LETTER TO JMG

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

P Maillet, H Bonnefoi, G Vaudan-Vutskits, B Pajk, T Cufer, W D Foulkes, P O Chappuis, A-P Sappino

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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, oculocutaneous 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.[1–5] Oncoproteins, including the tumour suppressors p53, BRCA1, and CHK2, are regulated by ATM.[6] 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.[7 8] 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.[9] In this model, up to 8% of breast cancer diagnosed in women under the age of 40 may arise in *ATM* mutation carriers, compared with 2% of cases diagnosed between 40 and 59 years. However, recent data suggest that this model may overestimate the true allele frequency in women with breast cancer.[10–12] Moreover, direct molecular examination of *ATM* in selected breast cancer patients outside AT families has led to conflicting results. Fitzgerald *et al*[13] 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, Broeks *et al*[14] identified seven germline *ATM* truncating mutations among 82 patients who developed breast cancer <45 years of age or bilateral disease. 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.[15 16] Moreover, it has been suggested that some *ATM* mutations are highly penetrant for breast cancer, such as T7271G and IVS10-6T>G.[17 18] 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: 56 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 $MgCl_2$. 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.[19]

Single strand conformation polymorphism (SSCP)/heteroduplex (HTX) analysis was performed as previously described.[20] 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 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

Type	Exon	Nucleotide change	Predicted effect	No in breast cancer cases (n=94)	No in controls (n=140)
M	15	2119T>C	S707P	3	0
M	30	IVS30–2A>G	Splicing defect	1	0
M	31	4388T>G	F1463C	1	0
UV	15	1960G>A	Q654K	1	0
UV	20	IVS20+28insA	Unknown	1	0
UV	25	IVS25–15insT	Unknown	1	0
UV	56	IVS56+23insT	Unknown	1	0
UV	59	IVS 59–20del4	Unknown	1	1
UV	63	IVS63+24delTT	Unknown	1	0
Total				11	1

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 798, p=0.0002). If only 2119T>C, IVS30–2A>G, and 4388T>C 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).
IVS refers to introns and nucleotides therein that are numbered such that the splice acceptor AG is numbered –2, –1 and the splice donor GT is numbered +1, +2.
M: mutation. UV: variant of unknown biological significance.

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 germline *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.[15 21] Dörk *et al*[16] screened a series of 1000 breast cancer patients and 500 controls for several *ATM* missense mutations and showed that the S707P mutation was significantly more frequent in the group of breast cancer patients, particularly among those who developed bilateral disease (p<0.001). The substitution of a phenylalanine for a serine amino acid at position 1463 (F1463S) is known to be deleterious to ATM function in patients with B cell non-Hodgkin's lymphomas.[22] The phenylalanine at position 1463 of the ATM protein is well conserved throughout evolution. As we found that this amino acid was substituted by a cysteine at the same protein position, we consider that the amino acid change F1463C is a pathogenic mutation. The IVS30-2A>G causes a skip of exon 31 and leads to a truncated ATM protein with a new stop codon at position 1423 (data not shown). While others have reported several truncating mutations in a similar study population,[14] we identified a single *ATM* truncating mutation and our observation corroborates previous works that showed that this type of genetic alteration is rare in patients with sporadic breast cancer.[15 21] 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.[23]

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.[24] In addition, somatic alterations of *ATM* have been reported in lymphoproliferative disorders.[22 25 26] 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).[27 28] 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.[29] 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.[17]

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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Authors' affiliations
P Maillet, G Vaudan-Vutskits, A-P Sappino, Unit of Identification of Genetic Predispositions to Cancer, Division of Oncology, Geneva University Hospital, Switzerland
H Bonnefoi, Department of Gynaecology and Obstetrics, Geneva University Hospital, Switzerland
B Pajk, T Cufer, Institute of Oncology, Ljubljana, Slovenia
W D Foulkes, Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Quebec, Canada
P O Chappuis, Divisions of Oncology and Medical Genetics, Geneva University Hospital, Switzerland

Correspondence to: Dr P Maillet, Unit of Identification of Genetic Predispositions to Cancer, CMU/Laboratory of Oncology 5216, 1 rue Michel Servet, CH-1211 Geneva 4, Switzerland; Philippe.Maillet@medecine.unige.ch

REFERENCES

1 **Savitsky K**, Sfez S, Tagle DA, Ziv Y, Sartiel A, Collins FS, Shiloh Y, Rotman G. The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. *Hum Mol Genet* 1995;**4**:2025-32.
2 **Savitsky K**, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkeazi M, Pecker I, Frydman M, Harnik R, Pantajali SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NGJ, Taylor AMR, Arlett CF, Miki T, Weissman SM, Lovett M, Collins FS, Shiloh Y. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 1995;**268**:1749-53.
3 **Uziel T**, Savitsky K, Platzer M, Ziv Y, Helbitz T, Nehls M, Boehm T, Rosenthal A, Shiloh Y, Rotman G. Genomic organization of the ATM gene. *Genomics* 1996;**33**:317-20.
4 **Platzer M**, Rotman G, Bauer D, Uziel T, Savitsky K, Bar-Shira A, Gilad S, Shiloh Y, Rosenthal A. Ataxia-telangiectasia locus: sequence analysis of 184 kb of human genomic DNA containing the entire ATM gene. *Genome Res* 1997;**7**:592-605.
5 **Kastan MB**, Lim DS. The many substrates and functions of ATM. *Nat Rev Mol Cell Biol* 2000;**1**:179-86.
6 **Khanna KK**, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat Genet* 2001;**27**:247-54.
7 **Swift M**, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia. *N Engl J Med* 1987;**316**:1289-94.
8 **Swift M**, Morrell D, Massey RB, Chase CL. Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N Engl J Med* 1991;**325**:1831-6.
9 **Easton DF**. Cancer risks in A-T heterozygotes. *Int J Radiat Biol* 1994;**66**:S177-82.
10 **Inskip HM**, Kinlen LJ, Taylor AM, Woods CG, Arlett CF. Risk of breast cancer and other cancers in heterozygotes for ataxia-telangiectasia. *Br J Cancer* 1999;**79**:1304-7.
11 **Janin N**, Andrieu N, Ossian K, Lauge A, Croquette MF, Griscelli C, Debre M, Bressac-de-Paillerets B, Aurias A, Stoppa-Lyonnet D. Breast cancer risk in ataxia telangiectasia (AT) heterozygotes: haplotype study in French AT families. *Br J Cancer* 1999;**80**:1042-5.
12 **Olsen JH**, Hahnemann JM, Borresen-Dale AL, Brondum-Nielsen K, Hammarstrom L, Kleinerman R, Kaariainen H, Lonnqvist T, Sankila R, Seersholm N, Tretli S, Yuen J, Boice JD Jr, Tucker M. Cancer in patients with ataxia-telangiectasia and in their relatives in the nordic countries. *J Natl Cancer Inst* 2001;**93**:121-7.
13 **FitzGerald MG**, Bean JM, Hegde SR, Unsal H, MacDonald DJ, Harkin DP, Finkelstein DM, Isselbacher KJ, Haber DA. Heterozygous *ATM* mutations do not contribute to early onset of breast cancer. *Nat Genet* 1997;**15**:307-10.
14 **Broeks A**, Urbanus JHM, Floore AN, Dahler E, Klijn JGM, Rutgers EJT, Devilee P, Russell NS, van Leeuwen FE, van't Veer LJ. *ATM*-heterozygous germline mutations contribute to breast cancer-susceptibility. *Am J Hum Genet* 2000;**66**:494-500.
15 **Teraoka SN**, Malone KE, Doody DR, Suter NM, Ostrander EA, Daling JR, Concannon P. Increased frequency of *ATM* mutations in breast carcinoma patients with early onset disease and positive family history. *Cancer* 2001;**92**:479-87.
16 **Dörk T**, Bendix R, Bremer M, Rades D, Klöpper K, Nicke M, Skawran B, Hector A, Yamini P, Steinmann D, Weise S, Stuhrmann M, Karstens JH. Spectrum of *ATM* gene mutations in a hospital-based series of unselected breast cancer patients. *Cancer Res* 2001;**61**:7608-15.
17 **Stankovic T**, Kidd AM, Sutcliffe A, McGuire GM, Robinson P, Weber P, Bedenham T, Bradwell AR, Easton DF, Lennox GG, Haites N, Byrd PJ, Taylor AM. *ATM* mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. *Am J Hum Genet* 1998;**62**:334-45.
18 **Chenevix-Trench G**, Spurdle AB, Gatei M, Kelly H, Marsh A, Chen X, Donn K, Cummings M, Nyholt D, Jenkins MA, Scott C, Pupo GM, Dork T, Bendix R, Kirk J, Tucker K, McCredie MR, Hopper JL, Sambrook J, Mann GJ, Khanna KK. Dominant negative *ATM* mutations in breast cancer families. *J Natl Cancer Inst* 2002;**94**:205-15.
19 **Vorechovsky I**, Rasio D, Luo L, Monaco C, Hammarström L, Webster AD, Zaloudik J, Barbanti-Brodano G, James M, Russo G, Croce CM, Negrini M. The *ATM* gene and susceptibility to breast cancer: analysis of 38 breast tumors reveals no evidence for mutation. *Cancer Res* 1996;**56**:2726-32.
20 **Maillet P**, Chappuis PO, Vaudan G, Dobbie Z, Müller H, Hutter P, Sappino AP. A polymorphism in the *ATM* gene modulates the penetrance of hereditary non-polyposis colorectal cancer. *Int J Cancer* 2000;**88**:928-31.
21 **Izatt L**, Greenman J, Hodgson S, Ellis D, Watts S, Scott G, Jacobs C, Liebmann R, Zvelebil MJ, Mathew C, Solomon E. Identification of germline missense mutations and rare allelic variants in the *ATM* gene in early-onset breast cancer. *Genes Chrom Cancer* 1999;**26**:286-94.
22 **Vorechovsky I**, Luo L, Dyer MJS, Catovsky D, Amlot PL, Yaxley JC, Foroni L, Hammarstrom L, Webster AD, Yuille MA. Clustering of missense mutations in the ataxia-telangiectasia gene in a sporadic T-cell leukaemia. *Nat Genet* 1997;**17**:96-9.
23 **Scott SP**, Bendix R, Chen P, Clark R, Dork T, Lavin MF. Missense mutations but not allelic variants alter the function of ATM by dominant interference in patients with breast cancer. *Proc Natl Acad Sci USA* 2002;**99**:925-30.
24 **Waha A**, Sturne C, Kessler A, Koch A, Kreyer E, Fimmers R, Wiestler OD, von Deimling A, Krebs D, Schmutzler RK. Expression of the *ATM* gene is significantly reduced in sporadic breast carcinomas. *Int J Cancer* 1998;**78**:306-9.
25 **Yuille MA**, Coignet LJ, Abraham SM, Yaqub F, Luo L, Matutes E, Brito-Babapulle V, Vorechovsky I, Dyer MJ, Catovsky D. *ATM* is usually rearranged in T-cell prolymphocytic leukaemia. *Oncogene* 1998;**16**:789-96.
26 **Stankovic T**, Weber P, Stewart G, Bedenham T, Murray J, Byrd PJ, Moss PA, Taylor AM. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukaemia. *Lancet* 1999;**353**:26-9.
27 **Gatti RA**, Tward A, Concannon P. Cancer risk in *ATM* heterozygotes: a model of phenotypic and mechanistic differences between missense and truncating mutations. *Mol Genet Metab* 1999;**68**:419-23.
28 **Meyn MS**. Ataxia-telangiectasia, cancer and the pathobiology of the *ATM* gene. *Clin Genet* 1999;**55**:289-304.
29 **Lavin MF**, Concannon P, Gatti RA. Eighth International Workshop on Ataxia-Telangiectasia (ATW8). *Cancer Res* 1999;**59**:3845-9.