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Discriminatory accuracy and potential clinical utility of genomic profiling for breast cancer risk in BRCA-negative women

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Abstract

Several single nucleotide polymorphisms (SNPs) are associated with an increased risk of breast cancer. The clinical utility of genotyping individuals at these loci is not known. Subjects were 519 unaffected women without BRCA mutations. Gail, Claus, and IBIS models were used to estimate absolute breast cancer risks. Subjects were then genotyped at 15 independent risk loci. Published per-allele and genotype-specific odds ratios were used to calculate the composite cumulative genomic risk (CGR) for each subject. Affected age- and ethnicity-matched BRCA mutation-negative women were also genotyped as a comparison group for the calculation of discriminatory accuracy. The CGR was used to adjust absolute breast cancer risks calculated by Gail, Claus and IBIS models to determine the proportion of subjects whose recommendations for chemoprevention or MRI screening might be altered (reclassified) by such adjustment. Mean lifetime breast cancer risks calculated using the Gail, Claus, and IBIS models were 19.4, 13.0, and 17.7%, respectively. CGR did not correlate with breast cancer risk as calculated using any model. CGR was significantly higher in affected women (mean 3.35 vs. 3.12, $P = 0.009$). The discriminatory accuracy of the CGR alone was 0.55 (SE 0.019; $P = 0.006$). CGR adjustment of model-derived absolute risk estimates would have altered clinical recommendations for chemoprevention in 11–19% of subjects and for MRI screening in 8–32%. CGR has limited discriminatory accuracy. However, the use of a genomic risk term to adjust model-derived estimates has the potential to alter individual recommendations. These observations warrant investigation to evaluate the calibration of adjusted risk estimates.

Keywords

Breast cancer risk; Single nucleotide polymorphism; Risk prediction model

Introduction

Inheritable factors are responsible for a significant fraction of the variation in individual breast cancer risk [1]. Germline mutations in a number of genes confer moderate to high risks of breast cancer [2]. These mutations are uncommon and only explain a small proportion of the hereditary risk. Candidate gene and genome-wide association studies (GWAS) have identified a number of single nucleotide polymorphisms (SNPs) that are associated with risk [3–13]. These associations are highly statistically significant, but quantitatively very modest. For most SNPs, per allele odds ratios are less than 1.5. For this reason, it is not clear that genotyping individuals for these SNPs will be clinically useful. In fact, initial modeling analyses and a recent study of a large number of women participating in prospective cohort studies conclude that prediction models incorporating genomic SNP profiles will have limited to no advantage over traditional empiric models [14–16].

Consideration of the role of genomic profiling in breast cancer risk assessment has been limited to the question of whether this technology would be useful in the general population. There are limited data assessing the potential impact in women at familial risk for breast cancer. To this end, we evaluated a group of unaffected women who received uninformative negative BRCA1/2 genetic test results after seeking genetic counseling because of a family

history suggesting a possible hereditary risk for breast cancer. We sought to correlate the genomic risk predicted from SNP genotyping with the absolute risk estimated by empiric models and to assess the discriminatory accuracy of the genomic risk profile in distinguishing these women from age- and ethnicity-matched affected BRCA-negative women. Finally, we evaluated whether incorporating a genomic risk term into a model-based risk assessment would alter clinical recommendations for the use of chemoprevention and breast magnetic resonance imaging (MRI).

Materials and methods

Subjects

Subjects of this investigation were women who presented to the Clinical Genetics Service of Memorial Sloan-Kettering for breast cancer risk assessment between September 1996 and December 2008. All were participants in IRB-approved studies of clinical outcomes after genetic testing. At the time of consent, participants provided detailed information regarding their family histories of cancer and completed baseline questionnaires cataloging their breast cancer risk factors. After pre-test genetic counseling, women underwent testing for mutations in BRCA1 and BRCA2. All women of Ashkenazi Jewish descent underwent testing for the common Ashkenazi founder mutations. Non-Ashkenazi women and a subset of Ashkenazi women underwent full sequence analysis (Myriad Genetic Laboratories, Salt Lake City, UT, USA).

Review of the study database identified 541 unrelated BRCA-negative unaffected women who had given permission for future use of residual DNA. For the determination of discriminatory accuracy, we identified a comparison group of affected BRCA-negative women from the same follow-up study. For each unaffected subject, an ethnicity-matched woman with breast cancer was identified who was the same age at diagnosis as the unaffected woman had been at the time of genetic testing (± 2 years). Clinical records and study questionnaires were reviewed to confirm disease status at testing and to extract information for empiric risk calculation. After excluding subjects who were incorrectly classified, had insufficient information for risk calculations, or had no DNA available, there were 519 pairs of unaffected and affected women.

Genotyping

Single nucleotide polymorphisms to be genotyped were selected from a review of the published literature (Table 1). Each of these SNPs has been reported in at least one published study to reach genome-wide levels of statistical significance for association with breast cancer risk. SNPs were only selected for consideration if they had been replicated in independent data sets, either within the original publication or in a confirmatory publication, and were not linked to another risk SNP. Genotypes for subjects (unaffected and affected) were determined using Sequenom iPLEX Gold chemistry on the MassArray system [17]. Two SNPs (rs1982073 and rs2107425) were not included in the optimal multiplex design. These were genotyped using TaqMan allelic discrimination system under standard conditions (Applied Biosystems). Genotyping was successful at each SNP in 98% of cases, but was unsuccessful at one or more loci in 61/1081 subjects (5.9%). Hardy-Weinberg equilibrium was confirmed using PLINK (v.1.07) [18].

Absolute breast cancer risk prediction

For unaffected women, absolute breast cancer risk was estimated using the Gail, Claus, and Tyrer-Cuzick (IBIS) models [19–24]. Calculations were performed using the Cancer Gene program, version 5 [25, 26], and Tyrer-Cuzick calculations were performed using the

downloadable IBIS calculator. Absolute risks were calculated for 5 year and “lifetime” horizons, with “lifetime” being defined as per the specific model.

Genotypic risk calculation

Aggregate genotype-associated risks were calculated as suggested by Gail and others [14, 15, 27]. For each SNP, per allele odds ratios (OR) were taken from the combined analyses of the original publication, whenever possible (Table 1). For 2 SNPs (rs11249433 and rs999737), per allele OR were not reported in the original publications, and genotype-specific OR were used. A combined genomic risk (CGR) was calculated as follows:

$$\text{CGR}(X) = \prod_{i=1}^{15} (\text{OR}_i)^{X_i}$$

where X is the number of minor alleles at SNP i in a given subject and OR_i is the per allele odds ratio at that SNP, derived from the published literature. The calculation assumes that SNP effects are multiplicative (log additive), that the SNPs are in linkage equilibrium, and that the odds ratio closely approximates relative risk. Genotype-specific risks were substituted for $(\text{OR}_i)^{X_i}$ for rs11249433 and rs999737. CGR were only calculated for individuals who were successfully genotyped at all risk loci.

Genotype-specific adjustment of model estimates

To incorporate the CGR into the absolute risk estimate, we assumed that the CGR was independent of the modeled risk and that the effect of the CGR on modeled risk was multiplicative. Demonstration that CGR does not correlate with model-calculated risk was the objective of the first part of the study. The adjusted absolute risk was defined as the product of the model-derived absolute risk estimate and the ratio of the individual CGR to the population average CGR

$$\text{Risk}_{\text{Adj}} = \text{Risk}_{\text{Model}} \times \left(\frac{\text{CGR}}{\text{CGR}_{\text{AVE}}} \right)$$

CGR_{AVE} was calculated by Monte Carlo simulation using published per-allele odds ratios (or genotype-specific OR) to derive genotype-specific odds ratios as described above, and published risk allele frequencies to calculate population genotype prevalence (assuming Hardy–Weinberg equilibrium).

Analysis

Non-parametric tests (Mann–Whitney, Kruskal–Wallis) were used to evaluate whether CGR was associated with specific Gail model risk factors. Correlations between CGR and model-predicted risks were evaluated by calculation of Spearman’s rho. The same procedure was used to evaluate correlations between the number of risk alleles and empiric modeled risk. For SNPs whose per allele OR was less than 1, the major allele was considered the “risk allele.” Receiver operating characteristic (ROC) curves were constructed to evaluate the discriminatory accuracy of the CGR and the number of risk of alleles. The area under the ROC curve was calculated as the c statistic, which represents the probability that a randomly selected affected individual in the data set will have a higher CGR (or number of risk alleles) than an unaffected individual [28, 29]. Statistical significance was evaluated by means of asymptotic P values, calculated under non-parametric assumptions. Analyses were

performed using SPSS, version 16.0 (SPSS, Inc.). All *P* values are two-sided and values of less than 0.05 are considered significant.

Results

The characteristics of the subjects are presented in Table 2. For unaffected subjects, the mean 5-year breast cancer risk estimates calculated by the Gail, Claus, and IBIS models were 2.1% (95% CI 2.0, 2.3), 1.8% (95% CI 1.7–1.9%), and 2.5% (95% CI 2.2–2.8%), respectively. Mean lifetime risk estimates from the Gail, Claus, and IBIS models were 19.4% (95% CI 18.7–20.1%), 13.0% (95% CI 12.1–13.8%), and 17.7% (95% CI 16.7–18.6%). The different models produced significantly different absolute risk estimates for both 5 year ($P < 0.001$) and lifetime ($P < 0.001$) horizons. Age at testing was directly correlated with 5-year risk as estimated by all models, and inversely correlated with lifetime risk.

The median number of risk alleles was 14 (range 7–22) in women successfully genotyped at all loci, with no significant difference between affected and unaffected women (Fig. 1, $P = 0.10$). The median CGR was 2.88 (1.17–9.45) in unaffected women and 3.09 (1.18–9.90) in affected women ($P = 0.006$). CGR was strongly correlated with the number of risk alleles ($\rho = 0.833$, $P < 0.001$).

There was no significant correlation between the CGR and either 5 year or lifetime risk estimates calculated using any of the empiric risk models. There was also no significant correlation between the number of risk alleles and risk estimates derived from any model over either time span. No significant correlations between risk estimates and the number of risk alleles or CGR were noted when the analysis was repeated excluding non-white women.

The discriminatory capacity of the CGR (ROC curve) is presented in Fig. 2. The c statistic for CGR was 0.55 (95% CI 0.52–0.59, $P = 0.006$). The c statistic for the integer count of risk alleles as the discriminant was 0.53 (95% CI 0.499–0.570, $P = 0.1$). Discriminatory accuracy was nearly identical if non-white women were excluded from the analysis (for CGR, $C = 0.55$, $P = 0.01$; for risk allele count, $C = 0.53$, $P = 0.12$).

To assess the possible clinical relevance of genomic risk profiling, we evaluated the impact of using a genomic risk term to adjust absolute risk estimates. Such adjustment resulted in the reclassification of a substantial proportion of subjects, manifested as post-adjustment change in the quintile of estimated absolute risk (Fig. 3). Up to 60% of subjects in a given quintile of risk were reclassified after genomic adjustment of their risk estimate.

Women with a 5-year absolute breast cancer risk of 1.67% or greater are candidates for pharmacologic risk reduction with tamoxifen or raloxifene [30, 31]. The proportions of unaffected subjects in this study who exceeded this threshold according to Gail, Claus, and IBIS model estimates were 53% (253/477), 40% (160/397), and 57% (270/475), respectively. The proportions eligible according to adjusted risk estimates were 51.4% (245/477), 39.5% (157/397), and 55.6% (264/475) (Table 3). Although the overall proportions of subjects eligible for tamoxifen were not different after genomic adjustment of model risk, individual recommendations varied in a substantial proportion of cases. Of the women who would not have been considered eligible for SERM treatment based upon model-derived absolute risk estimates, 11% (24/224) (Gail), 12% (28/237) (Claus), and 19% (39/205) (IBIS) would have been eligible after adjustment of their individual estimate using a CGR term. Conversely, 13% (32/253) (Gail), 19% (31/160) (Claus), and 17% (45/270) (IBIS) of women who could have been offered a SERM based on the model-derived estimate had a calculated risk below the treatment threshold after adjustment.

Breast MRI screening is recommended to women with a “lifetime” risk estimate of 20% or greater [32]. By this criterion, 40% (189/476)(Gail), 17% (69/398)(Claus), and 34% (163/475)(IBIS) of the subjects of this study would have been eligible for MRI screening (Table 3). Using genomic risk-adjusted estimates, 42% (200/476)(adjusted Gail), 20% (79/398)(adjusted Claus), and 34% (162/475)(adjusted IBIS) of subjects would have been eligible. However, as for tamoxifen recommendations, incorporation of a genomic term would have reduced the risk estimate below the recommendation threshold in a significant number of individuals, even though the overall proportion recommended MRI would not have changed (29% (55/189) of individual subjects eligible for MR screening by the Gail model, 25% (17/69) of those eligible by the Claus model, and 32% (52/163) of those eligible by the IBIS model). Conversely, genomic adjustment would have led to a recommendation for MRI screening in 23% (66/287) (adjusted Gail), 8% (27/329)(adjusted Claus), and 16% (51/312)(adjusted IBIS) of those who would not have received such a recommendation based upon the model estimate alone.

Discussion

Highly penetrant mutation in genes such as BRCA1 and BRCA2 are only responsible for a small fraction of hereditary breast cancer predisposition. Genome-wide association studies have identified a number of common SNPs that are associated with very modest increases in risk. The clinical relevance of these findings is not clear. Gail used quantitative modeling to predict that including limited numbers of these SNPs in risk prediction models would add little to the discriminatory accuracy of existing models [14]. Wacholder and colleagues described the impact of including a genomic risk term in a model to predict risk in 5590 case subjects and 5998 controls from four cohort studies and one case-control study [16]. In this analysis, genotype information only improved the discriminatory accuracy of the nongenetic risk prediction model from 58.0 to 61.8%. In this study, the discriminatory accuracy of the genetic model alone was 59.7%, slightly better than our value of 55%.

Although overall discriminatory accuracy is an important measure of a test, other aspects of test performance are important when assessing clinical utility [28]. Inclusion of a genomic risk term may not improve discriminatory accuracy, yet still lead to significant reclassification of individual risk level in relationship to other individuals in the study population. In our study, depending on the model and time horizon analyzed, a substantial proportion (up to 60%) of subjects moved into a different quintile of risk when a genomic risk term was used to adjust their risk estimate. Similarly, Wacholder et al. [16] found that slightly more than half of the subjects in their study moved into a different quintile of risk when their risk was calculated using a model incorporating genetic factors.

Reclassification of an individual to a higher absolute level of risk may be important if that reclassification leads the individual's risk to exceed a predetermined action threshold. For example, recommendations for SERM chemoprevention and MRI screening are arbitrarily set at 1.67%/5 years and 20% lifetime risk, respectively. Relatively small increments (or decrements) in absolute risk estimates could modify whether or not these interventions are recommended by clinicians or reimbursed by third-party payers. Gail calculated that including genotype information would lead to relatively little change in chemoprevention recommendations for women in the general population [15]. However, in populations at an increased risk of BC, even small changes in absolute risk estimates may be relevant (or result in reclassification) as a larger proportion of such patients have baseline risk estimates close to the predetermined action threshold. Consistent with this hypothesis, we found that using a genomic risk term to adjust model-derived risk estimates did not substantially alter the overall proportion of women in the study population who would be recommended tamoxifen or MRI. However, adjustment of individual estimates would lead to changes in

individual recommendations for tamoxifen chemoprevention or MRI screening in a significant fraction of women, consistent with our observations with regard to interquartile movement of individual subjects. It is likely that the difference between our calculations and those of Gail result from the fact that women in the present study were ascertained due to a family history that justified BRCA mutation testing. As a result, their baseline risk estimates were likely higher than in an unselected population. The absolute change in risk after multiplicative adjustment of the baseline estimate was substantial enough to modify clinical recommendations in a correspondingly larger fraction of the study population.

There are a number of limitations to the design of the present study. Follow-up of the unaffected group for subsequent breast cancer is not complete, so we were not able to evaluate whether including a genomic risk term improved the ratio of observed cases relative to those predicted to occur by the empiric model alone (model calibration). Other published studies, including that of Wacholder et al. [28] suffer from a similar limitation and this is clearly an important question for future studies. We also were unable to calculate certain parameters of model performance, such as the integrated discrimination improvement, as pre-diagnosis Gail and Claus risk estimates were not available for the affected women in our study. The genomic risk term itself is sensitive to a number of assumptions. The term will vary depending upon the per-allele odds ratio (OR) assigned to each allele, which may vary among populations. The OR used for this investigation were taken from the primary publications describing the risk alleles, but these may be overestimates of the OR in other groups of subjects due to the phenomenon of the “winner’s curse” [33]. Per-allele OR may also vary in subjects of differing ethnicities. The calculated average CGR, and thus the ratio that is used to adjust the empiric estimate, is dependent upon assumptions about allele and genotype frequencies and per allele OR in the population under study. Finally, empiric models vary widely in the absolute risk estimates that determine prevention recommendations in the absence of genomic information, and the calibration of all such models is somewhat limited in the familial risk setting [19]. Genomic adjustment is not likely to materially improve a poorly calibrated model-derived estimate.

Variation in the factors described above will result in variation in the proportion of women whose clinical recommendations are altered by genomic adjustment of a baseline model-derived risk estimate. This study uses a “real world” ascertainment of women who underwent BRCA mutation testing to demonstrate that, under specific supportable assumptions, genomic adjustment results in modification of eligibility for breast cancer prevention options in a small but relevant proportion of women. However, further studies are clearly needed before genomic risk assessment can be used in the management of women at risk. In particular, large prospective studies are necessary to identify the optimal means of using genomic risk terms to improve the calibration of model-derived estimates. Such studies would establish whether genomic risk assessment could improve the accuracy of risk assessment, even in the face of limited discriminatory accuracy. Such studies will also need to document whether or not genomic adjustment of modeled estimates to alter clinical recommendations more effectively targets preventive interventions by correctly reclassifying subjects. Until such studies are completed, the role of genomic risk assessment in the management of women at risk remains uncertain.

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Fig. 1.
Risk allele distribution in unaffected and affected subjects

Fig. 2. Receiver operating characteristic curve illustrating discriminatory accuracy of combined genomic risk (CGR)

Table 1

Genotyped single nucleotide polymorphisms (SNPs)

SNP	Gene (or region)	Per-allele	OR Risk allele frequency in unaffected subjects	Published RAF
rs2107425 [5]	H19	0.96 (0.93,0.99)	0.31	0.31
rs1982073 [4]	TGFB1 L10P	1.08(1.04,1.11)	0.48	0.38
rs1045485 [4]	CASP8 D302H	0.88(0.84,0.92)	0.08	0.13
rs1094167 [12, 13]	MRPS30 (5p12)	1.19(1.13,1.26)	0.20	0.24
rs11249433 [13]	1p11.2	1.16 (1.09,1.24) het 1.30 (1.19,1.41) homo	0.42	0.39
rs13281615 [5, 6, 13]	8q24	1.08 (1.05,1.11)	0.45	0.40
rs13387042 [10, 11, 13]	2q35	1.20 (1.14,1.26)	0.56	0.50
rs2180341 [7, 9]	6q22.33 (ECHDC1;RNF146)	1.41 (1.25,1.59)	0.23	0.21
rs2981582 [5, 8, 12, 13]	FGFR2 (10q)	1.26(1.23,1.30)	0.41	0.38
rs3803662 [5, 11, 13]	TNRC9 (16q)	1.20(1.16,1.24)	0.37	0.25
rs3817198 [5, 13]	LSP1 (11p)	1.07(1.04,1.11)	0.41	0.30
rs4973768 [3]	SLC4A7/NEK10 (3p24)	1.11 (1.08,1.13)	0.51	0.46
rs6504950 [3]	COX11 (17q)	0.95(0.92,0.97)	0.22	0.27
rs889312 [5, 13]	MAP3K1	1.13(1.10,1.16)	0.31	0.28
rs999737 [13]	RAD51L1	0.94 (0.88,0.99) het, 0.70 (0.62,0.80) homo	0.83	0.76

Table 2

Subject characteristics

N	Unaffected 519	Affected 519
Age at testing (mean, median, range)	46.9, 45.5, 24–86	46.8, 46, 25–85
Ethnicity		
AJ	331	331
White, non-Hispanic	148	148
Black	16	16
Hispanic	15	16
Asian	7	7
Persian	2	2
Gail Risk (mean, median, range)		
5 yr	2.1, 1.7, 0.1–12.5	
LT	19.4, 18.8, 2.7–60.7	
Claus Risk (mean, median, range)		
5 yr	1.8, 1.5, 0.1– 6.1	
LT	13.0, 11.0, 0.1– 42.7	
IBIS risk (mean, median, range)		
5 yr	2.5, 2.0, 0.15–27.5	
LT	17.7, 16.6, 2.0–87.0	
Number of risk alleles (mean, median)	14.03, 14.0	14.32, 14.0
Combined genomic risk (mean, median)	3.12, 2.88	3.35, 3.09

Table 3
Reclassification of eligibility for tamoxifen or MRI after incorporation of genomic risk term

Recommendation	Gail		Claus		IBIS	
	Before CGR *	After CGR	Before CGR	After CGR	Before CGR	After CGR
Tamoxifen (≥1.67% 5-year)	253/477 (53%)	245/477 (51%)	160/397 (40%)	157/397 (40%)	270/475 (57%)	264/475 (56%)
MRI (≥20% “lifetime”)	189/476 (40%)	200/476 (42%)	69/398 (17%)	79/398 (20%)	163/475 (34%)	162/475 (34%)

CGR combined genomic risk