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

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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 optimal annealing temperature, 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 exhibiting an 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 Thermosequenase 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, IVS22-771>C, L1046L, P1054R, K1454N, P1526P, D1853N, IVS48-69ins3, IVS62-55T>C) both in patients and controls. A previously
undescribed variant (IVS59-20del4) 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.

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. In addition, somatic alterations of ATM have been reported in lymphoproliferative disorders. 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). Both kinds of alteration are expected to be functionally relevant; for instance, monoallelic “impairing” mutations in ATM such as those found in cancers could 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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