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Copy number variant in the candidate tumor suppressor gene *MTUS1* and familial breast cancer risk

Bernd Frank[1,2,*], Justo Lorenzo Bermejo[1], Kari Hemminki[1,3], Christian Sutter[4], Barbara Wappenschmidt[5,6], Alfons Meindl[7], Marion Kiechle-Bahat[7], Peter Bugert[8], Rita K.Schmutzler[5,6], Claus R.Bartram[4] and Barbara Burwinkel[1,2]

[1]Division of Molecular Genetic Epidemiology and, [2]Helmholtz-University Group Molecular Epidemiology, German Cancer Research Center, DKFZ, Heidelberg, Germany, [3]Center for Family Medicine, Karolinska Institute, Huddinge, Sweden, [4]Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany, [5]Division of Molecular Gynaeco-Oncology, Department of Gynaecology and Obstetrics, Clinical Center University of Cologne, Germany, [6]Center of Molecular Medicine Cologne, University Hospital of Cologne, Germany, [7]Department of Gynaecology and Obstetrics, Klinikum rechts der Isar, Technical University, Munich, Germany and [8]Institute of Transfusion Medicine and Immunology, Red Cross Blood Service of Baden-Württemberg-Hessia, Faculty of Clinical Medicine, University of Heidelberg, Mannheim, Germany

[*]To whom correspondence should be addressed. Tel: +49 6221 421461; Fax: +49 6221 421455;
Email: b.frank@dkfz.de

Copy number variants (CNVs), insertions, deletions and duplications, contribute considerably to human genetic variation and disease development. A recent study has characterized 100 CNVs including a deletion in the mitochondrial tumor suppressor gene 1 (*MTUS1*) lacking the coding exon 4. *MTUS1* maps to chromosome 8p, a region frequently deleted and associated with disease progression in human cancers, including breast cancer (BC). To investigate the effect of the *MTUS1* CNV on familial BC risk, we analyzed 593 BC patients and 732 control individuals using a case–control study design. We found a significant association of the deletion variant with a decreased risk for both familial and high-risk familial BC (odds ratio (OR) = 0.58, 95% confidence interval (CI) = 0.37–0.90, *P* = 0.01 and OR = 0.41, 95% CI = 0.23–0.74, *P* = 0.003), supporting its role in human cancer. To our knowledge, the present study is the first to determine the impact of a CNV in a tumor suppressor gene on cancer risk.

Introduction

Within the past years, numerous submicroscopic structural copy number variants (CNVs) or copy number polymorphisms have been identified as a novel form of genetic variation in the human genome (1–3). They are suggested to arise from a recurrent rearrangement by non-allelic homologous recombination, resulting in duplication, inversion or deletion of intermediate sequences (2). About 5% of the human genome are enriched with intrachromosomal duplications, predisposing to aberrant recombination (2). CNVs are considered to be substantially related to human diversity and may influence susceptibility to complex diseases such as neurological disease, autism, obesity and cancer (1–4). Chromosomal regions such as 6cen, 8pter and 15q13-14 are deemed copy number variation hot spots comprising up to four CNV clusters (1). By applying an array-based comparative genomics approach, Hinds *et al.* (5) examined 100 deletion polymorphisms ranging from 70 bp to 7 kb. These included a common deletion in the mitochondrial tumor suppressor gene 1 (*MTUS1*) encompassing the complete coding exon 4.

Abbreviations: BC, breast cancer; CI, confidence interval; CNV, copy number variant; *MTUS1*, mitochondrial tumor suppressor gene 1; OR, odds ratio; PCR, polymerase chain reaction.

MTUS1 has been detected and characterized as mitochondrial tumor suppressor gene being ubiquitously expressed in normal tissue but transiently up-regulated during initiation of cellular quiescence and differentiation processes (6). mRNA expression studies in different tumors and tumor cell lines supplied final evidence of the tumor suppressor function of MTUS1. Native *MTUS1* mRNA expression was shown to be markedly reduced in tissue from pancreatic tumor and the pancreatic tumor cell line MIA PaCa-2, whereas recombinant expression in MIA PaCa-2 cells inhibited proliferation (6). The regulation of cellular proliferation by a mitochondrial protein may be caused by different mitochondrial functions, such as the maintenance of energy supply, the production of reactive oxygen intermediates and other cell cycle regulators, e.g. TP53 (6). *MTUS1* which is also designated *MTSG1*, *GK1* (7) or *ATIP1* (angiotensin II AT2 receptor-interacting protein) maps to chromosome 8p21.3-22, a region frequently deleted and associated with disease progression in a wide range of human cancers, including bladder, colorectal, esophageal, head and neck squamous cell, hepatocellular, lung, ovarian, pancreatic, prostate and especially breast carcinomas (8–14). In a variety of cellular models, MTUS1/ATIP1 is an early mediator of angiotensin II (AT2) receptor activation. Together with AT2, it antagonizes AT1 receptor function, inhibiting epidermal growth factor signaling via epidermal growth factor receptor autophosphorylation, growth factor-induced extracellular regulated kinase activity, phosphorylation of signal transducer and activator of transcription 3 (STAT3), activation of protein kinase C, involving apoptosis and proliferation (15–20).

To investigate the effect of this CNV on familial breast cancer (BC) risk, we refined the break point region and size of the *MTUS1* deletion and performed a case–control study.

Materials and methods

Study population

The analysis of the *MTUS1* deletion was performed on 593 German BC families and 732 control individuals.

The cases comprised unrelated, female index patients (19–87 years of age, median 45) without mutations in the high-penetrance genes *BRCA1* and *BRCA2*. They were collected during the years 1996–2005 through the Institute of Human Genetics (Heidelberg, Germany), the Department of Gynaecology and Obstetrics (Cologne, Germany) and the Department of Medical Genetics (Munich, Germany). According to the German Consortium for Hereditary Breast and Ovarian Cancer (21), the BC cases were divided into six categories based on family history: (A1) families with two or more BC cases including at least two cases with onset below the age of 50 years (232 cases); (A2) families with at least one male BC case (four cases); (B) families with at least one BC and one ovarian cancer case (137 cases); (C) families with at least two BC cases including one case diagnosed before the age of 50 years (153 cases); (D) families with at least two BC cases diagnosed after the age of 50 years (49 cases) and (E) single cases of BC diagnosed before the age of 35 (18 cases). The categories A1 and B were considered as high-risk categories due to stringent family history inclusion criteria and high *BRCA1/2* mutation frequencies in German breast/ovarian cancer families compared with the remaining categories (21). Thus, high-risk families have more cases of BC than one would expect by chance alone. Among the familial BC cohort, 370 unrelated, German women were at high risk (23–87 years of age, median 44).

The control series consisted of healthy, unrelated female and ethnically matched blood donors (26–68 years of age, median 49). They were recruited in 2004 and 2005 by the Institute of Transfusion Medicine and Immunology (Mannheim, Germany) from the southwestern area of Germany that corresponds to the geographical origin of the patients. According to the German guidelines for blood donation, all blood donors were examined to rule out cardiovascular, malignant and other diseases by a standard questionnaire, cardiac auscultation, blood pressure and pulse measurement. Blood donors with previous malignant disease were excluded from blood donation.

The study was approved by the Ethics Committee of the University of Heidelberg (Heidelberg, Germany), and written informed consent was obtained from all individuals.

Genotyping

Detection and genotyping of the *MTUS1* deletion was carried out by polymerase chain reaction (PCR) amplification (Figure 1). The identity of PCR products was confirmed by sequencing deletion-specific and wild type-specific amplicons as described previously (22). PCR and sequencing primer sequences are available upon request.

Statistical analysis

The differences in genotype frequencies between BC cases and controls were evaluated for statistical significance by χ^2 tests; when the expected number of cases was smaller than five, Fisher's exact tests were applied. Likelihood ratio tests were used to compare a general three genotype model with dominant and recessive models.

Genotype-specific odds ratios (ORs), 95% confidence intervals (CI) and *P* values were computed by unconditional logistic regression using the Statistical Analysis System software (version 9.1; SAS Institute, Cary, NC). $P < 0.05$ was deemed significant. Hardy–Weinberg equilibrium test was undertaken using Pearson's goodness-of-fit χ^2 test with one degree of freedom.

To assess the relevance of our finding, we calculated the proportion of cases in the general population attributable to the identified gene–disease association (population attributable fraction) (23,24).

Power calculation was carried out with the power and sample size software PS (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize).

Results and discussion

Recent studies have reported CNVs as relevant contributors to human diversity and cancer susceptibility (1–3). Hinds *et al.* (5) have described several CNVs, including a deletion in the mitochondrial tumor suppressor gene *MTUS1*, using a high-density oligonucleotide array design. In order to evaluate the influence of this CNV on familial BC risk, we confirmed the deletion variant lacking the entire exon 4 by means of PCR amplification and sequencing (Figure 1), refining the deletion size from 1312 bp (5) to 1128 bp (Figure 2).

In the subsequently performed case–control study, genotype frequencies in controls were in agreement with the Hardy–Weinberg equilibrium and in line with the data by Hinds *et al.* (5). The three *MTUS1* genotypes differed statistically in the risk of both familial ($\chi^2 = 6.19$, $P = 0.045$) and high-risk familial ($\chi^2 = 8.62$, $P = 0.003$) BC. Results from the likelihood ratio test ($\chi^2 = 0.263$, $P_{\text{three genotype model versus dominant model}} = 0.608$) supported a dominant model for the association of the *MTUS1* deletion and familial BC. ORs were 0.58 (95% confidence interval = 0.37–0.90, $P = 0.01$, Table I) for familial and 0.41 (95% confidence interval = 0.23–0.74, $P = 0.003$, Table I) for high-risk familial BC. Adjustment for sex and age did not change the results. Assuming dominant penetrance and the estimated protective effects to be true, the identified deletion would result in a population attributable fraction of 5.9%. This is in agreement with the fact that relatively common variants associated with slight changes in disease risk would explain many cases in the general population (23,24). Note that small biases in risk estimates have a large impact on estimated population attributable fractions, and replication of the present study is obligatory. One reason for the stronger effect on high-risk familial BC may be the increased sharing of environmental exposures, gene variants and their interactions within these families, predisposing to an enhanced BC susceptibility.

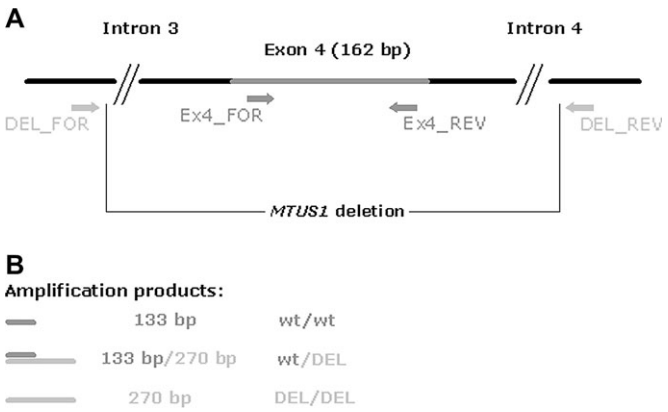

Fig. 1. (A) Schematic diagram of the *MTUS1* gene deletion that comprises the complete exon 4 and parts of introns 3 and 4. Deletion assessment was done in two separate PCRs with one set of amplification primers, respectively. The PCR primers DEL_FOR and DEL_REV are located in intron 3 and intron 4, whereas Ex4_FOR and Ex4_REV are exon-specific. **(B)** *MTUS1* PCR amplification products. DEL_FOR/DEL_REV primers flank the deleted region and generate a 270 bp product in the presence of the deletion. Ex4_FOR/Ex4_REV amplify an exon 4-specific 133 bp product, indicating absence of the deletion. Whereas homozygous wild type carriers (wt/wt) exclusively exhibit the 133 bp fragment, homozygous deletion carriers (DEL/DEL) exclusively exhibit the 270 bp fragment. Heterozygous deletion carriers (wt/DEL) are characterized by both PCR products.

The strengths of the present study are represented by a large sample size and a homogeneous study cohort of a single ethnic group. Only *BRCA1* and *BRCA2* mutation-negative familial BC cases were included in order to avoid effects caused by these high-penetrance susceptibility genes. Our study comprised women selected for familial BC since the power of an association study based on cases with a family history of the disease is considerably higher compared with a study using unselected cases (25). With our present sample size, we had a power of 80% at a significance level of 0.05 to detect an OR ≤ 0.51 for familial BC and an OR ≤ 0.44 for high-risk familial BC, respectively.

We hypothesize that the deletion of *MTUS1* exon 4 increases tumor suppressor activity. Interestingly, the deleted exon contains a polyproline-rich motif (26). Proline-rich regions usually participate in interactions with both SH3 and functional WW domains (27). Macias *et al.* (27) suggest that both SH3 and the well-conserved WW domains carry 'crude specificity constraints', implying that wild type and deleted MTUS1 interact with distinct intracellular partners and exhibit different cellular function, such as tumor suppression.

As MTUS1/ATIP1 inhibits insulin activation, insulin receptor autophosphorylation and mediates AT2 signaling, thus antagonizing AT1 receptor function (15–20), the *MTUS1* CNV may affect diabetes and hypertension risk as well.

The phenomenon that exon deletions provoke an enhanced or alternate protein activity has been described in further studies on the effects of deletion variants (28–30). For example, the ability of the nuclear receptor coactivator 3 (NCOA3) (also termed AIBI) Δexon 3 isoform to promote transcription was shown to be significantly higher than that of the full-length protein, sensitizing cells to estrogen, progesterone, and growth factors like epidermal growth factor (28). Similarly, alternative splicing of *bcl-x*, a *bcl-2* family member, generated an apoptosis-inducing protein (Bcl-x_S) (29), and exon skipping in *Mcl-1* yielded a death-inducing gene product, Mcl-$1_{S\Delta TM}$ (30).

Generally, mutations and polymorphisms in coding sequences of a gene may cause functional alteration of the gene product, which in turn may be associated with certain disease phenotypes. Functional studies may be helpful to clarify the implication of the *MTUS1* exon 4 deletion, and it will be elucidating to investigate its effect on the risk of other human cancers. This study lacked related phenotypic and functional assays (e.g. transient expression assays in cell lines), which limited the inquiry into the functional consequence of the *MTUS1* exon 4 deletion. However, such case–control studies showing significant findings may lead to subsequent functional studies that will enlighten the underlying mechanism of BC development associated with the given genetic variant. Validation of the findings with functional evaluation and larger studies with rigorous study designs of other ethnic populations are needed.

During the past two decades, there have been many analyses of copy number polymorphisms in cellular detoxification glutathione *S*-transferase genes on cancer risk (reviewed in 31). This is the first case–control study to investigate the effect of a CNV in a tumor suppressor gene on cancer risk, showing an association of an *MTUS1* deletion with familial and high-risk familial BC. Investigating the impact of further CNVs on human cancer risk will be revealing.

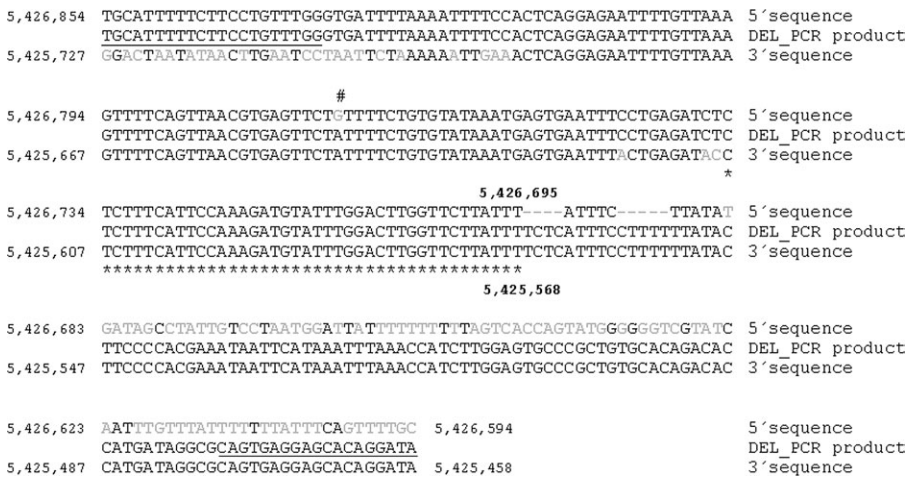

Fig. 2. Multiple *MTUS1* sequence alignment of the deletion-specific PCR product (generated with DEL_FOR and DEL_REV primers) sequence and the respective 5′ and 3′ sequences of full-length *MTUS1* derived from GenBank NT_030737.9. The presence of the highly homologous flanking 5′ and 3′ repeats predisposed these regions to recurrent rearrangement by non-allelic homologous recombination, leading to the deletion of the intervening sequence. PCR primer sequences are underlined. Letters in black indicate identity, letters in gray indicate mismatches and asterisks underscore the homologous deletion break point region. NT_030737.9 positions 5,426,695 and 5,425,568 were used to define the deletion size of 1128 bp.
#We assume that the mismatch is due to a sequence error in NT_030737.9.

Table I. Genotype frequencies for the *MTUS1* deletion variant in unrelated, female German *BRCA1/2* mutation-negative familial and high-risk familial BC patients and healthy, unrelated female control subjects, ORs with 95% CI and *P* values

	Cases *N* (%)	Controls *N* (%)
Familial BC		
wt/wt	562 (94.8)	668 (91.3)
wt/DEL	30 (5.1)	63 (8.6)
DEL/DEL	1 (0.2)	1 (0.1)
(DEL/DEL + wt/DEL)	31 (5.2)	64 (8.7)
OR (95% CI), *P* value	0.58 (0.37–0.90), 0.01	
(DEL/DEL + wt/DEL) versus (wt/wt)		
High-risk familial BC		
wt/wt	355 (96.2)	668 (91.3)
wt/DEL	14 (3.8)	63 (8.6)
DEL/DEL	0 (0)	1 (0.1)
(DEL/DEL + wt/DEL)	14 (3.8)	64 (8.7)
OR (95% CI), *P* value	0.41 (0.23–0.74), 0.003	
(DEL/DEL + wt/DEL) versus (wt/wt)		

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Conflict of Interest Statement: None declared.

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