TO THE EDITOR: An activating somatic mutation involving the JH2 pseudokinase domain of Janus kinase 2 (JAK2 [V617F]) has been associated with myeloproliferative disorders. The mutation is detectable in 65 percent\(^1\) to 97 percent\(^2\) of cases of polycythemia vera. Kralovics and colleagues (April 28 issue)\(^1\) report a significant association between homozygosity for the JAK2 (V617F) mutation, which occurred in approximately one quarter of the patients with polycythemia vera, and increased duration of disease in polycythemia vera, essential thrombocytemia, and myelofibrosis with myeloid metaplasia. A similar observation was made by others,\(^3\) raising the possibility that homozygosity for the mutant allele is a time-dependent clonal evolution.

We tested this hypothesis by performing mutational analysis in archived bone marrow cells from patients showing homozygosity in DNA derived from peripheral-blood granulocytes. Among 220 patients with either polycythemia vera or myelofibrosis with myeloid metaplasia who were seen at the Mayo Clinic and not included in previous publications,\(^3\) granulocyte-based mutation screening identified 21 patients who were homozygous for JAK2 (V617F) — 13 who had polycythemia vera and 8 who had myelofibrosis with myeloid metaplasia. In the case of 5 of these 21 patients, including 2 with polycythemia vera, the study was performed at the time of diagnosis. However, laboratory records in the two patients with polycythemia vera revealed a preexisting increase in the hematocrit from baseline that had been unrecognized for at least two years. Stored bone marrow from six patients, collected 1.5 to 9.5 years before the current analysis, showed variable degrees of heterozygosity in four patients at different times during their clinical course (Fig. 1). The pattern of change over time, especially as depicted in Patient 3, favors a time-dependent increase in clonal dominance rather than a two-step molecular event. This possibility is consistent with

![Figure 1](https://www.nejm.org/doi/10.1056/NEJMc056530)

**Figure 1. Mutational Analysis of JAK2 (V617F) in Serial Bone Marrow Specimens from Six Patients with Myeloproliferative Disorders.**

Bone marrow from Patients 1, 2, 3, and 6 shows variable degrees of homozygosity during their clinical course. Arrows indicate the nucleotide substitution of T for G.
the occurrence of a mixed-clonality pattern in purified CD34+ cell fractions in patients whose granulocytes show homozygosity4 and the in vitro demonstration of a JAK2 (V617F)–induced proliferative advantage in cell lines.1,5 Furthermore, as noted above, the occurrence of subclinical clonal myelopoesis might partly explain why homozygosity is not always documented at the time of clinical diagnosis.

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The authors reply: We proposed a two-step model for the role of JAK2 (V617F) in the clonal evolution of myeloproliferative disorders. The first step consists of a G-to-T mutation in one allele of the JAK2 gene that is acquired as a somatic mutation in a hematopoietic progenitor cell or stem cell. This cell gives rise to a clone that is heterozygous for JAK2 (V617F) and expands to replace hematopoietic cells without the JAK2 mutation. The second step consists of a mitotic recombination in one of the progenitor cells or stem cells heterozygous for the JAK2 mutation that generates uniparental disomy and homozygosity for JAK2 (V617F) in one of the two daughter cells. This daughter cell gives rise to a clone that is homozygous for JAK2 (V617F) and expands to replace homozygous hematopoietic cells. The results of the test described by Tefferi and colleagues provide evidence that confirms the predictions of our model. A strict two-step process applies at the level of individual cells (i.e., each cell can be either heterozygous or homozygous, provided that gene amplification at the JAK2 locus is not involved). In contrast, when cell populations are analyzed, the transition from heterozygosity to homozygosity will be a continuous process, since the proportion of homozygous cells in this mixed population of cells will gradually increase until the homozygous cells fully dominate hematopoiesis. Analyses of mixed-cell populations, such as those of bone marrow or blood, taken during this transition period are expected to show a time-dependent increase of clonal dominance rather than a two-step transition. Thus, the data from Tefferi and colleagues do not contradict our model but instead confirm its predictions. We have applied a quantitative allele-specific polymerase-chain-reaction technique to determine the ratios of wild-type and mutant JAK2 alleles, and we obtained very similar results to those reported here by Tefferi and colleagues.

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An Intervention Involving Traditional Birth Attendants in Pakistan

To the editor: The findings reported by Jokhio et al. (May 19 issue)1 are encouraging in that the intervention involving traditional birth attendants significantly reduced perinatal mortality. Disappointingly, however, it had no significant effect on maternal mortality. Some data suggest that traditional birth attendants may be counterproductive in reducing maternal mortality.2 In the developing world, 440