



Genetic Predisposition to In Situ and Invasive Lobular Carcinoma of the Breast

Elinor Sawyer^{1,9*}, Rebecca Roylance^{2,9}, Christos Petridis¹, Mark N. Brook³, Salpie Nowinski¹, Efterpi Papouli⁴, Olivia Fletcher⁵, Sarah Pinder¹, Andrew Hanby⁶, Kelly Kohut², Patricia Gorman², Michele Caneppele², Julian Peto⁷, Isabel dos Santos Silva⁷, Nichola Johnson⁵, Ruth Swann⁸, Miriam Dwek⁸, Katherine-Anne Perkins⁸, Cheryl Gillett¹, Richard Houlston³, Gillian Ross⁹, Paolo De Ieso⁹, Melissa C. Southey¹⁰, John L. Hopper¹¹, Elena Provenzano¹², Carmel Apicella¹¹, Jelle Wesseling¹³, Sten Cornelissen¹³, Renske Keeman¹³, Peter A. Fasching^{14,15}, Sebastian M. Jud¹⁵, Arif B. Ekici¹⁶, Matthias W. Beckmann¹⁵, Michael J. Kerin¹⁷, Federick Marme^{18,19}, Andreas Schneeweiss^{18,19}, Christof Sohn¹⁸, Barbara Burwinkel^{18,20}, Pascal Guénel^{21,22}, Therese Truong^{21,22}, Pierre Laurent-Puig²³, Pierre Kerbrat²⁴, Stig E. Bojesen²⁵, Børge G. Nordestgaard²⁵, Sune F. Nielsen²⁵, Henrik Flyger²⁶, Roger L. Milne²⁷, Jose Ignacio Arias Perez²⁸, Primitiva Menéndez²⁹, Javier Benitez³⁰, Hermann Brenner³¹, Aida Karina Dieffenbach³¹, Volker Arndt³¹, Christa Stegmaier³², Alfons Meindl³³, Peter Lichtner³⁴, Rita K. Schmutzler³⁵, Magdalena Lochmann³³, Hiltrud Brauch^{36,37}, Hans-Peter Fischer³⁸, Yon-Dschun Ko³⁹, The GENICA Network^{36,37,40,41,42}, Heli Nevanlinna⁴³, Taru A. Muraanen⁴³, Kristiina Aittomäki⁴⁴, Carl Blomqvist⁴⁵, Natalia V. Bogdanova⁴⁶, Thilo Dörk⁴⁷, Annika Lindblom⁴⁸, Sara Margolin⁴⁹, Arto Mannermaa^{50,51}, Vesa Kataja^{50,51}, Veli-Matti Kosma^{50,51}, Jaana M. Hartikainen^{50,51}, Georgia Chenevix-Trench⁵², kConFab Investigators⁵³, Diether Lambrechts^{54,55}, Caroline Weltens⁵⁶, Erik Van Limbergen⁵⁶, Sigrid Hatse⁵⁶, Jenny Chang-Claude⁵⁷, Anja Rudolph⁵⁷, Petra Seibold⁵⁷, Dieter Flesch-Janys⁵⁷, Paolo Radice^{58,59}, Paolo Peterlongo⁵⁹, Bernardo Bonanni⁶⁰, Sara Volorio⁶¹, Graham G. Giles^{62,63}, Gianluca Severi^{62,63}, Laura Baglietto^{62,63}, Catriona A. Mclean⁶⁴, Christopher A. Haiman⁶⁵, Brian E. Henderson⁶⁵, Fredrick Schumacher⁶⁵, Loic Le Marchand⁶⁶, Jacques Simard⁶⁷, Mark S. Goldberg^{68,69}, France Labrèche⁷⁰, Martine Dumont⁶⁷, Vessela Kristensen^{71,72}, Robert Winqvist⁷³, Katri Pylkäs⁷³, Arja Jukkola-Vuorinen⁷⁴, Saila Kauppila⁷⁴, Irene L. Andrulis^{75,76}, Julia A. Knight^{77,78}, Gord Glendon⁷⁵, Anna Marie Mulligan⁷⁹, Peter Devilee⁸⁰, Rob A. E. M. Tollenaar⁸¹, Caroline M. Seynaeve⁸², Mieke Kriege⁸², Jonine Figueroa⁸³, Stephen J. Chanock⁸³, Mark E. Sherman⁸³, Maartje J. Hooning⁸⁴, Antoinette Hollestelle⁸⁴, Ans M. W. van den Ouweland⁸⁵, Carolien H. M. van Deurzen⁸⁶, Jingmei Li⁸⁷, Kamila Czene⁸⁸, Keith Humphreys⁸⁸, Angela Cox⁸⁹, Simon S. Cross⁹⁰, Malcolm W. R. Reed⁸⁹, Mitul Shah⁹¹, Anna Jakubowska⁹², Jan Lubinski⁹², Katarzyna Jaworska-Bieniek^{92,93}, Katarzyna Durda⁹², Anthony Swerdlow⁹⁴, Alan Ashworth⁹⁵, Nicholas Orr⁹⁵, Minouk Schoemaker³, Fergus J. Couch⁹⁶, Emily Hallberg⁹⁷, Anna González-Neira⁹⁸, Guillermo Pita⁹⁸, M. Rosario Alonso⁹⁸, Daniel C. Tessier⁹⁹, Daniel Vincent⁹⁹, Francois Bacot⁹⁹, Manjeet K. Bolla¹⁰⁰, Qin Wang¹⁰⁰, Joe Dennis¹⁰⁰, Kyriaki Michailidou¹⁰⁰, Alison M. Dunning⁹¹, Per Hall⁸⁸, Doug Easton¹⁰⁰, Paul Pharoah^{91,100}, Marjanka K. Schmidt¹³, Ian Tomlinson¹⁰¹, Montserrat Garcia-Closas^{3,5}

1 Research Oncology, Division of Cancer Studies, Kings College London, Guy's Hospital, London, United Kingdom, **2** Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom, **3** Division of Genetics and Epidemiology, Institute of Cancer Research, London, United Kingdom, **4** Biomedical Research Centre, King's College London, Guy's Hospital, London, United Kingdom, **5** Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, United Kingdom, **6** Leeds Institute of Molecular Medicine, St James's University Hospital, Leeds, United Kingdom, **7** London School of Hygiene and Tropical Medicine, London, United Kingdom, **8** Department of Molecular and Applied Biosciences, University of Westminster, London, United Kingdom, **9** The Royal Marsden NHS Foundation Trust, London, United Kingdom, **10** Department of Pathology, The University of Melbourne, Melbourne, Australia, **11** Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Melbourne, Australia, **12** NIHR Cambridge Biomedical Research Centre, Addenbrookes Hospital, Cambridge, United Kingdom, **13** Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands, **14** David Geffen School of Medicine, Department of Medicine Division of Hematology and Oncology, University of California at Los Angeles, Los Angeles, California, United States of America, **15** Department of Gynecology and Obstetrics, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany, **16** Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany, **17** Surgery, Clinical Science Institute, National University of Ireland, Galway, Ireland, **18** Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg, Germany, **19** National Center for Tumor Diseases, University of Heidelberg, Heidelberg, Germany, **20** Molecular Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany, **21** Inserm (National Institute of Health and Medical Research), CESP (Center for Research in Epidemiology and Population Health), U1018, Environmental Epidemiology of Cancer, Villejuif, France, **22** University Paris-Sud, UMR5 1018, Villejuif, France, **23** Université Paris Sorbonne Cité, UMR-S775 Inserm, Paris, France, **24** Centre Eugène Marquis, Department of Medical Oncology, Rennes, France, **25** Copenhagen General Population Study and Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark, **26** Department of Breast Surgery, Herlev Hospital, Copenhagen University Hospital, Copenhagen, Denmark, **27** Genetic & Molecular Epidemiology Group, Human Cancer Genetics Program, Spanish National Cancer Research Centre (CNIO), Madrid, Spain,

28 Servicio de Cirugía General y Especialidades, Hospital Monte Naranco, Oviedo, Spain, **29** Servicio de Anatomía Patológica, Hospital Monte Naranco, Oviedo, Spain, **30** Human Genetics Group, Human Cancer Genetics Program, Spanish National Cancer Research Centre [CNIO], Madrid, Spain, **31** Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany, **32** Saarland Cancer Registry, Saarbrücken, Germany, **33** Division of Gynaecology and Obstetrics, Technische Universität München, Munich, Germany, **34** Institute of Human Genetics, Technische Universität, Munich, Germany, **35** Centre for Familial Breast and Ovarian Cancer and Centre for Integrated Oncology, University Hospital Cologne, Cologne, Germany, **36** Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany, **37** University of Tübingen, Tübingen, Germany, **38** Institute of Pathology, Medical Faculty of the University of Bonn, Bonn, Germany, **39** Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn, Germany, **40** Institute for Occupational Medicine and Maritime Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, **41** Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany, **42** Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bochum, Germany, **43** Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland, **44** Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland, **45** Department of Oncology, Helsinki University Central Hospital, Helsinki, Finland, **46** Department of Radiation Oncology, Hannover Medical School, Hannover, Germany, **47** Gynaecology Research Unit, Hannover Medical School, Hannover, Germany, **48** Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, **49** Department of Oncology - Pathology, Karolinska Institutet, Stockholm, Sweden, **50** School of Medicine, Institute of Clinical Medicine, Pathology and Forensic Medicine, University of Eastern Finland, Joensuu, Finland, **51** Cancer Center, Kuopio University Hospital, Kuopio, Finland, **52** Department of Genetics, QIMR Berghofer Institute of Medical Research, Brisbane, Australia, **53** Peter MacCallum Cancer Center, Melbourne, Australia, **54** Vesalius Research Center (VRC), VIB, Leuven, Belgium, **55** Department of Oncology, University of Leuven, Leuven, Belgium, **56** University Hospital Gashuisberg, Leuven, Belgium, **57** Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany, **58** Unit of Molecular Bases of Genetic Risk and Genetic Testing, Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale Tumori (INT), Milan, Italy, **59** IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy, **60** Division of Cancer Prevention and Genetics, Istituto Europeo di Oncologia (IEO), Milan, Italy, **61** IFOM, Fondazione Istituto FIRC di Oncologia Molecolare and Cogentech Cancer Genetic Test Laboratory, Milan, Italy, **62** Cancer Epidemiology Centre, The Cancer Council Victoria, Melbourne, Australia, **63** Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, Melbourne, Australia, **64** Department of Pathology, The Alfred Hospital, Prahran, Victoria, Australia, **65** Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, United States of America, **66** Epidemiology Program, Cancer Research Center, University of Hawaii, Honolulu, Hawaii, United States of America, **67** Cancer Genomics Laboratory, Centre Hospitalier Universitaire de Québec Research Center and Laval University, Quebec, Canada, **68** Department of Medicine, McGill University, Montreal, Quebec, Canada, **69** Division of Clinical Epidemiology, McGill University Health Centre, Royal Victoria Hospital, Montreal, Quebec, Canada, **70** Département de médecine sociale et préventive, Département de santé environnementale et santé au travail, Université de Montréal, Montreal, Quebec, Canada, **71** Department of Genetics, Institute for Cancer Research, Oslo University Hospital, Radiumhospitalet, Oslo, Norway, **72** Faculty of Medicine (Faculty Division Ahus), UiO, Oslo, Norway, **73** Laboratory of Cancer Genetics and Tumor Biology, Department of Clinical Chemistry and Biocenter Oulu, University of Oulu, NordLab/Oulu University Hospital, Oulu, Finland, **74** Department of Oncology, Oulu University Hospital, University of Oulu, Oulu, Finland, **75** Ontario Cancer Genetics Network, Fred A. Litwin Center for Cancer Genetics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada, **76** Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada, **77** Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada, **78** Division of Epidemiology, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada, **79** Laboratory Medicine Program, University Health Network, Toronto, Ontario; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada, **80** Department of Human Genetics & Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands, **81** Department of Surgical Oncology, Leiden University Medical Center, Leiden, The Netherlands, **82** Family Cancer Clinic, Department of Medical Oncology, Erasmus MC-Daniel den Hoed Cancer Center, Rotterdam, The Netherlands, **83** Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland, United States of America, **84** Department of Medical Oncology, Family Cancer Clinic, Erasmus MC Cancer Institute, Rotterdam, The Netherlands, **85** Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands, **86** Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands, **87** Human Genetics Division, Genome Institute of Singapore, Singapore, **88** Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, **89** CRUK/YCR Sheffield Cancer Research Centre, Department of Oncology, University of Sheffield, Sheffield, United Kingdom, **90** Academic Unit of Pathology, Department of Neuroscience, University of Sheffield, Sheffield, United Kingdom, **91** Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, United Kingdom, **92** Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland, **93** Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland, **94** Division of Genetics and Epidemiology and Division of Breast Cancer Research, The Institute of Cancer Research, Sutton, Surrey, United Kingdom, **95** Breakthrough Breast Cancer Research Centre, Division of Breast Cancer Research, The Institute of Cancer Research, London, United Kingdom, **96** Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, United States of America, **97** Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, United States of America, **98** Human Genotyping-CEGEN Unit, Human Cancer Genetics Program, Spanish National Cancer Research Centre [CNIO], Madrid, Spain, **99** McGill University and Génome Québec Innovation Centre, Montréal, Québec, Canada, **100** Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom, **101** Wellcome Trust Centre for Human Genetics and Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom

Abstract

Invasive lobular breast cancer (ILC) accounts for 10–15% of all invasive breast carcinomas. It is generally ER positive (ER+) and often associated with lobular carcinoma *in situ* (LCIS). Genome-wide association studies have identified more than 70 common polymorphisms that predispose to breast cancer, but these studies included predominantly ductal (IDC) carcinomas. To identify novel common polymorphisms that predispose to ILC and LCIS, we pooled data from 6,023 cases (5,622 ILC, 401 pure LCIS) and 34,271 controls from 36 studies genotyped using the iCOGS chip. Six novel SNPs most strongly associated with ILC/LCIS in the pooled analysis were genotyped in a further 516 lobular cases (482 ILC, 36 LCIS) and 1,467 controls. These analyses identified a lobular-specific SNP at 7q34 (rs11977670, OR (95%CI) for ILC = 1.13 (1.09–1.18), $P = 6.0 \times 10^{-10}$; P -het for ILC vs IDC ER+ tumors = 1.8×10^{-4}). Of the 75 known breast cancer polymorphisms that were genotyped, 56 were associated with ILC and 15 with LCIS at $P < 0.05$. Two SNPs showed significantly stronger associations for ILC than LCIS (rs2981579/10q26/FGFR2, P -het = 0.04 and rs889312/5q11/MAP3K1, P -het = 0.03); and two showed stronger associations for LCIS than ILC (rs6678914/1q32/LGR6, P -het = 0.001 and rs1752911/6q14, P -het = 0.04). In addition, seven of the 75 known loci showed significant differences between ER+ tumors with IDC and ILC histology, three of these showing stronger associations for ILC (rs11249433/1p11, rs2981579/10q26/FGFR2 and rs10995190/10q21/ZNF365) and four associated only with IDC (5p12/rs10941679; rs2588809/14q24/RAD51L1, rs6472903/8q21 and rs1550623/2q31/CDCA7). In conclusion, we have identified one novel lobular breast cancer specific predisposition polymorphism at 7q34, and shown for the first time that common breast cancer polymorphisms predispose to LCIS. We have shown that many of the ER+ breast cancer predisposition loci also predispose to ILC, although there is some heterogeneity between ER+ lobular and ER+ IDC tumors. These data provide evidence for overlapping, but distinct etiological pathways within ER+ breast cancer between morphological subtypes.

Citation: Sawyer E, Roylance R, Petridis C, Brook MN, Nowinski S, et al. (2014) Genetic Predisposition to In Situ and Invasive Lobular Carcinoma of the Breast. *PLoS Genet* 10(4): e1004285. doi:10.1371/journal.pgen.1004285

Editor: Greg Gibson, Georgia Institute of Technology, United States of America

Received: October 4, 2013; **Accepted:** February 17, 2014; **Published:** April 17, 2014

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: GLACIER: Genotyping was funded by the Breast Cancer Campaign (grant number 2010NovPR61, www.breastcancercampaign.org). Sample and data collection by Cancer Research UK. Core funding came from the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St. Thomas' NHS Foundation Trust and King's College London and the Wellcome Trust Centre for Human Genetics (provided by the Wellcome Trust, 090532/Z/09/Z). The views expressed are those of the author(s) and not necessarily those of the NHS, NIHR or the Department of Health. iCOGs was partly supported by the Canadian Institutes of Health Research for the "CIHR Team in Familial Risks of Breast Cancer" program (JS & DE), and the Ministry of Economic Development, Innovation and Export Trade of Quebec – grant # PSR-SIIRI-701 (JS, DE, PH). JS is chair holder of the Canada Research Chair in Oncogenetics. Part of this work was supported by the European Community's Seventh Framework Programme under grant agreement number 223175 (grant number HEALTH-F2-2009-223175) (COGS). The ABCFS, NC-BCFR and OFBCR work was supported by the United States National Cancer Institute, National Institutes of Health (NIH) 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), Northern California Cancer Center (U01 CA69417), University of Melbourne (U01 CA69638). Samples from the NC-BCFR 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. 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. JLH is a National Health and Medical Research Council (NHMRC) Australia Fellow and a Victorian Breast Cancer Research Consortium Group Leader. MCS 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 number NKI 2007-3839 and 2009-4363. The work of the BBCC was partly funded by ELAN-Fond of the University Hospital of Erlangen. The BBCC 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). The BCAC is funded by CR-UK (C1287/A10118 and C1287/A12014). Meetings of the BCAC have been funded by the European Union COST programme (BM0606). DE is a Principal Research Fellow of CR-UK. BIGGS: 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 Johan Boserup and Lise Boserup Fund, the Danish Medical Research Council and Herlev Hospital. The CNIO-BCS was supported by the Genome Spain Foundation, 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 PI081120). DietComplyf: The University of Westminster's ABC Research Unit acknowledges funding from the charity Against Breast Cancer (Registered Charity Number 1121258). 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 was supported by Deutsche Krebshilfe (107 352). 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, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Bochum, as well as the Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn, Germany. The HEBCS was financially supported by the Helsinki University Central Hospital Research Fund, Academy of Finland (132473), the Finnish Cancer Society, the Nordic Cancer Union and the Sigrid Juselius Foundation. 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 and Bert von Kantzow 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, the Academy of Finland and by the strategic funding of the University of Eastern Finland. kConFab is supported by grants from the National Breast Cancer Foundation, the NHMRC, the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia and the Cancer Foundation of Western Australia. The kConFab Clinical Follow Up Study was funded by the NHMRC [145684, 288704, 454508]. Financial support for the AOCs was provided by the United States Army Medical Research and Materiel Command [DAMD17-01-1-0729], the Cancer Council of Tasmania and Cancer Foundation of Western Australia and the NHMRC [199600]. GCT is supported by the NHMRC. LMBC is supported by the 'Stichting tegen Kanker' (232-2008 and 196-2010). DL is supported by the FWO and the KULPFV/10/016-SymBioSysll. The MARIE study was supported by the Deutsche Krebshilfe e.V. [70-2892-BR I], the Hamburg Cancer Society, the German Cancer Research Center and the genotype work in part by 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 "5 x 1000"). MCBCS investigators were supported by the NIH grant CA128978, an NIH Specialized Program of Research Excellence (SPORE) in Breast Cancer [CA116201] and the Breast Cancer Research Foundation, and generous gifts 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. The MEC was supported by NIH grants CA63464, CA54281, CA098758 and CA132839. MTLGEBCS: The Quebec Breast Cancer Foundation supported the case-control study. The NBCS was supported by grants from the Norwegian Research council FUGE-NFR 181600/V11 to VK. The OBCS was supported by the Finnish Cancer Foundation, the Academy of Finland, the University of Oulu, and the Oulu University Hospital. OFBCR: This work was supported by the Canadian Institutes of Health Research "CIHR Team in Familial Risks of Breast Cancer" program, and grant UM1 CA164920 from the National Cancer Institute/NIH (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 US 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 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. 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, Heidelberg, Germany. SZBCS: KJB is a fellow of International PhD program, Postgraduate School of Molecular Medicine, Warsaw Medical University, supported by the Polish Foundation of Science. The UKBGS is funded by Breakthrough Breast Cancer and the Institute of Cancer Research (ICR). 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 declared that no competing interests exist.

* E-mail: elinor.sawyer@kcl.ac.uk

☞ These authors contributed equally to this work.

☞ Membership of the GENICA Network and kConFab Investigators is provided in the acknowledgments.

Author Summary

Invasive lobular breast cancer (ILC) accounts for 10–15% of invasive breast cancer and is generally ER positive (ER+). To date, none of the genome-wide association studies that have identified loci that predispose to breast cancer in general or to ER+ or ER-negative breast cancer have focused on lobular breast cancer. In this lobular breast cancer study we identified a new variant that appears to be specific to this morphological subtype. We also ascertained which of the known variants predisposes specifically to lobular breast cancer and show for the first time that some of these loci are also associated with lobular carcinoma *in situ*, a non-obligate precursor of breast cancer and also a risk factor for contralateral breast cancer. Our study shows that the genetic pathways of invasive lobular cancer and ER+ ductal carcinoma mostly overlap, but there are important differences that are likely to provide insights into the biology of lobular breast tumors.

Introduction

Invasive lobular breast cancer (ILC) accounts for 10–15% of all invasive breast carcinomas and it has distinct etiological, clinical and biological characteristics compared with the more common invasive ductal/no special type carcinoma (IDC) [1]. Lobular cancers show stronger associations with the use of hormone replacement therapy (HRT) than IDC, [2] and its incidence follows a similar temporal pattern as the use of combined HRT [3]. ILC is characterized by E-cadherin loss and the malignant cells therefore infiltrate the breast stroma in single files with little associated stromal reaction. This makes it difficult to detect these tumors by palpation or mammography, and they are often larger at presentation than IDCs [4]. ILCs are generally of histological grade 2 and estrogen receptor positive (ER+), with the exception of the pleomorphic subgroup. They typically have a different pattern of metastatic spread to IDCs, tending to infiltrate the peritoneum, ovary and gastrointestinal system. There is some evidence that they are less chemo-sensitive than IDC and that the 10-year survival rate of women with ILC is lower than that of ER+ IDCs [5,6].

ILC is often associated with lobular carcinoma *in situ* (LCIS), a form of non-invasive breast cancer that is difficult to detect clinically and typically found incidentally on biopsy. The increased breast biopsy rate associated with screening mammography has led to an increase in the diagnosis of LCIS. LCIS shares many of the same genetic aberrations as ILC, suggesting that it is a precursor lesion in an analogous manner to ductal carcinoma *in situ* (DCIS) and IDC [7]. Women who have had LCIS are 2.4 times more likely to develop invasive breast cancer compared to the general population, with an excess of ILC (23–80% of cases) [8,9]. However only 50–70% of invasive cancers associated with LCIS have lobular morphology [10, unpublished data from GLACIER study]. The remaining cancers have a IDC or mixed ductal-lobular appearance, but again are generally ER+ (95% of IDC and mixed ductal-lobular cancers associated with LCIS in the GLACIER study were ER+). Unlike DCIS, LCIS is also a risk factor for developing invasive cancer in the contralateral breast [8].

Genome-wide association studies (GWAS) in breast cancer have identified loci that predispose to invasive breast cancer in general, or specifically to ER+ or ER-negative disease [11–25]. However, no previous study has focused specifically on lobular

carcinomas. Only one common single nucleotide polymorphism (SNP; rs11249433 at 1p11.2) has been shown to be more strongly associated with lobular than ductal histology [26]. For the remaining SNPs predisposing to ER+ tumors, it is unclear whether the studies have lacked statistical power to identify differential associations by histology, or whether associations tend to be non-differential by morphology after accounting for ER status.

The aim of this study was to identify new breast cancer susceptibility loci specific to lobular carcinoma, and to evaluate the heterogeneity of associations of known loci by morphology. This involved pooling genotyping data from over 6,000 cases of lobular carcinoma (ILC and/or LCIS) and over 34,000 controls genotyped using the iCOGS chip, a custom SNP array that comprises 211,155 SNPs enriched at predisposition loci for breast and other cancers [24].

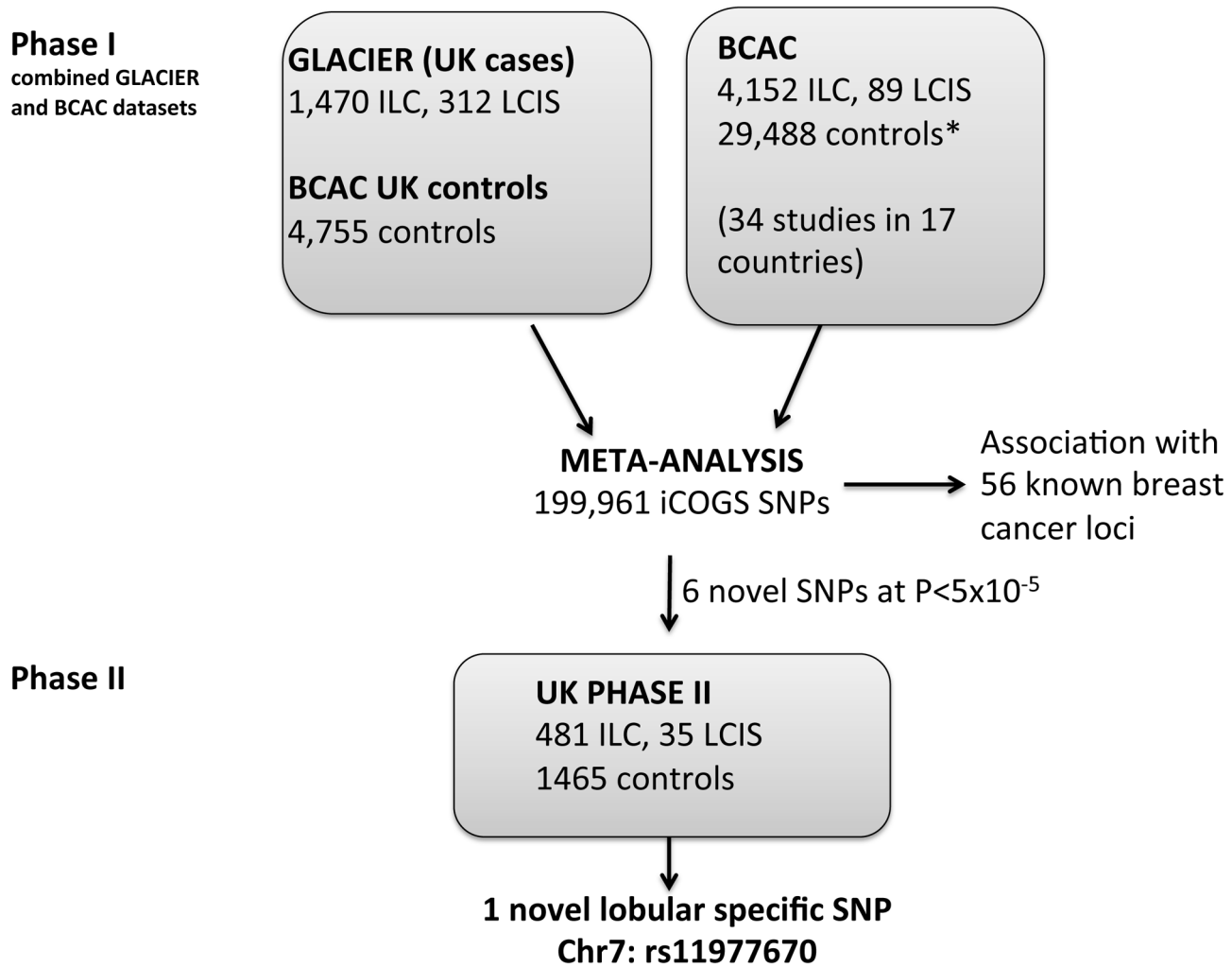
Results

In a phase I analysis, we evaluated risk associations between SNPs on the iCOGS chip and risk of ILC and LCIS using 1,782 lobular cases (1,470 ILC with or without LCIS, 312 pure LCIS) from GLACIER, a UK study of lobular breast cancer, and 4,755 UK controls from the Breast Cancer Association Consortium, BCAC (Figure 1). There was little evidence for systematic inflation of the test statistics, based on 37,544 uncorrelated SNPs that had not been selected on the basis of breast cancer risk ($\lambda = 1.04$; Figure S1). Data were combined by meta-analysis with a further 4,241 cases (4,152 ILC, 89 LCIS) and 29,519 controls of European ancestry, derived from 34 studies in BCAC, and previously typed on the iCOGS chip (Tables S1 and S2). This resulted in a total of 6,023 cases (5,622 ILC, 401 LCIS) and 34,271 controls with data on 199,961 iCOGS SNPs (after quality control exclusions and with minor allele frequency (MAF) >0.01) included in the meta-analysis.

Search for new lobular breast cancer predisposition loci

All SNPs reaching genome-wide significance ($P < 5 \times 10^{-8}$) in the meta-analysis were correlated with one of the known breast cancer predisposition loci. In order to identify new loci that predispose to lobular carcinoma, we selected six uncorrelated SNPs (rs11977670, rs2121783, rs2747652, rs3909680, rs9948182, rs7034265) that were only weakly correlated ($r^2 < 0.25$) with known loci and that showed the best evidence of association (P between 5×10^{-8} and 5×10^{-5}) in the overall lobular case-control analysis (ILC and LCIS). These SNPs were genotyped in a Phase II including 516 cases (481 ILC, 35 LCIS) and 1,467 controls, all from white European donors (Figure 1).

One of the six SNPs, rs11977670 at 7q34, reached genome-wide significance in a pooled analysis of phase I and II ILC cases and controls (OR = 1.13, 95%CI = 1.09–1.18, $P = 6.0 \times 10^{-10}$, Table 1, Figure 2). rs11977670 showed a similar association with LCIS (P -het for ILC vs LCIS = 0.198), and a very weak or no association with IDC (OR = 1.02, 95%CI = 1.00–1.05, $P = 0.070$; P -het for ILC vs IDC = 1.3×10^{-5}), indicating that this is a lobular specific predisposition locus (Table 2). The risk allele appeared to act in a dominant rather than additive manner: OR_{AG} = 1.21, 95%CI = 1.14–1.30; OR_{AA} = 1.27, 95%CI = 1.17–1.38; P for departure from log-additivity = 0.009; Table S3. rs11977670 was not significantly associated with age at onset of ILC ($P_{trend} = 0.16$) and risk alleles were not significantly over-represented in cases with a positive family history (FH) ($P = 0.90$, FH+ vs FH–). None of the other 5 SNPs genotyped



*Excludes a subset of UK cases in GLACIER comparison

Figure 1. Lobular cancer study design.

doi:10.1371/journal.pgen.1004285.g001

were associated with lobular breast cancer at a genome-wide significance level, with the strongest association being for rs2121783 at 3p13 (OR = 1.11, 95%CI = 1.07–1.15, $P = 4.5 \times 10^{-7}$; Table S4).

rs11977670 at 7q34 (position:139942304, GRCh Build 37) is intergenic, 65 kb from the nearest gene, *JHDM1D*, a histone demethylase and 500 kb from *BRAF*, a gene frequently mutated in melanoma. It is also in close proximity to a predicted novel U1 spliceosomal RNA that contains two U1 specific promoter motifs (Figure S2). ENCODE data on normal human mammary epithelial cells (HMEC), and breast carcinoma (MCF-7), were used to establish chromatin states in the region and showed that rs11977670 lies in region marked by H3K27 acetylation, Figure S3.

Using expression data from the Cancer Genome Atlas Network (TCGA database) [27], we assessed expression of the nine genes within 0.5 Mb of rs11977670 by breast cancer subtype (ER+ ILC, 40 cases; ER+ IDC, 341 cases; and ER-negative IDC, 108 cases; Figure S4). Three genes showed differential expression in ER+ ILC compared to ER+ IDC (*BRAF*, $P = 0.006$; *NDUFB2*, $P = 0.02$, *SLC37A3*, $P = 0.05$), however none reached statistical significance when correcting for multiple testing. Another two genes, *JHDM1D*

and *ADCK2*, showed a difference in expression between ER-negative and ER+ cancers, but this was not lobular-specific. To further investigate which genes may be influenced by SNPs tagged by rs11977670, germline genotype data for rs13225058 (A/G), a surrogate for rs11977670 (G/A) ($r^2 = 0.79$) was taken from the TCGA database (SNP6.0 Affymetrix array) and compared to expression of these genes, correcting for copy number variation, in 335 ER+ primary breast cancers where both genotype and expression data was available. A significant difference, after correcting for multiple testing, was found in expression between the AA and GG genotype for two genes *JHDM1D* ($P = 0.0005$) and *SLC37A3* ($P = 0.004$), Figure S5a. Confining the analysis to the 36 ILC cases with data in TCGA showed no significant genotype specific expression due small numbers although there was the suggestion of a trend towards overexpression with the GG genotype (2 cases), Figure S5b. 48 of the cases also had expression data on adjacent normal breast tissue, but due to the small numbers no significant genotype specific expression changes were detected, Figure S6. There was no evidence of copy number variation around rs11977670 and no evidence of an excess of somatic mutations in *JHDM1D*, *SLC37A3* or *BRAF* in ILC.

Table 1. rs11977670, chromosome 7:139942304 G>A, and association ILC in populations of European ancestry.

Study/Consortia	N Studies	Cases	Controls	MAF	OR (95% CI)*	P
Phase I						
GLACIER	1	1,470	4,755	0.437	1.16 (1.07, 1.26)	6.1×10^{-4}
BCAC	34	4,150	29,488	0.429	1.10 (1.05, 1.16)	4.0×10^{-5}
Combined	35	5,620	34,243	0.430	1.12 (1.07, 1.16)	1.4×10^{-7}
Phase II						
UK PHASE II	1	479	1,452	0.426	1.38 (1.19, 1.60)	2.9×10^{-5}
Phase I+II						
	36	6,099	5,695	0.430	1.13 (1.09, 1.18)	6.0×10^{-10}

* per allele.

doi:10.1371/journal.pgen.1004285.t001

Assessment of the 75 known breast cancer susceptibility loci for association with ILC and LCIS

Most (56 of 75) known common breast cancer susceptibility loci were associated with ILC at $P < 0.05$ with the effect in the same direction as previously reported (Table S5), and 13 of these reached genome-wide significance ($P < 5 \times 10^{-8}$, Table 3). The strongest associations were with SNPs close to *FGFR2* (rs2981579, OR = 1.38, $P = 5.1 \times 10^{-53}$), *TOX3* (rs3803662, OR = 1.33, $P = 1.1 \times 10^{-35}$), at 1p11.2 (rs11249433, OR = 1.25, $P = 2.7 \times 10^{-25}$) and 11q13.3 (rs554219, OR = 1.33, $P = 1.6 \times 10^{-22}$). All 13 loci had previously been shown to be associated with ER+ breast cancer and one locus, rs11249433 (1p11.2), with lobular histology in subgroup analysis. Of the remaining 19 SNPs with $P \geq 0.05$, 18 had ORs in the same direction as previously reported for overall breast cancer (Sign test $P = 0.0001$), suggesting that these SNPs are also likely to predispose to LCIS. Only one of the seven ER-negative specific loci on the iCOGS array showed a significant association with ILC (rs12710696, $P = 0.037$). In case-only analyses, no SNP showed an association with family history of breast cancer or young age at onset of ILC.

For the 75 known breast cancer susceptibility loci, case-control analysis for the 401 cases of pure LCIS (without invasion) and 24,045 controls, revealed 15 out of 75 SNPs associated with LCIS at $P < 0.05$ (Table 3). The strongest associations were for rs865686 (9q31.2, $P = 2.2 \times 10^{-3}$); rs3803662 (*TOX3*, $P = 1.2 \times 10^{-4}$), c11_pos69088342/rs75915166 (11q13.3, $P = 7.8 \times 10^{-4}$) and rs1243482 (*MLLT10*, 10p12.31, $P = 7.8 \times 10^{-4}$) that is partially correlated ($r^2 = 0.69$) with rs7072776, a recently identified ER+ breast cancer predisposition locus that showed a weaker association with LCIS (OR = 1.17, 95% CI = 1.00–1.36, $P = 0.05$; Table S5). Forty-seven of the remaining 60 SNPs at $P > 0.05$ had ORs in the same direction as for ILC. This is greater than one would expect by chance (Sign Test $P = 1.2 \times 10^{-5}$) suggesting many of these SNPs predispose to LCIS, but the study did not have enough power to detect these associations with the small sample size.

A global test in case-only analysis (ILC vs LCIS) indicated no significant differences in associations of the 75 SNPs between LCIS and ILC (likelihood ratio test (75 df) = 0.438). However, individual SNP analyses suggested some differences. Two loci showed stronger associations with ILC than pure LCIS: rs2981579, *FGFR2* (P -het = 0.02); and rs889312, 5q11.2 (P -het = 0.03). Case-only analysis also suggested that two ER-negative specific SNPs [23,25] were more strongly associated with LCIS than ILC: rs6678914, 1q32.1 (P -het = 0.0007) and rs17529111, 6q14.1 (P -het = 0.04) Table 3. The remaining SNPs showed no significant heterogeneity between ILC and LCIS.

Assessment of the 75 known susceptibility SNPs for differential effects on ILC and IDC

In order to identify lobular specific SNPs, we performed a case-only analysis of 3,201 ER+ ILC cases and 15,024 ER+ IDC cases from BCAC. Analysis was confined to ER+ cases since 94% of ILC cases were ER+ (compared to 78% of IDC in BCAC). A global test indicated significant differences in SNP associations between ILC and IDC (likelihood ratio test (75 df) $P = 5.9 \times 10^{-6}$). The SNP showing the largest difference between ILC and IDC was rs11249433 at chr 1p11.2 (P -het = 2.7×10^{-8} ; Table 4), a SNP previously associated with lobular histology. At $P < 0.05$, a further two loci were associated more strongly with ILC than IDC: rs2981579, *FGFR2* (P -het = 5.3×10^{-3}) and rs10995190, 10q21.2 (P -het = 0.002). This analysis also identified four IDC-specific SNPs at $P < 0.05$: rs10941679, 5p12 (P -het = 1.5×10^{-4}); rs2588809, *RAD51L1* (P -het = 0.001); rs6472903, 8q21.11 (P -het = 0.004); rs1550623, *CDC47* (P -het = 0.031) Table S6.

Assessment of the 75 known susceptibility SNPs for effects on mixed ILC-IDC cancer predisposition

Case-control analysis of 690 mixed ductal-lobular carcinomas revealed 25 loci that showed an association with these mixed cancers at $P < 0.05$. The top hits were at *FGFR2* (rs2981579, OR = 1.37, $P = 1.6 \times 10^{-7}$), rs941764 (*CCDC88C*, OR = 1.25, $P = 3.6 \times 10^{-4}$) and rs10995190 (*ZNF365*, OR = 0.74, $P = 3.9 \times 10^{-4}$). The case-only analysis above showed that two of these SNPs are more strongly associated with ILC than IDC (rs2981579, rs10995190). rs941764 showed no association with ILC and only weak association with ER+ IDC, Table S6.

Discussion

Our analyses of a total of 6,539 lobular cancers (including 436 cases of pure LCIS) and 35,710 controls has identified for the first time a lobular-specific SNP, rs11977670 (*JHDMID*; OR = 1.13 $P = 4.2 \times 10^{-10}$, that showed little evidence of association with IDC ($P = 0.07$) or DCIS ($P = 0.23$). Identification of the target of this association will require fine mapping of the region, followed by functional assays to determine which gene(s) the key SNPs regulate. The preliminary *in silico* functional analysis suggests that SNPs in this region may be influencing expression of *JHDMID* (a histone demethylase) and *SLC37A3* (a sugar-phosphate exchanger). For *JHDMID* this appears to be a recessive effect, in contrast to the susceptibility data, which suggests a dominant effect. There are little data on the role of these genes in cancer. There is some evidence that increased expression of *JHDMID* can

rs11977670: Lobular (invasive) cases vs controls

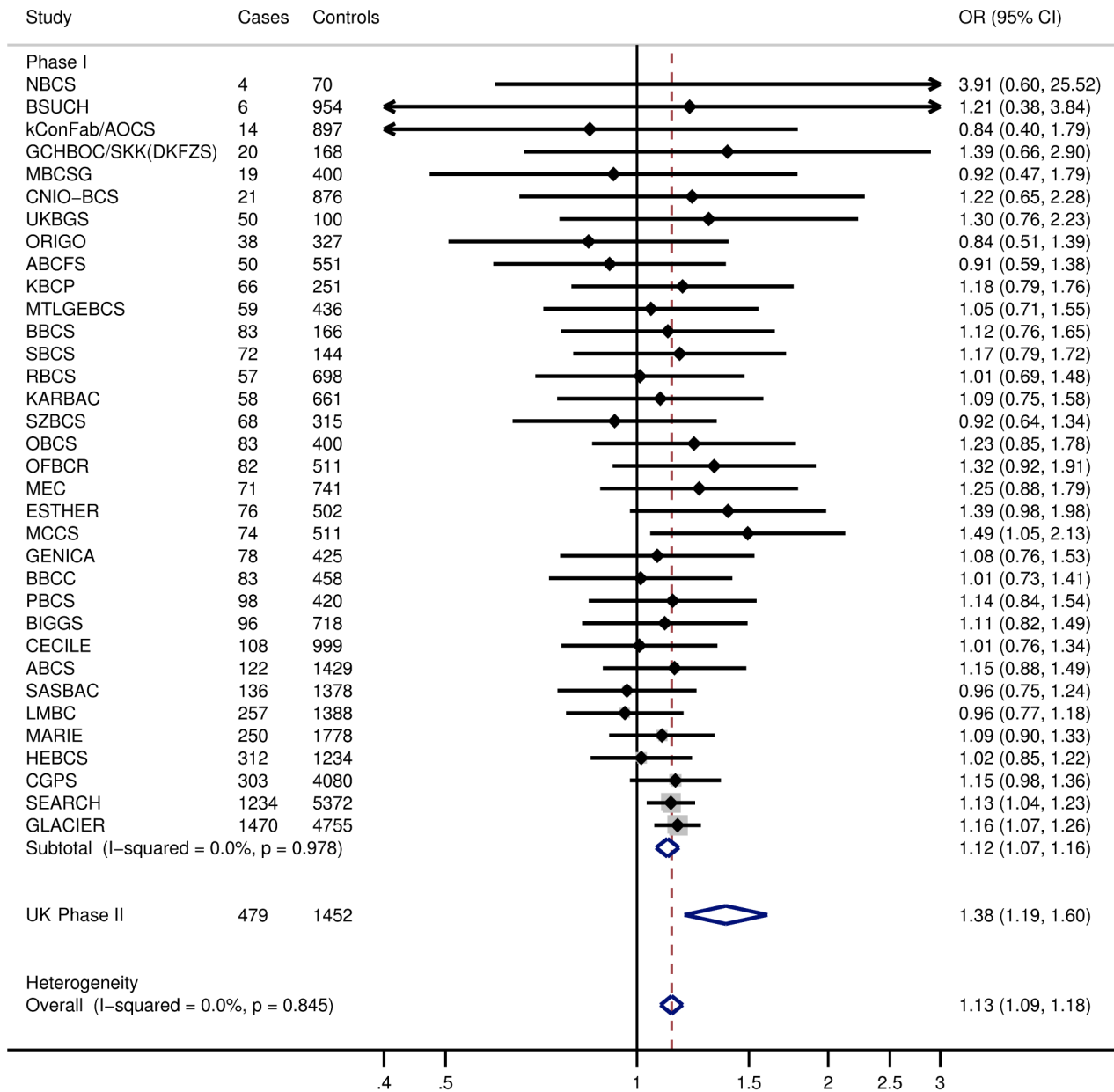


Figure 2. Forest plot for rs11977670.
doi:10.1371/journal.pgen.1004285.g002

suppress tumor growth by regulating angiogenesis [28] and decreased expression promotes invasiveness, which is contrary to what one would expect from the risk data [29]. This inconsistency does shed some doubt on these results and further analysis of the region is required before any firm conclusion can be made. Studies of syndecan-1-deficient breast cancer cells, which show increased cell motility and invasiveness, demonstrate decreased expression of both JHDM1D and E-cadherin [29], suggesting the two genes may interact. Somatic mutations in *CDH1* (E-Cadherin) are frequent in ILC and rare germline frameshift mutations in *CDH1* have been described in ILC, particularly in families with hereditary diffuse gastric cancer (HDGC), but also in cases of familial ILC

with no HDGC [30,31]. However, none of the 56 SNPs in *CDH1* that were typed on the iCOGS chip showed any association with lobular cancer at $P < 0.05$.

It should also be noted that this study is not a true genome wide association study for lobular breast cancer as the SNPs on the iCOGS chips were chosen on the basis of some prior evidence of association with breast cancer as a whole. Although ILC would have been a small proportion of the samples in the discovery sets for these SNPs it is possible that other lobular specific loci exist that have not been included on the iCOGS chip. This is particularly true for LCIS, which would only have been included in the discovery set as a parallel phenotype when associated with invasive disease.

Table 2. Association with risk of breast cancer for rs11977670 stratified by breast cancer tumour subtypes (Pooled analysis, BCAC, GLACIER, UK PHASE II).

Tumour type	N Studies	Cases	Controls	OR (95% CI)	P	P het
All tumours combined	43	48,286	43,776	1.05 (1.03, 1.07)	5.1×10^{-7}	
Ductal invasive	34	23,549	29,488	1.02 (1.00, 1.05)	0.070	
ER pos	32	15,010	29,250	1.03 (1.00, 1.06)	0.047	
ER neg	33	4,266	29,068	0.97 (0.93, 1.02)	0.208	
DCIS	22	847	23,372	1.06 (0.96, 1.17)	0.231	0.900
Lobular invasive	36	6,099	35,695	1.13 (1.09, 1.18)	6.0×10^{-10}	1.3×10^{-5}
ER pos	34	4,135	35,457	1.13 (1.08, 1.19)	2.1×10^{-7}	1.8×10^{-4}
ER neg	32	268	33,791	1.22 (1.03, 1.45)	0.022	0.009
LCIS	16	437	25,488	1.10 (0.96, 1.26)	0.179	0.198
Lobular (invasive+LCIS)	36	6,536	35,695	1.13 (1.09, 1.18)	4.2×10^{-10}	
ER pos	34	4,202	35,457	1.13 (1.08, 1.18)	3.1×10^{-7}	
ER neg	32	278	33,791	1.20 (1.01, 1.42)	0.035	

^aDuctal (invasive) vs Ductal (in situ) in subjects where ER status exists, adjusted for ER status.

^bDuctal (invasive) vs Lobular (invasive) in subjects where ER status exists adjusted for ER status, UK population-based studies grouped together.

^cDuctal (invasive) vs Lobular (invasive), ER positive only.

^dDuctal (invasive) vs Lobular (invasive), ER negative only.

^eLobular (invasive) vs Lobular (in situ) in subjects where ER status exists, adjusted for ER status.

doi:10.1371/journal.pgen.1004285.t002

Table 3. SNPs associated with ILC ($P < 5 \times 10^{-8}$) or LCIS ($P < 0.05$) in a pooled lobular analysis (GLACIER and BCAC).

Cytoband	Gene	RS number	MAF		ILC vs Controls*		LCIS vs Controls**		P	P-het
			Controls	OR (95% CI)	OR (95% CI)	P				
10q26.13	<i>FGFR2</i>	rs2981579	0.40	1.38 (1.32, 1.44)	5.1 × 10 ⁻⁵²	1.19 (1.03, 1.37)	0.019	0.04		
16q12.1	<i>TOX3</i>	rs3803662	0.26	1.33 (1.27, 1.39)	1.1 × 10 ⁻³⁵	1.35 (1.16, 1.57)	1.2 × 10 ⁻⁴	0.99		
1p11.2		rs11249433	0.40	1.25 (1.20, 1.30)	2.7 × 10 ⁻²⁵	1.15 (1.00, 1.33)	0.050	0.94		
11q13.3		rs554219	0.12	1.33 (1.26, 1.41)	1.6 × 10 ⁻²²	1.31 (1.08, 1.60)	0.007	0.80		
9q31.2		rs865686	0.38	0.83 (0.79, 0.86)	1.0 × 10 ⁻¹⁷	0.72 (0.61, 0.84)	2.2 × 10 ⁻⁵	0.12		
2q35		rs13387042	0.49	0.84 (0.80, 0.87)	5.7 × 10 ⁻¹⁷	0.90 (0.78, 1.04)	0.145	0.21		
11q13.3		rs75915166	0.06	1.40 (1.29, 1.51)	1.2 × 10 ⁻¹⁶	1.55 (1.20, 2.01)	7.8 × 10 ⁻⁴	0.54		
11q13.3		rs614367	0.14	1.24 (1.18, 1.31)	7.2 × 10 ⁻¹⁵	1.32 (1.10, 1.58)	0.003	0.46		
10q21.2	<i>ZNF365</i>	rs10995190	0.16	0.80 (0.75, 0.85)	1.7 × 10 ⁻¹³	0.69 (0.55, 0.87)	0.002	0.10		
5q11.2	<i>MAP3K1</i>	rs889312	0.28	1.18 (1.13, 1.23)	9.1 × 10 ⁻¹³	1.05 (0.89, 1.22)	0.576	0.03		
10q22.3	<i>ZMIZ1</i>	rs704010	0.38	1.14 (1.10, 1.19)	3.7 × 10 ⁻¹⁰	1.15 (0.99, 1.33)	0.063	0.49		
10p12.31		rs1243182	0.32	1.14 (1.09, 1.19)	6.1 × 10 ⁻⁹	1.29 (1.11, 1.49)	7.8 × 10 ⁻⁴	0.49		
4q34.1	<i>ADAM29</i>	rs6828523	0.12	0.82 (0.77, 0.88)	1.6 × 10 ⁻⁸	0.96 (0.77, 1.21)	0.751	0.21		
8q24.21		rs13281615	0.41	1.13 (1.08, 1.18)	2.1 × 10 ⁻⁸	1.13 (0.97, 1.30)	0.116	0.86		
2q35	<i>DIRC3</i>	rs16857609	0.26	1.10 (1.05, 1.15)	3.5 × 10 ⁻⁵	1.25 (1.07, 1.46)	0.006	0.625		
2q14.2		rs4849887	0.10	0.91 (0.84, 0.97)	0.007	0.71 (0.54, 0.93)	0.012	0.11		
6q14.1		rs17529111	0.22	1.06 (1.01, 1.11)	0.020	1.25 (1.06, 1.48)	0.009	0.04		
2p24.1		rs12710696	0.36	1.05 (1.00, 1.09)	0.037	1.17 (1.01, 1.35)	0.034	0.10		
5p15.33	<i>TERT, hTERT</i>	rs7726159	0.34	1.03 (0.99, 1.07)	0.195	1.22 (1.05, 1.42)	0.008	0.254		
1q32.1	<i>LGR6</i>	rs6678914	0.41	1.02 (0.98, 1.06)	0.415	0.77 (0.67, 0.90)	8.0 × 10 ⁻⁴	0.0007		
8q21.11		rs6472903	0.18	0.98 (0.93, 1.03)	0.459	0.81 (0.66, 0.99)	0.036	0.11		
5p15.33	<i>TERT</i>	rs10069690	0.26	1.01 (0.97, 1.06)	0.615	1.18 (1.01, 1.38)	0.040	0.19		

*5622 cases, 34272 controls.

**401 cases, 24045 controls.

doi:10.1371/journal.pgen.1004285.t003

Table 4. SNPs showing differential lobular and ductal associations with breast cancer risk in BCAC subjects (ER+ tumours only).

Cytoband	Genes	RS number	MAF	IDC vs Controls*		ILC vs Controls**		P	P-het
				OR (95% CI)	P	OR (95% CI)	P		
1p11.2		rs11249433	0.400	1.09 (1.06, 1.13)	2.3×10^{-9}	1.28 (1.22, 1.35)	7.1×10^{-20}	2.7×10^{-8}	
5p12		rs10941679	0.254	1.17 (1.13, 1.21)	4.0×10^{-21}	1.03 (0.97, 1.10)	0.318	1.5×10^{-4}	
10q26.13	<i>FGFR2</i>	rs2981579	0.404	1.31 (1.27, 1.35)	1.2×10^{-71}	1.42 (1.35, 1.50)	3.4×10^{-38}	5.3×10^{-3}	
14q24.1	<i>RAD51L1</i>	rs2588809	0.158	1.12 (1.08, 1.17)	8.7×10^{-9}	0.99 (0.92, 1.07)	0.870	0.001	
10q21.2	<i>ZNF365</i>	rs10995190	0.158	0.87 (0.84, 0.91)	4.1×10^{-11}	0.76 (0.71, 0.83)	1.2×10^{-11}	0.002	
8q21.11		rs6472903	0.178	0.89 (0.85, 0.92)	2.9×10^{-9}	1.00 (0.93, 1.07)	0.895	0.004	
2q31.1	<i>CDC47</i>	rs1550623	0.156	0.93 (0.89, 0.96)	2.1×10^{-4}	1.01 (0.94, 1.08)	0.839	0.031	

*15023 cases, 29273 controls.

**3201 cases, 29273 controls.

doi:10.1371/journal.pgen.1004285.t004

75 of the known common breast cancer susceptibility loci were assessed for association with ILC and LCIS. As cases of ILC were included in the discovery sets that generated these susceptibility loci and lobular breast cancer is generally ER+ (94% of the ILC cases in this study were ER+) with the majority of ILCs classified as luminal tumors [32], it is not surprising that the majority of SNPs that we found to be associated with ILC were known to also predispose to ER+ breast cancer. However, some loci were only associated with ER+ IDC and not with ILC, particularly rs10941679 at 5p12, previously shown to predispose more strongly to ER-positive, lower-grade cancers [33], $P_{\text{het}} = 2.7 \times 10^{-8}$. Others showed a much stronger association with ILC than IDC, particularly rs11249433 at 1p11.2, as previously described [26]. These data suggest specific etiological pathways for the development of different histological subtypes of breast cancer, in addition to common pathways that predispose to multiple tumor subtypes.

Despite the small number of pure LCIS cases without invasive disease, our analyses have shown for the first time that many of the SNPs that predispose to ILC also predispose to LCIS. Although only 15 of the known breast cancer SNPs were associated with LCIS risk at $P < 0.05$, 47 of the remaining 60 SNPs at $P > 0.05$ had ORs in the same direction as for ILC (Sign Test $P = 1.2 \times 10^{-5}$) suggesting that many more SNPs are likely to be associated with pure LCIS but did not reach statistical significance individually because of the relatively few LCIS cases without associated ILC in our sample set. This is not unexpected if LCIS is an intermediate phenotype for ILC. However, a small number of SNPs had differential effects on LCIS or ILC risk. Specifically, rs6678914 at 1q32.1 (*LGR6*), known to be an ER-negative specific SNP [25], that appeared to be associated with LCIS but not ILC ($P_{\text{het}} = 0.0007$), and rs17529111 at 6q14 preferentially associated with ER-negative tumors [23] that had a stronger association with LCIS than ILC ($P_{\text{het}} = 0.04$). We also identified SNPs in *FGFR2* and at 5q11.2 (*MAP3K1*) that appear only to predispose to ILC, but have little effect on LCIS suggesting that SNPs affect different parts of the lobular carcinoma pathway. These findings are surprising and as based on small numbers need confirmation in future studies.

Some of the SNPs associated with both ILC and LCIS showed a stronger effect size in LCIS compared to ILC (for example SNPs at *TOX3*, 9q31.2, 11q13.3, *ZNF365* and *MLLT10*). It is possible that the SNPs that showed an association with both LCIS and ILC predispose to the development of LCIS rather than ILC, and that the effect size is smaller in ILC as not all cases of LCIS will become invasive cancer. SNPs that predispose strongly to LCIS were also associated with ER+ IDCs but again with stronger effect sizes in LCIS, consistent with the fact that 30–40% of invasive tumors associated with LCIS will not be ILC but will be IDC, mixed ductal-lobular or other morphology.

One SNP, rs1243182 (*MLLT10*), that showed a strong association with LCIS (LCIS: $P = 7.8 \times 10^{-4}$, OR = 1.29; ILC: $P = 6.1 \times 10^{-9}$, OR = 1.14; ILC+LCIS: $P = 3 \times 10^{-10}$, OR = 1.15, IDC: $P = 1.4 \times 10^{-5}$, OR = 1.07, is partially correlated ($r^2 = 0.69$) with rs7072776, a recently identified ER+ breast cancer predisposition locus, which showed no association with LCIS in this study. It is also strongly correlated with rs1243180 ($r^2 = 0.80$), an ovarian cancer predisposition variant [34] and rs11012732 ($r^2 = 0.57$), which predisposes to meningioma [35]. The ovarian SNP, rs1243180, also showed a strong association with lobular cancer (ILC+LCIS: $P = 5.54 \times 10^{-10}$, OR = 1.13). Conditional analysis confirmed that this was not independent of rs1243182. rs11012732 was not genotyped on the iCOGS chip. The increased risk of ovarian carcinoma after breast cancer is well documented in epidemiological studies [36]. Of note, there are also reports

suggesting an association between breast cancer and meningioma [37].

In conclusion, we have identified a novel lobular-specific predisposition SNP at 7q34 close to *JHDM1D* that does not appear to be associated with IDC. Most known breast cancer predisposition SNPs also predispose to ILC, with some differential effects between ILC and IDC. In addition, many SNPs predisposing to invasive cancer are also likely to increase the risk for LCIS. Overall, our analyses show that genetic predisposition to IDC and lobular lesions (both ILC and LCIS) overlap to a large extent, but there are important differences that are likely to provide insights into the biology of lobular breast tumors.

Methods

Ethics statement

All studies were performed with ethical committee approval, Table S7, and subjects participated in the studies after providing informed consent.

Study populations

Phase I. Cases and controls came from 34 studies forming part of the Breast Cancer Association Consortium (BCAC) included in the COGS Project [13] (Table S1), and GLACIER (A study to investigate the Genetics of Lobular Carcinoma *In situ* in Europe MREC 06/Q1702/64), a UK case-only study of lobular breast cancer. BCAC studies recruited all types of breast cancer. Pathological information in BCAC was collected by the studies individually but combined and checked through standardized data control in a central database. A total of 4,152 ILC and 89 LCIS cases were identified by the central BCAC pathology database (see Table S2 for number of cases by study).

The GLACIER study recruited patients from participating centers throughout the UK with the aim of identifying predisposition genes for LCIS and/or ILC. Any patients aged 60 or less at the time of diagnosis, with a current or past history of LCIS (with or without invasive disease of any histological subtype) were eligible. A total of 2,539 cases were recruited: 2,167 were identified from local pathology reports in 97 UK hospitals, 346 cases were identified through the British Breast Cancer Study (BBCS) using UK Cancer Registry data and 26 cases from the Royal Marsden Breast Tissue Bank. Cryptic relatedness analysis showed no evidence of overlap between these samples and the BCAC samples. All these cases were genotyped with the iCOGS chip and compared to 5,000 UK controls selected from four UK studies participating in BCAC and already typed on the iCOGS chip. Controls were randomly selected prior to analysis so that each of these UK studies, including GLACIER, had a case:control ratio of at least 1:2 (Table S8). These controls were excluded from case-control comparisons with BCAC cases from the originating study. This report includes only cases of pure LCIS or ILC with or without LCIS. Cases of LCIS with IDC or mixed lobular and ductal carcinoma in GLACIER were excluded in order to perform meta-analyses with the BCAC studies which do not have information on the presence or absence of LCIS associated with an invasive cancer. After excluding individuals based on genotyping quality (see Genotyping and Analysis) and non-European ancestry, data for the GLACIER study available for analyses included 1,782 cases (1,470 ILC (with or without LCIS), 312 pure LCIS) and 4,755 controls.

Phase II. A further 516 cases (481 ILC, 35 LCIS) and 1,465 controls were analyzed as part of Phase II. Controls were

recruited through the GLACIER study, but were not genotyped in Phase I on the iCOGS chip to reduce costs, and were all white West European. Cases came from the following studies: 232 cases from GLACIER, 176 from BBCS, 71 from DietCompLyf [38], 39 from King's Health Partners Cancer Biobank (KHP-CB). All cases were white West European, apart from the 39 samples from the KHP-CB where there were no associated ethnicity data. For studies that had also participated in Phase I, we selected samples so there was no overlap with the samples in Phase I.

Genotyping and analysis

Phase I. After DNA extraction from peripheral blood, GLACIER samples were genotyped on the iCOGS custom Illumina iSelect, which contains 211,155 SNPs, at King's College, London. The remaining cases and controls were genotyped as part of the COGS project described in detail elsewhere [13]. The GLACIER cases were analyzed using the same QC criteria as the COGS project. Briefly, genotypes were called using Illumina's proprietary GenCall algorithm and 10,000 SNPs were manually inspected to verify the algorithm calling. Individuals were excluded if genotypically not female, had overall call rate <95% or were ethnic outliers (248 cases) as identified by multi-dimensional scaling, combining the genotyping data with the three Hapmap2 populations. SNPs with a Gencall rate of <0.25, call rate <95% (call rate <99% if MAF <0.1) and HWE <10⁻⁷ or evidence of poor clustering on inspection of cluster plots were excluded. All SNPs with MAF <0.01 were excluded for this analysis. A cryptic relatedness analysis of the whole dataset was performed using 46,918 uncorrelated SNPs and there was no evidence of any duplicates.

For GLACIER cases and controls, principal component analysis (PCA) was carried out on a subset of 46,918 uncorrelated SNPs and used to exclude individuals or groups distinct from the main cluster using the first five principal components (PCs), Figure S7. Following removal of outliers (166 cases and 245 controls), the PCA was repeated and the first five PCs included as covariates in the analysis. The adequacy of the case-control matching was evaluated using quantile-quantile plots of test statistics and the inflation factor (λ) calculated using only uncorrelated SNPs that were not selected by BCAC and were not within one of the four common fine-mapping regions, to minimize selection for SNPs associated with breast cancer, Figure S1. As the majority of the SNPs on the iCOGS array were selected from GWAS of breast, ovarian and prostate cancer the SNPs selected for this analysis were taken from the set of SNPs selected by the prostate consortium, with the assumption that these SNPs were more likely to be representative of common SNPs in terms of population structure in our study than those selected by the breast or ovarian consortia.

For each SNP, we estimated a per-allele log-odds ratio (OR) and standard error by logistic regression, including the 5 PCs as covariates, using PLINK v1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>).

Genotyping and analysis of BCAC studies is described in detail elsewhere [24], in brief data were analyzed using the Genotype Library and Utilities (GLU) package to estimate per-allele ORs and standard errors for each SNP using unconditional logistic regression. All analyses were performed in subjects of European ancestry (determined by PC analyses) and adjusted for study and seven principal components.

Case-control odds ratio (OR) for ILC or LCIS cases vs controls from BCAC and GLACIER were combined using inverse variance-weighted fixed-effects meta-analysis, as implemented in

METAL [39]. Case-only analyses were also carried out to compare genotype frequencies for ILC vs LCIS (GLACIER and BCAC) and ILC vs IDC (BCAC studies only), and were used as a test for heterogeneity of ORs by tumor subtype. Any study without data on both histological subtypes was dropped from the case-only analysis.

Phase II. SNPs showing the strongest evidence for association with lobular tumors ($P < 5 \times 10^{-5}$) in the meta-analysis (after excluding previously reported loci) were genotyped at LGC Genomics (formerly KBiosciences) in Phase II samples. Duplicate samples genotyped on the iCOGS chip were included to assess the concordance of the two genotyping methods. Cluster plots for rs11977670 are shown in Figure S8.

A pooled analysis of ILC including Phase I (GLACIER and BCAC) and Phase II data was performed. Data were analyzed using STATA v.12 to estimate per-allele ORs and standard errors for each SNP using unconditional logistic regression. Differences in the strength of the associations with ILC, IDC and LCIS were assessed using case-only analyses. A sign test was used to test whether the number of SNPs showing associations in the same direction in two different subtypes (i.e. LCIS vs ILC, and IDC vs ILC) was significantly greater than expected by chance. A likelihood ratio test was used as a global test of the null hypothesis of no differences between subtypes for any of the ORs of the 75 known loci evaluated. Stratum-specific estimates of per-allele OR by categories of age and family history of disease were obtained from logistic regression models and differences in ORs across strata were tested using an interaction term.

Bioinformatics

In order to establish the SNP's functional role, a window of 10 kb both up and downstream was formed around the marker and pairwise r^2 values calculated using 1000 genome CEU population data. Three SNPs were identified as being in LD ($r^2 > 0.5$) with rs11977670 and were compared to next generation sequence technologies to elucidate the overlap between chromatin states (ENCODE Project). Two cell lines, normal human mammary epithelial (HMEC), and breast carcinoma (MCF-7), were used to establish these chromatin states, i.e. active or engaged enhancers (H3K27ac), nucleosome-depleted regions (DNase I and FAIRE), and RNA polymerase linked regions (Pol II). Expression data from the Cancer Genome Atlas Network for each gene within a 1 Mb window of rs11977670 was analyzed looking for differential expression in each breast cancer subtype (ER+ ILC, 40 cases; ER+ IDC, 341 cases; and ER-negative IDC, 108 cases). Allele data for surrogate SNP rs13225058 was obtained for all ER+ cases from TCGA. These 335 cases were used to produce genotype specific gene expression data in R. Differences in gene expression between the three genotypes were tested for using one-way-anova, verified by t-test and visually by boxplot. Linear regression was performed across all three genotypes using copy number variation as a co-variate. Level 3 copy number variation data (hg19 build) was obtained from the TCGA data portal.

Supporting Information

Figure S1 Quantile-quantile plot for GLACIER. A: QQ plot based on the 37544 uncorrelated SNPs not selected on the basis of breast cancer risk ($\lambda = 1.04$). B: QQ plot for all SNPs in dataset ($\lambda = 1.09$). (PPTX)

Figure S2 LD block containing rs1197790. (PPTX)

Figure S3 rs1197790 falls in a high H3K27ac region using ENCODE data from normal human mammary epithelial (HMEC), and breast carcinoma (MCF-7) cell lines to establish chromatin states in the region. (PPTX)

Figure S4 Gene expression data taken from TCGA for genes in a 1 Mb window of rs11977670. Three genes showed differential expression in ER+ ILC compared to ER+ IDC (*BRAF*, $P = 0.006$; *NDUFB2*, $P = 0.02$, *SLC37A3*, $P = 0.05$). (PPTX)

Figure S5 **a:** Genotype specific gene expression in ER+ Breast Cancers. Gene expression and genotype data was taken from TCGA and compared using a surrogate for rs11977670, rs13225058 ($r^2 = 0.79$) for 335 ER+ cancers. A significant difference between the AA and GG genotype was only found for two genes, *JHDM1D* and *SLC37A3*. **b:** Genotype specific gene expression in 36 Invasive Lobular Cancers. Gene expression and genotype data was taken from TCGA and compared using a surrogate for rs11977670, rs13225058 ($r^2 = 0.79$). (PPTX)

Figure S6 Genotype specific gene expression in 48 cases of normal breast tissue associated with ER+ breast cancer. Gene expression and genotype data was taken from TCGA and compared using a surrogate for rs11977670, rs13225058 ($r^2 = 0.79$) for 48 cases with normal breast tissue. (PPTX)

Figure S7 Results of principal components analysis (PCA) – GLACIER cohort. A: PCA with the 3 HapMap populations. B: PCA after exclusion of outliers (414 cases and 245 controls). (PPTX)

Figure S8 Cluster plots for rs11977670 is on chromosome 7 (139942304). A: Phase I – iCOGS Array – GLACIER (Illumina). B: Phase I – iCOGS Array – BCAC (Illumina). C: Phase II-KASPAR (LGC Genomics). (PPTX)

Table S1 Participating studies from the BCAC. (DOCX)

Table S2 Number of lobular breast cancer cases per study. (DOCX)

Table S3 Genotype-specific odds ratios for rs11977670 and risk of lobular-specific breast cancer (based on pooled analysis of phase I and II). (DOCX)

Table S4 Results for borderline SNPs not reaching GWS after Phase II. (XLSX)

Table S5 Pooled lobular analysis of known SNPs (BCAC and GLACIER). (XLSX)

Table S6 Lobular and ductal associations with breast cancer risk in BCAC subjects (ER pos only). (XLSX)

Table S7 Details of ethical approval boards for each study. (DOCX)

Table S8 Lobular cases and controls from UK BCAC studies. Controls from each of these studies were randomly selected to obtain a control group for GLACIER cases in a 1:2 case to control ratio. (DOCX)

Acknowledgments

The *in silico* functional results published here in whole or part based upon data generated by The Cancer Genome Atlas pilot project established by the NCI and NHGRI (Information about TCGA and the investigators and institutions who constitute the TCGA research network can be found at <http://cancergenome.nih.gov>) and the ENCODE Project Consortium, (Myers RM, Stamatoyannopoulos J, Snyder M, Dunham I, Hardison RC, Bernstein BE, Gingeras TR, Kent WJ, Birney E *et al.* A user's guide to the encyclopedia of DNA elements (ENCODE). *PLoS Biol.* 2011 Apr;9(4):e1001046. Epub 2011 Apr 19. PMID: 21526222; PMCID: PMC3079585)

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.

In particular, we thank: Maria Troy, Maggie Angelakos, Judi Maskiell, Gillian Dite, Annegien Broeks, Frans Hogervorst, Senno Verhoef, Emiel Rutgers, Ellen van der Schoot, Femke Atsma, Eileen Williams, Elaine Ryder-Mills, Kara Sargus, Niall McInerney, Gabrielle Colleran, Andrew Rowan, Angela Jones, Peter Bugert, Medical Faculty Mannheim, staff and participants of the Copenhagen General Population Study, Dorthe Uldall Andersen, Maria Birna Arnadóttir, Anne Bank, Dorthe Kjeldgård Hansen, Charo Alonso, Guillermo Pita, Nuria Álvarez, Daniel Herrero, Primitiva Menendez, José Ignacio Arias Pérez, Pilar Zamora, the Human Genotyping-CEGEN Unit (CNIO), Hartwig Ziegler, Sonja Wolf, Volker Hermann, Heide Hellebrand, Stefanie Engert, GC-HBOC (Supported by Deutsche Krebshilfe), Karl von Smitten, Tuomas Heikkinen, Dario Greco, Irja Erkkilä, Peter Hillemanns, Hans Christiansen and Johann H. Karstens, Eija Myöhänen, Helena Kemiläinen, Heather Thorne, Eveline Niedermayr, the AOCs Management Group (D Bowtell, G Chenevix-Trench, A deFazio, D Gertig, A Green, P Webb), the ACS Management Group (A Green, P Parsons, N Hayward, P Webb, D Whiteman), Sabine Behrens, Ursula Eilber, Muhabbet Celik, Janet Olson, Susan Slager, Celine Vachon, Siranoush Manoukian, Bernad Peissel, Daniela Zaffaroni of the Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Monica Barile, Irene Feroce of the Istituto Europeo di Oncologia (IEO), the personnel of the Cogentech Cancer Genetic Test Laboratory, Lesley Richardson, Marie-Claire Goulet, Mervi Grip, Meeri Otsukka, Kari Mononen, Teresa Selander, Nayana Weerasooriya, E. Krol-Warmerdam, J. Blom, Dr. J. Molenaar, Louise Brinton Stephen Chanock, Neonila Szeszenia-Dabrowska, Beata Peplonska, Witold Zatonski, Pei Chao, Michael Stagner, Petra Bos, Jannet Blom, Ellen Crepin, Anja Nieuwlaet, Annette Heemskerck, the Erasmus MC Family Cancer Clinic, Sue Higham, Helen Cramp, and Dan Conlley, the SEARCH and EPIC teams, Breakthrough Breast Cancer 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 studies.

Consortia members

GENICA Network. Hiltrud Brauch, Wing-Yee Lo, Christina Justenhoven: Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tübingen, Germany.

References

- Yoder BJ, Wilkinson EJ, Massoll NA. (2007) Molecular and morphologic distinctions between infiltrating ductal and lobular carcinoma of the breast. *Breast J* 13(2): 172–79
- Reeves, G. K., Beral, V., Green, J., Gathani, T. & Bull, D. (2006) Hormonal therapy for menopause and breast-cancer risk by histological type: a cohort study and meta-analysis. *Lancet Oncol* 7: 910–918.
- Kotsopoulos J, Chen WY, Gates MA, Tworoger SS, Hankinson SE, *et al.* (2010) Risk factors for ductal and lobular breast cancer: results from the nurses' health study. *Breast Cancer Res* 12, R106.
- Pestalozzi BC, Zahrieh D, Mallon E, Gusterson BA, Price KN, *et al.* (2008) Distinct clinical and prognostic features of infiltrating lobular carcinoma of the breast: combined results of 15 International Breast Cancer Study Group clinical trials. *J Clin Oncol* 26(18): 3006–3014.
- Cristofanilli M, Gonzalez-Angulo A, Sneige N, Kau SW, Broglio K, *et al.* (2005) Invasive lobular carcinoma classic type: response to primary chemotherapy and survival outcomes. *J Clin Oncol* 23(1):41–48
- Tubiana-Hulin M, Stevens D, Lasry S, Guinebretière JM, Bouita L, *et al.* (2006) Response to neoadjuvant chemotherapy in lobular and ductal breast carcinomas. *Ann Oncol* 17(8):1228–1233.
- Hwang ES, Nyante SJ, Yi Chen Y, Moore D, DeVries S, *et al.* (2004) Clonality of lobular carcinoma in situ and synchronous invasive lobular carcinoma. *Cancer* 100(12): 2562–2572.
- Chuba PJ, Hamre MR, Yap J, Severson RK, Lucas D *et al.* (2005) Bilateral risk for subsequent breast cancer after lobular carcinoma-in-situ: analysis of surveillance, epidemiology, and end results data. *J Clin Oncol* 23(24): 5534–5541.
- Fisher ER, Land SR, Fisher B, Mamounas E, Gilarski L, *et al.* (2004) Pathologic findings from the NSABBP: twelve-year observations concerning lobular carcinoma in situ. *Cancer* 15;100(2):238–244.
- Sasson AR, Fowble B, Hanlon AL, Torosian MH, Freedman G *et al.* (2001) Lobular carcinoma in situ increases the risk of local recurrence in selected patients with stages I and II breast carcinoma treated with conservative surgery and radiation. *Cancer* 91:1862–1869.
- Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, *et al.* (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature* 447, 1087–1093.
- Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, *et al.* (2007) A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat Genet* 39, 870–874.

Yon-Dschun Ko, Christian Baisch: Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn.

Hans-Peter Fischer: Germany Institute of Pathology, University of Bonn, Bonn.

Ute Hamann; Germany Molecular Genetics of Breast Cancer, DKFZ, Heidelberg, Germany.

Thomas Bruening, Beate Pesch, Sylvia Rabstein, Anne Lotz: Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Bochum, Germany Volker Harth: Institute for Occupational Medicine and Maritime Medicine, University Medical Center Hamburg-Eppendorf, Germany.

kConFab Investigators. See <http://www.kconfab.org/Organisation/Members.aspx>

Author Contributions

Conceived and designed the experiments: ES RR IT MGC. Performed the experiments: CP EPa AGN GP MRA DCT DV FB JD AMD. Analyzed the data: ES CP MNB SN MKB QW KM IT MGC. Contributed reagents/materials/analysis tools: ES RR CP MNB SN EPa OF SP AHa KK PGo MC JP IdSS NJ RS MDw KAP CG RH GR PDI MCS JLH EPc CA JW SC RK PAF SMJ ABE MWB MJK FM ASc CSo BBu PGu TT PLP PK SEB BGN SFN HF RLM JIAP PM JB HBre AKD VA CSt AMe PL RKS ML HBra HPF YDK HN TAM KA CB NVB TD AL SM AMa VKa VMK JMH GCT DL CW EVL SH JCC AR PS DEFJ PR PPe BBo SV GGG GS LB CAM CAH BEH FS LLM JS MSG FL MDu VKr RW KP AJV SK ILA JAK GG AMM PD RAEMT CMS MK JF SJC MES MJH AHo AMWvdO CHMvd JLi KC KH AC SSC MWRR MSh AJ JLu KJB KD ASw AA NO MSc FJC EH AGN GP MRA DCT DV FB MKB QW JD KM AMD PH DE PPh MKS IT MGC. Wrote the paper: ES IT MGC RR PH PPh MKS. Histopathology Review: SP AHa. Provided critical review of the manuscript: ES RR CP MNB SN EPa OF SP AHa KK PGo MC JP IdSS NJ RS MDw KAP CG RH GR PDI MCS JLH EPc CA JW SC RK PAF SMJ ABE MWB MJK FM ASc CSo BBu PGu TT PLP PK SEB BGN SFN HF RLM JIAP PM JB HBre AKD VA CSt AMe PL RKS ML HBra HPF YDK HN TAM KA CB NVB TD AL SM AMa VKa VMK JMH GCT DL CW EVL SH JCC AR PS DEFJ PR PPe BBo SV GGG GS LB CAM CAH BEH FS LLM JS MSG FL MDu VKr RW KP AJV SK ILA JAK GG AMM PD RAEMT CMS MK JF SJC MES MJH AHo AMWvdO CHMvd JLi KC KH AC SSC MWRR MSh AJ JLu KJB KD ASw AA NO MSc FJC EH AGN GP MRA DCT DV FB MKB QW JD KM AMD PH DE PPh MKS IT MGC. Approved the final version of the manuscript: ES RR CP MNB SN EPa OF SP AHa KK PGo MC JP IdSS NJ RS MDw KAP CG RH GR PDI MCS JLH EPc CA JW SC RK PAF SMJ ABE MWB MJK FM ASc CSo BBu PGu TT PLP PK SEB BGN SFN HF RLM JIAP PM JB HBre AKD VA CSt AMe PL RKS ML HBra HPF YDK HN TAM KA CB NVB TD AL SM AMa VKa VMK JMH GCT DL CW EVL SH JCC AR PS DEFJ PR PPe BBo SV GGG GS LB CAM CAH BEH FS LLM JS MSG FL MDu VKr RW KP AJV SK ILA JAK GG AMM PD RAEMT CMS MK JF SJC MES MJH AHo AMWvdO CHMvd JLi KC KH AC SSC MWRR MSh AJ JLu KJB KD ASw AA NO MSc FJC EH AGN GP MRA DCT DV FB MKB QW JD KM AMD PH DE PPh MKS IT MGC.

13. Stacey SN, Manolescu A, Sulem P, Rafnar T, Gudmundsson J, et al. (2007) Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet* 39, 865–869 (2007).
14. Stacey SN, Manolescu A, Sulem P, Thorlacius S, Gudjonsson SA et al. (2008) Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet* 40: 703–706.
15. Ahmed S, Thomas G, Ghousaini M, Healey CS, Humphreys MK, et al. (2009) Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet* 41, 585–590.
16. Zheng W, Long J, Gao YT, Li C, Zheng Y, Xiang YB, et al. (2009) Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat Genet* 41: 324–328.
17. Thomas G, Jacobs KB, Kraft P, Yeager M, Wacholder S, et al. (2009) A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat Genet* 41: 579–584.
18. Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A et al. (2010) Genome-wide association study identifies five new breast cancer susceptibility loci. *Nat Genet* 42: 504–507.
19. Antoniou AC, Wang X, Fredericksen ZS, McGuffog L, Tarrell R, et al. (2010) A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. *Nat Genet* 42: 885–892.
20. Fletcher O, Johnson N, Orr N, Hosking FJ, Gibson LJ, et al. (2011) Novel breast cancer susceptibility locus at 9q31.2: results of a genome-wide association study. *J Natl Cancer Inst* 103: 425–435.
21. Haiman CA, Chen GK, Vachon CM, Canzian F, Dunning A, et al. (2011) A common variant at the TERT-CLPTM1L locus is associated with estrogen receptor-negative breast cancer. *Nat Genet* 43: 1210–1214.
22. Ghousaini M, Fletcher O, Michailidou K, Turnbull C, Schmidt MK, et al. (2012) Genome-wide association analysis identifies three new breast cancer susceptibility loci. *Nat Genet* 44: 312–318.
23. Siddiq A, Couch FJ, Chen GK, Lindström S, Eccles D, et al. (2012) A meta-analysis of genome-wide association studies of breast cancer identifies two novel susceptibility loci at 6q14 and 20q11. *Hum Mol Genet* 21: 5373–5384.
24. Michailidou K, Hall P, Gonzalez-Neira A, Ghousaini M, Dennis J, et al. (2013) Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat Genet* 45: 353–361
25. Garcia-Closas M, Couch FJ, Lindstrom S, Michailidou K, Schmidt MK, et al. (2013) Genome-wide association studies identify four ER negative-specific breast cancer risk loci. *Nat Genet* 45(4):392–398
26. Figueroa JD, Garcia-Closas M, Humphreys M, Platte R, Hopper JL et al. (2011) 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* 20(23): 4693–706.
27. Cancer Genome Atlas Network. (2012) Comprehensive molecular portraits of human breast tumours. *Nature* 490(7418): 61–70.
28. Osawa T, Muramatsu M, Wang F, Tsuchida R, Kodama T, et al. (2011) Increased expression of histone demethylase JHDM1D under nutrient starvation suppresses tumor growth via down-regulating angiogenesis. *PNAS* 108(51): 20725–20729.
29. Ibrahim SA, Yip GW, Stock C, Pan JW, Neubauer C, et al. (2012) Targeting of syndecan-1 by microRNA miR-10b promotes breast cancer cell motility and invasiveness via a Rho-GTPase- and E-cadherin-dependent mechanism. *Int J Cancer* 131(6): E884–896.
30. Pharoah PD, Guilford P, Caldas C, International Gastric Cancer Linkage Consortium. (2001) Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology* 121(6):1348–53.
31. Xie ZM, Li LS, Laquet C, Penault-Llorca F, Uhrhammer N, et al. (2011) Germline mutations of the E-cadherin gene in families with inherited invasive lobular breast carcinoma but no diffuse gastric cancer. *Cancer* 117(14): 3112–3117.
32. Weigelt B, Horlings HM, Kreike B, Hayes MM, Hauptmann M, et al. (2008) Refinement of breast cancer classification by molecular characterization of histological special types. *J Pathol* 216(2): 141–150
33. Milne RL, Goode EL, Garcia-Closas M, Couch FJ, Severi G et al. (2011) Confirmation of 5p12 as a susceptibility locus for progesterone-receptor-positive, lower grade breast cancer. *Cancer Epidemiol Biomarkers Prev* 10: 2222–2231
34. Pharoah PD, Tsai YY, Ramus SJ, Phelan CM, Goode EL et al. (2013) GWAS meta-analysis and replication identifies three new susceptibility loci for ovarian cancer. *Nat Genet* 45: 362–370.
35. Dobbins SE, Broderick P, Melin B, Feychting M, Johansen C et al. (2011) Common variation at 10p12.31 near MLLT10 influences meningioma risk. *Nat Genet* 43(9):825–7.
36. Curtis RE, Benjamin ER, Hankey BF, Hoover RN. New Malignancies Following Breast Cancer. Seer Cancer Registry. Available: <http://www.seer.cancer.gov/publications/>
37. Custer BS, Koepsell TD, Mueller BA. (2002) The association between breast carcinoma and meningioma in women. *Cancer* 94(6):1626–1635
38. Swann R, Perkins KA, Velentzis LS, Ciria C, Dutton SJ, et al. (2013) The DietCompLyf study: A prospective cohort study of breast cancer survival and phytoestrogen consumption. *Maturitas* 3: 232–240.
39. Willer CJ, Li Y, Abecasis GR (2010) METAL: fast and efficient meta-analysis of genome wide association scans. *Bioinformatics* 26: 2190–2191