PLOS | ONE

RESEARCH ARTICLE

RAD51B in Familial Breast Cancer

Liisa M. Pelttari[1], Sofia Khan[1], Mikko Vuorela[2], Johanna I. Kiiski[1], Sara Vilske[1], Viivi Nevanlinna[1], Salla Ranta[1], Johanna Schleutker[3,4,5], Robert Winqvist[2,6], Anne Kallioniemi[3,7], Thilo Dörk[8], Natalia V. Bogdanova[9], Jonine Figueroa[10], Paul D. P. Pharoah[11,12], Marjanka K. Schmidt[13], Alison M. Dunning[11], Montserrat García-Closas[10,14], Manjeet K. Bolla[12], Joe Dennis[12], Kyriaki Michailidou[12], Qin Wang[12], John L. Hopper[15], Melissa C. Southey[16], Efraim H. Rosenberg[13], Peter A. Fasching[17,18], Matthias W. Beckmann[17], Julian Peto[19], Isabel dos-Santos-Silva[19], Elinor J. Sawyer[20], Ian Tomlinson[21], Barbara Burwinkel[22,23], Harald Surowy[22,23], Pascal Guénel[24,25], Thérèse Truong[24,25], Stig E. Bojesen[26,27,28], Børge G. Nordestgaard[27,28], Javier Benitez[29,30], Anna González-Neira[29], Susan L. Neuhausen[31], Hoda Anton-Culver[32], Hermann Brenner[33,34,35], Volker Arndt[33], Alfons Meindl[36], Rita K. Schmutzler[37,38,39], Hiltrud Brauch[34,40,41], Thomas Brüning[42], Annika Lindblom[43], Sara Margolin[44], Arto Mannermaa[45,46,47], Jaana M. Hartikainen[45,46,47], Georgia Chenevix-Trench[48], kConFab/AOCS Investigators[48,49¶], Laurien Van Dyck[50,51], Hilde Janssen[52], Jenny Chang-Claude[53,54], Anja Rudolph[53], Paolo Radice[55], Paolo Peterlongo[56], Emily Hallberg[57], Janet E. Olson[57], Graham G. Giles[15,58], Roger L. Milne[15,58], Christopher A. Haiman[59], Fredrick Schumacher[59], Jacques Simard[60], Martine Dumont[60], Vessela Kristensen[61,62,63], Anne-Lise Borresen-Dale[61,62], Wei Zheng[64], Alicia Beeghly-Fadiel[64], Mervi Grip[65], Irene L. Andrulis[66,67], Gord Glendon[66], Peter Devilee[68,69], Caroline Seynaeve[70], Maartje J. Hooning[70], Margriet Collée[71], Angela Cox[72], Simon S. Cross[73], Mitul Shah[11], Robert N. Luben[74], Ute Hamann[75], Diana Torres[75,76], Anna Jakubowska[77], Jan Lubinski[77], Fergus J. Couch[78], Drakoulis Yannoukakos[79], Nick Orr[80], Anthony Swerdlow[14,81], Hatef Darabi[82], Jingmei Li[82], Kamila Czene[82], Per Hall[82], Douglas F. Easton[11,12], Johanna Mattson[83], Carl Blomqvist[83], Kristiina Aittomäki[84], Heli Nevanlinna[1]*

1 Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, **2** Laboratory of Cancer Genetics and Tumor Biology, Cancer Research and Translational Medicine, Biocenter Oulu, University of Oulu, Oulu, Finland, **3** BioMediTech, University of Tampere, Tampere, Finland, **4** Department of Medical Biochemistry and Genetics, University of Turku, Turku, Finland, **5** Tyks Microbiology and Genetics, Department of Medical Genetics, Turku University Hospital, Turku, Finland, **6** Laboratory of Cancer Genetics and Tumor Biology, Northern Finland Laboratory Centre NordLab, Oulu, Finland, **7** Fimlab Laboratories, Tampere, Finland, **8** Gynaecology Research Unit, Hannover Medical School, Hannover, Germany, **9** Radiation Oncology Research Unit, Hannover Medical School, Hannover, Germany, **10** Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, United States of America, **11** Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, United Kingdom, **12** Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom, **13** Netherlands Cancer Institute, Antoni van Leeuwenhoek hospital, Amsterdam, The Netherlands, **14** Division of Genetics and Epidemiology, The Institute of Cancer Research, London, United Kingdom, **15** Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global health, The University of Melbourne, Melbourne, Australia, **16** Department of Pathology, The University of Melbourne, Melbourne, Australia, **17** Department of Gynaecology and Obstetrics, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nuremberg, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany, **18** David Geffen School of Medicine, Department of Medicine, Division of Hematology and Oncology, University of California Los Angeles, Los Angeles, California, United States of America, **19** Department of Non-Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom, **20** Research Oncology, Guy's Hospital, King's College London, London, United Kingdom, **21** Wellcome Trust Centre for Human Genetics and Oxford NIHR Biomedical Research Centre, University of Oxford, Oxford, United Kingdom, **22** Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg, Germany, **23** Molecular Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany, **24** Environmental Epidemiology of Cancer, Center for Research in Epidemiology and Population Health, INSERM, Villejuif, France, **25** University Paris-Sud, Villejuif, France, **26** Copenhagen General Population Study, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark, **27** Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark,

CrossMark
click for updates

OPEN ACCESS

Citation: Pelttari LM, Khan S, Vuorela M, Kiiski JI, Vilske S, Nevanlinna V, et al. (2016) *RAD51B* in Familial Breast Cancer. PLoS ONE 11(5): e0153788. doi:10.1371/journal.pone.0153788

Editor: Klaus Brusgaard, Odense University Hospital, DENMARK

Received: October 20, 2015

Accepted: April 4, 2016

Published: May 5, 2016

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The HEBCS was financially supported by the Helsinki University Hospital Research Fund, Academy of Finland (266528), the Finnish Cancer Society, The Nordic Cancer Union and the Sigrid Juselius Foundation. LMP received funding from Orion-Farmos Research Foundation, Maud Kuistila Memorial Foundation, Biomedicum Helsinki Foundation, Alfred Kordelin Foundation, Paulo Foundation and the Finnish Cultural Foundation. BCAC is funded by Cancer Research UK [C1287/A10118, C1287/A12014] and by the European Community´s Seventh Framework Programme under grant agreement number 223175 (grant number HEALTH-F2-2009-223175) (COGS). Funding for the iCOGS infrastructure came from: the European Community's Seventh Framework Programme under grant agreement n° 223175 (HEALTH-F2-2009-223175) (COGS), Cancer Research UK (C1287/A10118, C1287/A 10710, C12292/A11174, C1281/A12014, C5047/A8384, C5047/A15007, C5047/A10692, C8197/A16565), the National Institutes of Health (CA128978) and Post-Cancer GWAS initiative (1U19 CA148537, 1U19 CA148065 and 1U19 CA148112 - the GAME-ON initiative), the Department of Defence (W81XWH-10-1-0341), the Canadian Institutes of Health Research (CIHR) for the CIHR Team in Familial Risks of Breast Cancer, Komen Foundation for the Cure, the Breast Cancer Research Foundation, and the Ovarian Cancer Research Fund. The Australian Breast Cancer Family Study (ABCFS) was supported by grant UM1 CA164920 from the National Cancer Institute (USA). 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 Breast Cancer Family Registry (BCFR), nor does mention of trade names, commercial products, or organizations imply endorsement by the USA Government or the BCFR. The ABCFS was also supported by the National Health and Medical Research Council of Australia, the New South Wales Cancer Council, the Victorian Health Promotion Foundation (Australia) and the Victorian Breast Cancer Research Consortium. J.L.H. is a National Health and Medical Research Council (NHMRC) Australia Fellow and a Victorian Breast Cancer Research Consortium Group Leader. M.C.S. is a NHMRC Senior Research Fellow and a Victorian Breast Cancer Research Consortium Group Leader. The ABCS study was supported by the Dutch Cancer Society [grants NKI 2007-3839; 2009 4363]. The work of the BBCC was partly funded by ELAN-Fond of the University Hospital of Erlangen. The BBCS is funded by Cancer Research UK and Breakthrough Breast Cancer and acknowledges NHS funding to the NIHR Biomedical Research Centre, and the National Cancer Research Network (NCRN). ES is supported by NIHR Comprehensive Biomedical Research Centre, Guy's & St. Thomas' NHS Foundation Trust in partnership with King's College London, United Kingdom. IT is supported by the Oxford Biomedical Research Centre. The BSUCH study was supported by the Dietmar-Hopp Foundation, the Helmholtz Society and the German Cancer Research Center (DKFZ). The CECILE study was funded by Fondation de France, Institut National du Cancer (INCa), Ligue Nationale contre le Cancer, Ligue contre le Cancer Grand Ouest, Agence Nationale de Sécurité Sanitaire (ANSES), Agence Nationale de la Recherche (ANR). The CGPS was supported by the Chief Physician

28 Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, **29** Human Cancer Genetics Program, Spanish National Cancer Research Centre, Madrid, Spain, **30** Centro de Investigación en Red de Enfermedades Raras, Valencia, Spain, **31** Beckman Research Institute of City of Hope, Duarte, California, United States of America, **32** Department of Epidemiology, University of California Irvine, Irvine, California, United States of America, **33** Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany, **34** German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany, **35** Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany, **36** Division of Gynaecology and Obstetrics, Technische Universität München, Munich, Germany, **37** Center for Hereditary Breast and Ovarian Cancer, University Hospital of Cologne, Cologne, Germany, **38** Center for Integrated Oncology (CIO), University Hospital of Cologne, Cologne, Germany, **39** Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany, **40** Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany, **41** University of Tübingen, Tübingen, Germany, **42** Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum, Bochum, Germany, **43** Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, **44** Department of Oncology—Pathology, Karolinska Institutet, Stockholm, Sweden, **45** Cancer Center, Kuopio University Hospital, Kuopio, Finland, **46** Institute of Clinical Medicine, Pathology and Forensic Medicine, University of Eastern Finland, Kuopio, Finland, **47** Imaging Center, Department of Clinical Pathology, Kuopio University Hospital, Kuopio, Finland, **48** Department of Genetics, QIMR Berghofer Medical Research Institute, Brisbane, Australia, **49** Peter MacCallum Cancer Center, The University of Melbourne, Melbourne, Australia, **50** Vesalius Research Center, VIB, Leuven, Belgium, **51** Laboratory for Translational Genetics, Department of Oncology, University of Leuven, Leuven, Belgium, **52** Leuven Multidisciplinary Breast Center, Leuven Cancer Institute, University Hospitals Leuven, Leuven, Belgium, **53** Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany, **54** University Cancer Center Hamburg (UCCH), University Medical Center Hamburg-Eppendorf, Hamburg, Germany, **55** Unit of Molecular Bases of Genetic Risk and Genetic Testing, Department of Preventive and Predictive Medicine, Fondazione IRCCS (Istituto Di Ricovero e Cura a Carattere Scientifico) Istituto Nazionale dei Tumori (INT), Milan, Italy, **56** IFOM, Fondazione Istituto FIRC (Italian Foundation of Cancer Research) di Oncologia Molecolare, Milan, Italy, **57** Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, United States of America, **58** Cancer Epidemiology Centre, Cancer Council Victoria, Melbourne, Australia, **59** Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, United States of America, **60** Genomics Center, Centre Hospitalier Universitaire de Québec Research Center, Laval University, Québec City, Canada, **61** Department of Genetics, Institute for Cancer Research, Oslo University Hospital Radiumhospitalet, Oslo, Norway, **62** K.G. Jebsen Center for Breast Cancer Research, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway, **63** Department of Clinical Molecular Biology, Oslo University Hospital, University of Oslo, Oslo, Norway, **64** Division of Epidemiology, Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America, **65** Department of Surgery, Oulu University Hospital, University of Oulu, Oulu, Finland, **66** Lunenfeld-Tanenbaum Research Institute of Mount Sinai Hospital, Toronto, Canada, **67** Department of Molecular Genetics, University of Toronto, Toronto, Canada, **68** Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands, **69** Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands, **70** Department of Medical Oncology, Family Cancer Clinic, Erasmus MC Cancer Institute, Rotterdam, The Netherlands, **71** Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands, **72** Sheffield Cancer Research, Department of Oncology, University of Sheffield, Sheffield, United Kingdom, **73** Academic Unit of Pathology, Department of Neuroscience, University of Sheffield, Sheffield, United Kingdom, **74** Clinical Gerontology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom, **75** Molecular Genetics of Breast Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany, **76** Institute of Human Genetics, Pontificia Universidad Javeriana, Bogota, Colombia, **77** Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland, **78** Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, United States of America, **79** Molecular Diagnostics Laboratory, IRRP, National Centre for Scientific Research "Demokritos", Athens, Greece, **80** Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, United Kingdom, **81** Division of Breast Cancer Research, The Institute of Cancer Research, London, United Kingdom, **82** Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, **83** Department of Oncology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, **84** Department of Clinical Genetics, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

¶ Membership of the kConFab/AOCS Investigators is listed in the Acknowledgments.
* heli.nevanlinna@hus.fi

Johan Boserup and Lise Boserup Fund, the Danish Medical Research Council and Herlev Hospital. The CNIO-BCS was supported by the Instituto de Salud Carlos III, the Red Temática de Investigación Cooperativa en Cáncer and grants from the Asociación Española Contra el Cáncer and the Fondo de Investigación Sanitario (PI11/00923 and PI12/00070). The CTS was initially supported by the California Breast Cancer Act of 1993 and the California Breast Cancer Research Fund (contract 97-10500) and is currently funded through the National Institutes of Health (R01 CA77398). Collection of cancer incidence data was supported by the California Department of Public Health as part of the statewide cancer reporting program mandated by California Health and Safety Code Section 103885. HAC receives support from the Lon V Smith Foundation (LVS39420). The ESTHER study was supported by a grant from the Baden Württemberg Ministry of Science, Research and Arts. Additional cases were recruited in the context of the VERDI study, which was supported by a grant from the German Cancer Aid (Deutsche Krebshilfe). The GC-HBOC (German Consortium of Hereditary Breast and Ovarian Cancer) is supported by the German Cancer Aid (grant no 110837, coordinator: Rita K. Schmutzler). The GENICA was funded by the Federal Ministry of Education and Research (BMBF) Germany grants 01KW9975/5, 01KW9976/8, 01KW9977/0 and 01KW0114, the Robert Bosch Foundation, Stuttgart, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum (IPA), Bochum, as well as the Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn, Germany. The HMBCS was supported by a grant from the Friends of Hannover Medical School and by the Rudolf Bartling Foundation. Financial support for KARBAC was provided through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet, the Swedish Cancer Society, The Gustav V Jubilee foundation and Bert von Kantzows foundation. The KBCP was financially supported by the special Government Funding (EVO) of Kuopio University Hospital grants, Cancer Fund of North Savo, the Finnish Cancer Organizations, and by the strategic funding of the University of Eastern Finland. kConFab is supported by a grant from the National Breast Cancer Foundation, and previously by the National Health and Medical Research Council (NHMRC), the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the Cancer Foundation of Western Australia. Financial support for the AOCS

Abstract

Common variation on 14q24.1, close to *RAD51B*, has been associated with breast cancer: rs999737 and rs2588809 with the risk of female breast cancer and rs1314913 with the risk of male breast cancer. The aim of this study was to investigate the role of *RAD51B* variants in breast cancer predisposition, particularly in the context of familial breast cancer in Finland. We sequenced the coding region of *RAD51B* in 168 Finnish breast cancer patients from the Helsinki region for identification of possible recurrent founder mutations. In addition, we studied the known rs999737, rs2588809, and rs1314913 SNPs and *RAD51B* haplotypes in 44,791 breast cancer cases and 43,583 controls from 40 studies participating in the Breast Cancer Association Consortium (BCAC) that were genotyped on a custom chip (iCOGS). We identified one putatively pathogenic missense mutation c.541C>T among the Finnish cancer patients and subsequently genotyped the mutation in additional breast cancer cases (n = 5259) and population controls (n = 3586) from Finland and Belarus. No significant association with breast cancer risk was seen in the meta-analysis of the Finnish datasets or in the large BCAC dataset. The association with previously identified risk variants rs999737, rs2588809, and rs1314913 was replicated among all breast cancer cases and also among familial cases in the BCAC dataset. The most significant association was observed for the haplotype carrying the risk-alleles of all the three SNPs both among all cases (odds ratio (OR): 1.15, 95% confidence interval (CI): 1.11–1.19, $P = 8.88 \times 10^{-16}$) and among familial cases (OR: 1.24, 95% CI: 1.16–1.32, $P = 6.19 \times 10^{-11}$), compared to the haplotype with the respective protective alleles. Our results suggest that loss-of-function mutations in *RAD51B* are rare, but common variation at the *RAD51B* region is significantly associated with familial breast cancer risk.

Introduction

Breast cancer is the most frequent cancer among women worldwide and also the leading cause of cancer-related death [1]. Several susceptibility loci for breast cancer have been identified and most of the currently known high- and moderate-penetrance predisposition genes have a role in DNA repair. The major high-penetrance breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* are important for DNA double-strand break (DSB) repair through homologous recombination (HR) [2]. Proteins encoded by the moderate-penetrance genes *ATM*, *BRIP1*, and *CHEK2* interact with BRCA1 in DNA damage repair whereas PALB2 associates with both BRCA1 and BRCA2 [2,3]. In the HR repair of DSBs, the RAD51 recombinase has a key role. Binding of RAD51 to single-stranded DNA at the break site initiates the repair of a DSB [4]. In humans, there are five RAD51 paralogs RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3, and they promote the binding of RAD51 to the DNA. Rare pathogenic mutations in *RAD51* paralogs *RAD51C* and *RAD51D* have been identified in breast and ovarian cancer families and confer a high risk specifically for ovarian cancer [5–7] whereas a homozygous missense mutation in *RAD51C* (*FANCO*) was found in a Fanconi anemia patient [8]. A homozygous *XRCC2* mutation has also been detected in a Fanconi anemia patient [9] and rare mutations in the gene in breast cancer families were identified in an exome sequencing study [10]; however, the association of *XRCC2* with breast cancer risk could not be confirmed in a large follow-up study [11].

was provided by the United States Army Medical Research and Materiel Command [DAMD17-01-1-0729], Cancer Council Victoria, Queensland Cancer Fund, Cancer Council New South Wales, Cancer Council South Australia, The Cancer Foundation of Western Australia, Cancer Council Tasmania and the National Health and Medical Research Council of Australia (NHMRC; 400413, 400281, 199600). G.C.T. and P.W. are supported by the NHMRC. RB was a Cancer Institute NSW Clinical Research Fellow. LMBC is supported by the "Stichting tegen Kanker" (232-2008 and 196-2010). Diether Lambrechts is supported by the FWO and the KULPFV/10/016-SymBioSysII. The MARIE study was supported by the Deutsche Krebshilfe e.V. [70-2892-BR I, 106332, 108253, 108419], the Hamburg Cancer Society, the German Cancer Research Center (DKFZ) and the Federal Ministry of Education and Research (BMBF) Germany [01KH0402]. MBCSG is supported by grants from the Italian Association for Cancer Research (AIRC) and by funds from the 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 “5x1000”). The MCBCS was supported by the NIH grants CA128978, CA116167, CA176785 an NIH Specialized Program of Research Excellence (SPORE) in Breast Cancer [CA116201], and the Breast Cancer Research Foundation and a generous gift from the David F. and Margaret T. Grohne Family Foundation and the Ting Tsung and Wei Fong Chao Foundation. MCCS cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further supported by Australian NHMRC grants 209057, 251553 and 504711 and by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry (VCR). The MEC was support by NIH grants CA63464, CA54281, CA098758 and CA132839. The work of MTLGEBCS was supported by the Quebec Breast Cancer Foundation, the Canadian Institutes of Health Research for the “CIHR Team in Familial Risks of Breast Cancer” program – grant # CRN-87521 and the Ministry of Economic Development, Innovation and Export Trade – grant # PSR-SIIRI-701. The NBCS has received funding from the K.G. Jebsen Centre for Breast Cancer Research; the Research Council of Norway grant 193387/V50 (to A-L Børresen-Dale and V.N. Kristensen) and grant 193387/H10 (to A-L Børresen-Dale and V.N. Kristensen), South Eastern Norway Health Authority (grant 39346 to A-L Børresen-Dale) and the Norwegian Cancer Society (to A-L Børresen-Dale and V.N. Kristensen). The NBHS was supported by NIH grant R01CA100374. Biological sample preparation was conducted the Survey and

Variants in the *RAD51B* region, also known as *RAD51L1*, have been associated with breast cancer risk in genome-wide association studies (GWAS) [12–14]. The major-allele of the common polymorphism rs999737 in intron 10 of *RAD51B* and the minor-allele of rs2588809 in intron 7 have been associated with an increased risk of female breast cancer [12,14]. The rs999737 has also been associated with breast cancer risk among *BRCA1* mutation carriers whereas no association was found for rs999737 or rs2588809 with breast cancer subtypes among *BRCA1* or *BRCA2* mutation carriers [15,16]. Another common polymorphism located in the intron 7 of the gene, rs1314913, has been associated with the risk of male breast cancer [13]. *RAD51B* is located at 14q24.1 and is expressed widely, with the highest levels in tissues that are active in recombination [17]. The RAD51B and RAD51C proteins form a stable complex which interacts weakly with RAD51 and promotes the assembly of RAD51 nuclear foci [18,19]. Furthermore, RAD51B is part of a larger BCDX2 complex that is formed with RAD51C, RAD51D, and XRCC2 [4]. The BCDX2 complex acts upstream of RAD51 recruitment to DNA damage foci [20]. Haploinsufficiency of *RAD51B* leads to aberrant HR repair of DNA and causes centrosome fragmentation and aneuploidy in human cells which suggests that loss of the proper biallelic expression of *RAD51B* may lead to chromosome instability in tumor cells [21].

In the Finnish population, recurrent founder mutations have been observed in many of the known breast and ovarian cancer susceptibility genes, including the RAD51 paralogs *RAD51C* and *RAD51D* [22,23]. Thus, the Finnish population is a valuable resource for the identification of new susceptibility alleles.

To identify putative recurrent founder mutations and to study the role of *RAD51B* in female and male breast cancer predisposition and especially in familial breast cancer, we comprehensively screened the *RAD51B* gene in 172 Finnish cancer patients. One identified missense mutation was *in silico* predicted to be pathogenic and was subsequently screened in a larger set of breast cancer cases and population controls from four datasets. In addition, the known *RAD51B* risk SNPs rs999737, rs2588809, and rs1314913 and *RAD51B* haplotypes were studied in the previously published 40 studies participating in the Breast Cancer Association Consortium (BCAC) including 44,791 breast cancer cases and 43,583 controls.

Materials and Methods

Screening of the *RAD51B* gene

The coding region and the exon-intron boundaries of the *RAD51B* gene (RefSeq NG_023267.1, NM_133509.3) were screened in 172 cancer patients from Southern Finland: in 87 female breast cancer patients with a family history of female breast cancer or breast and ovarian cancer, in 4 familial ovarian cancer patients, and in 4 female breast cancer patients with a family history of male and female breast cancer from the Helsinki region of Finland as well as in 77 male breast cancer patients (33 from the Helsinki region and 44 from the Tampere region) (S1 Text). All the female breast or ovarian cancer families and 60 of the male breast cancer families and patients were previously screened negative for *BRCA1/2* mutations whereas 21 patients had not been tested for *BRCA1/2* mutations. Genomic DNA isolated from blood was amplified by PCR and subsequently sequenced using ABI BigDyeTerminator 3.1 Cycle Sequencing kit (Life Technologies) (S1 Table). The sequencing results were analyzed with Variant Reporter Software v1.0 (Life Technologies).

Genotyping of c.541C>T

The identified c.541C>T, p.(Arg181Trp) missense change was genotyped in additional familial and unselected breast cancer patients and population controls from the Helsinki (cases = 2203,

Biospecimen Shared Resource, which is supported by P30 CA68485. The OBCS was supported by research grants from the Finnish Cancer Foundation, the Academy of Finland (grant number 250083, 122715 and Center of Excellence grant number 251314), the Finnish Cancer Foundation, the Sigrid Juselius Foundation, the University of Oulu, the University of Oulu Support Foundation and the special Governmental EVO funds for Oulu University Hospital-based research activities. The Ontario Familial Breast Cancer Registry (OFBCR) was supported by grant UM1 CA164920 from the National Cancer Institute (USA). 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 Breast Cancer Family Registry (BCFR), nor does mention of trade names, commercial products, or organizations imply endorsement by the USA Government or the BCFR. The ORIGO study was supported by the Dutch Cancer Society (RUL 1997-1505) and the Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL CP16). The PBCS was funded by Intramural Research Funds of the National Cancer Institute, Department of Health and Human Services, USA. The pKARMA study was supported by Märit and Hans Rausings Initiative Against Breast Cancer. The RBCS was funded by the Dutch Cancer Society (DDHK 2004-3124, DDHK 2009-4318). The SASBAC study was supported by funding from the Agency for Science, Technology and Research of Singapore (A*STAR), the US National Institute of Health (NIH) and the Susan G. Komen Breast Cancer Foundation. The SBCS was supported by Yorkshire Cancer Research S295, S299, S305PA and Sheffield Experimental Cancer Medicine Centre. SEARCH is funded by a programme grant from Cancer Research UK [C490/A10124] and supported by the UK National Institute for Health Research Biomedical Research Centre at the University of Cambridge. SKKDKFZS is supported by the DKFZ. The SZBCS was supported by Grant PBZ_KBN_122/P05/2004. The TNBCC was supported by: a Specialized Program of Research Excellence (SPORE) in Breast Cancer (CA116201), a grant from the Breast Cancer Research Foundation, a generous gift from the David F. and Margaret T. Grohne Family Foundation, the Stefanie Spielman Breast Cancer fund and the OSU Comprehensive Cancer Center, the Hellenic Cooperative Oncology Group research grant (HR R_BG/04) and the Greek General Secretary for Research and Technology (GSRT) Program, Research Excellence II, the European Union (European Social Fund – ESF), and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - ARISTEIA. The UKBGS is funded by Breakthrough

controls = 1278), Tampere (cases = 704, controls = 800), and Oulu (cases = 452, controls = 273) regions of Finland and in 1900 cases and 1235 controls from Belarus (S1 Text). The Helsinki and Tampere datasets were genotyped by sequencing the exon 6 and the Oulu and Belarus datasets by high-resolution-melting (HRM) analysis (S1 Table). Written informed consent was obtained from all the participants and the study was approved by the Ethics Committees of Helsinki University Hospital, Tampere University Hospital, Oulu University Hospital, and by the institutional Ethics Commissions at the Minsk Mother and Child Hospital and at Hannover Medical School.

iCOGS genotyping

The common *RAD51B* polymorphisms rs2588809, rs1314913, and rs999737 were studied in 44,791 invasive breast cancer cases and 43,583 controls from 40 studies (including partially the Helsinki, Oulu, and Belarus studies) participating in the Breast Cancer Association Consortium (BCAC) (S2 Table) [14]. The SNPs were genotyped on the iCOGS array as part of the Collaborative Oncological Gene-environment Study (COGS) as previously described [14] and genotypes for the c.541C>T missense were imputed with SHAPEIT and IMPUTEv2 by using the 1000Genomes project as the reference panel [24]. The analyses were restricted to cases with European ancestry. All participants gave written informed consent and all the studies were approved by the respective Institutional Review Boards or Ethics Committees (S2 Table).

Bioinformatics and statistical methods

The pathogenicity of the variants identified in the sequencing of *RAD51B* was predicted using the MutationTaster software as it considers both exonic and intronic variants [25]. In addition, PON-P that utilizes results from SIFT, PhD-SNP, PolyPhen-2, SNAP, and I-Mutant 3 was used to predict the pathogenicity of the missense variants [26]. Secondary structure prediction was done with RaptorX [27] and protein-protein interaction with PredictProtein [28]. Statistical analyses were performed in R version 3.0.2 (http://www.r-project.org/). To study the association of the c.541C>T mutation with breast cancer risk, two-sided *P*-values were calculated using Pearson's chi-squared test or, if the expected number of cell count was less than five, Fisher's exact test. For meta-analysis, the estimates were combined using a fixed-effects meta-analysis, using the inverse variance-weighted method. Student's t-test was used to compare the age at diagnosis between the mutation carriers and non-carriers. Per-allele odds ratios (OR) and confidence intervals (CI) for the common *RAD51B* polymorphisms were estimated with logistic regression. Multivariate regression models including any two of the three SNPs at a time were used to study the independence of the association signals. Haplotype-specific ORs and CIs were estimated with the HaploStats package in R. A haplotype carrying the major-alleles of rs2588809 and rs1314913 and the minor-allele of the rs999737 was used as a reference. Study and principal components were used as covariates in all analyses with the BCAC dataset to correct for potential population stratification. The analyses for the genotyped SNPs were adjusted for seven and the analysis for the imputed SNP for nine principal components as previously described [14,24]. Separate analyses were performed for all breast cancer cases and for subsets of cases with first-degree family history of breast cancer, and cases with estrogen receptor (ER) positive and negative tumors. Studies where family history information was predominantly missing were excluded from the familial analyses. The imputed c.541C>T missense variant was analyzed with SNPTEST. First, an association test stratified with study was performed to obtain imputation information scores for the individual studies. The final analysis, restricted to BCAC studies with information score ≥ 0.5, was performed with study and the nine principal components as covariates. The online tool LocusZoom was used to generate a

Breast Cancer and the Institute of Cancer Research (ICR), London. ICR acknowledges NHS funding to the NIHR Biomedical Research Centre. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have read the journal's policy and have the following competing interests: A. Mannermaa is a member of the PLOS ONE Editorial Board. This does not alter the authors' adherence to PLOS ONE Editorial policies and criteria.

regional association plot for visualization of the SNP associations and the extent of linkage disequilibrium (LD) [29].

Results

We sequenced the coding region and the exon-intron boundaries of the *RAD51B* gene in 168 breast (female and male) and 4 familial ovarian cancer patients from Southern Finland. Nine intronic and six missense variants were identified (Table 1). The c.541C>T, p.(Arg181Trp), missense change was the only variant that was predicted to be pathogenic by both MutationTaster and PON-P software and was selected for further genotyping. Based on Finnish subjects in the ExAC dataset, the minor-allele frequency (MAF) for the c.541C>T variant is estimated to be 1.2% whereas in the non-Finnish Europeans the MAF is 0.05% (Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [May, 2015]). According to RaptorX secondary structure prediction software the arginine in position 181 is located in beta-sheet with the likelihood of 83.4% but, as determined by PredictProtein, the amino acid is not predicted to directly participate in protein-protein interactions.

The c.541C>T missense variant was genotyped in additional 3359 female and male breast cancer patients and families, and in 2351 population controls from Southern (Helsinki and Tampere datasets) and Northern Finland (Oulu dataset). Altogether, when combining the two stages of mutation testing, c.541C>T was screened in 2331 patients from Helsinki, 748 patients from Tampere, and 452 patients from Oulu. There was no evidence of association in any of the population-based case-control studies (Table 2). There was a suggestive association among breast cancer families with at least three first or second-degree relatives affected with breast or ovarian cancer, compared to population controls, in the Helsinki dataset (OR: 2.31, 95% CI: 1.20–4.48, $P = 0.010$) (Table 2). The mean age at breast cancer diagnosis was similar between carriers and

Table 1. Variants identified in the screening of the *RAD51B* gene (RefSeq NM_133509.3).

DNA change	protein change	rs-number	position	AA [a]	Aa [b]	Aa [c]	MAF [d]	MAF_{ExAC} [e]	MutationTaster	PON-P
c.84+28T>G		rs17783124	intron 2	71	84	17	0.343	0.287	polymorphism	
c.84+82T>C		rs28604984	intron 2	71	84	17	0.343		polymorphism	
c.84+120G>A		rs28623567	intron 2	71	84	17	0.343		polymorphism	
c.199-33G>T		rs184815928	intron 3	170	2	0	0.006	0.007	polymorphism	
c.199-30dupA		rs34564590	intron 3	70	85	17	0.346	0.286	polymorphism	
c.199-21C>T		rs35183950	intron 3	171	1	0	0.003	0.002	polymorphism	
c.515T>G	p.Leu172Trp	rs34094401	exon 6	167	5	0	0.015	0.027	polymorphism	unclassified
c.539A>G	p.Tyr180Cys	rs28910275	exon 6	145	27	0	0.078	0.057	polymorphism	unclassified
c.541C>T	p.Arg181Trp	rs199981178	exon 6	169	3	0	0.009	0.012	disease causing	pathogenic
c.572+95_96delGG			intron 6	171	1	0	0.003		polymorphism	
c.728A>G	p.Lys243Arg	rs34594234	exon 7	167	4	1	0.017	0.008	disease causing	unclassified
c.854-103A>G		rs10146321	intron 8	104	64	4	0.209		polymorphism	
c.957+125C>A		rs10146772	intron 9	48	83	41	0.480		polymorphism	
c.1063G>A	p.Ala355Thr	rs61758785	exon 11	170	2	0	0.006	0.007	polymorphism	neutral
c.1094C>G	p.Pro365Arg	rs28908468	exon 11	140	32	0	0.093	0.115	polymorphism	NA

Counts of [a] common homozygotes

[b] heterozygotes and

[c] rare homozygotes in this study

[d] Minor-allele frequency (MAF) in this study

[e] MAF in the Finnish population in the ExAC database

doi:10.1371/journal.pone.0153788.t001

Table 2. Frequencies and ORs for the c.541C>T mutation among the different patient subgroups in the Helsinki, Tampere, Oulu, and Belarus series.

HELSINKI dataset	Total	CC	%	CT	%	OR (95% CI)	*P*
Controls	1278	1257	98.36%	21	1.64%		
All female BC cases	2293	2240	97.69%	53	2.31%	1.42 (0.85–2.36)	0.179
Familial BC (≥3 affected)[a]	430	414	96.28%	16	3.72%	2.31 (1.20–4.48)	0.010
Familial BC (2 affected) [b]	523	513	98.09%	10	1.91%	1.17 (0.55–2.50)	0.690
Unselected BC	1728	1690	97.80%	38	2.20%	1.35 (0.79–2.30)	0.277
Male BC [c]	37	36	97.30%	1	2.70%	1.66 (0.22–12.70)	0.469
Familial OC	4	4	100%	0	0%	-	1
TAMPERE dataset	**Total**	**CC**	**%**	**CT/TT [d]**	**%**	**OR (95% CI)**	***P***
Controls	800	778	97.25%	22	2.75%		
Unselected BC	645	620	96.12%	25	3.88%	1.43 (0.80–2.55)	0.230
Male BC [e]	103	102	99.03%	1	0.97%	0.35 (0.05–2.60)	0.503
OULU dataset	**Total**	**CC**	**%**	**CT**	**%**	**OR (95% CI)**	***P***
Controls	273	267	97.80%	6	2.20%		
All female BC cases	452	443	98.01%	9	1.99%	0.90 (0.32–2.57)	0.850
Familial BC (≥3 affected)[a]	83	82	98.80%	1	1.20%	0.54 (0.06–4.57)	1
Familial BC (2 affected) [b]	49	48	97.96%	1	2.04%	0.93 (0.11–7.87)	1
Unselected BC	320	313	97.81%	7	2.19%	1.00 (0.33–3.00)	0.993
BELARUS dataset	**Total**	**CC**	**%**	**CT**	**%**	**OR (95% CI)**	***P***
Controls	1235	1235	100%	0	0%	-	
BC cases	1900	1897	99.85%	3	0.15%	-	0.284

BC = breast cancer; OC = ovarian cancer

[a] Families with ≥3 BC or OC among first- or second-degree relatives

[b] Two first-degree relatives affected with BC or OC

[c] Includes 33 male BC cases and 4 female BC cases with family history of male BC

[d] The Tampere dataset included one case with homozygous mutation

[e] Male BC cases only

doi:10.1371/journal.pone.0153788.t002

non-carriers among the familial patients (54.2 for carriers and 53.1 for non-carriers, $P = 0.725$) and among all cases (55.9 for carriers and 56.4 for non-carriers, $P = 0.744$). However, no association was observed for familial breast cancer in the Oulu dataset, and there was no evidence of association when all datasets were combined (OR: 1.35, 95% CI: 0.66–2.73, $P = 0.410$). The variant was also genotyped in 1900 breast cancer cases and 1235 population controls from Belarus but only three carriers were identified among cases and none among controls (Table 2).

We further studied the missense variant c.541C>T and the previously reported common risk SNPs rs2588809, rs1314913, and rs999737 in a large breast cancer dataset from BCAC comprising 40 studies with 44,791 invasive breast cancer cases and 43,583 controls of predominantly European ancestry. The subjects were genotyped on an Illumina Infinium custom chip (iCOGS) for over 200,000 SNPs [14], including the rs2588809, rs1314913, and rs999737 SNPs. Genotypes for over 11 million SNPs, including the c.541C>T missense variant, were imputed by using the 1000Genomes project as a reference panel [24]. For the missense variant, we first performed an association test stratified by study including all the 40 BCAC studies. The overall information score for the c.541C>T was 0.673. To increase the imputation accuracy of this rare variant, studies with information score less than 0.5 in the stratified analysis were excluded from the final analysis (S3 Table). Altogether 26,969 cases and 27,092 controls from 23 studies were included and the information score for the variant in the final analysis was 0.755. The

Table 3. Frequencies and ORs for the SNPs rs2588809, rs1314913, and rs999737 for all breast cancer cases, familial cases and ER-positive and -negative cases in the BCAC dataset.

Rs-number	MAF $_{controls}$	MAF $_{cases}$	OR_{ALL} (95%CI)	P_{ALL}	OR_{FAM} (95%CI)	P_{FAM}	OR_{ER+} (95%CI)	P_{ER+}	OR_{ER-} (95%CI)	P_{ER-}
rs2588809	0.158	0.169	1.08 (1.05–1.10)	4.62×10^{-8}	1.10 (1.05–1.16)	9.72×10^{-5}	1.09 (1.06–1.12)	1.65×10^{-8}	1.00 (0.95–1.05)	8.90×10^{-1}
rs1314913	0.144	0.154	1.07 (1.04–1.10)	2.74×10^{-7}	1.10 (1.04–1.16)	2.45×10^{-4}	1.09 (1.05–1.12)	1.36×10^{-7}	0.98 (0.93–1.03)	3.85×10^{-1}
rs999737	0.231	0.215	1.09 (1.06–1.11)	7.78×10^{-13}	1.15 (1.10–1.20)	1.08×10^{-9}	1.10 (1.07–1.13)	7.70×10^{-12}	1.05 (1.00–1.10)	3.03×10^{-2}

ALL = all breast cancer cases; FAM = familial breast cancer cases; ER+ = ER-positive; ER- = ER- negative.

doi:10.1371/journal.pone.0153788.t003

MAF of the c.541C>T missense variant was 0.2% among all cases, familial cases, and controls and it did not associate with breast cancer among all cases (OR: 0.97, 95% CI: 0.76–1.25, $P = 0.538$) or familial cases (OR: 1.04, 95% CI: 0.65–1.66, $P = 0.794$). The highest frequency was observed in the Finnish studies HEBCS, KBCP, and OBCS with the overall MAF ranging from 0.6% to 0.7%. The HEBCS and OBCS studies are overlapping with the Finnish Helsinki and Oulu datasets that were genotyped for the variant.

Breast cancer associations for two of the genotyped SNPs, rs2588809 and rs999737, in the BCAC dataset have been published before [14,24,30] and the results were replicated here. The minor-alleles of rs2588809 and rs1314913 and the major-allele of rs999737 were associated with an increased risk of breast cancer with ORs between 1.07–1.08 among all breast cancer cases and also among familial cases with ORs between 1.10–1.15 (Table 3). The associations were also significant among the subset of cases with ER-positive tumors but only rs999737 showed association among the ER-negative subset (Table 3). Rs2588809 and rs1314913 are strongly correlated ($r^2 = 0.816$), whereas rs999737 is not correlated with them ($r^2 = 0.003$ with rs2588809 and $r^2 = 0.070$ with rs1314913) (S1 Fig). In multivariate logistic regression models, both rs2588809 and rs1314913 remained significant after adjustment with rs999737 (OR: 1.07, 95% CI: 1.04–1.09, $P = 1.74 \times 10^{-6}$ and OR: 1.06, 95% CI: 1.03–1.09, $P = 9.24 \times 10^{-6}$, respectively). Likewise, rs999737 remained an independent predictor for risk after adjustment with rs2588809 or rs1314913 (OR: 1.08, 95% CI: 1.06–1.11, $P = 2.91 \times 10^{-11}$and $P = 2.54 \times 10^{-11}$, respectively). Neither rs2588809 nor rs1314913 remained significant when adjusted with each other (OR: 1.07, 95% CI: 1.00–1.16, $P = 0.066$ and OR: 1.00, 95% CI: 0.93–1.08, $P = 0.977$, respectively).

To evaluate the allele combination associated with the highest risk, we performed haplotype analysis for rs2588809, rs1314913, and rs999737. The reference haplotype CCT, carrying the major-alleles of rs2588809 and rs1314913 and the minor-allele of rs999737 had an estimated frequency of 21.0% among controls and 19.4% among cases. The strongest association was observed for the TTC haplotype that carries the risk-alleles of all the three SNPs with an odds ratio of 1.15 for all cases (95% CI: 1.11–1.19, $P = 8.88 \times 10^{-16}$) and 1.24 for familial cases (95% CI: 1.16–1.32, $P = 6.19 \times 10^{-11}$) (Table 4). This haplotype had an estimated frequency of 12.6% among controls, 13.5% among all cases, and 14.1% among familial cases. The most common haplotype, with over 60% frequency among cases and controls, was the haplotype CCC carrying the risk-allele of rs999737 and also this haplotype associated with an increased risk of breast cancer among all the cases (OR: 1.09, 95% CI: 1.06–1.12, $P = 1.86 \times 10^{-10}$) and the familial cases (OR: 1.14, 95% CI: 1.09–1.20, $P = 1.30 \times 10^{-7}$) when compared to the CCT haplotype.

Discussion

To study the role of *RAD51B* in breast cancer predisposition, we screened the coding sequence in 172 Finnish breast or ovarian cancer patients. We also studied the common susceptibility variants in the genomic region of the gene in a large dataset from BCAC including 44,791

Table 4. Estimated haplotype frequencies and haplotype-specific ORs among the BCAC dataset. The haplotypes are based on genotypes for SNPs rs2588809, rs1314913, and rs999737.

	All breast cancer cases				Familial breast cancer cases			
Haplotype	**Freq controls**	**Freq cases**	**OR (95% CI)**	***P***	**Freq controls**	**Freq cases**	**OR (95% CI)**	***P***
CCT (reference)	0.210	0.194			0.211	0.188		
TTC	0.126	0.135	1.15 (1.11–1.19)	8.88×10^{-16}	0.126	0.141	1.24 (1.16–1.32)	6.19×10^{-11}
CCC	0.632	0.636	1.09 (1.06–1.12)	1.86×10^{-10}	0.628	0.635	1.14 (1.09–1.20)	1.30×10^{-7}
TCC	0.011	0.013	1.15 (1.04–1.27)	5.48×10^{-3}	0.012	0.013	1.17 (0.98–1.41)	8.36×10^{-2}
TTT	0.018	0.018	1.11 (0.99–1.23)	7.12×10^{-2}	0.018	0.017	1.06 (0.86–1.31)	5.70×10^{-1}

Freq = frequency

doi:10.1371/journal.pone.0153788.t004

breast cancer cases and 43,583 controls. Among the Finnish patients, we identified one putatively pathogenic missense variant, c.541C>T, p.(Arg181Trp), that was further screened in a larger set of unselected and familial breast cancer patients and population controls.

In the Helsinki dataset, the c.541C>T mutation had a suggestive association with familial breast cancer but this association was not confirmed in the other Finnish sample sets. In Belarus, the mutation was rare and was identified only in three cases but not among controls. For the large BCAC dataset that had been genotyped on the iCOGS chip, c.541C>T genotypes were imputed. The missense was very rare with a 0.2% MAF and no association with breast cancer was seen among all breast cancer cases or in the subset of familial patients.

Out of the common variants at the *RAD51B* region, rs999737 and rs2588809 have been associated with female breast cancer and rs1314913 with male breast cancer [12–14,30]. For rs999737 and rs2588809 the results from the previous BCAC studies for all cases and by ER status were replicated here [14,30]. The rs1314913 SNP has been previously associated with male breast cancer with an OR of 1.57 ($P = 3.02 \times 10^{-13}$) [13]. It has not been previously reported in female breast cancer, and showed here similar associations as rs2588809 with OR of 1.07 among all cases and 1.09 among ER-positive cases. The rs1314913 SNP is correlated with rs2588809 ($r^2 = 0.816$) but not with rs999737 ($r^2 = 0.070$). Our results suggest two independent associations at the region as rs999737 adjusted with rs1314913 or rs2588809 showed significant association whereas neither rs1314913 nor rs2588809 remained significant after adjustment with each other, suggesting both may be tagging another, causative allele.

All three variants were also associated with familial breast cancer. The strongest association was observed for a haplotype carrying the risk-alleles of all the three SNPs both among all cases and familial cases with ORs of 1.15 and 1.24, respectively. This haplotype is observed at a frequency of 12.6% among controls and 13.5% among cases.

To date, only three studies have reported truncating germline mutations in *RAD51B*: two deleterious mutations were detected among ovarian cancer cases, one splicing mutation in a breast and ovarian cancer family, and one nonsense mutation in a melanoma family [31–33]. In an Australian, study no pathogenic mutations were identified among 188 breast cancer families [34]. These reports together with our results suggest that loss-of-function mutations in *RAD51B* are very rare yet we cannot exclude such mutations and possibly a higher breast cancer risk associated with these. However, the common variants in the *RAD51B* region are associated with an increased breast cancer risk. Further fine-mapping studies are needed to identify the causative variants underlying the associations, and functional studies to determine whether *RAD51B* or another gene in the region is the target of these associations. *RAD51B* is a plausible candidate gene for the association as it functions in HR repair of DNA damage like most of the known breast cancer genes.

In conclusion, no pathogenic *RAD51B* mutations were identified among 172 Finnish breast or ovarian cancer patients. However, we cannot rule out rare risk-variants in the Finnish or other populations. The minor-alleles of the common polymorphisms rs2588809 and rs1314913 and the major-allele of rs999737 were associated with breast cancer risk in the large BCAC dataset. The strongest association was observed for a risk haplotype carrying the risk-alleles of all the three SNPs with an OR of 1.15 among all cases and 1.24 among familial cases compared to the haplotype with the respective protective alleles.

Supporting Information

S1 Fig. Regional association plot for the *RAD51B* SNPs rs2588809, rs1314913, and rs999737 and the c.541C>T missense variant. Each variant is represented with a dot and the color of the dot represents the extent of LD (r^2) with rs2588809. Association among all breast cancer cases in the BCAC dataset is represented at the–log10 scale. The *x* axis shows the genomic positions of the variants based on hg19 build. The right *y* axis shows the estimated recombination rate at the region (centiMorgans/megabase, cM/Mb). LD and recombination rate were estimated using the 1000Genomes Nov 2014 EUR as the reference population.
(PDF)

S1 Table. Primers and PCR conditions for the screening of the *RAD51B* gene and the c.541C>T missense mutation.
(DOCX)

S2 Table. Description of the BCAC studies. BCAC studies genotyped on the iCOGS array, ethics approval committees/institutional review boards and the numbers of controls, all invasive cases, cases with first-degree family history of breast cancer, and cases with ER-positive and negative tumors included in this study. All subjects are genetically European.
(DOCX)

S3 Table. The imputed c.541C>T variant in the BCAC dataset. Minor-allele frequencies (MAF) of the imputed c.541C>T missense variant in the BCAC studies and the information scores from the association analysis stratified by study. Studies with information score <0.5 were excluded from the final analysis.
(DOCX)

S1 Text. Supplementary Materials and Methods.
(DOCX)

Acknowledgments

We thank all the individuals who took part in these studies and all the researchers, clinicians, technicians and administrative staff who have enabled this work to be carried out. The **HEBCS** thanks Drs Kirsimari Aaltonen, Karl von Smitten, and Tuomas Heikkinen, and research nurses Irja Erkkilä and Virpi Palola for their help with patient samples and data. The Finnish Cancer Registry is gratefully acknowledged for the cancer diagnostic data and Molecular Medicine Sequencing Laboratory for the sequencing service. The **ABCFS** thanks Maggie Angelakos, Judi Maskiell, and Gillian Dite. The **ABCS** thanks Sten Cornelissen, Frans Hogervorst, Senno Verhoef, and Sanquin, The Netherlands. The **BBCS** thanks Eileen Williams, Elaine Ryder-Mills, and Kara Sargus. The **BIGGS** thanks Niall McInerney, Gabrielle Colleran, Andrew Rowan, and Angela Jones. The **BSUCH** thanks Peter Bugert and Medical Faculty Mannheim. The **CGPS** thanks the staff and participants of the Copenhagen General Population Study and Dorthe Uldall Andersen, Maria Birna Arnadottir, Anne Bank, and Dorthe Kjeldgård Hansen for excellent technical assistance.The **CNIO-BCS** thanks Guillermo Pita, Charo Alonso, Daniel Herrero, Nuria Álvarez, Pilar Zamora, Primitiva Menendez, and the Human Genotyping-CEGEN Unit (CNIO). The **CTS** Steering Committee includes Leslie Bernstein, Susan Neuhausen, James Lacey, Sophia Wang, Huiyan Ma, Yani Lu, and Jessica Clague DeHart at the Beckman Research Institute of City of Hope, Dennis Deapen, Rich Pinder, Eunjung Lee, and Fred Schumacher at the University of Southern California, Pam Horn-Ross, Peggy Reynolds, Christina Clarke Dur and David Nelson at the Cancer Prevention Institute of California, and Hoda Anton-Culver, Argyrios Ziogas, and Hannah Park at the University of California Irvine. The **ESTHER** thanks Hartwig Ziegler, Sonja Wolf, Volker Hermann, Christa Stegmaier, and Katja Butterbach. The **GC-HBOC** thanks Stefanie Engert, Heide Hellebrand, and Sandra Kröber. The **GENICA** Network includes Hiltrud Brauch, Wing-Yee Lo, and Christina Justenhoven, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tübingen, Germany; Yon-Dschun Ko, Christian Baisch, Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn, Germany; Hans-Peter Fischer, Institute of Pathology, University of Bonn, Germany; Ute Hamann, Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany; Thomas Brüning, Beate Pesch, Sylvia Rabstein, Anne Lotz, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Ruhr University Bochum (IPA), Bochum, Germany; and Volker Harth, Institute of Occupational Medicine and Maritime Medicine, University Medical Center Hamburg-Eppendorf, Germany. The **HMBCS** thanks Natalia Antonenkova, Peter Hillemanns, Hans Christiansen and Johann H. Karstens. The **KBCP** thanks Eija Myöhänen and Helena Kemiläinen. **kConFab/AOCS** 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 for their contributions to this resource, and the many families who contribute to kConFab. The **LMBC** thanks Gilian Peuteman, Dominiek Smeets, Thomas Van Brussel and Kathleen Corthouts. The **MARIE** thanks Dieter Flesch-Janys, Petra Seibold, Judith Heinz, Nadia Obi, Alina Vrieling, Sabine Behrens, Ursula Eilber, Muhabbet Celik, Til Olchers and Stefan Nickels. The **MBCSG** thanks Siranoush Manoukian, Bernard Peissel and Daniela Zaffaroni of the Fondazione IRCCS Istituto Nazionale dei Tumori (INT); Bernardo Bonanni, Monica Barile and Irene Feroce of the Istituto Europeo di Oncologia (IEO) and the personnel of the Cogentech Cancer Genetic Test Laboratory. The **MTLGEBCS** would like to thank Martine Tranchant (CHU de Québec Research Center), Marie-France Valois, Annie Turgeon and Lea Heguy (McGill University Health Center, Royal Victoria Hospital; McGill University) for DNA extraction, sample management and skillful technical assistance. J.S. is Chairholder of the Canada Research Chair in Oncogenetics. The following are **NBCS** Collaborators: Dr. Kristine K.Sahlberg, PhD (Department of Research, Vestre Viken Hospital, Drammen, Norway and Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital-Radiumhospitalet, Oslo, Norway), Dr. Lars Ottestad, MD (Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital-Radiumhospitalet, Oslo, Norway), Prof. Em. Rolf Kåresen, MD (Institute of Clinical Medicine, University of Oslo, Oslo, Norway and Department of Breast- and Endocrine Surgery, Division of Surgery, Cancer and Transplantation, Oslo University Hospital, Oslo, Norway), Dr. Anita Langerød, PhD (Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital-Radiumhospitalet, Oslo, Norway), Dr. Ellen Schlichting, MD (Section for Breast- and Endocrine Surgery, Department of Cancer, Division of Surgery, Cancer and Transplantation Medicine, Oslo University Hospital, Oslo, Norway), Dr. Marit Muri Holmen, MD (Department of Radiology and Nuclear Medicine, Oslo University Hospital, Oslo, Norway), Prof. Toril Sauer, MD (Department of Pathology at Akershus University hospital, Lørenskog, Norway and Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway), Dr. Vilde Haakensen, MD (Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital-Radiumhospitalet, Oslo, Norway), Dr. Olav Engebråten, MD (Department of Tumor Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway, Department of Oncology, Division of Surgery and Cancer and Transplantation Medicine, Oslo University Hospital, Oslo, Norway and Institute for Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway), Prof. Bjørn Naume, MD (Department of Oncology, Division of Surgery and Cancer and Transplantation Medicine, Oslo University Hospital-Radiumhospitalet, Oslo, Norway and K.G. Jebsen Centre for Breast Cancer, Institute for Clinical Medicine, University of Oslo, Oslo, Norway.), Dr. Cecile E. Kiserud, MD (National Advisory Unit on Late Effects after Cancer Treatment, Department of Oncology, Oslo University Hospital, Oslo, Norway and Department of Oncology, Oslo University Hospital, Oslo, Norway), Dr. Kristin V. Reinertsen, MD (National Advisory Unit on Late Effects after Cancer Treatment, Department of Oncology, Oslo University Hospital, Oslo, Norway and Department of Oncology, Oslo University Hospital, Oslo, Norway), Assoc. Prof. Åslaug Helland, MD (Department of Genetics, Institute for Cancer Research and Department of Oncology, Oslo University Hospital Radiumhospitalet, Oslo, Norway), Dr. Margit Riis, MD (Dept of Breast- and Endocrine Surgery, Oslo University Hospital, Ullevål, Oslo, Norway), Dr. Ida Bukholm, MD (Department of Breast-Endocrine Surgery, Akershus University Hospital, Oslo, Norway and Department of Oncology, Division of Cancer Medicine, Surgery and Transplantation, Oslo University Hospital, Oslo, Norway), Prof. Per Eystein Lønning, MD (Section of Oncology, Institute of Medicine, University of Bergen and Department of Oncology, Haukeland University Hospital, Bergen, Norway), OSBREAC (Oslo Breast Cancer Research Consortium) and Grethe I. Grenaker Alnæs, M.Sc. (Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital-Radiumhospitalet, Oslo, Norway). The **NBHS** thanks study partcipants and research staff for their contributions and commitment to this study. The **OBCS** thanks Katri Pylkäs, Arja Jukkola-Vuorinen, Meeri Otsukka, Leena Keskitalo, Annika Väntänen, and Kari Mononen. The **OFBCR** thanks Teresa Selander and Nayana Weerasooriya. The **ORIGO** thanks E. Krol-Warmerdam, and J. Blom for patient accrual, administering questionnaires, and managing clinical information. The **PBCS** thanks Louise Brinton, Mark Sherman, Neonila Szeszenia-Dabrowska, Beata Peplonska, Witold Zatonski, Pei Chao, and Michael Stagner. The **pKARMA** thanks the Swedish Medical Research Counsel. The **RBCS** thanks Petra Bos, Jannet Blom, Ellen Crepin, Elisabeth Huijskens, Annette Heemskerk, and the Erasmus MC Family Cancer Clinic. The **SASBAC** thanks the Swedish Medical Research Counsel. The **SBCS** thanks Sue Higham, Helen Cramp, Ian Brock, Sabapathy Balasubramanian, and Dan Connley. The **SEARCH** thanks the SEARCH and EPIC teams. The **SKKDKFZS** thanks all study participants, clinicians, family doctors, researchers and technicians for their contributions and commitment to this study. The **TNBCC** thanks Robert Pilarski and Charles Shapiro who were instrumental in the formation of the OSU Breast Cancer Tissue Bank. We thank the Human Genetics Sample Bank for processing of samples and providing OSU Columbus area control samples. The **UKBGS** thanks Breast Cancer Now and the Institute of Cancer Research for support and funding of the Breakthrough Generations Study, and the study participants, study staff, and the doctors, nurses and other health care providers and health information sources who have contributed to the study. The Institute of Cancer Research acknowledges NHS funding to the Royal Marsden/ICR NIHR Biomedical Research Centre. The iCOGS study would not have been possible without the contributions of the following: Andrew Berchuck (OCAC), Rosalind A. Eeles, Ali Amin Al Olama, Zsofia Kote-Jarai, Sara Benlloch (PRACTICAL), Antonis Antoniou, Lesley McGuffog, and Ken Offit (CIMBA), Andrew Lee, and Ed Dicks, Craig Luccarini and the staff of the Centre for Genetic Epidemiology Laboratory, the staff of the CNIO genotyping unit, Jacques Simard and Daniel

C. Tessier, Francois Bacot, Daniel Vincent, Sylvie LaBoissière and Frederic Robidoux and the staff of the McGill University and Génome Québec Innovation Centre, Sune F. Nielsen, Borge G. Nordestgaard, and the staff of the Copenhagen DNA laboratory, and Julie M. Cunningham, Sharon A. Windebank, Christopher A. Hilker, Jeffrey Meyer and the staff of Mayo Clinic Genotyping Core Facility.

Consortia members

KConFab Investigators (http://www.kconfab.org/Organisation/Members.aspx):

Morteza Aghmesheh, Medical Oncology Department, Illawarra Cancer Care Centre, Wollongong Hospital; David Amor, Genetic Health Services Victoria, Royal Children's Hospital; Lesley Andrews, Hereditary Cancer Clinic, Prince of Wales Hospital; Yoland Antill, Dept. Haem and Medical Oncology, Peter MacCallum Cancer Centre; Shane Armitage, Molecular Genetics Lab, Royal Brisbane and Women's Hospital; Leanne Arnold, Molecular Genetics Lab, Royal Brisbane and Women's Hospital; Rosemary Balleine, Department of Translational Oncology, Department of Medical Oncology, Westmead Hospital; Agnes Bankier, HEIDELBERG WEST, The Austin Hospital; Patti Bastick, St George Hospital, Medical Oncology Dept,; Jonathan Beesley, Queensland Institute of Medical Research; John Beilby, Pathology Centre, Queen Elizabeth Medical Centre; Barbara Bennett, Hereditary Cancer Clinic, Prince of Wales Hospital; Ian Bennett; Geoffrey Berry, Dept of Public Health and Community Medicine, University of Sydney; Anneke Blackburn, John Curtin School of Medical Research, Australian National University; Michael Bogwitz, Familial Cancer Centre, The Royal Melbourne Hospital; Meagan Brennan, NSW Breast Cancer Institute; Melissa Brown, Department of Biochemistry, University of Queensland; Michael Buckley, Molecular and Cytogenetics Unit, Prince of Wales Hospital; Matthew Burgess, Clinical Genetics Service; Jo Burke, Royal Hobart Hospital; Phyllis Butow, Medical Psychology Unit, Royal Prince Alfred Hospital; Keith Byron, Australian Genome Research Facility, Walter & Eliza Hall Medical Research Institute, Royal Melbourne Hospital; David Callen, Dame Roma Mitchell Cancer Research Laboratories, University of Adelaide/Hanson Institute; Ian Campbell, Peter MacCallum Cancer Centre; Deepa Chauhan, School of Psychology, University of Sydney; Georgia Chenevix-Trench, Queensland Institute of Medical Research, Royal Brisbane Hospital; Alice Christian, Genetics Department, Central Region Genetics Service, Wellington Hospital; Christine Clarke, Westmead Institute for Cancer Research, University of Sydney, Westmead Hospital; Alison Colley, Department of Clinical Genetics, Liverpool Health Service; Dick Cotton, Mutation Research Centre, St Vincent's Hospital; Ashley Crook, Department of Clinical Genetics, Royal North Shore Hospital; James Cui, Epidemiology and Preventive Medicine, Monash University; Bronwyn Culling, Molecular and Clinical Genetics, Royal Prince Alfred Hospital; Margaret Cummings, Department of Pathology, University of Queensland Medical School; Sarah-Jane Dawson, Molecular Genetics Department, Cambridge University; Anna DeFazio, Dept. Gynaecological Oncology, Westmead Institute for Cancer Research, Westmead Hospital; Martin Delatycki, Clinical Genetics, Austin Health, Heidelberg Repatriation Hospital; Rebecca Dickson, Royal North Shore Hospital; Joanne Dixon, Central Regional Genetic Services, Wellington Hospital; Alexander Dobrovic, Molecular Pathology, Department of Pathology, Peter MacCallum Cancer Centre; Tracy Dudding, Hunter Genetics, Hunter Area Health Service; Ted Edkins, Clinical Chemistry, Princess Margret Hospital for Children; Stacey Edwards, Department of Biochemistry and Molecular Biology, University of Queensland; Maurice Eisenbruch, Department of Multicultural Health, University of Sydney; Gelareh Farshid, Tissue Pathology, IMVS; Susan Fawcett, Family Cancer Clinic, Monash Medical Centre; Andrew Fellows, Molecular Diagnostic Development, Pathology Department, Peter MacCallum Cancer Centre; Georgina Fenton, South West Family

Cancer Clinic, Liverpool Hospital; Michael Field, Royal North Shore Hospital; Frank Firgaira, GTG; James Flanagan, Epigenetics Unit, Department of Surgery and Oncology, Imperial College London; Jean Fleming, Eskitis Institute of Cell & Molecular Therapies, School of Biomolecular and Biomedical Sciences, Griffith University; Peter Fong, Medical Oncology Department, Regional Cancer and Blood Services, Auckland City Hospital; John Forbes, Surgical Oncology, University of Newcastle, Newcastle Mater Hospital; Stephen Fox, Pathology Department, Peter MacCallum Cancer Centre; Juliet French, School of Molecular and Microbial Sciences, University of Queensland; Michael Friedlander, Department of Medical Oncology, Prince of Wales Hospital; Clara Gaff, Victorian Clinical Genetics Service, Royal Melbourne Hospital; Mac Gardner, Genetic Health Services Victoria, Royal Children's Hospital; Mike Gattas, Queensland Clinical Genetic Service, Royal Children's Hospital; Peter George, Clinical Biochemistry Unit, Canterbury Health Labs; Graham Giles, Cancer Epidemiology Centre, Anti Cancer Council of Victoria; Grantley Gill, Department of Surgery, Royal Adelaide Hospital; Jack Goldblatt, Genetic Services Of WA, King Edward Memorial Hospital; Sian Greening, Illawarra Cancer Centre, Wollongong Hospital; Scott Grist, Department of Haematology and Genetic Pathology; Eric Haan, Department of Medical Genetics, Women's and Children's Hospital; Kate Hardie, School of Chemistry and Molecular Biosciences, University of Queensland; Marion Harris, Familial Cancer Clinic, Peter MacCallum Cancer Centre; Stewart Hart, Breast and Ovarian Cancer Genetics, Monash Medical Centre; Nick Hayward, Queensland Institute for Medical Research, Royal Brisbane Hospital; Sue Healey, Queensland Institute of Medical Research (QIMR); Louise Heiniger, Medical Psychology Research Unit, The University of Sydney; John Hopper, Centre for M.E.G.A. Epidemiology, University of Melbourne; Evelyn Humphrey, Royal Hobart Hospital; Clare Hunt, Southern Health Familial Cancer Centre, Monash Medical Centre; Paul James Genetic Health Sevices, Monash Medical Centre; Mark Jenkins Centre for M.E.G.A. Epidemiology, The University of Melbourne; Alison Jones, Molecular Genetics Lab, Royal Brisbane and Women's Hospital; Rick Kefford, Medical Oncology, Westmead Hospital; Alexa Kidd, Clinical Genetics Departments, Central Regional Genetics Service; Belinda Kiely, NHMRC Clinical Trials Centre, University of Sydney; Judy Kirk, Familial Cancer Service, Department of Medicine, Westmead Hospital; Jessica Koehler, Hereditary Cancer Clinic, Prince of Wales Hospital; James Kollias, Breast Endocrine and Surgical Unit, Royal Adelaide Hospital; Serguei Kovalenko, Genetic Technologies Limited; Sunil Lakhani, UQ Centre for Clinical Research, University of Queensland, The Royal Brisbane & Women's Hospital Herston; Amanda Leaming, Wesley Breast Clinic, Chasely Street, Auchenflower; Jennifer Leary, Familial Cancer Laboratory, Westmead Hospital; Jacqueline Lim, Dept of Psychological Medicine, Royal North Shore Hospital; Geoff Lindeman, Breast Cancer Laboratory, Walter and Eliza Hall Institute, PO Royal Melbourne Hospital; Lara Lipton, Medical Oncology and Clinical Haematology Unit, Western Hospital; Liz Lobb, Medical Psychology Research Unit, The University of Sydney; Graham Mann, Westmead Institute for Cancer Research, Westmead Millennium Institute; Deborah Marsh, Kolling Institute of Medical Research, Royal North Shore Hospital; Sue Anne McLachlan, Department of Oncology, St Vincent's Hospital; Bettina Meiser, Hereditary Cancer Clinic, Prince of Wales Hospital; Cliff Meldrum, Molecular Pathology Dept, Peter MacCallum Cancer Centre; Roger Milne, Centro Nacional de Investigaciones Oncologicas; Gillian Mitchell, Family Cancer Clinic, Peter MacCallum Cancer Centre; Beth Newman, School of Public Health Road, Queensland University of Technology; Sophie Nightingale, Western Health and Peter MacCallum Cancer Centre; Shona O'Connell, Southern Health Familial Cancer Centre; ImeldaO 'Loughlin, St Vincent's Breast Clinic; Richard Osborne, Dept of Public Health and Community Medicine; Nick Pachter, Familial Cancer and Clinical Genetics, Royal Melbourne Hospital; Briony Patterson, Tas Clinical Genetics Service, Royal Hobart Hospital; Lester Peters, Radiation Oncology, Peter

MacCallum Cancer Centre; Kelly Phillips, Department of Medical Oncology, Peter MacCallum Cancer Centre; Melanie Price, Medical Psychology, University of Sydney; Lynne Purser, The Centre for Genetics Education NSW Health; Tony Reeve, Cancer Genetics Laboratory, University of Otago; Jeanne Reeve, Northern Regional Genetic Service, Auckland Hospital; Robert Richards Dept of Cytogenetics and Molecular Genetics, Women and Children's Hospital; Edwina Rickard, Familial Cancer centre, Westmead Hospital; Bridget Robinson, Oncology Service, Christchurch Hospital; Barney Rudzki, Molecular Pathology Department, The University of Melbourne; Mona Saleh, Centre for Genetic Education, Prince of Wales Hospital; Elizabeth Salisbury, Anatomical Pathology, UNSW, Prince of Wales Hospital; Joe Sambrook, Peter MacCallum Cancer Centre; Christobel Saunders, School of Surgery and Pathology, QE11 Medical Centre; Jodi Saunus, Breast Pathology, University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital; Robyn Sayer, Gynaecological Cancer Centre, Royal Hospital for Women; Elizabeth Scott, South View Clinic; Rodney Scott, Hunter Area Pathology Service, John Hunter Hospital; Clare Scott, Research Department, WEHI, Royal Melbourne Hospital; Ram Seshadri, Department of Haematology, Flinders Medical Centre; Adrienne Sexton, Familial Cancer Centre, Royal Melbourne Hospital; Raghwa Sharma, Dept of Tissue Pathology, Westmead Hospital; Andrew Shelling, Obstetrics and Gynaecology, University of Auckland; Peter Simpson, The University of Queensland; Melissa Southey, Genetic Epidemiology Laboratory, Department of Pathology, University of Melbourne; Amanda Spurdle, Cancer Unit, Queensland Institute of Medical Research; Graeme Suthers, South Australian Clinical Genetics Service, Centre for Medical Genetics, Women and Children's Hospital; Pamela Sykes, Molecular Pathology, Flinders Medical Centre; Donna Taylor, Department of Radiology, Royal Perth Hospital; Jessica Taylor, Familial Cancer and Genetics Medicine, Royal Melbourne Hospital; Benjamin Thierry, Ian Wark Research Institute, University of South Australia; Ella Thompson, Cancer Genetics, Research Department, Peter MaCallum Cancer Centre; Heather Thorne, Research Department, Peter MacCallum Cancer Centre; Sharron Townshend, Genetic Services of Western Australia; Alison Trainer, University of NSW, Prince of Wales Hospital; Lan Tran, Medical Psychology Unit, University of Sydney; Kathy Tucker, Heredity Cancer Clinic, Prince of Wales Hospital; Janet Tyler, Hereditary Cancer Clinic, Prince of Wales Hospital; Jane Visvader, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital; Logan Walker, Molecular Cancer Epidemiology Laboratory, Queensland Institute of Medical Research, Royal Brisbane Hospital; Ian Walpole, Genetic Services of WA, King Edward Memorial Hospital; Robin Ward, Department of Medical Oncology, Prince of Wales Hospital; Paul Waring, Department of Pathology, University of WA; Bev Warner, Cabrini Hospital; Graham Warren, St Vincent's Breast Clinic; Rachael Williams, Family Cancer Clinic, St Vincent's Hospital; Judy Wilson, Royal North Shore Hospital; Ingrid Winship, Department of Genetics, Royal Melbourne Hospital; Kathy Wu, Familial Cancer Centre, Westmead Hospital; Mary Ann Young, Familial Cancer Clinic, Peter MacCallum Cancer Centre

Contact: Georgia Chenevix-Trench, email: georgiaT@qimr.edu.au

AOCS. (http://www.aocstudy.org/org_coll.asp):

R Stuart-Harris (Canberra Hospital); F Kirsten (Bankstown); J Rutovitz (Hornsby); P Clingan, A Glasgow (Illawarra Area Health Services); A Proietto, S Braye, G Otton (John Hunter Hospital); J Shannon (Nepean Hospital); T Bonaventura, J Stewart (Newcastle Mater Misericordiae); S Begbie (Port Macquarie Base Hospital); M Friedlander (Prince of Wales Hospital); D Bell, S Baron-Hay, A Ferrier (*dec.*), G Gard, D Nevell, N Pavlakis, S Valmadre, B Young (Royal North Shore Hospital); C Camaris, R Crouch, L Edwards, N Hacker, D Marsden, G Robertson (Royal Hospital for Women); P Beale, J Beith, J Carter, C Dalrymple, R Houghton, P Russell, L Anderson (Royal Prince Alfred Hospital); M Links (St George Hospital); J Grygiel (St Vincent's Hospital); J Hill (Wagga Wagga Base Hospital); A Brand, K Byth, R

Jaworski, P Harnett, R Sharma, G Wain (Westmead Hospital); D Purdie, D Whiteman (QIMR); B Ward, D Papadimos (Mater Misericordiae Hospitals); A Crandon, M Cummings, K Horwood. A Obermair, L Perrin, D Wyld (Royal Women's Hospital); J Nicklin (Royal Women's Hospital and Wesley Hospital); SA- M Davy, MK Oehler, C Hall, T Dodd, T Healy, K Pittman (Royal Adelaide Hospital, Burnside Memorial Hospital); D Henderson (Flinders Medical Centre); J Miller, J Pierdes (Queen Elizabeth Hospital); A Achan (ICPMR); P Blomfield, D Challis, R McIntosh, A Parker (Royal Hobart Hospital); B Brown, R Rome (Freemasons Hospital); D Allen, P Grant, S Hyde, R Laurie M Robbie, (Mercy Hospital for Women), D Healy, T Jobling, T Manolitsas, J McNealage, P Rogers, B Susil, E Sumithran, I Simpson, (Monash Medical Centre); I Haviv, K Phillips, D Rischin, S Fox, D Johnson, S Lade, P Waring, M Loughrey, N O'Callaghan, B Murray, L Mileshkin, P Allan (Peter MacCallum Cancer Centre); V Billson, J Pyman, D Neesham, M Quinn, A Hamilton, O McNally (Royal Women's Hospital); C Underhill (Border Medical Oncology); R Bell (Andrew Love Cancer Centre); LF Ng (Ballarat Base Hospital); R Blum (Bendigo Health Care Group); V Ganju (Peninsula Health); I Hammond, Y Leung (School for Women's and Infants' Health, University of Western Australia and the Western Australian Gynaecologic Cancer Service); A McCartney (*dec.*), C Stewart (King Edward Memorial Hospital); M Buck (Mount Hospital); N Zeps (WARTN)

Contact: Georgia Chenevix-Trench, email: georgiaT@qimr.edu.au

Author Contributions

Conceived and designed the experiments: LMP HN. Performed the experiments: LMP SK MV JIK SV VN SR. Analyzed the data: LMP SK MV. Contributed reagents/materials/analysis tools: LMP SK MV JIK SV VN SR JS RW AK TD NVB JF PDPP MKS AMD MGC MKB JD KM QW JLH MCS EHR PAF MWB JP IdSS EJS IT BB HS PG TT SEB BGN JB AGN SLN HAC H. Brenner VA A. Meindl RKS H. Brauch TB AL SM A. Mannermaa JMH GCT kConFab/AOCS Investigators LVD HJ JCC AR PR PP EH JEO GGG RLM CAH FS J. Simard MD VK ALBD WZ ABF MG ILA GG PD CS MJH MC AC SSC MS RNL UH DT AJ J. Lubinski FJC DY NO AS HD J. Li KC PH DFE JM CB KA HN. Wrote the paper: LMP SK MV RW TD PDPP MKS AMD GCT UH DFE HN. Provided critical review of the manuscript: LMP SK MV JIK SV VN SR JS RW AK TD NVB JF PDPP MKS AMD MGC MKB JD KM QW JLH MCS EHR PAF MWB JP IdSS EJS IT BB HS PG TT SEB BGN JB AGN SLN HAC H. Brenner VA A. Meindl RKS H. Brauch TB AL SM A. Mannermaa JMH GCT kConFab/AOCS Investigators LVD HJ JCC AR PR PP EH JEO GGG RLM CAH FS J. Simard MD VK ALBD WZ ABF MG ILA GG PD CS MJH MC AC SSC MS RNL UH DT AJ J. Lubinski FJC DY NO AS HD J. Li KC PH DFE JM CB KA HN. Approved the final version of the manuscript: LMP SK MV JIK SV VN SR JS RW AK TD NVB JF PDPP MKS AMD MGC MKB JD KM QW JLH MCS EHR PAF MWB JP IdSS EJS IT BB HS PG TT SEB BGN JB AGN SLN HAC H. Brenner VA A. Meindl RKS H. Brauch TB AL SM A. Mannermaa JMH GCT kConFab/AOCS Investigators LVD HJ JCC AR PR PP EH JEO GGG RLM CAH FS J. Simard MD VK ALBD WZ ABF MG ILA GG PD CS MJH MC AC SSC MS RNL UH DT AJ J. Lubinski FJC DY NO AS HD J. Li KC PH DFE JM CB KA HN.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015; 65: 87–108. doi: 10.3322/caac.21262
2. Stratton MR, Rahman N. The emerging landscape of breast cancer susceptibility. Nat Genet. 2008; 40: 17–22. PMID: 18163131
3. Tischkowitz M, Xia B. PALB2/FANCN: recombining cancer and Fanconi anemia. Cancer Res. 2010; 70: 7353–7359. doi: 10.1158/0008-5472.CAN-10-1012 PMID: 20858716

4. Suwaki N, Klare K, Tarsounas M. RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis. Semin Cell Dev Biol. 2011; 22: 898–905. doi: 10.1016/j.semcdb.2011.07.019 PMID: 21821141
5. Meindl A, Hellebrand H, Wiek C, Erven V, Wappenschmidt B, Niederacher D, et al. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nat Genet. 2010; 42: 410–414. doi: 10.1038/ng.569 PMID: 20400964
6. Loveday C, Turnbull C, Ramsay E, Hughes D, Ruark E, Frankum JR, et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nat Genet. 2011; 43: 879–882. doi: 10.1038/ng.893 PMID: 21822267
7. Loveday C, Turnbull C, Ruark E, Xicola RM, Ramsay E, Hughes D, et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. Nat Genet. 2012; 44: 475–476. doi: 10.1038/ng.2224 PMID: 22538716
8. Vaz F, Hanenberg H, Schuster B, Barker K, Wiek C, Erven V, et al. Mutation of the RAD51C gene in a Fanconi anemia-like disorder. Nat Genet. 2010; 42: 406–409. doi: 10.1038/ng.570 PMID: 20400963
9. Shamseldin HE, Elfaki M, Alkuraya FS. Exome sequencing reveals a novel Fanconi group defined by XRCC2 mutation. J Med Genet. 2012; 49: 184–186. doi: 10.1136/jmedgenet-2011-100585 PMID: 22232082
10. Park DJ, Lesueur F, Nguyen-Dumont T, Pertesi M, Odefrey F, Hammet F, et al. Rare mutations in XRCC2 increase the risk of breast cancer. Am J Hum Genet. 2012; 90: 734–739. doi: 10.1016/j.ajhg.2012.02.027 PMID: 22464251
11. Hilbers FS, Wijnen JT, Hoogerbrugge N, Oosterwijk JC, Collee MJ, Peterlongo P, et al. Rare variants in XRCC2 as breast cancer susceptibility alleles. J Med Genet. 2012; 49: 618–620. doi: 10.1136/jmedgenet-2012-101191 PMID: 23054243
12. Thomas G, Jacobs KB, Kraft P, Yeager M, Wacholder S, Cox DG, et al. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). Nat Genet. 2009; 41: 579–584. doi: 10.1038/ng.353 PMID: 19330030
13. Orr N, Lemnrau A, Cooke R, Fletcher O, Tomczyk K, Jones M, et al. Genome-wide association study identifies a common variant in RAD51B associated with male breast cancer risk. Nat Genet. 2012; 44: 1182–1184. doi: 10.1038/ng.2417. PMID: 23001122
14. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013; 45: 353–361. doi: 10.1038/ng.2563 PMID: 23535729
15. Couch FJ, Wang X, McGuffog L, Lee A, Olswold C, Kuchenbaecker KB, et al. Genome-wide association study in BRCA1 mutation carriers identifies novel loci associated with breast and ovarian cancer risk. PLoS Genet. 2013; 9: e1003212. doi: 10.1371/journal.pgen.1003212 PMID: 23544013
16. Kuchenbaecker KB, Neuhausen SL, Robson M, Barrowdale D, McGuffog L, Mulligan AM, et al. Associations of common breast cancer susceptibility alleles with risk of breast cancer subtypes in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res. 2014; 16: 3416-014-0492-9. doi: 10.1186/s13058-014-0492-9
17. Albala JS, Thelen MP, Prange C, Fan W, Christensen M, Thompson LH, et al. Identification of a novel human RAD51 homolog, RAD51B. Genomics. 1997; 46: 476–479. doi: 10.1006/geno.1997.5062 PMID: 9441753
18. Sigurdsson S, Van Komen S, Bussen W, Schild D, Albala JS, Sung P. Mediator function of the human Rad51B-Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. Genes Dev. 2001; 15: 3308–3318. doi: 10.1101/gad.935501 PMID: 11751636
19. Lio YC, Mazin AV, Kowalczykowski SC, Chen DJ. Complex formation by the human Rad51B and Rad51C DNA repair proteins and their activities in vitro. J Biol Chem. 2003; 278: 2469–2478. doi: 10.1074/jbc.M211038200 PMID: 12427746
20. Chun J, Buechelmaier ES, Powell SN. Rad51 Paralog Complexes BCDX2 and CX3 Act at Different Stages in the BRCA1-BRCA2-Dependent Homologous Recombination Pathway. Mol Cell Biol. 2013; 33: 387–395. doi: 10.1128/MCB.00465-12 PMID: 23149936
21. Date O, Katsura M, Ishida M, Yoshihara T, Kinomura A, Sueda T, et al. Haploinsufficiency of RAD51B causes centrosome fragmentation and aneuploidy in human cells. Cancer Res. 2006; 66: 6018–6024. doi: 10.1158/0008-5472.CAN-05-2803 PMID: 16778173
22. Pelttari LM, Heikkinen T, Thompson D, Kallioniemi A, Schleutker J, Holli K, et al. RAD51C is a susceptibility gene for ovarian cancer. Hum Mol Genet. 2011; 20: 3278–3288. doi: 10.1093/hmg/ddr229 PMID: 21616938

23. Pelttari LM, Kiiski J, Nurminen R, Kallioniemi A, Schleutker J, Gylfe A, et al. A Finnish founder mutation in RAD51D: analysis in breast, ovarian, prostate, and colorectal cancer. J Med Genet. 2012; 49: 429–432. doi: 10.1136/jmedgenet-2012-100852 PMID: 22652533
24. Michailidou K, Beesley J, Lindstrom S, Canisius S, Dennis J, Lush MJ, et al. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nat Genet. 2015; 47: 373–380. doi: 10.1038/ng.3242 PMID: 25751625
25. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010; 7: 575–576. doi: 10.1038/nmeth0810-575 PMID: 20676075
26. Olatubosun A, Väliaho J, Härkönen J, Thusberg J, Vihinen M. PON-P: integrated predictor for pathogenicity of missense variants. Hum Mutat. 2012; 33: 1166–1174. doi: 10.1002/humu.22102 PMID: 22505138
27. Kallberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, et al. Template-based protein structure modeling using the RaptorX web server. Nat Protoc. 2012; 7: 1511–1522. doi: 10.1038/nprot.2012.085 PMID: 22814390
28. Rost B, Yachdav G, Liu J. The PredictProtein server. Nucleic Acids Res. 2004; 32: W321–6. doi: 10.1093/nar/gkh377 PMID: 15215403
29. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, et al. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics. 2010; 26: 2336–2337. doi: 10.1093/bioinformatics/btq419 PMID: 20634204
30. Figueroa JD, Garcia-Closas M, Humphreys M, Platte R, Hopper JL, Southey MC, et al. Associations of common variants at 1p11.2 and 14q24.1 (RAD51L1) with breast cancer risk and heterogeneity by tumor subtype: findings from the Breast Cancer Association Consortium. Hum Mol Genet. 2011; 20: 4693–4706. doi: 10.1093/hmg/ddr368 PMID: 21852249
31. Golmard L, Caux-Moncoutier V, Davy G, Al Ageeli E, Poirot B, Tirapo C, et al. Germline mutation in the RAD51B gene confers predisposition to breast cancer. BMC Cancer. 2013; 13: 484-2407-13-484. doi: 10.1186/1471-2407-13-484
32. Wadt KA, Aoude LG, Golmard L, Hansen TV, Sastre-Garau X, Hayward NK, et al. Germline RAD51B truncating mutation in a family with cutaneous melanoma. Fam Cancer. 2015; 14: 337–340. doi: 10.1007/s10689-015-9781-4 PMID: 25600502
33. Song H, Dicks E, Ramus SJ, Tyrer JP, Intermaggio MP, Hayward J, et al. Contribution of Germline Mutations in the RAD51B, RAD51C, and RAD51D Genes to Ovarian Cancer in the Population. J Clin Oncol. 2015. JCO.2015.61.2408.
34. Johnson J, Healey S, Khanna KK, kConFab, Chenevix-Trench G. Mutation analysis of RAD51L1 (RAD51B/REC2) in multiple-case, non-BRCA1/2 breast cancer families. Breast Cancer Res Treat. 2011; 129: 255–263. doi: 10.1007/s10549-011-1539-6 PMID: 21533530