Calreticulin mutations in Chinese with primary myelofibrosis

Bing Li,[1] Junqing Xu,[1] Jingya Wang,[1] Robert Peter Gale,[2] Zefeng Xu,[1] Yajuan Cui,[1] Lin Yang,[3] Ruixian Xing,[1] Xiaofei Ai,[4] Tiejun Qin,[1] Yue Zhang,[1,3] Peihong Zhang,[5] and Zhijian Xiao[1,3]

[1]MDS and MPN Centre, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China; [2]Haematology Research Center, Division of Experimental Medicine, Department of Medicine, Imperial College London, UK; [3]State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China; [4]Molecular Diagnostic Laboratory, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China; and [5]Department of Pathology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China

ABSTRACT

We tested 357 Chinese with primary myelofibrosis for mutations in *CALR*, *JAK2* and *MPL*. *CALR* mutations were detected in 76 subjects (21%). There were 24 (32%) type-1 (L367fs*46) and 49 (64%) type-2 (K385fs*47) mutations. Seventy-two of 168 subjects (43%) without a *JAK2* or *MPL* mutation had a *CALR* mutation. Subjects with a type-2 *CALR* mutation had lower hemoglobin concentrations (P=0.001), lower WBC counts (P<0.001), a higher percentage of blood blasts (P=0.009), and higher conventional (P<0.001) and Chinese-adjusted Dynamic International Prognostic Scoring System (P<0.001) scores compared with subjects with *JAK2* mutations. Subjects with a type-2 *CALR* mutation were also likely to have abnormal platelet levels (<100 x 10^9/L, P=0.01 or >450 x 10^9/L, P=0.042) and no splenomegaly (P=0.004). Type-2 *CALR* mutation or no detectable mutation was an independent high-risk factor for survival in multivariate analyses. These data suggest the ratio between type-1 and type-2 mutations is reversed in Chinese with primary myelofibrosis compared with populations of subjects with primary myelofibrosis of predominately European descent. The unfavorable prognostic impact of *CALR* mutations in Chinese with primary myelofibrosis is only seen in those with type-2 mutations. These data underscore the need to evaluate the prognostic impact of genetic mutations in different populations.

Introduction

Calreticulin (*CALR*) mutations occur in approximately 60-90% of subjects with primary myelofibrosis (PMF) without mutations in *JAK2* or MPL.[1-4] Typically, these *CALR* mutations involve exon 9 and are somatic insertions or deletions (indels). Type-1 (L367fs*46) and type-2 (K385fs*47) *CALR* mutations are the most frequent. *CALR* mutations are associated with younger age, more severe anemia, higher WBC and platelet counts, lower DIPSS-plus scores, and better survival compared to subjects with *JAK2* mutations.[3] Some recent data suggest *CALR* mutations may only have a favorable prognostic impact in subjects with type-1 mutations.[5]

We previously reported important differences in clinical and laboratory features in Chinese with PMF compared with PMF in persons of predominately European descent. These differences led us to revise the Dynamic International Prognostic Scoring System (DIPSS) prognostic staging system that we termed DIPSS-Chinese.[6] Our data are consistent with the notion that PMF in Chinese develops on a different genetic background than in persons of predominately European descent and this has phenotypic consequences.

This observation led us to analyze frequency, clinical correlates and the prognostic impact of *CALR* mutations in Chinese with PMF.

Methods

A bone marrow sample was collected at diagnosis or referral from 357 consecutive subjects with PMF who had given informed consent according to the Declaration of Helsinki. Histological material was re-reviewed by a blinded pathologist and diagnosis was based on World Health Organization (WHO) criteria.[7] DIPSS, DIPSS-Plus and modified DIPSS scores for Chinese with PMF (DIPSS-Chinese) were calculated as previously described.[6,8,9] Evaluable cytogenetic data were available for 194 subjects and these were further categorized using the DIPSS-plus scoring system. Follow-up data were available for 311 subjects. Subjects were treated as reported.[6] Last follow up was date of last contact, date of death or 10 January 2014. Median follow up of survivors was 28 months (range 1-385). The study was approved by the Ethical Committee of the Institute of Hematology at the Chinese Academy of Medical Sciences, according to the guidelines of the Declaration of Helsinki. *JAK2* and *MPL* mutations were tested at diagnosis as described.[10-12] The minimal detection limit for *JAK2* and *MPL* mutations is a 2.5% mutation burden. Oligonucleotide primers targeting exon 9 of *CALR* were used to amplify a 375 bp product: (Forward 5'-GTGGGGCGTAACAAAGGTGA-3' and Reverse 5'-AGAGACATTATTTGGCGCGG-3'). PCR products were purified and sequenced bi-directionally. The minimal detection limit for *CALR* mutations is a 5% mutational burden. Mutations were identified using Mutation Surveyor Software (Applied Biosystems Genetic Analyzers). Correlations between sample groups and clinical and laboratory data were calculated with the χ^2 test for qualitative variables with discrete categories and the Mann-Whitney U-test or Kruskal-Wallis analysis of variance for continuous variables. Survival distributions were estimated by the Kaplan-Meier method and were compared between subgroups using the log rank test. Cox proportional hazards regression model was used to assess the correlation between variables and survival. Two-tailed P≤0.05 was considered significant.

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Correspondence: zjxiao@hotmail.com

Results and Discussion

CALR mutations were detected in 76 subjects (21%): *JAK2V617F* mutations in 178 (50%) and *MPL* mutation in 11 (3%). Ninety-six subjects (27%) had no detectable mutation in *CALR*, *JAK2* or *MPL* and are referred to as subjects with no mutation. There were 24 (32%) type-1 and 49 (64%) type-2 *CALR* mutations (*Online Supplementary Figure S1*). Seventy-two of 168 subjects (43%) without a *JAK2* or *MPL* mutation had a *CALR* mutation. Four subjects with *CALR* and *JAK2* mutations are excluded from subsequent analyses. *Online Supplementary Table S1* lists base-line clinical and laboratory variables of the 353 study subjects (except for 4 subjects with concomitant *CALR* and *JAK2* mutations) categorized by mutation profile.

Subjects with type-1 and type-2 *CALR* mutations were compared to those with *JAK2* mutations (Table 1). Subjects with type-1 *CALR* mutations were younger (P=0.006), had lower WBC counts (P<0.001), more frequent hemoglobin concentrations below 100 g/L (P=0.044), and more frequent platelet counts over 450 x 10^9/L (P=0.019). Subjects with type-2 *CALR* mutations were younger (P<0.001), had lower hemoglobin concentrations (P=0.001), lower WBC counts (P<0.001), a higher percentage of blood blasts (P=0.009), and higher DIPSS (P<0.001) and DIPSS-Chinese (P<0.001) scores. They were also likely to have abnormal platelet counts (<100 x 10^9/L, P=0.01 or >450 x 10^9/L, P=0.042) and no splenomegaly (P=0.004).

In univariate survival analysis, subjects with type-2 *CALR* mutation and those with no detectable mutation had significantly shorter survival compared with subjects with *JAK2* mutations (Figure 1A). There was no significant difference in survival between subjects with type-1 *CALR* mutation and those with JAK2 mutations (*Online Supplementary Figure S2A*). Subjects with type-2 *CALR* mutations or no detectable mutation had comparable survival rates (*Online Supplementary Figure S2B*). In global survival analysis, subjects with type-2 *CALR* mutations or no detectable mutation had shorter survival compared to those with *JAK2*, *MPL* or type-1 *CALR* or other less common *CALR* mutations (Figure 1B). In multivariate analysis adjusted for DIPSS-Chinese, type-2 *CALR* mutation or no

Table 1. Clinical and laboratory features of 340 subjects with PMF and type-1 or -2 CALR or *JAK2* mutations or no detectable mutation.

Variables	*JAK2 V617F* mutated (n=174)	*CALR* type 1 mutated (n=22)	*CALR* type 2 mutated (n=48)	No mutation (n=96)	*P* Type 1 *vs.* Type 2	*P* Type 1 *vs.* *JAK2*	*P* Type 2 *vs.* *JAK2*	*P* No mutation *vs.* *JAK2*
Age (years)	59 (15-89)	51 (26-79)	54 (26-79)	52 (21-82)	NS**	*P*=0.006	*P*=0.031	*P*<0.001
Age>65 years n (%)	47 (27%)	2 (9%)	10 (21%)	13 (14%)	NS	NS	NS	*P*=0.011
Males n (%)	86 (50%)	9 (41%)	26 (54%)	52 (54%)	NS	NS	NS	NS
Hemoglobin (g/L)	111 (27-195)	98 (35-150)	90 (44-150)	77 (36-174)	NS	NS	*P*=0.001	*P*<0.001
WBC (x10^9/L)	13.9 (0.7-1044)	6.9 (2.2-25.3)	6.5 (1.9-415)	4.0 (0.6-170.1)	NS	*P*<0.001	*P*<0.001	*P*<0.001
Platelets (x10^9/L)	253 (23-1373)	319 (46-1325)	121 (6-1246)	88 (8-1141)	NS	NS	NS	*P*<0.001
Blood blast (%)	0 (0-20)	0 (0-3)	0 (0-10)	0 (0-9)	NS	NS	*P*=0.009	NS
Spleen (cm)	8 (0-26)	6 (0-20)	4 (0-24)	2 (0-35)	NS	NS	*P*=0.02	*P*<0.001
Hemoglobin <100g/L; n (%)	57 (32.8%)	12 (55%)	33 (69%)	65 (68%)	NS	*P*=0.044	*P*<0.001	*P*<0.001
WBC>25x10^9/L; n (%)	36 (21%)	1 (5%)	4 (8%)	7 (7%)	NS	*P*=0.037	*P*=0.049	*P*=0.004
Platelets<100x10^9/L; n (%)	37 (21%)	5 (23%)	19 (40%)	54 (57%)	NS	NS	*P*=0.01	*P*<0.001
Platelets>450x10^9/L; n (%)	31 (18%)	9 (40.9%)	15 (31%)	17 (18%)	NS	*P*=0.019	*P*=0.042	NS
Constitutional symptoms; n (%)	31 (18%)	4 (18%)	8 (17%)	23 (24%)	NS	NS	NS	NS
Blood blasts ≥1%;n (%)	42 (24%)	8 (36%)	20 (42%)	15 (16%)	NS	NS	*P*=0.017	NS
Palpable spleen; n (%)	143 (82%)	17 (77%)	30 (63%)	51 (53%)	NS	NS	*P*=0.004	*P*<0.001
DIPSS risk group; n (%)					*P*=0.010	NS	*P*<0.001	NS
Low	52 (30%)	5 (23%)	9 (19%)	16 (17%)				
Intermediate-1	88 (51%)	14 (64%)	14 (29%)	56 (58%)				
Intermediate-2	31 (18%)	3 (14%)	24 (50%)	22 (23%)				
High	3 (2%)	0	1 (2%)	2 (2%)				
DIPSS-Chinese risk group; n (%)					*P*=0.076	NS	*P*<0.001	*P*<0.001
Low	105 (60%)	11 (50%)	14 (29%)	28 (29%)				
Intermediate	66 (38%)	11 (50%)	30 (63%)	55 (57%)				
High	3 (2%)	0	4 (8%)	13 (14%)				
DIPSS-plus risk group* n (%)					NS	NS	NS	*P*=0.006
Low	24 (27%)	3 (23%)	5 (22%)	6 (11%)				
Intermediate-1	26 (29%)	2 (15%)	2 (9%)	8 (15%)				
Intermediate-2	27 (30%)	6 (46%)	11 (48%)	24 (45%)				
High	12 (14%)	2 (15%)	5 (22%)	15 (28%)				

*178 subjects had evaluable cytogenetic results and can be categorized by DIPSS-plus; **NS: not significant.

detectable mutation was independently correlated to risk of death (HR: 2.15; 95%CI: 1.32-3.51; *P*=0.002).

Our data indicate approximately 40% of Chinese with PMF and no detectable mutations in *JAK2* or *MPL* have a *CALR* mutation. Type-1 and type-2 were the most common *CALR* mutations. This frequency is substantially lower than that reported in subjects with PMF of predominantly European descent. What remains unknown is how this parallels the different frequencies of *JAK2* (49% *vs.* 58%) and *MPL* (3% *vs.* 8%) mutations. Although some of these differences may reflect the different sensitivities of the respective assays used, the different genetic backgrounds on which PMF develops may also be important.[6]

To study this issue further, we compared clinical features of our 353 Chinese subjects with PMF to a cohort reported from the Mayo Clinic[3] (Table 2). We also noted differences in the impact of *CALR* mutations, especially type-2, on clinical features and survival of Chinese *versus* that of subjects of predominantly European descent with PMF. Chinese with type-2 *CALR* mutations had adverse clinical and laboratory features, including lower hemoglobin concentrations, a higher percentage of blood blasts, abnormal platelet levels, and less splenomegaly (splenomegaly is a favorable prognostic variable in Chinese populations; *see below*). These features resulted in higher conventional and Chinese DIPSS scores in subjects with type-2 *CALR* mutations. Subjects with no detectable mutation were also likely to be clustered in the higher risk group. Subjects with type-2 *CALR* mutations and those with no detectable mutation had significantly shorter survival than subjects with *JAK2* mutations in univariate analysis. In contrast, we found less or no significant difference between subjects with type-1 *CALR* mutations *versus* those with *JAK2* mutations in clinical or laboratory variables or survival. These data explain the differences in survival between Chinese with *CALR* mutations and subjects of predominantly European descent who had adverse type-1 and type-2 *CALR* mutations.[1,3]

In a previous study, we had reported that splenomegaly at diagnosis correlated with longer survival in Chinese with PMF.[6] The opposite is the case in subjects with PMF of predominantly European descent. Consequently, we analyzed the correlation between *CALR* mutations and splenomegaly in our subjects. Those with type-2 *CALR* mutations and those with no detectable *JAK2* mutation were less likely to have splenomegaly than those with *JAK2* mutations; they also had shorter survival. However, subjects with type-1 *CALR* and those with *JAK2* mutations had similar frequencies of splenomegaly and similar survival rates. There was no significant difference in frequency of splenomegaly or survival between subjects with type-2 *CALR* mutations and those with no detectable mutation. Type-2 *CALR* mutations or no detectable mutation was an independent unfavorable prognostic factor in multivariate analysis.

In summary, our study shows *CALR* mutations occur in Chinese with PMF who lack mutations in *JAK2* or *MPL*. However, this frequency of *CALR* mutations and the ratio of *CALR* mutation types differ from those reported in subjects with PMF of predominantly European descent. These data raise the possibility that one or more undiscovered mutations may be found in the 48% of Chinese with PMF

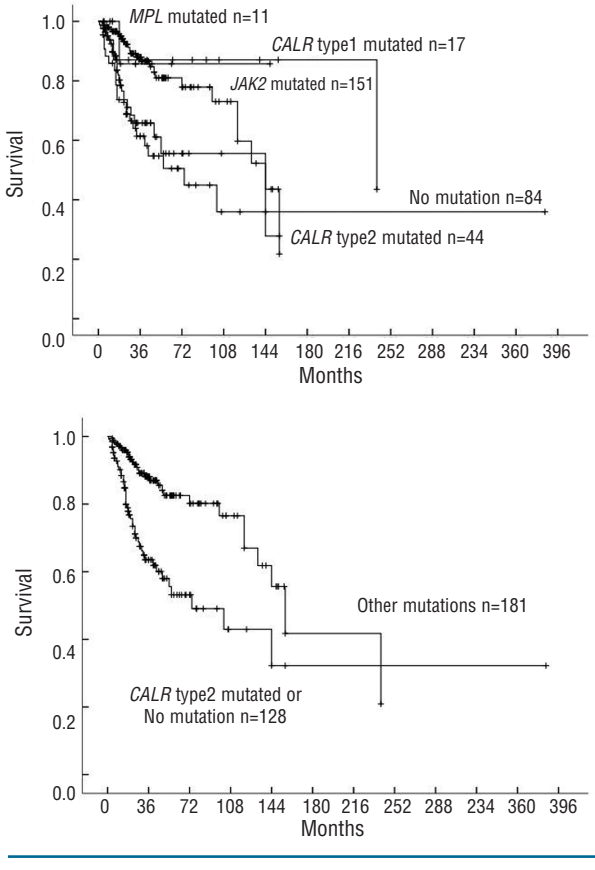

Figure 1. Survival of 309 subjects with PMF classified by *CALR*, *JAK2*, *MPL* or no detectable mutation. (A) *CALR* type-1 *vs.* -2 *vs. JAK2 vs. MPL vs.* no detectable mutation; (*P*<0.001); (B) *CALR* type-2 or no detectable mutation *vs. JAK2*, *MPL*, *CALR* type-1 and infrequent *CALR* mutation (referred to other mutations, *P*<0.001).

Table 2. Comparison of features between Chinese subjects and subjects reported from the Mayo Clinic.[3]

	Mayo Clinic	Chinese	*P*
N	253	353	
Age >65 years	111 (44%)	75 (21%)	<0.001
Males	165 (65%)	179 (51%)	<0.001
Hemoglobin <100g/L	123 (48%)	178 (50%)	0.295
WBC >25x10⁹/L	39 (15%)	49 (14%)	0.308
Platelets <100x10⁹/L	51(20%)	118 (30%)	<0.001
Platelets >450x10⁹/L	50 (20%)	75 (20%)	0.299
Constitutional symptoms	89 (35%)	71 (20%)	<0.001
Blood blasts ≥1%	137 (54%)	94 (27%)	<0.001
DIPSS-plus risk group*			0.012
Low	32 (13%)	38 (20%)	
Intermediate-1	45 (18%)	40 (21%)	
Intermediate-2	94 (37%)	75 (40%)	
High	81 (32%)	37 (20%)	
JAK2^{V617F}	147 (58%)	174 (49%)	<0.001
CALR	63 (25%)	72 (20%)	<0.001
Type-1**	49 (78%)	22 (31%)	<0.001
Type-2**	9 (14%)	48 (67%)	<0.001
MPL	21 (8%)	11 (3%)	<0.001
No detectable mutation	22 (9%)	96 (27%)	<0.001

**190 subjects had evaluable cytogenetic results and can be categorized by DIPSS-plus; **percent of type-1or -2 mutated in all CALR mutated.*

without mutations in *JAK2* and *MPL* and we are currently using exomic- and whole genome sequencing in our mutation-negative population to explore this. In Chinese with PMF, and in contrast to subjects of predominately European descent, the unfavorable prognostic impact of *CALR* mutations is limited to those with type-2. Type-2 *CALR* mutation or no detectable mutation in *CALR, JAK2* or *MPL* is an independent high-risk molecular signature In Chinese with PMF. Consequently, screening of Chinese with PMF for *CALR* mutations may be useful for diagnosis and estimating survival.[13]

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Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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