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ATM mutations are rare in familial chronic lymphocytic leukemia

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It is now recognized that a subset of B-cell chronic lymphocytic leukemia (CLL) is familial. The genetic basis of familial CLL is poorly understood, but recently germ line mutations in the Ataxia Telangiectasia *(ATM)* gene have been proposed to confer susceptibility to CLL. The evidence for this notion is, however, not unequivocal. To examine this proposition further we have screened the *ATM* gene for mutations in CLLs from 61 individuals in 29 families. Truncating *ATM* mutations, including a known *ATM* mutation, were detected in 2 affected individuals, but the mutations did not cosegregate with CLL in the families. In addition, 3 novel *ATM* missense mutations were detected. Common *ATM* missense mutations were not overrepresented. The data support previous observations that *ATM* mutation is associated with B-CLL. However, *ATM* mutations do not account for familial clustering of the disease. (Blood. 2002;100:603-609)

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Introduction

Leukemia affects between 1% and 2% of Western populations.[1] B-cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia, accounting for around 30% of cases.[2] The incidence rate of CLL increases logarithmically from age 35 with a median age at diagnosis of 65 years.[3]

Epidemiologic studies and published case reports of families indicate that a subset of the CLL is ascribable to an inherited genetic predisposition. A dominantly acting gene or genes with pleiotropic effects appear to be the most likely genetic model of inheritance since CLL appears to segregate with other lymphoproliferative disorders (LPDs) in many families.[4] However, no gene has been shown unequivocally to be causative.

Ataxia Telangiectasia (A-T), is one of a group of recessive syndromes characterized by excessive spontaneous chromosomal breakage associated with an elevated risk of hematologic malignancies.[5] Specifically, in A-T there is an increased risk of lymphomas and leukemias, including those in the B-cell lineage.[6]

The Ataxia Telangiectasia *(ATM)* gene maps to chromosome 11q23[7] and specifies a 12-kilobase (kb) mRNA encoding an approximately 350-kd protein.[8] The 3′ end of the gene has some homology to phosphoinositide 3–kinases and to *Saccharomyces cerevisiae* telomerase 1 (TEL1) that controls telomere length and maintenance of genome integrity.[9] Homozygous germ line mutations in *ATM* are associated with increased radiosensitivity and genomic instability. The mutation rate is increased, double-strand breaks show reduced rejoining fidelity, there is a high frequency of cytogenetic rearrangements, and homologous recombination is increased and error prone.[10-13]

A-T cells also show defects in cell-cycle checkpoints. These properties provide the rationale for proposing a model[14] in which *ATM* has a role as a DNA damage-response gene probably responding to a subset of DNA double-strand breaks.

There is evidence that A-T carriers may display an increased cancer risk.[15] Some reports have suggested that A-T heterozygotes display an increased chromosomal instability and hypersensitivity to carcinogens, thereby increasing the probability of acquiring oncogenic somatic mutations.[16-18] The relationship between cancer risk and A-T heterozygosity has been most extensively studied in relation to breast cancer.[19] CLL has been reported in A-T families,[15] suggesting that heterozygosity may also confer an increased risk of this disease. In a retrospective study of cancer incidence in 110 A-T families, the risk of hematologic and lymphoid malignancies was increased in blood relatives of A-T patients and CLL accounted for all but one of the leukemias seen in adult blood relatives. However, these observations did not attain statistical significance. It has recently been shown that the *ATM* gene is mutant in approximately 20% of CLL samples from unrelated patients and that some patients have heterozygous germ line mutations.[20-23]

The prevalence of B-cell malignancy in A-T coupled with the possible increase in risk of leukemia in relatives of A-T patients has led a number of researchers to question whether germ line *ATM* mutations are involved in familial cases of CLL. In a small study of 32 CLL patients, Stankovic et al[20] reported that 6% of cases harbored germ line *ATM* mutations.[20] Bevan et al[24] recently examined the role of *ATM* in familial CLL through a linkage analysis. While there was no evidence for linkage, the study did not preclude *ATM* underlying a subset of familial CLL.

ATM represents an attractive candidate CLL predisposition gene but direct evidence for its role is lacking. To investigate further the relationship between *ATM* and CLL we have screened tumor samples from 61 individuals in 29 CLL families for mutations.

Patients, materials, and methods

Patient selection

Families with 2 or more individuals affected with CLL were ascertained through hematologists in the United Kingdom, Norway, Israel, Italy,

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Table 1. Sequences of primers flanking *ATM* exons

ATM exon	Primer names	Forward sequence (5′→3′)	Reverse sequence (5′→3′)
3	AT3F/R	TCC TCT GAG ATA TAT GTT CCC TGA C	GCT CAT TCA CTG ATA GAT GCA AA
4	AT4F/R	TTC ACA CCT CTT TCT CTC TAT ATA TGC	TCA CAC ATT TCA AGG AAA AAT TG
5	AT5F/R	TCA ATT TTT CCT TGA AAT GTG TG	TGC AAG GCA TAA TGA TAT ATA GGA A
6	AT6F/R	GCT CTT TGT GAT GGC ATG AA	AAA AAA AAA AAA AAC TCA CGC G
7	AT7F/R	AGT TGC CAT TCC AAG TGT CTT	GGT GAA GTT TCA TTT CAT GAG G
8	AT8IF/R	CCT TTT TCT GTA TGG GAT TAT GGA	CCG TCA GTC TGA GAA CAG CA
8	AT8IIF/R	CAT GCT GTT ACC AAA GGA TGC	TGA TGG ATC AAT GTT TTA TTT TAA GG
9	AT9F/R	CCC CCT GTT ATA CCC AGT TG	TGA ATG AAG AAG CAA ATT CAA AA
10	A10F/R	GGA GCT AGC AGT GTA AAC AGA	AAA TGT GAC ATG ACC TAC TTA CTG
11	A11F/R	GAT ACG AGA TCG TGC TGT TCC	GGA TTC CAC TGA AAG TTT TCT G
12	AT12IF/R	TCC TTT TAG TTT GTT AAT GTG ATG GA	CCC TGA ATT ATG GCT CCA AG
12	AT12IIF/R	TTT GGT GTA TTA CCT TTC GTG GT	TGA AAA TGA TCA GGG ATA TGT GA
13	AT13F/R	GCT TGC TTT TCA CAA TTG TCC T	AAA CAG CAG CAT GCT AAT GAA C
14	AT14F/R	TTC TTT ACA TGG CTT TTG GTC T	TAA GAT GCA GCT ACT ACC CAG C
15	AT15IF/R	GGC AAA GCA TTA GGT ACT TGG	TGG TGG ACA GAG AAG CCA AT
15	AT15IIF/R	ATT GGC TTC TCT GTC CAC CA	TTC CTG CCC CTA TTT CTC CT
16	AT16IF/R	CCA GGA TAT GCC ACC TTT AAC	TCC TAC CTT GGC TTT CTG GA
16	AT16IIF/R	GTC CGG TGT TCA CGT CTT TT	AAG AGA AAG GGT TAA CCT GCA T
17	AT17F/R	TAA AAA GCA ATA CTA AAC TA	ATG AGT TGT GAC AAT CCC ACT G
18	AT18F/R	TCT GCC GAG AAT AAT TGT TTT T	TGT TGT GAG ATG CAT CCT TAT T
19	AT19F/R	TGA CTA CAG CAT GCT CCT GC	CAA TGA GGC CTC TTA TAC TGC C
20	AT20F/R	TGT GCC CTT CTC TTA GTG TT	TCC AAG AGC TTC TTC ATT TAA C
21	AT21F/R	AAA TGA TTT GTG GAT AAA CCT GAT T	TGG TCA CGA CGA TAC AAA GAA
22	AT22F/R	AAT AAC TGA TGT GTT CTG TTA AGC	GCA AAA GAA AAA TAG GAC CA
23	AT23F/R	TCA GTG AGT TTT CTG AGT GCT TTT	ACT CAT TAA CAA ACA AAG ACT GCT TTA
24	AT24F/R	TTA ACC ACA GTT CTT TTC CCG TA	CCA TCT GCA GCA TTC CAA ATA
25	AT25F/R	ATG CTT TGG AAA GTA GGG TTT G	TAT GGG ATA TTC ATA GCA AGC A
26	AT26F/R	TGG AGT TCA GTT GGG ATT TT	TTC ACA GTG ACC TAA GGA AGC
27	AT27F/R	TTT TTC TTA ACA CAT TGA CTT TTT GG	CTG GTG AGG GGA CTT GCT AA
28	AT28IF/R	TTA ATG CTG ATG GTA TTA AAA CAG	CTT TTC CAG TCC TCT TGA ATC
28	AT28IIF/R	TTC AAG AGG ACT GGA AAA GTC	CAT TCA GGG AAT GAA AAG TAC A
29	AT29F/R	TGC CTT TTG AGC TGT CTT GA	GAC ATT GAA GGT GTC AAC CAA
30	AT30F/R	TTT TCA TTT TGG AAG TTC ACT GG	AAA TTT TTC ATT TGT TTA CTT TTC CTC
31	AT31IF/R	AAG TGT ATT TAT TGT AGC CGA GT	CTC CTC CTA AGC CAC TTT TT
31	AT31IIF/R	AAA AAG TGG CTT AGG AGG AG	CGG ACA GAG TGA GTC TTT GT
32	AT32F/R	TTT TCC AGA ACT TAC TGG TTG TTG	CCA TTT TGA AGA TGA GTC AGA AAA
33	AT33F/R	TGG CTT ACT TTA AAA TTA TTT CTC TCC	TGC TAG AGC ATT ACA GAT TTT TGA A
34	AT34F/R	AAC CAA TAC GTG TTA AAA GCA AGT T	CAG GTA GAA ATA GCC CAT GTC A
35	AT35F/R	CAA AAA GTG TTG TCT TCA TGC T	TAT GTG ATC CGC AGT TGA CTG
36	AT36F/R	TGA TCT CTT ACC TAT GAC TCT ACT GA	TGT GAA GTA TCA TTC TCC ATG A
37	AT37F/R	TTT GAA ATT TTT TCA GTG GAG G	TTT AAC AGT CAT GAC CCA CAG C
38	AT38F/R	TAC ATT TTC TAA TCC CTT TCT TTC	TGC AGT ATC ACA GCA CTC TTT
39	AT39F/R	ACT ATT GGG TGG ATT TGT T	CCA TCT TAA ATC CAT CTT TC
40	AT40F/R	CTG GGA CTG AGG GGA GAT A	CAT GTT AAA ATT CAG CCG ATA GTT
41	AT41F/R	CTA CCA TTG TAT TCT ATA TCA ACA TGC	TGC CAA CAT TAA TTT TAA AAA CC
42	AT42F/R	CAG GAG CTT CCA AAT AGT ATG T	GGC ATC TGT ACA GTG TCT ATA A
43	AT43F/R	TTG GGA GTT ACA TAT TGG TAA TGA	GCT TTG GGT TTT ACA CAC ACA T
44	AT44F/R	TTG CAT TTT TCT AAA CAA CGG TA	CCA ACA TAC TGA AAT AAC CTC AGC
45	AT45F/R	TCT CTG GTT TTC TGT TGA TAT C	CAG TTG TTG TTT AGA ATG AGG A
46	AT46F/R	AAA TTT TGT CCT TTG GTG AAG C	TTT CAG AAA AGA AGC CAT GAC A
47	AT47F/R	ATT TCC CTG AAA ACC TCT TCT T	GGT AAC AGA AAA GCT GCA CTT T
48	AT48F/R	CAT TTC TCT TGC TTA CAT GAA CTC	AGT CAA GTG GTA AGA TGA CAT AGT
49	AT49F/R	GCT GCT TTC ATT ATT ATT ATT CAT GGT	CAG TAA AAC ACT AAT CCA GCC AAT
50	AT50F/R	GGG CAG TTG GGT ACA GTC AT	GAT CTT GAT GAA AAG ATG AAG CA
51	AT51IF/R	AAA TTG GTT GTG TTT TCT TGA	ACT TCC TCT TTG GCT CTT TT
51	AT51IIF/R	CTC ATT AGC CCG GTT TTC AG	GAC CAA GTC ACT CTT TCT ATG CAA
52	AT52F/R	TGT TAA AGT TCA TGG CTT TTG TG	TTG GGC TGA GTA ACA CTT GC
53	AT53F/R	TTG CTT AGA TGT GAG AAT ATT TGA A	TTG TGG TTT GAT TTT CAG GTT T
54	AT54F/R	TGC AGG CAT ACA CGC TCT AC	TGC ATT TGC TAA GGC CAG TA
54	AT54IIF/R	GAT CAC CCC CAT CAC ACT TT	CCA GCC TTG AAC CGA TTT TA
55	AT55F/R	TCT GAG AAG TTT AAA TGT TGG GTA	GAG TAA CAC AGC AAG AAA GTA ACG
56	AT56F/R	CTT GAC CTT CAA TGC TGT TCC	TGC CAA TAT TTA GCC AAT TTT G
57	AT57F/R	TTT AAG TGC AAA TAG TGT ATC TGA CC	AAG GGC TAA GCC AGA GAA GG
58	AT58F/R	GAG TGC CCT TTG CTA TTC TCA	CCA ACC AAA TGG CAT CTT TT

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Table 1. Sequences of primers flanking *ATM* exons (continued)

ATM exon	Primer names	Forward sequence (5′→3′)	Reverse sequence (5′→3′)
59	AT59F/R	GAT AGC TGA ATG ATC ATC AAA TG	AGC TGT CAG CTT TAA TAA GCC A
60	AT60IF/R	CTG TTC ATC TTT ATT GCC CCT A	ACT GCG CGT ATA AGC CAA TC
60	AT60IIF/R	GAA GTC TTC ATG GAT GTT TGC	ATC CCC CTG CAA CTC AGA AT
61	AT61F/R	CTC AAC ATG GCC GGT TAT G	CAA ACA ACA TTC CAT GAT GAC C
62	AT62F/R	AGC TGT CAA ACC TCC TAA CTT CA	CCC AGC CCA TGT AAT TTT GA
63	AT63F/R	TTG ACA ACA TTG GTG TGT AAC A	GCC ACA TCC CCC TAT GTT AA
64	AT64F/R	CCA TGT GAC TGG CTT ATT TGT ATG	TGA AAA ACT GAC AAC AGG ACC TT
65	AT65F/R	TCA CCT CAC TGA AAC CTT TGT G	AAT TTC TAA AGG CTG AAT GAA AGG

Germany, Portugal, and Poland. The diagnosis of CLL in all cases was based on standard hematologic and immunologic criteria. Samples were obtained from family members with informed consent and ethical review board approval from the Royal Marsden Hospital's National Health Service Trust. DNA was salt extracted from peripheral EDTA venous blood samples using a standard sucrose lysis method. No separation of tumor B cells from other cells was undertaken prior to DNA extraction.

Mutation detection

The 63 coding exons and exon 2 of *ATM* were analyzed by polymerase chain reaction (PCR) amplification of DNA and single-strand conformational polymorphism (SSCP) electrophoresis of ^{32}P–deoxycytosine triphosphosphate (dCTP)–labeled products through glycerol polyacrylamide gels as described,[25] except that primers were redesigned to ensure that PCR products were approximately 250 base pairs or shorter. Table 1 details the nucleotide sequences of each of the 65 sets of PCR primers. Samples with bandshifts were sequenced with the same PCR primers in forward and reverse directions using the Li-Cor method (MWG, Milton Keynes, United Kingdom; http://www.mwg-biotech.com). Identified nucleotide changes were coded according to the genomic nucleotide sequence at GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) accession no. U82828 for introns and NM 000051 for each exon (renumbered with base 190 corresponding to base 1 in order to provide data in the same form as that used in the Virginia Mason *ATM* Mutation Database).[26]

Results

Twenty-nine families with 2 or more affected individuals with CLL were studied. Table 2 shows the pedigree structure of each of the families and the ages at diagnosis of CLL in family members.

DNA from blood samples was examined for nucleotide changes in the *ATM* gene by PCR-SSCP. All coding exons were examined using primers in the flanking introns or, where an exon was long, using 2 pairs of primers ensuring the middle 2 primers amplified overlapping segments of the exon.

The *ATM* nucleotide changes detected in the 61 affected individuals from the 29 CLL families are detailed in Table 2. Allele frequencies estimated for each of the mutations detected in the study are given in Table 3. This table also shows the allele frequencies of each of the mutations reported in published studies together with reported designations of each nucleotide change. In some instances different authors have interpreted the same nucleotide change as a mutation, a polymorphism, or present in a carrier. To be consistent we have classified nucleotide changes as "substitution" or "truncating" (leading to amino acid substitution or to truncation of the *ATM* polypeptide) or other (outside exons and their splice site consensus sequences or within an exon but not leading to change in amino acid usage).

In the samples analyzed, 11 nucleotide changes were identified that have not previously been reported. Altered SSCP patterns corresponding to these nucleotide changes are shown in Figure 1. Three of the nucleotide changes lead to amino acid substitutions. Two of the nucleotide changes lead to truncation of *ATM* due to either an insertion or deletion of 2 base pairs within respective exons of the gene. Of the 7 other nucleotide changes detected, 2 result in no change in amino acid usage and 5 were intronic nucleotide changes residing at least 27 base pairs away from intron-exon boundaries. No nucleotide changes identified created or destroyed consensus splice sites.

Among previously unreported nucleotide changes, intervening sequence (IVS)4-36insTG was the most common in this series, detected in 10% (12/120) of alleles. The abnormal band pattern due to IVS4-36insTG was only detected when autoradiograms were briefly exposed to film (~15 minutes). The pattern involved a new dark band virtually comigrating with a normal dark band. There were no detectable new bands among the less intense bands. The subtle nature of change in bands associated with IVS4-36insTG may explain why this common polymorphism has not previously been detected. The allelic frequencies of other previously unreported nucleotide changes were less than 4%.

In 61 of the samples analyzed, 9 amino acid substitutions or truncating changes were detected that might affect the function of the *ATM* protein. Six of these changes were observed in single cases but for none of these cases was the same change seen in any affected relative. Among these 6 changes, 2 were detected in the same case: 5756delAA and 7271insGT were detected in an affected sibling in family 43. Figure 1 shows the comparative relative intensities of the exon 40 and exon 51 bands corresponding to these mutations. Examination of autoradiograms indicates that 5756delAA constituted around 20% of the sample while 7271insGT constituted about 50% of the sample. Two amino acid substitutions, 378T>A (Asp126Glu) and 5042T>C (Ile1681Thr) were each detected in 2 affected individuals. 378T>A was detected in the index case of family 63, but not in his affected sibling, and in the index case of family 98, but not in his affected sibling. 5042T>C was detected in 2 of the 3 affected siblings in family 5. One mutation, (5557G>A), which leads to an amino acid substitution (Asp1853Asn), was detected in 15 alleles giving a frequency comparable to that previously reported in healthy individuals from the general population.

Discussion

The hypothesis that *ATM* represents a predisposition locus for CLL is attractive a priori. Strong evidence for such a notion would be provided by cosegregation in families of microsatellite markers and of mutations. However, this depends on *ATM* mutations conferring a markedly elevated risk. Furthermore, the power of any set of families to demonstrate linkage will be severely reduced if the disease is genetically heterogeneous. If *ATM* only confers modest

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Table 2. *ATM* nucleotide changes detected in familial CLL cases

Family	CLL case	Age at diagnosis, y	*ATM* nucleotide changes	
			Truncating or amino acid substitution	Other
5	Father	nk	ne	ne
	Uncle	nk	ne	ne
	Sister	54	5042T>C (Ile1681Thr)	IVS25-13insA†; 4578C>T‡; IVS63+60G/A‡
	Brother	46	nd	IVS4-36insTG; IVS25-13insA†; 4578C>T; IVS63+60G/A‡
	Brother	47	5042T>C (Ile1681Thr)	IVS25-13insA†§
6	Mother	69	nd	IVS25-13insA†; IVS40+27G/A; IVS63+60G/A‡
	Daughter	42	5557G>A (Asp1853Asn)‡	IVS4-36insTG§; IVS24-10delT; IVS25-13insA†; IVS63+60G/A‡
	Son	40	5557G>A (Asp1853Asn)‡	IVS4-36insTG§; IVS24-10delT; IVS25-13insA†; IVS63+60G/A‡
8	Brother	70	6919C>T (Leu2307Phe)	IVS63+60G/A‡§; IVS25-13insA†
	Brother	76	nd	IVS25-13insA†; IVS63+60G/A‡
	Brother	nk	ne	ne
11	Sister	69	nd	nd
	Sister	61	nd	nd
13	Sister	70	2572T>C (Phe858Leu)‡	IVS25-13insA†; IVS63+60G/A‡
	Brother	58	nd	162T>C§; IVS25-13insA†; IVS63+60G/A‡
15	Brother	63	8266A>T (Lys2756Xaa)‡	IVS25-13insA†; 4578C>T; IVS63+60G/A‡
	Sister	61	nd	IVS25-13insA†§
18	Sister	74	nd	IVS25-13insA†; IVS63+60G/A‡
	Sister	73	nd	IVS25-13insA†; IVS63+60G/A‡
20	Brother	64	nd	IVS25-13insA†; IVS63+60G/A‡
	Brother	66	nd	IVS63+60G/A§‡
39	Sister	60	5557G>A (Asp1853Asn)‡	IVS24-10delT; IVS25-13insA†; IVS63+60G/A‡
	Sister	51	4258C>T (Leu1420Phe)‡	IVS4-36insTG§; IVS63+60G/A‡§
	Sister	nk	ne	ne
40	Brother	71	nd	IVS25-13insA†§
	Sister	48	nd	IVS25-13insA†; IVS63+60G/A‡
41	Brother	35	nd	IVS25-13insA†; IVS63+60G/A‡
	Sister	51	nd	IVS25-13insA†§
42	Uncle	59	nd	IVS25-13insA†§
	Second cousin	43	nd	IVS25-13insA†; IVS63+60G/A‡
43	Sister	72	nd	nd
	Sister	67	5756delAA; 7271insGT	nd
45	Brother	61	nd	IVS25-13insA†; IVS63+60G/A‡
	Sister	52	nd	IVS25-13insA†§
46	Sister	61	nd	IVS62+8A/C
	Sister	59	nd	IVS62+8A/C
48	Sister	45	nd	IVS25-13insA†; IVS63+60G/A‡
	Sister	51	nd	IVS25-13insA†; IVS63+60G/A‡
49	Brother	69	nd	IVS25-13insA†§
	Sister	80	nd	IVS25-13insA†; IVS63+60G/A‡
60	Brother	55	nd	nd
	Sister	49	nd	nd
63	Brother	57	378T>A (Asp126Glu)	IVS25-13insA†
	Sister	43	nd	IVS25-13insA†; IVS63+60G/A‡
64	Brother	62	5557G>A (Asp1853Asn)‡	IVS15-48T/C; IVS24-10delT
	Brother	64	5557G>A (Asp1853Asn)‡§	IVS16+78G/A
78	Uncle	54	nd	IVS25-13insA†§
	Uncle	50	ne	ne
	Nephew	32	nd	IVS4-36insTG§; 4578C>T; IVS63+60G/A‡§
85	Brother	60	nd	IVS25-13insA†; IVS63+60G/A‡
	Brother	49	2572T>C (Phe858Leu)	nd
96	Brother	65	5557G>A (Asp1853Asn)‡	IVS24-10delT; IVS25-13insA†§
	Brother	61	nd	IVS4-36insTG§; IVS63+60G/A‡§
98	Brother	67	378T>A(Asp126Glu); 2572T>C (Phe858Leu) 5557G>A (Asp1853Asn)‡	IVS16+78G/A; IVS24-10delT; IVS25-13insA†
	Brother	64	5557G>A (Asp1853Asn)‡	IVS4-36insTG; IVS16+78G/A; IVS24-10delT; IVS25-13insA†
115	Brother	53	2572T>C (Phe858Leu); 5557G>A (Asp1853Asn)‡	IVS24-10delT
	Brother	51	5557G>A (Asp1853Asn)‡	IVS24-10delT; IVS63+60G/A‡§
117	Sister	65	nd	nd
	Sister	64	nd	nd

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Table 2. *ATM* nucleotide changes detected in familial CLL cases (continued)

Family	CLL case	Age at diagnosis, y	*ATM* nucleotide changes	
			Truncating or amino acid substitution	Other
118	Father	79	nd	nd
	Daughter	46	nd	IVS63+60G/A‡
138	Sister	64	5557G>A (Asp1853Asn)‡	IVS24-10delT; IVS25-13insA†§
	Brother	74	nd	IVS25-13insA†
162	Brother	54	5557G>A (Asp1853Asn)‡	IVS24-10delT; IVS25-13insA†; IVS63+60G/A‡
	Brother	61	5557G>A (Asp1853Asn)‡§	IVS25-13insA†§

ne indicates not examined; nd, not detected; and nk, not known.
†Nucleotide change reported at Genbank (http://www.ncbi.nlm.nih.gov/).
‡Nucleotide change reported at Virginia Mason *ATM* mutation database (http://www.vmresearch.org/atm.htm).
§Apparent homozygosity.

risks, mutations will not generate multiple-case families and linkage will not be detected, especially if phenocopies are common. There should, however, be evidence of overrepresentation of mutations in cases compared to the general population. The mutation analysis reported here on the *ATM* gene in tumor samples from 61 individuals in 29 CLL families was undertaken to assess further the question of whether *ATM* represents a predisposition locus for CLL.

In our study, 2 of the 61 patients with CLLs in the 29 families had truncating *ATM* mutations. One patient with CLL had 8266A>T (Lys2756Xaa). This mutation has been reported in A-T families.[33-35] The second patient with CLL had 2 truncating mutations, 5766delAA and 7271insGT. It is highly probable that just one of these mutations is somatic. To assess whether this class of *ATM* mutation confers an increase in risk of CLL per se, the observed number of mutations needs to be compared with that expected by chance. The number of mutations expected is not simply a function of the number of affected individuals analyzed but is also a function of the familial relationship between affected individuals within each family. The expected number of mutations computed on this basis is 0.46. Therefore, observation of 2 mutations equates to a 4.4-fold overrepresentation, albeit, nonsignificant (95% CI, 0.5-15.9). Stankovic et al[20] suggested that germ line mutations in *ATM* confer susceptibility to CLL following the finding that 2 of 32 sporadic CLL cases harbored constitutional mutations. One of the mutations identified was a truncating mutation and the other was a missense mutation (Pro1054Arg, 3161C>G) which the authors designated as pathogenic. The significance of this observation depends critically on the carrier frequency of pathogenic *ATM* mutations in the general population. Most estimates based on the prevalence of A-T suggest that the population carrier frequency of pathogenic mutations in *ATM* is around 1%.[36] Assuming this to be the case, then the observation of 2 mutations in 32 cases is significant ($P = .04$). Pro1054Arg has however, been designated as a polymorphism by some workers.[37] If this is the case, then the observation of 1 pathogenic constitutional mutation in 32 CLL cases does not constitute an overrepresentation ($P = .28$). However, combining this data with ours, the observation of 3 truncating mutations indicates overrepresentation at the 0.04 level.

It is conceivable that *ATM* may impact more significantly on the risk of CLL if some nontruncating mutations have pathogenic potential. Gatti et al[38] have recently proposed that cancer susceptibility may be associated not with truncating *ATM* mutations, but rather with missense *ATM* mutations. In the present study, 6 missense mutations were identified; 3 have been reported previously (Asp1853Asn, Phe858Leu, and Leu1420Phe) and 3 are new (Asp126Glu, Ile1681Thr, and Leu2307Phe). Asp1853Asn (5557G>A) was detected in 13 cases of CLL from 8 of the 29 families studied. Some evidence suggests that 5557A may be associated with a modest increase in cancer risk.[39] The evidence

Table 3. Allele frequencies of *ATM* nucleotide changes in this and previous studies and designation of nucleotide changes

Nucleotide change (amino acid substitution)	Allele frequencies		Type of nucleotide change	Reported designation of nucleotide change	Reference
	This study	Previous studies*			
IVS4-36insTG	12/122	—	Intronic		
162T>C	2/122	—	No substitution		
378T>A (Asp126Glu)	2/120	—	Substitution		
IVS15-48T/C	1/118	—	Intronic		
IVS16+78G/A	4/118	—	Intronic		
2572T>C (Phe858Leu)	4/120	0.02 (C)	Substitution	Polymorphism carrier mutation	27-29
IVS24-10delT	11/122	0.18 (delT)	Intronic	Polymorphism	30
IVS25-13insA	50/120	0.37 (ins A)	Intronic	Polymorphism	27
4258C>T (Leu1420Phe)	1/118	0.02	Substitution	Polymorphism carrier mutation	28, 29, 31
4578C>T	4/120	—	No substitution		
5042T>C (Ile1681Thr)	2/122	—	Substitution		
5557G>A (Asp1853Asn)	15/122	0.18(A)	Substitution	Polymorphism risk factor	27, 32
5756delAA	1/122	NA	Truncating		
IVS40+27G/A	1/122	—	Intronic		
6919C>T (Leu2307Phe)	1/122	—	Substitution		
7271insGT	1/122	NA	Truncating		
8266A>T (Lys2756Xaa)	1/122	NA	Truncating	Mutation	33-35
IVS63+60G/A	36/122	0.37(A)	Intronic	Polymorphism	

NA indicates not applicable.
*Only provided for *ATM* polymorphisms/variants defined in the Virginia Mason database (http://www.vmresearch.org/atm.htm).

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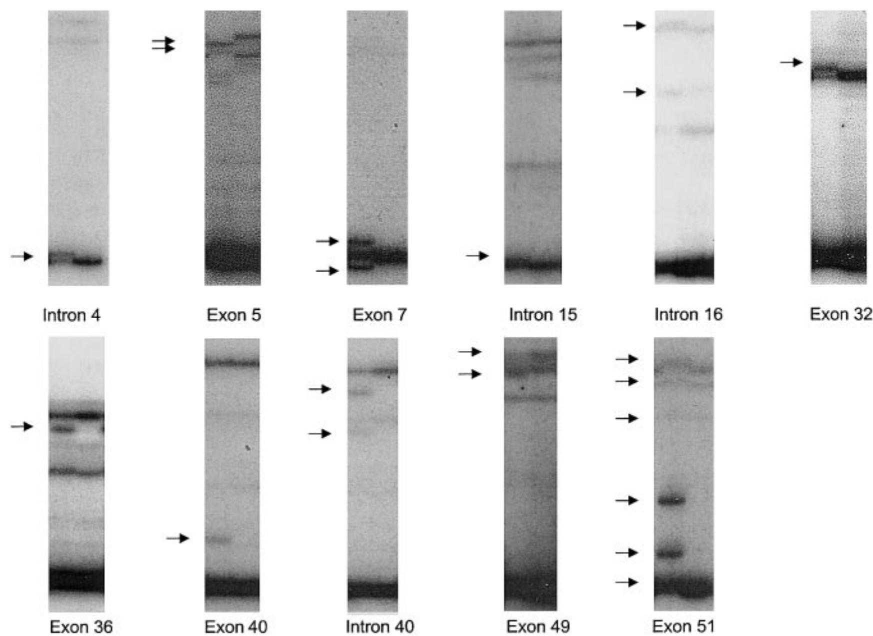

Figure 1. SSCP bandshifts corresponding to novel *ATM* nucleotide changes identified. Out of 61 samples, 11 novel nucleotide changes were identified. Bandshifts (arrowed) due to these nucleotide changes detected on autoradiograms after SSCP are shown adjacent to normal samples. The nucleotide changes were: IVS4-36insTG (intron 4); 162T>C (exon 5); 378T>A (exon 7); IVS15-48T/C (intron 15); IVS16 + 78G/A (intron 16); 4578C>T (exon 32); 5042T>C (exon 36); 5756delAA (exon 40); IVS40 + 27G/A (intron 40); 6919C>T (exon 49); 7271insGT (exon 51).

from our study does not support such a postulate in the context of CLL. Although in 4 families the CLLs from both affected individuals harbored at least one copy of this allele, in the other 4 families the mutation did not segregate with disease. Furthermore, there was no evidence of overrepresentation of the 5557A allele. Overall the frequency of the 5557A allele was comparable to the population estimate of 18%. 2572T>C (Phe858Leu) was detected in 4 unrelated individuals, failing to segregate with CLL. The frequency of the 2572C allele was also similar to the population estimate of 2% in published reports. The recent proposal[40] that 2572C only confers an elevated cancer risk in the presence of 5557A is not supported by our study as this combination was not observed to segregate with CLL. Asp126Glu (378T>A) was also not observed to segregate with CLL, being detected in 2 unrelated individuals. While the residue is conserved between human and murine ATM protein, 126Glu is unlikely to confer risk as the amino acid change is conservative. Ile1681Thr (5042T>C) was detected in 2 of the 5 affected individuals in family 5. 5042C is unlikely to confer risk as the amino acid change is conservative and Ile1681 is not conserved between human and murine ATM proteins.

Leu1420Phe (4258C>T) and Leu2307Phe (6919C>T) mutations were detected in single CLLs. Leu1420Phe (4258C>T) has been reported as conferring risk of A-T. Leu2307Phe is a nonconservative change and thus may confer risk of CLL. In the absence of robust functional assays for each domain of the multifunctional *ATM* protein it is difficult to specify pathogenicity of these variants. However, the absence of cosegregation of amino acid changes in the families we have studied implies that the nonconservative amino acid changes will by themselves confer small genotypic risks. If a sequence variant is detected in just one family member we cannot exclude in the present study the possibility that some mutations are somatic. As a result, the *ATM* sequence variants we report here may overrepresent the germ line variability of *ATM* in this collection of CLL samples.

As with most common cancers susceptibility to CLL is likely to exhibit genetic heterogeneity. Furthermore, excluding large multiple-case families segregating CLL in a clear mendelian fashion, nuclear families with small numbers of cases may display a degree of within-family heterogeneity whereby, in addition to phenocopies, affected individuals in the same family may be caused by different susceptibility genes.

We report additional data that provide support for previous observations that *ATM* mutations are associated with CLL. However, on the basis of the data presented here, *ATM* is unlikely to represent a major susceptibility gene for familial CLL as the familial relative risk ascribable to mutations is unlikely to be more than approximately 1.1. To determine whether specific *ATM* variants represent predisposition mutations, large cohorts of CLL patients and controls will be required. However, the spectrum and the frequency of *ATM* variants makes the evaluation of specific nucleotide changes as risk factors for CLL inherently difficult, as has been seen in studies seeking to establish *ATM* as a risk factor for breast cancer.

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Appendix

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