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Genotype-Phenotype Relationships in Ataxia-Telangiectasia and Variants

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Summary

Ataxia-telangiectasia (A-T) is an autosomal recessive disorder characterized by cerebellar degeneration, immunodeficiency, chromosomal instability, radiosensitivity, and cancer predisposition. A-T cells are sensitive to ionizing radiation and radiomimetic chemicals and fail to activate cell-cycle checkpoints after treatment with these agents. The responsible gene, ATM, encodes a large protein kinase with a phosphatidylinositol 3-kinase–like domain. The typical A-T phenotype is caused, in most cases, by null ATM alleles that truncate or severely destabilize the ATM protein. Rare patients with milder manifestations of the clinical or cellular characteristics of the disease have been reported and have been designated "A-T variants." A special variant form of A-T is A-T_{Fresno}, which combines a typical A-T phenotype with microcephaly and mental retardation. The possible association of these syndromes with ATM is both important for understanding their molecular basis and essential for counseling and diagnostic purposes. We quantified ATM-protein levels in six A-T variants, and we searched their ATM genes for mutations. Cell lines from these patients exhibited considerable variability in radiosensitivity while showing the typical radioresistant DNA synthesis of A-T cells. Unlike classical A-T patients, these patients exhibited 1%–17% of the normal level of ATM. The underlying ATM genotypes were either homozygous for mutations expected to produce mild phenotypes or compound heterozygotes for a mild and a severe mutation. An A-T_{Fresno} cell line was found devoid of the ATM protein and homozygous for a severe ATM mutation. We conclude that certain "A-T variant" phenotypes represent ATM mutations, including some of those without telangiectasia. Our findings extend the range of phenotypes associated with ATM mutations.

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Introduction

Ataxia-telangiectasia (A-T; MIM 208900) is a complex, multisystem disorder inherited in an autosomal recessive manner. The major manifestations of A-T are progressive cerebellar degeneration leading to severe neuromotor dysfunction, oculocutaneous telangiectasia, profound immunodeficiency of both humoral and cellular compartments, gonadal dysgenesis, growth retardation in some patients, high levels of serum alpha-fetoprotein, predisposition to malignancies (primarily lymphoreticular), and acute radiosensitivity. A-T cells show chromosomal instability, premature senescence, accelerated telomere shortening, sensitivity to the cytotoxic and clastogenic effects of ionizing radiation and radiomimetic chemicals, and defective activation of cell-cycle checkpoints by these agents (Sedgwick and Boder 1991; Shiloh 1995, 1997; Gatti 1996; Lavin and Shiloh 1997).

The responsible gene, ATM, has been mapped to chromosome 11q22-23 (Gatti et al. 1988; Lange et al. 1995) and subsequently has been identified by use of a positional cloning approach (Savitsky et al. 1995*a,* 1995*b;* GenBank accession numbers U67092 and U67093 [http://www.ncbi.nlm.nih.gov/]). This gene occupies 150 kb of genomic DNA structured into 66 exons that encode a 13-kb transcript (Savitsky et al. 1995*b,* 1997; Uziel et al. 1996). The ATM protein is a large, 370-kD phosphoprotein localized mainly in the nucleus (Chen and Lee 1996; Lakin et al. 1996; Brown et al. 1997; Watters et al. 1997). ATM is a member of a family of proteins, identified in various organisms, that share a carboxy-terminal region similar to the catalytic subunit of phosphatidylinositol 3-kinases (PI 3-kinases). These proteins are involved in cellular responses to DNA damage, and some of them play regulatory roles in cell-cycle progression or damage-induced cell-cycle checkpoints. Mutations in some of the corresponding genes lead to genomic instability and sensitivity to DNA damaging agents (reviewed by Savitsky et al. 1995*b;* Jorgensen and Shiloh 1996;Shiloh and Rotman 1996 Hoekstra 1997; Lavin and Shiloh 1997;). Evidence is accumulating that ATM indeed plays an important role in several signal-transduction pathways associated with stress responses

or normal cellular growth (Hoekstra 1997; Khanna et al. 1997; Rotman and Shiloh, in press).

Extensive screening of the ATM gene or transcript in A-T patients has revealed a large variety of mutations, most of which are unique to single families. The great majority of these mutations are expected to completely inactivate the ATM protein, mostly by truncations. The A-T phenotype is thus determined by homozygosity or compound heterozygosity for ATM null alleles (Baumer et al. 1996; Byrd et al. 1996; Gilad et al. 1996*a*, 1996*b*, and in press; Telatar et al. 1996; Vorechovsky et al. 1996; Wright et al. 1996; Concannon and Gatti, in press).

Despite some variability in several features of the disease, patients with the classical A-T phenotype usually have been grouped together and have not been subdivided into clinical subtypes. This approach has been retrospectively supported by the uniform nature of ATM mutations underlying classical A-T, which did not lead to the establishment of genotype-phenotype relationships within this group. Occasionally, however, milder cases of the disease—"A-T variants"—have been reported. Such patients may show either later age at onset or more-moderate severity of the ataxia, intermediate cellular radiosensitivity, and longer life span than is seen in most A-T patients (Stankler and Bennett 1973; Ying and Docteau 1981; Fiorilli et al. 1984; Taylor et al. 1987, 1993; de Jong and Tijssen 1988; Maserati et al. 1988; Ziv et al. 1989; Chessa et al. 1992; Lanzi et al. 1992; Sanal et al. 1993; Willems et al. 1993; Antoccia et al. 1995).

In addition to variants exhibiting milder forms of A-T, other syndromes have been reported that include some A-T signs combined with other clinical features (reviewed by Taylor et al. [1993] and Bundey [1994]). Most notable is the Nijmegen breakage syndrome (NBS), which combines chromosomal instability, cancer predisposition, and the typical A-T cellular phenotype with microcephaly and, sometimes, mental retardation (Weemaes et al. 1994; Wegner et al., in press). NBS patients do not show ataxia or telangiectasia, and the responsible gene is separate from ATM (Stumm et al. 1995; Gatti 1996; Saar et al. 1997). Curry et al. (1989) identified in identical twin girls a rare combination of the classical A-T phenotype with microcephaly and mental retardation; they designated this syndrome "AT_{Fresno}." This syndrome segregates with 11q22-23 markers (Gatti 1996). We have attempted to determine whether this phenotypic variation correlates with ATM mutations and levels of ATM protein expression. The clinical and cellular characteristics, ATM protein levels, and underlying ATM mutations were examined in a series of mild A-T variants, and a search for ATM mutations was performed on a cell line derived from one of the AT_{Fresno} sisters.

Material and Methods

Cell Lines

Lymphoblastoid cell lines (LCLs) were established from peripheral blood lymphocytes of patients, as described elsewhere (Littlefield et al. 1981). The normal lymphoblast line GM02184 was obtained from the National Institute of General Medical Sciences' Human Mutant Cell Repository (Corriell Institute for Medical Research, Camden, NJ). Lymphoblasts were grown in RPMI 1640 medium, and fibroblasts were grown in Dulbecco's modified Eagle's medium, each supplemented with 15% fetal bovine serum (Beit Ha'Emek Biological Industries and Gibco BRL).

Cellular Sensitivity to Ionizing Radiation

LCLs were seeded at a density of 2×10^5 cells/ml; were irradiated with various doses of gamma rays, by use of a J.L. Shepherd Mark I cesium-137 irradiator; and were returned to the incubator. Cell density was measured daily for 7 d after irradiation, and cell growth was assessed relative to an unirradiated culture, for each cell line. Relative cell growth was plotted as a function of treatment dose, and a sensitivity score was calculated for each patient's cell line, on the basis of the ratio between the dose leading to 50% growth inhibition in that cell line and the dose leading to 50% growth inhibition in control cells.

Radioresistant DNA Synthesis (RDS)

The cells were prelabeled with [2-^{14}C]thymidine and were irradiated with various doses of gamma rays (for experimental details, see Ziv et al. 1995). The rate of DNA synthesis after irradiation was quantified by measuring the incorporation of tritiated thymidine into chromosomal DNA during 1 h, beginning 1 h after irradiation.

Western Blotting Analysis of the ATM Protein

Cellular extracts were fractionated by PAGE and were transferred to nitrocellulose membranes, as described by Gilad et al. (1996*a*) and Ziv et al. (1997). The ATM protein was detected with a polyclonal antiserum (X2361) raised against a recombinant protein spanning amino acids 2361–2547 of the ATM protein. An anti-actin antibody (Sigma) was used to quantitate variations in protein amounts loaded in different lanes. Immunoreactive bands were visualized by use of the Super Signal system (Pierce), and the signals were quantitated by densitometry using the BioImaging Densitometry system 202D (Dinco and Renium).

Table 1

Phenotypic Characterization of Six A-T Variants

Clinical/Laboratory Feature	AT53RM	AT9RM[a]	AT31RM[b]	A(-T)2RM[bc]	AT42RM	AT35RM
Age (years)	12	20	38	22	33	31
Sex	F	F	F	F	M	F
Telangiectasia	+	+	+	−	+	+
Age at onset of ataxia (years)	9	4	2	2	1.5	4
Choreoathetosis	+	+	+	+	+	+
Ocular apraxia	+	+	+	+	+	+
Nystagmus	+	+	+	+	+	+
Recurrent infections	−	−	−	−	−	−
Immunodeficiency	+	+	−	+[c]	−	−
Increased alpha-fetoprotein	+	+	+	+	+	+
Malignancy	−	−	−	−	−	−
Chromosomal instability	+	+	NT[d]	+	+	+
Clonal translocations	+	+	NT[d]	−	+	+

[a] This patient and her affected sister have been described in detail by Chessa et al. (1992) and Antoccia et al. (1995).

[b] NT = not tested.

[c] For detailed phenotypic description, see the work of Maserati et al. (1988) and Lanzi et al. (1992).

[d] Reduced IgE and normal IgA.

Search for ATM Mutations

The ATM transcript was screened for mutations by use of reverse transcriptase–PCR (RT-PCR) followed by restriction-endonuclease fingerprinting. Suspect regions of the ATM transcript were sequenced to identify specific mutations. This protocol has recently been described in detail by Gilad et al. (1998).

Results

Definition of the Variant Phenotype

There is no uniform definition of the A-T variant phenotype. Six mild A-T variants examined in this study manifested most or all of the hallmarks of the A-T phenotype, but each one deviated from classical A-T in at least one phenotypic feature (table 1); This deviation could be either a later age at onset and slower progression of the neurological signs of the disease (patient AT53RM), reduced radiosensitivity (patient AT9RM [Chessa et al. 1992]), absence of telangiectasia (patient A[-T]2RM), or longer life span (patients AT31RM, AT42RM, and AT35RM). All these patients are Italian.

Cellular Responses to Ionizing Radiation

Two of the hallmarks of the A-T cellular phenotype are increased sensitivity to the cytotoxic effect of ionizing radiation and reduced inhibition of semiconservative DNA synthesis after radiation treatment (reviewed by Thacker [1994] and Shiloh [1995]). The latter phenomenon, termed "radioresistant DNA synthesis" (RDS) (Houldsworth and Lavin 1980; Painter and Young 1980), represents a defect in the induction of an S-phase checkpoint by radiation damage.

The common assay of cellular survival after treatment with DNA-damaging agents measures clonogenic growth of the cells. In view of the difficulty in the application of this assay to newly established LCLs, their radiosensitivity was assessed by means of a growth-inhibition assay. Ionizing radiation inhibited cellular growth in the cell lines of all six patients, to a greater extent than it did in the control cell line, although the response was variable. In their postirradiation growth pattern, some of the patients' cell lines resembled typical A-T cells, whereas others showed higher or lower sensitivities (fig. 1 and table 2). Variable extents of RDS were also observed, ranging from above to below the values for the typical A-T cell lines (fig. 2 and table 2). RDS and radiosensitivity were not correlated, but the small sample size does not allow statistically significant analysis.

ATM-Protein Level

Western blot analysis and densitometry were used to assess the levels of ATM protein in the cell lines. The readings of the ATM signals were normalized against the actin readings (fig. 3). Whereas two classical A-T cells typically showed no immunoreactive signal with an ATM antiserum, the variant cell lines exhibited variable levels of the ATM protein, in the range of ~1%–17% of the normal level (fig. 3 and table 2). Notably, there was no apparent correlation between ATM-protein levels and the two parameters of the cellular phenotype—radiosensitivity and RDS (table 2).

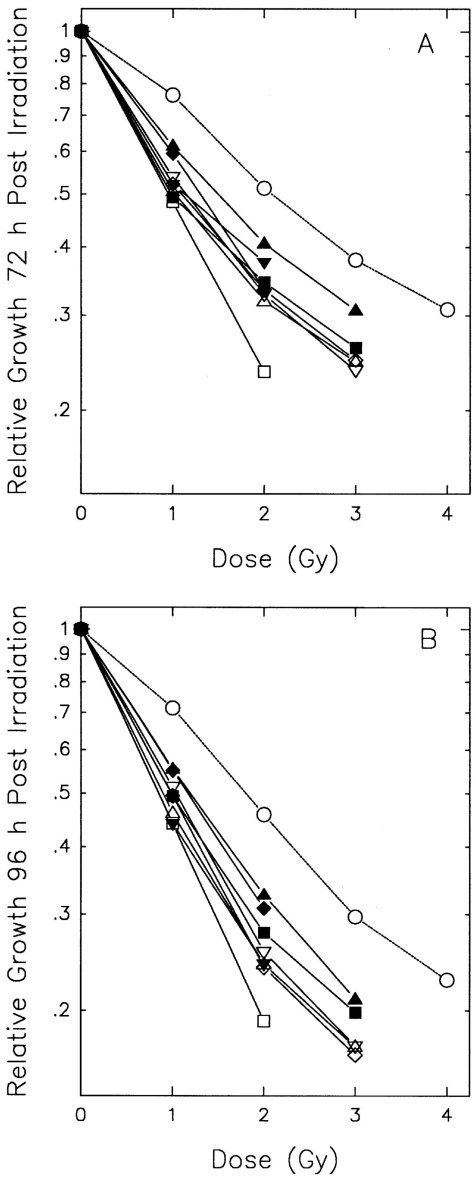

Figure 1 Radiation survival of LCLs, as measured by relative growth at 72 h (*A*) and 96 h (*B*) postirradiation, in a control (GM02184 [○]), in classical A-T (AT65RM [△] and L-3 [◆]), and in A-T variants (AT53RM [▽], AT42RM [▼], AT9RM [□], AT35RM [■], AT31RM [▲], and A[-T]2RM [◇]).

ATM Mutations

The variable levels of ATM protein in the variant cell lines indicated ATM mutations. A search for mutations in these cell lines revealed several ATM defects that had not previously been found in patients with classical A-T (table 3).

The truncating mutation 9139C→T at codon 3047, identified in patient AT9RM, shortens the large ATM protein by just 10 amino acids. To date, this is the smallest extent of truncation noted among ATM mutant alleles. The truncated protein is clearly less stable than the normal protein (fig. 3 and table 2), and its biochemical activity may be reduced, since the catalytic domain of the protein is expected to be in the carboxy-terminal PI 3-kinase–like region (Brown et al. 1995).

The splicing mutation 3576G→A seems to be a leaky mutation that leaves a residual amount of normally spliced ATM transcript. The mutation leads to a base substitution at the last position of exon 26, which results in a defective but not completely abolished splice site (fig. 4) (Mount 1982; Maquat 1996). In both patients homozygous for this mutation—patents AT31RM and A(-T)2RM—the cellular amount of ATM protein is ~5% of the normal level, probably reflecting both the amount of full-length ATM transcript produced by this allele and the stability of its protein product (fig. 4). It is important to note, however, that the two patients homozygous for the same mutation differ in a central feature of the A-T phenotype—that is, the appearance of telangiectasia.

The 3576G→A mutation appears also in two other A-T variants—patients AT42RM and AT35RM—each of whom is a compound heterozygote for this mutation and another, unknown mutation. Heterozygosity for the 3576G→A mutation was revealed by sequencing genomic PCR products spanning the mutation site. RT-PCR followed by sequence analysis of the corresponding region in the ATM transcript failed, however, to detect, in either patient, the transcript of the other ATM allele. This may be due to mutation(s) that either preclude the expression of those alleles or severely destabilize their RNA products. Both patients therefore demonstrate the outcome of compound heterozygosity for a null allele and a milder, leaky mutation.

The 3576G→A mutation thus appears in four Italian families, pointing to a founder effect. Patients AT31RM and A(-T)2RM live in two villages located 35 km apart in central Italy, whereas patients AT35RM and AT42RM come from two adjacent villages in southern Italy.

Patient AT53RM is a compound heterozygote exhibiting a relatively high level of the ATM protein (~12% of the normal level). The IVS47-9G→A mutation in one of this patient's ATM alleles activates a cryptic splice site within intron 47 and, a priori, could be leaky. The amino acid substitution and amino acid deletion caused by the second mutation, 5435del3, probably destabilize the ATM protein. On the basis of this patient alone, it is hard to determine the relative share of each mutation in the residual amount of protein found in the patient's cells. However, a clue to the outcome of the IVS47-9G→A mutation was obtained when this mutation was identified in a classical A-T patient, AT65RM, who is a second cousin of patient AT53RM (fig. 5). In this patient, the other allele contains a typical truncation mutation. Since the classical A-T phenotype is determined

Table 2

Radiation Response and ATM-Protein Level in Classical and Variant A-T Cell lines

Patient Group	Radiation-Dose Ratio Yielding 50% Growth at 72 h[a]	Thymidine-Incorporation Ratio at 25 Gy[b]	ATM-Protein Level (%[c])
Classical A-T:			
L-3[d]	.62	1.55	0
AT65RM	.49	1.39	0
A-T variants:			
AT9RM	.47	1.92	16.8
AT31RM	.66	2.05	4.8
A(-T)2RM	.55	1.68	5.4
AT42RM	.47	1.74	1.0
AT35RM	.55	2.16	1.0
AT53RM	.66	1.69	11.7

[a] The ratio between the doses required for inhibition of cellular growth by 50%, in this cell line and in normal cells.

[b] The ratio between thymidine incorporation following treatment with 25 Gy in this cell line and that in normal cells.

[c] Of normal protein level.

[d] Moroccan-Jewish A-T patient homozygous for a stop codon mutation at position 35 of the ATM protein (Gilad et al. 1996*a*).

by null alleles (Gilad et al. 1996*b*), and because AT65RM cells do not produce any ATM protein (fig. 3), it became apparent that the IVS47-9G→A mutation is probably not leaky and in fact represents a null allele. This leaves the entire amount of the ATM protein in patient AT53RM to be produced by the 5435de13 allele. The family depicted in figure 5 therefore represents an example of segregation of three ATM mutations in one family, two with severe A-T mutations and one with a milder mutation that leaves a fraction of the ATM protein probably with some residual activity.

An ATM Mutation in an A-T$_{Fresno}$ Patient

The fibroblast cell line AT25LA derived from one of the A-T$_{Fresno}$ sisters is as radiosensitive as a typical A-T cell line (Curry et al. 1989). No immunoreactive material was identified in this cell line by use of an anti-ATM antibody (not shown). Screening the ATM transcript in this cell line revealed homozygosity for a typical A-T mutation, which abolished a splice site at intron 33 of the ATM gene and led to skipping of exon 32 (table 3). The resultant large deletion in the protein probably severely destabilizes the ATM molecule. This result establishes that the A-T component in the phenotype of the twin sisters described by Curry et al. (1989) is indeed caused by an ATM mutation of the type that usually leads to classical A-T.

Discussion

There is no universally accepted definition of the A-T variant phenotype. The considerable variability of many of the clinical features of A-T sometimes makes it difficult to distinguish between classical and variant patients (Taylor et al. 1993; Bundey 1994). This distinction is often retrospective, particularly when the patient reaches an advanced age or exhibits slow progres-

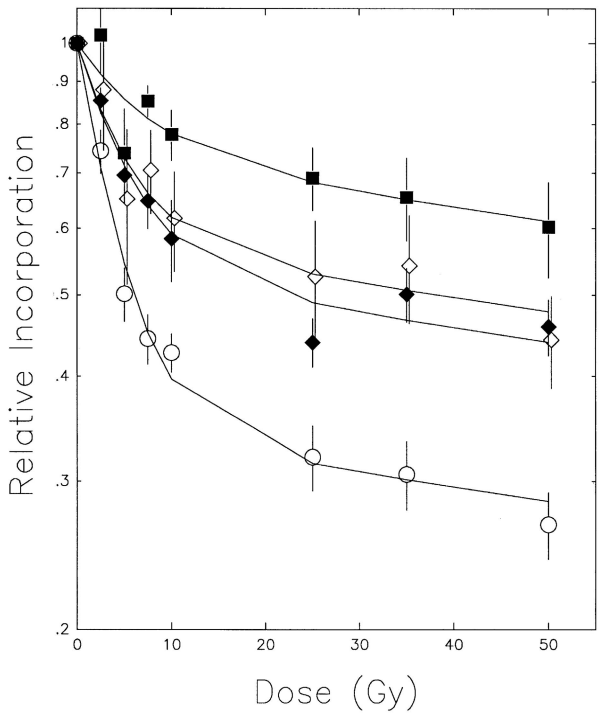

Figure 2 RDS in LCLs: relative nucleotide incorporation, with increasing radiation dose, for a normal control (GM02184 [○]), in classical A-T (L-3 [◆]), and in A-T variants (AT35RM [■] and A[-T]2RM [◇]).

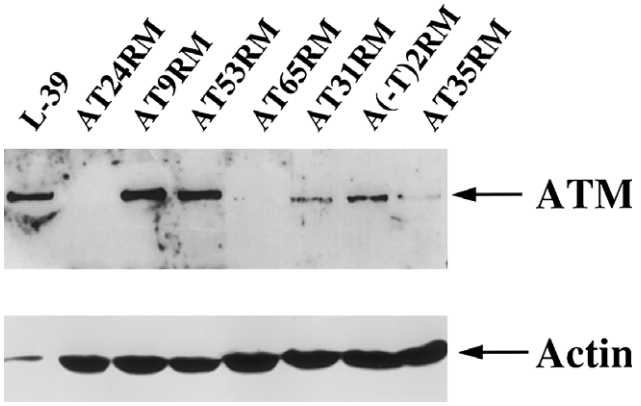

Figure 3 Western blotting analysis of cellular extracts prepared from various LCLs. The same blot was reacted with antibodies against ATM (*top panel*) and actin (*bottom panel*) (see Material and Methods). Equal amounts of protein were loaded in the lanes representing patients, and 10% of that amount was loaded in the control lane (lane L-39). Variable amounts of the ATM protein are observable in A-T variants, whereas no ATM can be detected in two classical patients (lanes AT24RM and AT65RM).

sion of the disease. Sometimes the clinical phenotype is typical of A-T patients but the cellular phenotype is less severe. The definition of patients as variants may therefore be subjective. However, it is important to identify atypical phenotypes that are allelic to A-T, since the localization of the responsible mutation within the ATM gene provides a reliable basis for genetic counseling, carrier detection within the affected family, and prenatal diagnosis. One quick way to establish the connection between a possible A-T–variant phenotype and the ATM gene is western blotting analysis of the ATM-protein level. A significantly reduced level would indicate involvement of the ATM gene.

The A-T–variant patients described in this study were defined as such on the basis of an overall picture of their disease, which usually displayed a milder form of at least one of the disease features. It should be noted that none of these patients suffers from recurrent sinopulmonary infections, which characterize 50%–60% of classical A-T patients. It is well established, however, that, even among classical patients, there is no correlation between the degree of immune deficiency and the appearance of recurrent infections (Roifman and Gelfand 1985). Previously reported variants typically did not exhibit this characteristic.

One of the A-T features that consistently appears in the variants, including those without telangiectasia, is chromosomal instability, often with the typical clonal translocations involving the sites of the immune-system genes (for examples, see the work of Ying and Docteau [1981] and Maserati et al. [1988]); this is the case with the variants described here as well. In view of the extended life span of these patients, compared with typical A-T cases, it is reasonable to assume that they are less cancer prone than are classical A-T patients. Thus, this phenotype might reflect a possible dissociation between the chromosomal instability and predisposition to lymphoid malignancies in A-T.

Although telangiectasia is regarded as an essential feature for the diagnosis of A-T (Sedgwick and Boder 1991), this is one of the most variable features of the A-T phenotype (Bundey 1994; Sanal et al. 1993; Taylor et al. 1993). Clinicians may not consider cerebellar ataxia as a possible representation of A-T unless it is accompanied by telangiectasia. Several cases with clinical and cellular characteristics of A-T but without telangiectasia have been reported (Ying and Docteau 1981; Byrne et al. 1984; Taylor et al. 1987; Maserati et al. 1988; Stell et al. 1989; Lanzi et al. 1992; Willems et al. 1993; de Graaf et al. 1994). In the present study, patient A(-T)2RM presents an important example of a phenotype that is caused by an ATM mutation but that does not include telangiectasia. Friedman and Weitberg (1993) proposed a new umbrella category of "ataxia with immune deficiency." Patients with this double combination often also present chromosomal instability and other A-T cellular features (see references cited above). In view of these considerations and our finding of an ATM mutation in patient A(-T)2RM, we propose that A-T features such as chromosomal instability, immunodeficiency, and high serum alpha-fetoprotein levels be searched for in patients with sporadic or unclassified cerebellar ataxias. If an "A-T–like" phenotype is found, a search for an ATM mutation would be appropriate.

A-T variants have also been defined by testing their cellular phenotype. In this study we investigated two hallmarks of this phenotype—radiosensitivity and RDS. Both features have been studied extensively in fibroblast and LCLs from A-T patients. LCLs are immortalized, but they faithfully retain the cellular A-T features observed in primary fibroblast lines—including radiosensitivity, RDS, and defective activation of the G1/S and G2/M cell-cycle checkpoints by ionizing radiation (for examples, see Houldsworth and Lavin 1980; Beamish and Lavin 1984; Beamish et al. 1996). Even the intermediate radiosensitivity of A-T heterozygotes is clearly observed in cellular-survival assays applied to lymphoblasts (Chen et al. 1978). It should also be noted that the LCLs in our experiments were at relatively low passage levels. Although the growth inhibition used in the present study may be less sensitive to subtle differences in radiosensitivity, which can be detected by clonogenic growth of fibroblasts, results strikingly similar to ours recently have been reported in a study of fibroblast lines from a series of British A-T variants: a wide range of radiosensitivity was observed, with no correlation with the severity of the clinical phenotype (Taylor 1997).

Table 3

ATM Mutations Identified in Patients

Patient Group	Transcript Mutation[a]	Genomic Mutation[b]	Predicted Protein Alteration	Genotype
A-T variants:				
AT9RM	9139C→T	Base substitution in exon 65	Arg→ter; truncation at codon 3047	Homozygosity
AT31RM	3403del174	3576G→A	In-frame deletion of 58 amino acids at codon 1135	Homozygosity
A(-T)2RM	3403del174	3576G→A	In-frame deletion of 58 amino acids at codon 1135	Homozygosity
AT42RM	3403del174	3576G→A	In-frame deletion of 58 amino acids at codon 1135 amino acids at codon 1135	Compound heterozygosity[c]
AT35RM	3403del174	3576G→A	In-frame deletion of 58 amino acids at codon 1135	Compound heterozygosity[c]
AT53RM	6572ins7	IVS47-9G→A	Truncation at codon 2198	Compound heterozygosity
AT53RM	5435del3	3-bp deletion in exon 38	A1812V and in-frame deletion of 1813^{Phe}	Compound heterozygosity
Classical A-T:				
AT65RM	8814del11	11-bp deletion in exon 63	Truncation at codon 2951	Compound heterozygosity
AT65RM	6572ins7	IVS47-9G→A	Truncation at codon 2198	Compound heterozygosity
AT_{Fresno}:				
AT25LA	4612del165	IVS33+2T→C	In-frame deletion of 55 amino acids at codon 1538	Homozygosity

[a] Presented according to the nomenclature proposed byAntonarakis et al. and the Nomenclature Working Group (1998); the first nucleotide of the ATM open reading frame was designated "+1" (GenBank accession number U33841[http://www.ncbi.nlm.nih.gov/]).

[b] Exon numbers are according to Uziel et al. (1996).

[c] The other ATM allele is not expressed.

We also noted, in the patients' cell lines, a lack of correlation between the degree of cellular radiosensitivity and RDS. The sample size did not allow us to draw significant statistical conclusions, but it should be noted that the two phenomena are not necessarily correlated and can be dissociated from each other by certain manipulations (reviewed by Jorgensen and Shiloh 1996; Lavin and Shiloh 1997).

A-T patients with radiosensitivity that is reduced compared with that in classical patients have been reported. Interestingly, postirradiation DNA synthesis in such patients could be either normal, similar to that in classical patients, or intermediate (Fiorilli et al. 1984; Taylor et al. 1987). Patient AT9RM, studied here, showed a fairly typical clinical phenotype but, because of both (*a*) intermediate radiosensitivity of her lymphocytes (Chessa et al. 1986) and fibroblasts (Chessa et al. 1992) and (*b*) normal postirradiation DNA synthesis observed in her fibroblasts (Chessa et al. 1992), was classified as a variant. Antoccia et al. (1995) noted nearly normal postirradiation DNA synthesis in the LCL established from this patient. In our hands, however, this LCL shows both radiosensitivity in the A-T range and RDS typical for A-T cell lines. This cell line was characterized by a dynamic clonal evolution, indicated by the spread of a t(2;14)(p12;q32) translocation (Chessa et al. 1989), and this may also have contributed to its current phenotype, which resembles that of classical A-T cells. These results underscore differences in such parameters, between cell lines and between laboratories, particularly when the parameters are measured across extended time periods, and they call for caution when they are used to evaluate a patient's phenotype. Indeed, Chessa et al. (1992) and Antoccia et al. (1995) stressed the lack of correlation between the severity of the clinical phenotype in A-T and the degree of both radiosensitivity and RDS observed in patients' cell lines.

Patients A(-T)2RM and AT31RM are homozygous for the same ATM mutation, yet they differ phenotypically in a cardinal feature of A-T—the telangiectasia. This

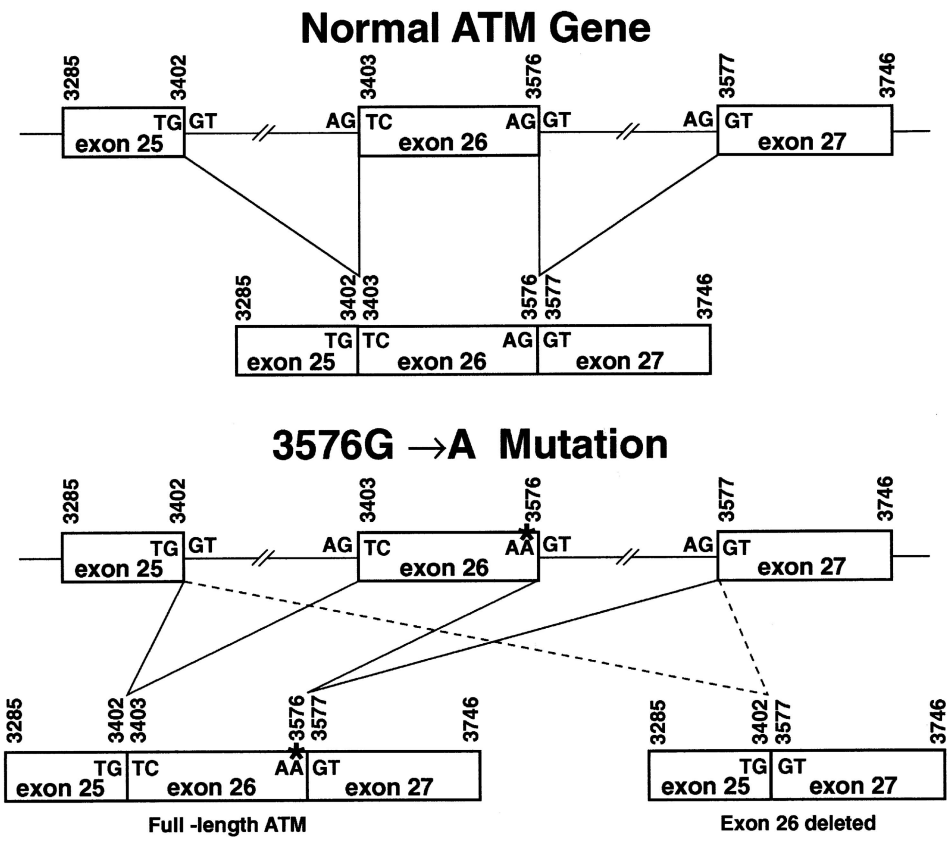

Figure 4 Consequences of the leaky splicing mutation 3576G→A

observation points to the involvement of other physiological factors in the determination of the extent—and even existence—of the disease features. The mutation common to these patients is leaky, allowing expression of the ATM protein at ~5% of its normal level. Another leaky ATM mutation was found in compound heterozygosity with severe mutations in a group of British A-T variants. That mutation caused activation of a cryptic splice site within the ATM gene (McConville et al. 1996).

A severe defect in the same splice site, affected by the 3576G→A mutation, has been identified in a Turkish A-T patient, F-2095, in whom one of the ATM alleles contains a IVS26+1delG mutation (Gilad et al. 1996*b*). That mutation, which eliminates the GT consensus dinucleotide at the beginning of intron 26, abolishes this splice site completely and leads to an ATM null allele.

Patients AT42RM and AT35RM were classified as variants primarily because of their longer life span, compared with that for typical A-T patients. A-T variants who reached the ages of 44 and 58 years were reported by de Jonge and Tijssen (1988) and Ying and Docteau (1981). The latter authors assumed that their patient represented an allelic form of classical A-T. In view of our findings, this is highly likely. Patients AT42RM and AT35RM are compound heterozygotes for the 3576G→A mutation and a null allele that is not expressed. In that respect, they are similar to the British A-T variants reported by McConville et al. (1996), who are compound heterozygotes for severe and leaky ATM mutations. It is of interest that the phenotype of patients AT42RM and AT35RM resembles that of patient AT31RM, who is homozygous for the 3576G→A mutation. In many autosomal recessive disorders, the interaction between severe and mild mutations gives rise to clinical variability (for examples, see the work of Gravel et al. [1995] and Guttler and Guldberg [1996]).

Patient AT9RM shows the highest ATM-protein level in this series. Had this level been determined by a regulatory mutation, and had the protein itself not been altered, the resulting phenotype would be expected to be relatively mild. This patient's mutation led, however, to a minute truncation of the protein, which was probably responsible for reduction in both its catalytic activity and stability, reductions that, together, led to a rather pronounced phenotype. This mutation highlights the importance of the carboxy-terminal domain of the ATM protein, which probably harbors the catalytic site of this protein kinase.

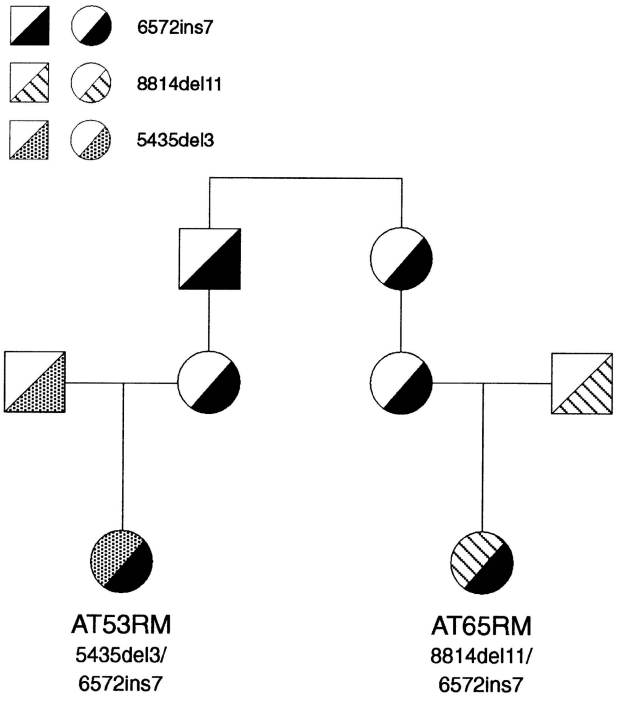

Figure 5 Segregation of three ATM mutations in two families

A-T_{Fresno} (Curry et al. 1989) represents a typical A-T phenotype with additional features. A distinction should be made between such syndromes and A-T variants who have milder forms of the classical disease but who have no additional signs. The typical A-T mutation identified in the A-T_{Fresno} cell line indicates, however, that the A-T component in this phenotype is caused by an ATM mutation of the type that characterizes classical A-T, whereas the microcephaly and mental retardation represent a different genetic or environmental etiology.

The mutations identified in this study in A-T variants affect the ATM-protein function and level less severely than do the null mutations typical of classical A-T. Nevertheless, even these mutations leave only residual active protein. The mutations found in A-T patients and in A-T variants may still represent only a portion of the ATM defects in the population. ATM mutations of a more minor nature must underlie various other phenotypes, making far from complete the known spectrum of genotype-phenotype combinations associated with this gene.

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