Association of genetic variants in the Rho guanine nucleotide exchange factor AKAP13 with familial breast cancer

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The A-kinase anchor protein 13 (AKAP13, alias BRX and lbc) tethers cAMP-dependent protein kinase to its subcellular environment and catalyses Rho GTPases activity as a guanine nucleotide exchange factor. The crucial role of members of the Rho family of GTPases in carcinogenesis is well established and targeting Rho proteins with anti-neoplastic compounds has become a major effort in the fight against cancer. Thus, genetic alterations within the candidate cancer susceptibility gene AKAP13 would be expected to provoke a constitutive Rho signalling, thereby facilitating the development of cancer. Here, we analysed the potential impact of four polymorphic non-conservative amino acid exchanges (Arg494Trp, Lys526Gln, Asn1086Asp and Gly1246Ser) in AKAP13 on familial breast cancer. We performed a case–control study using genomic DNA of BRCA1/2 mutation-negative German female index patients from 601 unrelated families, among a subset of 356 high-risk families, and 1053 German female unrelated controls. The newfound Lys526Gln polymorphism revealed a significant association with familial breast cancer (OR = 1.58, 95% CI = 1.07–2.35) and an even stronger association with high-risk familial breast cancer (OR = 1.85, 95% CI = 1.19–2.88). Haplotype analyses were in line with genotype results displaying a similar significance as analyses of individual polymorphisms. Due to the pivotal role of AKAP13 in the Rho GTPases signalling network, this variant might affect the susceptibility to other cancers as well.

Abbreviations: AKAP13, A-kinase anchor protein 13; BRCA1/2, breast cancer susceptibility genes; BRX, breast cancer nuclear receptor-binding auxiliary protein; ER, oestrogen receptor; GEF, guanine nucleotide exchange factors; HWE, Hardy–Weinberg-equilibrium; onco-lbc, oncogene lymphoid blast crisis; PKA, protein kinase A; proto-lbc, proto lymphoid blast crisis; SNPs, single nucleotide polymorphisms.

Introduction

Breast cancer is the most common cause of cancer related death in women worldwide (1). About 10% of all breast cancers are associated with family history (2). Twin studies suggest that genetic defects account for 27% of the total variability in breast cancer propensity (3). Familial aggregation of breast cancer risk is mainly due to heritable causes (4). The two major high penetrance breast cancer susceptibility genes BRCA1 and BRCA2 are responsible for ~25% of the excess familial breast cancer risk in the investigated German study population (5). Other susceptibility genes, such as ATM, TP53 and ARNTS, (6–8) account only for a small portion of familial breast cancers, thus further susceptibility genes remain to be discovered.

A-kinase anchor protein 13 (AKAP13, alias BRX, lbc, Ht31, AKAP-Lbc and HA-3) belongs to a group of structurally diverse proteins, which have the common function of binding to a cyclic AMP-dependent kinase (protein kinase A; PKA) holoenzyme, thereby providing a mechanism that positions PKA in close proximity to its physiological substrates (9,10). The proto-oncogene BRX (breast cancer nuclear receptor-binding auxiliary protein), a truncated form of AKAP13, has been identified by probing a breast cancer expression library and functions as an oestrogen receptor (ER) co-factor (11,12). The proto lymphoid blast crisis oncogene (proto-lbc), a truncated form of BRX, and the corresponding oncogene (onco-lbc) have been identified by Toksoz and Williams (13) from acute-phase chronic myeloid leukaemia patients. Both proteins function as guanine nucleotide exchange factors (GEF), stimulating the replacement of GDP bound to Rho GTPases with GTP, characterized by the presence of tandem dbl oncogene homology and pleckstrin-homology domains. The oncogen-lbc cDNA is a chimera derived from fusion of the proto-oncogene on chromosome 15q with an unrelated chromosome 7q36 sequence. It has been shown to be tumorigenic in nude mice and it has led to oncogenic transformation of NIH 3T3 fibroblasts (14–17). Recent studies have demonstrated that AKAP13 is capable of both anchoring PKA and activating Rho GTPases (18).

Rho GTPases mediate key cellular processes in response to diverse stimuli, such as cell growth, transcriptional activation and cytoarchitecture (19–21). The relationship of Rho proteins involved in cell transformation and human cancers is well established (22,23). Several studies have shown an enhanced activity of Rho GTPases in human cancers (24–27). Moreover, Rho proteins affect tumorigenesis at different stages, e.g. they promote G1 to S transition independently or as downstream components of other pro-tumorigenic signals in many cell types (22,28). RhoA GTPase is overexpressed in breast cancer cells and it is significantly involved in tumour invasion and progression (27,29). It triggers different intercellular pathways that lead to the activation of transcription factors, such as serum response factor and nuclear factor NF-κB, as well as
transcription factors dependent on the activation of JNK1 and p38. Rock kinases mediate most of the cytoskeletal changes induced by RhoA and they play also essential roles with respect to its neoplastic transformation ability (23). Targeting Rho proteins with antineoplastic compounds, such as statins, has become a major endeavour in the fight against cancer, and as a result treatment of highly metastatic breast cancer cell-lines with cerivastatin has reduced their metastatic and proliferative potential in a RhoA-dependent manner (30–32).

AKAP13 is an important upstream effector of RhoA catalysing its GDP–GTP exchange reaction required for RhoA signalling, which makes it a candidate breast cancer susceptibility gene. This is the first case–control genotyping study investigating the effects of AKAP13 polymorphisms on cancer risk. We assessed the putative influence of three known (Arg494Trp, Asn1086Asp and Gly246I3er) and one novel (Lys526Gln) polymorphic non-conservative amino acid exchanges in AKAP13 on familial breast cancer risk.

### Material and methods

#### Study Population

A randomly chosen set of 23 German familial breast cancer cases was initially screened to verify annotated SNPs (dbSNP database, NCBI) and to identify potential new SNPs in AKAP13 by DNA sequencing (Table I). Further analyses were performed on genomic constitutional DNA of 601 female unrelated German controls. These two categories were chosen because (A) for a detailed description of family inclusion criteria see below), and 1053 cancer families, among a subset of 356 high-risk families (risk category A1 and mutation-negative female index patients from 601 unrelated German breast cancer families, among a subset of 356 high-risk families (risk category A1 and B; for a detailed description of family inclusion criteria see below), and 1053 female unrelated German controls. These two categories were chosen because of stringent family history inclusion criteria and they have shown the highest BRCA1/2 mutation frequencies in a German study population [35% for A1 and 52% for B (5)].

All breast cancer cases were unrelated women (18–87 years of age; mean 45.4) who had been tested BRCA1/2 mutation-negative by applying the DHPLC method on all exons, followed by direct sequencing of conspicuous exons (5). They were collected during the years 1997–2005 by three centres of the German Consortium for Hereditary Breast and Ovarian Cancer (Institute of Human Genetics in Heidelberg, Germany, Department of Gynaecology and Obstetrics in Cologne, Germany and the Department of Medical Genetics in Munich, Germany). Index patients were first diagnosed with breast cancer and then referred to a family registry. All breast cancer patients agreed to participate in the present study.

The control population included healthy unrelated female blood donors (18–68 years of age, mean 40.5) collected by the Institute of Transfusion Medicine and Immunology (Mannheim, Germany) and shared the same ethnic background, the same sex and approximately the same age with the breast cancer patients. According to the German guidelines for blood donation all blood donors were examined by a standard questionnaire and all gave their consent that blood samples can be used for research studies. They were selected randomly during the years 2004–2005 for this study and no further inclusion criteria were applied during recruitment. Large cohorts of healthy blood donors matched with the cases regarding sex, age and ethnic origin represent proper controls for genotyping case–control analyses (33,34). The study was approved by the Ethics Committee of the University of Heidelberg (Heidelberg, Germany).

According to the German Consortium for Hereditary Breast and Ovarian Cancer, all breast cancer cases were classified into six categories based on family history (5): (A1) families with two or more cases of breast cancer including at least two cases with onset under the age of 50 years; (A2) families with at least one male breast cancer case; (B) families with one or more cases of breast cancer and at least one ovarian cancer; (C) families with two or more cases of breast cancer including one case diagnosed before the age of 50 years; (D) families with two or more cases of breast cancer diagnosed after the age of 50 years; and (E) a single case of breast cancer with diagnosis before the age of 35 years. Thus, we accumulated familial cases and early onset cases, which are more likely to be due to a genetic cause in our study population.

#### Table I. Allele frequencies of sequenced AKAP13 SNPs using 23 randomly chosen German breast cancer samples, primer sequences for SNP verification, and TaqMan primers and probes for allelic discrimination assays of the genotyping case–control study

<table>
<thead>
<tr>
<th>db SNP</th>
<th>rs No.</th>
<th>Variation</th>
<th>Amino acid exchange</th>
<th>Allele frequencies of sequenced AKAP13 SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. SNP verification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2061821</td>
<td>1525C&gt;T</td>
<td>Thr525Met</td>
<td>F: CTTCTGGAATGCCCACACACAGAC</td>
<td>17/44 (0.39)</td>
</tr>
<tr>
<td>rs2061822</td>
<td>1650C&gt;T</td>
<td>Arg494Trp</td>
<td>R: TGTCCTTTAGACATGAGATGA</td>
<td>14/46 (0.30)</td>
</tr>
<tr>
<td>rs2061823</td>
<td>1746A&gt;C</td>
<td>Lys526Gln</td>
<td>4/46 (0.09)</td>
<td></td>
</tr>
<tr>
<td>rs2061824</td>
<td>1890T&gt;C</td>
<td>Arg624Val</td>
<td>17/46 (0.37)</td>
<td></td>
</tr>
<tr>
<td>rs745191</td>
<td>2041G&gt;T</td>
<td>Gly624Val</td>
<td>17/46 (0.37)</td>
<td></td>
</tr>
<tr>
<td>rs4834073</td>
<td>3297C&gt;T</td>
<td>Ser1086Cys</td>
<td>17/46 (0.37)</td>
<td></td>
</tr>
<tr>
<td>rs4834074</td>
<td>3354G&gt;C</td>
<td>Ala1062Pro</td>
<td>17/46 (0.37)</td>
<td></td>
</tr>
<tr>
<td>rs4834075</td>
<td>3426A&gt;G</td>
<td>Asn1086Asp</td>
<td>17/46 (0.37)</td>
<td></td>
</tr>
<tr>
<td>rs2241268</td>
<td>7516G&gt;A</td>
<td>Gly246I3er</td>
<td>14/46 (0.30)</td>
<td></td>
</tr>
<tr>
<td>rs2241267</td>
<td>3540T&gt;C</td>
<td>Intron</td>
<td>0/46 (0.00)</td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>354525A&gt;C</td>
<td>Intron</td>
<td>6/46 (0.13)</td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>354609A&gt;G</td>
<td>Intron</td>
<td>22/46 (0.48)</td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>354727C&gt;T</td>
<td>Intron</td>
<td>10/46 (0.22)</td>
<td></td>
</tr>
<tr>
<td>B. Genotyping case–control study</td>
<td></td>
<td></td>
<td>TaqMan primer sequence</td>
<td>TaqMan probe</td>
</tr>
<tr>
<td>rs2241268</td>
<td>7516G&gt;A</td>
<td>Gly246I3er</td>
<td>F: AGAAGCCCCATTTGACCA</td>
<td>VIC-ATGTTGCTGGTCCCG</td>
</tr>
<tr>
<td>rs2061822</td>
<td>1650C&gt;T</td>
<td>Arg494Trp</td>
<td>0/46 (0.00)</td>
<td></td>
</tr>
<tr>
<td>rs4834073</td>
<td>3426G&gt;A</td>
<td>Asn1086Asp</td>
<td>0/46 (0.00)</td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>1746A&gt;G</td>
<td>Lys526Gln</td>
<td>0/46 (0.00)</td>
<td></td>
</tr>
</tbody>
</table>

*The polymorphisms chosen for further analysis are in bold.

*bAllele frequency of 22 and 23, respectively, randomly chosen German breast cancer cases (48 chromosomes).

*Position according to NM_006738.

*These polymorphisms showed complete linkage disequilibrium.

*Position according to NM_000015.
SNP verification
In order to verify annotated single nucleotide polymorphisms (SNPs) from the SNP database (NCBI) and to identify potential new SNPs in AKAP13, PCRs and sequencing of the respective regions were performed using genomic DNA of 23 randomly chosen German breast cancer cases. Primer design was based on the GenBank sequence of AKAP13, (accession nos NC_000015 and NM_006738, Table I). PCRs were carried out according to a protocol described previously (35).

Genotyping
The polymorphisms Arg494Trp, Asn1086Asp and Gly2461Ser and the novel polymorphism Lys526Gln were analysed using TaqMan allelic discrimination (Table I) according to earlier descriptions (35).

Statistical analysis
Calculations for Hardy–Weinberg-equilibrium (HWE) were carried out by using the HWE tool offered by the Institute of Human Genetics, TU Munich (http://ihg.gsdf.de/cgi-bin/hw/hwa1.pl). Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were computed by means of unconditional logistic regression using the Statistical Analysis System software (Version 8.2; SAS Institute, Cary, NC). P-values were calculated using two-sided χ²-test. With the analysed sample size and a calculated risk genotype carrier frequency of 5.2% among the controls we achieved a power of 90% to detect an OR of 1.96 using the power and sample size calculation software PS version 2.1.31 (http://www.mc.vanderbilt.edu/prevmed/ps/index.htm) (36).

Haplotype analysis
Haplotypes of AKAP13 polymorphisms Arg494Trp, Asn1086Asp, Gly2461Ser and the novel Lys526Gln variant were determined using the SNPHAP 1.3 software by David Clayton (http://archimedes.well.ox.ac.uk/pise/snphap-simple.html). Each individual was assumed to carry the most likely pair of haplotypes and the haplotype distributions were estimated based on the controls. Individuals with haplotype frequencies <0.3% were excluded from further analyses (0.9% of the study population) because they had no appreciable effect on the ORs. ORs and corresponding 95% CIs were calculated by comparing the distribution of each haplotype with the sum of the remaining haplotypes between cases and controls.

LD (linkage disequilibrium) analyses
Pairwise LD was calculated between the investigated SNPs among the control population using ID1 based on the ‘Haplovie’ program by Mark Daly (37) (http://www.broad.mit.edu/mpg/haplovie/documentation.php). SNPs with a minor allele frequency of ≤0.03 among the controls were excluded from LD analyses.

Results
In order to verify SNPs in AKAP13 derived from the NCBI SNP database, we sequenced a randomly chosen set of 23 German breast cancer cases (46 chromosomes). We confirmed nine coding and one intronic polymorphisms within the 23 breast cancer patients; only one intronic polymorphism (rs2241267) was not found in our 23 samples. Furthermore, we identified one new coding (Lys526Gln) and two new intronic SNPs (Table I). Our study focused on the potential impact of polymorphic non-conservative amino acid exchanges on familial breast cancer risk. Thus, we analysed the following four variations in the subsequent genotype association study: Arg494Trp, the newfound Lys526Gln variant, Asn1086Asp and Gly2461Ser. Asn1086Asp was chosen from a group of five other completely linked coding SNPs (identical genotype and allele frequencies among 23 individuals: Thr452Met, Ala539 Ala, Cys574Arg, Ser1040Ser and Ala1062Pro). Additionally, these SNPs including Asn1086Asp were in complete linkage disequilibrium (D' = 1 for all SNPs). All other SNPs, including non-coding, coding synonymous and intronic SNPs, were not subjected to further genotype association analyses (Table I). We performed a case–control study using genomic constitutional DNA of BRCA1/2 mutation-negative female index patients from 601 unrelated families, among a subset of 356 high-risk families, and 1053 female unrelated German controls. Genotype distributions in cases and controls were consistent with the HWE and 5% sequencing controls were in complete agreement with the TaqMan genotyping results. The extent of pairwise LD between the investigated SNPs was estimated by using ID1 (D' = 0.98 between Arg494Trp and Asn1086Asp, D' = 0.94 between

Table II. Genotype frequencies of AKAP13 polymorphisms

<table>
<thead>
<tr>
<th>A. All breast cancer cases</th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg494Trp (C&gt;T)</td>
<td>CC (%)</td>
<td>CT (%)</td>
<td>TT (%)</td>
<td>OR* [CT+TT] versus [CC]</td>
<td>95% C.I.</td>
</tr>
<tr>
<td>Controls</td>
<td>482 (45.8)</td>
<td>460 (43.7)</td>
<td>111 (10.5)</td>
<td>0.89</td>
<td>0.73–1.09</td>
</tr>
<tr>
<td>Cases</td>
<td>290 (48.6)</td>
<td>249 (41.8)</td>
<td>57 (9.6)</td>
<td>1.57</td>
<td>1.05–2.35</td>
</tr>
<tr>
<td>Lys526Gln (A&gt;C)</td>
<td>AA (%)</td>
<td>AC (%)</td>
<td>CC (%)</td>
<td>OR* [AC+CC] versus [AA]</td>
<td>95% C.I.</td>
</tr>
<tr>
<td>Controls</td>
<td>991 (94.8)</td>
<td>54 (5.2)</td>
<td>0 (0.0)</td>
<td>1.05</td>
<td>0.74–1.11</td>
</tr>
<tr>
<td>Cases</td>
<td>549 (92.1)</td>
<td>46 (7.7)</td>
<td>1 (0.2)</td>
<td>1.58</td>
<td>1.07–2.35</td>
</tr>
<tr>
<td>Cochran–Armitage trend test</td>
<td>AA (%)</td>
<td>AG (%)</td>
<td>GG (%)</td>
<td>OR* [AG+GG] versus [AA]</td>
<td>95% C.I.</td>
</tr>
<tr>
<td>Asn1086Asp (A&gt;G)</td>
<td>Controls</td>
<td>443 (42.6)</td>
<td>465 (44.8)</td>
<td>131 (12.6)</td>
<td>0.95</td>
</tr>
<tr>
<td>Cases</td>
<td>261 (44.0)</td>
<td>257 (44.0)</td>
<td>76 (12.8)</td>
<td>1.53</td>
<td>1.05–2.23</td>
</tr>
<tr>
<td>Gly2461Ser (G&gt;A)</td>
<td>GG (%)</td>
<td>GA (%)</td>
<td>AA (%)</td>
<td>OR* [GA+AA] versus [GG]</td>
<td>95% C.I.</td>
</tr>
<tr>
<td>Controls</td>
<td>539 (51.9)</td>
<td>410 (39.5)</td>
<td>90 (8.6)</td>
<td>0.90</td>
<td>0.74–1.11</td>
</tr>
<tr>
<td>Cases</td>
<td>317 (54.4)</td>
<td>221 (37.9)</td>
<td>45 (7.7)</td>
<td>1.38</td>
<td>1.03–1.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. High-risk breast cancer cases (risk category A1 and B)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys526Gln (A&gt;C)</td>
<td>AA (%)</td>
<td>AC (%)</td>
<td>CC (%)</td>
<td>OR* [AC+CC] versus [AA]</td>
<td>95% C.I.</td>
</tr>
<tr>
<td>Controls</td>
<td>991 (94.8)</td>
<td>54 (5.2)</td>
<td>0 (0.0)</td>
<td>1.05</td>
<td>0.74–1.11</td>
</tr>
<tr>
<td>Cases</td>
<td>320 (90.9)</td>
<td>31 (8.8)</td>
<td>1 (0.3)</td>
<td>1.58</td>
<td>1.07–2.35</td>
</tr>
</tbody>
</table>

*OR of genotype comparisons.
OR of allele comparisons.
Haplotypes with frequencies <0.3% were excluded. 

Asn1086Asp and Gly2461Ser and D' = 0.95 between Arg494Trp and Gly2461Ser). Lys526Gln was excluded from LD calculations because of its low minor allele frequency among controls (0.026).

Concerning Arg494Trp, Asn1086Asp and Gly2461Ser, genotype frequencies between breast cancer cases and control samples were similar, showing no significant association with familial breast cancer risk (Table II), not even when stratifying cases and controls according to age (<50 or >50 years, data not shown). Adjustment for age did not change the respective ORs (data not shown).

The analysis of the novel Lys526Gln polymorphism, however, revealed a significant association with familial breast cancer and an even stronger association with high-risk familial breast cancer (risk categories A1 and B, Table II). The results of the Cochran–Armitage trend test point to a dose-dependent association between the rare allele C of Lys526Gln and increased familial breast cancer risk (Table II). Stratification of familial and high-risk familial cases and controls according to different age groups (<50 or >50 years) did not influence the risk of this polymorphism (data not shown). Age adjustment had no appreciable effect on the ORs.

In order to calculate haplotype frequencies of Arg494Trp, Lys526Gln, Asn1086Asp and Gly2461Ser in AKAP13 we used the SNPHAP program. Each individual was assumed to carry the most likely pair of haplotypes and the haplotype distributions were estimated based on the controls. Individuals with haplotype frequencies <0.3% were excluded from further analyses (0.9% of the study population). Of the calculated haplotype pairs, 96.4% had a probability of ≥0.98. ORs and corresponding 95% CIs were calculated by comparing the distribution of each haplotype with the sum of the remaining haplotypes between cases and controls (Table III). Only the haplotype CCGG, carrying the risk allele C of Lys526Gln at position 2, was significantly more frequent among breast cancer cases than among controls, resulting in an association with familial breast cancer (Table III). This association was even stronger in high-risk familial breast cancer cases (OR = 1.88, 95% CI = 1.21–2.93, data not shown). Stratification of familial and high-risk familial cases and controls according different age groups (<50 or >50 years, data not shown) did not influence the risk of the haplotypes. Age adjustment had no appreciable effect on the ORs.

## Discussion

The crucial role of AKAP13 in key pathways involved in tumorigenesis, such as the ER, the cAMP/PKA and the RhoA signalling cascades has been demonstrated in several studies (11–13,15,17,21,23,38). To investigate the potential influence of polymorphisms in AKAP13 on familial breast cancer risk we verified annotated database polymorphisms and identified new polymorphisms in this gene. Our study focused on the putative impact of polymorphic non-conservative amino acid exchanges on familial breast cancer risk. Thus, we analysed the impact of Arg494Trp, the newfound Lys526Gln, Asn1086Asp and Gly2461Ser variants on familial breast cancer risk. We performed a genotype association analysis on constitutional genomic DNA of female index patients from 601 unrelated German breast cancer families, among a subset of 356 high-risk breast cancer families, and 1053 female unrelated German controls. Familial index patients were chosen for our study because it has been shown that the use of familial cases in case–control studies significantly increases the power to detect rare alleles contributing to breast cancer risk (39). Only BRCA1/2 mutation-negative familial breast cancer cases were included in the study to avoid all effects derived from mutations in these two high penetrance susceptibility genes (5). Concerning Arg494Trp, Asp1086Asn and Gly2461Ser, no significant differences in genotype frequencies between breast cancer cases and controls were observed, although the last was located in a region of the gene which has been suggested to modulate oestrogen-dependent activation of oestrogen receptors α and β (11,12). Since Asn1086Asp was in complete linkage disequilibrium with five other coding SNPs, associations of these respective variants with familial breast cancer can be ruled out.

We revealed a 1.58-fold risk for familial breast cancer associated with carriers of the rare allele C of Lys526Gln (OR = 1.58, 95% CI = 1.07–2.35, Table II). The risk association was even stronger in high-risk familial breast cancers (category A1 and B, OR = 1.85, 95% CI = 1.19–2.88, Table III), suggesting this risk variant to be more disadvantageous in an unfavourable genetic constellation. Furthermore, the results of the Cochran–Armitage trend test indicated a dose-dependent association between the rare allele C of Lys526Gln with breast cancer and high-risk breast cancer (Table II). These two categories were chosen as high-risk categories because of stringent family history inclusion criteria and they have shown the highest BRCA1/2 mutation frequencies in a German study population (35% for A1 and 52% for B) (5). Thus, one can assume that the BRCA1/2 mutation-negative breast cancer cases of these two categories analysed in this study are more likely a result of genetic causes.

The extent of pairwise LD between Arg494Trp, Asp1086Asn and Gly2461Ser was estimated by using ID' (D' = 0.98, 0.94 and 0.95, respectively). Although Lys526Gln was excluded from LD calculations due to its low minor allele frequency among controls it is in close proximity to Arg494Trp. As a result, one can estimate that the alleles of this SNP are located in the haplotype predicted for Arg494Trp and Asp1086Asn (D' = 0.98).

Haplotype analyses of the four polymorphisms did not reveal any new association with breast cancer besides the one of haplotype CCGG, which contains the risk allele C of the associated Lys526Gln. Thus, we did not further adjust for multiple comparisons (Table III).

This study represents an approach to identify functional candidate SNPs in a gene with strong biological relevance. Thus, adjustment for multiple comparisons was not taken into account even though four different SNPs were analysed.
The consistency of the effects of Lys526Gln in the genotype and haplotype analyses, the observation that the effect was even stronger in the subgroup of high-risk cases as well as the dose-dependency of the association are strong additional arguments against a chance finding.

The rare Lys526Gln polymorphism represents a non-conservative amino acid exchange from a positively charged residue with an amino group to a neutral residue with an amid group, and is located in the N-terminus of AKAP13. According to ‘SIFT’ version 2.1 (an algorithm that predicts whether an amino acid substitution affects protein function by Pauline Ng (40), http://blocks.fhcrc.org/sift/SIFT.html) the Lys526Gln amino acid exchange alters the secondary structure of AKAP13 and is predicted to affect the function of the protein. Additional analyses of AKAP13 using the DNASTAR Lasergene 5.0 software (DNASTAR, USA) confirmed the influence of the amino acid substitution on the secondary structure of the protein. Immunocytochemical analyses targeting the N-terminus of the full length transcript of AKAP13 have shown its uniform distribution throughout the cell, but it was excluded from the nucleus in HeLa-S3 cells (18), suggesting that its function as nuclear co-activator is rather confined in contrast to the shorter variant BRX (11).

Diviani and co-workers have demonstrated that the truncation of the N-terminal region of AKAP13 can significantly increase its basal RhoA-GEF activity, suggesting an inhibitory function of the N-terminus. Subsequently, they have identified that binding of 14-3-3, a member of a protein family that mediates signal transduction by binding to phosphoserine-containing proteins, to AKAP13 in the N-terminal part (between residue 1561 and 1570) inhibits its RhoA-GEF activity in vitro and in vivo. This interaction is modulated by phosphorylation of AKAP13 due to anchored PKA at serine 1565 (10,18).

However, the regions beyond the residue 1388 in the N-terminal part of AKAP13, in which the Lys526Gln polymorphism is located, have not been subject of detailed functional studies and their impact on a suggested auto-inhibitory function, regulation and integrity of the protein as well as their potential role as binding sites for other regulatory proteins remains to be elucidated. Previous studies on other GEFs have shown that auto-inhibitory sites in the N-terminus attenuate GEF activities of the respective genes through interaction with the DH domain to maintain a low level of tonic GEF activity (41,42).

Considering these facts, we hypothesize that the amino acid exchange from Lys to Gln at position 526 of AKAP13 associated with an increased breast cancer risk might lead to an impaired function of a potential auto-inhibitory site in the N-terminus of AKAP13 provoking a consecutive activation of the RhoA pathway and sensitizing the cell for carcinogenesis. However, functional studies are required to clarify the particular effect of the Lys526Gln variant in AKAP13 on breast cancer. Analysing other genes involved in the PKA/AKAP13/RhoA signalling network will help to elucidate their potential role as low penetrance susceptibility genes for breast cancer.

In summary, the present study revealed a significant association between the rare AKAP13 Lys526Gln variant and increased risk for developing breast cancer in a German familial breast cancer population. Due to the crucial role of AKAP13 in the PKA and RhoA signalling network, this variant might affect the susceptibility to other cancers as well and might also influence the response to anticancer drugs targeting Rho proteins.

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References


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