Sex differences in the *JAK2*^{V617F} allele burden in chronic myeloproliferative disorders

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ABSTRACT

Background
The *JAK2*^{V617F} allele burden is a variable measure, determined by the frequency of mitotic recombination events and the expansion of *JAK2*^{V617F} clones. Since variability in the *JAK2*^{V617F} allele burden is partly responsible for the distinct phenotypes seen in the myeloproliferative disorders, the objective of this study was to identify modifiers of the allele burden.

Design and Methods
Blood samples were obtained between May 2005 and January 2009 from 272 patients with essential thrombocytosis, polycythemia vera, and myelofibrosis. The *JAK2*^{V617F} allele burden was measured by an allele-specific quantitative polymerase chain reaction using DNA from purified neutrophils. Repeated measures, on average 2 years apart, were available for 104 patients.

Results
Sex, age at diagnosis, and disease duration all independently influenced the *JAK2*^{V617F} allele burden. When considering all patients with myeloproliferative disorders, women had significantly lower allele burdens than men (P=0.04). In those patients with repeated measures, the increase in allele burden per year between the first and second evaluations was significantly less in females than in males. Among those who experienced disease evolution, females were 4.5 times more likely to have evolution from essential thrombocytosis to polycythemia vera, but 0.23 times as likely to have evolution from essential thrombocytosis to myelofibrosis.

Conclusions
Sex is an independent factor accounting for variability in the *JAK2*^{V617F} allele burden. We speculate that lower allele burdens in females reflect a lower frequency of mitotic recombination events in females than in males, and should be considered when evaluating the relationship of allele burden to disease phenotype and also in evaluating responses to *JAK2*^{V617F}-inhibitors. Because sex may influence genotype and/or clonal expansion, underpinning the variability in *JAK2*^{V617F} allele burden, it will be important to explore factors that determine susceptibility to mitotic recombination events.

Key words: *JAK2*^{V617F} allele, phenotypes, myeloproliferative disorders.

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Introduction

The myeloproliferative disorders – essential thrombocytosis, polycythemia vera, and primary myelofibrosis – share the same acquired genetic lesion, *JAK2*^{V617F}, but differ with respect to epidemiology and natural history. There are also clinical and pathological differences among the three disorders, raising the question of how a shared genetic lesion can result in three distinct phenotypes. We have shown that the *JAK2*^{V617F} allele burden at both the neutrophil and CD34^{+} cell level varies among patients with myeloproliferative disorders, and is a consequence of both the degree of dominance of the *JAK2*^{V617F} clone and the specific *JAK2*^{V617F} genotype (heterozygous *versus* homozygous) as determined by acquired uniparental disomy.[1,2] Variability of the *JAK2*^{V617F} allele burden is a major modifier of the phenotype of myeloproliferative disorders, and within polycythemia vera, a modifier of white blood cell count and extramedullary disease.[2-5]

Since variability of *JAK2*^{V617F} allele burden has diagnostic and prognostic implications, factors that may explain this variability are of particular importance. The objective of this study was to identify modifiers that account for variability in the *JAK2*^{V617F} allele burden. We identified sex as a potential modifier, based initially on observations of sex differences in our cohort of patients with myeloproliferative disorders. Though polycythemia vera has been described as occurring predominantly in males,[6-12] our cohort of polycythemia vera patients contained more females than males (female-male ratio, 1.7 to 1). In fact, other studies have reported similar findings. Johansson *et al.*[13] reported on the incidence rates over time in a cohort from Goteburg, Sweden; over a 16-year period, of 205 patients diagnosed with polycythemia vera, 118 were female (female-male ratio, 1.36 to 1). The annual gender-specific incidence rates were 2.4 per 10^5 male inhabitants compared to 3.08 per 10^5 female inhabitants.[13] Confirming historical data,[6] we have observed that polycythemia vera is manifested at a younger age in women than in men. We have also observed differences in disease distribution, including an over-representation of women among those with essential thrombocytosis, and an under-representation of women among those with primary myelofibrosis.[2] These observations from our cohort are consistent with literature supporting a female predominance in essential thrombocytosis and a male predominance in primary myelofibrosis.[11,12]

After noting these sex differences in disease distribution, we evaluated whether there were also differences in the *JAK2*^{V617F} allele burden between males and females. Our initial observations suggested that female patients with essential thrombocytosis and polycythemia vera had lower *JAK2*^{V617F} allele burdens than males with the same disorders; we also noted a selective expression of *JAK2*^{V617F} in the platelets of female patients as opposed to male patients.[14,15]

As a result of these observations, we hypothesized that sex may be an important modifier of *JAK2*^{V617F} allele burden, associated with age and disease duration. To test this hypothesis, we examined the interaction between sex and factors that were believed to be associated with the quantitative *JAK2*^{V617F} allele burden in 272 male and female patients with myeloproliferative diseases in the Johns Hopkins Center for Chronic MPD cohort.

Design and Methods

Study design and participants

This study was based on 272 consecutive patients with *JAK2*^{V617F}-positive myeloproliferative diseases – essential thrombocytosis, polycythemia vera, and myelofibrosis (including post-essential thrombocytosis and post-polycythemia vera cases) – referred to the Johns Hopkins Center for Chronic MPD between May 2005 and January 2009. The diagnoses of polycythemia vera and essential thrombocytosis were based on the Polycythemia Vera Study Group criteria;[16,17] the diagnosis of primary myelofibrosis was based on Italian Consensus criteria.[3,18,19] Myelofibrosis patients with an antecedent myeloproliferative disease were characterized as having post-essential thrombocytosis myelofibrosis or post-polycythemia vera myelofibrosis.[18] All patients gave written consent to venipuncture and allowed their clinical and laboratory data to be recorded in a database for later analysis; the development of this database was approved by the Johns Hopkins University Institutional Review Board.

Primary and secondary outcomes

The primary outcome was the *JAK2*^{V617F} allele burden, expressed as a continuous variable. The *JAK2*^{V617F} allele burden was measured using an allele-specific, quantitative real-time polymerase chain reaction assay sensitive to 5% of the wild-type or mutant *JAK2* allele; intra-assay replicates did not vary by more than 5%, as previously described.[3] Of 272 patients, 104 had repeat measures of the *JAK2*^{V617F} allele burden available for analysis. The primary interest was effect modification by sex on the associations between the primary outcome covariates including disease subgroup, age at diagnosis, disease duration and follow-up time (follow-up time reflected the difference in years between the two allele burden measures); all of these covariates were assessed at the time of *JAK2*^{V617F} determination. Secondary outcomes included disease distribution and the risk and type of disease evolution, hypothesized to be differentiated by sex. Genotype was included as a covariate in the secondary outcome analysis, and was considered as a binary variable. A homozygous genotype was defined based on a *JAK2*^{V617F} allele burden of 55% or more, while allele burdens less than 55% were considered to reflect a heterozygous genotype.

Statistical analysis

Distributions of outcomes and covariates were described by sex, using means and standard deviations for continuous variables that were relatively symmetric, medians and interquartile ranges for continuous variables that were skewed, and number and percentages for categorical variables. The Mann-Whitney rank sum test was used for hypothesis testing of sex differences for continuous variables as appropriate. Testing for sex differences in mean proportions was performed using the z test, or the χ^2 statistic when appropriate, while Fisher's exact test was used when observed and/or expected cell counts in the contingency table were small. Lowess plots were used to explore potential departures from linear associations between the primary outcome and continuous covariates. Linear splines were used to provide segment-linear approximation of a potentially non-linear association to ease interpretation of modeling results. The generalized estimating equations approach was used to account for potential correlations among repeated outcome measures in the linear models to determine the association between the *JAK2*^{V617F} allele burden and sex, age at diagnosis, disease duration, and the follow-up time variable. The regression coefficient for the follow-up time variable captured the mean annual change in allele burden between evaluation 1 and evaluation 2 afforded by information from those with repeated outcome measures. The Shapiro-Wilks test was used to

detect non-normality of the studentized residuals as part of the model checking procedure. Multivariable logistic regression was used to identify variables that associated with the odds of disease evolution, and multinomial logistic regression (estimating relative risk ratios) was used to identify variables that were associated with the type of disease evolution. Data were analyzed using the STATA 10.1 program (STATA Corp, College Station, TX, USA).

Results

Baseline characteristics

The 272-patient cohort consisted of 60 patients with essential thrombocytosis, 165 with polycythemia vera, 30 with primary myelofibrosis, 8 with post-essential thrombocytosis myelofibrosis, and 9 with post-polycythemia vera myelofibrosis (Table 1). The majority of the patients were female (61%) with the highest percentage of females in the subgroups with essential thrombocytosis (70%) and polycythemia vera (64%). Females were more likely to distribute in the essential thrombocytosis (42/165: 25%) and polycythemia vera (106/165: 64%) subgroups as compared to males who more likely to distribute within the polycythemia vera (59/107: 55%) and myelofibrosis (30/107: 28%) subgroups (P=0.001). Across the entire cohort, the median age difference at diagnosis of a myeloproliferative disease between males and females was 4 years (P=0.004). In the subgroup with polycythemia vera, men were 6 years older at diagnosis (P=0.01), but the difference in age at diagnosis was not significant between males and females with essential thrombocytosis or myelofibrosis. Among all patients with myeloproliferative disease, median disease duration was shorter in males than in females (3 *versus* 4 years, respectively, P=0.09), but this was only of marginal statistical significance. There were no significant differences in disease duration when looking by disease subgroup. Among 104 (70 female, 34 male) participants with repeated measures of *JAK2*^{V617F} allele burden, the median interval between measures was 2 years for females and 3 years for males; there were no statistically significant differences in this interval across the entire cohort or within any of the disease classes.

The median allele burden differed between males and females. For the entire cohort, the mean allele burden was significantly higher in males (63%) than in females (53%) (P=0.041). From a disease-specific perspective, males with polycythemia vera had significantly higher allele burdens compared to females with polycythemia vera (74% *versus* 63%, respectively; P=0.007). The allele burdens were not statistically different when analyzing males and females with essential thrombocytosis, but females with myelofibrosis had higher allele burdens than their male counterparts (75% *versus* 55%; P=0.018) in this univariate analysis.

The majority of the male patients with myeloproliferative diseases (58%) had a homozygous genotype, defined by a *JAK2*^{V617F} allele burden of 55% or more, compared to a smaller proportion of female patients who were considered to be homozygous (47%, P=0.077). Within the polycythemia vera subgroup, 80% of male patients had a homozygous genotype, whereas only 61% of the female patients were homozygous (P=0.016). The proportions of patients with a homozygous genotype did not differ significantly among those with essential thrombocytosis or myelofibrosis, although in the latter subgroup, 71% of females were homozygous compared to only 47% of male patients (Table 1). Among 104 patients with repeated measures, the genotype, defined according to allele burden, was unchanged in 94 patients. Of the 10 patients whose genotype changed, the allele burdens decreased below the 55% threshold in four patients (1 female with essential thrombocytosis, 1 female with polycythemia vera, 1 male with polycythemia vera and 1 male with myelofibrosis); of the remaining six patients whose allele burdens increased above the 55% threshold at the second evaluation, all had polycythemia vera and three were female and three were male (*data not shown*).

The association between sex, disease duration, and JAK2^{V617F} allele burden

To further evaluate differences in *JAK2*^{V617F} allele burden as a function of disease duration, we compared allele burdens in males and females, stratified by time from diagnosis. Disease duration was stratified based on the range of the disease duration in the entire cohort of patients and included those with disease durations below the 25th percentile (<1 year from diagnosis), the middle 50% (≥1 years and <8 years from diagnosis) and above the 75th percentile (≥8 years from diagnosis). The allele burdens in males and females measured within 1 year after diagnosis did not differ statistically (51% *versus* 49% respectively, P=0.715). However, when comparing males and females who were analyzed 8 years or more after diagnosis, males had higher allele burdens than females (74% *versus* 54%, respectively; P=0.06), although this difference was of only marginal statistical significance (Table 1). The effect of disease duration on the allele burden between males and females was also analyzed within the disease subgroups. Among polycythemia vera patients, the allele burdens were similar within 1 year from diagnosis (P=0.173), but were higher in males than in females when measured 8 years or more after diagnosis (82% *versus* 63%, respectively; P=0.076), although this difference was of marginal statistical significance because of the sample size (Table 1). A similar analysis was performed in male and female patients with essential thrombocytosis, but there were no statistical differences when comparing allele burdens at any of the three intervals from diagnosis. The allele burdens in males and females with myelofibrosis differed statistically between 1 and 8 years after diagnosis (53% *versus* 75%; P=0.037), but not beyond this time point (Table 1).

Determinants of JAK2^{V617F} variability: generalized estimating equations regression model

A generalized estimating equations regression model was constructed to predict the mean *JAK2*^{V617F} allele burden as a function of sex in all the *JAK2*^{V617F}-positive patients (Table 2). Because of baseline imbalances in age at diagnosis and disease duration between males and females, these covariates were included in the model; furthermore, given that the relationships between the continuous covariates (age at diagnosis, disease duration) and the *JAK2*^{V617F} allele burden were non-linear, spline terms were generated to appropriately model these variables with knots at age 60 and a disease duration of 12 years. A variable representing the change in allele burden between the first and second evaluations was incorporated to analyze repeated measures of the allele burden. Interaction terms were included for sex and disease subgroup, sex and disease

duration, and disease subgroup and disease duration.

Results from the generalized estimating equations regression analysis are presented in Table 2. Linear contrasts of regression coefficients were used to estimate 95% confidence intervals (95% CI) and *P* values for the expected allele burden by disease subgroup and sex for patients at evaluation 1, for those who were over 60 years old at diagnosis and whose disease had been present for 12 years or more; the other presented values reflect the corresponding expected changes in the allele burden per year of increase in disease duration, age at diagnosis, and time between the first and second evaluations.

Determinants of JAK2^{V617F} variability: consistent associations in all disease classes

Regardless of disease class, age at diagnosis was independently associated with the *JAK2*^{V617F} allele burden, but the relationship differed depending on age; for those patients who were diagnosed at younger than 60 years of age, the adjusted allele burden was predicted to increase by 0.4% (95% CI 0.1, 0.6; *P*=0.001) for every year increase in age at diagnosis, whereas if patients were older than 60 years of age at diagnosis, the adjusted allele burden actually decreased by 0.5% (95% CI -0.9, -0.04; *P*=0.03) per year increase in age at diagnosis. There was a significant increase in allele burden from evaluation 1 to evaluation 2. In those male patients with the same disease duration at evaluation 1, each additional year during follow-up was associated with a 1.8% increase (95% CI 1.0, 2.7%; *P*=<0.001) in the *JAK2*^{V617F} allele burden, adjusting for disease class and age at diagnosis. However, this increase in the allele burden during each year of follow-up differed by sex, as the magnitude of increase was less in female patients, only increasing by 0.7% (95% CI 0.2, 1.3; *P*=0.007) per year increase during follow-up. To place this modeling finding in context, over 10 years of follow-up the estimated mean allele burdens rose by 18% in males compared to 7% in females. There was no statistically significant interaction between evaluation time and disease class.

Differing magnitudes of association among disease classes

Among the remaining variables, the expected magnitude of change attributed to covariates differed by disease class. Among the patients with essential thrombocytosis, the adjusted *JAK2*^{V617F} allele burden was 42% in males, compared to 37% in females, but the difference due to sex was not statistically significant (*P*=0.36). The level of mean *JAK2*^{V617F} allele burden associated with each year of increase of disease duration was not significantly different whether the disease had been present for more than 12 years or not. In patients with polycythemia vera, however, the adjusted *JAK2*^{V617F} allele burden was statistically higher in males than in females (70% *versus* 62%, respectively, *P*=0.032). Unlike the situation in patients with essential thrombocytosis, there were statistically significant associations between disease duration and the expected change in allele burden, although these relationships did not significantly differ by sex. In the first 12 years of disease, the allele burden was expected to rise by 1.4% in males (*P*=0.045) for each 1 year increase in disease duration and 1.5% in females (*P*=0.002); however, in a patient whose disease had been present for more than 12 years, the magnitude was less, with a 0.9% increase in males (*P*=0.029) and a 0.6% increase in females (*P*=0.006) for each 1-year increase in disease duration, adjusted for all other covariates. Finally, there were also sex differences in the expected *JAK2*^{V617F} allele burden in patients with myelofibrosis, but the relationship was reversed, in that females had statistically higher allele burdens than males (65% *versus* 51%, respectively; *P*=0.013). The magnitude of change attributed to disease duration was also greater in myelofibrosis patients than in those with essential

Table 1. Differences in the myeloproliferative disorders cohort by sex.

	Female	Male	*P* value
Number (%)			
Any myeloproliferative disorder	165 (60.7)	107 (39.3)	**0.001
Essential thrombocytosis	42 (70)	18 (30)	
Polycythemia vera	106 (64)	59 (36)	
*Myelofibrosis	17 (36)	30 (64)	
Age at diagnosis, years: median [IQR]			
Any myeloproliferative disorder	53 (37-63)	57 (48-67)	0.004
Essential thrombocytosis	50 (37-59)	48 (31-63)	0.936
Polycythemia vera	50 (35-64)	56 (50-67)	0.01
Myelofibrosis	59 (53-63)	61 (51-68)	0.504
Disease duration, years: median [IQR]			
Any myeloproliferative disorder	4 (1-8)	3 (1-8)	0.088
Essential thrombocytosis	4 (2-8)	3 (1-9)	0.315
Polycythemia vera	4 (1-9)	2 (1-7)	0.284
Myelofibrosis	3 (1-6)	2 (0-7)	0.612
Follow-up, years: median [IQR] (N)			
Any myeloproliferative disorder	2 (1-4) (70)	3 (2-4) (34)	0.538
Essential thrombocytosis	2 (1-3.5) (20)	2 (2-4) (3)	0.476
Polycythemia vera	3(2-5) (46)	3 (2-4) (23)	0.99
Myelofibrosis	2 (1.5-2) (4)	2.5 (1.5-3) (8)	0.283
% Allele burden: median [IQR]			
Any myeloproliferative disorder	53 (42-69)	63 (46-84)	0.041
Essential thrombocytosis	34 (22-43)	44 (34-48)	0.134
Polycythemia vera	63 (50-71)	74 (57-88)	0.007
Myelofibrosis	75 (55-100)	55 (46-79)	0.018
% Allele burden: median [IQR] (N) <1 year from diagnosis			
Any myeloproliferative disorder	51 (37-62) (23)	49 (41-67) (20)	0.715
Essential thrombocytosis	23 (19-50) (6)	38 (38-51)(3)	0.3
Polycythemia vera	52 (47-63) (16)	68 (45-76)(9)	0.173
Myelofibrosis	61 (1)	46 (41-53)(8)	0.245
% Allele burden: median [IQR] (N) ≥1 and <8 years			
Any myeloproliferative disorder	54 (41-70) (88)	63 (48-83) (58)	0.187
Essential thrombocytosis	34 (29-39) (21)	44 (18-46) (9)	0.248
Polycythemia vera	66 (50-71) (54)	70 (58-86) (36)	0.096
Myelofibrosis	75 (55-100) (13)	53 (48-77) (13)	0.037
% Allele burden: median [IQR] (N) ≥8 years			
All myeloproliferative disorders	54 (43-71) (53)	74 (49-93) (29)	0.06
Essential thrombocytosis	33 (20-52) (14)	42 (34-49) (6)	0.508
Polycythemia vera	63 (49-78) (36)	82 (69-100) (14)	0.076
Myelofibrosis	82 (55-100) (3)	90 (57-100) (9)	1.00
% Homozygous genotype			
Any myeloproliferative disorder	47	58	***0.077
Essential thrombocytosis	0	6	***0.128
Polycythemia vera	61	80	***0.016
Myelofibrosis	71	47	***0.113

**Myelofibrosis includes 30 cases of primary myelofibrosis, 8 post essential thrombocytosis myelofibrosis, and 9 post polycythemia vera myelofibrosis cases; **χ^2 statistic 13.81, reflects dependence of disease distribution and sex *** P values for mean difference in proportions (ζ test).*

thrombocytosis or polycythemia vera. In those whose disease had been present less than 12 years, each 1-year increase was associated with a 4.3% increase in males ($P<0.001$) and a 4.4% increase in females ($P<0.001$). In those whose disease had lasted longer than 12 years, the expected increase in the *JAK2*^{V617F} allele burdens associated with per year longer in disease duration were lesser in magnitude (1% in males and 0.8% in females) and did not reach statistical significance.

Associations between sex, disease class, disease duration, genotype and the JAK2^{V617F} allele burden

The associations between sex, disease class, disease duration, genotype, and the measures of *JAK2*^{V617F} allele burden (at evaluations 1 and 2) are integrated in Figure 1. The allele burdens in patients with essential thrombocytosis were lower than those in patients with polycythemia vera or myelofibrosis, were not significantly different by sex, were below 55%, indicating predominant heterozygosity, and showed the smallest magnitude of change through the course of the disease duration (Figure 1, Panel A).

The allele burdens in polycythemia vera were higher in males than in females, and the majority of allele burdens were greater than 55%, indicating predominant homozygosity; based on the univariate analysis, the prevalence of homozygosity was greater in males than in females. Furthermore, the magnitude of change in the allele burden through the course of disease duration was greater than in essential thrombocytosis, and the increase in allele burden was most apparent in the first 12 years of the disease. The slope for this change in allele burden appears greater in males than in females, but this was not significantly different in the generalized estimating equations regression model, adjusting for sex, age at diagnosis and disease duration (Figure 1, Panel B).

The allele burdens in myelofibrosis were higher in females than in males, and among females, more likely to be greater than 55%. The magnitude of change in the allele burden through the course of disease was greater than in polycythemia vera and essential thrombocytosis, and the rate of rise was greatest in the first 12 years of disease; thereafter, the allele burdens decrease. However, there were only a limited number of observations from patients with long-standing disease, allowing a less precise estimate of the slope for that range of disease duration (Figure 1, Panel C).

Sex differences in disease evolution

Finally, sex differences in the risk of and type of disease evolution were explored between the 16 males and 34 females with antecedent essential thrombocytosis or polycythemia vera who had a history of disease evolution (essential thrombocytosis to polycythemia vera or myelofibrosis and polycythemia vera to myelofibrosis) based on clinical criteria and those patients who had no such history of disease evolution at the time of *JAK2*^{V617F} allele burden assessment. A multiple logistic regression model was constructed to identify risk factors associated with the odds of disease evolution among those whose disease had evolved, as compared to those whose disease had not yet evolved, but no sex differences in this risk were found in a model that included sex, and was adjusted for disease duration (1st *versus* 2nd 12 years of diagnosis), age at diagnosis (before *versus* after 60 years of age), and genotype (*data not shown*). Among 33 patients who had disease evolution from essential thrombocytosis to poly-

Table 2. Determinants of variability of the *JAK2*^{V617F} allele burden.

	Females			Males		
	Adjusted *JAK2* allele burden	Expected % change to *JAK2*^{V617F} allele burden (95% CI)	*P* value	Adjusted *JAK2* allele burden	Expected % change to *JAK2*^{V617F} allele burden (95% CI)	*P* value
Essential thrombocytosis	37 (27, 46)			42 (31, 53)		**0.36
Disease duration < 12 years		1.0 (-0.3, 2.4)	0.13		0.9 (-0.8, 2.6)	0.31
Disease duration > 12 years		0.2 (-0.6, 1.1)	0.57		0.5 (-0.6, 1.6)	0.4
Age at diagnosis <60		0.4 (0.1, 0.6)	0.001		***	0.001
Age at diagnosis >60		-0.5 (-0.9, -0.04)	0.03		***	0.03
Evaluation 1 to 2		0.7 (0.2, 1.3)	0.007		1.8 (1.0, 2.7)	<0.001
Polycythemia vera	62 (55, 68)			70 (63, 77)		**0.032
Disease duration < 12 years		1.5 (0.5, 2.5)	0.002		1.4 (0.03, 2.7)	0.045
Disease duration > 12 years		0.6 (0.2, 1.1)	0.006		0.9 (0.09, 1.7)	0.029
Age at diagnosis <60		0.4 (0.1, 0.6)	0.001		***	0.001
Age at diagnosis >60		-0.5 (-0.9, -0.04)	0.03		***	0.03
Evaluation 1 to 2		0.7 (0.2, 1.3)	0.007		1.8 (1.0, 2.7)	<0.001
Myelofibrosis	65 (54, 76)			51 (41, 60)		**0.013
Disease duration <12 years		4.4 (2.3, 6.5)	<0.001		4.3 (2.5, 6.0)	<0.001
Disease duration >12 years		0.8 (-0.3, 1.8)	0.15		1.0 (-0.02, 2.0)	0.054
Age at diagnosis <60		0.4 (0.1, 0.6)	0.001		***	0.001
Age at diagnosis >60		-0.5 (-0.9, -0.04)	0.03		***	0.03
Evaluation 1 to 2		0.7 (0.2, 1.3)	0.007		1.8 (1.0, 2.7)	<0.001

**Generalized estimating equation regression used; 95% CI and P values come from evaluating linear contrasts of coefficients; **P value for the mean difference between females and males within disease class, adjusted for all other covariates; ***the values are identical to those of females since the sex-age at diagnosis interaction was not modeled.*

cythemia vera, 28 (85%) were women; however, when considering eight patients whose disease evolved from essential thrombocytosis to myelofibrosis, six (75%) were men (Fisher's exact test, *P*=0.002); among those whose disease transformed from polycythemia vera to myelofibrosis, five were men (56%). Multinomial logistic regression was used to analyze sex differences in the type of transformation in a multivariable way (Table 3). The relative risk ratio of transformation from essential thrombocytosis to polycythemia vera (compared to no evolution) was 4.5 times higher in females than in males (95% CI 1.6, 12.7; *P*=0.004), adjusted for age at diagnosis and disease duration. In contrast, the adjusted relative risk ratio of transformation from essential thrombocytosis to myelofibrosis was 0.23 times that of males (95% CI 0.04, 1.3; *P*=0.09), with this association being only marginally significant. From this regression model, transformation from essential thrombocytosis to myelofibrosis was 77% less likely in females than in males.

Discussion

Analysis of 272 patients with myeloproliferative diseases in the Johns Hopkins MPD Cohort revealed sex differences in the *JAK2*^{V617F} allele burden. In particular, we found a significantly lower *JAK2*^{V617F} allele burden in women with polycythemia vera than in men, an association that remained after adjusting for age at diagnosis and disease duration. In those patients with repeated measures of allele burden, each year of follow-up was associated with a significant increase in allele burden after accounting for disease duration at first evaluation, and the magnitude was greater in males than in females. In addition to sex, the age at diagnosis and disease duration explained some of the variability in the *JAK2*^{V617F} allele burden. There were also sex-differences in disease distribution, as essential thrombocytosis and polycythemia vera were more common in women, and polycythemia vera and myelofibrosis were more common in men. Furthermore, among those with an antecedent myeloproliferative disease, females with essential thrombocytosis were 4.5 times more likely to develop polycythemia vera than males, but only 0.23 times as likely to have disease evolution to myelofibrosis, compared to males with essential thrombocytosis.

Reports of sex differences in the *JAK2*^{V617F} allele burden throughout the myeloproliferative diseases are limited, but several studies deserve mention. Kittur *et al.*[20] found an association between male sex and a higher burden of *JAK2* mutated alleles in essential thrombocytosis; allele burdens were 5% higher in males than in females with essential thrombocytosis in this study, although the difference was not statistically significant, likely due to the smaller number of patients (only 18 males) with essential thrombocytosis studied. Larsen *et al.*[21] also found a higher *JAK2*^{V617F} allele burden in males than in females, similar to results seen in our univariate analysis. Among patients with polycythemia vera, we found that, compared to females, males had a higher *JAK2*^{V617F} allele burden. Larsen *et al.* also reported an increase in *JAK2*^{V617F}-positive essential thrombocytosis in women, compared to the homozygous disease states of polycythemia vera and primary myelofibrosis, which were found more frequently in men. Our results are consistent with their report.

Girodon *et al.*[22] described sex differences in the allele burden in response to hydroxyurea. A lower post-therapy *JAK2*^{V617F} allele burden was observed for female essential thrombocytosis patients receiving hydroxyurea therapy; also, a statistically significant decrease in *JAK2*^{V617F} allele

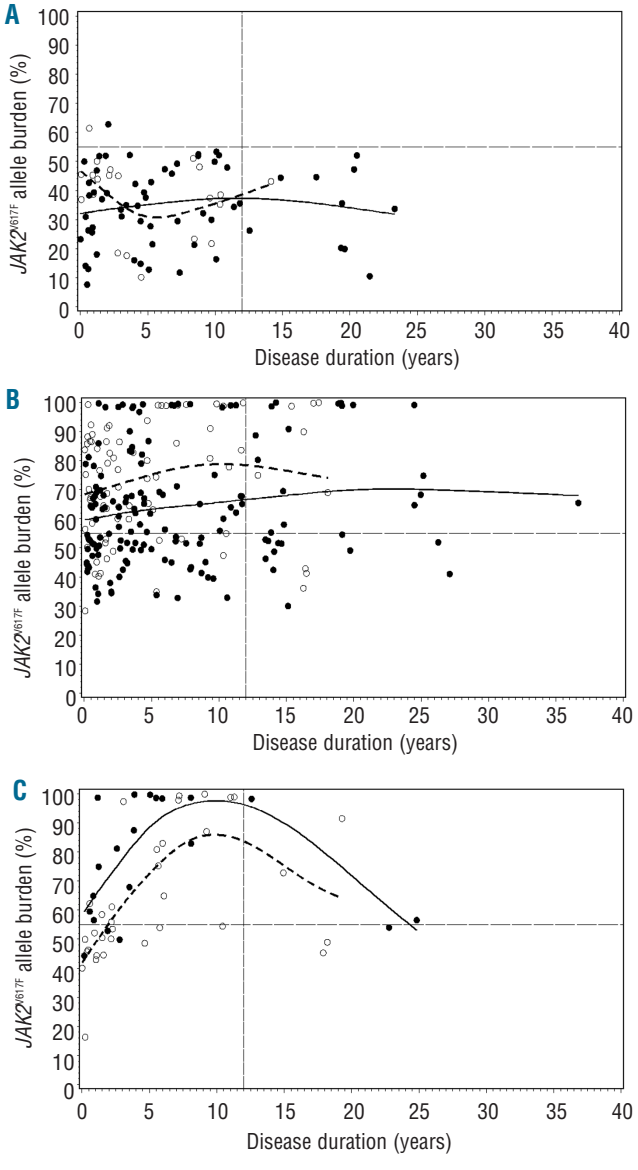

Figure 1. Associations between sex, disease class, disease duration, genotype and the *JAK2*^{V617F} allele burden. Essential thrombocytosis patients (panel A) had the lowest *JAK2*^{V617F} allele burdens of the three disease classes, often lower than 55% (horizontal line) with predominantly heterozygous clones and the smallest magnitude of change in allele burden through the course of the disease. In polycythemia vera (panel B), the allele burdens were higher in males than in females, and the majority of allele burdens were greater than 55%, indicating predominant homozygosity, and the magnitude of change in the allele burden through the course of the disease was greater than in essential thrombocytosis. The increase in allele burden was most apparent in the first 12 (vertical line) years of the disease. In myelofibrosis (panel C), the allele burdens were higher in females than in males, and among females, more likely to be greater than 55%. Also, the magnitude of change in the allele burden through the course of the disease was greater than in polycythemia vera and essential thrombocytosis, and the rate of rise was greatest in the first 12 years of disease; thereafter, the allele burdens decreased. The line reflects slope for females (black circles); dashed lines reflects slope for males (empty circles). The trend line was estimated with a Lowess smoothing procedure.

Table 3. Multinomial logistic regression: predicting the type of evolution.

Type of evolution (Compared to base outcome of no evolution)	Relative risk ratio	*P* value	95% confidence interval
Essential thrombocytosis to polycythemia vera			
Female sex	4.5	0.004	1.6, 12.7
Disease duration < 12 years (per 1 year increase)	0.78	0.001	0.68, 0.90
Disease duration > 12 years (per 1 year increase)	1.0	0.72	0.83, 1.3
Age < 60 years (1 year increase)	0.98	0.44	0.95, 1.0
Age > 60 years (1 year increase)	1.0	0.94	0.92, 1.1
Homozygous genotype	1.4	0.42	0.63, 3.1
Essential thrombocytosis to myelofibrosis			
Female sex	0.23	0.09	0.04, 1.3
Disease duration < 12 years (per 1 year increase)	0.88	0.29	0.7, 1.1
Disease duration > 12 years (per 1 year increase)	1.2	0.29	0.88, 1.5
Age < 60 years (1 year increase)	1.0	0.87	0.92, 1.1
Age > 60 years (1 year increase)	1.0	0.80	0.89, 1.2
Homozygous genotype	0.55	0.45	0.12, 2.6
Polycythemia vera to myelofibrosis			
Female sex	0.69	0.60	0.17, 2.8
Disease duration < 12 years (per 1 year increase)	0.88	0.23	0.71, 1.1
Disease duration > 12 years (per 1 year increase)	1.3	0.08	0.97, 1.6
Age <60 years (1 year increase)	1.2	0.06	0.99, 1.4
Age >60 years (1 year increase)	0.77	0.07	0.58, 1.0
Homozygous genotype	2.8	0.23	0.53, 14.4

burden was observed in female polycythemia vera patients receiving hydroxyurea therapy but not in male patients. As a result of these findings, Girodon *et al.*[22] raised the possibility of a differential sensitivity of the mutated clone to hydroxyurea in female and male patients with myeloproliferative diseases. In our clinical cohort, hydroxyurea use was assessed cross-sectionally, and not at diagnosis, so its effect on the allele burden could not be truly discerned. We observed in our cohort that allele burdens were higher in those taking hydroxyurea, but cannot rule out the possibility of confounding by indication – that disease stage or severity demanded cytoreductive treatment. While lack of adjustment for hydroxyurea use could be considered a limitation of this study, the cross-sectional nature of its assessment and the possibility of its use being confounded by indication would preclude any direct comparison of our results to those of Girodon *et al.* Whether or not hydroxyurea affects *JAK2*^{V617F} allele burden remains unclear.[23]

Our observations come from a relatively large sample of patients with myeloproliferative diseases with well-defined phenotypes, and a valid assay for *JAK2*^{V617F} determination. We also incorporated repeat measures of *JAK2*^{V617F} into our regression model, which captured sex differences in the allele burden between the first and second evaluations. Despite these strengths, our study, like any other observational study, can only identify associations, rather than implying causal relationships. In addition, there remains a potential for residual confounding, as other unknown factors may account for variability in *JAK2*^{V617F} allele burden.

Our study suggests that age, disease duration, and sex influence the variability in *JAK2*^{V617F} allele burden. In all disease classes, age at diagnosis was independently associated with the *JAK2*^{V617F} allele burden, but the relationship changed after 60 years of age; a biological mechanism for this observation is unclear, but suggesting that age is a surrogate for genomic instability is insufficient. Disease duration was also associated with allele burden, but the association differed by disease class, and was more pronounced in polycythemia vera and myelofibrosis, possibly because of the higher proportions of homozygous clones in these patients[2,4] than in patients with essential thrombocytosis.[24] Finally, sex influenced variability of the *JAK2*^{V617F} allele burden, as allele burdens were lower in all female patients with myeloproliferative diseases than in male patients with myeloproliferative diseases, especially among the polycythemia vera group; furthermore, among those patients for whom repeated measures were available, the magnitude of allele burden increase was less in female patients. Though a biological mechanism for these findings is not yet clear, we speculate that the lower *JAK2*^{V617F} allele burdens may reflect a lower frequency of mitotic recombination events, resulting in fewer homozygous *JAK2*^{V617F} clones and less clonal expansion, leading to a predominant distribution within the essential thrombocytosis and polycythemia vera phenotypes.

Our findings suggest that in the genotype-phenotype discrepancy among *JAK2*^{V617F}-positive myeloproliferative diseases, sex should be taken into account when evaluating patients with regard to the diagnosis, prognosis, and

disease complications. Because sex may influence genotype and/or clonal expansion underpinning the variability in *JAK2*^{V617F} allele burden, it will be important to explore factors that determine susceptibility to mitotic recombination events. Future studies are, therefore, indicated, including genome-wide association studies to identify single nucleotide polymorphisms that associate with heterozygous or homozygous *JAK2*^{V617F} disease states.

Authorship and Disclosures

BS, NP, JS, and AM designed the research and wrote the paper; DW, OR and MI designed and performed the research; NYW provided guidance with the statistical analysis and critical editing of the revision of the manuscript.

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