Assessment of an *APOBEC3B* truncating mutation, c.783delG, in patients with breast cancer

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Abstract

Purpose: APOBEC3B belongs to the family of DNA editing enzymes. A copy number variant targeting the genomic *APOBEC3A-APOBEC3B* locus has a significant impact on breast cancer risk, but the relative contribution of *APOBEC3B* is uncertain. In this study, we investigate a loss-of-function mutation that selectively targets *APOBEC3B*, for its association with breast cancer risk.

Methods: We performed exome sequencing on genomic DNA samples of 6 Byelorussian patients with familial breast cancer. We then studied through mutation-specific genotyping four hospital-based breast cancer case-control series from Belarus, Russia, Germany and Iran respectively, comprising a total of 3,070 breast cancer patients and 2,878 healthy females. Results were evaluated using fixed-effects meta-analyses.

Results: Exome sequencing uncovered a frameshift mutation, *APOBEC3B**c.783delG, that was recurrent in the study populations. Subsequent genotyping identified this mutation in 23 additional breast cancer cases and 9 healthy female controls, with an adjusted Odds Ratio 2.29 (95% CI 1.04; 5.03, p=0.04) in the combined analysis. There was an enrichment of the c.783delG mutation in patients with breast cancer diagnosed below 50 years of age (OR 3.22, 95%CI 1.37; 7.56, p=0.007).

*Conclusions: APOBEC3B**c.783delG shows evidence of modest association with breast cancer and seems to contribute to earlier onset of the disease. These results may need to be reconciled with proposals to consider APOBEC3B as a possible therapeutic target in breast cancer.

Keywords: breast carcinoma; genetic susceptibility; DNA editing; founder mutation.

Background

APOBEC3B belongs to the family of DNA editing enzymes and is one of seven cytidine deaminase genes found in a cluster on chromosome 22 [1,2]. APOBEC3 proteins function within the innate immune system to restrict viral infection and retrotransposition, but they may also cause damage to the cellular genome. As deaminases, APOBEC3 proteins convert cytosines to uracils in single-stranded DNA and mediate localized hypermutation, a process termed "kataegis" [3,4]. APOBEC3B activity has specifically been identified as a prevailing enzymatic source of mutation in breast cancer [5]. APOBEC3B is induced through DNA replication stress [6], and it appears to be required for estrogen receptor action in breast cancer through transient cytidine deamination at the regulatory regions of ER target genes that promotes their expression [7].

In previous case-control studies, a copy number variant (CNV) at the *APOBEC3B* gene locus has been associated with breast cancer [8,9]. Loss of one or both *APOBEC3B* copies was associated with odds ratios of 1.31 and 1.76, respectively [8]. The *APOBEC3B* CNV has further been correlated with immune response-related gene sets and with a higher abundance of tumour-infiltrating immune cells [10,11]. The CNV results from the deletion of approximately 29.5 kb of sequence between *APOBEC3B* and the adjacent gene, *APOBEC3A*. APOBEC3A and APOBEC3B are both endogenous human DNA mutators, but their phenotypes are different and non-overlapping [12]. APOBEC3A is the more genotoxic of the two and tightly regulated in its expression, whereas APOBEC3B is more abundant but generates less DNA double-strand breaks. The enzymatic difference has been attributed to evolutionary divergence at 18 amino acid positions [12]. While the whole *APOBEC3B* coding sequence is omitted on the risk allele of the *APOBEC3B* CNV, its 3´-UTR is fused to *APOBEC3A*, and the more stable transcript of this fusion gene constitutes increased activity of APOBEC3A that is thought to drive the increased breast cancer risk [13]. It is unknown whether the loss of the APOBEC3B enzyme alone would contribute to cancer susceptibility and prognosis. This question is relevant since APOBEC3B is being considered as a possible therapeutic target in breast cancer [5, 14, 15].

In the present study, we describe a truncating mutation in *APOBEC3B* that was selected from exome sequencing and does not target *APOBEC3A*. This mutation was investigated for its potential contribution to breast cancer risk in a total of 3,070 breast cancer patients and 2,878 healthy females from Germany, Belarus, Russia and the Iran.

Patients and methods

Patients

We investigated four breast cancer case-control series from Belarus, Germany, Russia, and Iran. The three European Series have been previously used for genetic association studies [16-19].

The Hannover-Minsk Breast Cancer Study (HMBCS) consists of 1,927 breast cancer patients diagnosed in the Republic of Belarus during the years 1998-2008 [17]. Patients were recruited at the Byelorussian Institute for Oncology and Medical Radiology Aleksandrov N.N. in Minsk or at one of five regional oncology centers in Gomel, Mogilev, Grodno, Brest or Vitebsk. The Belarus series mainly consisted of consecutive patients unselected for family history, with the exception of an additional 28 cases with familial breast cancer ascertained at the center in Minsk. Median age at diagnosis in the Belarus cohort was 48 years, and 16% of patients reported a first-degree family history of breast cancer. Byelorussian population controls were 1,245 healthy volunteers from the same population who had no personal history of breast cancer at the time when entering the study and were not known blood relatives of the study patients. Based on available genomic DNA and stringent quality control, a total of 1,778 Byelorussian patient samples and 1,216 Byelorussian control samples were included into the genotyping study. Over 98% of the study individuals were known to be of Byelorussian descent.

The Hannover Breast Cancer Study (HaBCS) includes a hospital-based series of 1,012 unselected German breast cancer patients who were treated at the Department of Radiation Oncology at Hannover Medical School from 1996-2001. Median age at diagnosis in the Hannover cohort was 57 years, and 11% of patients reported a first-degree family history of breast cancer. Population controls were taken from a cohort of 1,002 healthy female German blood donors recruited in 2005 at the Transfusion Department of Hannover Medical School. In total, genomic DNA samples were available from 721 German patients and 921 German controls for inclusion into the genotyping study. Over 97% of the study individuals were known to be of German descent.

The Hannover-Ufa Breast Cancer Study (HUBCS) consisted of 1,059 breast cancer patients unselected for family history who had been diagnosed during the years 2000-2007 at the oncological center in Ufa (Bashkortostan). Breast cancer patients in this series belonged to different ethnic groups mainly living in the Volga Ural region of Russia. Median age at diagnosis was 51 years (range 25-85 years), and 7% of patients reported a first-degree relative

diagnosed with breast cancer. Healthy population controls included 1,069 volunteers from the same geographic regions, with a similar ethnic distribution and age distribution (median age 46 years, range 18-84 years). For the association study, cases and controls were stratified by their ancestry, and only 340 cases and 494 controls of known Russian descent were included into the genotyping study.

The Iranian Breast Cancer Study (IBCS) is a novel study involving 241 female patients with breast cancer who were treated at the Omid and Imam Reza Educational Hospital of Mashhad University of Medical Sciences, Reza Radiotherapy Oncology Center, or associated clinics in Mashhad between the years 2012 and 2016. The median age at diagnosis was 45 years (range 20-77 years) and 13.6% of the cases reported a first-degree family history of breast cancer. The control group comprised 253 healthy female volunteers who were recruited from the same population in the year 2016 at the ACECR Central Laboratory (Mashhad Branch). The median age for the healthy group was 40 years. Genomic DNA samples were available from all 241 Iranian breast cancer cases and 253 healthy controls for inclusion into this case-control study. The IBCS included only individuals of Persian ethnicity.

Our study was carried out with informed consent of the probands and was approved by local ethical boards at the respective institutions.

Genetic analyses

Genomic DNA was isolated from peripheral white blood cells by routine phenol-chloroform extraction. Exome sequencing was performed on six genomic DNA samples (3 µg) from Byelorussian patients with familial breast cancer at 50x coverage. For this purpose, exonic sequences were enriched using the SureSelect XT Huma All Exon V5 library (Agilent Technologies, Santa Clara, CA, USA) and were sequenced on a HiSeq2500 platform (Illumina Inc., San Diego, CA, USA). Raw exome sequencing data were called, de-multiplexed and aligned according to the GATK pipeline and variants were annotated using the Annovar tool. Mutations were filtered according to their minor allele frequencies in the NCBI SNP and/or 1000Genomes databases and according to their predicted effects. A list of variants in any gene of interest is available upon request. Truncating mutations in DNA repair and processing genes were then confirmed by Sanger sequencing using BigDye chemistry and a Genetic Analyser 3100 Avant (Applied Biosystems, Foster City, CA, USA). Primers for validation sequencing of the *APOBEC3B** c.783delG mutation were 5´-GGCTAAGAATCTTCTCTGTG-3´ and 5´- CAGGAGATGAACCAAGTGAC-3´, respectively.

Direct mutation analysis of *APOBEC3B**c.783delG was performed by means of PCR amplification using the primers 5´-TCTAGGCTAAGAATCTTCTCTG-3´ and 5´-ACCATCGTAGGTCATGATGG-3´. The 302 bp PCR product was then subjected to restriction enzyme fragment length analysis using *Scr*FI to produce cleavage products of 150, 63, 35, 24, 18 and 12 bp in case of the wildtype sequence. The presence of the *APOBEC3B**c.783delG mutation gave rise to an additional product of 87 bp while inhibiting the 63 and 24 bp products. The diagnostic 87 bp and 63 bp bands were well distinguished by means of 3% agarose gel electrophoresis. Call rates were 98.1% for the HMBCS samples from Belarus, 95.6% for the HaBCS samples from Germany, 90.1% for the HUBCS samples from Russia and 97.8% for the IBCS samples from Iran.

Statistical analyses

The prevalence of the *APOBEC3B**c.783delG mutation was compared in breast cancer cases and healthy population controls for all genotyped studies. Odds ratios were calculated from two-by-two tables using Fisher´s exact test, and adjusted Mantel-Haenszel odds ratios were obtained in fixed-effects meta-analyses using the metan command in STATA12.0. For the meta-analysis, we added an increment of 0.001 to each field in order to account for one zero field in HUBCS. p-values were calculated from chi(2)-values of Fisher´s exact test for single studies, from chi(2)-values for heterogeneity between studies, or from z-values in fixed effects meta-analyses. All p values are two-sided. c.783delG was the only *APOBEC3B* mutation investigated in the study populations, and results with $p<0.05$ were considered significant.

Results

In an exploratory analysis of six whole exome data sets from Byelorussian breast cancer patients within the Hannover-Minsk Breast Cancer Study, we identified a truncating variant in the *APOBEC3B* gene, c.783delG, in one of the sequenced patients (Figure 1A). We followed this mutation because it was one of only two truncating mutations in a known DNA repair gene (the other one was in *BRCA2*) that were not present in dbSNP at the time of analysis. *APOBEC3B**c.783delG is now recorded with the identifier rs368511533 and has been listed by the Exome Aggregation Consortium (ExAC) at an overall MAF 0.008. This frameshift mutation is predicted to result in a premature truncation of the APOBEC3B protein (p.Val262Phefs).

Using an allele-specific RFLP assay with *Scr*FI (Figure 1B), we screened for the prevalence of c.783delG in four large breast cancer case-control studies including 1,772 Byelorussian and 1,216 Byelorussian controls from HMBCS, 340 cases and 494 controls from HUBCS (restricted to study individuals of known Russian ethnicity), 721 German cases and 922 German controls from HaBCS and 237 Iranian patients and 246 controls from IBCS.

The c.783delG mutation was detected in additional 13 cases and 4 controls from Belarus, 0 cases and 3 controls from Ufa, 5 cases and 1 control from Germany, and in 5 cases and 1 control from Iran (Table 1). A combined fixed-effects meta-analyses indicated a borderline significant difference between cases and controls, yielding a Mantel-Haenszel pooled OR 2.29 (95%CI 1.04; 5.03, p=0.04). There was no evidence for heterogeneity between studies (p_{het}=0.67). All mutation carriers were heterozygous and all had invasive breast cancers. The mutation was associated with ductal morphology (p=0.02) and with ER-positive tumours (p=0.03). It was not significantly enriched in familial breast cancer (OR 2.79, 95% CI 0.78; 9.97, p=0.12). When patients were stratified by age at diagnosis, we observed a nominally significant association with early onset of breast cancer, defined by an age at diagnosis below age 50 (OR 3.22, 95% CI 1.37; 7.56, p=0.007) (Table 1). One of the *APOBEC3B**c.783delG carriers (in HaBCS) had a mutation in *BRCA1* which might have influenced her early age at diagnosis. However, omitting this single mutation carrier from the analysis still yielded a nominally significant association with early-onset breast cancer in the combined analysis (p=0.02).

Discussion

Exome sequencing has made important contributions to the discovery of genetic breast cancer risk factors [20-24], but any suspected role of a mutation for disease needs to be validated in subsequent case-control studies. In the present study, we investigated a truncating mutation in *APOBEC3B* as a candidate breast cancer susceptibility allele. Earlier work had implicated a copy number variant at the *APOBEC3B* gene locus as a risk factor for breast cancer, conferring an about 1.7-fold increase in risk per deletion allele [8,9]. It has been proposed that the effect of this allele is mediated by increased expression and mutagenic activity from the neighbouring *APOBEC3A* gene that is fused to the 3´-UTR of *APOBEC3B* in deletion carriers [13]. Indeed, tumour samples with *APOBEC3B* deletion show predominantly APOBEC3A-like mutational signatures, whereas samples with wild-type *APOBEC3B* show a roughly equal split between APOBEC3A-like and APOBEC3B-like signatures [25]. However, another study

did not find upregulation of *APOBEC3A* or any of the other *APOBEC* cytosine deaminase family members in frozen tissue samples from deletion carriers, suggesting that there was no detectable feedback loop at the mRNA level that results in co-regulation of any of the members of this family [11]. It has also been argued that APOBEC3A when expressed endogenously, is confined to myeloid lineage cell types and located in the cytoplasm, so that APOBEC3B is considered the main factor [26]. Unlike the common CNV, that is frequently termed "*APOBEC3B* deletion", the *APOBEC3B**c.783delG mutation that we describe here, does not affect the sequence of *APOBEC3A* and therefore constitutes an opportunity to study *APOBEC3B* as a genetic risk factor on its own.

Perhaps unexpectedly, we did observe an overall effect of the *APOBEC3B**c.783delG mutation on breast cancer risk, and there was a nominally significant association with early-onset breast cancer as well as with ER-positive and ductal disease in the stratified analyses. The mutation results in a frameshift within exon 6 and premature termination 42 codons downstream (p.Val262PhefsTer42, NM_004900). If translated, this mutation occurs adjacent to the active site at Glu255 and eliminates downstream metal-binding sites, which almost certainly leads to a loss of enzymatic function. The frameshift could be bypassed through an alternative splicing event that skips the first 25 codons of exon 6, but this would also eliminate the active site. *APOBEC3B**c.783delG is the only relatively common *APOBEC3B* truncation identified and, at the average frequency of ~1/330 in our investigated populations, it might be worth to be genotyped in further cancer series. Consistent with our frequency data, the most recent database of the Exome Aggregation Consortium now lists this mutation at a minor allele frequency of MAF 0.003 in Europeans, but it appears to have a higher frequency in Africans (http://exac.broadinstitute.org/variant/22-39387395-TG-T).

APOBEC3B has been proposed as a strong candidate for targeted intervention, based on its non-essential nature and the observation that *APOBEC3B* overexpression is associated with C-to-T mutation biases and increased mutational loads [5, 14, 15]. Counteracting APOBEC3B may remove a chronic source of mutagenesis since tumours with high levels of *APOBEC3B* have twice as many mutations as those that express low levels and are more likely to have mutations in *TP53* [5]. Its inhibition has been predicted to decrease tumor mutation rates and diminish the likelihood of undesirable mutation-dependent outcomes such as recurrence, metastasis, and the development of therapy resistant tumors [14]. In a naturally mutant context, one may thus have expected some protective effect of the *APOBEC3B**c.783delG mutation on breast cancer risk. Our results indicate that this is not observed, rather we find some evidence for an increased risk for breast cancer in three of four study populations. More

data will be needed before APOBEC3B inhibition can be exploited as a potentially valuable preventive means [15].

In conclusion, our study identified a truncating variant at the *APOBEC3B* locus that appears to modestly contribute to overall breast cancer risk and was associated with an earlier onset of breast cancer. These results may need to be reconciled with proposals to consider APOBEC3B as a possible therapeutic target in breast cancer.

Ethical Standards

The experiments in the present study comply with the current laws of the country in which they were performed.

Conflict of interest

The authors have no disclosures and declare there is no conflict of interest in the writing, preparing, or finalizing of this manuscript.

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References

1. Harris RS, Petersen-Mahrt SK, Neuberger MS (2002) RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. Mol Cell 10:1247-1253.

2. Jarmuz A, Chester A, Bayliss J, Gisbourne J, Dunham I, Scott J, Navaratnam N (2002) An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics 79: 285–296.

3. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, Jones D, Hinton J, Marshall J, Stebbings LA, Menzies A, Martin S, Leung K, Chen L, Leroy C, Ramakrishna M, Rance R, Lau KW, Mudie LJ, Varela I, McBride DJ, Bignell GR, Cooke SL, Shlien A, Gamble J, Whitmore I, Maddison M, Tarpey PS, Davies HR, Papaemmanuil E, Stephens PJ, McLaren S, Butler AP, Teague JW, Jönsson G, Garber JE, Silver D, Miron P, Fatima A, Boyault S, Langerød A, Tutt A, Martens JW, Aparicio SA, Borg Å, Salomon AV, Thomas G, Børresen-Dale AL, Richardson AL, Neuberger MS, Futreal PA, Campbell PJ, Stratton MR; Breast Cancer Working Group of the International Cancer Genome Consortium (2012) Mutational processes molding the genomes of 21 breast cancers. Cell 149(5):979-993.

4. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale AL, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjörd JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinski M, Jäger N, Jones DT, Jones D, Knappskog S, Kool M, Lakhani SR, López-Otín C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt AN, Valdés-Mas R, van Buuren MM, van 't Veer L, Vincent-Salomon A, Waddell N, Yates LR; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, Stratton MR (2013) Signatures of mutational processes in human cancer. Nature 500(7463):415-421.

5. Burns MB, Lackey L, Carpenter MA, Rathore A, Land AM, Leonard B, Refsland EW, Kotandeniya D, Tretyakova N, Nikas JB, Yee D, Temiz NA, Donohue DE, McDougle RM, Brown WL, Law EK, Harris RS (2013) APOBEC3B is an enzymatic source of mutation in breast cancer. Nature 494(7437):366–370.

6. Kanu N, Cerone MA, Goh G, Zalmas LP, Bartkova J, Dietzen M, McGranahan N, Rogers R, Law EK, Gromova I, Kschischo M, Walton MI, Rossanese OW, Bartek J, Harris RS, Venkatesan S, Swanton C (2016) DNA replication stress mediates APOBEC3 family mutagenesis in breast cancer. Genome Biol 17(1):185.

7. Periyasamy M, Patel H, Lai CF, Nguyen VT, Nevedomskaya E, Harrod A, Russell R, Remenyi J, Ochocka AM, Thomas RS, Fuller-Pace F, Győrffy B, Caldas C, Navaratnam N, Carroll JS, Zwart W, Coombes RC, Magnani L, Buluwela L, Ali S (2015) APOBEC3B-Mediated Cytidine Deamination Is Required for Estrogen Receptor Action in Breast Cancer. Cell Rep 13(1):108-121.

8. Long J, Delahanty RJ, Li G, Gao YT, Lu W, Cai Q, Xiang YB, Li C, Ji BT, Zheng Y, Ali S, Shu XO, Zheng W (2013) A common deletion in the APOBEC3 genes and breast cancer risk. J Natl Cancer Inst 105:573–579.

9. Xuan D, Li G, Cai Q, Deming-Halverson S, Shrubsole MJ, Shu XO, Kelley MC, Zheng W, Long J (2013) APOBEC3 deletion polymorphism is associated with breast cancer risk among women of European ancestry. Carcinogenesis 34:2240–2243.

10. Cescon DW, Haibe-Kains B, Mak TW (2015) APOBEC3B expression in breast cancer reflects cellular proliferation, while a deletion polymorphism is associated with immune activation. Proc Natl Acad Sci USA 112(9):2841–2846.

11. Wen WX, Soo JS, Kwan PY, Hong E, Khang TF, Mariapun S, Lee CS, Hasan SN, Rajadurai P, Yip CH, Mohd Taib NA, Teo SH (2016) Germline APOBEC3B deletion is associated with breast cancer risk in an Asian multi-ethnic cohort and with immune cell presentation. Breast Cancer Res 27;18(1):56.

12. Caval V, Bouzidi MS, Suspène R, Laude H, Dumargne MC, Bashamboo A, Krey T, Vartanian JP, Wain-Hobson S (2015) Molecular basis of the attenuated phenotype of human APOBEC3B DNA mutator enzyme. Nucleic Acids Res 43(19):9340-9349.

13. Caval V, Suspène R, Shapira M, Vartanian JP, Wain-Hobson S (2014) A prevalent cancer susceptibility APOBEC3A hybrid allele bearing APOBEC3B 3'UTR enhances chromosomal DNA damage. Nat Commun. 2014 Oct 9;5:5129.

14. Harris RS (2015) Molecular mechanism and clinical impact of APOBEC3B-catalyzed mutagenesis in breast cancer. Breast Cancer Res 17:8.

15. Land AM, Wang J, Law EK, Aberle R, Kirmaier A, Krupp A, Johnson WE, Harris RS (2015). Degradation of the cancer genomic DNA deaminase APOBEC3B by SIV Vif. Oncotarget 6(37):39969-39979.

16. Bogdanova N, Cybulski C, Bermisheva M, Datsyuk I, Yamini P, Hillemanns P, Antonenkova NN, Khusnutdinova E, Lubinski J, Dörk T (2009) A nonsense mutation (E1978X) in the ATM gene is associated with breast cancer. Breast Cancer Res Treat 118:207-211.

17. Bogdanova NV, Antonenkova NN, Rogov YI, Karstens JH, Hillemanns P, Dörk T (2010) High frequency and allele-specific differences of BRCA1 founder mutations in breast cancer and ovarian cancer patients from Belarus. Clin Genet 78:364–372.

18. Prokofyeva D, Bogdanova N, Dubrowinskaja N, Bermisheva M, Takhirova Z, Antonenkova N, Turmanov N, Datsyuk I, Gantsev S, Christiansen H, Park-Simon TW, Hillemanns P, Khusnutdinova E, Dörk T (2013) Nonsense mutation p.Q548X in BLM, the gene mutated in Bloom's syndrome, is associated with breast cancer in Slavic populations. Breast Cancer Res Treat 137(2):533-9.

19. Noskowicz M, Bogdanova N, Bermisheva M, Takhirova Z, Antonenkova N, Khusnutdinova E, Bremer M, Christiansen H, Park-Simon TW, Hillemanns P, Dörk T (2014) Prevalence of PALB2 mutation c.509_510delGA in unselected breast cancer patients from Central and Eastern Europe. Fam Cancer 13(2):137-142.

20. Thompson ER, Doyle MA, Ryland GL, Rowley SM, Choong DY, Tothill RW, Thorne H; kConFab, Barnes DR, Li J, Ellul J, Philip GK, Antill YC, James PA, Trainer AH, Mitchell G, Campbell IG (2012) Exome sequencing identifies rare deleterious mutations in DNA repair genes *FANCC* and *BLM* as potential breast cancer susceptibility alleles. PLoS Genet 8(9):e1002894.

21. Kiiski JI, Pelttari LM, Khan S, Freysteinsdottir ES, Reynisdottir I, Hart SN, Shimelis H, Vilske S, Kallioniemi A, Schleutker J, Leminen A, Bützow R, Blomqvist C, Barkardottir RB, Couch FJ, Aittomäki K, Nevanlinna H (2014) Exome sequencing identifies FANCM as a susceptibility gene for triple-negative breast cancer. Proc Natl Acad Sci USA 111(42):15172-15177.

22. Sun J, Wang Y, Xia Y, Xu Y, Ouyang T, Li J, Wang T, Fan Z, Fan T, Lin B, Lou H, Xie Y (2015) Mutations in RECQL Gene Are Associated with Predisposition to Breast Cancer. PLoS Genet 11(5):e1005228.

23. Cybulski C, Carrot-Zhang J, Kluźniak W, Rivera B, Kashyap A, Wokołorczyk D, Giroux S, Nadaf J, Hamel N, Zhang S, Huzarski T, Gronwald J, Byrski T, Szwiec M, Jakubowska A, Rudnicka H, Lener M, Masojć B, Tonin PN, Rousseau F, Górski B, Dębniak T, Majewski J, Lubiński J, Foulkes WD, Narod SA, Akbari MR (2015) Germline RECQL mutations are associated with breast cancer susceptibility. Nat Genet 47(6):643-646.

24. Ellingson MS, Hart SN, Kalari KR, Suman V, Schahl KA, Dockter TJ, Felten SJ, Sinnwell JP, Thompson KJ, Tang X, Vedell PT, Barman P, Sicotte H, Eckel-Passow JE, Northfelt DW, Gray RJ, McLaughlin SA, Moreno-Aspitia A, Ingle JN, Moyer AM, Visscher DW, Jones K, Conners A, McDonough M, Wieben ED, Wang L, Weinshilboum R, Boughey JC, Goetz MP (2015) Exome sequencing reveals frequent deleterious germline variants in cancer susceptibility genes in women with invasive breast cancer undergoing neoadjuvant chemotherapy. Breast Cancer Res Treat 153(2):435-443.

25. Chan K, Roberts SA, Klimczak LJ, Sterling JF, Saini N, Malc EP, Kim J, Kwiatkowski DJ, Fargo DC, Mieczkowski PA, Getz G, Gordenin DA (2015) An APOBEC3A hypermutation signature is distinguishable from the signature of background mutagenesis by APOBEC3B in human cancers. Nat Genet 47(9):1067-1072.

26. Burns MB, Leonard B, Harris RS (2015) APOBEC3B: pathological consequences of an innate immune DNA mutator. Biomed J 38(2):102-110.

Table and figure legends:

Figure 1: Molecular detection of *APOBEC3Bc.783delG**

Detection of *APOBEC3B**c.783delG by direct sequencing (upper panel) and restriction fragment length polymorphism (RFLP) analysis (bottom figure).
Upper panel: Direct sequencing of *APOBEC3B* exon 6 in a wildtype control (above) and a patient heterozygous for *APOBEC3B**c.783delG. The site of the guanine deletion is indicated by an arrow.
Bottom subfigure: 3% agarose gel showing, from left to right: (1) PCR product from a non-carrier after cleavage with *Scr*FI, (2,3) PCR product from heterozygous *APOBEC3B**c.783delG carriers after cleavage with *Scr*FI, (4) uncleaved PCR product, (5) size marker.

Table 1: *APOBEC3Bc.783delG in four breast cancer case-control studies**

Genotyped samples and carrier frequencies for *APOBEC3B**c.783delG in patients with breast cancer and in healthy female controls from Belarus, Germany, Russia and Iran. Patients were further stratified by age at diagnosis with early onset defined as < 50 years, or by familial breast cancer defined as a first-degree family history of breast cancer. OR, Odds ratio. For single studies, OR and p-values were calculated using Fisher´s exact tests. For the combined analysis, the Mantel-Haenszel pooled OR was derived from a fixed effects meta-analysis, and the p-value was drawn from the z-value of the meta-analysis. OR in subgroups was calculated using all controls from the same population as the reference group. n.a., not applied due to zero fields. CI, confidence interval. HMBCS, Hannover-Minsk Breast Cancer Study. HaBCS, Hannover Breast Cancer Study. HUBCS, Hannover-Ufa Breast Cancer Study. IBCS, Iranian Breast Cancer Study. *Six Byelorussian patients from the initial exome sequencing study, one of whom was a *APOBEC3B**c.783delG carrier, are not included in this analysis.