

## Brief report

Analysis of the Ten-Eleven Translocation 2 (*TET2*) gene in familial myeloproliferative neoplasms

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**The *JAK2*<sup>V617F</sup> mutation does not elucidate the phenotypic variability observed in myeloproliferative neoplasm (MPN) families. A putative tumor suppressor gene, *TET2*, was recently implicated in MPN and myelodysplastic syndromes through the identification of acquired mutations affecting hematopoietic stem cells. The present study analyzed the *TET2* gene in 61 MPN cases from 42 families.**

**Fifteen distinct mutations were identified in 12 (20%) *JAK2*<sup>V617F</sup>-positive or -negative patients. In a patient with 2 *TET2* mutations, the analysis of 5 blood samples at different phases of her disease showed the sequential occurrence of *JAK2*<sup>V617F</sup> and *TET2* mutations concomitantly to the disease evolution. Analysis of familial segregation confirmed that *TET2* mutations were not inherited but**

**somatically acquired. *TET2* mutations were mainly observed (10 of 12) in patients with primary myelofibrosis or patients with polycythemia vera or essential thrombocythemia who secondarily evolved toward myelofibrosis or acute myeloid leukemia. (Blood. 2009;114:1628-1632)**

## Introduction

Families of myeloproliferative neoplasms (MPNs) are characterized by a clinical and genetic heterogeneity. First, within MPN families, distinct clinical entities are observed, the 3 main ones being polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).<sup>1,2</sup> Second, disease evolution can be highly variable within families presenting with the same type of MPN.<sup>2,3</sup> The acquired *JAK2*<sup>V617F</sup> mutation present in most PV and half of ET and PMF sporadic or familial cases does not totally explain the phenotypic variability.<sup>2-7</sup> The existence of additional molecular events, either germline or acquired, may explain the MPN predisposition and the distinct phenotypes observed within MPN families.<sup>8-10</sup>

Recently, acquired mutations of the Ten-Eleven Translocation 2 gene, (*TET2*), were reported in approximately 12% of sporadic MPN (PV, ET, and PMF) as well as in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).<sup>11</sup> By in vitro clonal assays, Delhommeau et al showed that *TET2* defects target both multipotent and committed progenitors and were associated with a selective advantage of early hematopoiesis.<sup>11</sup> These recent findings led us to analyze *TET2* in familial MPN cases to determine whether

*TET2* could be a predisposition factor, to estimate the prevalence of acquired *TET2* events in MPN cases, and to describe the clinical profile of patients with *TET2* mutations.

## Methods

Families with at least 2 affected patients with MPN were collected through a national network.<sup>2</sup> The diagnoses of MPN were reviewed based on the 2008 World Health Organization criteria.<sup>12</sup> The study was approved by the French Comité de Protection des Personnes. All participants gave their written informed consent in accordance with the Declaration of Helsinki.

We analyzed 61 patients from 42 MPN families, 40 European and 2 African (F3 and F4). Thirty-three patients displayed a simple phenotype consisting of PV (14), ET (12), or PMF (7) with no observed hematologic evolution of the disease after a mean follow-up period of 12 years. Twenty-eight other patients had experienced an evolution in their MPD phenotype with a mean follow-up of 14.3 years: PV evolving into myelofibrosis (post-PV MF, 6) and/or into AML (12), ET evolving into MF (4) and/or AML (5), or PMF turning into AML (1).

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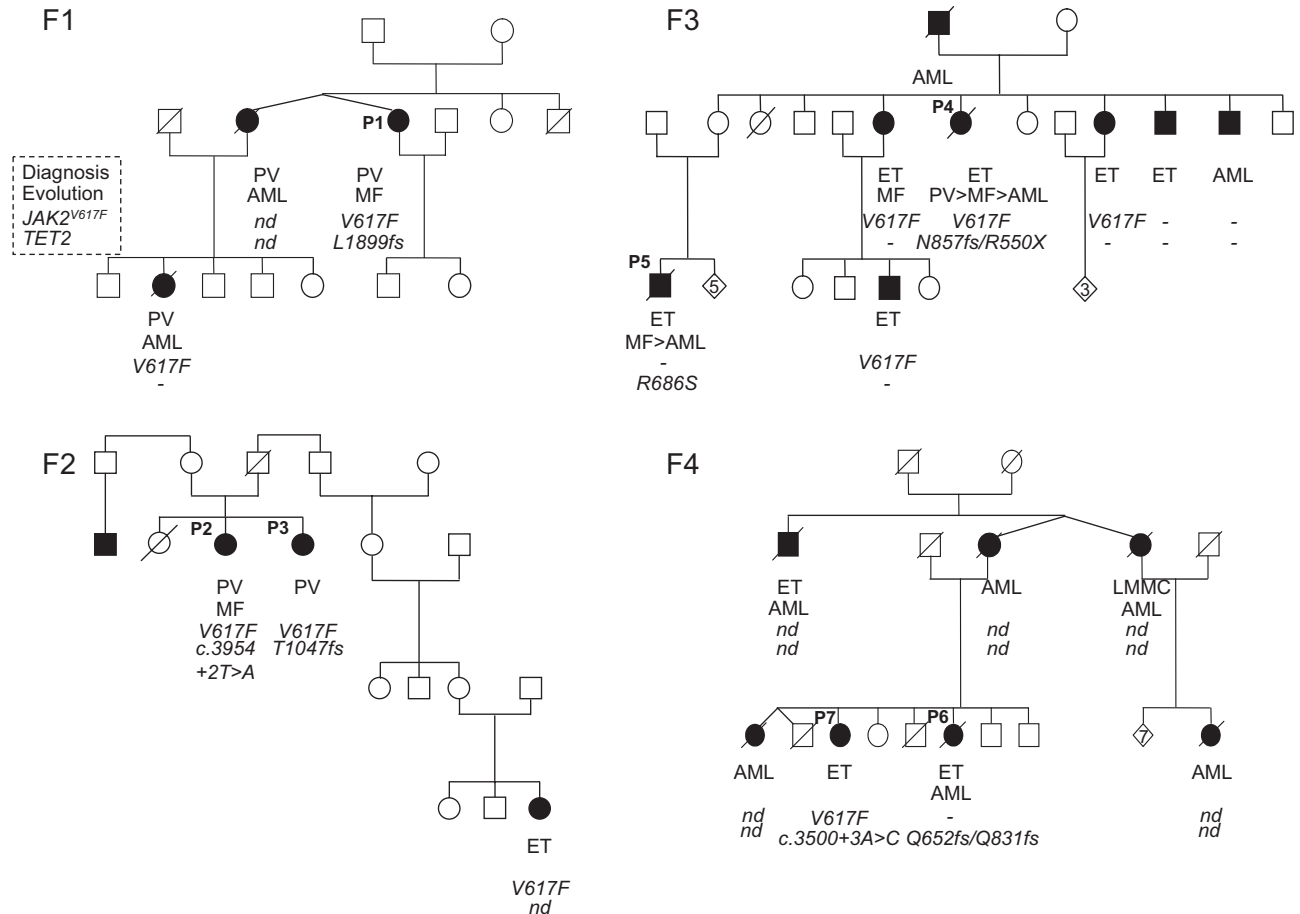
**Table 1. Clinical profile of the 12 patients with TET2 mutations**

	P1 (F1)	P2 (F2)	P3 (F2)	P4 (F3)	P5 (F3)	P6 (F4)	P7 (F4)	P8	P9	P10	P11	P12
Sex	Female	Female	Female	Female	Male	Female	Female	Female	Male	Female	Male	Male
Diagnosis	PV	PV	PV	ET > PV	ET	ET	ET	ET	PMF	PMF	PMF	PV
Age at diagnosis, y	75.3	62.1	58.4	37.5	34.8	47	48.6	45.3	78	77.2	64.3	54.6
Overall duration of the disease, y	5.9	16.3	16.4	18.1	7.3	1.2	6.2	29.2	3.5	4.3	3.6	13.2
Outcome	Alive	Alive	Alive	Deceased	Deceased	Deceased	Alive	Alive	Deceased	Deceased	Deceased	Alive
<b>Complications</b>												
Disease duration at first complication, y	4.7	13	—	15.7	7.1	1.0	—	16.0	—	—	—	9.4
Myelofibrosis, yes/no	Yes	Yes	No	Yes	Yes	No	No	Yes	—	—	—	Yes
Leukemic transformation, yes/no	No	No	No	Yes	Yes	Yes	No	No	—	—	—	No
<b>JAK2<sup>V617F</sup></b>												
Disease duration at first observation, y	4.6	14.4	14.5	8.4	3.6	1.0	5.7	20.3	0.2	2.9	2.7	3.4
Allele burden, %	95	65	50	25	0	0	40	90	35	35	65	80
<b>TET2 mutations</b>												
Disease duration at first observation, y	4.6	14.4	14.5	8.4/15.1*	3.6	1.0	5.7	20.3	0.2	2.9	2.7	3.4
TET2 allele burden, %	50	25	20	5/40†	40	50/35	50	45/60	45/60	45	45	20
Location	Exon 11	Intron 7	Exon 3	Exon 3	Exon 3	Exon 3	Intron 4	All exons	Exons 3/11	Exon 3	Exon 8	Exon 11
Nucleotide change	c.5695delC	c.3954+2T>A	c.3138delT	c.1648C>T/c.2570delA	c.2058A>T	c.1955delA/c.2490dupA	c.3500+3A>C	c.1_6009del	c.1720C>T/c.4999_5014del16	c.694C>T	c.4019T>C	c.5603A>G
Protein change	p.Leu1899fs	p.?	p.Thr1047fs	p.Arg550X/Asn857fs	p.Arg686Ser	p.Gln652fs/p.Gln831fs	p.?	p.?	p.Gln574X/p.Leu1667fs	p.Gln232X	p.Leu1340Pro	p.His1868Arg

— indicates not applicable.

\*Disease duration at first observation for each mutation.

† TET2 allele burden at first observation for each mutation.



**Figure 1. Pedigrees of 4 MPN families (F1, F2, F3, and F4) showing that *TET2* mutations do not segregate with the MPN phenotype.** Filled symbols represent patients; their clinical phenotype is indicated below. Under each symbol, the first top line represents the phenotype at the time of diagnosis; the second line, the evolution (blank where there is none); the third line, the *JAK2*<sup>V617F</sup> status (V617F when the mutation was found; – otherwise); and the fourth line, any *TET2* mutation. Mutations are annotated in amino acid one-letter code. nd indicates not done.

### *TET2* molecular analysis

Amplifications of *TET2* (NM\_001127208.1) were performed either on genomic DNA extracted from mononuclear blood cells and from buccal swabs or directly on hematopoietic colonies after heating at 95°C for 10 minutes. Purified polymerase chain reaction (PCR) products were sequenced using the BigDye Terminator chemistry (Applied Biosystems) and run on an ABI3100 capillary sequencer.

The search for large genomic rearrangement was performed by quantitative multiplex PCR of short fluorescent fragments and applied to patients homozygous for all *TET2* polymorphisms to exclude hemizyosity of the region. Primer pairs were designed for exons 3, 6, and 11. PCR products were separated on an ABI3100 capillary sequencer. Analysis using GeneMapper, Version 4.0 (Applied Biosystems), is based on the comparison of the peak heights generated from the tested DNA sample and the control DNA. A heterozygous exon deletion will lead to a 2-fold reduction of the height of the corresponding peak. Primer sequences for *TET2* sequencing and quantitative multiplex PCR of short fluorescent fragments are shown in supplemental Table 1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

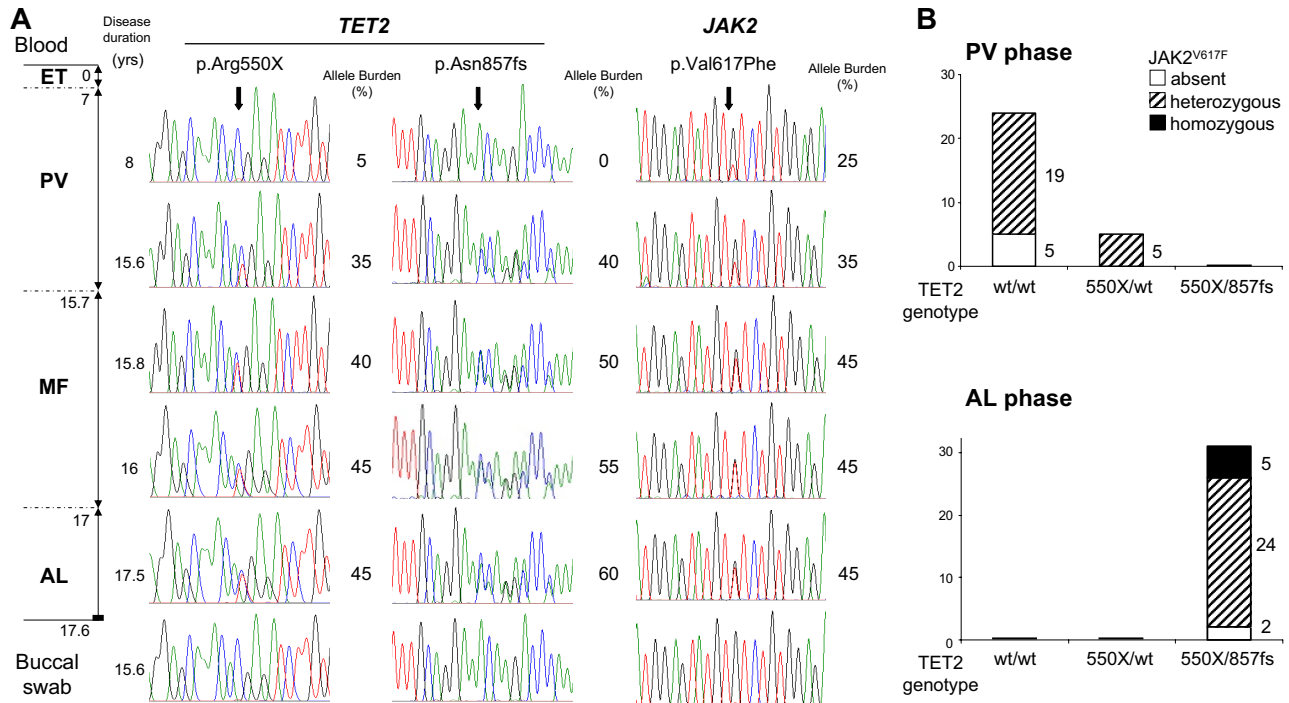
### Cell culture

Peripheral blood granulocytes were isolated using standard protocols. Thawed cells were sorted on the CD34 and CD38 antigens using a FACSDiva cell sorter. CD34<sup>+</sup>CD38<sup>+</sup> cells were seeded in methylcellulose medium supplemented with erythropoietin, interleukin-3, and stem cell factor. Hematopoietic colonies were picked at day 14.

## Results and discussion

Sixty-one patients were screened for mutations in the 6009-bp coding sequence of the *TET2* gene, composed of 11 exons spanning 130 kb. Fifteen distinct molecular defects, including 11 (73%) truncating mutations, 3 (20%) missense mutations, and one whole gene deletion, were identified (Table 1). They were spread throughout the gene. The 11 truncating mutations consisted of 3 nonsense mutations, 6 out-of-frame insertions/deletions, and 2 splice site mutations. The 3 missense mutations affected amino acids that were conserved in at least one of the *TET2* paralogs, *TET1* and *TET3*, and in *TET2* orthologs.<sup>13,14</sup> Two, p.Leu1340Pro and p.His1868Arg, were located in the 2 highly conserved *TET2* functional domains (1134-1444 and 1842-1921) of the *Drosophila* ortholog.<sup>13</sup> Furthermore, all 3 missense mutations were absent from 165 control individuals of ethnically matched populations, leading us to consider them as putative mutations. The proportion and the type of *TET2* mutations identified in familial MPNs were similar to the ones found in sporadic MPN cases.<sup>11,15</sup> *TET2* mutations have also been reported in other myeloid malignancies.<sup>11,15-19</sup>

All mutations identified were each found in a single patient, and segregation with the phenotype could never be observed in the families (Figure 1). Moreover, in 3 families (F2, F3, and F4), different *TET2* mutations were identified in affected members.



**Figure 2. Sequential study of *TET2* mutations and *JAK2*<sup>V617F</sup> in patient P4 (F3) in mononuclear cells and committed progenitors.** (A) Sequence electrophorograms are shown for each *TET2* mutation and for *JAK2*<sup>V617F</sup>. (Left diagram) The different phases of the disease with their time lapse from diagnosis. (Right diagram) The disease duration (in years) at each sample date. Allele burdens of *TET2* mutations and *JAK2*<sup>V617F</sup>, semiquantitatively estimated by sequencing as Bellanne-Chantelot et al.,<sup>2</sup> are indicated. (B) Histograms show for PV and acute leukemia (AL) phases the 3 distinct *TET2* genotypes (each bar corresponding to a specific genotype, wt/wt, 550X/wt, 550X/857fs). The *JAK2*<sup>V617F</sup> mutation was absent, heterozygous, or homozygous. The number of genotyped clones is indicated for each group.

Sequence analysis of buccal swabs available for patients P1 (F1) and P4 (F3) showed the absence of the *TET2* mutations. Therefore, the analysis of the 42 families showed that *TET2* is not a major predisposing gene factor in familial MPN and that identified *TET2* defects were acquired events.

Two distinct *TET2* mutations were found in 3 unrelated patients (P4, P6, and P9, Table 1). We picked 3 individual colonies derived from CD34<sup>+</sup>CD38<sup>+</sup> cells from the blast phase of patient P4; and using allele-specific PCR, we found that each *TET2* mutation affected a different allele of the same colony (data not shown). In addition, we showed in mononuclear cells from patient P6 that the 2 exon 3 mutations were not located in cis. These observations are in favor of a biallelic inactivation of *TET2*, in agreement with the hypothesis of a tumor suppressor role of *TET2*.<sup>11</sup>

In patient P4 for whom 5 blood samples were available throughout the last 3 steps of her evolution, PV, MF, and AML (Figure 2A), sequence analysis showed that *JAK2*<sup>V617F</sup> and the *TET2* p.Arg550X mutation were present at the PV stage and later on. The p.Asx857fs mutation only started being detectable in the second sample, 7 years later and 5 months before the diagnosis of MF. The sequential analysis showed that the burden of *JAK2* and *TET2* mutant alleles grew in time, concomitantly with the development of the disease. Blood progenitor cells were available at the PV and blast phases (Figure 2B). During the PV stage, endogenous erythroid colonies carried the p.Arg550X mutation (5 of 29) but p.Asx857fs was never observed and 19 of 29 clones were found *JAK2*<sup>V617F</sup>-positive in the absence of any *TET2* mutation. These results suggest the initial occurrence of *JAK2*<sup>V617F</sup> followed by *TET2* p.Arg550X and subsequently by the *TET2* p.Asx857fs defect. After leukemic transformation, all colonies but 2 (29 of 31) carried *JAK2*<sup>V617F</sup> and both *TET2* mutations. We genotyped the rs16922579 SNP located in intron 9 of *JAK2* and heterozygous in patient P4. A homozygous G/G genotype was found in *JAK2*<sup>V617F</sup>-

negative colonies, whereas G/A and A/A genotypes were observed in *JAK2*<sup>V617F</sup> heterozygous and homozygous colonies, respectively. These preliminary results suggest a loss of the *JAK2*<sup>V617F</sup> allele through mitotic recombination during disease progression. In contrast, in 2 other studies, mitotic recombination was excluded in 2 informative patients who transformed to AML and for whom all blast cells were *JAK2*<sup>V617F</sup>-negative.<sup>20,21</sup>

The *TET2* defects with an allele burden semiquantitatively estimated by sequencing varied from 20% to 60% and were identified in 12 patients (12 of 61, 20%) diagnosed with PV (4 of 32), ET (5 of 21), and PMF (3 of 8; Table 1). All but 2 patients were positive for the *JAK2*<sup>V617F</sup> mutation. Altogether, 20% of the *JAK2*<sup>V617F</sup>-positive patients were found mutated for *TET2* (10 of 49) and 17% of the *JAK2*<sup>V617F</sup>-negative patients (2 of 12). The 2 negative cases were ET patients who developed very active AML and died rapidly (P5 and P6, Table 1). In contrast to results recently reported, we did not observe an older age for patients with *TET2* mutations (supplemental Table 1).<sup>15</sup>

Twenty-nine percent (10 of 35) of patients with PMF or with hematologic complications (post-PV/ET MF or AML) were found mutated in *TET2* compared with 7.7% (2 of 26) of patients without any diagnosed hematologic complications after a mean disease duration of 12 years.

We first performed a univariate analysis to compare the incidence of transformation between the 2 groups (supplemental Table 1). The results indicated a trend toward hematologic complications in *TET2*-mutated patients, although this difference was not statistically significant ( $P = .055$ ). This was confirmed by a multivariate analysis (supplemental Table 1). Similarly, a trend toward malignant proliferation was recently reported in MDS/MPN syndromes.<sup>18</sup> Studies on larger cohorts of MPN cases would be required to estimate the prevalence of *TET2* events in patients with more aggressive outcome.

Recent data showed that TET1 catalyzes conversion of 5-methylcytosine to 5-hydroxy-methylcytosine.<sup>22</sup> TET gene family may play an important role in epigenetic regulation of stem cell functions. In addition, it was shown that deletion of *TET2* induced a clonal advantage at the level of early hematopoietic progenitors.<sup>11</sup> This may explain that patients with a defect in *TET2* are prone to progress to MF. Moreover, *TET2* defects may be a primary or secondary event, before or after *JAK2*<sup>V617F</sup> as reported for the 20q- deletion.<sup>21</sup> In future studies, it will be important to understand whether different kinetics of occurrence of these 2 independent genetic defects have the same impact on the clinical course of the disease and are both associated with a clonal dominance at early hematopoietic stages.

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## Authorship

Contribution: C.B.-C., F.D., W.V., and A.N. drew the original study design; D.B., C.C., A. Delannoy, A. Devidas, M.G.-P., F.I., A.N., G.P., Y.P., W.V., and the French Group of Familial Myeloproliferative Disorders recruited the patients; A.N. recorded all clinical and hematologic data; C.S.-M., G.L., and C.J. performed molecular analyses; F.D., S.D., I.P., and O.B. assisted with interpretation of research; C.B.-C. and C.S.-M. analyzed data and wrote the manuscript; and A.N., F.D., S.D., and W.V. critically reviewed the manuscript. All authors contributed to the amendment of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of the French Group of Familial Myeloproliferative Disorders appears in the supplemental Appendix.

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