Breast Cancer Res Treat (2011) 129:947–954
DOI 10.1007/s10549-011-1546-7

EPIDEMIOLOGY

Evidence for a link between *TNFRSF11A* and risk of breast cancer

Núria Bonifaci · Marta Palafox · Pasquale Pellegrini · Ana Osorio · Javier Benítez · Paolo Peterlongo · Siranoush Manoukian · Bernard Peissel · Daniela Zaffaroni · Gaia Roversi · Monica Barile · Alessandra Viel · Frederique Mariette · Loris Bernard · Paolo Radice · Bella Kaufman · Yael Laitman · Roni Milgrom · Eitan Friedman · María E. Sáez · Fina Climent · María Teresa Soler · Orland Diez · Judith Balmaña · Adriana Lasa · Teresa Ramón y Cajal · María-Dolores Miramar · Miguel de la Hoya · Pedro Pérez-Segura · Trinidad Caldés · Víctor Moreno · Ander Urruticoechea · Joan Brunet · Conxi Lázaro · Ignacio Blanco · Miguel Angel Pujana · Eva González-Suárez

Received: 12 January 2011 / Accepted: 20 April 2011 / Published online: 4 May 2011

Abstract Intracellular signaling mediated by the receptor activator of nuclear factor-κB [Rank, encoded by the tumor necrosis factor receptor superfamily, member 11a (*Tnfrsf11a*) gene] is fundamental for mammary gland development in mice, regulating the expansion of stem and progenitor cell compartments. Conversely, Rank overexpression in mice promotes abnormal proliferation and impairs differentiation, leading to an increased incidence of tumorigenesis. Here, we show that a common genetic variant near the 5′-end of *TNFRSF11A*, rs7226991, is associated with breast cancer risk in the general population and among carriers of mutations in the *breast cancer 2, early onset* (*BRCA2*) gene. Akin to the results of the Cancer and Genetics Markers of Susceptibility initiative, combined analysis of rs7226991 in two Spanish case–control studies (1,365 controls and 1,323 cases in total) revealed a significant association with risk: odds ratio (OR) = 0.88, 95% confidence interval (CI) 0.78–0.98, $P_{trend} = 0.025$. Subsequent examination of *BRCA1* ($n = 1{,}017$) and *BRCA2* ($n = 885$) mutation carriers revealed a consistent association in the latter group: weighted hazard ratio ($_{w}$HR) = 0.70; 95% CI 0.55–0.88; and $P_{trend} = 0.003$; compared to *BRCA1* mutation carriers, $_{w}$HR = 0.91; 95% CI 0.76–1.10; and $P_{trend} = 0.33$. The results of this study need to be replicated in other populations and with larger numbers of *BRCA1/2* mutation carriers.

Keywords Breast cancer · *BRCA1/2* mutation carriers · RANK · *TNFRSF11A*

Núria Bonifaci and Marta Palafox contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s10549-011-1546-7) contains supplementary material, which is available to authorized users.

N. Bonifaci · V. Moreno · M. A. Pujana (✉)
Biomarkers and Susceptibility Unit, and Biomedical Research Center Network for Epidemiology and Public Health, Cancer Prevention and Control Program, Catalan Institute of Oncology, Bellvitge Institute for Biomedical Research (IDIBELL), Gran Via 199, L'Hospitalet, Barcelona 08908, Spain
e-mail: mapujana@iconcologia.net

M. Palafox · P. Pellegrini · E. González-Suárez (✉)
Cancer Epigenetics and Biology Program (PEBC), Bellvitge Institute for Biomedical Research (IDIBELL), Gran Via 199, L'Hospitalet, Barcelona 08908, Spain
e-mail: egsuarez@idibell.cat

A. Osorio · J. Benítez
Human Cancer Genetics Program and Biomedical Research Center Network for Rare Diseases, Spanish National Cancer Research Centre, Madrid, Spain

P. Peterlongo · P. Radice
Unit of Molecular Bases of Genetic Risk and Genetic Testing, Department of Preventive and Predictive Medicine, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Nazionale Tumori, and IFOM Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy

S. Manoukian · B. Peissel · D. Zaffaroni · G. Roversi
Unit of Medical Genetics, Department of Preventive and Predictive Medicine, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Nazionale Tumori, Milan, Italy

M. Barile
Division of Cancer Prevention and Genetics, Istituto Europeo di Oncologia, Milan, Italy

Introduction

As shown in murine models, intracellular activity mediated by the Tnf family member Rankl and its receptor Rank (encoded by *Tnfsf11* and *Tnfrsf11a* genes, respectively) is essential for mammary gland development, differentiation, and function control. Rank overexpression in mice disrupts these processes by promoting abnormal proliferation of mammary epithelial cells [1], whereas absence of endogenous Rankl compromises cell survival and proper proliferation [2]. In both scenarios, non-functional mammary glands lacking lobuloalveolar structures were observed [1, 2]. Accordingly, paracrine signaling through Rankl has recently been described as responsible for the expansion of stem and progenitor cell compartments during pregnancy and ovarian cycles [3, 4].

Consistent with the fundamental mammary processes in which they are involved, increased signaling of Rankl and Rank promotes breast carcinogenesis. Accelerated preneoplasic lesions and augmented mammary tumor formation were observed in Rank transgenic mice, both after multiparity and following treatment with carcinogen and progestin [5, 6]. Reciprocally, selective pharmacologic inhibition of endogenous Rankl attenuated mammary tumor development in transgenic and wild-type mice treated with carcinogen or progestin and also in Neu overexpressing mice [6]. In addition, a lower incidence of progestin-induced mammary tumors was reported in mice lacking Rank in the mammary epithelium [5]. These observations suggest that Rankl/RANKL and Rank/RANK are principal mediators of the protumorigenic effects of progesterone and support the hypothesis that increased signaling through their pathway promotes breast carcinogenesis. This body of evidence led us to investigate the link between *TNFRSF11A*, the human ortholog of *Tnfrsf11a*, and breast cancer risk.

Materials and methods

Study samples, genotyping, and statistical analysis

Two case–control studies of the Spanish population—one led by the Spanish National Cancer Research Centre (CNIO), which had previously been used in international consortia [7, 8], and the other led by Neocodex [9, 10]—were used in this study. Both case–control studies included sporadic breast cancer cases, unselected for age or family history. Data on menopause status or osteoporosis disease were not collected. The CNIO study had recruited hospital controls and the Neocodex study population controls. The participation acceptance rates exceeded 70% in both studies. The *BRCA1/2* mutation carriers were enrolled through nine participating centers across three countries: Israel, Chaim Sheba Medical Center (CSMC); Italy, Consortium of Italian Studies on Hereditary Breast Cancer (CONSIT TEAM), which included carriers enrolled at the Centro di Riferimento Oncologico (CRO), Istituto Europeo di Oncologia (IEO), and Istituto Nazionale Tumori (INT); and Spain, including CNIO, Hospital Clínico San Carlos (HCSC), Hospital Sant Pau (HSP), Hospital Vall d'Hebron (HVH), and Catalan Institute of Oncology (ICO). Details of the number of carriers enrolled (affected or unaffected) and the number of families represented are provided in Electronic Supplementary Table S1. Eligibility to participate

A. Viel
Division of Experimental Oncology 1, Centro di Riferimento Oncologico, Istituto di Ricovero e Cura a Carattere Scientifico, Aviano, PN, Italy

F. Mariette
Consortium for Genomics Technology (Cogentech), Milan, Italy

L. Bernard
Department of Experimental Oncology, Istituto Europeo di Oncologia, Milan, Italy

B. Kaufman
The Institute of Oncology, Chaim Sheba Medical Center, Ramat Gan, Israel

Y. Laitman · R. Milgrom · E. Friedman
The Susanne Levy Gertner Oncogenetics Unit, Institute of Human Genetics, Chaim Sheba Medical Center, Ramat Gan, Israel

E. Friedman
Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

M. E. Sáez
Department of Structural Genomics, Neocodex, Sevilla, Spain

F. Climent · M. T. Soler
Department of Pathology, University Hospital of Bellvitge, Bellvitge Institute for Biomedical Research (IDIBELL), L'Hospitalet, Barcelona, Spain

O. Diez
Vall d'Hebron Institute of Oncology, University Hospital Vall d'Hebron, Barcelona, Spain

J. Balmaña
Department of Medical Oncology, University Hospital Vall d'Hebron, Barcelona, Spain

A. Lasa · T. Ramón y Cajal
Department of Medical Oncology, Hospital Santa Creu i Sant Pau, Barcelona, Spain

M.-D. Miramar
Genetics Unit, Clinical Biochemistry Service, Miguel Servet, University Hospital, Zaragoza, Spain

was restricted to female carriers of *BRCA1* or *BRCA2* mutations (classified as pathogenic according to Breast Cancer Information Core criteria) who were 18-years-old or older at recruitment. Collected information included birth year; age at diagnosis of breast and/or ovarian cancer; tumor bilaterality; family history (first- and second-degree relatives with breast and/or ovarian cancer); and estrogen receptor α (ERα)-tumor status (in >60% of cases). Data on menopause status and osteoporosis disease were not collected. All participants provided written informed consent and each of the studies was approved by the corresponding local ethics committees. Genotyping was performed at the corresponding centers for the population studies (CNIO and Neocodex) and at Cogentech (for carriers enrolled by CRO, IEO, and INT), CNIO, CSMC, and ICO (for individuals enrolled by HCSC, HSP, HVH, and ICO within a Spanish Consortium for Modifiers of *BRCA1/2*). Genotypes were assessed using a pre-designed TaqMan assay (Applied Biosystems) or, for the Neocodex study, melting curve analyses with fluorescence resonance energy transfer (FRET) probes in a LightCycler® 480 instrument (Roche). The quality controls comprised ≥98% genotyping calls for controls and cases in the population studies, and for unaffected and affected *BRCA1/2* mutation carriers across all centers. They also comprised 2–4% of sample replicates in all studies/centers with >98% concordance of genotypes. Data for the controls in both Spanish studies were in Hardy–Weinberg equilibrium (χ^2 goodness-of-fit test; $P > 0.05$). Hardy–Weinberg equilibrium for *BRCA1/2* mutation carrier sets from each center was evaluated by random selection of one individual from each participating family, and the results showed equilibrium in all sets. Unconditional and conditional logistic regression analyses were used to analyze data from a case–control study. Hazard ratios were estimated using Cox regression models under standard regression analysis and under a weighted cohort approach to enable the retrospective study design and non-random sampling of affected and unaffected mutation carriers [11]. Weights were assigned separately for carriers of mutations in *BRCA1* or *BRCA2*, and by age interval, and the *P* values were calculated from the robust score test. Analyses were stratified by birth-year cohort (pre-1940; 1940–1949; 1950–1959; and post-1959), ethnicity, and center. A robust variance estimate was used to account for familial correlations. The time from birth to diagnosis of breast cancer was modeled by censoring at the first of the following events: bilateral prophylactic mastectomy; breast cancer diagnosis; ovarian cancer diagnosis; death; and last date known to be alive. Subjects were considered to be affected if they were censored at breast cancer diagnosis; otherwise, they were considered to be unaffected. All statistical analyses were carried out using R software [12].

M. de la Hoya · T. Caldés
Molecular Oncology Laboratory, Hospital Clínico San Carlos, Madrid, Spain

P. Pérez-Segura
Medical Oncology, Hospital Clínico San Carlos, Madrid, Spain

A. Urruticoechea · M. A. Pujana
Translational Research Laboratory, Catalan Institute of Oncology, Bellvitge Institute for Biomedical Research (IDIBELL), L'Hospitalet, Barcelona, Spain

J. Brunet
Hereditary Cancer Program, Catalan Institute of Oncology, Girona Biomedical Research Institute, Girona, Spain

C. Lázaro · I. Blanco
Hereditary Cancer Program, Catalan Institute of Oncology, Bellvitge Institute for Biomedical Research (IDIBELL), L'Hospitalet, Barcelona, Spain

Results and discussion

Association in the general population

The results of the genome-wide association study conducted by the Cancer and Genetics Markers of Susceptibility (CGEMS) initiative [13] suggested that common genetic variation near the 5′-end of *TNFRSF11A* is associated with breast cancer risk (lowest $P_{trend} = 0.004$) (Fig. 1a). The three single nucleotide polymorphisms (SNPs) that showed the highest associations at this region were in linkage disequilibrium ($r^2 > 0.80$ in HapMap Caucasian individuals) within a block encoding for an uncharacterized gene, *KIAA1468* (Fig. 1a). The SNP rs7226991 is located within an evolutionarily conserved and potential regulatory sequence located ~4 kb proximal to the 5′-exon of *TNFRSF11A* (Fig. 1a). Potential expression regulation by this sequence is suggested by the results of DNAse I [14] and TAF1 chromatin immunoprecipitation [15] assays, combined with the analysis of evolutionary conservation [16] (Fig. 1a).

Based on the above observations, rs7226991 was genotyped in two case–control studies of the Spanish population (CNIO and Neocodex, see "Materials and methods" section). After quality control and Hardy–Weinberg equilibrium checks, the results from both studies showed per-allele effects consistent with the CGEMS results: for CNIO, OR = 0.84 and 95% CI 0.72–0.98; for Neocodex, OR = 0.93 and 95% CI 0.79–1.11; and for CGEMS, OR = 0.84 and 95% CI 0.74–0.95. Combined analysis of the Spanish studies (1,365 controls and 1,323 cases in total) revealed a significant association: OR = 0.88; 95% CI 0.78–0.98; and $P_{trend} = 0.025$, adjusted by age and center (Table 1). The dominant model was similarly significant: OR = 0.84; 95% CI 0.72–0.98; and $P = 0.026$. In

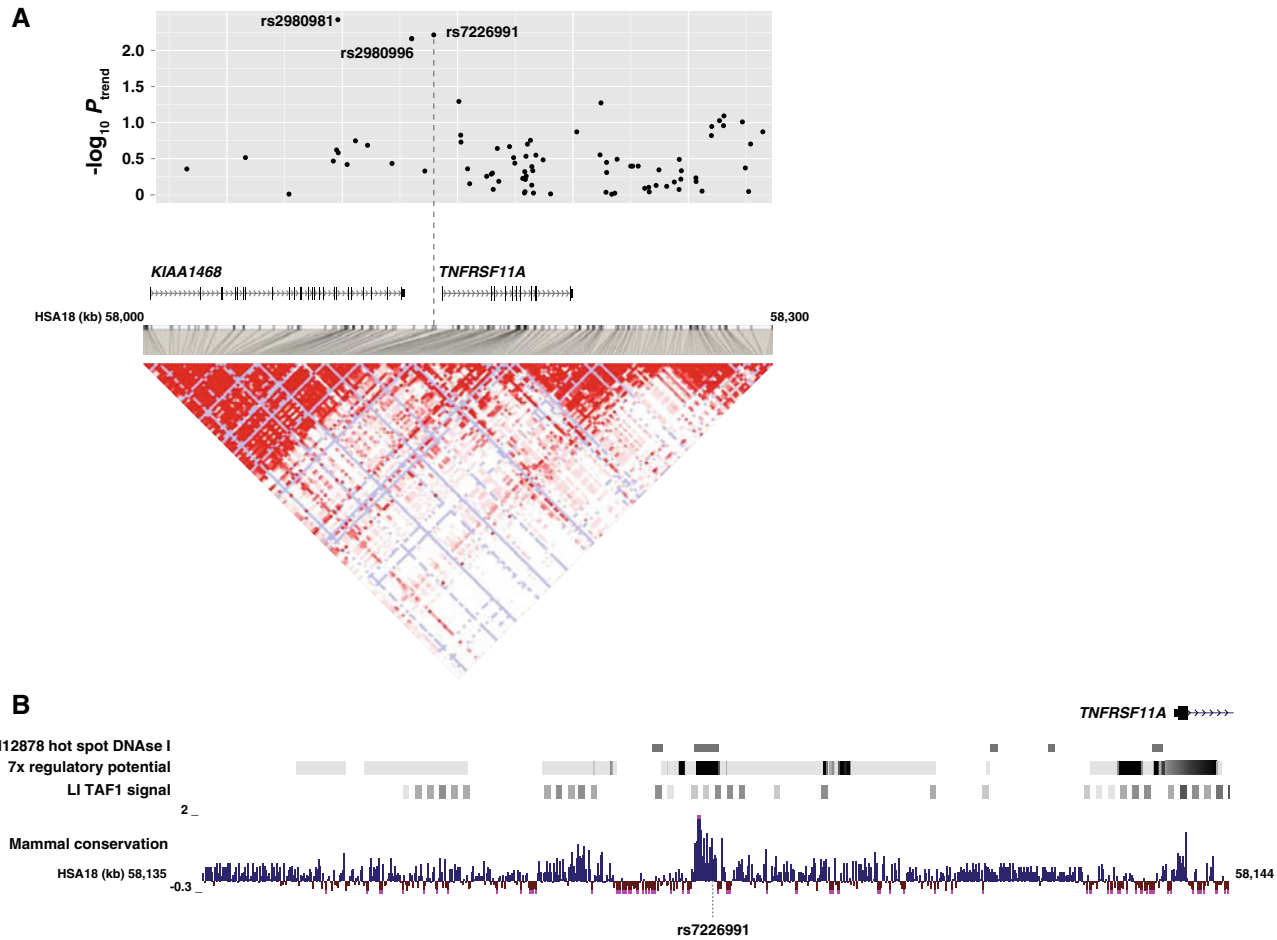

Fig. 1 Genetic variation near the 5′-end of *TNFRSF11A* and risk of breast cancer. **a** SNPs with previous evidence suggestive of association with breast cancer risk in the CGEMS study [13], genes and linkage disequilibrium structure around *KIAA1468/TNFRSF11A* in HapMap Caucasians (data release 27). The *top graph* shows the $-\log_{10} P_{\text{trend}}$ values per SNPs genotyped in the CGEMS study, highlighting the strongest three signals. **b** Potential regulatory region near the 5′-end of *TNFRSF11A* and including rs7226991

addition, the effect might be similar among breast cancer patients stratified according to ERα tumor status: negative, OR = 0.85 and 95% CI 0.69–1.04; and positive, OR = 0.88 and 95% CI 0.76–1.02. The dominant model was marginally significant for ERα-positive: OR = 0.83; 95% CI 0.68–1.00; and $P = 0.049$ (Electronic Supplementary Table S2). Together, these results suggest the identification of a low-penetrance breast cancer allele located near the 5′-end of *TNFRSF11A*, influencing cancer risk in diverse populations.

Association in *BRCA2* mutation carriers

Since several common breast cancer-predisposition alleles identified through genome-wide association studies and firmly replicated across populations also associate with breast cancer risk among *BRCA1* and/or *BRCA2* mutation carriers [17], we next evaluated the effect of rs7226991 within these groups. After the data had been checked for quality controls and Hardy–Weinberg equilibrium, Cox regression analysis revealed that rs7226991 associates with breast cancer risk in the total set of *BRCA1/2* mutation carriers consistent with the above observations: $n = 1{,}902$, HR = 0.85; 95% CI 0.77–0.95; and $P_{\text{trend}} = 0.003$. As observed in the general population, the dominant model was similarly significant: HR = 0.82; 95% CI 0.72–0.93; and $P = 0.002$. Next, examination of *BRCA1* and *BRCA2* mutation carriers independently revealed a significant association among the latter group: for 1,017 *BRCA1* mutation carriers, HR = 0.91; 95% CI 0.79–1.05; and $P_{\text{trend}} = 0.21$; whereas for 885 *BRCA2* mutation carriers, HR = 0.79; 95% CI 0.68–0.92; and $P_{\text{trend}} = 0.002$.

To assess the robustness of our results we performed several sensitivity analyses. Data were analyzed using a weighted cohort approach to enable retrospective study design and, in particular, non-random sampling of affected and unaffected mutation carriers [11]. This analysis yielded similar results to those reported above: for *BRCA1*

Table 1 rs7226991 association with breast cancer risk in Spain

Genotype	Controls		Cases		OR	95% CI
	n	%	*n*	%		
CNIO						
G/G	332	45.9	411	51.4	1.00	
G/A	310	42.8	318	39.8	0.83	0.67–1.03
A/A	82	11.3	70	8.8	0.72	0.50–1.01
Total	724		799			
Trend					0.84	0.72–0.98
						$P = 0.024^{\dagger}$
Neocodex						
G/G	290	45.2	251	47.9	1.00	
G/A	281	43.9	216	41.2	0.88	0.69–1.13
A/A	70	10.9	57	10.9	0.93	0.63–1.37
Total	641		524			
Trend					0.93	0.79–1.11
						$P = 0.45^{\dagger}$
Combined						
G/G	622	45.6	662	50.0	1.00	
G/A	591	43.3	534	40.4	0.85	0.73–1.00
A/A	152	11.1	127	9.6	0.79	0.61–1.03
Total	1,365		1,323			
Trend					0.88	0.78–0.98
						$P = 0.025$*
Dominant					0.84	0.72–0.98
						$P = 0.026$*

† Adjusted by age

* Adjusted by age and center

mutation carriers, weighted HR ($_{w}$HR) = 0.91; 95% CI 0.76–1.10; and $P_{trend} = 0.33$; whereas for *BRCA2* mutation carriers, $_{w}$HR = 0.70; 95% CI 0.55–0.88; and $P_{trend} = 0.003$. Again, the dominant model was similarly significant among *BRCA2* mutation carriers: $_{w}$HR = 0.65; 95% CI 0.49–0.87; and $P = 0.004$. Importantly, the effect in the three populations was consistent across all participating centers (Fig. 2 and Electronic Supplementary Table S3). The results were also similar when prevalent cases (defined as those diagnosed more than 5 years before recruitment) were excluded from the analyses: for $n = 746$ *BRCA2* mutation carriers, $_{w}$HR = 0.79; 95% CI 0.65–0.95; and $P_{trend} = 0.013$; $_{w}$HR = 0.78; 95% CI 0.62–0.98; and $P_{dominant} = 0.032$. Finally, there was no evidence of heterogeneity in the $_{w}$HRs among studies under the multiplicative model ($P > 0.50$). Together, the results from CGEMS, and the results from studies of the Spanish general population and of *BRCA2* mutation carriers, support that common genetic variation near the 5′-end of *TNFRSF11A* is associated with breast cancer risk. Nevertheless, a role for *KIAA1468*—recently described as being mutated in a lobular breast tumor [18]—cannot be ruled out until the causative mutation and its functional consequences have been discovered.

Other common variants at the *TNFRSF11A* locus and/or at functionally related loci, including *TNFSF11*, have been associated with differences in age at menarche or at natural menopause [19], bone mineral density and osteoporosis [20], and Paget's disease of bone [21]. The associated variants in these studies are in low linkage disequilibrium with rs7226991 ($r^2 < 0.25$ in HapMap Caucasian individuals), which suggests that different mutations at the *TNFRSF11A* locus might lead to different phenotypes or diseases. However, our analyses were limited by the lack of information on menopause status and osteoporosis disease of the participants. Given the importance of hormonal factors in these conditions and in breast cancer, adjusted analyses would be required together with fine mapping and identification of the potential mutations of *TNFRSF11A*/RANK.

The genetic association shown here, together with the current knowledge on Rank function in mice studies, would support the idea that differentiation of stem or progenitor

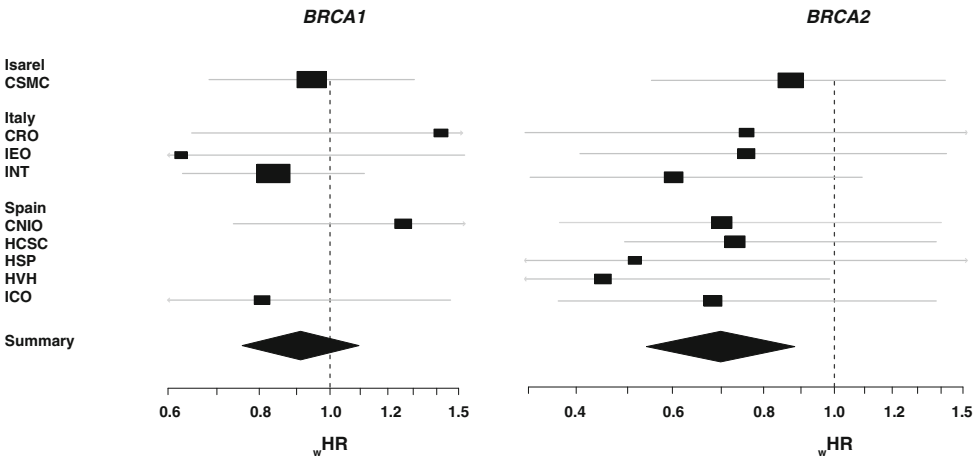

Fig. 2 rs7226991 and breast cancer risk among *BRCA1/2* mutation carriers. Estimates ($_{w}$HR) of association with cancer risk among *BRCA1* (*left panel*) and *BRCA2* (*right panel*) mutation carriers. The graphs show $_{w}$HRs and 95% CIs. The size of the *rectangles* is proportional to the corresponding study precision

cells is perturbed in breast carcinogenesis, among other shared or distinct processes [22]. In line with these observations, human RANK-mediated signaling can activate several mitogen-activated protein kinases downstream [23, 24], which in turn may link with the control of differentiation and/or proliferation by other breast cancer susceptibility genes affected by low-penetrance mutations (e.g., *fibroblast growth factor receptor 2* (*FGFR2*) and *mitogen-activated protein kinase kinase kinase 1* (*MAP3K1*) [7, 13, 25]). Similarly, BMP-2 (bone morphogenetic protein 2) can induce RANKL-mediated signaling [26, 27], and its receptor, BMPR1B, has recently been identified as a potential breast cancer susceptibility gene product [28]. However, we did not detect significant associations between the *TNFRSF11A* expression levels and rs7226991 alleles or genotypes in a series of 70 paired tumors and peritumoral tissue samples (data not shown), and there is no evidence for a *TNFRSF11A* expression quantitative trait locus acting on cis searching in published studies (data not shown). While the potential functional alteration linked to rs7226991 might be complex [29, 30], the association results shown here should be replicated in other case–control population studies using larger numbers of *BRCA1/2* mutation carriers, as well as including the analysis of other variants highlighted by the CGEMS results in the region.

Conclusions

Taking CGEMS results as a starting point, we have shown that a common genetic variant near the 5′-end of *TNFRSF11A* may be associated with breast cancer risk in the Spanish population and among *BRCA2* mutation carriers from different populations. While both associations need to be replicated, they could enhance the knowledge of the genetic basis and altered signal pathways that influence risk of breast cancer.

Acknowledgments We wish to thank all study participants, clinicians, and centers for their valuable contributions, and the CGEMS initiative for making their genome-wide association study results available. The CONSIT TEAM thanks Marco A Pierotti, Carla B Ripamonti, and Marilena Morganti of the Fondazione IRCCS Istituto Nazionale dei Tumori, and Bernardo Bonanni of the Istituto Europeo di Oncologia. The authors acknowledge the following grants for support of this work: a 2009 Spanish Society of Medical Oncology grant to J. Brunet; Ramón Areces Foundation XV, FIS (09/02483), and Generalitat de Catalunya 2009-SGR283 grants to MAP; the Biomedical Research Center Network for Epidemiology and Public Health Group 55 to MAP and VM; the Spanish Association Against Cancer (AECC) and FIS (08/1120) grants to J. Benítez; the RTICC 06/0020/0021 to HCSC; and MICINN (SAF2008-01975), Concern Foundation, and Scientific Foundation of the Spanish Association Against Cancer grants to EG-S. The CONSIT TEAM is supported by grants from Ministero della Salute (Extraordinary National Cancer Program 2006 "Alleanza contro il Cancro" and "Progetto Tumori Femminili"), Ministero dell'Universita' e Ricerca (RBLAO3-BETH to PR), Fondazione Italiana per la Ricerca sul Cancro (Special Project "Hereditary tumors") to PR, Associazione Italiana per la Ricerca sul Cancro (4017) to PP, and by funds from Italian citizens who allocated the 5 × 1000 share of their tax payment in support of the Fondazione IRCCS Istituto Nazionale Tumori, as provided for in Italian laws (INT institutional strategic projects "5 × 1000").

Conflict of interest The authors declare that they have no competing interests.

References

1. González-Suárez E, Branstetter D, Armstrong A, Dinh H, Blumberg H, Dougall WC (2007) RANK overexpression in transgenic mice with mouse mammary tumor virus promoter-controlled RANK increases proliferation and impairs alveolar differentiation in the mammary epithelia and disrupts lumen formation in cultured epithelial acini. Mol Cell Biol 27(4):1442–1454
2. Fata JE, Kong YY, Li J, Sasaki T, Irie-Sasaki J, Moorehead RA, Elliott R, Scully S, Voura EB, Lacey DL, Boyle WJ, Khokha R, Penninger JM (2000) The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. Cell 103(1):41–50
3. Asselin-Labat ML, Vaillant F, Sheridan JM, Pal B, Wu D, Simpson ER, Yasuda H, Smyth GK, Martin TJ, Lindeman GJ, Visvader JE (2010) Control of mammary stem cell function by steroid hormone signalling. Nature 465(7299):798–802
4. Joshi PA, Jackson HW, Beristain AG, Di Grappa MA, Mote PA, Clarke CL, Stingl J, Waterhouse PD, Khokha R (2010) Progesterone induces adult mammary stem cell expansion. Nature 465(7299):803–807
5. Schramek D, Leibbrandt A, Sigl V, Kenner L, Pospisilik JA, Lee HJ, Hanada R, Joshi PA, Aliprantis A, Glimcher L, Pasparakis M, Khokha R, Ormandy CJ, Widschwendter M, Schett G, Penninger JM (2010) Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. Nature 468(7320):98–102
6. González-Suárez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, Pinkas J, Branstetter D, Dougall WC (2010) RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. Nature 468(7320):103–107
7. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schurmann P, Dork T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X, Mannermaa

A, Kosma VM, Kataja V, Hartikainen J, Day NE, Cox DR, Ponder BA (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447(7148): 1087–1093
8. Milne RL, Ribas G, González-Neira A, Fagerholm R, Salas A, González E, Dopazo J, Nevanlinna H, Robledo M, Benítez J (2006) *ERCC4* associated with breast cancer risk: a two-stage case-control study using high-throughput genotyping. Cancer Res 66(19):9420–9427
9. Gayán J, Galan JJ, González-Pérez A, Sáez ME, Martínez-Larrad MT, Zabena C, Rivero MC, Salinas A, Ramírez-Lorca R, Morón FJ, Royo JL, Moreno-Rey C, Velasco J, Carrasco JM, Molero E, Ochoa C, Ochoa MD, Gutiérrez M, Reina M, Pascual R, Romo-Astorga A, Susillo-González JL, Vázquez E, Real LM, Ruiz A, Serrano-Ríos M (2010) Genetic structure of the Spanish population. BMC Genomics 11:326
10. Lorenzo C, Serrano-Rios M, Martinez-Larrad MT, Gabriel R, Williams K, Gonzalez-Villalpando C, Stern MP, Hazuda HP, Haffner SM (2001) Was the historic contribution of Spain to the Mexican gene pool partially responsible for the higher prevalence of type 2 diabetes in mexican-origin populations? The Spanish Insulin Resistance Study Group, the San Antonio Heart Study, and the Mexico City Diabetes Study. Diabetes Care 24(12):2059–2064
11. Antoniou AC, Goldgar DE, Andrieu N, Chang-Claude J, Brohet R, Rookus MA, Easton DF (2005) A weighted cohort approach for analysing factors modifying disease risks in carriers of high-risk susceptibility genes. Genet Epidemiol 29(1):1–11
12. The R Development Core Team (2011) A language and environment for statistical computing. R Foundation for Statistical Computing. www.r-project.org
13. Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE, Wacholder S, Wang Z, Welch R, Hutchinson A, Wang J, Yu K, Chatterjee N, Orr N, Willett WC, Colditz GA, Ziegler RG, Berg CD, Buys SS, McCarty CA, Feigelson HS, Calle EE, Thun MJ, Hayes RB, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover RN, Thomas G, Chanock SJ (2007) A genome-wide association study identifies alleles in *FGFR2* associated with risk of sporadic postmenopausal breast cancer. Nat Genet 39(7):870–874
14. Sabo PJ, Hawrylycz M, Wallace JC, Humbert R, Yu M, Shafer A, Kawamoto J, Hall R, Mack J, Dorschner MO, McArthur M, Stamatoyannopoulos JA (2004) Discovery of functional non-coding elements by digital analysis of chromatin structure. Proc Natl Acad Sci USA 101(48):16837–16842
15. Kim TH, Barrera LO, Zheng M, Qu C, Singer MA, Richmond TA, Wu Y, Green RD, Ren B (2005) A high-resolution map of active promoters in the human genome. Nature 436(7052): 876–880
16. King DC, Taylor J, Elnitski L, Chiaromonte F, Miller W, Hardison RC (2005) Evaluation of regulatory potential and conservation scores for detecting cis-regulatory modules in aligned mammalian genome sequences. Genome Res 15(8):1051–1060
17. Antoniou AC, Spurdle AB, Sinilnikova OM, Healey S, Pooley KA, Schmutzler RK, Versmold B, Engel C, Meindl A, Arnold N, Hofmann W, Sutter C, Niederacher D, Deissler H, Caldes T, Kampjarvi K, Nevanlinna H, Simard J, Beesley J, Chen X, Neuhausen SL, Rebbeck TR, Wagner T, Lynch HT, Isaacs C, Weitzel J, Ganz PA, Daly MB, Tomlinson G, Olopade OI, Blum JL, Couch FJ, Peterlongo P, Manoukian S, Barile M, Radice P, Szabo CI, Pereira LH, Greene MH, Rennert G, Lejbkowicz F, Barnett-Griness O, Andrulis IL, Ozcelik H, Gerdes AM, Caligo MA, Laitman Y, Kaufman B, Milgrom R, Friedman E, Domchek SM, Nathanson KL, Osorio A, Llort G, Milne RL, Benitez J, Hamann U, Hogervorst FB, Manders P, Ligtenberg MJ, van den Ouweland AM, Peock S, Cook M, Platte R, Evans DG, Eeles R, Pichert G, Chu C, Eccles D, Davidson R, Douglas F, Godwin AK, Barjhoux L, Mazoyer S, Sobol H, Bourdon V, Eisinger F, Chompret A, Capoulade C, Bressac-de Paillerets B, Lenoir GM, Gauthier-Villars M, Houdayer C, Stoppa-Lyonnet D, Chenevix-Trench G, Easton DF (2008) Common breast cancer-predisposition alleles are associated with breast cancer risk in *BRCA1* and *BRCA2* mutation carriers. Am J Hum Genet 82(4):937–948
18. Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A, Delaney A, Gelmon K, Guliany R, Senz J, Steidl C, Holt RA, Jones S, Sun M, Leung G, Moore R, Severson T, Taylor GA, Teschendorff AE, Tse K, Turashvili G, Varhol R, Warren RL, Watson P, Zhao Y, Caldas C, Huntsman D, Hirst M, Marra MA, Aparicio S (2009) Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature 461(7265):809–813
19. Lu Y, Liu P, Recker RR, Deng HW, Dvornyk V (2010) *TNFRSF11A* and *TNFSF11* are associated with age at menarche and natural menopause in white women. Menopause 17(5):1048–1054
20. Rivadeneira F, Styrkarsdottir U, Estrada K, Halldorsson BV, Hsu YH, Richards JB, Zillikens MC, Kavvoura FK, Amin N, Aulchenko YS, Cupples LA, Deloukas P, Demissie S, Grundberg E, Hofman A, Kong A, Karasik D, van Meurs JB, Oostra B, Pastinen T, Pols HA, Sigurdsson G, Soranzo N, Thorleifsson G, Thorsteinsdottir U, Williams FM, Wilson SG, Zhou Y, Ralston SH, van Duijn CM, Spector T, Kiel DP, Stefansson K, Ioannidis JP, Uitterlinden AG (2009) Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet 41(11):1199–1206
21. Albagha OM, Visconti MR, Alonso N, Langston AL, Cundy T, Dargie R, Dunlop MG, Fraser WD, Hooper MJ, Isaia G, Nicholson GC, del Pino Montes J, Gonzalez-Sarmiento R, di Stefano M, Tenesa A, Walsh JP, Ralston SH (2010) Genome-wide association study identifies variants at *CSF1*, *OPTN* and *TNFRSF11A* as genetic risk factors for Paget's disease of bone. Nat Genet 42(6):520–524
22. Foulkes WD (2004) BRCA1 functions as a breast stem cell regulator. J Med Genet 41(1):1–5
23. Matsumoto M, Sudo T, Saito T, Osada H, Tsujimoto M (2000) Involvement of p38 mitogen-activated protein kinase signaling pathway in osteoclastogenesis mediated by receptor activator of NF-kappa B ligand (RANKL). J Biol Chem 275(40):31155–31161
24. Leibbrandt A, Penninger JM (2008) RANK/RANKL: regulators of immune responses and bone physiology. Ann NY Acad Sci 1143:123–150
25. Stacey SN, Manolescu A, Sulem P, Thorlacius S, Gudjonsson SA, Jonsson GF, Jakobsdottir M, Bergthorsson JT, Gudmundsson J, Aben KK, Strobbe LJ, Swinkels DW, van Engelenburg KC, Henderson BE, Kolonel LN, Le Marchand L, Millastre E, Andres R, Saez B, Lambea J, Godino J, Polo E, Tres A, Picelli S, Rantala J, Margolin S, Jonsson T, Sigurdsson H, Jonsdottir T, Hrafnkelsson J, Johannsson J, Sveinsson T, Myrdal G, Grimsson HN, Sveinsdottir SG, Alexiusdottir K, Saemundsdottir J, Sigurdsson A, Kostic J, Gudmundsson L, Kristjansson K, Masson G, Fackenthal JD, Adebamowo C, Ogundiran T, Olopade OI, Haiman CA, Lindblom A, Mayordomo JI, Kiemeney LA, Gulcher JR, Rafnar T, Thorsteinsdottir U, Johannsson OT, Kong A, Stefansson K (2008) Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet 40(6):703–706
26. Hofbauer LC, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S (1998) Osteoprotegerin production by human osteoblast lineage cells is stimulated by vitamin D, bone morphogenetic protein-2, and cytokines. Biochem Biophys Res Commun 250(3):776–781
27. Usui M, Xing L, Drissi H, Zuscik M, O'Keefe R, Chen D, Boyce BF (2008) Murine and chicken chondrocytes regulate

osteoclastogenesis by producing RANKL in response to BMP2. J Bone Miner Res 23(3):314–325
28. Saetrom P, Biesinger J, Li SM, Smith D, Thomas LF, Majzoub K, Rivas GE, Alluin J, Rossi JJ, Krontiris TG, Weitzel J, Daly MB, Benson AB, Kirkwood JM, O'Dwyer PJ, Sutphen R, Stewart JA, Johnson D, Larson GP (2009) A risk variant in an miR-125b binding site in *BMPR1B* is associated with breast cancer pathogenesis. Cancer Res 69(18):7459–7465
29. Pomerantz MM, Ahmadiyeh N, Jia L, Herman P, Verzi MP, Doddapaneni H, Beckwith CA, Chan JA, Hills A, Davis M, Yao K, Kehoe SM, Lenz HJ, Haiman CA, Yan C, Henderson BE, Frenkel B, Barretina J, Bass A, Tabernero J, Baselga J, Regan MM, Manak JR, Shivdasani R, Coetzee GA, Freedman ML (2009) The 8q24 cancer risk variant rs6983267 shows long-range interaction with *MYC* in colorectal cancer. Nat Genet 41(8): 882–884
30. Tuupanen S, Turunen M, Lehtonen R, Hallikas O, Vanharanta S, Kivioja T, Bjorklund M, Wei G, Yan J, Niittymaki I, Mecklin JP, Jarvinen H, Ristimaki A, Di-Bernardo M, East P, Carvajal-Carmona L, Houlston RS, Tomlinson I, Palin K, Ukkonen E, Karhu A, Taipale J, Aaltonen LA (2009) The common colorectal cancer predisposition SNP rs6983267 at chromosome 8q24 confers potential to enhanced Wnt signaling. Nat Genet 41(8):885–890