Journal of Genetics (2022)101:11
https://doi.org/10.1007/s12041-021-01354-7

RESEARCH NOTE

Genetic alterations in patients with chronic leucocytosis and persistent thrombocytosis

NAOKI MORI[1,2]*, MARI OHWASHI-MIYAZAKI[1], KENTARO YOSHINAGA[1], TOSHIE OGASAWARA[2], SHOKO MARSHALL[2], MASAYUKI SHISEKI[1], HIROSHI SAKURA[2] and JUNJI TANAKA[1]

[1]*Department of Hematology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan*
[2]*Department of Internal Medicine, Tokyo Women's Medical University, Medical Center East, 2-1-10 Nishiogu, Arakawa-ku, Tokyo 116-8567, Japan*
*For correspondence. E-mail: moridh1@twmu.ac.jp.

Received 5 December 2020; revised 15 October 2021; accepted 18 October 2021

Abstract. To elucidate the relevance of genetic alterations, we analysed 17 genes known to be involved in haematological neoplasms in patients with chronic leucocytosis and patients with persistent thrombocytosis. Mutations of the *JAK2*, *SETBP1* and *ASXL1* genes were found in 1/13, 1/13, and 2/13 patients with leucocytosis, respectively. Mutations of the *JAK2*, *CALR*, *SETBP1* and *ASXL1* genes were found in 1/5, 1/5, 1/5 and 2/5 patients with thrombocytosis, respectively. One leucocytosis patient with a *JAK2* V617F mutation developed polycythaemia vera. Another leucocytosis patient developed Philadelphia chromosome-negative chronic myeloid leukaemia (Ph(−) CML) accompanied by t(9;12)(q34.1;p13.?3) (Mori *et al*. 2016). Another leucocytosis patient with mutations of the *SETBP1* and *ASXL1* genes progressed to blast crisis of Ph(−) CML accompanied by i(17)(q10). Chronic leucocytosis patients who had genetic alterations tended to develop haematological neoplasms, while thrombocytosis unexpectedly resolved in two persistent thrombocytosis patients with genetic alterations.

Keywords. chronic idiopathic leucocytosis; thrombocytosis; mutation; haematological malignancies.

Introduction

Chronic idiopathic leucocytosis (CIL) is a rare persistent neutrophilia with no specific cause (Ward and Reinhard 1971; Weir *et al.* 2011). Although leucocytosis is a frequent clinical problem, most patients with leucocytosis have infection, inflammation or injury; therefore, it is regarded as reactive leucocytosis (Ward and Reinhard 1971; Weir *et al.* 2011). Use of medication (steroids, beta agonists and lithium) and splenectomy are also known in the aetiology of leucocytosis. Persistent thrombocytosis is also caused by infection, iron deficiency, and splenectomy, but unexplained persistent thrombocytosis is rare. In addition to reactive leucocytosis and thrombocytosis, myeloproliferative neoplasms (MPN) are an important cause of chronic leucocytosis and persistent thrombocytosis.

Frequent V617F mutations on the *JAK2* gene have been reported in patients with MPN including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) (Baxter *et al.* 2005; James *et al.* 2005; Kralovics *et al.* 2005; Levine *et al.* 2005). We also found *JAK2* V617F mutations in one of 11 patients with idiopathic erythrocytosis (Yoshinaga *et al.* 2008). Mutations of the *CSF3R*, *SETBP1* and *ETNK1* genes have been found in chronic neutrophilic leukaemia and atypical chronic myeloid leukaemia (aCML) (Maxson *et al.* 2013; Piazza *et al.* 2013; Gambacorti-Passerini *et al.* 2015). Further, an autosomal mutation was found in the *CSF3R* gene in a family with chronic neutrophilia (Plo *et al.* 2009). However, mutation spectrum associated with CIL and unexplained persistent thrombocytosis have not been well studied. There are reports in the literature proposing leucocytosis and the *JAK2* V617F mutation as disease-related prognostic factors in thrombocytosis (Vannucchi and Barbui 2007). To elucidate the relevance of genetic alterations, we analysed 17 genes known to be involved in haematological neoplasms in patients with chronic leucocytosis and patients with persistent thrombocytosis.

Published online: 18 January 2022

Table 1. Characteristics of chronic leucocytosis and persistent thrombocytosis patients.

Case	Age	Sex	WBC $\times 10^9$/L	RBC $\times 10^{12}$/L	Hb g/dL	Platelets $\times 10^9$/L	Neutrophils %	BM cellularity	Progression	Complication	Smoking	BMI	Karyotype	Mutation
CL1	47	Female	12.5	4.9	15.1	293	77.0	Slightly hypo	Resolved	Gastric cancer, RA	−	20.5	46,XX	
CL2	60	Female	14.1	3.3	10.6	328	82.2	ND			+	19.5	ND	
CL3	41	Male	11.0	4.9	16.0	264	60.0	Slightly hypo		Lymphoma	+	31.7	46,XY	
CL4	58	Male	13.6	4.4	13.7	211	85.0*	Slightly hypo		Kidney cancer	−	24.4	46,XY	
CL6	81	Female	2.7	5.6	13.6	479	75.0	Normo	PV		−	NA	46,XX	*JAK2*
CL7	77	Male	14.7	5.0	13.2	246	79.5*	Hyper	Ph(−) CML		+	19.4	46,XY → 46,XY,t(9;12)(q34.1;p13.?3)	*ETV6/ABL1* (Mori *et al.* 2016)
CL8	59	Female	12.3	5.0	15.1	375	70.5	Normo			+	25.6	46,XX	
CL9	65	Male	10.8	5.5	16.3	355	53.6	Normo		Lymphoma	−	26.6	46,XY	
CL11	69	Male	13.4	5.5	17.2	336	53.0	ND			+	NA	ND	
CL12	73	Female	11.6	4.9	14.1	140	73.0	NA		ITP	+	19.8	46,XX	
CL14	58	Female	11.1	5.0	15.0	305	61.0	ND		Dissection	+	17.7	ND	
CL15	78	Female	12.8	4.5	13.7	126	77.0	NA		ITP	−	22.1	NA	*ASXL1*
CL16	84	Male	20.7	4.4	11.8	373	84.0*	Hyper		Ph(−) CML	+	18.6	46,XY→46,XY,i(17)(q10)	*SETBP1*, *ASXL1*
PT1	63	Female	1.7	4.1	12.9	424	26.5*	Slightly hypo	Resolved	History of AA	−	NA	46,XX,dup(1)(q21q32)[5]/46,XX[13]	
PT2	71	Female	6.6	4.7	14.3	677	55.0	Hypo	Resolved		−	24.4	46,XX	*CALR*, *ASXL1*
PT3	37	Male	7.1	5.1	15.3	1099	72.5	Hyper			+	20.8	46,XY,del (7)(q21.?3q31.3?2)	*SETBP1*
PT4	74	Male	942	3.2	9.3	2611	71.6	Hyper			NA	17.0	46,XY	*JAK2*, *ASXL1*
PT5	29	Female	5.5	4.2	12.6	514	65.0	NA			NA	NA	46,XX	

WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; BM, bone marrow; BMI, body mass index; RA, rheumatoid arthritis; ND, not done; PV, polycythemia vera; Ph(−) CML, Philadelphia chromosome-negative chronic myeloid leukaemia; NA, not available; ITP, idiopathic thrombocytopenic purpura; AA, aplastic anaemia. *With myelocytes.

Materials and methods

Patients

Thirteen patients with chronic leucocytosis (CL1-16) and five patients with persistent thrombocytosis (PT1-5) were retrospectively analysed in this study (table 1). Patients who satisfied the following criteria were included in the study: leucocytosis (white blood cell count more than two standard deviations above the mean, or a value greater than 11.0 × 10^9/L, predominantly neutrophils), the absence of any apparent cause of leucocytosis, and documentation of the leucocytosis over a prolonged period of time (Ward and Reinhard 1971). The period of observation was one year or longer in 12 of the 13 patients. Patients with unexplained persistent thrombocytosis (greater than 400 × 10^9/L) and haematological diseases showing thrombocytosis who did not satisfy the criteria of ET (World Health Organization Classification 2008) were also analysed.

Samples

Neutrophils or mononuclear cells were collected after obtaining written informed consent. Genomic DNA was extracted using a QIAamp DNA blood mini kit (Qiagen, Valencia, USA). Total RNA was extracted with TRIZOL (Invitrogen, Carlsbad, USA) in nine patients with leucocytosis. This research complied with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study protocol was approved by the ethical committee of Tokyo Women's Medical University.

AS-PCR

Allele-specific polymerase chain reaction (AS-PCR) was performed to screen *JAK2* V617F mutation (Baxter *et al.* 2005).

Direct sequencing

Sequencing was performed in both directions with a 3730xL DNA Analyzer (Life Technologies, Carlsbad, USA). Mutations within hot spots were analysed by direct sequencing in *CSF3R*, *JAK2*, *CALR*, *SETBP1*, *ETNK1*, *CBL*, *TET2*, *ASXL1*, *EZH2*, *IDH1/IDH2*, *DNMT3A*, *U2AF1*, *MPL* and *CEBPA* genes.

ETV6/ABL1 and BCR/ABL1 fusions

Reverse transcriptase PCR (RT-PCR) analysis was performed between *ETV6* and *ABL1* genes in nine patients with

chronic leucocytosis (Mori *et al.* 2016). The *BCR/ABL1* fusion gene was analysed by RT-PCR or fluorescence *in situ* hybridization (FISH) in eight patients with leucocytosis and/or thrombocytosis.

Results

Chronic leucocytosis patients

One patient had an elevated red blood cell (RBC) count and she developed PV after administration of iron (CL6, table 1). Another patient had leucocytosis for 20 years consistent with CIL (CL7). Another patient with a normal karyotype (CL16) progressed to blast crisis of Ph(−) CML accompanied by i(17)(q10). Of the remaining 10 patients with possible CIL, the disease resolved in one of them after diagnosis and resection of an adrenal gland adenoma (CL1), and nine had stable disease. Eight of the 13 patients were active cigarette smokers. Obesity (body mass index >26) was observed in three of 11 patients.

Persistent thrombocytosis patients

One patient had a past history of aplastic anaemia, and dup(1)(q21q32) was recently detected (PT1, table 1). Another patient was initially suspected as having ET (PT2). However, thrombocytosis unexpectedly resolved in both patients. Bone marrow (BM) examination showed del (7)(q21.?3q31.3?2) in PT3, and myelodysplastic syndrome/MPN was very likely. This patient eventually progressed to acute myeloid leukaemia. PT4 had marked leucocytosis and thrombocytosis which were reminiscent of CML, but BM examination and a lack of t(9;22)(q34;q11) or *BCR/ABL1* fusions led to a diagnosis of pre-PMF.

Mutations in chronic leucocytosis patients

Mutations of the *JAK2*, *SETBP1* and *ASXL1* genes were found in one, one, and two of the 13 patients with chronic leucocytosis, respectively (figure 1; table 1). The patient with an elevated RBC count had a mutation c.1849G>T (p.Val617Phe) of the *JAK2* gene (*JAK2* V617F mutation) and developed PV (CL6, figure 1a). We previously reported that a novel translocation resulted in the *ETV6-ABL1* fusion gene in a patient with Ph(−) CML (Mori *et al.* 2016). The Ph(−) CML stage eventually progressed from the CIL stage in CL7. CL16 had a missense mutation c.2609G>A (p.Gly870Asp) of the *SETBP1* gene (exon 3) and c.1934dupG (p.Gly646TrpfsTer12) in the *ASXL1* gene (exon 12) (figure 1, b&c). In contrast, another patient with a missense mutation c.1954G>A (p.Gly652Ser) in the *ASXL1* gene (exon 12) had stable disease (CL15, figure 1c).

Mutations in persistent thrombocytosis patients

Mutations of the *JAK2*, *CALR*, *SETBP1* and *ASXL1* genes were found in one, one, one and two of the five patients with persistent thrombocytosis, respectively (figure 1; table 1). Mutations were not found in PT1, while PT2 had c.1092-1143del (p.Leu367ThrfsTer46, type 1 mutation) in the *CALR* gene and c.2468delT (p.Leu823Ter) in the *ASXL1* gene (exon 14) (figure 1, d&e). PT3 had a missense mutation c.2602G>A (p.Asp868Asn) of the *SETBP1* gene (exon 3) (figure 1f), but not the *JAK2*, *CALR* or *MPL* genes. The mutation of the *SETBP1* gene in PT3 was identical to those reported in Schinzel–Giedion syndrome, but he did not have any features of that syndrome (Hoischen *et al.* 2010; Piazza *et al.* 2013). PT4 had the *JAK2* V617F mutation and c.3085dupG (p.Val1029GlyfsTer5) in the *ASXL1* gene (exon 14) (figure 1e).

ETV6/ABL1 and BCR/ABL1 fusions

No *ETV6/ABL1* fusions were found by RT-PCR analysis in the nine patients with chronic leucocytosis except CL7 (figure 1g) (Mori *et al.* 2016). The *BCR/ABL1* fusion gene was not detected by FISH or RT-PCR in the eight patients with leucocytosis and/or thrombocytosis (figure 1, h&i).

Discussion

CIL is considered a rare clinical condition. Previous studies showed smoking and obesity were associated with CIL (Ward and Reinhard 1971; Herishanu *et al.* 2006; Weir *et al.* 2011). A previous study indicated that no haematological neoplasms other than CML were associated with CIL. Of the patients with MPN reviewed at the time of their initial diagnosis, only CML occasionally presented with a clinical picture consistent with CIL (Weir *et al.* 2011). Our study showed that patients with PV and Ph(−) CML in the early stage may present with a clinical picture consistent with CIL.

Previous studies recommended that persistent neutrophilia be evaluated by history, complete blood cell counts, and review of blood smears, while indications for additional tests were based on abnormalities of RBCs, platelets, monocytes, eosinophils, basophils, myelocytes, FISH or PCR for *BCR-ABL1*, and BM examination (Weir *et al.* 2011).

This study adds a mutation spectrum in CIL and persistent thrombocytosis, and most of these have not been studied in patients with these conditions. *BCR/ABL1* fusions were previously reported in some patients with chronic leucocytosis (Weir *et al.* 2011), whereas *ETV6-ABL1* fusions have not been studied. Mutations of the *JAK2*, *ASXL1* or *SETBP1* gene have not been reported in CIL or persistent thrombocytosis. Mutations of the *CALR* gene were reported in ET and PMF (Nangalia *et al.* 2013), but not in unexplained persistent thrombocytosis.

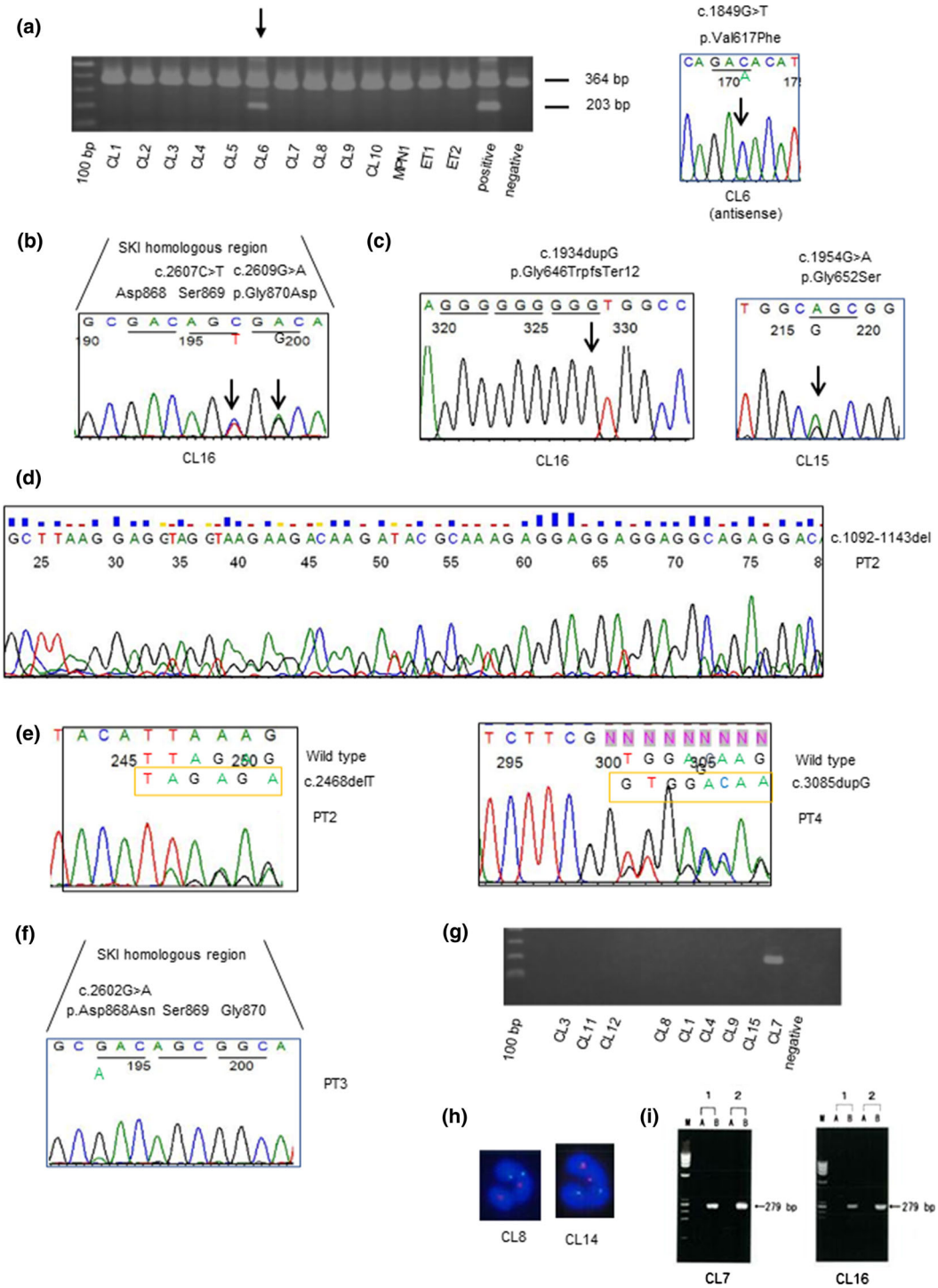

Figure 1. Genetic alterations in chronic leucocytosis patients and persistent thrombocytosis patients. (a) AS-PCR of the *JAK2* gene showed mutations in CL6. Sequence analysis revealed a mutation c.1849G>T (p.Val617Phe) of the *JAK2* gene in CL6 (antisense). (b) Direct sequencing showed a missense mutation c.2609G>A (p.Gly870Asp) of the *SETBP1* gene (exon 3) in CL16. (c) Subcloning and sequencing showed c.1934dupG (p.Gly646TrpfsTer12) in the *ASXL1* gene (exon 12) in CL16. Direct sequencing showed a missense mutation c.1954G>A (p.Gly652Ser) of the *ASXL1* gene (exon 12) in CL15. (d) Direct sequencing of the *CALR* gene showed c.1092-1143del (p.Leu367ThrfsTer46, type 1 mutation) in PT2. (e) Direct sequencing showed c.2468delT (p.Leu823Ter) in the *ASXL1* gene (exon 14) in PT2. Direct sequencing showed c.3085dupG (p.Val1029GlyfsTer5) in the *ASXL1* gene (exon 14) in PT4. (f) Direct sequencing showed a missense mutation c.2602G>A (p.Asp868Asn) of the *SETBP1* gene (exon 3) in PT3. (g) RT-PCR analysis of *ETV6/ABL1* fusions in chronic leucocytosis patients. No fusions were found except in CL7 (Mori *et al.* 2016). (h) FISH analysis was performed using the LSI BCR/ABL ES Dual Color Translocation Probe Kit (Vysis, Downers Grove, USA). No fusion signal was observed between the *BCR* (green) and *ABL1* (orange) genes in CL8 and CL14. (i) RT-PCR analysis of *BCR/ABL1* fusions in chronic leucocytosis patients. 1, negative control; 2, patient; M, marker; A, *BCR-ABL1*; B, *β-actin*. No fusions were found in CL7 and CL16.

Although mutations associated with CIL were not fully elucidated in this small study, chronic leucocytosis patients who had mutations of the *JAK2*, *ASXL1* or *SETBP1* genes and cytogenetic abnormalities tended to develop haematological neoplasms.

Persistent thrombocytosis is thought to be less common than chronic leucocytosis. In the present study, cytogenetic abnormalities had recently been detected in PT1 and PT2 was compatible with ET except for the subsequent normalization of platelet counts. Moreover, although PT5 did not have any evidence of clonality, PT5 may have had triple negative ET. Therefore, the presence of persistent 'idiopathic' thrombocytosis is uncertain in comparison with CIL.

Recent studies revealed clonal haematopoiesis in elderly persons (Xie *et al.* 2014; Genovese *et al.* 2014; Jaiswal *et al.* 2014). Since mutations in the *ASXL1* and *JAK2* genes have been reported to occur with ageing, the patient who had *ASXL1* mutation but showed a stable leucocyte count may have had clonal haematopoiesis (CL15). Further prospective studies will elucidate the significance of these mutations in such patients.

Acknowledgement

This work was in part supported by JSPS KAKENHI grant number JP19K07447.

Author contributions

NM and KY designed the study. NM, KY, TO, SM and MS recruited patients and collected data. NM and MO-M performed the experiments and analysed the data. NM drafted the manuscript and MO-M, KY, TO, SM, MS, HS and JT critically revised the manuscript.

References

Baxter E. J., Scott L. M., Campbell P. J., East C., Fourouclas N., Swanton S. *et al*. 2005 Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* **365**, 1054–1061.

Gambacorti-Passerini C. B., Donadoni C., Parmiani A., Pirola A., Redaelli S., Signore G. *et al*. 2015 Recurrent ETNK1 mutations in atypical chronic myeloid leukemia. *Blood* **125**, 499–503.

Genovese G., Kähler A. K., Handsaker R. E., Lindberg J., Rose S. A., Bakhoum S. F. *et al*. 2014 Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N. Engl. J. Med.* **371**, 2477–2487.

Herishanu Y., Rogowski O., Polliack A. and Marilus R. 2006 Leukocytosis in obese individuals: possible link in patients with unexplained persistent neutrophilia. *Eur. J. Haematol.* **76**, 516–520.

Hoischen A., van Bon B. W., Gilissen C., Arts P., van Lier B., Steehouwer M. *et al*. 2010 De novo mutations of SETBP cause Schinzel-Giedion syndrome. *Nat. Genet.* **42**, 483–485.

Jaiswal S., Fontanillas P., Flannick J., Manning A., Grauman P. V., Mar B. G. *et al*. 2014 Age-related clonal hematopoiesis associated with adverse outcome. *N. Engl. J. Med.* **371**, 2488–2498.

James C., Ugo V., Le Couédic J. P., Staerk J., Delhommeau F., Lacout C. *et al*. 2005 A unique clonal JAK2 mutation leading to constitutive signaling causes polycythaemia vera. *Nature* **434**, 1144–1148.

Kralovics R., Passamonti F., Buser A. S., Teo S.-S., Tiedt R., Passweg J. R. *et al*. 2005 A gain-of function mutation of JAK2 in myeloproliferative disorders. *N. Engl. J. Med.* **352**, 1779–1790.

Levine R. L., Wadleigh M., Cools J., Ebert B. L., Wernig G., Huntly B. J. P. *et al*. 2005 Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* **7**, 387–397.

Maxson J. E., Gotlib J., Pollyea D. A., Fleischman A. G., Agarwal A., Eide C. A. *et al*. 2013 Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. *N. Engl. J. Med.* **368**, 1781–1790.

Mori N., Ohwashi-Miyazaki M., Okada M., Yoshinaga K., Shiseki M. and Tanaka J. 2016 Translocation (9;12)(q34.1;p13.?3) resulted in ETV6-ABL1 fusion in a patient with Philadelphia chromosome-negative chronic myelogenous leukemia. *Acta Haematol.* **136**, 240–243.

Nangalia J., Massie C. E., Baxter E. J., Nice F. L., Gundem G., Wedge D. C. *et al*. 2013 Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N. Engl. J. Med.* **369**, 2391–2405.

Piazza R., Valletta S., Winkelmann N., Redaelli S., Spinelli R., Pirola A. *et al*. 2013 Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. *Nat. Genet.* **45**, 18–24.

Plo I., Zhang Y., Le Couédic J. P., Nakatake M., Boulet J. M., Itaya M. *et al*. 2009 An activating mutation in the CSF3R gene induces a hereditary chronic neutrophilia. *J. Exp. Med.* **206**, 1701–1707.

Vannucchi A. M. and Barbui T. 2007 Thrombocytosis and thrombosis. *Hematology Am. Soc. Hematol. Educ. Program* **2007**, 363–370.

Ward H. N. and Reinhard E. H. 1971 Chronic idiopathic leukocytosis. *Ann. Intern. Med.* **75**, 193–198.

Weir A. B., Lewis J. B. Jr. and Arteta-Bulos R. 2011 Chronic idiopathic neutrophilia: experience and recommendations. *South Med. J.* **104**, 499–504.

Xie M., Lu C., Wang J., McLellan M. D., Johnson K. J., Wendl M. C. *et al*. 2014 Age-related cancer mutations associated with clonal hematopoietic expansion. *Nat. Med.* **20**, 1472–1478.

Yoshinaga K., Mori N., Wang Y. H., Tomita K., Shiseki M. and Motoji T. 2008 JAK2 V617F mutation is rare in idiopathic erythrocytosis: a difference from polycythemia vera. *Int. J. Hematol.* **88**, 82–87.

Corresponding editor: B. K. Thelma