Association of a Common AKAP9 Variant With Breast Cancer Risk: A Collaborative Analysis


Data from several studies have suggested that polymorphisms in A-kinase anchor proteins (AKAPs), which are key components of signal transduction, contribute to carcinogenesis. To evaluate the impact of AKAP variants on breast cancer risk, we genotyped six nonsynonymous single-nucleotide polymorphisms (SNPs) in cancer-related genes that were predicted to be deleterious and found two (M463I, 1389G>T and N2792S, 8375A>G) to be associated with an allele dose-dependent increase in risk of familial breast cancer in a German population. We extended the analysis of AKAP9 M463I, which is in strong linkage disequilibrium with AKAP9 N2792S, to 9523 breast cancer patients and 13,770 healthy control subjects from seven independent European and Australian breast cancer studies. All statistical tests were two-sided. The collaborative analysis confirmed the association of M463I with increased breast cancer risk. Among all breast cancer patients, the combined adjusted odds ratio (OR) of breast cancer for individuals homozygous for the rare allele TT (frequency = 0.19) compared with GG homozygotes was 1.17 (95% confidence interval [CI] = 1.08 to 1.27, P = .0003), and the OR for TT homozygotes plus GT heterozygotes compared with GG homozygotes was 1.10 (95% CI = 1.04 to 1.17, P = .001). Among the combined subset of 2,795 familial breast cancer patients, the respective ORs were 1.27 (95% CI = 1.12 to 1.45, P = .0003) and 1.16 (95% CI = 1.06 to 1.27, P = .001).

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Germline mutations in the high-penetrance genes BRCA1 and BRCA2 account for up to 25% of the familial risk of breast cancer (1,2). The excess familial risk may be largely subject to polygenic inheritance due to the combined effects of multiple low-penetrance genetic variants (3). Most association studies have focused on non-synonymous single-nucleotide polymorphisms (SNPs) in cancer-related genes that are expected to be directly associated with disease. Whereas genes whose products are involved in DNA repair (BRCA1, BRCA2, ATM, CHEK2, TP53, BRIP1, and PALB2) and metabolism of carcinogens (GSTM1, GSTT1, NAT1, and NAT2) and estrogen (COMT, CYP1A1, CYP1B1, CYP19A1, and NCOA3) have been studied extensively (4–11), the role of A-kinase anchoring protein (AKAP)—encoding genes has remained largely underinvestigated.

AKAP family members are structurally different yet functionally related proteins that bind and anchor the Ser/Thr protein kinase A (PKA) to specific subcellular sites, thereby confining PKA activity to potential substrates, such as cyclic adenosine monophosphate (cAMP) (12,13). PKA overexpression, which is a hallmark of the vast majority of human tumors, leads to the

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There is accumulating evidence that AKAP expression and gene variation are directly associated with cancer development. AKAP3 mRNA expression appears to be associated with poor prognosis in epithelial ovarian cancer (20), and genetic alterations of AKAP9, AKAP11, AKAP12, and AKAP13 are associated with the etiology of colorectal and lung cancer (21,22) and oral (23), gastric (24), prostate, and breast (25,26) cancers.

In this study, all 52 nonsynonymous AKAP SNPs that have been identified to date were tested for their functional impact by means of literature/database searches (PubMed/Ensembl) and in silico programs (27). Six putative protein-damaging polymorphisms were selected for analysis of breast cancer risk. Using a case–control study design, we genotyped AKAP3 E118G, AKAP5 P100L, AKAP6 F2171Y, AKAP9 M463I, AKAP9 N2792S, and AKAP12 E920G in 1110 familial case patients and 1131 control subjects from Germany (step I). Familial, BRCAl/2 mutation–negative case patients were used to increase the statistical power. The increased power of using familial case patients is estimated to reduce the sample size required to find a small relative risk by two- to fourfold (28).

We found an association with increased familial breast cancer risk for AKAP9 M463I and N2792S carriers. To further examine the relevance of these findings, we subsequently analyzed AKAP9 M463I, which is in strong linkage disequilibrium (LD) with AKAP9 N2792S, in 9523 breast cancer case patients and 13770 control subjects from Germany, the United Kingdom, and Australia (step II).

A detailed description of the seven contributing studies is shown in Supplementary Data (available online). In brief, the participating studies were the following: Australian Breast Cancer Family Study (ABCFS) (5,29,30), British Breast Cancer Study (BBCS) (4,21,22,30,31), Gene Environment Interaction and Breast Cancer in Germany (GENICA) (9,32), German Familial Breast Cancer Study (GFBCS) (33), Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer/Australian Ovarian Cancer Study (kConFaB/AOCS) (5,34–36), Mammacarcinoma Riskfactor Investigation (MARIE), and Studies of Epidemiology and Risk Factors in Cancer Heredity (SEARCH) (37–39).

All studies were approved by the appropriate local Institutional Review Board or Human Research Ethics Committees, and written informed consent was obtained from all participants. For all studies, we defined familial breast cancer patients as having at least one affected first-degree family member and/or bilateral breast cancer and/or were younger than 40 years (28,30). According to this definition, familial breast cancer case patients are individuals who are potentially genetically enriched for the detection of low-penetration genes that act in a complex genetic trait (2,8). Thus, patients with bilateral breast cancer are (statistically) equivalent to patients in families with three members (themselves and two other members) who have breast cancer (28).

Of the nonsynonymous SNPs identified in AKAPI–14, we selected candidate nonsynonymous SNPs that 1) showed in silico evidence to be probably damaging to the function of the respective protein by applying the in silico tools SIFT (http://blocks.fhcrc.org/sift) and PolyPhen (http://coot.embl.de/PolyPhen) (27), 2) occurred with an allele frequency greater than 0.05, and 3) had not been previously analyzed for an association with breast cancer risk. Applying these criteria, we found seven putative functionally relevant SNPs. Due to technical reasons, we did not analyze the AKAPI A18V (rs17761023) variant, which was predicted (Ensembl) to reside within a transmembrane domain responsible for protein cleavage.

Initial genotyping of the AKAP variants (step I: GFBCS study, Table I) was carried out by using TaqMan allelic discrimination, as previously described (41). TaqMan primers and probes were provided by the assay-by-design service (Applied Biosystems, Foster City, CA) and designed on the basis of GenBank sequences NT_009759 (AKAP3 E118G, rs2072355), NT_026437 (AKAP5 P100L, rs2230491), NT_026437 (AKAP6 F2171, rs4678999), NT_007933 (AKAP9 M463I, rs6964587 and AKAP9 N2792S, rs6960867), and NT_025741 (AKAP12 E920G, rs13212161). The extended analyses (step II) of rs6964587 (AKAP9 M463I) were conducted using TaqMan (GENICA, GFBCS, MARIE, and SEARCH studies), iPLEX (Sequenom, San Diego, CA) (ABCFS, kConFaB/AOCS, and MARIE studies), and
Table 1. Functional predictions and associations of six AKAP gene variants with familial breast cancer risk in the German Familial Breast Cancer Study*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>SNP ID</th>
<th>Predicted function†</th>
<th>Genotype</th>
<th>Familial case patients, No. (%)</th>
<th>Control subjects, No. (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKAP3</td>
<td>E118G</td>
<td>rs2072355</td>
<td>probably damaging†</td>
<td>AA</td>
<td>710 (66.2)</td>
<td>718 (64.7)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>317 (29.6)</td>
<td>357 (32.2)</td>
<td>0.90 (0.75 to 1.08)</td>
<td>.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GG</td>
<td>45 (4.2)</td>
<td>35 (3.2)</td>
<td>1.30 (0.83 to 2.05)</td>
<td>.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GG + AG</td>
<td>362 (33.8)</td>
<td>392 (35.3)</td>
<td>0.93 (0.78 to 1.11)</td>
<td>.45</td>
</tr>
<tr>
<td>AKAP5</td>
<td>P100L</td>
<td>rs2230491</td>
<td>probably damaging†</td>
<td>CC</td>
<td>833 (76.5)</td>
<td>849 (75.2)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CT</td>
<td>238 (21.9)</td>
<td>253 (22.4)</td>
<td>0.96 (0.78 to 1.17)</td>
<td>.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT</td>
<td>18 (1.7)</td>
<td>27 (2.4)</td>
<td>0.68 (0.37 to 1.24)</td>
<td>.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT + CT</td>
<td>256 (23.5)</td>
<td>280 (24.8)</td>
<td>0.93 (0.77 to 1.13)</td>
<td>.48</td>
</tr>
<tr>
<td>AKAP6</td>
<td>F2171Y</td>
<td>rs4647899</td>
<td>damaging‡</td>
<td>TT</td>
<td>551 (50.9)</td>
<td>584 (52.3)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>TA</td>
<td>439 (40.5)</td>
<td>451 (40.4)</td>
<td>1.03 (0.87 to 1.23)</td>
<td>.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>95 (8.8)</td>
<td>82 (7.3)</td>
<td>1.23 (0.89 to 1.69)</td>
<td>.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AA + TA</td>
<td>534 (49.2)</td>
<td>533 (47.7)</td>
<td>1.06 (0.90 to 1.26)</td>
<td>.48</td>
</tr>
<tr>
<td>AKAP9</td>
<td>M463I</td>
<td>rs6964587</td>
<td>possibly damaging†</td>
<td>GG</td>
<td>387 (35.7)</td>
<td>449 (40.3)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>GT</td>
<td>519 (47.9)</td>
<td>517 (46.4)</td>
<td>1.17 (0.97 to 1.40)</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT</td>
<td>177 (16.3)</td>
<td>149 (13.4)</td>
<td>1.39 (1.07 to 1.80)</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT + GT</td>
<td>696 (64.3)</td>
<td>666 (59.7)</td>
<td>1.22 (1.02 to 1.45)</td>
<td>.03</td>
</tr>
<tr>
<td>AKAP9</td>
<td>N2792S</td>
<td>rs6960867</td>
<td>possibly damaging†</td>
<td>AA</td>
<td>385 (35.7)</td>
<td>448 (40.5)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>524 (48.8)</td>
<td>511 (46.2)</td>
<td>1.19 (0.99 to 1.43)</td>
<td>.06</td>
</tr>
<tr>
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<td></td>
<td>GG</td>
<td>169 (15.7)</td>
<td>148 (13.4)</td>
<td>1.33 (1.03 to 1.72)</td>
<td>.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GG + AG</td>
<td>693 (64.3)</td>
<td>659 (59.5)</td>
<td>1.22 (1.03 to 1.45)</td>
<td>.02</td>
</tr>
<tr>
<td>AKAP12</td>
<td>E920G</td>
<td>rs13212161</td>
<td>probably damaging†</td>
<td>AA</td>
<td>903 (84.1)</td>
<td>944 (83.6)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>165 (15.4)</td>
<td>171 (15.1)</td>
<td>1.01 (0.80 to 1.27)</td>
<td>.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GG</td>
<td>6 (0.6)</td>
<td>14 (1.2)</td>
<td>0.45 (0.17 to 1.17)</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GG + AG</td>
<td>171 (15.9)</td>
<td>186 (16.4)</td>
<td>0.97 (0.77 to 1.21)</td>
<td>.77</td>
</tr>
</tbody>
</table>

* SNP = single-nucleotide polymorphism; OR = odds ratio; CI = confidence interval; AKAP = A-kinase anchoring protein; P = two-sided P value for the 2-df χ² test (general model) and the 1-df χ² test (dominant model). The individual studies were adjusted for age.
† Functional predictions based on the in silico tool PolyPhen (http://coot.embl.de/PolyPhen) (27) (August 1, 2006).
‡ Functional predictions based on the in silico tool SIFT (http://blocks.fhcrc.org/sift) (27) (August 1, 2006).

and customized Illumina Sentrix Bead Arrays (BBCS). For quality control, more than 3% of randomly selected samples in each study were subjected to repeated analysis, yielding a concordance rate of 100%. Genotyping call rates for all studies were >95%.

Deviations of the genotype frequencies in the control subjects from those expected under Hardy–Weinberg equilibrium (HWE) were assessed using Pearson’s goodness-of-fit χ² test with 1 df. All analyses were performed using the Statistical Analysis System software (version 9.1; SAS Institute Inc., Cary, NC). The extent of heterogeneity across studies was examined by Cochrane χ² test or Q test using the Comprehensive Meta-analysis Version 2 (Biostat, Englewood, NJ, 2005). Power calculation was carried out with the power and sample size software PS (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). LD calculation was performed by the Haploview software (42). All statistical tests were two-sided.

In the genotyping analysis of the six initial AKAP variants (step I), we found no association of the AKAP3 E118G, AKAP5 P100L, AKAP6 F2171Y, and AKAP12 E920G variants with familial breast cancer in a German population (GFBCS, Table 1). AKAP9 M463I and N2792S were marginally associated with an increased risk of familial breast cancer (for M463I, TT + GT vs GG: OR = 1.22, 95% confidence interval [CI] = 1.02 to 1.45, P = .03; and for N2792S, GG + AG vs AA: OR = 1.22, 95% CI = 1.03 to 1.45, P = .02; Table 1). The minor allele frequencies in control subjects of the SNPs analyzed were 0.19 (AKAP3 E118G), 0.03 (AKAP5 P100L), 0.27 (AKAP6 F2171Y), 0.37 (AKAP9 M463I), 0.37 (AKAP9 N2792S), and 0.09 (AKAP12 E920G). The null findings for AKAP3 rs2072355, AKAP5 rs2230491, and AKAP12 rs13212161 are supported by the data published in the Cancer Genetic Markers of Susceptibility database (adjusted P values for these three SNPs were .87, .76, and .27, respectively). This National Cancer Institute (NCI) enterprise conducts whole-genome association studies to identify breast cancer susceptibility genes using Illumina HumanHap550 assays on approximately 1200 case patients and control subjects (US female nurses; https://caintegrator.nci.nih.gov/cgems/). Their whole-genome association studies, however, did not include AKAP6 rs4647899 or AKAP9 rs6964587 or rs6960867.

According to the GFBCS data, AKAP9 M463I and N2792S polymorphisms showed...
strong LD in control subjects ($D^2 = .98$, $r^2 = .97$), covering an LD block of 367 kb in size (42). We chose AKAP9 M463I for the advanced analysis and included case patients and control subjects from the ABCFS, BBCS, GENICA, kConFaB/AOCS, MARIE, and SEARCH studies (step II). The genotype distributions in control subjects were consistent with HWE in all seven studies.

We found a statistically significant association of AKAP9 M463I T allele with an increased breast cancer risk in a dose-dependent manner ($P_{pooled} = .0002$). The combined adjusted odds ratios (step II) were 1.17 (TT vs GG, 95% CI = 1.12 to 1.45, $P = .0003$) (Figure 1, D), 1.12 (GT vs GG, 95% CI = 1.02 to 1.24, $P = .02$), and 1.16 (TT + GT vs GG, 95% CI = 1.06 to 1.27, $P = .001$) (Figure 1, C). The phenomenon of increasing risks with familial aggregation has been observed previously (3, 5, 28, 43) and indicates that susceptibility alleles that confer low risk are slightly more common in patients with a family history of breast cancer than in patients with sporadic breast cancer, leading to differences in allele frequencies between familial case patients and control subjects (28). With the present overall sample size (step II), we had a power of 90% at a statistical significance level of .05 to detect an odds ratio greater than 1.09 for breast cancer and greater than 1.16 for familial breast cancer with respect to M463I.

No heterogeneity across studies was observed ($P = .49$), and the T allele frequencies of AKAP9 M463I in control subjects ranged from 0.37 to 0.39 (mean = 0.38). Assuming dominant penetrance and the estimated effects to be true, AKAP9 M463I would account for a population attributable fraction of approximately 5.6% (5). Odds ratios were greater than 1.0 for all studies but one—the Australian familial breast cancer group kConFaB/AOCS. This result is most likely due to chance because the upper confidence intervals for the kConFaB/AOCS subgroup analysis include the risk estimates observed for all studies combined and because results for the other Australian study (the ABCFS) were similar to the overall findings.

According to the functional predictions using the programs PolyPhen and SIFT, AKAP9 M463I is deleterious (27). To some extent, in silico prognoses about the functional impact of nonsynonymous amino acid exchanges are speculative. However,
these algorithms have been shown to be approximately 80% successful in benchmarking studies (44). Our findings that the AKAP9 M463I T allele is associated with breast cancer support both in silico predictions. It is also of interest that this variant has previously been found to be associated with colorectal and lung cancer risk (21,22). However, it is possible that the AKAP9 N2792S G allele—due to strong LD and also being predicted to be deleterious—accounts for the observed association or that AKAP9 M463I and N2792S provoke risk enhancement mutually, resulting in shifted PKA localization and function. Alternatively, it is possible that neither of these variants are directly associated with breast cancer but are in LD with one or more other, perhaps rare, variants that are associated with risk in this or neighboring genes.

Ciampi et al. (45) reported an in-frame fusion between the C-terminal catalytic domain (exons 9–18) of the Ser/Thr kinase BRAF and the N-terminus (exons 1–8) of AKAP9, leading to constitutive BRAF and MAPK pathway activation in thyroid papillary carcinomas (44–47). In the AKAP9–BRAF fusion protein, AKAP9, which is normally localized to the centrosome and Golgi, lacks the C-terminal centrosomal domain and thus loses its centrosomal localization in cancer cells. Furthermore, wild-type AKAP9 could not be immunohistochemically detected within the centrosomes of cancer cells in all patients with the AKAP9–BRAF mutant, suggesting competitive inhibition of wild-type AKAP9 by AKAP9–BRAF (43). Hence, the deregulation of the multitude of cellular AKAP9 functions in cells that harbor AKAP9–BRAF mutants may affect a variety of physiologic processes.

We hypothesize that the T allele of AKAP9 M463I has oncogenic potential by altering PKA compartmentalization. As part of the AKAP9–BRAF fusion protein, this variant (located in exon 8) may also affect BRAF protein activation and give rise to carcinogenesis.

A limitation of our study is that the functional effect of AKAP9 463I (and/or AKAP9 2792S) has not been shown. If the AKAP9 463I and/or the 2792S variant are functionally responsible for the risk association, and considering the high allele frequency of AKAP9 463I (and AKAP9 2792S) the observed association is likely to occur also in other populations. However, if AKAP9 463I (and AKAP9 2792S) are not functionally relevant and are in LD with a perhaps rare causative variant, the association might not be observed or might be even stronger in other ethnic groups. Further studies investigating populations of different ethnic groups will help to clarify this issue.

In summary, by combining the results of seven independent case–control studies, we found that the T allele of AKAP9 M463I was associated with an increased breast cancer risk. The risk association of the AKAP9 M463I T allele was slightly stronger when the analysis was confined to familial breast cancer.

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