Breast cancer risk prediction using a polygenic risk score in the familial setting: a prospective study from the Breast Cancer Family Registry and kConFab

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Supplementary information is available at the Genetics in Medicine website.
Abstract

Purpose—This study examined the utility of sets of Single Nucleotide Polymorphisms (SNPs) in familial but non-BRCA-associated breast cancer (BC).

Methods—We derived a polygenic risk score (PRS) based on 24 known BC risk SNPs for 4,365 women from the BCFR and kConFab familial BC cohorts. We compared scores in women based on cancer status at baseline. 2,599 women unaffected at enrollment were followed for an average of 7.4 years. Cox proportional hazards regression was used to analyze the association of PRS with BC risk. The BOADICEA risk prediction algorithm was used to measure risk based on family history alone.

Results—The mean (SD) PRS baseline was 2.25 (0.35) for the affected and 2.17 (0.35) for unaffected women from combined cohorts (p<10^−6). During follow-up, 205 BCs occurred. The hazard ratios for continuous PRS (per SD), and upper vs. lower quintiles were 1.38 (95% CI: 1.22–1.56) and 3.18 (95% CI: 1.84–5.23) respectively. Based on their PRS-based predicted risk, management for up to 23% of women could be altered.

Conclusion—including BC-associated SNPs in risk assessment can provide more accurate risk prediction than family history alone and can influence recommendations for cancer screening and prevention modalities for high-risk women.

Keywords
risk prediction; breast cancer; non-BRCA associated; polygenic risk score; cancer screening

INTRODUCTION

In the recent initiative towards “precision medicine” announced by the National Institutes of Health\(^1\), the use of genetic information for the identification of high-risk groups for targeted
screening and/or prevention is gradually becoming part of routine medical care. Once limited to pathogenic mutations in high-risk genes such as BRCA1, BRCA2, p53, and the mismatch repair genes associated with Lynch Syndrome, the last decade has seen the identification of additional genes for which pathogenic variants are associated with perhaps two- to five-fold increased risks of cancer, as well as an ever-increasing set of common SNPs, each of which is associated with a relative risk of 1.05 to 1.3 of developing breast cancer. Although these SNPs are not useful for risk prediction when considered individually, theoretical calculations indicate that a combined score based on genotypes at a large number of such loci could have substantial predictive value for risk stratification in the general population, as well as in BRCA1 and BRCA2 carriers. The combination of high-risk genes such as BRCA1 and BRCA2 and the known SNPs described above is estimated to explain less than half of the familial aggregation of breast cancer. This notwithstanding, such sets of SNPs may have clinically useful predictive power in the familial setting, due to the increased risk of breast cancer conferred by a woman’s family history alone. To date, there has only been a single study examining the utility of such SNP panels in the familial context, and none in a prospective fashion. Sawyer et al. looked at differences in a PRS based on 22 SNPs between BRCA1/2 carriers and BRCA1/2 negative women with breast cancer from a familial cancer clinic in Australia, and a set of controls. They found that non-carrier cases had a higher PRS than BRCA1/2 carriers and that a higher proportion of non-BRCA1/2 cases individuals with a PRS in the top quartile had breast cancer diagnosed before age 30 compared to the lowest quartile. The goal of the present study was to examine the utility of panels of SNPs in the context of familial breast cancer, where women are already at elevated risk due to their family history and to determine if such SNP panels could stratify women into clinically useful risk groups. Currently, various advisory bodies have proposed guidelines for the use of magnetic resonance imaging (MRI) in addition to mammography for women at high risk. For example, the American Cancer Society proposes lifetime risk thresholds of 20 – 25% for MRI while the UK NICE guidelines use a threshold of 30%. Here we examine women in families not known to have BRCA1/2 mutations from two different familial breast cancer resources – the Breast Cancer Family Registry (BCFR) cohort and the Kathleen Cuningham Consortium Foundation for Research into Familial Breast Cancer (kConFab). This study is novel in two ways: first, it examines women who are already at increased familial risk, and second it prospectively analyzes women who were unaffected at cohort enrollment.

MATERIALS AND METHODS

SNP selection and genotyping

BCFR—For BCFR subjects, a total of 24 SNPs were successfully genotyped (Supplemental Table S1 online). These correspond to the loci known to be associated with breast cancer at the start of the study and do not include the more recent loci discovered as part of the iCOGS analyses. These SNPs were genotyped using a capture-based next generation sequencing method developed by one of us (A.M.) specifically for this study.

kConFab—In kConFab, SNPs were genotyped in two phases using two different technologies. In the first phase, 18 SNPs were typed using iPLEX, and in the second phase,
an additional 90 SNPs were typed using Fluidigm technology. In order to have comparable scores for the two data sets to allow a combined analysis, we chose 24 SNPs, which were either the same SNP or in complete or strong ($R^2 > 0.9$) linkage disequilibrium (LD) with the SNP genotyped in BCFR. Supplemental Table S1 online shows the minor allele frequency and odds ratio (OR) for the SNPs genotyped in each cohort.

**Subjects**—The BCFR is a National Cancer Institute sponsored resource of familial breast cancer ([www.bcfamilyregistry.org](http://www.bcfamilyregistry.org))\(^{10,11}\). It consists of over 15000 families enrolled since 1995 from six sites in the U.S. (Utah, Northern California, New York, Philadelphia), Australia, and Canada, with data collection on lifestyle factors, tumor histopathology, and increasingly, genetic information. Three of the sites incorporated a clinic-based ascertainment strategy, while the other three were population-based. Recruitment and genetic studies were approved by the University of Utah IRB, and the local IRBs of the BCFR centers from which we received blood samples and data. Written informed consent was obtained from each participant. Families were selected for this study on the basis of availability of DNA samples in the family and age (at least one woman diagnosed with BC under age 60 years prior to enrollment and one or more unaffected women over age 30 years at baseline with a DNA sample available). In total, 2,467 women were successfully genotyped for at least 20 of the 24 SNPs; of these, 96% had at least 22 valid genotypes called. After exclusion of 376 women without the required dates of birth, enrollment, and follow-up end-points, 2,091 women from 707 families were included in the analyses. Of these, 991 women in 481 families who were unaffected with BC and were less than 70 years of age at baseline were included in the prospective analyses.

The second data set analyzed as part of this project was based on the kConFab\(^{12}\) resource that has enrolled BC families since 1997 and systematically followed up women every 3 years.\(^{13}\) Details on the resource and the ascertainment criteria have been described elsewhere ([www.kconfab.org](http://www.kconfab.org)). Subjects were selected for genotyping based solely on their phenotype at baseline, without regard for any subsequent cancers. For this study, eligibility was restricted to families with at least one family member genotyped for the SNPs of interest. Families were systematically screened for and excluded if found to contain a mutation in BRCA1, BRCA2, PALB2 or ATM. In this study we included 2,732 women from 535 families who had sufficient genotype data to compute PRS. After excluding women who did not meet the inclusion criteria, there were 2,274 women from 523 families eligible for analysis. Of these, 1,608 women from 488 families were included in the prospective cohort based on the same inclusion criteria as for BCFR described above. All participants in this study signed informed consent and the study was approved by the Human Research Ethics Committee of the Peter MacCallum Cancer Center, as well as at all participating centers.

**Statistical Methods**

**Calculation of PRS**—We created a PRS for each genotyped individual based on their genotypes at each of the 24 loci, defined for the $j_{th}$ individual as $PRS_j = \sum_{i=1}^{24} n_{ij} \ln(R_i)$, where $n_{ij}$ is the number of risk alleles carried by the $j_{th}$ individual at the $i_{th}$ SNP, $n_{ij} = \{0, 1, 2\}$ and $R_i$ is the per-allele Relative Risk (estimated by the per allele Odds Ratio (OR) in Europeans from large published studies\(^{3}\)) associated with the $i_{th}$ SNP. When SNP genotypes
were missing for an individual (maximum of four missing genotypes per individual), they were included in the overall PRS by weighting each genotype by its expectation given the MAF at that locus and their relatives’ genotypes (if any) as estimated from 10,000 replicates of the data set using the simulation program SLINK\textsuperscript{14}.

For the 24 SNPs used here, the theoretical expected value of the PRS is 2.123 with variance of 0.117, based on the ORs and MAF for each SNP.

Assessment of family history—As part of the Prof-SC cohort\textsuperscript{11} the BOADICEA model\textsuperscript{15} was used to predict BC risk in over 18,000 unaffected women from the BCFR and KConFab cohorts. Although originally designed to predict probabilities of an individual carrying a \textit{BRCA1} or \textit{BRCA2} mutation, BOADICEA also predicts a woman’s risk of breast and ovarian cancer both for the next 10 years and until age 80 (remaining lifetime risk) and has shown to an accurate predictor of breast cancer risk in a prospective study\textsuperscript{16}. Specifically we used the predicted 10-year risk of BC as calculated by BOADICEA as a summary measure of each woman’s familial risk given her age and the ages/age at diagnosis and cancer (breast, ovarian, prostate and pancreatic) status of all their relatives and incorporates any available \textit{BRCA1/2} genetic testing results. Of the 2,599 women in the prospective analysis, BOADICEA scores were available for 2457 (95%) women. Lastly, we used the BOADICEA remaining lifetime risk as a baseline for modification by PRS as described below to examine lifetime risk changes as a function of the SNP-based PRS.

Statistical Analysis of PRS scores—We compared PRS scores in women who were affected and unaffected with BC at entry into the BCFR or kConFab cohorts. In this analysis, all women with a PRS were included without regard to previous history of other cancers (e.g., ovarian cancer). To adjust for the slight differences in the specific SNPs used in the two cohorts and to express the estimated hazard ratios (HRs) per standard deviation, we normalized the PRS scores by subtracting the theoretical mean from each score and dividing by the theoretical standard deviation prior to analysis. The primary analyses were prospective in which women who were unaffected by BC and who had not undergone bilateral prophylactic mastectomy (BPM) prior to cohort enrolment were eligible for follow-up with the primary endpoint development of invasive BC or DCIS during the follow-up period. Women were censored at the earliest of 1) diagnosis of BC (invasive or DCIS); 2) BPM; 3) death; or 4) last follow-up questionnaire (or last date known to be alive and cancer free). The characteristics of the 2,599 women who form the prospective cohort are presented in Table 1. We used Cox proportional hazards models to evaluate the effect of PRS on BC risk in this cohort. In these analyses, we used both the continuous PRS score as an independent predictor as well as a comparison of the upper and lower quintile of such scores (calculated separately for BCFR and kConFab cohorts). The main analyses were stratified by study center (the six BCFR sites and kConFab) and all analyses used a robust variance estimator based on family membership to adjust the variance for correlations in scores and overall cancer risks in related individuals. Interactions with family history, age, and study center were done using multivariable Cox models including main effects and an interaction term.
In order to examine the effect of the PRS on the estimated lifetime risk of breast cancer as assessed by BOADICEA (LRB) we estimated a SNP-based cumulative risk for each woman in the sample by $1 - \exp(-LRB \times HR_i)$ where $HR_i = \exp(\beta \times PRS_i)$ and $\beta$ is the natural logarithm of the estimated HR for continuous PRS in the prospective cohort and $PRS_i$ is the standardized PRS for the $i$th woman in the cohort.

All statistical analyses were done using STATA 12.0 (StataCorp, College Station TX).

RESULTS

We first compared the PRS in all subjects at baseline. A total of 1,496 women affected with breast cancer (1,084 BCFR; 412 kConFab) and 2,869 (1,007 BCFR; 1,862 kConFab) unaffected women were available for analysis. There were highly significant differences between the mean PRS in affected women at baseline compared to unaffected women in each cohort as well as the combined set ($p=3 \times 10^{-5}$, $1 \times 10^{-6}$, and $1 \times 10^{-10}$, respectively). The mean PRS in unaffected women of 2.170 is slightly higher than the theoretical mean of 2.123, which is expected given their selection from a positive family history. PRS scores were quite comparable between the two cohorts, especially in unaffected women. Table 1 shows the characteristics of the prospective cohort. The overall breast cancer incidence was higher in the BCFR cohort ($p=0.0012$) but this is likely because women in the BCFR were on average older at start of follow-up than those in kConFab (46.4 vs. 42.6; $p<10^{-5}$) and may have had a less stringent family history criterion for entry than that for the BCFR. The results of the Cox proportional hazards models in the analysis of prospective data are shown in Table 2. In both of the cohorts and for both the continuous and upper vs. lower quintile PRS score, the PRS was associated with highly significant increased risk with a HR for upper vs. lower quintile of 3.18. HRs by quintile, for each study are shown in Supplemental Table S2 online. The HRs for the continuous PRS were not significantly different between the BCFR and kConFab study cohorts ($p=0.13$) for study*PRS interaction, but were borderline significant for the upper vs. lower quintile ($p=0.05$), nor did the HR vary significantly as a function of age at baseline ($p=0.88$ and $p=0.71$ for the two cohorts, respectively). We tested the validity of the proportional hazards assumption implicit in the Cox models; neither the quintiles defined by PRS ($p=0.85$) nor the continuous PRS score ($p=0.64$) showed departure from the proportional hazards assumption. In a sensitivity analyses we excluded women who had been affected with any cancer at baseline (including ovarian) and censored women at date of diagnosis of any non-breast cancer occurring during follow-up. Results were only slightly changed from those above.

We used Kaplan-Meier survival analysis to look at the cumulative risks of BC for the lower quintile, three middle quintiles, and upper quintile as shown in Figure 1. Risks to age 70 were 51% (95% CI: 42% – 60%) for women in the highest quintile of PRS compared to 21% (14% – 31%) in the lowest. Similar plots for each of the two cohorts individually are presented in Supplemental Figure S1 online.

Analysis of PRS and family history

In order to explore the joint relationship of the PRS and family history on risk, we added the BOADICEA 10-year risk score to the Cox models and looked at the effect of the PRS score
adjusted for family history. For the set of individuals with these scores, the HR associated with the PRS in the combined dataset was 1.36 ($p=2\times 10^{-6}$) while with the BOADICEA 10-year score in the model the HR was only slightly reduced (1.34 ($p=1\times 10^{-5}$)). The BOADICEA 10-year risk estimate was also a significant predictor (HR=1.1; $p=9\times 10^{-4}$) of BC risk. There was no evidence of an interaction between the PRS and BOADICEA 10-year risk ($p=0.31$). Supplemental Figure S2 online shows the Kaplan-Meier plots for the lowest, middle, and highest tertiles of the baseline BOADICEA 10-year risk.

Figure 2 displays a plot of the BOADICEA lifetime risk plotted against the estimated remaining lifetime risk based on the BOADICEA score and the individual PRS with indicators of the 20% and 25% risk categories which would be considered cutoffs for recommending screening breast MRI. Table 3 shows the numbers of women in each of the risk quadrants for the two thresholds. For example, assuming the 20% threshold for MRI screening, 249 women out of 1,585 (16%) moved from below the threshold to above this threshold.

**DISCUSSION**

The results of this study show that using even a subset of the current ~96 breast cancer-associated SNPs can provide a potentially useful stratification of women into risk groups. However, the SNPs that we did not include in our study are, in general rarer, and/or have smaller effect size so we believe we have captured a significant proportion of the known genetic variance of BC due to common alleles of small effect. Based on the theoretical standard deviation of the score calculated from 77 SNPs in Mavaddat et al. We calculate that our PRS score captures about 2/3 of the genetic variance represented in the more recent panel. It is likely that inclusion of more complete sets of SNPs would further increase the discriminatory power. To our knowledge this is the first prospective study (familial or otherwise) to demonstrate the ability of such SNP panels to predict breast cancer outcome. Sawyer et al. estimated an HR of 2.08 for the lowest quartile compared to the highest quartile in assessing the risk of contralateral BC using a PRS based on 22 SNPs. However, this was a retrospective analysis in which women who presented with bilateral BC were compared with unilateral cases. This compares with the HR of 3.18 for highest and lowest quintile in our prospective analysis based on a PRS composed of 24 SNPs. Comparing familial BC cases to controls, the Sawyer study found an Area Under the Curve (AUC) of 0.64 for predicting BC based on their PRS; in our prospective analysis we found an AUC of 0.59 (95% CI 0.55 – 0.63). The absolute risks associated with women in the highest quintile of PRS were quite high, but it must be noted that these women in the BCFR were selected for genotyping based on having a family history, and women/families enrolled in kConFab are selected on the basis of their family history.

Both the American Cancer Society and the National Comprehensive Cancer Network guidelines propose that women with a lifetime risk for BC above 20 to 25% should receive MRI screening. Using the BOADICEA algorithm to predict lifetime risk and assuming the 25% threshold, 14% of women in this familial cohort would theoretically have a change in management (i.e., screening or prevention recommendations); with the lower threshold of 20%, this figure increases to 23%. However, these estimates are based on the HRs for the
PRS estimated from the data and thus would not be, strictly speaking, valid estimates of risk and are specific to the risk distribution in this set of selected families. However, this does demonstrate how the PRS can be used to more effectively target screening/prevention choices in BRCA1/2-negative women with a family history of the BC.

In summary, we have shown that SNP panels can be a useful adjunct to genetic testing for high penetrance genes in women with a family history of BC. Inclusion of risk scores based on BC associated SNPs in risk assessment can provide more accurate risk prediction than family history alone and can influence recommendations for cancer screening and prevention modalities for high-risk women.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

This research was supported by a grant from Cancer Australia’s Priority-driven Collaborative Cancer Research Scheme no. 566791 to G.M., by NIH grant U19 CA 148065-01 (DRIVE, part of the GAME-ON initiative) and NIH grant R01CA155767, to D.E.G. The Breast Cancer Family Registry is supported by NIH grants R01 CA159868 and UM1 CA164920 from the USA National Cancer Institute. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products or organizations imply endorsement by the USA Government or the BCFR. K.A.P. is an Australian National Breast Cancer Foundation Practitioner Fellow. I.L.A. holds the Anne and Max Tanenbaum Chair in Molecular Medicine at Mount Sinai Hospital and the University of Toronto.

We wish to thank Heather Thorne, Eveline Niedermayr, all the kConFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the investigators and staff of the kConFab Clinical Follow Up Study, which has received funding from the NHMRC, the National Breast Cancer Foundation, Cancer Australia, and the National Institute of Health (USA), for their contributions to this resource. kConFab is supported by a grant from the National Breast Cancer Foundation, and previously by the National Health and Medical Research Council (NHMRC), the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the Cancer Foundation of Western Australia.

Most importantly, the authors would like to thank all the families enrolled in the BCFR and kConFab resources for their willingness to participate in research without whom the work presented here would not be possible.

**References**


Figure 1.
Kaplan-Meier plot of breast cancer risk in the prospective cohort for the upper, middle three, and lower quintiles of the PRS. P-value shown corresponds to log-rank test comparing the three curves.
Figure 2.
Scatter plot of BOADICEA lifetime risk against estimated lifetime risk based on the combination of BOADICEA score and the individual PRS. In the bottom panel solid horizontal and vertical line indicate the 20% threshold of lifetime risk while dashed lines denote the 25% threshold. Each red dot corresponds to an individual woman in the prospective cohort. Those in the upper left and lower right quadrants would be those who potentially could have a change in screening recommendations based on current guidelines.
<table>
<thead>
<tr>
<th>Study</th>
<th>Women</th>
<th>Person Years</th>
<th>Median (Mean) follow-up (yrs)</th>
<th>N(BC)</th>
<th>Age at Dx Mean (range)</th>
<th>Incidence Rate</th>
</tr>
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<tr>
<td>BCFR</td>
<td>991</td>
<td>10789.66</td>
<td>12.3 (10.9)</td>
<td>138</td>
<td>54.4 (29–79)</td>
<td>0.013</td>
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<tr>
<td>kConFab</td>
<td>1608</td>
<td>8420.32</td>
<td>5.0 (5.2)</td>
<td>67</td>
<td>51.9 (30–75)</td>
<td>0.008</td>
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<tr>
<td>Combined</td>
<td>2599</td>
<td>19209.98</td>
<td>6.2 (7.4)</td>
<td>205</td>
<td>53.6 (29–79)</td>
<td>0.011</td>
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<tr>
<td>Study</td>
<td>Analysis</td>
<td>Hazard Ratio</td>
<td>95% CI</td>
<td>p-value</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>BCFR</td>
<td>Continuous PRS</td>
<td>1.30</td>
<td>(1.12, 1.51)</td>
<td>$6.3 \times 10^{-4}$</td>
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</tr>
<tr>
<td></td>
<td>Upper vs. Lower Quintile</td>
<td>2.38</td>
<td>(1.37, 4.13)</td>
<td>$2.0 \times 10^{-3}$</td>
<td></td>
<td></td>
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<tr>
<td>kConFab</td>
<td>Continuous PRS</td>
<td>1.59</td>
<td>(1.29, 1.96)</td>
<td>$1.2 \times 10^{-5}$</td>
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<td>Upper vs. Lower Quintile</td>
<td>10.82</td>
<td>(2.73, 42.86)</td>
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<tr>
<td>Combined</td>
<td>Continuous PRS</td>
<td>1.38</td>
<td>(1.22, 1.56)</td>
<td>$2.9 \times 10^{-7}$</td>
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<td></td>
<td>Upper vs. Lower Quintile</td>
<td>3.18</td>
<td>(1.84, 5.23)</td>
<td>$4.7 \times 10^{-6}$</td>
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Table 3

Number of women below and above MRI screening threshold based on BOADICEA remaining lifetime risk and BOADICEA and PRS score. Two thresholds for screening are shown: 20% and 25%.

<table>
<thead>
<tr>
<th>BOADICEA Lifetime risk</th>
<th>BOADICEA +PRS Risk</th>
<th>Number of women</th>
<th>Percent change</th>
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<tbody>
<tr>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>1336</td>
<td>249/1585 = 15.7%</td>
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<tr>
<td>&lt;0.2</td>
<td>&gt;=0.2</td>
<td>249</td>
<td></td>
</tr>
<tr>
<td>&gt;=0.2</td>
<td>&lt;0.2</td>
<td>312</td>
<td>312/873 = 35.7%</td>
</tr>
<tr>
<td>&gt;=0.2</td>
<td>&gt;=0.2</td>
<td>561</td>
<td></td>
</tr>
<tr>
<td><strong>Overall change</strong></td>
<td></td>
<td><strong>561/2458 = 23%</strong></td>
<td></td>
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<tr>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>1944</td>
<td>232/2176 = 10.7%</td>
</tr>
<tr>
<td>&lt;0.25</td>
<td>&gt;=0.25</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td>&gt;=0.25</td>
<td>&lt;0.25</td>
<td>119</td>
<td>119/282 = 42.2%</td>
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<tr>
<td>&gt;=0.25</td>
<td>&gt;=0.25</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td><strong>Overall Change</strong></td>
<td></td>
<td><strong>351/2458 = 14%</strong></td>
<td></td>
</tr>
</tbody>
</table>