

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

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260 **Abstract**

261 Previous studies have suggested that polymorphisms in *CASP8* on chromosome 2 are
262 associated with breast cancer risk. To clarify the role of *CASP8* in breast cancer susceptibility
263 we carried out dense genotyping of this region in the Breast Cancer Association Consortium
264 (BCAC). Single nucleotide polymorphisms (SNPs) spanning a 1Mb region around *CASP8*
265 were genotyped in 46,450 breast cancer cases and 42,600 controls of European origin from 41
266 studies participating in the BCAC as part of a custom genotyping array experiment (iCOGS).
267 Missing genotypes and SNPs were imputed and, after quality exclusions, 501 typed and 1,232
268 imputed SNPs were included in logistic regression models adjusting for study and ancestry
269 principal components. The SNPs retained in the final model were investigated further in data
270 from nine genome-wide association studies (GWAS) comprising in total 10,052 case and
271 12,575 control subjects. The most significant association signal observed in European
272 subjects was for the imputed intronic SNP rs1830298 in *ALS2CRI2* (telomeric to *CASP8*),
273 with per allele odds ratio and 95% confidence interval [OR (95% CI)] for the minor allele of
274 1.05 (1.03-1.07), $p=1 \times 10^{-5}$. Three additional independent signals from intronic SNPs were
275 identified, in *CASP8* (rs36043647), *ALS2CRI1* (rs59278883) and *CFLAR* (rs7558475). The
276 association with rs1830298 was replicated in the imputed results from the combined GWAS
277 ($p=3 \times 10^{-6}$), yielding a combined OR (95% CI) of 1.06 (1.04-1.08), $p=1 \times 10^{-9}$. Analyses of
278 gene expression associations in peripheral blood and normal breast tissue indicate that *CASP8*
279 might be the target gene, suggesting a mechanism involving apoptosis.

280

281 **Introduction**

282 Breast cancer is a complex disease with high, moderate and low penetrance germ-line variants
283 involved in its aetiology (1). In recent years around 80 low penetrance breast cancer alleles
284 have been identified, with modest odds ratios, ranging from 1.05-1.4, and together accounting
285 for around 15% of familial breast cancer risk (2, 3). It is likely that there are many more loci

286 with even smaller effect sizes that remain to be identified, accounting for a further 14-15% of
287 familial risk (2). One of the first low penetrance breast cancer variant associations to be
288 convincingly replicated by large case-control studies was the single nucleotide polymorphism
289 (SNP) rs1045485 encoding the mis-sense alteration D302H in the caspase 8 apoptosis-related
290 cysteine peptidase (*CASP8*) gene at chromosome region 2q33 (4, 5). This association was
291 first identified by a candidate gene study and replicated in 2007 by the Breast Cancer
292 Association Consortium (BCAC), in a study of more than 17,000 cases and 16,000 controls (4,
293 5). The minor C allele, common in Europeans and rare in Asians, was found to be associated
294 with a 10% reduction in risk of breast cancer (5). However, further fine-mapping studies have
295 shown that other variants in the region are associated with an increased risk of breast cancer,
296 and in the recent large-scale genotyping study carried out by the BCAC as part of the COGS
297 (Collaborative Oncology Gene- environment Study), rs1045485 showed only weak evidence
298 of association with breast cancer risk (2, 6, 7). In addition, in 2010 a UK genome-wide
299 association study (GWAS) of 3,659 cases and 4,897 controls found suggestive evidence of
300 association [OR (95% CI) 1.14 (1.06-1.22); $p=1.5 \times 10^{-4}$] with an independent variant in the
301 region; rs10931936, a *CASP8* intronic SNP, that is only weakly correlated with rs1045485
302 ($r^2=0.083$) (8).

303

304 In order to clarify the breast cancer risk association(s) at this locus, we have analysed 501
305 SNPs across a 1Mb region surrounding *CASP8*, for 89,050 women, as part of a custom-
306 designed Illumina genotyping chip – the iCOGS array. We present here the results of this
307 fine-mapping analysis, together with a meta-analysis across iCOGS and the combined data
308 from nine breast cancer GWAS, followed by an examination of associations between the key
309 SNPs and RNA expression levels.

310

311 **Results**

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

313 A summary of the breast cancer risk associations of 1,733 typed and imputed SNPs across a
314 1Mb region surrounding *CASP8*, based on the iCOGS European data, is shown in Figure 1.
315 The most significant associations were for SNPs in the *CASP8* and *ALS2CR12* (amyotrophic
316 lateral sclerosis 2 (juvenile) chromosome region, candidate 12) genes (Figure 1 and Table S2).
317 The strongest signals came from imputed SNP rs1830298 in *ALS2CR12*, with MAF (minor
318 allele frequency) of 0.29 and an estimated OR (95% CI) per copy of the minor allele of 1.05
319 (1.03-1.07), $p=1.1 \times 10^{-5}$, and the genotyped SNP rs10197246, (MAF=0.28), with odds ratio
320 (95% CI) 1.05 (1.02-1.07), $p=2.5 \times 10^{-5}$. These two SNPs are highly correlated and likely
321 reflect the same signal ($r^2=0.9$).

322

323 Two previously reported susceptibility SNPs, *CASP8* D302H (rs1045485) and rs10931936,
324 were weakly replicated in iCOGS European data (Table S2), with minor allele OR in the same
325 direction; however, the iCOGS OR estimates were much weaker than those from the original
326 reports (5, 8). The minor C allele of rs1045485 (MAF=0.11) yielded an OR (95% CI) of 0.97
327 (0.94-1.0), $p=0.03$, in contrast to 0.88 (0.84-0.92) reported in Cox et al (5). Similarly, the
328 rs10931936 minor allele (MAF=0.28) was associated with a 4% increased breast cancer risk
329 [OR (95% CI)=1.04 (1.02-1.06), $p=1.9 \times 10^{-4}$], compared to the 12% increase presented in
330 Turnbull *et al.* (8). The latter SNP is strongly correlated with the iCOGS best hit rs1830298
331 ($r^2=0.96$), but there is very little correlation between rs1045485 and rs1830298 ($r^2=0.055$).

332

333 ***Identification of possible independent signals in iCOGS European data***

334 The SNPs in the main association peak have similar ORs for breast cancer, are strongly
335 correlated with one another ($r^2>0.66$) and confined to an 82 Kb region spanning the *CASP8*
336 and *ALS2CR12* genes, and are therefore likely to reflect a single association signal, but this

337 does not preclude the possibility of other signals in the region. To test this hypothesis, we
338 carried out a regression analysis testing the association of individual SNPs adjusted for the
339 top hit rs1830298, in the iCOGS European data set (Table S3). Interestingly, whilst this
340 resulted in the loss of the signal from the main peak in *CASP8/ALS2CR12*, residual
341 associations remained (for example 43 SNPs with $p \leq 1 \times 10^{-3}$), suggesting that there may be
342 further signals present in the region, albeit weaker (Table S3 and Figure S1). To investigate
343 this further, we carried out penalised logistic regression analysis of all 1,733 SNPs to identify
344 the best subset of SNPs that explain the association, using HyperLasso (9). This identified 59
345 models containing combinations of 27 SNPs (Table S2), but many of these models were
346 equivalent after taking into account linkage disequilibrium between SNPs. To obtain the most
347 parsimonious model, we carried out stepwise forward logistic regression on the 27 SNPs,
348 which resulted in a model containing four SNPs; rs1830298 (*ALS2CR12*; $p_{\text{conditional}} = 9.3 \times 10^{-3}$,
349 MAF=0.29), rs36043647 (*CASP8*; $p_{\text{conditional}} = 1.9 \times 10^{-4}$, MAF=0.06), rs59278883 (*ALS2CR11*;
350 $p_{\text{conditional}} = 6.1 \times 10^{-4}$, MAF=0.07) and rs7558475 (*CFLAR*; *CASP8* and *FADD*-like apoptosis
351 regulator; $p_{\text{conditional}} = 9.2 \times 10^{-4}$, MAF=0.07). We refer to these four SNPs, marking four
352 independent sets of correlated highly associated variants (iCHAVs), as index SNPs.

353

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

355 We first examined the results for the four index SNPs, together with the previous hits
356 rs1045485 and rs10931936, in the combined nine GWAS meta-analysis, and then carried out
357 a further meta-analysis combining the iCOGS European data with the combined nine GWAS
358 for these SNPs (total sample size 56,502 cases and 55,175 controls; Tables S4 and S5). We
359 found that the top index SNP, rs1830298, replicated in the combined GWAS data alone
360 ($p = 2.7 \times 10^{-6}$), and reached genome-wide significance ($p = 1.1 \times 10^{-9}$) in the meta-analysis
361 containing both the iCOGS and combined GWAS data (Table S5 and Figure 2). The
362 genotyped proxy rs10197246 also reached genome-wide significance ($p = 1.7 \times 10^{-8}$). When we

363 examined the other three index SNPs in the combined GWAS data, we found a replicated
364 association ($p=1.8 \times 10^{-3}$) for rs59278883, a null result for rs36043647 ($p=0.58$) and borderline
365 evidence for rs7558475 ($p=0.05$) (Table S5 and Figure 2). However these three index SNPs
366 all showed some evidence of association in the meta analysis of iCOGS and combined
367 GWAS (Table S5 and Figure 2), providing some support for the existence of four signals in
368 the region. Consistent with its strong correlation with rs1830298, a similar but slightly weaker
369 signal was found for rs10931936 in the combined analysis ($p=1.0 \times 10^{-7}$). Weak evidence for
370 association was observed for *CASP8 D302H* rs1045485 ($p=1.1 \times 10^{-3}$).

371

372 *Analysis of index SNPs in different ethnic groups*

373 We next explored these four associations in the available Asian and African-American
374 populations genotyped as part of COGS (Figure 3 and Table S6). Figure 3 shows the study-
375 specific OR for rs1830298 by the three ethnic groups. The rs1830298 OR were homogeneous
376 among European studies ($p_{\text{het}}=0.54$, $I^2=0$) and African-American studies ($p_{\text{het}}=0.40$, $I^2=0$), but
377 were more heterogeneous amongst the nine Asian studies ($p_{\text{het}}=0.025$, $I^2=54$), although the
378 combined effect size in Asians was similar to that seen in Europeans [OR (95% CI)=1.04
379 (0.95-1.13); $p=0.44$], and slightly stronger in African-Americans [OR (95% CI)=1.12 (0.96-
380 1.30); $p=0.16$]. Although estimates in both Asian and African-American populations were not
381 statistically significant, the OR were consistent with the European data, and the pooled OR
382 (95% CI) was 1.05 (1.03-1.07); $p=4.1 \times 10^{-6}$ for all populations combined. The MAF of *CASP8*
383 rs36043647 was much lower in Asians, in whom the association was in the opposite direction
384 to that in Europeans and African-Americans, with an OR (95% CI) of 1.69 (1.13-2.51),
385 $p=0.009$, for the minor allele (Table S6). We did not observe any association of rs59278883
386 and rs7558475 in Asian and African-American populations (Table S6).

387

388 *Subtype and survival analysis in iCOGS*

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

397

398 SNP effects were also evaluated for overall survival and breast-cancer-specific survival.

399 There were 4,191 deaths among 39,140 breast cancer patients with known vital status in the
400 European dataset. Of these deaths, 1,979 died from breast cancer. We did not observe any
401 associations between the index SNPs or previous hit SNPs with either overall or breast
402 cancer-specific survival, and all hazard ratios (HR) were close to unity (data not shown).

403

404 *In silico functional and eQTL annotations*

405 We examined available *in silico* functional and expression quantitative trait loci (eQTL) data
406 for the four iCHAVs. Of interest in iCHAV1, rs3769823 is a missense alteration encoding
407 K14R in the 4th exon of *CASP8*, which encodes the N-terminus of protein isoform 9. In
408 addition, this SNP and rs3769821 are both located in a region of deoxyribonuclease I (DNase
409 I) hypersensitivity and histone H3K27 acetylation in breast cell lines (Figure 5). The minor
410 alleles of both of these SNPs, together with four others in iCHAV1 for which data were
411 available, were associated with a reduction in *CASP8* mRNA levels in peripheral blood
412 samples in the eQTL meta-analysis of Westra et al ($p \leq 9.4 \times 10^{-5}$; Table S7, Figure 5) (10). The
413 cancer genome atlas (TCGA) dataset only had data available for two SNPs from iCHAV1,

414 and both were associated with a reduction in *CASP8* mRNA in normal breast tissue ($p \leq 1 \times 10^{-3}$;
415 Table S7, Figure 5). No strong eQTL associations were seen for other genes in the region in
416 either the Westra *et al.* or the TCGA data. Taken together, these data suggest that one or more
417 variants in *iCHAV1* may affect levels of *CASP8* gene expression. As shown in Figure 5,
418 *iCHAVs* 3 and 4 overlap enhancer sites identified in Hnisz *et al.*; a *CASP8* enhancer in MCF7
419 cells and a *CFLAR* enhancer in human mammary epithelial cells (HMEC), respectively (11).
420 However, there was limited eQTL data available for these *iCHAVs*, with no evidence of any
421 significant eQTLs (Table S7).

422

423 Discussion

424 In our analysis of the genomic region surrounding *CASP8* for association with breast cancer,
425 the strongest signal came from an imputed SNP, rs1830298, in the *ASL2CR12* gene
426 (*iCHAV1*). A strongly correlated genotyped SNP, (rs10197246; $r^2=0.9$, 23.5 Kb telomeric in
427 the same gene), yielded a similar association signal ($p=1.1 \times 10^{-5}$ and 2.5×10^{-5} respectively). In
428 each case, the rare allele (MAF=0.28) was associated with an increase in the risk of breast
429 cancer of 5% [OR(95% CI) 1.05 (1.03, 1.07) and 1.05 (1.02, 1.07) respectively]. The odds
430 ratios for both SNPs are consistent in Europeans, Asians and African-Americans, (although
431 not statistically significant in the smaller non-European cohorts), and were replicated in the
432 combined GWAS data, achieving a genome-wide level of significance when the iCOGS and
433 GWAS data were combined ($p=1.1 \times 10^{-9}$ and $p=1.7 \times 10^{-8}$ respectively). This association is
434 consistent between ER positive and negative disease, and between invasive and *in situ* cancers
435 (Figure 4). The previously published result for rs10931936 in the UK GWAS is consistent
436 with its correlation with rs1830298 (8).

437

438 Several of the SNPs in *iCHAV1* were associated with *CASP8* eQTLs. The minor alleles of
439 SNPs in this group, associated with increased risk of breast cancer, are associated with

440 reduced *CASP8* mRNA levels in both peripheral blood lymphocytes and normal breast tissue
441 (Table S7, Figure 5). These data suggest that *CASP8* may be the target gene of iCHAV1, and
442 are consistent with a hypothesis in which the effect of the risk alleles is via reduced levels of
443 apoptosis, thus promoting tumour initiation. However, further functional studies are required
444 to demonstrate a direct interaction between iCHAV1 and the *CASP8* promoter and to
445 investigate the allele-specific functional effects of these SNPs in different tissue types.

446

447 Our results also suggest three other independent signals in the region; the most significant
448 SNPs for these three signals are in *CASP8* (iCHAV2), *ALS2CR11* (iCHAV3), and in the anti-
449 apoptotic gene *CFLAR* (iCHAV4); see Figure 2 and Table S5. The signals for iCHAVs 3 and
450 4 were replicated in the combined GWAS, but since they did not achieve genome-wide levels
451 of significance even in the very large data sets analysed here, they are harder to interpret.
452 However, it is interesting that both these iCHAVs overlap enhancer regions (Figure 5).

453

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

465

466 The association for the top *CASP8* index SNP, rs1830298, represents one of the smaller effect
467 sizes identified to date for breast cancer. However it is worth noting that the *CASP8* region
468 has recently been reported to be associated with other cancers at genome-wide levels of
469 significance, including melanoma and chronic lymphocytic leukaemia (CLL) (12, 13). The
470 alleles associated with increased risk in melanoma are correlated with rs1830298, but the
471 signal in CLL appears to be due to uncorrelated SNPs in the region. This difference may
472 reflect the different cell type of origin and it will be interesting to determine the relative
473 importance and function of alleles of the *CASP8* gene family in immune cell lineages,
474 compared to that in epithelial cancers.

475

476 **Materials and Methods**

477 *Study samples*

478 The iCOGS and nine breast cancer GWAS data sets have been described in detail previously
479 (2). Briefly, the COGS includes a total of 103,991 women from 50 studies participating in the
480 BCAC whose DNA samples were genotyped with the iCOGS array. These were 89,050
481 Europeans (46,450 cases; 42,600 controls), 12,893 Asians (6,269 cases; 6,624 controls), and
482 2,048 African-Americans (1,116 cases and 932 controls). The numbers of subjects by study
483 are detailed in Table S1. Approximately 93% of cases had invasive breast cancer (Table S1).
484 The combined nine breast cancer GWAS data set comprised 10,052 cases and 12,575 controls
485 of European ancestry from USA, UK, Australia, Germany, Finland, Sweden and the
486 Netherlands (2).

487

488 *Ethics statement*

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

491

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

503

504 *Genotyping & quality control*

505 Genotyping, allele calling, quality control and principal components analysis for COGS are
506 described in detail in Michailidou *et al.* (2). Genotyping was carried out at four centres using
507 the Illumina Infinium iCOGS array, including 2% duplicates from each participating study.
508 Final genotype calls were made using Illumina's proprietary GenCall algorithm. SNPs were
509 excluded from analysis if the overall call rate was $< 95\%$, duplicate concordance rate was
510 $< 98\%$, or if deviation from Hardy-Weinberg equilibrium in controls was significant at
511 $p < 1 \times 10^{-7}$ (2). Subjects were excluded from analysis for the following reasons: genotypically
512 non female; overall call rate $< 95\%$; low or high heterozygosity ($P < 1 \times 10^{-6}$); discordant
513 replicates or cryptic duplicates. Genotype data and ancestry principal components (seven

514 principal components for the European and two each for the Asian and African-American
515 populations) were thus available for 103,991 individuals.

516

517 *Statistical analysis*

518 The iCOGS *CASP8* region genotype data were split into four groups for efficiency of
519 imputation of missing genotypes and untyped SNPs. These comprised 36,793 European
520 ancestry subjects from North American and UK studies in group 1, with 26,129 and 26,128 of
521 the remaining European subjects in groups 2 and 3 respectively, and 14,941 Asians and
522 African-Americans in group 4. Imputations were carried out separately by group based on the
523 1000 genomes phase I reference panel with singleton variants excluded, using IMPUTE2
524 version 2.3 (14, 15). SNPs were included in the subsequent analyses if the mean information
525 score of the European groups was ≥ 0.9 , and untyped imputed SNPs were only included if
526 their MAF was $\geq 3\%$; these criteria resulted in inclusion of 501 typed and 1,232 imputed SNPs
527 in the final analysis. The imputation accuracy for rs1830298 was verified in whole genome
528 sequence data from 197 individuals; the correlation between the observed and imputed
529 genotypes was 0.974. The imputation step increases the number of common SNPs captured at
530 $r^2 > 0.9$ from 76% (1198/1583) to 84% (1333/1583).

531

532 The main analyses were based on the data for individuals of European ancestry. For each SNP,
533 allelic dosage of the minor allele was estimated, and included in a logistic regression model,
534 to estimate OR and corresponding 95% CI. Covariates for each study plus the seven ancestry
535 principal components were included in the model (2). These analyses were implemented in R.
536 P-values from the Wald test are reported in the text (uncorrected for multiple testing). FDR
537 values in Table S2 were calculated according to the Benjamini & Hochberg method, as
538 implemented in the R `p.adjust` function (16). Penalised logistic regression models (based on
539 the normal exponential gamma probability density) were implemented in HyperLasso (9),

540 including all 501 typed and 1,232 imputed SNPs, to identify the best subsets of SNPs to
541 account for the observed association data. Based on the sample size and a type I error of 0.001,
542 a lambda of 0.05 and penalty of 491 were specified in HyperLasso, according to equation 7 in
543 Hoggart *et al.* (9). Candidate SNPs were then compiled from the resulting HyperLasso models
544 and included in a stepwise forward logistic regression procedure with penalty $k=10$ in the step
545 function in R to identify the most parsimonious model, as described previously (17). The
546 SNPs retained in the final model are referred to as index SNPs.

547

548 Index SNPs were further examined by means of meta-analysis of iCOGS European, Asian
549 and African-American data, and also with individual SNP results from the combined nine
550 breast cancer GWAS (2). Due to an overlap of 1,955 samples that exist in both the iCOGS
551 and the combined GWAS data, we removed these samples from the iCOGS data before
552 carrying out the meta-analysis. The meta-analysis was carried out using the MetaFor package
553 in R, with inverse-variance weights and the DerSimonian-Laird estimator for the random
554 effects model (18). We used the threshold of $p=5 \times 10^{-8}$ to define genome-wide significance (2).

555

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

562

563 *In silico functional and eQTL annotations*

564 We defined independent sets of correlated highly associated variants (iCHAVs) with
565 likelihood (determined from the individual-SNP logistic regression analysis) relative to an

566 index SNP of greater than 1/100 and degree of correlation with the index SNP of greater than
567 0.65. The ENCODE integrated regulation data for each SNP were retrieved from the UCSC
568 Genome Browser by use of ANNOVAR (19). Predicted enhancers and target genes were
569 retrieved from Hnisz *et al.* (11). Expression QTL data were obtained by interrogation of the
570 GTEx Portal, the online results of the peripheral blood eQTL meta-analysis based on 5,311
571 samples from 7 studies by Westra and colleagues (10), and from breast cancer cases in the
572 TCGA Project. For the latter, RNAseq data in the form of fragments per Kb of transcript per
573 million mapped reads (FPKM) were available for uninvolved breast tissue from 97 TCGA
574 breast cancer cases. Peripheral blood DNA SNP genotypes for these individuals were
575 extracted from the TCGA Level 2 Affymetrix 6.0 array birdseed files. Mean FPKM were
576 compared between individuals homozygous for the common allele and those carrying one or
577 two copies of the rare allele by use of an unpaired, unequal variance T-test in Stata.
578

579

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937

938 **Legends to Figures**

939 **Figure 1**

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

941 The upper panel plots SNPs based on their chromosomal coordinates on the x axis and their p
942 values on the $-\log_{10}$ scale on the y axis. Circle and diamond symbols represent typed and
943 imputed SNPs respectively. The colours indicate the pairwise r^2 with index SNP for iCHAV1,
944 rs1830298 (highlighted in purple); r^2 are calculated based on the European panel in the 1000
945 genomes project. The ranges of iCHAVs 1-4 are indicated with coloured shading. Genes
946 within the region are indicated in the lower panel, with arrows indicating transcript direction,
947 dense blocks for exons and lines for introns. The plot was generated using LocusZoom (20).

948

949 **Figure 2**

950 **Associations of the 4 index SNPs corresponding to iCHAVs 1-4, and the two previous**
951 **associations, in iCOGS European subjects and GWAS data.**

952 Squares denote the per-allele OR for the minor allele based on iCOGS and nine GWAS data,
953 with the size of the square proportional to the sample size. Diamonds represent the pooled
954 estimates of ORs under the fixed effect model after exclusion of the 1955 samples from the
955 iCOGS data that were also in the combined GWAS data. Index SNPs correspond to iCHAVs
956 as follows: rs1830298; iCHAV1, rs36043647; iCHAV2, rs59278883; iCHAV3, rs7558475
957 iCHAV4.

958

959 **Figure 3**

960 **Study-specific OR for the minor allele of rs1830298 in iCOGS European, Asian and**
961 **African-American subjects.**

962 Squares denote the individual study per-allele OR and diamonds indicate the combined effects,
963 with the size of the symbol indicating sample size. Fixed effect models (FE model) were used

964 to combine the study ORs if p for the Cochran's Q test (p_{het}) was greater than 0.05, otherwise
965 random effect models (RE model) were used. Pooled OR across the 3 populations is shown,
966 with p_{het} and I-squared for heterogeneity in parenthesis.

967

968 **Figure 4**

969 **Associations between rs1830298 and clinical subtypes of breast cancer in iCOGS**

970 **European subjects.**

971 Squares denote the individual study per-allele OR with the size of the symbol indicating
972 sample size. Cases in each subtype group were compared to all controls.

973

974 **Figure 5**

975 **Summary of the *CASP8/ALS2CR12* locus.**

976 The locations of iCHAVs and lead SNPs are shown relative to genes. eQTL SNPs are
977 displayed as red marks. ENCODE DNaseI hypersensitive sites derived from various mammary
978 cell types are depicted as gray marks. H3K27ac histone modification ChIP-seq data is shown
979 as well as predicted enhancers and target genes from Hnisz et al (11).

980

- 981**
- 982** **Abbreviations**
- 983** BCAC: Breast Cancer Association Consortium
- 984** CI: confidence interval
- 985** CLL: chronic lymphocytic leukaemia
- 986** COGS: Collaborative Oncology Gene- environment Study
- 987** DNase: deoxyribonuclease
- 988** eQTL: expression quantitative trait locus/loci
- 989** ER: estrogen receptor
- 990** FPKM: fragments per Kb of transcript per million mapped reads
- 991** GWAS: genome-wide association studies
- 992** HMEC: human mammary epithelium cells
- 993** HR: hazard ratio
- 994** iCHAV independent sets of correlated highly associated variants
- 995** iCOGS: Collaborative Oncology Gene- environment Study genotyping array
- 996** Kb: kilobase
- 997** MAF: minor allele frequency
- 998** Mb: megabase
- 999** OR: odds ratio
- 1000** SNP: Single nucleotide polymorphism
- 1001** TCGA: The Cancer Genome Atlas
- 1002**