show that among PRV-1-positive ET patients, those with normal c-Mpl levels appear to be at highest risk of thromboembolic events. The results of this retrospective analysis must now be corroborated in a large prospective trial of newly diagnosed patients such as that being implemented by the MPD Research Consortium

Philipp S. Goerttler, Edith März,* Peter L. Johansson,° Björn Andreasson,° Jack Kutti,° Alison R. Moliterno,# Roberto Marchioli,¶ Jerry L. Spivak,# Heike L. Pahl* for the MPD Research Consortium*

**Department of Experimental Anaesthesiology, University Hospital Freiburg; °Haematology Section, Department of Medicine, Sahlgrenska University Hospital, University of Göteborg, Göteborg, Sweden; #Division of Hematology, Johns Hopkins University School of Medicine, Baltimore, MD, USA; @Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy*

Acknowledgments: the authors wish to thank Prof. Dr. K. Geiger for his continuing support. This work was supported by grants from the Else Kröner Fresenius-Stiftung and by the Alfried Krupp Förderpreis für Junge Hochschullehrer, both awarded to H.L.P.

Key words: essential thrombocythemia, complications, c-Mpl, PRV-1.

Correspondence: Heike L. Pahl, Ph. D., Center for Clinical Research, Breisacher Str. 66, 79106 Freiburg, Germany. Phone: international +49.761.2706340. Fax: international +49.7612706341. E-mail: heike.pahl@klinikum.uni-freiburg.de

References

1. Barbui T, Barosi G, Grossi A, Gugliotta L, Liberato LN, Marchetti M, et al. Practice guidelines for the therapy of essential thrombocythemia. A statement from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. Haematologica 2004;89:215-32.
2. Harrison CN, Gale RE, Machin SJ, Linch DC. A large proportion of patients with a diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombotic complications. Blood 1999; 93:417-24.
3. Andreasson B, Harrison C, Lindstedt G, Linch D, Kutti J. Monoclonal myelopoiesis and subnormal erythropoietin concentration are independent risk factors for thromboembolic complications in essential thrombocythemia. Blood 2003; 101:783.
4. Teofili L, Pierconti F, Di Febo A, Maggiano N, Vianelli N, Ascani S, et al. The expression pattern of c-mpl in megakaryocytes correlates with thrombotic risk in essential thrombocythemia. Blood 2002; 100:714-7.
5. Johansson P, Ricksten A, Wennstrom L, Palmqvist L, Kutti J, Andreasson B. Increased risk for vascular complications in PRV-1 positive patients with essential thrombocythaemia. Br J Haematol 2003;123:513-6.
6. Griesshammer M, Klippel S, Strunck E, Temerinac S, Mohr U, Heimpel H, et al. PRV-1 mRNA expression discriminates two types of Essential Thrombocythemia. Ann Hematol 2004; 83:364-70.
7. Kralovics R, Buser AS, Teo SS, Coers J, Tichelli A, Van Der Maas AP, et al. Comparison of molecular markers in a cohort of patients with chronic myeloproliferative disorders. Blood 2003;102:1869-71.
8. Moliterno AR, Hankins D, Spivak JL. Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. N Engl J Med 1998;338:572-80.
9. Klippel S, Strunck E, Temerinac S, Bench AJ, Meinhardt G, Mohr U, et al. Quantification of PRV-1 mRNA distinguishes Polycythemia vera from Secondary Erythrocytosis. Blood 2003; 102:3569-74.
10. Berk PD, Goldberg JD, Donovan PB, Fruchtman SM, Berlin NI, Wasserman LR. Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. Semin Hematol 1986;23:132-43.

Acute Myeloid Leukemia

Mutations of *PTPN11* are rare in adult myeloid malignancies

The *PTPN11* gene encodes the phospho-tyrosyine phosphatase protein SHP-2. Constitutional mutations of this gene are involved in Noonan's syndrome, a developmental disorder in which children have a predisposition to develop a myeloid disorder called juvenile myelomonocytic leukemia. Recently, studies have shown that somatic mutations of *PTPN11* can be found in children with myeloid malignancies. We evaluated the incidence of acquired mutation of *PTPN11* in 76 adults with acute or chronic myeloid malignancies and summarized our results together with others published recently.

haematologica 2005; 90:853-854
(*http://www.haematologica.org/journal/2005/6/853.html*)

The *PTPN11* gene (protein-tyrosine phosphatase, nonreceptor-type, 11), localized in 12q24, encodes a protein with tyrosine phosphatase activity called SHP-2 (Src homology 2 domain-containing phosphotyrosine phosphatase 2). The SHP-2 protein has 3 functional domains: the PTPase catalytic domain and two SH2 domains (src homology 2), one in the C-terminal part (C-SH2) and the other in the N-terminal part (N-SH2) of the protein. In its inactive form, the PTPase catalytic domain is masked by N-SH2; binding of phospho-tyrosyl peptides on N-SH2 induces a conformational change uncovering the catalytic site. SHP-2 is implicated in signal transduction pathways, particularly those induced by growth factors; activation of SHP-2 generally leads to activation of the RAS/RAF/ERK pathway.[1] The SHP-2 protein is strongly expressed in blood cells and is implicated in the response to KIT-ligand, interleukin-3, granulocyte-monocyte colony-stimulating factor and erythropoietin. The *PTPN11* alterations so far reported in human diseases were exclusively missense mutations. In Noonan's syndrome, *PTPN11* carries constitutional mutations preferentially localized in exon 3, the coding sequence for the domain of N-SH2 implicated in the inhibition of the PTPase catalytic site.[2] Children with this syndrome have a developmental disorder as well as a predisposition to develop juvenile myelomonocytic leukemia (JMML). Furthermore, in children, somatic mutations of *PTPN11* have been reported in 34% of non-syndromic JMML, 18.5% of refractory anemia with excess blasts (RAEB) in transformation, 4% of acute myeloid leukemia (AML), and 6.5% of acute lymphocytic leukemia (Table 1); 98% of those mutations were localized in exon 3.[3-7] In this study, we performed mutational analysis of exon 3 of the *PTPN11* gene to assess the incidence of mutations in adult myeloid disorders. We tested 84 patients: 38 had chronic myelomonocytic leukemia (CMML), 18 RAEB, 4 RAEB in transformation, 4 AML with monosomy 7, 12 AML post-myelodysplastic syndrome and, as a control, 8 non-syndromic JMML. After DNA extraction from bone marrow or blood samples, exon 3 of *PTPN11* was amplified as previously described.[3] Amplification products were purified and sequenced. As expected, 3 of the 8 cases of JMML tested carried mutations (G60R, D61V and G503A). Among the 76 remaining patients, only one had a *PTPN11* mutation (missense mutation: D61N). This patient had AML with monosomy

Table 1. Incidence of *PTPN11* mutations (mutated cases/studied cases).

Children						
JMML	*NS-JMML*	*RAEB-T*	*Other MDS*	*AML*	*ALL*	*Ref.*
1/4	–	–	–	–	–	Johan *et al.*[5]
21/62	5/5	5/27	0/23	1/24	–	Tartaglia *et al.*[3]
–	–	–	–	4/69	23/362	Tartaglia *et al.*.[4]
16/49	2/2	–	–	2/95	–	Loh *et al.*[6]
–	–	–	–	11/278	–	Loh *et al.*[7]
3/8	–	–	–	–	–	this study
33% (41/123)	100% (7/7)	18,5% (5/27)	0% (0/23)	4% (18/466)	6,5% (23/362)	Total

Adults					
RAEB-T	*Other MDS*	*AML*	*CMML*	*CML*	*Ref.*
0/2	0/70	1/64	0/35	–	Johan *et al.*[5]
2/7	–	4/38	1/4	0/11	Loh *et al.*[6]
0/15	0/26	0/49	0/1	–	Watkins *et al.*[8]
0/4	0/18	1/16	0/38	–	this study
7% (2/28)	0% (0/114)	3,5% (6/167)	1% (1/87)	0% (0/11)	Total

JMML: juvenile myelomonocytic leukemia; NS-JMML: Noonan's syndrome - JMML; RAEB-T: refractory anemia with excess of blasts in transformation; MDS: myelodysplasia; AML: acute myeloid leukemia; ALL: acute lymphoid leukemia; CMML: chronic myelomonocytic leukemia; CML: chronic myeloid leukemia; –: no case.

7. As previously described by Loh *et al.* in pediatric AML7, monosomy 7 is frequently associated with *PTPN11* mutations. Our results confirm that alteration of *PTPN11* is a rare event in the leukemogenesis of adult myeloid malignancies. Indeed, in published studies[5,6,8] (Table 1), only 1% of CMML, 3.5% of AML and 7% of RAEB-T had somatic mutations of *PTPN11*. Interestingly, *PTPN11* mutations occurred preferentially in leukemia with a monocytic component and/or in the presence of monosomy 7.[4,6,7] Moreover, as in Noonan's syndrome, all somatic mutations described were missense mutations and they were almost exclusively localized in exon 3, rarely in exon 13. As demonstrated by Tartaglia *et al.*,[2] such mutations modify the zone of interaction between N-SH2 and PTPase domains and release enzymatic activity of SHP-2.

Contrary to pediatric myeloid malignancies in which different mechanisms can induce activation of the RAS-MEK-ERK pathway (*RAS* mutations, *NF1* deletions or *PTPN11* mutations), in adult myeloid malignancies this activation only exceptionally involves *PTPN11* mutations and therefore seems related mostly to *RAS* mutations.

Hugues Leroy,° Hélène Cavé, Nathalie Philippe, Sabrina Pereira,* Pierre Fenaux,# Claude Preudhomme°*

*°Laboratoire d'Hématologie A, CHRU Lille, INSERM U524 Lille, France; *Laboratoire de Biochimie Génétique, Hôpital Robert Debré (AP-HP), Paris, France; #Service d'Hématologie Clinique, Hôpital Avicenne-Paris 13 University, France*

Funding: this work was supported by the "Fondation de France" and by the "Ligue contre le cancer, Comité Nord".

Key words: PTPN11, SHP2, somatic mutation, adult, myeloid malignancies.

Correspondence: Claude Preudhomme, MD, PhD, Laboratoire d'Hématologie A, Hôpital Calmette, C.H.R.U., Place de Verdun , 59000 Lille, France. Phone: international + 33.3. 20444783. Fax: international + 33.3.20445510. E-mail: cpreudhomme@chru-lille.fr

References

1. Cunnick JM, Meng S, Ren Y, Desponts C, Wang HG, Djeu JY, et al. Regulation of the mitogen-activated protein kinase signaling pathway by SHP2. J Biol Chem 2002;277:9498-504.
2. Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001;29:465-8.
3. Tartaglia M, Niemeyer CM, Fragale A, Song X, Buechner J, Jung A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet 2003;34:148-50.
4. Tartaglia M, Martinelli S, Cazzaniga G, Cordeddu V, Iavarone I, Spinelli M, et al. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. Blood 2004; 104:307-13.
5. Johan MF, Bowen DT, Frew ME, Goodeve AC, Wilson GA, Peake IR, et al. Mutations in PTPN11 are uncommon in adult myelodysplastic syndromes and acute myeloid leukaemia. Br J Haematol 2004;124:843-4.
6. Loh ML, Vattikuti S, Schubbert S, Reynolds MG, Carlson E, Lieuw KH, et al. Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood 2004;103:2325-31.
7. Loh ML, Reynolds MG, Vattikuti S, Gerbing RB, Alonzo TA, Carlson E, et al. PTPN11 mutations in pediatric patients with acute myeloid leukemia: results from the Children's Cancer Group. Leukemia 2004;18:1831-4.
8. Watkins F, Fidler C, Boultwood J, Wainscoat JS. Mutations in PTPN11 are rare in adult myelodysplastic syndromes and acute myeloid leukemia. Am J Hematol 2004;76:417.

Malignant Lymphomas

The relative levels of cyclin D1a and D1b alternative transcripts in mantle cell lymphoma may depend more on sample origin than on *CCND1* polymorphism

The relative levels of cyclin D1 (*CCND1*) (a) and (b) transcripts were determined by real-time reverse transcription polymerase chain reaction (RT-PCR) and found to vary according to the tissue origin in both control and tumor samples. A five-fold overexpression of both isoforms was observed in 28/38 cases of mantle cell lymphoma (MCL) and of only one isoform in 10/38 MCL. No correlation was observed between expression of cyclin D1 isoforms and *CCND1* genotype at position 870.

haematologica 2005; 90:854-856
(*http://www.haematologica.org/journal/2005/6/854.html*)