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ORIGINAL ARTICLE

Mutational analysis of *JAK2*, *CBL*, *RUNX1*, and *NPM1* genes in familial aggregation of hematological malignancies

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Abstract Familial aggregation of hematological malignancies has been reported highlighting inherited genetic predisposition. In this study, we targeted four candidate genes: *JAK2* and *RUNX1* genes assuring a prominent function in hematological process and *CBL* and *NPM1* as proto-oncogenes. Their disruption was described in several sporadic hematological malignancies. The aim of this study is to determine whether *JAK2*, *CBL*, *RUNX1*, and *NPM1* germline genes mutations are involved in familial hematological malignancies. Using direct sequencing, we analyzed *JAK2* (exons 12 and 14); *CBL* (exons 7, 8 and 9); NPM1 (exon 12) and the entire *RUNX1* in 88 independent families belonging to Tunisian and French populations. Twenty-one sporadic acute leukemias were included in this study. We reported a heterozygous intronic c.1641+6 T>C *JAK2* variant (rs182123615) found in two independent familial cases diagnosed with gastric lymphoma and Hodgkin lymphoma. The in silico analysis suggested a potential impact on splicing, but the functional splicing minigene reporter assay on rs182123615 variant showed no aberrant transcripts. In one sporadic acute myeloblastic leukemia, we reported an insertion 846 in. TGTT in exon 12 of *NPM1* gene that may impact the normal reading frame. The rs182123615 *JAK2* variant was described in several contexts including myeloproliferative neoplasms and congenital erythrocytosis and was supposed to be pathogenic. Through this current study, we established the assessment of pathogenicity of rs182123615 and we classified it rather as rare polymorphism.

Keywords *JAK2* · *CBL* · *RUNX1* · *NPM1* · Familial hematological malignancies

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Introduction

Cancer results from the accumulation of several gene alterations. Approximately 5 % of all cancers correspond to the inherited form among them blood cancer. Familial aggregations of hematological malignancies were described in several studies underlying heritable germline genes mutations [1, 2]. The investigation of this inherited predisposition leads to the identification of driver gene mutations responsible for the tumorigenesis process.

Until now, only a few genetic alterations leading to familial hematological malignancies have been reported, describing mutations with low penetrance susceptibility through several association studies. In our investigation, we have underlined four genes widely described in several hematological malignancies in familial and sporadic context namely *JAK2*, *CBL*, *RUNX1*, and *NPM1* [3].

JAK2 and *RUNX1* have a prominent function in hematological process. The encoded JAK2 protein activates the transcriptional JAK-STAT signaling pathway involved in proliferation and survival of hematopoietic, immune, cardiac, and other cells [4, 5]. While *RUNX1*, also known as AML1 gene, acts as a key regulator of the expression of various hematopoietic genes [6, 7]. The disruption of the normal process involving both *JAK2* and *RUNX1* genes may lead to tumorigenesis. Indeed, *JAK2* mutations may activate the JAK-STAT pathway resulting in oncogenic events observed in myelodysplastic syndrome [8], in primary acute myeloid leukemia (AML) and Philadelphia-negative myeloproliferative disorders [9, 10], majorly in 95 % of polycythemia vera and in 50 % of essential thrombocythemia and primary myelofibrosis. The most prevalent mutation is *JAK2* V617F which occurs within the pseudokinase domain leading to the inhibition of kinase activity [11]. Somatic *RUNX1* mutations were reported in several forms of cancers involving myelodysplastic syndrome and AML [12, 13] while germline mutations were found in autosomal familial platelet disorder predisposing to myelodysplastic syndromes/AML development [14].

Both *CBL* and *NPM1* genes were classified as proto-oncogenes. They may promote growth factor independence and cellular proliferation leading to a tumorigenesis effect in several cell lines [15]. The *CBL* gene encodes for E3 ubiquitin-protein ligase involved in cell signaling and protein ubiquitination. Most mutations occur in exons 7, 8 and 9 encoding the zinc binding Ring-finger and the linker domains [16]. Heterogeneous somatic *CBL* mutations were reported especially in chronic myelomonocytic leukemia [15, 17], while germline mutations were identified only in juvenile myelomonocytic leukemia [18, 19].

The *NPM1* or nucleophosmin gene encodes a molecular chaperone moving between the nucleus and the cytoplasm, facilitating the transport of ribosomal proteins through the nuclear membrane [20]. *NPM1* mutations result in aberrant cytoplasmic dislocation of the mutant protein. This event appears to be critical for leukemogenesis [21, 22]. *NPM1* mutations often occur in exon 12 but occasionally are found in other exons [23].

Most *CBL* and *NPM1* mutations were described in myeloid malignancies including AML [24–27] and myelodysplastic syndrome [28].

The occurrence of *JAK2*, *CBL*, *RUNX1*, and *NPM1* mutations in several hematological neoplasms incite us to hypothesize that some patients with familial hematological malignancies might harbor mutations in these genes. The aim of this study is to establish the involvement of these four genes in 98 familial cases with aggregated hematological malignancies with or without solid tumors and 21 sporadic acute leukemia cases belonging to Tunisian and French populations.

Methods

Patients

JAK2 (exons 12 and 14); *CBL* (exons 7, 8 and 9); *NPM1* (exon 12); and the entire *RUNX1* genes were analyzed in 98 patients belonging to 88 independent families: 13 Tunisian and 75 French families recruited via a French national cooperative network focusing on familial hematological malignancies and the GenHem INSERM/DGRS Franco-Tunisian project.

The hematological malignancies cases included chronic or acute, lymphoid or myeloid leukemias, Hodgkin's or non Hogdkin's lymphomas, and myeloproliferative or myelodysplastic syndromes.

The studied cohort consists of 80 patients belonging to 71 familial forms of hematological malignancies (at least two cases of hematological malignancies with or without solid tumors in first, second or third degree relatives); 17 patients from 17 families with aggregation of tumors including one case of hematological malignancy in first, second or third degree relatives and 1 patient who had a multiple primitive tumor with hematological malignancy but without family history.

Thirteen Tunisian sporadic acute lymphoblastic leukemia (ALL) and 8 sporadic acute myeloblastic leukemia (AML) cases were included in this study only for *JAK2*, *CBL*, and *NPM1* genes investigation.

A control Tunisian population was recruited consisting of 100 healthy blood donors. Blood samples were obtained after the donors had given their informed consent.

Gene sequencing

To perform the mutational analysis, genomic DNA was extracted from peripheral blood cells obtained during complete

remission according to standard protocols of treatments. Informed consent was obtained from the patients, relevant family members (healthy relatives) or their legal guardian as required by the Helsinki Declaration. Genomic DNA was extracted from whole blood with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The exon coding region and the intron-exon junction of hot spot regions: the *JAK2* exons 12, 14; *CBL* exons 7,8,9; *NPM1* exon 12 and the entire *RUNX1* gene were amplified using standard PCR methods. The primer sequences are available upon request. The amplified PCR products were column-purified and both strands were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit v1.1 (Applied Biosystems- Foster City, USA) and loaded onto an ABI Prism 3500 sequencer (Applied Biosystems). The sequence chromatograms obtained were compared with the published human *JAK2*, *NPM1*, *CBL* and *RUNX1* gene sequence using the SeqScape software program v2.5 (Applied Biosystems).

In silico analysis

To predict the effects of non-synonymous SNP (nsSNP) at amino acid levels, bioinformatic tools provided in the Alamut pack V2.5 http://www.interactive-biosoftware.com and condel http://useast.ensembl.org/tools.html were used.

The SIFT (Sorting Intolerant from Tolerant) method predicts the effect of the substituted amino acid on protein function based on sequence homology and the physical properties of amino acids. Normalized probabilities of substitutions are calculated under default settings, and probabilities ≤0.05 are taken to be deleterious. While PolyPhen-2 (Polymorphism Phenotyping v2) tool predicts the impact of amino acids substitutions on the structure and function on protein based on phylogenetic and structural information characterizing the obtained genetic variation.

Bioinformatic predictions of splicing alterations

Four different algorithms were used to predict the effect of genetic variation affecting splicing sites in any human sequence, based on the evaluation of consensus values of potential splice sites and search for branch points. The used tools are the following: Splice Site Finder-like (SSF, see http://www.interactive-biosoftware.com), MaxEntScan(MES, http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html), splice site prediction by Neural Network (NNS, http://www.fruitfly.org/seq_tools/splice.html), and Human Splicing Finder (HSF, http://www.umd.be/HSF/). SSF, MES, NNS, and HSF were interrogated simultaneously using the integrated software Alamut v.2.5 (Interactive Biosofware; http://www.interactive-biosoftware.com). No thresholds were used for this analysis.

Splicing minigene reporter assay

The splicing minigene assay previously described [29] was performed to test the effect of the variant *JAK2* c.1641+6 T>C on splicing of *JAK2* exon 12. Wild-type and mutant genomic segments were amplified by PCR from patient genomic DNA using forward primer JAK2Ex12F-*Bam*HI: (5′-GACCGGATCCTCAAAGTTCAATGAGTTGACCCC-3′) and reverse primer JAK2Ex12R-*Mlu*I: (5′-GACCACGCGTACATCTAACACAAGGTTGGCA-3′), carrying 5′ tails with *BamH*I and *Mlu*I restriction sites, respectively (underlined). The PCR-amplified genomic segments encompassed the exon 12 (128 bp) of *JAK2* and part of the flanking intronic 5′ and 3′ regions (170 and 163 bp, respectively). After digestion with *Bam*HI and *Mlu*I enzymes, the PCR products were inserted into the *Bam*HI and *Mlu*I cloning sites in the intron of pCAS2, a two-exon splicing reporter minigene vector (Fig. 4b). The insert was then sequenced to identify the wild-type and variant minigene constructs and to ensure that no extra mutations were added during amplification or cloning. The wild-type and mutant minigene constructs were then transiently transfected into HeLa cells using the FuGENE 6 transfection reagent, according to manufacturer's instructions (Roche Applied Science). Cells were collected 24 h post-transfection, and the splicing patterns of the wild-type and mutant minigenes were analyzed by RT-PCR, electrophoresis, and sequencing of gel-purified RT-PCR products, as previously described [29].

Results

I-Genes sequencing

RUNX1 gene analysis

The entire *RUNX1* gene was sequenced in 98 familial cases of aggregated hematological malignancies (Table 1). Three variants were found, the first variant c.654 C>T p.Ser218Ser was found in three Tunisian familial cases: one independent patient diagnosed with AML at the age of 18, and two second degree related patients diagnosed with Hodgkin disease and CML at the age of 17 and 35, respectively.

The second variant c.167 T>C p.Leu56Ser was found in 2 unrelated female patients belonging to French familial cases. The first patient was diagnosed with breast cancer at the age of 66, the second one was diagnosed with breast cancer at the age of 53 and myeloproliferative disorders at the age of 54.

The third variant p.Pro463Pro was identified in 2 unrelated patients belonging to French familial cases. The first one was diagnosed with myeloma at the age of 35 and the second one was diagnosed with myelodysplastic syndrome at the age of

Table 1 Patients data with identified *JAK2*, *RUNX1*, and *NPM1* genes mutations

Gene	Patient N°	Sex	Geographic origin	Age at diagnosis (year)	Diagnosis	Mutations	Familial history
JAK2	P1	F	Tunisia	29	Gastric lymphoma	1641+ 6 T > C	FHM without solid tumors
	P2	M	Tunisia	32	Hodgkin lymphoma	1641+ 6 T > C	FHM without solid tumors
RUNX1	P3	F	France	66	Brest cancer	p.Leu56Ser	FHM with solid tumors
	P4	F	France	53/54	Brest cancer/Thrombocythemia	p.Leu56Ser	FHM with solid tumors
	P5	F	France	35	Myeloma	p.Pro463Pro	FHM with solid tumors
	P6	F	France	50	Myelodysplastic syndrome	p.Pro463Pro	FHM with solid tumors
	P7	F	Tunisia	18	Acute myeloid leukemia	p.Ser218Ser	FHM without solid tumors
	P8	M	Tunisia	17	Hodgkin lymphoma	p.Ser218Ser	FHM without solid tumors
	P9	M	Tunisia	35	Chronic myeloid leukemia	p.Ser218Ser	FHM without solid tumors
NPM1	P10	M	Tunisia	52	Acute myeloid leukemia	846 in. TGTT	Sporadic acute myeloid leukemia

FHM familial hematological malignancies, *M* Male, *F* Female

50. No concomitant mutations were observed in *JAK2*, *CBL*, and *NPM1* genes in all these patients.

CBL gene analysis

CBL exons 7-8-9 were investigated in which most mutations occur. Only one intronic variant 1095 + 19 G > T (rs2510152) was detected at homozygous and heterozygous levels with high frequency of 85 % covering several forms of sporadic and familial hematological malignancies. The in silico analysis using Splice Site Finder-Like, MaxEntScan, NNSPLICE and Human Splicing Finder (HSF, http://www.umd.be/HSF) in Alamut V2.5 (http://www.interactive-biosoftware.com), did not predict any damaging effects.

NPM1 gene analysis

The exon 12 of *NPM1* gene analysis did not reveal any mutation in all familial cases analyzed. One Tunisian patient diagnosed with AML at the age of 52 without any familial history, showed the presence of a nucleotide insertion occurring in exon 12, the 846 in. TGTT (Fig. 1, Table 1). This mutation may have a damaging effect on protein function. No concomitant mutations were observed in *JAK2*, *CBL* and *RUNX1* genes investigated in this patient.

JAK2 gene analysis

The sequencing of *JAK2* exons 12 and 14 and its flanking intronic regions revealed the presence of only one heterozygous variant c.1641 + 6 T > C (Fig. 2), in two unrelated patients (Table 1) belonging to Tunisian familial cases.

The first patient was a female diagnosed with gastric lymphoma at the age of 29 and the second one was a 32-year-old male (proband IV2, Fig. 3) diagnosed with nodular sclerosis Hodgkin lymphoma type II stage I. After being cured, he relapsed 5 years and a half later, showing stage III Bb Hodgkin lymphoma (HL), he developed bone metastases and died 1 year later. His cousin was diagnosed with HL stage IV B at the age of 36 and. After the failure of the treatment, she relapsed twice, 2 and 3 years later, and died due to the disease progress, unfortunately she was not included in this study since the DNA was not available.

When permissions were available, we also screened the patient's healthy relatives: 11 members (III2, IV1, IV3, IV4, IV5, IV7, IV8, V1, V2, V3 and V4) were tested for c.1641 + 6 T > C *JAK2* variant (Fig. 3). It was carried by 5/11 relatives: the healthy brother, wife and son (IV1, IV3, V2) carry the c.1641 + 6 T > C *JAK2* variant at heterozygous form, and his healthy son and daughter (V1, V3) carry it at homozygous form. In order to investigate the occurrence of the c.1641 + 6 T > C *JAK2* variant, 198 control chromosomes recruited among consent Tunisian healthy blood donors were screened for the exon 12 *JAK2* and its adjacent introns regions. We detected the variant in two control cases at homozygous form and one case at heterozygous state.

II-Evaluation of the effect of the variant *JAK2* c.1641 + 6 T > C on splicing

We evaluated the effect of the variant *JAK2* c.1641 + 6 T > C on the strength of the natural 5′ splice site of exon 12 as well as on the potential creation of splice site, by using four bioinformatic prediction algorithms (SSF, MES, NNS, HSF), interrogated simultaneously using the integrated software Alamut V2.3 (http://www.interactive-biosoftware.com). All 4 algorithms predicted a decrease (−7.6 % for SSF, −47.3 % for MES, −66.7 % for NNS, −2.7 % for HSF) of the strength of the natural 5′ splice site of *JAK2* exon 12 (Fig. 4a). These bioinformatics predictions suggested a potential impact of the variant *JAK2* c.1641 + 6 T > C on splicing.

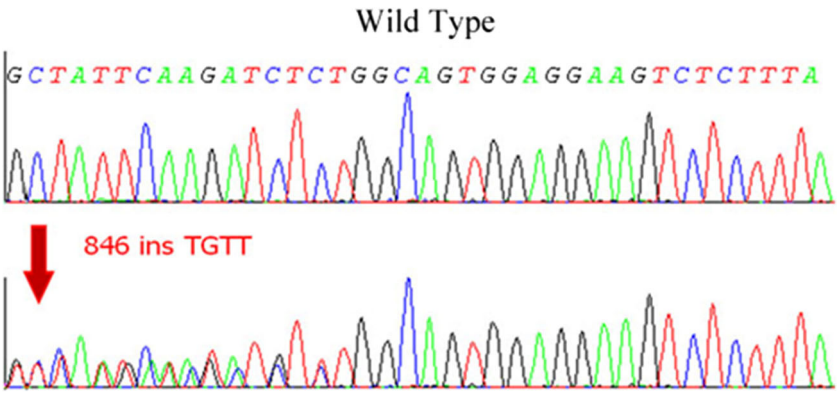

Fig. 1 846 in. TGTT in *NPM1* gene (sporadic AML case)

Then, we tested the effect of this variant on *JAK2* exon 12 splicing by performing an ex vivo functional assay that relies on the use of a minigene vector (Fig. 4b). HeLa cells were transfected with pCAS2-*JAK2*-exon12-derived minigenes and the splicing pattern of the mutant minigene was compared to the wild-type by RT-PCR and sequencing analysis. As expected, the wild-type minigene produced transcripts containing *JAK2* exon 12 (Fig. 4c). On the other hand, the corresponding mutant minigene generated a similar splicing pattern to the wild-type one, and no aberrant transcripts with *JAK2* exon 12 skipping were detected (Fig. 4c).

Discussion

A positive family history of blood cancer is recognized as one of the most important risk factors predisposing to potential development of hematological malignancies. In this study, we targeted the *JAK2*, *CBL*, *RUNX1*, and *NPM1* genes, their prominent function is well defined in hematological process and were described in several hematological malignancies. The aim was to search germline genes mutations involved in familial hematological malignancies. For that reason, we have screened these four genes in 98 patients belonging to 88 independent Tunisian and French families.

The *RUNX1* gene alterations have been commonly described in AML and less frequently in other hematological malignancies. We reported here several variants: a germline missense substitution c.167 T>C p.Leu56Ser (rs111527738) that occurred in exon 4, was found in 2 unrelated female patients 2/98 (2 %) diagnosed with breast cancer and breast cancer/thrombocythemia respectively. According to ExAC database, this variant was found in European (Non-Finnish) population with the frequency of (1.6 %). This substitution was previously described in cytogenetically normal AML [30]. A recent study targeting a novel AML cell line with a normal karyotype (CG-SH cells) showed the presence of this variant with a frequency of 10 % of analyzed cells. The in silico analysis via PolyPhen and condel predicts a potential damaging effect while SIFT predicts a tolerated effect [31]. These three algorithms present a high sensitivity but low specificity for analyzing genetic variants, further evidence should be established to support pathogenicity of p.Leu56Ser substitution. Two silent nucleotide changes, Ser218Ser and Pro463Pro, were found in 5 familial cases. They were previously classified as polymorphism with low frequency. Despite the fact of *RUNX1* gene was described in several familial contexts as AML and myelodysplastic syndrome, we do not report any mutation in our 98 familial cohort. Recent studies correlate the *RUNX1* gene expression to a poor prognosis in breast cancer [32]. Since we reported the p.Leu56Ser variation in two unrelated patients diagnosed with breast cancer and a deleterious effect predicted by in silico approaches, it will be interesting to establish an association study of this variant and its prognosis impact on breast cancer.

The *NPM1* gene was described as potential high penetrance gene in heredity breast cancer and myelogenous leukemia. In our investigation, we did not detect genetic variantion in all analyzed familial cases with hematological malignancies and cosegregated solid tumors especially breast cancer. However, in one patient diagnosed with sporadic AML without karyotype abnormality, we identified a C terminal frameshift *NPM1* mutation: 846 in. TGTT which may impact the normal reading frame of the gene. More than 30 different 4pb insertions or

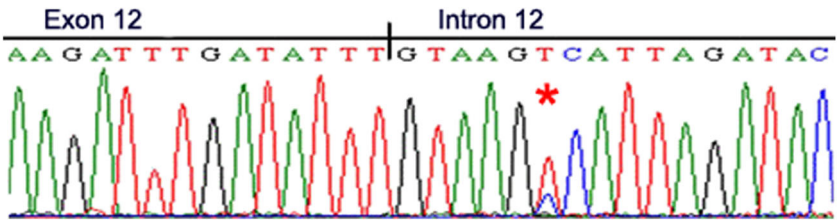

Fig. 2 c.1641+6 T>C *JAK2* variant

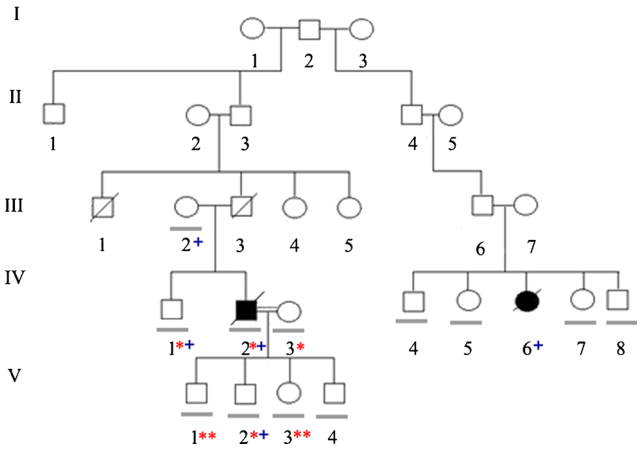

Fig. 3 Family pedigree *underlined*: analyzed relatives for *JAK2* variant. *heterozygous form of *JAK2* c.1641 + 6 T > C; **homozygous form of c. 1641 + 6 T > C; +Variant PRF1: Ala211Val

insertions/deletions in *NPM1* were described leading to a longer protein with a new nuclear export signal motif. These insertions/deletions may harbor the normal protein function, which appear to be critical for leukemogenesis, since *NPM1* is involved in crucial cell functions and its disruption result in aberrant cytoplasmic dislocation of the mutant protein [33, 34]. In this patient, no concomitant mutations were found in *CBL*, *JAK2*, *GATA2, PRF1* and *P53* genes investigated (data not shown), empathizing the pathogenicity of this *NPM1* insertion.

The analysis of the proto-oncogene *CBL* did not reveal any mutation in familial or sporadic hematological malignancies cases despite the involvement of this gene in a variety of myeloid neoplasms, including de novo AML [27]. Only one intronic *CBL* variant 1095 + 19 G > T (rs2510152) was detected with high frequency at homozygous and heterozygous state. It was classified as polymorphism found in several populations at a frequency of 10 to 50 % according to NCBI database. The Human Splicing Finder software analysis showed no impact of this variant on the splice site. Several proto-oncogenes were reported in familial cancer context, the absence of *CBL* mutation in 98 familial cases of hematological malignancies may suppose its non involvement.

As the V617F mutation in *JAK2* gene was the most recurrent mutation described in several cancers, we did not detect it in 98 hematological malignancies included in our investigation. We reported an intronic *JAK2* variant c.1641 + 6 T > C found in two Tunisian independent familial cases diagnosed with gastric lymphoma and Hodgkin lymphoma. This variant (rs182123615) was previously described in several pathologies including myeloproliferative neoplasms [35] and congenital erythrocytosis [36]. No functional assay to establish the assessment of pathogenicity of this variant has been reported until now.

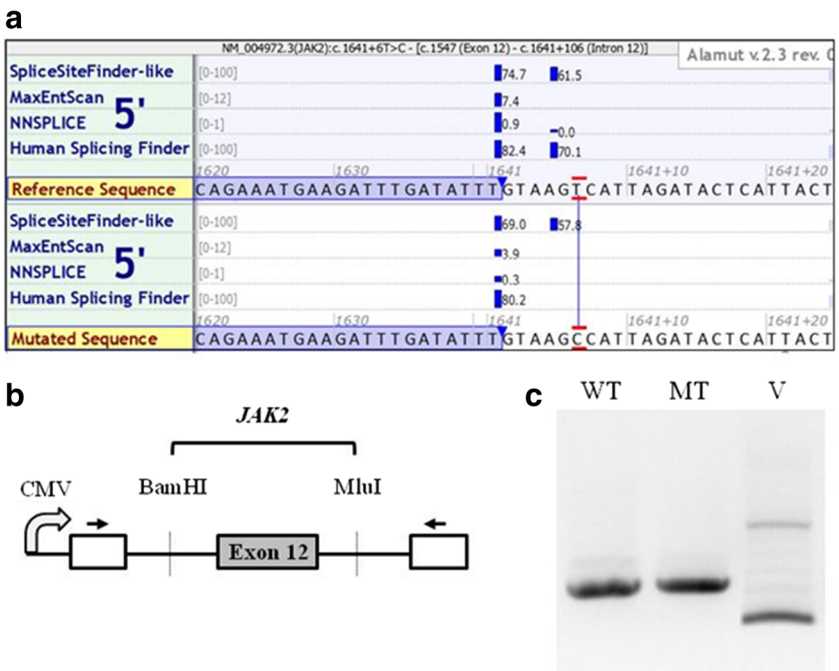

Fig. 4 Analysis of the effect of *JAK2* c.1641 + 6 T > C on splicing. **a** Bioinformatics predictions of the effect on exon 12 splicing of *JAK2* c.1641 + 6 T > C. Four bioinformatics prediction algorithms were interrogated simultaneously using the integrated software Alamut v2.3 (http://www.interactive-biosoftware.com). **b** Schematic representation of the pCAS2-JAK2-exon 12 minigene used in the functional splicing minigene reporter assay. *Boxes* represent exons and *lines* in between indicate introns. The minigene was generated by inserting the exon 12 of *JAK2* and flanking intronic sequences into the intron of pCAS2. *Arrows* above the exons indicate the positions of primers used in RT-PCR analysis. **c** 2 % Agarose gel electrophoresis of RT-PCR transcript as splicing pattern of wild-type (*WT*) and mutant (*MT*) *JAK2* c.1641 + 6 T > C exon 12 minigenes. The minigene carrying no insert (*V*) was used as control. The gel was stained with ethidium bromide

Because of the degenerative nature of the splice sites, intronic variants outside conserved GT/AG positions at the 5′ and the 3′ intron boundaries of the acceptor and donor splice sites have been usually classified as variants of unknown significance, unless there is some functional evidence of their pathogenicity. In the clinical setting, alterations involving regulatory signals may lead to a pathogenic effect. In this study we investigated the intronic variant c.1641 + 6 T > C (rs182123615) to determine the functional impact on splicing. A familial segregation analysis of this *JAK2* variant was enrolled in relatives of patient diagnosed with Hodgkin lymphoma and carrying this identified variant. Among 11 healthy relatives, 3/11 carried c.1641 + 6 T > C at heterozygous form and 2/11 at homozygous form. In a previous study, we have reported in the same Tunisian family a segregation of perforine *PRF1* Ala211Val missense substitution carried by the proband (IV-2) diagnosed with Hodgkin Lymphoma, his cousin (IV-6) and 3 healthy relatives (III-2, IV-1, V-2). We have shown that the functional assay on Ala211Val variant has no impact on lytic function of perforin [37]. The absence of functional implication of the *PRF1* and *JAK2* variants may not avert suspicion of their implication as low risk factors in hematological malignancies. In proband IV2 carrying the *JAK2* variant, we have also sequenced *ASXL1*, *NPM1*, *CBL*, *IDH1*, *IDH2*, *YY1*, *CASP8*, *CASP10*, *FAS*, *FASL*, *CEBPA*, *AML1*, *CRAF*, *BRAF*, *TP53*, *ARTLS1*, and *GATA2* genes and also in several familial cases including in this study to check potential concomitant mutations. No mutations were found in these candidate genes (data not shown).

The in silico analysis of *JAK2* variant was in favor of a deleterious splicing effect. The MaxEntScan predicted a 47 % decrease of the score of natural splice sites which may affect consensus splice sites and leads to a possible pathogenic effect. Despite in silico prediction, the functional splicing minigene reporter assay did not reveal any effect on splicing domain. Hence, the minigene constructs and the used cell line did not show any effect on mRNA processing. The presence of c.1641 + 6 T > C *JAK2* variant in healthy probands and controls with a frequency of 1.5 % may suppose that it is not pathogenic and can be classified as rather a rare polymorphism. The in silico prediction does not reflect the in vivo situation. This may be explained by the degenerate nature of sequences which bind the splice sites. That is why Alamut classified the rs182123615 as a deleterious variant.

We aimed through this study to identify genetic inherited factors contributing in the background of familial hematological malignancies through molecular screening of four candidate genes widely evoked in several familial cancers and hematological malignancies. The investigation of *RUNX1*, *CBL*, and *NPM1* genes does not revealed potential driver mutation leading to oncogenic processes, except the c.1641 + 6 T > C *JAK2* variation found in Tunisian familial cases. This variant was reported in several studies and was described as potentially deleterious. Through this study, we establish its clinical assessment and we classified as rather a polymorphism than as a mutation. Despite the few genetic variations found in familial aggregation of hematological malignancies, they cannot be used alone as prognostic factors, they might contribute to the background of genetic factors which have to be revealed. Identification of a pathogenic mutation in clinically affected individuals allows to perform genetic counseling specially in familial context disease. We expect to enlarge our investigation in other genes reported in hematological malignancies such as *IDH1*, *IDH2*, and *ASXL1*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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