ATM-Heterozygous Germline Mutations Contribute to Breast Cancer–Susceptibility

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Summary

Approximately 0.5%–1% of the general population has been estimated to be heterozygous for a germline mutation in the *ATM* gene. Mutations in the *ATM* gene are responsible for the autosomal recessive disorder ataxia-telangiectasia (A-T) (MIM 208900). The finding that *ATM*-heterozygotes have an increased relative risk for breast cancer was supported by some studies but not confirmed by others. In view of this discrepancy, we examined the frequency of *ATM* germline mutations in a selected group of Dutch patients with breast cancer. We have analyzed *ATM* germline mutations in normal blood lymphocytes, using the protein-truncation test followed by genomic-sequence analysis. A high percentage of *ATM* germline mutations was demonstrated among patients with sporadic breast cancer. The 82 patients included in this study had developed breast cancer at age <45 and had survived ⩾5 years (mean 15 years), and in 33 (40%) of the patients a contralateral breast tumor had been diagnosed. Among these patients we identified seven (8.5%) *ATM* germline mutations, of which five are distinct. One splice-site mutation (IVS10-6T→G) was detected three times in our series. Four heterozygous carriers were patients with bilateral breast cancer. Our results indicate that the mutations identified in this study are "A-T disease–causing" mutations that might be associated with an increased risk of breast cancer in heterozygotes. We conclude that *ATM* heterozygotes have an approximately ninefold-increased risk of developing a type of breast cancer characterized by frequent bilateral occurrence, early age at onset, and long-term survival. The specific characteristics of our population of patients may explain why such a high frequency was not found in other series.

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Introduction

Individuals with the autosomal recessive disorder ataxia-telangiectasia (A-T) (MIM 208900) are homozygous or compound heterozygous for a mutation in the *ATM* gene (Savitsky et al. 1995; Lavin and Shiloh 1997). More than 300 different disease-causing mutations, located throughout this gene's open reading frame (ORF), have been identified thus far. The disease has an estimated incidence of 1/40,000–1/100,000 live births and is characterized by several clinical features—for example, cerebellar ataxia, dilated blood vessels in the eyes and skin (telangiectasia), immune deficiency, chromosomal instability, predisposition to cancer, and increased sensitivity to ionizing radiation (Gatti et al. 1991; Segdwick and Boder 1991; Shiloh 1995; Lavin and Shiloh 1996, 1997). The typical phenotype is caused by *ATM* null alleles that either truncate (frameshift or stop-codon mutations) or severely destabilize (mutations affecting mRNA splicing) the ATM protein (Gilad et al. 1998; Sandoval et al. 1999; Teraoka et al. 1999).

A number of epidemiological studies have indicated that female *ATM*-heterozygous carriers have an excess risk of breast cancer, with an estimated relative risk of 3.9 (95% confidence interval [CI] 2.0–6.0) (Swift et al. 1987, 1991; reviewed in Easton 1994; Inskip et al. 1999; Janin et al. 1999). Several recent studies have been conducted to verify this hypothesis. Athma et al.'s (1996) study of families with A-T showed that female *ATM*-mutation carriers have an increased relative risk for breast cancer: 3.8. In spite of that, studies of patients with breast cancer have so far failed to confirm the findings in families with A-T. Fitzgerald et al. (1997), analyzing a series of young patients with breast cancer, suggested that *ATM*-heterozygous germline mutations do not contribute to early-onset (age <40 years) sporadic breast cancer. Moreover, in a third recent report, Chen et al. (1998) conclude that the contribution of heterozygous *ATM* mutations to familial breast cancer is minimal.

In the present study we examined whether *ATM* germline mutations can be found in a selected group of Dutch

patients with breast cancer. We determined the *ATM* mutation frequency in women who had developed breast cancer at a young age. Using the protein-truncation test (PTT) as the mutation-detection method, which was followed by genomic sequencing, we expected to have a mutation-detection sensitivity of ~75% (Broeks et al. 1998). We had reported elsewhere that there was no common *ATM* founder mutation present in a small series of 12 Dutch patients with A-T and that the mutations had been found throughout the ORF of the *ATM* gene, with no apparent hotspots.

Subjects and Methods

Study Population

The patients in this study were selected from a large cohort of patients with breast cancer who had been treated at either The Netherlands Cancer Institute (Amsterdam) or The Dr. Daniel den Hoed Cancer Center (Rotterdam), during 1970–90. Patients with contralateral breast cancer ($n = 33$) were eligible if their first breast cancer had been diagnosed at age <45 and ⩾5 years before the diagnosis of contralateral disease. Patients with unilateral disease ($n = 49$) also had to have been diagnosed with breast cancer at age <45 and had to have survived ⩾5 years without developing contralateral disease. On the basis of their birth names, all 82 patients were inferred to be of Dutch ethnic origin. Information regarding a family history of breast cancer was obtained by means of a questionnaire mailed to all patients. The extent of family history of breast cancer was divided into three categories (one case–i.e., the index case—had to be diagnosed at age <45, paternal inheritance was considered to be first degree): (1) hereditary—three family members with breast cancer in two generations, who were first-degree relatives of each other; (2) sporadic—either a single breast cancer case or only one second-degree relative with breast cancer; and (3) familial—all others. Each of these patients was asked to donate a 20-ml blood sample for DNA and RNA isolation, and informed consent was obtained. To determine the frequency of specific *ATM* mutations in the Dutch population, DNA samples from 268 healthy individuals of Dutch ethnic origin (179 males and 89 females of which 51 were age <45 years) were obtained from the Blood Bank at Leiden.

Generation of Genomic DNA and Total RNA and Preparation of cDNA

Peripheral blood lymphocytes were used to isolate genomic DNA and total RNA, with DNAzol (Gibco) and RNAzol B (Ampro) methods, respectively, according to the manufacturers' protocol. A 25–1.25-μg sample of total RNA was used in the cDNA reaction, which was conducted by means of a Superscript II kit, according to the manufacturer's (Gibco BRL) protocol.

PTT

The ATM ORF was divided into 10 overlapping fragments of ~1–2 kb each, with an overlap of 400–500 bp to avoid the possibility that truncations close to the ends of the fragments could be missed. Forward primers all include a T7-promoter sequence for the transcriptional initiation of T7 RNA polymerase and an ATG codon for translational initiation. Nested PCR was necessary only for amplification of region I. Primer sequences are available on request.

The PCR of each region was performed in a total volume of 30 μl containing 1 μl of cDNA template, 3 μl PCR buffer 6 (Stratagene), 0.2 mM of each dNTP, 15 pmol of each (forward and reverse) primer, 1.5 μl of dimethyl sulfoxide, 1 unit of *Taq* DNA-polymerase (Gibco BRL), and 0.25 units of *PWO* DNA-polymerase (Boehringer). The PCR regions were amplified in a PTC200 apparatus (MJ Research) for 1 min at 94°C (for denaturing); 35 cycles of 10 s at 92°C, 1 s at 70°C (with a decrease of 0.5°C/s until 55°C was attained), 10 s at 55°C (with an increase of 0.7°C/s until 72°C was attained), 2.5 min at 72°C; and, finally, 5 min at 72°C. The outer PCR of region I was performed in 15 cycles in a total volume of 30 μl, under the same conditions as described above, and 1 μl of the product of the first PCR was used for the nested inner PCR. PCR products were analyzed by agarose gel-electrophoresis.

From each sample, 2 μl of PCR product was used for the in vitro transcription-translation reaction using the TNT T7 Coupled Reticulocyte Lysate System (L4610; Promega). The reaction was performed according to protocol in a 6.25-μl total volume containing 2 μl of PCR product, 3.1 μl of rabbit reticulocyte lysate, 0.25 μl of TNT-buffer, 0.125 μl of amino acid mixture (minus methionine), 0.5 μl of [^{35}S]-methionine (10 mCi/ml), 0.125 μl (40 units/μl) of RNAse inhibitor, and 0.125 μl of T7 RNA polymerase. The reaction mixture was incubated for 60 min at 30°C. The translation products were separated on a 15% SDS-polyacrylamide gel, and the gels were fixed, incubated in fluorographic reagent (Amplify; Amersham), dried, and subjected to autoradiography. Each gel contained a ^{14}C-labeled high-molecular-weight rainbow marker (Amersham) as a size standard.

Nucleotide-Sequence Analysis

All aberrant PTT patterns were analyzed by sequence analysis, first on cDNA and finally on genomic DNA, to identify candidate mutations. Sequence analysis was performed by means of the "DNA Sequencing Kit" bigdye primer cycle sequencing ready reaction with 21M13 and M13 reverse primers (PE Biosystems). Sequencing

products were analyzed with the ABI 377 automated sequencing apparatus and corresponding software.

IVS10-6T→G Mutation–Specific Assay

A 100- ng sample of genomic DNA was amplified, in a total volume of 30 μl containing 3 μl of PCR buffer with 3 mM $MgCl_2$, 15 pmol of each primer, an intron 10 forward primer (IVS10-146: 5′-ctg agg cag gag aat cac ttg) and an exon 11 reverse primer (nucleotide 1213: 5′-tct gtg act tct gaa ggt gat c), 0.7 μl of Ampli*Taq* gold, and 3 μl (2 mM each) of each dNTP, in the PTC200 (MJ research) apparatus, for 6 min at 94°C; 20 cycles of 30 s at 94°C, 20 s at 65°C (with a decrease of 0.5°C/s/cycle until 55°C was attained), 2.5 min at 72°C, followed by 20 cycles annealing at 55°C; and, finally, 5 s at 72°C). PCR fragments were digested with 5 units of *Rsa*I for 2 h at 37°C and were analysed on a 2% agarose gel.

IVS10-6T→G Splicing

PCR with forward primer FSQ1 (nucleotide −2: 5′-gtg tgt tct gaa att gtg aac) and reverse primer RSQ2 (nucleotide 1370: 5′-cgt aac aca tat ggt gta cg) resulted in two fragments—one including exon 11 and the other excluding it—of 1,373 and 1,203 bp, respectively. These products were separated on a 1.2% agarose gel and were isolated from gel. The products were individually sequenced, and a polymorphism at nucleotide position 37, 37C/T, was used as an allele-specific marker; nucleotide 37C is linked to the normal sequence IVS10-6T, whereas nucleotide 37T is linked to the mutation IVS10-6G.

Tumor-Tissue Histology and Immunohistochemistry

For each paraffin-embedded tumor, six 10-μm paraffin slides were used for genomic-DNA isolation (whereas three 10-μm paraffin slides are used in the standard protocol [Sambrook et al. 1989]), and an hematoxylin-eosin–stained slide was used for histopathological examination.

Loss of Heterozygosity (LOH)

LOH of the microsatellite marker D11S2179 (located in *ATM*) in tumor material was determined by comparison of peak-height ratios of normal tissue with those of tumor tissue. The peak height of the longer allele peak was divided by the peak height of the shorter allele peak. The ratio obtained in the tumor DNA was divided by the ratio obtained in normal-tissue DNA. LOH was determined by ratios >2 or <0.5, depending on which allele was lost. Microsatellite analysis was performed by PCR in a total volume of 30 μl containing 5–10 ng of genomic DNA, 3 μl PCR buffer 6 (Stratagene), 0.2 mM of each dNTP, 9 pmol of each primer (listed in The Genome Database), and 1.5 units of *Taq* polymerase, in a multicycler PTC200 apparatus (MJ Research). The PCR products were amplified in 30 cycles of 30 s for 94°C, 1 min at 62°C, and 1 min at 72°C; were electrophoresed on an ABI 373 or 377 automated sequencing apparatus; and were analysed by means of GENESCAN 2.1. Subsequently, sequence analysis of the tumor DNA was used to determine whether the wild-type or mutant allele was lost.

Results

Seven ATM Germline Mutations in 82 Selected Patients with Breast Cancer

Among 82 patients with sporadic breast cancer, we have identified in 7 (8.5%) *ATM* germline mutations, of which five are distinct (tables 1 and 2); these mutations either already had been described as A-T disease causing mutations elsewhere (Ataxia-Telangiectasia Mutation Database; T. Dörk, personal communication), as causing the disease in patients with A-T or are truncating mutations (table 2; patients ATMb56 and ATMb73) and therefore most likely are pathological mutations.

All patients with breast cancer who were included in our study were diagnosed with breast cancer at age <45 and had survived for 5 years after diagnosis (mean duration of follow-up was 15 years), and in 33 (40%) of these patients a contralateral breast tumor was diagnosed ⩾5 years after the first diagnosis of breast cancer. The proportion of women in the *ATM*-mutated group who had hereditary or familial versus sporadic breast cancer were roughly similar to those in the group of women who did not have an *ATM* germline mutation (table 3A). Additional characteristics of these patients and their tumors are listed in table 3. Patients with an *ATM* germline mutation were slightly older at the first diagnosis of their first breast cancer and had a slightly longer follow-up period, and a higher proportion of them had bilateral disease (tables 1 and 3A). However, none of these differences was statistically significant, possibly because of the small number of patients. Furthermore, no specific clinicopathological characteristics (e.g., histology or tumor stage) of *ATM*-heterozygous patients were apparent (table 3B).

Table 1

Frequency of All *ATM* Germline Mutations and of the *ATM* IVS10-6T→G Splice-site Mutation in Patients and Controls

Group	All *ATM* Mutations	*ATM* IVS10-6T→G
Breast cancer at age <45 years:		
Unilateral	3/49	2/49
Bilateral	4/33	1/33
Total	7/82 (8.5%)	3/82 (3.7%)
Controls	.5%–1.0%[a]	2/268 (.7%)

[a] Data are from the literature.

Table 2

Seven *ATM* Germline Mutations in Patients With Breast Cancer at Age <45 Years

Patient	Genomic Mutation[a]	Predicted Alteration in ATM Protein[b]
Bilateral breast cancer:		
ATMb56	3114A→T (exon 23)	Stop codon, truncation at amino acid 1039
ATMb57	IVS10-6T→G (exon 11)	Out-of-frame deletion, truncation at amino acid 419
ATMb58	IVS14+2T→G (exon 14)	In-frame deletion codons 601-633
Unilateral breast cancer:		
ATMb73	1660delA (exon 13)	Truncation at amino acid 555
ATMc211	1562delAG (exon 12)	Truncation at amino acid 564
ATMc214	IVS10-6T→G (exon 11)	Out-of-frame deletion, truncation at amino acid 419
ATMc232	IVS10-6T→G (exon 11)	Out-of-frame deletion, truncation at amino acid 419

[a] Nucleotide positions indicated are numbered in accordance with reference sequence ATG = 1 (Platzer et al. 1997).

[b] Length is 3,056 amino acids.

ATM Germline Mutation IVS10-6T→G

The splice-site mutation IVS10-6T→G, localized in the highly conserved pyrimidine tract of the 3′ splice-acceptor site of intron 10, was detected in three unrelated patients with breast cancer in this study (3.7% of the sample; see table 1). We assessed the frequency of this particular mutation in the general population, using a mutation-specific assay (see the Subjects and Methods section). In the general population, we detected this mutation in 2 (both males) of 268 healthy controls, which results in an estimated frequency of ~0.7% (95% CI 0%–1.7%) for this mutation alone. Haplotype analysis of the five individuals with this mutation, using seven microsatellite markers located in the *ATM* region (Laake et al. 1999), revealed that the *ATM* IVS10-6T→G mutation is present on at least two different alleles, each sharing a different set of at least three of these seven markers (authors' unpublished observations).

The IVS10-6T→G mutation leads to incorrect splicing of exon 11 and to exon skipping, resulting in a frameshift and subsequent truncation of the protein at amino acid residue 419. Assaying for a mutation-linked polymorphism at nucleotide position 37, we determined that a small proportion (<10%) of the mutant allele is spliced correctly, resulting in some full-length mRNA (data not shown). The wild-type allele did not display any incorrect splicing.

LOH: Not a Prerequisite for Initiation of Breast Cancer in ATM Heterozygotes

To obtain further insight into the precise role of ATM in tumorigenesis in *ATM*-heterozygous patients, we investigated whether the loss of the wild-type *ATM* allele in these tumors was a prerequisite for tumor development. We analyzed LOH of the *ATM* locus—that is, chromosomal region 11q22-23—in four breast tumors in three *ATM* heterozygotes, using the microsatellite marker D11S2179, located within the *ATM* gene. One patient (ATMb56) with bilateral breast cancer showed LOH in the first tumor (data not shown); however, sequence analysis revealed that it was the mutant allele that was lost in the tumor—in, remarkably, only a proportion (~50%) of the tumor cells. Two other informative tumors, one each in patients ATMb57 and ATMb58, showed no LOH.

Discussion

We have identified a high percentage of *ATM* germline mutations among selected patients with breast cancer. The mutations that are described in this study are most likely *ATM* null alleles, since most of them either truncate the ATM protein or have been described, elsewhere (Ataxia-Telangiectasia Mutation Database; T. Dörk, personal communication), as occurring in patients who are homozygous or compound heterozygous for A-T. Our results do not show an overrepresentation of hereditary breast cancer among patients with breast cancer and heterozygous *ATM* germline mutations, thereby excluding the likelihood of the presence of a breast cancer–susceptibility gene, such as BRCA1 or BRCA2, that would account for predisposition to breast cancer.

One mutation, the exon 11 splice-site mutation IVS10-6T→G, was found frequently among the patients with breast cancer in this study (frequency 3.7%, vs. 0.7% in the general population). Despite this high frequency in the Dutch population, the IVS10-6T→G mutation has not been detected thus far in a small series of Dutch patients with A-T (Broeks et al. 1998). However, a patient with A-T who was homozygous for the exon 11 splice-site mutation has been identified in Germany (T. Dörk, personal communication). Our data suggest that this particular *ATM* mutation might be associated with an increased risk of breast cancer in *ATM* heterozygotes.

None of the tumors of the *ATM* heterozygotes tested for LOH showed LOH of the wild-type allele. Thus, it seems that a mutant *ATM* germline allele plays a role in tumor initiation but that complete loss of the normal

Table 3

Patient and Histopathological Characteristics of All Patients with Breast Cancer and of *ATM* Heterozygotes

A. All Patients with Breast Cancer

Characteristic	Patients without *ATM* Mutation (n = 75)	Patients with *ATM* Mutation (n = 7)
Mean age [range] at diagnosis (years):		
First breast cancer	38.0 (22.1–45.0)	40.5 (35.7–43.3)
Second breast cancer (n = 33)	46.3 (27.3–55.7)	50.6 (43.7–59.3)
Interval [range] between diagnosis of first tumor and diagnosis of second tumor (years)	9.9 (4.6–19.9)	11.0 (6.8–19.6)
Mean follow-up period [range] since diagnosis (years):		
First tumor	15.0 (7.5–25.7)	16.8 (13.9–24.1)
Second tumor (n = 33])	5.3 (.7–16.8)	6.4 (4.4–8.4)
Type of breast cancer in family:[a]		
Hereditary	10%	14%
Familial	16%	14%
Sporadic	74%	72%

B. *ATM* Heterozygotes

Patient (Mutation) and Diagnosis of Breast Cancer	Age (years)	Histology	TNM Stage	Interval (years)	Follow-Up Period since First Diagnosis of Breast Cancer
ATMb56 (3114A→T):					
First diagnosis	40	Ductal carcinoma in situ	$T_0N_0M_0$		14.3
Second diagnosis	46	Infiltrating ductal carcinoma/ductal carcinoma in situ	$T_2N_1M_0$	6.8	
ATMb57 (IVS10-6T→G):					
First diagnosis	39	Ductal/lobular carcinoma in situ	$T_0N_0M_0$		24.1
Second diagnosis	59	Ductal carcinoma in situ	$T_0N_0M_0$	19.6	
ATMb58 (IVS14+2T→G):					
First diagnosis	35	Infiltrating ductal carcinoma	$T_1N_0M_0$		16.4
Second diagnosis	43	Infiltrating ductal carcinoma	$T_2N_0M_0$	8	
ATMb73 (1660delA):					
First diagnosis	42	Infiltrating ductal carcinoma	$T_2N_1M_0$		14.8
Second diagnosis	52	Infiltrating ductal carcinoma	$T_1N_0M_0$	9.7	
ATMc211 (1562delAG):					
First diagnosis	39	Infiltrating lobular carcinoma	$T_1N_0M_0$		17.8
ATMc214 (IVS10-6T→G):					
First diagnosis	43	Infiltrating ductal carcinoma	$T_1N_1M_0$		13.9
ATMc232 (IVS10-6T→G):					
First diagnosis	42	Infiltrating ductal carcinoma	$T_2N_xM_0$		16.2

[a] For category descriptions, see the Subjects and Methods section. Data were available for 7 mutation carriers and for 70 non– mutation carriers.

protein is not a prerequisite for tumor initiation. Loss of the mutant allele, as was found in patient ATMb56, could be explained by LOH directed by a locus in close proximity to *ATM*, which would be important at a later stage during tumor development (Laake et al. 1997, 1999).

Our results support the hypothesis that *ATM* heterozygotes do have an increased risk of development of breast cancer. The specific characteristics of our the patients whom we studied (i.e., early age at onset, frequent occurrence of contralateral disease, and long-term survival) may explain why such a high frequency of *ATM* germline mutations was not found in other studies of patients with breast cancer (Fitzgerald et al. 1997; Chen et al. 1998). For a precise assessment of their risk, data are needed on the prevalence of *ATM*-heterozygous mutations in a large group of women without breast cancer. In the absence of such data, we have used the commonly accepted upper estimate of 1% heterozygotes in the Dutch population (based on an upper estimate of 1/40,000 A-T live births in The Netherlands; Jaspers, personal communication) to estimate that *ATM* heterozygotes have an approximately ninefold-increased risk, compared with nonheterozygotes, of developing a breast tumor with specific characteristics (early age at onset, increased risk of contralateral disease, and long-term survival).

It is well known that A-T is associated with high sensitivity to ionizing radiation and that fibroblast strains or cultures from *ATM* heterozygotes display moderate levels of radiation sensitivity; on average, they are more sensitive than are normal controls (Paterson et al. 1979; West et al. 1995). All patients with breast cancer who were included in the present study received low- dosage

diagnostic radiation at a young age, as part of a screening program for tuberculosis. Moreover, all these patients had received radiation treatment for their first breast tumor, and a nonsignificant trend toward a higher incidence of *ATM* mutations was noted in women with bilateral breast cancer. Thus, radiation might indeed be an induction trigger for development of breast cancer in *ATM* heterozygotes, and these data strongly suggest that the role of *ATM* heterozygosity in the pathogenesis of radiogenic cancers needs further investigation. Although our findings indicate an approximately ninefold-increased risk of heterozygous *ATM* mutation carriers for breast cancer, we did not observe an extended family history of breast cancer in these patients. However, since an induction trigger might be a prerequisite for tumor development, their relatives might not have been exposed to such an environmental factor, as has been suggested as well by a study of the incidence of breast cancer in female relatives with A-T (Swift et al. 1991 Inskip et al. 1999). We hope that in the near future we can provide more insight into the quantitative contribution of *ATM* heterozygosity to the risk of radiation-related cancers.

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Electronic-Database Information

The accession number and URL for data in this article are as follows:

Ataxia-Telangiectasia Mutation Database, http://www.vmresearch.org/atm.htm

Genome Database, The, http://www.gdb.org

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for A-T [MIM 208900])

References

Athma P, Rappaport R, Swift M (1996) Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. Cancer Genet Cytogenet 92: 130–134

Broeks A, de Klein A, Floore AN, Muijtjens M, Kleijer WJ, Jaspers NG, van 't Veer LJ (1998) *ATM* germline mutations in classical ataxia-telangiectasia patients in the Dutch population. Hum Mut 12:330–337

Chen J, Birkholtz GG, Lindblom P, Rubio C, Lindblom A (1998) The role of ataxia-telangiectasia heterozygotes in familial breast cancer. Cancer Res 58:1376–1379

Easton DF (1994) Cancer risks in A-T heterozygotes. Int J Radiat Biol Suppl 66:S177–S182

FitzGerald MG, Bean JM, Hegde SR, Unsal H, MacDonald DJ, Harkin DP, Finkelstein DM, et al (1997) Heterozygous *ATM* mutations do not contribute to early onset of breast cancer. Nat Genet 15:307–310

Gatti RA, Boder E, Vinters HV, Sparkers RS, Norman A, Lange K (1991) Ataxia-telangiectasia: an interdisciplinary approach to pathogenesis. Medicine 70:99–117

Gilad S, Chessa L, Khosravi R, Russell P, Galanty Y, Piane M, Gatti RA, et al (1998) Genotype-phenotype relationships in ataxia-telangiectasia and variants. Am J Hum Genet 62: 551–561

Inskip HM, Kinlen LJ, Taylor AMR, Woods CG, Arlett CF (1999) Risk of breast cancer and other cancers in heterozygotes for ataxia-telangiectasia. Br J Cancer 79:1304–1307

Janin N, Andrieu N, Ossian K, Laugé A, Croquette MF, Griscelli C, Debré M, et al (1999) Breast cancer risk in ataxia-telangiectasia (A-T) heterozygotes: haplotype study in French A-T families. Br J Cancer 80:1042–1045

Laake K, Launonen V, Niederacher D, Gudlaugsdottir S, Seitz S, Rio P, Champème MH, et al (1999) Loss of heterozygosity at 11q23.1 and survival in breast cancer: results of a large European study. Genes Chromosom Cancer 25:212–221

Laake K, Odegard A, Andersen TI, Bukholm IK, Karesen R, Nesland JM, Ottestad L, et al (1997) Loss of heterozygosity at 11q23.1 in breast carcinomas: indication for involvement of a gene distal and close to *ATM*. Genes Chromosom Cancer 18:175–180

Lavin MF, Shiloh Y (1996) Ataxia-telangiectasia: a multifaceted genetic disorder associated with defective signal transduction. Curr Opin Immunol 8:459–464

Lavin MF, Shiloh Y (1997) The genetic defect in ataxia-telangiectasia. Annu Rev Immunol 15:177–202

Paterson MC, Anderson AK, Smith BP, Smith PJ (1979) Enhanced radiosensitivity of cultured fibroblasts from ataxia-telangiectasia heterozygotes manifested by defective colony-forming ability and reduced DNA repair replication after hypoxic gamma irradiation. Cancer Res 39:3725–3734

Platzer M, Rotman G, Bauer D, Uziel T, Savitsky K, Bar-Shira A, Gilad S, et al (1997) Ataxia-telangiectasia locus: sequence analysis of 184 kb of human genomic DNA containing the entire *ATM* gene. Genome Res 7:592–605

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manuel, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sandoval N, Platzer M, Rosenthal A, Dörk T, Bendix R, Skawran B, Stuhrmann M, et al (1999) Characterization of *ATM* gene mutations in 66 ataxia-telangiectasia families. Hum Mol Genet 8:69–79

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, et al (1995) A single ataxia-telangiectasia gene with a product similar to PI-3 kinase. Science 268: 1749–1753

Segdwick RP, Boder E (1991) Ataxia-telangiectasia. In: Vianney De Jong JMB (ed) Hereditary neuropathies and spinocerebellar atrophies. Elsevier Science, Amsterdam, pp 347–423

Shiloh Y (1995) Ataxia-telangiectasia: closer to unravelling the mystery. Eur J Hum Genet 3:116–138

Swift M, Morell D, Massey RB, Chase CL (1991) Incidence of cancer in 161 families affected by ataxia-telangiectasia N Engl J Med 325:1831–1836

Swift M, Reitnauer PJ, Morell D, Chase CL (1987) Breast and other cancers in families with ataxia-telangiectasia. N Engl J Med 316:1289–1294

Teraoka SN, Telatar M, Becker-Cantania S, Liang T, Onengüt S, Tolun A, Chessa L, et al (1999) Splicing defects in the Ataxia-telangiectasia gene, *ATM:* underlying mutations and consequences. Am J Hum Genet 64:1617–1631

West CM, Elyan SA, Berry P, Cowan R, Scott D (1995) A comparison of the radiosensitivity of lymphocytes from normal donors, cancer patients, individuals with ataxia-telangiectasia (A-T) and A-T heterozygotes. Int J Radiat Biol 68: 197–203