Common alleles at 6q25.1 and 1p11.2 are associated with breast cancer risk for \textit{BRCA1} and \textit{BRCA2} mutation carriers

Antonis C Antoniou\textsuperscript{1,*}, Christiana Kartsonaki\textsuperscript{1}, Olga M. Sinilnikova\textsuperscript{3,4}, Penny Soucy\textsuperscript{5}, Lesley McGuffog\textsuperscript{1}, Sue Healey\textsuperscript{7}, Andrew Lee\textsuperscript{1}, Paolo Peterlongo\textsuperscript{8,11}, Siranoush Manoukian\textsuperscript{12}, Bernard Peissel\textsuperscript{12}, Daniela Zaffaroni\textsuperscript{12}, Elisa Cattaneo\textsuperscript{10}, Monica Barile\textsuperscript{14}, Valeria Pensotti\textsuperscript{15}, Barbara Pasini\textsuperscript{16}, Riccardo Dolcetti\textsuperscript{17}, Giuseppe Giannini\textsuperscript{18}, Anna Laura Putignano\textsuperscript{19,20}, Liliana Varesco\textsuperscript{21}, Paolo Radice\textsuperscript{9}, Phuong L. Mai\textsuperscript{22}, Mark H. Greene\textsuperscript{22}, Irene L. Andrulis\textsuperscript{23,24,25,27}, Gord Glendon\textsuperscript{27}, Hilmi Ozcelik\textsuperscript{22,23}, Mads Thomassen\textsuperscript{28}, Anne-Marie Gerdes\textsuperscript{29}, Torben A. Kruse\textsuperscript{28}, Uffe Birk Jensen\textsuperscript{30}, Dorthe G. Crüger\textsuperscript{31}, Maria A. Caligo\textsuperscript{32}, Yael Laitman\textsuperscript{33}, Roni Milgrom\textsuperscript{33}, Bella Kaufman\textsuperscript{34}, Shani Paluch-Shimon\textsuperscript{34}, Eitan Friedman\textsuperscript{33}, Niklas Loman\textsuperscript{35}, Katja Harbst\textsuperscript{35}, Annika Lindblom\textsuperscript{36}, Brita Arver\textsuperscript{37}, Hans Ehrencreuna\textsuperscript{38}, Beatrice Melin\textsuperscript{39}, SWE-BRCA\textsuperscript{35,36}, Katherine L. Nathanson\textsuperscript{40}, Susan M. Domchek\textsuperscript{40}, Timothy Rebbeck\textsuperscript{40}, Ania Jakubowska\textsuperscript{41}, Jan Lubinski\textsuperscript{41}, Jacek Gronwald\textsuperscript{41}, Tomasz Huzarski\textsuperscript{41}, Tomasz Byrski\textsuperscript{41}, Cezary Cybulski\textsuperscript{41}, Bohdan Gorski\textsuperscript{41}, Ana Osorio\textsuperscript{42,43}, Teresa Ramón y Cajal\textsuperscript{44}, Florentia Fostira\textsuperscript{45}, Raquel Andrés\textsuperscript{46}, Javier Benitez\textsuperscript{42,43}, Ute Hamann\textsuperscript{47}, Frans B. Hogervorst\textsuperscript{48}, Matti A. Rookus\textsuperscript{49}, Maartje J. Hooming\textsuperscript{50}, Marcel R. Nelen\textsuperscript{51}, Rob B. van der Luijt\textsuperscript{52}, Theo A.M. van Os\textsuperscript{53}, Christi J. van Asperen\textsuperscript{54}, Peter Devilee\textsuperscript{55,56}, Hanne E.J. Meijers-Heijboer\textsuperscript{57}, Encarna B. Gómez García\textsuperscript{58,59}, HEBON\textsuperscript{49}, Susan Peock\textsuperscript{1}, Margaret Cook\textsuperscript{1}, Debra Frost\textsuperscript{1}, Radka Platte\textsuperscript{1}, Jean Leyland\textsuperscript{1}, D. Gareth Evans\textsuperscript{60}, Fiona Laloo\textsuperscript{60}, Ros Eeles\textsuperscript{61}, Louise Izatt\textsuperscript{62}, Julian Adlard\textsuperscript{63}, Rosemarie Davidson\textsuperscript{64}, Diana Eccles\textsuperscript{65}, Kai-ren Ong\textsuperscript{66}, Jackie Cook\textsuperscript{67}, Fiona Douglas\textsuperscript{68}, Joan Paterson\textsuperscript{69}, M. John Kennedy\textsuperscript{70}, Zosia Miedzybrodzka\textsuperscript{71}, EMBRACE\textsuperscript{1}, Andrew Godwin\textsuperscript{72}, Dominique Stoppa-Lyonnet\textsuperscript{73,74,75}, Bruno Buecher\textsuperscript{73}, Muriel Belotti\textsuperscript{73}, Carole Tirapo\textsuperscript{73}, Sylvie Mazoyer\textsuperscript{4}, Laure Barjhoux\textsuperscript{4}, Christine Lasset\textsuperscript{76,77}, Dominique Leroux\textsuperscript{78,79}, Laurence Faivre\textsuperscript{80,81}, Myriam Bronner\textsuperscript{82}, Fabienne Prieur\textsuperscript{83}, Catherine Nogues\textsuperscript{84}, Etienne Rouleau\textsuperscript{85}, Pascal Pujol\textsuperscript{86,87}, Isabelle Cupier\textsuperscript{86,88}, Marc Frénay\textsuperscript{89}, CEMO Study Collaborators\textsuperscript{3}, John L. Hopper\textsuperscript{90}, Mary B. Daly\textsuperscript{91}, Mary B. Terry\textsuperscript{92}, Esther M. John\textsuperscript{93,94}, Sandra S. Buys\textsuperscript{95}, Yosuf Yassin\textsuperscript{96}, David Goldgar\textsuperscript{97}, Breast Cancer Family Registry, Christian F. Singer\textsuperscript{98}, Muy-Kheng Tea\textsuperscript{98}, Georg Pfeiler\textsuperscript{98}, Anne Catharina Dressler\textsuperscript{98}, Thomas v.O. Hansen\textsuperscript{99}, Lars Jønson\textsuperscript{99}, Bent Ejlertsen\textsuperscript{100}, Rosa Bjork Barkardottir\textsuperscript{101,102}, Tomas Kirchhoff\textsuperscript{103}, Kenneth Offit\textsuperscript{103}, Marion Piedmonte\textsuperscript{104}, Gustavo Rodriguez\textsuperscript{106}, Laurie Small\textsuperscript{107}, John Bogess\textsuperscript{108}, Stephanie Blank\textsuperscript{109}, Jack Basil\textsuperscript{110}, Masoud Azodi\textsuperscript{111}, Amanda Ewart Toland\textsuperscript{112}, Marco Montagna\textsuperscript{113}, Silvia Tognazz\textsuperscript{113}, Simona Agata\textsuperscript{113}, Evgeny Imyanitov\textsuperscript{114}, Ramunas Janavicius\textsuperscript{115,116}, Conxi Lazaro\textsuperscript{117},

*To whom correspondence should be addressed at: Strangeways Research Laboratory, University of Cambridge, Worts Causeway, Cambridge CB1 8RN, UK. Tel: +44 1223 740163; Fax: +44 1223 740159; Email: antonis@srl.cam.ac.uk

© The Author 2011. Published by Oxford University Press. All rights reserved.
For Permissions, please email: journals.permissions@oup.com

1Department of Public Health and Primary Care, Centre for Cancer Genetic Epidemiology and 2Department of Oncology, University of Cambridge, Cambridge, UK, 3Centre Hospitalier Universitaire de Lyon/Centre Léon Bérard, Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Lyon, France, 4Equipe labellisée LIGUE 2008, UMR5201 CNRS, Centre Léon Bérard, Université de Lyon, Lyon, France, 5Cancer Genomics Laboratory and 6Canada Research Chair in Oncogenetics, Cancer Genomics Laboratory, Centre Hospitalier Universitaire de Québec and Laval University, 2705 Laurier Boulevard, T3-57, Quebec City, QC, Canada, 7Queensland Institute of Medical Research, Brisbane, Australia, kConFab: Kathleen Cunningham Consortium for Research into Familial Breast Cancer, Peter MacCallum Cancer Center, Melbourne, Australia, 9Unit of Molecular Bases of Genetic Risk and Genetic Testing, Department of Preventive and Predictive Medicine and 10Unit of Medical Genetics, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale Tumori (INT), Milan, Italy, 11IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy, 12Unit of Medical Genetics, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale Tumori (INT), Milan, Italy, 13Department of Medicine, Surgery and Dentistry, Università degli Studi di Milano, Medical Genetics, Milan, Italy, 14Division of Cancer Prevention and Genetics, Istituto Europeo di Oncologia (IEO), Milan, Italy, 15Cogentech, Consortium for Genomics Technology, Milan, Italy, 16Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy, 17Cancer Bioimmunotherapy Unit, Centro di Riferimento Oncologico, IRCCS, Aviano (PN), Italy, 18Department of Experimental Medicine, University La Sapienza, Rome, Italy, 19Department of Clinical Physiopathology, University of Florence, Florence, Italy, 20Fiogen Foundation for Pharmacogenomics, Sesto Fiorentino, Italy, 21Unit of Hereditary Cancer, Department of Epidemiology, Prevention and Special Functions, Istituto Nazionale per la Ricerca sul Cancro (IST), Genoa, Italy, 22Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, US National Cancer Institute, Rockville MD, USA, 23Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, 24Department of Molecular Genetics, Department of Laboratory Medicine and Pathobiology and 25Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, 26Cancer Care Ontario, Toronto, ON, Canada, 27Clinical Genetics, Odense University Hospital, Odense C, Denmark, 28Clinical Genetics, Rigshospital, Copenhagen, Denmark, 29Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark, 30Clinical Genetics, Vejle Hospital, Vejle, Denmark, 31Section of Genetic Oncology, Department of Laboratory Medicine, University and University Hospital of Pisa, Pisa, Italy, 32The Susanne Levy Gertner Oncogenetics Unit, Sheba Medical Center, Tel Hashomer, Israel, 33The Breast cancer Unit, Institute of Oncology, Sheba Medical Center, Tel-Hashomer, Israel, 34Department of Oncology, Lund University Hospital, Lund, Sweden, 35Department of Oncology, Karolinska University Hospital, S-17176 Stockholm, Sweden, 36Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, S-751 85 Uppsala, Sweden, 37Department of Radiation Sciences, Oncology, Umeå University, S-901 85 Umeå, Sweden, 38University of Pennsylvania, Philadelphia, PA, USA, 39International Hereditary Cancer Centre, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland, 40Human Genetics Group, Human Cancer Genetics Programme, Spanish National

†Joint senior authors.
Cancer Research Centre, Madrid, Spain, 45Spanish Network on Rare Diseases (CIBERER), 44Oncoology Service, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, 45Molecular Diagnostics Laboratory, I.R.R.P., National Center for Scientific Research ‘Demokritos’, Athens, Greece, 46Oncology unit, Hospital clinico Universitaro “Lozano Blesa”, Zaragoza, Spain, 47Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany, 48Family Cancer Clinic and 49Department of Epidemiology, Netherlands Cancer Institute, Amsterdam, The Netherlands, 50Department of Medical Oncology, Family Cancer Clinic, Erasmus University Medical Center, Rotterdam, The Netherlands, 51Department of Human Genetics 849, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, 52Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands, 53Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands, 54Department of Clinical Genetics, 55Department of Human Genetics and 56Department of Pathology, Leiden University Medical Center Leiden, Leiden, The Netherlands, 57Department of Clinical Genetics, VU Medical Center, Amsterdam, The Netherlands, 58Department of Clinical Genetics and GROM, School for Oncology and 59Developmental Biology, MUMC, Maastricht, The Netherlands, 60Genetic Medicine, Manchester Academic Health Sciences Centre, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK, 61Oncogenetics Team, The Institute of Cancer Research and Royal Marsden NHS Foundation Trust, Surrey, UK, 62Clinical Genetics, Guy’s and St. Thomas’ NHS Foundation Trust, London, UK, 63Yorkshire Regional Genetics Service, Leeds, UK, 64Ferguson-Smith Centre for Clinical Genetics, Yorkhill Hospitals, Glasgow, UK, 65Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, UK, 66West Midlands Regional Genetics Service, Birmingham Women’s Hospital Healthcare NHS Trust, Edgbaston, Birmingham, UK, 67Sheffield Clinical Genetics Service, Sheffield Children’s Hospital, Sheffield, UK, 68Institute of Human Genetics, Centre for Life, Newcastle Upon Tyne Hospitals NHS Trust, Newcastle upon Tyne, UK, 69Department of Clinical Genetics, East Anglian Regional Genetics Service, Addenbrooke’s Hospital, Cambridge, UK, 70Academic Unit of Clinical and Molecular Oncology, Trinity College Dublin and St James’s Hospital, Dublin, Eire, 71North of Scotland Regional Genetics Service, NHS Grampian & University of Aberdeen, Foresterhill, Aberdeen, UK, 72Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, USA, 73Service de Génétique Oncologique et 74Unité INSERM U830, Institut Curie, Paris, France, 75Faculté de Médecine, Université Paris Descartes, Paris, France, 76CNRS UMR5558, Université Lyon 1, Lyon, France, 77Unité de Prévention et d’Épidémiologie Génétique, Centre Léon Bérard, Lyon, France, 78Department of Genetics, Centre Hospitalier Universitaire de Grenoble, Grenoble, France, 79Institut Albert Bonniot, Université de Grenoble, Grenoble, France, 80Centre de Génétique, Centre Hospitalier Universitaire de Dijon, France, 81Centre de Lutte Contre le Cancer Georges François Leclerc, Dijon, France, 82Laboratoire de Génétique Médicale, Nancy Université, Centre Hospitalier Régional et Universitaire, Vandoeuvre-les-Nancy, France, 83Service de Génétique Clinique Chromosomique et Moléculaire, Centre Hospitalier Universitaire de St Etienne, St Etienne, France, 84Oncogénétique Clinique and 85Laboratoire d’Oncogénétique, Hôpital René Huguenin/Institut Curie, Saint-Cloud, France, 86Unité d’Oncogénétique, CHU Arnaud de Villeneuve, Montpellier, France, 87INSERM 896, CRCM Val d’Aurelle, Montpellier, France, 88Unité d’Oncogénétique, CRLCC Val d’Aurelle, Montpellier, France, 89Centre Antoine Lacassagne, Nice, France, 90The University of Melbourne, Melbourne, Australia, 91Women’s Cancer Program, Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA, 92Columbia University New York, New York, NY, USA, 93Cancer Prevention Institute of California (formerly the Northern California Cancer Center), Fremont, CA, USA, 94Stanford Cancer Center, Stanford University School of Medicine, Stanford, CA, USA, 95Huntsman Cancer Institute, University of Utah Health Sciences Centre, Salt Lake City, UT, USA, 96Dana-Farber Cancer Institute, Boston, MA, USA, 97Department of Dermatology, University of Utah, Salt Lake City, UT, USA, 98Department of Obstetrics/Gynaecology, Medical University of Vienna, Vienna, Austria, 99Genomic Medicine, Department of Clinical Biochemistry and 100Department of Oncology, Rigshospitalet, Copenhagen University Hospital, Allerød, Denmark, 101Laboratory of Cell Biology, Department of Pathology, Landspitali-University Hospital, Tromsø, Norway, 102Faculty of Medicine, University of Iceland, Reykjavik, Iceland, 103Clinical Genetics Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, 104GOG Statistical and Data Center and 105Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY 14263, USA, 106Evanston Northwestern Healthcare, Evanston, IL, USA, 107Maine Medical Center, Scarborough, ME, USA, 108University of North Carolina, Chapel Hill, NC, USA, 109New York University School of Medicine, New York, NY, USA, 110St. Elizabeth Medical Center, Edgewood, KY, USA, 111Yale University School of Medicine, New Haven, CT.
USA, 112Comprehensive Cancer Center, Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Columbus, OH, USA, 113Immunology and Molecular Oncology Unit, Istituto Oncologico Veneto IOV - IRCCS, Padua, Italy, 114N.N. Petrov Institute of Oncology, St.-Petersburg, 197758, Russia, 115Department of Molecular and Regenerative Medicine, Vilnius university hospital Santariskiu Clinics, Hematology, Oncology and Transfusion Medicine Center, Vilnius, Lithuania, 116State Research Institute Inovative medicine center, Vilnius, Lithuania, 117Hereditary Cancer Program, Catalan Institute of Oncology, Barcelona, Spain, 118Women’s Cancer Research Institute at the Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA, 119Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary, 120Cancer Research Initiatives Foundation, Sime Darby Medical Centre, Subang Jaya, Malaysia, 121University Malaya Cancer Research Institute, University Malaya, Kuala Lumpur, Malaysia, 122UCLA Cancer Genetics Program, CLA Jonsson Comp Cancer Center, Santa Monica, CA, USA, 123UCSF Cancer Risk Program, University of California, San Francisco, CA, USA, 124UCSF Department of Medicine, 125Department of Epidemiology and 126Department of Biostatistics, University of California, San Francisco, CA, USA, 127Department of Genetics, University of Pretoria, Pretoria, South Africa, 128Oncogenetics Laboratory, University Hospital Vall d’Hebron, Barcelona, Spain, 129Vall d’Hebron Institute of Oncology (VHIO), Barcelona, Spain, 130Centre of Familial Breast and Ovarian Cancer, Department of Gynaecology and Obstetrics and 131Centre for Integrated Oncology (CIO), University hospital of Cologne, Cologne, Germany, 132Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany, 133Department of Gynaecology and Obstetrics, Division of Tumor Genetics, Klinikum rechts der Isar, Technical University Munich, Munich, Germany, 134Department of Gynaecology and Obstetrics, Ludwig-Maximillians University Munich, Munich, Germany, 135Institute of Human Genetics, Campus Virchow Klinikum, Charite Berlin, Germany, 136Department of Gynaecology and Obstetrics, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany, 137Department of Gynaecology and Obstetrics, University Hospital Ulm, Ulm, Germany, 138Department of Gynaecology and Obstetrics, University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, 139Department of Gynaecology and Obstetrics, University Hospital Schleswig-Holstein, Campus Kiel, Christian-Albrechts University Kiel, Kiel, Germany, 140Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover, Germany, 141Centre of Familial Breast and Ovarian Cancer, Department of Medical Genetics, Institute of Human Genetics, University Würzburg, Würzburg, Germany, 142Molecular Oncology Laboratory, Hospital Clinico San Carlos, Madrid, Spain, 143Department of Obstetrics and Gynecology and 144Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland, 145Unité de recherche en santé des populations, Centre des maladies du sein Deschênes-Fabia, Centre de recherche FRSQ du Centre hospitalier affilié universitaire de Québec, Québec, QC, Canada, 146Program in Cancer Genetics, Departments of Human Genetics and Oncology, McGill University, Montreal, QC, Canada, 147Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, Montreal, QC, Canada, 148Department of Population Sciences, Beckman Research Institute of the City of Hope, Duarte, CA, USA, 149Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA and 150Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Received March 24, 2011; Revised May 9, 2011; Accepted May 16, 2011

Two single nucleotide polymorphisms (SNPs) at 6q25.1, near the ESR1 gene, have been implicated in the susceptibility to breast cancer for Asian (rs2046210) and European women (rs9397435). A genome-wide association study in Europeans identified two further breast cancer susceptibility variants: rs11249433 at 1p11.2 and rs999737 at RAD51L1 at 1q42.1. Although previously identified breast cancer susceptibility variants have been shown to be associated with breast cancer risk for BRCA1 and BRCA2 mutation carriers, the involvement of these SNPs to breast cancer susceptibility in mutation carriers is currently unknown. To address this, we genotyped these SNPs in BRCA1 and BRCA2 mutation carriers from 42 studies from the Consortium of Investigators of Modifiers of BRCA1/2. In the analysis of 14 123 BRCA1 and 8053 BRCA2 mutation carriers of European ancestry, the 6q25.1 SNPs ($r^2 = 0.14$) were independently associated with the risk of breast cancer for BRCA1 mutation carriers [hazard ratio (HR) = 1.17, 95% confidence interval (CI): 1.11–1.23, $P$-trend = $4.5 \times 10^{-9}$ for rs2046210; HR = 1.28, 95% CI: 1.18–1.40, $P$-trend = $1.3 \times 10^{-8}$ for
INTRODUCTION

Genome-wide association studies (GWASs) have identified multiple common alleles that are associated with breast cancer risk in the general population (1–7). Such alleles provide plausible candidates as modifiers of cancer risk for BRCA1 and BRCA2 mutation carriers. Nine of these polymorphisms have been investigated as risk modifiers to date (8–10); single nucleotide polymorphisms (SNPs) in FGFR2, TOX3, MAP3K1, LSP1, 2q35, SLC4A7 and 5p12 have been shown to be associated with breast cancer risk for BRCA2 mutation carriers, but only SNPs in TOX3 and 2q35 were associated with the risk for BRCA1 mutation carriers. The differential patterns of associations between BRCA1 and BRCA2 mutation carriers appear to be in line with the differential effects of these polymorphisms for oestrogen receptor-positive and oestrogen receptor-negative breast cancer in the general population (10,11). More recently, a GWAS restricted to BRCA1 mutation carriers identified a locus at 19p13 which modified breast cancer risk for BRCA1 mutation carriers and the risk of oestrogen receptor (ER) negative and triple negative (oestrogen, progesterone receptor (PR) and Human Epidermal growth factor receptor 2 (HER2) negative) breast cancer in the general population (12). A separate GWAS in BRCA2 mutation carriers suggested that another locus at ZNF365 may modify the risk of breast cancer for BRCA2 mutation carriers (13). Candidate gene studies have also suggested that a SNP in CASP8 is also associated with the risk of breast cancer for BRCA1 mutation carriers (14). Each of these polymorphisms confers modest relative risks for breast cancer, but evidence so far suggests that they interact multiplicatively on the breast cancer risk for mutation carriers and the range of the combined risks of these SNPs is ≏ 6-fold (10). Since BRCA1 and BRCA2 mutations confer high risks of breast cancer, these relative risks result in substantial differences in the absolute risk of developing breast cancer between SNP genotype categories, and such differences could potentially influence the clinical management of mutation carriers (15). However, several other variants identified through population-based GWAS have not yet been evaluated as modifiers of cancer risk for BRCA1 and BRCA2 mutation carriers. Identifying further modifiers of risk could enhance risk prediction and will lead to a better understanding of the biology of tumour development in BRCA1 and BRCA2 mutation carriers.

Using data from the Shanghai Breast Cancer Study, Zheng et al. (7) identified a polymorphism at 6q25.1 through a GWAS on the risk of breast cancer among Chinese women. SNP rs2046210 was located upstream of the gene encoding for ER α-ESR1: 29 kb upstream of the first untranslated exon and 180 kb upstream of the first coding exon. Each copy of the minor allele of the SNP was estimated to confer an Odds Ratio (OR) of 1.29 among Chinese women and the authors reported a stronger association with ER-negative than ER-positive breast cancer. The same study also found an association between rs2046210 and the risk of breast cancer for European women, but a subsequent larger study among Europeans suggested that the association in Europeans is primarily due to another weakly correlated SNP in the region (rs9397435) (16). In a separate GWAS, Thomas et al. identified two further SNPs associated with the risk of breast cancer in the Cancer and Genetic Markers of Susceptibility (CGEMS) study: rs11249433 at 1p11.2 in a linkage disequilibrium block neighbouring NOTCH2 and FGGR1B, and rs999737 at 14q24.1 in RAD51L1 (6). SNP rs11249433 was mainly associated with ER-positive disease.

To evaluate the associations between these four SNPs and breast cancer risk for BRCA1 and BRCA2 mutation carriers, we genotyped these SNPs in BRCA1 and BRCA2 mutation carriers from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA).

RESULTS

Characteristics of the eligible mutation carriers, after quality control exclusions, are summarised in Table 1. The primary analysis included only mutation carriers of self-reported white European ancestry, and included data from 11604 women considered affected (first breast cancer diagnosis) and 10572 considered as unaffected (censored at bilateral prophylactic mastectomy, ovarian cancer or age at last observation).

The association results with breast cancer risk are summarized in Table 2. The minor allele of SNP rs2046210, at 6q25.1, was associated with an increased risk of breast cancer for BRCA1 mutation carriers (per-allele HR = 1.17, 95% CI: 1.11–1.23, P-trend = 7.5 × 10⁻⁹). In contrast, there was little evidence of association with the risk of breast cancer for BRCA2 mutation carriers (per-allele HR = 1.06, 95% CI: 0.99–1.14, P-trend = 0.09). There was no evidence for heterogeneity in the HRs across studies for BRCA1 mutation carriers (P-heterogeneity = 0.47), but there was marginal evidence for heterogeneity for BRCA2 mutation carriers (P-heterogeneity = 0.03; Fig. 1). This was mainly due to data from the HUNBOCS study. After excluding this study from the analysis, there was no longer evidence for heterogeneity.
Similarly, there was no evidence of heterogeneity in the frequencies of the minor allele of this SNP (minor allele frequency 0.031) across studies when considered individually (P-heterogeneity = 0.12; Fig. 2). There was no evidence that the HR for BRCA1 mutation carriers varied by age (P-trend = 0.34), but there was evidence that the HR for BRCA2 mutation carriers decreased with age (P-trend = 0.0025). The estimated age-specific HRs for rs9397435 among BRCA2 mutation carriers were all >1 for ages <50, but there was no evidence of an increased risk for ages >50 years (Supplementary Material, Table S2).

SNPs rs9397435 and rs2046210 are located in the same region at 6q25.1 and were only weakly correlated (pair-wise r² = 0.14 based on the current data set). In an analysis for the joint effects of these SNPs on breast cancer risk for BRCA1 mutation carriers (based on 9347 carriers with genotypes at both SNPs), the most parsimonious model included the effects of both SNPs (P for inclusion = 1.4 × 10⁻⁵ and 0.0037 for rs2046210 and rs9397435, respectively; 2-degree of freedom (df) P = 5.8 × 10⁻¹⁰ for the inclusion of both SNPs compared with the null model).

The minor allele of SNP rs11294433 at 1p11.2 was associated with the risk of breast cancer for both BRCA1 and BRCA2 mutation carriers, but the evidence of association was stronger for BRCA1 (per-allele HR = 1.28, 95% CI: 1.18–1.40, P-trend = 1.3 × 10⁻⁸) than for BRCA2 (HR = 1.14, 95% CI: 1.14, 95% CI: 1.01–1.28, P-trend = 0.031). There was some evidence of heterogeneity in the HRs across studies when considered individually (P-heterogeneity = 0.023). However, this was mainly due to studies with small numbers of mutation carriers and the low frequency of the minor allele of this SNP (minor allele frequency among unaffected = 6.7%). Repeating the analysis by grouping all studies within each country, there was no evidence of heterogeneity in the country-specific HRs (P-heterogeneity = 0.26; Fig. 2). Similarly, there was no evidence of heterogeneity in the HRs across countries for BRCA2 mutation carriers (P-heterogeneity = 0.12; Fig. 2). There was no evidence that the HR for BRCA1 mutation carriers varied by age (P-trend = 0.34), but there was evidence that the HR for BRCA2 mutation carriers decreased with age (P-trend = 0.0025). The estimated age-specific HRs for rs9397435 among BRCA2 mutation carriers were all >1 for ages <50, but there was no evidence of an increased risk for ages >50 years (Supplementary Material, Table S2).

SNPs rs9397435 and rs2046210 are located in the same region at 6q25.1 and were only weakly correlated (pair-wise r² = 0.14 based on the current data set). In an analysis for the joint effects of these SNPs on breast cancer risk for BRCA1 mutation carriers (based on 9347 carriers with genotypes at both SNPs), the most parsimonious model included the effects of both SNPs (P for inclusion = 1.4 × 10⁻⁵ and 0.0037 for rs2046210 and rs9397435, respectively; 2-degree of freedom (df) P = 5.8 × 10⁻¹⁰ for the inclusion of both SNPs compared with the null model).

The minor allele of SNP rs11294433 at 1p11.2 was associated with the risk of breast cancer for both BRCA1 and BRCA2 mutation carriers, but the evidence of association was stronger for BRCA1 (per-allele HR = 1.28, 95% CI: 1.18–1.40, P-trend = 1.3 × 10⁻⁸) than for BRCA2 (HR = 1.14, 95% CI: 1.14, 95% CI: 1.01–1.28, P-trend = 0.031). There was some evidence of heterogeneity in the HRs across studies when considered individually (P-heterogeneity = 0.023). However, this was mainly due to studies with small numbers of mutation carriers and the low frequency of the minor allele of this SNP (minor allele frequency among unaffected = 6.7%). Repeating the analysis by grouping all studies within each country, there was no evidence of heterogeneity in the country-specific HRs (P-heterogeneity = 0.26; Fig. 2). Similarly, there was no evidence of heterogeneity in the HRs across countries for BRCA2 mutation carriers (P-heterogeneity = 0.12; Fig. 2). There was no evidence that the HR for BRCA1 mutation carriers varied by age (P-trend = 0.34), but there was evidence that the HR for BRCA2 mutation carriers decreased with age (P-trend = 0.0025). The estimated age-specific HRs for rs9397435 among BRCA2 mutation carriers were all >1 for ages <50, but there was no evidence of an increased risk for ages >50 years (Supplementary Material, Table S2).

SNPs rs9397435 and rs2046210 are located in the same region at 6q25.1 and were only weakly correlated (pair-wise r² = 0.14 based on the current data set). In an analysis for the joint effects of these SNPs on breast cancer risk for BRCA1 mutation carriers (based on 9347 carriers with genotypes at both SNPs), the most parsimonious model included the effects of both SNPs (P for inclusion = 1.4 × 10⁻⁵ and 0.0037 for rs2046210 and rs9397435, respectively; 2-degree of freedom (df) P = 5.8 × 10⁻¹⁰ for the inclusion of both SNPs compared with the null model).

The minor allele of SNP rs11294433 at 1p11.2 was associated with the risk of breast cancer for both BRCA1 and BRCA2 mutation carriers, but the evidence of association was stronger for BRCA1 (per-allele HR = 1.28, 95% CI: 1.18–1.40, P-trend = 1.3 × 10⁻⁸) than for BRCA2 (HR = 1.14, 95% CI: 1.14, 95% CI: 1.01–1.28, P-trend = 0.031). There was some evidence of heterogeneity in the HRs across studies when considered individually (P-heterogeneity = 0.023). However, this was mainly due to studies with small numbers of mutation carriers and the low frequency of the minor allele of this SNP (minor allele frequency among unaffected = 6.7%). Repeating the analysis by grouping all studies within each country, there was no evidence of heterogeneity in the country-specific HRs (P-heterogeneity = 0.26; Fig. 2). Similarly, there was no evidence of heterogeneity in the HRs across countries for BRCA2 mutation carriers (P-heterogeneity = 0.12; Fig. 2). There was no evidence that the HR for BRCA1 mutation carriers varied by age (P-trend = 0.34), but there was evidence that the HR for BRCA2 mutation carriers decreased with age (P-trend = 0.0025). The estimated age-specific HRs for rs9397435 among BRCA2 mutation carriers were all >1 for ages <50, but there was no evidence of an increased risk for ages >50 years (Supplementary Material, Table S2).

SNPs rs9397435 and rs2046210 are located in the same region at 6q25.1 and were only weakly correlated (pair-wise r² = 0.14 based on the current data set). In an analysis for the joint effects of these SNPs on breast cancer risk for both BRCA1 and BRCA2 mutation carriers, but the evidence of association was stronger for BRCA1 (per-allele HR = 1.28, 95% CI: 1.18–1.40, P-trend = 1.3 × 10⁻⁸) than for BRCA2 (HR = 1.14, 95% CI: 1.14, 95% CI: 1.01–1.28, P-trend = 0.031). There was some evidence of heterogeneity in the HRs across studies when considered individually (P-heterogeneity = 0.023). However, this was mainly due to studies with small numbers of mutation carriers and the low frequency of the minor allele of this SNP (minor allele frequency among unaffected = 6.7%). Repeating the analysis by grouping all studies within each country, there was no evidence of heterogeneity in the country-specific HRs (P-heterogeneity = 0.26; Fig. 2). Similarly, there was no evidence of heterogeneity in the HRs across countries for BRCA2 mutation carriers (P-heterogeneity = 0.12; Fig. 2). There was no evidence that the HR for BRCA1 mutation carriers varied by age (P-trend = 0.34), but there was evidence that the HR for BRCA2 mutation carriers decreased with age (P-trend = 0.0025). The estimated age-specific HRs for rs9397435 among BRCA2 mutation carriers were all >1 for ages <50, but there was no evidence of an increased risk for ages >50 years (Supplementary Material, Table S2).

SNPs rs9397435 and rs2046210 are located in the same region at 6q25.1 and were only weakly correlated (pair-wise r² = 0.14 based on the current data set). In an analysis for the joint effects of these SNPs on breast cancer risk for both BRCA1 and BRCA2 mutation carriers, but the evidence of association was stronger for BRCA1 (per-allele HR = 1.28, 95% CI: 1.18–1.40, P-trend = 1.3 × 10⁻⁸) than for BRCA2 (HR = 1.14, 95% CI: 1.14, 95% CI: 1.01–1.28, P-trend = 0.031). There was some evidence of heterogeneity in the HRs across studies when considered individually (P-heterogeneity = 0.023). However, this was mainly due to studies with small numbers of mutation carriers and the low frequency of the minor allele of this SNP (minor allele frequency among unaffected = 6.7%). Repeating the analysis by grouping all studies within each country, there was no evidence of heterogeneity in the country-specific HRs (P-heterogeneity = 0.26; Fig. 2). Similarly, there was no evidence of heterogeneity in the HRs across countries for BRCA2 mutation carriers (P-heterogeneity = 0.12; Fig. 2). There was no evidence that the HR for BRCA1 mutation carriers varied by age (P-trend = 0.34), but there was evidence that the HR for BRCA2 mutation carriers decreased with age (P-trend = 0.0025). The estimated age-specific HRs for rs9397435 among BRCA2 mutation carriers were all >1 for ages <50, but there was no evidence of an increased risk for ages >50 years (Supplementary Material, Table S2).
studies were combined and analysed as a single locus. There were no significant associations between this SNP and the risk of breast cancer for either **BRCA1** or **BRCA2** mutation carriers (**BRCA1**: per-allele HR \(= 0.96\), 95% CI: 0.90–1.03, \(P\)-trend = 0.27; **BRCA2**: per-allele HR = 0.96, 95% CI: 0.88–1.04, \(P\)-trend = 0.30). The HR estimates were consistent across the studies for both **BRCA1** (\(P\)-heterogeneity \(= 0.11\)) and **BRCA2** mutation carriers (\(P\)-heterogeneity \(= 0.42\)). There was no evidence that the HRs varied by age for **BRCA1** (\(P\) \(= 0.50\)) or **BRCA2** (\(P\) \(= 0.60\)) mutation carriers.

The associations were not altered after excluding long-term survivors (Supplementary Material, Table S3) and there was no evidence of differences in the associations between class 1 and class 2 **BRCA1** mutation carriers (\(P\) for difference \(> 0.15\) for all SNPs, Supplementary Material, Table S3).

**BRCA1** and **BRCA2** mutations also confer high risks of ovarian cancer. To determine whether the three polymorphisms modify ovarian cancer risk in mutation carriers, we analysed the associations within a competing risk analysis framework by estimating simultaneously the HRs for breast and ovarian cancer. There was no evidence of association with the risk of ovarian cancer for any of the SNPs (Table 3). The estimated HRs for breast cancer were similar to those from the primary analysis. SNPs rs2046210 and rs9397435 remained significantly associated with the risk of breast cancer for **BRCA1** mutation carriers (\(P\)-trend \(= 6.7 \times 10^{-8}\) and \(7.8 \times 10^{-7}\)) and there was a slightly stronger evidence of association between SNP rs11249433 and the risk of breast cancer for **BRCA2** mutation carriers (\(P\)-trend \(= 0.0052\)).

### Table 2. SNP genotype distributions and associations with breast cancer risk

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Genotype</th>
<th>Unaffected, Unaffected, (N) (%)</th>
<th>Affected(^a), Affected, (N) (%)</th>
<th>HR</th>
<th>95% CI</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6q25.1 (rs2046210) <strong>BRCA1</strong></td>
<td>CC</td>
<td>2282 (43.0)</td>
<td>2067 (37.5)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>2361 (44.5)</td>
<td>2669 (48.4)</td>
<td>1.23</td>
<td>1.14–1.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>659 (12.4)</td>
<td>779 (14.1)</td>
<td>1.32</td>
<td>1.18–1.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per-allele</td>
<td></td>
<td></td>
<td>1.17</td>
<td>1.11–1.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.5 \times 10^{-9}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.5 \times 10^{-9}</td>
<td></td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td>CC</td>
<td>1144 (40.8)</td>
<td>1321 (39.1)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>1312 (46.7)</td>
<td>1574 (46.5)</td>
<td>1.02</td>
<td>0.92–1.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>351 (12.5)</td>
<td>486 (14.4)</td>
<td>1.16</td>
<td>1.00–1.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per-allele</td>
<td></td>
<td></td>
<td>1.06</td>
<td>0.99–1.14</td>
<td>0.13</td>
</tr>
<tr>
<td>6q25.1 (rs9397435) <strong>BRCA1</strong></td>
<td>AA</td>
<td>5361 (86.5)</td>
<td>5282 (82.9)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>802 (12.9)</td>
<td>1043 (16.4)</td>
<td>1.31</td>
<td>1.19–1.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>38 (0.6)</td>
<td>49 (0.8)</td>
<td>1.37</td>
<td>0.92–2.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per-allele</td>
<td></td>
<td></td>
<td>1.28</td>
<td>1.18–1.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.3 \times 10^{-8}</td>
<td></td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td>AA</td>
<td>2786 (84.1)</td>
<td>3141 (82.6)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>510 (15.4)</td>
<td>631 (16.6)</td>
<td>1.11</td>
<td>0.98–1.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>17 (0.5)</td>
<td>32 (0.8)</td>
<td>1.56</td>
<td>0.91–2.67</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per-allele</td>
<td></td>
<td></td>
<td>1.14</td>
<td>1.01–1.28</td>
<td>0.031</td>
</tr>
<tr>
<td>1p11.2 (rs11249433) <strong>BRCA1</strong></td>
<td>TT</td>
<td>1833 (34.4)</td>
<td>1961 (35.1)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>2584 (48.5)</td>
<td>2732 (48.9)</td>
<td>1.00</td>
<td>0.90–1.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>911 (17.1)</td>
<td>890 (15.9)</td>
<td>0.92</td>
<td>0.83–1.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per-allele</td>
<td></td>
<td></td>
<td>0.97</td>
<td>0.92–1.02</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td>TT</td>
<td>1016 (35.9)</td>
<td>1135 (33.2)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>1377 (48.7)</td>
<td>1698 (49.6)</td>
<td>1.07</td>
<td>0.96–1.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>434 (15.4)</td>
<td>590 (17.2)</td>
<td>1.20</td>
<td>1.04–1.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per-allele</td>
<td></td>
<td></td>
<td>1.09</td>
<td>1.02–1.17</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>RAD51L1</strong> (rs999737/rs10483813) <strong>BRCA1</strong></td>
<td>CC/TT</td>
<td>2725 (62.3)</td>
<td>2849 (63.6)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC/AT</td>
<td>1461 (33.4)</td>
<td>1439 (32.1)</td>
<td>0.93</td>
<td>0.86–1.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT/AA</td>
<td>186 (4.3)</td>
<td>195 (4.3)</td>
<td>1.01</td>
<td>0.84–1.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per-allele</td>
<td></td>
<td></td>
<td>0.96</td>
<td>0.90–1.03</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td>CC/TT</td>
<td>1609 (61.1)</td>
<td>1950 (62.2)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC/AT</td>
<td>869 (33.0)</td>
<td>1039 (33.1)</td>
<td>0.98</td>
<td>0.88–1.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT/AA</td>
<td>154 (5.9)</td>
<td>147 (4.7)</td>
<td>0.86</td>
<td>0.69–1.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per-allele</td>
<td></td>
<td></td>
<td>0.96</td>
<td>0.88–1.04</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Analysis restricted to mutation carriers of European ancestry.

\(^a\)Breast cancer.

\(^b\)Genotyped using iPLEX.

\(^c\)Genotyped using Taqman, pair-wise \(r^2 = 1\) between rs999737 and rs10483813 based on HapMap data.

Downloaded from https://academic.oup.com/hmg/article-abstract/20/16/3304/648450 by guest on 31 December 2018
DISCUSSION

Several common variants identified through GWASs in the general population and BRCA1 and BRCA2 mutation carriers have been demonstrated to be associated with the risk of breast cancer for BRCA1 and/or BRCA2 mutation carriers (8–10,12,13). In this study, we evaluated the associations of four additional variants, identified through population-based GWAS or subsequent follow-up mapping studies, with the risk of breast and ovarian cancer for BRCA1 and BRCA2 mutation carriers.

We found strong evidence that SNP rs2046210 at 6q25.1 was associated with the risk of breast cancer for BRCA1 mutation carriers, but there was no clear evidence of association with the risk of breast cancer for BRCA2 mutation carriers (P for difference: 0.027). The observed association with BRCA1 breast cancer risk was unaltered after the exclusion of prevalent breast cancer cases, and did not vary by the predicted functional effect of the mutations. This polymorphism was identified through a GWAS in Chinese women (7), in whom the authors estimated a per-allele OR of 1.29 (95% CI: 1.21–1.37) for breast cancer in this population. This OR estimate was greater than our estimated HR for BRCA1 mutation carriers. However, the Chinese study reported a further replication of their findings among women of European ancestry for whom the per-allele OR was estimated to be 1.15, similar to the HR based on our analysis of BRCA1 mutation carriers who were also of self-reported European ancestry. A more recent study by the same group also found evidence for association with breast cancer in an independent sample of European-ancestry American cases and controls (17). Both studies (7,17) also reported a stronger association with ER-negative disease than ER-positive in particular among Asian women, although the SNP was associated with both disease subtypes. This is consistent with our finding that this SNP is predominantly associated with the risk of breast cancer for BRCA1 mutation carriers, the majority of whom present with ER-negative tumours (18), and hence conforms to the general pattern we have observed previously that the breast cancer susceptibility SNPs confer a similar relative risk in carriers to that in the general population, once receptor status is taken into account (10,11). A more recent study by Stacey et al. (16) evaluated the associations of SNP rs2046210 in a larger set of women of European ancestry, but failed to replicate the association. The authors concluded that this SNP does not confer a substantial risk of breast cancer in Europeans and they postulated that this is due to the different linkage disequilibrium structures between the causal variant and SNP rs2046210 in Europeans. They found a different SNP, rs9397435 (pair-wise $r^2 = 0.08$ based on CEU HapMap data, $r^2 = 0.71$ based on CHB + JPT HapMap data), accounting for the association in both the
Europeans and Chinese. SNP rs9397435 was also strongly associated with the risk of breast cancer for \textit{BRCA1} mutation carriers in our data set and exhibited weak association with the risk for \textit{BRCA2} mutation carriers ($P = 0.10$). Our joint analysis of SNPs rs9397435 and rs2046210 among \textit{BRCA1} mutation carriers demonstrated that a model that includes both SNPs fits significantly better than a model that includes either SNP on its own, and are not therefore consistent with the conclusions of Stacey \textit{et al.} (16) who suggest that the association is primarily due to SNP rs9397435. Our results suggest that either the observed associations are driven by another causative variant that is partially associated with both SNPs, or that more than one causative variant is located in this region. Further comprehensive genotyping of variants across the region will be required to determine which of these hypotheses is correct. A potential explanation for the observed differences between our study and that of Stacey \textit{et al.} (16) could be the fact that the \textit{BRCA1} tumours are predominantly ER-negative, whereas the majority of cases in Stacey \textit{et al.} were ER-positive. This result could have been observed if rs2046210 was mainly associated with ER-negative breast cancer and rs9397435 was associated with both ER-negative and ER-positive breast cancer. Stacey \textit{et al.} (16) did not present the associations of rs2046210 by tumour ER status, but reported that rs9397435 was associated with both ER-positive and ER-negative breast cancer. Stacey \textit{et al.} (16) also found that rs9397435 was associated with an earlier age at diagnosis in Europeans from the general population.

Our results also suggest that rs2046210 is associated with higher relative risks of breast cancer at younger ages among \textit{BRCA1} mutation carriers. A similar pattern was observed for rs9397435 among \textit{BRCA2} mutation carriers. Stacey \textit{et al.} (16) also found that rs9397435 was associated with an earlier age at diagnosis in Europeans from the general population.

SNPs rs2046210 and rs9397435 are located close to \textit{ESR1}, which encodes ER $\alpha$ mediator of oestrogen action (19). Elevated oestrogen levels have been associated with increased breast cancer risk (20), and although it is assumed that the action of oestrogen is via ER in ER-positive tumours, two studies have recently provided evidence that the size and repopulating ability of the mammary stem cell compartment in mice are controlled by 17$\beta$-estradiol and progesterone via a paracrine-signalling mechanism from steroid receptor-positive luminal cells to steroid receptor-negative stem cells (21,22). This may explain the apparently paradoxical observation that a SNP in \textit{ESR1} could modify the risk of breast cancer in \textit{BRCA1} carriers, in which the tumour phenotype is usually ER-negative. The cell of origin of basal ER-negative tumours in \textit{BRCA1} mutation carriers is likely to be a luminal progenitor cell that is dependent on steroid hormone signalling (23). There is also indirect evidence that steroid hormones regulate breast cancer stem cells in humans where the same paracrine regulation probably occurs, perhaps mediated via the Receptor Activator of NF-$\kappa$B) (RANK) ligand (24). Other studies have also provided evidence that oophorectomy decreases the risk of breast cancer in \textit{BRCA1} mutation carriers (26,27) and tamoxifen treatment may decrease the risk of contralateral breast cancer for \textit{BRCA1} mutation carriers. Both of these findings suggest the potential ER involvement in \textit{BRCA1} associated disease (28).
There are currently limited data in the literature on the impact of these variants on expression levels of *ESR1* in breast tumour samples. Stacey *et al.* found some evidence that breast tumours from 11 GG homozygote carriers of rs9397435 expressed higher mean levels of *ESR1* compared with tumours from over one thousand carriers of the ‘A’ allele (16). However, Dunbier *et al.* (29) recently reported that *ESR1* is co-expressed in tumour biopsies along with three uncharacterized open reading frames located upstream of *ESR1*. It is therefore currently uncertain whether rs9397435 or correlated causal variant(s) affect breast cancer risk through modulating *ESR1* expression levels or those of additional genes in the region. If the *ESR1* gene is found to be the target, this would provide direct evidence that ER signalling is important in the development of ER-negative breast cancer (and breast cancer in *BRCA1* carriers in particular).

We also found evidence that SNP rs11249433 at 1p11.2 was associated with the risk for *BRCA2* mutation carriers for *BRCA2* mutation carriers. However, the OR estimate from the original breast cancer GWAS (0.94) is only slightly different from the HR estimates for both *BRCA1* and *BRCA2* carriers (0.96) and is included in the CIs for both estimates (5). If the relative risk associated with each copy of the minor allele of this SNP is between 0.90 and 1.00, we have limited power to detect these associations given our sample size. *RAD51L1* is known to be essential to DNA repair via homologous recombination; therefore, if the breast cancer association seen in the general population was mediated through *RAD51L1*, an absence of an association in *BRCA1* and *BRCA2* mutation carriers (i.e. a ‘negative interaction’ with BRCA status) could also be plausible. It is interesting to note, however, that a rare allele in the *RAD51* gene, in the same pathway, was previously associated with an increased risk of breast cancer for *BRCA2* mutation carriers (25). Future studies with a larger number of mutation carriers, and analysis of the causal variant once it has been identified, may help to clarify the involvement of this locus in breast cancer for mutation carriers.

Including the SNPs from the present study, five loci are now known to modify the risk of breast cancer for *BRCA1* mutation carriers.

![Figure 3](https://academic.oup.com/hmg/article-abstract/20/16/3304/648450)
carriers (CASP8, TOX3, 2q35, 19p13 and 6q25.1) (8–10, 12,14) and nine loci are known to modify the risk of breast cancer for BRCA2 mutation carriers (FGFR2, TOX3, MAP3K1, LSP1, 2q35, SLC4A7, 5p12, ZNF365 and 1p11.2) (8–10,13). These loci are estimated to account for ≏3.0% of the genetic variance in the risk of breast cancer in BRCA1 mutation carriers and 5.6% of the variance in BRCA2 mutation carriers. Although these variants account for a small proportion of the variability in risk, it has been demonstrated that these SNPs have implications for the absolute risk prediction in mutation carriers (10), and could therefore be relevant in the genetic counselling of women carrying mutations (15). There are also suggestions from candidate gene studies that other variants may modify cancer risks for mutation carriers, which are currently being investigated in larger sample sizes (31,32). The three associated polymorphisms presented here, in conjunction with previously identified risk-modifying polymorphisms and other risk-modifying factors, can be used to improve risk prediction in BRCA1 and BRCA2 mutation carriers.

Data from the general population indicate that chemopreventive agents have different effects on the risk of ER-positive and ER-negative breast cancer (33). Ongoing and future CIMBA studies will aim to clarify the involvement of these polymorphisms in ER-positive and ER-negative breast cancer risk, as well as other tumour subtypes, in BRCA1 and BRCA2 mutation carriers, which should lead to further improvements in risk prediction. Since BRCA1 and BRCA2 mutations confer high risks of breast cancer, these SNPs, taken together with other risk factors such as mammographic breast density (34), will result in substantial differences in the absolute risk of developing breast cancer between combined SNP and risk factor categories

Table 3. Competing risk analysis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Unaffected, N (%)</th>
<th>Breast cancer, N (%)</th>
<th>Ovarian cancer, N (%)</th>
<th>Breast cancer</th>
<th>Ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>6q25.1 (rs2046210)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1658 (42.5)</td>
<td>2046 (37.4)</td>
<td>645 (44.7)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TC</td>
<td>1740 (44.6)</td>
<td>2648 (48.4)</td>
<td>642 (44.5)</td>
<td>1.23</td>
<td>0.98</td>
</tr>
<tr>
<td>TT</td>
<td>505 (12.9)</td>
<td>777 (14.2)</td>
<td>156 (10.8)</td>
<td>1.30</td>
<td>0.89</td>
</tr>
<tr>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td>9.8 × 10⁻⁸</td>
<td>0.54</td>
</tr>
<tr>
<td>Per-allele</td>
<td></td>
<td></td>
<td></td>
<td>6.7 × 10⁻⁸</td>
<td>0.31</td>
</tr>
<tr>
<td>BRCA2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>988 (40.8)</td>
<td>1317 (39.1)</td>
<td>160 (40.4)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TC</td>
<td>1130 (46.6)</td>
<td>1567 (46.5)</td>
<td>189 (47.7)</td>
<td>1.01</td>
<td>0.99</td>
</tr>
<tr>
<td>TT</td>
<td>305 (12.6)</td>
<td>485 (14.4)</td>
<td>47 (11.9)</td>
<td>1.14</td>
<td>0.88</td>
</tr>
<tr>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td>0.22</td>
<td>0.76</td>
</tr>
<tr>
<td>Per-allele</td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
<td>0.57</td>
</tr>
<tr>
<td>6q25.1 (rs9397435)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>4116 (86.1)</td>
<td>5245 (82.9)</td>
<td>1282 (87.5)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>AG</td>
<td>633 (13.2)</td>
<td>1034 (16.3)</td>
<td>178 (12.1)</td>
<td>1.28</td>
<td>0.90</td>
</tr>
<tr>
<td>GG</td>
<td>33 (0.7)</td>
<td>49 (0.8)</td>
<td>5 (0.3)</td>
<td>1.25</td>
<td>0.47</td>
</tr>
<tr>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td>2.8 × 10⁻⁶</td>
<td>0.15</td>
</tr>
<tr>
<td>Per-allele</td>
<td></td>
<td></td>
<td></td>
<td>7.8 × 10⁻⁷</td>
<td>0.10</td>
</tr>
<tr>
<td>BRCA2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>2410 (83.9)</td>
<td>3131 (82.5)</td>
<td>386 (85.4)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>AG</td>
<td>466 (15.5)</td>
<td>631 (16.6)</td>
<td>64 (14.2)</td>
<td>1.11</td>
<td>0.93</td>
</tr>
<tr>
<td>GG</td>
<td>15 (0.5)</td>
<td>32 (0.8)</td>
<td>2 (0.4)</td>
<td>1.50</td>
<td>0.65</td>
</tr>
<tr>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
<td>0.75</td>
</tr>
<tr>
<td>Per-allele</td>
<td></td>
<td></td>
<td></td>
<td>0.92</td>
<td>0.52</td>
</tr>
<tr>
<td>1p11.2 (rs11249433)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1363 (34.8)</td>
<td>1945 (35.1)</td>
<td>486 (33.5)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CT</td>
<td>1863 (47.5)</td>
<td>2714 (49.0)</td>
<td>739 (50.9)</td>
<td>1.02</td>
<td>1.12</td>
</tr>
<tr>
<td>CC</td>
<td>695 (17.7)</td>
<td>880 (15.9)</td>
<td>226 (15.6)</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>Per-allele</td>
<td></td>
<td></td>
<td></td>
<td>0.96</td>
<td>0.70</td>
</tr>
<tr>
<td>BRCA2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>889 (36.4)</td>
<td>1129 (33.1)</td>
<td>133 (33.7)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CT</td>
<td>1182 (48.4)</td>
<td>1695 (49.7)</td>
<td>198 (50.1)</td>
<td>1.10</td>
<td>1.12</td>
</tr>
<tr>
<td>CC</td>
<td>372 (15.2)</td>
<td>588 (17.2)</td>
<td>64 (16.2)</td>
<td>1.23</td>
<td>1.23</td>
</tr>
<tr>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.44</td>
</tr>
<tr>
<td>Per-allele</td>
<td></td>
<td></td>
<td></td>
<td>1.11</td>
<td>0.20</td>
</tr>
<tr>
<td>RAD51L1 (rs999737/rs10483813)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC/TT</td>
<td>2014 (61.9)</td>
<td>2828 (63.6)</td>
<td>732 (63.4)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TC/AT</td>
<td>1100 (33.8)</td>
<td>1426 (32.1)</td>
<td>374 (32.4)</td>
<td>0.92</td>
<td>0.95</td>
</tr>
<tr>
<td>TT/AA</td>
<td>138 (4.2)</td>
<td>194 (4.4)</td>
<td>49 (4.2)</td>
<td>1.02</td>
<td>1.00</td>
</tr>
<tr>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
<td>0.76</td>
</tr>
<tr>
<td>Per-allele</td>
<td></td>
<td></td>
<td></td>
<td>0.96</td>
<td>0.90</td>
</tr>
<tr>
<td>BRCA2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC/TT</td>
<td>1384 (61.1)</td>
<td>1942 (62.1)</td>
<td>233 (62.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TC/AT</td>
<td>753 (33.2)</td>
<td>1036 (33.2)</td>
<td>119 (31.6)</td>
<td>0.98</td>
<td>0.95</td>
</tr>
<tr>
<td>TT/AA</td>
<td>130 (5.7)</td>
<td>147 (4.7)</td>
<td>24 (6.4)</td>
<td>0.88</td>
<td>1.16</td>
</tr>
<tr>
<td>2-df test</td>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>Per-allele</td>
<td></td>
<td></td>
<td></td>
<td>0.96</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Associations with breast and ovarian cancer risk for BRCA1 and BRCA2 mutation carriers. Analysis restricted to mutation carriers of European ancestry.
and
was performed in two stages. Stage 1 involved SNPs
complementary Material, Table S1. Genotyping for the four SNPs
history in the study.
the individual with the most complete version of the family
were included only once in the analysis. To avoid inclusion
the extent of true overlap in subjects and families appearing
of the individuals in question, in order to determine precisely
identified, we contacted the relevant centres for further information
find hidden duplicates. When a potential duplicate was ident-
SNP genotype data available within the CIMBA database to
potential duplicate individuals. Where possible we also used
non-European ancestry. We investigated possible overlap of
performed which were restricted to mutation carriers of
'more of European ancestry', but additional analyses were
primary analysis was restricted to women self-reported as
information of rs11249433. As an additional genotyping quality-control
was also available on the country of
residence, which was defined to be the country where
the carrier family was recruited to the study. Related individ-
women were included in the analysis if they carried mutations
that were pathogenic according to generally recognized cri-
teria (25) (Breast Cancer Information Core). Further details
of the CIMBA initiative can be found elsewhere (36).
Women who carried pathogenic mutations in both BRCA1
and BRCA2 were excluded from the current analysis. The primary analysis was restricted to women self-reported as
'white of European ancestry', but additional analyses were
performed which were restricted to mutation carriers of
non-European ancestry. We investigated possible overlap of
carriers between studies by comparing the year of birth,
exact mutation description, and the reported ages, to identify
potential duplicate individuals. Where possible we also used
SNP genotype data available within the CIMBA database to
find hidden duplicates. When a potential duplicate was ident-
ied, we contacted the relevant centres for further information
about these individuals, in a manner that protected the identity
of the individuals in question, in order to determine precisely
the extent of true overlap in subjects and families appearing
more than once in the data set. Duplicated mutation carriers
were included only once in the analysis. To avoid inclusion
of families extending over several studies, we included only
the individual with the most complete version of the family
history in the study.

Genotyping
The genotyping platforms used by each study are shown in Sup-
plementary Material, Table S1. Genotyping for the four SNPs
was performed in two stages. Stage 1 involved SNPs
rs2046210, rs11249433 and the RAD51L1 SNPs rs999737 and
rs10483813. DNA samples from 11 studies were genotyped
using the iPLEX Mass Array platform at a single genotyping
centre. All remaining studies used the S' endonuclease assay
(Taqman), with reagents supplied by Applied Biosystems and
tested centrally. A Taqman assay could not be adequately
designed for SNP rs999737 and studies using this platform gen-
yotyped the surrogate SNP rs10483813 (pair-wise $r^2 = 1$ with
rs999737 based on HapMap data). Stage 2 involved SNP
rs9397435 and all samples were genotyped using the iPLEX
Mass Array platform at four genotyping centres. All centres
included at least 2% of the samples in duplicate, no template
controls in every plate and a random mixture of affected and
unaffected carriers. Samples that failed for more than two of
the SNPs genotyped (or ≥20% of the SNPs typed if more
than three SNPs were analysed using multiplex genotyping)
were excluded from the analysis. A study was included in the
analysis only if the call rate was over 95% after samples that
failed at multiple SNPs had been excluded. The concordance
between duplicates had to be at least 98%. To assess the accu-
rracy of genotyping across genotyping centres, all centres geno-
typed 95 DNA samples from a standard test plate (Coriell
Institute) for all three SNPs. If the genotyping was inconsistent
for more than one sample in the test plate, the study was
excluded from the analysis of that SNP. On the basis of these cri-
teria, two studies were excluded from the analysis of rs2046210,
eight studies were excluded from the analysis of rs999737/ 
rs10483813 and three studies were excluded from the analysis of
rs11249433. As an additional genotyping quality-control
check, we also evaluated the deviation from Hardy–Weinberg
equilibrium (HWE) for unrelated subjects separately for each
SNP and study. Seven studies had HWE P-values in the range
0.003–0.05 (one study for the rs2046210 SNP, two for
rs9397435 and four studies for rs11249433). Upon examination
of the cluster plots for these studies and SNPs, none revealed any
unusual patterns and these studies were included in all the ana-
yses. After the above exclusions, a total of 22 176 unique
mutation carriers (14 123 BRCA1 and 8053 BRCA2) from 42
studies had an observed genotype for at least one of the SNPs
and were therefore included in the primary analysis (Sup-
plementary Material, Table S1).

Statistical analysis
The aim of the primary analysis was to evaluate the associ-
ation between each genotype and the risk of breast cancer.
The phenotype of each individual was therefore defined by
their age at diagnosis of breast cancer or their age at the last
follow-up. For this purpose, individuals were censored at the
age of the first breast cancer diagnosis, ovarian cancer diagno-
sis or bilateral prophylactic mastectomy or the age at the last
observation. Mutation carriers censored at ovarian cancer
diagnosis were considered unaffected. Since mutation carriers
were not sampled randomly with respect to their disease
status, standard methods of survival analysis (such as Cox
regression) may lead to biased estimates of the hazard ratios
(HRs) (37). We therefore conducted the analysis by modelling
the retrospective likelihood of the observed genotypes con-
ditional on the disease phenotypes as previously described
(25). The effect of each SNP was modelled either as a per-
allele HR (multiplicative model) or as separate HRs for

MATERIALS AND METHODS
Subjects
All carriers participated in clinical or research studies at the
host institutions under ethically approved protocols and data
were analysed anonymously. Subjects were BRCA1
and BRCA2 mutation carriers recruited by 42 study centres
in 22 countries through the CIMBA initiative (Supplementary
Material, Table S1). The large majority of carriers were
recruited through cancer genetics clinics offering genetic
testing, and enrolled into national or regional studies. Some
carriers were identified by population-based sampling of
cases, and some by community recruitment (e.g. in Ashkenazi
Jewish populations). Eligibility to participate in CIMBA is
restricted to female carriers of pathogenic BRCA1 or BRCA2
mutations who were 18 years old or older at recruitment.
Information collected included the year of birth; mutation
description, including nucleotide position and base change;
age at the last follow-up; ages at breast and ovarian cancer
diagnoses; and age or date at bilateral prophylactic mastect-
omy. Information was also available on the country of
residence, which was defined to be the country where
the carrier family was recruited to the study. Related individ-
uals were identified through a unique family identifier.
Women were included in the analysis if they carried mutations
that were pathogenic according to generally recognized cri-
teria (25) (Breast Cancer Information Core). Further details
of the CIMBA initiative can be found elsewhere (36).
Women who carried pathogenic mutations in both BRCA1
and BRCA2 were excluded from the current analysis. The primary analysis was restricted to women self-reported as
'white of European ancestry', but additional analyses were
performed which were restricted to mutation carriers of
non-European ancestry. We investigated possible overlap of
carriers between studies by comparing the year of birth,
exact mutation description, and the reported ages, to identify
potential duplicate individuals. Where possible we also used
SNP genotype data available within the CIMBA database to
find hidden duplicates. When a potential duplicate was ident-
ified, we contacted the relevant centres for further information
about these individuals, in a manner that protected the identity
of the individuals in question, in order to determine precisely
the extent of true overlap in subjects and families appearing
more than once in the data set. Duplicated mutation carriers
were included only once in the analysis. To avoid inclusion
of families extending over several studies, we included only
the individual with the most complete version of the family
history in the study.
heterozygotes and homozygotes, and these were estimated on the logarithmic scale. The HRs were assumed to be independent of age (i.e. we used a Cox proportional-hazards model). The assumption of proportional hazards was tested by adding a ‘genotype × age’ interaction term to the model in order to fit models in which the HR changed with age. Where there was significant evidence of a ‘genotype × age’ interaction, we fitted models that allowed for age-specific HRs. These allowed for age-specific HRs to be estimated simultaneously in 10-year intervals (20–29, 30–39, …, 70–79). Thus, these models included six log-HR parameters. We examined between-study heterogeneity by comparing the models that allowed for study-specific log-HRs against models in which the same log-HR was assumed to apply to all studies. Analyses were carried out with the pedigree analysis software MENDEL (38), and details of this approach have been described previously (25). Under the retrospective likelihood approach, the baseline age-specific incidence rates in the Cox proportional-hazards model were chosen such that the overall breast cancer incidence rates, averaged over all genotypic categories, agree with external estimates of incidence for BRCA1 and BRCA2 mutation carriers. All analyses were stratified by study group and country of residence and used calendar-year- and cohort-specific breast cancer incidence rates for BRCA1 and BRCA2 (39).

To evaluate the combined effects of the ESR1 SNPs on the risk of breast cancer, we fit retrospective likelihood models where the breast cancer incidence λ(t) was assumed to be of the form λ(t) = λ0(t) exp(β1x1 + β2x2), where λ0(t) is the baseline incidence, β1 is the per-allele log-HR for SNP1, β2 is the per-allele log-HR for SNP2, and x1 and x2 represent the number of minor alleles at SNP 1 and 2, respectively (0,1,2), while allowing for linkage disequilibrium between the loci. To test whether the fit of the model is significantly improved by the inclusion of a locus into the model, we tested for the significance of parameters β1 and β2.

To investigate whether our results were influenced by any of our assumptions, we performed additional sensitivity analyses. If any of the SNPs were associated with disease survival, the inclusion of prevalent cases may influence the HR estimates. Current data indicate that 5-year survival after a breast cancer diagnosis is now over 80% (Cancer Research—UK, Breast cancer survival statistics). Therefore, we repeated our analysis by excluding mutation carriers diagnosed more than 5 years prior to the age at recruitment into the study. To examine whether SNP associations differed by type of mutations, we classified BRCA1 mutations according to their potential functional effect (40–42). Class 1 mutations comprised loss-of-function mutations, expected to result in a reduced transcript or protein level due to mRNA nonsense-mediated decay and/or degradation or instability of truncated proteins, translation re-initiation but no production of stable protein, or the absence of expression because of the deletion of transcription regulatory regions. Class 2 mutations were those likely to generate potentially stable mutant proteins that might have dominant negative action, partially preserved normal function or loss of function. Class 2 mutations include missense substitutions, in-frame deletions and insertions, as well as truncating mutations with premature stop codons occurring in the last exon. Mutations whose consequences at the transcript or protein level could not be inferred were not considered for this classification. These were mainly mutations located in splice sites but not characterized for their effect at the transcript level, or large deletions or insertions with undetermined boundaries.

We further evaluated the associations of these SNPs with the risk of ovarian cancer within a competing risk analysis framework (12,43), by estimating HRs simultaneously for breast and ovarian cancers. In this model, each individual was at risk of developing either breast or ovarian cancer, by assuming that the probabilities of developing each disease were independent conditional on the underlying genotype. A different censoring process was used in this case, whereby individuals were followed up to the age of the first breast or ovarian cancer diagnosis and were considered to have developed the corresponding disease. No follow-up was considered after the first cancer diagnosis. Individuals were censored for breast cancer at the age of bilateral prophylactic mastectomy and for ovarian cancer at the age of bilateral oophorectomy and were assumed to be unaffected for the corresponding disease. The remaining individuals were censored at the age at the last observation and were assumed to be unaffected for both diseases.

All analyses were stratified by study group and country of residence and used calendar-year- and cohort-specific breast cancer incidence rates for BRCA1 and BRCA2 (39). For sensitivity analyses, strata with a small number of mutation carriers were grouped. We used a robust variance-estimation approach to allow for the non-independence among related carriers (44). Data on the two completely correlated SNPs (rs999737 and rs10483813) were combined and treated as a single locus in the analysis of associations.

WEB RESOURCES

SUPPLEMENTARY MATERIAL
Supplementary Material is available at HMG online.

ACKNOWLEDGEMENTS
A.C.A. is a CR-UK Senior Cancer Research Fellow, D.F.E. is CR-UK Principal Research Fellow and G.C.T. is a NHMRC Senior Principal Research Fellow.
Study-specific acknowledgments:
The Baltic Familial Breast and Ovarian Cancer Consortium (BFBOCC)
Dr Laima Tihomirova, the Genome Database of the Latvian Population, Latvian Biomedical Research and Study Centre provided data and DNA samples for BFBOCC. This work is supported by the Research Council of Lithuania grant LIG-19/2010 to Ramunas Janavicius.
**BRCA-gene mutations and breast cancer in South African women (BMBSA)**

The study is supported by grants from the Cancer Association of South Africa to Elizabeth J. van Rensburg.

**Breast Cancer Family Registry (BCFR)**

This work was supported by the National Cancer Institute, National Institutes of Health under RFA-CA-06-503 and through cooperative agreements with members of the Breast Cancer Family Registry (BCFR) and Principal Investigators, including Cancer Care Ontario (U01 CA69467), Columbia University (U01 CA69398), Fox Chase Cancer Center (U01 CA69631), Huntsman Cancer Institute (U01 CA69446), Cancer Prevention Institute of California (formerly the Northern California Cancer Center) (U01 CA69417), University of Melbourne (U01 CA69638), and Research Triangle Institute Informatics Support Center (RFP No. N02PC45022-46).

Samples from the FCCC, HCI and C PIC were processed and distributed by the Coriell Institute for Medical Research. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government or the BCFR.

**Copenhagen Breast Cancer Study (CBCS)**

We thank Susanne Kjaergaard and Mette Klarskov for clinical data. The study was supported by the NEYE Foundation.

**CONSIT TEAM**

CONSIT TEAM is supported by grants from Ministero della Salute (Extraordinary National Cancer Program 2006 ‘Alleanza contro il Cancro’ to LV and PR and ‘Progetto Tumori Femminili’ to PR), 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, according to Italian laws (INT-Institutional strategic projects ‘5 × 1000’) to P.P.

CONSIT TEAM thanks Marco A. Pierotti, Carla B. Ripamonti and Marilena Mongari of the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; Bernardo Bonanni of the Istituto Europeo di Oncologia, Milan, Italy; Alessandra Viel of the Centro di Riferimento Oncologico, Aviano, Italy and Antonella Savarese of the Istituto Regina Elena, Rome, Italy.

**Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)**


**Deutsches Krebsforschungszentrum (DKFZ) study**

The DKFZ study was supported by the DKFZ.

**The German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC)**

GC-HBOC is supported by a grant of the German Cancer Aid (grant 107054). We thank Christian Sutter (Center Heidelberg) Sabine Preisler-Adams (Center Münster), Britta Fiebig (Center Regensburg), Wolfram Heinritz (Center Leipzig) and Dieter Schäfer (Center Frankfurt) for providing samples and Juliane Köhler for her excellent technical assistance.

__Genetic Modifiers of cancer risk in BRCA1/2 mutation carriers (GEMÖ) study: Cancer Genetics Network ‘Groupe.__

__Human Molecular Genetics, 2011, Vol. 20, No. 16__

3317
The study is supported by the Ligue National Contre le Cancer; Association for International Cancer Research Grant (AICR-07-0454); and the Association ‘Le cancer du sein, parions-en!’ Award. We wish to thank all the GEMO collaborating groups for their contribution to this study. GEMO Collaborating Centers are: Coordinating Centre, Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Centre Hospitalier Universitaire de Lyon/Centre Léon Bérard, & UMR5201 CNRS, Université de Lyon, Lyon: Olga Sinilnikova, Laure Barjhoux, Carole Verny-Pierre, Sophie Giraud, Mélanie Leone, Sylvie Mazoyer; and Service de Génétique et Cancer’, Fédération Nationale des Centres de Lutte contre le Cancer, France.

The study was supported by the Icelandic Association ‘Walking for Breast Cancer Research’ and by the Landspitali University Hospital Research Fund.

Istituto Oncologico Veneto—Hereditary Breast Ovarian Cancer Study (IOV/HBOCS)

This study was supported by ‘Ministro della Salute’ (grant numbers RFS 2006-5-341353, ACC2/R6.9 and ‘Progetto Tumori Femminili’).

Kathleen Cunningham Consortium for Research into Familial Breast Cancer (kConFab)

We wish to thank Heather Thorne, Eveline Niedermayr, all the kConFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the Clinical Follow-Up Study (funded by NHMRC grants 145684, 288704 and 454508) for their contributions to this resource, and the many families who contribute to kConFab. kConFab is supported by grants from the National Breast Cancer Foundation, the National Breast Cancer Foundation, and the Sigrid Juselius Foundation. We thank Tuomas Heikkinen and RN Irja Erkkilä for their help with the patient data and study samples.
the National Health and Medical Research Council (NHMRC) and by the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the Cancer Foundation of Western Australia. ABS is supported by an NHMRC Senior Research Fellowship.

McGill
This work was supported by the Jewish General Hospital Weekend to End Breast Cancer. MT holds a Fonds de la Recherche en Santé du Québec clinician-scientist award.

National Cancer Institute (NCI)
The research of Drs PL Mai and MH Greene was supported by the Intramural Research Program of the US National Cancer Institute, and by support services contracts NO2-CP-11019-50 and NO2-CP-65504 with Westat, Inc, Rockville, MD.

Mayo Clinic (MAYO)
This work was supported by grants from the Breast Cancer Research Foundation (BCRF), Komen Foundation for the Cure, Department of Defense ovarian cancer research award (W81XWH-10-1-0341) and US National Cancer Institute, National Institutes of Health grant CA128978.

N.N. Petrov Institute of Oncology (NNPIO)
The work is supported by the Russian Foundation for Basic Research (grants 08-04-00369-a, 09-04-90402 and 10-04-92110-a), the Commission of the European Communities (grant PITN-GA-2009-238132) and through a Royal Society International Joint grant (JP090615).

Ontario Cancer Genetics Network (OCGN)
This work was supported by Cancer Care Ontario and the US National Cancer Institute, National Institutes of Health under RFA # CA-06-503 and through cooperative agreements with members of the Breast Cancer Family Registry (BCFR) and Principal Investigators. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government or the BCFR. We wish to thank Teresa Selandier, Nayana Weerasooriya and members of the Ontario Cancer Genetics Network for their contributions to the study.

The Ohio State University Clinical Cancer Genetics (OSU CCG)
This work was supported by the Ohio State University Comprehensive Cancer Center. We thank Leigha Senter and Kevin Sweet for patient accrual and data management, the Human Genetics Sample bank for sample preparation and the OSU CCC Nucleic Acids Shared Resource for genotyping plate reads.

Pisa Breast Cancer Study (PBCS)
MAC from University Hospital of Pisa was supported by Istituto Toscano Tumori grant

SEABASS
SEABASS is a collaborative effort between Cancer Research Initiatives Foundation (Malaysia), University Malaya (Malaysia), National University Hospital (Singapore), University Kebangsaan Malaysia (Malaysia), Hospital Kuala Lumpur (Malaysia) and Putrajaya Hospital (Malaysia). The research has received funding from CARIF and University Malaya.

Swedish Breast Cancer Study (SWE-BRCA)
SWE-BRCA collaborators: Per Karlsson, Margareta Nordling, Annika Bergman and Zakaria Einbeig, Gothenburg, Sahlgrenska University Hospital; Marie Stenmark-Askalm and Sigrun Liedgren, Linköping University Hospital; Ake Borg, Niklas Loman, Håkan Olsson, Ulf Kristoffersson, Helena Jernström, Katja Harbst and Karin Henriksson, Lund University Hospital; Annika Lindblom, Brita Arver, Anna von Wachenfeldt, Annelie Liljegren, Gisela Barbany-Bustinza and Johanna Rantalä, Stockholm, Karolinska University Hospital; Beatrice Melin, Henrik Grönborg, Eva-Lena Stattin and Monica Emanuelsson, Umeå University Hospital; Hans Ehrencrona, Richard Rosenquist Brandell and Niklas Dahl, Uppsala University Hospital.

University of California Irvine Study (UCI)
Now at the Beckman Research Institute of the City of Hope. This work was supported by NIH grant R01-CA74415 (to SLN). SLN is partially supported by the Morris and Horowitz Families Endowed Professorship.

UK and Gilda Radner Familial Ovarian Cancer Registries (UKGRFOCR)
UKFOCR was supported by a project grant from CR-UK to Paul Pharoah. We thank Simon Gayther, Susan Ramus, Carole Pye, Patricia Harrington and Eva Wozniak for their contributions towards the UKFOCR. We would like to acknowledge the Roswell Park Alliance Foundation for their continued support of the Gilda Radner Ovarian Family Cancer Registry. GRFOCR would like to acknowledge Kirsten Moysich (Department of Cancer Prevention and Control) and Kunle Oduseni (Departments Gynecologic Oncology and Immunology).

University California San Francisco study (USCF)
Dr Beattie was supported by a grant from the National Institutes of Health, National Cancer Institute, Bay Area Breast SPORE (P50 CA058207)

UPENN
SMD receives funding from the MacDonald Family Foundation, and KLN from Breast Cancer Research Foundation. TR is funded by NIH grants R01-CA102776 and R01-CA083855.

Cedars-Sinai Medical Center (WCRI)
This work was supported by the American Cancer Society Early Detection Professorship and Entertainment Industry Foundation.

Conflict of Interest statement. None declared.

FUNDING
This work was supported by Cancer Research UK grants C12292/A11174 and C1287/A10118. The research leading to these results has received funding from the European Community’s Seventh Framework Programme under grant agreement no. 223175 (HEALTH-F22009-223175).

REFERENCES


