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EPIDEMIOLOGY

Genetic variants in *FGFR2* and *MAP3K1* are associated with the risk of familial and early-onset breast cancer in a South-American population

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Abstract Genome-Wide Association Studies have identified several loci associated with breast cancer (BC) in populations of different ethnic origins. One of the strongest associations was found in the *FGFR2* gene, and *MAP3K1* has been proposed as a low-penetrance BC risk factor. In this study, we evaluated the associations among *FGFR2* SNPs rs2981582, rs2420946, and rs1219648; and *MAP3K1* rs889312, with BC risk in 351 *BRCA1/2*-negative Chilean BC cases and 802 controls. All the SNPs studied were significantly associated with increased BC risk in familial BC and in non-familial early-onset BC, in a dose-dependent manner. Subjects with 3 risk alleles were at a significantly increased risk of BC compared with subjects with 0–2 risk alleles, in both familial BC and early-onset non-familial BC (OR = 1.47, 95 % CI 1.04–2.07, $P = 0.026$ and OR = 2.04 95 % CI 1.32–3.24, $P < 0.001$, respectively). In the haplotype analysis, the *FGFR2* rs2981582 T / rs2420946 T / rs1219648 G haplotype (ht2) was associated with a significantly increased BC risk compared with the rs2981582 C / rs2420946 C / rs1219648 A haplotype in familial BC and in non-familial early-onset BC (OR = 1.32, 95 % CI 1.06–1.65, $P = 0.012$; OR = 1.46, 95 % CI 1.11–1.91, $P = 0.004$, respectively). When the *FGFR2* ht2 and *MAP3K1* rs889312 were evaluated as risk alleles, the risk of BC increased in a dose-dependent manner as the number of risk alleles increased (P trend <0.0001), indicating an additive effect. Nevertheless, there is no evidence of an interaction between *FGFR2* ht2 and the *MAP3K1* rs889312 C allele. These findings suggest that genetic variants in the *FGFR2* and *MAP3K1* genes may contribute to genetic susceptibility to BC.

Keywords Breast cancer · Polymorphism · *FGFR2* · *MAP3K1*

Introduction

Breast cancer (BC) is the most common cancer among women worldwide. One of every eight women will develop

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BC during their lives [1]. In Chile, BC has the first-highest mortality rate among cancers (15.8/100,000 women), and its incidence has increased in all age groups analyzed [2].

Family-based studies indicate that an important proportion of BC is due to inherited susceptibility of known high-penetrance genes (e.g., *BRCA1*, *BRCA2*, *ATM*, and others). Nevertheless, mutations in *BRCA1* and *BRCA2* are responsible for only 16–20 % of familial BC risk; therefore, the genetic basis of 80 % of familial cases remains unexplained [3]. Researchers have proposed a polygenic model as the most likely explanation for the bulk of the genetic component. Under this model, which includes high-, moderate-, and low-penetrance genes, multiple loci across the genome contribute to disease susceptibility [4]. Recent Genome-Wide Association Studies (GWAS) have identified genetic variations that may play a role as BC risk factors in populations of diverse ethnicities. Easton et al. [5], in a large-scale GWAS, identified five novel, independent, low-penetrance susceptibility loci that were strongly associated with BC in European women. Four of these (*FGFR2*, *TOX3*, *MAP3K1*, and *LSP1*) contain plausible causative genes.

Fibroblast Growth Factor Receptor 2 (FGFR2) is a tyrosine kinase receptor that belongs to the FGFR family involved in tumorigenesis. FGFR2 is a transmembrane protein that acts as a mitogenic gene activator for invasion, mobility, or angiogenesis, depending on cell type or environment [6]. *FGFR2* is overexpressed in BC cell lines and amplified and overexpressed in breast tumors [7, 8]. The human gene *FGFR2* is located in 10q26 and has 21 exons [9]. Easton et al. [5] reported in a GWAS study that rs2981582, located on intron 2 of *FGFR2*, is associated with increased BC risk. Hunter et al. [10] reported that four single-nucleotide polymorphisms (SNPs) (rs2420946, rs1219648, rs2981579, and rs11200014), also located in intron 2, were associated with BC risk. Huijts et al. [11] confirmed this association. Interestingly, the five SNPs described showed high linkage disequilibrium (LD) in people with European ancestry (all pairwise $r^2 > 0.90$). Liang et al. [12] performed genotyping analysis of rs2981582, rs1219648, and rs2420946 SNPs in a case–control study of 1,073 healthy controls and 1,048 BC cases, of which 28.8 % had familial BC. They found that each of the three SNPs was significantly associated with increased BC risk in a dose-dependent manner. The authors concluded that genetic variation in *FGFR2* contributes to BC risk in Chinese women, with no statistical difference in risk between the subgroup with familial BC and sporadic BC. Raskin et al. [13] genotyped four *FGFR2* SNPs (rs11200014, rs1219648, rs2420946, and 2981579) in 1,529 women with BC, including Ashkenazi Jews, Sephardic Jews, and Arabs, of which 14 % had familial BC, and 1,528 healthy controls. They found significant association between BC risk and all four studied SNPs in *FGFR2* (*P* trend for all SNPs <0.0001). Meyer et al. [14] studied the effect of a haplotype constituted by 8 SNPs distributed in a 7.5 kb region of intron 2 of *FGFR2*. They found that *FGFR2* presented higher levels of expression in tumors homozygous for minor frequency alleles than those homozygous for higher frequency alleles (Wilcox $P < 0.028$).

Hemminki et al. [15] confirmed the contribution of *FGFR2* SNPs in German women with familial *BRCA1/2*-negative BC. Barnholz-Sloan et al. [16] reported an association between *FGFR2* SNPs and BC risk in African-American and younger women. The meta-analysis by Jia et al. [17] suggested that rs2981582, rs1219648, and rs2420946 polymorphisms in *FGFR2* are associated with elevated BC risk. Recently, Fu et al. [18] reported an association between *FGFR2* SNPs and early-onset non-familial BC.

Mitogen-Activated Protein Kinase Kinase Kinase 1 (*MAP3K1*) gene was identified in two GWAS of BC [5, 10]. It acts in the mitogen-activated protein kinase (MAPK) signaling pathway that includes Ras, Raf, Mek, and Erk and is responsible for regulating the transcription of important cancer genes. GWAS have identified the SNP rs889312, which lies in a linkage disequilibrium block of approximately 280 kb, which encodes a serine/threonine kinase protein and forms part of the MAPK cell-signaling pathway implicated in cellular response to mitogens [5]. To date, several studies have evaluated the association between the *MAP3K1* gene rs889312 polymorphism and BC risk. Garcia-Closas et al. [19] reported that the *MAP3K1* rs889312 SNP was associated with significant increase in risk of ER-negative breast tumors. Slattery et al. [20] reported that *MAP3K1* was not associated with BC risk among Hispanic and non-Hispanic white women living in the Southwest United States. Lu et al. [21] performed a meta-analysis suggesting that the *MAP3K1* rs889312 C allele is a low-penetrance risk factor for developing BC.

Most previous studies on genetic variants in *FGFR2* and *MAP3K1* genes have been done in populations of diverse ethnicities. Nevertheless, the contribution of these variants to BC in South-American women is unknown. On the other hand, few studies included younger women or early-onset non-familial BC [16, 18]. In the Chilean population, 18 % of the BC patients with family history of breast and ovarian cancer carry *BRCA1/2* point mutations [22], and none of the non-familial early-onset patients studied was a carrier of *BRCA1/2* point mutations. The mutations in other susceptibility genes are not frequent enough to explain the remaining BRCA-negative familial BC cases [3]. Under the assumption of a polygenic trait, we evaluated SNPs and haplotypes in *FGFR2* and *MAP3K1* for their association with familial BC and early-onset non-familial BC.

Methods

Families

A total of 351 BC patients belonging to 351 high-risk *BRCA1/2*-negative Chilean families were selected from the files of the Servicio de Salud del Area Metropolitana de Santiago, Corporación Nacional del Cáncer (CONAC) and other private services of the Metropolitan Area of Santiago. All index cases were tested for *BRCA1* and *BRCA2* mutations as described [22, 23]. Briefly, the whole coding sequence and exon–intron boundaries of *BRCA1* and *BRCA2* genes were amplified by polymerase chain reaction (PCR) using previously described primers [23]. The fragments obtained were analyzed for sequence variants using conformational sensitive gel electrophoresis (CSGE) [24]. Amplified samples were denaturated at 95 °C for 5 min and 65 °C for 30 min to generate heteroduplex. The products were diluted 1:2 in sucrose buffer and loaded in a partially denaturing MDE® gel (Cambrex, UK) at constant power of 7 W during different time periods depending on the size of the fragment. Gels were silver-stained and dried on a vacuum gel dryer. All sequence variants detected by CSGE were identified by reamplification of the original DNA sample and direct sequencing was performed in an ABI Prism 310 automated fluorescence-based cycle sequencer and a Rhodamine dye terminator system (Applied Biosystems, Foster City, CA). The exon 11 of *BRCA1/2* genes were analyzed by direct sequencing in all index cases. Pedigrees were constructed on the basis of an index case considered to have the highest probability of being a deleterious mutation carrier. None of the families met the strict criteria for other known syndromes involving BC, such as Li-Fraumeni, ataxia-telangiectasia, or Cowden disease.

Table 1 shows the specific characteristics of the families selected according to the inclusion criteria. All families participating in the study self reported Chilean ancestry dating from several generations, after extensive interviews with several members of each family from different generations. In the selected families, 13.1 % (46/351) had cases of bilateral BC; 8.3 % (29/351) had cases of both BC and OC; and 2.6 % (9/351) had male BC. In the BC group, the mean age of diagnosis was 42.3 years, and 77.2 % had age of onset <50 years. BC was verified by the original pathology report for all probands.

Table 1 Inclusion criteria for the families included in this study

Inclusion criteria	Families *n* (%)
Three or more family members with breast and/or ovarian cancer	85 (24.2)
Two family members with breast and/or ovarian cancer	129 (36.8)
Single affected individual with breast cancer ≤age 35	67 (19.1)
Single affected individual with breast cancer age 36–50	70 (19.9)
Total	351 (100)

This study was approved by the Institutional Review Board of the School of Medicine of the University of Chile. Informed consent was obtained from all participants.

Control population

The sample of healthy Chilean controls ($n = 802$) was recruited from CONAC files. DNA samples were taken from unrelated individuals with no personal or familial history of cancer and who had given consent for anonymous testing. These individuals were interviewed and informed as to the aims of the study. DNA samples were obtained according to all ethical and legal requirements. The control sample was matched to the cases for age and socioeconomic strata.

Mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes of 351 cases belonging to the high-risk selected families and 802 controls. Samples were obtained according to the method described by Chomczynski and Sacchi [25].

Genotyping of rs2981582 (*FGFR2*), rs1219648 (*FGFR2*), rs2420946 (*FGFR2*), and rs889312 (*MAP3K1*) was carried out using pre-designed TaqMan SNP Genotyping Assays (Applied Biosystems) (assay ID C___2917302_10, C___2917314_20, C___2917305_10, and C___8886795_10, respectively). The reaction was performed in a 10uL final volume containing 5 ng of genomic DNA, 1X TaqMan Genotyping MasterMix, and 1X TaqMan SNP Genotyping Assay. Polymerase chain reaction was carried out in a StepOne Plus RealTime PCR System (Applied Biosystems). The thermal cycles were initiated for 10 min at 95 °C, followed by 40 cycles each of 92 °C for 15 s and 60 °C for 1 min. Each genotyping run contained DNA controls confirmed by sequencing. The alleles were assigned using the software SDS 2.2 (Applied Biosystems). As a quality control, we repeated the genotyping on ~10 % of the samples, and all genotype scoring was performed and checked separately by two reviewers unaware of the case–control status.

Statistical analyses

The Hardy–Weinberg equilibrium assumption was assessed in the control sample using a goodness-of-fit χ^2 test (HWChisq function included in "HardyWeinberg" package v 1.4.1). Fisher's exact test was used to test the

association of *FGFR2* and *MAP3K1* genotypes and/or alleles in cases and controls. The odds ratio (OR) and its 95 % confidence interval (CI) were calculated to estimate the strength of the association in cases and controls (oddsratio.fisher function included on "epitools" package v0.5–6). A two-sided *P* value <0.05 was used as the criterion for significance. Haplotype estimation was carried out using UNPHASED v 3.1.5 software which uses a maximum likelihood approach [26]. The linkage disequilibrium among polymorphisms was measured using HaploView v4.2 [27]. The Cochran-Armitage trend test was performed to test additive genetic effect model (CATT function included on "Rassoc" package v 1.03). A χ^2 test for trend was performed to examine additive combined effects of *FGFR2* risk haplotypes and *MAP3K1* rs889312 risk allele (Stata/SE 10.0 for Unix -StataCorp, TX, USA-using "ptrend" package). The interaction on the additive scale was assessed by measuring the relative excess risk due to interaction (RERI) [28]. The confidence interval (CI) and *P* value were calculated according to Hosmer et al. [29] (expected value under the null hypothesis = 0). The interaction on the multiplicative scale was assessed by logistic regression analysis (Stata/SE 10.0 for Unix -StataCorp, TX, USA) and by calculating the ratio of the combined OR divided by the independent ORs of the SNPs considered in this study (expected value under the null hypothesis = 1). A *P* value <0.05 was used as the criterion for statistical significance. All statistical analyses were performed using the R statistical environment (available at http://www.r-project.org/), unless indicated otherwise.

Results

Table 2 shows the allele and genotype distributions of *FGFR2* rs2981582 C/T, rs2420946 C/T, rs1219648 A/G, and *MAP3K1* rs889312 A/C polymorphisms in the whole sample of *BRCA1/2*-negative cases ($n = 351$) and in the subgroups of cases belonging to families with two or more family members with BC and/or OC ($n = 214$) (subgroup A), and single affected women with BC without family history of BC or OC and age of diagnosis before 50 years ($n = 137$) (subgroup B) and in controls ($n = 802$). The observed genotype frequencies for these four SNPs were all in agreement with Hardy–Weinberg equilibrium in the controls ($P = 0.15$; 0.32; 0.43; and 0.62 for rs2981582, rs2420946, rs1219648, and rs889312, respectively).

The genotype and allele distribution of the three *FGFR2* SNPs and *MAP3K1* rs889312 A/C were significantly different in the whole sample *BRCA1/2*-negative cases, and in subgroups A and B, with respect to controls ($P < 0.05$). The *FGFR2* rs2981582, rs2420946, and rs1219648; and *MAP3K1* rs889312 were associated with a signifi-

Table 2 Genotype and allelic frequencies of *FGFR2* and *MAP3K1* SNPs in *BRCA1/2*-negative breast cancer cases and controls

Genotype or allele	Controls (%) ($n = 802$)	All BC cases ($n = 351$)			Families with ≥ 2 BC and/or OC cases ($n = 214$)			Single affected, diagnosis ≤ 50 years ($n = 137$)		
		BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]
FGFR2 rs2981582										
C/C	295 (36.8)	93 (26.5)	–	1 (ref)	62 (29.0)	–	1 (ref)	31 (22.6)	–	1 (ref)
C/T	366 (45.6)	178 (50.7)	0.004	1.54 [1.13–2.10]	101 (47.2)	0.134	1.31 [0.91–1.90]	77 (56.2)	0.002	2.00 [1.26–3.23]
T/T	141 (17.6)	80 (22.8)	0.001	1.80 [1.23–2.62]	51 (23.8)	0.015	1.72 [1.10–2.68]	29 (21.2)	0.020	1.95 [1.09–3.50]
P trend[b]			0.0007			0.011			0.007	
C/T + T/T	507 (63.2)	258 (73.5)	0.0007	1.61 [1.21–2.16]	152 (71.0)	0.036	1.43 [1.02–2.02]	106 (77.4)	0.001	1.99 [1.28–3.15]
C	956 (0.60)	364 (0.52)	–	1(ref)	225 (0.53)	–	1 (ref)	139 (0.51)	–	1 (ref)
T	648 (0.40)	338 (0.48)	0.0006	1.37 [1.14–1.64]	203 (0.47)	0.010	1.33 [1.07–1.66]	135 (0.49)	0.007	1.43 1.10–1.87]
FGFR2 rs2420946										
C/C	285 (35.5)	91 (25.9)	–	1 (ref)	60 (28.0)	–	1 (ref)	31 (22.6)	–	1 (ref)
C/T	374 (46.6)	175 (49.9)	0.012	1.46 [1.07–2.00]	101 (47.2)	0.182	1.28 [0.88–1.86]	74 (54.0)	0.008	1.81 [1.43–2.94]
T/T	143 (17.8)	85 (24.2)	0.0009	1.86 [1.28–2.70]	53 (24.8)	0.011	1.76 [1.13–2.74]	32 (23.4)	0.009	2.05 [1.16–3.63]

Table 2 continued

Genotype or allele	Controls (%) ($n = 802$)	All BC cases ($n = 351$)			Families with ≥ 2 BC and/or OC cases ($n = 214$)			Single affected, diagnosis ≤ 50 years ($n = 137$)		
		BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]
P trend[b]			0.0004			0.009			0.005	
C/T + T/T	517 (64.5)	260 (74.1)	0.0013	1.57 [1.18–2.11]	154 (72.0)	0.042	1.41 [1.01–2.01]	106 (77.4)	0.002	1.88 [1.21–2.99]
C	944 (0.59)	357 (0.51)	–	1 (ref)	221 (0.52)	–	1 (ref)	136 (0.50)	–	1 (ref)
T	660 (0.41)	345 (0.49)	0.0004	1.38 [1.15–1.66]	207 (0.48)	0.008	1.34 [1.07–1.67]	138 (0.50)	0.005	1.45 [1.11–1.89]
FGFR2 rs1219648										
A/A	286 (35.7)	90 (25.6)	–	1 (ref)	60 (28.0)	–	1 (ref)	30 (21.9)	–	1 (ref)
A/G	368 (45.9)	181 (51.6)	0.003	1.56 [1.15–2.13]	106 (49.5)	0.079	1.37 [0.95–1.99]	75 (54.7)	0.004	1.94 [1.22–3.16]
G/G	148 (18.5)	80 (22.8)	0.004	1.71 [1.18–2.50]	48 (22.4)	0.057	1.54 [0.98–2.42]	32 (23.4)	0.011	2.06 [1.16–3.66]
P trend[b]			0.002			0.035			0.005	
A/G + G/G	516 (64.3)	261 (74.4)	0.0008	1.60 [1.21–2.18]	154 (72.0)	0.042	1.42 [1.01–2.02]	107 (78.1)	0.002	1.98 [1.27–3.15]
A	940 (0.59)	361 (0.51)	–	1 (ref)	225 (0.53)	–	1 (ref)	135 (0.49)	–	1 (ref)
G	664 (0.41)	341 (0.49)	0.001	1.34 [1.12–1.61]	203 (0.47)	0.026	1.28 [1.02–1.59]	139 (0.51)	0.004	1.46 [1.12–1.90]
Dichotomized risk loci										
0–2	300	93	–	1 (ref)	62	–	1 (ref)	31	–	1 (ref)
3	502	258	0.0003	1.66 [1.25–2.21]	152	0.026	1.47 [1.04–2.07]	106	0.0007	2.04 [1.32–3.24]
MAP3K1 rs889312										
A/A	333 (41.5)	120 (34.2)	–	1 (ref)	74 (34.6)	–	1 (ref)	46 (33.6)	–	1 (ref)
A/C	362 (45.1)	158 (45.0)	0.200	1.21 [0.91–1.62]	96 (44.9)	0.345	1.19 [0.84–1.70]	62 (45.3)	0.351	1.24 [0.81–1.91]
C/C	107 (13.3)	73 (20.8)	0.0008	1.89 [1.29–2.76]	44 (20.6)	0.007	1.84 [1.17–2.91]	29 (21.2)	0.016	1.96 [1.13–3.37]
P trend[b]			0.001			0.008			0.013	
A/C + C/C	469 (58.5)	231 (65.8)	0.022	1.37 [1.04–1.79]	140 (65.4)	0.071	1.34 [0.97–1.87]	91 (66.4)	0.090	1.40 [0.95–2.11]
A	1028 (0.64)	398 (0.57)	–	1 (ref)	244 (0.57)	–	1 (ref)	154 (0.56)	–	1 (ref)
C	576 (0.36)	304 (0.43)	0.0009	1.36 [1.13–1.64]	184 (0.43)	0.008	1.35 [1.08–1.68]	120 (0.44)	0.015	1.39 [1.06–1.82]

BC breast cancer, *OC* ovarian cancer, *OR* odds ratio, *CI* confidence interval

[a] Fisher's exact test

[b] Cochran-Armitage trend test

Table 3 Frequencies of inferred haplotypes of *FGFR2* rs2981582, rs2420946, and rs1219648 in *BRCA1/2*-negative breast cancer cases and controls

Haplotype[a]	Controls (n = 1,604)	All BC cases (n = 702)			Families with ≥2 BC and/or OC cases (n = 428)			Single affected, diagnosis ≤50 years (n = 274)		
		BC cases	*P* value[b]	OR [95 CI]	BC cases	*P* value[b]	OR [95 % CI]	BC cases	*P* value[b]	OR [95 % CI]
ht1, C-C-A	0.577	0.504	–	Ref.	0.514	–	Ref	0.489	–	Ref.
ht2, T-T-G	0.397	0.477	0.0005	1.37 [1.14–1.65]	0.467	0.012	1.32 [1.06–1.65]	0.493	0.004	1.46 [1.11–1.91]
Others[c]	0.026	0.019	0.643	0.82 [0.40–1.60]	0.019	0.713	0.82 [0.33–1.80]	0.018	0.763	0.84 [0.25–2.18]

ht haplotype, *BC* breast cancer, *OC* ovarian cancer, *OR* odds ratio, *CI* confidence interval

[a] In the order of FGFR2 rs2981582, rs2420946, and rs1219648

[b] Fisher's exact test

[c] Include haplotypes that had a frequency <2 %

cantly increased BC risk in familial BC and in non-familial early-onset BC. Furthermore, in the whole sample, the homozygous minor allele genotypes were associated with increased BC risk (rs 2981582 (TT genotype OR = 1.80 [95 % CI 1.23–2.62], $P = 0.001$), rs2420946 (TT genotype OR = 1.86 [95 % CI 1.28–2.70], $P < 0.001$), rs 1219648 (GG genotype OR = 1.71 [95 % CI 1.18–2.50], $P = 0.004$), and rs889312 (CC genotype OR = 1.89 [95 % CI 1.29–2.76], $P < 0.001$). We also observed increased BC risk in the whole sample for carriers of the minor allele frequency for the four SNPs studied (Table 2). In subgroup A, which includes cases with family history of BC, a significant association was observed between BC risk and the homozygous minor allele genotype for *FGFR2* rs2981582 (TT genotype OR = 1.72 [95 % CI 1.10–2.68], $P = 0.015$), *FGFR2* rs2420946 (TT genotype OR = 1.76 [95 % CI 1.13–2.74], $P = 0.011$), and *MAP3K1* rs889312 (CC genotype OR = 1.84 [95 % CI 1.17–2.91], $P = 0.007$). Table 2 also shows that in subgroup B, the homozygous minor allele genotypes for the four SNPs studied were associated with increased risk of breast cancer. Furthermore, the *P* trend test for the genotypes between cases and controls shows that the association for allele variants were dose dependent for each locus in the whole sample and in the subgroup A and B (Table 2). Breast cancer risk was significantly increased in carriers with 3 risk loci compared with those with 0–-2 risk loci in the whole sample of *BRCA1/2*-negative cases and in the subgroups A and B (OR = 1.66 [95 % CI 1.25–2.21], $P = 0.0003$; OR = 1.47 [95 % CI 1.04–2.07], $P = 0.026$, and OR = 2.04 [95 % CI 1.32–3.24], $P < 0.001$, respectively).

Linkage disequilibrium analyses showed that all three variants of *FGFR2* were in LD with each other ($r^2 = 0.93$ for rs1219648 and rs2981582; $r^2 = 0.92$ for rs2420946 and rs2981582; and $r^2 = 0.94$ for rs1219648 and rs2420946). Therefore, we performed haplotype inference on these three polymorphisms. As shown in Table 3, just two common haplotypes accounted for >95 % of all haplotypes construed by these three SNPs. Compared with the most common haplotype CCA (ht1), the TTG (ht2) haplotype, which contains the three risk alleles, was associated with an increased breast cancer risk in the whole sample and in subgroups A and B (Table 3).

Table 4 shows the distribution of combined genotypes of the *FGFR2* haplotypes and *MAP3K1* rs889312. We observed that the combined genotypes ht1/ht2–C/C significantly increased the breast cancer risk in the three groups analyzed, and the OR were higher with respect to the risk conferred for the haplotype ht2 alone or for the genotype CC (*MAP3K1*) alone. ORs were also statistically significant higher for the combined genotypes ht2/ht2–C/C in the three groups studied. Moreover, in the subgroup B, which includes single affected women with early-onset

Table 4 Distribution of combined genotypes of *FGFR2* haplotypes and *MAP3K1* rs889312 in *BRCA1/2*-negative breast cancer cases and controls

Composite genotype		Controls ($n = 802$) (%)	All BC cases ($n = 351$)			Families with ≥2 BC and/or OC cases ($n = 214$)			Single affected, diagnosis ≤50 years ($n = 137$)		
FGFR2	MAP3K1		BC cases (%)	OR [95 % CI]	*P* value[a]	BC cases (%)	OR [95 % CI]	*P* value[a]	BC cases (%)	OR [95 % CI]	*P* value[a]
ht1/ht1	A/A	119 (15.5)	30 (8.8)			19 (9.2)			11 (8.3)		
ht1/ht1	A/C	116 (15.1)	35 (10.3)	1.20 [0.67–2.16]	0.576	25 (12.1)	1.35 [0.67–2.74]	0.413	10 (7.5)	0.93 [0.34–2.52]	1.000
ht1/ht1	C/C	41 (5.4)	23 (6.8)	2.22 [1.10–4.46]	0.024	14 (6.8)	2.13 [0.90–4.95]	0.059	9 (6.8)	2.36 [0.80–6.79]	0.109
ht1/ht2	A/A	148 (19.3)	60 (17.7)	1.61 [0.95–2.75]	0.065	38 (18.4)	1.61 [0.85–3.11]	0.141	23 (17.3)	1.68 [0.75–3.98]	0.201
ht1/ht2	A/C	163 (21.3)	83 (24.5)	2.02 [1.22–3.39]	0.004	44 (21.4)	1.69 [0.91–3.23]	0.088	38 (28.6)	2.52 [1.20–5.69]	0.011
ht1/ht2	C/C	43 (5.6)	30 (8.8)	2.75 [1.43–5.35]	0.001	18 (8.7)	2.61 [1.17–5.81]	0.011	12 (9.0)	3.00 [1.12–8.13]	0.016
ht2/ht2	A/A	51 (6.7)	24 (7.1)	1.86 [0.94–3.66]	0.068	15 (7.3)	1.84 [0.80–4.16]	0.113	9 (6.8)	1.90 [0.65–5.40]	0.205
ht2/ht2	A/C	64 (8.4)	36 (10.6)	2.22 [1.21–4.12]	0.008	23 (11.2)	2.24 [1.08–4.71]	0.022	13 (9.8)	2.19 [0.85–5.74]	0.076
ht2/ht2	C/C	21 (2.7)	18 (5.3)	3.37 [1.49–7.62]	0.002	10 (4.9)	2.96 [1.07–7.87]	0.031	8 (6.0)	4.07 [1.26–12.71]	0.009
Others[b]		36 (4.5)	12 (3.6)	1.32 [0.56–2.99]	0.544	8 (3.9)	1.39 [0.48–3.67]	0.472	4 (3.1)	1.20 [0.26–4.38]	0.754

BC breast cancer, *OC* ovarian cancer, *OR* odds ratio, *CI* confidence interval

[a] Fisher's exact test

[b] Includes *FGFR2* haplotypes that had a frequency <2 %

breast cancer, we observed higher OR values for the combined genotypes ht1–ht2–CC and ht2–ht2–CC with respect to the whole sample or subgroup A (Table 4).

Since the *FGFR2* ht2 and *MAP3K1* rs889312 C allele were associated with increased breast cancer risk, we considered the *FGFR2* ht2 and *MAP3K1* rs889312 C as risk alleles and then evaluated their combined effects by dividing the subjects into five groups based on the number of risk alleles [subjects with 0 (group 1), 1 (group 2), 2 (group 3), 3 (group 4), and 4 (group 5)]. As shown in Table 5, the distribution of the combined genotypes in the whole sample and in subgroups A and B significantly differed from that in controls ($P = 0.003$, 0.017, and 0.0025, respectively), and the risk of breast cancer increased in a dose-dependent manner as the number of risk alleles increased (P trend <0.0001). Considering group 1 as the reference group, the OR of group 5 for breast cancer was 3.40 (95 % CI 1.61–7.17, $P = 0.001$) for the whole sample, 2.98 (95 % CI 1.22–7.30, $P = 0.017$) for familial BC, and 4.12 (95 % CI 1.48–11.45, $P = 0.007$) for early-onset non-familial BC. These results indicate an additive effect of the *FGFR2* ht2 and *MAP3K1* rs889312 C allele on increased breast cancer risk.

Considering the additive effect observed between *FGFR2* ht2 and *MAP3K1* C allele on increased risk of familial and early-onset non-familial BC, we then evaluated the interaction between the two loci on an additive and multiplicative scale. The estimated measures of interaction were not significant ($P > 0.05$).

Discussion

Mutations in *BRCA1* and *BRCA2* are associated with susceptibility to BC and OC. At present, however, these mutations account for only a portion of familial cases, and consequently there is an intensive search for additional susceptibility targets. GWAS have recently identified genetic variants associated with BC in populations of European and Asian ancestry [5, 10]. However, the contribution of these variants as predictors in South-American women is unknown. In the present study, we evaluated the impact of *FGFR2* and *MAP3K1* polymorphisms on familial and in non-familial early-onset BC negative for point mutations in *BRCA1/2* from a Chilean population. To this end, we studied the association between three SNPs (rs2981582, rs2420946, and rs1219648) of *FGFR2* and rs889312 in the *MAP3K1* gene in a case–control study.

The *FGFR2* gene encodes a receptor tyrosine kinase and is a tumor suppressor gene that can be amplified and overexpressed in BC cells. Meyer et al. [14] have shown that the rs2981582 and rs1219648 SNPs alter the binding of two transcription factors, Oct-1/Runx2 and C/EBPb, resulting in an increase of *FGFR2* gene expression both in

Table 5 Combined effects of *FGFR2* haplotypes (rs2981582 C>T, rs2420946 C>T, and rs1219648 A>G) and *MAP3K1* rs889312 A>C on the risk of breast cancer

Number of risk alleles[a]	Controls (n = 802) (%)	All BC cases (n = 333)			Families with ≥2 BC and/or OC cases (n = 204)			Single affected, diagnosis ≤50 years (n = 129)		
		BC cases (%)	OR [95 %CI]	P value[b]	BC cases (%)	OR [95 %CI]	P value[b]	BC cases (%)	OR [95 %CI]	P value[b]
0 risk allele	119 (15.5)	30 (8.8)			19 (9.2)			11 (8.3)		
1 risk allele	264 (34.5)	95 (28.0)	1.43 [0.90–2.27]	0.133	63 (30.4)	1.49 [0.86–2.61]	0.157	32 (24.1)	1.31 [0.64–2.69]	0.460
2 risk allele	255 (33.3)	130 (38.3)	2.02 [1.29–3.18]	0.002	73 (35.3)	1.79 [1.03–3.11]	0.037	57 (42.9)	2.42 [1.22–4.78]	0.011
3 risk allele	107 (14.0)	66 (19.5)	2.45 [1.48–4.05]	0.001	42 (20.3)	2.46 [1.35–4.49]	0.003	25 (18.8)	2.52 [1.19–5.38]	0.016
4 risk allele	21 (2.7)	18 (5.3)	3.40 [1.61–7.17]	0.001	10 (4.8)	2.98 [1.22–7.30]	0.017	8 (6.0)	4.12 [1.48–11.45]	0.007
P trend[c]				<0.0001			0.266			0.0001
Global P[d]				0.0003			0.017			0.0025

BC breast cancer, *OC* ovarian cancer, *OR* Odds Ratio, *CI* Confidence Interval

[a] 0 risk allele: ht1/ht1 + A/A; 1 risk allele: ht1/ht1 + A/C, ht1/ht2 + A/A; 2 risk alleles: ht1/ht1 + C/C, ht1/ht2 + A/C, ht2/ht2 + A/A; 3 risk alleles: ht1/ht2 + C/C, ht2/ht2 + A/C; 4 risk alleles: ht2/ht2 + C/C

[b] Fisher's exact test

[c] χ^2 test for trend

[d] χ^2 test for independence

cell lines and in breast tissue. A number of case–control studies have been conducted to investigate the association between *FGFR2* polymorphisms located in intron 2 and in promoter of this gene with BC susceptibility [30]. Specifically, case–control studies have shown that SNPs in intron 2 of *FGFR2* are strongly associated with risk of BC in European [5, 10], Asian [5, 12], African-American [31], Ashkenazi Jewish [32], Israeli [13], and Chinese populations [12, 33]. Therefore, the association and functional studies, and the meta-analysis by Jia et al. [17], suggest that risk alleles of SNPs rs2981582, rs1219648, and rs2420946 are low-penetrant risk factors for developing BC.

In this study, we found that the SNPs in the second intron of *FGFR2*, rs2981582, rs2420946, and rs1219648 were significantly associated with increased risk of familial BC and early-onset non-familial BC in Chilean population. This result is in accordance with Esteban Cardeñosa et al. [34], who in Spanish population found statistically significant differences between familial BC/OC and healthy controls for rs2981582 polymorphism, particularly in non-carriers of *BRCA1/2* mutations. The Chilean population is the result of the admixture between Amerindian peoples (40 %) and the Spanish population (60 %) [35, 36]. The majority of the case–control studies have been done in sporadic BC. Nevertheless, Latif et al. [37] reported that susceptibility variants in *FGFR2* are associated with increased BC risk in individuals with family history of BC, and that the level of risk is dependent on the family history. This study also established that the risk conferred by *FGFR2* variants is similar to those of individuals from case–control series of sporadic BC. Otherwise, the risk factors for early-onset BC remain to be determined. A study conducted in an American population showed that in this group of cases, only 10 % carried deleterious *BRCA1* or *BRCA2* mutations, and 1 % were non-familial [38]. These data indicate that mutations in *BRCA1* and *BRCA2* genes account for only a very small proportion of early-onset non-familial BC, and that other susceptibility genes may exist. Fu et al. [18] reported that polymorphisms in the second intron of the *FGFR2* gene, including rs2981582, rs1219648, and rs2420946, are associated with risk of early-onset BC in Chinese Han women. In our experience, none of the 137 women with early-onset BC without family history of breast or ovarian cancer were carriers of *BRCA1/2* mutations; therefore, it is likely that polymorphisms in intron 2 of *FGFR2* play a role in tumorigenesis in this subgroup of women.

In addition, each of the three SNPs was significantly associated with increased BC risk in a dose-dependent manner, with increasing risk as the number of variant alleles increased, both in the subgroup with familial BC and in the subgroup of non-familial BC with early age of diagnosis. The presence of three risk alleles was associated

with 1.47- (subgroup A) and 2.04- (subgroup B) fold increased risk of BC compared with the presence of 0–2 risk alleles. These results indicate that the *FGFR2* SNPs have an additive effect on an increased BC risk. These results are consistent with those reported by Liang et al. [12] in Chinese women with sporadic BC.

Single-nucleotide polymorphisms in the second intron of *FGFR2*, including rs2981582, rs2420946, and rs1219648, are in a linkage disequilibrium block strongly related to increased BC risk [10]. The three *FGFR2* SNPs were in perfect LD in our population. Consistent with the single-locus analysis, carriers of the *FGFR2* haplotype TTG had a significantly greater risk compared with those of the common haplotype CCA. This findings suggest that the SNPs of *FGFR2* intron 2 might be useful markers for determining genetic susceptibility to familial and early-onset non-familial BC. In conclusion, this is the first study to demonstrate that genetic variants in intron 2 of *FGFR2* are significantly associated with increased risk of BC in a South-American population.

The *MAP3K1* gene acts in the MAP-signaling pathway and is responsible for regulation of transcription of important cancer genes. GWAS have identified the SNP rs889312, located close to the *MAP3K1* gene. The rs889312 lies in a linkage disequilibrium block of approximately 280 kb, which encodes a serine/threonine kinase protein and forms part of the MAPK cell-signaling pathway implicated in cellular response to mitogens [5]. Some studies have examined the association of this SNP with BC risk; however, the results were generally inconclusive. Nevertheless the meta-analysis published by Lu et al. [21] suggests that rs889312 C allele is a low-penetrant risk factor for developing BC. This meta-analysis includes seven studies of BC patients from European, Asian, African-American, African, and Australian backgrounds. To date, this variant has not been investigated in Spanish or South-American populations. In our case–control study, we found that *MAP3K1* rs889312A/C was significantly associated with increased risk of familial BC and early-onset non-familial BC in Chilean population. These results are consistent with those published by Latif et al. [37], which concluded that *MAP3K1* rs889312 is associated with increased risk of cancer in individuals with a family history of BC. Furthermore, Slattery et al. [20] reported that the minor allele frequency (MAF) of *MAP3K1* rs889312 was significantly greater among Hispanic women in the United States. Therefore, these authors support the hypothesis that genetic factors differ by race and ethnicity as they relate to BC. Also, this suggests that examining ethnicity/race associations as a component of validating and replicating associations is critical to understanding the complexity of disease associations among genetically admixed populations. The contemporary Chilean population stems from the admixture of Amerindian peoples with the Spanish settlers arriving in the 16th and 17th centuries. The relationships among ethnicity in the Amerindian admixture, genetic markers, and socioeconomic strata have been extensively studied in Chile [35, 36]. Therefore, our results are the first to contribute to identifying the *MAP3K1* rs889312 as a polymorphism associated with increased risk of BC in a South-American admixed population.

We also analyzed the effect of the combined genotypes of the *FGFR2* haplotype and *MAP3K1* rs889312. In the subgroup with familial BC, the *FGFR2* ht2 was associated with a 1.32-fold increased risk of BC, and the presence of at least one *MAP3K1* rs889312 C allele was associated with a 1.35-fold increased risk of BC. When these two risk alleles were combined, BC risk increased in a dose-dependent manner, and the presence of four risk alleles was associated with a 2.98-fold increased BC risk as compared with the presence of zero risk alleles. These results indicate that the *FGFR2* and *MAP3K1* SNPs have an additive effect on an increased risk of familial BC. Similar results were obtained when the same analysis was performed in subgroup B, which included non-familial BC with early age of diagnosis. In this sub-group, the combined genotype ht2/ht2–C/C was associated with a 4.07-fold increased of BC, and we also observed an additive effect of *FGFR2* ht2 and *MAP3K1* rs889312 C allele on an increased BC risk. The *FGFR2* gene is a growth factor receptor in tumorigenesis. It participates in the signal transduction pathway within which it activates the MAP kinase pathway, which initiates with the activation of the *MAP3K1* gene. This pathway finalizes with the expression of genes important for angiogenesis, proliferation, and cell migration. Therefore, it is possible that *FGFR2* and *MAP3K1* SNPs may have an additive effect on BC risk. However, since the interaction analysis between the *FGFR2* and *MAP3K1* SNPs was not significant, additional studies are needed to confirm this finding.

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References

1. Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. CA Cancer J Clin 62:10–29. doi:10.3322/caac.20138
2. Departamento de Estadísticas e Información en Salud (2009) Defunciones por tumores malignos según sexo, chile 2000–2009. [http://deis.minsal.cl/vitales/defunciones_serie/Defunciones_Mortalidad_Tumores_Malignos_edad_2000-2009.htm] Accessed 15 Sep 2012

3. Stratton MR, Rahman N (2008) The emerging landscape of breast cancer susceptibility. Nat Genet 40:17–22. doi:10.1038/ng.2007.53.2
4. Pharoah PDP, Antoniou AC, Easton DF, Ponder BAJ (2008) Polygenes, risk prediction, and targeted prevention of breast cancer. N Engl J Med 358:2796–2803. doi:10.1056/NEJMsa0708739
5. Easton DF, Pooley KA, Dunning AM, Pharoah PDP, Thompson D, Ballinger DG et al (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447:1087–1093. doi:10.1038/nature05887
6. Wesche J, Haglund K, Haugsten EM (2011) Fibroblast growth factors and their receptors in cancer. Biochem J 437:199–213. doi:10.1042/BJ20101603
7. Penault-Llorca F, Bertucci F, Adélaïde J, Parc P, Coulier F, Jacquemier J et al (1995) Expression of FGF and FGF receptor genes in human breast cancer. Int J Cancer 61:170–176. doi:10.1002/ijc.2910610205
8. Koziczak M, Holbro T, Hynes NE (2004) Blocking of FGFR signaling inhibits breast cancer cell proliferation through down-regulation of D-type cyclins. Oncogene 23:3501–3508. doi:10.1038/sj.onc.1207331
9. Ingersoll RG, Paznekas WA, Tran AK, Scott AF, Jiang G, Jabs EW (2001) Fibroblast growth factor receptor 2 (FGFR2): genomic sequence and variations. Cytogenet Cell Genet 94:121–126. doi:10.1159/000048802
10. Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE et al (2007) A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 39:870–874. doi:10.1038/ng2075
11. Huijts PEA, Vreeswijk MPG, Kroeze-Jansema KHG, Jacobi CE, Seynaeve C, Krol-Warmerdam EMM et al (2007) Clinical correlates of low-risk variants in FGFR2, TNRC9, MAP3K1, LSP1 and 8q24 in a dutch cohort of incident breast cancer cases. Breast Cancer Res 9:R78. doi:10.1186/bcr1793
12. Liang J, Chen P, Hu Z, Zhou X, Chen L, Li M et al (2008) Genetic variants in fibroblast growth factor receptor 2 (FGFR2) contribute to susceptibility of breast cancer in Chinese women. Carcinogenesis 29:2341–2346. doi:10.1093/carcin/bgn235
13. Raskin L, Pinchev M, Arad C, Lejbkowicz F, Tamir A, Rennert HS et al (2008) FGFR2 is a breast cancer susceptibility gene in Jewish and Arab Israeli populations. Cancer Epidemiol Biomarkers Prev 17:1060–1065. doi:10.1158/1055-9965.EPI-08-0018
14. Meyer KB, Maia A, O'Reilly M, Teschendorff AE, Chin S, Caldas C et al (2008) Allele-specific up-regulation of FGFR2 increases susceptibility to breast cancer. PLoS Biol 6:e108. doi:10.1371/journal.pbio.0060108
15. Hemminki K, Müller-Myhsok B, Lichtner P, Engel C, Chen B, Burwinkel B et al (2010) Low-risk variants FGFR2, TNRC9 and LSP1 in german familial breast cancer patients. Int J Cancer 126:2858–2862. doi:10.1002/ijc.24986
16. Barnholtz-Sloan JS, Shetty PB, Guan X, Nyante SJ, Luo J, Brennan DJ et al (2010) FGFR2 and other loci identified in genome-wide association studies are associated with breast cancer in african-american and younger women. Carcinogenesis 31:1417–1423. doi:10.1093/carcin/bgq128
17. Jia C, Cai Y, Ma Y, Fu D (2010) Quantitative assessment of the effect of FGFR2 gene polymorphism on the risk of breast cancer. Breast Cancer Res Treat 124:521–528. doi:10.1007/s10549-010-0872-5
18. Fu F, Wang C, Huang M, Song C, Lin S, Huang H (2012) Polymorphisms in second intron of the FGFR2 gene are associated with the risk of early-onset breast cancer in Chinese Han women. Tohoku J Exp Med 226:221–229. doi:10.1620/tjem.226.221
19. Garcia-Closas M, Chanock S (2008) Genetic susceptibility loci for breast cancer by estrogen receptor status. Clin Cancer Res 14:8000–8009. doi:10.1158/1078-0432.CCR-08-0975
20. Slattery ML, Baumgartner KB, Giuliano AR, Byers T, Herrick JS, Wolff RK (2011) Replication of five GWAS-identified loci and breast cancer risk among Hispanic and non-hispanic white women living in the southwestern united states. Breast Cancer Res Treat 129:531–539. doi:10.1007/s10549-011-1498-y
21. Lu P, Yang J, Li C, Wei M, Shen W, Shi L et al (2011) Association between mitogen-activated protein kinase kinase kinase 1 rs889312 polymorphism and breast cancer risk: evidence from 59,977 subjects. Breast Cancer Res Treat 126:663–670. doi:10.1007/s10549-010-1151-1
22. Gonzalez-Hormazabal P, Gutierrez-Enriquez S, Gaete D, Reyes JM, Peralta O, Waugh E et al (2011) Spectrum of BRCA1/2 point mutations and genomic rearrangements in high-risk breast/ovarian cancer Chilean families. Breast Cancer Res Treat 126:705–716. doi:10.1007/s10549-010-1170-y
23. Jara L, Ampuero S, Santibáñez E, Seccia L, Rodríguez J, Bustamante M et al (2006) BRCA1 and BRCA2 mutations in a South American population. Cancer Genet Cytogenet 166:36–45. doi:10.1016/j.cancergencyto.2005.08.019
24. Körkkö J, Annunen S, Pihlajamaa T, Prockop DJ, Ala-Kokko L (1998) Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 95:1681–1685. doi:10.1073/pnas.95.4.1681
25. Chomczynski P, Sacchi N (2006) The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nat Protoc 1:581–585. doi:10.1038/nprot.2006.83
26. Dudbridge F (2008) Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum Hered 66:87–98. doi:10.1159/000119108
27. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265. doi:10.1093/bioinformatics/bth457
28. Rothman KL (1986) Modern epidemiology. Little Brown & Co, Boston
29. Hosmer DW, Lemeshow S (1992) Confidence interval estimation of interaction. Epidemiology 3:452–456
30. Zhou L, Yao F, Luan H, Wang Y, Dong X, Zhou W et al (2012) Three novel functional polymorphisms in the promoter of FGFR2 gene and breast cancer risk: a HuGE review and meta-analysis. Breast Cancer Res Treat. doi: 10.1007/s10549-012-2300-5
31. Udler MS, Meyer KB, Pooley KA, Karlins E, Struewing JP, Zhang J et al (2009) FGFR2 variants and breast cancer risk: fine-scale mapping using African American studies and analysis of chromatin conformation. Hum Mol Genet 18:1692–1703. doi:10.1093/hmg/ddp078
32. Gold B, Kirchhoff T, Stefanov S, Lautenberger J, Viale A, Garber J et al (2008) Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. Proc Natl Acad Sci USA 105:4340–4345. doi:10.1073/pnas.0800441105
33. Chen F, Lv M, Xue Y, Zhou J, Hu F, Chen X et al (2012) Genetic variants of fibroblast growth factor receptor 2 (FGFR2) are associated with breast cancer risk in Chinese women of the Han nationality. Immunogenetics 64:71–76. doi:10.1007/s00251-011-0564-2
34. Esteban Cardeñosa E, de Juan Jiménez I, Palanca Suela S, Chirivella González I, Segura Huerta A, Santaballa Beltran A et al (2012) Low penetrance alleles as risk modifiers in familial and sporadic breast cancer. Fam Cancer 11:629–636. doi:10.1007/s10689-012-9563-1
35. Cruz-Coke R (1976) Origen y evolución étnica de la población chilena. Rev Med Chil 104:365–368
36. Valenzuela C, Harb Z (1977) Socioeconomic assortative mating in Santiago, Chile: as demonstrated using stochastic matrices of mother-child relationships applied to abo blood groups. Soc Biol 24:225–233
37. Latif A, Hadfield KD, Roberts SA, Shenton A, Lalloo F, Black GCM et al (2010) Breast cancer susceptibility variants alter risks

in familial disease. J Med Genet 47:126–131. doi:10.1136/jmg.2009.067256
38. Malone KE, Daling JR, Neal C, Suter NM, O'Brien C, Cushing-Haugen K et al (2000) Frequency of BRCA1/BRCA2 mutations in a population-based sample of young breast carcinoma cases. Cancer 88:1393–1402. doi:10.1002/(SICI)1097-0142(20000315)88:6<1393:AID-CNCR17>3.0.CO;2-P