Evaluation of the role of Finnish ataxia-telangiectasia mutations in hereditary predisposition to breast cancer

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Biallelic mutations in the ataxia-telangiectasia mutated (ATM) gene result in ataxia-telangiectasia (A-T). Studies on A-T families have shown that obligate female carriers have increased risk of developing breast cancer. Here we have evaluated the role of known Finnish ATM germ line mutations as possible breast cancer predisposing alleles outside A-T families by analyzing their prevalence in large cohorts of familial and unselected breast cancer cases. Of seven different alterations, two were observed in the studied breast cancer material. ATM 6930insA (causing protein truncation) was seen in 3/541 familial and 5/1124 unselected cases, but not among healthy population controls (0/1107). 7570G>C (Arg2912Gly) associated previously with breast cancer susceptibility and suggested to be causative also for A-T was detected in 2/541 of familial cases, but not in unselected cases (0/1124) or controls (0/1107). In total, heterozygous ATM mutation carriers were observed in 6/541 familial (P = 0.006, odds ratio (OR) 12.4, 95% confidence interval (CI) 1.5–103.3) and 7/1124 unselected cases (P = 0.07, OR 6.9, 95% CI 0.9–56.4), compared with 1/1107 in controls. Additionally, 8734A>C, whereas 8734A>G showed only a partial defect in the phosphorylation of ATM substrates, and 6903insA seemed to be a null allele.

Introduction

Familial breast cancer is a heterogeneous disorder. Apart from BRCA1 and BRCA2, the lack of convincing evidence of additional major susceptibility genes suggests that the remaining cases are due to mutations in several other genes, perhaps with lower disease penetrance (1). ATM has long been considered a good candidate gene. It encodes a protein which is a major activator of cellular responses to DNA double-strand breaks through subsequent phosphorylation of central players in the DNA damage response pathways, including BRCA1, p53, Chk2 and NBS1 (2).

Biallelic ataxia-telangiectasia mutated (ATM) mutations result in ataxia-telangiectasia (A-T), a recessive disorder characterized by progressive neurodegeneration, cell cycle checkpoint defects, radiosensitivity and increased risk of cancer (3). Studies on A-T families have suggested that obligate female carriers have an increased risk of breast cancer (4,5). However, the role of ATM as a breast cancer susceptibility gene outside the A-T families has been controversial, as many of the case–control studies have failed to show an elevated frequency of truncating ATM mutations in breast cancer patients. This has been explained not only by the use of breast cancer cases unselected for family history, which might be an inefficient way to detect ATM mutations, but also by that only ATM mutations with specific functional consequences predispose to cancer (6). It has been suggested that dominant-negative mutations, missense changes in particular, which give rise to stable kinase-inactive or non-phosphorylatable proteins are the ones mainly responsible for the increased cancer risk in A-T carriers (7,8). Yet, two recent studies in A-T families did not identify mutation-specific differences in cancer risk (9,10).

In order to search for possible cancer susceptibility alleles in ATM, we recently performed a full mutation analysis of the coding regions and splice sites of the gene in 121 Northern Finnish breast cancer families, previously evaluated also for the presence of Finnish A-T-related ATM mutations (11–13). Altogether, the analysis of this geographically constrained cohort revealed only two different heterozygous mutations, 7570G>C (in two individuals) and 6903insA (in one individual), both of which have been identified previously in A-T patients. This suggests that breast cancer susceptibility alterations in ATM mainly are restricted to those reported in A-T. Some other studies on familial cases have also provided evidence that A-T-causing mutations are breast cancer susceptibility alleles in the general population (14–16). These findings prompted us to perform a more comprehensive analysis on the impact of ATM mutations, originally identified in Finnish A-T patients, in hereditary predisposition to breast cancer.

A large new cohort of 541 BRCA1 and BRCA2 mutation-negative breast cancer families were screened for the presence of the following A-T mutations: IVS14 + 3-4delAT (exon 14 skipped), IVS37 + 9A>G (insertion Val, Ser, Stop), 6779-6780delTA (truncation), 6903insA (truncation), 7570G>C (marked previously as 7522G>C, Ala2524Pro), 8710-8715delGAGACA (deletion of Glu and Thr) and 9139C>T (Arg3047Stop). The frequencies of the observed ATM alleles were compared with those of 1124 breast cancer patients unselected for family history together with 1107 unaffected population controls. The obtained results indicate contribution of germ line ATM aberrations in cancer predisposition also outside A-T families. In addition, we provide evidence for functional consequences of three observed breast cancer-associated ATM mutations.

Materials and methods

Subjects

Index cases of 541 BRCA1 and BRCA2 mutation-negative families were screened for known Finnish A-T-related mutations and for the additional

Abbreviations: AI, allelic imbalance; A-T, ataxia-telangiectasia; ATM, ataxia-telangiectasia mutated; CI, confidence interval; IR, ionizing radiation; LCL, lymphoblast cell line; OR, odds ratio.

Joint first authorship.
ATM germ line mutation, 8734A>G, observed during this study. Inclusion criteria for the families were as follows: (i) three or more affected in the family (258 cases); (ii) two affected first-degree relatives (251 cases) or (iii) two affected second-degree relatives (5 cases). The frequencies of the observed mutations were compared with those of genetically matched 1124 unselected breast cancer cases and 1107 healthy controls. All patients provided informed consent for obtaining pedigree data and DNA specimens. Approval for the study was obtained from the Ethical Boards of the involved University Hospital health care districts and the Finnish Ministry of Social Affairs and Health.

Mutation detection
Screening was performed by conformation-sensitive gel electrophoresis and minisequencing (17,18). All findings were confirmed by re-amplification of the original DNA sample and direct sequencing. Conformation-sensitive gel electrophoresis primers for exons 14, 37, 62 and 65 have been reported previously (19). Sequences for exons 48, 49, 53 and for minisequencing are available upon request.

Microsatellite marker analysis
D11S1819, D11S2179, D11S1778, D11S1294 and D11S1818 markers were used to determine the haplotype of observed mutation alleles, and to study possible allelic imbalance (AI) in the tumors of one 6903insA and two 7570G carriers. The polymerase chain reaction products were analyzed with the Li-Cor IR² 4200S DNA Analysis system (Li-Cor, Lincoln, NE) using an IRD800-labeled forward primer. Allele intensity ratios were quantified with the Gene Profiler 4.0 analysis program (Scanalytics, Fairfax, VA). AI was calculated by the formula AI = (T2 × N1)/(T1 × N2), where T1/2 represents tumor and N1/2 the corresponding normal alleles. A value >1.67 or <0.60 was considered to indicate AI, meaning that the intensity of one allele had decreased >40%.

Cell cultures
Lymphoblast cell lines (LCLs) were established from one 7570G>C (BR04108), one 8734A>G (BR0510) and two 6903insA carriers (BR0996, BR0997). Two healthy (BR0409, C3ABR) and two affected non-carrier LCLs (BR0197, BR0122), together with two A-T LCLs (AT1ABR, L3), were used as reference. BR0409 was derived from the same family as BR0996 and BR0997. AT1ABR was established in Brisbane, Australia. L3 is an LCL established from a North African Jewish A-T patient and was obtained from Dr Yousef Shilloh (Tel Aviv University, Tel Aviv, Israel). LCLs were grown in RPMI 1640 medium containing 20% fetal calf serum, l-glutamine and antibiotics.

ATM expression and kinase activity analysis
The effects of the mutations on ATM expression and kinase activity were evaluated by western blot analysis. Cellular extracts were prepared by resuspending the cells in lysis buffer and incubating the mixture on ice for 30 min. Supernatants were collected after centrifugation at 14 000g for 15 min at 4°C. ATM was immunoprecipitated with anti-ATM polyclonal antibody against the N-terminus of protein (residues 250–522). Immunoprecipitates were resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and western blotted with the same anti-ATM antibody. For assessment of in vivo ATM kinase activity, 40 μg of extracts from mock or irradiated cell (4 Gy) was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted with appropriate antibody. The phosphorylation of two ATM substrates, p53 (Ser15) and Chk1 (Ser317), and the ATM autophosphorylation in vivo were determined by western blot analysis. Cellular extracts were prepared by resuspending the cells in lysis buffer and incubating the mixture on ice for 30 min. Supernatants were collected after centrifugation at 14 000g for 15 min at 4°C. ATM was immunoprecipitated with anti-ATM polyclonal antibody against the N-terminus of protein (residues 250–522). Immunoprecipitates were resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and western blotted with the same anti-ATM antibody. For assessment of in vivo ATM kinase activity, 40 μg of extracts from mock or irradiated cell (4 Gy) was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted with appropriate antibody. The phosphorylation of two ATM substrates, p53 (Ser15) and Chk1 (Ser317), and the ATM autophosphorylation in vivo were determined by western blot analysis.

Cell survival after IR
The cell survival was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyterazolium bromide assay. Cells (10 000) of each LCL were plated in 100 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyterazolium bromide solution, 1 mg/ml in phosphate-buffered saline, was added to each well and incubated at 37°C for 4 h. The cells were pelleted by centrifugation and re-suspended in 150 μl dimethyl sulfoxide. Plates were incubated for 1 h at room temperature and measured at 570 nm. Cell survival fraction was calculated relative to the number of viable cells in the non-irradiated culture at 96 h. Experiments were performed at least twice on each LCL.

6903insA mRNA expression analysis
mRNA was isolated from 6903insA carrier LCLs with the FastTrack® 2.0 Kit (Invitrogen, Carlsbad, CA) and cDNA was synthesized using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Hanover, MD). The presence of 6903insA transcript in the mRNA pool was evaluated by direct sequencing with cDNA-specific primers: forward 5’-CCTGATGAAAAGGAAATGG-3’ and reverse 5’-GCCCACAAA CCCCAGACATT-3’.

Statistical analysis
The differences in carrier frequencies were analyzed by Fisher’s exact test (SPSS version 12.0 for Windows, SPSS). All P-values were two sided.

Results
ATM 6903insA and ATM 7570G>C are present in both familial and unselected breast cancer cases
Of the seven Finnish A-T-related ATM mutations, 6903insA and 7570G>C (Ala2524Pro) were the only ones observed in the analyzed breast cancer patients. ATM 6903insA was observed in the index patients of three families (p088, p1025 and s134) (Figure 1A). The study of additional family members in p088, however, showed incomplete segregation with the disease. The prevalence of 6903insA was also tested in 1124 unselected breast cancer cases and five carriers, diagnosed between the age 41 and 58, were observed. Cancer registry inquiries revealed that at least one of the parents of four of these cases had had cancer (lymphoma, uterine, bladder, esophageal and stomach). All eight 6903insA carriers originated from the Tampere region (Figure 2) and shared the same haplotype (data not shown). 6903insA was not observed in the tested controls (0/1107). ATM 7570G>C allele was observed in the index patient of family 5063 (Figure 1B). Interestingly, she had tested positive also for the CHK2 1100delC alteration (20). Maternally the index originated from central Finland and paternally from eastern Finland. The analysis of 1124 unselected breast cancer patients revealed two 7570G>C carriers, diagnosed at the age of 44 and 56 years, both originating from the Oulu region (Figure 2). One 7570G>C carrier was identified also among healthy controls. Because of the seemingly different geographical origins of some of the currently identified mutation carriers, a microsatellite marker analysis was used to assess whether 7570G>C derived from a common founder. Samples from eight different families, including two A-T and two breast cancer families identified in previous studies (11,12), were analyzed. All carriers shared the same haplotype (data not shown), thus confirming a common origin of the mutation.

Breast-cancer-associated ATM 8734A>G allele is observed in two families
When screening for the A-T-related 8710-8715delGAGACATA mutation in exon 2 by conformation-sensitive gel electrophoresis, another alteration, 8734A>G (Arg2912Gly), was observed in the index patients of two families (p337 and p293) (Figure 1C). 8734A>G has previously been associated with breast cancer susceptibility (15,21), and has been suggested to be causative also for A-T (15). Consequently, it was included in the study. Both 8734A>G positive families originated from the Tampere region (Figure 2) and shared the same haplotype (data not shown). However, the segregation of 8734A>G with cancer was incomplete as several unaffected carriers occurred in both families, and in family p337, one mutation-negative breast cancer patient was observed. 8734A>G was not observed among unselected cases or in controls.

In the current study, ATM mutations, 6903insA, 7570G>C and 8734A>G, were observed altogether in 6/541 familial cases [P = 0.006, odds ratio (OR) 12.4, 95% confidence interval (CI) 1.5–103.3] and in 7/1124 unselected cases (P = 0.07, OR 6.9, 95% CI 0.9–56.4), compared with only one 7570G>C carrier in 1107 healthy controls (Table I). Thus far, in Finland, a total of 630 familial and 1209 breast cancer patients have been analyzed for known germ line ATM mutations (current study, 12). The frequency of the observed three ATM alleles is significantly higher in familial (9/630, P = 0.0003, OR 18.9, 95% CI 2.4–149.7) and in unselected breast cancer cases (7/1209, P = 0.03, OR 7.6, 95% CI 0.9–61.9) compared with 1/1307 in healthy controls.
Loss of the wild-type allele is not implicated in the tumorigenesis of ATM carriers

AI analysis was performed on available tumor samples of one 6903insA and two 7570G>C carriers. None of the tumors showed loss of the wild-type allele (data not shown).

The two missense mutations have no effect on protein stability

Heterozygous LCLs were used for the functional characterization of the 7570G>C (BR04108), 8734A>G (BR0510) and 6903insA (BR0996, BR0997) mutations. The amount of ATM from 7570G>C and 8734A>G LCLs was comparable with that of the control subject.

Fig. 1. Families displaying (A) 6903insA, (B) 7570G>C and (C) 8734A>G mutations. Filled/open symbols indicate cancer/non-cancer status, respectively. Age at diagnosis, when known, is shown after the cancer type (Bil Br, bilateral breast; Br, breast; Col, colorectal; Hp, hypopharyngeal; Lu, lung; Pan, pancreas; Pro, prostate; Sto, stomach). Index cases are marked with an arrow, and subjects tested for a specific mutation ‘+’ if positive and ‘−’ if negative. The subject marked with an asterisk tested positive also for the CHK2 1100delC alteration (20).

Fig. 2. The geographical origin of Finnish A-T families (circle) (11), breast cancer families (triangle) and unselected breast cancer cases (star) displaying known ATM germ line mutations. Minus (−) depicts absence of a certain mutation. Three previously reported mutation-positive breast cancer families (two with 7570G>C and one with 6903insA) are also included (12).
(C3ABR) homozygous for wild-type ATM (Figure 3A). For 6903insA, leading to premature translation stop at codon 2372, no truncated protein was observed and the level of full-length ATM expression was reduced to half relative to the reference LCLs (Figure 3B). However, sequencing analysis demonstrated that 6903insA transcripts were still present in the tested mRNA pool.

7570G>C shows dominant-negative effect on ATM kinase activity
Possible downstream effects of the observed mutations were evaluated by assessing the phosphorylation of two ATM substrates, p53 (Ser15) and Chk1 (Ser317), and the ATM autophosphorylation site Ser1981 for the missense mutations 7570G>C and 8734A>G, before and after exposure to IR (Figure 3A and C). In extracts from the C3ABR control cell line, ATM was activated rapidly, as judged by enhanced phosphorylation of p53 on Ser15 and Chk1 on Ser317. In contrast, phosphorylation was barely detectable in the ATM non-expressing L3 cell line. DNA damage-induced phosphorylation of p53 and Chk1 was also dramatically lower in the 7570G>C carrier LCL (BR04108), whereas the 8734A>G carrier LCL (BR0510) was defective only in Chk1 phosphorylation (Figure 3C).

Table I. Heterozygous ATM germ line mutations in Finnish breast cancer families, unselected breast cancer cases and controls, observed in the current study

<table>
<thead>
<tr>
<th>ATM mutation</th>
<th>Carrier frequency (%)</th>
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<tbody>
<tr>
<td>Familial cases</td>
<td>Unselected cases</td>
</tr>
<tr>
<td>6903insA</td>
<td>0.6% (3/541)</td>
</tr>
<tr>
<td>7570G&gt;C</td>
<td>0.2% (1/541)</td>
</tr>
<tr>
<td>8734A&gt;G</td>
<td>0.4% (2/541)</td>
</tr>
<tr>
<td>Overall</td>
<td>1.1% (6/541)</td>
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*Observed in A-T patients.

6903insA carrier LCLs show radiosensitivity
Since deficiency in ATM leads to increase in cellular sensitivity to IR, the possible effects of 7570G>C, 8734A>G and 6903insA on radiosensitivity were evaluated. Two LCLs (L3 and AT1ABR) from A-T patients served as positive controls. As expected, the A-T cell lines displayed extreme sensitivity to IR when compared with healthy control LCLs (C3ABR, BR0409). The survival of the 7570G>C carrier and 8734A>G carrier LCL was of the same order as the controls (data not shown), whereas the survival of the two 6903insA heterozygous cell lines (BR0996 and BR0997) was indistinguishable from that of A-T cell lines at most dose points (Figure 4). Affected non-carrier LCLs (BR0197, BR0122) showed radiosensitivity comparable with the healthy control cell lines.

Table II. Clinical data for Finnish patients with breast cancer and A-T mutations

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>6903insA</th>
<th>7570G&gt;C</th>
<th>8734A&gt;G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>&lt;50</td>
<td>&gt;50</td>
<td>=50</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Histological type</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

Fig. 3. ATM expression and kinase activity of mutation heterozygous LCLs. (A) ATM expression and Ser1981 phosphorylation in 7570G>C (BR04108) and 8734A>G (BR0510) carrier LCLs before (−) and after (+) exposure to IR. Both cell lines show normal ATM protein levels, but only BR04108 exhibits defective IR-induced ATM autophosphorylation. DNA-PKcs = control for protein loading. (B) Reduced expression of ATM in 6903insA carrier LCLs (BR0996 and BR0997). A band of ~200 kDa precipitating non-specifically with the antibody has been used as control for protein loading. (C) In vivo analysis of ATM kinase activity with p53 and Chk1 as substrates, before (−) and after (+) exposure to IR. Kinase activity is shown by western blotting (WB) with anti-p53 Ser15 and anti-Chk1 Ser317, and with additional anti-p53 and anti-Chk1 antibodies that detects the total pool, both phosphorylated and unphosphorylated, of these proteins. Irradiation of BR04108 (7570G>C) cells results in grossly reduced phosphorylation of both p53 and Chk1. BR0510 (8734A>G) cells, on the other hand, show only defective Chk1 phosphorylation. The origin of the supplementary bands in BR0997 is unknown. LCLs used are the following: BR04108 = 7570G>C carrier, BR0510 = 8734A>G carrier, BR0996 and BR0997 = 6903insA carriers, BR0197 and BR0122 = affected non-carrier LCLs, BR0409 and C3ABR = healthy non-carrier cell lines, L3 = A-T LCL.
We have assessed the relevance of seven ATM mutations, originally identified in Finnish A-T patients, in hereditary predisposition to breast cancer. The results suggest that two of these mutations, 6903insA and 7570G>C, are breast cancer susceptibility alleles also outside A-T families. The other five known alleles occurring in A-T were not observed, which might be due to their low frequency. Yet, it is also possible that some of these alleles do not predispose to breast cancer, as it has been suggested that the risk might also depend on the location of truncating mutations (9). Besides the 6903insA and 7570G>C alleles, another ATM mutation, 8734A>G, was observed in two families. It has previously been associated with breast cancer susceptibility (15,21), and has been suggested to be causative also for A-T (15).

In Finland, 6903insA, 7570G>C and 8734A>G have been observed altogether in 9630 breast cancer families (P = 0.0003, OR 18.9, 95% CI 2.4–149.7) (current study, 12). The study of additional family members, however, showed incomplete segregation, as both unaffected mutation carriers and mutation-negative breast cancer patients were observed. In addition, 6903insA and 7570G>C have also been observed in breast cancer patients without known familial background of the disease (7/1209) (P = 0.03, OR 7.6, 95% CI 0.9–61.9), and 7570G>C also in one healthy control further suggesting incomplete penetrance for these mutations. Consequently, the breast cancer risk associated with the observed ATM mutations is likely to depend on environmental factors and/or susceptibility alleles in other genes, as suggested by the polygenic model for breast cancer susceptibility (1). This is consistent with the recent study, which found an ~2-fold increase in risk of breast cancer associated with ATM mutations causing A-T. This risk appears to be similar to that of low-penetration susceptibility allele CHK2 1106delC (16). Overall, the observed ATM mutations seem to explain only a small fraction of hereditary susceptibility to breast cancer in Finland, as they have been observed in 1.4% of the familial and 0.6% of the unselected cases studied so far. The relatively low frequency of these alleles is not, however, surprising when considering the fact that at least two of them are also pathogenic for A-T, thereby limiting their population frequency. Nevertheless, as the observed alleles show geographical clustering, their predisposing effect of this mutation is not a dominant-negative one. Instead of the complete loss of normal protein, it has been suggested that mutations with dominant-negative effect are the ones mainly responsible for the increased risk of breast cancer in ATM carriers. Subsequently, the functional consequences of all three observed breast cancer-associated ATM mutations were investigated. Analysis of the 7570G>C heterozygous cell line showed that substitution of the evolutionarily conserved (for instance, Mus musculus, Xenopus laevis and Tel1p protein of Schizosaccharomyces pombe) Ala2524 residue with proline in the FRAP/ATM/TRRAP (FAT) domain leads to a stable protein with defective kinase activity. Although FAT contains no catalytic sequences, it occurs only in combination with FRAP/ATM/TRRAP C-terminal (FATC), and it has been suggested that these domains fold together in a configuration that ensures proper function of the interposed kinase (24). Failure in correct folding could inactivate kinase functions, which could explain the pathogenicity of 7570G>C. The defective phosphorylation of Ser1981, and the two ATM downstream targets p53 Ser15 and Chk1 Ser317, seems to be related to the dominant-negative effect of 7570G>C. This effect has been shown also for another A-T-related and breast cancer-associated mutation, 7271T>G (Val2424Gly), located in the FAT domain (14,25). However, whereas the A-T patients homozygous for 7271T>G have been reported to have only mild clinical symptoms (25,26), no difference in the disease phenotype of the two Finnish A-T patients, one being compound heterozygote and the other homozygous for 7570G>C, was observed (12).

The other observed A-T mutation, 6903insA, causes a frameshift. No truncated protein was present in the carrier LCLs and the total amount of endogenous ATM was reduced to about half. This seems sufficient for normal function of the ATM checkpoint signaling pathway, but not to ensure normal level of cell survival after IR-induced damage. This differential impact on cellular radiosensitivity has been reported previously by over-expression of ATM fragment containing the leucine zipper domain that can act in a dominant-negative manner to influence cell survival, but not p53 stabilization and cell cycle checkpoints (27). If a truncated protein was expressed, although below the level of the used detection method, it would contain the leucine zipper and could potentially act in a dominant-negative way to influence cell survival. Alternatively, different biological endpoints and functions could have different threshold requirements for ATM, which also has been reported previously (28,29). Thus, although one cellular pathway that might promote tumorigenesis is altered, others may function apparently normally. Accordingly, instead of the dominant-negative effect, haploinsufficiency might be a more plausible explanation for the cancer susceptibility associated with 6903insA. The geographically confined high frequency among breast cancer patients and absence among healthy controls strongly suggest that 6903insA mutation is associated with increased risk of developing cancer. Consequently, breast cancer susceptibility is not restricted to ATM mutations with dominant-negative effect on the kinase activity. This view is supported by the results from two recent studies of A-T families, showing no mutation-specific differences in cancer risk (9,10).

Besides the two A-T-related alleles, another potentially pathogenic ATM mutation, 8734A>G, was observed. 8734A>G leads to Arg2912Gly substitution in the kinase domain, altering a highly conserved residue between different species and also in most other members of phosphoinositide 3-kinase family kinases. However, the LCL heterozygous for 8734A>G showed no defects in the phosphorylation of ATM Ser1981 or p53 Ser15, whereas faulty Chk1 Ser317 phosphorylation was observed. Thus, Arg2912Gly substitution does not impair the phosphorylation of all ATM substrates, and the cancer predisposing effect of this mutation is not a dominant-negative one. Nevertheless, Arg2912Gly may impair some other protein–protein interactions required for optimal ATM kinase activity. Interestingly, it has been shown that upon irradiation, the phosphorylation of Chk1 Ser317 by ATM is also dependent on NBS1 (30), and it has been suggested that NBS1 assists ATM in targeting some of its substrates. Consistent with this, it has been reported that phosphorylation of p53 by ATM occurs through an NBS1-independent mechanism (30,31).
Even though the role of 8734A>G as a breast cancer susceptibility allele has been reported previously (15,21), and is further supported by the present results, so far it has not been reported in A-T cases. Based on the current functional evidence, there might be a simple explanation for this: although 8734A>G appears to predispose to cancer, it may not be pathogenic enough to result in A-T clinical phenotype when paired with itself or other mutant ATM alleles.

In conclusion, current results support the association of two A-T-related ATM mutations, 6903insA and 7570G>C, in addition to 8734A>G, with breast cancer susceptibility. The results also provide evidence for founder effects in the geographical distribution of these alleles. The clustering of 6903insA and 8734A>G to the Tampere region seems particularly strong, and together, these mutations regionally contribute 3% of the studied familial breast cancer cases. Of the observed changes, 7570G>C and 8734A>G lead to amino acid substitutions, but only 7570G>C showed dominant-negative effect on kinase activity. For ATM 6903insA carriers, haploinsufficiency might be a more plausible explanation for the predisposition to cancer. Consequently, the results indicate that the ATM gene-dosage effect is sufficient to exert a cellular phenotype that promotes tumorigenesis.

Acknowledgements
We thank Drs Guillermo Blanco, Ulla Puistola, Jaakko Ignatius and Hanna-leena Eerola, and nurses Outi Kajulu and Minna Merikivi for their help in patient contacts. We thank Drs Anne-Lise Botresen-Dale and Jiri Bartek for helpful discussions and Dr Veli Isomaa for support in protein analysis. The technical assistance of Ms Arja Tapio, Ms Kati Outila, Ms Kati Rouhento and Ms Sirpa Stick is greatly appreciated. This study was supported by the Academy of Finland, University of Oulu, Oulu University Hospital, Finnish Cancer Society, Cancer Foundation of Northern Finland, Nordic Cancer Union, Maud Kuistila Memorial Foundation, Clinical Research Fund of Helsinki University Central Hospital and Sigríð Juselius Foundation. In particular, we thank all patients participating in this study.

Conflict of Interest Statement: None declared.

References

Received October 11, 2006; revised November 23, 2006; accepted November 24, 2006.