Disruption of the *ASXL1* gene is frequent in primary, post-essential thrombocytosis and post-polycythemia vera myelofibrosis, but not essential thrombocytosis or polycythemia vera: analysis of molecular genetics and clinical phenotypes

Brady L. Stein,[1] Donna M. Williams,[2] Christine O'Keefe,[3] Ophelia Rogers,[2] Roxann G. Ingersoll,[4] Jerry L. Spivak,[2] Amit Verma,[5] Jarek P. Maciejewski,[3] Michael A. McDevitt,[2] and Alison R. Moliterno[2]

[1]Medicine, Northwestern University Feinberg School of Medicine, USA; [2]Medicine, Johns Hopkins University School of Medicine, USA; [3]Translational Hematology and Oncology Research, Cleveland Clinic/Taussig Cancer Institute, USA;[4]Genetics Research Core Facility, Johns Hopkins University School of Medicine, USA, and [5]Albert Einstein College of Medicine, NY, USA

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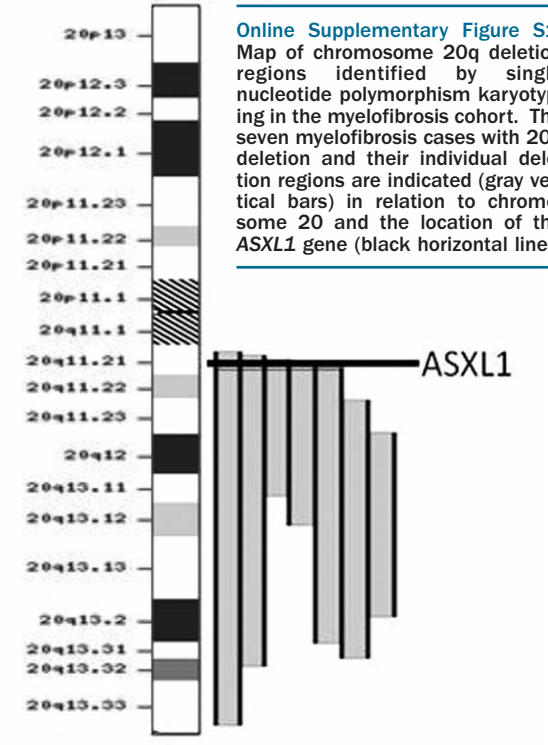
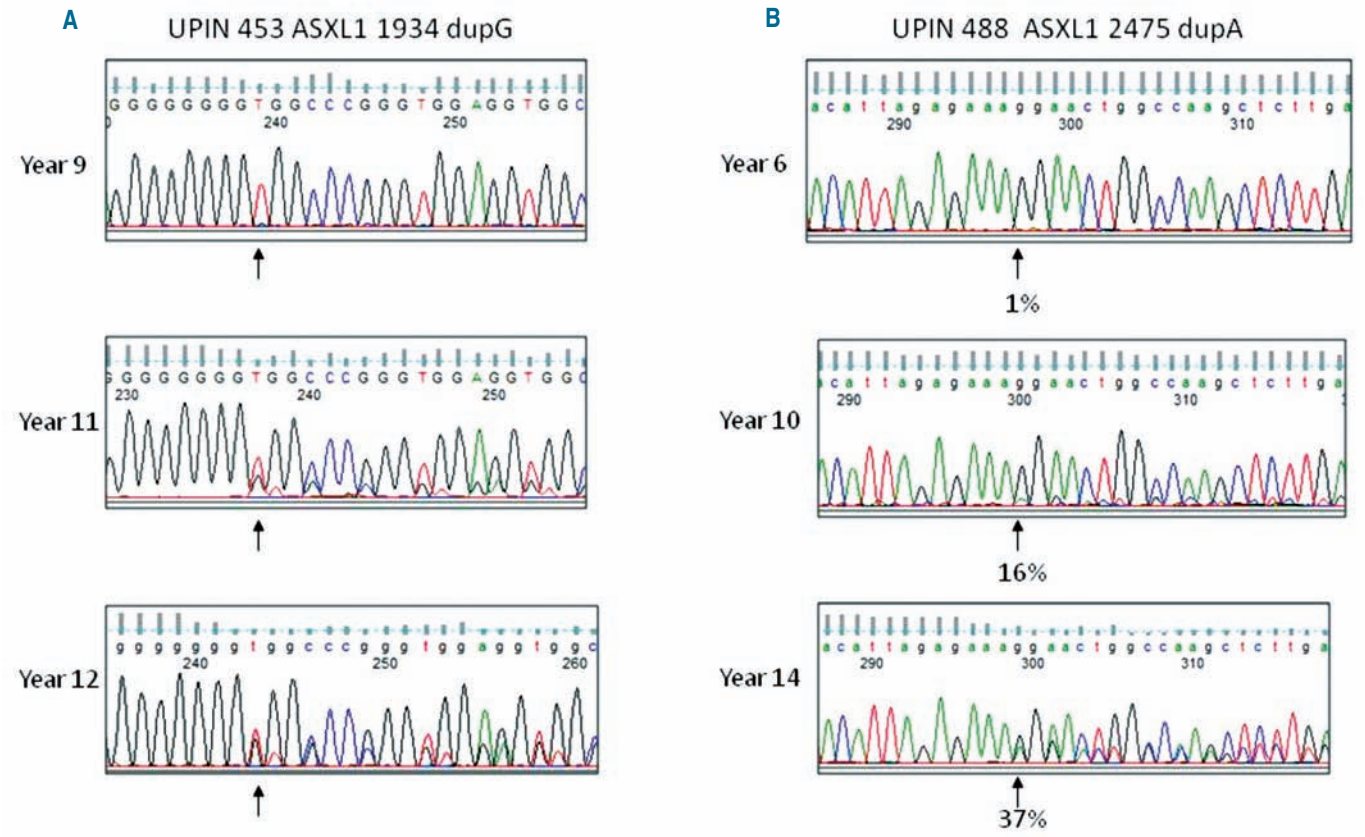
Online Supplementary Table S1. Pyrosequencing primers.

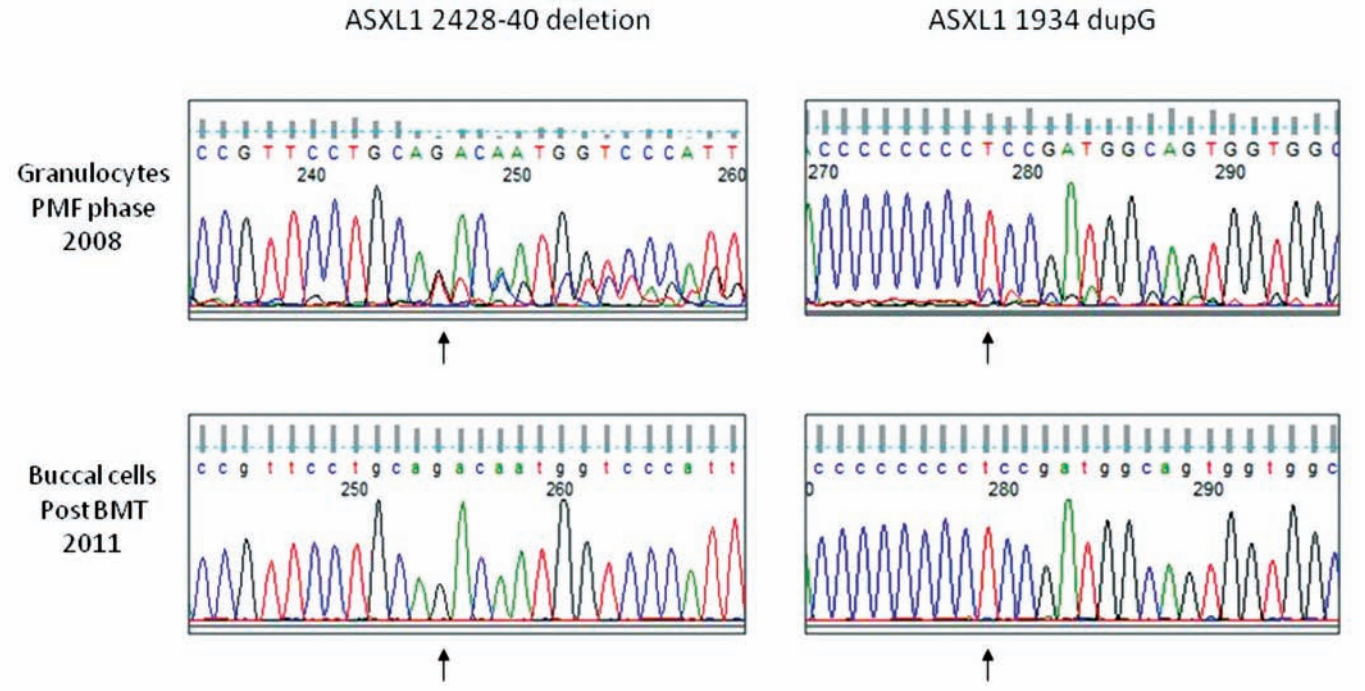
Lesion	Forward Primer	Reverse Primer	Sequencing Primer
JAK2 V617F	GCAGAGAGAATTTTCTGAACTAT	CTCTGAGAAAGGCATTAGAAAG	GGTTTTAAATTATGGAGTATGT
ASXL1 1954G->A	AGGTCCGAGGGGCGAGAG	CCACAGGCCTCACCACCAT	GGTGGCCCGGGTGGA
ASXL1 1963-1976del	AGGTCCGAGGGGCGAGAG	CCACAGGCCTCACCACCAT	GGTGGCCCGGGTGGA
ASXL1 2475dupA	ATTCCGTCTCTAGTGGGAGATGA	CCTTCATAGTGGGATGACTGTCAA	GAGATGATACATTAGAGAAA
ASXL1 2846-47del	GCATTGCCTGGGGATTTG	GGCACAGTCCAGAGTGAAGTAAGG	TGACAGCTGAGGAGG



Online Supplementary Figure S1. Map of chromosome 20q deletion regions identified by single nucleotide polymorphism karyotyping in the myelofibrosis cohort. The seven myelofibrosis cases with 20q deletion and their individual deletion regions are indicated (gray vertical bars) in relation to chromosome 20 and the location of the *ASXL1* gene (black horizontal line).



Online Supplementary Figure S2. Electropherograms identifying *ASXL1* lesions over time. (A) The *ASXL1* lesion 1934 dupG identified in increasing intensity after year 9 in UPIN 453. (B) The *ASXL1* nonsense lesion 2475 dupA identified in increasing intensity after year 6 in UPIN 488 (Manuscript Figure 1A). Arrows indicate the start of the sequence variation; percentages indicate the pyrosequencing result of the mutant allele from the same sample which generated the electropherogram in the case of *ASXL1* 2475 dupA.



Online Supplementary Figure S3. Electropherograms identifying two separate *ASXL1* nonsense lesions. Electropherograms from granulocyte DNA from UPIN 1327 obtained in 2008 during the PMF phase of the MPN are shown in the upper panels, with arrows indicating the start of the respective frameshift lesions. Electropherograms from buccal cell DNA obtained from the same patient in 2011, after bone marrow transplantation with donor engraftment and PMF remission (lower panels) do not identify frameshift lesions apparent in the upper panels. The *ASXL1* 1934 dupG sequence is in reverse orientation.