Gain of function, loss of control – A molecular basis for chronic myeloproliferative disorders

The concept of myeloproliferative disorders was introduced in 1951 by Dameshek[1] to describe a group of clinical entities that included chronic myeloid leukemia, polycythemia vera, essential thrombocythemia and idiopathic myelofibrosis. The molecular basis of chronic myeloid leukemia was defined in the 1980s, while that of more rare myeloproliferative disorders such as the hypereosinophilic syndrome[2] has been elucidated more recently. This issue of Haematologica/The Hematology Journal reports a comprehensive review article on mutated protein tyrosine kinases in hematologic malignancies, including chronic myeloproliferative disorders.[3]

After years of lack of knowledge concerning the pathogenesis of these conditions, four articles[4–7] have been published in a period of six weeks (March 19–April 28, 2005) describing a unique gain-of-function mutation of the Janus kinase 2 (*JAK2*) gene in patients with polycythemia vera, essential thrombocythemia and idiopathic myelofibrosis. Three additional articles on the same topic have appeared online recently.[8–10]

What was known about the pathogenesis of chronic myeloproliferative disorders before the JAK2 V617F mutation was found

Adamson and co-workers[11] provided the first evidence for the stem-cell nature of polycythemia vera and the clonal origin of this disease, and subsequent studies confirmed that chronic myeloproliferative disorders represent clonal proliferations of hematopoietic stem cells.[12] The clonal nature is found in nearly all patients with polycythemia vera and idiopathic myelofibrosis, while a variable proportion of female patients with essential thrombocythemia show polyclonal patterns of X chromosome inactivation.[13–15] It remains to be determined whether these latter patients with an apparently polyclonal hematopoiesis do indeed have a myeloproliferative disorder or whether they should be considered to have a different disease.

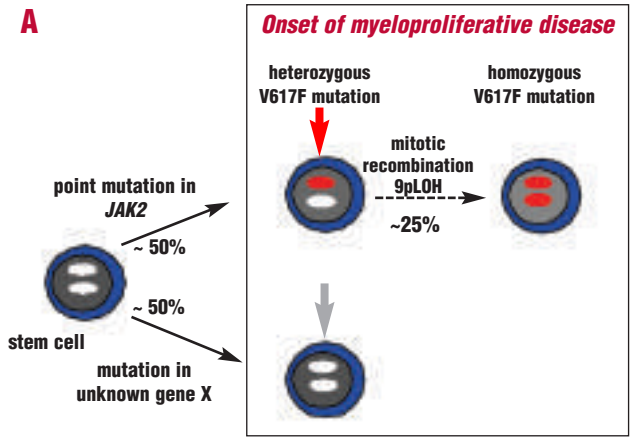
Another key observation was that when marrow cells from patients with polycythemia vera are cultured in semi-solid medium, a number of erythroid colonies form in the absence of exogenous erythropoietin.[16] Spontaneous growth of erythroid progenitor cells from peripheral blood mononuclear cells soon became a hallmark of polycythemia vera,[17,18] and comparative studies showed that this was the most reliable auxiliary test for diagnosis of this condition.[19] Spontaneous growth of erythroid progenitor cells was also found in a proportion of patients with essential thrombocythemia and idiopathic myelofibrosis, suggesting that this biological abnormality was a more general characteristic of chronic myeloproliferative disorders.

Several observations, particularly in polycythemia vera, suggested that these conditions might be caused by a gain-of-function mutation. When ferrokinetics was used to investigate erythropoiesis in the early 1970s, the typical ferrokinetic pattern in polycythemia vera was a small increase in total erythroid activity and a decrease in ineffective erythropoiesis: the slightly expanded, more efficient erythropoiesis fully explained the increases in hemoglobin and hematocrit values. The expansion of red cell mass in polycythemia vera is typically associated with low levels of endogenous erythropoietin,[20,21] indicating that red cell production is increased despite lower amounts of erythropoietin in the erythroid marrow. Finally, the increased sensitivity of hematopoietic progenitors from patients with polycythemia vera is not restricted to erythropoietin, but is also observed with other growth factors,[22–25] pointing to an abnormality in a common signal transduction pathway.

We previously characterized mutations that cause disease through increased efficiency of mRNA translation, and described translational pathophysiology as a novel mechanism of human disease.[26] Hereditary hyperferritinemia/cataract syndrome arises from various point mutations or deletions within a protein-binding sequence in the 5'-UTR of the L-ferritin mRNA.[27] These molecular lesions reduce the affinity of the protein-binding sequence for the iron regulatory proteins that normally inhibit ferritin mRNA translation, thereby causing increased production of L-ferritin. Hereditary thrombocythemia can be caused by mutations in upstream AUG codons in the 5'-UTR of the thrombopoietin (*THPO*) gene which normally function as translational repressors:[28] their inactivation leads to excessive production of thrombopoietin and elevated platelet counts.

From identification of a chromosomal aberration to detection of the JAK2 V617F mutation

Three different approaches led to the discovery of the *JAK2* mutation, including analysis of *in vitro* growth of erythroid colonies and their responsiveness to tyrosine kinase inhibitors,[4] high throughput sequencing of kinases in patients with myeloproliferative disorders,[6] and mapping of a recurrent chromosomal alteration on the short arm of chromosome 9.[7] Since the last strategy also provided the basis for understanding why some patients are homozygous for *JAK2* V617F, we briefly summarize the key results. In 2002, Kralovics and co-workers characterized a region on chromosome 9p frequently involved in loss of heterozygosity (LOH) in polycythemia vera.[29] 9pLOH was subsequently found in about one third of patients with polycythemia vera and a few patients with essential thrombocythemia.[19] In a collaborative study involving Basel and Pavia, candidate genes of a large number of patients with myeloproliferative disorders were mapped using microsatellites to define a minimal genomic region shared by all patients with 9pLOH. The identified region of 6.2-Mbp contained the *JAK2* gene,[7] which is essential for signaling through a variety of cytokine receptors.[30] Sequencing of the coding region of *JAK2* in patients with 9pLOH revealed a G to T transversion that changed a valine to a phenylalanine at position 617 (V617F).[7] Interestingly, the patients with 9pLOH were homozygous for the *JAK2* V617F mutation, whereas patients without 9pLOH were either heterozygous for V617F, or did not have the mutation. The most likely mechanism causing 9pLOH was identified as mitotic recombination, since two copies of the *JAK2* gene locus were found in granulocytes from all patients with 9pLOH.[7] Thus, mitotic recombination in a cell that car-



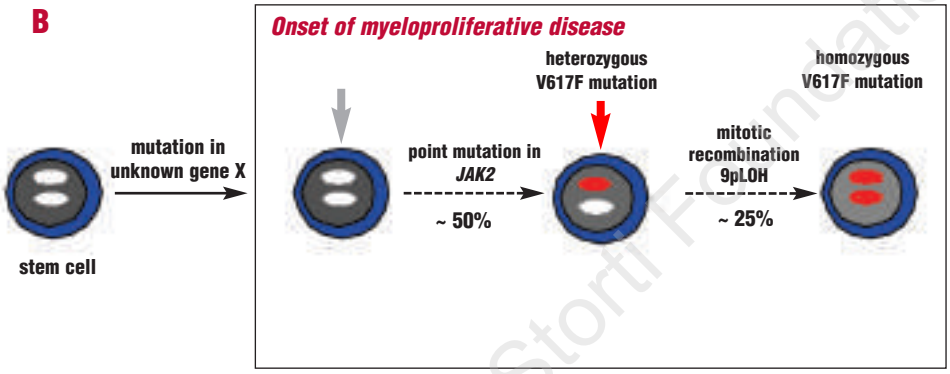


Figure 1. Models of the potential pathogenetic role of the *Jak2* V617F mutation in myeloproliferative disorders. Two models (A and B), differing for the time at which the disease phenotype appears, are shown. In model A, a point mutation in a hematopoietic progenitor or stem cell (red arrow) changes a valine in position 617 of *JAK2* to a phenylalanine (V617F). The *JAK2* V617F mutation alone causes the onset of the myeloproliferative phenotype in about 50% of the patients (red arrow). In model B, the *JAK2* V617F mutation occurs after the onset of the myeloproliferative phenotype, which is caused by mutation(s) in as yet unknown gene(s) (gray arrow). Chromosomes carrying the *JAK2* V617F allele are drawn in red; chromosomes with the wild type *JAK2* allele are white. Cells that are heterozygous for the V617F mutation have a proliferative advantage over cells homozygous for the wild type allele. Mitotic recombination between homologous chromosomes 9 in a cell heterozygous for V617F results in loss of heterozygosity (9pLOH). One of the daughter cells is homozygous for V617F and gains additional proliferative advantage. This cell establishes a subclone that outcompetes cells heterozygous for V617F and cells homozygous for wild type *JAK2*.

ries *JAK2* V617F on one of the two chromosomes 9p will result in uniparental disomy and transition from *JAK2* V617F heterozygosity to homozygosity (Figure 1). Homologous mitotic recombination is vital in the repair of damage that occurs during DNA replication[31] but is also a frequent genetic mechanism in the inactivation of tumor-suppressor genes in solid tumors. Recently, partial uniparental disomy due to somatic recombination has been described in acute myeloid leukemia.[32] Additional studies showed that *JAK2* V617F was a somatic mutation present in myeloid hematopoietic cells but not in T lymphocytes or hair-follicle cells.

Functional consequences of the *JAK2* mutation on cell growth and survival

JAK2 plays an essential role in hematopoiesis by mediating signals from several hematopoietic cytokines, including erythropoietin, thrombopoietin and granulocyte colony-stimulating factor.[33,34] The effects of the *JAK2* V617F mutation appear to depend on the cellular context. In non-hematopoietic 293T cells the *JAK2* V617F protein is strongly phosphorylated in the absence of exogenous stimuli. In hematopoietic cell lines, such as BaF3 or UT7 cells, the effect of the mutation is more subtle and involves a reduced propensity to apoptosis and a modest proliferative advantage.[7] The mutant *JAK2* V617F protein was only slightly more efficient than the wild-type one, and its activity was clearly less dramatic than that of the TEL-JAK2 fusion protein, which results from the t(9;12)(p24;p13) chromosomal translocation in T-cell childhood acute lymphoblastic leukemia.[35] The TEL-JAK2 fusion protein causes constitutive activation of its tyrosine kinase activity and confers cytokine-independent proliferation to the interleukin-3-dependent BaF3 cell line. The mutant JAK2 V617F protein primarily resulted in a better transduction of the signals induced by growth factors at low concentrations. Interestingly, mice transplanted with bone marrow cells that were transduced by retrovirus expressing the *JAK2* V617F mutant developed erythrocytosis.[4] No information on other lineages is available. Further studies will be required to understand the precise mechanism by which the mutation contributes to myeloproliferation. In any case, gain of function involves loss of control and overproduction of mature cells.

It is intriguing that the identical amino acid exchange occurred in all patients examined to date and no other *JAK2* alleles have been found. In contrast, translational pathophysiology arises from various point mutations or deletions.[26] Another open question is how the *JAK2* V617F mutation is connected to the responsiveness of some patients with polycythemia vera to imatinib mesylate. In fact, a constitutively active *JAK2* can cooperate with either c-kit or flt-3 in signaling to induce self-renewal of multipotent hematopoietic cells.[36]

Table 1. Frequency of the *JAK2* V617F mutation in patients with chronic myeloproliferative disorders.

Authors	Polycythemia vera	Essential thrombocythemia	Idiopathic myelofibrosis
DNA sequencing			
James *et al.*[4]	40/45 (89%)	9/21 (43%)	3/7 (43%)
Levine *et al.*[6]	121/164 (74%)	37/115 (32%)	16/46 (35%)
Kralovics *et al.*[7]	83/128 (65%)	21/93 (23%)	13/23 (57%)
Baxter *et al.*[5]	*53/73 (73%)	6/51 (12%)	7/16 (44%)
Zhao *et al.*[9]	20/24 (83%)	n.d.	n.d.
Jones *et al.*[10]	58/81 (81%)	24/59 (41%)	15/35 (43%)
Total	375/506 (74%)	97/339 (29%)	55/127 (43%)
Allele-specific PCR			
Baxter *et al.*[5]	71/73 (97%)	29/51 (57%)	8/16 (50%)

Clinical consequences of the JAK2 V617F mutation

By pooling all currently published data that were generated by DNA sequencing, the frequency of *JAK2* V617F is 74% (375/506) in polycythemia vera , 29 % (97/339) in essential thrombocythemia and 43% (55/127) in idiopathic myelofibrosis (Table 1). Occasionally, the mutation has been found in cases of atypical chronic myeloid leukemia, myelodysplastic syndrome, hypereosinophilic syndrome and systemic mastocytosis.[8,10] The mutation was absent in 617 healthy individuals,[27] in patients with chronic myeloid leukemia and in patients with secondary erythrocytosis.

A much higher frequency of *JAK2* V617F in myeloproliferative disorders was described in one study that used allele-specific polymerase chain reaction (PCR) analysis in addition to sequencing (Table 1). Sequencing is less sensitive (at least 25% of cells must carry the mutation to be detected) than allele-specific PCR (already 2% of cells with the mutation can be detected). However, we should be cautious of considering patients who carry the *JAK2* mutation in a very small proportion of their granulocytes (e.g., 2-3%) as being *JAK2* V617F-positive. It is unlikely that the *JAK2* mutation present in such a small percentage of cells can be the cause of myeloproliferative disease in these patients, and it is conceivable that it is a secondary event present only in a subclone of cells. In the future, in addition to scoring the presence or absence of *JAK2* V617F, we will need quantitative data for each patient in order to provide information about what percentage of chromosomes in a given cell population (e.g., granulocytes) carries the *JAK2* mutation.

Patients with myeloproliferative disorders who carried *JAK2* V617F had a longer median disease duration and were more likely to develop complications such as secondary myelofibrosis, bleeding or thrombosis than were patients without the mutation.[7] A trend to more leukemic transformation was noted but was not statistically significant. Furthermore, the presence of the *JAK2* V617F mutation correlated with the presence of endogenous erythroid colonies.[7] More extensive studies will be needed to examine these correlations further. For these studies, we will need to take into account the variable percentage of cells positive for *JAK2* V617F in patients with myeloproliferative disorders. Correlations can only be expected if the *JAK2* mutation is present in a relevant fraction of the patient's cells and this threshold will need to be defined.

Polycythemia vera, essential thrombocythemia and idiopathic myelofibrosis have different hematologic and clinical phenotypes, but the *JAK2* mutation is not restricted to a single clinical entity. The majority of patients with polycythemia vera are positive for the *JAK2* V617F mutation, but there are patients in whom the mutation is definitely absent or present in only a small fraction of the cells. This raises questions about the pathogenetic role of the mutation. Nevertheless, the presence of the *JAK2* V617F mutation will be a key diagnostic features of polycythemia vera in the future. The role of the *JAK2* V617F mutation in essential thrombocythemia is less clear. Only a minority of patients with this disease have a mutation in *JAK2* and very few progress to 9pLOH and homozygosity. Similarly, about half of the patients with idiopathic myelofibrosis do not have a *JAK2* mutation. Thus, there must be other molecular lesions responsible for myeloproliferation in these patients. One could argue that patients with essential thrombocytosis or idiopathic myelofibrosis who are positive for the *JAK2* V617F mutation might be *misdiagnosed* cases of thrombocythemia that will later evolve into polycythemia vera or of myelofibrosis that developed from a clinically unapparent polycythemia, respectively. Since there are currently no data to verify these hypotheses, discussions about the *correct diagnosis* at times take a religious character. Clearly, none of the molecular or biological assays described to date have respected the boundaries of the clinically defined entities. Identifying the additional molecular lesion(s) responsible for disease initiation and progression will be of fundamental importance and will eventually make the debates about the *right* diagnostic approach obsolete.

Possible pathogenetic roles of the JAK2 V617F mutation in myeloproliferative disorders

Although the currently available data clearly demonstrate that *JAK2* V617F participates in the pathogenesis of myeloproliferative disorders, the mutation's precise place in the hierarchical order of events remains to be established. In the simplest model, *JAK2* V617F is the primary cause of myeloproliferative disorders in about 50% of the patients (Figure 1A). According to this model, the *JAK2* mutation is responsible for the onset of clinical disease as a single step event. Gain of function and loss of control would be the essential features of myeloproliferation caused by *JAK2* V617F. In the other half of the patients, the myeloproliferative disease is caused by a mutation in an as yet unknown gene. This model is supported by the fact that irradiated mice transplanted with retrovirally transduced bone marrow cells expressing *JAK2* V617F developed erythrocytosis.[4] However, retroviral transduction can result in high levels of expression, which may cause phenotypes that are not seen when the protein is expressed at more physiological levels. Furthermore, this model hardly explains why clinically different diseases should arise from the same mutation. An alternative model proposes that *JAK2* V617F is not the primary cause, but rather a secondary event associated with disease progression (Figure 1B). According to this model, a somatic mutation in an as yet

unknown gene or genes causes the onset of the disease. Different mutations and/or genes could be involved in initiating the different clinical entities of myeloproliferative disorders. Acquisition of *JAK2* V617F would make the disease more aggressive, which could explain the reported higher frequency of complications in this group. This model is also compatible with the observed shorter median disease duration in patients without *JAK2* V617F and with the frequent presence of small numbers of cells with the *JAK2* mutation that are detectable only with allele-specific PCR. The prediction of the first model is that drugs specifically targeting the mutant *JAK2* protein might cure the disease, whereas if the second model were correct, such a therapy would merely reset the disease to an earlier stage. These and other open questions will be exciting to address and for the first time we have the tools to do this.

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