The functional genetic variant Ile646Val located in the kinase binding domain of the A-kinase anchoring protein 10 is associated with familial breast cancer

Michael Wirtenberger1,2,*,†, Julia Schmutzhard1,2,†, Kari Hemminki1,3, Alfons Meindl4, Christian Sutter5, Rita K.Schmutzler6, Barbara Wappenschmidt6, Marion Kiechle4, Norbert Arnold7, Bernhard H.F.Weber7, Dieter Niederacher9, Claus R.Bratram2 and Barbara Burwinkel1,2

1Division of Molecular Genetic Epidemiology, 2Helmholtz-University Group Molecular Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany, 3Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden, 4Department of Gynaecology and Obstetrics, Klinikum rechts der Isar at the Technical University, Munich, 5Institute of Human Genetics, University of Heidelberg, Heidelberg, 6Division of Molecular Gynaeco-Oncology, Department of Gynaecology and Obstetrics, Center of Molecular Medicine Cologne (CMMC), University Hospital of Cologne, 7Division of Oncology, Department of Gynaecology and Obstetrics, University Hospital Schleswig-Holstein, Kiel, 8Institute of Human Genetics, University of Regensburg, Regensburg and 9Division of Molecular Genetics, Department of Gynaecology and Obstetrics, Clinical Center University of Düsseldorf, Düsseldorf, Germany

*To whom correspondence should be addressed at: Division of Molecular Genetic Epidemiology C050, German Cancer Research Centre (DKFZ), Im Neuenheimer Feld 580, 69120 Heidelberg, Germany. Tel: +49 6221 421811; Fax: +49 6221 421810; Email: m.wirtenberger@dkfz.de

Overexpression of cAMP-dependent protein kinase A (PKA) is a hallmark of the great majority of human cancers including breast cancer. A-kinase anchoring proteins (AKAPs) coordinate the specificity of PKA signalling by localizing the kinase to its subcellular sites. We tested the hypothesis whether the functional amino acid exchange Ile646Val, located in the kinase-binding domain of AKAP10, is a low-penetrance familial breast cancer risk factor. Ile646Val alters the binding of AKAP10 to PKA and is associated with morbidity. The analysis of 787 BRCA1/2 mutation-negative familial breast cancer patients and 993 controls revealed an association of the AKAP10 Ile646Val polymorphism with increased familial breast cancer risk [odds ratio (OR) = 1.25, 95% confidence interval (CI) 1.03–1.51, P = 0.024]. Our previous study has shown that AKAP13 Lys526Gln is associated with familial breast cancer (OR = 1.58). Here, we discovered that carriers of both variants, AKAP10 Ile646Val and AKAP13 Lys526Gln, are at a further enhanced breast cancer risk (OR = 2.41, 95% CI 1.30–4.46, P = 0.005). PKA is a major target of therapeutic anticancer strategies. Phosphorylation of the estrogen receptor (ER) α by PKA induces resistance against the anti-estrogen tamoxifen. Our results indicate for the first time the importance of AKAP10 Ile646Val for familial breast cancer susceptibility. Due to the impact of Ile646Val on the subcellular localization of PKA, it will be interesting to investigate whether this polymorphism influences the effectiveness of PKA and tamoxifen based therapeutic anticancer concepts.

Abbreviations: PKA, protein kinase A; AKAPs, A-kinase-anchoring proteins; ER, estrogen receptor; ORs, odds ratios; CI, confidence interval.

Introduction

Breast cancer is the most common cause of cancer-related death in women worldwide and after lung cancer, the second most frequent cancer in the world (1). According to the polygenic model of inherited breast cancer, unfavourable combinations of polymorphic genetic variants in low-penetrance susceptibility genes jointly contribute to the excess familial breast cancer risk. Most of these susceptibility genes have not been discovered yet (2,3).

Protein kinase A (PKA) is a key regulatory protein, which responds to extracellular signals transmitted by hormones and neurotransmitters that activate G-protein coupled receptors (4–6). Activated PKA phosphorylates serine and threonine residues on various substrates including enzymes, membrane receptors, ion channels and transcription factors to regulate their function in cellular processes, such as energy metabolism (7), steroidogenesis (7,8), cell proliferation, differentiation and cell death (9–11).

A-kinase-anchoring proteins (AKAPs) coordinate the specificity of PKA signalling by localizing the kinase to subcellular sites through binding to a specific docking domain at the N-terminus of the R subunits (12–14). AKAP10 (alias D-AKAP2) is a dual-specific AKAP, binding to both type I and type II R subunit isoforms of PKA via an C-terminal PKA kinase-binding domain (15). A binding site in the C-terminus (12 amino acids downstream from the kinase-binding domain) has been suggested to serve as a motif for targeting to membrane receptor complexes (16,17). Two putative regulators of G-protein signalling domains, located in the N-terminus of the protein, could coordinate upstream G-protein signalling events with downstream PKA signalling (18).

Overexpression of PKAI, as compared to PKAII, is a hallmark of the great majority of human tumours, correlating with worse clinicopathological features in several tumour types, including breast cancer [reviewed in (19,20)]. PKAI overexpression is associated with cell proliferation, neoplastic transformation and with G1→S cell cycle transition. Extracellular PKA has been shown to be 10-fold upregulated in the serum of cancer patients and serves as a cancer biomarker (21). The kinase is involved in transduction of mitogenic signals originated by different growth factors, including transforming growth factor and epithelial growth factor (22,23). Many different therapeutic anticancer strategies have been developed that selectively target PKA (24–30).

Kammerer and co-workers have shown that the amino acid exchange Ile646Val, located in the C-terminal kinase-binding domain of AKAP10, alters the binding of AKAP10 to PKAI. It has been suggested that this variant influences the
subcellular localization of PKAI, leading to an observed association with morbidity and mortality (31).

Recently, we have shown that the non-conservative Lys526Gln variant of AKPA13, another member of the AKAP protein family, is associated with increased familial breast cancer risk (32).

The present study examined for the first time the putative impact of a functional amino acid exchange in AKAP10 on breast cancer risk using a large German study cohort.

Materials and methods

Study population

Genotyping analyses were performed on genomic DNA of BRCA1/2 mutation-negative index patients from 787 German breast cancer families, including 64 bilateral breast cancer cases and 993 unrelated German controls. All breast cancer cases were classified into six categories (33): (A1) families with two or more cases of breast cancer including at least two cases with onset under the age of 50 years (354 cases); (A2) families with at least one male breast cancer case (9 cases); (B) families with one or more cases of breast and at least one ovarian cancer (127 cases); (C) families with two or more cases of breast cancer including one case diagnosed before the age of 50 years (244 cases); (D) families with two or more cases of breast cancer diagnosed after the age of 50 years (29 cases); (E) a single case of breast cancer with diagnosis before the age of 35 years (24 cases). Thus, we accumulated familial cases and early onset cases, which are more likely to be due to a genetic cause, in our study population. The breast cancer cases comprised unrelated women that had been tested BRCA1/2 mutation-negative by applying the denaturing high performance liquid chromatography (DHPLC) method on all exons, followed by direct sequencing of conspicuous exons (33). The samples were collected during the years 1997–2005 by six centers of the German Consortium for Hereditary Breast and Ovarian Cancer (centers of Heidelberg, Würzburg, Cologne, Kiel, Düsseldorf and Munich, see authors affiliations). Index patients were first diagnosed with breast cancer and then referred to a family registry. All breast cancer patients gave an informed consent.

The control population included healthy and unrelated female blood donors collected by the Institute of Transfusion Medicine and Immunology (Mannheim), sharing the ethnic background and sex with the breast cancer patients. The age distribution in the controls and cases was nearly identical (controls: mean age 45.6 years, median age 46 years; cases: mean age 45.1 years, median age 45 years). According to the German guidelines for blood donation, all blood donors were examined by a standard questionnaire and gave their informed consent. They were randomly selected during the years 2004–2005 for this study and no further inclusion criteria were applied during recruitment. The study was approved by the Ethics Committee of the University of Heidelberg (Heidelberg, Germany).

Genotyping

The polymorphic, non-conservative amino acid exchange Ile646Val of AKAP10 was analysed using TaqMan allelic discrimination assays according to earlier descriptions (34). The following primers and probes were used: forward primer: 5’-GAAGAAGCTAAGGAGATTGC-3’, reverse primer: 5’-GGTTGTACATACTGAGCCGTG-3’, VIC-probe: 5’-VIC-ATAGTCAGTGACGTTATG-3’, FAM-probe: 5’-FAM-ATAGTCAGTGACGTTATG-3’. The SNP assays were validated by re-genotyping 10% of all samples. The SNP assays were consistent with the Hardy–Weinberg equilibrium (HWE) (controls: mean age 45.6 years, median age 46 years; cases: mean age 45.1 years, median age 45 years). According to the German guidelines for blood donation, all blood donors were examined by a standard questionnaire and gave their informed consent. They were randomly selected during the years 2004–2005 for this study and no further inclusion criteria were applied during recruitment. The study was approved by the Ethics Committee of the University of Heidelberg (Heidelberg, Germany).

Statistical analysis

Hardy–Weinberg equilibrium test was undertaken using the chi-square ‘goodness-of-fit’ test. Genotype-specific odds ratios (ORs), 95% confidence intervals (CIs) and P-values were computed by unconditional logistic regression using a tool offered by the Institute of Human Genetics, Technical University Munich, Munich, Germany (http://gth.gsf.de/eqi-bin/hw/hwjava.pl) and SAS version 9.1 (SAS Institute Inc., Cary, NC). P-values were calculated using two-sided chi-square test. Given our sample size, we calculated a power of 80% (\(\alpha = 0.05\)) to detect an OR of 1.33 using the power and sample size calculation software PS version 2.1.31 (http://www.mc.vanderbilt.edu/prevecmd/ps/index.htm) (35).

Results

The present study focussed on the putative impact of the functional amino acid exchange Ile646Val of AKAP10 on familial breast cancer risk. We performed a case-control study using genomic DNA of BRCA1/2 mutation-negative female index patients from 787 unrelated families, including 64 bilateral breast cancer cases, and 993 female unrelated German controls. Genotype distributions in cases and controls were consistent with the HWE. The SNP assays were validated by re-genotyping 10% of all samples and a concordance rate of 100% was attained. Given our large sample size, we achieved a power of 80% (\(\alpha = 0.05\)) to detect effects with an OR of 1.33. Logistic regression analyses of AKAP10 Ile646Val (2073A>G) revealed a significant association of heterozygous and homozygous G variant allele carriers with familial breast cancer (OR = 1.25, 95% CI 1.03–1.51, \(P = 0.024\), Table I). The OR for bilateral cases was 1.76 (95% CI 1.01–3.05, \(P = 0.045\), Table I). The minor allele frequency of Ile646Val among cases and controls was 0.37 and 0.35, respectively (OR = 1.11, 95% CI 0.97–1.28, \(P = 0.128\), Table I). The minor allele frequency of Ile646Val among bilateral cases was 0.44 (OR = 1.46, 95% CI 1.01–2.09, \(P = 0.042\), Table I). We divided all breast cancer cases according to the different family history groups, but no statistically significant associations within these subgroups were observed. Stratification of cases and controls according to different age groups (<50 years or ≥50 years) did not influence the risk of the investigated polymorphisms and age adjustment had no appreciable effect on the ORs.

Discussion

This is the first study focussing on the possible impact of the functional amino acid exchange Ile646Val (2073A>G) in AKAP10 on cancer risk. The strength of the present study is the large sample size and the resulting high statistical power. The high statistical power of this study was further enhanced by exclusively using selected familial breast cancer cases, since it has been shown that the power of association studies based on cases with a familial history of the disease was at least twice as high as the power of a study using unselected cases (36,37). By the exclusive use of BRCA1/2 mutation-negative familial breast cancer cases all effects derived from mutations in these high-penetration susceptibility genes were excluded (33).

Carriers of the Val646 variant were more frequent among cases than among controls resulting in an increased familial


Table I. Genotype frequencies of AKAP10 Ile646Val in the German study population

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotypes</th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKAP10—all cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile646Val AA (%)</td>
<td>295 (37.5)</td>
<td>423 (42.6)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2073A&gt;G AG (%)</td>
<td>396 (50.3)</td>
<td>447 (45.0)</td>
<td>1.28</td>
<td>1.04–1.56</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>rs203462 GG (%)</td>
<td>96 (12.2)</td>
<td>123 (12.4)</td>
<td>1.14</td>
<td>0.84–1.55</td>
<td>0.389</td>
<td></td>
</tr>
<tr>
<td>AG+GG (%)</td>
<td>492 (62.5)</td>
<td>570 (57.4)</td>
<td>1.25</td>
<td>1.03–1.51</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>MAF</td>
<td>0.37</td>
<td>0.35</td>
<td>1.11</td>
<td>0.97–1.28</td>
<td>0.128</td>
<td></td>
</tr>
<tr>
<td>AKAP10—bilateral cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile646Val AA (%)</td>
<td>19 (29.7)</td>
<td>423 (42.6)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2073A&gt;G AG (%)</td>
<td>34 (53.1)</td>
<td>447 (45.0)</td>
<td>1.69</td>
<td>0.95–3.02</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>rs203462 GG (%)</td>
<td>11 (17.2)</td>
<td>123 (12.4)</td>
<td>1.99</td>
<td>0.92–4.29</td>
<td>0.080</td>
<td></td>
</tr>
<tr>
<td>AG+GG (%)</td>
<td>45 (73.8)</td>
<td>570 (57.4)</td>
<td>1.76</td>
<td>1.03–3.05</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>MAF</td>
<td>0.44</td>
<td>0.35</td>
<td>1.46</td>
<td>1.01–2.09</td>
<td>0.042</td>
<td></td>
</tr>
</tbody>
</table>

* Position according to NM_007202.
* OR, 95% CI and P from [G] versus [A].
* MAF, minor allele frequency.
breast cancer risk. The association was stronger in bilateral familial breast cancer cases. Recent case-control studies have reported an increase in genotype effects when bilateral breast cancer cases have been examined (32,38–40). However, the results of bilateral cases must be interpreted with caution, since this subgroup consisted of only 64 cases. The observed effect was stronger and only significant for heterozygous carriers of *Ile*646*Val*. There is accumulating evidence that molecular heterosis is common in humans and occurs in ~50% of all gene associations (41). Since the power within the subgroups is limited and the OR of the heterozygotes ([AG], Table I) is not highly different from the OR of the rare homozygotes and heterozygotes ([AG+GG], Table I), a heterosis effect is debatable. However, the higher OR of the homozygotes ([GG], Table I) compared to the OR of the heterozygotes ([AG], Table I) among the bilateral cases points to a chance finding rather than a true heterosis effect in the total study population.

*Ile*646*Val* is located in the A-kinase-binding domain of AKAP10. It has been shown that this polymorphism influences the binding to PKA in an isoform-specific manner. The Val646 variant bound ~3-fold stronger to the protein kinase A RII subunit than the *Ile*646 variant (31). Moreover, it has been shown that the G allele, which determines the *Val* variant, is significantly associated with morbidity and a cardiac phenotype with shorter PR intervals in the electrocardiogram. Thus, it has been suggested that the *Val* variant has an enhanced ability to compartmentalize RIIα PKA, which increases the function of PKAI and leads to an disadvantageous health effect (31).

Overexpression of PKAI has been determined in a variety of human cancers, including breast cancer [reviewed in (19,20)]. Analyses of two separate cohorts of breast cancer patients have revealed that high levels of PKAI expression were associated with poor prognosis in terms of both disease recurrence and overall survival (42,43). We hypothesize that the increased ability of the AKAP10 Val646 variant to bind and localize PKA to its subcellular substrates is a disadvantage for the cell in carcinogenesis, resulting in a stronger mitogen influence of PKA.

The concept of selectively targeting cAMP-dependent PKAI in therapeutic, antitumour strategies has become very attractive. This has taken various forms, including the use of site-selective cAMP analogues (30), transfection of inducible vectors for RII subunits (29), antisenses oligonucleotides for RII (25–28) and designing of isoform-specific peptide disruptors of PKA localization (24).

In a previous study we have shown a significant association of homozygous and heterozygous variant allele carriers of the novel AKAP13 Lys526Gln (1746A>C) polymorphism with familial breast cancer risk (Table II) (32). A genotype-combination analysis using concordant samples of this and the present study revealed an increased breast cancer risk of patients carrying the risk genotypes of AKAP13 Lys526Gln and AKAP10 Ile646Val, compared to the effect of a single variant. The results of the genotype-combination analysis must be interpreted with caution, since they were based on a small number of samples (Table III).

The putative functional influence of AKAP10 Ile646Val on binding and localizing PKA to its subcellular substrates might also influence anti-estrogen therapy outcome. The mitogen effect of the ovarian steroid estrogen, which is primarily mediated by the estrogen receptor (ER) α, is a strong risk factor for breast cancer development [reviewed in (44,45)]. Recently, it has been shown that phosphorylation of serine-305 in the hinge region of the ER α by PKA induces resistance to the commonly used anti-estrogen tamoxifen by converting the ER antagonist to an agonist due to conformational changes of the ER (46). Furthermore, it has been reported that a gain of function mutation in the ER α itself (K303R), leading to estrogen hypersensitivity, was further enhanced by PKA dependent phosphorylation (47).

Our results revealed AKAP10 Ile646Val to be associated with an increased familial breast cancer risk. Due to the proposed impact of Ile646Val on coordinating the subcellular localization of PKA, it will be interesting to investigate whether this polymorphism influences the development of PKA mediated tamoxifen resistance and the effectiveness of PKA based therapeutic anticancer concepts.

### Acknowledgements

The authors are grateful to Bowang Chen and Justo L. Bermejo for statistical analyses as well as Dagmar Beisse for performing TaqMan assays. The German breast cancer samples were collected within a project funded by the Deutsche Krebshilfe, supported by the Center of Molecular Medicine, Cologne (CMMC) and coordinated by Rita K. Schmutzler. This study was supported by the EU, LSHC-CT-2004-503465.

### Conflict of Interest statement

None declared.

### References


### Table II. Genotype frequencies of AKAP13 Lys526Gln (1746A>C*) obtained in a previous study (32)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotypes</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKAP13</td>
<td>AA (%)</td>
<td>549 (92.1)</td>
<td>991 (94.8)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Lys526Gln</td>
<td>46 (7.7)</td>
<td>54 (5.2)</td>
<td>1.54</td>
<td>0.92–2.13</td>
</tr>
<tr>
<td>AKAP10</td>
<td>1746A&gt;C*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC (%)</td>
<td>646 variant bound</td>
<td>336322</td>
<td>1.23 (0.97–1.56)</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>CC (%)</td>
<td>16/13</td>
<td>17/32</td>
<td>2.41 (1.30–4.46)</td>
<td>0.005</td>
</tr>
<tr>
<td>MAF</td>
<td>0.04</td>
<td>0.03</td>
<td>1.58b</td>
<td>1.07–2.35b</td>
<td>0.022b</td>
</tr>
</tbody>
</table>

MAF...minor allele frequency.

aAccording to NM_007202.
bAccording to NM_006738.


Received June 14, 2006; revised August 11, 2006; accepted August 24, 2006