A novel t(1;9)(p36;p24.1) JAK2 translocation and review of the literature

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Short Title: JAK2 Translocations

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1. Abstract

The JAK2V617F point mutation has been implicated in the pathogenesis of the vast majority of myeloproliferative neoplasms (MPNs), but translocations involving JAK2 have increasingly been identified in a subset of patients with JAK2V617F-negative MPNs. Here we present a case of a patient diagnosed with JAK2V617F-negative polycythemia vera (PV) that transformed to MPN-blast phase (MPN-BP). Cytogenetic and FISH analysis revealed a novel translocation of t(1;9)(p36;p24.1) and a PEX14-JAK2 gene fusion, as a result of the translocation, was identified. The t(1;9)(p36;p24.1) has not been previously described and represents a new addition to the list of known translocations involving JAK2 that have been identified in hematologic malignancies. Although the prognostic and treatment implications of JAK2 translocations in MPNs is not yet clear, positive outcomes have been described in early case reports of the use of JAK inhibitors in these patients. Further research into the role of JAK2 translocations in the pathogenesis and outcomes of hematologic malignancies is warranted.
2. Introduction

The Janus Kinase (JAK) proteins are a family of cytoplasmic tyrosine kinases involved in the JAK-STAT signaling pathway and are essential in maintaining normal hematopoiesis. The JAK2 gene, located on chromosome 9p24, encodes for a receptor predominantly responsive to type I cytokine ligands, including erythropoietin (EPO), thrombopoietin (TPO) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Ligand binding to JAK2 leads to autophosphorylation and activation of signal transducers and activators of transcription (STAT) proteins, which mediate the expression of genes involved in hematopoietic cell production [1].

Constitutive activation of the JAK-STAT pathway through the acquired point activating mutation on exon 14 (JAK2V617F) has been implicated in the pathogenesis of myeloproliferative neoplasms (MPNs). This mutation is present in 98% of polycythemia vera (PV), 40-50% of essential thrombocythemia (ET) and 50-60% of primary myelofibrosis (PMF) [2,3], and is present in 20% of patients with non-classical MPNs [4]. Additionally, a subset of patients with JAK2V617F-negative MPNs is found to have translocations involving JAK2 which result in gene fusion products that lead to JAK2 amplification or constitutive activation of the tyrosine kinase. These translocations are not limited to the MPNs, but have also been identified in de novo leukemias of both myeloid and lymphoid lineages (Table 2).

Here we describe a case of a patient with JAK2V617F-negative PV who was found to have a novel JAK2 translocation, not previously described. We also provide a review of the known JAK2 translocations associated with PV and other MPNs.
A 52 year old woman initially presented in 2008 with symptoms of headaches, dizziness, fatigue, shortness of breath and numbness and tingling of the hands and feet and was found to have a hematocrit (HCT) of 61% with normal white blood count (WBC) and platelet (PLT) count. The bone marrow biopsy showed a hypercellular marrow (95%) with increased megakaryocytes in clusters, without reticulin staining. The patient was found to meet World Health Organization’s (WHO) diagnostic criteria for JAK2V617F mutation negative PV and was initiated on treatment with aspirin and therapeutic phlebotomy, to maintain the Hct <42%.

A year later, the patient developed leukocytosis with a WBC 30 x10^9/L and thrombocytosis with a PLT count of 600,000/L. She was started on hydroxyurea at a dose of 500 mg daily, with subsequent improvement in leukocytosis and thrombocytosis, but developed treatment emergent anemia. Repeat bone marrow biopsy and aspirate at that time showed a hypercellular marrow (100%) with trilineage hematopoiesis, marked granulocytic hyperplasia, increased immature forms, with markedly increased eosinophils (31%) and 7% blasts. Peripheral blood flow cytometry showed a small CD33+ and CD34+ myeloblast population (1.4%).

Several months following initiation of hydroxyurea, the patient developed constitutional symptoms. The WBC was found to be elevated to 72x 10^9/L and peripheral blasts were 21%, consistent with transformation to MPN-blast phase (MPN-BP), a form of secondary acute myeloid leukemia (AML).
Decitabine was initiated. After receiving 4 cycles of decitabine, the patient underwent hematopoietic stem cell transplantation (HSCT) in August 2010 with cells from a 10/10 matched unrelated donor, with successful engraftment. Post-transplant bone marrow biopsy, however, showed persistent hypercellularity (80-90%) and persistent myeloblast populations ranging from 1.1% to 8%, consistent with a residual disease. Unfortunately, the patient’s post-transplant course was complicated by graft-versus-host disease of the gastrointestinal system and central nervous system, and ultimately the patient succumbed to gram-negative sepsis on day +106 post-transplant.

3. Materials & Methods

Cytogenetic and FISH Analysis

Conventional cytogenetic preparations and fluorescent in situ hybridization (FISH) analyses were performed as described previously [5, 6]. To detect the exact region involved in the novel translocation 12 FISH probes were used: 9p24.1 (RP11-3H3), 9p24.1 (RP11-28A9)(BACPAC Resources Oakland, CA, USA), 1p36.22 (RP11-483P2), 1p36.22 (RP11-1107P2), RP11-1134M20, RP11-483P2, RP11-1107P2 (Empire Genomics, Buffalo, NY, USA), 9q34 (ABL1), 22q11.2 (BCR), 1p36 (p58), 1p36 (CEP108/T7), 1q25, WCP19, telomere 1p, telomere 19p (Abbott Molecular, Abbott Park, IL, USA). FISH chimerism was detected using XX/XY probes (Abbott Molecular). All FISH probes, including BACs, were fluorescently labeled by the manufacturer excluding RP11-3H3 and RP11-28A9, these BACs were fluorescently labeled using the Nick translation kit (Abbott Molecular) following the manufacturer’s’ procedure [5]. DNA cel isolated from bone marrow was subjected to whole genome sequencing (WGS) and analyzed by standard pipelines at the Sanger Institute, Hinxton, UK [7].
Confirmation of a PEX14-JAK2 genomic DNA fusion was performed using a forward primer in PEX14 exon 102 (5’CCACCAACTGGATCCTGGAGT) and reverse primer in JAK2 exon 19 (5’AACCCCAGGGCACCTATCCT), on 25ng DNA using the Expand Long-Template LT-PCR system 2 (Roche, Burgess Hill, UK) at an annealing temperature of 64°C and an elongation time of 4 min. Sequencing across the breakpoint was performed using primer PEX14 exon 9 (5’GTTCCTCCATCCCCATCAG), using an Applied Biosystems 3130 (Foster City, CA, USA).

The presence of PEX14-JAK2 fusion mRNA was confirmed on random hexamer reverse transcribed cDNA, using forward primer PEX14 exon 8 and a reverse primer in JAK2 exon 21 (5’TTTTAGATTACGCCGACCAGCA) using the Expand High Fidelity PCR System, an annealing temperature of 64°C and an elongation time of 1 min. The product was sequenced in both directions using the same primers.

4. Results

Summary of conventional cytogenetic analysis is shown in Table 1. Initial bone marrow cytogenetic analysis, a year after the diagnosis of PV, showed 50% of evaluated metaphase cells to have t(1;9)(p36;p24.1) karyotype. Subsequent metaphase FISH analyses using bacterial artificial chromosome (BAC) FISH probes (RP11-3H3 and RP11-28A9) revealed that the 3’ portion of JAK2 was translocated to 1p36 while the 5’ portion remained on 9p24.1, indicating a JAK2 structural rearrangement (Figure 1). To investigate the exact breakpoint on chromosome 1, we used a FISH BAC probe, and as shown in Figure 1, BAC FISH probe RP11-4832P2 normally localized on chromosome 1p36 was detected on 9p24.1 (aqua) whereas BAC RP11-1107P2 remained on 1p36. Therefore, the breakpoint on chromosome 1, involved in the JAK2 translocation, was determined to be within band p36.22 on chromosome 1 [7].
Five months from the initial analysis, 100% of cells had t(1;9) and 10% developed a subclone consisting of balanced t(7;17)(q22.1;25.3) and trisomy 1q in the form of unbalanced der(15)t(1;15)(q12;q26).

Following HSCT, the host cells were never eradicated (Table 1). A year after the initial cytogenetic analysis, the original abnormal host clone and a subclone were present in 100% of cells with additional chromosomal abnormalities consistent with complex subclonal evolution.

To characterize the t(1;9) in detail, we performed WGS analysis of patient bone marrow DNA to identify the translocation breakpoints. Focusing on the analysis of JAK2, we identified two split reads that mapped to PEX14 exon 9 and JAK2 intron 18. PEX14 maps to 1p36.22, and was thus a strong candidate to be fused to JAK2. To confirm a PEX14-JAK2 fusion, we amplified patient and control DNA using primers located in PEX14 exon 8 and JAK2 exon 19. A product was obtained from the t(1;9) case only (not shown), which, upon sequencing, confirmed a break within PEX14 exon 9 and JAK2 intron 18 (Figure 2). Amplification from cDNA also yielded a specific product from the t(1;9) case but not controls (Figure 3), sequencing of which showed a fusion between a truncated PEX14 exon 9 and JAK2 exon 19 (Figure 2). Comparison of the cDNA and genomic sequences indicates that two nucleotides (TA) from JAK2 intron 8 were retained in the mature mRNA that result in maintenance of the correct reading frame (Figure 3). The TA dinucleotide is immediately followed by GT which must have acted as a splice donor site.

Table 1: Results: Summary of Cytogenetic and FISH results (attached)
5. Discussion

The most common JAK2 abnormality identified in MPNs is the JAK2V617F, which is seen in the vast majority of patients with PV, as well as approximately half of the cases of ET and PMF. Subsequent to the 2005 discovery of the acquired somatic point activating mutation by four independent groups, knowledge of the genetic underpinnings of MPNs increased exponentially, along with information on the role that mutations play in diagnosis, prognosis and therapeutic approach [8, 9, 10, 11].

In stark contrast to this wealth of knowledge about the JAK2V617F-positive MPNs, there is a relative dearth of information on how to approach the small subset of patients with MPNs who lack this point mutation, but contain JAK2 translocations at 9p24.1.

Translocations at 9p24.1 and the resultant gene fusion products have been identified in a wide spectrum of hematologic malignancies of both myeloid and lymphoid origin, including acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), chronic eosinophilic lymphoma (CEL), and the BCR-ABL1 negative MPNs (Table 2). In addition to this, several translocations involving 9p24.1 with subsequent gene fusion products involving JAK2 have been seen in solid malignancies, such as breast cancer [12] and small cell lung cancer [13].
It is likely that JAK2 translocations are more common in hematologic malignancies than previously recognized. In a 2010 publication in European Journal of Haematology, Patnaik and colleagues screened over twenty four thousand patient cytogenetic reports and found five patients harboring translocations at 9p24 with gene fusion products involving JAK2 [3]. All five of the translocations described in this subset of patients had not previously been reported in the literature until this 2010 publication: t(8;9)(q22;p24), t(9;17)(p24;q23), t(4;9)(q25;p24), t(2;9)(p21;p24), and t(8;9)(q13;p24). Four of the five patients carried diagnoses of JAK2V617F-positive MPNs (PMF and PV), and the patient with t(8;9)(q13;p24) was diagnosed with diffuse large B-cell lymphoma, without JAK2V617F. In each of these translocations, the JAK2 fusion partner could not be identified.

Though the above study found JAK2 translocations only in patients with JAK2V617F-positive MPNs, structural rearrangements, including JAK2 translocations, have been found frequently in chromosomal analysis of samples from patients with JAK2V617F-negative MPNs [14].

The discovery of several of these translocations in JAK2V617F-negative MPNs has shed light on theories of the pathogenesis of the diseases and has helped identify unique patterns of disease. A prime example of this is the identification of t(8;9)(p22;p24), resulting in a PCM1-JAK2 fusion gene product in a variety of hematologic diseases, including many in the spectrum of MPNs, such as atypical chronic myeloid leukemia (aCML), CEL, myelodysplastic syndrome/myeloproliferative neoplasm-unclassified (MDS/MPN-U) and MF. Identifying the common translocation in cases of these disparate disorders has led to the recognition that patients with t(8;9)(p22;p24) are more likely to be male, tend to have prominent erythroid dysplasia and they have high rates of peripheral blood and bone marrow eosinophilia.
PCM1-JAK2 was identified in both myeloid and lymphoid neoplasms, leading to the conclusion that malignancies with acquired t(8;9)(p22;p24) result from disorders of the pluripotent hematopoietic stem cell [16]. Increased recognition of the role of PCM1-JAK2 in hematologic malignancies has led to a relatively new categorization of “myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFR, PDGFRB, or FGFR1, or with PCM1-JAK2” in the 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia [17].

Similarly, both myeloid and lymphoid neoplasms with ETV6-JAK2 fusions have been identified. Animal modeling using this translocation has led to a proposed pathogenic mechanism involving the rearranged genes, which appears to cause constitutive activation of several STATs within the JAK-STAT pathway [18]. A number of cases have also been noted in which JAK2 fuses with the BCR gene on 9p24, most notably associated with Philadelphia chromosome positive CML, resulting in aCML and unclassified MPNs [19, 20].

Here we present a case of a novel translocation t(1;9)(p36;p24.1) involving JAK2 and peroxisomal biogenesis factor 14 (PEX14) found in a patient with JAK2V617F-negative PV, which transformed to an aggressive form of MPN-BP. PEX14 is a membrane protein involved in protein docking on the peroxisomes, with a role in peroxisome formation and degradation. It also has a unique function as a transcriptional co-repressor and a polypeptide transport modulator. Upregulated expression of PEX14 has been demonstrated in tissue from carcinomas of the lung, rectum, ovary and esophagus, but the protein’s precise role in these malignancies remains unclear and information about its role in hematologic malignancies is absent [21]. Interestingly, like many other cases of JAK2V617F-negative,
JAK2-translocation positive MPNs, this patient’s disease was notable for prominent bone marrow eosinophilia of undetermined significance.

JAK2 was identified as a fusion gene partner with a gene on chromosome 1 in only one other case of a hematologic malignancy; a TPM3-JAK2 fusion was found in a case of T-cell acute lymphoblastic leukemia [22].

Although commonalities between hematologic malignancies with select JAK2 rearrangements have been identified as result of increased attention to the cytogenetic underpinnings of these diseases, the prognostic significance and therapeutic implications of the translocations are not fully elucidated. As the disease of our patient progressed to MPN-BP, cytogenetic analysis showed a gain of 1q. We recently reported this to be associated with progression of MPNs to MF and AML [23]. In vitro studies utilizing cell lines containing JAK2 rearrangements suggest a promising role of JAK inhibitors in halting malignant cell proliferation [24, 22]. Early case reports of ruxolitinib treatment in patients with MPNs containing JAK2 translocations (namely, PCM1-JAK2 and BCR-JAK2) also show positive outcomes in cytogenetic response and hematologic remission, although the durability of remission is variable and individual differences in outcomes are not well-studied [25, 26, 27]. As data accumulate about newly identified JAK2 translocations, further connections can be made between the specific translocations, disease course and prognosis. Further information is needed to understand the translocations’ oncogenicity and the potential for novel targeted therapies aimed at targeting the specific JAK2 partners for this minority of MPN patients. In the future, MPNs may be stratified further into distinct entities that take into account specific JAK2 translocations.
Table 2: JAK2 Translocations and Associated Gene Fusion Products

(see attached)
6.1. Statement of Ethics

The authors have no conflicts of interest to declare.

6.2. Disclosure Statement

The authors have no conflicts of interest to declare.

6.3. Author Contributions

Hannah Levavi and Bridget Marcellino wrote the manuscript. Joseph Tripodi and Diana Gruenstein performed cytogenetic and FISH studies. Amy V Jones and Nicholas C. P. Cross did molecular studies and analyses. John Mascarenhas was involved in clinical care and studies, and Vesna Najfeld conceived and organized the work, and helped in preparing the manuscript.
7. References


8. Figure Legends

Table 1
MPD = myeloproliferative disorder; PV = polycythemia vera; PB = peripheral blood; BM = bone marrow; CSF = cerebrospinal fluid; ND = not done
* status post gender-mismatched allogeneic stem cell transplant (allo-SCT)
† day +58 status post allo-SCT
€ day +58 status post allo-SCT

Table 2
aCML = atypical chronic myeloid leukemia; CML = chronic myeloid leukemia; MPN-U = myeloproliferative neoplasm unspecified; MPN = myeloproliferative neoplasm; MDS = myelodysplastic syndrome; CEL = chronic eosinophilic leukemia; PMF = primary myelofibrosis; PV-AML = polycythemia vera-acute myeloid leukemia; T-ALL = T-cell acute lymphoblastic leukemia; Pre-B ALL = precursor B cell acute lymphoblastic leukemia; MLL-R ALL = MLL rearrangements acute lymphoblastic leukemia

Figure 1
The first row shows a partial karyotype of chromosomes 1 and 9. Metaphase fluorescence in situ Hybridization (FISH) was performed using bacterial artificial chromosome (BAC) FISH probes. RP11-3H3 (labeled in aqua) normally localized to the 5’ portion of JAK2 on 9p24.1 was translocated to 1p36 while the telomere of chromosome 1p (labeled in green) translocated to the derivative chromosome 9. The chromosomal breakpoint on chromosome 1 was determined to be within band 1p36.22.

Figure 2
Sequence trace of the PEX14-JAK2 amplicon from genomic DNA (top panel) plus alignment of genomic sequences of PEX14, PEX14-JAK2 and JAK2 in the relevant region (bottom panel). The two nucleotides from JAK2 intron 8 that are retained in the mature mRNA are indicated in italics and the cryptic splice donor site in bold.

Figure 3
Specific amplification of cDNA from the t(1;9) case using primers to PEX14 exon 8 and JAK2 exon 21. Sequence of the mRNA junction with PEX14 sequence in plain type, JAK2 in bold and the two intron-derived nucleotides in italics.
Figure 2

**PEX14**

TCTCACCTGTCAAGCAACGAGTCCACGTCGTCCTCGGCTGGGAAG

**PEX14-JAK2**

TCTCACCTGTCAAGCAACGAGTCTAGTGAGTTGTAAATTATAGTA

**JAK2**

AACCTAATTTTAGTTTTCCATTAGGTAGTTGTAATAATTATAGTA
Figure 1

- Telomere 1p
  - RP11-3H3
- RP11-1134M20
  - RP11-483P2
  - RP11-1107P2

1  der(1)  9  der(9)
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<th>I-FISH for Chimerism (% of abnormal cells)</th>
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<td>PB 46XX,t(1;9)(p36;p24.1)[18]/46,idem,t(7;17)(q22.1;q25.3),der(15)t(1;5)(q12;q26) [2] (trisomy 1q)</td>
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Figure 3

A

Ladder  t(1;9)  N1  N2  N3  Blank

B

ΔPEX14 exon 9  JAK2 exon 19

CCT  GTC  AGC  AAC  GAG  TCT  AT  TAT  GAA  CTA  TTA
P  V  S  N  E  S  N  Y  E  L  L