Brief Report

Germline genetic factors influence the outcome of interferon-α therapy in polycythemia vera

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KEY POINTS

- A diplotype spanning the coding region of the *IFNL4* gene influences molecular response to IFN-α therapy in polycythemia vera.

- Determining *IFNL4* diplotype status rather than typing tagSNPs may allow for optimizing patient management during IFN-α treatment of polycythemia vera.

Interferon-α (IFN-α)–based treatments can induce hematologic and molecular responses (HRs and MRs, respectively) in polycythemia vera (PV); however, patients do not respond equally. Germline genetic factors have been implicated in differential drug responses. We addressed the effect of common germline polymorphisms on HR and MR after treatment of PV in the PROUD-PV and CONTINUATION-PV studies in a total of 122 patients who received ropeginterferon alfa-2b. Genome-wide association studies using longitudinal data on HR and MR over a 36-month follow-up did not reveal any associations at the level of genome-wide statistical significance. Furthermore, we performed targeted association analyses at the interferon lambda 4 (*IFNL4*) locus, well known for its role in hepatitis C viral clearance and recently reported to influence HR during treatment of myeloproliferative neoplasms. We did not observe any association of *IFNL4* polymorphisms with HR in our study cohort; however, we demonstrated a statistically significant effect of the functionally causative *IFNL4* diplotype (haplotype pair, including the protein-coding variants rs368234815/rs117648444) on MR ($P = 3.91 \times 10^{-4}$; odds ratio, 10.80; 95% confidence interval, 2.39-69.97) as reflected in differential *JAK2*V617F mutational burden changes according to *IFNL4* diplotype status. Stratification of patients with PV based on IFNL4 functionality may allow for optimizing patient management during IFN-α–based therapy. (*Blood*. 2021;137(3):387-391)

Introduction

Interferon-α (IFN-α)–based therapies can induce sustained hematologic responses (HRs) and durable molecular responses (MRs) in polycythemia vera (PV) and other myeloproliferative neoplasms (MPNs).[1] During treatment of PV, an HR reflects normalization of blood values, whereas a MR marks a substantial reduction of the malignant clone in the peripheral blood, when quantified by the allelic burden of the *JAK2*V617F mutation that drives the disease in 95% of patients with PV.[1-4] IFN-α has been consistently reported to have a disease-modifying capacity whereby it selectively targets malignant cells, which induces durable MRs in some patients, whereas other patients respond insufficiently.[5-7] Previous studies have suggested that certain features of disease-driving somatic mutations and genomic aberrations do not predict the response to treatment with IFN-α.[6-10] The role of germline polymorphisms on a genome-wide scale has not been studied comprehensively in MPNs treated with IFN-α. In patients with hepatitis C, however, germline variation at the interferon lambda 4 (*IFNL4*) locus[11,12] (Figure 1A) has been reported to strongly affect both spontaneous and IFN-α–induced viral clearance.[13-15]

In this study, we addressed the effect of germline genetic factors on the outcome of treatment with ropeginterferon alfa-2b (ropeg), a monopegylated IFN-α, in a cohort of patients with PV (N = 122). We performed genome-wide association studies (GWAS) as an unbiased approach and additionally evaluated the potential influence of *IFNL4* polymorphisms on HR and MR during treatment of PV.

Study design

Data on HR and MR were collected in patients with PV in the ropeg arm of the PROUD-PV (www.clinicaltrials.gov, NCT01949805) and CONTINUATION-PV (NCT02218047) clinical trials[16] (N = 122). For replication, we included data on MR from the PEGINVERA (NCT01193699) study[9] (n = 27). We restricted the HR evaluation to complete HRs only, whereas the MR analysis comprised both complete MRs and partial MRs, defined according to European LeukemiaNet criteria.[17] Genome-wide genotypes were derived from Affymetrix SNP 6.0 microarrays. Additional *IFNL4* variants were sequenced and subsequently phased with SHAPEIT.[18] Association analyses were performed with PLINK[19] and R.[20] Details on study design and patient characteristics have been described elsewhere[16];
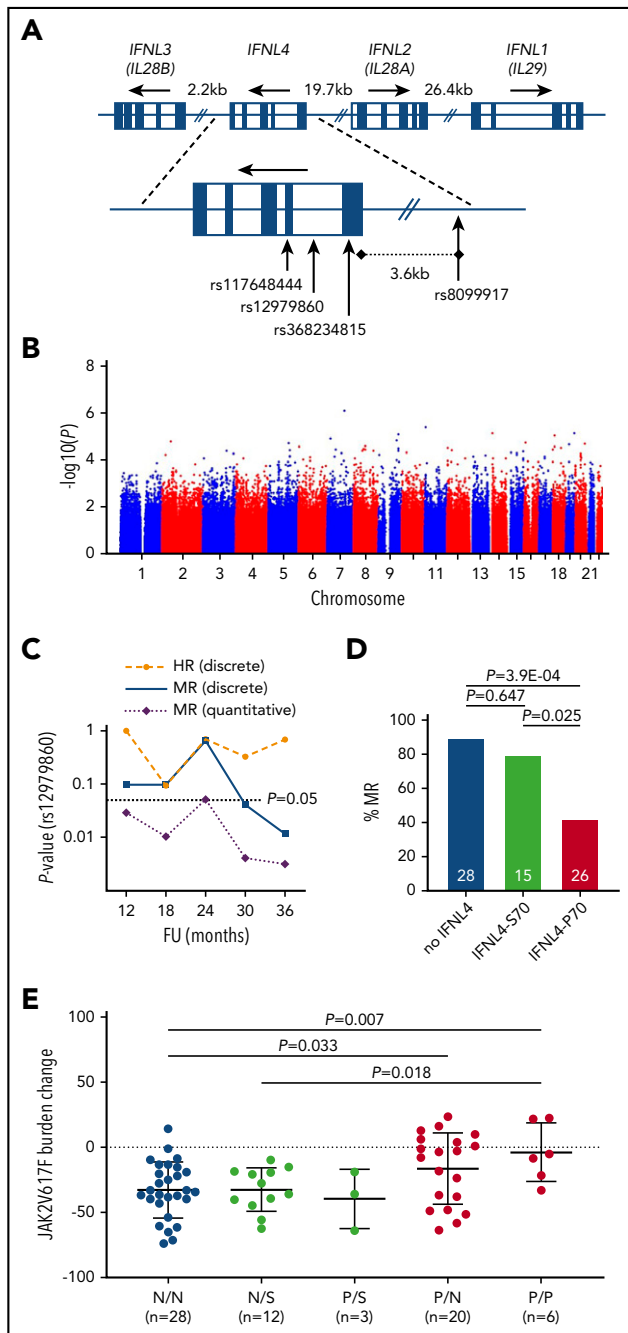
Figure 1. Germline genetic variation within the protein-coding region of IFNL4 affects MR during IFN-α treatment of PV. (A) Genomic organization of the IFNL locus on human chromosome 19q13.2. (B) An association plot derived from GWAS performed for MR after a 12-month follow-up of ropeg treatment (n = 102 patients). The plot is representative of all GWAS performed on both HR and MR data after a 12-, 18-, 24-, 30-, and 36-month follow-up. (C) Longitudinal *P* values for the association of the *IFNL4* tagSNP rs12979860 with response to ropeg over a 36-month follow-up (FU). (D) Fraction (%) of MRs in the 3 main functional categories: patients who produced no functional IFNL4 (no IFNL4), those who produced impaired IFNL4-S70 (IFNL4-S70 alone+IFNL4-S70/P70), and those who produced fully functional IFNL4-P70 alone. Numbers within the bars represent total patients in the categories. (E) *JAK2*V617F mutant allele burden changes (absolute change from baseline value) at a 36-month follow-up in patients stratified according to *IFNL4* diploid functional status. N, no IFNL4; S, IFNL4-S70; P, IFNL4-P70.

additional methods are detailed in the supplemental Information (available on the *Blood* Web site).

Results and discussion

To test for potential associations between germline genetic variation and response to IFN-α, we performed GWAS for HRs and MRs at a 12-, 18-, 24-, 30-, and 36-month follow-up after ropeg treatment (supplemental Table 1). After standard quality controls, as implemented in GWAS (supplemental Figures 1 and 2), genome-wide tagging single-nucleotide polymorphisms (tagSNPs) were tested for allelic association in a case-control setup. For MR, we also applied a test to determine quantitative trait association, using changes in *JAK2*V617F mutational burden as continuous variables. None of those analyses revealed any association that reached genome-wide statistical significance after Bonferroni correction for multiple testing (ie, $P < 5.00 \times 10^{-8}$; Figure 1B). Although the statistical power of GWAS is limited for small sample numbers, a cohort of similar size has revealed variants that have a strong impact on IFN-α treatment of hepatitis C.[13] Our results suggest that no germline factors influence IFN-α treatment outcome in PV with an effect size equivalent to that of IFN-α in hepatitis C. These results indicate that all patients with PV may be eligible for ropeg therapy, independent of their genetic makeup.

Although GWAS has the potential to agnostically identify associations, conservative correction for multiple testing limits the power of detecting true positive associations. This can be overcome by hypothesis-based targeted testing. Our targeted analysis focused on variants implicated in response to hepatitis C virus.[13-15] Two such variants, specifically the noncoding SNPs rs8099917 and rs12979860, have been reported to affect HR during IFN-α treatment of a Swedish mixed PV and essential thrombocythemia cohort.[21] To replicate those findings regarding HR and to investigate a potential effect on MR, we performed a series of association analyses for longitudinal time points up to the 36-month follow-up in our cohort. For hepatitis C virus clearance, Terczyńska-Dyla et al[22] demonstrated that a diplotype (ie, a specific combination of 2 haplotypes) that includes 2 exonic *IFNL4* variants covers most of the causality. Rs368234815_TT disrupts the open reading frame (IFNL4 loss of function; no IFNL4), whereas the rs117648444_G variant generates the impaired IFNL4-S70 protein in contrast to the fully functional IFNL4-P70 (Figure 1A). Interestingly, it is the functional IFNL4-P70 that exhibits a negative impact on viral clearance.[22] For both variants, we included the individual genotypes, as well as the phased diplotypes into our analyses.

When testing our cohort for genotypic association of rs12979860 with HR and MR in a case-control setup, we could not replicate the previously reported findings on HR; however, for MR we observed a statistically significant association at a 36 months follow-up ($P = .0065$; odds ratio [OR], 6.10; 95% confidence interval [CI], 1.49-36.64; Figure 1C; Table 1). Notably, when testing for changes of *JAK2*V617F burdens as a quantitative trait, we found the association to be present at formal statistical significance at all stages of follow-up (Figure 1C), suggesting superior statistical power in testing quantitative burden as compared with discrete response categories defined on arbitrary thresholds.

Table 1. Association of *IFNL4* polymorphisms with HRs and MRs at 36-month follow-up of ropeg treatment

Variant/diplotype	Genotype/diplotype status	n	NR, n	R, n	% R	OR (95% CI)	P
Influence of IFNL4 individual variants on HR in ropeg treatment of PV							
rs8099917	Non-TT	29	8	21	72.4		
	TT	49	7	42	85.7	2.26 (0.62-8.45)	.2336
rs12979860	Non-CC	48	10	38	79.2		
	CC	31	5	26	83.9	1.36 (0.37-5.70)	.7710
rs368234815	Non-TT	48	10	38	79.2		
	TT	31	5	26	83.9	1.36 (0.37-5.70)	.7710
rs117648444	GG	58	13	45	77.6		
	GA	21	2	19	90.5	2.71 (0.53-27.11)	.3303
Influence of IFNL4 individual variants on MR in ropeg treatment of PV							
rs8099917	Non-T/T	28	14	14	50.0		
	T/T	40	7	33	82.5	4.60 (1.39-16.69)	.0071
rs12979860	Non-C/C	41	18	23	56.1		
	C/C	27	3	24	88.9	6.10 (1.49-36.64)	.0065
rs368234815	Non-TT/TT	41	18	23	56.1		
	TT/TT	27	3	24	88.9	6.10 (1.49-36.64)	.0065
rs117648444	G/G	53	18	35	66.0		
	G/A	15	3	12	80.0	2.04 (0.46-12.68)	.3609
Influence of IFNL4 diplotypes on HR in ropeg treatment of PV							
IFNL4-P70 alone*	P/N and P/P	27	8	19	70.4	Reference	
IFNL4-S70 and S70/P70*	N/S and P/S	21	2	19	90.5	3.89 (0.66-42.31)	.1518
No IFNL4*	N/N	32	5	27	84.4	2.24 (0.55-10.16)	.2236
No IFNL4†	N/N	32	5	27	84.4	Reference	
IFNL4-S70 and S70/P70†	N/S and P/S	21	2	19	90.5	1.74 (0.25-20.11)	.6897
Influence of IFNL4 diplotypes on MR in ropeg treatment of PV							
IFNL4-P70 alone*	P/N and P/P	26	15	11	42.3	Reference	
IFNL4-S70 and S70/P70*	N/S and P/S	15	3	12	80.0	5.23 (1.06 35.87)	.0254
No IFNL4*	N/N	28	3	25	89.3	10.80(2.39-69.97)	3.91E-04
no IFNL4†	N/N	28	3	25	89.3	Reference	
IFNL4-S70 and S70/P70†	N/S and P/S	15	3	12	80.0	0.49 (0.06-4.20)	.6474

P values by Fisher's exact test.

N, no IFNL4; NR, nonresponder; P, IFNL4-P70; R, responder; S, IFNL4-S70.

*Contribution of IFNL4-S70 (IFNL4-S70 alone + IFNL4-S70/P70) and no-IFNL4, compared with that of IFNL4-P70 alone.

†Contribution of IFNL4-S70 (IFNL4-S70 alone + IFNL4-S70/P70), compared with that of no-IFNL4.

Because the strength of association increased with treatment duration (Figure 1C), we performed detailed analyses at a 36-month follow-up, including the noncoding tagSNPs (rs8099917 and rs12979860) and the coding diplotype variants (rs368234815 and rs117648444). First, we studied the association of these *IFNL4* variants individually. HR was not significantly influenced by the variants tested (Table 1); however, we observed a significant association of *IFNL4* variants with MR. Specifically, rs117648444 alone showed no association, whereas rs368234815 and rs12979860 individually exerted a strong effect on MR, the 2 variants being in complete linkage disequilibrium in our cohort. Furthermore, rs8099917 tagged the causative *IFNL4* variants to a lesser extent (Table 1), similar to what has been reported in other studies.[11]

Next, we evaluated the rs368234815/rs117648444 diplotype for association with HR and MR. Of the 9 existing diplotype combinations derived from phased genotypes, in accordance with described population allele frequencies (supplemental Table 2), only 5 were observed in our cohort (supplemental Table 3). These diploid combinations were further grouped into 3 main functional categories: patients who did not produce any functional IFNL4 (no IFNL4), producers of the impaired IFNL4-S70, and producers of the fully functional IFNL4-P70. Only a small percentage of patients (<6% in HR and MR categories; supplemental Table 3) carried both the S70 and P70 alleles. Analyses based on IFNL4 functionality did not reveal any associations with HR. However, for MR a strong difference in response rates between the patients with no IFNL4 (89.3% responders) and

those with IFNL4-P70 (43.3% responders) was observed ($P = 3.91 \times 10^{-4}$; OR, 10.80; 95% CI, 2.39-69.97; Table 1; Figure 1D). Notably, the effect of the impaired variant IFNL4-S70 resembled that of no IFNL4 (Figure 1D). This finding was also reflected in the quantitative response profiles based on *JAK2*V617F burden, which allowed for stratification of patients by MR according to *IFNL4* diploid functional status (Figure 1E; supplemental Figure 3).

To validate the association of the *IFNL4* diplotype with MR in an independent cohort of patients with PV, we repeated the analyses of quantitative trait association of MR data in conjunction with phased diplotypes in 27 patients enrolled in the PEGINVERA[9] prospective clinical trial. Despite the modest size of this replication cohort, we observed a consistent trend toward improved MRs after ropeg treatment in patients lacking functional IFNL4 that reached borderline statistical significance at the 24-month follow-up ($P = .068$; supplemental Figure 4).

Germline *IFNL4* variants have also been demonstrated to affect therapy-independent, spontaneous hepatitis C viral clearance.[23,24] In our PV discovery cohort (n = 122), we did not observe *IFNL4* diplotype-dependent differences in JAKV617F burden levels before trial enrollment (supplemental Figure 5), implying that IFN-α treatment has a strong mechanistic role in the effect of IFNL4 on MR.

Our results demonstrate a robust association of *IFNL4* diplotype status with MR but not with HR, which may reflect a direct effect of IFN-α on the *JAK2*V617F burden. More than one-third of patients in our study cohort carried at least 1 allele coding for the fully functional IFNL4-P70 protein variant (supplemental Table 3). Because deep MRs are indispensable for curative therapy, it is important to investigate whether increased treatment duration can overcome the dampening effect of functional IFNL4 on MR. Longitudinal monitoring of the *JAK2*V617F burden in conjunction with determination of the phased *IFNL4* diplotype status, rather than typing tagSNPs, may allow for optimization of patient management during ropeg treatment. Functional studies of this genetic predisposition may provide insight into the interplay between intrinsic expression of type III IFNs and IFN-α therapy.

Acknowledgments

Authorship

Contribution: R.J. designed and performed experiments and analyzed the data; H.G. contributed to the clinical study design, collection of clinical data, and preparation of the manuscript; E.F., E.B., J.D.M.F., J.W., B.G., and M.S. performed experiments and clinical sampling; F.S. contributed to data analysis and visualization; M.Z., K.K., and C.K. contributed to study coordination, clinical data management, and preparation of the manuscript; R.K. designed experiments and oversaw analyses; and R.J. and R.K. designed the genetic study, performed data interpretation, and wrote the paper.

Conflict-of-interest disclosure: R.K. has received honoraria from and served on the advisory board of AOP Orphan Pharmaceuticals AG; has received honoraria from Pharma Essentia; and has equity ownership in MyeloPro Diagnostics and Research GmbH. H.G. has been a consultant to and received honoraria and research funding from AOP Orphan Pharmaceuticals AG; has received honoraria from Novartis, Celgene, and Janssen-Cilag; has been a consultant to Roche, MyeloPro Diagnostics and Research GmbH; and has received personal fees from Pharma-Essentia. M.Z., K.K., and C.K. are employed by AOP Orphan Pharmaceuticals AG. The remaining authors declare no competing financial interests.

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Footnotes

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