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The relationship of *JAK2*^{V617F} and acquired UPD at chromosome 9p in polycythemia vera

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Letter to the Editor

JAK2^{V617F} mutation is the most common somatic event observed in patients with myeloproliferative neoplasms (MPN)¹⁻⁴. *JAK2*^{V617F} is found in over 95% of patients with polycythemia vera (PV), 55% of essential thrombocythosis and 65% of primary myelofibrosis. Homozygous *JAK2*^{V617F} is present in about half of PV patients whereas it is rarely seen in other MPNs^{3, 5, 6}. Patients bearing homozygous *JAK2*^{V617F} tend to have a longer duration of disease², higher hemoglobin levels, increased incidence of pruritis⁷ and are more likely to progress to post-PV myelofibrosis⁸. Homozygous *JAK2*^{V617F} is shown to result from mitotic recombination, leading to acquired uniparental disomy (aUPD) on chromosome 9p⁹.

It was reported that *JAK2* 46/1 (GGCC) haplotype may predispose carriers to *JAK2* mutation¹⁰⁻¹², and *JAK2*^{V617F} facilitates the acquisition of homozygous *JAK2*^{9, 13}. Challenging this view was a single study reporting 9p aUPD in two PV subjects with wild-type *JAK2*¹⁴, suggesting that in these two individuals, 9p aUPD might have preceded *JAK2*^{V617F}. However, the relationship between *JAK2*^{V617F} and 9p aUPD, the frequency and stability of existing PV genotypes has yet to be systematically defined in a larger PV cohort. To address this, we performed whole-exome sequencing and SNP array in 31 PV patients, and further validated our findings in two additional cohorts totaling 59 patients

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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(**Supplementary Methods**). In addition, we investigated the stability of PV genotypes using 36 longitudinal samples.

The allelic fraction of $JAK2^{V617F}$ in granulocytes (GNC), measured by sequencing, varied from 0.01 to 1.0 (**Figure 1**). 9p aUPD spanning $JAK2$ locus was present in 48% of patients in the discovery set. The fraction of genomes harboring 9p aUPD in a given GNC sample, varied from 0.08 to 1.0 (**Figure 1**). Based on the quantitative relationship between $JAK2^{V617F}$ allelic fraction and the fraction of genomes harboring 9p aUPD, we defined 4 PV subgroups and validated this finding in extended two cohorts (**Figure 1**). Across the three cohorts, 42% of patients harbored $JAK2^{V617F}$ in a heterozygous state without detectable 9p aUPD (Subgroup I); 45% of patients had $JAK2^{V617F}$ with an allelic fraction in direct proportion to the level of 9p aUPD (Subgroup II; homozygous $JAK2^{V617F}$); 10% of patients harbored 9p aUPD at approximately twice the level of the $JAK2^{V617F}$ allelic burden (Subgroup III; aUPD with heterozygous $JAK2^{V617F}$). Although any single patient in Subgroup III, taken in isolation, could be explained as a mixture of cells from Subgroup I and II, the best explanation for the pattern of Subgroup III across these patients is that $JAK2^{V617F}$ and 9p aUPD reside in the same cell with heterozygous $JAK2^{V617F}$ in all such patients. Thus, Subgroup III reported in this study comprises a novel subtype that has not been previously described. Three patients (3%) exhibited trisomy of 9p, generating 2 copies of $JAK2^{V617F}$ allele (Subgroup IV). The number of patients in Subgroup IV was too small and thus this group was not evaluated further.

We then investigated the stability of each PV genotype using longitudinal samples. The $JAK2^{V617F}$:9p aUPD ratio remained stable in 11 of 17 patients, although the $JAK2^{V617F}$ allele burden itself varied (**Figure 2A**). In contrast, 6 of 17 patients changed their $JAK2^{V617F}$ Subgroup membership. Patients PV1, PV16, and PV27 progressed from prognostically more favorable heterozygous $JAK2^{V617F}$ to less favorable homozygous $JAK2^{V617F}$ clones (**Figures 2B-C**). For example, patient PV27 was a mixture of Subgroup-I and -II clones at the first timepoint but four years later only Subgroup-II clone was apparent. However, patients PV2, PV28 and PV31 transformed their $JAK2$ genotypes and gained new clones (**Figures 2D-E**). The Subgroup-II clone in PV2 was eliminated, possibly as a result of treatment, and a new Subgroup-III clone emerged. It was interesting that PV28 and PV31 transformed to myelofibrosis (MF) whereas none of the other 14 patients with stable or progressed $JAK2$ genotype showed phenotype transformation. It has been suggested that patients with homozygous $JAK2^{V617F}$ were more likely to develop post-PV MF², a propensity we also observed in the Subgroup-II patients (**Supplementary Figure 1**). Among those 11 Subgroup-II patients that transformed to MF, we have longitudinal samples collected for two patients PV28 and PV31. We observed that, in both patients, there was an outgrowth of a new Subgroup-I clone (**Figure 2D**). However, we could not establish the sequential order of these two events.

We propose four pathways leading to PV (**Figure 2F**) to explain these results. PV stem cells or their self-renewing progenitors, in the majority of patients, acquire $JAK2^{V617F}$ first. Approximately half of them remain stable in this configuration (Subgroup I).

Others in this pathway duplicate *JAK2* by one of two different mechanisms: a minor fraction duplicated *JAK2*^{V617F}-bearing chromosome to produce trisomy 9p (Subgroup IV). Almost half of the patients in Subgroup I duplicate *JAK2*^{V617F} allele via mitotic recombination to produce 9p aUPD (Subgroup II). We observed progression from Subgroup I to II in two patients, but the factors controlling the progression could not be ascertained by our analysis. 10% of patients acquire 9p aUPD first followed by *JAK2*^{V617F} mutation, yielding patients in Subgroup III. Although the 9p aUPD cell with wild-type *JAK2* has been reported in one case study¹⁴, we did not observe 9p aUPD alone in our unselected 3 cohorts, suggesting it might be asymptomatic until acquisition of *JAK2*^{V617F}. We found that, in a single female patient which was not included in these 3 cohorts, her granulocytes showed almost complete 9p aUPD with a low *JAK2*^{V617F} allelic burden (0.24), indicating the majority of PV clone was composed of 9p aUPD (**Supplementary Figure 2**). Further, this female was heterozygous for X-chromosome *FHL1* gene and her granulocytes were clonal using a transactional clonality assay¹⁵. This patient is probably in a transient state from 9p aUPD with wild-type *JAK2* to Subgroup III. Since we observed more patients in Subgroup III, it suggests that acquiring the *JAK2*^{V617F} mutation would provide a proliferative advantage. It was reported that *JAK2* 46/1 (GGCC) haplotype may predispose carriers to *JAK2* mutation¹⁰⁻¹², but we didn't observe any significant difference in the frequency of this haplotype across 3 subgroups (**Supplementary Figure 3**).

The 9p aUPD region, in any given patient, includes many genes (**Supplementary Figure 4**). In the mitotic recombination event(s) leading to generation of aUPD, both common and rare alleles may be lost. We reasoned that genes with recurrent loss of wild-type alleles within aUPD regions underwent selection for the PV phenotype. Forty-eight genes lost their wild-type alleles in at least 3 patients (**Supplementary Figure 5**). Among them, 9 genes were related to cell division, 7 in transcriptional regulation, 4 in epigenetic regulation and 3 genes were potential tumor suppressors. *KDM4C* and *SMARCA2*, which are involved in histone modification and chromatin remodeling, are among them. Moreover, 6 of the top 10 genes (**Supplementary Table 1**) play a functional role in regulation of cell division. Among them, *DOCK8*, a gene involved in intracellular signaling networks, also had a somatic loss-of-function mutation and a novel germline variant. Recent evaluation of recurrent loss of heterozygosity in 3131 tumors in the absence of clear driver mutations led Elledge and coworkers to hypothesize the existence of cancer gene islands¹⁶. These are regions harboring multiple cancer genes that act in a coordinated fashion within the cancer cell to produce a strong tumorigenic effect. Thus, our observations may suggest that genes in 9p aUPD region other than *JAK2* could also contribute to PV pathophysiology.

Heretofore, the zygosity of the *JAK2*^{V617F} has been estimated by the allele burden with an arbitrary threshold of >0.5 for homozygous and <0.5 for heterozygotes. This approach assumed a pure tumor cell population, which often is not the case. In this study, we account for tumor cell purity by comparing *JAK2*^{V617F} to the level of 9p aUPD in the cell, rather than the absolute allele burden, leading to more accurate determination of mutant *JAK2* zygosity. With this more accurate measure of *JAK2* mutant zygosity, we observe that most patients can be classified in to one of 3 subgroups (**Figure 1**), setting the stage for more

precise analysis of prognostic value of *JAK2* zygosity, as well as improved resolution of the clonal status of a patient's tumor.

For the first time, we investigated the clonal stability of these PV genotypes. Two thirds of the patients were apparently stable in their PV clone for at least two years. Patients of all subgroups were represented among the stable patients. One third of the patients exhibited clonal changes, with emergence of both prognostically more or less favorable *JAK2*^{V617F} patterns (**Figure 2B-E**). However, our samples size is too small to investigate the factors controlling the stability of clones but the role of treatment regimen in clonal stability is clearly an important question. Further study of the clinical properties of a larger cohort of Subgroup III patients, compared to Subgroup I and II, is required to elucidate the contribution of 9p and the cancer genes therein in PV pathogenesis. This novel perspective on the molecular basis of the evolution of PV should lead to a better understanding of the roles of *JAK2*^{V617F} and 9p aUPD in this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal *JAK2* mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005; 434(7037): 1144–8. [PubMed: 15793561]
2. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of *JAK2* in myeloproliferative disorders. *The New England journal of medicine*. 2005; 352(17):1779–90. [PubMed: 15858187]
3. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase *JAK2* in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer cell*. 2005; 7(4):387–97. [PubMed: 15837627]
4. Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: *JAK2*, *MPL*, *TET2*, *ASXL1*, *CBL*, *IDH* and *IKZF1*. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K.* 2010; 24(6):1128–38.
5. Vannucchi AM, Antonioli E, Guglielmelli P, Rambaldi A, Barosi G, Marchioli R. Clinical profile of homozygous *JAK2* 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. *Blood*. 2007; 110(3):840–6. [PubMed: 17379742]
6. Wang K, Swierczek S, Hickman K, Hakonarson H, Prchal JT. Convergent mechanisms of somatic mutations in polycythemia vera. *Discovery medicine*. 2011; 12(62):25–32. [PubMed: 21794206]
7. Tefferi A, Lasho TL, Schwager SM, Strand JS, Elliott M, Mesa R, et al. The clinical phenotype of wild-type, heterozygous, and homozygous *JAK2*V617F in polycythemia vera. *Cancer*. 2006; 106(3):631–5. [PubMed: 16369984]
8. Passamonti F, Rumi E, Pietra D, Elena C, Boveri E, Arcaini L, et al. A prospective study of 338 patients with polycythemia vera: the impact of *JAK2* (V617F) allele burden and leukocytosis on

- fibrotic or leukemic disease transformation and vascular complications. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K.* 2010; 24(9):1574–9.
9. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Experimental hematology*. 2002; 30(3):229–36. [PubMed: 11882360]
 10. Jones AV, Chase A, Silver RT, Oscier D, Zoi K, Wang YL, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nature genetics*. 2009; 41(4):446–9. [PubMed: 19287382]
 11. Kilpivaara O, Mukherjee S, Schram AM, Wadleigh M, Mullally A, Ebert BL, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nature genetics*. 2009; 41(4):455–9. [PubMed: 19287384]
 12. Olcaydu D, Harutyunyan A, Jager R, Berg T, Gisslinger B, Pabinger I, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nature genetics*. 2009; 41(4):450–4. [PubMed: 19287385]
 13. Klampfl T, Harutyunyan A, Berg T, Gisslinger B, Schalling M, Bagienski K, et al. Genome integrity of myeloproliferative neoplasms in chronic phase and during disease progression. *Blood*. 2011; 118(1):167–76. [PubMed: 21531982]
 14. Vilaine M, Olcaydu D, Harutyunyan A, Bergeman J, Tiab M, Ramee JF, et al. Homologous recombination of wild-type JAK2, a novel early step in the development of myeloproliferative neoplasm. *Blood*. 2011; 118(24):6468–70. [PubMed: 22161852]
 15. Swierczek SI, Agarwal N, Nussenzweig RH, Rothstein G, Wilson A, Artz A, et al. Hematopoiesis is not clonal in healthy elderly women. *Blood*. 2008; 112(8):3186–93. [PubMed: 18641369]
 16. Solimini NL, Xu Q, Mermel CH, Liang AC, Schlabach MR, Luo J, et al. Recurrent hemizygous deletions in cancers may optimize proliferative potential. *Science*. 2012; 337(6090):104–9. [PubMed: 22628553]

Figure 1. The mutational pattern of $JAK2^{V617F}$ and 9p aUPD

The mutational pattern of $JAK2^{V617F}$ and 9p aUPD in the discovery study (n=31) and two validation studies obtained from the archived PV DNA specimen from the same institution (n=40 and n=19, respectively) was shown. I, II, III indicates 3 subgroups defined by the mutational pattern. Two patients in the discovery study with triploid $JAK2$ was excluded from the analysis. Black dots and open circles, DNA copy number status determined by Affymetrix SNP6 array. Cross, DNA copy number status determined by Illumina610 SNP array. P values of Pearson's correlation test were shown. Line fit was done by JMP algorithm.

Figure 2. The stability of *JAK2* genotypes

Longitudinal samples were collected from 17 patients during a 6-year period from 2007 to 2013. **(A)** Patients with stable *JAK2* genotypes. **(B, C)** Patients with progressed *JAK2* genotypes. Patients were progressed from prognostically more favorable to less favorable *JAK2* genotypes. **(D, E)** Patients with transformed *JAK2* genotypes. Patient transformed their *JAK2* genotypes and gained new clones. **(F)** The pathway of acquisition *JAK2*^{V617F} and 9p aUPD in PV. G and T indicate the wild-type allele and the mutant allele of the codon 617 of *JAK2* gene, respectively. For translated protein, G corresponds to valine and T corresponds to phenylalanine. The width of the arrows is proportional to the relative percentage of patients which carrying specific mutational pattern calculated by pooled patients from all three cohorts (n=90). The arrows are unidirectional. Therefore patients that transform from one end state to another must represent the outgrowth of a new clone.