Acquired *ASXL1* mutations are common in patients with inherited *GATA2* mutations and correlate with myeloid transformation

Robert R. West,[1] Amy P. Hsu,[2] Steven M. Holland,[2] Jennifer Cuellar-Rodriguez,[2] and Dennis D. Hickstein[1]

[1]Experimental Transplantation and Immunology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA; and [2]Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

©2013 Ferrata Storti Foundation. This is an open-access paper. *doi:10.3324/haematol.2013.090217*
Manuscript received on April 24, 2013. Manuscript accepted on September 27, 2013.
Correspondence: westrob@mail.nih.gov

Supplemental Table 1. PCR primers used for *ASXL1* genotyping

Primer	Orientation	Sequence
1	5' - fwd	GTTTTGCTTTACAGTCCCTAGGTCAG
2	5' – fwd	GGTCAGATCACCCAGTCAGTTAAAAC
3	3' - rev	GTCCTCCCAAACCTCAGTAGC
4	3' – rev	GGGCTAGGAGATGCCTCCC
5	5' – fwd	GCATACCCAGGCCGGAAC
6	3' – rev	CCTATGAGGGAAAGTGATACTAGACAAG
7	5' – fwd	CAGTTCCACACCTGAATCCTCACC
8	3' – rev	CCAAGGTGAATGGTGACATGC
9	5' – fwd	GCAGTGACAAAGGGATCTTCG
10	3' – rev	GCAGTTCTCTTCCTTTAGTTGTGA
11	5' – fwd	CAGTGGCATGGTTGATGGAAG
15	3' – rev	CATTGATATAATACTCTTTAGGCAGGAGCAC
12A	5' - fwd	GTGCACCACACAGATTTATTTTGTTC
12B	3' - rev	GATTATAGAGCCCTTAACTCTGGGGC

Primers used for PCR amplification and DNA sequencing are based on the *ASXL1* sequence given for NCBI Reference Sequence: NG_027868.1

Supplemental Table 2. Chronological patient number, DNA sample sources, and previously published pedigree designation.

#	DNA source	Pedigree	Reference
1	PB granulocyte/mononuclear cells BM granulocyte/mononuclear cells	35.III.3	2
2	BM granulocyte/mononuclear cells	25.II.1	40
3 [A]	peripheral blood leukocytes	1.II.5	3,5,40
4 [A]	peripheral blood leukocytes	1.II.1	2,3,5
5 [B]	peripheral blood leukocytes	4.II.1	5,40,41
6 [B]	peripheral blood leukocytes	4.I.1	3,5,40
7	peripheral blood leukocytes	8.I.1	3,5,40,42
8	peripheral blood leukocytes	3.I.1	2,5
9	peripheral blood leukocytes PB granulocyte/mononuclear cells	5.II.1	2,5
10	peripheral blood leukocytes	12.I.1	3,5,41
11 [C]	peripheral blood leukocytes	13.II.1	3,5,40,43
12 [C]	peripheral blood leukocytes	13.I.1	3,5,40,43
13	peripheral blood leukocytes	11.II.1	5,40
14	blood smear BM granulocyte/mononuclear cells	10.I.1	3,5
15	blood smear	20.I.3	3,42
16	blood smear	18.I.2	3
17	blood smear	17.I.1	5,41
18	blood smear, BM granulocyte/mononuclear cells	24.I.1	3
19	peripheral blood leukocytes	26.I.1	40,41
20 [C]	peripheral blood leukocytes	34.II.2	not pub.
21 [C]	peripheral blood leukocytes	34.I.1	not pub.
22	BM granulocyte/mononuclear cells	40.I.1	40
23 [A]	peripheral blood leukocytes	33.III.1	40
24 [A]	peripheral blood leukocytes	33.III.3	40
25	peripheral blood leukocytes	6.II.1	5,40,41,44
26 [D]	peripheral blood leukocytes BM granulocyte/mononuclear cells	31.II.1	not pub.
27 [D]	BM granulocyte/mononuclear cells	31.II.2	not pub.
28	peripheral blood leukocytes	27.I.1	40
29	peripheral blood leukocytes	29.I.1	40
30 [E]	PB granulocyte/mononuclear cells	39.I.1	not pub.
31 [E]	PB granulocyte/mononuclear cells	39.I.2	not pub.
32	BM granulocyte/mononuclear cells	15.I.1	3,5,40,41
33	peripheral blood leukocytes PB granulocyte/mononuclear cells	38.I.1	41
34	peripheral blood leukocytes	43.I.1	not pub.
35	BM granulocyte/mononuclear cells	9.III.1	3,5
36	peripheral blood leukocytes	28.I.1	not pub.
37	peripheral blood leukocytes	37.I.1	40
38	peripheral blood leukocytes	44.I.1	not pub.
39	peripheral blood leukocytes	22.I.1	5,40
40 [B]	peripheral blood leukocytes	30.II.1	40
41 [B]	peripheral blood leukocytes	30.I.1	40
42	PB granulocyte/mononuclear cells	41.I.1	40
43	blood smear BM granulocyte/mononuclear cells	42.I.1	not pub.

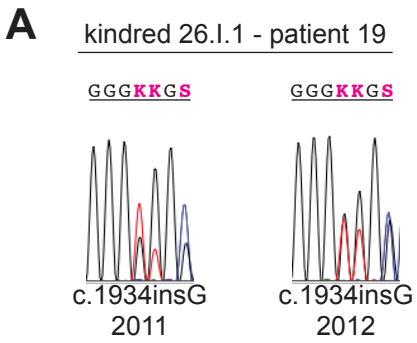
44	peripheral blood leukocytes	46.I.1	not pub.
45	peripheral blood leukocytes	7.I.1	5,40
46	peripheral blood leukocytes	14.I.1	5
47	peripheral blood leukocytes	21.II.1	3,42
48	peripheral blood leukocytes	48.III.1	not pub.

A, sisters; B, daughter/father; C, son/mother; D, brothers; E, monozygotic twins. PB, peripheral blood; BM, bone marrow.

Supplemental Table 3. Single nucleotide polymorphisms observed in the *ASXL1* gene, exons 12 and 13, among GATA2 deficiency patients

dbSNP number	alleles	residue change
rs79865730	**C/T**	$Thr^{655}Thr$
rs6058693	**G/A**	$Val^{751}Ile$
novel*	**A/G**	$Leu^{764}Leu$
rs6058694	**C/T**	$Pro^{815}Leu$
rs62206933	**C/T**	$His^{995}His$
rs75887545	**C/G**	$Ser^{1166}Arg$
rs117901891	**G/A**	$Leu^{1173}Leu$
rs74638057	**C/T**	$Ser^{1231}Phe$
rs4911231	**T/C**	$Ser^{1253}Ser$
rs6057581	**C/T**	$Leu^{1325}Phe$
rs2295764	**A/G**	3'UTR

Ten single nucleotide polymorphisms (SNPs) found in the dbSNP database were identified among the GATA2 deficiency patients, and one novel SNP. dbSNP number is from the Database of Single Nucleotide Polymorphisms (dbSNP), Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: {138, phase 1}). * This SNP is not in dbSNP, but it is the third position of the codon, giving a synonymous coding sequence change. It is also not at a splice acceptor/donor site. Therefore, it was not considered to be a pathological *ASXL1* mutation.



Supplemental Figure 1. DNA sequence trance for *ASXL1* mutation c.1934insG in patient 19. Patient 19 had an increase in th signal for the c.1934insG mutation over time, which correlated with an increasing blast count and the development of proliferative CMML.