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1 **ANALYSIS OF LOCUS-SPECIFIC LINE-1 AND ALU ELEMENT DNA**
2 **METHYLATION REVEALS NOVEL EARLY EPIGENETIC CHANGES IN CHRONIC**
3 **LYMPHOCYTIC LEUKAEMIA**

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18 **BACKGROUND:** Retrotransposons, such as LINE-1 (L1) and Alu elements, comprise
19 more than 25% of the human genome. Their ability to retrotranspose throughout the
20 genome is normally suppressed by epigenetic mechanisms. However, this repression
21 is frequently lost in solid tumours through internal and external stimuli, and
22 consequently somatic retrotransposition can be an initiating event in carcinogenesis.
23 The epigenome in chronic lymphocytic leukaemia (CLL) is shaped by the maturation
24 stage of the cell of origin, and its evolution during disease progression is correlated
25 with the acquisition of genetic abnormalities associated with poor patient prognosis.
26 Early work has demonstrated that L1 and Alu hypomethylation are associated with the
27 acquisition of 17p deletions in CLL, but to date there has been no comprehensive or
28 locus-specific analysis of retrotransposon DNA methylation.

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30 **AIMS:** To develop an approach to enable locus-specific analysis of L1 and Alu
31 subfamily DNA methylation using the Illumina Infinium 450K microarray platform
32 (H450K) and apply this to study aberrant methylation of L1 and Alu elements in CLL.

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34 **METHODS:** H450K probes mapping to retrotransposons were identified using
35 RepeatMasker. The probeset was applied to a publicly-available dataset from a study
36 of 138 CLL patients and 13 healthy individuals available from the International Cancer
37 Genome Consortium. Leading hits were further analysed in Gene Expression
38 Omnibus (GEO) datasets from 1,169 healthy individuals, 764 acute lymphoblastic
39 leukaemia (ALL) patients, 174 acute myeloid leukaemia (AML) patients, and 31 diffuse
40 large B-cell and Burkitt's lymphoma patients, and also prospective samples from 82
41 future CLL cases (<18 years from diagnosis) and 82 age-matched controls within the
42 Melbourne Collaborative Cohort Study.

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44 **RESULTS:** We identified 9,549 probes mapping to 117 L1 subfamilies, and 12,806
45 mapping to 37 Alu subfamilies. In normal B-cells from healthy individuals, DNA
46 methylation at these sites was routinely high (mean β : 0.75), with greater variation
47 observed in older subfamilies (L1M and AluJ) in comparison to the youngest
48 (L1H/L1PA and AluY), especially at CpGs within 200 bases of TSS. We identified
49 10,782 CpG sites within L1 and Alu sequences that were differentially methylated
50 between CLL patients and healthy individuals (P_{fdr}<0.05), of which 55 were

51 hypomethylated in >90% of CLL patients but never in healthy individuals.
52 Hypomethylation of Alu elements was associated with evolutionary age, with older
53 subfamilies (AluJ) displaying greater changes than younger ones (AluY).
54 Hypomethylation of 17 leading hits was highly confined to CLL, never observed in
55 healthy individuals and infrequently in ALL, AML and lymphoma. In prospective
56 samples, methylation at each of the 17 loci, located across the genome, was highly
57 correlated within individual patients. In contrast to diagnosed CLL patients,
58 hypomethylation at the loci was observed in only 9 future CLL cases (11%). Notably,
59 however, this was more commonly observed in samples taken <7 years before
60 diagnosis (7 of 24, 29%) than in those taken more than 7 years before diagnosis (2 of
61 58, 3%).

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63 **CONCLUSIONS:** We have identified locus-specific hypomethylation events of L1 and
64 Alu elements that are highly frequent and specific to CLL, and which are present prior
65 to diagnosis for some patients. Further work is required to establish how these
66 epigenetic changes correspond to modulation of global DNA methylation patterns in
67 leukaemogenesis.