Common variants in breast cancer risk loci predispose to distinct tumor subtypes

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Abstract

Background: Genome-wide association studies (GWAS) have identified multiple common breast cancer susceptibility variants. Many of these variants have differential associations by estrogen receptor (ER), but how these variants relate with other tumor features and intrinsic molecular subtypes is unclear.

Methods: Among 106,571 invasive breast cancer cases and 95,762 controls of European ancestry with data on 173 breast cancer variants identified in previous GWAS, we used novel two-stage polytomous logistic regression models to evaluate variants in relation to multiple tumor features (ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and grade) adjusting for each other, and to intrinsic-like subtypes.

Results: Eighty-five of 173 variants were associated with at least one tumor feature (false discovery rate <5%), most commonly ER and grade, followed by PR and HER2. Models for intrinsic-like subtypes found nearly all of these variants (83 of 85) associated at P<0.05 with risk for at least one luminal-like subtype, and approximately half (41 of 85) of the variants were associated with risk of at least one non-luminal subtype, including 32 variants associated with triple-negative (TN) disease. Ten variants were associated with risk of all subtypes in different magnitude. Five variants were associated with risk of luminal A-like and TN subtypes in opposite directions.

Conclusion: This report demonstrates a high level of complexity in the etiology heterogeneity of breast cancer susceptibility variants and can inform investigations of subtype-specific risk prediction.

Key Words: Breast Cancer, etiologic heterogeneity, genetic predisposition, common breast cancer susceptibility variants

Introduction

Breast cancer represents a heterogenous group of diseases with different molecular and clinical features[1]. Clinical assessment of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and histological grade are routinely determined to inform treatment strategies and prognostication[2]. Combined, these tumor features define five intrinsic-like subtypes (i.e., luminal A-like, luminal B–like/HER2-negative, luminal B-like/HER2-positive, HER2-positive/non-luminal, and triple negative) that are correlated with intrinsic subtypes defined by gene expression panels[2, 3]. Most known breast cancer risk or protective factors are related to luminal or hormone receptor (ER or PR) positive tumors, whereas less is known about the etiology of triple-negative (TN) tumors, an aggressive subtype[4, 5].

Breast cancer genome-wide association studies (GWAS) have identified over 170 common susceptibility variants, most of them single nucleotide polymorphisms (SNPs), of which many are differentially associated with ER-positive than ER-negative disease[6-8]. These include 20 variants that primarily predispose to ER-negative or TN disease[7, 8]. However, few studies have evaluated variant associations with other tumor features, or simultaneously studied multiple, correlated tumor markers to identify source(s) of etiologic heterogeneity[7, 9-13]. We recently developed a two-stage polytomous logistic regression method that efficiently characterizes etiologic heterogeneity while accounting for tumor marker correlations and missing tumor data[14, 15]. This method can help describe complex relationships between susceptibility variants and multiple tumor features, helping to clarify breast cancer subtype etiologies and increasing the power to generate more accurate risk estimates between

susceptibility variants and less common subtypes. We recently demonstrated the power of this method in a GWAS to identify novel breast cancer susceptibility accounting for tumor heterogeneity[15].

In this report, we sought to expand our understanding of etiologic heterogeneity among breast cancer subtypes, by applying the two-stage polytomous logistic regression methodology to a large study population from the Breast Cancer Association Consortium (BCAC) for detailed characterization of risk associations with 173 breast cancer risk variants identified by GWAS[6, 7] by tumor subtypes defined by ER, PR, HER2 and tumor grade.

Methods

Study Population and Genotyping

The study population and genotyping are described in previous publications[6, 7] and in the **Supplementary Methods**. We included invasive cases and controls from 81 BCAC studies with genotyping data from two Illumina genome-wide custom arrays, the iCOGS and OncoArray (106,571 cases (OncoArray: 71,788; iCOGS: 34,783) and 95,762 controls (OncoArray: 58,134; iCOGS: 37,628); **Supplementary Table 1**). We evaluated 173 breast cancer risk variants that were identified in or replicated by prior BCAC analyses to be associated with breast cancer risk at a p-value threshold $p<5.0x10^{-8}$ [6, 7]. Most of these variants (n=153) were identified because of their association with risk of overall breast cancer, and a small number of variants (n=20) were identified because of their association specific to ER-negative breast cancer (**Supplementary Table 2**). These 173 variants have not previously been simultaneously investigated for evidence of tumor heterogeneity with multiple tumor

markers[6, 7, 15, 16]. Genotypes for the variants marking the 173 susceptibility loci were determined by genotyping with the iCOGS and the OncoArray arrays and imputation to the 1000 Genomes Project (Phase 3) reference panel.

Statistical Analysis

An overview of the analytic strategy is shown in **Figure** 1 and a detailed discussion of the statistical methods, including the two-stage polytomous logistic regression, are provided in the **Supplementary Methods** and elsewhere[14, 15]. Briefly, we used two-stage polytomous regression models that allow modelling of genetic association of breast cancer accounting for underlying heterogeneity in associations by combinations of multiple tumor markers using a parsimonious decomposition of subtype-specific case-control odds-ratio parameters in terms of marker-specific case-case odd-ratio parameters[14, 15]. We introduced further parsimony by using mixed-effect formulation of the model that allows ER-specific case-case parameters to be treated as fixed and similar parameters for other markers (PR, HER2 and grade) as random. We used an expectation–maximization (EM) algorithm[17] for parameter estimation under this model to account for missing data in tumor characteristics. A detailed description of the two-stage polytomous regression models used in this manuscript is presented in the **Supplementary Methods** and in separate manuscripts[14, 15].

Our primary aim was to identify which of 173 known breast cancer susceptibility variants showed heterogenous risk associations by ER, PR, HER2 and grade. This was tested using a global heterogeneity test by ER, PR, HER2 and/or grade, with a mixed-effect two-stage polytomous model (**model 1**), fitted separately for each variant. The global null hypothesis was

that there was no difference in risk of breast cancer associated with the variant genotype across any of the tumor features being evaluated. We accounted for multiple testing (173 tests, one for each of variant) of the global heterogeneity test using a false discovery rate (FDR) <0.05% under the Benjamini-Hochberg procedure[18].

For the variants showing evidence of global heterogeneity after FDR adjustment, we further evaluated which of the tumor features contributed to the heterogeneity by fitting a fixed-effects two-stage model (**model 2**) that simultaneously tested for associations with each tumor feature (this model was fitted for each variant separately). We used a threshold of P<0.05 for marker-specific tumor heterogeneity tests to describe which specific tumor marker(s) contributed to the observed heterogeneity, adjusting for the other tumor markers in the model. This p-value threshold was used only for descriptive purposes, as the primary hypotheses were tested using the FDR-adjusted global test for heterogeneity described above.

We conducted additional analyses to explore evidence of heterogeneity. We fitted a fixed-effect two-stage model (**model 3**) to estimate case-control odd ratios (ORs) and 95% confidence intervals (CI) between the variants and five intrinsic-like subtypes defined by combinations of ER, PR, HER2 and grade: (1) luminal A-like (ER+ and/or PR+, HER2-, grade 1 or 2); (2) luminal B-like/HER2-negative (ER+ and/or PR+, HER2-, grade 3); (3) luminal B-like/HER2-positive (ER+ and/or PR+, HER2+); (4) HER2-positive/non-luminal (ER- and PR-, HER2+), and (5) TN (ER-, PR-, HER2-). We also fitted a fixed-effect two-stage model to estimate case-control ORs and 95% confidence intervals (CI) with tumor grade (**model 4**; defined as grade 1, grade 2, and grade 3) for the variants associated at P<0.05 only with grade in case-case comparisons from model 2.

To help describe sources of heterogeneity from different tumor characteristics in models 2 and 3, we performed cluster analyses based on Euclidean distance calculated from z-statistics that were estimated by the individual marker-specific tumor heterogeneity tests (model 2) and the case-control associations with risk of intrinsic-like subtypes (model 3) . The clusters were used only to help present our findings and were not intended to suggest strictly defined categories. Clustering was performed in R using the function Heatmap as implemented by the package "Complex Heatmap" version 3.1[19].

We performed sensitivity analyses, in which we estimated the ORs and 95% CI between the variants and the intrinsic-like subtypes by implementing a standard polytomous model restricted to cases with complete tumor marker data. For all analyses we analyzed OncoArray and iCOGS array data separately, adjusting for the first 10 principal components for ancestry-informative variants, and then meta-analyzed the results.

Results

The mean (SD) ages at diagnosis (cases) and enrollment (controls) were 56.6 (12.2) and 56.4 (12.2) years, respectively. Among cases with information on the corresponding tumor marker, 81% were ER-positive, 68% PR-positive, 83% HER2-negative and 69% grade 1 or 2 (**Table 1;** see **Supplementary Table 1** for details by study). **Supplementary Table 3** shows the correlation between the tumor markers. ER was positively correlated with PR (r^2=0.61) and inversely correlated with HER2 (r^2=-0.16) and grade (r^2=-0.39). The most common intrinsic-like subtype was luminal A-like (54%), followed by TN (14%), luminal B-like/HER2-negative (13%), Luminal B-like/HER2-positive (13%) and HER2-positive/non-luminal (6%; **Table 1**).

Figure 1 shows an overview of the analytic strategy and results from three main

analyses performed separately for each variant: 1) global test for heterogeneity by all tumor markers (model 1; primary hypothesis), 2) marker-specific tumor test for heterogeneity for each marker, adjusting for the others (model 2), and 3) estimation of case-control ORs (95%CIs) by intrinsic-like subtypes (model 3).

1) Global test for heterogeneity by tumor markers (primary hypothesis)

Mixed-effects two-stage models (model 1) were fitted for each of the 173 variants separately and included terms for ER, PR, HER2 and grade to test of global heterogeneity by any of the tumor features (case-case comparison). This model identified 85 of 173 (49.1%) variants with evidence of heterogeneity by at least one tumor feature (FDR<5%; **Figure 1-2; Supplementary Figure 1**). **Figure 2** shows a heatmap of the second stage z-values from the fixed-effects two-stage polytomous model for the marker-specific heterogeneity tests (case-case comparison from model 2) for the 173 breast cancer susceptibility variants and ER, PR, HER2, and grade.

2) Marker-specific tumor test for heterogeneity for each marker, adjusting for other markers

Fixed-effects two-stage models (model 2) were used to test which of the correlated tumor markers was responsible for the observed global heterogeneity in the 85 variants (case-case comparison). These analyses identified ER and grade as the two features that most often contributed to the observed heterogeneity (45 and 33 variants had marker-specific P<0.05 for ER and grade, respectively), and 29 variants were associated with more than one tumor feature (**Figure 1-2**, **Supplementary Figure 1)**. Eighteen of these 85 variants showed no associations

with any individual tumor marker at P<0.05 (**Supplementary Figure 1**). Twenty-one variants were associated at P<0.05 only with ER, 12 variants only with grade, 4 variants only with PR and one variant only with HER2 (**Supplementary Figure 1,** see footnotes).

3) Estimation of case-control ORs (95%CIs) by intrinsic-like subtypes (model 3)

Fixed-effects two-stage models for intrinsic-like subtypes (model 3) were fitted for each of the 85 variants with evidence of global heterogeneity to estimate ORs (95% CIs) for risk associations with each subtype (case-control comparisons). **Supplementary Figure 2** shows a summary of these analyses for the 85 variants, clustered by case-control z-value of association between susceptibility variants and breast cancer intrinsic-like subtypes, and **Supplementary Figure 3** shows forest plots for associations with risk by tumor subtypes. Nearly all (83 of 85) variants were associated with risk (P<0.05) for at least one luminal-like subtype, and approximately half (41 of 85) of the variants were associated with risk of at least one non-luminal subtype, including 32 variants that were associated with TN disease (**Figure 1**, **Supplementary Figure 2 footnote 'h'**). Ten variants were associated with risk of all subtypes (**Figure 1**, **Supplementary Figure 2 footnote 'j'**). Below we describe examples of groups of variants associated with different patterns of associations with intrinsic subtypes (**Figure 3 a-d**).

Two correlated (r^2=0.73) variants at 10q26.13 (rs2981578 and rs35054928) and 16q12.1-rs4784227 had the strongest evidence of association with risk of luminal-like subtypes (**Figure 3a**, **Supplementary Figure 2)**. The two variants at 10q26.13 showed no evidence of associations with TN subtypes, and a weaker association with HER2-positive/non-luminal subtype (**Figure 3a**, **Supplementary Figure 2**). In contrast, 16q12.1-rs4784227 was strongly

associated with risk for all luminal-like subtypes and, weaker so, with risk of HER2-positive/non-luminal and TN subtypes (**Figures 3a**, **Supplementary Figure 2**).

Three variants 19p13.11-rs67397200, 5p15.33-rs10069690 and 1q32.11-rs4245739 showed the strongest evidence of associations with risk of TN disease (**Figure 3b**, **Supplementary Figure 2**).

Two weakly correlated variants in 6q25 (r^2=0.17), rs9397437 and rs3757322, and a third variant in 6q25, rs2747652, which was not correlated (r^2<0.01) with rs9397437 or rs3757322, showed strong evidence of being associated with risk of all subtypes. rs9397437 and rs3757322 had strong evidence of associations with risk of TN and risk of luminal-like subtypes (**Figures 3c** and **Supplementary Figure 2**). rs2747652 was most strongly associated with risk of HER2-positive subtypes (**Figures 3c**, **Supplementary Figure 2**).

Five variants were associated with risk of luminal A-like disease in an opposite direction to their association with risk of TN disease (**Figure 3d**, **Supplementary Figure 2**). 1q32.1-rs6678914, 2p23.2-rs4577244, and 19p13.11-rs67397200 had weaker evidence of associations with risk of luminal A-like disease compared to associations with risk of TN disease, and 10p12.31-rs7072776 and 22q12.1-rs17879961 (I157T) had stronger evidence of an association with risk of luminal A-like disease compared to their association with risk of TN disease (**Figure 3d**, **Supplementary Figure 2**, for rs67397200 see **Figure 3b).**

4) Estimation of case-control ORs (95%CIs) by tumor grade (model 4)

Case-control associations by tumor grade for the 12 variants associated at P<0.05 only with grade in case-case comparisons are shown in **Supplementary Figure 4**. 13q13.1-

rs11571833, 1p22.3-rs17426269 and 11q24.3-rs11820646 showed stronger evidence for predisposing to risk of high-grade subtypes, and the remaining variants showed stronger evidence for predisposing to risk of low-grade subtypes.

When limiting analyses to cases with complete tumor marker data, results from case-control analyses were similar, but less precise than results from the two-stage polytomous regression model using the EM algorithm to account for missing tumor marker data (**Supplementary Table 4**).

Discussion

This study demonstrates the extent and complexity of genetic etiologic heterogeneity among 173 breast cancer risk variants by multiple tumor characteristics, using novel methodology in the largest and the most comprehensive investigation conducted to date. We found compelling evidence that about half of the investigated breast cancer susceptibility loci (85 of 173 variants) predispose to tumors with different characteristics. We identified tumor grade, along with confirming ER status, as important determinants of etiologic heterogeneity. Associations with individual tumor features translated into differential associations with the risk of intrinsic-like subtypes defined by their combinations.

Many of the variants with evidence of global heterogeneity predisposed to risk of multiple subtypes, but with different magnitudes. For example, three variants identified in early GWAS for overall breast cancer, *FGFR2* (rs35054928 and rs2981578)[20, 21] and 8q24.21

(rs13281615)[20], were associated with luminal-like and HER2-positive/non-luminal subtypes, but not with TN disease. rs4784227 located near *TOX3[20, 22]* and rs62355902 located in a *MAP3K1[20]* regulatory element, were associated with risk of all five subtypes. Of the five variants found associated in opposite directions with luminal A-like and TN disease, we previously reported rs6678914 and rs4577244 to have opposite effects between ER-negative and ER-positive tumors[7]. rs17879961 (I157T), a likely causal[16] mis-sense variant located in a *CHEK2* functional domain that reduces or abolishes substrate binding[23], was previously reported to have opposite directions of effects on lung adenocarcinoma and lung squamous cell carcinoma and for lung cancer between smokers and non-smokers[24, 25]. However, further studies of these five variants are required to follow-up and clarify the mechanisms for these apparent cross-over effects.

In prior ER-negative GWAS, we identified 20 variants that predispose to ER-negative disease, of which five variants were only or most strongly associated with risk of TN disease (rs4245739, rs10069690, rs74911261, rs11374964, and rs67397200)[7, 8]. We confirmed these five variants to be most strongly associated with TN disease. The remaining previously identified 15 variants all showed associations with risk of non-luminal subtypes, especially TN disease, and for all but four variants (rs17350191, rs200648189, rs6569648, and rs322144) evidence of global heterogeneity was observed.

Little is known regarding PR and HER2 as sources of etiologic heterogeneity independent of ER status. Of the four variants that showed evidence of heterogeneity only according to PR, rs10759243[6, 26], rs11199914[27] and rs72749841[6] were previously found primarily associated with risk of ER-positive disease, and rs10816625 was found to be associated with risk

of ER-positive/PR-positive tumors, but not other ER/PR combinations[12]. rs10995201 was the only variant found in case-case comparisons to be solely associated with HER2 status, although the evidence was not strong, requiring further confirmation. Previously, rs10995201 showed no evidence of being associated with ER status[28]. Most variants associated with PR or HER2, had not been investigated for PR or HER2 heterogeneity while adjusting for ER[9-13]. We previously reported rs10941679 to be associated with PR-status, independent of ER, and also with grade[10]. We also found suggestive evidence of PR-specific heterogeneity for 16q12-rs3803662[13], which is in high LD (r^2= 0.78) with rs4784227 (*TOX3*), a variant strongly associated with PR status. Our findings for rs2747652 are also consistent with a prior BCAC fine-mapping analysis across the *ESR1* locus, which found rs2747652 to be associated with risk of the HER2-positive/non-luminal subtype and high grade independent of ER[9]. rs2747652 overlaps an enhancer region and is associated with reduced *ESR1* and *CCDC170* expression[9].

Histologic grade is a composite of multiple tumor characteristics including mitotic count, nuclear pleomorphism, and degree of tubule or gland formation[29]. Among the 12 variants identified with evidence of heterogeneity by grade only, rs17426269, rs11820646, and rs11571833 were found to be most strongly associated with risk of grade 3 disease. rs11571833 lies in the *BRCA2* coding region and produces a truncated form of the protein[30] and has been shown to be associated with both risk of TN disease and risk of serous ovarian tumors, both of which tend to be high-grade[31]. To our knowledge, rs17426269 and rs11820646 have not been investigated in relation to grade heterogeneity. The remaining 9 variants were all more strongly associated with grade 1 or grade 2 disease. Six of these variants were previously reported to be

associated primarily with ER-positive disease[6, 27, 32, 33], highlighting the importance of accounting for multiple tumor characteristics to better illuminate heterogeneity sources.

We identified 18 variants with evidence of global heterogeneity (FDR<5%), but no significant (marker-specific P<0.05) associations with any of the individual tumor characteristic(s). This is likely explained by the fact that the test for association with specific tumor markers using fixed-effects models are less powerful than mixed-effects models used to test the primary hypothesis of global heterogeneity by any tumor marker[14].

To help describe and visualize the strength of the evidence for common heterogeneity patterns, we performed clustered analyses of z-values for tumor marker-specific heterogeneity tests and case-control associations with risk of intrinsic-like subtypes. Because they are based on z-values, these clusters reflect differences in sample size and statistical power to detect associations between variants and specific tumor subtypes. Thus, clusters should not be interpreted as strictly defined categories.

A major strength of our study is our large sample size of over 100,000 breast cancer cases with tumor marker information, and a similar number of controls, making this the largest, most comprehensive breast cancer heterogeneity investigation. Our application of the two-stage polytomous logistic regression enabled adjusting for multiple, correlated tumor markers and accounting for missing tumor marker data. This is a more powerful and efficient modeling strategy for identifying heterogeneity sources among highly correlated tumor markers, compared with standard polytomous logistic regression[14, 15]. In simulated and real data analyses, we have demonstrated that in the presence of heterogenous associations across

subtypes, the two-stage model is more powerful than polytomous logistic regression for detecting heterogeneity. Moreover, we have demonstrated that in the presence of correlated markers, the two-stage model, incorporating all markers simultaneously, has much better ability to distinguish the true source(s) of heterogeneity compared to testing for heterogeneity by analysis of one marker at a time[14, 15]. In prior analyses, we showed that the two-stage polytomous regression is a powerful approach to identify susceptibility variants that display tumor heterogeneity[15]. Notably, in this prior investigation we excluded the genomic regions in which the 173 variants that were investigated in this work are located[15].

Our study also has some limitations. First, many breast cancer cases from studies included in this report had missing information on one or more tumor characteristics. ER tumor status data was available for 81% of cases, but missing data for the other tumor markers ranged from 27% to 46%. To address this limitation, we implemented an EM algorithm that allowed a powerful analysis to incorporate cases with missing tumor characteristics under the assumption that tumor characteristics are *missing at random* (MAR), i.e., the underlying reason for missing data may depend on observed tumor markers or/and covariate values, but not on the missing values themselves[34]. If this assumption is violated it can lead to an inflated type-one error[14]. However, in the context of genetic association testing, the missingness mechanism would also need to be related to the genetic variants under study, which is unlikely. Our study focused on investigating ER, PR, HER2, and grade as heterogeneity sources, and future studies with more detailed tumor characterization could reveal additional etiologic heterogeneity sources.

In summary, our findings provide insights into the complex etiologic heterogeneity patterns of common breast cancer susceptibility loci. These findings may inform future studies, such as fine-mapping and functional analyses to identify the underlying causal variants, clarifying biological mechanisms that drive genetic predisposition to breast cancer subtypes. Moreover, these analyses provide precise estimates of relative risk for different intrinsic-like subtypes that could improve the discriminatory accuracy of subtype-specific polygenic risk scores [35].

Abbreviations

GWAS: Genome-wide association studies

ER: estrogen receptor

PR: progesterone receptor

HER2: human epidermal growth factor receptor 2

FDR: false discovery rate

TN: triple-negative

BCAC: Breast Cancer Association Consortium

EM: expectation–maximization

OR: odd ratios

95% CI: 95% confidence interval

Declarations

Ethics approval and consent to participate

All the studies included in these analyses were approved by local IRBs.

Consent for publication

Not applicable

Availability of data and materials

Requests for data can be made to M.K.B. (bcac@ medschl.cam.ac.uk;

http://bcac.ccge.medschl.cam.ac.uk/bcacdata/).

Competing interests

The authors have no competing interests to declare.

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Author Contributions

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References

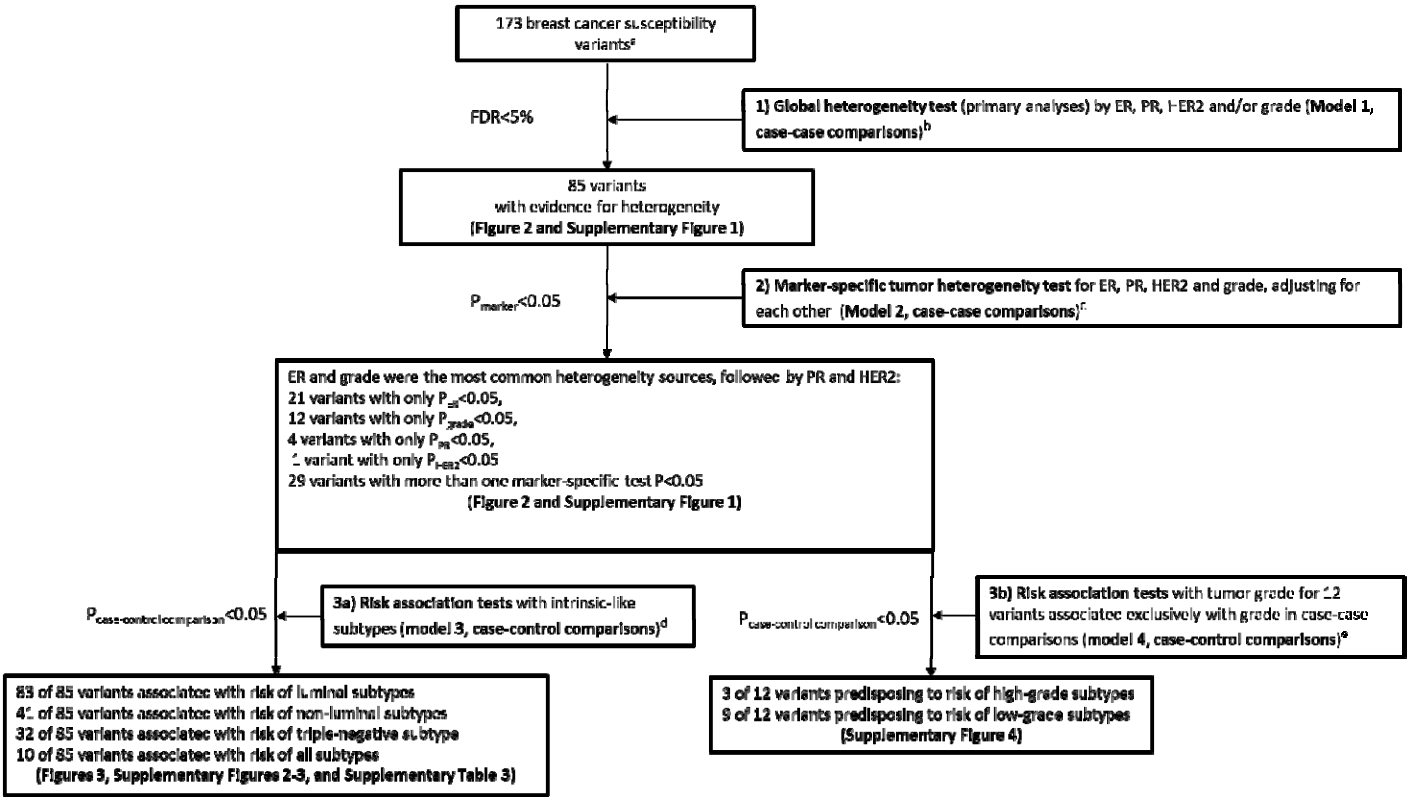
1. Cancer Genome Atlas N: **Comprehensive molecular portraits of human breast tumours**. *Nature* 2012, **490**(7418):61-70.
2. Curigliano G, Burstein HJ, E PW, Gnant M, Dubsky P, Loibl S, Colleoni M, Regan MM, Piccart-Gebhart M, Senn HJ *et al*: **De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017**. *Ann Oncol* 2017, **28**(8):1700-1712.
3. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B, Senn HJ, Panel m: **Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013**. *Ann Oncol* 2013, **24**(9):2206-2223.
4. Barnard ME, Boeke CE, Tamimi RM: **Established breast cancer risk factors and risk of intrinsic tumor subtypes**. *Biochim Biophys Acta* 2015, **1856**(1):73-85.
5. Yang XR, Chang-Claude J, Goode EL, Couch FJ, Nevanlinna H, Milne RL, Gaudet M, Schmidt MK, Broeks A, Cox A *et al*: **Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies**. *Journal of the National Cancer Institute* 2011, **103**(3):250-263.
6. Michailidou K, Lindstrom S, Dennis J, Beesley J, Hui S, Kar S, Lemacon A, Soucy P, Glubb D, Rostamianfar A *et al*: **Association analysis identifies 65 new breast cancer risk loci**. *Nature* 2017, **551**(7678):92-94.
7. Milne RL, Kuchenbaecker KB, Michailidou K, Beesley J, Kar S, Lindstrom S, Hui S, Lemacon A, Soucy P, Dennis J *et al*: **Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer**. *Nature genetics* 2017, **49**(12):1767-1778.
8. Garcia-Closas M, Couch FJ, Lindstrom S, Michailidou K, Schmidt MK, Brook MN, Orr N, Rhie SK, Riboli E, Feigelson HS *et al*: **Genome-wide association studies identify four ER negative-specific breast cancer risk loci**. *Nature genetics* 2013, **45**(4):392-398, 398e391-392.
9. Dunning AM, Michailidou K, Kuchenbaecker KB, Thompson D, French JD, Beesley J, Healey CS, Kar S, Pooley KA, Lopez-Knowles E *et al*: **Breast cancer risk variants at 6q25 display different phenotype associations and regulate ESR1, RMND1 and CCDC170**. *Nature genetics* 2016, **48**(4):374-386.
10. Milne RL, Goode EL, Garcia-Closas M, Couch FJ, Severi G, Hein R, Fredericksen Z, Malats N, Zamora MP, Arias Perez JI *et al*: **Confirmation of 5p12 as a susceptibility locus for progesterone-receptor-positive, lower grade breast cancer**. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2011, **20**(10):2222-2231.
11. Figueroa JD, Garcia-Closas M, Humphreys M, Platte R, Hopper JL, Southey MC, Apicella C, Hammet F, Schmidt MK, Broeks A *et al*: **Associations of common variants at 1p11.2 and 14q24.1 (RAD51L1) with breast cancer risk and heterogeneity by tumor subtype: findings from the Breast Cancer Association Consortium**. *Human molecular genetics* 2011, **20**(23):4693-4706.
12. Orr N, Dudbridge F, Dryden N, Maguire S, Novo D, Perrakis E, Johnson N, Ghoussaini M, Hopper JL, Southey MC *et al*: **Fine-mapping identifies two additional breast cancer susceptibility loci at 9q31.2**. *Human molecular genetics* 2015, **24**(10):2966-2984.
13. Broeks A, Schmidt MK, Sherman ME, Couch FJ, Hopper JL, Dite GS, Apicella C, Smith LD, Hammet F, Southey MC *et al*: **Low penetrance breast cancer susceptibility loci are associated with**

specific breast tumor subtypes: findings from the Breast Cancer Association Consortium. *Human molecular genetics* 2011, **20**(16):3289-3303.

14. Zhang H, Zhao N, Ahearn TU, Wheeler W, García-Closas M, Chatterjee N: **A mixed-model approach for powerful testing of genetic associations with cancer risk incorporating tumor characteristics**. *Biostatistics* 2020.
15. Zhang H, Ahearn TU, Lecarpentier J, Barnes D, Beesley J, Qi G, Jiang X, O'Mara TA, Zhao N, Bolla MK *et al*: **Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses**. *Nature genetics* 2020, **52**(6):572-581.
16. Fachal L, Aschard H, Beesley J, Barnes DR, Allen J, Kar S, Pooley KA, Dennis J, Michailidou K, Turman C *et al*: **Fine-mapping of 150 breast cancer risk regions identifies 191 likely target genes**. *Nature genetics* 2020, **52**(1):56-73.
17. Dempster AP, Laird NM, Rubin DB: **Maximum Likelihood from Incomplete Data Via Em Algorithm**. *J Roy Stat Soc B Met* 1977, **39**(1):1-38.
18. Benjamini Y, Hochberg Y: **Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing**. *Journal of the Royal Statistical Society Series B (Methodological)* 1995, **57**(1):289-300.
19. Gu Z, Eils R, Schlesner M: **Complex heatmaps reveal patterns and correlations in multidimensional genomic data**. *Bioinformatics* 2016, **32**(18):2847-2849.
20. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R *et al*: **Genome-wide association study identifies novel breast cancer susceptibility loci**. *Nature* 2007, **447**(7148):1087-1093.
21. Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE, Wacholder S, Wang Z, Welch R, Hutchinson A *et al*: **A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer**. *Nature genetics* 2007, **39**(7):870-874.
22. Stacey SN, Manolescu A, Sulem P, Rafnar T, Gudmundsson J, Gudjonsson SA, Masson G, Jakobsdottir M, Thorlacius S, Helgason A *et al*: **Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer**. *Nature genetics* 2007, **39**(7):865-869.
23. Li J, Williams BL, Haire LF, Goldberg M, Wilker E, Durocher D, Yaffe MB, Jackson SP, Smerdon SJ: **Structural and functional versatility of the FHA domain in DNA-damage signaling by the tumor suppressor kinase Chk2**. *Mol Cell* 2002, **9**(5):1045-1054.
24. McKay JD, Hung RJ, Han Y, Zong X, Carreras-Torres R, Christiani DC, Caporaso NE, Johansson M, Xiao X, Li Y *et al*: **Large-scale association analysis identifies new lung cancer susceptibility loci and heterogeneity in genetic susceptibility across histological subtypes**. *Nature genetics* 2017, **49**(7):1126-1132.
25. Wang Y, McKay JD, Rafnar T, Wang Z, Timofeeva MN, Broderick P, Zong X, Laplana M, Wei Y, Han Y *et al*: **Rare variants of large effect in BRCA2 and CHEK2 affect risk of lung cancer**. *Nature genetics* 2014, **46**(7):736-741.
26. Li X, Zou W, Liu M, Cao W, Jiang Y, An G, Wang Y, Huang S, Zhao X: **Association of multiple genetic variants with breast cancer susceptibility in the Han Chinese population**. *Oncotarget* 2016, **7**(51):85483-85491.
27. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, Schmidt MK, Chang-Claude J, Bojesen SE, Bolla MK *et al*: **Large-scale genotyping identifies 41 new loci associated with breast cancer risk**. *Nature genetics* 2013, **45**(4):353-361, 361e351-352.
28. Darabi H, McCue K, Beesley J, Michailidou K, Nord S, Kar S, Humphreys K, Thompson D, Ghoussaini M, Bolla MK *et al*: **Polymorphisms in a Putative Enhancer at the 10q21.2 Breast Cancer Risk Locus Regulate NRBF2 Expression**. *American journal of human genetics* 2015, **97**(1):22-34.

29. Elston CW, Ellis IO: **Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up**. *Histopathology* 1991, **19**(5):403-410.
30. Mazoyer S, Dunning AM, Serova O, Dearden J, Puget N, Healey CS, Gayther SA, Mangion J, Stratton MR, Lynch HT *et al*: **A polymorphic stop codon in BRCA2**. *Nature genetics* 1996, **14**(3):253-254.
31. Meeks HD, Song H, Michailidou K, Bolla MK, Dennis J, Wang Q, Barrowdale D, Frost D, McGuffog L, Ellis S *et al*: **BRCA2 Polymorphic Stop Codon K3326X and the Risk of Breast, Prostate, and Ovarian Cancers**. *Journal of the National Cancer Institute* 2016, **108**(2).
32. Darabi H, Beesley J, Droit A, Kar S, Nord S, Moradi Marjaneh M, Soucy P, Michailidou K, Ghoussaini M, Fues Wahl H *et al*: **Fine scale mapping of the 17q22 breast cancer locus using dense SNPs, genotyped within the Collaborative Oncological Gene-Environment Study (COGs)**. *Scientific reports* 2016, **6**:32512.
33. Michailidou K, Beesley J, Lindstrom S, Canisius S, Dennis J, Lush MJ, Maranian MJ, Bolla MK, Wang Q, Shah M *et al*: **Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer**. *Nature genetics* 2015, **47**(4):373-380.
34. Little RJA, Rubin DB: **Statistical analysis with missing data**. In: *Wiley series in probability and statistics.* Third edition edn. Hoboken, NJ: Wiley,; 2019: 1 online resource.
35. Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, Lee A, Tyrer JP, Chen TH, Wang Q, Bolla MK *et al*: **Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes**. *American journal of human genetics* 2019, **104**(1):21-34.

Figure 1. Overview of the analytic strategy and results from the investigation of 173 known breast cancer susceptibility variants for evidence of heterogeneity according to the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and grade



[a] We evaluated 173 breast cancer risk variants identified in or replicated by prior BCAC GWAS [6, 7], see **Methods** and **Supplementary Methods** sections for more details.

[b] Model 1 (primary analyses): Mixed-effect two-stage polytomous model (ER as fixed-effect, and PR, HER2 and grade as random-effects) for global heterogeneity tests (i.e. case-case comparisons from stage 2 of the two-stage model) between each individual risk variant and any of the tumor features (separate models were fit for each variant).

[c] Model 2: Fixed-effect two-stage polytomous model for marker-specific tumor heterogeneity tests (i.e. case-case comparisons from stage 2 of the two-stage model) between each individual variant and each of the tumor features (ER, PR, HER2, and grade), mutually adjusted for each other (separate models were fit for each variant).

[d] Model 3: Fixed effect two-stage polytomous model for risk associations with intrinsic-like subtypes (i.e. case-control comparisons from stage 1 of the two-stage model): luminal A-like, luminal B-like/HER2-negative, luminal B-like/HER2-positive, HER2-positive/non-luminal, and triple negative.

[e] Model 4: Fixed effect two-stage polytomous model for risk associations with tumor grade (i.e. case-control comparisons from stage 1 of the two-stage model) for the 12 variants associated at P<0.05 only with grade in case-case comparisons (from model 2): grade 1, grade 2, and grade 3.

Figure 2. Heatmap of the z-values from the fixed-effects two-stage polytomous model for marker-specific heterogeneity tests (case-case comparison from model 2) for association between each of the 173 breast cancer susceptibility variants and estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) or grade, adjusting for principal components and each tumor marker. Columns represent individual variants. For more detailed information on the context of figure see **Supplementary Figure 1.**

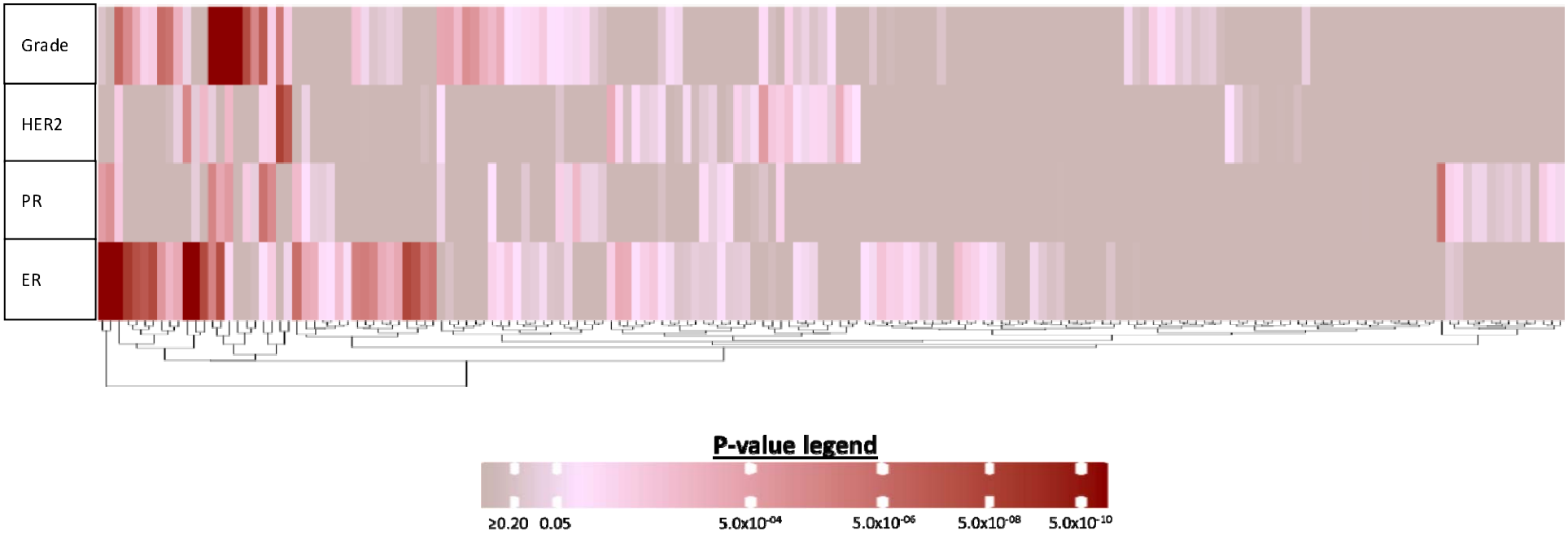
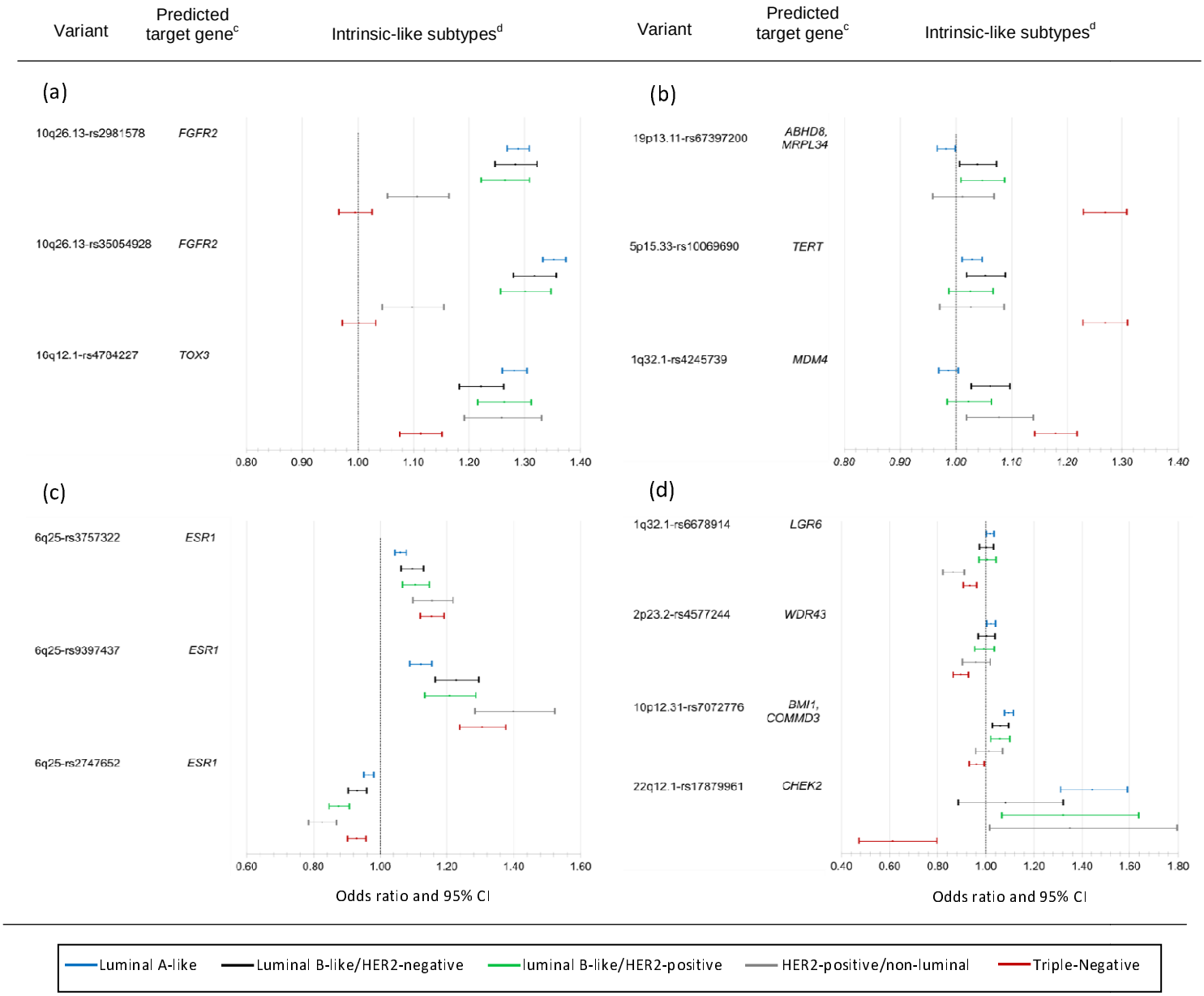


Figure 3. Results from fixed-effects two-stage polytomous models for risk associations[a] with intrinsic-like subtypes (model 3) for variants with evidence of heterogeneity by tumor markers in the two-stage model (model1)[b]; panels show examples of variants (a) most strongly associated with luminal-like subtypes, (b) most strongly associated with TN subtypes, (c) associated with all subtypes with varying strengths of association, and (d) associated with luminal A-like and TN subtypes in different directions. See **Supplementary Figure 3** for more details.



[a] Per-minor allele odds ratio (95% confidence limits).

[b] Model 1, mixed-effects two-stage polytomous model testing for global heterogeneity according to estrogen receptor (ER), progesterone receptor (PR), huma epidermal growth factor receptor 2 (HER2) and grade

[c] Predicted target genes as reported in Fachal L, et al. Nature genetics 2020; 52 (1), 56-73

[d] Luminal A-like (ER+ and/or PR+, HER2-, grade 1 & 2); Luminal B-like/HER2-negative (ER+ and/or PR+, HER2-, grade 3); luminal B-like/HER2-positive (ER+ and/or PR+, HER2+); HER2-positive/non-luminal (ER- and PR-, HER2+), and triple-negative (ER-, PR-, HER2-)

Table 1. Distribution of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and grade and the intrinsic-like subtypes[a] among cases of invasive breast cancer in studies from the Breast Cancer Consortium Association.

Tumor Marker	N (%)
ER	
Negative	16,900 (19%)
Positive	70,030 (81%)
Unknown	19,641
PR	
Negative	24,283 (32%)
Positive	51,603 (68%)
Unknown	30,685
HER2	
Negative	47,693 (83%)
Positive	9,529 (17%)
Unknown	49,349
Grade	
1	15,583 (20%)
2	37,568 (49%)
3	24,382 (31%)
Unknown	29,038
Intrinsic-like subtypes	
Luminal A-like	27,510 (54%)
Luminal B-like/HER2-negative	6,804 (13%)
Luminal B-like/HER2-positive	6,511 (13%)
HER2-positive/non-luminal	2,797 (6%)
Triple-negative	7,178 (14%)
Unknown	55,771

a Luminal A-like (ER+ and/or PR+, HER2-, grade 1 & 2); Luminal B-like/HER2-negative (ER+ and/or PR+, HER2-, grade 3); Luminal B-like/HER2-positive (ER+ and/or PR+, HER2+); HER2-positive/non-luminal (ER- and PR-, HER2+), and triple-negative (ER-, PR-, HER2-)