A nonsense mutation (E1978X) in the *ATM* gene is associated with breast cancer

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Abstract Blood relatives of patients with ataxia-telangiectasia (A-T) have an increased risk to develop breast cancer. Allelic heterogeneity has made it difficult to confirm the role of *ATM*, the gene mutated in A-T, for breast cancer susceptibility in the general population. We now report that a nonsense mutation, p.E1978X (c.5932G>T), is both a classical A-T mutation and a breast cancer susceptibility allele in Eastern European populations. In a case-control study from Belarus, the E1978X mutation was identified in 10/1,891 Byelorussian breast cancer cases (0.5%) compared with 1/1,019 population controls [odds ratio (OR): 5.4; 95% confidence interval (95% CI),

0.7–42.4, $P = 0.1$]. A second case-control study from Russia identified the E1978X mutation in two Russian and one Ukrainian cases out of 611 breast cancer patients but not in any Russian or Ukrainian controls ($P = 0.1$). In a third case-control study from Poland, E1978X was observed in 7/3,910 Polish breast cancer cases (0.2%) compared with 1/2,010 cancer-free population controls (OR: 3.6; 95% CI: 0.4–29.3, $P = 0.4$). In the combined analysis, E1978X was significantly associated with breast cancer (Mantel-Haenszel OR: 5.6, 95% CI: 1.3–21.4, $P = 0.01$). Taken together, this study provides first evidence for the association of a common A-T causing mutation with breast cancer in Eastern European founder populations.

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Introduction

Ataxia-telangiectasia (A-T) is an autosomal recessive syndrome characterized by ionizing radiation sensitivity, cerebellar neurodegeneration, immunodeficiency, and a markedly increased risk of cancer [1]. A-T is caused by biallelic mutations in *ATM*, a gene encoding a large protein kinase with a central role in the signaling of radiation-induced DNA damage [2]. There is strong evidence from epidemiological studies that female blood relatives of A-T patients face an increased risk to develop breast cancer compared with spouses [3–10], that this risk depends on their inherited *ATM* allele [6], and that it may be modified by the nature and location of the underlying *ATM* gene mutation [10]. However, the large size and the very

heterogeneous mutational spectrum of the *ATM* gene have made it difficult to prove its role as a breast cancer susceptibility gene in the general population, and the results of several previously published breast cancer case–control studies have been inconsistent [11–17]. Recently, a comprehensive sequencing study of 443 breast cancer cases from the UK has demonstrated an accumulation of diverse A-T causing mutations in breast cancer cases as compared to 521 controls, consistent with an average two to threefold increase in risk for breast cancer for female carriers of a monoallelic, A-T causing mutation [18]. One prediction from this result would be that any more common A-T causing mutation should be identifiable as a single breast cancer susceptibility allele in the respective founder population given sufficiently powerful studies. Here we report on the association of the p.E1978X (c.5932G>T) mutation, a common truncating mutation in A-T patients [19–21], with breast cancer in three large case–control series from Eastern European populations.

Patients and methods

Patients

We investigated three large case–control series from Belarus, Russia, and Poland. The first series from Belarus consisted of 1,891 breast cancer patients diagnosed in the Republic of Belarus during the years 1998–2007. 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 a total of 305 patients (16%) reported a first-degree relative with breast cancer. Byelorussian population controls were 1,019 healthy volunteers from the same population who had no personal history of breast cancer at the time when entering the study.

The second series from Russia consisted of breast cancer patients unselected for family history who were diagnosed during the years 2000–2007 at the oncological center in Ufa (Bashkortostan). Breast cancer patients in this series belong to different ethnic groups living in the Volga Ural region of Russia, and we selected 314 Russian, 158 Tatar, and 49 Ukrainian females for the purpose of this study. Median age at diagnosis was 51 years, and 7% of patients reported a first-degree relative diagnosed with breast cancer. Healthy population controls included 542 volunteers from the same geographic regions (376 Russians and 166

Tatars). In addition to the 49 samples from Russian patients with Ukrainian descent, we also included a small series of 90 Ukrainian breast cancer patients and 37 population controls ascertained at the R.E.Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology, National Academy of Sciences in Kyiv, Ukraine. The Ukrainian series was enriched for familial cases ($n = 30$), and median age at diagnosis was 47 years.

The third series consisted of 3,910 cases of breast cancer diagnosed in Szczecin ($n = 1,919$) and other sites throughout Poland ($n = 1,991$) between 1996 and 2003. This series included 1,813 cases of breast cancer unselected for age, and an additional group of 2,097 cases of breast cancer diagnosed at age 50 or below. Therefore, the Polish series was enriched for early onset cases, and median age of diagnosis was 47 years. All cases were unselected for family history and 9.1% of patients reported a first-degree relative with breast cancer. The Polish control group included 2,010 cancer-free adult women from the same population. Our study was carried out with informed consent of the probands and was approved by local ethics commissions.

Mutation analyses

Genomic DNA was isolated from peripheral white blood cells by routine phenol–chloroform extraction. The exon 42 of the *ATM* gene was amplified by polymerase chain reaction using flanking intronic primers as previously described [20]. PCR products were digested with *MseI* and separated by 3% agarose gel electrophoresis. During the course of the study, an additional duplex assay using allele-specific primers was established using previously genotyped samples and was then applied to the Polish case–control series. For this purpose, genomic DNA was amplified with the primers 5'-GGTGTTCTTGTGACAAACAGAAGTC TTGCATCTT-3' and 5'-GGGTCAGTCCTTCGTCTTTC AGATAAGGAAGTC-3' that generate a 341 bp PCR product specifically in the presence of the E1978X mutation (annealing 63°C). A fragment of the *VHL* gene was co-amplified as an internal PCR control, and PCR products were separated and analyzed by 2% agarose gel electrophoresis. Positive and negative controls were included in each assay, and in positive samples the presence of E1978X was confirmed by direct sequencing of PCR products using BigDye chemistry and an Avant 3100 Genetic Analyser (Applied Biosystems).

Statistical analyses

The prevalence of the E1978X mutation was compared in cases and healthy population controls. Odds ratios (OR) were calculated from two-by-two tables and statistical significance was assessed with Fisher's exact test. Crude

Table 1 Frequency distribution of E1978X carriers in cases and controls from three Eastern European studies

Study population	Cases no. (%)	Controls no. (%)	Odds ratio (95% CI)	P
Belarus	10/1,891 (0.5%)	1/1,019 (0.1%)	5.4 (0.7–42.3)	0.1
Russia/Ukraine	3/611 (0.5%)	0/579	NA	0.1
Russians	2/314 (0.6%)	0/376	NA	0.4
Tatars	0/158	0/166	NA	NA
Ukrainians	1/139 (0.7%)	0/37	NA	0.6
Poland	7/3,910 (0.2%)	1/2,010 (0.05%)	3.6 (0.4–29.3)	0.4
Combined	20/6,412 (0.3%)	2/3,608 (0.06%)	5.6 (1.3–23.4)^a	0.01

Proportional distribution of E1978X heterozygotes between cases and controls in three different studies

^a Mantel–Haenszel Odds Ratio is provided in the combined analysis

NA not applicable due to calculation with zero

and adjusted Mantel–Haenszel OR were calculated using EpiCalc v1.02 Software Package (Gilman J, Myatt M 1998, Brixton Books). All *P* values are two-sided. E1978X was the first A-T causing mutation investigated in the study populations, and results with *P* < 0.05 were considered significant.

Results

We first investigated the frequency of the E1978X mutation in a case–control study from Belarus which comprised 1,891 breast cancer patients and 1,019 healthy population controls. The E1978X allele (nucleotide substitution c.5932G>T, Fig. 1) was identified in 10 patients and in one control individual (OR: 5.4, 95% CI: 0.7–42.3, *P* = 0.1). All mutation carriers were heterozygotes. Median age at diagnosis among mutations carriers was 43 years compared with 48 years in the total case series (*P* = 0.06). One patient heterozygous for p.E1978X reported a first-degree relative also affected with breast cancer, none of the mutation carriers had bilateral breast cancer. Five of ten patients had ductal and four had lobular breast cancer, one patient had an unclassified adenocarcinoma. Two patients also carried a mutation in the *BRCA1* gene, and one was a double heterozygote for E1978X and a *CHEK2* mutation.

We wished to further test and extend these observations in a second study that combined a case–control

series of 521 patients and 542 controls from Ufa, Russia, and a smaller series of 90 breast cancer patients and 37 population controls from Kiev, Ukraine. Cases and controls were stratified by ethnicity, and the E1978X mutation was screened as before in the Belarus series. A heterozygous state for E1978X was confirmed in 2/314 Russian patients (0.6%) and 1/139 Ukrainian patients (0.7%) whereas the mutation was not found in any of the 413 Russian or Ukrainian controls, nor in any individual of Tatar ancestry. The results were in line with a higher frequency of E1978X in breast cancer cases, although this second study again was too small to reach statistical significance (*P* = 0.1).

We finally assessed the frequency distribution of the E1978X mutation in a large case–control series from Poland. The mutation was identified in 7 out of 3,910 unselected breast cancer patients and in 1 out of 2,010 cancer-free control females (OR: 3.6; 95% CI: 0.4–29.3, *P* = 0.4). Median age at onset in the seven patients carrying E1978X was 47 years. One of the seven patients had a positive family history of breast cancer, and she also carried a *BRCA1* truncation. Five patients had ductal and two had lobular breast cancer. Four cancers were ER negative and three were ER positive. Three tumors had developed lymph node metastases. Thus, while this study again did not reach statistical significance on its own, it showed the same trend towards a higher frequency of the *ATM* nonsense mutation in breast cancer patients.

Fig. 1 Direct sequencing of the E1978X mutation in exon 42 of the *ATM* gene. The sequence of the sense strand is shown; *left*: wildtype sequence, *right*: heterozygous carrier of E1978X (c.5972G>T). The *arrow* marks the position of the mutation, which changes the glutamine codon GAA to a nonsense codon TAA

Taken together, in a total of 6,412 cases and 3,608 controls, the E1978X mutation was observed in 20 breast cancer patients and 2 control individuals, and a combined analysis yielded a Mantel–Haenszel Odds ratio 5.6 (95% CI: 1.3–23.4, $P = 0.01$).

Discussion

E1978X is a truncating mutation that has originally been identified in A-T patients of Polish descent [19] and has subsequently been reported at a particularly high proportion in Russian A-T families [21]. Its frequency distribution outside of A-T families and its role for breast cancer has not been investigated, however. Our results indicate an approximately fivefold higher prevalence of this nonsense mutation in breast cancer cases compared with population controls which we regard as substantial evidence for a role of E1978X as a predisposing breast cancer susceptibility allele in the studied populations. The data would be in line with the assumption that the contribution of this mutation to the incidence of breast cancer is highest in Eastern European populations such as in Belarus, Ukraine, Russia, and somewhat lower in Poland, though it is not necessarily confined to these populations. Founder mutations have previously been identified in some candidate breast cancer susceptibility genes such as *CHEK2*, *NBN*, *RAD50*, or *PALB2* [22–26], and have proven very useful to confirm the proposed role of these genes in breast cancer susceptibility. In case of *ATM*, such common truncating mutations have been lacking, thus far, and the results presented in our study may help to fill a long-standing gap in this respect. Another recent study of an *ATM* frame-shift insertion in Finland, c.6903insA, has provided similar evidence for an association with breast cancer as this mutation was seen in 3/541 familial and 5/1,124 unselected Finnish cases, but not among 1,107 healthy population controls [27]. Supportive evidence has also been obtained for the possible association of an A-T causing missense mutation, c.7271T>G, with breast cancer [28]. Although the magnitudes of risk conferred by these mutations remain to be fully defined in additional and larger series, our present data appear to be consistent with both the epidemiological insights from A-T families [3–10] and the sequencing study by Renwick and coworkers [18]. While the latter had been focussing on *BRCA1* and *BRCA2* mutation negative families, our data show that pathogenic *BRCA1* and *ATM* mutations concur in some patients, which may deserve attention in future investigations.

In regard that the association of A-T mutations with breast cancer has been a matter of debate over the past 30 years, we have now shown that a single truncating mutation in the *ATM* gene that causes A-T in the

homozygous (or compound heterozygous) state, is clearly associated with breast cancer in a large case–control study of Eastern European populations. Further research will be required to investigate whether this *ATM* mutation also plays some role in other malignancies.

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