order to double-check our results, we developed a PCR–restriction fragment length polymorphism (RFLP) method to analyze these 4 polymorphisms. For the detection of 608TTG->GTT, we used primer 5’-AATACAGTTTTATACCTGAAC-3’ (forward: nt 1 to 23) and 5’-CTTGATCCAGGCTGCTGTC-3’ (reverse: nt 219 to 190). A mismatched base (underlined) was introduced at the 3’-end base of the reverse primer to create a TaqI restriction enzyme cutting site in combination with the base 608T of wild type after PCR and no TaqI site for the 608GTG polymorphism. For the detection of 561CAG->CCG, we used the same forward primer, and 5’-TTCCATTAAAGAATAAGC-3’ (reverse: nt 174 to 153). A mismatched base (underlined) was introduced at position 3 from the 3’-end of the reverse primer to create an AluI site in combination with the base 561A of wild type after PCR and no AluI site for the 561CGG polymorphism. For the detection of 537CCT->CCT, we used the same forward primer and 5’-CTGATCTTTATAATCCAAACGTTC-3’ (reverse: nt 153 to 129). A mismatched base (underlined) was introduced at position 2 from the 3’-end of reverse primer to create an NlaIII site in combination with the base 536C and base 537A of wild type after PCR reaction, and no NlaIII site for the 537CCT polymorphism. For the detection of 527ATT->CTT, we used primer 5’-CCCTGCAATGTGATGTC-3’ (forward: nt 97 to 116) and 5’-GGAATTCAGGGTTGATCCATG-3’ (reverse: nt 312 to 291). A mismatched base was introduced at position 3 from 5’-end of forward primer to create an NlaIV site in combination with the base 527C of wild type after PCR, and no NlaIV site for the 527CTT polymorphism. The PCR condition was performed as described,2,3 except the annealing temperature was done at 55°C, 57°C, 56°C, and 55°C for 608, 561, 537, and 527, respectively. All the above mentioned primer sequences can be found in GenBank accession number L16879. They are specific for the CYP2C9 gene.

In total, 300 cases were analyzed with these 4 PCR-RFLP methods (Figure 2), and the polymorphisms found by Leung et al were not found in our analysis.

In conclusion, we demonstrated that no genetic polymorphism in exon 4 of cytochrome CYP2C9 is found in Taiwanese, which is different from the results reported by Leung et al. We suggest that further study of the Chinese population is necessary.

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To the editor:

Lack of alteration in GATA-1 expression in CD34+ hematopoietic progenitors from patients with idiopathic myelofibrosis

Idiopathic myelofibrosis (IM), also known as myelofibrosis with myeloid metaplasia, is a myeloproliferative disorder of clonal origin characterized by extramedullary hematopoiesis with a leukoerythroblastic blood picture, tear-drop erythrocytes, and progressive splenomegaly associated with bone marrow fibrosis.1 A number of data suggested that alterations of megakaryocyte...
with accumulation of abnormal megakaryocytes in the hematopoietic tissues, could participate in the development of the disease.\(^2\)

We read with great interest the recent article by Vannucchi et al\(^3\) as we have been involved in studies on the pathogenesis of IM for several years. In this paper, the authors reported that mice harboring the GATA-1\(^{low}\) mutation develop a frank IM-like disease characterized by the presence of tear-drop poikilocytes and progenitors in the blood, collagen fibers in the marrow and in the spleen, and hematopoietic foci in the liver, and they suggested that the human IM might result from deregulated expression or mutations of GATA-1. Actually, transcription factor GATA-1, which interacts with the transcriptional cofactor, friend of GATA-1 (FOG),\(^4\) exerts a critical role in erythroid and megakaryocytic cell differentiation.\(^5,6\) Given the prominent place of the megakaryocyte (Mk) population in the pathogenesis of IM, we recently investigated alterations in the expression and/or functionality of these factors in hematopoietic cells from 20 patients. Using semiquantitative reverse transcriptase–polymerase chain reaction (RT-PCR), we observed an increased level of GATA-1 expression in patients’ peripheral blood mononuclear cells (PBMC) compared with PBMC from normal blood; such an increase likely reflects the presence of a high number of circulating megakaryocytes and their precursors in patients’ blood.\(^7\) Concerning CD34\(^+\) progenitor cells (Figure 1A), we did not observe any significant difference in GATA-1 expression level between CD34\(^+\) progenitor cells purified from patients’ blood (as myelofibrosis precluded successful bone marrow aspiration) and CD34\(^+\) cells isolated from unmotivated peripheral blood and bone marrow of healthy individuals (yield of purity \(\geq 97\%)\).

In contrast, the expression of cofactor FOG-1\(^8\) was significantly increased \((P = .02)\) in CD34\(^+\) cells from patients when compared with CD34\(^+\) cells from normal bone marrow and normal unmotivated peripheral blood. As GATA-1 also associates in a multiprotein complex with the stem cell leukemia gene (SCL),\(^9\) a crucial actor in early hematopoiesis and differentiation along the erythroid and megakaryocytic pathways,\(^10\) we sought for potential alteration in SCL expression. Our studies showed that SCL gene transcription is altered in patients’ CD34\(^+\) progenitor cells and that the sublocalization of SCL protein is different in patients’ megakaryocytic cells and megakaryocytes from healthy subjects, ie both nuclear and cytoplasmic versus solely nuclear, respectively.

Altogether, the interesting data by Vannucchi et al and our findings highlight the possible contribution of a deregulated expression of any of these interacting transcription factors and/or of altered interactions between these nuclear proteins to the pathogenesis of idiopathic myelofibrosis.

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### Response:

**Little steps toward the identification of the myelofibrotic locus**

Idiopathic myelofibrosis (IM) is a myeloproliferative disorder of clonal origin thought to be originated by either somatic or inherited mutations at a locus, the IM locus, that would confer proliferative advantage to a bipotent (erythroid [E], and megakaryocytic [MK]) progenitor cell.\(^1,2\) This cell would eventually undergo transformation leading the disease toward its final leukemic phase. Almost all of the IM cases express at...
diagnosis one major cytogenetic defect, mostly del(13q), del(20q), or
partial trisomy 1q. IM, however, is not characterized by any “unique”
chromosomal abnormality. As such, the chromosomal abnormalities
found to be associated with IM are considered secondary genetic events
in the multistep progression of a disease whose primary genetic cause,
the IM locus, is still to be identified.

The genes that are currently under investigation as candidates for the
IM locus are represented by classic tumor suppressor genes, such as the
retinoblastoma (RB1) and the p53 genes or the p16 gene and the RAS
family of proto-oncogenes.4,5 Alternatively, genes that are involved in
the control of proliferation of early hematopoietic cells, such as stem cell
factor (SCF) and its receptor c-kit, and elements of the b-fibroblast
growth factor (b-FGF) pathway7 are also being considered. Among
those, SCF/c-kit are slightly favored because of the observation that the
progenitor cells circulating in myeloproliferative disorders present both
elevated expression6 and point mutation.9,10 of c-kit. However, none of
the many mouse mutants in the SCF and c-kit locus available has been
described up to now to develop myelofibrosis. Furthermore it is not clear
how any of these genes would specifically induce the abnormalities in
the E and Mk pathway, which are the landmarks of the disease.

Hypothesis-driven research has suggested genes in the thrombopoietin
(TPO) pathway, the growth factor which specifically regulates Mk
production in vivo,11 as possible candidates for the IM locus. This
hypothesis gained considerable favor when it was demonstrated that IM
can be experimentally induced in mice by in vivo manipulation of the
levels of TPO.6,12-14 However, a later study failed to find autocrine TPO
production or mutations in the TPO receptor (Mpl) in 4 cases of human IM,15 leaving the relationship between possible presence of
alterations in the TPO/Mpl pathway and the increased numbers of Mk
observed in these patients still to be ascertained.

GATA-1 is a gene specifically involved in the regulation of the
number of both E and Mk in vivo.16 Our paper,17 showing that
GATA-1low (GATA-1tm2Sho) mutant mice develop a frank myelofi
brotic syndrome with age, opens the possibility that genes involved in
GATA-1 function might be directly responsible for the develop-
ment of the disease. The letter by Martyr et al, showing that
CD34+ cells circulating in the patients express lower levels of
FOG-1 (one of the GATA-1 obligatory partners), further encourages
research in this direction. Both reports are far away from having
identified the IM locus. In fact, since the mouse develops IM very
late in life, it is possible that in this case the GATA-1low mutation has
a permissive role by inducing the hyper-proliferation of the
progenitor cells that allows them to accumulate secondary mutants.
On the other hand, since the letter by Dr Martyr et al does not
provide any evidence for genetic abnormalities in the FOG-1 locus,
it is possible that the alterations it describes are pleiotropic to
an underlying genetic defect.

It must be said that many excellent experimental papers on the
pathophysiology of IM have been recently published. Among
those, especially worthy of mention is the report that forced
expression of TPO transforms normal, but not transforming growth
factor–βnull (TGF-βnull), stem cells into IM-inducing clones.18 All
the data that are being accumulated, plus the existence of national
and international registries of the disease, which have been
carefully established in the mean time, raise great hope that by
unifying the experimental efforts we might be finally very close to
grasp the etiology of human IM.

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To the editor:

Deferiprone and hepatic fibrosis

We write to request that Wanless and colleagues correct the record
on the efficacy and safety of deferiprone for treatment of transfu-
sional iron overload by providing the data on hepatic iron omitted
from their publication in Blood. This rectification would document
the failure of deferiprone therapy to prevent iron accumulation in
thalassemia major and make evident the inherent bias against