The *JAK2*V617F mutation is detectable at very low level in peripheral blood of healthy donors

Leukemia (2006) **20,** 1622. doi:10.1038/sj.leu.2404292;
published online 15 June 2006

The *JAK2*V617F mutation has been recently described in several subsets of Philadelphia-negative myeloproliferative diseases (MPD) (for a review see McClure *et al.*[1]). However, its precise role and position in the multistep genetic events leading to such MPD is still unknown. It may be that this mutation represents an early molecular onset causing the MPD phenotype or occurs as a mutation associated with disease progression.[2] Its identification in normal patients would favour the first hypothesis, and could thus help in understanding the function of the *JAK2*V617F mutation in genesis of mutated MPD. Using a sensitive quantitative polymerase chain reaction (PCR) approach, we thought to search for *JAK2*V617F mutation in a cohort of normal donors, and have detected it in around 10% of healthy volunteers.

Three blood samples were taken from 57 healthy donors – aged from 23 to 52 years – after informed consent was obtained. The samples were immediately coded to ensure anonymity of the donors. Five patients were rejected from the study because of a slightly abnormal blood count analysis performed on the first tube. Red blood cell lysis as well as DNA and RNA extractions were performed at day 1 from the second tube, and at day 2 from the third one. The PCR method used has been described elsewhere (in press in *Clinical Chemistry*). In brief, we used a quantitative PCR approach, which combines both molecular beacon probe and locked nucleic acid (LNA) oligonucleotide in a single and sealed tube. Molecular beacon was used to detect specifically the *JAK2*V617F-mutated allele while LNA oligonucleotide significantly and specifically limited the amplification of the wild-type (WT) *JAK2* sequence. This blocking property for the WT target allowed thus to enrich the PCR products with *JAK2*V617F-mutated amplicons and to reach a quantitative sensitivity of 0.01% of mutated alleles.

A low level of *JAK2*V617F mutation – corresponding to less than 10 copies of *JAK2*V617F alleles (based on a dilution of the homozygous *JAK2*V617F HEL cell line) – was detected on nucleic acids (genomic DNA and/or cDNA) extracted from second and third tubes in five out of 52 tested patients (Figure 1). Sequence analysis performed on the positive PCR products confirmed the existence of the V617F mutation (Figure 1, inset).

We were thus able to detect the *JAK2*V617F mutation in a small number of hematopoietic cells in healthy individuals unlike previously reported publications.[1] This discrepancy is more likely owing to the better quantitative sensitivity obtained with our method. The present observations shed light on the role of *JAK2*V617F mutation in the genesis of MPD and suggest that it is a very early molecular event, as proposed by Kralovics *et al.*[2] in one of their hypothesis regarding the putative pathogenetic mechanisms of *JAK2*V617F mutation (model A). Since our five positive healthy donors had a perfectly normal blood count, it is likely that this mutation is not associated with disease progression but would rather occur before the appearance of the MPD phenotype. Also, this mutational event is probably not sufficient *per se* to induce a MPD. This point is supported by the weak annual incidence of MPD (0.5–6.5 per 100 000 inhabitants)[3] compared to at least 10% (5/52) of healthy subjects harbouring the *JAK2*V617F mutation.

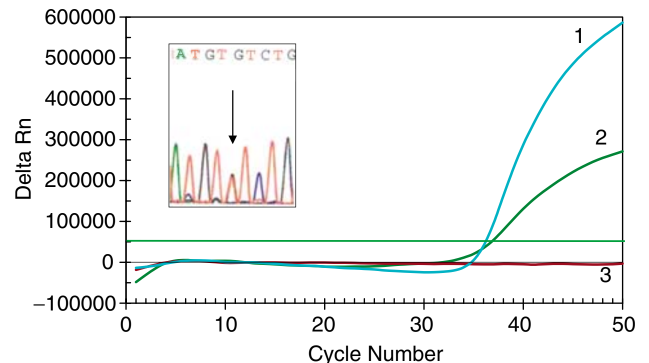

Figure 1 Detection of the *JAK2*V617F mutation at very low level in healthy individuals. An efficient and specific detection of *JAK2*V617F-mutated allele was obtained with the use of a method combining both molecular beacon and LNA technologies. Molecular beacon was used to specifically detect *JAK2*V617F-mutated allele while LNA oligonucleotide significantly limited the amplification of WT *JAK2* sequences. This combination allowed to detect up to 0.01% of *JAK2*V617F-mutated alleles. Amplification plot 1 corresponds to a 0.03% dilution of the homozygous *JAK2*V617F HEL cell line (representing theoretically 10 copies of *JAK2*V617F alleles). Amplification plot 2 illustrates the detection at very low level of *JAK2*V617F mutation in a normal patient, whereas the curve 3 represents a negative healthy donor. The green line corresponds to fluorescent detection threshold. *Inset* shows the nucleic acid sequence corresponding to amplification plot 2. Despite the very low percentage of *JAK2*V617F alleles in this healthy donor, the use of LNA oligonucleotide allowed to enrich sufficiently the PCR tube with mutated PCR products in order to get a sequence of good quality. The arrow indicates the position of the guanine/thymine (G/T) substitution.

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References

1 McClure R, Mai M, Lasho T. Validation of two clinically useful assays for evaluation of JAK2 V617F mutation in chronic myeloproliferative disorders. *Leukemia* 2006; **20**: 168–170.
2 Kralovics R, Passamonti F, Buser AS, Teo S-S, Tiedt R, Passweg JR *et al.* A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; **352**: 1779–1790.
3 Johansson P, Kutti J, Andréasson B, Safai-Kutti S, Vilen L, Wedel H *et al.* Trends in the incidence of chronic Philadelphia chromosome negative (Ph−) myeloproliferative disorders in the city of Goteborg, Sweden, during 1983–99. *J Intern Med* 2004; **256**: 161–165.