Published in final edited form as:
Leuk Res. 2010 August ; 34(8): 969–973. doi:10.1016/j.leukres.2010.02.033.

Spectrum of mutations in RARS-T patients includes *TET2* and *ASXL1* mutations

Hadrian Szpurka[1], **Anna M. Jankowska**[1], **Hideki Makishima**[1], **Juraj Bodo**[2], **Nelli Bejanyan**[1], **Eric D. Hsi**[2], **Mikkael A. Sekeres**[3], and **Jaroslaw P. Maciejewski**[1]

[1]Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

[2]Department of Clinical Pathology, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

[3]Hematologic Oncology and Blood Disorders; Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

Abstract

While a majority of patients with refractory anemia with ring sideroblasts and thrombocytosis harbor JAK2V617F and rarely MPLW515L, JAK2/MPL-negative cases constitute a diagnostic problem. 23 RARS-T cases were investigated applying immunohistochemical phospho-STAT5, sequencing and SNP-A-based karyotyping. Based on the association of *TET2/ASXL1* mutations with MDS/MPN we studied molecular pattern of these genes. Two patients harbored *ASXL1* and another 2 *TET2* mutations. Phospho-STAT5 activation was present in one mutated *TET2* and *ASXL1* case. JAK2V617F/MPLW515L mutations were absent in *TET2/ASXL1* mutants, indicating that similar clinical phenotype can be produced by various MPN-associated mutations and that additional unifying lesions may be present in RARS-T.

Keywords

RARS-T; *TET2*; *ASXL1*; JAK2 V617F; MPL W515L; STAT5

Introduction

Refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) has been considered a provisional subtype within the diagnostic entity of myelodysplastic/myeloproliferative neoplasms (MDS/MPN). As the JAK2 V617F mutation is present in a significant proportion of RARS-T patients(1-7), many investigators consider this entity to be more closely related to

Corresponding Author: Jaroslaw P. Maciejewski, M.D., Ph.D; Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, R40; 9500 Euclid Avenue; Cleveland, OH, 44195, USA, Tel: 216-445-5962, Fax: 216-636-2498. maciejj@ccf.org.

Authors' contributions

HS designed and performed molecular assays, wrote the manuscript; AMJ performed molecular assays and analyzed results; HM performed molecular assays, JB performed immunohistochemistry, NB classification of patients and database; EDH reviewed pathologic specimens, interpretation of results, MAS classified and identified patients, corrected manuscript; JPM conceived the idea, designed the trial.

Conflict of interest: None.

MPN, as reflected in the newest version of the 2008 revised WHO classification of myeloid neoplasms [8]. Some cases of RARS-T revealed the MPL W515L mutation typical for classical MPN [9;10]. In addition, we have previously postulated that an aberrant STAT5 phosphorylation pattern may be a characteristic feature of true RARS-T, while negative cases may result from an unrelated etiology [11]. Consequently, a significant minority of patients with RARS-T that does not harbor *JAK2/MPL* mutations may simply represent RARS cases with other thrombocytosis-inducing conditions, or be due to unrelated molecular lesions producing a similar phenotype. Conversely, morphologically indistinguishable phenotypes may be due to different gene mutations functionally connected by convergent networks.

Using single nucleotide polymorphism (SNP) arrays we have recently observed a frequent area of somatic uniparental disomy (UPD) at 4q24, most commonly encountered in patients with chronic myelomonocytic leukemia (CMML), mixed MDS/MPN, some typical MDS, secondary acute myeloid leukemia (sAML) and one report on RARS-T [12-18]. Overlapping microdeletions on 4q24 pointed towards the *TET2* gene, in which mutations were identified by us and other groups in different myeloid malignancies, most significantly MPN and MDS/MPN [15;16;19]. Recent studies have also identified mutations of *ASXL1* (additional sex combs like 1) gene in MDS and MPN [20;21] that is located in the chromosomal region 20q11. *ASXL1*, along with *TET2*, is a member of WNT pathway and possibly regulates histone modifications [21]. Based on the association with MDS/MPN, and the established correlation of RARS-T with JAK2 V617F and MPL W515L mutations, we therefore sought to evaluate the mutational status of *TET2* and *ASXL1* in RARS-T patients.

Marerials and Methods

Patients

Bone marrow and blood samples were collected from 23 patients with RARS-T. Informed consent was obtained according to protocols approved by the IRB of the Cleveland Clinic. The RARS-T patient characteristics are presented in Table 1. Metaphase cytogenetic (MC) analysis was performed on marrow aspirates according to standard methods.

DNA extraction

The Genomic DNA Purification Kit (Gentra Systems, Inc., MN) was used for DNA isolation according to manufacturer instruction.

TET2 and *ASXL1* sequencing. JAK2 V617F and MPL W515L mutation detection by ARMS-PCR

The status of the JAK2 V617F and MPL W515L mutations were determined by a DNA tetra-primer ARMS assay as previously described [11]. Genomic DNA was used for *TET2* sequencing as previously described [15]. *ASXL1* primers will be described elsewhere. Briefly, PCR primers were designed to amplify and sequence all coding region of *TET2* and *ASXL1*. When needed, multiple set of primers overlapping by 100bp was used to ensure complete coverage. Sequencing was performed by standard technique using ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA).

Immunohistochemistry

Staining was performed as previously described [1] using mouse monoclonal anti-phospho-STAT5a/b (Y694/99; Advantex BioReagents LLP, Conroe, TX) at 1:500 dilution. Phospho-STAT5 staining was defined as positive if more than 10% of the megakaryocytes examined showed nuclear staining [1].

SNP-A analysis

Analysis was performed as previously described [11]. Signal intensity and SNP genotypes were determined using GTYPE software (v.4.0). Copy number and loss of heterozygosity (LOH) were investigated using Copy Number Analyzer for Affymetrix GeneChip Mapping (CNAGv.2.0). Somatic changes were identified by analyzing paired sorted CD3+ lymphocytes.

Results and Discussion

The previously reported cohort of 20 patients with RARS-T [11] has been extended to newer cases and all patients were analyzed for the presence of *ASXL1* and *TET2* mutations. The extended group of 23 patients with phenotypic features consistent with RARS-T (Tab. 1) was subjected to MC and SNP-A analysis. All patients showed the presence of ring sideroblasts (>15%), some degree of reticulin fibrosis and varying degrees of thrombocytosis (>450*10^9/l). SNP-A facilitated detection of previously cryptic lesions: 13/22 patients showed an abnormal SNP-A-based karyotype (only 4 of these defects were also detected by MC). The new lesions seen by SNP-A included various deletions of chromosome 2p and 5q, as well as areas of UPD, including 1p, 2p, 3q, 6p, 8p, and 10p (Tab. 1). The presence of UPD9p and UPD1p suggests that homozygous mutations are involved in the progression of the disease [11;22;23]; patients having the JAK2 V617F mutation in a homozygous constellation can be characterized by a more aggressive types of MPN [24], and an increased platelet level may be preferentially associated with the W515L [25]. None of the patients showed a somatic LOH at 4q24 or 20q11, suggesting that biallelic *TET2* or *ASXL1* mutations were not involved in the pathogenesis of RARS-T. Simultaneously, lack of UPD11q suggested that *CBL* mutations were absent.

Mutational analysis showed that JAK2 V617F mutations were present in 8 of 23, and MPL W515L mutations in 3 of 23 patients. One of the patients was double positive for JAK2 V617F/MPL W515L (Tab. 1, Fig. 1). The abnormal activation of STAT5 always correlated with the presence of the upstream mutations. However, 4 patients demonstrated abnormal megakaryocytic STAT5 phosphorylation, despite the absence of both JAK2 V617F and MPL W515L mutations. Within this group, a monoallelic *TET2* mutation, delC 1480Sfs and monoallelic ASXL1 L1395V were identified. Additionally, we also found a group of 7 patients without JAK2 V617F or MPL W515L mutations, and also without association of aberrant phospho-STAT5 staining typical for other cases. One of these patients had a monoallelic TET2 V1718L mutation. Interestingly, another patient harbored a novel ASXL1 Q1102D mutation, but the phospho-STAT5 staining was not available. These findings indicate involvement of both *TET2* and *ASXL1* mutations in RARS-T pathogenesis, and also suggest that RARS-T cases with MPN-associated mutations may not show obligatory phospho-STAT5 staining. Of note is that Pt. 21 and 22 had increased staining of granulocytic and erythroid precursors.

We also performed phospho-STAT3 staining including patients with activated STAT5 (Pt. 1, 7, 8, 10,) and those without activation (Pt. 19, 21, 22). Our results show that despite the pSTAT5 status, STAT3 pathway remains inactive in RARS-T patients. Furthermore, we performed *CBL* ring finger domain mutational screening in the remaining cohort of patients that showed both wild type JAK2 V617F, MPL W515L, *TET2, ASXL1* and either normal or abnormal STAT5 phosphorylation. No mutation was found, and this finding was consistent with the fact that most mutated *CBL* cases are biallelic and associated with UPD11q; in the case of our RARS-T cohort UPD11q was not detected. The majority of patients were characterized by lack of splenomegaly, decreased white blood cell (WBC) counts, increased thrombocytosis, and a normal karyotype, although SNP-A analysis revealed additional lesions including gain of chromosome 2p, 11p and 21q, as well as deletion of chromosome X. We are not able to explain the pathogenesis of RARS-T in these patients. Theoretically, it is possible that instead of inactivating *TET2/ASXL1* mutations, in some patients, expression of these genes is impaired.

However, we were not able to detect any significant methylation of C residues in *TET2* [15] or *ASXL1* promoter (data not shown). Because of the lack of material we were unable to formally check mRNA levels of these genes; in CMML however wt TET2 mRNA levels were not decreased.

The *TET*2 gene comprises 11 exons [26;27] and is widely expressed in myeloid cells [15]. Along with the other members of the family (TET1 and TET3), TET2 contains two highly conserved regions. *TET2* gene mutations were recently identified in different myeloid malignancies, but their impact on prognosis remains unresolved, and the mechanisms by which *TET2* leads to transformation remain unclear. Based on its homology to *TET1*, it is possible that *TET2* may play a role in epigenetic regulation. *TET1* has been shown to be involved in the mixed-lineage leukemia (*MLL*) gene in the chromosomal translocation t(10;11)(p12;q23) (26;27), as well as in conversion of methylcytosine to hydroxymethylcytosine, thereby preventing maintenance hypermethylation, as implicated by a recent report [28]. Mutations in *TET2* could, by this mechanism, lead to inactivation of a specific tumor suppressor gene and activation of pro-proliferative pathways, resulting in activation of STAT5 in some instances. Conversely, the presence of a DSBH-2OG-depenedent dioxygenase domain in TET2 may indicate other, not yet identified, function. Another member of the WNT family, *ASXL1*, encodes a poorly characterized protein regulating chromatin remodeling [21], contains a C-terminal PHD (plant homeodomain) finger and belong to the polycomb and mixed lineage leukemia/trithorax chromatin modifier complexes. A mutation in *ASXL1* gene could possibly truncate the protein, removing its PHD domain and thus compromising the function of the associated chromatin modifiers.

TET2 mutations were identified as a pre-JAK2 mutation in 14% of JAK2 V617F positive MPN patients [14]. When hematopoietic progenitors from patients who had MPN features were analyzed, both mutations were present in clones containing lymphoid and myeloid cells, and the JAK2 V617F mutation was observed in the presence of the *TET2* mutation. In addition, recent studies of *ASXL1* in myeloid malignancies suggest that acquisition of *ASXL1* mutations might precede the acquisition of *JAK2* in some MPN patients, similar to *TET2* [20;21]. However, in our cohort of patients with RARS-T, we did not find patients with concurrent mutations affecting both *JAK2* and *TET2* or *ASXL1*. Moreover, patients that carried *TET2* or *ASXL1* mutations were negative for MPL W515L. Based on identification of *TET2* and *ASXL1* at the time of diagnosis, we hypothesize that *TET2* and *ASXL1* mutations constitute an early marker of RARS-T evolution that precedes either *JAK2 or MPL* mutations typically occurring in the later stages of disease In addition, lack of a clear relationship between activated STAT5 signaling and *TET2/ASXL1* mutations could suggest that *TET2/ASXL1* are not the causative mutations that activate signaling, and argues more for the *TET2/ASXL1* serving as a cooperating allele in a pathway parallel to JAK2/STAT5. This however needs to be evidenced by further studies.

The majority of RARS-T patients harbor JAK2 V617F. However the number of positive JAK2 V617F mutants has dropped in our cohort of RARS-T patients. This might be a consequence of the latest WHO criteria and classification of RARS-T [29] where the platelet count threshold was decreased to $>450*10^9$/l. To our knowledge, *JAK2* mutation analysis has been reported in 45 patients with RARS-T [30]. Overall, among 33 RARS patients with platelet counts between $400*10^9$/l and $600*10^9$/l, only 3 (9%) patients were positive for JAK2 V617F vs. 27/45 (60%) of patients with thrombocytosis above $600*10^9$/l(30). Additionally some patients may harbor MPL W515L mutations which, along with JAK2 V617F mutants, strongly activate STAT5 phosphorylation. Overall, the *TET2* mutations were present in 2/22 (9%) and *ASXL1* in 2/20 (10%) of RARS-T patients.

In summary, we describe herein novel *TET2* and *ASXL1* mutations, which might contribute to the pathogenesis of RARS-T in some patients, and eventually could serve as additional important markers in prognosis and disease progression.

Acknowledgments

This work was supported by NIH R01 HL082983 (JPM), U54 RR019391 (JPM), K24 HL077522 (JPM), Award from AA & MDS International Foundation and a charitable donation from the Robert Duggan Cancer Research Fund.

References

1. Szpurka H, Tiu R, Murugesan G, Aboudola S, Hsi ED, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. Blood 2006 Oct 1;108(7):2173–81. [PubMed: 16741247]
2. Wang SA, Hasserjian RP, Loew JM, Sechman EV, Jones D, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis harbors JAK2 mutation and shows overlapping myeloproliferative and myelodysplastic features. Leukemia 2006 Sep;20(9):1641–4. [PubMed: 16871284]
3. Boissinot M, Garand R, Hamidou M, Hermouet S. The JAK2-V617F mutation and essential thrombocythemia features in a subset of patients with refractory anemia with ring sideroblasts (RARS). Blood 2006 Sep 1;108(5):1781–2. [PubMed: 16926301]
4. Ceesay MM, Lea NC, Ingram W, Westwood NB, Gaken J, et al. The JAK2 V617F mutation is rare in RARS but common in RARS-T. Leukemia 2006 Nov;20(11):2060–1. [PubMed: 16932338]
5. Renneville A, Quesnel B, Charpentier A, Terriou L, Crinquette A, et al. High occurrence of JAK2 V617 mutation in refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Leukemia 2006 Nov;20(11):2067–70. [PubMed: 16990759]
6. Gattermann N, Billiet J, Kronenwett R, Zipperer E, Germing U, et al. High frequency of the JAK2 V617F mutation in patients with thrombocytosis (platelet count>600×109/L) and ringed sideroblasts more than 15% considered as MDS/MPD, unclassifiable. Blood 2007 Feb 1;109(3):1334–5. [PubMed: 17244688]
7. Schmitt-Graeff AH, Teo SS, Olschewski M, Schaub F, Haxelmans S, et al. JAK2V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica 2008 Jan;93(1):34–40. [PubMed: 18166783]
8. Schmitt-Graeff A, Thiele J, Zuk I, Kvasnicka HM. Essential thrombocythemia with ringed sideroblasts: a heterogeneous spectrum of diseases, but not a distinct entity. Haematologica 2002 Apr;87(4):392–9. [PubMed: 11940483]
9. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood 2006 Nov 15;108(10):3472–6. [PubMed: 16868251]
10. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med 2006 Jul;3(7):e270. [PubMed: 16834459]
11. Szpurka H, Gondek LP, Mohan SP, Hsi ED, Theil KS, et al. UPD1p indicates the presence of MPL W515L mutation in RARS-T, a mechanism analogous to UPD9p and JAK2 V617F mutation. Leukemia 2009 Mar;23(3):610–4. Epub 2008 Sep 25. [PubMed: 18818701]
12. Mohamedali AM, Smith AE, Gaken J, Lea NC, Mian SA, et al. Novel TET2 Mutations Associated With UPD4q24 in Myelodysplastic Syndrome. J Clin Oncol 2009 Aug 20;27(24):4002–6. Epub 2009 Jun 15. [PubMed: 19528370]
13. Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet 2009 Jul;41(7):838–42. Epub 2009 May 31. [PubMed: 19483684]
14. Delhommeau F, Dupont S, Della VV, James C, Trannoy S, et al. Mutation in TET2 in myeloid cancers. N Engl J Med 2009 May 28;360(22):2289–301. [PubMed: 19474426]

15. Jankowska AM, Szpurka H, Tiu RV, Makishima H, Afable M, et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. Blood 2009 Jun 18;113(25):6403–10. Epub 2009 Apr 16. [PubMed: 19372255]

16. Tefferi A, Lim KH, Abdel-Wahab O, Lasho TL, Patel J, et al. Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML. Leukemia 2009 Jul;23(7):1343–5. Epub 2009 Mar 19. [PubMed: 19295549]

17. Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, Patel J, et al. Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood 2009 Jul 2;114(1):144–7. Epub 2009 May 6. [PubMed: 19420352]

18. Flach J, Dicker F, Schnittger S, Kohlmann A, Haferlach T, et al. Mutations of JAK2 and TET2, but not CBL are detectable in a high portion of patients with refractory anemia with ring sideroblasts and thrombocytosis (RARS-T). Haematologica. 2009 Nov 10;

19. Tefferi A, Pardanani A, Lim KH, Abdel-Wahab O, Lasho TL, et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. Leukemia 2009 May; 23(5):905–11. [PubMed: 19262601]

20. Gelsi-Boyer V, Trouplin V, Adelaide J, Bonansea J, Cervera N, et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. Br J Haematol 2009 Jun;145(6):788–800. [PubMed: 19388938]

21. Carbuccia N, Murati A, Trouplin V, Brecqueville M, Adelaide J, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. Leukemia 2009 Nov;23(11):2183–6. [PubMed: 19609284]

22. Gondek LP, Dunbar AJ, Szpurka H, McDevitt MA, Maciejewski JP. SNP Array Karyotyping Allows for the Detection of Uniparental Disomy and Cryptic Chromosomal Abnormalities in MDS/MPD-U and MPD. PLoS ONE 2007;2(11):e1225. [PubMed: 18030353]

23. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. Exp Hematol 2002 Mar;30(3):229–36. [PubMed: 11882360]

24. Vannucchi AM, Antonioli E, Guglielmelli P, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal. Leukemia 2008 Jul;22(7):1299–307. [PubMed: 18496562]

25. Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, et al. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. Blood 2008 Aug 1;112(3): 844–7. [PubMed: 18519816]

26. Ono R, Taki T, Taketani T, Taniwaki M, Kobayashi H, et al. LCX, leukemia-associated protein with a CXXC domain, is fused to MLL in acute myeloid leukemia with trilineage dysplasia having t(10;11) (q22;q23). Cancer Res 2002 Jul 15;62(14):4075–80. [PubMed: 12124344]

27. Lorsbach RB, Moore J, Mathew S, Raimondi SC, Mukatira ST, et al. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). Leukemia 2003 Mar;17(3):637–41. [PubMed: 12646957]

28. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 2009 May 15;324 (5929):930–5. [PubMed: 19372391]

29. Swerdlow, SH.; Campo, E.; Harris, NL.; Jaffe, ES.; Pileri, SA., et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC; 2008.

30. Zipperer E, Wulfert M, Germing U, Haas R, Gattermann N. MPL 515 and JAK2 mutation analysis in MDS presenting with a platelet count of more than 500 x 10(9)/l. Ann Hematol 2008 May;87(5): 413–5. [PubMed: 18040685]

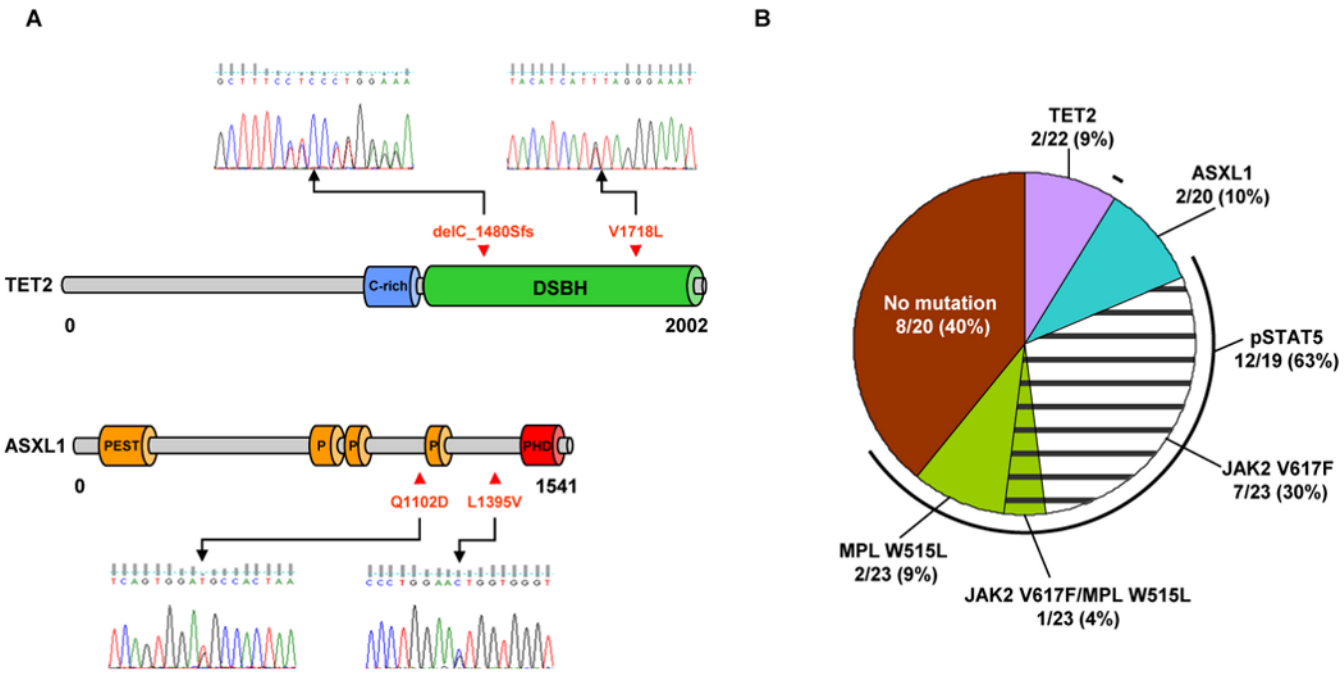

Figure 1.

A) Schematic representation of the topographic distribution of the individual mutations in the TET2 (isoform A NM_0011270208) and ASXL1 proteins. Genomic sequencing revealed frame shift delC_1480Sfs and missense mutation V1718L in *TET2* as well two missense mutations L1395V and Q1102D in *ASXL1* gene. B) Frequency of *TET2 and ASXL1* mutations, JAK2 V617F, MPL W515L and abnormal phospho-STAT5 activation in RARS-T patients. 2 monoallelic *TET2* mutations were found in 9% of patients; 2 monoallelic *ASXL1* mutations in 10%, 7 monoallelic and 1 biallelic *JAK2* V617F mutations in 35%; *MPL* W515L mutations, 1 mono- and 2 biallelic, in 13%. Abnormal phospho-STAT5 activation was found in 63% of cases. The frequencies were calculated based on 22, 20, 23, 23, and 19 patients, respectively. Abbreviations: C-rich - cysteine-rich region; DSBH - double-stranded β helix; PEST, P - proline (P), glutamic acid (E), serine (S), and threonine (T); PHD - the plant homeodomain finger.

Table 1

Clinical and pathologic features of patients with RARS-T.

Patient No.	Age	Plt. Count, (× 10^9/L)	Fibrosis	WBC (× 10^9/L)	Splenomegaly	*TET2*	*ASXL1*	JAK2 V617F	MPL W515L	pSTAT5	Cytogenetics	SNP-A karyotyping		
												Gain	Loss	UPD
1*†‡	78	277§	2+	19.5	N	-	-	-	+	+	46,XY[20]	-	-	-
2*†	73	664	2+	10.14	Y	delC 1480Sfs	NA	-	-	+	46,XY[20]	-	-	-
3†	79	660	4+	23.84	Y	-	-	+	+	+	46,XX,inv(9)(p11;q12)[20]	-	2p16.2; 22q11.23	1p11.2p-ter
4†	76	587	1+	28.06	Y	-	-	+	-	+	46,XY[20]	-	-	9p13.1-pter
5†	71	766	4+	26.2	Y	-	L1395V	-	-	+	46,XY[20]	-	-	-
6†	57	600	4+	37.09	Y	-	-	+	-	+	46,XX[20]	-#	-#	-#
7†	78	894	2+	3.81	N	-	-	+	-	+	46,XX[20]	-	-	-
8†	58	771	0	9.99	N	-	-	-	-	+	46,XX[20]	-	-	-
9†	87	625	2+	15.4	Y	-	-	-	+	+	46,XY[20]	-	-	1p13.3-pter 2p12-pter 6p21.2-p22.1 6p16.3-q22.33 8p21.3-q24.13 10p13-p14
10†	78	483	NA	22.48	N	-	-	-	-	+	46, XY,inv(3)(q21;q26.2)[20]	11p25	-	-
11*†‡	61	329π	2+	90.34	Y	-	-	+	-	+	46,XY[20]	-	-	-
12*†	85	598	1+/2+	21.8	N	-	NA	+	-	+	46,XX[20]	7q21.2	-	-
13†	71	496	0	4.14	N	-	NA	+	-	NA	5q-	20q13.12	-	1p32-pter
14*†	72	512	0	8.1	N	-	-	+	-	NA	45,X,inv(10)(q21.2;q24.3)[3] 46,XX,inv(10)(q21.2;q24.3)[17]	-	-	-
15†	66	773	NA	5.39	N	-	-	-	-	-	45,X,-X[4]/46,XX[16]	2p11.23	Xq25	-
16†	69	616	2+	4.26	Y	NA	-	-	-	-	46,XY,del(5)(q22;q33)[20]	-	5q21.1q31.3	1p35.1-pter
17†	63	611	0	4.99	N	-	-	-	-	-	46,XX[20]	21q22.3	-	-
18†‡	73	667	2+	33.46	N	V1718L	-	-	-	-	47,XX,+8[20]	8	-	-
19	83	469	NA	4.6	N	-	Q1102D	-	-	NA	46,XX[20]	NA	NA	NA
20	63	614	NA	14.36	Y	-	-	-	-	-	46,XX[20]	-	-	2p22.3-pter 3q21.3qter

Patient No.	Age	Plt. Count, (× 10^9/L)	Fibrosis	WBC (× 10^9/L)	Splenomegaly	*TET2*	*ASXL1*	JAK2 V617F	MPL W515L	pSTAT5	Cytogenetics	SNP-A karyotyping		
												Gain	Loss	UPD
21	61	457	0	5.79	N	-	-	-	-	-	46,XX,add(15)(p11.1),add(22)(p11.2)[3]/47,idem, +19 [19]	+19	-	-
22	83	636	1+	5.30	N	-	-	-	-	-	46,XY[20]	-	-	-
23	80	761	0	8.27	N	-	-	-	-	NA	46,XX[20]	-	-	3q21.3qter

*,†,‡Patients were part of a previous series where mutation analysis was confined to JAK2 V617F/MPL W515L mutations and pSTAT5 analysis [1,11,15]; NA - not available;

§documented history of thrombocytosis over previous 6 months (to 1353×10^9/l);

πdocumented history of thrombocytosis over previous 6 months ($546–1213\times10^9$/l);

#used 50K array.