Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders


Summary

Background Human myeloproliferative disorders form a range of clonal haematological malignant diseases, the main members of which are polycythaemia vera, essential thrombocythaemia, and idiopathic myelofibrosis. The molecular pathogenesis of these disorders is unknown, but tyrosine kinases have been implicated in several related disorders. We investigated the role of the cytoplasmic tyrosine kinase JAK2 in patients with a myeloproliferative disorder.

Methods We obtained DNA samples from patients with polycythaemia vera, essential thrombocythaemia, or idiopathic myelofibrosis. The coding exons of JAK2 were bidirectionally sequenced from peripheral-blood granulocytes, T cells, or both. Allele-specific PCR, molecular cytogenetic studies, microsatellite PCR, Affymetrix single nucleotide polymorphism array analyses, and colony assays were undertaken on subgroups of patients.

Findings A single point mutation (Val617Phe) was identified in JAK2 in 71 (97%) of 73 patients with polycythaemia vera, 29 (57%) of 51 with essential thrombocythaemia, and eight (50%) of 16 with idiopathic myelofibrosis. The mutation is acquired, is present in a variable proportion of granulocytes, alters a highly conserved valine present in the negative regulatory JH2 domain, and is predicted to dysregulate kinase activity. It was heterozygous in most patients, homozygous in a subset as a result of mitotic recombination, and arose in a multipotent progenitor capable of giving rise to erythroid and myeloid cells. The mutation was present in all erythropoietin-independent erythroid colonies.

Interpretation A single acquired mutation of JAK2 was noted in more than half of patients with a myeloproliferative disorder. Its presence in all erythropoietin-independent erythroid colonies demonstrates a link with growth factor hypersensitivity, a key biological feature of these disorders.

Relevance to practice Identification of the Val617Phe JAK2 mutation lays the foundation for new approaches to the diagnosis, classification, and treatment of myeloproliferative disorders.

Introduction

The human myeloproliferative disorders represent a range of clonal haematological malignant diseases, with three main members: polycythaemia vera, essential thrombocythaemia, and idiopathic myelofibrosis. These three disorders are all thought to reflect transformation of a multipotent haemopoietic stem cell,1 but their molecular pathogenesis remains obscure. The absence of definitive diagnostic tests and the scarcity of randomised controlled trials make management of these diseases especially challenging.2

The myeloproliferative disorders are characterised by overactive haemopoiesis, with increased production of red cells and platelets the major feature of polycythaemia vera and essential thrombocythaemia, respectively. The main clinical complication in these two disorders is thrombosis, although haemorrhage can also happen. In the longer term, a few patients with polycythaemia vera and essential thrombocythaemia might develop myelofibrosis or acute myeloid leukaemia. Late-stage idiopathic myelofibrosis is characterised by bone-marrow fibrosis, cytopenia, and splenomegaly, and can also transform to acute myeloid leukaemia.

Erythroid progenitor cells from patients with polycythaemia vera form colonies in the absence of erythropoietin.3 Subsequently, investigators showed that both erythroid and myeloid progenitor cells from these patients are hypersensitive to several different growth factors4,5 and that similar abnormalities are present in a proportion of patients with essential thrombocythaemia and idiopathic myelofibrosis.6,7 These observations suggest that the primary abnormality in myeloproliferative disorders might be in a signalling component downstream of multiple growth-factor receptors.8

Several lines of evidence suggest that kinases, particularly tyrosine kinases, represent attractive candidate genes. The BCR-ABL tyrosine kinase fusion protein causes the related disorder chronic myeloid leukaemia,9 and several tyrosine kinases are altered in patients with other myeloid malignant diseases,10 including FLT3 in acute myeloid leukaemia, PDGFRβ in chronic myelomonocytic leukaemia, PDGFRα in chronic eosinophilic leukaemia, and KIT in systemic mastocytosis.

JAK2 is a cytoplasmic tyrosine kinase with a key role in signal transduction from multiple haemopoietic growth-factor receptors and so is an especially attractive candidate gene.11,12 As part of our continuing assessment of protein kinase genes in patients with a myeloproliferative disorder, we sequenced the coding exons of
JAK2 in peripheral-blood granulocytes from patients with polycythaemia vera, essential thrombocythaemia, or idiopathic myelofibrosis.

Methods
Study population
Our study was approved by Addenbrooke’s NHS Trust local research ethics committee. We recruited patients from the myeloproliferative disorders clinic at Addenbrooke’s Hospital and other haematology clinics. Written informed consent was obtained from every patient. We prospectively gathered data for baseline characteristics and diagnostic information, and we defined clinical events according to standard criteria by retrospective case-note review. Patients’ diagnoses were defined according to published criteria.

Procedures
We took a blood sample from every patient. Peripheral-blood granulocytes, T cells, and bone marrow cells were purified and DNA extracted as described previously. Control DNA samples came from a population of patients with type 1 diabetes.

Mutation screening
We designed primers to amplify coding exons and splice junctions of the gene. Primer sequences are available from the authors. Genomic DNA was amplified and PCR products were directly sequenced in both directions on ABI 3730 or 3100 machines by BigDye terminator sequencing (Applied Biosystems, Foster City, CA, USA). We analysed sequencing traces both manually and with Mutation Surveyor (version 2.0; Softgenetics, State College, PA, USA). Paired granulocyte and T cell samples were analysed using a 10K single nucleotide polymorphism (SNP) array (Affymetrix, High Wycombe, UK) to prove they were from the same individual and to determine allele copy number and loss of heterozygosity.

Allele-specific PCR
80 ng of patient’s DNA was amplified in a 36-cycle PCR reaction at an annealing temperature of 58°C. We used 1 μmol/L of a common reverse primer and 0·5 μmol/L of two forward primers (panel). The first forward primer is specific for the mutant allele and contains an intentional mismatch at the third nucleotide from the 3’ end to improve specificity (giving a 203-bp product); the second amplifies a 364-bp product from both mutant and wild-type alleles and serves as an internal PCR control.

Haemopoietic colony assays
We mixed 25 mL of heparinised peripheral blood in a 1/1 ratio with Hank’s balanced salt solution and layered this mixture over lymphocyte separation medium (Axis Shield Diagnostics, Huntingdon, UK). Mononuclear cells were obtained and washed in Hank’s balanced salt solution. We plated cells at 5×10⁴ per mL in Methocult H4531 (Stem Cell Technologies, London, UK), according to the manufacturer’s instructions, with and without erythropoietin at 2 IU/mL (Eprex, Janssen-Cilag, High Wycombe, UK). We incubated plates at 37°C in a 5% carbon dioxide 95% air mixture in a humidified incubator for 8–10 days. Haemopoietic colonies were scored by standard morphological criteria using an inverted microscope.

Mutation screening of haemopoietic colonies
Individual colonies were harvested into 100 μL deionised water and heated at 95°C for 10 min to release DNA, before being amplified in the same manner as the patient DNA. The amplified DNA was sequenced on an ABI 3730 or 3100 machine and then analysed using Mutation Surveyor.

Panel: Primers used in the study
Mutation screening of JAK2
Available from authors
Allele-specific PCR
Reverse: 5’ CTGAAATAGTCTACGTGTTTCACTTTCA3’
Forward (specific): 5’ AGCATTGGTTTAAATTATGGAGTATATT3’
Forward (internal control): 5’ ATCTATAGTCATGCTGAAAGTAGGAGAAAG3’

Mutation screening of haemopoietic colonies
JAK2 exon 12 sequence
Forward: 5’ GGTTTTCCCTCAGAAAGTTGA3’
Reverse: 5’ TCATTGCTTTTCTTTTCAATAAA3’

Human SCL control
Forward: 5’ TCCTGAGGCCATGGGGAAT3’
Reverse: 5’ CCGAGAGGCAATGGGAGTA3’

Figure 1: Sequence traces showing wild-type sequence and G→T mutation in JAK2
Wild-type pattern in a patient with idiopathic myelofibrosis. Mixed and mutant sequences in two patients with polycythaemia vera. Arrows indicate the relevant base.
genomic DNA. The human JAK2 exon 12 sequence was amplified from 5 μL of colony DNA by PCR with the primers shown in the panel. As a control, a human SCL (HUGO-approved name TAL1) intron 1 sequence was independently amplified from 5 μL colony DNA with the primers shown in the panel. After 45 PCR cycles with an annealing temperature of 57ºC the resulting 460-bp amplified fragment was digested with BsaXI (New England Biolabs, Hitchin, UK) for 4 h at 37ºC then analysed on a 2% agarose gel. The mutant allele remained undigested whereas the wild-type allele was digested into 241 bp, 189 bp, and 30 bp fragments. The 496 bp SCL genomic fragment was digested into 356 bp, 110 bp and 30 bp fragments.

Loss of heterozygosity analysis of chromosome 9p
Published primer sequences were used for markers D9S925, D9S288, and D9S199 (http://www.ncbi.nlm.nih.gov/genome/sts/), with the reverse primer in each case fluorescently labelled at 5′ end with 6-carboxyfluorescein. For every patient, we amplified 50 ng of DNA from peripheral-blood granulocytes and T cells with standard PCR conditions and an annealing temperature of 56ºC. Products were diluted 1 in 25 with water and mixed with Genescan 350ROX size standard (Applied Biosystems), according to the manufacturer’s instructions, and run on an ABI3100 genetic analyser.

Fluorescence in-situ hybridisation (FISH)
We obtained bacterial artificial chromosome (BAC) RP11-192B11 from the Wellcome Trust Sanger Institute clone resource (Hinxton, UK). We prepared BAC DNA as described previously; spectrum orange dUTPs were incorporated with the nick translation kit (Abbott Diagnostics, Maidenhead, UK), according to the manufacturer’s instructions. 400 ng of BAC probe and 250 ng spectrum green chromosome 9 centromeric probe (Abbott Diagnostics) were hybridised to fixed patient bone-marrow metaphases, as described previously.

Statistical analysis
We compared the clinical characteristics and complications of patients with and without a JAK2 mutation with the χ² test or Fisher’s exact test for categorical variables, the Wilcoxon rank-sum test for continuous variables, and the log-rank test for time-to-event variables (clinical complications after diagnosis).

Role of the funding source
The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
In 66 of 140 patients with a myeloproliferative disorder, a G→T alteration was detected in exon 12 of JAK2 by sequence analysis of peripheral-blood granulocytes—53 of 73 patients with polycythaemia vera, six of 51 with essential thrombocythaemia, and seven of 16 with idiopathic myelofibrosis (figure 1; table 1). This alteration was within the pseudokinase (JH2) domain and changed a highly conserved valine at position 617 to phenylalanine (figure 2). In 30 patients with the Val617Phe mutation, the sequence pattern obtained from purified T cells was wild-type (figure 1), showing that the mutation was acquired. In 19 (26%) patients with polycythaemia vera and three (19%) of those with idiopathic myelofibrosis, the mutant T allele was predominant (>80% of total peak height). By contrast, no patient with essential thrombocythaemia had this pattern. Sequence analysis of all JAK2 coding exons in 20 patients (15 with polycythaemia vera, one with essential thrombocythaemia, and four with idiopathic
myelofibrosis) detected several common SNPs, but no other mutations were recorded. The Val617Phe mutation was not detected in 90 control samples by either sequencing or allele-specific PCR (table 1).

In some patients with a myeloproliferative disorder, only a proportion of peripheral-blood granulocytes might derive from malignant progenitor cells. Sequencing can, therefore, underestimate the proportion of patients carrying a JAK2 mutation since it will reproducibly detect a heterozygous mutation only if it is present in more than 40% of cells (i.e., >20% of alleles). We established a sensitive allele-specific PCR assay capable of detecting a heterozygous mutation present in 3% of cells (data not shown). This assay detected the Val617Phe mutation in a substantial proportion of patients with a wild-type sequence pattern, but not in 90 controls (figure 3; table 1). Combining the sequencing and mutation-specific PCR results, the Val617Phe mutation was detected in 71 of 73 (97%) patients with polycythaemia vera, 29 of 51 (57%) with essential thrombocythaemia, and eight of 16 (50%) with idiopathic myelofibrosis (table 1).

The mutant sequence pattern (figure 1) might indicate a hemizygous mutation (with deletion of the normal allele) or a homozygous mutation. Metaphase FISH and microsatellite PCR were used to distinguish these possibilities in three cytogenetically normal patients with polycythaemia vera and a mutant sequence pattern in their granulocytes. Microsatellite PCR showed extensive loss of heterozygosity on chromosome 9p but a BAC adjacent to JAK2 hybridised to both chromosome 9 homologs (figure 4). These results indicate that in these patients, mitotic recombination has resulted in homozygosity for the mutation. This conclusion was independently verified by hybridisation of granulocyte DNA from two additional patients to a SNP array, which showed extensive loss of heterozygosity on chromosome 9p in the presence of a diploid copy number (data not shown).

To assess the prevalence of the Val617Phe mutation in haematopoietic progenitor cells, individual colonies were grown from four patients and analysed with a restriction enzyme-based assay (figure 5; table 2). The G→T mutation destroys a BsaXI site present in the wild-type
JAK2 sequence. This approach, therefore, allows both normal and mutant alleles to be visualised and can distinguish between colonies carrying heterozygous and homozygous mutations. The Val617Phe mutation was identified in granulocyte-macrophage colonies and erythroid colonies, showing that it arises in a multipotent progenitor capable of giving rise to both erythroid and myeloid lineages. Sequence analysis of peripheral-blood granulocytes from patients 1 and 2 showed a mixed pattern, which might represent either a population of cells, most or all of which carry a heterozygous mutation, or the presence of a smaller proportion of cells carrying a homozygous mutation. In accordance with the first interpretation, all colonies carrying the mutation were heterozygous. In colonies grown in the presence of exogenous growth factors, the Val617Phe mutation was present in a significantly higher proportion of erythroid colonies than granulocyte-macrophage colonies (table 2). Of particular note, the Val617Phe mutation was present in all erythropoietin-independent erythroid colonies, consistent with a role for activated JAK2 in the growth factor-independent phenotype.

Our results show that patients with essential thrombocythaemia and idiopathic myelofibrosis show genetic heterogeneity, with about half lacking the Val617Phe mutation. Comparison of clinical and laboratory features between patients with and without the mutation did not reveal any significant differences (table 3). Although numbers are small, acquired chromosomal abnormalities, thrombotic events, and myelofibrotic transformation occurred in individuals with essential thrombocythaemia who were positive and negative for the mutation. Only two of 73 patients with polycythaemia vera did not have the Val617Phe mutation. Review of the presentation and clinical course of these two patients did not reveal any atypical features.

DNA samples were available at diagnosis or within a year of diagnosis from 27 patients with polycythaemia vera. Seven of these (26%) were homozygous for the Val617Phe mutation (ie, had a predominantly mutant sequence pattern) compared with 19 (26%) of the whole study cohort with polycythaemia vera. These data show that homozygosity for the Val617Phe mutation is present at an early stage and is not restricted to longstanding disease.

Familial clustering of patients with a myeloproliferative disorder is uncommon but well documented. Five patients (four with polycythaemia vera and one with essential thrombocythaemia) with a Val617Phe mutation had one or more first-degree relatives with a myeloproliferative disorder, raising the possibility of familial inheritance.

### Table 2: Pattern of mutation frequency in different haemopoietic colonies from four patients with myeloproliferative disorders who showed a mixed pattern of mutation on sequencing

<table>
<thead>
<tr>
<th></th>
<th>CFU-GM Mutant</th>
<th>Wild-type</th>
<th>BFU-E Mutant</th>
<th>Wild-type</th>
<th>EEC Mutant</th>
<th>Wild-type</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, essential thrombocythaemia</td>
<td>24</td>
<td>21</td>
<td>38</td>
<td>7</td>
<td>NP</td>
<td>NP</td>
<td>0.003</td>
</tr>
<tr>
<td>2, essential thrombocythaemia</td>
<td>10</td>
<td>25</td>
<td>28</td>
<td>17</td>
<td>25</td>
<td>0</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>3, polycythaemia vera</td>
<td>ND</td>
<td>ND</td>
<td>8</td>
<td>16</td>
<td>17</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4, polycythaemia vera</td>
<td>NP</td>
<td>NP</td>
<td>10</td>
<td>11</td>
<td>22</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are number of colonies of each genotype. CFU-GM=granulocyte-macrophage colonies. BFU-E=erythroid colonies. EEC=erythropoietin-independent erythroid colonies. ND=not determined. NP=none present. *p refers to the null hypothesis that the mutation is present in all three colony types in the same proportion. Pairwise comparison between CFU-GM and BFU-E was also significant (p<0.005).
The molecular basis for essential X-chromosome inactivation patterns, erythropoietin-conclusion that accords with previous studies of that these disorders are genetically heterogeneous, a substantial proportion of granulocytes are polyclonal and therefore normal. Identification of the Val617Phe mutation will also stimulate clinical development of small-molecule inhibitors of JAK2, which could provide novel approaches to treatment.

Sequence analysis suggests that malignant cells in most patients positive for Val617Phe are heterozygous for the mutation, a conclusion confirmed by analysis of individual haemopoietic colonies from such individuals. However, sequence analysis shows that the mutant peak predominates in a quarter of patients with polycythaemia vera. Our data indicate that in these individuals, mitotic recombination has resulted in homozygosity for the mutation. These results accord with those of a previous report showing that loss of heterozygosity on chromosome 9p happens in about 30% of patients with polycythaemia vera, and have at least two implications. First, they suggest that, in a subset of patients, at least two sequential changes have taken place at an early stage of clinical disease, with acquisition of a Val617Phe mutation as the first event and mitotic recombination the second event. Second, they indicate that the mutation might be dose-dependent, with cells carrying two copies having a selective advantage. Our results show that the first event is acquired in most patients but do not exclude the possibility that it could be inherited in a few patients. It remains unclear why the same mutation can be associated with three different disease phenotypes. Possible explanations include interindividual differences in genetic background, in the acquisition of additional genetic changes, or in the target cell for transformation.

There are only two explanations for the presence of a somatic mutation in the majority of cells of any malignant disease: the mutation might contribute to the pathogenesis of the latter possibility is highly unlikely as an explanation for the Val617Phe mutation, since most patients with a myeloproliferative disorder had the same alteration. This reasoning strongly suggests that the JAK2 mutation contributes to the pathogenesis of myeloproliferative disorders. Furthermore, we have shown that only erythroid progenitors carrying the mutation were able to grow in the absence of erythropoietin, whereas both mutant and normal progenitors were able to grow in the presence of constitutional mutation of JAK2 in these families. However, sequence analysis showed that the Val617Phe mutation was not present in buccal cells, T cells, or both in the five probands. These results indicate that a constitutional Val617Phe mutation is not a common cause of familial clustering.

**Discussion**

The myeloproliferative disorders were classified as a spectrum of related diseases in 1951 by Dameshek. The prescience of this insight is emphasised by the results presented here, which show that a single mutation is shared by patients with polycythaemia vera, essential thrombocytopenia, and idiopathic myelofibrosis and underlie the many similarities between these disorders. The observation that the Val617Phe alteration is seen in only 50% of patients with essential thrombocytopenia or idiopathic myelofibrosis shows that these disorders are genetically heterogeneous, a conclusion that accords with previous studies of X-chromosome inactivation patterns, erythropoietin-independent colonies, and polycythaemia rubra vera 1 (PRV1) expression. The molecular basis for essential thrombocytopenia and idiopathic myelofibrosis in patients without a Val617Phe alteration remains unclear.

To distinguish myeloproliferative disorders from reactive conditions, particularly secondary thrombocytosis and erythrocytosis, can be difficult and so detection of the Val617Phe mutation could become a widely used diagnostic test. The sensitive allele-specific PCR assay is likely to be especially useful since we show that, in some patients, the mutation is carried only by a subset of granulocytes. This observation accords with findings of several groups, who have used X-chromosome inactivation patterns to show that, in some patients (particularly those with essential thrombocytopenia), a substantial proportion of granulocytes are polyclonal and therefore normal.

**Table 3: Clinical characteristics at diagnosis of patients with and without the JAK2 mutation**

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>Val617Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essential thrombocytopenia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 (17)</td>
<td>55 (16)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (55%)</td>
<td>15 (52%)</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>141 (13)</td>
<td>141 (12)</td>
</tr>
<tr>
<td>White-cell count ($\times 10^9/L$)</td>
<td>9.4 (2.7)</td>
<td>10.9 (4.4)</td>
</tr>
<tr>
<td>Platelet count ($\times 10^9/L$)</td>
<td>926 (242)</td>
<td>1024 (346)</td>
</tr>
<tr>
<td>Splenic palpable</td>
<td>5 (23%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Abnormal karyotype</td>
<td>4 (18%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Complications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombosis</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Transformation to myelofibrosis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Transformation to polycythaemia vera</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>Val617Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Idiopathic myelofibrosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60 (9)</td>
<td>65 (8)</td>
</tr>
<tr>
<td>Female</td>
<td>5 (63%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>102 (28)</td>
<td>107 (26)</td>
</tr>
<tr>
<td>White-cell count ($\times 10^9/L$)</td>
<td>13.1 (8.9)</td>
<td>21.6 (12.3)</td>
</tr>
<tr>
<td>Platelet count ($\times 10^9/L$)</td>
<td>599 (258)</td>
<td>364 (194)</td>
</tr>
<tr>
<td>Splenic size (cm below costal margin by palpation)</td>
<td>7.4 (4.0)</td>
<td>6.4 (6.9)</td>
</tr>
<tr>
<td>Abnormal karyotype</td>
<td>0</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Complications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Major haemorrhage</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Data are mean (SD) or number of patients (%). No significant differences noted between patients with and without the mutation.
Mechanisms of Disease

erythropoietin. This finding links the JAK2 mutation to the phenotype of growth factor independence.

JAK2 is a widely expressed cytoplasmic tyrosine kinase, which has a central role in transduction of signals from multiple growth-factor receptors. An activating mutation is therefore, completely consistent with the growth factor hypersensitiviy shown by haemopoietic progenitor cells from patients with a myeloproliferative disorder. Moreover, a TEL-JAK2 fusion protein with constitutive kinase activity is associated with rare cases of lymphoid or myeloid leukaemia.15,16 Several lines of evidence suggest that the Val617Phe mutation results in dysregulated JAK2 kinase activity. First, the mutation is located in the JAK2 pseudokinase (JH2) domain, which negatively regulates activity of the kinase (JH1) domain.29,30 Second, structural modelling suggests that valine 617 and cysteine 618 are both important for maintenance of the kinase domain of JAK2 in an inactive conformation.31 Replacement of valine 617 with a bulky phenylalanine is predicted to destabilise this inhibitory interaction, leading to increased kinase activity. Finally, a mutation within the JH2 domain activates the kinase activity of a drosophila JAK homolog and results in a leukaemia-like disorder.32

Dysregulated JAK2 kinase activity might account for some of the previously identified biological markers for the myeloproliferative disorders, such as BCL-X (BCL2L1),13 PRV1,14 and MPL.15 No mutations have been detected in the corresponding genes and so the perturbed expression of these molecules seems to be a secondary consequence of the primary abnormality. It is therefore pertinent to note that JAK2 activates STAT5, which in turn upregulates transcription of BCL-X.3 Similarly, the JAK/STAT pathway has been implicated in megakaryocytic and erythroid differentiation in patients with myeloproliferative disorders.3,14 By contrast, increased phosphorylation of JAK2 was not detected in platelets from patients with polycythaemia vera.3 The reason for this finding is unclear but could include a fairly subtle effect of the Val617Phe mutation on kinase activity, existence of cell-specific feedback regulation, or presence of normal platelets and clonally derived platelets in some patients with myeloproliferative disorders.

For more than a quarter of a century, the myeloproliferative disorders have been known to be clonal haematological malignancies, but the identity of underlying target genes has remained elusive. Here, we have shown that a single acquired point mutation in JAK2 is present in virtually all patients with polycythaemia vera and in about half of those with either essential thrombocythaemia or idiopathic myelofibrosis. These results have important implications for the classification, diagnosis, and treatment of these diseases and provide insight into their pathogenesis.

Acknowledgments

We thank B Gottgens and G Follows for helpful discussions; K Andreas, B Manasse, and G Bryant for cytogenetic analysis; I Joy for technical assistance; and D G Gilliland and R Skoda for sharing data before publication. This work was supported in part by the UK Leukaemia Research Fund and the Wellcome Trust.

References

Mechanisms of Disease