Hereditary thrombocytosis caused by *MPL*^{Ser505Asn} is associated with a high thrombotic risk, splenomegaly and progression to bone marrow fibrosis

Luciana Teofili,[1] Fiorina Giona,[2] Lorenza Torti,[1] Tonia Cenci,[3] Bianca Maria Ricerca,[1] Carlo Rumi,[1] Vittorio Nunes,[2] Robin Foà,[2] Giuseppe Leone,[1] Maurizio Martini,[3] and Luigi Maria Larocca[3]

[1]Department of Hematology and [3]Department of Pathology, Catholic University, Rome, and [2]Division of Hematology, Department of Cellular Biotechnologies and Hematology, "La Sapienza" University, Rome, Italy

Funding: supported by Prin 2006, Ministero Università e Ricerca Scientifica (Rome, Italy) and by Fondi d'Ateneo, Progetti D1 2006-2007, Università Cattolica (Rome, Italy).

Acknowledgments: the authors are indebted to Prof. V. De Stefano and to Dr. L. Laurenti (Department of Hematology, Catholic University, Rome, Italy) for having contributed some of the patients with the MPL^{Ser505Asn} *mutation. The authors gratefully acknowledge Dr. Sara Mariotti (Department of Hematology, Catholic University, Rome, Italy) for sample processing.*

Manuscript received on February 16, 2009. Revised version arrived on July 6, 2009. Manuscript accepted on July 28, 2009.

Correspondence:
Luigi M. Larocca, MD
Istituto di Anatomia Patologica, Università Cattolica, Largo Gemelli 8, 00168 Roma, Italy.
E-mail: llarocca@rm.unicatt.it

The online version of this article has a supplementary appendix.

ABSTRACT

Background

The *MPL*^{Ser505Asn} mutation has been reported to be a cause of hereditary thrombocythemia. Recently, we detected this mutation in a large proportion of children with familial thrombocythemia, suggesting that in Italy the incidence of *MPL*^{Ser505Asn} mutation could be underestimated.

Design and Methods

We extended the search for this mutation to all patients with essential thrombocythemia who had a positive family history for thrombocytosis or essential thrombocythemia. We identified eight Italian families positive for the *MPL*^{Ser505Asn} mutation. Clinical and hematologic data were available for members of seven families, including 21 patients with a proven mutation and 20 relatives with thrombocytosis.

Results

Fifteen major thrombotic episodes, nine of which were fatal, were recorded among 41 patients. The thrombotic manifestation was stroke in four cases, myocardial infarction in seven cases, fetal loss in two cases, deep vein thrombosis of the leg in one case and Budd Chiari syndrome in one case. Almost all patients over 20 years old had splenomegaly and bone marrow fibrosis, while these were rarely observed in patients under 20 years old, suggesting that these manifestations are associated with aging. Finally, the life expectancy of family members with thrombocytosis was significantly shorter than that of members without thrombocytosis (P=0.003).

Conclusions

Patients with familial thrombocytosis caused by a *MPL*^{Ser505Asn} mutation have a high risk of thrombosis and, with aging, develop splenomegaly and bone marrow fibrosis, significantly affecting their life expectancy.

Key words: *MPL*^{Ser505Asn}, hereditary thrombocytosis, splenomegaly, bone marrow fibrosis.

Citation: Teofili L, Giona F, Torti L, Cenci T, Ricerca BM, Rumi C, Nunes V, Foà R, Leone G, Martini M, and Larocca LM. Hereditary thrombocytosis caused by MPL^{Ser505Asn} *is associated with a high thrombotic risk, splenomegaly and progression to bone marrow fibrosis. Haematologica. 2010; 95:65-70. doi:10.3324/haematol.2009.007542*

Introduction

Hereditary thrombocytoses are caused by molecular alterations in the thrombopoietin gene (*THPO*) or in the gene for the thrombopoietin receptor (*MPL*).[1] Several point mutations of *THPO*, involving the 5'-untranslated region of the *THPO* mRNA, have been reported.[2-8] This region contains the upstream open reading frames that inhibit mRNA translation. All the mutations remove the inhibitory upstream open reading frame, leading to increased translation of the *THPO* mRNA. Patients carrying mutations of the 5'-untranslated region have a high platelet count due to the elevated serum levels of thrombopoietin[2-7] but are not usually considered at high thrombotic risk.[9] Nevertheless, Liu *et al.* recently reported that patients with thrombocytosis due to a G to C transversion in the splice donor of intron 3 of *THPO* have a risk of vascular complications similar to that of patients with essential thrombocythemia.[8]

In 2004, Ding *et al.* described the pedigree of a Japanese family with thrombocythemia caused by a G to A nucleotide substitution at position 1073 in exon 10 of *MPL*, leading to the exchange of serine for asparagine at position 505 (*MPL*^{Ser505Asn}).[10] The authors clearly demonstrated that cells expressing *MPL*^{Ser505Asn} showed autonomous phosphorylation of both Mek1/2 and STAT5 down signaling transduction pathways, but the clinical course of the disease in the affected individuals was not reported.[10] Recently, we evaluated a large cohort of Italian children with familial thrombocythemia and detected the germ line *MPL*^{Ser505Asn} mutation in many of them,[11-13] suggesting that this molecular defect might be rather frequent in our country. For this reason, we extended the search for the *MPL*^{Ser505Asn} mutation also to adult patients with essential thrombocythemia who had a positive family history of essential thrombocythemia or thrombocytosis. Here we describe the results of this extended search.

Design and Methods

Study population

All patients were observed at the Divisions of Hematology of *La Cattolica* and *La Sapienza* Universities of Rome for suspected or ascertained essential thrombocythemia. The only criterion for inclusion in the study was a positive family history for essential thrombocythemia or thrombocytosis. Splenomegaly was defined as a palpable organ below the left costal margin and was confirmed by ultrasound scans. In all cases of venous thrombosis, the diagnosis was ascertained by ultrasound Doppler or computed tomography scanning, as appropriate. The presence of other underlying causes of thrombophilia (antithrombin III, protein C or protein S deficiency, factor V Leiden, prothrombin G20210A mutation, autoimmune diseases, cancer) was ruled out. The hematologic and clinical findings of patients harboring the *MPL*^{Ser505Asn} mutation were evaluated in comparison with those recorded in patients with non-familial essential thrombocythemia. For this purpose, we evaluated a control group of 72 patients with non-familial essential thrombocythemia (52 adults and 20 children; median age 55 years, range 5-86) consecutively observed at the same institutions. Among this control group, 43 patients harbored the *JAK2*^{V617F} mutation, while the remaining 29 had wild-type *JAK2*; all the patients in this group had the wild-type *MPL* gene.

A family history was collected from all patients and the survival of family members with thrombocytosis was compared to that of the members without thrombocytosis. All blood samples were collected after informed consent and the study was approved by the local institutional review boards.

Molecular analysis

Genomic DNA was isolated using standard procedures from peripheral blood or buccal swab samples. Mutation analysis of the *MPL* (exon 10) gene was carried out by sequencing, as described elsewhere.[11] The presence of *THPO* 5'untranslated region mutations and of *JAK2*^{V617F} mutations was excluded. Moreover, clonality of hematopoiesis was examined in all female patients by the human androgen receptor assay (HUMARA) and by HUMARA methylation-specific polymerase chain reaction analysis. The methods used to analyze *MPL*, *THPO* 5'untranslated region and *JAK2*^{V617F} mutations and clonality have been described in detail elsewhere.[11]

Flow cytometry analysis

In order to investigate the state of activation of leukocytes in patients with the *MPL*^{Ser505Asn} mutation, we measured the expression of CD11b on the neutrophil membrane, by using mouse anti-human CD11b/Mac.1 antibody (BD, Pharmingen), as described previously.[14] The results are expressed as mean fluorescence intensity (MFI) in arbitrary units and compared to those obtained in ten normal controls and in five control patients with essential thrombocythemia.

Statistical analysis

Statistical analyses were performed with GraphPad software using the Kruskal-Wallis and Mann-Whitney tests for continuous variables, and Fisher's exact test or the χ^2 test for categorical variables, as appropriate. p values less than 0.05 were considered statistically significant. The Kaplan-Meier method was used to estimate univariate survival curves, and the log-rank test was adopted to compare the survival curves.

Results

Forty-four patients with ascertained or suspected thrombocythemia with a positive family history for essential thrombocythemia or thrombocytosis were investigated for the presence of the *MPL*^{Ser505Asn} mutation. Among these 44 patients, 24 (11 males and 13 females) were found to carry the mutation. These 24 patients belonged to eight families and were all native to regions of central Italy. Twelve of them are part of a previously reported series of children with myeloproliferative diseases (Table 1 and Figure 1, patients C_2, C_3, I_2, I_3, I_4, I_5, T_{11}, T_8, T_9, S_3, M_9, M_{11}).[11-13] Furthermore, the same patients and patients S_2, B_{13} and B_8, (Table 1 and Figure 1) were included in the study by Liu *et al.*, who showed a common founder effect in these families.[15] In this study we included seven out of the eight identified families, whose pedigrees are shown in Figure 1. One family was excluded because hematologic and clinical data were not available.

Inheritance pattern of the MPL^{Ser505Asn} mutation

All patients with the *MPL*^{Ser505Asn} mutation were heterozy-

Table 1. Hematologic and clinical findings at the time of the first observation or at the last follow-up* in patients carrying the *MPL*^{Ser505Asn} mutation.

Pt	Sex	Age	Follow-up (years)	WBC N.V. 4.1-9.8 $\times10^9$/L	Platelets N.V. 140-450 $\times10^9$/L	Hb N.V. 12-16 g/dL	Splenomegaly (cm)	Thrombosis (age)	Bone marrow fibrosis (grade)	Therapy
C_2	M	18	24	4.7*	712*	14.4*	Yes (NA)§	No	No	ASA
C_3	M	1	6	16.2°	1126	13.6	No	No	ND	No
C_4	M	1	1	12.1°	1726	12.1	No	No	ND	No
I_2	M	18	32	9.7	1210	15.5	No	No	No	ASA/HU
I_3	F	17	8	9.5	695	13.7	No	No	No	No
I_4	F	16	12	7.3	963	11.1	No	No	No	No
I_5	M	11	9	8.1	783	14.3	No	No	No	No
T_{11}	M	1	3	10.9°	1553	13.9	No	No	ND	No
T_8	F	14	20	7.5	1060	13.7	Yes (NA)§	No	No	ASA
T_9	F	3	18	6.3	627	12.9	No	No	No	No
T_1	M	35	34	8.3*	552*	10.9*	NA#	No	NA	No
S_1	F	72	4†	20.5	846	10.6	Yes (NA)	Stroke§ (76)	Yes (MF1)*	ASA/HU
S_2	F	43	12	5.8*	408*	10.0*	Yes (14,5)	DVT(41) TIA (43)	Yes (MF2)*	ASA/INF/HU
S_3	M	17	4	7.5	776	15.0	No	No	ND	ASA
F_1	F	76	4†	7.0	722	12.8	Yes (16)	Stroke§ (80)	Yes (MF1)	ASA/HU
F_3	F	54	1	4.2	426	10.1	Yes (18,5)	No	No	ASA
M_9	F	16	12	8.7	1019	14.7	Yes (18)	No	Yes (MF2)	ASA/INF
M_{10}	F	20	3	4.5	738	11.1	Yes (15)	No	Yes (MF1)	ASA
B_{13}	M	31	2	6.2*	605*	15.0*	Yes (NA)	MI (31)	Yes (MF1)	ASA
B_{14}	M	40	2	6.8	842	15.5	Yes (15)	No	Yes (MF1)	ASA
B_8	M	59	18	3.7*	317*	11.9*	Yes (NA)	No	Yes (MF2)*	ASA/HU

N.V.: normal values; WBC: white blood cell count; Hb: hemoglobin. DVT: deep vein thrombosis, TIA: transient ischemic attack, MI: myocardial infarction. N.A.: not available. N.D.: not done; ASA: aspirin; HU: hydroxyurea; INF: interferon. †This patient had a traumatic rupture of the spleen 4 years prior to the diagnosis of the hematologic disorder. §Indicates patients developing splenomegaly during the follow-up. ‡Indicates thrombosis that occurred during the follow-up. °Normal value for age: 6-17.5x10^9/L.

gous for the mutated gene and exhibited thrombocytosis. None of 13 investigated relatives with normal platelet counts showed the mutation. These data confirm the dominant autosomal pattern of inheritance of the *MPL*^{Ser505Asn} mutation.[10] Furthermore, hematopoiesis was polyclonal in all affected females. In order to confirm the germ line nature of the defect, DNA from buccal swabs from eight patients with the mutation was analyzed and all these samples proved to be positive for the mutation.

Hematologic and clinical findings of patients with MPL^{Ser505Asn}

The hematologic and clinical findings recorded at the time of the first observation or at the last follow-up in 21 evaluable family members (11 males and 10 females) carrying the *MPL*^{Ser505Asn} mutation are shown in Table 1. The median age at the time of the first observation was 18 years (range, 1-76) and the median time of follow-up was 9 years (range, 1-34). A slight increase of white blood cell count was noted in one patient who had undergone splenectomy because of traumatic rupture of the spleen (patient S_1). Mild anemia was found at diagnosis in four patients: one of them (patient T_1) had impaired renal function, and the other three (patients S_1, F_3, and M_{10}) had enlarged spleen volume and/or significant bone marrow fibrosis. Anemia was documented in two patients during the follow-up (patients S_2 and B_8). Both patients developed splenomegaly and bone marrow fibrosis, and received antiproliferative therapy (Table 1).

Splenomegaly was detected at diagnosis in nine out of 20 cases (patient T_1 was not evaluable because of traumatic rupture of the spleen before the diagnosis of the hematologic disorder). Of these nine patients, eight (S_1, S_2, M_{10}, F_1, F_3, B_8, B_{13}, and B_{14}, Table 1) were older than 20 years (P<0.0001 at Fisher's exact test). Two additional patients (T_8 and C_2, Table I) developed splenomegaly during the follow-up.

Results of bone marrow biopsies were available for 16 patients previously diagnosed as having essential thrombocythemia. The grade of fibrosis and hypercellularity were defined as previously stated.[16] In young patients, the histological picture was characterized by hypercellular bone marrow with an increased number of neutrophils and atypical megakaryocytes, in the absence of reticulin fibrosis (Table 1 and Figure 2, A-B). However, in adult and elderly patients, overt bone marrow fibrosis, with several atypical megakaryocytes forming dense clusters and progressive increase of reticulin with many intersections and focal bundles of collagen, was detected (Table 1 and Figure 2, C-F). In three patients (S_2, F_3, and M_9, Table 1), the bone marrow biopsy was performed at diagnosis and thereafter during the course of the disease: an increase of reticulin fibrosis was documented in all three cases.

Overall, in our series of 21 affected family members, four patients (19%) experienced a major thrombosis: patients S_1 and F_1 had fatal strokes at the age of 76 and 80, respectively, patient B_{13} had a myocardial infarct at the age of 31, and patient S_2 suffered from deep vein thrombosis of the legs at the age of 41 and then, 2 years later, had a transient ischemic attack.

With regards to therapy, low dose aspirin was given at diagnosis or during the follow-up to 15 out of the 21 patients. The indication for antiplatelet therapy in all treated children was headache. Hemorrhagic complications

were not observed, even though extreme thrombocytosis (platelet count >2000×10⁹/L) was recorded during the follow-up in two children. Six patients also received antiproliferative therapy, which consisted of hydroxycarbamide in four cases and interferon in two other patients. The reasons for starting antiproliferative treatment were a high risk of thrombosis in patients over 65 years old (patients F_1, S_1 and B_8), previous thrombosis at diagnosis (patient S_2), reported discomfort from splenomegaly (patient M_{10}), and a high platelet count (patient I_2). Three female patients completed a total of seven uncomplicated pregnancies; in two cases, antiplatelet drugs were administered throughout the pregnancy, replaced by low dose heparin in the peri-partum period.

The hematologic parameters at diagnosis and the incidences of thrombotic complications and splenomegaly recorded in patients with *MPL*^{Ser505Asn} were compared with those observed in 72 patients with non-familial essential thrombocythemia grouped according to the presence of the *JAK2*^{V617F} mutation (Table 2). The white blood cell count was lower in patients with *MPL*^{Ser505Asn} than in patients with either *JAK2*^{V617F} or the wild-type *JAK2* (*JAK2*^{WT}) (P=0.04; Table 2). The concentration of hemoglobin was lower in *MPL*^{Ser505Asn} patients than in *JAK2*^{V617F} patients (P=0.02) but not *JAK2*^{WT} patients (Table 2). Finally, no differences were found in platelet counts, or the incidences of thrombotic complications or splenomegaly between patients with *MPL*^{Ser505Asn} and those with essential thrombocythemia.

Family history

The family pedigrees of the 21 *MPL*^{Ser505Asn} patients are illustrated in Figure 2. The detailed history of each family is presented in the *Online Supplementary Appendix*. Thrombocytosis was referred in the history of 26 additional family members but medical histories were available for only 20 out of these. Overall, among 41 individuals (21 patients with documented *MPL*^{Ser505Asn} mutation and 20 family members with reported thrombocytosis) 15 major thrombotic episodes occurred in 14 members. In particular, Budd Chiari syndrome occurred in one patient aged 17 (T_{10}, Figure 1); deep vein thrombosis of the legs occurred in one patient aged 41 (S_2, Figure 2); eclampsia was recorded in one patient (M_4, Figure 2) and this woman's daughter had a fetal loss (M_8, Figure 2); four patients, aged 80, 72 and 76 and 43 years, had a stroke (B_{10}, F_1, S_1 and S_2, Figure 2), while seven patients had a myocardial infarction (M_1, M_2, M_5, B_1, B_6, B_{11} and B_{13}, Figure 2). The median age at the time of the myocardial infarction was 52 years (range, 31-81).

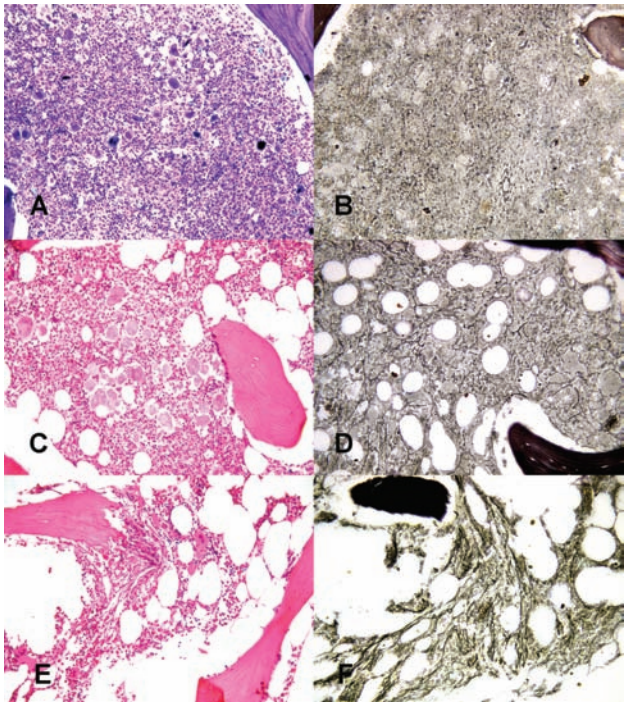

Figure 1. Hematoxylin-eosin and silver staining of bone marrow biopsies from three patients aged 16 (A and B), 43 (C and D) and 69 years (E and F), affected by hereditary thrombocythemia due to the *MPL*^{Ser505Asn} mutation. A: shows a slightly hypercellular bone marrow with an increase in the number of neutrophils and atypical megakaryocytes with deviations from the normal nuclear:cytoplasmic ratio and the frequent occurrence of bare megakaryocytic nuclei. B: reticulin fibrosis is absent. C: shows a hypercellular bone marrow with an increase in the number of atypical megakaryocytes that form dense clusters. D: loose network of reticulin with many intersections. E, F: shows a bone marrow biopsy mainly characterized by a diffuse increase in reticulin with focal bundles of collagen and with numerous distorted megakaryocytes. A-F 250x.

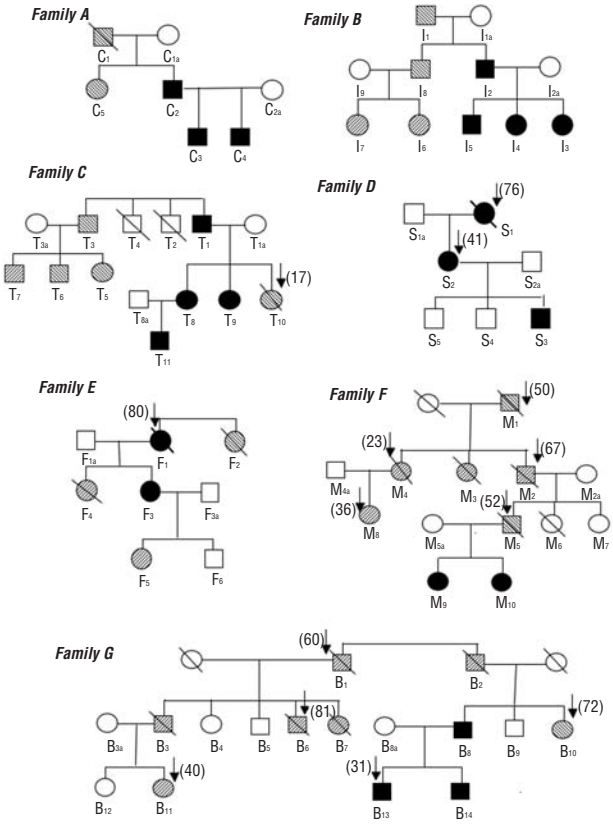

Figure 2. Pedigrees of the seven Italian families carrying the *MPL*^{Ser505Asn} mutation. Squares denote men, circles women and a slash indicates a person who has died. Solid symbols indicate affected members, shaded symbols members with thrombocytosis not investigated for the mutation and white symbols members with a normal platelet count. Arrows indicate those patients who experienced one or more thrombotic complications (between brackets the age at the time of the thrombosis).

Among the 15 family members who died, nine died of thrombosis, three patients died of undefined complications of myelofibrosis (2 cases) or essential thrombocythemia, one patient died of liver cirrhosis, one patient died of gastric cancer and one patient died of an unknown cause. The overall survival and thrombosis-free survival of family members with thrombocytosis were compared to those recorded in 23 relatives without thrombocytosis. For this purpose, only relatives with a detailed medical history were included in the analysis. As illustrated in Figure 3 both overall survival and thrombosis-free survival appear to be significantly shortened in individuals with thrombocytosis (P=0.003 and P=0.0009, for overall survival and thrombosis-free survival, respectively).

Neutrophil CD11b expression

The pathogenesis of thrombotic complications occurring in myeloproliferative diseases has been ascribed, at least in part, to the activation of polymorphonucleated cells.[14,17] We evaluated the expression of CD11b in the granulocytes of three patients carrying the *MPL*^{Ser505Asn} mutation and found that CD11b expression in these patients was significantly higher than that in normal controls and similar to that in patients with essential thrombocythemia (the MFI±SEM was 12.1±1.4 in patients with the mutation, 7.2±0.5 in ten controls and 11.4±1.7 in five patients with essential thrombocythemia;P<0.01 by the Kruskal-Wallis test).

Discussion

This study defines for the first time the course of the disease associated with the *MPL*^{Ser505Asn} mutation. The clinical manifestations, recorded in seven affected Italian families through three generations, highlight that the disease resulting from this mutation has distinctive features and is quite different from other hereditary thrombocytoses,[2-8] although patients carrying the *MPL*^{Ser505Asn} mutation have the same high thrombotic risk as patients with essential thrombocythemia.[18] This finding is in agreement with the results of a recent study by Liu *et al.* in patients with hereditary thrombocytosis,[9] whereas in this set of patients microcirculatory disturbances rather than major thromboses were mostly reported.[9] It is noteworthy that in our series of patients the thrombotic disease was fatal in nine cases and that most patients were not receiving antiplatelet therapy at the time of the thrombosis. Thus, in the light of the evidence that the *MPL*^{Ser505Asn} mutation affects both overall and thrombosis-free survival, patients with this mutation would probably benefit from treatment with low dose aspirin. The increased thrombotic risk in patients with the *MPL*^{Ser505Asn} mutation is in accordance with the detection of functionally similar mutations in both essential thrombocythemia and primary myelofibrosis[9-22] and suggests that this defect, like the *JAK2*^{V617F} mutation, is able to induce platelet or leukocyte activation.[14,17] In accordance with this hypothesis, the expression of CD11b in granulocytes of three patients with the *MPL*^{Ser505Asn} was similar to that observed in patients with essential thrombocythemia and significantly higher than in normal controls.

Our second major finding was that the *MPL*^{Ser505Asn} mutation induces splenomegaly with aging. Indeed, the detection of an enlarged spleen at diagnosis seemed to be significantly dependent on the age of patients, and, ultimately, on disease duration. Accordingly, a progressive increase of spleen volume was documented during the follow-up. Finally, the bone marrow showed a histological picture that closely resembled that of primary myelofibrosis, because of the hypercellularity and atypical megakaryocytes present in the early stages of the disease, and because of progression

Table 2. Hematologic and clinical findings in patients with hereditary *MPL*^{Ser505Asn} mutation, in comparison with those of patients with essential thrombocythemia grouped according to *JAK2* mutation status.

	MPL^{Ser505Asn} (A) n=21	JAK2^{V617F} (B) n=43	*JAK2*^{WT} (C) n=29	(A) *vs.* (B) *p*	(A) *vs.* (C) *p*
Plt×10^9/L: Median (Range)	795 (426-2500)	722 (491-2640)	900 (483-2537)	0.46	0.36
WBC×10^9/L: Mean Median (Range)	8.3 (4.2-20.5)	10.6 (3.3-22.0)	10.5 (4.3-23.5)	0.03	0.03
Hb g/dL: Median (Range)	12.9 (10.1-15.5)	14.2 (9.7-17.5)	13.5 (9.8-15.6)	0.02	0.17
Thrombosis n (%)	4 (19)	12 (28)	5 (17)	0.39	
Splenomegaly n (%)	9 (45)	9 (21)	7 (24)	0.12	

Plt: platelets; WBC: white blood cell count; Hb: hemoglobin.

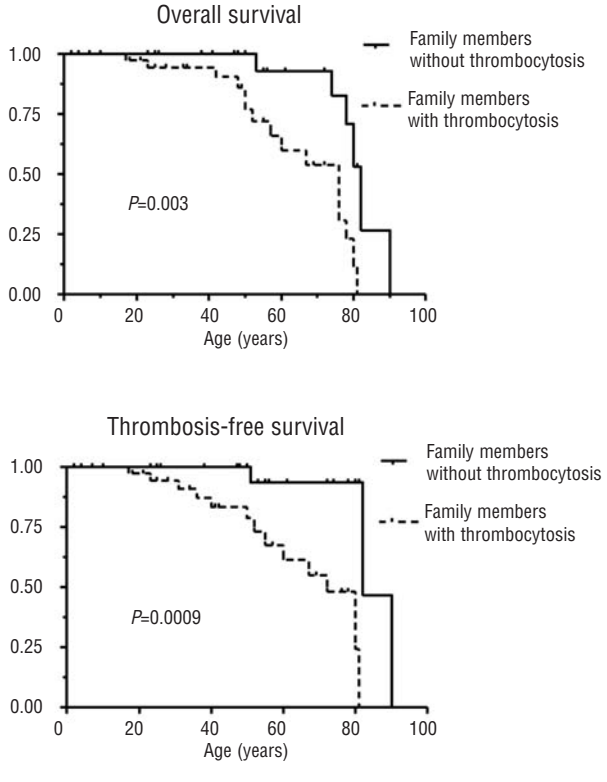

Figure 3. Overall and thrombosis-free survival in 41 family members with documented or reported thrombocytosis in comparison with survival in 23 unaffected familial members.

towards a significant fibrosis with aging (Figure 1). Interestingly, Beer *et al.* reported that patients with essential thrombocythemia carrying the acquired *MPL*$^{W515L/K}$ mutation more frequently had bone marrow fibrosis as compared to patients with *MPL*^{WT}.[22]

Two germ line *MPL* mutations have been so far reported: the *MPL*^{Baltimore} polymorphism[23] and the *MPL*$^{p.Pro106Leu}$ mutation.[24] Both these mutations involve the extracellular domain of MPL, affecting its binding ability to thrombopoietin. In fact, these patients have high serum levels of thrombopoietin,[23] probably because of decreased thrombopoietin clearance. Interestingly, thrombosis, splenomegaly and bone marrow fibrosis have never been reported in patients with these mutations. In contrast, the *MPL*^{Ser505Asn} mutation appears functionally similar to the *MPL*^{W515L} mutation: while the former involves juxtamembrane domains and the latter intracellular domains, both can autonomously activate downstream signal transduction pathways.[10,25] Indeed, it is not surprising that the clinical course of an *MPL*^{Ser505Asn} mutation-related disease is consistent with that of a myeloproliferative disorder presenting with thrombocytosis already at the time of birth, causing a significant thrombotic risk also at young age, and inducing splenomegaly, bone marrow fibrosis and progressive anemia with aging. The clinical penetrance of this genetic defect appears to be variable, considering that some family members carry the mutation but have few or no clinical manifestations. However, given the progressive nature of the disease and its possible evolution to myelofibrosis, patients with the *MPL*^{Ser505Asn} mutation might benefit from kinase inhibitors designed for patients with *MPL*$^{W515L/K}$ and *JAK2*-mutated myeloproliferative diseases.[26]

Authorship and Disclosures

LT designed the study, analyzed data and wrote the manuscript; FG contributed to the study design, analyzed data and critically reviewed the manuscript; MM contributed to the study design and, with TC, performed molecular analyses; CR performed the flow cytometry analysis; LT, BMR and VN enrolled patients and recorded clinical data; GL and RF critically reviewed the manuscript; LML designed the study and wrote the manuscript.

The authors reported no conflicts of interest.

References

1. Skoda R, Prchal JT. Lessons from familial myeloproliferative disorders. Semin Hematol. 2005;42(4):266-73.
2. Wiestner A, Schlemper RJ, van der Maas AP, Skoda RC. An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythaemia. Nat Genet. 1998;18(1):49-52.
3. Ghilardi N, Wiestner A, Skoda RC. Thrombopoietin production is inhibited by a translational mechanism. Blood. 1998; 92(11):4023-30.
4. Kondo T, Okabe M, Sanada M, Kurosawa M, Suzuki S, Kobayashi M, et al. Familial essential thrombocythemia associated with one-base deletion in the 5'-untranslated region of the thrombopoietin gene. Blood. 1998;92(4):1091-6.
5. Ghilardi N, Wiestner A, Kikuchi M, Ohsaka A, Skoda RC. Hereditary thrombocythaemia in a Japanese family is caused by a novel point mutation in the thrombopoietin gene. Br J Haematol. 1999;107(2):310-6.
6. Ghilardi N, Skoda RC. A single-base deletion in the thrombopoietin (TPO) gene causes familial essential thrombocythemia through a mechanism of more efficient translation of TPO mRNA. Blood. 1999; 94(4):1480-2.
7. Cazzola M, Skoda RC. Translational pathophysiology: a novel molecular mechanism of human disease. Blood. 2000; 95(11):3280-8.
8. Liu K, Kralovics R, Rudzki Z, Grabowska B, Buser AS, Olcaydu D, et al. A de novo splice donor mutation in the thrombopoietin gene causes hereditary thrombocythemia in a Polish family. Haematologica. 2008;93(5): 706-14.
9. Dror Y, Blanchette VS. Essential thrombocythaemia in children. Br J Haematol. 1999;107(4):691-8.
10. Ding J, Komatsu H, Wakita A, Kato-Uranishi M, Ito M, Satoh A, et al. Familial essential thrombocythemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. Blood. 2004; 103(11): 4198-200.
11. Teofili L, Giona F, Martini M, Cenci T, Guidi F, Torti L, et al. Markers of myeloproliferative diseases in childhood polycythemia vera and essential thrombocythemia. J Clin Oncol. 2007;25(9):1048-53.
12. Teofili L, Giona F, Martini M, Cenci T, Guidi F, Torti L, et al. The revised WHO diagnostic criteria for Ph-negative myeloproliferative diseases are not appropriate for the diagnostic screening of childhood polycythemia vera and essential thrombocythemia. Blood. 2007;110(9):3384-6.
13. Teofili L, Foà R, Giona F, Larocca LM. Childhood polycythemia vera and essential thrombocythemia: does their pathogenesis overlap with that of adult patients? Haematologica. 2008;93(2):169-72.
14. Arellano-Rodrigo E, Alvarez-Larrán A, Reverter JC, Villamor N, Colomer D, Cervantes F. Increased platelet and leukocyte activation as contributing mechanisms for thrombosis in essential thrombocythemia and correlation with the JAK2 mutational status. Haematologica. 2006; 91(2):169-75.
15. Liu K, Martini M, Rocca B, Amos CI, Teofili L, Giona F, et al. Evidence for a founder effect of the MPL-S505N mutation in 8 Italian pedigrees with hereditary thrombocythemia. Haematologica. 2009;94(10): 1368-74.
16. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica. 2005;90(8):1128-32.
17. Falanga A, Marchetti M, Vignoli A, Balducci D, Russo L, Guerini V, et al. V617F JAK-2 mutation in patients with essential thrombocythemia: relation to platelet, granulocyte, and plasma hemostatic and inflammatory molecules. Exp Hematol. 2007;35(5): 702-11.
18. Wolanskyj AP, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. Mayo Clin Proc. 2006;81(2):159-66.
19. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006; 3(7):e270.
20. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood. 2006;108(10):3472-6.
21. Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, Barosi G, et al. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. Blood. 2008;112(3):844-7.
22. Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford D, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood. 2008;112(1):141-9.
23. Moliterno AR, Williams DM, Gutierrez-Alamillo LI, Salvatori R, Ingersoll RG, Spivak JL. Mpl Baltimore: a thrombopoietin receptor polymorphism associated with thrombocytosis. Proc Natl Acad Sci USA. 2004; 101(31):11444-7.
24. El-Harith el-HA, Roesl C, Ballmaier M, Germeshausen M, Frye-Boukhriss H, von Neuhoff N, et al. Familial thrombocytosis caused by the novel germ-line mutation p.Pro106Leu in the MPL gene. Br J Haematol. 2009 144(2):185-94.
25. Chaligné R, Tonetti C, Besancenot R, Roy L, Marty C, Mossuz P, et al. New mutations of MPL in primitive myelofibrosis: only the MPL W515 mutations promote a G1/S-phase transition. Leukemia. 2008;22(8): 1557-66.
26. Pesu M, Laurence A, Kishore N, Zwillich SH, Chan G, O'Shea JJ. Therapeutic targeting of Janus kinases. Immunol Rev. 2008; 223:132-42.