Mutation status of essential thrombocythemia and primary myelofibrosis defines clinical outcome

Julia Asp,[1,2] Björn Andréasson,[3,4] Ulrika Hansson,[5] Carina Wasslavik,[2] Johanna Abelsson,[3] Peter Johansson,[3,4] and Lars Palmqvist[1,2]

[1]Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine, the Sahlgrenska Academy, University of Gothenburg; [2]Department of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg; [3]Haematology and Coagulation Section, Department of Medicine, Sahlgrenska University Hospital, Gothenburg; [4]Hematology Section, Department of Medicine, NU Hospital Group, Uddevalla; and [5]Department of Clinical Pathology and Genetics, Sahlgrenska University Hospital, Gothenburg, Sweden

Correspondence: julia.asp@gu.se

doi:10.3324/haematol.2015.138958

Online supplement

Mutation Status of Essential Thrombocythemia and Primary Myelofibrosis Defines Clinical Outcome

Julia Asp, Björn Andréasson, Ulrika Hansson, Carina Wasslavik, Johanna Abelsson, Peter Johansson, and Lars Palmqvist

Methods

Patients

The study was performed in accordance to the Declaration of Helsinki after ethical approval by the Research Ethics Board at the University of Gothenburg and the Sahlgrenska Academy, Gothenburg, Sweden. All patients diagnosed with ET or PMF according to the WHO 2008 classification between 2008 and 2013 in Western Sweden at the Sahlgrenska University Hospital or NU Hospital Group and reported to the Swedish national cancer register, INCA, were selected (n=186). 170 patients were included; 129 ET patients (73 females and 56 males, median age 67 years, range 27–90 years) and 41 PMF patients (21 females and 20 males, median age 70 years, range 32–85 years). 16 patients did not answer the request to participate in the study. Clinical, laboratory and outcome data was available for all patients. A critical review of diagnosis was performed in triple negative patients by re-analyzing bone marrow in addition to review of clinical data.

Mutation analysis of JAK2, CALR and MPL

Genomic DNA from blood taken at initial referral or time of diagnosis was used for analysis. *JAK2* V617F mutation status was determined for all patients. Ipsogen JAK2 MutaQuant Kit (Qiagen, Hilden, Germany) was used in the majority of cases. *CALR* mutation status was analyzed with fragment analysis in all cases negative for *JAK2* mutation.[1] Detected *CALR* mutations were verified by Sanger sequencing. The *MPL* W515 mutation was analyzed using Ipsogen MPL W515K/L MutaScreen Kit (Qiagen) in cases negative for both *JAK2* V617F and *CALR* mutations.

Screening for myeloid mutations

Genomic DNA from all patients negative for *JAK2* V617F, *CALR* and *MPL* W515K/L mutations (n=20) in addition to all ET patients with *MPL* mutations (n=3, all with the W515L mutation) and age matched controls from ET patients harboring the *JAK2* (n=18) or *CALR* (n=18) mutations were screened for additional mutations. The TruSight Myeloid Sequencing panel (Illumina, San Diego, CA, USA) was used on the MiSeq instrument (Illumina). Secondary analysis was performed by MiSeq Reporter using BWA mapper and somatic variant caller. Results were filtered using Variant Studio (Illumina). Global filtering according to 1000 genomes was set to >3%, coverage was at least 100 reads and at least 10 reads for the variant. Variants causing missense, frameshift, altered stop/initation codon, in-frame insertion/deletion or variants affecting splice site were regarded as mutations. Variants with quality >Q30 and allele frequencies of at least 5% were considered positive for mutation. In addition to patient samples, three samples of DNA extracted from blood donors or individuals with normal blood morphology were included as controls. Mutations appearing in these samples were considered as normal polymorphisms occurring in our population cohort or sequencing artefacts and were excluded from further analysis. BAM-files from the secondary analysis were used to further analyze the regions with identified mutations by Integrative Genomics Viewer (www.broadinstitute.org). Variants in areas with difficult reads were excluded. Results were visualized using a Circos plot.[2]

Statistical analysis

Statistical significance was determined using Kruskal-Wallis non-parametric test or 2-tailed Fisher exact test with Analyze-it, v.2.30 (Analyse-it Software Ltd, Leeds, UK). A value of $p<0.05$ was considered statistically significant.

Survival analysis

The probabilities of OS were estimated by the Kaplan-Meier method and differences in survival distributions were compared with Gehan-Breslow-Wilcoxon test using GraphPad Prism, v.6.07 (GraphPad Software Inc, La Jolla, CA, USA). OS was defined as time from diagnosis to last follow-up or death from any cause. Follow up was done from diagnosis to end of study (2015-05-31) and the median follow-up time was 46 months for ET patients and 41 month for PMF patients.

References

1. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379-2390.
2. Krzywinski M, Schein J, Birol I, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639-1645.

Table S1 **ET with different mutations (median values and ranges)**

	***JAK2* V617F** **(n=82)**	***CALR*** **(n=28)**	***MPL*** **(n=7)**	**TN** **(n=8)**
Gender (F/M)	49/33	13/15	3/4	6/2
Age	66.5 (27-90)	67.5 (49-88)	**80 (70-82)***	64.5 (39-85)
Hemoglobin (g/L)	**143 (117-170)****	137 (108-152)	**117 (99-128)****	134.5 (113-155)
Hematocrit (EVF)	**0.44 (0.37-0.52)****	0.43 (0.37-0.48)	**0.38 (0.31-0.40)****	0.40 (0.35-0.47)
WBC (10^9/L)	9.4 (4.8-16.9)	8.7 (2.9-12.5)	9.0 (5.6-19.8)	9.4 (5.6-15.6)
Platelets (10^9/L)	736 (467-1776)	**991 (407-1824)****	1011 (592-1888)	690 (570-2061)
EPO (2.6-18.5 IU/L)	**4.9 (1.7-30.6)*****	10.1 (3.3-40.8)	**15.0 (6.2-26.3)****	10.9 (6.2-14.6)
Vascular complications (yes/no)	33/49	**5/23**$^{+}$	4/3	3/5
Transformation (yes/no)	1/81	1/27	**2/5**$^{+}$	0/8

Kruskal-Wallis non-parametric test * p≤0.01, ** p≤0.001, *** p≤0.0001, Fischer exact test $^{+}$p<0.05

Table S2 PMF with different mutations (median values and ranges)

	JAK2 **V617F** **(n=24)**	***CALR*** **(n=7)**	***MPL*** **(n=3)**	**TN** **(n=7)**
Gender (F/M)	12/12	4/3	1/2	4/3
Age	71 (41-85)	61 (48-82)	82 (73-83)	63 (50-83)
Hemoglobin (g/L)	120 (79-146)	119 (90-146)	111 (104-116)	91 (60-156)
Hematocrit (EVF)	0.38 (0.23-0.49)	0.38 (0.29-0.45)	(0.32-0.35)	(0.21-0.22)
WBC (10^9/L)	8.4 (3.2-28.0)	8.7 (5.6-42.7)	11.0 (5.3-19.8)	7.1 (1.0-17.8)
Platelets (10^9/L)	392 (37-1244)	878 (32-1412)	504 (384-937)	64 (5-833)
EPO (2.6-18.5 IU/L)	9.9 (3.3-578)	19.0 (10.1-30.1)	33.2 (7.4-40.7)	44.8 (4.8-81.1)
Vascular complications (yes/no)	7/17	2/5	0/3	2/5
Transformation (yes/no)	1/23	0/7	0/3	**3/4***

Fischer exact test $^{*}p<0.05$

Table S3 Findings from TruSight Myeloid Sequencing panel

Mutation load in %

			ET																																																		PMF							
			TN								*CALR*																	*JAK2*																		*MPL*							TN							*MPL*
			Patient no.																																																								
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59
CALR (NM 004343.3)	c.1154 1155insTTGTC	p.Lys385AsnfsTer47											24	31	38		37		35	8	41	25		17	34	26																																		
JAK2 (NM 004972.3)	c.1849G>T	p.Val617Phe																											8	10	25	29	14	17	6	33	12	9	14	6	13	35	46	22	32	32															
MPL (NM 005373.2)	c.1514G>A	p.Ser505Asn																																															27										
	c.1543T>C	p.Trp515Arg																																																	66								
	c.[1543T>C; 1546C>G]	p.[Trp515Arg; Gln516Glu]																																																35									
	c.1543 1544delinsGC	p.Trp515Ala																																														72											44
	c.1544G>T	p.Trp515Leu																																											5	39	28												
EZH2 (NM 004456.4)	c.2110+6T>G	-																																			50																						
	c.553G>C	p.Asp185His																																												52													
	c.965A>G	p.Asn322Ser															49																																										
ATRX (NM 000489.3)	c.5464G>A	p.Ala1822Thr																																																								5	
	c.5579A>G	p.Asn1860Ser																100																																									
	c.6479T>C	p.Val2160Ala																																																6									
ASXL1 (NM 015338.5)	c.1867C>T	p.Gln623Ter							41																																																		
	c.2278C>T	p.Gln760Ter													11																																												
	c.2444T>C	p.Leu815Pro					100																																																				
	c.2873C>A	p.Ser958Ter																																										42															
	c.2880G>A	p.Trp960Ter																							33																																		
	c.3000 3001insA	p.Thr1001AsnfsTer4																																											27														
	c.3083C>A	p.Ser1028Ter																																								9																	
KDM6A (NM 021140.2)	c.1402T>C	p.Cys468Arg																	48																																								
	c.1527+3G>A	-			100																																																						
	c.2240T>A	p.Leu747His																																									40																
	c.3190G>T	p.Glu1064Ter																																																									
	c.385-2A>C																																																8									
	c.4075C>T	p.Gln1359Ter																																																6									
DNMT3A (NM 022552.4)	c.1117C>G	p.Leu373Val																																																									5
	c.1182delC	p.Asp394GlufsTer13																																													15												
	c.2193 2195delCTT	p.Phe732del																																													25												
	c.2644C>T	p.Arg882Cys																		19																																							
	c.2645G>A	p.Arg882His																																44																			38						
	c.517G>T	p.Gly173Cys																																																								6	
	c.718G>T	p.Glu240Ter																																																							68		
	c.89A>C	p.Glu30Ala																																									53																
TET2 (NM 001127208.2)	c.100C>T	p.Leu34Phe		50														53																																									
	c.1064G>A	p.Gly355Asp											50																							52																		51							
	c.1088C>T	p.Pro363Leu					47																																																				
	c.1273delA	p.Thr425LeufsTer2																																							46																		
	c.1737delA	p.Lys580AsnfsTer21																																																42									
	c.1897A>T	p.Met633Leu																																																51									
	c.2399 2400delAT	p.His800GlnfsTer15																																																					30			
	c.2604T>G	p.Phe868Leu																															51																									
	c.3454G>A	p.Gly1152Arg																																																			38					
	c.3659 3660delCC	p.Thr1220MetfsTer2														26																																											
	c.4072T>G	p.Cys1358Gly																																												30													
	c.4097G>A	p.Arg1366His																														9																											
	c.4257 4258insTTG	p.Pro1419 Leu1420insLeu																																41																									
	c.4567C>T	p.Gln1523Ter																																					34																			
	c.5162T>G	p.Leu1721Trp					53						50																																														
	c.521C>A	p.Pro174His																					50																50																				
	c.5284A>G	p.Ile1762Val					49						48																								50									51													
	c.5605G>T	p.Gly1869Trp																																																					27			
	c.5666C>A	p.Pro1889His																																														8											
IDH1 (NM 005896.2)	c.211G>A	p.Val71Ile																																															51										
	c.395G>A	p.Arg132His																																											27														
IDH2 (NM 002168.2)	c.419G>A	p.Arg140Gln																																																		45							
	c.418C>T	p.Arg140Trp																																															39										
RAD21 (NM 006265.2)	c.407A>G	p.Asn136Ser																																																								5	
STAG2 (NM 001042749.1)	c.1821+2T>A	-																																																6									
	c.3682T>C	p.Phe1228Leu																																																								5	
SMC3 (NM 005445.3)	c.1238T>C	p.Ile413Thr																															5																									
	c.2080G>C	p.Ala694Pro																																																								11	
SMC1A (NM 006306.2)	c.2557A>G	p.Lys853Glu																																																							5		
ZRSR2 (NM 005089.3)	c.1314 1315insAGCCGG	p.Gly438 Ser439insSerArg																																																			45						
	c.212T>A	p.Leu71Ter																																																								10	
	c.1385G>A	p.Arg462Gln																																																6									
	c.873G>A	p.Trp291Ter																																										59															
SRSF2 (NM 001195427.1)	c.284C>A	p.Pro95His							40																																				32									31					
	c.284C>T	p.Pro95Leu																																																					24			20
	c.284 307delCCCCGGACTCACACCACA	p.Pro95 Arg103del																																															43										
U2AF1 (NM 001025203.1)	c.101C>T	p.Ser34Phe													8																																												
	c.470A>C	p.Gln157Pro																																																		40							
ETV6 (NM 001987.4)	c.602T>C	p.Leu201Pro									47														48																																		
BCORL1 (NM 021946.4)	c.1508C>T	p.Pro503Leu																																																								5	
	c.3158A>G	p.Lys1053Arg															100																																										
	c.625G>A	p.Gly209Ser																																			49																						
BCOR (NM 001123385.1)	c.4977-4G>T	-					50						49																								45									53													
	c.651G>A	p.Met217Ile																															48																									
	c.946C>T	p.Pro316Ser																																																			49					
RUNX1 (NM 001754.4)	c.167T>C	p.Leu56Ser																															54																									
CUX1 (NM 001202543.1)	c.1702G>C	p.Ala568Pro																																																			19					
	c.174+8G>A	-																																												99													
	c.2014C>T	p.Arg672Ter																																															41										
	c.295G>A	p.Val99Ile																									57																																		
	c.3466+2T>G	-																																																		28							
	c.392 393delAA	p.Glu131AspfsTer11																																																					15			
	c.550G>A	p.Ala184Thr																																																								6	
	c.640+6A>G	-					48						51																								100																						
GATA1 (NM 002049)	c.7T>C	p.Phe3Leu																																																								6	
GATA2 (NM 032638.4)	c.481C>G	p.Pro161Ala															58					46																			58																			
	c.490G>A	p.Ala164Thr																																												57													
	c.616G>A	p.Glu206Lys																														5																												
PHF6 (NM 032458.2)	c.871G>A	p.Gly291Arg																																																		8							
CEBPA (NM 004364.3)	c.563 564insCTC	p.Pro188 Pro189insSer																																							7																		
	c.566C>A	p.Pro189His																																							8																		
	c.568T>C	p.Ser190Pro																																							7																		
	c.584 589dupACCCGC	p.His195 Pro196dup																																							33																		
MYD88 (NM 001172567.1)	c.577C>T	p.Arg193Trp	47																																																								
NPM1 (NM 002520.6)	c.859 860insTCTG	p.Trp288CysfsTer12																																																			36				33		
CBLC (NM 012116.3)	c.1303C>T	p.Pro435Ser																						32																																			
KIT (NM 000222.2)	c.1621A>C	p.Met541Leu											49																								50																						
CSF3R (NM 156039.3)	c.2092C>T	p.Arg698Cys																		51																																							
	c.2093G>A	p.Arg698His					32																																																				
	c.2509G>A	p.Asp837Asn																																														19											
NOTCH1 (NM 017617.3)	c.5011G>A	p.Val1671Ile																																									56																
	c.6853G>A	p.Val2285Ile																																												47													
CBL (NM 005188.3)	c.1186T>C	p.Cys396Arg																																																					23			
	c.1246T>G	p.Cys416Gly																																																		43							
	c.1259G>A	p.Arg420Gln																																																		7							
CDKN2A (NM 001195132.1)	c.442G>A	p.Ala148Thr																																																					48		48	
TP53 (NM 000546.5)	c.215C>G	p.Pro72Arg					54						99																																														
PTEN (NM 000314.4)	c.768G>T	p.Glu256Asp																																																								9	
SETBP1 (NM 015559.2)	c.2612T>C	p.Ile871Thr																																																			9					