ORIGINAL ARTICLE

A Gain-of-Function Mutation of *JAK2* in Myeloproliferative Disorders

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ABSTRACT

BACKGROUND

Polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis are clonal myeloproliferative disorders arising from a multipotent progenitor. The loss of heterozygosity (LOH) on the short arm of chromosome 9 (9pLOH) in myeloproliferative disorders suggests that 9p harbors a mutation that contributes to the cause of clonal expansion of hematopoietic cells in these diseases.

METHODS

We performed microsatellite mapping of the 9pLOH region and DNA sequencing in 244 patients with myeloproliferative disorders (128 with polycythemia vera, 93 with essential thrombocythemia, and 23 with idiopathic myelofibrosis).

RESULTS

Microsatellite mapping identified a 9pLOH region that included the Janus kinase 2 (*JAK2*) gene. In patients with 9pLOH, *JAK2* had a homozygous G→T transversion, causing phenylalanine to be substituted for valine at position 617 of *JAK2* (V617F). All 51 patients with 9pLOH had the V617F mutation. Of 193 patients without 9pLOH, 66 were heterozygous for V617F and 127 did not have the mutation. The frequency of V617F was 65 percent among patients with polycythemia vera (83 of 128), 57 percent among patients with idiopathic myelofibrosis (13 of 23), and 23 percent among patients with essential thrombocythemia (21 of 93). V617F is a somatic mutation present in hematopoietic cells. Mitotic recombination probably causes both 9pLOH and the transition from heterozygosity to homozygosity for V617F. Genetic evidence and in vitro functional studies indicate that V617F gives hematopoietic precursors proliferative and survival advantages. Patients with the V617F mutation had a significantly longer duration of disease and a higher rate of complications (fibrosis, hemorrhage, and thrombosis) and treatment with cytoreductive therapy than patients with wild-type *JAK2*.

CONCLUSIONS

A high proportion of patients with myeloproliferative disorders carry a dominant gain-of-function mutation of *JAK2*.

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The myeloproliferative disorders are a heterogeneous group of diseases characterized by excessive production of blood cells by hematopoietic precursors. In addition to thrombotic and hemorrhagic complications, leukemic transformation can occur.[1] Typically, the myeloproliferative disorders encompass four related entities[2]: chronic myelogenous leukemia (CML), polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis. Clonal hematopoiesis is a key feature of these disorders.[3-5] The lesion is believed to involve the hematopoietic stem cell, since all myeloid lineages and, frequently, the B-cell lineage are monoclonal. T cells, however, are polyclonal.[4,6]

Progenitor cells in polycythemia vera form erythroid colonies in the absence of exogenous erythropoietin. These endogenous erythroid colonies[7] have been used in an auxiliary diagnostic assay to distinguish polycythemia vera from secondary erythrocytosis,[8] but they also occur in some cases of essential thrombocythemia and idiopathic myelofibrosis. More important, the presence of endogenous erythroid colonies is a hallmark of abnormal in vitro growth of hematopoietic progenitors, and this finding has been the basis of many studies of signaling by cytokine receptors of hematopoietic cells. These receptors transduce signals by activating members of the Janus kinase (JAK) family of proteins, which phosphorylate cytoplasmic targets, including the signal transducers and activators of transcription (STATs).[9] Constitutive activation of the STAT3 protein has been found in 30 percent of patients with polycythemia vera,[10] and a decrease in the level of the thrombopoietin receptor protein in platelets is a feature of both polycythemia vera and essential thrombocythemia.[11,12] Only the late steps of differentiation of endogenous erythroid colonies in polycythemia vera are erythropoietin-independent, and they can be blocked by inhibitors of JAK2, phosphatidylinositol 3′ kinase, or kinases of the Src family.[13] Recently, the tyrosine kinase inhibitor imatinib mesylate has been reported to produce clinical responses in patients with polycythemia vera.[14,15] These data suggest the involvement of a kinase in the pathogenesis of myeloproliferative disorders.

Cytogenetic abnormalities occur in only 10 to 15 percent of patients with myeloproliferative disorders.[16-18] The most common abnormalities are deletions in chromosome 20q.[19,20] Fluorescence in situ hybridization and comparative genomic hybridization suggested a role for chromosome 9p.[20-23] Using genome-wide microsatellite screening, we identified loss of heterozygosity (LOH) on the short arm of chromosome 9 (9pLOH) in six patients with polycythemia vera[24]; using microsatellite markers within the LOH region, we found 9pLOH in 13 of 43 patients with polycythemia vera and 1 of 15 patients with essential thrombocythemia.[24,25] Markers from the 9pLOH region did not cosegregate with the phenotype in four families with polycythemia vera, suggesting that a somatic event causes 9pLOH.[26] In the present study, we increased the number of microsatellite markers in order to map a minimal genomic region shared by all patients with 9pLOH and myeloproliferative disorders to identify potential candidate genes.

METHODS

SUBJECTS

We evaluated 244 patients with myeloproliferative disorders from Switzerland and Italy: 128 patients with polycythemia vera, 93 with essential thrombocythemia, and 23 with idiopathic myelofibrosis.[25,27,28] We studied 41 healthy persons, 9 patients with chronic myelogenous leukemia, and 11 patients with secondary erythrocytosis as controls. The study was approved by the local ethics committees, and all samples were obtained after subjects provided written informed consent. In addition, we used DNA from 30 archival samples. The diagnostic criteria of the World Health Organization (WHO) were followed for all Swiss patients, whereas the Polycythemia Vera Study Group (PVSG) criteria were used for all Italian patients.[29-31] The main difference between the two classifications is the use of bone marrow histologic findings as a diagnostic criterion in the WHO classification to distinguish early stages of idiopathic myelofibrosis from essential thrombocythemia or polycythemia vera. The presence of endogenous erythroid colonies is a major WHO criterion and a minor PVSG criterion.

ISOLATION OF CELLS AND DNA

Granulocytes were isolated,[24] and analysis of cytospin preparations verified that the purity exceeded 90 percent. Peripheral-blood CD4+ T cells were isolated by means of magnetic sorting (Miltenyi Biotech). Peripheral-blood mononuclear cells (PBMCs) were prepared with the use of Ficoll gradient centrifugation. Buccal mucosal cells were obtained with cytologic brushes, and hair-follicle DNA was prepared from plucked hair. Genomic DNA was isolated with the use of the QIAmp DNA Blood Mini Kit (Qiagen).

DETECTION OF LOH

LOH was detected by means of fluorescence microsatellite polymerase-chain-reaction (PCR) analysis with the use of primer sequences from public databases (listed in the Supplementary Appendix, available with the complete text of this article at www.nejm.org). The samples were analyzed on a DNA genetic analyzer (model 3100, Applied Biosystems). LOH was considered to be present if one allele showed a reduction in the peak fluorescence intensity of more than 90 percent in granulocytes, but two alleles were present in nonclonal tissues (T cells, PBMCs, buccal mucosa, or hair-follicle cells) from the same patient.

ANALYSIS OF THE NUMBER OF GENE COPIES

The number of copies of chromosome 9p in granulocyte DNA was determined with the use of quantitative PCR by comparing two single-copy loci: one in exon 14 of *JAK2* and an uncharacterized gene on chromosome 13. The primers and details of these assays are provided in the Supplementary Appendix.

JAK2 SEQUENCING

We sequenced the *JAK2* complementary DNA (cDNA) with reverse-transcriptase (RT) PCR using seven pairs of overlapping primers described in the Supplementary Appendix (sequence information is available on request).

DNA CONSTRUCTS

The mutant *JAK2* cDNA was amplified by RT-PCR with the use of granulocyte RNA from a patient who was homozygous for the mutation. The Supplementary Appendix gives details of the primers and cloning into vectors.

PROLIFERATION ASSAYS

The mouse interleukin 3–dependent cell line BaF3 and the human thrombopoietin-dependent cell line UT-7/TPO (kindly provided by Dr. N. Komatsu) were transfected by electroporation with the wild-type *JAK2* (plasmid murine stem-cell virus [pMSCV]–Jak2) and mutant *JAK2* (pMSCV-V617F-Jak2) constructs. The Supplementary Appendix gives details of the assays for proliferation and cell viability.

IMMUNOPRECIPITATION AND WESTERN BLOTTING

BaF3 cells were incubated in a culture medium (RPMI) containing 10 percent fetal-calf serum in the absence of interleukin-3 for 12 hours at 37°C, whereupon various concentrations of interleukin-3 were added for 15 minutes. Cell lysates were prepared as previously described.[32] Immunoprecipitation and immunoblotting were carried out with the use of polyclonal antibodies against JAK2 (Upstate) and STAT5 (Santa Cruz) and the phosphotyrosine-specific mouse monoclonal antibody 4G10 (Upstate).

STATISTICAL ANALYSIS

We used the chi-square or Fisher's exact test where appropriate to compare categorical variables among the groups, which were categorized according to mutational status (heterozygous, homozygous, or wild type), and the Mann–Whitney U test or Kruskal–Wallis test to compare continuous variables among the groups. For some analyses, the heterozygous and the homozygous groups of patients were pooled and compared with patients without *JAK2* mutations.

RESULTS

FINE MAPPING OF THE COMMON 9pLOH REGION

Using 10 microsatellite markers covering chromosome 9p, we found 9pLOH in granulocytes from 51 of 244 patients with myeloproliferative disorders (21 percent), but not in those from any of the control subjects: 41 healthy subjects, 9 patients with CML, and 11 patients with secondary erythrocytosis. The frequency of 9pLOH was 34 percent among patients with polycythemia vera, 22 percent among patients with idiopathic myelofibrosis, and 3 percent among patients with essential thrombocythemia. The size of the chromosomal region showing LOH varied, but the telomeric region of chromosome 9p was always involved (Fig. 1A). By aligning the LOH regions of all 51 patients with 9pLOH, we identified a 6.2-Mbp interval common to all patients that extended from the telomere to marker D9S1852 and contained the gene for the tyrosine kinase JAK2 (Fig. 1B). Since JAK2 mediates signaling through several hematopoietic cytokine receptors, we considered *JAK2* an attractive candidate gene.

9pLOH AND A G→T MUTATION IN THE CODING REGION OF *JAK2*

The coding region of *JAK2* in all 51 patients with 9pLOH had a G→T transversion that changed a valine to a phenylalanine at position 617 (V617F; GenBank accession number AY973037) (Table 1 and Fig. 2). All patients with 9pLOH are expected to be homozygous (both alleles mutated) or hemizygous (one allele mutated and the other absent) for

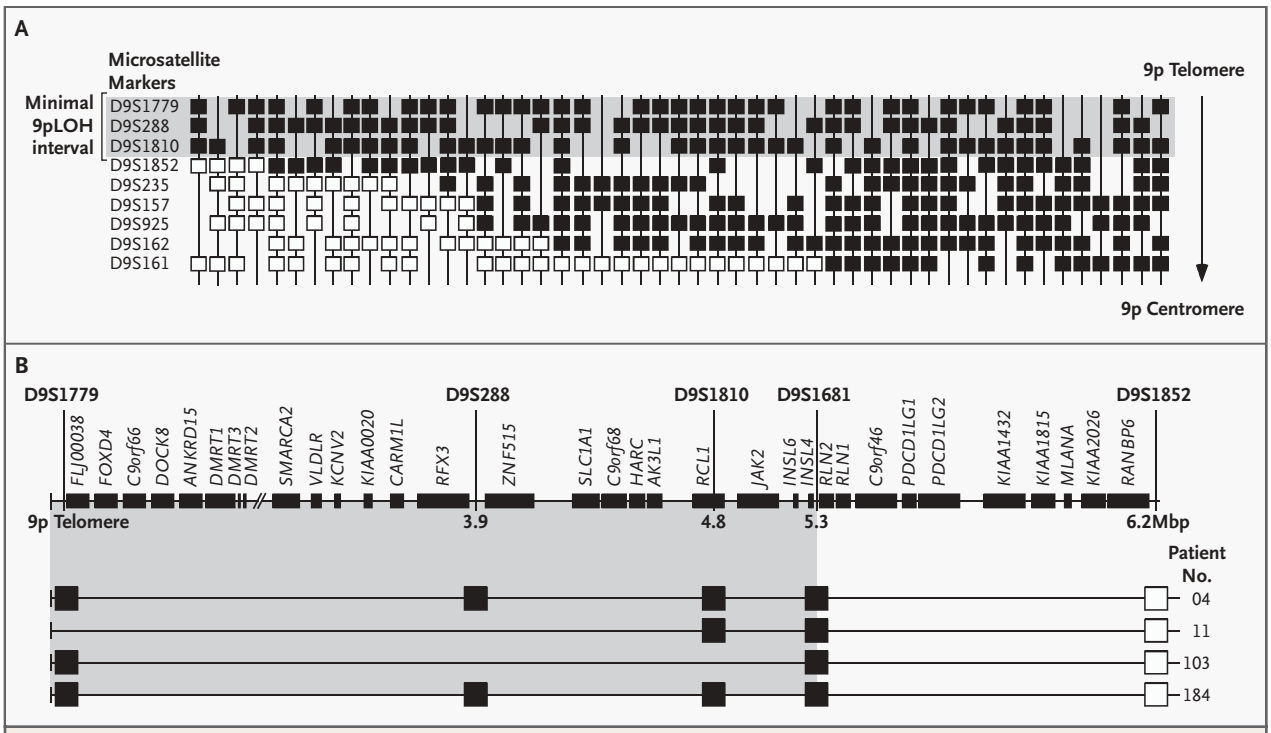

Figure 1. Mapping of the Minimal 9pLOH Region in Patients with Myeloproliferative Disorders.

Panel A shows the mapping results for 51 patients with 9pLOH. Solid squares indicate LOH in granulocytes detected by the corresponding microsatellite marker; open squares represent the absence of LOH. Vertical lines represent individual patients. The patients' results are arranged from left to right in the order of increasing size of the LOH region. The minimal LOH region is delineated by the gray background color. For clarity, markers that were uninformative have been omitted. Panel B shows a physical map of genes within the common 9pLOH region (not drawn to scale). The positions of microsatellite markers used to identify the common LOH region (gray zone) are shown as vertical lines. Numbers indicate the physical distance from the chromosome 9p telomere in megabase pairs (Mbp). Black boxes represent genes. The results of microsatellite mapping in four patients with the shortest LOH region are shown below the map. Solid squares indicate LOH, and open squares represent the absence of LOH. The mitotic recombination breakpoint in these patients occurred in the 0.9-Mbp region between the markers D9S1681 and D9S1852.

the V617F mutation. However, in eight patients, the findings were compatible with the presence of heterozygosity (one allele was mutated, and the other was wild type) (Table 1). Our definition of LOH allows 10 percent of granulocytes without 9pLOH to be present in a given sample and the ratios of G and T peak intensities in sequencing chromatograms cannot be used to quantify allelic ratios. It is therefore likely that an admixture of granulocytes with wild-type or heterozygous V617F genotypes caused the apparent heterozygosity in these eight patients. Of the remaining 193 patients without 9pLOH, 34 percent were heterozygous for V617F and 66 percent were homozygous for the wild-type allele; none were homozygous for V617F (Table 1). The V617F mutation was absent in 71 healthy controls, 11 patients with secondary erythrocytosis, and 9 patients with CML (Table 1).

The mutation was present in 65 percent of patients with polycythemia vera, as compared with 57 percent of those with idiopathic myelofibrosis ($P=0.72$) and 23 percent of those with essential thrombocythemia ($P<0.001$) (Table 1). Homozygosity for V617F was underrepresented among patients with essential thrombocythemia, as compared with patients with polycythemia vera and patients with idiopathic myelofibrosis, and was linked to the presence of 9pLOH.

The median duration of disease was significantly longer among patients with myeloproliferative disorders who were homozygous for the V617F mutation than among those who were heterozygous for the mutation (48 months [range, 0 to 576] vs. 23 months [range, 0 to 252], $P<0.02$ by the Mann–Whitney U test). This difference is compatible with the existence of a two-step process to acquire ho-

Table 1. Frequency of the *JAK2* V617F Mutation.

Group	Total No. of Subjects	*JAK2* Genotype in Granulocytes		
		G/G (wild type)	G/T (heterozygous)	T/T (homozygous)
		number of subjects (percent)		
Patients with myeloproliferative disorders	244	127 (52)	74 (30)	43 (18)
With 9pLOH	51	0	8 (16)	43 (84)
Without 9pLOH	193	127 (66)	66 (34)	0
Patients with polycythemia vera	128	45 (35)	48 (38)	35 (27)
With 9pLOH	43	0	8 (19)	35 (81)
Without 9pLOH	85	45 (53)	40 (47)	0
Patients with essential thrombocythemia	93	72 (77)	18 (19)	3 (3)
With 9pLOH	3	0	0	3 (100)
Without 9pLOH	90	72 (80)	18 (20)	0
Patients with idiopathic myelofibrosis	23	10 (43)	8 (35)	5 (22)
With 9pLOH	5	0	0	5 (100)
Without 9pLOH	18	10 (56)	8 (44)	0
Patients with CML*	9	9 (100)	0	0
Patients with secondary erythrocytosis	11	11 (100)	0	0
Healthy controls	71	71 (100)	0	0

* CML denotes chronic myelogenous leukemia.

mozygosity. Interestingly, patients with myeloproliferative disorders who had wild-type *JAK2* had the shortest duration of disease (15 months; range, 0 to 330; P=0.05 for the comparison with heterozygous patients).

IDENTIFICATION OF A SOMATIC MUTATION IN MYELOPROLIFERATIVE DISORDERS

We sequenced DNA from T cells, PBMCs, or nonhematopoietic tissues from 89 patients with the V617F mutation. We could not detect the mutant *JAK2* allele in these control tissues, a result consistent with the existence of a somatic mutation. In two patients, we detected the V617F mutation in buccal mucosa cells, but since samples of buccal mucosa can be contaminated with blood, we analyzed hair-follicle cells from these patients and found only the wild-type *JAK2* sequence (Fig. 2C).

CAUSE OF 9pLOH

The 9pLOH in myeloproliferative disorders could result from deletions of the telomeric portions of chromosome 9p or mitotic recombination between chromatids of homologous 9p chromosomes (Fig. 3A). In the case of a deletion, we would expect to find only one copy of DNA for the deleted region, whereas two copies should be expected in the event of mitotic recombination (Fig. 3A). To evaluate these possibilities, we used quantitative PCR to determine the number of copies of *JAK2* and, as a control, the number of copies of a single-copy gene on chromosome 13. Of 33 patients with 9pLOH, all had two copies of chromosome 9p (Fig. 3B). This result argues against deletions as the cause of 9pLOH and makes mitotic recombination the most likely mechanism. We also analyzed parental chromosomes of two patients with 9pLOH. In one (Patient 50), the maternally derived chromosome 9p was lost, whereas in the other (Patient 116), the paternal chromosome 9p was missing (Fig. 3C). Thus, either maternal or paternal chromosome 9p can be lost, suggesting that genomic imprinting is not involved in the expansion of cells with 9pLOH.

PROLIFERATIVE AND SURVIVAL ADVANTAGE AFFORDED BY V617F

To investigate whether the V617F mutation has functional consequences, we analyzed data on en-

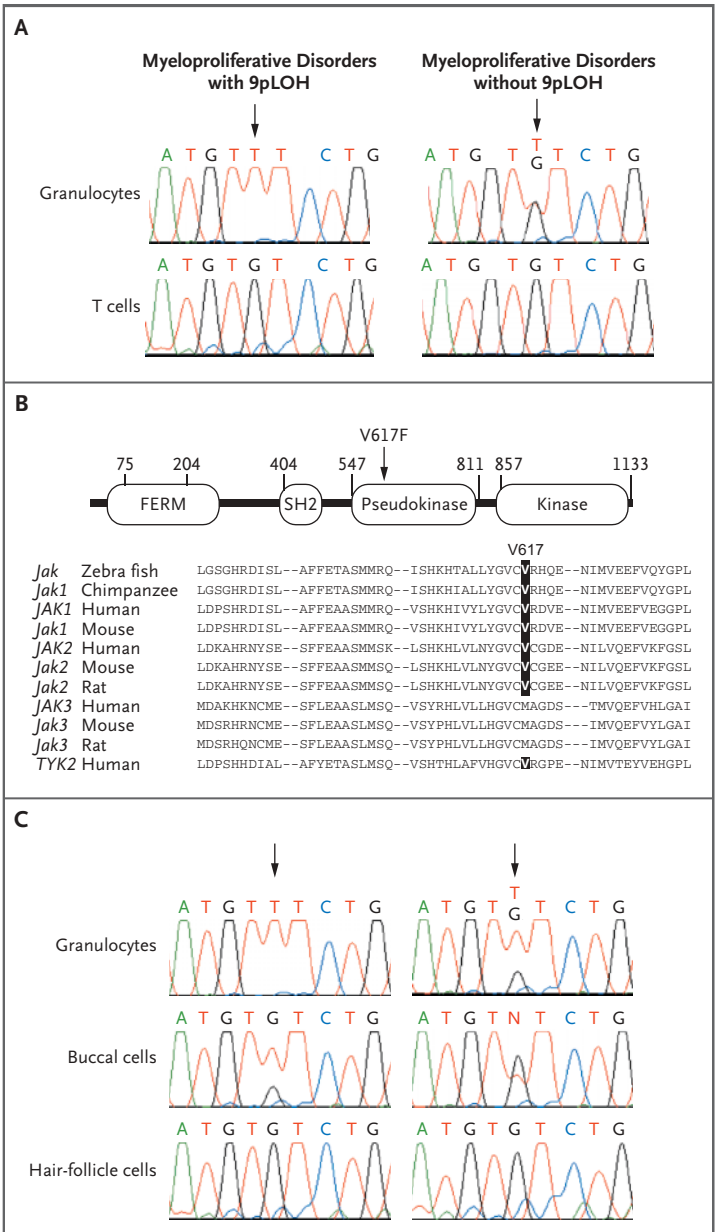

Figure 2. The *JAK2* Mutation in Patients with Myeloproliferative Disorders.

Panel A shows a homozygous G→T transversion in *JAK2* (arrow) in a patient with 9pLOH (left) and a heterozygous mutation in a patient without 9pLOH (right). The mutation was present in DNA from granulocytes, but absent in T cells, consistent with the existence of an acquired somatic origin of the mutation. Panel B shows the domain structure of the JAK2 protein. Numbers indicate the amino acid position within the protein. The arrow indicates the position of the V617F mutation. The alignment of sequences of the JAK family of proteins is shown below. Panel C shows the somatic origin of the *JAK2* mutation. DNA from granulocytes and buccal mucosa cells showed the G→T transversion, but DNA from hair follicles demonstrated absence of the mutation. FERM denotes band 4.1(f), ezrin, radixin, and moesin; and SH2 SRC homology 2.

dogenous erythroid colonies in 180 patients with myeloproliferative disorders. Endogenous erythroid colonies were present in 77 of 87 patients with the V617F mutation, whereas only 57 of 93 patients without the mutation had them ($P<0.001$ by Fisher's exact test). Next, we expressed the JAK2 V617F protein in the mouse interleukin-3–dependent cell line BaF3 (Fig. 4). These transfected cells were hypersensitive to low concentrations of interleukin-3 and were more numerous (elevated baseline value) than control cells in the absence of interleukin-3 (Fig. 4A). The same results were obtained in four independent experiments and in analogous experiments with BaF3 cells that were doubly transfected with the erythropoietin receptor gene and *JAK2* and in V617F-transfected thrombopoietin-dependent UT-7/TPO human cells (data not shown).

Interleukin-3, erythropoietin, and thrombopoietin signaling is dependent on JAK2.[33,34] Differences in cell viability were apparent when the transfected BaF3 cell lines were maintained in serum-containing medium in the absence of interleukin-3 (Fig. 4B). This result suggests that the transfected *JAK2* mutant increased survival of the cells in the absence of the cytokine. Since the proliferation assay measures the numbers of cells after three days of culture, the results obtained in the absence of interleukin-3 in Figure 4A can be compared with those of the viability curves on day 3 in Figure 4B: both show approximately twice the number of viable mutant cells as control cells. When kept under the same conditions for more than 10 days, BaF3 cells transfected with the *JAK2* V617F survived, and their numbers increased by a factor of 100, whereas cells transfected with the wild-type *JAK2* or the vector control died (Fig. 4C).

We also examined the phosphorylation of JAK2 and STAT5 proteins under the same conditions as in the proliferation assays (Fig. 4D). JAK2 phosphorylation was only slightly increased in the absence of or at low concentrations of interleukin-3. A more pronounced difference was detected in the phosphorylation of STAT5, one of the principal protein substrates of JAK2 (Fig. 4D). At higher interleukin-3 concentrations, these differences disappeared. The differences in signaling mirrored the differences in growth and survival observed at the same cytokine concentrations. Thus, the presence of *JAK2* V617F conferred a proliferative and survival advantage by rendering the cells more sensitive to incoming stimulatory signals.

CORRELATIONS WITH CLINICAL DATA

Possible associations between the V617F mutation and clinical features were retrospectively analyzed in 244 patients. The only significant differences between patients who were heterozygous and those who were homozygous for the V617F mutation were the above-mentioned increase in the median duration of disease and the correlation between 9pLOH and homozygosity for the mutation. Therefore, we limited our comparison to patients with the V617F mutation and those without the mutation (Table 2). Significantly more patients with the mutation than without the mutation had complications (secondary fibrosis, hemorrhage, and thrombosis) and had received cytoreductive treatment. Of five patients with leukemic transformation, four had the V617F mutation (P=0.20).

DISCUSSION

Fine mapping of the 9pLOH region in patients with myeloproliferative disorders identified a 6.2-Mbp genomic interval that contains the gene for the tyrosine kinase JAK2 (Fig. 1). This gene, *JAK2*, was a strong candidate for causing clonal expansion of hematopoietic progenitors in myeloproliferative disorders because of its essential function in hematopoiesis.[33,34] We found a G→T transversion that results in a change in a single amino acid, V617F, in the pseudokinase domain of JAK2 (Fig. 2 and Table 1). V617F is not a polymorphism, since it was absent from 142 chromosomes of healthy people and is not recorded in the databases of single-nucleotide polymorphisms. Moreover, we did not detect the V617F mutation in 11 patients with secondary erythrocytosis or 9 patients with CML.

We found conclusive evidence that V617F is a somatic mutation in hematopoietic cells. We cannot exclude the possibility of germ-line mutations in some cases of familial myeloproliferative disorders, although chromosome 9p was previously excluded by linkage analysis in four families with polycythemia vera.[26] The frequency of the V617F mutation was highest among patients with polycythemia vera and lowest among patients with essential thrombocythemia (Table 1). Essential thrombocythemia is the most heterogeneous myeloproliferative disorder, with a substantial proportion of patients showing polyclonal hematopoiesis.[4,6,35] The low frequency of 9pLOH in this disease might reflect the low mitotic activity in the essential-thrombocythemia progenitor or stem-cell pool.

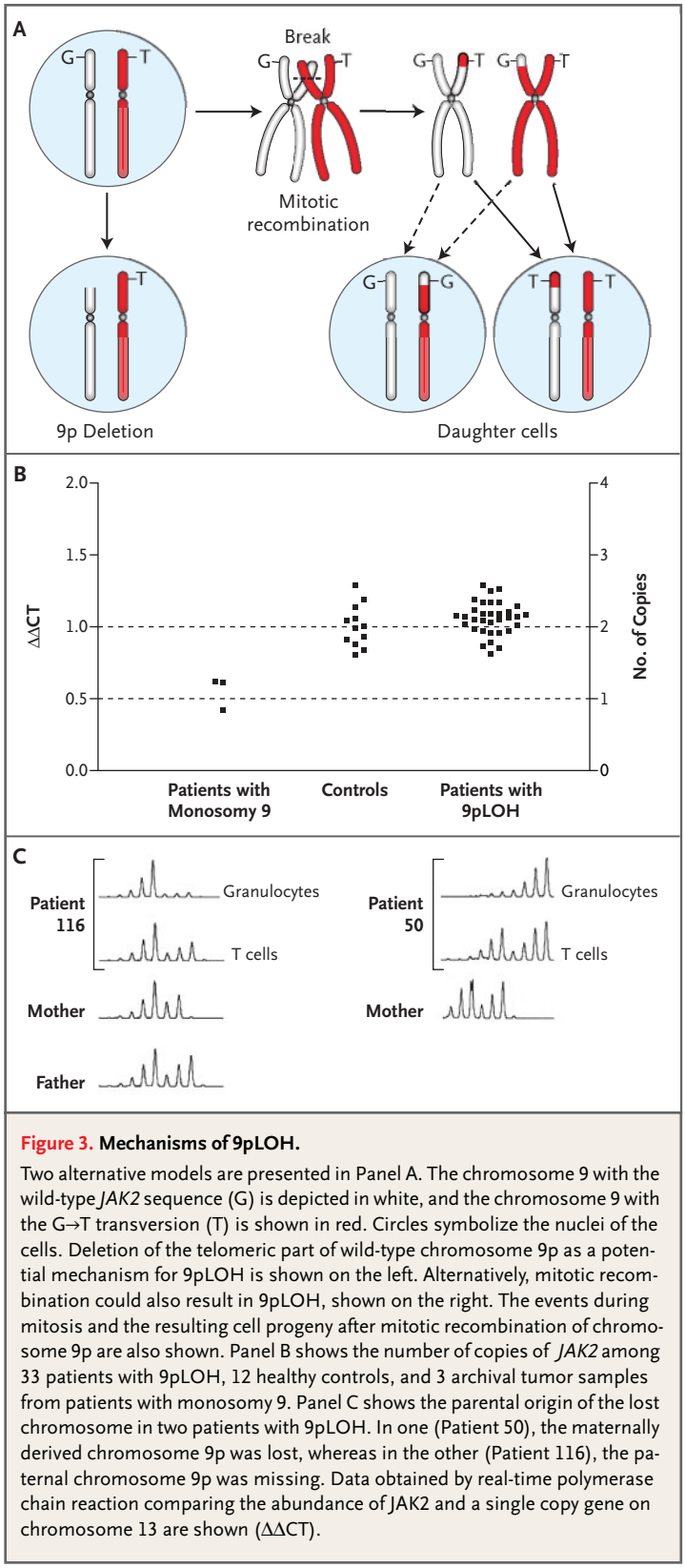

Figure 3. Mechanisms of 9pLOH.

Two alternative models are presented in Panel A. The chromosome 9 with the wild-type *JAK2* sequence (G) is depicted in white, and the chromosome 9 with the G→T transversion (T) is shown in red. Circles symbolize the nuclei of the cells. Deletion of the telomeric part of wild-type chromosome 9p as a potential mechanism for 9pLOH is shown on the left. Alternatively, mitotic recombination could also result in 9pLOH, shown on the right. The events during mitosis and the resulting cell progeny after mitotic recombination of chromosome 9p are also shown. Panel B shows the number of copies of *JAK2* among 33 patients with 9pLOH, 12 healthy controls, and 3 archival tumor samples from patients with monosomy 9. Panel C shows the parental origin of the lost chromosome in two patients with 9pLOH. In one (Patient 50), the maternally derived chromosome 9p was lost, whereas in the other (Patient 116), the paternal chromosome 9p was missing. Data obtained by real-time polymerase chain reaction comparing the abundance of JAK2 and a single copy gene on chromosome 13 are shown (ΔΔCT).

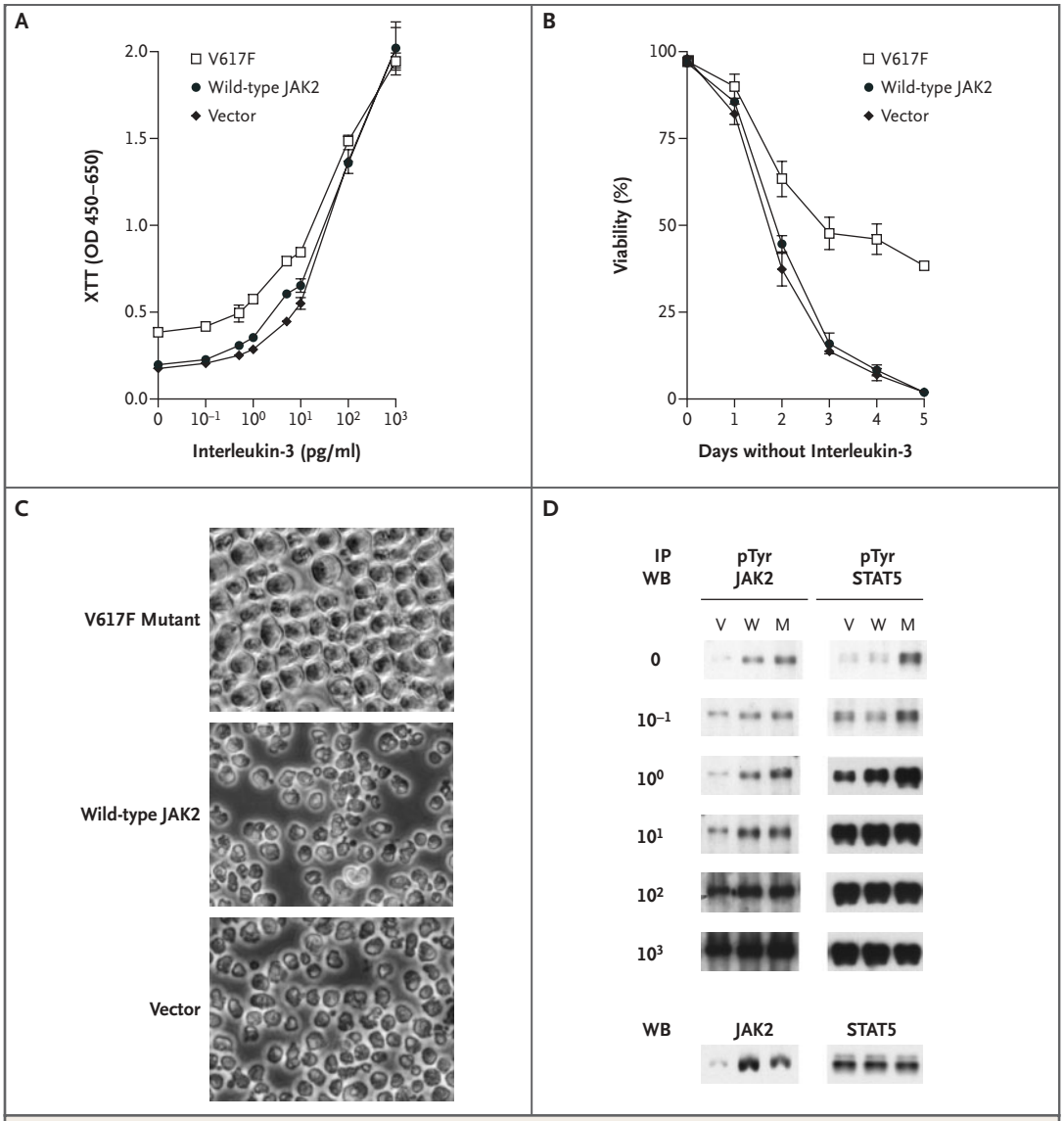

Figure 4. Functional Effects of the *JAK2* V617F Mutation in Stably Transfected Murine Interleukin-3–Dependent BaF3 Cells.

Panel A shows the proliferation of BaF3 cells transfected with the V617F mutant JAK2, the wild-type JAK2, or the empty vector in the absence of interleukin-3 (a concentration of 0) and the presence of increasing concentrations of interleukin-3, as determined by the tetrazolium salt (XTT) assay. The mean (±SD) of triplicate results is shown (in some cases, the error bars are hidden behind the symbols). Increased optical density (OD) of the XTT dye corresponds to increased numbers of cells. Panel B shows the survival of stably transfected BaF3 cells in the absence of interleukin-3. The percentage of viable cells was determined by the exclusion of the dye trypan blue from the cells. The mean (±SD) of triplicate results is shown. Panel C shows stably transfected BaF3 cells maintained in the absence of interleukin-3 for 10 days. BaF3 cells transfected with JAK2 V617F are viable, whereas almost all cells transfected with the wild-type JAK2 or the empty vector are dead. Panel D shows the activation of JAK2 and STAT5 in response to interleukin-3. BaF3 cells transfected with the empty vector (V), the wild-type JAK2 (W), or the JAK2 V617F mutant (M) were incubated for 12 hours without interleukin-3 but with 10 percent fetal-calf serum and were then stimulated for 15 minutes with increasing concentrations of interleukin-3, as indicated. Immunoprecipitation (IP) was carried out with the phosphotyrosine-specific mouse monoclonal antibody 4G10 (pTyr) followed by Western blotting (WB) with the use of antibodies against JAK2 or STAT5, as indicated. Levels of expression of JAK2 and STAT5 proteins in the lysates used for immunoprecipitation were visualized by immunoblotting with the use of the corresponding antibodies and are shown at the bottom.

Table 2. Characteristics Associated with the *JAK2* V617F Mutation.

Characteristic	*JAK2* V617F Mutation		P Value
	Absent (N=127)	Present (N=117)	
Diagnosis — no. (%)*			0.001
Polycythemia vera	45 (35)	83 (65)	
Essential thrombocythemia	72 (77)	21 (23)	
Idiopathic myelofibrosis	10 (44)	13 (57)	
Age — yr			0.001
Median	52	60	
Range	16–83	18–89	
Sex — no. (%)			0.19
Male	61 (48)	66 (56)	
Female	66 (52)	51 (44)	
Complications — no. (%)			0.005
Any	27 (21)	44 (38)	
None	100 (79)	73 (62)	
Secondary fibrosis — no. (%)†			0.004
Yes	3 (3)	13 (12)	
No	114 (97)	91 (88)	
Hemorrhage — no. (%)			0.02
Yes	1 (1)	8 (7)	
No	126 (99)	109 (93)	
Thromboembolic events — no. (%)			0.03
Yes	19 (15)	31 (26)	
No	108 (85)	86 (74)	
Acute leukemia — no. (%)			0.20
Yes	1 (1)	4 (3)	
No	126 (99)	113 (97)	
Cytoreductive treatment at time of sampling — no. (%)‡			0.003
Yes	47 (38)	66 (57)	
No†	77 (62)	49 (43)	

* Percentages are of all the patients with each diagnosis.
† Only patients with polycythemia vera and essential thrombocythemia were evaluated for secondary fibrosis.
‡ Information for some patients was not available.

It is remarkable that we found an identical somatic G→T transversion in *JAK2* in 117 unrelated patients, or 48 percent of the patients with myeloproliferative disorders (Table 1). The V617F mutation is located in the pseudokinase domain of JAK2 (Fig. 2B), a region that inhibits JAK2 kinase activity.[36,37] Mutations affecting this domain are associated with malignant transformation of hematopoietic cells in drosophila.[38]

BaF3 and UT-7/TPO cells that were transfected with the mutant *JAK2* gene were hypersensitive to low concentrations of interleukin-3 and erythropoietin and thrombopoietin, respectively. In the presence of serum, transfected BaF3 cells showed increased survival, proliferation, and phosphorylation of JAK2 and STAT5, even in the absence of interleukin-3 (Fig. 4). This result suggests that other cytokines in serum at low concentrations are most likely sufficient to maintain the viability and proliferation of cells expressing *JAK2* V617F. Similarly, the growth of endogenous erythroid colonies in myeloproliferative disorders also depends on the presence of serum.[39]

The effect of the *JAK2* V617F mutation on the

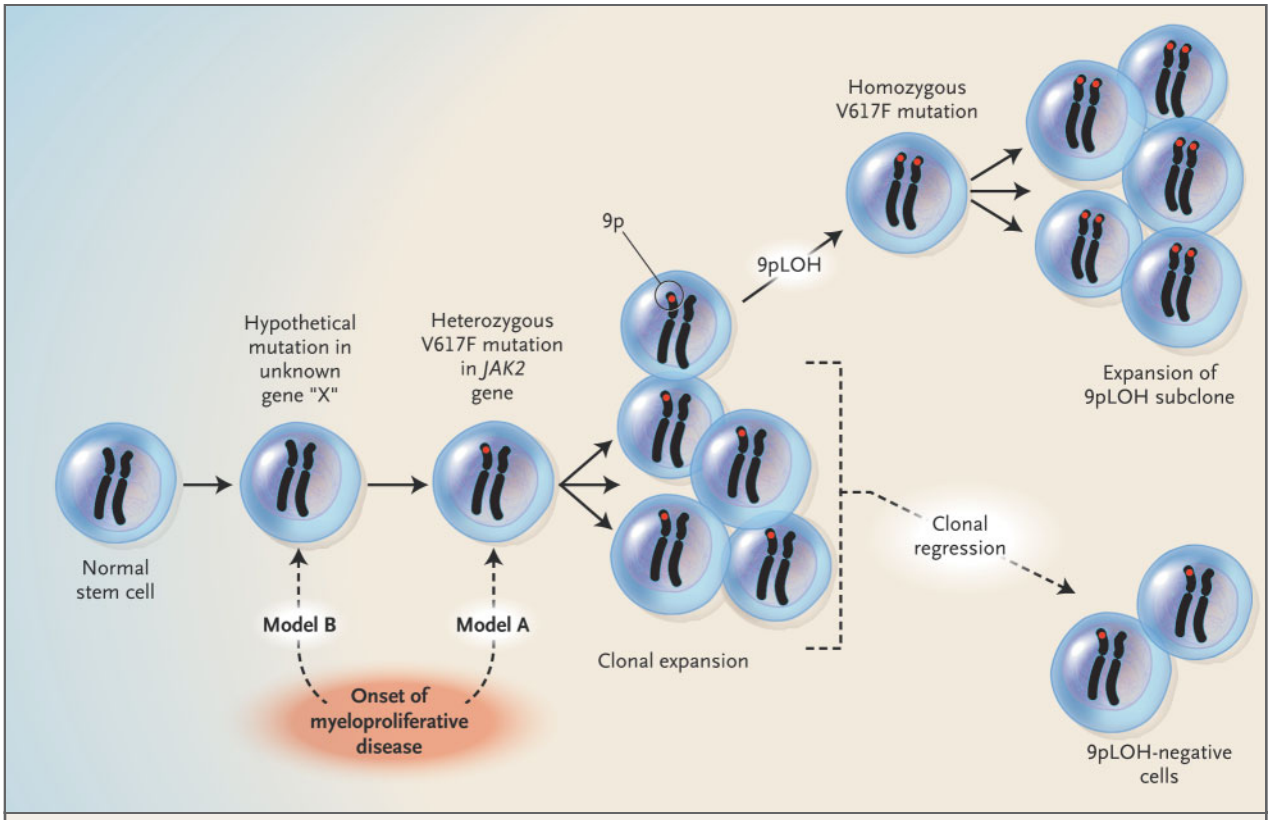

Figure 5. Possible Roles of the *JAK2* V617F Mutation in Myeloproliferative Diseases.

In model A, a mutation (V617F) of one allele of *JAK2* on chromosome 9p (red dot), alone or in combination with a hypothetical preexisting mutation in an unknown gene ("X"), initiates the onset of the myeloproliferative disease (dashed arrow). In model B, the heterozygous V617F mutation on 9p occurs after the initiation of the myeloproliferative disease (dashed arrow), which was provoked by one or more mutations in an unknown gene or genes. Cells that are heterozygous for the V617F mutation have a proliferative advantage over cells bearing only the wild-type allele. Mitotic recombination between homologous regions of the two chromosomes 9 in a cell heterozygous for V617F results in loss of heterozygosity of 9p (9pLOH). One of the daughter cells is homozygous for V617F and gains an additional proliferative advantage. This cell establishes a subclone that outcompetes both cells that are heterozygous for V617F and cells that are homozygous for wild-type *JAK2*.

proliferation of BaF3 cells was less dramatic than that of the TEL-JAK2 fusion protein found in acute lymphoblastic leukemias.[40] The relatively subtle effect of the V617F mutation fits well with the mild and indolent nature of the clonal proliferation observed in myeloproliferative disorders. Hypersensitivity to insulin-like growth factor, thrombopoietin, interleukin-3, and other cytokines has been described in hematopoietic progenitor cells from patients with myeloproliferative disorders.[39,41-43] How the V617F mutation sustains the increased phosphorylation of JAK2 in the presence of serum, but in the absence of interleukin-3, and the factor or factors in serum that mediate this effect remain unknown. Nevertheless, the functional relevance of the V617F mutation is supported by the finding that endogenous erythroid colonies were present in 89 percent of patients with V617F and the close association between homozygosity for the V617F mutation and 9pLOH, which demonstrates the survival advantage of such homozygous hematopoietic cells.

The gene-dosage analysis suggests that mitotic recombination between chromatids of homologous chromosomes 9p is the most likely mechanism leading to 9pLOH (Fig. 3). The absence of homozygosity for the V617F mutation in patients with myeloproliferative disorders who do not have 9pLOH suggests that a transition from heterozygosity to homozygosity by means of a mechanism that is independent of mitotic recombination is rare. Mitotic recombination is a frequent genetic mechanism in the inactivation of tumor-suppressor genes in solid tumors,[44-46] but not in malignant diseases of the hematopoietic system.[47]

Our data suggest two possible pathogenetic mechanisms of the *JAK2* V617F mutation. In one (model A in Fig. 5), the heterozygous *JAK2* V617F mutation alone or in combination with one or more preexisting somatic mutations causes the myeloproliferative-disorder phenotype in approximately 50 percent of patients. A somatic mutation in an as yet unknown gene would be responsible for the phenotype in the other patients (not shown). This model does not explain the presence of the V617F mutation in several myeloproliferative disorders. Perhaps the genetic background or additional somatic mutations in hematopoietic cells alter the effects of the V617F mutation on the phenotype. In model B, *JAK2* V617F occurs after the appearance of the myeloproliferative-disorder phenotype as a mutation associated with disease progression but is not necessary or sufficient to cause the phenotype (Fig. 5). In this model, patients with myeloproliferative disorders without the *JAK2* mutation have an early stage of the disease. One prediction of this model is that the duration of disease should be shortest in patients without the *JAK2* mutation and longest in those who are homozygous for the V617F mutation, as we observed. In both models, the transition from heterozygosity to homozygosity for the mutation represents clonal evolution. Our data indicate that mitotic recombination in patients with 9pLOH produces a daughter cell that is homozygous for the V617F mutation and can outcompete the parental cells that are heterozygous (Fig. 3A and 5).

Evaluation of the clinical data (Table 2) revealed significant correlations between the presence of the V617F mutation and the frequency of complications (secondary fibrosis, hemorrhage, and thrombosis). These correlations could be a consequence of the longer duration of disease in patients with the *JAK2* mutation or could be linked to a more aggressive phenotype, perhaps owing to an increased responsiveness to cytokines. Patients with the V617F mutation were older than those without the mutation, and a higher proportion had received cytoreductive therapy at the time of sample collection. The increased frequency of cytoreductive therapy does not necessarily imply the presence of a more aggressive disease and could be based on age alone, according to widely accepted therapeutic guidelines.

Taken together, our data suggest that the V617F mutation in *JAK2* is a dominant gain-of-function mutation that contributes to the expansion of the myeloproliferative-disorder clone. The V617F mutation could form the basis for a new molecular classification of myeloproliferative disorders. Furthermore, in view of the invariant nature of the *JAK2* mutation, small molecules could be developed that specifically target the mutated protein in patients with myeloproliferative disorders.

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