Ataxia-telangiectasia (AT) is a recessive disorder caused by mutations in the ATM gene (ataxia-telangiectasia mutated) located on chromosome 11q22-23 (OMIM 208900). AT is characterised by progressive cerebellar ataxia, ocucutaneous telangiectasia, immunodeficiency, radiosensitivity, and cancer predisposition with a predominance of lymphoid tumours and less frequently other tumours including breast cancer. The 13 kb mRNA of ATM is assembled from 66 exons distributed across a genomic region of 150 kb. It codes for a 350 kDa protein with a C-terminus phosphatidylinositol 3-kinase domain involved in the recognition and repair of radiation induced DNA double strand breaks. Oncoproteins, including the tumour suppressors p53, BRCA1, and CHK2, are regulated by ATM. Epidemiological evidence suggests that ATM heterozygotes, representing 0.5–1% of the general population, have a 5 to 8-fold increased risk of developing breast cancer. These estimations raised the possibility that germline mutations of ATM may account for ∼5% of all breast cancer cases. Furthermore, since breast cancer reported in obligate carriers among AT family members affects predominantly younger women, an age specific relative risk model has been proposed. In this model, up to 8% of breast cancer diagnosed in young women is attributed to ATM mutations. In a recent study, Broeks et al identified seven germline ATM truncating mutations among 82 patients who developed breast cancer before the age of 40 and only looked for truncating mutations. These results suggested that the model may overestimate the true allele frequency in women with breast cancer. Moreover, direct molecular examination of ATM in selected breast cancer patients outside AT families has led to conflicting results. Fitzgerald et al showed that ATM mutations were present in only 2/401 (0.5%) women with early onset breast cancer, but they only looked for truncating mutations. In a recent study, Brooks et al identified seven germline ATM truncating mutations among 82 patients who developed breast cancer before the age of 40. The susceptibility to breast cancer related to ATM is not confined to truncating mutations, but an increased risk has also recently been attributed to various different missense mutations. Moreover, it has been suggested that some ATM mutations are highly penetrant for breast cancer, such as T7271G and IVS10-6T>G. However, the overall contribution of ATM variants to breast cancer is not known.

To determine further the contribution of ATM as a breast cancer predisposing gene, we designed a study to establish the frequency of ATM mutations in a highly selected, but not unusual group of women diagnosed with invasive breast cancer before the age of 40 and documented to have no first or second degree family history of breast cancer.

**METHODS**

**Patient selection**

Ninety-four patients with breast cancer diagnosed before the age of 40 were recruited from three centres: 96 from Geneva University Hospital (Switzerland), 23 from the Institute of Oncology, Ljubljana (Slovenia), and 15 from McGill University affiliated hospitals, Montreal, Canada. Medical and family histories were obtained by direct interviews and diagnosis was confirmed by review of pathological records. The mean age at diagnosis was 35.9 years, ranging from 25 to 39.9 years. After having signed a consent form, all women agreed to provide a single blood sample for an anonymised genetic analysis. Forty-five healthy female blood donors (mean age of 36.2 years, range 23-45 years), without a family or personal history of cancer, were selected as controls from the Geneva population. The sequence alterations identified among the breast cancer cases were screened in an additional group of 95 random blood donors from Geneva. The study was approved by the local ethical committee of the three centres.

**ATM mutation analysis**

DNA was isolated from whole blood using the QIAamp DNA Blood Mini kit according to the manufacturer’s recommendations (Qiagen, Hilden, Germany). PCR reactions were performed in a Biometra T3 thermocycler (Biometra, Göttingen, Germany) in a 50 µl volume with 100 ng genomic DNA, 20 pmol of each primer in 1× Taq PCR Master Mix (Qiagen, Hilden, Germany) containing 3 mmol/l MgCl₂. After initial denaturation at 94°C for five minutes, each of the 35 cycles of amplification consisted of 30 seconds at 94°C, 30 seconds at 58°C, and 30 seconds at 72°C, followed by final extension of five minutes at 72°C. The oligonucleotide primer pairs used to amplify all the ATM coding exons have been described previously, with conditions for each pair.

Single strand conformation polymorphism (SSCP)/heteroduplex (HTX) analysis was performed as previously described. Briefly, 10 µl of PCR products containing 10 µl non-denaturing loading buffer were boiled for five minutes, chilled on ice for 10 minutes, and loaded on a 6% MDE acrylamide gel (FMC Bioproducts, Rockland, ME, USA). The gel was silver stained (BioRad, Hercules, CA, USA) after electrophoresis at 500 V for 2.5 hours in 0.6× TBE buffer cooled at 12°C.

All ATM segments excepting the aberrant SSCP/HTX pattern were reamplified under the same conditions except primers containing SP6 (forward) and T7 (reverse) sequence added at the 5′ ends of each PCR primer. These PCR products were sequenced using a Thermo Sequenase fluorescent labelled primer sequencing kit (Amersham Pharmacia Biotech, Uppsala, Sweden) with SP6 and T7 primers. Sequence products were analysed with the Li-Cor DNA Analyzer Gene ReadIR 4200 apparatus (Li-Cor, Lincoln, NE, USA) on Sequagel XR acrylamide gel (National Diagnostic, Atlanta, GA, USA) according to the manufacturer’s instructions.

**RESULTS AND DISCUSSION**

We found a series of well known polymorphisms (IVS4+37insAA, IVS17-56G>A, F858L, IVS22-77T>C, L1046L, P1054R, K1454N, P1526P, D1853N, IVS48-69ins3, IVS62-55T>C) both in patients and controls. A previously...
Table 1  ATM alterations found in early onset breast cancer patients and controls

<table>
<thead>
<tr>
<th>Type</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Predicted effect</th>
<th>No in breast cancer cases (n=94)</th>
<th>No in controls (n=140)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>15</td>
<td>2119T&gt;C</td>
<td>S707P</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>30</td>
<td>IVS30-2A&gt;G</td>
<td>Splicing defect</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>31</td>
<td>4388T&gt;G</td>
<td>F1463C</td>
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<td>0</td>
</tr>
<tr>
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<td>15</td>
<td>1960G&gt;A</td>
<td>δα54K</td>
<td>1</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>25</td>
<td>IVS25-15insT</td>
<td>Unknown</td>
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<td>0</td>
</tr>
<tr>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>UV</td>
<td>59</td>
<td>IVS 59-20delA</td>
<td>Unknown</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>IVS63+24delTT</td>
<td>Unknown</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>

The odds ratio (OR) of the frequency of all ATM variants not known to be polymorphisms in the breast cancer cases compared with the controls is 18.4 (95% CI 2.6 to 79.8, p=0.0002). If only 2119T>C, IVS30-2A>G, and 4388T>G are counted as disease associated mutations [see text] then these variants are significantly over-represented in the cases compared with the controls (p=0.011 [two sided Fisher’s exact test].

M: mutation. UV: variant of unknown biological significance.

undescribed variant (IVS59-20delA) was found once in the cases and once in the controls. The functional relevance of this alteration has not yet been determined. We detected 10 germ-line ATM sequence variants among 94 breast cancer patients (10.6%, 95% confidence interval (CI) 5.2 to 18.7%) not identified in the control group of 140 healthy blood donors (p=0.0006) (table 1). Five of these 10 ATM alterations (Q654K, IVS20+28insA, IVS25-15insT, IVS56+23insT, IVS63+24delTT) were variants with an unknown biological significance and the size of our control group could not formally rule out the possibility that any one of these variants is a polymorphism. Two were nucleotide substitutions resulting in missense mutations, S707P (found three times) and F1463C, and one was a splicing mutation, IVS30-2A>G. Three of these eight distinct alterations can be considered as likely pathogenic mutations. The S707P missense mutation found in three unrelated patients has already been reported in sporadic breast cancer patients.

In keeping with previous studies, we identified five uncommon ATM variants in breast cancer patients that were not found in controls and have not been previously described. The functional significance of these alterations is currently undefined and therefore the question remains open as to whether to refer to them as “variants of unknown biological significance” or as harmless polymorphisms. Though we failed to detect abnormal patterns of ATM mRNA splicing in the five intronic ATM variants (data not shown), these variants may still alter ATM function, for instance through modulations of ATM mRNA level production. A recently described ATM functional assay may help to distinguish functional changes in the ATM gene from polymorphisms.

Several studies have explored the structure and function of the ATM gene in neoplastic tissues. The 11q23 locus encompassing the ATM gene is often deleted in breast carcinoma and reduction in the levels of ATM mRNA and protein has also been observed in this type of tumour. Interestingly, by revealing missense mutations and complex intragenic rearrangements, the spectrum of somatic mutations found in these malignancies differs from that of classical AT patients, leading to the suggestion that there may exist two classes of ATM mutations, that is, the “null” mutations (complete/near complete loss of function) and the “impairing” mutations (reduced function).

Of both kinds of alteration are expected to be functionally relevant; for instance, monoallelic “impairing” mutations in ATM such as those found in cancers would compete with the remaining wild type copy of ATM to form functional multiprotein complexes. These mutations would act as dominant negative mutations interfering with the cell capacity to maintain DNA integrity. A recently described missense mutation (T7271G) in an AT family with a mild clinical phenotype and high cancer incidence would lend credit to this hypothesis.

Our study is limited by its small sample size, the retrospective design, and the SSCP/heteroduplex technique used to screen for ATM genetic alterations, which was not optimally sensitive to the identification of missense mutations. Despite these limitations, our findings add to the growing number of reports indicating that subtle constitutional alterations of ATM may impart an increased risk of developing breast cancer and therefore act as a low penetrance, high prevalence gene in the general population.

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Constitutional alterations of the ATM gene in early onset sporadic breast cancer

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