Identification and characterization of novel associations in the CASP8/ALS2CR12 region on chromosome 2 with breast cancer risk


1The authors wish it to be known that, in their opinion, the first 2 and 4 final authors should be regarded as having contributed equally to this work.
Caroline Seynaeve\textsuperscript{118}, Christi J. Van Asperen\textsuperscript{117}, Montserrat García-Closas\textsuperscript{22,119}, Jonine Figueroa\textsuperscript{122}, Jolanta Lissowska\textsuperscript{12}, Louise Brinton\textsuperscript{122}, Kamila Czene\textsuperscript{74}, Hatem Darabi\textsuperscript{74}, Mikael Eriksson\textsuperscript{74}, Judith S. Brand\textsuperscript{74}, Maartje J. Hooning\textsuperscript{118}, Antoinette Hollestelle\textsuperscript{118}, Ans M.W. Van Den Ouweland\textsuperscript{124}, Agnes Jager\textsuperscript{118}, Jingmei Li\textsuperscript{126}, Jianjun Liu\textsuperscript{125}, Keith Humphreys\textsuperscript{74}, Xiao-Ou Shu\textsuperscript{106}, Wei Lu\textsuperscript{126}, Yu-Tang Gao\textsuperscript{127}, Hui Cai\textsuperscript{106}, Simon S. Cross\textsuperscript{128}, Malcolm W. R. Reed\textsuperscript{1}, William Blot\textsuperscript{106,129}, Lisa B. Signorello\textsuperscript{106,129}, Qiuyin Cai\textsuperscript{106}, Paul D.P. Pharoah\textsuperscript{4,5}, Barbara Perkins\textsuperscript{5}, Mitul Shah\textsuperscript{5}, Fiona M. Blows\textsuperscript{5}, Daeehee Kang\textsuperscript{130,133}, Keun-Young Yoo\textsuperscript{132}, Dong-Young Noh\textsuperscript{131}, Mikael Hartman\textsuperscript{134,135,137}, Hui Miao\textsuperscript{134,137}, Kee Seng Chia\textsuperscript{134,137}, Thomas Choudary Putti\textsuperscript{136}, Ute Hamann\textsuperscript{33}, Craig Luccarini\textsuperscript{5}, Caroline Baynes\textsuperscript{5}, Shahana Ahmed\textsuperscript{5}, Mel Maranian\textsuperscript{5}, Catherine S. Healey\textsuperscript{5}, Anna Jakubowska\textsuperscript{138}, Jan Lubinski\textsuperscript{138}, Katarzyna Jaworska-Bieniek\textsuperscript{138}, Katarzyna Durda\textsuperscript{138}, Suleeporn Sangrajrang\textsuperscript{139}, Valerie Gaborieau\textsuperscript{140}, Paul Brennan\textsuperscript{140}, James Mckay\textsuperscript{140}, Susan Slager\textsuperscript{129}, Amanda E. Toland\textsuperscript{141}, Drakoulis Yannoukakos\textsuperscript{142}, Chen-Yang Shen\textsuperscript{143,145}, Chia-Ni Hsiung\textsuperscript{143}, Pei-Ei Wu\textsuperscript{144}, Shian-ling Ding\textsuperscript{146}, Alan Ashworth\textsuperscript{22}, Michael Jones\textsuperscript{119}, Nick Orr\textsuperscript{23}, Anthony J Swerdlow\textsuperscript{119,120}, Helen Tsimiklis\textsuperscript{11}, Enes Makalic\textsuperscript{12}, Daniel F. Schmidt\textsuperscript{12}, Quang M. Bui\textsuperscript{12}, Stephen J. Chanock\textsuperscript{122}, David J. Hunter\textsuperscript{147}, Rebecca Hein\textsuperscript{34,148}, Norbert Dahmen\textsuperscript{149}, Lars Beckmann\textsuperscript{150}, Kirsirami Aaltonen\textsuperscript{62,65}, Taru A. Muranen\textsuperscript{62,65}, Tuomas Heikkinen\textsuperscript{62,65}, Astrid Iwamoto\textsuperscript{125}, Nazneen Rahman\textsuperscript{119}, Clare A. Turnbull\textsuperscript{119}, The Breast and Ovarian Cancer Susceptibility (BOCS) Study\textsuperscript{119}, Quinten Waisfisz\textsuperscript{151}, Hanne E. M. Meijers-Heijboer\textsuperscript{151}, Muriel A. Adank\textsuperscript{151}, Rob B. Van Der Luijt\textsuperscript{152}, Per Hall\textsuperscript{174}, Georgia Chenevix-Trench\textsuperscript{6}, Alison Dunning\textsuperscript{5}, Douglas F. Easton\textsuperscript{4,5,†} and Angela Cox\textsuperscript{1,∗,†}

\textsuperscript{1}Department of Oncology, University of Sheffield Medical School, Sheffield S10 2RX, UK, \textsuperscript{2}Department of Neurosurgery, Chang Gung Memorial Hospital, Taoyuan County 333, Taiwan, \textsuperscript{3}Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT 84108-1266, USA, \textsuperscript{4}Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, \textsuperscript{5}Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge CB1 8RN, UK, \textsuperscript{6}Department of Genetics, \textsuperscript{7}QIMR Berghofer Medical Research Institute, Brisbane 4006, Australia, \textsuperscript{8}Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, \textsuperscript{9}Department of Pathology, \textsuperscript{10}Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, Melbourne School of Population Health, \textsuperscript{11}Genetic Epidemiology Laboratory, Department of Pathology, \textsuperscript{12}Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, Melbourne School of Population Health, The University of Melbourne, Melbourne, VIC 3010, Australia, \textsuperscript{13}Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam 1066 CX, the Netherlands, \textsuperscript{14}Division of Health Sciences, Warwick Medical School, Warwick University, Coventry CV4 7AL, UK, \textsuperscript{15}Institute of Population Health, University of Manchester, Manchester M13 9QQ, UK, \textsuperscript{16}Ministry of Public Health, Nonthaburi 11000, Thailand, \textsuperscript{17}University Breast Center Franconia, Department of Gynecology and Obstetrics, \textsuperscript{18}Institute of Human Genetics, University Hospital Erlangen, Friedrich Alexander University Erlangen-Nuremberg, Erlangen D-91054, Germany, \textsuperscript{19}David Geffen School of Medicine, Department of Medicine Division of Hematology and Oncology, University of California, Los Angeles, CA 90095, USA, \textsuperscript{20}Non-communicable Disease Epidemiology Department, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK, \textsuperscript{21}Breakthrough Breast Cancer Research Centre, \textsuperscript{22}Breakthrough Breast Cancer Research Centre, Division of Breast Cancer Research, \textsuperscript{23}Breakthrough Breast Cancer Research Centre and Division of Breast Cancer Research, The Institute of Cancer Research, London SW3 6JB, UK, \textsuperscript{24}Division of Cancer Studies, Kings College London, Guy’s Hospital, London SE1 9RT, UK, \textsuperscript{25}Wellcome Trust Centre for Human Genetics and Oxford Biomedical Research Centre, University of Oxford, Oxford OX3 7BN, UK, \textsuperscript{26}School of Medicine, National University of Ireland, Galway, Ireland, \textsuperscript{27}Department of Obstetrics and

\textsuperscript{*}To whom correspondence should be addressed at: Sheffield University Medical School, Beech Hill Road, Sheffield S10 2RX, UK. Tel: +44 1142712373; Fax: +44 1142711602; Email: a.cox@sheffield.ac.uk
Previous studies have suggested that polymorphisms in CASP8 on chromosome 2 are associated with breast cancer risk. To clarify the role of CASP8 in breast cancer susceptibility, we carried out dense genotyping of this region in the Breast Cancer Association Consortium (BCAC). Single-nucleotide polymorphisms (SNPs)
spanning a 1 Mb region around CASP8 were genotyped in 46 450 breast cancer cases and 42 600 controls of European origin from 41 studies participating in the BCAC as part of a custom genotyping array experiment (iCOGS). Missing genotypes and SNPs were imputed and, after quality exclusions, 501 typed and 1232 imputed SNPs were included in logistic regression models adjusting for study and ancestry principal components. The SNPs retained in the final model were investigated further in data from nine genome-wide association studies (GWAS) comprising in total 10 052 case and 12 575 control subjects. The most significant association signal observed in European subjects was for the imputed intronic SNP rs1830298 in ALS2CR12 (telomeric to CASP8), with per allele odds ratio and 95% confidence interval [OR (95% confidence interval, CI)] for the minor allele of 1.05 (1.03–1.07), \( P = 1 \times 10^{-5} \). Three additional independent signals from intronic SNPs were identified, in CASP8 (rs36043647), ALS2CR11 (rs59278883) and CFLAR (rs7558475). The association with rs1830298 was replicated in the imputed results from the combined GWAS (\( P = 3 \times 10^{-5} \)), yielding a combined OR (95% CI) of 1.06 (1.04–1.08), \( P = 1 \times 10^{-9} \). Analyses of gene expression associations in peripheral blood and normal breast tissue indicate that CASP8 might be the target gene, suggesting a mechanism involving apoptosis.

**INTRODUCTION**

Breast cancer is a complex disease with high, moderate and low penetrance germ-line variants involved in its etiology (1). In recent years, ~80 low penetrance breast cancer alleles have been identified, with modest odds ratios, ranging from 1.05 to 1.4, and together accounting for around 15% of familial breast cancer risk (2,3). It is likely that there are many more loci with even smaller effect sizes that remain to be identified, accounting for a further 14–15% of familial risk (2). One of the first low penetrance breast cancer variant associations to be convincingly replicated by large case–control studies was the single-nucleotide polymorphism (SNP) rs1045485 encoding the missense alteration D302H in the caspase 8 apoptosis-related cysteine peptidase (CASP8) gene at chromosome region 2q33 (4,5). This association signal, but this does not preclude the possibility of further region, candidate 12) genes (Fig. 1; Supplementary Material, Table S2). The strongest signals came from imputed SNP rs1830298 in ALS2CR12, with minor allele frequency (MAF) of 0.29 and an estimated OR (95% CI) per copy of the minor allele of 1.05 (1.03–1.07), \( P = 1.1 \times 10^{-5} \), and the genotyped SNP rs10197246 (MAF = 0.28), with odds ratio (95% CI) 1.05 (1.02–1.07), \( P = 2.5 \times 10^{-5} \). These two SNPs are highly correlated and likely reflect the same signal (\( r^2 = 0.9 \)).

Two previously reported susceptibility SNPs, CASP8 D302H (rs1045485) and rs10931936, were weakly replicated in iCOGS European data (Supplementary Material, Table S2), with minor allele OR in the same direction; however, the iCOGS OR estimates were much weaker than those from the original reports (5,8). The minor C allele of rs1045485 (MAF = 0.11) yielded an OR (95% CI) of 0.97 (0.94–1.0), \( P = 0.03 \), in contrast to 0.88 (0.84–0.92) reported in Cox et al. (5). Similarly, the rs10931936 minor allele (MAF = 0.28) was associated with a 4% increased breast cancer risk [OR (95% CI) = 1.04 (1.02–1.06), \( P = 1.9 \times 10^{-4} \)], compared with the 12% increase presented in Turnbull et al. (8). The latter SNP is strongly correlated with the iCOGS best hit rs1830298 (\( r^2 = 0.96 \)), but there is very little correlation between rs1045485 and rs1830298 (\( r^2 = 0.055 \)).

**RESULTS**

**Breast cancer risk associations in the CASP8 region on chromosome 2**

A summary of the breast cancer risk associations of 1733 typed and imputed SNPs across a 1 Mb region surrounding CASP8, based on the iCOGS European data, is shown in Figure 1. The most significant associations were for SNPs in the CASP8 and ALS2CR12 (amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 12) genes (Fig. 1; Supplementary Material, Table S2). The strongest signals came from imputed SNP rs1830298 in ALS2CR12, with minor allele frequency (MAF) of 0.29 and an estimated OR (95% CI) per copy of the minor allele of 1.05 (1.03–1.07), \( P = 1.1 \times 10^{-5} \), and the genotyped SNP rs10197246 (MAF = 0.28), with odds ratio (95% CI) 1.05 (1.02–1.07), \( P = 2.5 \times 10^{-5} \). These two SNPs are highly correlated and likely reflect the same signal (\( r^2 = 0.9 \)).

Identification of possible independent signals in iCOGS European data

The SNPs in the main association peak have similar ORs for breast cancer, are strongly correlated with one another (\( r^2 > 0.66 \)) and confined to an 82 kb region spanning the CASP8 and ALS2CR12 genes, and are therefore likely to reflect a single association signal, but this does not preclude the possibility of other signals in the region. To test this hypothesis, we carried out a regression analysis testing the association of individual
SNPs adjusted for the top hit rs1830298, in the iCOGS European dataset (Supplementary Material, Table S3). Interestingly, while this resulted in the loss of the signal from the main peak in CASP8/ALS2CR12, residual associations remained (e.g. 43 SNPs with $P \leq 1 \times 10^{-3}$), suggesting that there may be further signals present in the region, albeit weaker (Supplementary Material, Table S3 and Fig. S1). To investigate this further, we carried out penalized logistic regression analysis of all 1733 SNPs to identify the best subset of SNPs that explain the association, using HyperLasso (10). This identified 59 models containing combinations of 27 SNPs (Supplementary Material, Table S2), but many of these models were equivalent after taking into account linkage disequilibrium between SNPs. To obtain the most parsimonious model, we carried out stepwise forward logistic regression on the 27 SNPs, which resulted in a model containing four SNPs: rs1830298 ($ALS2CR12$; $p_{\text{conditional}} = 9.3 \times 10^{-3}$, MAF = 0.29), rs36043647 (CASP8; $p_{\text{conditional}} = 1.9 \times 10^{-4}$, MAF = 0.06), rs59278883 ($ALS2CR11$; $p_{\text{conditional}} = 6.1 \times 10^{-4}$, MAF = 0.07) and rs75584755 (CFLAR; CASP8- and FADD-like apoptosis regulator; $p_{\text{conditional}} = 9.2 \times 10^{-4}$, MAF = 0.07). We refer to these four SNPs, marking four independent sets of correlated highly associated variants (iCHAVs), as index SNPs.

**Meta-analysis of iCOGS and combined nine GWAS data**

We first examined the results for the four index SNPs, together with the previous hits rs1045485 and rs10931936, in the combined nine GWAS meta-analysis, and then carried out a further meta-analysis combining the iCOGS European data with the combined nine GWAS for these SNPs (total sample size 56502 cases and 55175 controls; Supplementary Material, Tables S4 and S5). We found that the top index SNP, rs1830298, replicated in the combined GWAS data alone ($P = 2.7 \times 10^{-6}$), and reached genome-wide significance ($P = 1.1 \times 10^{-5}$) in the meta-analysis combining both the iCOGS and combined GWAS data. Index SNPs correspond to iCHAVs as follows: rs1830298; iCHAV1, rs36043647; iCHAV2, rs59278883; iCHAV3, rs7558475; iCHAV4.

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**Figure 1.** Breast cancer associations within the 1 Mb region surrounding CASP8.

The upper panel plots SNPs based on their chromosomal coordinates on the x-axis and their $P$-values on the $-\log_{10}$ scale on the y-axis. Circle and diamond symbols represent typed and imputed SNPs, respectively. The colors indicate the pairwise $r^2$ with index SNP for iCHAV1, rs1830298 (highlighted in purple); $r^2$ is calculated based on the European panel in the 1000 genomes project. The ranges of iCHAVs 1–4 are indicated with colored shading. Genes within the region are indicated in the lower panel, with arrows indicating transcript direction, dense blocks for exons and lines for introns. The plot was generated using LocusZoom (9).

**Figure 2.** Associations of the four index SNPs corresponding to iCHAVs 1–4, and the two previous associations, in iCOGS European subjects and GWAS data. Squares denote the per-allele OR for the minor allele based on iCOGS and nine GWAS data, with the size of the square proportional to the sample size. Diamonds represent the pooled estimates of ORs under the fixed effect model after exclusion of the 1955 samples from the iCOGS data that were also in the combined GWAS data. Index SNPs correspond to iCHAVs as follows: rs1830298; iCHAV1, rs36043647; iCHAV2, rs59278883; iCHAV3, rs7558475; iCHAV4.
null result for rs36043647 ($P = 0.58$) and borderline evidence for rs7558475 ($P = 0.05$) (Supplementary Material, Table S5; Fig. 2). However, these three index SNPs all showed some evidence of association in the meta-analysis of iCOGS and combined GWAS (Supplementary Material, Table S5 and Fig. 2), providing some support for the existence of four signals in the region. Consistent with its strong correlation with rs1830298, a similar but slightly weaker signal was found for rs10931936 ($P = 1.0 \times 10^{-7}$). Weak evidence for association was observed for CASP8 D302H rs1045485 ($P = 1.1 \times 10^{-3}$).

Analysis of index SNPs in different ethnic groups

We next explored these four associations in the available Asian and African-American populations genotyped as part of COGS (Fig. 3; Supplementary Material, Table S6). Figure 3 shows the study-specific OR for rs1830298 by the three ethnic groups. The rs1830298 OR were homogeneous among European studies ($p_{\text{het}} = 0.54$, $I^2 = 0$) and African-American studies ($p_{\text{het}} = 0.40$, $I^2 = 0$), but were more heterogeneous among the nine Asian studies ($p_{\text{het}} = 0.025$, $I^2 = 54$), although the combined effect size in Asians was similar to that seen in Europeans [OR (95% CI) = 1.04 (0.95–1.13); $P = 0.44$], and slightly stronger in African Americans [OR (95% CI) = 1.12 (0.96–1.30); $P = 0.16$]. Although estimates in both Asian and African-American populations were not statistically significant, the ORs were consistent with the European data, and the pooled OR (95% CI) was 1.05 (1.03–1.07); $P = 4.1 \times 10^{-6}$ for all populations combined. The MAF of CASP8 rs36043647 was much lower in Asians, in whom the association was in the opposite direction to that in Europeans and African Americans, with an OR (95% CI) of 1.69 (1.13–2.51), $P = 0.009$, for the minor allele (Supplementary Material, Table S6). We did not observe any association of rs59278883 and rs7558475 in Asian and African-American populations (Supplementary Material, Table S6).

Subtype and survival analysis in iCOGS

To investigate whether these SNP associations vary with clinical subtypes of breast cancer, we explored potential subtype-specific associations by comparing different subtypes to all controls in the iCOGS European data. The OR estimates by tumor estrogen receptor (ER) status, triple negative status and invasiveness of breast cancer were all similar and close to the OR of 1.05 seen in overall breast cancer for rs1830298 (Fig. 4). Similarly, no significant differences in OR were seen when cases were stratified by family history, tumor grade, tumor stage, tumor size and lymph node status (Supplementary Material, Fig. S2). A broadly similar picture was seen for the other index SNPs (Supplementary Material, Figs S2 and S3).

SNP effects were also evaluated for overall survival and breast cancer-specific survival. There were 4191 deaths among 39 140 breast cancer patients with known vital status in the iCOGS European dataset. Of these deaths, 1979 died from breast cancer. We did not observe any associations between the index SNPs or previous hit SNPs with either overall or breast cancer-specific survival, and all hazard ratios (HR) were close to unity (data not shown).
In silico functional and expression quantitative trait loci annotations

We examined available in silico functional and expression quantitative trait loci (eQTL) data for the four iCHAVs. Of interest in iCHAV1, rs3769823 is a missense alteration encoding K14R in the 4th exon of CASP8, which encodes the N-terminus of protein isoform 9. In addition, this SNP and rs3769821 are both located in a region of deoxyribonuclease I hypersensitivity and histone H3K27 acetylation in breast cell lines (Fig. 5). The minor allele of both of these SNPs, together with four others in iCHAV1 for which data were available, were associated with a reduction in CASP8 mRNA levels in peripheral blood samples in the eQTL meta-analysis of Westra et al. (P ≤ 9.4 × 10^{-5}; Supplementary Material, Table S7; Fig. 5) (11). The cancer genome atlas (TCGA) dataset only had data available for two SNPs from iCHAV1, and both were associated with a reduction in CASP8 mRNA in normal breast tissue (P ≤ 1 × 10^{-3}; Supplementary Material, Table S7; Fig. 5). No strong eQTL associations were seen for other genes in the region in either the Westra et al. or the TCGA data. Taken together, these data suggest that one or more variants in iCHAV1 may affect levels of CASP8 gene expression. As shown in Figure 5, iCHAVs 3 and 4 overlap enhancer sites identified in Hnisz et al.; a CASP8 enhancer in MCF7 cells and a CFLAR enhancer in human mammary epithelial cells, respectively (12). However, there was limited eQTL data available for these iCHAVs, with no evidence of any significant eQTLs (Supplementary Material, Table S7).

DISCUSSION

In our analysis of the genomic region surrounding CASP8 for association with breast cancer, the strongest signal came from an imputed SNP, rs1830298, in the ASL2CR12 gene (iCHAV1). A strongly correlated genotyped SNP (rs10197246; r^2 = 0.9, 23.5 kb telomeric in the same gene), yielded a similar association signal (P = 1.1 × 10^{-5} and 2.5 × 10^{-5}, respectively). In each case, the rare allele (MAF = 0.28) was associated with an increase in the risk of breast cancer of 5% [OR(95% CI) 1.05 (1.03, 1.07) and 1.05 (1.02, 1.07), respectively]. The odds ratios for both SNPs are consistent in Europeans, Asians and African Americans (although not statistically significant in the smaller non-European cohorts), and were replicated in the combined GWAS data, achieving a genome-wide level of significance when the iCOGS and GWAS data were combined (P = 1.1 × 10^{-5} and P = 1.7 × 10^{-5}, respectively). This association is consistent between ER-positive and -negative disease, and between invasive and in situ cancers (Fig. 4). The previously published result for rs10931936 in the UK GWAS is consistent with its correlation with rs1830298 (8).

Several of the SNPs in iCHAV1 were associated with CASP8 eQTLs. The minor alleles of SNPs in this group, associated with increased risk of breast cancer, are associated with reduced CASP8 mRNA levels in both peripheral blood lymphocytes and normal breast tissue (Supplementary Material, Table S7; Fig. 5). These data suggest that CASP8 may be the target gene of iCHAV1, and are consistent with a hypothesis in which the effect of the risk alleles is via reduced levels of apoptosis, thus promoting tumor initiation. However, further functional studies are required to demonstrate a direct interaction between iCHAV1 and the CASP8 promoter and to investigate the allele-specific functional effects of these SNPs in different tissue types.

Our results also suggest three other independent signals in the region; the most significant SNPs for these three signals are in CASP8 (iCHAV2), ASL2CR11 (iCHAV3) and the anti-apoptotic gene CFLAR (iCHAV4); see Figure 2; Supplementary Material, Table S5. The signals for iCHAVs 3 and 4 were replicated in the combined GWAS, but since they did not achieve genome-wide levels of significance even in the very large datasets analyzed here, they are harder to interpret. However, it is interesting that both these iCHAVs overlap enhancer regions (Fig. 5).

As previously noted, we find only very weak support for an association of rs1045485/D302H in the iCOGS data (P = 0.03) (2), although the odds ratio in the combined GWAS data was more consistent with the original report [OR (95% CI) = 0.90 (0.85, 0.96), P = 0.0007] (5). At present, the reasons for the discrepancy with the original report are not clear. D302H is only weakly correlated with any of the four index SNPs identified here (max r^2 = 0.06 with rs1830298). However, it is correlated with rs28845859 (r^2 = 0.67); the latter SNP is associated with reduced breast cancer risk in the iCOGS data (OR 0.95, P = 1.9 × 10^{-4}; Supplementary Material, Table S2) and
combined GWAS \((P = 4.0 \times 10^{-5})\). We found no significant differences between subtypes, although the associated effect for D302H was stronger (and borderline significant) for triple negative disease, despite the smaller sample size (Supplementary Material, Fig. S3). Further investigation with a larger sample of triple negative cases may help clarify this point.

The association for the top \(CASP8\) index SNP, rs1830298, represents one of the smaller effect sizes identified to date for breast cancer. However, it is worth noting that the \(CASP8\) region has recently been reported to be associated with other cancers at genome-wide levels of significance, including melanoma and chronic lymphocytic leukemia (CLL) (13,14). The alleles associated with increased risk in melanoma are correlated with rs1830298, but the signal in CLL appears to be due to uncorrelated SNPs in the region. This difference may reflect the different cell type of origin and it will be interesting to determine the relative importance and function of alleles of the \(CASP8\) gene family in immune cell lineages, compared with that in epithelial cancers.

MATERIALS AND METHODS

Study samples

The iCOGS and nine breast cancer GWAS datasets have been described in detail previously (2). Briefly, the COGS includes a total of 103,991 women from 50 studies participating in the BCAC whose DNA samples were genotyped with the iCOGS array. These were 89,050 Europeans (46,450 cases; 42,600 controls), 12,893 Asians (6,629 cases; 6,624 controls) and 20,484 African Americans (11,16 cases and 932 controls). The numbers of subjects by study are detailed in Supplementary Material, Table S1. Approximately 93% of cases had invasive breast cancer (Supplementary Material, Table S1). The combined nine breast cancer GWAS dataset comprised 10,052 cases and 12,575 controls of European ancestry from United States, UK, Australia, Germany, Finland, Sweden and the Netherlands (2).

Ethics statement

Each study was approved by the relevant local/institutional Research Ethics Committee, and all subjects gave written informed consent to take part.

SNP selection for fine-scale mapping on the iCOGS array

The region for analysis on chromosome 2 was defined such that it contained all SNPs correlated \((r^2 \geq 0.1)\) with the SNPs previously reported to be associated with breast cancer, namely \(CASP8 D302H\) (rs1045485) and rs10931936 (5,8). This identified a 1 Mb region from 201,566,128 to 202,566,128 (hg19). In March 2010 when the iCOGS array was designed, 2,191 SNPs had been catalogued in this region by the 1000 genomes and HapMap3 projects. Of these, 1,723 SNPs had an MAF \(\geq 2\%\), and of these 1,723, there were 988 SNPs with Illumina assay design scores of \(\geq 0.8\). We selected a total of 280 SNPs correlated at \(r^2 \geq 0.1\) with rs1045485 or rs10931936, plus 288 tagSNPs which tagged the remaining 708 SNPs at \(r^2 \geq 0.9\). Another 45 SNPs in the region, nominated by other consortia members, were included as part of the genotyping array that comprised 211,155 SNPs in total (2).

Genotyping and quality control

Genotyping, allele calling, quality control and principal components analysis for COGS are described in detail in Michailidou et al. (2). Genotyping was carried out at four centers using the Illumina Infinium iCOGS array, including 2% duplicates from each participating study. Final genotype calls were made using Illumina’s proprietary GenCall algorithm. SNPs were excluded from analysis if the overall call rate was <95%, duplicate concordance rate was <98%, or if deviation from Hardy–Weinberg equilibrium in controls was significant at \(P < 1 \times 10^{-7}\) (2). Subjects were excluded from analysis for the following reasons: genotypically non-female; overall call rate <95%; low or high heterozygosity (\(P < 1 \times 10^{-6}\)); discordant replicates or cryptic duplicates. Genotype data and ancestry principal components (seven principal components for the European and two each for the Asian and African-American populations) were thus available for 103,991 individuals.

Statistical analysis

The iCOGS \(CASP8\) region genotype data were split into four groups for efficiency of imputation of missing genotypes and untyped SNPs. These comprised 36,793 European ancestry subjects from North American and UK studies in Group 1, with 26,129 and 26,128 of the remaining European subjects in Groups 2 and 3, respectively, and 14,941 Asians and African Americans in Group 4. Imputations were carried out separately by group based on the 1000 genomes phase I reference panel with singleton variants excluded, using IMPUTE2 version 2.3 (15,16). SNPs were included in the subsequent analyses if the mean information score of the European groups was \(\geq 0.9\), and untyped imputed SNPs were only included if their MAF was \(\geq 3\%\); these criteria resulted in inclusion of 501 typed and 1232 imputed SNPs in the final analysis. The imputation accuracy for rs1830298 was verified in whole-genome sequence data from 197 individuals; the correlation between the observed and imputed genotypes was 0.974. The imputation step increases the number of common SNPs captured at \(r^2 > 0.9\) from 76% (1198/1583) to 84% (1333/1583).

The main analyses were based on the data for individuals of European ancestry. For each SNP, allelic dosage of the minor allele was estimated, and included in a logistic regression model, to estimate OR and corresponding 95% CI. Covariates for each study plus the seven ancestry principal components were included in the model (2). These analyses were implemented in R. \(P\)-values from the Wald test are reported in the text (uncorrected for multiple testing). FDR values in Supplementary Material, Table S2 were calculated according to the Benjamini & Hochberg method, as implemented in the R p.adjust function (17). Penalized logistic regression models (based on the normal exponential gamma probability density) were implemented in HyperLasso (10), including all 501 typed and 1232 imputed SNPs, to identify the best subsets of SNPs to account for the observed association data. Based on the sample size and a type I error of 0.001, a \(z\) of 0.05 and penalty of 491 were specified in HyperLasso, according to equation 7 in Hoggart.
et al. (10). Candidate SNPs were then compiled from the resulting HyperLasso models and included in a stepwise forward logistic regression procedure with penalty \(k = 10\) in the step function in R to identify the most parsimonious model, as described previously (18). The SNPs retained in the final model are referred to as index SNPs.

Index SNPs were further examined by means of meta-analysis of iCOGS European, Asian and African-American data, and also with individual SNP results from the combined nine breast cancer GWAS (2). Due to an overlap of 1955 samples that exist in both the iCOGS and the combined GWAS data, we removed these samples from the iCOGS data before carrying out the meta-analysis. The meta-analysis was carried out using the MetaFor package in R, with inverse-variance weights and the DerSimonian-Laird estimator for the random effects model (19). We used the threshold of \(P = 5 \times 10^{-8}\) to define genomewide significance (2).

The index SNPs were also examined for associations with breast cancer specific and overall survival in Cox’s proportional hazard models, including age at diagnosis, study and seven principal components as covariates, and accounting for the left-censoring time between study entry and diagnosis. Further adjustment was carried out for stage, grade, tumor size and lymph node involvement for SNPs with nominally significant associations with survival \((P < 0.05)\). These analyses were implemented in R.

**In silico functional and eQTL annotations**

We defined independent sets of iCHAVs with likelihood (determined from the individual-SNP logistic regression analysis) relative to an index SNP of \(>1/100\) and degree of correlation with the index SNP of \(>0.65\). The ENCODE integrated regulation data for each SNP were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20). Predicted enhancers and target genes were retrieved from the UCSC Genome Browser by use of ANNOVAR (20).

**SUPPLEMENTARY MATERIAL**

Supplementary Material is available at HMG online.

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