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Short Communication

A complex and cryptic intrachromosomal rearrangement generating the FIP1L1_PDGFRA in adult acute myeloid leukemia

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ABSTRACT

Myeloid neoplasms with eosinophilia and abnormalities of the *PDGFRA* gene can benefit from therapy with tyrosine kinase inhibitors, therefore revealing the *PDGFRA* rearrangement is essential to ensure the best choice of treatment. The most common *PDGFRA* partner is the *FIP1L1* gene, generating the oncoprotein *FIP1L1/PDGFRA (F/P).* In the majority of cases the *F/P* fusion gene originates from intrachromosomal rearrangement at band 4q12, and occasionally from chromosomal translocations. In both cases, the interstitial chromosomal deletion of a region involving the *CHIC2* gene has been reported, which is cryptic by conventional karyotyping but detectable by Fluorescence In Situ Hybridization (FISH) analyses. Herein, we report an acute myeloid leukemia (AML) case presenting with eosinophilia; the *F/P* fusion gene originated from a new, cryptic and complex intrachromosomal rearrangement of 4q12. Classical FISH assay revealed abnormal hybridization signals, but the presence of the *F/P* chimaeric gene was demonstrated by molecular analysis. We performed molecular characterization of the chromosomal rearrangement and targeted Next-Generation Sequencing (NGS) analysis with a myeloid gene panel, revealing the presence of pathogenic genomic variants affecting the TET2 and ETV6 genes. These mutations were present as subclones at the disease onset and their clone size increased at relapse.

Introduction

According to the WHO classification of haematopoietic malignancies, disorders associated with eosinophilia and abnormalities of the *PDGFRA* gene belong to a sub-category of myeloid and lymphoid neoplasms [1]. As patients with the *PDGFRA* gene alteration respond to therapy with tyrosine kinase inhibitors (TKIs), it is particularly important to detect such a rearrangement by cytogenetics, Fluorescence In Situ Hybridization (FISH) and/or molecular analyses [2].

The *PDGFRA* gene can rearrange with many partners but the most common is the *FIP1L1* gene, generating the oncoprotein *FIP1L1/PDGFRA (F/P)* [2,3]. The majority of *F/P* fusion gene cases originate from intrachromosomal rearrangement at chromosome band 4q12, generating an interstitial chromosomal deletion of ~800 kb involving the *CHIC2* gene, which is cryptic by conventional karyotyping but detectable by FISH analyses [4–6]. In a minority of cases translocations have also been identified in patients with myeloid neoplasms, showing disruption of the *PDGFRA* gene, and the formation of novel chimeric genes that similarly respond to TKIs [2]. Among these cases, in very rare forms the *CHIC2* deletion has been reported, associated with the *PDGFRA* gene rearrangement [6].

In this study we describe an acute myeloid leukemia (AML) patient presenting with eosinophilia and the *F/P* fusion gene generated by a cryptic and complex intrachromosomal rearrangement of the 4q12. A *F/P* fusion gene generated by this kind of rearrangement has never before been described. In this work we also report the molecular characterization of the chromosomal rearrangement and the associated genomic alterations.

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Materials and methods

Clinical history

A 57-year-old man was referred to our center for hyperpyrexia, leukocytosis (33,000/μL) with eosinophilia (3000/μL) and thrombocytopenia (47,000/μL). Peripheral blood microscopic examination revealed a blast percentage of 4%. Bone marrow aspirate and biopsy revealed the presence of blasts (40%), increased eosinophil precursors and fibrosis. Cytogenetic analysis showed a normal karyotype. Molecular analysis showed the *FIP1L1-PDGFRA* fusion gene and the absence of NPM1 and FLT3 ITD/835 gene mutations. A diagnosis of AML with the *PDGFRA* rearrangement was made according to the 2016 WHO criteria. He was started on "3 + 7" induction treatment (daunorubicin and cytosine arabinoside) and achieved complete haematological remission (CHR). At that time molecular analysis showed the persistence of the *FIP1L1-PDGFRA* chimeric transcript, therefore the patient was started on imatinib treatment (200 mg/day). After one month from the CHR the patient developed hyperpyrexia, acute kidney failure and acute respiratory distress. Blood cell count analysis showed leukocytosis (75,000/μL) with eosinophilia (25,000/μL), anaemia (8.0 g/dL) and thrombocytopenia (25,000/μL). Peripheral blood smear analysis revealed 40% of blasts. He died after one week.

Cytogenetic analysis

Karyotyping was performed at diagnosis on bone marrow cells according to standard methods: the bone marrow (BM) cells were cultured for 24–48h, and chromosomes were G-banded with trypsin–Giemsa staining (GTG-banded) according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013 recommendations [7].

FISH analysis

FISH analyses were performed on a BM sample using bacterial artificial chromosomes (BACs) according to the University of California Santa Cruz (UCSC http://genome.ucsc.edu/; 2013 release) database. Chromosome preparations were hybridized in situ with probes labelled by nick translation, as previously reported [8–11]. A two-color FISH assay with BAC clones RP11-367N1 (4q12) and RP11-586A2 (4q12), specific for the *CHIC*2 and *KIT* genes, respectively, was used to investigate the 800-kb del(4)(q12) involving the region between the *FIP1L1* and *PDGFRA* genes (Fig. 1S). This assay, generally showing *CHIC2* deletion and *KIT* retention, is a surrogate marker for detecting the *F/P* fusion gene. Moreover, further FISH experiments were performed using specific probes to exclude the presence of a *RUNX1-RUNXT1, CBFB-MYH11* and 3q26 rearrangement, as previously reported [12–14].

Molecular analysis

Total RNA derived from BM cells of the patient's samples at onset and relapse was Reverse Transcribed (RT) to cDNA using the QuantiTect reverse transcription kit (Qiagen, Chatsworth, CA, USA). PCR analysis was performed according to the published methods [15].

For *PDGFRA* mutational analysis, the search for the mutations known to confer resistance to TKIs (T674I [15] and D842V [16]) was carried out with the following primers: *PDGFRA*-14F (5′GAACATTGTAAACTTGCTGGG)/*PDGFRA*-15R (5′cacCTCCGTGT GCTTTCAT), and *PDGFRA*-int17F (5′ggagtgggtggagtgagaac)/*PDGFRA*-int18R (5′ccagtgagggaagtgaggac). PCR products obtained from the samples at onset and relapse were purified and sequenced by Sanger sequencing.

For Ion Torrent sequencing the genomic DNA (gDNA) was extracted from BM mononuclear cells by density centrifugation in Ficoll. DNA quantification was performed using a QUBIT® 2.0 Fluorometer (Invitrogen). A customized panel encompassing the full coding regions or specific exons of 26 target genes involved in the pathogenesis of myeloid malignancies was used, as previously reported (Supplementary Table S1) [17]. Library preparation, quantification and sequencing were performed as previously reported [17]. Variants were annotated with Ion Reporter Software v5.10 (Thermo Fisher). Variants located in intronic regions or synonymous,or present with >1% global minor allele frequency (GMAF) in the 1000 Genomes (1000 G) Project were filtered out. Selected variants were investigated for a potential pathogenetic role using the SIFT and Polyphen scores and the Catalogue Of Somatic Mutations In Cancer (COSMIC) database. Sanger sequencing was performed for variants validation.

Bioinformatic segmental duplications analysis

In accordance with the GRCh37/hg19 human genome assembly, genomic coordinates of segmental duplications were retrieved from the UCSC Genome Browser (https://genome.ucsc.edu/index.html); the Segmental Duplication Database (http://humanparalogy.gs.washington.edu/) was queried to obtain information about inter and intrachromosomal segmental duplications density and distribution in chromosome 4.

Results

Molecular analysis by RT-PCR with *FIP1L1* and *PDGFRA* specific primers revealed the presence of the *F/P* fusion gene at both AML onset and relapse. Several splicing variants that joined different possible *FIP1L1* exons, starting from exon 7, to *PDGFRA* exon 12 and generating PCR products from 500 to about 900 bp long, were detected, similarly to other previously reported chronic eosinophilic leukemia (CEL) cases (Fig. 1A) [5,15,18].

FISH analysis performed using BAC clones RP11-367N1 (4q12) and RP11-586A1 (4q12), specific for the *CHIC*2 and *KIT* genes, respectively, revealed an abnormal hybridization signal of RP11-367N1 on chromosome 4q, approximately at the 4q31 region, and a normal signal of RP11-586A1 at 4q12 excluding the occurrence of the *CHIC2* gene deletion (Fig. 1B). Further FISH experiments with BAC clones RP11-631M9, RP11-1127P13 (red) and RP11-367N1, RP11-626H4 (green), entirely encompassing *FIP1L1* and *PDGFRA* genes, showed hybridization signals on chromosome 4 at 4q12 as expected, and splitting signals on der(4) chromosome, revealing the occurrence of breakpoints in correspondence with both the *FIP1L1* and *PDGFRA* genes. Reiterative FISH cohybridizations with BAC clones mapping at 4q12, in the genomic region centromeric and telomeric to *FIP1L1* and *PDGFRA* genes, respectively, showed normal signals at 4q12, whereas all BAC clones included in the region between the two genes hybridized distally, at 4q31 on the der(4) chromosome (Fig. 1B). A double inversion with breakpoints mapping first in *FIP1L1* and further in *PDGFRA* could explain the detected FISH pattern (Fig. 1C); on the other hand a fusion region duplication and insertion into distal 4q hypothesis should be excluded as it could not explain abnormally distal *CHIC2* gene localization on der(4) chromosome, with *KIT* gene hybridizing at 4q12 pericentric region. However, due to the paucity of material it was not possible to identify the precise breakpoint in correspondence with the 4q31 region.

By observing the density and distribution of 4q intrachromosomal segmental duplications in relation to the *FIP1L1* and *PDGFRA* genes (4q12), we detected two main clusters of about 1.5 Mb and 0.4 Mb, mapping to 4q13.2-13.3 and 4q31.21, at a distance of about 15Mb and 90Mb from the two genes, respectively. Two smaller

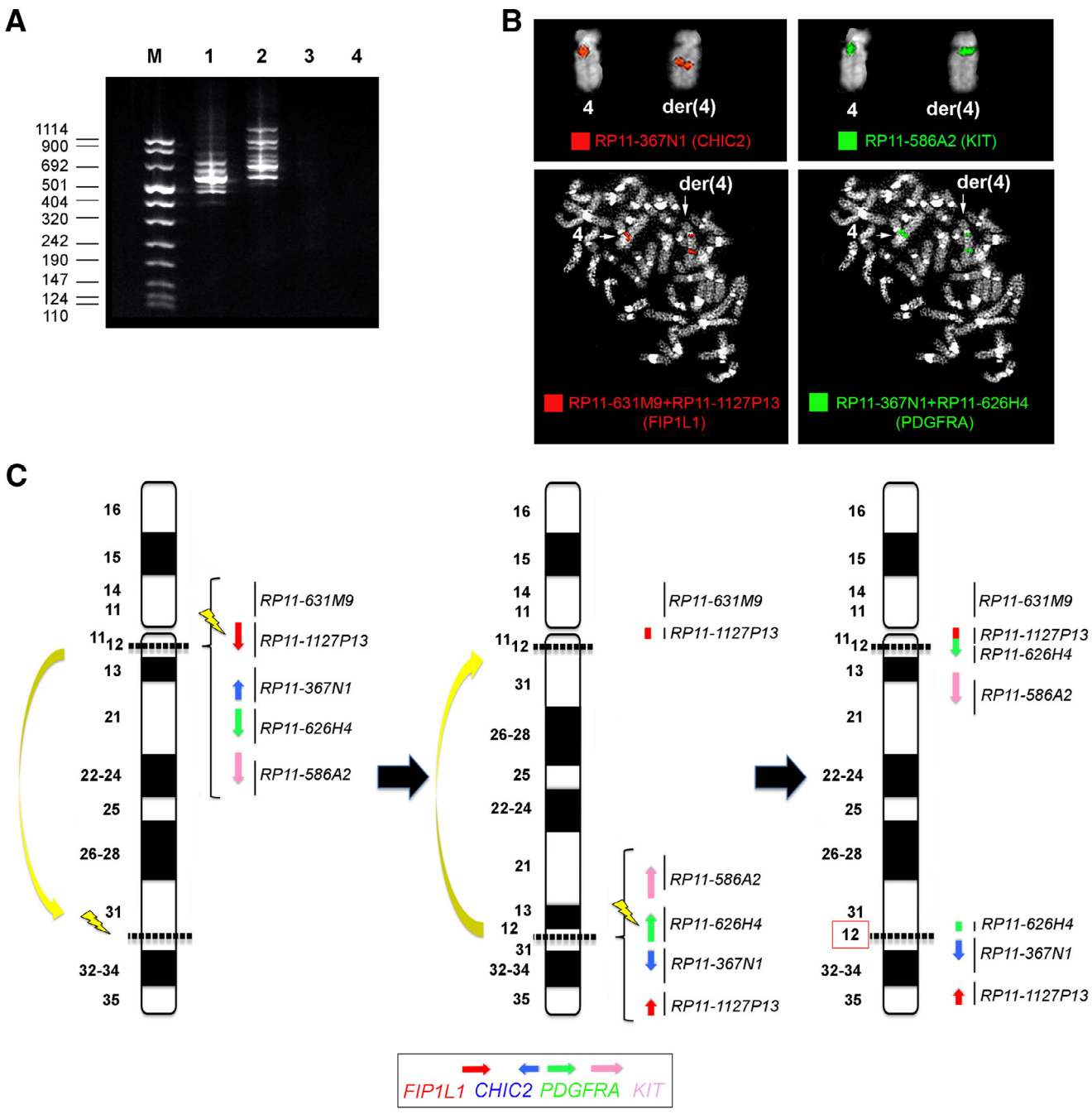

Fig. 1. **(A)** RT-PCR performed on BM samples of the AML patient at the disease onset (lane 1), a *F/P* positive CEL patient (lane 2), a negative cDNA control (lane 3), H_2O (lane 4). M: DNA Molecular Weight Marker VIII. **(B)** FISH experiments on a BM sample from the analyzed AML patient. BAC clones specific for *CHIC2* and *KIT* genes, respectively, showed an abnormally distal *CHIC2* gene localization on der(4) chromosome. Pools of BAC RP11-631M9, RP11-1127P13 (red) and RP11-367N1, RP11-626H4 (green) specific for *FIP1L1* and *PDGFRA* genes, respectively, showed spitting signals on der(4) chromosomes. **(C)** Chromosome diagram showing the hypothetical mechanism at the basis of the *F/P* fusion gene generation in the reported case. The hypothesis is that a first paracentric inversion occurred with breakpoints in correspondence with the *FIP1L1* gene and an unidentified region at 4q31; the second inversion occurred as a consequence of the *PDGFRA* gene break, but it could be hypothesized that the three breaks occurred concomitantly, generating only the *F/P* fusion gene without the reciprocal one. The mechanism of a double paracentric inversion is in accordance with the detected FISH pattern of BAC clones mapping next to the *FIP1L1* and *PDGFRA* genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

clusters also mapped to 4q26-27, extended 80 kb and 140 kb respectively, and located about 65 Mb from the two genes (Fig. 2S).

After filtering, targeted NGS analysis revealed two heterozygous mutations in the *TET2* and *ETV6* genes, both in the sample at AML onset and at relapse. A summary description of these variants is shown in Supplementary Table S2. All the mutations detected by NGS were confirmed by Sanger analysis. Moreover, *PDGFRA* Sanger mutational analysis revealed the appearance of the mutation T674I at AML relapse (Fig. 2).

Discussion

Several kinds of aberrations have been described for the *PDGFRA* gene, including translocations, insertions, deletions and complex rearrangements [19]; in all the reported cases the molecular consequences were represented by fusion genes of *PDGFRA* with different partners, among which more frequently *FIP1L1* [2,19].

Hematologic malignancies presenting with the *F/P* gene fusion show a wide range of clinical presentations including Myelopro-

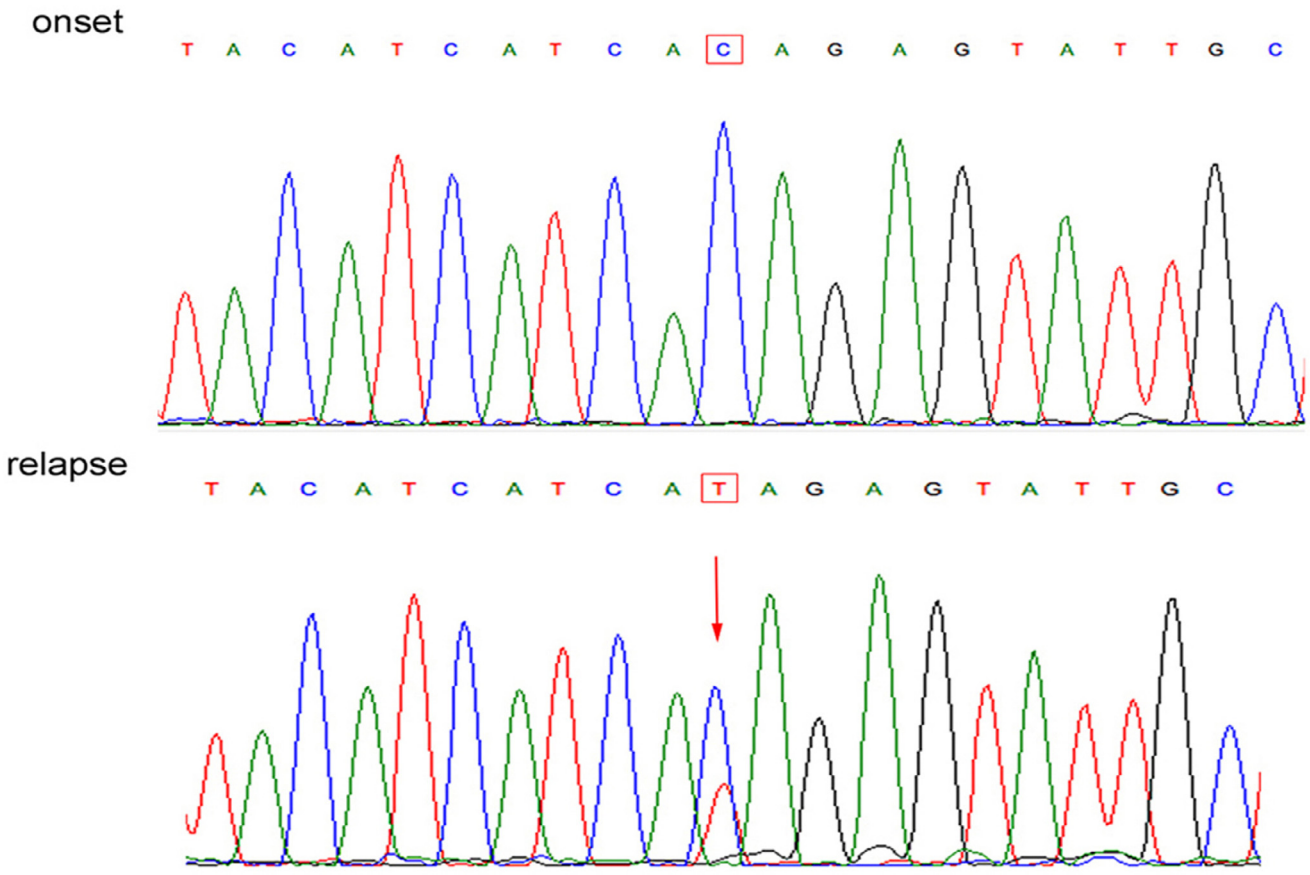

Fig. 2. Sanger sequencing revealed the acquisition of mutation T674I of *PDGFRA* in the sample at relapse.

liferative Neoplasms (MPNs) with eosinophilia, blastic phase of MPNs, eosinophilia-associated AML or T-cell lymphoblastic lymphoma. It is clear that the chimeric gene alone is insufficient to explain this phenotypic heterogeneity, and that at least a second hit probably occurred to generate the more aggressive forms [20].

Until now, FISH and PCR analyses have been considered equivalent techniques to detect the presence of the *F/P* gene, since all the reported cases presenting with the gene fusion were generated by the deletion of the *CHIC2* gene on the long arm of chromosome 4 [2]. When present, the *F/P* gene is an important molecular marker, which can be targeted by TKIs, and its detection is clinically essential to design tailored treatment [2].

We report an AML patient in which FISH analysis, performed to ascertain the *CHIC2* deletion, revealed its abnormal localization on the long arm of chromosome 4. As molecular RT-PCR analysis revealed the presence of the *F/P* fusion gene, further FISH analyses were performed and showed a complex rearrangement on chromosome 4q, likely generated by a double paracentric inversion, with breakpoints mapping first in *FIP1L1* and further in *PDGFRA*, responsible for the creation of the fusion gene.

Our AML patient received classic 3 + 7 induction associated to imatinib treatment, achieving a brief CHR. As resistance to TKIs can appear as a consequence of the acquisition of secondary mutations within the PDGFRA kinase domain, we searched for the presence of such mutations and found the T674I mutation at AML relapse.

We performed targeted NGS on the samples at AML onset and then relapse. This revealed two relevant point mutations affecting the *TET2* and *ETV6* genes. The former variant was a frameshift mutation. The *TET2* gene is frequently mutated in haematologic malignancies and most alterations are deletions or loss-of-function mutations [21]. *TET2* loss of function causes progressive defects in haematopoiesis, increasing haematopoietic stem cell self-renewal and myeloid lineage expansion, and impairing normal haematopoietic differentiation [21,22]. Moreover, the *TET2* gene mutation can be associated with CEL, not otherwise specified [23]. The latter observed variant was the ETV6 R399P, a missense mutation. R399 is a residue of the highly conserved ETS DNA-binding domain, reported as a hotspot for recurrent somatic mutation in cancer; the mutation abrogates DNA binding, altering subcellular localization and transcriptional regulation thus impairing hematopoiesis in a dominant-negative manner. The substitution of the residue R399 has been reported as germinal in cases of familial thrombocytopenia and hematologic neoplasm [24], and specifically the R399P somatic mutation has been reported in myelodysplastic syndromes [25].

To the best of our knowledge, our case is the first *F/P* positive patient with the concomitant alteration of *TET2* and *ETV6*. *ETV6* has been described as a partner of both *PDGFRA* and *PDGFRB* in some cases of myeloid malignancies associated with hypereosinophilia and its mutation has been reported rarely in *F/P* positive neoplasms [26]; similarly, *TET2* alteration has been occasionally found in *F/P* positive malignancies, but never together with *ETV6* mutations [26]. In our patient, the clone size of the revealed point mutations suggested that the *F/P* gene could likely represent the founder clone responsible for the disease onset and that other genomic instability conditions fostered the expansion of the *TET2* and *ETV6* mutated subclones. However, it is unclear whether these point mutations were concomitant mutations in the same cell, or emerged as distinct subclones that gained the upper hand in the disease evolution.

Non-allelic homologous recombination between segmental duplications located close to each other has been proposed to trigger recurrent rearrangements in myeloid neoplasms [27–30]. Although the mechanism underlying the genesis of the *F*/*P* gene fusion has not been fully elucidated, the scattered segmental duplications in the flanking regions of the *FIP1L1* and *PDGFRA* loci may possess the architectural elements responsible for the genomic instability associated with this region.

In conclusion, our report demonstrates that the *F*/*P* gene may be associated, in rare cases, with complex chromosomal rearrangements that demand FISH analyses associated to molecular studies to clarify the mechanism at the basis of the fusion gene generation. Furthermore, the NGS study of our case confirms the pathogenic complexity of the myeloid neoplasm associated with the *F*/*P* fusion gene.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.08.003.

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