ATM germline heterozygosity does not play a role in chronic lymphocytic leukemia initiation but influences rapid disease progression through loss of the remaining *ATM* allele

Anna Skowronska,[1]* Belinda Austen,[1]* Judith E. Powell,[2] Victoria Weston,[1] David G. Oscier,[3] Martin J.S. Dyer,[4] Estella Matutes,[5] Guy Pratt,[1] Christopher Fegan,[6] Paul Moss,[1] Malcolm A. Taylor,[1] and Tatjana Stankovic[1]

[1]School of Cancer Sciences, University of Birmingham; [2]School of Health and Population Sciences, University of Birmingham; [3]Haematology Department, Royal Bournemouth Hospital, Bournemouth; [4]MRC Toxicology Unit, Leicester University, Leicester; [5]Institute for Cancer Research and the Royal Marsden NHS, London; and [6]Department of Haematology, University Hospital of Wales, Cardiff, UK

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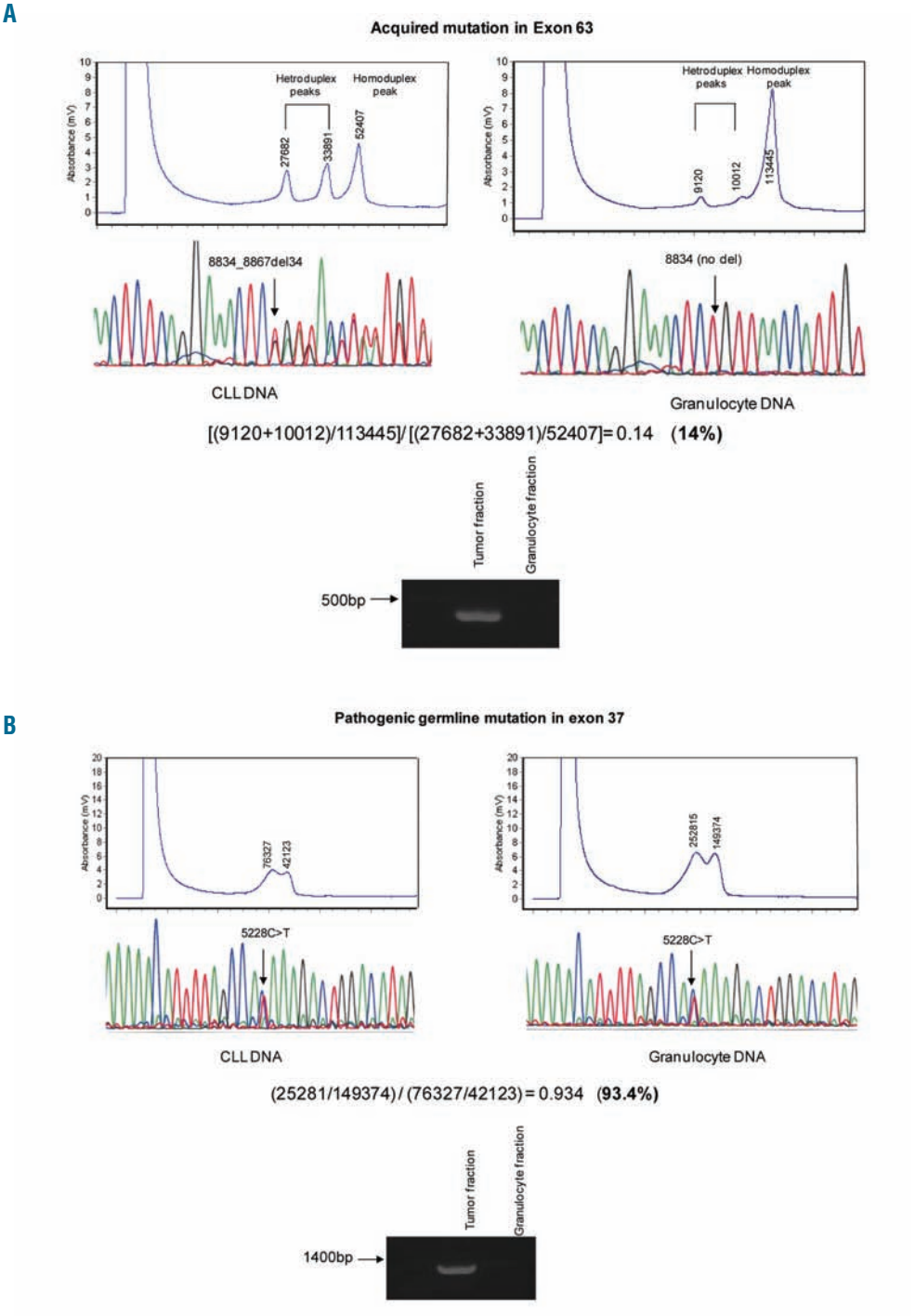

Online Supplementary Figure S1. Detection of acquired *ATM* sequence change using combined DHPLC and sequencing. (A) In the presence of a wild-type sequence, homoduplexes form a single peak pattern. If a sequence mismatch is present, heteroduplexes form a double, triple or quadruple peak pattern. To quantify the probability that a mutation was germline, the size of the heteroduplex peak area as a proportion of the homoduplex peak area in granulocytes DNA (germline) was compared with the same ratio in CLL DNA for the same individual. [(Area under heteroduplex peak / Area under homoduplex peak in granulocytes)/ (Area under heteroduplex peak / Area under homoduplex peak in CLL lymphocytes)]. A proportion above 90% was consistent with a germline mutation and below 40% with an acquired mutation. An example of an acquired mutation (8834_8867del34) in exon 63 is shown. A multiple peak pattern occurs only in CLL tumor cells and the relative proportion of heteroduplex peaks to homoduplex is just 14% confirming an acquired change. The absence of significant amplification in granulocyte DNA of clonal *IGH* VDJ rearrangement indicates no significant contamination of the granulocyte fraction with leukemia cells. (B) Amplification of *ATM* exon 37 produces a double peak pattern in both CLL tumor DNA and granulocyte DNA fractions in this patient. The ratio of the heteroduplex to homoduplex peak in the granulocyte DNA is 93.4% of the ratio of these peaks in the CLL DNA, confirming that the mutation is present in the germ line. Sequencing identifies the mutation as 5228C>T. The absence of amplification in granulocyte DNA and clonal *IGH* VDJ rearrangement in tumor DNA excludes contamination of granulocyte fraction with tumor cells.

Online Supplementary Table S1. Sequence changes found in CLL patients only.

	Sequence change	Amino acid change	Classification	Number of 11q CLL affected (n=140)	Number of non-11q CLL affected (n=178)
1	c.378T>A	p.(Asp126Glu)	Polymorphism	1	1
2	c.478_482delTCTCA	p.(Ser160fs)	Pathogenic Mutation	1	0
3	c.795T>C	No AA change	Polymorphism	2	0
4	c.998C>T	p.(Ser333Phe)	Polymorphism	1	1
5	c.1009C>A	p.(Arg337Ser)	Variant/Mutation	0	1
6	c.1048G>A	p.(Ala350Thr)	Variant/Mutation	0	1
7	c.1058_1059delGT	p.(Cys353fs)	Pathogenic Mutation	0	1
8	c.1066-6T>G	splicing site exon 11	Pathogenic Mutation	1	0
9	c.1120C>T	p.(Gln374X)	Pathogenic Mutation	1	0
10	c.1229T>C	p.(Val410Ala)	Variant/Mutation	0	1
11	c.1402delAA	p.(Lys468fs)	Pathogenic Mutation	1	0
12	c.1986T>C	No AA change	Polymorphism	1	0
13	c.2193delC	p.(Tyr731fs)	Pathogenic Mutation	0	1
14	c.2308G>T	p.(Glu770X)	Pathogenic Mutation	1	0
15	c.2417T>G	p.(Leu806Trp)	Variant/Mutation	1	0
16	c.2466+2T>G	splicing site exon 18	Pathogenic Mutation	0	1
17	c.2720_2723delGTGT	p.(Cys907fs)	Pathogenic Mutation	1	0
18	c.3284+6G>A	Non-coding	Polymorphism	0	1
19	c.3383A>G	p.(Gln1128Arg)	Variant/Mutation	1	0
20	c.3651delG	p.(Leu1217fs)	Pathogenic Mutation	1	0
21	c.3712_3716delTTATT	p.(Leu1238fs)	Pathogenic Mutation	1	0
22	c.3720_3736del17	p.(Asn1240fs)	Pathogenic Mutation	1	0
23	c.3883delCTT	p.(Lys1295del)	Pathogenic Mutation	1	0
24	c.4095_4109+4del19	p.(Lys1365fs)	Pathogenic Mutation	0	1
25	c.4591C>T	p.(Gln1531X)	Pathogenic Mutation	1	0
26	c.4802G>A	p.(Ser1601Asn)	Polymorphism	1	0
27	c.4947C>T	No AA change	Polymorphism	1	0
28	c.5006-2A>G	splicing site exon 36	Pathogenic Mutation	1	0
29	c.5224G>C	p.(Ala1742Pro)	Variant/Mutation	0	1
30	c.5228C>T	p.(Thr17431Ile)	Pathogenic Mutation	1	1
31	c.5352C>T	No AA change	Polymorphism	0	1
32	c.5857A>G	p.(Thr1953Ala)	Variant/Mutation	0	1
33	c.5882A>G	p.(Tyr1961Cys)	Variant/Mutation	1	0
34	c.5980A>G	p.(Lys1994Glu)	Variant/Mutation	1	0
35	c.6067G>A	p.(Gly2023Arg)	Variant/Mutation	2	0
36	c.6106T>A	p.(Tyr2036Asn)	Variant/Mutation	1	0
37	c.6375insT	p.(Glu2126fs)	Pathogenic Mutation	1	0
38	c.6815delA	p.(Glu2272fs)	Pathogenic Mutation	1	0
39	c.6820G>A	p.(Ala2274Thr)	Polymorphism	0	1
40	c.6989_6995del7	p.(Leu2330fs)	Pathogenic Mutation	1	0
41	c.7047C>G	p.(Cys2349Trp)	Variant/Mutation	1	0
42	c.7313C>A	p.(Thr2438Lys)	Variant/Mutation	0	1
43	c.7327C>G	p.(Arg2443Gly)	Variant/Mutation	1	0
44	c.7438C>T	p.(His2480Tyr)	Variant/Mutation	0	1
45	c.7638_7646del9del9	p.(Arg2547_Ser2549del)	Pathogenic Mutation	1	0
46	c.7883_7887del5	p.(Ile2628fs)	Pathogenic Mutation	1	0
47	c.8056T>C	p.(Phe2686Leu)	Variant/Mutation	1	0
48	c.8095C>T	p.(Pro2699Ser)	Variant/Mutation	0	1
49	c.8161G>A	p.(Asp2721Asn)	Variant/Mutation	1	0
50	c.8246_8252del7insT	p.(Lys2749_Thr2751delinsIle)	Pathogenic Mutation	1	0
51	c.8249T>C	p.(Leu2750ser)	Variant/Mutation	3	0

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52	c.8266A>T	p.(Lys2756X)	Pathogenic Mutation	0	1
53	c.8592C>T	No AA change	Polymorphism	0	1
54	c.8600G>A	p.(Gly2867Glu)	Variant/Mutation	1	0
55	c.8663T>C	p.(Ile2888Thr)	Variant/Mutation	0	1
56	c.8671+9T>G	Non-coding	Variant/Mutation	1	0
57	c.8672-1G>T	splicing site exon 62	Pathogenic Mutation	1	0
58	c.8672G>A	p.(Gly2891Asp)	Pathogenic Mutation	1	0
59	c.8834_8867del34	p.(Lys2945fs)	Pathogenic Mutation	0	1
60	c.8839A>T	p.(Thr2946Ser)	Variant/Mutation	1	0
61	c.8861A>G	p.(Tyr2954Cys)	Variant/Mutation	1	0
62	c.8977C>T	p.(Arg2993X)	Pathogenic Mutation	1	0
63	c.9022C>T	p.(Arg3008Cys)	Pathogenic Mutation	0	1
64	c.9023G>A	p.(Arg3008His)	Pathogenic Mutation	1	0
65	c.9032T>A	p.(Met3011Lys)	Variant/Mutation	0	1
66	c.9139C>T	p.(Arg3047X)	Pathogenic Mutation	1	0

Online Supplementary Table S2. Sequence changes present in the control group including changes common to both CLLs and controls.

	Sequence change	Amino Acid change	Classification	Controls affected % n=281	11q deleted CLL affected (11q)% n=140	non11q deleted CLL affected (no 11q) % n=178
1	c.146C>G	p.(Ser49Cys)	Polymorphism	1.4 (4)	0.7 (1)	0.6 (1)
2	c.162T>C	No AA change	Polymorphism	0.7 (2)	0	0
3	c.609C>T	No AA change	Polymorphism	0.4 (1)	0	0
4	c.735C>T	No AA change	Polymorphism	1.4 (4)	0.7 (1)	0
5	c.1727T>C	p.(Ile576Thr)	Variant/Mutation	0.4 (1)	0	0
6	c.1744T>C	p.(Phe582Leu)	Polymorphism	0.7 (2)	0.7 (1)	0
7	c.2119T>C	p.(Ser707Pro)	Polymorphism	3.2 (9)	1.4 (2)	1.1 (2)
8	c.2250+22A>C	Non-coding	Polymorphism	0.4 (1)	0	0
9	c.2572T>C	p.(Phe858Leu)	Polymorphism	5.0 (14)	2.1 (3)	5.6 (10)
10	c.2805G>C	No AA change	Polymorphism	0.4 (1)	0	0.6 (1)
11	c.2922-22del T	Non coding	Polymorphism	1.4 (4)	0	0
12	c.3161C>G	p.(Prp1054Arg)	Polymorphism	6.8 (19)	0	6.7 (12)
13	c.3403-12insA	Non-coding	Polymorphism	34.5 (97)	24.3 (34)	41.6 (74)
14	c.3419A>G	p.(Asn1140Ser)	Variant/Mutation	0.4 (1)	0	0
15	c.4119T>C	No AA change	Polymorphism	0.4 (1)	0	0
16	c.4138C>T	p.(His1380Tyr)	Polymorphism	0.4 (1)	0.7 (1)	0
17	c.4167A>G	No AA change	Polymorphism	0.4 (1)	0	0
18	c.4258C>T	p.(Leu1420Phe)	Polymorphism	4.3 (12)	2.9 (4)	5.1 (9)
19	c.4473C>T	No AA change	Polymorphism	0.7 (2)	0	0
20	c.4578C>T	No AA change	Polymorphism	8.5 (24)	2.1 (3)	10.7 (19
21	c.4724G>A	p.(Arg1575His)	Variant/Mutation	0.4 (1)	0	0
22	c.4980C>T	No AA change	Polymorphism	0.4 (1)	0	0
23	c.5071A>C	p.(Ser1691Arg)	Polymorphism	0.7 (2)	0.7 (1)	0
24	c.5497-15G>C	Non-coding	Polymorphism	0.4 (1)	0.7 (1)	0
25	c.5497-8T>C	Non-coding	Polymorphism	5.7 (16)	3.6 (5)	2.3 (4)
26	c.5557G>A	p.(Asp1853Asn)	Polymorphism	35.9 (101)	19.3 (27)	32.0 (57)
27	c.5793T>C	No AA change	Polymorphism	1.1 (3)	0.7 (1)	2.3 (4)
28	c.5821G>C	p.(Val1941Leu)	Variant/Mutation	0.7 (2)	0	1.1 (2)
29	c.5975A>C	p.(Lys1992Thr)	Variant/Mutation	0.4 (1)	0.7 (1)	0
30	c.6975+13insT	Non-coding	Polymorphism	0.4 (1)	0	0
31	c.7390T>C	p.(Cys2464Arg)	Variant/Mutation	0.4 (1)	0	0
32	c.7788+8G>T	Non-coding	Polymorphism	0.7 (2)	0	0
33	c.8786+8A>C	Non-coding	Polymorphism	2.5 (7)	3.6 (5)	9.6 (17)
34	c.8987+50A>T	Non-coding	Polymorphism	0.4 (1)	0	0
35	c.9200C>G	Non-coding	Polymorphism	0.7 (2)	0	0

Online Supplementary Table S3. Comparative Clinical Data for CLL patients.

	Pathogenic germline *ATM* mutations (n=8)	Pathogenic acquired *ATM* mutations (n=19)	*ATM* sequence variants^ (n=26)	*ATM* wild type* (n=260)	*P* value
Mean age at diagnosis	61.8	66.7	69.2	62.9	0.044
Stage of disease:	(n=8)	(n=19)	(n=26)	(n=248)	0.292
A	1	9	13	114	
B/C	7	10	13	134	
VH status	(n=8)	(n=18)	(n=26)	(n=232)	0.208
UM	6	12	19	128	
M	2	6	7	104	
Multiple (≥2) FISH abnormalities	(n=5)	(n=6)	(n=10)	(n=138)	0.051
	3	5	4	46	
5-year survival	(n=8)	(n=19)	(n=26)	(n=249)	0.005
(%)	60.0	68.4	49.2	76.3	
95% CI	(24.4-95.6)	(47.5-89.3)	(29.8-68.7)	(71.0-81.6)	
10-year survival	(n=8)	(n=19)	(n=26)	(n=249)	
(%)	0.0	27.1	16.8	42.0	
95% CI		(5.5-48.7)	(0.9-32.7)	(35.2 - 48.9)	

*^ This subgroup included patients with ATM sequence variants and no additional ATM pathogenic mutation. *This subgroup included patients with wild-type ATM gene or the presence of known polymorphisms only. + The 5 patients with pathogenic mutations of unknown origin were not included in this comparative analysis.*