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Ataxia-Telangiectasia: Identification and Detection of Founder-Effect Mutations in the ATM Gene in Ethnic Populations

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Summary

To facilitate the evaluation of ATM heterozygotes for susceptibility to other diseases, such as breast cancer, we have attempted to define the most common mutations and their frequencies in ataxia-telangiectasia (A-T) homozygotes from 10 ethnic populations. Both genomic mutations and their effects on cDNA were characterized. Protein-truncation testing of the entire ATM cDNA detected 92 (66%) truncating mutations in 140 mutant alleles screened. The haplotyping of patients with identical mutations indicates that almost all of these represent common ancestry and that very few spontaneously recurring ATM mutations exist. Assays requiring minimal amounts of genomic DNA were designed to allow rapid screening for common ethnic mutations. These rapid assays detected mutations in 76% of Costa Rican patients (3), 50% of Norwegian patients (1), 25% of Polish patients (4), and 14% of Italian patients (1), as well as in patients of Amish/Mennonite and Irish English backgrounds. Additional mutations were observed in Japanese, Utah Mormon, and African American patients. These assays should facilitate screening for A-T heterozygotes in the populations studied.

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Introduction

Ataxia-telangiectasia (A-T) is an autosomal recessive disorder with a frequency of 1/40,000–1/100,000 (Swift et al. 1986). The disorder is characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, cellular and humoral immune deficiency, chromosomal instability, sensitivity to ionizing radiation, and increased incidence of cancer (Boder and Sedgwick 1958; Sedgwick and Boder 1991; Gatti 1997). Heterozygotes are also at an increased risk of developing cancer, especially breast cancer (Swift et al. 1987, 1991; Pippard et al. 1988; Borresen et al. 1990; Easton 1994; Athma et al. 1996) and possibly T cell leukemia (Vorechovsky et al. 1997); however, they are otherwise asymptomatic. The gene responsible for the disease, ATM, was localized to chromosome 11q22-23 (Gatti et al. 1988; Lange et al. 1995) and was subsequently isolated by positional cloning (Savitsky et al. 1995*a*).

There is great interest in screening cancer-risk populations for mutations in the ATM gene (Vorechovsky et al. 1996*a,* 1997; FitzGerald et al. 1997). However, with 141 mutations already described in A-T homozygotes (Savitsky et al. 1995*a;* Baumer et al. 1996; Byrd et al. 1996; Gilad et al. 1996*a,* 1996*b;* Lakin et al. 1996; McConville et al. 1996; Telatar et al. 1996; Vorechovsky et al. 1996*b;* Wright et al. 1996; Watters et al. 1997), and with another 100 either still unpublished or described herein, the spectrum of mutations that is emerging creates significant technical problems for population screening. The ATM gene is large, containing 66 small exons encoding a 13-kb transcript. Most mutations are unique, and they are distributed uniformly along the length of the gene, with no obvious "hot spots." Approximately 70% of published mutations would truncate the ATM protein and should be detectable by protein-truncation testing (PTT).

In this report, we demonstrate that (1) PTT detected

Table 1

Primers Used in PTT Analysis and Rapid Detection of Ethnic Mutations

PTT Fragment or Exon	Nucleotide Sequence[a] (5′–3′)
PTT fragment (nucleotide position):	
Region e (76–1392):	
Forward	(T7)-GAAGTTGAGAAATTTAAGC
Reverse	AATGCAACTTCCGTAAGGC
Region f (1048–2817):	
Forward	(T7)-GCAGATATCTGT
Reverse	GTAGGTTCTAGCGTGCTAGA
Region g (2437–4092):	
Forward	(T7)-AATGACATTGCAGATATTT
Reverse	TCAGTGCTCTGACTGGCACT
Region a (4048–5435):	
Forward	(T7)-ACGTTACATGAGCCAG
Reverse	TCCAAATGTCATGATTTTCAC
Region b (5282–6529):	
Forward	(T7)-CTGGCCTATCTACAGC
Reverse	CAACCTGCTAAGTGTGGGAT
Region c (6322–7856):	
Forward	(T7)-CAGTGGGACCATTGC
Reverse	TTCTGACCATCTGAGGTCTCC
Region d (7651–9172):	
Forward	(T7)-GATCACCCCATCACA
Reverse	TCACACCCAAGCTTTCCATC
Exon:	
12:	
Forward	GCTTACTTGGAGCCATAATTC
Reverse	TGAAGGTCTGCAGGCTGAC
24:	
Forward	TGGGAAAAGACTTTCCTGTAAA
Reverse	CTATTGATTGACTCTGCAGCC
41:	
Forward	CTCTATGCAGAAATCTATGCAG
Reverse	ATACCCTTATTGAGACAATGCC
42:	
Forward	GTATATGTATTCAGGAGCTTC
Reverse	ATGGCATCTGTACAGTGTCT
43:	
Forward	CAGAACTGTATTTCAGAATCAT
Reverse	ACATAACTCCTTCATAAACAGA
50:	
Forward	AGTTGGGTACAGTCATGGTA
Reverse	GAAAAGATGAAGCATATTCATG
52:	
Forward	ATTAAGTGGAGAACCACATGATA
Reverse	ATGCCATTGACTTCAGAAAC
53:	
Forward	TTACTTGCTTAGATGTGAGA
Reverse	ATATGTTGGAATCTTCATTCCG
54:	
Forward	AAGCAAAATGAAAAATATGG
Reverse	AAGTGTGATGGGGGTGA

[a] "(T7)" indicates the sequence GGATCCTAATACGACTCACTATAGGAACAGACCACCATG.

66% (92/140) of mutant alleles, (2) almost all recurring mutations share haplotypes and represent "founder effects," and (3) it is possible to approach carrier testing by first identifying the most common mutations in selected ethnic populations and then developing rapid assays that use small amounts of genomic DNA and less costly methods. We have detected many relatively common founder-effect mutations in Norwegian, Costa Rican, Polish, Italian, and Amish/Mennonite populations, and we have analyzed other, less common mutations in patients of Irish, English, Japanese, Utah Mormon, and African American backgrounds. The rapid assays described herein should facilitate heterozygote screening in these populations.

Subjects and Methods

Subjects

We initially used PTT to analyze cDNAs from 70 unrelated patients with various ethnic backgrounds. We later used rapid assays, based on the PTT results, to detect specific mutations in DNAs from 8 Norwegian families, 27 Costa Rican patients (previously described by Uhrhammer et al. [1995]), 22 Polish patients, 28 Italian patients, and several Amish/Mennonite families, as well as in DNAs from 40 normal individuals (CEPH parents; northern European backgrounds [Dausset et al. 1990]).

Primers

The ATM cDNA sequence was divided into seven overlapping fragments (e, f, g, a, b, c, and d), and each fragment was subjected to PTT. Primers used for the PTT analysis of the entire ATM cDNA are shown in table 1. Primers used in rapid assays, also shown in table 1, were designed on the basis of sequences reported by Savitsky et al. (1995*a;* 1995*b*), Rasio et al. (1995), and personal communications from M. Platzer and A. Rosenthal (accession number U82828; Platzer et al. 1997). Primers for exons 42, 43, and 50 were designed by A. Tolun and S. Onengut.

Haplotype Analyses

Panels of 6–15 genetic markers located across the ATM gene region were used to create haplotypes (Gatti et al. 1994; Vanagaite et al. 1995; Uhrhammer et al. 1995).

Heteroduplex Analysis

Heteroduplexes were formed by mixing the PCR products of an A-T patient with PCR product of a normal individual. The DNA mixture was then denatured at 95°C for 5 min and was allowed to reanneal at 55°C

Figure 1 Spectrum of ATM mutations, based on 205 mutations. Boxes represent founder-effect mutations, identified by shared haplotypes.

for 45 min. Samples were run on a 12% polyacrylamide gel, under nondenaturing conditions, for 1.5 h at 120 V. Bands were visualized by staining the gel with ethidium bromide.

RNA Isolation, cDNA Synthesis, and PTT

These methods have been described elsewhere (Telatar et al. 1996); primers for the seven PTT-screening segments are described in table 1. Protein products of the coupled PTT reaction were run on 10%–20% gradient SDS-PAGE gels for 5 h at 250 V.

Nomenclature

ATM mutations were initially detected in cDNAs derived from lymphoblastoid cell lines of A-T patients. The underlying genomic mutation sites often differed from the cDNA sequence locations when splice sites were involved. Because the official nomenclature report states that the recommendations for describing splicing mutations "will provide a unique identifier but will not call attention to the effect on splicing" (Ad Hoc Committee on Mutation Nomenclature 1996, p. 201), in this report we describe all mutations first by the official nomenclature of nucleotide position (e.g., 6095G→A); second, by the first affected codon (e.g., codon 2003); and, last, by the effect of the mutation on the cDNA (e.g., del89nt): 6095G→A (codon 2003del89nt). We have included the first affected codon for most mutations because prior publications have used a variety of numbering systems for both nucleotides and exons, whereas codon numbering has remained unchanged. A website for ATM mutations (http://www.vmmc.org/vmrc/atm.htm) gives both further details on the nomenclature and updates on mutations reported previously.

Results

By using the PTT to screen cDNA, we detected 92 (66%) truncating mutations in 140 mutant chromosomes. This figure approximated the predicted number of truncating mutations (i.e., 70%) (Telatar et al. 1996).

Figure 1 depicts the spectrum of all published ATM mutations (Savitsky et al. 1995*a*, 1995*b*; Baumer et al. 1996; Byrd et al. 1996; Gilad et al. 1996*a*, 1996*b*; Lakin et al. 1996; McConville et al. 1996; Telatar et al. 1996; Vorechovsky et al. 1996*b*; Wright et al. 1996), including those reported herein, as well as ~50 unpublished mutations identified in our laboratories. Although the spectrum observed is generally diverse, we have noted several positions at which the same mutation has been described in multiple patients. So as not to bias the emerging spectrum of ATM mutations, those associated with shared haplotypes are represented only once, and a box has been placed above those locations in figure 1. These are considered founder-effect mutations and are summarized in table 2. We designed rapid assays for some of the more common mutations, using genomic DNA as the template for PCR amplification. These assays were also used to screen normal populations, to determine whether some mutations might in fact be common polymorphisms; none of these mutations were observed in 40 persons of northern European background.

Table 2

ATM Mutations in Ethnic Populations

Codon Number	cDNA Mutation	Genomic Mutation	Ethnic Origin
144	insA	432insA	Polish
522[a]	delAG	1563delAG	Amish
536	ins764nt	IVS12+1G→T	Italian
750	ins9nt	IVS16−10T→G	African American
834	insA	2502insA	Italian
937	insCTAG	2810insCTAG	African American
1026	del207nt	3214G→T	Polish
1029	insA	3084insA	Polish
1081	ATC→TGAT	3245ATC→TGAT	Norwegian
1135	del174nt	3576G→A	Italian
1537	ins11	IVS32−12A→G	Utah Mormon
1921[a]	ins137nt	IVS40+1126A→G	English Midland
1970	C→T	5908C→T	Costa Rican
1973	del88nt	5932G→T	Polish Mennonite
2003[a]	del89nt	6095G→A	Polish
2271[a]	delC	6810delC	Italian
2337[a]	delGT	7010delGT	Polish
2443[a]	C→T	7327C→T	African American
2481	del70nt	7449G→A	Costa Rican
2506[a]	del4nt	7517del4nt	Italian, French
2544[a]	del298nt	7926A→C	African American
2544[a]	del159nt	IVS53−2A→C	Polish
2546[a]	del9nt	7636del9nt	Irish English
2628[a]	del5nt	7883del5nt	Japanese
2832	C→T	8494C→T	Utah Mormon
2891[a]	del115nt	IVS62+1G→A	Utah Mormon
2922	insT	8766insT	Polish
2941[a]	ins4nt	8822ins4nt	Italian
2945[a]	delCT	8833delCT	Italian
2951	del17kb	IVS63del17kb	Costa Rican

[a] Published previously; see Results.

Norwegian Mutation: 3245ATC→TGAT (codon 1081)

Two Norwegian patients showed protein truncation in PTT-screening region g; one was homozygous for the same truncated band, and the other was heterozygous (fig. 2*a*). Sequence analysis revealed a complex frameshift mutation, 3245ATC→TGAT (codon 1081), in exon 24. Primers flanking the mutation were designed from genomic DNA sequence. Genomic DNA from eight unrelated Norwegian A-T patients (including the two patients mentioned above) and their siblings was amplified for this region, and the PCR products were subjected to heteroduplex analysis (fig. 3). Three patients were found to be homozygous, and two were found to be heterozygous, for this mutation. Haplotype analysis with seven markers demonstrated that all five patients who had the mutation shared the same haplotype. Thus, among Norwegian A-T patients, one mutation accounted for half (8 of 16) of the mutant alleles. An American patient of Norwegian ancestry was also found to have this mutation.

Costa Rican Mutations

In a previous study, 10 distinct haplotypes were observed in 27 Costa Rican A-T patients (Uhrhammer et al. 1995). Further testing with two newer markers (D11S1778 and D11S2179) has refined our ability to distinguish haplotypes A, A′, and B from one another. Other than this, the original haplotype assignments of A–J have been retained. The haplotypes A, B, C, and D account for, respectively, 56%, 7%, 13%, and 9% of the 27 Costa Rican patients studied. We have identified the mutations of haplotypes A, B, and C.

Haplotype A (5908C→T [codon 1970]).—PTT of a haplotype A patient demonstrated protein truncation in region b (fig. 2*b*). Sequence analysis identified the mutation as a C→T transition in exon 41, at position 5908 (codon 1970). The mutation abolishes a *Sau*3AI digestion site. Primers flanking the mutation were designed from genomic DNA sequence. When digested with *Sau*3AI, the PCR product (116 bp) of a normal individual produced two bands, of sizes 77 bp and 39 bp. The 5908C→T mutation abolishes the *Sau*3AI site, and only one band, of size 116 bp, was observed in patients who were homozygous for haplotype A. Genomic DNA from all Costa Rican patients was PCR amplified across this region and was digested with *Sau*3AI, and the products were electrophoresed on a 3:1 NuSieve agarose gel (fig. 4). Eleven patients were found to be homozygous, and

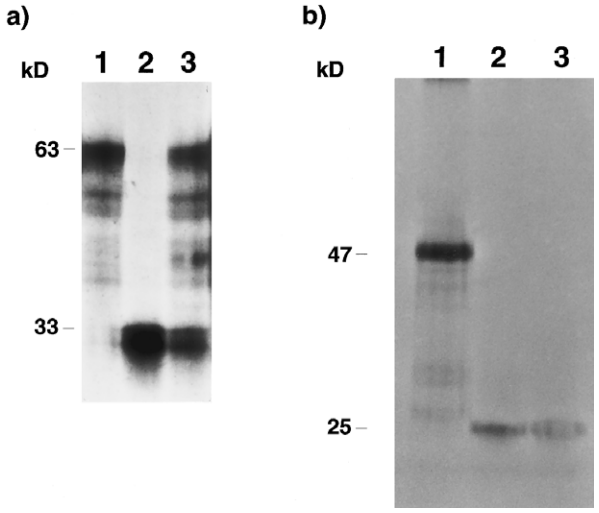

Figure 2 Protein truncation test results. *a,* Norwegian patients: PTT-screening region g. Lane 1 shows results for a normal control, and lanes 2 and 3 show results for patients with truncated protein of 33 kD (one homozygous, the other heterozygous). *b,* Costa Rican patients: PTT-screening region b. Lane 1 shows results for a normal control, and lanes 2 and 3 show results for homozygous patients with truncated protein of 25 kD.

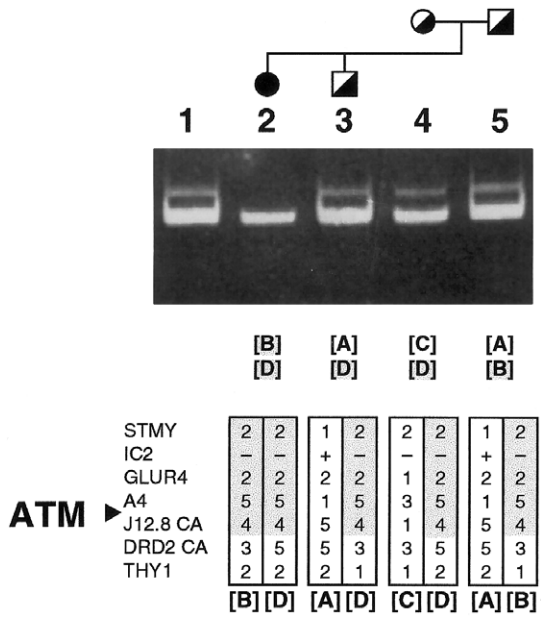

Figure 3 Heteroduplex and haplotype analysis of a Norwegian family. Lane 1 is a heteroduplex mixture of DNA from an affected child and a normal child, lane 2 is the affected child alone, and lanes 3–5 are heterozygotes. Bracketed letters indicate corresponding haplotypes. Parents (lanes 4 and 5) carry the same mutation; consanguinity was denied.

8 patients were found to be heterozygous, for the 5908C→T mutation. This result was in complete agreement with prior haplotyping data (Uhrhammer et al. 1995). Patients with the A′ haplotype had the same mutation as was seen in those with haplotype A. The frequency of the 5908C→T mutation in 27 Costa Rican patients was 56%.

Haplotype B (*IVS63del17kb*).—The haplotype B mutation (IVS63del16kb) is a 17-kb deletion that begins in intron 63 and ends beyond the 3′ UTR. Southern blot analysis with *Bgl*II digestion showed that DNA from a homozygous haplotype B patient fails to hybridize with probes for exon 64 or exon 65. The deletion begins and ends in two LINES repeats. Three Costa Rican patients (7.4%) shared the B haplotype. This is only the second "large" deletion described to date (Savitsky et al. 1995*a*) in the ATM gene.

Haplotype C (*7449G→A* [*codon 2481del70nt*]).—A haplotype C patient showed truncation in PTT-screening region c. Sequencing of genomic DNA identified the mutation (7449G→A [codon 2481del70nt]) as a homozygous G→A transition at position 7449. Because the mutation does not create or abolish any known restriction sites, we designed a forward primer that contains CC instead of GA, as indicated by underlines in table 1 (exon 52). PCR amplification with this primer introduces an *Xcm*I site (CCANNNNNNNNNTGG) in normal DNA, so that an 86-bp PCR product becomes two bands, 66 bp and 20 bp, in normal individuals; DNA from patients with the 7449G→A mutation remained undigested (data not shown). The location of the mutation is underlined in the *Xcm*I site above. Haplotype C was observed in 7 (13%) of 54 chromosomes. This result confirmed the original haplotyping by Uhrhammer et al. (1995).

Polish Mutations

We analyzed haplotypes of 22 Polish A-T patients for linkage disequilibrium (data not shown). Ten patients with representative haplotypes were screened by use of PTT. We identified eight distinct mutations. Rapid assays were designed for five of these, comprising 27% of the mutant alleles, and were used to screen all 22 Polish patients, several American patients of Polish ancestry, and 80 normal chromosomes of non-Polish origin (CEPH families). None of these mutations were found in normal individuals. Further testing for these mutations in the Polish population is under way (by E.B.-M.).

Haplotype A (*IVS53-2A→C* [*codon 2544del-159nt*]).—This mutation results in a deletion of exon 54, beginning at codon 2544. The genomic mutation alters the invariant splice-acceptor site at −2 from exon 54 (Wright et al. 1996). The mutation abolishes an *Alu*I site. Primers flanking the mutation were designed from genomic sequence. When normal DNA was digested

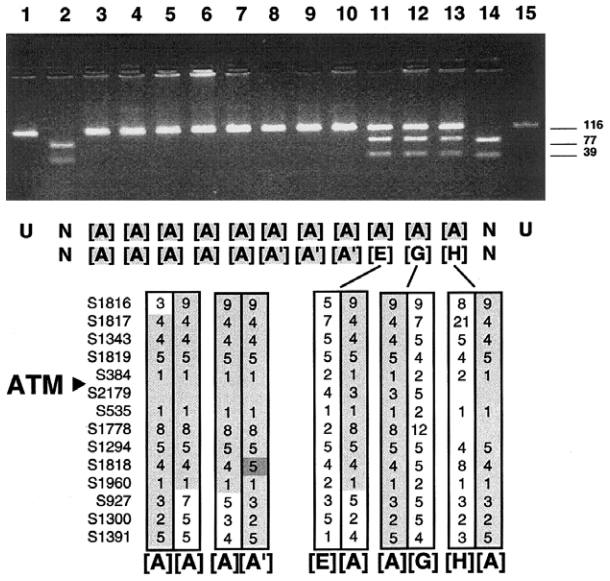

Figure 4 *Sau*3AI digestion of 11 PCR products from Costa Rican patients. Lanes 1 and 15 are undigested (U) samples, and lanes 2 and 14 are digested normals (N); lanes 3–10 are A-T patients who were homozygous for haplotype A and are homozygous for the 5908C→T mutation, as indicated by the undigested bands. Lanes 11–13 are compound heterozygotes showing both digested and undigested bands.

with *Alu*I, a 110-bp PCR product was divided into bands of 70 bp and 40 bp; the 110-bp PCR product of alleles with this mutation remained undigested (fig. 5*a*). Of seven A-T patients with deletions of exon 54 that were identified by use of reverse transcriptase–PCR based tests, four patients with this mutation were identified by use of genomic testing. All of these patients (W1, W15, W23, and W24) were Polish and shared haplotype A (fig. 5*f*). Among Polish A-T patients, this allele represented 9% of mutant alleles.

Haplotype B (*6095G→A* [*codon 2003del89nt*]).—This mutation involves a G→A substitution of the last nucleotide of exon 43, and it results in the deletion of exon 43 from the cDNA. The mutation abolishes a *Bfa*I site. Primers flanking the mutation were designed from genomic sequence. After digestion with *Bfa*I, a 301-bp PCR product from normal individuals showed two bands, 239 bp and 62 bp, whereas patients with the 6095G→A mutation showed only the undigested 301-bp product. Genomic DNA from 22 Polish A-T patients was screened for this mutation; 3 (W8, W19, and W22) were found to be heterozygous (fig. 5*b*). These three patients (3 [6.8%] of 44) shared haplotype B (fig. 5*f*).

Haplotype C (*7010delGT* [*codon 2337*]).—Two of 22 patients were found to be heterozygous for the haplotype C mutation. Deletion of GT at position 7010 in exon 50 creates a *Tfi*I site. Genomic DNAs were amplified with primers flanking exon 50, and the products were digested with *Tfi*I. A 231-bp PCR product remained undigested in normal individuals, whereas the two patients (W1 and W9) with this mutation produced two bands, 141 bp and 88 bp (fig. 5*c*). These two patients (2 [4.5%] of 44) shared haplotype C (fig. 5*f*).

Haplotype D (*5932G→T* [*codon 1973del88nt*]).—This mutation creates a stop codon (GAA[Glu]→TAA [stop]). In cDNA, exon 42—where this mutation occurs—is frequently deleted. The G→T transversion creates an *Mse*I site. Primers flanking the mutation were designed from genomic DNA sequence. When the normal 232-bp PCR product was digested with *Mse*I, two bands, 33 bp and 199 bp, were observed, whereas patients with the 5932G→T mutation produced three bands, of 33 bp, 40 bp, and 159 bp (fig. 5*d*). Genomic DNAs of 22 Polish A-T patients were screened, and 2 (W3 and W10) were found to be heterozygous for this mutation. These 2 patients (2 [4.5%] of 44) shared haplotype D (fig. 5*f*).

Mutation 3214G→T (*codon 1026del207nt*).—This mutation in exon 24 also results in the creation of a stop codon (GAA[Glu]→TAA[stop]). In cDNA, a variant form of mRNA was observed in which exons 23 and 24 were deleted, thereby splicing over this mutation and maintaining the correct reading frame. The mutation creates an *Mse*I site. PCR primers flanking the mutation were designed from genomic DNA sequences. After digestion with *Mse*I, a 94-bp PCR product remained undigested in normal individuals, whereas patients with this mutation produced two bands, 71 bp and 23 bp (fig. 5*e*). Genomic DNA of 22 Polish A-T patients was amplified for exon 24 (with the same primers used for detecting the 3245ATC→TGAT Norwegian mutation) and was digested with *Mse*I; only 1 Polish patient (W6) was found to be heterozygous for this mutation.

Thus, the Polish population appears to contain at least four founder-effect mutations, comprising 25% of the patients studied. Rapid assays were designed and tested for all four. We identified three additional mutations in Polish patients, for which rapid assays were not designed: 432insA (codon 144), 3084insA (codon 1029), and 8766insT (codon 2922); all were compound heterozygotes.

Italian Mutations

Gilad et al. (1996*a*, 1996*b*) reported a founder-effect deletion of 4 nt at position 7517 in exon 53 (7517del4nt [codon 2506]), found in six patients from central-southern Italy (fig. 1, box over exon 53). This mutation has also been reported in a French patient (Vorechovsky et al. 1996*b*). To determine the frequency of this mutation among other A-T patients of Italian ancestry, we designed, from genomic sequence, primers flanking the mutation, and we tested the PCR products by heteroduplex

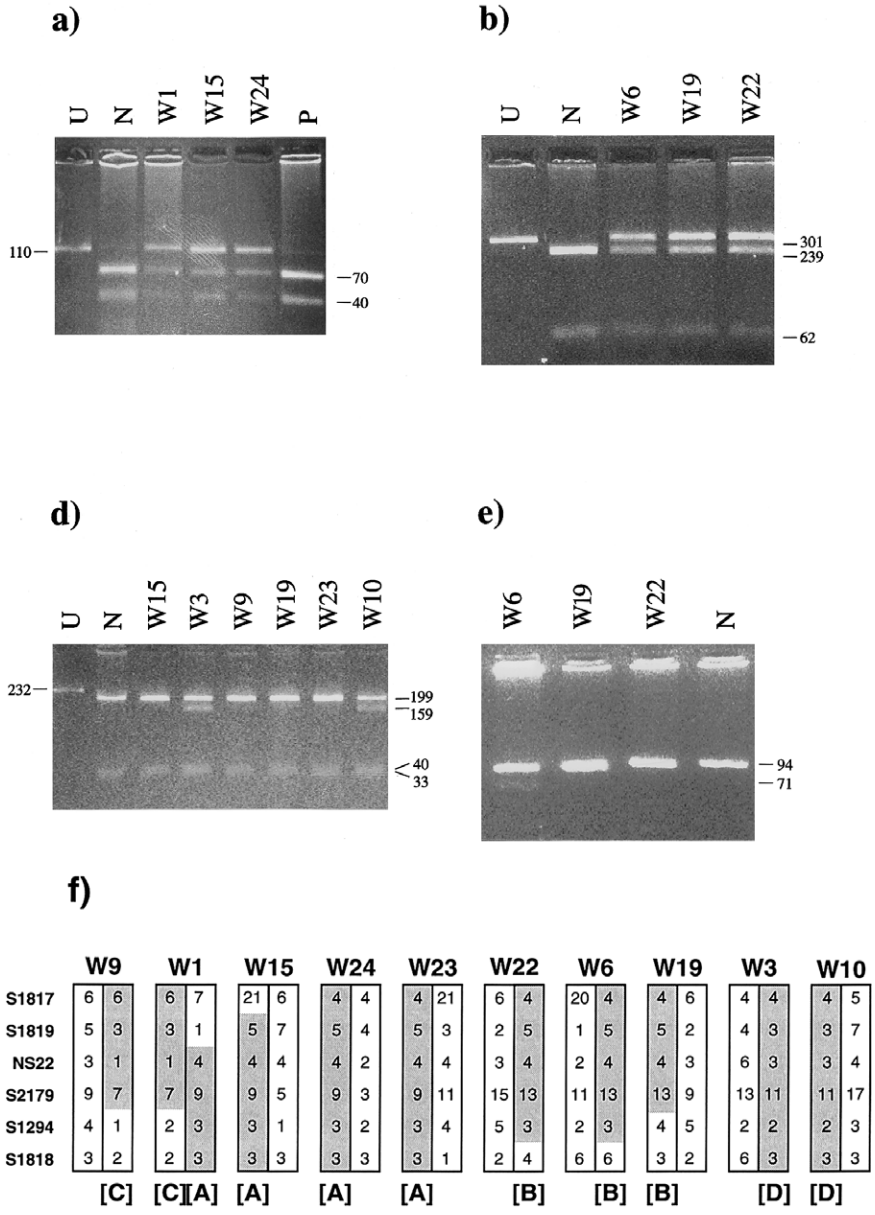

Figure 5 Comparison of Polish mutations and haplotypes. U = undigested DNA, and N = normal control. *a,* Haplotype A mutation, IVS53-2A→C. Lanes 3–5 are heterozygous patient samples, and lane 6 is a patient with a cDNA deletion of exon 54 who does not have the IVS53-2A→C genomic mutation. Haplotype analysis shows that patients in lanes 3–5 share haplotype A (data shown in *f*), whereas the patient in lane 6 does not share this haplotype (data not shown). *b,* Haplotype B mutation, 6095G→A(2003del89nt). Three heterozygous Polish patients (lanes 3–5) have the 6095G→A(2003del89nt) mutation in exon 43. *c,* Haplotype C mutation, 7010delGT. Lanes 1 and 2 are patients who have the 7010delGT mutation in exon 50. Lanes 3 and 4 are patients who do not have this mutation. *d,* Haplotype D mutation, 5932G→T. *Mse*I digestion assay was performed on samples from six Polish patients (lanes 3–8). Lanes 4 and 8 are patients with the 5932G→T mutation in exon 42. *e,* 3214G→T mutation. Assay was performed on samples from three Polish patients (lanes 1–3). Lane 1 is a patient with the 3214G→T mutation in exon 24. *f,* Haplotype analysis of the 10 Polish patients described above. Shaded areas represent four shared haplotypes (A–D).

analysis. Twenty-eight patients were screened. Two patients showed this mutation; both were homozygous (fig. 6) and shared the same two haplotypes at chromosome 11q23.1 (4 [7%] of 56 mutant alleles).

Screening by use of PTT identified five other mutations in A-T patients of Italian ancestry. We previously had reported three mutations, at 6810delC (codon 2271), 8822ins4nt (codon 2941), and 8833delCT (codon 2945) (Telatar et al. 1996). Three new mutations were detected by use of PTT screening: 3576G→A (codon 1135del174nt), IVS12+1G→T(codon 536ins764nt), and 2502insA (codon 834). The 3576G→A (codon

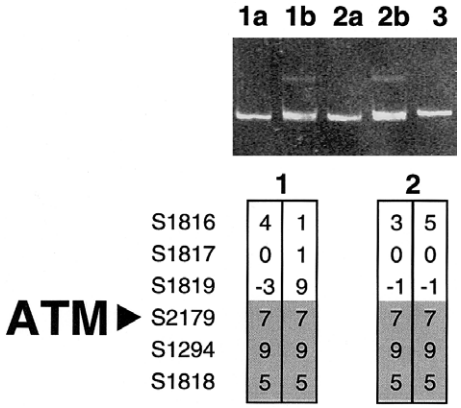

Figure 6 Heteroduplex and haplotype analysis of two homozygous Italian patients showing the 7517del4 mutation (Savitsky et al. 1995a). Lanes 1a and 2a are the patient samples; lanes 1b and 2b are the heteroduplex mixture of DNAs from normal individuals with those of patients 1a and 2a, respectively; and lane 3 is a normal control. Haplotype analysis has shown that both patients share both haplotypes at chromosome 11q23.1.

1135del174nt) mutation was observed in 4 (7%) of 56 mutant alleles. Rapid assays have not yet been designed for any of these mutations.

Amish/Mennonite Mutations

The mutation in the original Amish family used to localize the A-T gene (Gatti et al. 1988) was a deletion of AG at position 1563 in exon 12 (codon 522) (Savitsky et al. 1995*a*). PCR primers flanking the mutation were designed from genomic DNA sequence. The remaining 71 members of the pedigree were screened for the mutation by use of heteroduplex analysis (fig. 7). The three affected individuals in the pedigree were found to be homozygous for this mutation, as well as for all adjacent markers, as expected. All expected heterozygotes (Gatti et al. 1988; Lange et al. 1993) were confirmed by use of the heteroduplex assay.

We also screened three patients, from two families of Mennonite background, who were residents of British Columbia (Pohl et al. 1992) and Belize. We did not observe the 1563delAG (codon 522) mutation or the expected Amish haplotype in these families. Lymphoblastoid cell lines from these patients were further tested for radiosensitivity, and they showed the characteristic decreased postradiation colony survival of A-T cells (Huo et al. 1994) (data not shown). Subsequent PTT screening identified a second mutation, 5932G→T (codon 1973del88nt), which was common to both families. This mutation is described above in two Polish patients who shared a common haplotype. We found the same haplotype in the two Mennonite families of non-U.S. origin. Both Mennonite families have northern European

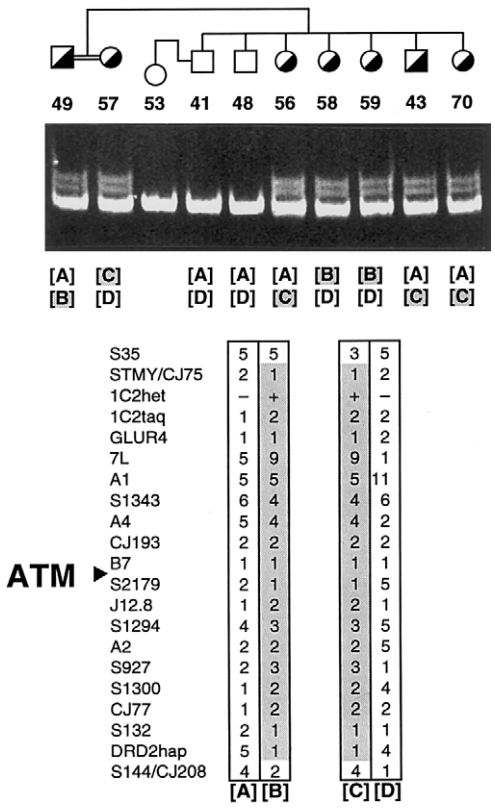

Figure 7 Heteroduplex and haplotype analysis of a consanguineous Amish family showing the inheritance of the 1563delAG mutation. Haplotype analysis has shown that the mutation is inherited through haplotypes B and C (Lange et al. 1993). Heteroduplex analysis confirms that any person in the family with either haplotype B or haplotype C is a carrier for the mutation.

surnames, and the data are compatible with the historical record of a Dutch-Polish Mennonite migration to southern Russia and then to North America at the end of the 18th century (Hostetler 1983, p. 17).

English Midlands Mutation: IVS40+1126A→G (codon 1921ins137nt)

This intriguing founder-effect mutation, affecting ~15% of U.K. patients, was described by McConville et al. (1996) in a group of 16 families (12 homozygotes, 6 compound heterozygotes) who shared a common "418" haplotype (defined by alleles 4, 1, and 8, respectively, of markers D11S1817, D11S1343, and D11S1819), as well as intermediate radiosensitivity, a milder phenotype, and lack of cancer susceptibility (Taylor et al. 1994). Although five families were Scottish (Taylor et al. 1994), all families traced their forebears to the Midlands region of England. An A→G transition within intron 40 creates a consensus splice-donor site (gtaaag→gtaagg) that, when used, results in the insertion of 137 nt into transcripts from this allele. However, de-

tectable amounts of normal transcript are also produced by this allele, with the potential to encode functional ATM protein. It has been suggested that this normal protein might be sufficient to moderate the phenotype (McConville et al. 1996). Using PTT, we screened 70 purportedly unrelated patients of various ethnic backgrounds, and we found two families with this mutation (data not shown); both had English family names. In one family, the onset of ataxia in two sibs occurred at 3 and 6 years of age; disease progress was slow. One sibling lived to the age of 44 years. In the other family, the age at onset of ataxia among the three affected sibs was early or typical, occurring at 8 mo, 3.5 years, and 9 mo; despite this, disease progress has been slow. Cancer has not been observed in either family. Thus, this fairly common English mutation may be associated with a mild phenotype and a low frequency of cancer, and this could distort the outcome of studies that screen cancer patients for this ATM mutation. Furthermore, special primers nested within intron 40 must be included to detect this mutation when one is screening genomic DNA.

Irish English Mutation: 7636del9nt (codon 2546)

This mutation in exon 54 has been described in 16 A-T patients (Savitsky et al. 1995*a;* Gilad et al. 1996*b*; McConville et al. 1996; Wright et al. 1996; present study), most of whom either lived in the United Kingdom or had Irish or English family names and lived in the United States or Australia. The mutation has also been observed in a Swedish breast cancer patient (Vorechovsky et al. 1996*a*). We have designed and tested a rapid assay for its detection. The 9-nt deletion removes a *Bfa*I site; two bands (78 bp and 32 bp) were observed in normal individuals, whereas patients with this mutation showed only a single band (101 bp) (data not shown). Using the same exon 54 primers used for detection of the IVS53-2A→C mutation seen in Polish patients, we observed this mutation in three additional A-T patients. We determined the haplotypes for 6 of the 16 patients with this mutation for whom DNA was available; 5 shared the same haplotype. Thus, our suggestion that this is a frequent mutation among unrelated A-T patients (Wright et al. 1996) now appears unlikely. More likely, it is primarily a founder-effect mutation of Irish English origin, comprising perhaps 10% of English patients. Using a similar rapid assay, Wright et al. (1996) failed to observe the mutation in >300 normal chromosomes.

Japanese Mutation

Gilad et al. (1996*b*) reported two ATM mutations in Japanese patients. One of these mutations, 7883del5nt (codon 2628), was homozygous in one patient and heterozygous in the second. We detected one copy of this mutation in one additional patient of Japanese ancestry. Thus, in a very limited survey, 7883del5nt accounts for four of six mutant alleles described in the Japanese population. Haplotypes have not been compared for a founder effect. This mutation is easily detected by use of either SSCP or heteroduplex analysis.

Utah Mormon Mutations

We have identified three unique mutations in three patients of Utah Mormon background. These patients do not share haplotypes. One patient was homozygous for IVS32-12A→G (codon 1537ins11nt); her mutation resulted in the production of at least four transcripts, all of which had an insertion of 11 nt in exon 33. In three of the transcripts, we also observed exon skipping of one or two exons (i.e., either exon 34 or exons 34 and 35). Two other patients were heterozygous for the following mutations: 8494C→T (codon 2832) and IVS62+1G→A (codon 2891del115nt).

African American Mutations

Four mutations have been identified in two African American patients. One patient was heterozygous for mutations IVS16-10T→G (codon 750ins9nt) and 2810insCTAG (codon 937); the other patient, previously described by Wright et al. (1996), was heterozygous for mutations 7327C→T (codon 2443) and 7926A→C (codon 2544del298nt).

Discussion

Since >200 ATM mutations in A-T patients have been defined, we can now realistically assess the mutation-detection methodology for this large gene. Virtually all patients who are not of a consanguineous background are compound heterozygotes. Few, if any, hot spots exist in the gene. Most patients express transcripts for both mutated alleles (Telatar et al. 1996). Approximately 70% of reported ATM mutations would result in a truncated protein. However, it remains unclear whether mutated ATM proteins, truncated or otherwise, are stable in most A-T cells.

A wide array of mutation-detection techniques have been tested. Since the gene is so large (~150 kb), most studies have used cDNA as the starting template. To date, the single most effective method of screening cDNA has been the PTT. We have detected 92 mutations in 140 chromosomes (66%). We have used conformation-sensitive gel electrophoresis, as a complementary method, to screen 600–800-nt segments of ATM cDNA. This has yielded ~35 mutations/100 chromosomes screened, including some that were identified by PTT as well (Liang et al., unpublished data). Several laboratories have used restriction-endonuclease fingerprinting to

screen cDNA segments of 1,000–2,000 nt, with good success (Liu and Sommer 1995; Savitsky et al. 1995*a;* Byrd et al. 1996; Gilad et al. 1996*b*). However, because all ATM exons are <372 nt (exon 12), none of these methods are optimal or, in some cases, applicable to screening genomic DNA. These assays also vary widely in cost; PTT and SSCP cost ~$100–$150/patient, as compared to ~$2,000–$4,000/patient for direct sequencing of the entire cDNA. Of course, these estimates also depend on the stage of the analyses at which mutations can be identified and at which testing can be terminated. The rapid assays described herein can be used to screen batches of 20–50 gDNAs, at a cost of <$5 each (not including the costs of DNA isolation).

The current study reflects an alternative approach to the technical challenges of population screening for A-T carriers. To circumvent the problem of testing prospectively for the many possible ATM mutations in carriers, we have sought to identify and to determine the frequencies of mutations in ethnic populations where founder effects or common mutations may be present. With this information and with the rapid genomic DNA–screening assays described in this report, we believe that carrier screening can be initiated in these limited populations.

On the basis of the mutations described above in A-T patients of common ethnic backgrounds, we investigated the spectrum of ATM mutations in 10 populations. Common mutations were found in Norwegians, Costa Ricans, Poles, Italians, and Japanese (on the basis of a small sample of Japanese patients). Rapid genomic screening assays were developed that detect 76% of mutant ATM alleles in Costa Ricans, 50% in Norwegians, 27% in Poles, and 14% in Italians. The mutation described most commonly in the literature, 7636del9, probably constitutes a founder effect (see above). It can be detected by either of two rapid assays (Wright et al. 1996; present study).

In the Amish population, our failure to find either the common haplotype or the 1563delAG (codon 522) mutation in Mennonite patients who were not members of the immediate Pennsylvania/Ohio/Indiana extended family was surprising. Of further interest, when all other A-T patients were tested with the rapid gDNA assay for this mutation, it was found in a non-Amish American family and in a Turkish family. Byrd et al. (1996) have also reported this mutation, in a family in the United Kingdom. Thus, the 1563delAG (codon 522) mutation accounts for 2% of the mutations described to date worldwide; however, haplotypes of these patients have not yet been compared to determine how many of them share common ancestry. We also found a different mutation, 5932G→T (codon 1973del88nt), in two non-U.S. Mennonite families and in two Polish patients, with shared haplotypes in all four families.

Each rapid assay allows large-scale screening of A-T homozygotes with as yet unidentified mutations, and these assays may be especially useful in analyzing patients of known ancestry. We have detected the Norwegian and Italian mutations in some American families of these ancestries, and this approach has allowed us to rapidly determine the frequency of particular mutations across a large sample of A-T homozygotes. Nonetheless, we and others are still detecting primarily new mutations, and we do not yet appear to be reaching a point of saturation for new mutations.

Savitsky et al. (1995*a*) have provided strong evidence against the existence of genetic heterogeneity. During the positional cloning of the ATM gene, an international A-T consortium genotyped >200 families across >30 cM at chromosome 11q22-23 (Lange et al. 1995). Approximately 25 of those families were set apart for one reason or another, usually because one member of the family either had or lacked a clinical feature, calling the status of the entire family into question. Such families had to be prospectively excluded from linkage analyses. Seven of the 176 families with firm diagnoses did not link to 11q23.1 (Gatti et al. 1994; Lange et al. 1995). One of these seven, CRAT 10 (Uhrhammer et al. 1995), now links, since a young child with two affected haplotypes has subsequently developed ataxia. Of the other six, and among many of the 25 "variant" families, at least one ATM mutation has been identified in approximately half. Several patients with ATFresno (Curry et al. 1989) also harbor mutations in the ATM gene. Thus, it appears unlikely that mutations in other genes will account for more than a very small fraction of patients with the characteristic A-T syndrome (Boder and Sedgwick 1958; Boder 1985; Sedgwick and Boder 1991; Woods and Taylor 1992; Lange et al. 1993; Bundey 1994; Gatti 1997).

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