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

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Abstract

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. 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. 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. 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.

Significance

Our results demonstrate complex etiologic heterogeneity patterns of common breast cancer susceptibility variants and can inform functional analyses to identify underlying causal variants and the development of subtype-specific polygenic risk scores.

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 $p<5.0x<10^{-8}$ (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 also tested for evidence of heterogenous risk associations by TN status by fitting an extended mixed-effect two-stage polytomous model (model 1-extended), using a global heterogeneity test by ER, PR, HER2, TN, and/or grade, and an extended fixed-effects two stage model polytomous model to test which tumor features, ER, PR, HER2, TN, and/or grade, contributed to the heterogeneity (model 2-extended).

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), 3) estimation of case-control ORs (95%CIs) by intrinsic-like subtypes (model 3), and 4) estimation of case-control ORs (95%CIs) by tumor grade (model 4).

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 Fig. 1**).

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

Fixed-effects two-stage models (model 2) was 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**, **Supplementary Fig. 1)**. Eighteen of these 85 variants showed no associations with any individual tumor marker at P<0.05 (**Supplementary Fig. 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 Fig. 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 p-value of association between susceptibility variants and breast cancer intrinsic-like subtypes, and **Supplementary Figures 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 Fig. 2 footnote 'h'**). Ten variants were associated with risk of all subtypes (**Figure 1**, **Supplementary Fig. 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 Fig. 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 Fig. 2**). In an extended two-stage model (model 2-extended) to specifically test for heterogeneity between TN vs non-TN subtypes, both rs2981578 and rs35054928 were strongly associated with TN status (**Supplementary Fig. 4**). 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 Fig. 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 Fig. 2**). In the model 2-extended these variants were significantly associated with TN status (**Supplementary Fig. 4**).

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 Fig. 2**). rs2747652 was most strongly associated with risk of the HER2-positive/non-luminal subtype (**Figures 3c**, **Supplementary Fig. 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 Fig. 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 Fig. 2**, for rs67397200 see **Figure 3b**). In model 2-extended, rs7072776, rs17879961, and rs67397200 were significantly associated with TN status (**Supplementary Fig. 4**).

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 Fig. 5**. 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 and TN 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 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 or TN status. Of the four variants that showed evidence of heterogeneity only according to PR, rs10759243(6,26), rs11199914(6) 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(27). 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(28). 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(29) 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(30). 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. Five of these variants were previously reported to be associated primarily with ER-positive disease(6,31,32), 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 p-values for tumor marker-specific heterogeneity tests and case-control associations with risk of intrinsic-like subtypes. Because they are based on p-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. 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(33). 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(34).

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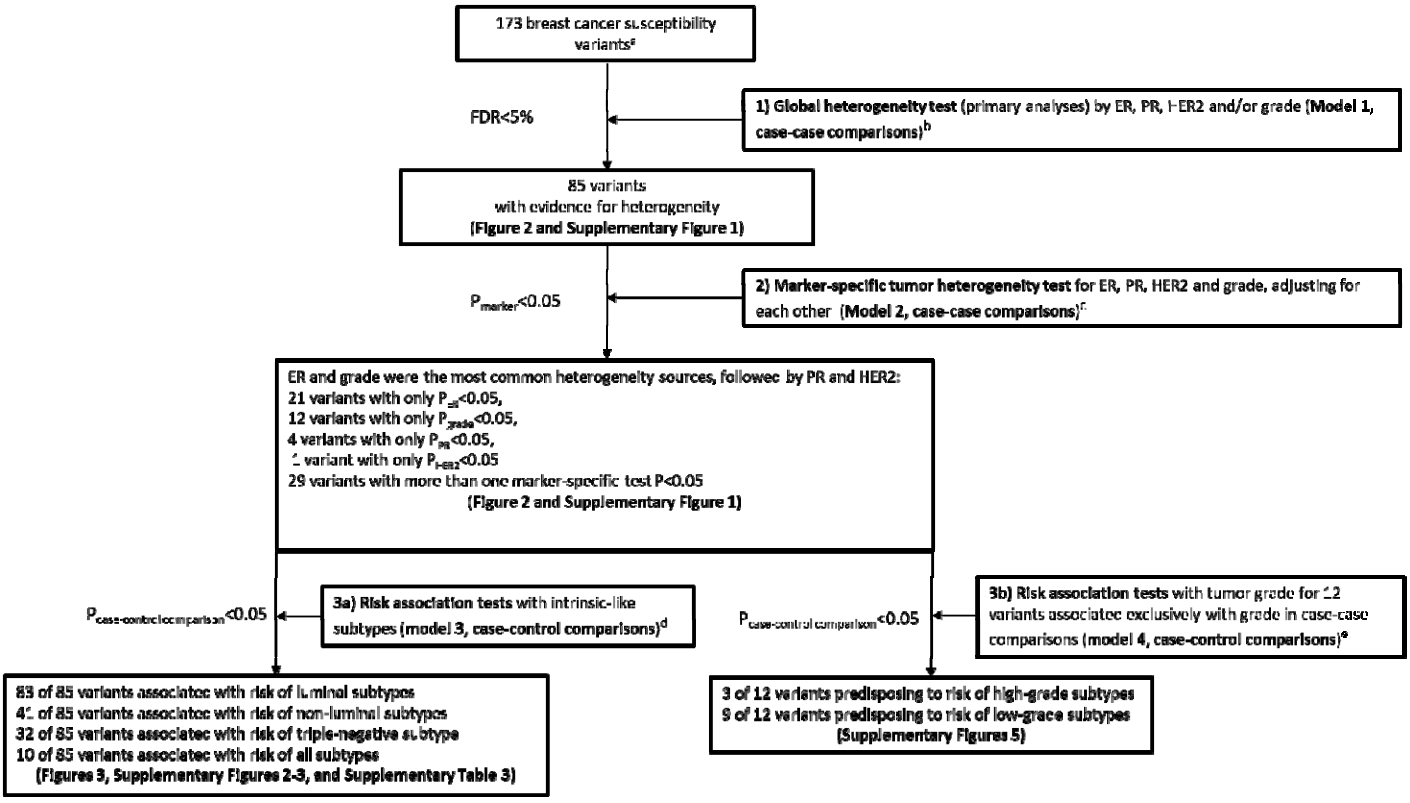
Figure 1. Overview of the analytic strategy and results from the investigation of 173 known breast cancer susceptibility variants for evidence of heterogeneity according to the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and grade



[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). In an extended analysis we fit, Model 1-extended, a mixed-effects two-stage polytomous model to test for global heterogeneity between each individual susceptibility variant and ER and triple-negative status (as fixed effects) and PR, HER2, and grade (as random effects).

[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). In an extended analysis we fit Model 2-extended, a fixed-effects two-stage polytomous model to test for marker-specific tumor heterogeneity between each individual susceptibility variant and each of ER, PR, HER2, grade, and triple-negative status, mutually adjusting for each other.

[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 P-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 Fig. 1.**

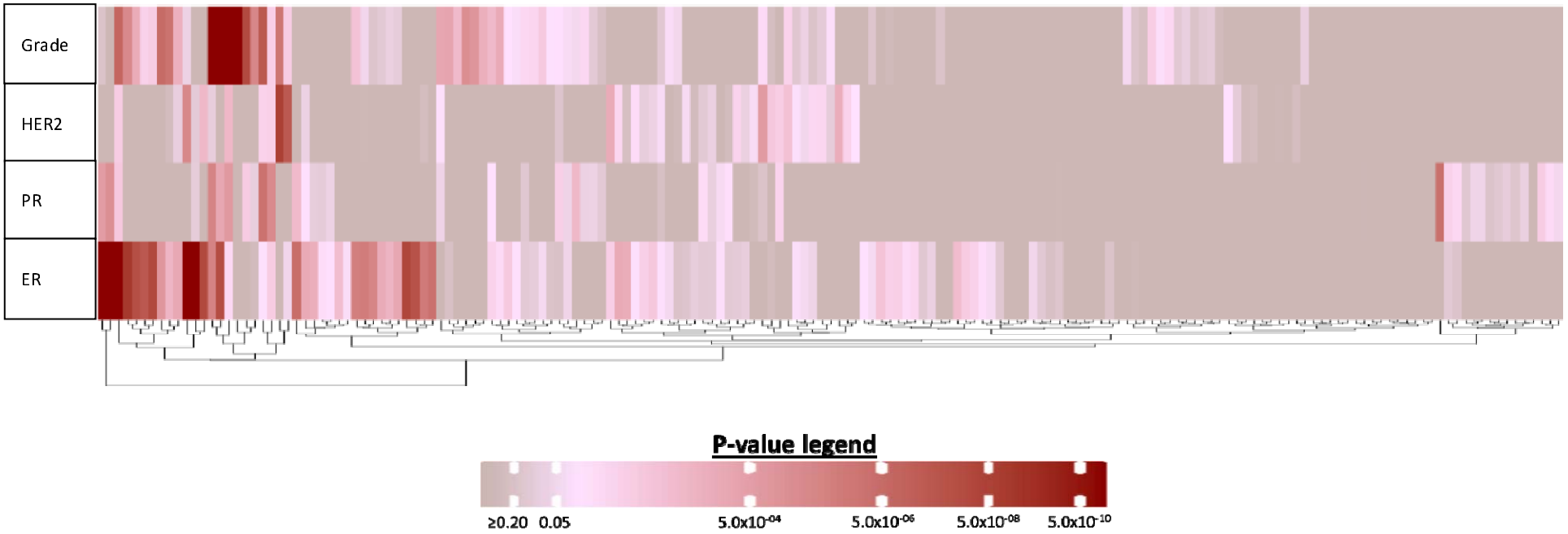
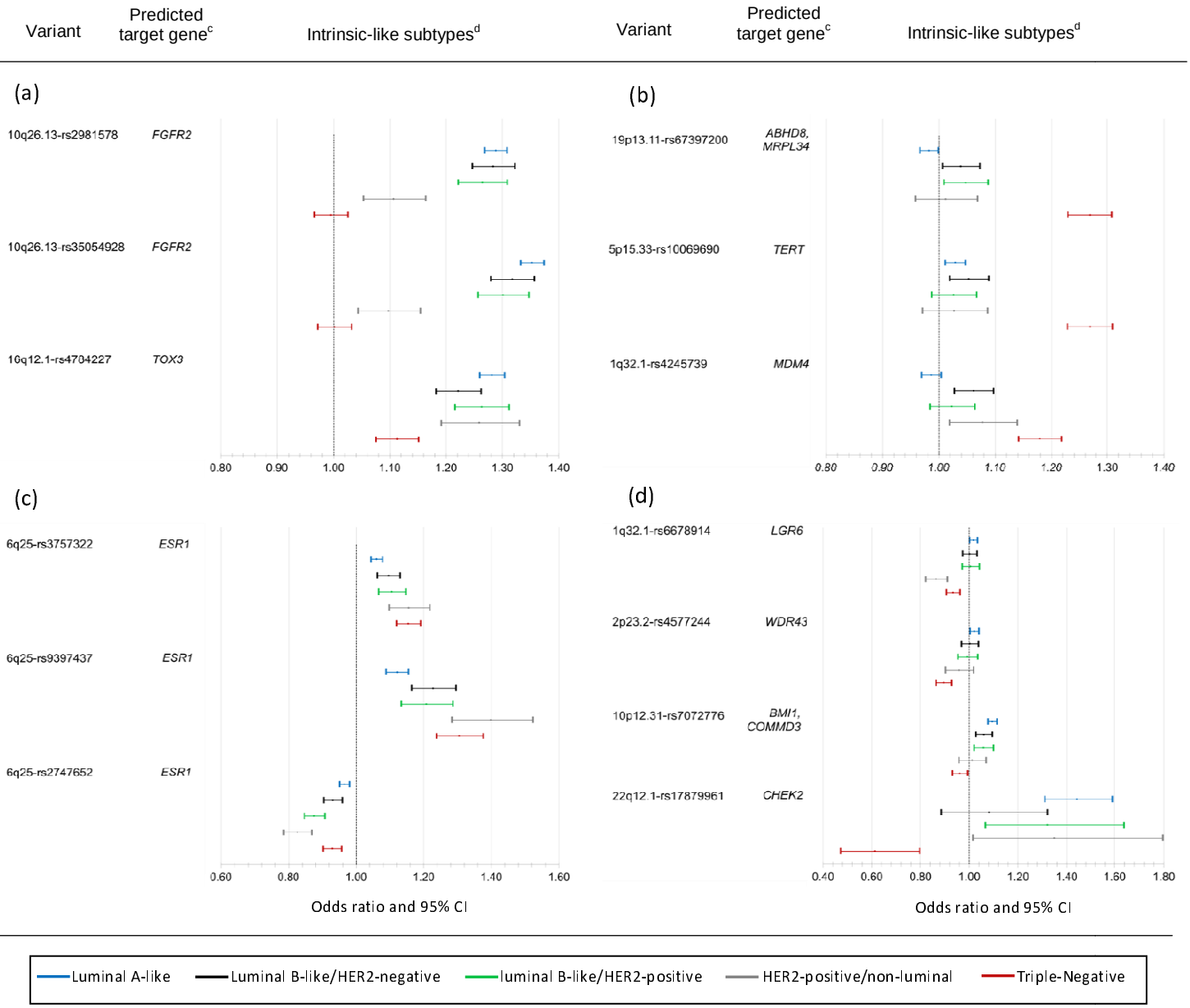


Figure 3. Results from fixed-effects two-stage polytomous models for risk associations[a] with intrinsic-like subtypes (model 3) for variants with evidence of heterogeneity by tumor markers in the two-stage model (model1)[b]; panels show examples of variants (a) most strongly associated with luminal-like 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-)