PALB2 sequencing in Italian familial breast cancer cases reveals a high-risk mutation recurrent in the province of Bergamo

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Purpose: Monoallelic germ-line deleterious mutations of *PALB2* (partner and localizer of *BRCA2*) are associated with breast cancer risk and have been found in several populations, with carrier frequencies of ~1–2%. Initially, these mutations were considered to have moderate penetrance, but accumulating evidence now indicates that they are associated with much higher risk.

Methods: In this study, we sequenced the *PALB2* coding regions unlinked to *BRCA* (breast cancer) genes in 575 probands from Italian breast cancer families recruited in Milan.

Results: We found 12 carriers (2.1%) of deleterious mutations, and none of the mutations was found in 784 controls collected in Milan.

One of these mutations, the c.1027C>T (p.Gln343X), was found to be recurrent in the province of Bergamo in northern Italy, being detected in 6/113 (5.3%) familial breast cancer cases and 2/477 (0.4%) controls recruited in this area (Fisher's exact test: $P < 0.01$).

Conclusions: Our data provide confirmatory findings that, in the Italian population also, deleterious mutations of *PALB2* are relatively frequent predisposing factors for breast cancer and may be associated with high risk of the disease.

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Key Words: association study; breast cancer risk; familial breast cancer cases; *PALB2*; recurrent mutation

INTRODUCTION

Breast cancer is a fairly common and heterogeneous disease, and both environmental and genetic factors are involved in its etiology. In recent years, genetic factors have been discovered at an increasing rate. Several genome-wide association studies described a few dozen single-nucleotide polymorphisms associated with low risk for breast cancer. A particularly extensive recent genome-wide association study identified numerous additional loci and suggested that these markers are promising for the identification of at-risk individuals in the general population.¹

In terms of risk prediction in mutation carriers and their families, germ-line mutations with high penetrance are clinically relevant. These account for a substantial proportion of familial clusters (up to 25%) and occur mainly in the *BRCA1* and *BRCA2* genes that are routinely tested in clinical practice. Moreover, a small fraction of cases are explained by high-risk

mutations in genes associated with syndromic diseases in which breast cancer is one of the clinical features, including *TP53* (Li–Fraumeni syndrome), *PTEN* (Cowden syndrome), and *STK11* (Peutz–Jeghers syndrome).²

Mutations with moderate/intermediate penetrance are of increasing importance for the estimate of familial breast cancer risk. These were originally found in genes tested as candidate breast cancer loci because the encoded proteins were involved in *BRCA* (breast cancer) gene pathways. These genes include *CHEK2*,³ *ATM*,⁴ and some of the genes causing Fanconi anemia (FA) syndrome. In addition to *BRCA2*, which was found to be the same as the FA gene *FANCD1*,⁵ two other FA-associated genes, *BRIP1/FANCF* and *PALB2/FANCF*, have a clear impact on breast cancer risk,^{6,7} although together they account for a much smaller fraction of cases than those ascribable to *BRCA* genes.² Mutations in the *RAD51C/FANCF* gene were originally found in breast/ovarian cancer families.⁸ However, subsequent

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studies indicated that *RAD51C* mutations may confer a higher risk for ovarian cancer than for breast cancer.⁹ Sequencing performed in other FA genes, including *FANCL*, *FANCM*, *FANCI*, and *SLX4/FANCP*, initially suggested that they have no impact on breast cancer risk.^{10–13} However, subsequent analyses allowed the identification of (i) three unique *SLX4*-truncating mutations,^{14–16} indicating these mutations are very rare in familial cases, and (ii) a nonsense mutation in *FANCM*, whose frequency in cases was, however, not significantly higher than that in controls.¹⁷ Finally, deleterious mutations in the FA gene *XRCC2* were detected in families with breast cancer ascertained mainly in Australia.¹⁸ The extent of the impact of this gene on breast cancer risk was questioned by a subsequent study.¹⁹

The *PALB2* (partner and localizer of *BRCA2*) gene, which encodes a direct interactor of *BRCA2*—namely, *RAD51*—and other proteins,²⁰ has been actively studied as a breast cancer risk factor, and sequencing of its coding regions revealed that deleterious mutations are present in several populations. The first study that indicated that *PALB2* is a breast cancer gene reported germ-line truncating mutations in 10/923 (1.1%) English familial breast cancer cases.⁷ Additional studies found carriers of truncating mutations with a frequency of ~1–2% in Spanish, Chinese, South African, Russian, German, Italian, African-American, Australasian, and Malaysian familial or early-onset breast cancer cases.^{10,20–24} A similar frequency was observed in population-based studies of Australians and mixed-ethnicity breast cancer cases.^{20,25,26} A mutation-carrier frequency of 3.4% was reported in familial cases from the United States. This frequency, apparently higher as compared with that reported in other studies, might be due to the examined sample being enriched in a few founder mutations.²⁷ No mutations were identified in Jewish families of either Ashkenazi or non-Ashkenazi origin.^{27,28}

To date, *PALB2* recurrent mutations have been reported in four populations. The Finnish c.1592delT (p.Leu531fs) mutation was investigated in two studies^{29,30} and identified in 3/113 (2.7%) and 19/947 (2.0%) familial cases and in 6/2,501 (0.2%) and 2/1,079 (0.2%) controls, respectively. The c.2323C>T (p.Gln775X) mutation was detected in 4/564 (0.7%) French-Canadian early-onset cases and in 0/6,443 newborn infant controls.³¹ The Polish c.509_510delGA (p.Arg170fs) mutation was found in 4/648 (0.6%) familial cases and in 1/1,310 (0.1%) controls.³² Finally, the c.3113G>A (p.Trp1038X) mutation was recurrent in Australians, found in 5/1,403 (0.4%) cases and in 0/764 controls from a population-based study²⁵ and in 8/871 (0.9%) high-risk familial cases.³³

PALB2 mutations were found with similar frequencies in all populations tested—the only exception being the Jewish population—and were found to be associated with increased risk of developing breast cancer. Although initially these mutations were referred to as risk factors with moderate penetrance, several studies demonstrated that at least some of these mutations confer a much higher risk.²⁰ In the current study, we aimed to assess the involvement of *PALB2* in Italian families with breast cancer by sequencing the entire gene-coding region in a large

series of cases. In all, 575 probands were analyzed and 12 carriers of deleterious mutations were detected. One of these mutations was found to be recurrent in families from the province of Bergamo in northern Italy.

MATERIALS AND METHODS

Study population

The study included individuals with evidence of a genetic predisposition to breast cancer who had been previously selected for mutation testing in *BRCA1* and *BRCA2*—based on the criteria reported in the **Supplementary Material** online—at the Medical Genetics Unit of the Fondazione IRCCS Istituto Nazionale dei Tumori (INT) and the Division of Cancer Prevention and Genetics of the Istituto Europeo di Oncologia (IEO) in Milan and at the Unit of Medical Oncology of the Azienda Ospedaliera Papa Giovanni XXIII of Bergamo (AO-BG). Only individuals who tested negative for disease-causing mutations in *BRCA* genes, as described in the **Supplementary Material** online, and who were affected with breast cancer as the first diagnosed malignancy (BRCAX cases) were considered. Normal controls were consecutive female blood donors recruited by the Unit of Immunohematology and Transfusion Medicine of INT and the Associazione Volontari Italiani Sangue of Milan and of Bergamo. All individuals participating in this study signed an informed consent to the use of their biological samples for research projects aimed at the identification and the study of genetic factors associated with breast cancer risk. The study was approved by the Ethics Committee of the Fondazione IRCCS Istituto Nazionale dei Tumori and the Bioethics Committee of Azienda Ospedaliera Papa Giovanni XXIII of Bergamo.

PALB2 mutation analysis

DNA samples were obtained from peripheral blood, using the Gentra Puregene extraction kit (Qiagen, Hilden, Germany). All 13 *PALB2* exons and intron/exon boundaries were amplified by PCR and Sanger sequencing in 575 cases recruited in Milan by using primers reported elsewhere.³⁴ A search for large genomic rearrangements was not performed. PCR fragments that included deleterious mutations identified in cases were also tested by sequencing in 784 controls from Milan. The c.72delG (p.Arg26fs) and the c.1027C>T (p.Gln343X) recurrent mutations were genotyped by Taqman assay in an additional 332 cases from Milan and 113 cases and 477 controls from Bergamo. Probes and experimental conditions are available on request.

In silico analyses

Analyses of candidate splicing aberrations were performed using different computational programs, including Automated Splice Site and Exon Definition Analyses (<http://splice.uwo.ca>), Splice View (http://zeus2.itb.cnr.it/~webgene/wwwspliceview_ex.html), and the five tools integrated in the Alamut application (Interactive Biosoftware, Roven, France; <http://www.interactive-biosoftware.com/software/alamut/overview>). The predicted

effect of missense mutations on protein structure and function was assessed using Polymorphism Phenotyping, version 2 (PolyPhen2; <http://genetics.bwh.harvard.edu/pph2/>); Sorting Intolerant From Tolerant (SIFT; <http://sift.jcvi.org/>); Single-Nucleotide Polymorphisms and Gene Ontology (SNPs&GO; <http://snps.biofold.org/snps-and-go/snps-and-go.html>); alignment Using Grantham Variation and Grantham Deviation (Align-GVGD; <http://agvgd.iarc.fr>); Protein Variation Effect Analyzer (PROVEAN; <http://provean.jcvi.org/index.php>); and Mutation Assessor (<http://mutationassessor.org>) tools.

In vitro analyses of the c.48G>A splicing mutation

Establishment and culture of the lymphoblastoid cell line derived from the mutation carrier, total RNA purification, and cDNA synthesis were performed as described.³⁵ PCR amplification was carried out with a forward primer in *PALB2* exon 1 (5'-GCTGCTCTTTTCGTTCTGTC-3') and a reverse primer in *PALB2* exon 3 (5'-GGTGAGAGATCCTGCTGAGAC-3'). An LCL negative for the c.48G>A mutation, three LCLs with mutations in other genes, and seven LCLs from blood donors were used as negative controls. The amplification products were visualized on an agarose gel and characterized by direct sequencing.

Statistical methods

The confidence interval of the proportion of each considered mutation was computed by means of exact methods. We used

the Fisher's exact test to assess possible associations between genotype frequency and disease status (cases and controls). With the purpose of comparing our results with those reported by other authors, we estimated the odds ratios (ORs) and their 95% confidence intervals by resorting to a logistic regression model with age as adjustment covariate. All statistical analyses were performed with the SAS software (version 9.2; SAS Institute, Cary, NC).

RESULTS

The *PALB2* coding region was sequenced in 575 BRCA2 cases recruited at INT and IEO in Milan, and a total of 34 different variants were detected. Of these, eight were common, previously annotated polymorphisms (Table 1) and 26 were rare or unique variants (Tables 2 and 3). Of these 26 variants, 7 mutations (3 frameshift and 4 nonsense) introduced a premature termination codon and 1, c.3497delG, was a frameshift mutation that changed the last 21 amino acids of the protein's C terminus, causing the loss of the stop codon and the addition of three new residues (Table 2). This change modifies the conserved WD40 protein domain interacting with BRCA2²⁰ and, therefore, is considered to be deleterious. Three of the deleterious mutations were previously described in a subanalysis of the same sample set.³⁶

The remaining 18 variants were analyzed in silico using the bioinformatics tools described in the Materials and Methods section to identify potential splicing mutations. The c.48G>A

Table 1 Frequencies of common *PALB2* polymorphisms in 575 cases and 784 controls

Variant	Protein change	Annotation number	Cases (%)			Controls (%)		
			Nor	Het	Hom	Nor	Het	Hom
c.-47G>A	None	rs8053188	554 (96.3)	21 (3.7)	0 (0.0)	744 (94.9)	39 (5.0)	1 (0.1)
c.212-58A>C	None	rs80291632	519 (90.3)	54 (9.4)	2 (0.3)	ND	ND	ND
c.1010T>C	p.Leu337Ser	rs45494092	568 (98.8)	7 (1.2)	0 (0.0)	776 (99.0)	8 (1.0)	0 (0.0)
c.1676A>G	p.Gln559Arg	rs45494092	449 (78.1)	121 (21.0)	5 (0.9)	613 (78.2)	164 (20.9)	7 (0.9)
c.2014G>C	p.Glu672Gln	rs45532440	528 (91.8)	46 (8.0)	1 (0.2)	699 (89.7)	79 (10.1)	6 (0.8)
c.2794G>A	p.Val932Met	rs45624036	563 (97.9)	12 (2.1)	0 (0.0)	773 (98.6)	11 (1.4)	0 (0.0)
c.2993G>A	p.Gly998Glu	rs45551636	527 (91.7)	48 (8.3)	0 (0.0)	ND	ND	ND
c.3300T>G	p.Thr1100Thr	rs45516100	522 (90.8)	51 (8.9)	2 (0.3)	ND	ND	ND

Het, heterozygote; Hom, homozygote; ND, not done; Nor, normal; *PALB2*, partner and localizer of *BRCA2*.

Table 2 *PALB2* deleterious mutations detected in 575 cases

Exon	Mutation	Protein change	Type	No. of families with the mutation
1	c.48G>A ^a	p.Cys11fs	Splicing	1
2	c.72delG ^b	p.Arg26fs	Frameshift	2
4	c.1027C>T ^b	p.Gln343Ter	Nonsense	3
4	c.1037_1041delAAGAA	p.Lys346fs	Frameshift	1
4	c.1108C>T	p.Gln370Ter	Nonsense	1
5	c.2074C>T	p.Gln692Ter	Nonsense	1
5	c.2167_2168delAT	p.Met723fs	Frameshift	1
8	c.2787_2788delTA	p.Tyr929Ter	Nonsense	1
13	c.3497delG ^b	p.Gly1166fs	Frameshift	1

PALB2, partner and localizer of *BRCA2*.

^aThis mutation was shown to be truncating only after in vitro analyses. ^bPreviously described in the same sample set in ref. 36.

Table 3 Frequency and predicted effect on the protein of rare or unique nontruncating PALB2 variants detected in 575 cases and 784 controls

Variant	Protein change	Annotation number	Cases	Controls	Polyphen2	Predicted effect on protein				Mutation assessor
						SIFT	SNPs&GO	Align-GVGD ^a	PROVEAN	
c.13C>T	p.Pro5Ser	TMP_ESP_16_23652466 ^b	2	0	Benign	Tolerated	Neutral	C65	Neutral	Low
c.243G>A	p.Lys81Lys	None	2	ND	NA	NA	NA	NA	NA	NA
c.292A>G	p.Ile98Val	None	1	ND	Benign	Tolerated	Neutral	C25	Neutral	Neutral
c.1001A>G	p.Tyr334Cys	rs200620434	1	1	Benign	Tolerated	Neutral	C65	Deleterious	Neutral
c.1194G>A	p.Val398Val	rs61755173	1	0	NA	NA	NA	NA	NA	NA
c.1572A>G	p.Ser524Ser	rs45472400	3	3	NA	NA	NA	NA	NA	NA
c.1684+42_1684+43insTGA	None	rs180177106	4	7	NA	NA	NA	NA	NA	NA
c.2091C>G	p.Gly697Gly	None	1	0	NA	NA	NA	NA	NA	NA
c.2379C>T	p.Gly793Gly	TMP_ESP_16_23641096 ^b	1	ND	NA	NA	NA	NA	NA	NA
c.2418G>T	p.Pro806Pro	None	1	ND	NA	NA	NA	NA	NA	NA
c.2587-38C>G	None	rs180177119	5	ND	NA	NA	NA	NA	NA	NA
c.2587-25A>G	None	None	1	ND	NA	NA	NA	NA	NA	NA
c.2590C>T	p.Pro864Ser	rs45568339	5	ND	Prob. damag.	Tolerated	Neutral	C65	Deleterious	Low
c.2792T>G	p.Leu931Arg	None	1	0	Poss. damag.	Affected prot. funct.	Disease	C65	Deleterious	Medium
c.2816T>G	p.Leu939Trp	rs45478192	2	2	Poss. damag.	Affected prot funct.	Disease	C55	Deleterious	Medium
c.2996+17T>C	None	rs180177128	2	ND	NA	NA	NA	NA	NA	NA
c.3428T>A	p.Leu1143His	rs62625284	2	1	Poss. damag.	Tolerated	Neutral	C65	Deleterious	Medium

Align-GVGD, Alignment Using Grantham Variation and Grantham Deviation; ESP, Exome Sequencing Project; NA, not applicable; ND, not done; PALB2, partner and localizer of BRCA2; Polyphen, Polymorphism Phenotyping; Poss damag, possibly damaging; Prob damag, probably damaging; Prot funct, protein function; PROVEAN, Protein Variation Effect Analyzer; SIFT, Sorting Intolerant From Tolerant; SNPs&GO, Single-Nucleotide Polymorphisms and Gene Ontology.

^aVariants are classified into seven classes (C0–C65), with estimated proportions of pathogenicity ranging from 0.01 (C0) to 0.81 (C65). ^bExome Variant Server, National Heart, Lung, and Blood Institute GO ESP, Seattle, WA (URL: <http://evs.gs.washington.edu/EVS/>) (August 2013).

mutation, located at the last base of exon 1, was predicted to affect the canonical mRNA splicing by causing the loss of the natural donor splice site by all five bioinformatics tools that correctly detected the site. To verify this prediction, a RT-PCR analysis was performed. A cDNA fragment spanning the junction of exons 1 and 2 was amplified from an LCL established from the mutation carrier, and an aberrant transcript, which was not present in a mutation-negative cDNA, was observed (Figure 1a). In addition, the aberrant transcript was not detected in three LCLs from individuals with mutations in other genes and seven LCLs from blood donors (data not shown), indicating that the observed alternative splicing is caused by the c.48G>A mutation and is not a naturally occurring *PALB2* isoform. Sequencing of the amplification products showed that this transcript lacked 17 nucleotides at the 3' end of exon 1 (Figure 1b). These results indicate that the c.48G>A mutation abolishes the canonical donor splice site and activates an alternative site within exon 1, causing the loss of the reading frame and the formation of a premature termination codon at the amino acid residue 36 (p.Cys11fs; Table 2). Furthermore, the c.2379C>T (p.Gly793Gly) variant was predicted to create a novel donor site by six of the seven bioinformatics tools considered. No source of RNA from the mutation carrier was available to verify these outputs.

The nine deleterious mutations were tested by sequencing the corresponding fragments in 784 blood donors recruited at INT and Associazione Volontari Italiani Sangue in Milan, and no carriers were identified. Through analysis of these fragments, we genotyped five of the common polymorphisms and nine of the rare variants identified in cases. The frequencies of the five polymorphisms in controls were found by Fisher's exact test to be not different from those observed in cases, in keeping with the notion that they are probably neutral polymorphisms (Table 1). Five of the nine rare variants were detected in controls (Table 3).

In silico analyses were also performed to investigate all the missense mutations (Table 3). In this analysis, two mutations—the c.2792T>G (p.Leu931Arg) and the c.2816G>T (p.Leu939Trp)—were classified as damaging or possibly damaging by all tools reported in the Materials and Methods section. Of note, both the leucine amino acids at positions 931 and 939 are conserved in all species, from *Homo sapiens* to *Danio rerio*, and are located in the WD40-repeat domain responsible for binding to BRCA2 and in a region that mediates the binding with RAD51.²⁰ Of these two mutations, only p.Leu931Arg was not annotated and not found in controls from this study or other previously published studies. Thus, of all *PALB2* missense mutations we detected, p.Leu931Arg seems to have the highest probability of being deleterious.

Each of the nine deleterious mutations was found in a single index case, except for the c.72delG and the c.1027C>T mutations, which were detected in two and three cases, respectively. Therefore, in our survey of 575 BRCA2 cases, a total of 12 families with *PALB2* deleterious mutations were detected, for an overall frequency of 2.1%.

Figure 1 Characterization of the mRNA transcripts produced as a result of the *PALB2* c.48G>A mutation. (a) RT-PCR results. M, molecular marker (Φ X-174 HaeIII); lane 1, no template; lane 2, genomic DNA used as negative control of the RT-PCR reaction; lane 3, cDNA (cDNA) from a *PALB2* wild-type lymphoblastoid cell line (LCL) used as a normal control for the RT-PCR reaction; lane 4, cDNA from the LCL carrying the heterozygous *PALB2* c.48G>A mutation. The sizes of the full-length (FL) and aberrant (Ex1_17bp del) transcripts are indicated. The additional band due to the improper annealing of the two transcripts is indicated by the asterisk. (b) Sequencing results. The mutated cDNA (upper panel) shows two overlapping sequences, one corresponding to the FL transcript (the unique fragment present in the normal control cDNA, lower panel) and one corresponding to the aberrant transcript due to the skipping of 17 nucleotides (GTGAGGAGAAGGAAAAG) at the 5' end of exon 1. The sequencing reaction was performed using the reverse primer.

We further genotyped the two c.72delG and the c.1027C>T recurrent mutations in 332 additional BRCA2 cases ascertained at INT and IEO, but no other carriers of these mutations were found, for an overall frequency of 0.22% (2/907) and 0.33% (3/907), respectively. However, we noticed that two of the three c.1027C>T mutation carriers self-reported

as being originally from the province of Bergamo in northern Italy. To explore the possibility that the c.1027C>T was recurrent in this area, 113 BRCAX cases recruited at AO-BG were genotyped, and 6 carriers were detected (5.3%). The mutation was also tested in 477 female blood donors recruited through Associazione Volontari Italiani Sangue in Bergamo, and 2 carriers (0.4%) were identified.

Hence, a total of 18 probands with deleterious *PALB2* mutations were found. The pedigrees are reported in **Supplementary Figure S1** online as they were reconstructed at genetic counseling (12 in the Milan group and 6 in the Bergamo group). In five families, relatives of the proband were also available for mutation testing.

DISCUSSION

The sequencing of all coding exons and splice sites of *PALB2* in 575 Italian BRCAX cases detected 34 variants. Eight were previously found common polymorphisms, 17 were rare or unique variants of unclear significance, and 9 were deleterious mutations. The last were identified in a total of 12 BRCAX cases (2.1%) and not observed in 784 healthy controls recruited in Milan. Interestingly, one deleterious mutation, c.1027C>T, had a frequency of 5.3% in 113 BRCAX cases recruited at Bergamo city hospital (AO-BG). This was more than 15 fold higher than the frequency of 0.33% observed among 907 BRCAX patients recruited in Milan, (including the 575 cases of the original set and 332 additional cases specifically genotyped for the mutation). The two proportions differ significantly as confirmed by their exact 95% confidence intervals: 1.97–11.20% and 0.07–0.96%, respectively.

Milan is the largest city in northern Italy and one of the urban areas with the greatest immigration from other regions of the country during the past decades. Moreover, both INT and IEO are among the largest and most recognized Italian institutions for cancer treatment and serve patients from all over the country. On the other hand, more than 80% of BRCAX patients recruited in Bergamo, including all carriers of the c.1027C>T mutation, were born in Bergamo or in its province. Hence, the increased frequency of a few specific cancer–predisposing mutations observed in cases from Bergamo, with respect to those from Milan, indicates that this region may be characterized by lower genetic heterogeneity as compared with the rest of the country and may therefore be possibly enriched in individuals from a genetically isolated population. This could be due, among other factors, to the particular conformation of the territory, characterized by secluded deep valleys, which might have straitened genetic admixture. Of note, two other mutations in breast cancer–predisposing genes, the c.190C>T (p.Cys64Arg) in *BRCA1* and the c.6132_6135delAGTC (p.Val1969fs) in *BRCA2*, were found to be recurrent in the Bergamo area (P.R., unpublished data). The preferential geographical distribution of the *PALB2* c.1027C>T mutation is further confirmed by the finding that, although absent in blood donors recruited in Milan ($n = 784$), it occurred in two of the 477 female blood donors recruited in Bergamo, for a frequency of 0.4%.

The overall frequency of deleterious mutations found in Milan cases, 12/575 carriers (2.1%) vs. none in 784 controls, suggested that *PALB2*-truncating mutations are relevant factors for breast cancer risk. An estimate of the risk conferred by *PALB2*-truncating variants was derived by comparing the frequencies of c.1027C>T in BRCAX (5.3%) and controls (0.4%) ascertained in Bergamo. These frequencies were found to differ significantly (Fisher's exact test: $P < 0.01$). With the purpose of comparing our results with those reported by other authors, we estimated the age-adjusted OR of these frequencies by resorting to a logistic regression model. The OR value of 13.4 (95% confidence interval: 2.7–67.4) that we found was similar to those previously observed for mutations recurrent in other countries. An OR of ~11 was estimated in Finnish familial cases and controls in two separate studies for the c.1592delT mutation.^{29,30} The French–Canadian c.2323C>T mutation was tested in early-onset cases and controls, yielding an OR of 103. This OR value was most likely overestimated, given that no mutation carriers were found among controls.³¹ Finally, cumulative risks similar to those observed in *BRCA2* mutation carriers (49% to age 50 and 91% to age 70) were estimated for the c.3113G>A truncating mutation based on analysis of the relatives of five carriers identified in a population-based *PALB2* screening.²⁵ Together with these previous observations, our data reinforce the growing knowledge that *PALB2* is a high-risk factor for breast cancer.

Our survey of a large number of Italian BRCAX cases provides further evidence of the association of *PALB2* mutations with breast cancer susceptibility and indicates that the proportion of at-risk families attributable to these mutations in Italy is roughly similar to that ascertained in other populations. In addition, this study, while confirming the high degree of heterogeneity of *PALB2* mutations both within the same and among different populations, is the first report describing a *PALB2* recurrent mutation in Italian individuals. This mutation, c.1027C>T (p.Gln343X), appears to preferentially occur in families from a restricted geographical area in the north of the country, and comparison of its frequency in cases versus controls indicates that it is associated with high risk for breast cancer. Further analyses are required to verify whether genotyping for this specific mutation can be proposed as a cost-effective strategy for the rapid identification of genetically predisposed subjects in this area.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/gim>

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DISCLOSURE

The authors declare no conflict of interest.

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