks describing nonsense mutations near the C terminus of BRCA2 between codon 3308 and 3408 are described as unclassified variants. Thus, the effect of this truncating mutation on cancer predisposition remains unclear.

In all cases a significant number of discrepancies between the reference sequence from GenBank (NCBI, Bethesda, MD) and the actual individual patient data were detected. Most of these discrepancies could be classified as normal polymorphisms. However, a significant number of the unclassified variants might also represent polymorphisms linked to a specific population. Thus, the definition of polymorphic changes in the population under study will be the most important task for geneticists in individual countries. These definitions may then help to reduce the number and frequency of detections of unclassified variants of BRCA1 and BRCA2.

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References

1 Futreal PA, Liu Q, Shattuck-Eidens D, et al. BRCA1 mutations in primary breast and ovarian carcinomas. Science 1994;266: 120–2.

2 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.

3 Tavtigian SV, Simard J, Rommens J, et al. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. Nat Genet 1996;12:333–7.

4 Garvin AM, Mueller H, Eppenberger-Castori S, Eppenberger UR, Scott RJ. Informed consent and BRCA1 mutation detection in archived breast tumor specimens [letter]. Lancet 1996; 347:1189.

5 Garvin AM, Eppenberger U, Muller H, Eppenberger-Castori S, Scott RJ. BRCA1 mutations found in archived early onset breast tumours. Eur J Cancer 1997;33:683–6.

6 Monteiro AN, August A, Hanafusa H. Common BRCA1 variants and transcriptional activation [letter]. Am J Hum Genet 1997;61:761–2.

7 Roa BB, Boyd AA, Volcik K, Richards CS. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. Nat Genet 1996;14:185–7.

8 Caligo MA, Ghimenti C, Cipollini G, et al. BRCA1 germline mutational spectrum in Italian families from Tuscany: a high frequency of novel mutations. Oncogene 1996;13:1483–8.

9 Simard J, Tonin P, Durocher F, et al. Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. Nat Genet 1994;8:392–8.

10 Peelen T, van Vliet M, Petrij-Bosch A, et al. A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. Am J Hum Genet 1997;60:1041–9.

11 Petrij-Bosch A, Peelen T, van Vliet M, et al. BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. Nat Genet 1997;17:341–5.

12 Pichert, G. and Stahel, R. A. Organizing cancer genetics programs: The Swiss model. J Clin Oncol 2000;18:65–9s.

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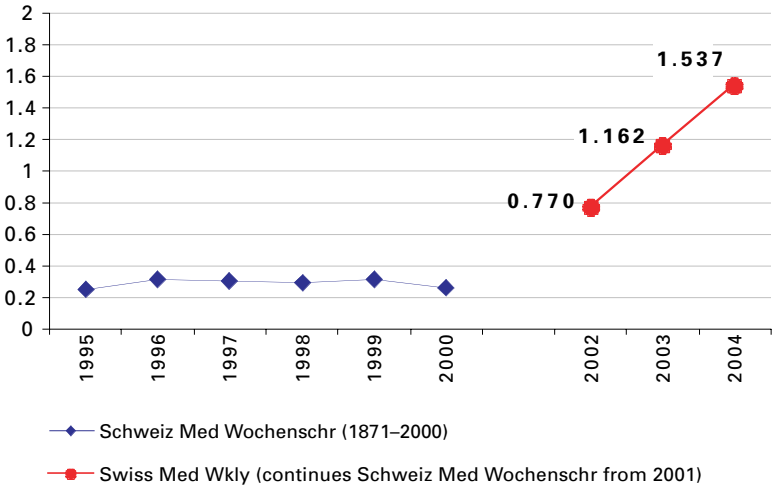

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