Extent of hematopoietic involvement by *TET2* mutations in *JAK2*^{V617F} polycythemia vera

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Online Supplementary Table S1. Mutation-specific primers used to quantify mutant TET2 alleles. Determination of allelic frequencies was reproducible (SD =1.4% (P1), 1.5% (P2), 1.9% (P3), 1.7% (P4)), and sensitive (0.3% (P1), 0.5% (P2), 0.7% (P3) and 0.5% (P4)) mutant allele detected in 50 ng of DNA.

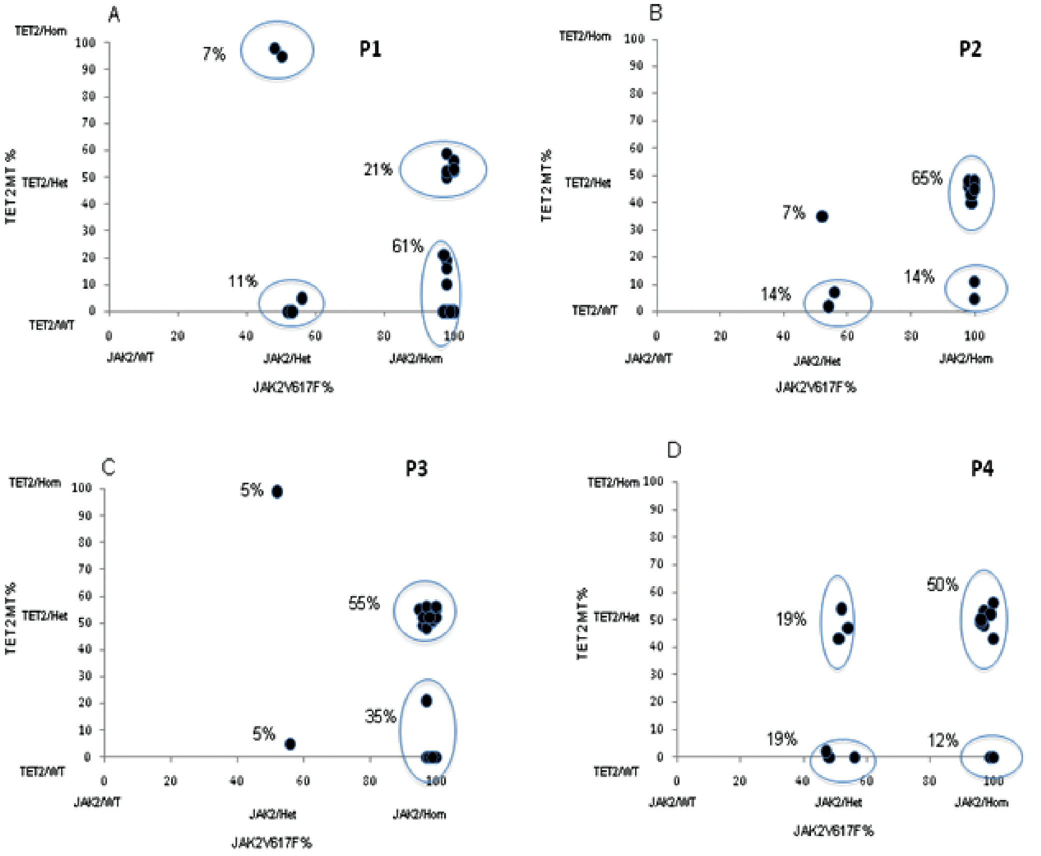
Primers/Probe	Sequence 5' to 3'
Patient 1 (P1)	
FAM-AS-TET2-P1-MGB	6FAM-CTTCCTTGGGATCTTG-MGBNFQ
R-WT-TET2-LNA-P1	CGATTATACATCAGGAAGTAAAC*A*tT
R-MT-TET2-LNA-P1	CGATTATACATCAGGAAGTAAAC*A*tA
F-TET2-P1	CTCCTTCTCTTTTGGTTGTTC
Patient 2 (P2)	
FAM-AS-TET2-P2-MGB	6FAM-CTCAAATCACAGAAGCAA-MGBNFQ
F-WT-TET2-LNA-P2	CGTTATTTGACCATAAGGCT*G*tT
F-MT-TET2-LNA-P2	CGTTATTTGACCATAAGGCT*G*tA
R-TET2-P2	GTTCTGCAGCAGTGGTTTGTCTAGTC
Patient 3 (P3)	
FAM-AS-TET2-P3-MGB	6FAM-AAGGCCTCAGAATAA-MGBNFQ
F-WT-TET2-LNA-P3	TAAACCTGAGGCACCA*C*gTT
F-MT-TET2-LNA-P3	TAAACCTGAGGCACCA*C*gTC
R-TET2-P3	CTGGCAGTTGTCCTGTAGCTCT
Patient 4 (P4)	
FAM-AS-TET2-P4-MGB	6FAM-CCAGACTAAAGTGGAAGAA-MGBNFQ
F-WT-TET2-LNA-P4	CAGACTTTTCCTCACCC*C*gA
F-MT-TET2-LNA-P4	CAGACTTTTCCTCACCC*C*gC
R-TET2-P4	CTGACTATAAGGGGAATTTCTACGATT

The mismatched nucleotide is depicted in lower case and the locked nucleic acid base is underlined and italicized.

Online Supplementary Table S2. Clonal analysis of granulocytes and erythroid progenitors after *in vitro* expansion. "*X-chromosome marker*" denotes the polymorphic X-chromosome gene informative (heterozygous) for clonality studies in PV *JAK2*2V617F-positive patients without known *TET2* mutations and PV patients with *JAK2*V617F and *TET2* mutations.

Patient	GNC	Terminal erythropoiesis	X-chromosome marker
PV patients with *JAK2*V617F and *TET2* mutations			
P1	Clonal	Clonal	*FHL1*
P2	Clonal	Clonal	*FHL1, IDS*
P3	Clonal	Clonal	*MPP1*
P4	Clonal	Clonal	*IDS, G6PD*
PV patients *JAK2*V617F-positive and *TET2*-negative mutations			
P5	Clonal	Polyclonal	*BTK*
P6	Clonal	Polyclonal	*FHL1*
P7	Clonal	Polyclonal	*BTK*
P8	Clonal	Polyclonal	*FHL1*
P9	Clonal	Polyclonal	*FHL1*
P10	Clonal	Polyclonal	*IDS*
P11	Clonal	Polyclonal	*FHL1*

Online Supplementary Figure S1. Analysis of single EPO-independent colonies for mutations in *TET2* and *JAK2*. Each colony is represented by a single dot and represents one of six different genotypes: wild-type (WT), heterozygous (Het), and homozygous (Hom) for *JAK2*V617F on the horizontal axis, and *TET2* mutations on vertical axis. Allelic ratio (T%) *JAK2*V617F on the horizontal axis, and allelic ratio (MT%) and *TET2* mutations (WT, Het. Hom) on the vertical axis. (A) PV patient (P1) with c.*3954+2T>A TET2* mutation. (B) *JAK2*V617F-positive PV patient (P2) with c.*3138delT TET2* mutation. (C) *JAK2*V617F-positive PV patient (P3) with c.*1378delT TET2* mutation. (D) *JAK2*V617F-positive PV patient (P4) with c.*2290dupC TET2* mutation.



Online Supplementary Figure S2. Expression of *TET2* tumor suppressor gene in patients with MPNs in circulating granulocytes and platelets. Relative gene expressions (fold changes) were calculated against arbitrary control. *denotes $P<0.05$ compared to mean of controls. PV subjects without known *TET2* mutations; P1-P4 PV subjects with known *TET2* mutations. (A) PV, ET and PMF granulocytes and normal controls. (B) PV, ET and PMF platelets and normal controls. All experiments were repeated and each experiment was performed in duplicate.