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Short Communication

Infrequent V617F mutation of the *JAK2* gene in myeloid leukemia and its absence in lymphoid malignancies in Japan

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Abstract

A unique mutation of the *JAK2* gene, V617F, has recently been identified in polycythemia vera, essential thrombocythemia and myeloid metaplasia with myelofibrosis. To determine the relevance of this mutation in other types of hematological neoplasms in Japan, we performed allele-specific polymerase chain reaction analysis on the *JAK2* gene. The V617F mutation was detected in one out of 130 myeloid neoplasms, but in none of 114 lymphoid malignancies and four biphenotypic acute leukemias. Although a favorable chromosomal alteration t(8;21)(q22;q22) was observed in one acute myeloid leukemia (AML) patient with the mutation, two courses of chemotherapy resulted in induction failure and short survival. Sequencing of *JAK2* cDNA revealed expression of the mutant allele in the patient. The V617F mutation might play a role in the pathogenesis of certain AML cases.

Key words: JAK2 gene, V617F mutation, signal transduction, acute myeloid leukemia, lymphoid malignancies.

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Myeloproliferative disorders (MPD) comprise a heterogeneous group of diseases including chronic myelocytic leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and myeloid metaplasia with myelofibrosis (MMM). With the exception of CML, characterized by the Philadelphia chromosome and the *BCR-ABL* fusion gene, genetic events causing these disorders have remained unidentified for a long time. The *Janus kinase 2 (JAK2)* gene encodes a tyrosine kinase involved in cytokine signal transduction. JAK phosphorylates cytoplasmic targets including signal transducers and activators of transcription (STAT). In hematological neoplasms, several chromosomal translocations involving the *JAK2* gene locus have been identified (Khwaja, 2006). Recently, a unique mutation of the *JAK2* gene, G to T transversion at nucleotide 1849 resulting in valine to phenylalanine substitution at amino acid position 617 (V617F), has been identified in MPD (Baxter *et al.*, 2005; James *et al.*, 2005; Kralovics *et al.*, 2005; Levine *et al.*, 2005a). This mutation was present in most patients with PV and in half of the patients with ET and MMM. Since the V617F mutation is located in the JH2 negative regulatory domain of the *JAK2* gene, it disrupts the auto-inhibitory activity of JAK2. In consequence, the mutation leads to constitutive tyrosine phosphorylation activity, promoting cytokine hypersensitivity and inducing erythrocytosis in a mouse model (James *et al.*, 2005).

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Recent studies have shown the V617F mutation to be present in some cases of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and chronic myelomonocytic leukemia (CMMoL) (Jelinek *et al.*, 2005; Levine *et al.*, 2005b). However, limited information is available to determine whether the mutation is specifically associated with myeloid neoplasms, and most of the studies are from Europe and North America. In order to assess the range and frequency of the mutation in Japanese patients with hematological neoplasms, we performed allele-specific polymerase chain reaction (PCR) analysis on the *JAK2* gene.

Bone marrow, peripheral blood or lymph node samples from 248 hematological neoplasms were analyzed after obtaining written informed consent (Table 1). Of the 248 samples, 130 were myeloid neoplasms, 114 were lymphoid neoplasms, and four were biphenotypic acute leukemias (BAL). The current study was conducted within the guidelines and with the approval of the institutional review board. The primer sequences for allele-specific PCR were previously published (Baxter *et al.*, 2005):

ALLF-S, 5'-AGCATTTGGTTTTAAATTATGGAGTATATT-3';

ALLF-IC, 5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3'; and

Table 1 - *JAK2* V617F mutations in hematological neoplasms.

	Number of samples	Number of V617F myeloid malignancies
Myeloid malignancies		
AML	38	1
MDS	38	0
RAEB-t[1]	2	0
MDS/AML	16	0
CML CP[2]	18	0
CML AP[3]	1	0
CML BC[4]	11	0
CMMoL	5	0
aCML[5]	1	0
Lymphoid malignancies		
MM	48	0
MGUS[6]	2	0
ALL	40	0
CLL	7	0
ML	8	0
Macroglobulinemia	2	0
ATL	7	0
Biphenotypic acute leukemia	4	0
Total	248	1

[1]RAEB-t, refractory anemia with excess of blasts in transformation; [2]CP, chronic phase; [3]AP, accelerated phase; [4]BC, blast crisis; [5]aCML, atypical chronic myeloid leukemia; [6]MGUS, monoclonal gammopathy with undetermined significance.

ALLR-S, 5'-CTGAATAGTCCTACAGTGTTTTC AGTTTCA-3'.

ALLF-S is specific for the mutant allele and contains an intentional mismatch at the third nucleotide from the 3' end, to improve specificity. After 5 min at 94 °C, 36 amplification cycles of 60 s at 94 °C, 60 s at 58 °C and 60 s at 72 °C were performed, with a subsequent 7 min extension at 72 °C. Electrophoresis was repeated two or three times in each sample, using independent PCR products. Primers ALLF-S and ALLR-S amplify 203-bp (base pair) products, while the size of products using primers ALLF-IC and ALLR-S is 364 bp. The 203-bp products indicated the V617F mutation, while the 364-bp products indicated the internal control. PV samples with the V617F mutation were used as positive control. Aberrant bands were detected in one of the 248 samples (AML02, Figure 1 lane 5).

To confirm the aberrant bands detected by allele-specific PCR, DNA from AML02 was subsequently sequenced in both directions on a MegaBase sequence system (Amersham, Buckingham, UK). PCR products were purified and ligated into pGEM-T vector (Promega, Madison, WI, USA). Sequencing revealed G to T transversion at nucleotide 1849, resulting in the V617F mutation (Figure 2). Several samples without aberrant bands by allele-specific PCR were also sequenced, but only wild-type sequences were obtained.

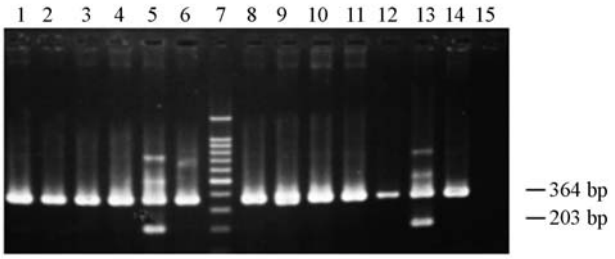

Figure 1 - Allele-specific PCR analysis of the *JAK2* gene in hematological neoplasms. Ten of 20 µL of PCR products were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide and visualized by ultraviolet illumination. The size of the products is indicated on the right. Aberrant bands were detected in one of the AML samples (lane 5). Lane 1, AML01; lane 2, MDS01; lane 3, ALL01; lane 4, BAL01; lane 5, AML02; lane 6, AML03; lane 7, 100 bp ladder; Lane 8, ALL02; lane 9, AML04; lane 10, AML05; lane 11, AML06; lane 12, CLL01, lane 13, PV01; lane 14, CML01, lane 15, water. Aberrant bands of PV01 are shown as positive control (lane 13).

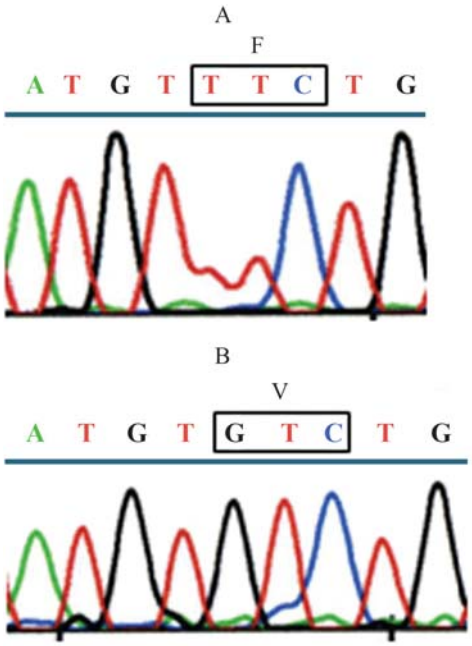

Figure 2 - Sequence analysis of the *JAK2* gene in AML. Sequencing identified G to T transversion at nucleotide 1849, resulting in the V617F mutation in the AML sample (A, AML02). Wild-type sequences are shown as control (B, MM01).

To determine the expression of the JAK2 V617F mutation, we performed reverse transcriptase-PCR (RT-PCR) analysis in AML02 (Mori *et al.*, 1990). The following sequences were used for the primers: RT-11F1, 5'-AAAGC CTTGGCCAAGGCACTT-3'; and RT-13R2, 5'-ATGCA TGGCCCATGCCAACTG-3'. After 5 min at 94 °C, 30 amplification cycles of 60 s at 94 °C, 60 s at 55 °C and 60 s at 72 °C were performed, with a subsequent 10 min extension at 72 °C. Primers RT-11F1 and RT-13R2 amplify

340-bp products. Sequencing of *JAK2* cDNA revealed the expression of the mutant allele in 20 out of 41 AML02 clones.

The V617F mutation of the *JAK2* gene was found in one of the 130 myeloid neoplasms, but not in the 114 lymphoid malignancies or in the four biphenotypic acute leukemias. The mutation was detected in the majority of Japanese patients with PV and ET (K Yoshinaga, N Mori, Y Wang, M Shiseki, T Motoji, unpublished data). In contrast to classic MPD, the V617F mutation was infrequent in myeloid leukemia and absent in lymphoid malignancies. Recent reports show that the JAK2 V617F mutation was not identified in either acute lymphoblastic leukemia (ALL) or chronic lymphocytic leukemia (CLL) (Levine *et al.*, 2005b). Our study showed that also in Japan the V617F mutation is absent in ALL and CLL. Furthermore, it was also undetectable in adult T-cell leukemia (ATL), macroglobulinemia, multiple myeloma (MM), and malignant lymphoma (ML).

AML02 was obtained from a patient with AML showing M2 morphology according to the French-American-British (FAB) classification at diagnosis. This patient was a 70-year-old woman with no history of preceding MPD or MDS. Peripheral blood tests revealed 50.1 x 10^9/L leukocytes with 76.5% blast cells, 7.8 g/dL hemoglobin, and 55 x10^9/L platelets. Although the favorable chromosomal alteration t(8;21)(q22;q22) accompanied by *AML1/ETO* transcripts was the only chromosomal abnormality observed in the patient, two courses of chemotherapy resulted in induction failure and short survival. The mutation was found in nine out of 14 AML02 clones. Since this sample was expected to contain more than 90% blast cells after mononuclear cell isolation, this case may have been heterozygous for the V617F mutation. Another possibility is that a substantial proportion of the leukemic cells from AML02 did not harbor the mutation.

The V617F mutation was detected in one of the 38 patients with AML (3%). Other recent studies found a similar incidence of the V617F mutation: 0/17 (Jones *et al.*, 2005), 5/90 (6%) (Scott *et al.*, 2005), 2/39 (5%) (Jelinek *et al.*, 2005), 4/222 (2%, three had preceding MPD) (Levine *et al.*, 2005b), 1/152 (0.7%) (Frohling *et al.*, 2006), and 2/112 (2%) (Lee *et al.*, 2006). The V617F mutation in AML was initially found in FAB M6 (1/53) (Frohling *et al.*, 2006) and FAB M7 (2/11) (Jelinek *et al.*, 2005), while the FAB subtype of AML was not described in other reports. In the current study, this mutation was also found to be present in one out of 12 patients with AML M2 (8%): one of the four patients with t(8;21)(q22;q22). After submission of our paper, expression of the JAK2 V617F mutation was reported in two out of 18 MDS/AML but not in 198 *de novo* AML cases (Nishii *et al.*, 2007). In contrast to their result, in the present study expression of the mutant allele was detected in AML02, as described above.

Our study revealed that the V617F mutation is infrequent in myeloid leukemia and absent in lymphoid malignancies in Japan. Nevertheless, it was detected in AML02, along with the favorable cytogenetic alteration t(8;21)(q22;q22). Previous studies have demonstrated that additional event(s) are required for the development of AML in the presence of t(8;21)(q22;q22) (Yuan *et al.*, 2001; Kuchenbauer *et al.*, 2006). Tyrosine kinases have been involved in several disorders including BCR-ABL in CML, PDGFRβ in CMMoL, and PDGFRα in chronic eosinophilic leukemia. Furthermore, activating mutations in the *FLT3* receptor tyrosine kinase gene are the most common genetic events in AML. Patients with FLT3 mutations have a poor prognosis, suggesting the important role of tyrosine kinase gene mutations in leukemogenesis. The relation between the V617F mutation and short survival in AML02 is unclear. The V617F mutation might confer a proliferative advantage to blast cells and play a role in the pathogenesis of certain AML cases. As suggested for FLT3 mutations, constitutive signaling in the absence of ligand may result in reduced apoptosis of the leukemic cells or may grant increased repair capacity following cell damage; either of these mechanisms could be considered as inducing chemoresistance (Kottaridis *et al.*, 2001). Further studies will clarify the incidence and significance of the V617F mutation in AML with t(8;21)(q22;q22).

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