JAK1 somatic mutation in a myeloproliferative neoplasm

The Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway plays a key oncogenic role in blood cancers, particularly myeloproliferative neoplasms (MPNs) and acute lymphoblastic leukemia (ALL). In normal cells, cytokine-induced activation of the JAK/STAT pathway stimulates proliferation, survival, differentiation, and functional activation, resulting in tightly regulated blood cell production according to need.[1] A recurrent mutation in *JAK2*, leading to the V617F missense mutation in the pseudo-kinase domain, is the most common molecular abnormality in MPNs.[2-5] It results in non-physiological ligand-independent signalling and excess blood cell production. The discovery of this key driver mutation has fast tracked the development of targeted agents that inhibit JAK2, resulting in therapeutic benefit.[6,7] Whilst more than 95% of cases of polycythemia vera (PV) harbour the JAK2V617F mutation, only about 50-60% of cases of essential thrombocythemia (ET) and primary myelofibrosis (PMF) do so. The rest carry mutations in *CALR*, *MPL*, and many other genes at much lower frequencies. For most triple-negative MPNs (i.e., wild type for *JAK2, CALR* and *MPL*), a molecular diagnosis is not available because access to clinical next generation sequencing (NGS) platforms is limited or expensive. In addition, there are clinical cases which are difficult to fit into the WHO criteria for MPNs; MPN/MDS, MPN-unclassifiable (MPN-u), and atypical CML (aCML) are good examples of such difficulties.

A precise molecular diagnosis can be helpful in the management of MPNs and related disorders. For example, mutations in the CSF3R are commonly found in chronic neutrophilic leukemia (CNL),[8] so it is likely to soon be incorporated into the WHO criteria for this disorder.[9] Additional mutations in genes involved in RNA splicing and epigenetic gene regulation are also common in MPNs, and have prognostic significance.[10] It is becoming increasingly important to monitor the clonal evolution of these additional mutations. For these reasons, targeted NGS panels of genes, or hotspots within genes, have been developed by a few groups to aid in the diagnosis and risk stratification of MPNs and related disorders.[11,12] We developed a comprehensive panel designed to discover mutations in most (86 genes) of the JAK-STAT pathway genes as well as all reported mutations in MPNs up until 2014. We included genes and hotspots previously reported to be mutated in MPNs, chronic myelomonocytic leukemia (CMML), CNL, mastocytosis,

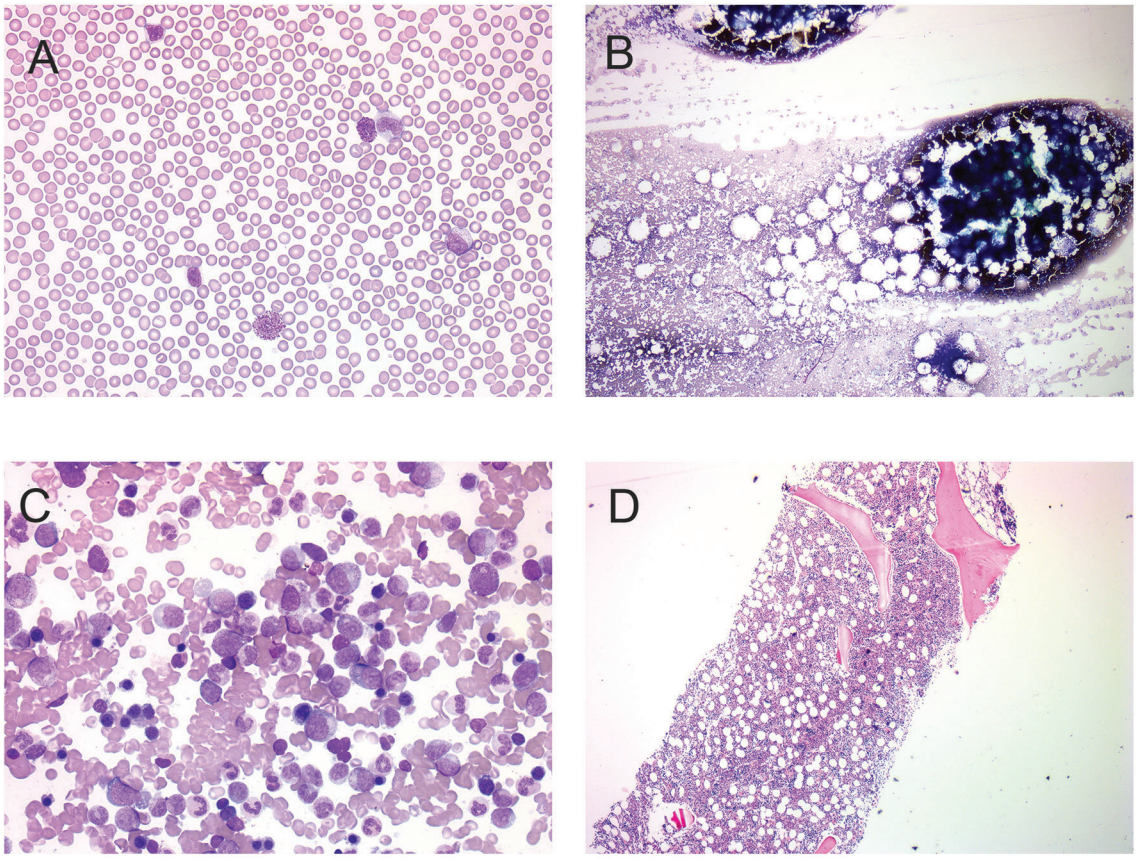

Figure 1. Blood film and bone marrow biopsy findings at diagnosis. A. Peripheral blood film demonstrating basophilia and thrombocytopenia. B. Hypercellular bone marrow fragment. C. Granulocytic hyperplasia and left shift evident on bone marrow aspirate. D. Trephine demonstrating megakaryocytic hyperplasia with several morphologically abnormal/small cells.

hyper-eosinophilic syndromes (HES), and related disorders,[11] but we also included known positive and negative regulators of the JAK-STAT pathway in the belief that mutations in these genes could also lead to MPNs in rare cases.[11]

We report the case of a 78-year-old woman who was referred for hematology consultation after a routine full blood examination (FBE) demonstrated significant abnormalities. She was clinically well at presentation, reporting no notable symptoms. In particular, she reported no bruising, bleeding, infections, fevers, weight loss, night sweats or lethargy. There was no significant past medical history, and the only regular medication was frusemide which she took for fluid retention. On examination, there was no splenomegaly, hepatomegaly or lymphadenopathy. FBE demonstrated a significantly elevated white cell count (WCC) of 21.6x10^9/L with left shift, including numerous circulating metamyelocytes and myelocytes, and occasional promyelocytes and blast cells. There was also basophilia (0.66x10^9/L), mild monocytosis (2.8x10^9/L), and mild thrombocytopenia (72x10^9/L) (Figure 1A). No dysplasia was noted. Lactate dehydrogenase (LDH) was elevated at 665U/L (reference range 120-250U/L) at time of presentation. In light of the FBE findings, the BCR-ABL1 translocation was searched for via RT-PCR and by FISH for the t(9;22) fusion; both tests were negative. A CT scan of the chest and abdomen was unremarkable, and molecular tests on DNA harvested from whole blood were negative for *JAK2 V617F, CALR* and *MPL* gene mutations.

A bone marrow examination demonstrated marked abnormalities. The marrow was markedly hypercellular (Figure 1B); granulopoiesis was moderately hyperplastic with a mild left shift; megakaryocytic hyperplasia was also present with occasional small forms and grade 1 fibrosis (i.e., reticulin staining) (Figure 1C-D). Blast cells were present in normal numbers. No immunophenotypic abnormality was detected by flow cytometry. As in the blood, FISH for the t(9;22) translocation was negative. Cytogenetics demonstrated a normal female karyotype, and FISH for FIP1LI-PDGFRA and PDGFRB was negative. RT-PCR analysis for rare BCR-ABL breakpoints [including e13a2(b2a2), e14a2(b3a2), e13a3 (b2a3), e14a3(b3a3) and e1a2/p190)] was also negative. A provisional diagnosis of MPN-u was made.

The patient was observed without specific treatment.

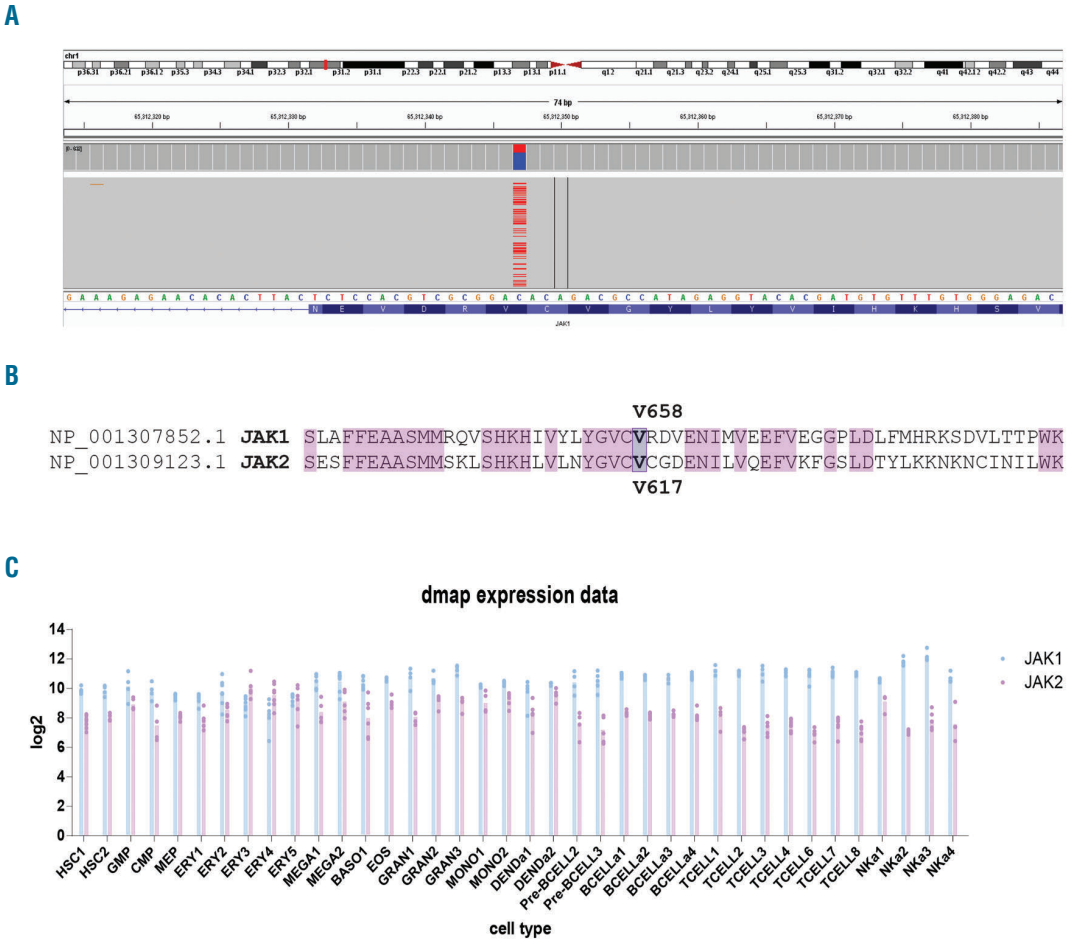

Figure 2. A gain of function mutation in JAK1 – V658I. A. IGV Browser view of the *JAK1* amplicon which encodes some of the pseudokinase domain. The heterozygous sequence variant leading to the V658I missense mutation is shown. B. Sequence alignment of the JAK1 and JAK2 pseudokinase domains with the critical valine residue indicated. C. Expression of *JAK1* and *JAK2* in normal human blood cell types. Data generated from Haemosphere using the "normal human haematopoiesis (DMAP)" dataset, derived from GSE24759.[18]

Over the next three months she reported mild bruising and fatigue. However, there was progression of FBE abnormalities; the WCC steadily increased to 54x10^9/L, and platelet count decreased to 54x10^9/L. The hemoglobin remained in the normal range during this time. LDH increased steadily, peaking at 1169U/L four months after presentation but spontaneously returned to ~600U/L without intervention. After a further eight months of stability there was a sudden change in the FBE; platelets decreased to 13x10^9/L (necessitating platelet transfusion), WCC increased to 82.5x10^9/L with leucoerythroblastic features, and there was an increasing myeloid left shift. The monocytosis gradually increased to 20x10^9/L but no dysplastic features were present. Spleen size remained normal at 13cm by abdominal ultrasound. The change in hematologic parameters was not associated with a change in clinical symptoms; the patient remained well, reporting only mild fatigue.

The initial bone marrow DNA specimen was referred for further molecular analysis using a custom designed AmpliSeq panel run on an Ion Torrent PGM machine.[11] Using this assay, just three single nucleotide variants were detected in >200kb of sequenced DNA: (i) a heterozygous SNV, c.1972G>A, in *JAK1* leading to a predicted missense mutation, V658I (Figure 2A); (ii) a heterozygous insertion, c.27_28insT, leading to a predicted frameshift in *DNMT3A*, which has not previously reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) database; it was considered most likely to be a sequencing error, and (iii) a SNV, c.1124G>T, in ETV6 which is predicted to lead to a conservative amino acid change, G375V. This has not been reported in COSMIC and is not in a functionally critical region of the protein, so was considered unlikely to be pathogenic. The DNA sequences of all the exons of *JAK2, MPL, CALR, CBL, SETBP1, SRSF1* and all the other genes on the panel, including those previously linked with aCML and CMML,[13] were normal.

Activating mutations of JAK1 have mostly been reported in T-cell prolymphocytic leukemia[14] and acute lymphoblastic leukemia[15] where it has been shown to confer a poorer prognosis as well as non-hematologic malignancies.[16] Transduction of V658F, V658L and V658I variants of JAK1 lead to factor- independent cell growth of BaF3 cells, whereas transduction of wild type JAK1 does not,[17] so the mutation is unquestionably pathogenic. The valine residue at amino acid 658 in JAK1 is functionally equivalent to V617 in JAK2 (Figure 2B); i.e., it is critically important for negative regulation of JAK kinase activity. To our knowledge, *JAK1* mutations have never been described in MPNs or CMML, so this is the first case report. *JAK1* is highly expressed in lymphoid and myeloid cell lineages, particularly granulocytes, but it is poorly expressed in megakaryocyte-erythroid progenitors (MEPs) and their erythroid and megakaryocytic progeny compared with JAK2 (Figure 2C).[18] It is therefore not surprising that gain-of-function mutations lead to a proliferation of myeloid cells rather than erythroid cells (i.e., PV) or platelets (i.e., ET).

We suggest that consideration of *JAK1* mutations should be undertaken in cases of MPN-U, aCML or CMML which do not otherwise have a molecular diagnosis. Since these diseases are likely to be molecularly heterogeneous, a broad NGS panel is most likely to yield the responsible driver mutation, so it is the preferred diagnostic test. We note that *JAK1* is not part of the TruSight® myeloid NGS panel offered by Illumina or part of some custom myeloid NGS panels.[12] Mutations would be missed unless the *JAK1* gene were included as it is in this custom panel[11] or a related one.[19] Alternatively, a full exome or clinical exome NGS approach could be employed, but this is associated with greater bioinformatics and clinical reporting challenges. We suggest that the JAK1 gene should be included in the future design of MPN and broader myeloid NGS panels in order to find cases like this one. This genetic information could be of clinical value since the disease is likely to respond to JAK inhibitors which have a strong binding affinity for JAK1 as well as JAK2, such as ruxolitinib.[20] On the other hand, the disease is not likely to respond well to JAK inhibitors with minimal anti-JAK1 cross activity, such as pacritinib.[21]

Suzanne O. Arulogun,[1] Hock-Lai Choong,[2] Debbie Taylor,[3] Paula Ambrosoli,[3] Graham Magor,[4] Ian M. Irving,[2,5] Tee-Beng Keng[1] and Andrew C. Perkins[3,4,5]

[1]Sullivan Nicolaides Pathology, Brisbane; [2]Townsville Hospital; [3]Mater Pathology, South Brisbane; [4]Mater Research, Translational Research Institute, University of Queensland, Woolloongabba and [5]ICON Cancer Care, South Brisbane, Australia.

Correspondence: andrew.perkins@mater.uq.edu.au
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