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Young Versus Old Age at Diagnosis Confers Distinct Genomic Profiles in Patients with Polycythemia Vera

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Polycythemia vera (PV) is a clonal disease characterized by overproduction of mature erythrocytes, impaired survival, and a propensity for transformation to myelofibrosis (MF) and acute myeloid leukemia (AML).¹ The *JAK2* V617F mutation occurs in > 95% of PV patients, leading to hyperactive JAK-STAT signaling which mediates proliferation, differentiation, and survival.¹ Recurrent mutations in other genes (e.g. *TET2*, *DNMT3A*, *ASXL1*, *IDH1/2*, and *EZH2*) have been identified in PV as well as other myeloid malignancies.¹ Mutations in a subset of these genes (e.g. *TET2*, *DNMT3A*, and *ASXL1*) have also been associated with clonal hematopoiesis of indeterminate potential (CHIP).² How these mutations modulate PV disease phenotype is not well understood.

PV is typically diagnosed in older patients (> 65 years old, PV_{old}) but can occur in younger patients (< 45 years old, PV_{young}). In a prior study, PV_{young} patients were reported to be predominantly female, with lower white blood cell count and *JAK2* V617F mutant allele burden, and were more likely to present with splenomegaly and splanchnic vein thrombosis (SVT) than PV_{old} patients.³ Transformation to MF/AML was similar for both groups, but the median time to transformation was longer in PV_{young}.³ The reasons for these findings are unclear. Somatic mutations have been shown to accumulate as a function of age, and a recent study demonstrated that mutation order can dictate specific clinical features in MPNs.^{4, 5} These observations led us to hypothesize that the mutational profile of PV_{young} may differ from PV_{old} and potentially contribute to distinct clinical phenotypes observed in these cohorts.

Samples from ten PV_{young} (< 45 years old) and nine PV_{old} (> 65 years old) *JAK2* V617F+ patients were identified for genomic analysis. Similar to Stein et. al, a trend towards more females and SVT events in the PV_{young} group was observed (Fig. 1a).³ When the cohort was expanded with clinical data from an additional nine young and nine old non-sequenced *JAK2* V617F+ patients, the PV_{young} group had significantly more females than PV_{old}, and enrichment of SVT in PV_{young} approached significance (Fig. 1a & Supp. Table S1).

Genomic DNA was isolated from either bulk peripheral blood mononuclear cells (PBMC) or granulocytes, in addition to matched normal tissue (skin or sorted CD3+ T cells) obtained

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The authors report no conflicts of interest.

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from each patient. Age at sample collection was on average within 1.5 years of diagnosis (Fig. 1a). Drug treatment for patients did not significantly differ with age (Supp. Table S1). Enhanced exome capture sequencing was performed utilizing a standard xGen panel plus additional probes for recurrently mutated genes in MPN and/or AML.

Over 99% of exons sequenced had at least 20x coverage, with a mean depth of 133 reads. Of the 19 patients sequenced, three samples were removed due to sample contamination and one sample was removed due to technical artifact issues, leaving seven PV_{young} and eight PV_{old} remaining for analysis. The *JAK2* V617F variant allele frequency (VAF) was not significantly different between PV_{old} and PV_{young} ($p = 0.3357$) (Supp. Fig. S1a). However, when clinical *JAK2* V617F data was collected from 16 separate patients from the expanded cohort, a significantly higher VAF in older patients was observed ($p = 0.0188$) (Supp. Fig. S1b).

A total of 107 somatic mutations were identified, of which 84 were non-synonymous (Supp. Table S2). A significantly higher mutation load was observed in PV_{old}, with a mean of 10.4 mutations versus 3.4 for PV_{young} ($p = 0.0154$) (Fig. 1b). No large-scale chromosomal aberrations were identified (data not shown).

Secondary mutations potentially contributing to disease phenotype were identified in each cohort. Non-synonymous coding variants predicted to be deleterious to protein function through either protein truncation or having a FATHMM score > 0.5 and occurring in a gene where variants have been reported in a myeloid malignancy in multiple studies were considered. The identification of these putative secondary driver mutations revealed a striking difference in PV_{young} versus PV_{old} patients. Secondary mutations were identified in 7/8 PV_{old} patients, namely in epigenetic regulators *ASXL1*, *TET2*, *DNMT3A*, transcription factors *FOXPI* and *NFE2*, and cell cycle regulator *CHEK2*. In contrast, secondary driver mutations were not identified in any PV_{young} patients (Fig. 1c & Table 1).

Mutation order in the PV_{old} cohort was inferred from VAF or serial sampling (patient 374024 who transformed to AML). Three mutations were predicted to arise before *JAK2*, with four mutations occurring coincident with *JAK2*, and two mutations arising after *JAK2* (Fig. 1d). For 4/8 PV_{old} patients where cryopreserved PBMCs were available, mutation validation via qPCR SNP genotyping was performed on lineage negative CD34+ single cell-derived colonies (Fig. 1e). In three patients, secondary mutations were confirmed as occurring before (336362-*ASXL1*) or coincident (513509-*CHEK2*; 673286-*FOXPI/NFE2*) with *JAK2* (Fig. 1e). For patient 149502, the identification of *ASXL1* in 7/12 colonies but absence of *JAK2* V617F in all colonies tested suggested that *ASXL1* was acquired prior to *JAK2* but could not exclude the potential existence of two independent clones (Fig. 1e). For PV_{old} patient 374024, sequencing of serial samples associated with disease transformation revealed loss of *JAK2/TET2*, while *ASXL1* remained present in a subclone containing new *RUNX1/NRAS* mutations. These findings imply acquisition of *ASXL1* prior to *JAK2*, with subsequent AML arising from the *ASXL1*-only clone (Supp. Fig. S2). These data show that in PV_{old} secondary mutations can be acquired before, coincident with, or after *JAK2*, with no specific predominant pattern.

The germline *JAK2* 46/1 haplotype is known to predispose to *JAK2* V617F+ MPN.⁶ Consistent with a previous report, 73.3% and 13.3% of patients were 46/1 heterozygous or homozygous, respectively, with no significant difference in distribution by age (Supp. Fig. S3a).⁶ When additional germline variants in genes known to potentially predispose to hematologic malignancies were identified (*JAK2*, *MECOM*, *TET2*, *RUNX1*, *TP53*, *ATM*, *GATA2*),⁷⁻⁹ however, PV_{young} averaged nearly twice the number of variants per patient as PV_{old}, although the difference was not significant ($p = 0.2918$) (Supp. Fig. 3b & Supp. Table S3).

The observed single driver landscape in PV_{young} suggests non-genomic factors may contribute to the clinically distinct phenotype. Inflammatory cytokines are overproduced in PV and increase with normal aging.^{10, 11} The profile of ten selected cytokines was examined in the plasma of 15 PV_{young} and 13 PV_{old} patients in addition to 17 age-matched normal donors. No significant differences in cytokine concentrations between young and old normal plasma were identified, although this may have been related to small sample size (Supp. Fig. S4a). Compared to age-matched normals, the same cytokines were significantly increased in PV_{young} and PV_{old} plasma, namely IFN- γ , IL-2R α , IL-6, IP-10, MIP-1 α , and TNF (Supp. Fig. S4b & S4c). This abnormal cytokine profile was accentuated with age, as the fold changes of IFN- γ , IL-2R α , IL-6, IP-10, TNF and VEGF (relative to age-matched normals) were significantly higher in old versus young patients (Supp. Fig. S4d). These data indicate that overproduction of inflammatory cytokines is a hallmark of PV that is further exacerbated by older age.

With recent research beginning to better characterize young MPN patients,^{12, 13} this study is the first to directly compare the mutation profile of young versus old PV patients. The finding that several secondary mutations identified in PV_{old} can also be found with CHIP raises the possibility that these mutations were related merely to aging and occurred independent of *JAK2* and the patient's underlying PV. However, in 3/4 patients with genotyping validation, the presence of mutations within the same clone as *JAK2* was confirmed. If these secondary mutations were entirely due to aging, one might expect that they would predominantly occur prior to *JAK2* V617F. However, a minority of the observed non-*JAK2* drivers (3/9) were likely acquired before *JAK2*. While these secondary mutations may nonetheless have been acquired incidentally, it remains plausible that they may contribute to the differences in clinical phenotype observed in old versus young PV patients.

The high prevalence of secondary driver mutations in PV_{old} is consistent with the previously reported shorter median time to MF/AML transformation in PV_{old} compared to PV_{young}.³ These findings are also consistent with our prior study in primary myelofibrosis demonstrating accrual of disease-modifying mutations in conjunction with disease transformation.¹⁴ In a study focused on mutation order in MPNs, the transcriptional effects of *JAK2* V617F were different depending on whether it occurred before or after *TET2*. In "JAK2-first" patients, single *JAK2* mutant clones were larger than non-mutant and double mutant clones, suggesting that the addition of a second mutation can limit *JAK2*-driven HSPC proliferation.⁴ It is likely in PV_{young} that the effects of *JAK2* V617F in a single mutant landscape are different than when it is accompanied by a second mutation, and may contribute to distinct clinical phenotypes.

In conclusion, our data indicates that younger individuals develop PV in the setting of a single somatic *JAK2* V617F mutation, whereas in older individuals *JAK2* is predominantly accompanied by a secondary driver mutation that might not be entirely explained by age-induced mutation accumulation and which may impact PV disease presentation. Larger scale studies will be needed to verify these observations. In addition, the role of inflammatory cytokines, and the potential contribution of epigenetic¹⁵ and gender-related factors^{3, 15} in PV clinical phenotype remains to be further elucidated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Tefferi A, Pardanani A. Myeloproliferative Neoplasms: A Contemporary Review. *JAMA Oncol* 2015 4; 1(1): 97–105. [PubMed: 26182311]
2. Link DC, Walter MJ. ‘CHIP’ping away at clonal hematopoiesis. *Leukemia* 2016 8; 30(8): 1633–1635. [PubMed: 27311932]
3. Stein BL, Saraf S, Sobol U, Halpern A, Shammo J, Rondelli D, et al. Age-related differences in disease characteristics and clinical outcomes in polycythemia vera. *Leuk Lymphoma* 2013 9; 54(9): 1989–1995. [PubMed: 23245211]
4. Ortmann CA, Kent DG, Nangalia J, Silber Y, Wedge DC, Grinfeld J, et al. Effect of mutation order on myeloproliferative neoplasms. *N Engl J Med* 2015 2 12; 372(7): 601–612. [PubMed: 25671252]
5. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell* 2012 7 20; 150(2): 264–278. [PubMed: 22817890]
6. Trifa AP, Banescu C, Tevet M, Bojan A, Dima D, Urian L, et al. TERT rs2736100 A>C SNP and *JAK2* 46/1 haplotype significantly contribute to the occurrence of *JAK2* V617F and *CALR* mutated myeloproliferative neoplasms - a multicentric study on 529 patients. *Br J Haematol* 2016 7; 174(2): 218–226. [PubMed: 27061303]
7. Hinds DA, Barnholt KE, Mesa RA, Kiefer AK, Do CB, Eriksson N, et al. Germ line variants predispose to both *JAK2* V617F clonal hematopoiesis and myeloproliferative neoplasms. *Blood* 2016 8 25; 128(8): 1121–1128. [PubMed: 27365426]
8. Bougeard G, Baert-Desurmont S, Tournier I, Vasseur S, Martin C, Brugieres L, et al. Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome. *J Med Genet* 2006 6; 43(6): 531–533. [PubMed: 16258005]
9. Godley LA. Inherited predisposition to acute myeloid leukemia. *Semin Hematol* 2014 10; 51(4): 306–321. [PubMed: 25311743]
10. Vaidya R, Gangat N, Jimma T, Finke CM, Lasho TL, Pardanani A, et al. Plasma cytokines in polycythemia vera: phenotypic correlates, prognostic relevance, and comparison with myelofibrosis. *Am J Hematol* 2012 11; 87(11): 1003–1005. [PubMed: 22965887]

11. Alvarez-Rodriguez L, Lopez-Hoyos M, Munoz-Cacho P, Martinez-Taboada VM. Aging is associated with circulating cytokine dysregulation. *Cell Immunol* 2012; 273(2): 124–132. [PubMed: 22316526]
12. Boddu P, Masarova L, Verstovsek S, Strati P, Kantarjian H, Cortes J, et al. Patient characteristics and outcomes in adolescents and young adults with classical Philadelphia chromosome-negative myeloproliferative neoplasms. *Ann Hematol* 2018 1; 97(1): 109–121. [PubMed: 29143068]
13. Szuber N, Vallapureddy RR, Penna D, Lasho TL, Finke C, Hanson CA, et al. Myeloproliferative neoplasms in the young: Mayo Clinic experience with 361 patients age 40 years or younger. *Am J Hematol* 2018 8 29.
14. Engle EK, Fisher DA, Miller CA, McLellan MD, Fulton RS, Moore DM, et al. Clonal evolution revealed by whole genome sequencing in a case of primary myelofibrosis transformed to secondary acute myeloid leukemia. *Leukemia* 2015 4; 29(4): 869–876. [PubMed: 25252869]
15. Mascarenhas J, Roper N, Chaurasia P, Hoffman R. Epigenetic abnormalities in myeloproliferative neoplasms: a target for novel therapeutic strategies. *Clin Epigenetics* 2011 8; 2(2): 197–212. [PubMed: 22704337]

Figure 1. Exome sequencing suggests JAK2 V617F is only driver present in young PV.

a) Clinical parameter summaries from the 19 PV patients that underwent exome sequencing (left) and an expanded cohort of 37 total patients (right). Mann-Whitney test was used to test difference between age groups for continuous variables, Fisher's Exact test for categorical variables. "IQR" = interquartile range, "SVT" = splanchnic vein thrombosis, "WBC" = white blood cell. b) Scatter plot showing the mean +/- standard deviation of overall mutational load of young and old PV patients. c) Scatter plot showing the mean +/- standard deviation of the number of putative non-JAK2 driver mutations in young and old PV

patients. Difference in mean mutation number for *b* and *c* assessed by Welch's t-test. d) Bar graph of the variant allele frequencies of driver mutations identified in old PV. Letters above bars signify whether the mutation was predicted to be acquired before ("B"), coincident with ("C"), or after ("A") *JAK2* V617F. e) Plots of genotyping results from custom SNP assays to validate the driver mutations in four old PV patients. Each point represents the genotype of single colony. "WT" = wildtype for mutation; "het" = heterozygous for mutation; "hom" = homozygous for mutation. Variant allele frequencies from bulk cells are displayed above the plots for each patient.

Table 1.

Primary and putative secondary driver mutations in young and old PV patients

Young PV sample	chr	start	ref	var	type	gene	strand	mut type	aa change	Tumor VAF
331060	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	16.5
465783	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	18.93
551599	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	45.51
638517	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	39.9
697576	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	13.6
732651	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	17.36
993645	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	11.03
Old PV sample	chr	start	ref	var	type	gene	strand	mut type	aa change	Tumor VAF
149502	20	31022264	G	A	SNP	ASXL1	1	nonsense	p.W583*	11.13
149502	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	5.14
208617	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	46.87
336362	20	31021250	C	T	SNP	ASXL1	1	nonsense	p.R417*	50
336362	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	33.25
374024	4	106196695	-	A	INS	TET2	1	frame_shift_ins	p.T1676fs	41.8
374024	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	39.62
374024	20	31022441	-	G	INS	ASXL1	1	frame_shift_ins	p.G645fs	39.78
513509	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	17.52
513509	22	29105992	A	C	SNP	CHEK2	-1	splice_site	e7+2	16.57
513884	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	17.91
513884	20	31023561	GCTGACA	-	DEL	ASXL1	1	frame_shift_del	p.A1016fs	6.71
570655	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	25.37
570655	2	25464537	C	T	SNP	DNMT3A	-1	missense	p.R659H	7.87
673286	9	5073770	G	T	SNP	JAK2	1	missense	p.V617F	92.24
673286	3	71027072	G	A	SNP	FOXP1	-1	nonsense	p.Q419*	50
673286	12	54686735	T	-	DEL	NFE2	-1	frame_shift_del	p.Y182fs	44.33

chr = chromosome; ref = reference; var = variant; mut type = mutation type; aa = amino acid; VAF = variant allele frequency; INS = insertion; DEL = deletion; SNP = single nucleotide polymorphism