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SH2B3 (LNK) rs3184504 polymorphism is correlated with *JAK2* V617F-positive myeloproliferative neoplasms

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Abstract

***Background**: Pathogenesis and phenotypic diversity in myeloproliferative neoplasms (MPN) cannot be fully explained by the currently known acquired mutations alone. Some susceptible germline variants of different genes have been proved to be associated with the development of these diseases. The goal of our study was to evaluate the association between the rs3184504 polymorphism of SH2B3 (LNK) gene (p.R262W, c.784T>C) and the risk of developing the four typical MPN - polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML). **Material and methods**: We investigated the SH2B3 rs3184504 T>C polymorphism by real-time PCR in 1901 MPN patients (575 with PV, 798 with ET, 251 with PMF, and 277 with CML), all of them harboring one of the specific driver mutations - JAK2 V617F or CALR in case of PV, ET and PMF, or BCR-ABL1 in case of CML, and 359 controls. **Results**: Overall, the TT homozygous genotype was significantly associated with BCR-ABL1-negative MPN (OR = 1.34; 95% CI = 1.03-1.74; crude p-value = 0.02; adjusted p-value = 0.04). The most significant association was seen in case of PV (OR = 1.54; 95% CI = 1.14-2.06; crude p-value = 0.004; adjusted p-value = 0.024). Also, SH2B3 rs3184504 correlated significantly with*

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JAK2 V617F-positive MPN (OR = 1.36; 95% CI = 1.04 -1.77; crude p-value = 0.02; adjusted p-value = 0.08), but not with those CALR-positive. ET (regardless of molecular subtype) and CML were not correlated with SH2B3 rs3184504. ***Conclusions****: The SH2B3 rs3184504 polymorphism is associated with risk of MPN development, especially PV. This effect is restricted to JAK2 V617F-positive PV and PMF only.*

Keywords: *myeloproliferative neoplasms, somatic mutations, genetic predisposition*

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Introduction

Myeloproliferative neoplasms (MPN) are a group of chronic hematopoietic stem-cell derived proliferative disorders characterized by the overproduction of terminally differentiated cells from myeloid lineages (1).

The revised 2016 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues includes in the category of MPN seven subcategories. The most common ones are chronic myeloid leukemia (CML), the only *BCR-ABL1*-positive MPN, and polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocythemia (ET), which are *BCR-ABL1*–negative MPN (2-4).

Most of the patients with *BCR-ABL1*-negative MPN harbor somatic driver mutations in one of three genes - *JAK2* (Janus kinase 2), *CALR* (Calreticulin), and *MPL* (myeloproliferative leukemia virus oncogene). They are all involved in the activation of JAK-STAT pathway and are mutually exclusive in the majority of patients with MPN. Mutations in *JAK2*, mostly V617F, are present in all three diseases (>90% in PV, 50% - 60% in ET, and PMF), while *MPL* and *CALR* mutations are almost exclusively found in ET and PMF patients (3,5).

However, there are important features relating to pathogenesis and phenotypic diversity that cannot be fully described by acquired mutations alone. Somatic acquisition of genetic mutations is not the only pathogenic mechanism involved in the development of MPN. Researchers are increasingly turning their attention to the individual genetic background, which is assumed to influence the predisposition to MPN. It has been hypothesized that a pre-existing germline predisposition facilitates one or more additional genetic events that lead to uncontrollable proliferation. In a large population-based case-control study, including more than 11000 MPN patients and their almost 25000 linkable first-degree relatives, a 5- to 7-fold elevated risk of developing MPNs among first-degree relatives of MPN patients was concluded (6).

In 2009, several published reports described a germline haplotype, called 46/1 or "GGCC", situated on chromosome 9p, which is present in the general population at a rough estimate of 45%. This haplotype predisposes to MPN-associated mutations, such as *JAK2* and *MPL*, but confers a less significant susceptibility to *CALR*-mutated MPN (7-12). From these observations, two major hypotheses arose, one suggesting that this germline variant causes a fertile ground on which mutations gain a stronger growth advantage, and the second theory which suggests a hypermutability status, due to the 46/1 haplotype, conferring an increased frequency of mutations at *JAK2* locus. To date, none of these hypotheses have been proven entirely correct, nor that they are mutually exclusive. Shortly afterwards, another polymorphism, this time in the *TERT* (telomerase) gene, emerged as a major predisposing factor to MPN, regardless of phenotype or molecular subtype (13). Further studies have

found additional variants in other genes, such as *MECOM, SH2B3, TET2, ATM, CHEK2, THRB-RARB, PINT,* that were shown to predispose to MPN (14,15).

LNK (lymphocyte adapter protein) is a member of SH2B (Src homology 2-B) adapter family of proteins encoded by *SH2B3* gene (band 12q24.12). This adaptor protein, highly expressed in hematopoietic stem cells and endothelial cells, serves as a molecular platform which coordinates multiple pathways signaling events. LNK plays an important role as a negative regulator of growth and cytokine receptor-induced proliferation and migration. In normal hematopoiesis, LNK inhibits STAT proteins activation. LNK exerts this inhibitory effect by binding itself to JAK2 protein upon thrombopoietin stimulation. On the other hand, LNK null mice develop an MPN-like phenotype, including leukocytosis, thrombocythemia, and splenomegaly. Their spleen and bone marrow also show megakaryocytic hyperplasia. Thus, LNK inactivation leads to dysregulation of the JAK-STAT pathway producing a proliferation of the myeloid elements which is the major feature of the MPN (16, 17).

Somatic *SH2B3* mutations were found at a relatively low frequency (5-7%) in sporadic MPN cases, their frequency increasing to about 13% in patients with leukemic transformation (18). Germline *SH2B3* mutations are also present in about 2% of familial MPN (19). Recently, one non-synonymous polymorphism of *SH2B3,* namely rs3184504 (p.R262W, c.784T>C), has been shown to associate with MPN, especially those *JAK2* V617F-positive (20-22).

The aim of this study was to determine whether the *SH2B3* rs3184504 polymorphism is associated with an enhanced potential of acquiring MPN-associated somatic driver mutations and developing the four major MPN - PV, ET, PMF, and CML.

Material and methods

Patients and controls

The study included 1901 patients with various MPN. There were 575 patients with PV, 798 patients with ET, 251 patients with PMF, and 277 patients with CML. The patients were diagnosed between 1984 and 2019 in different hematology hospitals and departments from Romania. The diagnosis of all patients was reviewed according to the latest WHO classification of myeloid neoplasms (4). Table 1 presents demographical data, and also the distribution of the driver mutations in MPN patients included in the study. Of note, we included only patients with a molecularly proven driver mutation (*JAK2* V617F, *CALR* or *BCR-ABL1* mutation). ET and PMF patients positive for *MPL* mutations were ruled out from consideration of being included in this study, because of their low number. Also ET and PMF patients lacking *JAK2* V617F, *CALR* or *MPL* mutations (the so-called "triple-negative") were not considered for inclusion in this study. Most of these patients were lacking bone marrow biopsy and also the status for MPN-associated additional mutations. Thus, their diagnosis was judged as uncertain. We also included 359 individuals who represented the control group. They were age and sex-matched to the patients. The controls were referred for routine blood workup to the Clinics of Hematology from Cluj-Napoca, Romania. Only individuals showing no hematological neoplasm were included in the control group. The study was approved by the Ethics Committee of Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca. Written consent regarding the genetic testing was also obtained from each participant to the study.

Genotyping methods

All the genetic assays were performed on DNA obtained from peripheral whole blood collected on EDTA, using various commercial kits (Wiz-

Table 1. Basic features displayed by MPN patients included in the study

Feature	PV group; N = 575	ET group; N = 798	PMF group; N = 251	CML group; N = 277
Male sex; n (%)	299 (52)	289 (36.2)	120 (47.8)	139 (50.2)
Age; median (range); years	64 (29-89)	61 (18-91)	66 (27-93)	52 (18-85)
JAK2 V617F mutation; n (%)	100 (0)	561 (70.3)	164 (65.3)	0 (0)
CALR mutations; n (%)	0 (0)	237 (29.7)	87 (34.7)	0 (0)
BCR-ABL1 fusion; n (%)	0 (0)	0 (0)	0 (0)	100 (100)

n = number of cases; range = interval between the smallest and largest value

ard Genomic DNA Purification kit, Promega, USA; Quick gDNA MiniPrep kit, Zymo Research, USA; PureLink Genomic DNA Mini Kit, Invitrogen, Thermo Fisher, USA). The somatic mutations, namely *JAK2* V617F, *CALR* exon 9 indels, and *BCR-ABL1*, were analyzed in all patients, mostly on diagnosis, or when the technique became available in our center, using previously described protocols. Specifically, *JAK2* V617F mutation was analyzed using a tetra-primer PCR until 2015, or detected and quantified using a real-time PCR assay thereafter (23,24). *CALR* exon 9 indels were assessed using a simplex PCR (25). *BCR-ABL1* major transcript was detected on diagnosis using a qualitative nested PCR (26), and quantified whenever necessary thereafter using an automated, cartridge-based real-time PCR system (BCR-ABL Ultra, GenExpert system, Cepheid, USA).

SH2B3 rs3184504 polymorphism was genotyped in all individuals using a TaqMan SNP (single nucleotide polymorphism) Genotyping Assay (assay number C___2981072_10), as recommended by the manufacturer (Applied Biosystems, Thermo Fisher, USA), using Quant Studio 3 or 7500 Fast Dx real-time PCR systems (Applied Biosystems, Thermo Fisher, USA).

Statistical analysis

Comparisons involving qualitative variables were performed using Fisher's exact test. In case of quantitative variables, different sets of non-parametric data were analyzed using Mann-Whitney test, wherever appropriate. Correction for multiple testing was accomplished by controlling the false discovery rate (FDR) at 0.05. The original p-values were accompanied by the computation of the adjusted p-values using by Benjamini-Hochberg procedure (27). All p-values (crude and adjusted) <0.05 were considered significant.

The statistical analysis was performed using the GraphPad Prism version 8 (GraphPad Software Inc., San Diego, CA, USA) and R platform, version 3.6.1 (28).

Results

Table 2 shows a detailed distribution of *SH2B3* rs3184504 genotypes and alleles in patients and controls included in the study.

Correlations between SH2B3 rs3184504 genotypes and alleles, and MPN phenotypes

First, we analyzed the relationship between *SH2B3* rs3184504 genotypes and alleles, and the four phenotypes included in the study, namely PV, ET, PMF, and CML.

In case of rs3184504 genotypes, we analyzed both dominant and recessive genetic models, but only the recessive model yielded statistically significant results. The homozygous TT genotype was significantly associated with PV (OR = 1.54; 95% CI = 1.14-2.06; crude p-value = 0.004; adjusted p-value = 0.024), and with PMF (OR = 1.50; 95% CI = 1.04-2.12; crude p-value = 0.02; adjusted p-value = 0.04). The ho-

Table 2. Distribution of *SH2B3* rs3184504 genotypes and alleles in MPN patients and controls

Genotypes/ alleles	PV group (N = 575)	ET group (N = 798)		PMF group (N = 251)		CML group (N = 277)	Controls (N = 359)
		JAK2 V617F+-positive (N = 561)	*CALR*-positive (N = 237)	*JAK2 V617F*-positive (N = 164)	*CALR*-positive (N = 87)		
CC	130 (22.6)	125 (22.3)	50 (21.1)	37 (22.6)	17 (19.5)	75 (27.1)	81 (22.6)
CT	251 (43.7)	283 (50.4)	119 (50.2)	71 (43.3)	43 (49.4)	140 (50.5)	189 (52.6)
TT	194 (33.7)	153 (27.3)	68 (28.7)	56 (34.1)	27 (31.1)	62 (22.4)	89 (24.8)
C allele	511 (44.4)	533 (47.5)	219 (46.2)	145 (44.2)	77 (44.3)	290 (52.3)	351 (48.9)
T allele	639 (55.6)	589 (52.5)	255 (53.8)	183 (55.8)	97 (55.7)	264 (47.6)	367 (51.1)

mozygous TT genotype also attained statistical significance when analyzing the whole cohort of *BCR-ABL1*-negative MPN (OR = 1.34; 95% CI = 1.03-1.74; crude p-value = 0.02; adjusted p-value = 0.04). There was no correlation with ET and CML phenotypes. We also analyzed the allelic model, but in this case the T allele showed a near significant association only in case of PV (OR = 1.19; 95% CI = 1-1.44; crude p-value = 0.06; adjusted p-value = 0.24), while all other comparisons showed non-significant results. We also analyzed the dominant model (CT+TT versus CC genotype), but in this case none of the comparisons performed yielded significant results. Table 3 details all the comparisons between *SH2B3* rs3184504 and MPN phenotypes

Correlations between SH2B3 rs3184504 genotypes and alleles, and MPN molecular subtypes

Then, we analyzed the relationship between *SH2B3* rs3184504 genotypes and alleles, and the molecular subtypes to which the MPN patients included in the study belong. We analyzed both dominant and recessive genetic models, but again only the recessive model yielded statistically significant results. Because all patients with PV harbored *JAK2* V617F mutation, identical values for TT genotype were obtained as in the case of PV phenotype (OR = 1.54; 95% CI = 1.14-2.06; crude p-value = 0.004; adjusted p-value = 0.024). The TT genotype was also associated with *JAK2* V617F-positive PMF, albeit less significantly (OR = 1.57; 95% CI = 1.04-2.33; crude p-value = 0.03; adjusted p-value = 0.08). Similar results for the TT genotype as in the case of *JAK2* V617F-positive PMF were also obtained when analyzing the whole cohort of MPN patients harboring *JAK2* V617F mutation (OR = 1.36; 95% CI = 1.04-1.77; crude p-value = 0.02; adjusted p-value = 0.08). However, the TT genotype was not associated with *CALR*-positive ET or PMF, *JAK2* V617F-positive ET, or CML. Again, the allelic model yielded non-significant results, except for PV (where all patients were *JAK2* V617-positive), in which the T allele showed the same near significant association (OR = 1.19; 95% CI = 1-1.44; crude p-value = 0.06; adjusted p-value = 0.24). We also analyzed the dominant model (CT+TT versus CC geno-

Table 3. Association between *SH2B3* rs3184504 genotypes and alleles, and MPN phenotypes

Comparison	TT versus CT+CC genotypes (recessive model)			T allele versus C allele (allelic model)		
	OR [95% CI]	Crude p-value	FDR adjusted p-value	OR [95% CI]	Crude p-value	FDR adjusted p-value
PV versus controls	1.54 [1.14-2.06]	0.004	0.024	1.19 [1-1.44]	0.06	0.24
ET versus controls	1.16 [0.87-1.54]	0.31	0.37	1.07 [0.45 -1.28]	0.44	0.44
PMF versus controls	1.50 [1.04-2.12]	0.02	0.04	1.20 [0.95-1.51]	0.11	0.24
CML versus controls	0.88 [0.61-1.27]	0.57	0.57	0.87 [0.69-1.08]	0.23	0.27
MPN *BCR-ABL1*-negative whole cohort (PV+ET+PMF)	1.34 [1.03-1.74]	0.02	0.04	1.13 [0.96-1.33]	0.12	0.24
MPN whole cohort (PV+ET+PMF+CML)	1.26 [0.97-1.63]	0.07	0.10	1.12 [0.95-1.31]	0.16	0.24

type), but in this case none of the comparisons performed yielded significant results. Table 4 presents in detail all the comparisons between *SH2B3* rs3184504 and MPN-associated molecular subtypes.

Correlations between SH2B3 rs3184504 polymorphism and various hematological and clinical features of patients with MPN

We also assessed whether *SH2B3* rs3184504 was correlated with hematological parameters displayed by MPN patients (hemoglobin, hematocrit, white blood cells count, platelets). The white blood cells count had higher values in patients with homozygous TT genotype. However, this was seen only in PV (median 12.95 x 10^6/L in patients with TT genotype versus 11.45 x 10^6/L in patients with CT+CC genotypes, Mann-Whitney test p-value = 0.03). All other comparisons yielded statistically non-significant results (p-value > 0.05).

The complete clinical information regarding the occurrence of major thrombosis was available in 375 patients with ET and 273 patients with PV. There were 97 patients with ET (25.9%) and 108 patients with PV (39.5%) having experienced major thrombosis on diagnosis. We considered the following events as major thrombosis: stroke/transient ischemic attack, acute coronary disease, acute limb ischemia, splenic infarction, and mesenteric infarction, deep venous thrombosis, splanchnic thrombosis, and cerebral sinus venous thrombosis. There was no correlation between *SH2B3* rs3184504 genotypes and alleles and the occurrence of major thrombosis.

Discussions

Our results support the supposition that the *SH2B3* rs3184504 polymorphism determines a predisposition for the development of MPN. Of all the tested genetic models (dominant, recessive, and allelic), only the recessive one yielded statistically significant correlations. We noted that the frequency of TT homozygous genotype was significantly higher in *BCR-ABL1*-negative MPN group than in controls. This parallels data obtained by Lesteven et al., the first study to report on the association between MPN and *SH2B3* rs3184504 (20). However, their study enrolled only patients with ET and myelofibrosis. Also, they reported significant associations be-

Table 4. Association between *SH2B3* rs3184504 genotypes and alleles, and MPN major molecular subtypes

Comparison	TT versus CT+CC genotypes (recessive model)			T allele versus C allele (allelic model)		
	OR [95% CI]	Crude p-value	FDR adjusted p-value	OR [95% CI]	Crude p-value	FDR adjusted p-value
PV *JAK2* V617F-positive versus controls	1.54 [1.14-2.06]	0.004	0.024	1.19 [1-1.44]	0.06	0.24
ET *JAK2* V617F-positive versus controls	1.13 [0.83-1.54]	0.44	0.50	0.86 [0.71-1.04]	0.13	0.32
PMF *JAK2* V617F-positive versus controls	1.57 [1.04-2.33]	0.03	0.08	1.20 [0.93-1.56]	0.16	0.32
MPN (PV+ET+PMF) *JAK2* V617F-positive versus controls	1.36 [1.04-1.77]	0.02	0.08	1.13 [0.96-1.33]	0.13	0.32
ET *CALR*-positive versus controls	1.22 [0.85-1.76]	0.29	0.38	1.11 [0.88-1.40]	0.37	0.37
PMF *CALR*-positive versus controls	1.36 [0.81-2.30]	0.27	0.387	1.20 [0.86-1.68]	0.31	0.35
ET+PMF *CALR*-positive versus controls	1.25 [0.89-1.76]	0.19	0.38	1.13 [0.92-1.40]	0.25	0.33
CML *BCR-ABL1*-positive versus controls	0.88 [0.61-1.27]	0.57	0.57	0.87 [0.69-1.08]	0.23	0.27

tween *SH2B3* rs3184504 and MPN for the allelic model, the T allele being the risk allele. They reported the most significant association in the case of myelofibrosis (20). We also report significant association between *SH2B3* rs3184504 and PMF. However, in our study, the strongest correlation was between *SH2B3* rs3184504 and PV. Olkhovskiy et al. reported significant correlation with PV only (22), while Chen et al. with all three *BCR-ABL1*-negative MPN - PV, ET and PMF (21). On the other hand, we did not see any correlation between *SH2B3* rs3184504 and CML, which is in agreement with data obtained by Olkhovskiy et al. (22). Chen et al. reported the opposite. In their study, the CC genotype had a remarkably high frequency in the CML group (21).

We then analyzed the correlation between *SH2B3* rs3184504 and the two major MPN molecular subtypes, namely *JAK2* V617F and *CALR*. We saw no association between *SH2B3* rs3184504 and *CALR* mutations, neither when analyzing the whole cohort of ET plus PMF *CALR*-positive patients, nor when analyzing each entity separately, ET and PMF *CALR*-positive, respectively. However, we observed significant association between *SH2B3* rs3184504 and the combined cohort of *JAK2* V617F-positive MPN patients. The association remained significant when analyzing each *JAK2* V617F-positive PV and PMF cohort of patients. Again, the strongest correlation was in the case of PV. Surprisingly, *SH2B3* rs3184504 was not associated with *JAK2* V617F-positive ET. Our findings parallel data reported by Lesteven et al. and Olkhovskiy et al. who reported positive association between *SH2B3* rs3184504 and *JAK2* V617F-positive MPN only (20,22). Chen et al. did not perform a separate analysis on *JAK2* V617F-negative MPN (21). It should be noted that none of the

three studies analyzed *CALR* mutations. However, since most of *JAK2* V617F-negative ET and PMF patients harbor *CALR* mutations, we may state that the lack of association between *SH2B3* rs3184504 and *JAK2* V617F-negative MPN translates actually into the lack of association between *SH2B3* rs3184504 and *CALR*-positive MPN. The partial differences between our findings and those reported by Lesteven et al., Chen et al. and Olkhovskiy et al. could be explained by the differences in cohort sizes and the ethnic origin of the patients included in the studies. For instance, the T allele of the *SH2B3* rs3184504 has a much lower frequency in Asians than in Caucasians (21).

Hinds et al. recently reported on the association between MPN and another *SH2B3* SNP, namely rs7310615, which is in strong linkage disequilibrium with rs3184504 (r^2 = 0.94) (15). The *SH2B3* rs7310615 was strongly associated with MPN. However, it was not associated preferentially with *JAK2* V617F-positive MPN, like our study revealed regarding rs3184504. Interestingly, their study revealed that the same polymorphisms predisposing to MPN, including *SH2B3* rs7310615, also predispose to clonal hematopoiesis of indeterminate potential (CHIP) (15).

SH2B3 variants seem to be associated not only with hematological malignancies, and in particular to MPN, but also with various other conditions. Several GWAS (genome wide association studies) have identified the *SH2B3* rs3184504 SNP to be associated with many chronic inflammatory and autoimmune disorders, including celiac disease, type I diabetes, asthma, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis. Besides this large spectrum of diseases, this SNP seems to be also involved in increasing the risk of hypercholesterolemia and cardiovascular disorders, like hypertension, myocardial infarction, and coronary artery disease (29). Moreover, an association was noted between the presence of *SH2B3* rs3184504 polymorphism and several clinically significant modifications of hematological parameters, such as increased numbers of peripheral white blood cells, eosinophils, erythrocytes and platelets, reviewed in detail by Maslah et al. (17). Taken together, these data sustain the supposition that *SH2B3* rs3184504 SNP could play a role in the predisposition to MPNs, as well as in the MPN-associated thrombotic events and cardiovascular diseases. Indeed, we observed that the TT homozygotes had increased number of white blood cells, compared with carriers of other genotypes. This effect was restricted to patients with PV, suggesting that *SH2B3* rs3184504 could have distinct effects on various MPN types. However, we failed to demonstrate any association between *SH2B3* rs3184504 and both arterial and venous thrombosis in our group of patients. This could be due to the relatively low number of MPN patients in whom we had complete clinical information concerning especially the cardiovascular diseases. Also, the pathogenesis of cardiovascular events seen in MPN, including the thrombotic events, is very complex, supposing different pathways.

This study expands our previous work and adds important data regarding the genetic predisposition to MPN in our population. We previously demonstrated significant association between *JAK2* 46/1 haplotype and *JAK2* V617F-positive MPN, *TERT* rs2736100 polymorphism and MPN, regardless of phenotype or molecular subtype, *MECOM* rs2201862 and MPN, regardless of molecular subtype, *HBS1L-MYB* rs9376092 and *JAK2* V617F-mutated ET, and *THRB-RARB* rs4858647 and PMF (11,12). Our findings indicate that *SH2B3* rs3184504 has a magnitude similar to *MECOM* rs2201862. However, while *MECOM* rs2201862 predisposes to MPN regardless of molecular subtype, *SH2B3* rs3184504 predisposes to *JAK2* V617F-positive MPN only.

The most important strength of our study is the large number of patients included in the cohorts

we analyzed, which assures the high relevance of our results. Also, the availability of the genetic information regarding the main somatic driver mutation (*JAK2* V617F and *CALR*) in all the patients is an important strength of the study. We consider that the main limit of our study is represented by the lack of data regarding other polymorphisms from the genome. We cannot rule out possible interactions between *SH2B3* rs3184504 and other polymorphisms. Also, we were not able to study the functional consequences of *SH2B3* rs3184504.

In conclusion, our study shows significant association between the TT homozygous genotype of *SH2B3* rs3184504 and *JAK2* V617F-positive MPN, but not *CALR*-positive MPN. Thus, we confirm on a large cohort of patients the contribution of *SH2B3* rs3184504 to the occurrence of *JAK2* V617F-positive MPN, placing it together with other polymorphisms defining the genetic predisposition to MPN, such as *JAK2* 46/1 haplotype, *TERT* rs2736100 or *MECOM* rs2201862. Further studies should clarify the intimate mechanism by which *SH2B3* rs3184504 predisposes to the acquisition of *JAK2* V617F mutation.

Abbreviations

MPN - myeloproliferative neoplasms
SH2B3 - Src homology 2B3
PV - polycythemia vera
ET - essential thrombocythemia
PMF - primary myelofibrosis
CML - chronic myeloid leukemia
JAK2 - Janus kinase 2
CALR - calreticulin
BCR-ABL1 - breakpoint cluster region - Abelson murine leukemia 1
MPL - myeloproliferative leukemia virus oncogene
STAT - signal transducer and activator of transcription
TERT - telomerase
MECOM - MDS1 and EVI1 complex locus protein EVI1
TET2 - ten-eleven translocation methylcytosine dioxygenase 2
ATM - ataxia-telangiectasia mutated gene
CHEK2 - checkpoint kinase 2
THRB - thyroid hormone receptor beta
RARB - retinoic acid receptor beta
PINT - p53-induced transcript
WHO - World Health Organization
EDTA - ethylenediaminetetraacetic acid
PCR - polymerase chain reaction
SNP - single nucleotide polymorphism
CHIP - clonal hematopoiesis of indeterminate potential
GWAS - genome wide association studies

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Authors' contribution

APT designed the study, performed genetic analysis, analyzed data and wrote the manuscript; DLL analyzed data and wrote the manuscript; CJ, FT, DRA, ȘGP, and RMP performed genetic analysis; AB and DD provided samples and analyzed clinical data of the patients; CB designed the study and wrote the manuscript.

APT and DLL contributed equally to the study and should be considered both first authors

Conflict of Interest

There is no potential conflict-of-interest related to this work.

References

1. Abdel-Wahab O. Genetics of the myeloproliferative neoplasms. Curr Opin Hematol. 2011;18(2):117-23. DOI: 10.1097/MOH.0b013e328343998e

2. Barbui T, Thiele J, Gisslinger H, Kvasnicka HM, Vannucchi AM, Guglielmelli P, et al. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. Blood Cancer J. 2018;8(2):15. DOI: 10.1038/s41408-018-0054-y
3. Tefferi A, Pardanani A. Myeloproliferative Neoplasms: A Contemporary Review. JAMA Oncol. 2015;1(1):97-105. DOI: 10.1001/jamaoncol.2015.89
4. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405. DOI: 10.1182/blood-2016-03-643544
5. Nangalia J, Green AR. Myeloproliferative neoplasms: from origins to outcomes. Blood. 2017;130(23):2475-83. DOI: 10.1182/blood-2017-06-782037
6. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Bjorkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 first-degree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. Blood. 2008;112(6):2199-204. DOI: 10.1182/blood-2008-03-143602
7. Jones AV, Chase A, Silver RT, Oscier D, Zoi K, Wang YL, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. Nat Genet. 2009;41(4):446-9. DOI: 10.1038/ng.334
8. Kilpivaara O, Mukherjee S, Schram AM, Wadleigh M, Mullally A, Ebert BL, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. Nat Genet. 2009;41(4):455-9. DOI: 10.1038/ng.342
9. Olcaydu D, Harutyunyan A, Jäger R, Berg T, Gisslinger B, Pabinger I, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. Nat Genet. 2009;41(4):450-4. DOI: 10.1038/ng.341
10. Jones AV, Campbell PJ, Beer PA, Schnittger S, Vannucchi AM, Zoi K, et al. The JAK2 46/1 haplotype predisposes to MPL-mutated myeloproliferative neoplasms. Blood. 2010;3;115(22):4517-23. DOI: 10.1182/blood-2009-08-236448
11. 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;174(2):218-26. DOI: 10.1111/bjh.14041
12. Trifa AP, Banescu C, Bojan AS, Voina CM, Popa S, Visan S, et al. MECOM, HBS1L-MYB, THRB-RARB, JAK2, and TERT polymorphisms defining the genetic predisposition to myeloproliferative neoplasms: A study on 939 patients. Am J Hematol. 2018;93(1):100-6. DOI: 10.1002/ajh.24946
13. Oddsson A, Kristinsson SY, Helgason H, Gudbjartsson DF, Masson G, Sigurdsson A, et al. The germline sequence variant rs2736100_C in TERT associates with myeloproliferative neoplasms. Leukemia. 2014;28(6):1371-4. DOI: 10.1038/leu.2014.48
14. Tapper W, Jones AV, Kralovics R, Harutyunyan AS, Zoi K, Leung W, et al. Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. Nat Commun. 2015;6:6691. DOI: 10.1038/ncomms7691
15. 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;128:1121-8. DOI: 10.1182/blood-2015-06-652941
16. Velazquez L. The Lnk adaptor protein: a key regulator of normal and pathological hematopoiesis. Arch Immunol Ther Exp (Warsz). 2012;60(6):415-29. DOI: 10.1007/s00005-012-0194-x
17. Maslah N, Cassinat B, Verger E, Kiladjian JJ, Velazquez L. The role of LNK/SH2B3 genetic alterations in myeloproliferative neoplasms and other hematological disorders. Leukemia. 2017;31(8):1661-70. DOI: 10.1038/leu.2017.139
18. McMullin MF, Cario H. LNK mutations and myeloproliferative disorders. Am J Hematol. 2016;91(2):248-51. DOI: 10.1002/ajh.24259
19. Rumi E, Cazzola M. Advances in understanding the pathogenesis of familial myeloproliferative neoplasms. Br J Haematol. 2017;178(5):689-98. DOI: 10.1111/bjh.14713
20. Lesteven E, Picque M, Conejero Tonetti C, Giraudier S, Varin-Blank N, Velazquez L, et al. Association of a single-nucleotide polymorphism in the SH2B3 gene with JAK2V617F-positive myeloproliferative neoplasms. Blood. 2014;123(5):794-6. DOI: 10.1182/blood-2013-10-532622
21. Chen Y, Fang F, Hu Y, Liu Q, Bu D, Tan M, et al. The Polymorphisms in LNK Gene Correlated to the Clinical Type of Myeloproliferative Neoplasms. PLoS One. 2016;11(4):e0154183. DOI: 10.1371/journal.pone.0154183
22. Olkhovskiy IA, Gorbenko AS, Stolyar MA, Vasiliev EV, Mikhalev MA, Tabakova KA. T784C LNK gene polymorphism and the risk of myeloproliferative disorders. Leuk Lymphoma. 2019;60(1):277-8. DOI: 10.1080/10428194.2018.1459604
23. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005;106:2162-8. DOI: 10.1182/blood-2005-03-1320
24. Jovanovic JV, Ivey A, Vannucchi AM, Lippert E, Oppliger Leibundgut E, Cassinat B, et al. Establishing optimal quantitative-polymerase chain reaction assays for routine diagnosis and tracking of minimal residual

disease in JAK2-V617F-associated myeloproliferative neoplasms: a joint European LeukemiaNet/MPN&MP-Nr-EuroNet (COST action BM0902) study. Leukemia. 2013;27:2032-9. DOI: 10.1038/leu.2013.219
25. Trifa AP, Cucuianu A, Popp RA. Familial Essential Thrombocythemia Associated with MPL W515L Mutation in Father and JAK2 V617F Mutation in Daughter. Case Rep Hematol 2014;2014:841787. DOI: 10.1155/2014/841787
26. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia. 1999;13(12):1901-28. DOI: 10.1038/sj.leu.2401592
27. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001;125(1-2):279-84. DOI: 10.1016/S0166-4328(01)00297-2
28. R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
29. Brian G. Kral, Lewis C. Becker, Chapter 8 - Genetics of Coronary Disease, Editor(s): Wilbert S. Aronow, John Arthur McClung, Translational Research in Coronary Artery Disease, Academic Press, 2016, Pages 81-101. DOI: 10.1016/B978-0-12-802385-3.00008-5