

## LETTER TO THE EDITOR

### Mutations and deletions of the *SUZ12* polycomb gene in myeloproliferative neoplasms

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Alterations of epigenetic marks have an important role in myeloid malignancies.<sup>1</sup> Mutations have been found in several epigenetic regulators including *ASXL1*, *DNMT3A*, *EZH2*, *IDH1/2* and *TET2*.<sup>2,3</sup> *DNMT3A*, *IDH1/2* and *TET2* are involved in the regulation of DNA methylation. *EZH2* belongs to a complex of proteins called 'polycomb repressor complex 2' (PRC2).<sup>4</sup> *ASXL1* is a regulator of PRC1, the other polycomb complex. Several other proteins belong to the two polycomb complexes; although alterations of *ASXL1*<sup>5</sup> and *EZH2*<sup>6,7</sup> are probably prominent in myeloid diseases, we suspected that these other PRC components might also be involved in some cases.

We searched for mutations in *SUZ12*, a gene encoding a PRC2 protein, in 186 whole blood samples from 125 myeloproliferative neoplasms (MPNs), 14 MPNs at the blast-phase stage and 47 chronic myelomonocytic leukemias (CMML). The MPNs comprised 33 polycythemia vera (PV) and 4 post-PV myelofibrosis (MF), 51 essential thrombocythemia (ET) and 9 post-ET MF, 22 primary myelofibrosis, 3 unclassifiable and 3 myelodysplastic syndrome/MPN cases. *SUZ12* is located on chromosome arm 17q (Figure 1a) and comprises 16 exons (Figure 1b). We determined the sequence of *SUZ12* exons 10 to 16 (the existence of a highly conserved *SUZ12* pseudogene prevented easy design of oligonucleotide primers for *SUZ12* exons 1 to 9). In the MPN samples, we also searched for mutations of *JAK2* (exon 14), *ASXL1* (exons 12), *DNMT3A* (exons 15–23), *IDH1* and *IDH2* (exon 4), *MPL* (exon 10) and *TET2* (all exons). Mutations were searched by Sanger DNA sequencing analysis except for the *JAK2* mutation, which was detected by semi-quantitative PCR. We searched for *SUZ12* deletions in 80 of the MPN samples and in the 47 CMML samples by using array-comparative genomic hybridization (aCGH) on high-density oligonucleotide microarrays (Hu-244A, Agilent Technologies, Massy, France), as described previously.<sup>8</sup> All patients signed an informed consent and the study was approved by our ethics committee.

We found mutations of *SUZ12* in two MPN patients: a PV, HD-0716 and a blast phase of post-ET MF, HD-1038 (Table 1). The two mutations were missense and apparently heterozygous (Figures 1c and d). In the PV case, sequencing of DNA from buccal swab or CD3-purified T-cells showed the absence of variation compared with reference, thus showing that the mutation was acquired (Figure 1e). The HD-0716 PV case was *JAK2* V617F-positive (the mutated allele burden was 15–30%) and *TET2*-mutated. After diagnosis, the patient, a 66-year-old man, was treated with hydroxyurea. After 2 years of treatment a blood sample processed and studied as the initial one showed that the *SUZ12* mutation was barely detectable (Figure 1d). The HD-1038 blast phase of a post-ET MF was diagnosed in an 80-year-old woman. The chronic stage was not available for study. At the acute stage, the sample was *JAK2* V617F-negative and *TET2*-mutated (c.3640C>T p.Arg1214Trp), the karyotype was complex and the aCGH profile showed, among other alterations, a heterozygous loss of *TET2*, 7q21-qter spanning

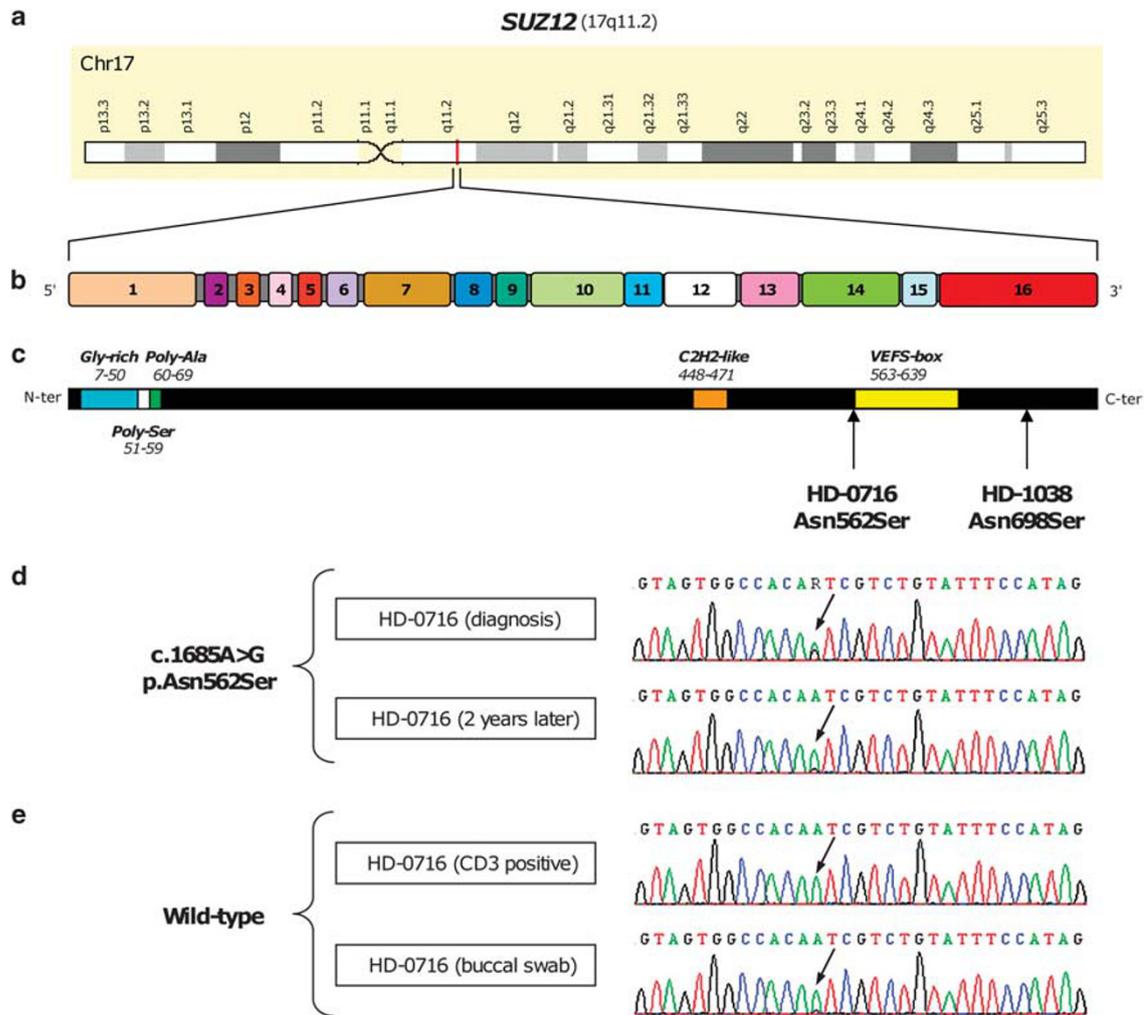
*EZH2*, 12p12-p13 spanning *AEBP2* and 17q11-q21 spanning *NF1* and the other *SUZ12* allele (Figure 2). No germline DNA was available. We did not detect any mutation of *ASXL1* in the two cases, whereas 17 other MPN cases were mutated (Table 1). We found 2 mutations in *DNMT3A*,<sup>9</sup> 2 in *IDH2*, 1 in *MPL* and 15 in *TET2*. No *SUZ12* mutation was found in the 47 CMML cases.

Deletions of the *NF1* gene at 17q11 are frequent in MPNs.<sup>10</sup> By using aCGH we found that the *SUZ12* gene, which lies close to *NF1*, is often included in these deletions. We found *SUZ12* deletions in three MPNs and in two CMML cases (Figure 2). This was the case for HD-1038. No aCGH profile was available for HD-0716. Two other MPN cases (HD-0535 and HD-0728) showed a deletion of a *SUZ12* allele but were not mutated (Table 1); however, as mentioned above, the first nine exons of the gene were not studied. In another MPN case (HD-0689), the deletion encompassed *NF1* but not *SUZ12* (Figure 2), suggesting that *NF1* was the actual targeted gene of the deletion.

Our study shows that a mutation can affect a PRC2 component other than *EZH2* in few cases of MPN. This mutation of *SUZ12* may substitute for or cooperate with a mutation of *EZH2* to compromise PRC2. This reinforces the current view that position epigenetic regulators as major players of leukemogenesis, together with signaling molecules and transcription factors. *SUZ12* mutations are rare but are actually in the same range as *DNMT3A*,<sup>9</sup> *IDH2* and *MPL*. Because we tested only the last exons of the gene our study may have underestimated the frequency of *SUZ12* mutations. Interestingly, in addition to mutations, *SUZ12* function could be affected by gene loss. The 17q11 region encompassing *NF1* and *SUZ12* is deleted in MPNs<sup>10</sup> and CMMLs. Our study suggests that both genes may participate to leukemogenesis, explaining why the *NF1-SUZ12* 17q11 region is often lost *en bloc*. It is also possible that other genes are involved; we sequenced the exons of *RAB11FIP4*, located between the *NF1* and *SUZ12*, in our series of MPN cases but found no mutation.

PRC2 is the major methyltransferase for H3K27 methylation, a modification of histone H3 that represses gene expression programs throughout development.<sup>4</sup> Mice with loss of function mutations in PRC2 components display enhanced activity of their hematopoietic stem cell/progenitor population and loss of *SUZ12* function in particular enhances hematopoietic stem cell activity.<sup>11</sup> It remains to be determined whether the mutations we have identified lead to a loss of function of the protein. In the HD-0716 case, the c.1685A>G p.Asn562Ser mutation may affect the VEFS-box known to be critical for *EZH2* interaction,<sup>12</sup> consequently disrupting PRC2 function. In the HD-1038 case, the deletion of an allele combined with the mutation of the other allele suggests a two-hit process associated with a tumor suppressor. Whether *SUZ12* mutations are present in other hematopoietic diseases should be determined. Whether other PRC2 components, such as *AEBP2*, *EED* and *JARID2*, are mutated and how they combined with other mutations in hematopoietic diseases should also be investigated.

Many genes contribute to leukemogenesis through mutations of their sequence; our study also shows that, beside the



**Figure 1** Mutation of the *SUZ12* gene in MPNs. (a) Localization of the *SUZ12* gene in chromosomal region 17q11. (b) Representation of the 16 *SUZ12* exons. (c) Representation of the 739-amino-acid-long *SUZ12* protein with known motifs and domains. Localization of the mutations is indicated below by arrowheads. (d) Nucleotide sequences for HD-0716 PV case. Sequence of the mutated *SUZ12* allele demonstrates change at the position indicated by an arrow in the sample taken at diagnosis. (e) The mutation was hardly detectable after 2 years of treatment and is absent from CD3 T-cells and buccal swab samples. The corresponding sequence is shown above the tracks.

**Table 1** Mutations and deletions of *SUZ12* in patients with myeloproliferative neoplasms

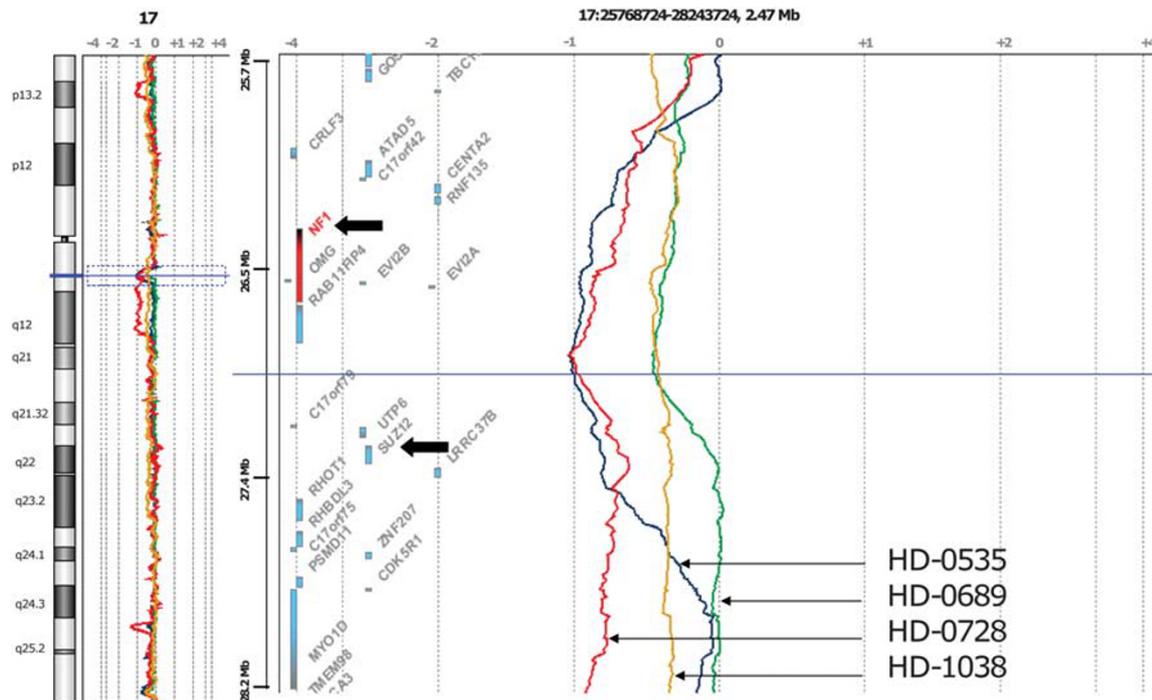
	<i>SUZ12</i> mutation	<i>SUZ12</i> loss	<i>ASXL1</i> mutation	<i>ASXL1</i> loss	<i>DNMT3A</i> mutation	<i>IDH1/2</i> mutation	<i>JAK2</i> mutation	<i>MPL</i> mutation	<i>TET2</i> mutation	<i>TET2</i> loss
Polycythemia vera	1/33	0/7	3/33	0/7	1/33	0/33	32/33	0/33	4/33	0/7
Essential thrombocythemia	0/51	0/19	2/51	0/19	0/51	0/51	32/51	0/51	3/51	0/19
Myelofibrosis	0/35	1/35	8/35	1/35	1/35	0/35	24/35	0/35	4/35	0/35
Other MPN	0/6	0/6	2/6	0/6	0/6	0/6	1/6	0/6	2/6	0/6
Blast phase MPN	1/14 <sup>a</sup>	2/13 <sup>a</sup>	2/14	0/13	0/14	2/14	4/14	1/14	2/14 <sup>a</sup>	2/13 <sup>a</sup>
Total MPNs	2/139 <sup>a</sup>	3/80 <sup>a</sup>	17/139	1/80	2/139	2/139	93/139	1/139	15/139 <sup>a</sup>	2/80 <sup>a</sup>

Abbreviation: MPN, myeloproliferative neoplasm.

<sup>a</sup>HD-1038 sample in common.

major contributors, most of which may be known already, there may be a number of rarely mutated contributors. This could render the molecular diagnosis of myeloid diseases more difficult to establish. Many of these rarely

mutated genes (for example, *SUZ12*) may affect the same functions and pathways (for example, polycomb repression) as the frequently mutated ones (for example, *EZH2*).



**Figure 2** aCGH profile of chromosome 17 in four MPN cases showing loss of the *NF1-SUZ12* region. HD-0535 (blue profile), a blast phase primary myelofibrosis (PMF), HD-0728 (red profile), a PMF and HD-1038 (yellow profile), a blast phase post-ET MF show loss, among other genes, of *NF1* and *SUZ12*, whereas in HD-0689 (green profile), a PMF, no loss of *SUZ12* is observed. Deletion of *NF1* but not *SUZ12* in HD-0689, and mutation of *SUZ12* in HD-1038 suggest both genes participate in leukemogenesis.

### Conflict of interest

The authors declare no conflict of interest.

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