Detection of leukemia-associated mutations in peripheral blood DNA of hematologically normal elderly individuals

J Score, A Chase, L A Forsberg, L Feng, K Waghorn, A V Jones, C Rasi, D C Linch, J P Dumanski, R E Gale, N C P Cross

Cite this article as: J Score, A Chase, L A Forsberg, L Feng, K Waghorn, A V Jones, C Rasi, D C Linch, J P Dumanski, R E Gale, N C P Cross, Detection of leukemia-associated mutations in peripheral blood DNA of hematologically normal elderly individuals, *Leukemia* accepted article preview 28 January 2015; doi: 10.1038/leu.2015.13.

Accepted article preview online 28 January 2015

Detection of leukemia-associated mutations in peripheral blood DNA of hematologically normal elderly individuals

Letter to the Editor

New sequencing technologies are facilitating the development of large panels of genes that can be screened for mutations in patients with suspected hematological malignancies. Emerging evidence, however, has indicated that some malignancy-associated mutations are detectable in the general population, raising questions about the utility of broad mutation screening of peripheral blood or bone marrow DNA as a diagnostic tool. Analysis of intensity data tracks from single nucleotide polymorphism (SNP) array analysis of peripheral blood DNA from older individuals (typically >60 years) in population cohorts has revealed large somatically-acquired chromosomal duplications, deletions and regions of acquired uniparental disomy (aUPD; also known as copy number neutral loss of heterozygosity) similar to those seen in individuals with a hematological malignancy[1-4]. Such variants are typically present in a proportion of peripheral blood cells at a specific time-point but their frequency can increase or decrease over time and even disappear, suggesting a dynamic pattern of clonal change[1]. Consistent with this data, mutations in genes that are often mutated in myeloid malignancy such as *TET2*, *DNMT3A, ASXL1* and *JAK2* have also been described in elderly individuals with no evidence of hematological malignancy[5-8]. Increasing age is accompanied by changes to the hematopoietic stem cell (HSC) compartment, a process known as myeloid shift, with a bias towards the myeloid lineage at the expense of the lymphoid lineage[9]. In addition, acquired skewing of X-chromosome inactivation patterns (XCIP) in elderly women suggests that there is a reduction in stem cell usage over time that might in principle be stochastic or driven by subclinical clonal expansion[10]. These combined factors may help to explain the higher frequency of myeloid neoplasia in the elderly population.

To investigate the presence of mutations in elderly populations further, we studied two independent cohorts, (i) the Uppsala Longitudinal Study of Adult Men (ULSAM), an ongoing epidemiologic study of all available men born between 1920-24 in Uppsala County, Sweden

that have been analyzed by SNP arrays[1, 4] and (ii) 56 elderly women with normal blood counts and paired DNA from both neutrophils and T cells [10].

We have previously reported the finding that mosaic loss of the Y chromosome in peripheral blood leukocytes is associated with shorter survival and higher risk of cancer based on the analysis of Illumina 2.5M Omni Chip SNP data of 1,153 ULSAM participants[4]. Further analysis of this SNP data identified 15 cases (1.3%) with regions of likely autosomal aUPD >10Mb in size (median size 27Mb; range 13-103Mb). Two observations each were seen at 11p, 19q and 22q and one observation each at 1p, 4q, 6p, 9p, 9q, 11q, 14q, 15q and 17p. Although no obvious recurrences were identified, many of these regions include somatically-mutated driver genes that are associated with aUPD in hematological malignancies [11]. Of the 15 individuals with aUPD, two had been diagnosed with a hematological disorder at the time of sampling: one with *JAK2* V617F positive polycythemia vera (9p aUPD) and the other with chronic lymphocytic leukemia (17p aUPD; *p53* status not tested). The 13 remaining individuals had normal blood counts.

Since aUPD of chromosome 4 is associated with inactivating *TET2* mutations,[11] the case with chromosome 4 aUPD (ULSAM-697) was screened for possible abnormalities in this gene. This revealed a 21bp deletion that removes the 3' end of exon 4 and is thus very likely to be a deleterious (Figure 1). The mutation was present in peripheral blood samples taken at 71, 88 and 90 years of age, but was clearly somatic since it was not detected in CD19+ B-cells (Figure 1). We have previously reported SNP array data showing that ULSAM-697 had readily apparent chromosome 4q aUPD in nucleated blood cells at the ages of 82, 88 and 90 but not at the age of 71.[1] Reinspection of the array data indicated that chromosome 4q aUPD was just visible at the age of 71, at a level of <10% nucleated cells. Overall, these data indicate that this individual thus had a large heterozygous mutant *TET2* clone at the age of 71 but by the age of 82 a prominent homozygous mutant *TET2* subclone had emerged. Peripheral blood counts at all time points were normal, and there was no other indication of a hematological disorder. Therefore, for nearly 20 years this individual carried a somatic *TET2* mutation, an initiating event for a hematological malignancy, that evolved to homozygosity without the development of any obvious hematological abnormality.

Of note, the level of aUPD4q in ULSAM-697 was very similar at 82 and 88 years (58% of nucleated blood cells) but had significantly decreased to 30% of cells at the age of 90.[1] Although it is difficult to accurately quantify deletions directly from sequence data, visual inspection of the mutant electropherograms suggests that the *TET2* mutation level rose and fell in line with the changing aUPD (Figure 1). Cell fractionation studies at the age of 90 showed that granulocytes and CD4+ T-cells had similar levels of aUPD4q to total nucleated cells but aUPD was not seen in CD19+ B-cells.[1] Remarkably, follow up analysis at the age of 91 (peripheral blood counts still normal) showed that the level of aUPD in nucleated blood cells had further decreased to just below 30% and was no longer detectable in CD4+ T-cells (data not shown).

MPL and *CBL* mutations have been associated with aUPD at 1p and 11q, respectively, but we did not detect mutations in the known mutational hotspots of these genes in the two affected ULSAM cases. For the other regions, mutational targets associated with aUPD have not yet been identified.[11]

Next, we analyzed a cohort of 56 hematologically normal older women, median age 81 years (range, 75-95 years), with paired DNA available from purified neutrophils and T-cells[10]. Initially we screened neutrophil DNA for *JAK2* V617F and mutations in *DNMT3A* (exons 18-23) or *TET2* (all coding exons) by high resolution melt followed by Sanger sequencing[12]. Subsequently the samples were analyzed on an Illumina Miseq using either Illumina Trueseq Custom Amplicon (TSCA) in-house designed panel of genes known to be mutated in myeloid neoplasia (all or selected exons of *ASXL1, CBL, CSF3R, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, PHF6, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, TET2, TP53, U2AF1*) or by the Illumina TruSight Myeloid Sequencing Panel (catalogue number FC-130-1010). T-cell DNA was only analyzed in cases with sequence variants in the target genes. Overall, combining the Sanger sequencing and MiSeq data, six of 56 (11%) individuals were found to carry relevant mutations: *DNMT3A* variants in two cases at allele frequencies of 24% and 34%, five *TET2* variants in four individuals at allele frequencies varying between 4% and 47%, and an *SRSF2* variant at 19% (Table 1). Of these, the *DNMT3A* p.R882C and *SRSF2* p.P95L mutations are both known to be recurrent and frequent in myeloid neoplasia, and one of the *TET2* mutations is a frameshift mutation and therefore highly likely to be

deleterious. All the abnormalities were either absent or markedly reduced in T-cells from the same patient, and the latter may simply reflect the low level presence of contaminating myeloid cells. Therefore, it is likely that all these mutations were somatically acquired.

Overall our results concur with the findings of other population based studies[5-8] that a subset of myeloid neoplasia-associated mutations are present in a significant minority of the elderly population who have no clinical manifestations of a hematological disorder. In one such study of whole-exome sequencing data from 17,182 individuals, 160 genes recurrently mutated in lymphoid and myeloid cancers were selected for targeted reanalysis, revealing 805 candidate somatic variants in 73 genes in 746 individuals, mostly without an associated hematological phenotype[8]. It is also now clear that the presence of a somatic mutation increases the risk of development of a hematological malignancy with reported hazard ratios of 10.1-12.89 [2, 7, 8]. In addition to conferring a risk of subsequent disease development, which our data suggest might be refined by analysis of different blood cell types, findings such as these clearly have implications for the interpretation of whole exome or genome sequencing in elderly individuals, and introduce some uncertainty into the assumption that the mutational landscape of the peripheral blood is representative of that of the germline.

Joannah Score[1,2], Andrew Chase[1,2], Lars A Forsberg[3], Lin Feng[1,] Katherine Waghorn[1,2], Amy V Jones[1,2], Chiara Rasi[3], David C Linch[4], Jan P Dumanski[3], Rosemary E Gale[4], Nicholas C P Cross[1,2].

[1]Wessex Regional Genetics Laboratory, Salisbury, UK
[2]Faculty of Medicine, University of Southampton, Southampton, UK
[3]Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden
[4]Dept. Of Haematology, UCL Cancer Institute, London, UK

Corresponding author:
Professor N.C.P. Cross
Wessex Regional Genetics Laboratory
Salisbury NHS Foundation Trust
Salisbury SP2 8BJ, UK

Tel: + (44) 1722 429080
Fax: + (44) 1722 331531
Email: ncpc@soton.ac.uk

None of the authors have a relevant conflict of interest.

Figure legend

Analysis of ULSAM-697. A. Schematic of the 21bp deletion in *TET2* exon 4/intron 4. Amino acids are shown in green, nucleotides in black and deleted nucleotides in purple. Underlined nucleotides show the donor splice site. B. Electropherograms taken from Mutation Surveyor (Softgenetics, State College, PA) of the deleted region amplified from DNA extracted from total nucleated blood cells taken at the ages of 71, 88 and 90 (a sample at the age of 82 was not available), plus CD19+ B-cells selected from peripheral blood at the age of 90. Each timepoint shows the sequence trace plus the mutation electropherogram derived from the comparison of the sample peak profile with a wild type reference peak profile run at the same time. Levels of chromosome 4q aUPD, as determined previously,[1] are indicated and the start of the deletion is indicated by a black arrow.

REFERENCES

1. Forsberg LA, Rasi C, Razzaghian HR, Pakalapati G, Waite L, Thilbeault KS*, et al.* Age-related somatic structural changes in the nuclear genome of human blood cells. *Am J Hum Genet* 2012; **90:** 217-228.

2. Laurie CC, Laurie CA, Rice K, Doheny KF, Zelnick LR, McHugh CP*, et al.* Detectable clonal mosaicism from birth to old age and its relationship to cancer. *Nat Genet* 2012; **44:** 642-650.

3. Jacobs KB, Yeager M, Zhou W, Wacholder S, Wang Z, Rodriguez-Santiago B*, et al.* Detectable clonal mosaicism and its relationship to aging and cancer. *Nat Genet* 2012; **44:** 651-658.

4. Forsberg LA, Rasi C, Malmqvist N, Davies H, Pasupulati S, Pakalapati G*, et al.* Mosaic loss of chromosome Y in peripheral blood is associated with shorter survival and higher risk of cancer. *Nat Genet* 2014; **46:** 624-628.

5. Busque L, Patel JP, Figueroa ME, Vasanthakumar A, Provost S, Hamilou Z*, et al.* Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet* 2012; **44:** 1179-1181.

6. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC*, et al.* Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* 2014; **20:** 1472-1478.

7. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF*, et al.* Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 2014; **371:** 2477-2487.

8. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG*, et al.* Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014; **371:** 2488-2498.

9. Pang WW, Price EA, Sahoo D, Beerman I, Maloney WJ, Rossi DJ*, et al.* Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proc Natl Acad Sci U S A* 2011; **108:** 20012-20017.

10. Gale RE, Fielding AK, Harrison CN, Linch DC. Acquired skewing of X-chromosome inactivation patterns in myeloid cells of the elderly suggests stochastic clonal loss with age. *Br J Haematol* 1997; **98:** 512-519.

11. Score J, Cross NC. Acquired uniparental disomy in myeloproliferative neoplasms. *Hematol Oncol Clin North Am* 2012; **26:** 981-991.

12. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV*, et al.* Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet* 2010; **42:** 722-726.

UPID	Gene	Neutrophil/T cell sequencing technology	Mutation in granulocytes (Miseq variant allele frequency)	Mutation in T-cells (Miseq variant allele frequency)
8470	*DNMT3A*	SS, MS/SS	G699D (714/2819 = 0.24)	Not detected
8478	*DNMT3A*	SS, MS/SS	R882C (210/616 = 0.34)	Yes, at much lower level by Sanger sequencing
8445	*TET2* *SRSF2*	SS, MS/SS SS, MS/SS MS/SS	R1261H (321/898 = 0.36) G1365R (60/128 = 0.47) P95L (16/82 = 0.19)	Not detected Not detected Not detected
8460	*TET2*	SS, MS/SS	176230delA (2/52 = 0.038)	Not detected
8463	*TET2*	SS, MS/SS	91477insA_ 91478_91481delAGGT (82/1084 = 0.076)	Not detected
8464	*TET2*	MS/MS	R1214W (232/1332 = 0.17)	(9/1771 = 0.005)

Table 1: Variants found in granulocyte and T cell DNA of hematologically normal women.

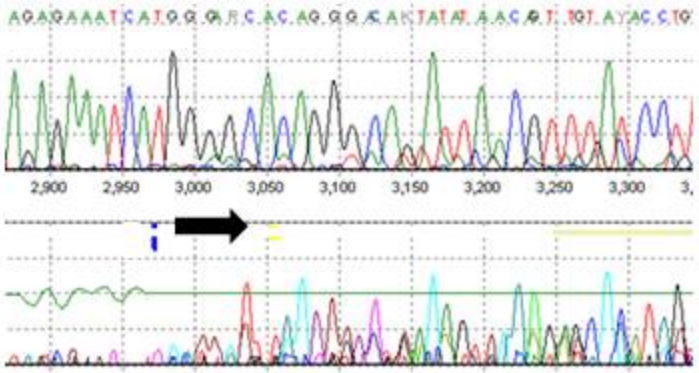
Sequencing technology: SS=Sanger sequencing; MS=MiSeq

A

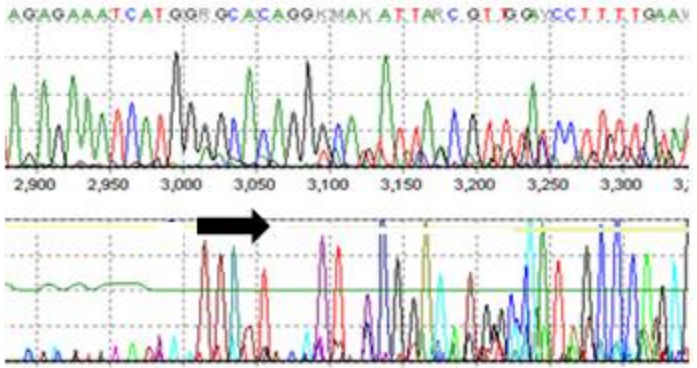
```
                    R  E  I  M  E  E  R
Wild-type  AGAGAAATCATGGAAGAAAGgtaattaacgcaaaggcacagggcaga

Mutant     AGAGAAATCATGGggcacagggcagattaacgtttatcct
                    R  E  I  M  G  H  R  A  D STOP
```

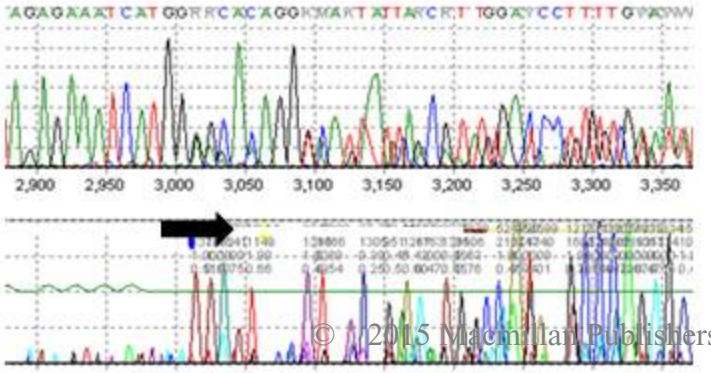
B



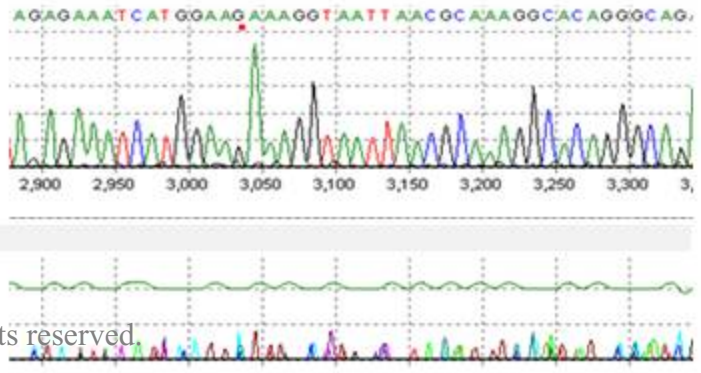
Age 71
(<10% aUPD4q)



Age 88
(58% aUPD4q)



Age 90
(30% aUPD4q)



Age 90
CD19+ cells
(0% aUPD4q)