SHORT REPORT

Recurrent somatic JAK-STAT pathway variants within a RUNX1-mutated pedigree

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Germline variants within the transcription factor *RUNX1* are associated with familial platelet disorder and acute leukemia in over 40% of carriers. At present, the somatic events triggering leukemic transformation appear heterogeneous and profiles of leukemia initiation across family members are poorly defined. We report a new *RUNX1* family where three sisters harboring a germline nonsense *RUNX1* variant, c.601C > T (p.(Arg201*)), developed acute myelomonocytic leukemia (AML) at 5 years of age. Whole-exome sequencing of tumor samples revealed all three siblings independently acquired variants within the JAK-STAT pathway, specifically targeting *JAK2* and *SH2B3* (a negative regulator of JAK2), while also sharing the 46/1 haplotype linked with sporadic *JAK2*-positive myeloproliferative neoplasms. In-depth chromosomal characterization of tumors revealed acquired copy number gains and uniparental disomy amplifying *RUNX1*, *JAK2* and *SH2B3* variants, highlighting the significance of co-operation between these disrupted pathways. One sibling, presenting with myelodysplasia at 14 years, had no evidence of clonal or subclonal *JAK2* or *SH2B3* variants, suggesting the latter were specifically associated with leukemic transformation in her sisters. Collectively, the clinical and molecular homogeneity across these three young siblings provides the first notable example of convergent AML evolution in a *RUNX1* pedigree, with the recurrent acquisition of JAK-STAT pathway variants giving rise to high-risk AML, characterized by chemotherapy resistance and relapse.

INTRODUCTION

Acquired *RUNX1* variants occur in ~ 10% of sporadic acute myelomo-nocytic leukemia (AML) cases and the clinico–pathologic associations within this subgroup of disease have been comprehensively defined.[1] Inherited or germline *RUNX1* variants are comparatively rare and typically cluster in the highly conserved runt homology domain, predisposing to familial platelet disorder and myelodysplasia (MDS) or acute leukemia.[2] Leukemic transformation occurs in over 40% of variant carriers, coin-ciding with a heterogeneous profile of somatic genetic lesions, although aberrations of the second *RUNX1* allele or amplification of the germline variant are frequent.[3,4] Notably, *CDC25C* variants were reported as a recurrent event in a Japanese *RUNX1*-mutated cohort,[5] however these results were not replicated in the US and European studies,[3,6,7] suggesting ethnicity may influence somatic variant acquisition. Exploring this notion further, we considered whether family members may develop similar patterns of disease, due to their common genetic background. Using comprehensive clinical and genetic profiling of multiple siblings with MDS/AML, we report striking phenotypic and molecular convergence of AML within a new Hungarian *RUNX1*-mutated family.

DESIGN AND METHODS

We used the SureSelect All Exon V5 kit (Agilent, Santa Clara, CA, USA) to perform whole-exome sequencing (WES) on bone marrow (BM) tumor DNA from siblings (II.1–II.4) and peripheral blood (PB) DNA from both healthy parents (I.1 and I.2), the latter providing a germline reference. Enriched exome libraries were sequenced to an average depth of ×96 (Supplementary Table S1) using the HiSeq 2000 (Illumina, San Diego, CA, USA) with additional analysis described in Supplementary Data.

An established pipeline incorporating MuTect (single-nucleotide variants, SNVs) and GATK (indels) was employed for variant calling in tumor samples. Analysis of chromosomal loss of heterozygosity, including copy number aberrations (CNAs) and acquired uniparental disomy (aUPD) is detailed in the Supplementary Data. Sequence variants and CNAs were verified using Sanger sequencing and multiplex ligation-dependent probe amplification (MRC Holland, Amsterdam, the Netherlands, SALSA X060-X2), respectively. The WES data have been deposited in the European Genome-Phenome Archive under the accession number EGAS00001001862. Deep sequencing of *JAK2* exon 14 and all coding exons of *SH2B3* was performed using the AmpliSeq approach on an Ion Torrent (Life Technologies, Carlsbad, CA, USA) instrument. The reference sequences used for variant annotations are listed in the Supplementary Data.

RESULTS

A nonsense *RUNX1* variant, c.601C > T (p.(Arg201*)), was detected in all four siblings and their mother, (Figures 1a and b; Supplementary Table S2). The first affected family members were dizygotic twins (II.1 and II.2) who presented in 2002 at 5 years of age, within a period of 14 days. They both had hepatosplenomegaly and monocytosis, with BM morphology revealing AML (FAB AML M4). After 6 years, sibling

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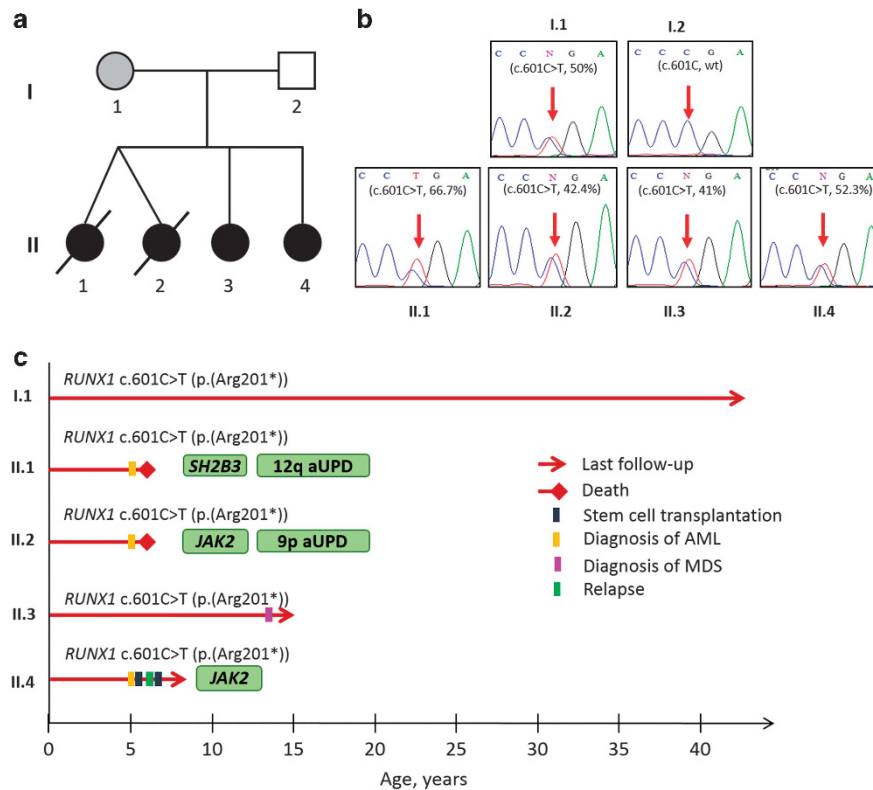
Figure 1 Profile of a novel *RUNX1*-mutated pedigree. (**a**) Novel *RUNX1* pedigree showing affected individuals (black shading) and asymptomatic carriage of germline variant (gray shading). (**b**) *RUNX1* c.601C>T (p.(Arg201*)) variant identified in four siblings and their mother, the corresponding WES VAFs are shown. (**c**) Clinical timeline of the pedigree showing siblings II.1, II.2 and II.4 presenting with AML at 5 years of age. All three siblings with AML acquired variants upregulating JAK-STAT signaling, while sibling II.3 remains under expectant follow-up with MDS. (*RUNX1* reference sequences: ENSG00000159216 / ENST00000300305).

II.4 presented with thrombocytopenia aged 1 year, followed by AML (FAB AML M4) at 5 years of age. In contrast, sibling II.3 developed mild anemia and thrombocytopenia, and was diagnosed with multilineage dysplasia (RCMD) at 14 years. Their mother (I.1, 44 years) remains an asymptomatic carrier, with normal PB indices and no history of mucosal bleeding or bruising to suggest platelet dysfunction (Figure 1c).
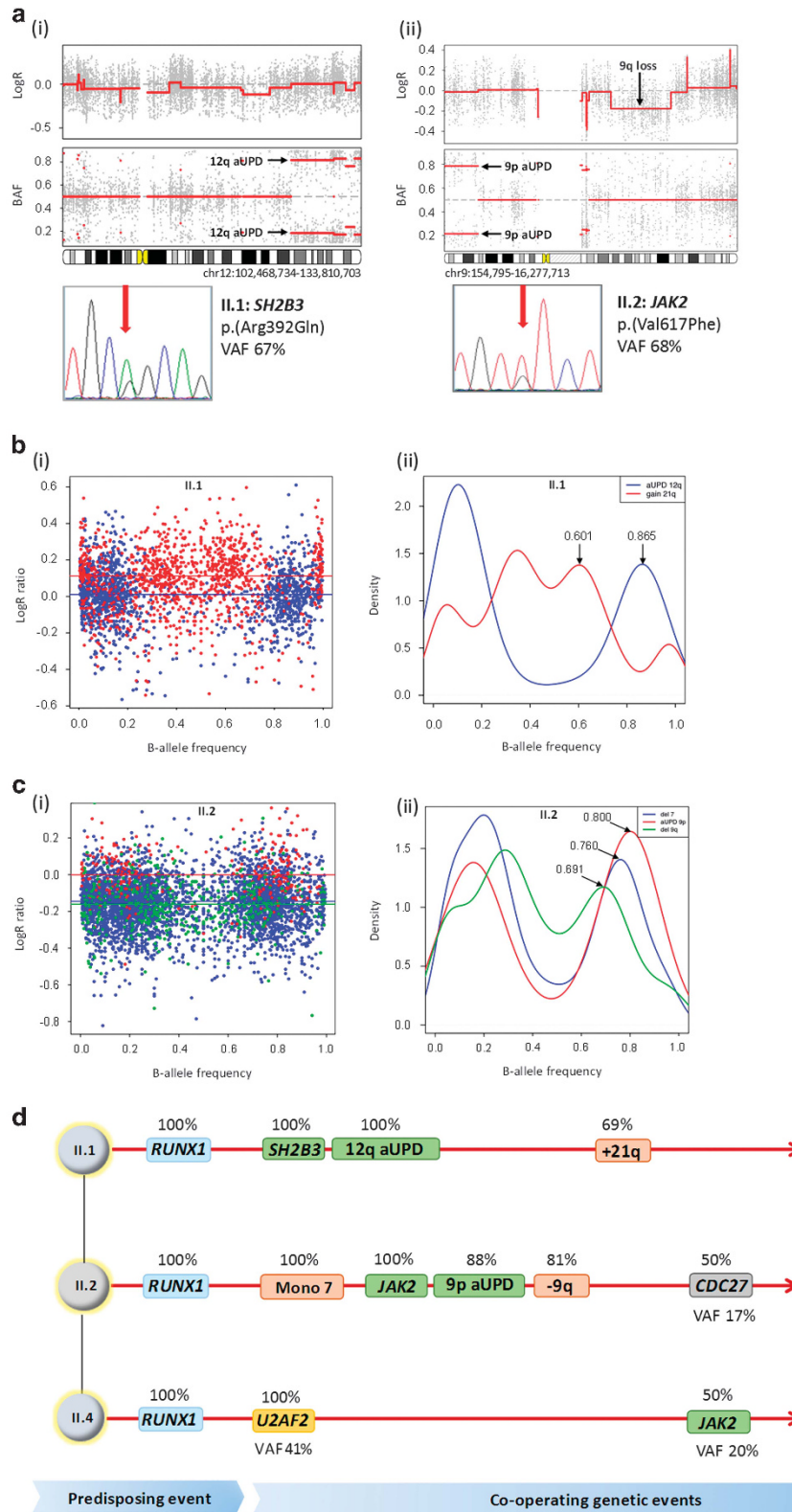
Cytogenetic analysis of tumor samples revealed gain of 21q (46,XX, der(21).ish amp(RUNX1)[15]/46,XX[5]) in II.1, amplifying the germline *RUNX1* variant (c.601C>T (p.(Arg201*))) WES variant allele frequency (VAF) 67%; additional events included monosomy 7 and deletion of 9q (45,XX,-7[2]/45,XX,idem,del(9)(q21q31)[17]/46,XX [1]) in II.2, while II.3 and II.4 had a normal karyotype. WES identified a median of 11 somatic non-synonymous variants (range 10–11), with 97% (*n* = 39) comprising SNVs, Supplementary Table S3. Targeted analysis of WES data from the mother and father confirmed the absence of variants in established pre-leukemic loci, confirming that additional germline lesions with known pathogenic relevance were not harbored by the siblings.

All three siblings with AML (II.2, II.3 and II.4) acquired variants upregulating JAK2 signaling, as shown in Figure 1c. In II.2 and II.4, *JAK2* c.1849G>T (p.(Val617Phe)) was detected, with the variant dosage amplified by 9p aUPD in II.2 (p.(Val617Phe) VAF 68%, Figure 2a(i)). Sibling II.1 acquired a unique variant (c.1175G>A (p.(Arg392Gln))) in *SH2B3*, with 12q aUPD increasing its allelic burden to 67% (Figure 2a(ii)). SH2B3 (also known as LNK) is a member of the SH2B family of adaptor proteins and negatively regulates JAK2 by interacting with the phosphorylated JAK2 tyrosine

residue 813 (p.Tyr813) via a conserved SH2 domain, regulating both WT and mutant JAK2 activity.[8] By analyzing the structure of murine Sh2b, we identified that SH2B3 p.Arg392 corresponds to Sh2b p.Arg555,[9] situated in the midst of a precisely conserved region spanning 15 amino acids and forming a salt bridge with the phosphopeptide binding pocket targeted by phosphorylated JAK2. *SH2B3* c.1175G>A (p.(Arg392Gln)) is a unique variant, predicted to be damaging using MutationTaster (*P* = 1.0000, 4 d.p., http://www.mutationtaster.org) and absent from both ExAC (http://exac.broadinstitute.org) and Thousand Genome databases (http://www.internationalgenome.org/data/). We hypothesize that p.(Arg392Gln) may therefore disrupt salt bridging with the phosphopeptide binding pocket in SH2B3, consequently reducing JAK2 inhibition, as previously described with missense variants in this domain.[10]

Additional somatic variants identified across the siblings included the anaphase promoting complex component, *CDC27*, in II.2 (c.1901T>C (p.(Ile634Thr)), VAF 16.7%) and II.3 (c.1477A>G (p.(Ile493Val)), VAF 7.5%); *RBBP8* (DNA double-strand break repair, c.959A>G (p.(Glu320Gly)), VAF 32.3%) and *CHD4* (nucleosome remodeling and histone deacetylase complex, c.3530G>A (p.(Arg1177His)), VAF 5.6%) in II.2 and *U2AF2* (spliceosome component, c.439C>G (p.(Gln147Glu)), VAF 41.2%) in sibling II.4. These molecular lesions are infrequently observed in sporadic MDS/AML, mirroring observations within other *RUNX1* pedigrees where only one to two recurrently mutated driver events are detected in tumors.[3,6,7,11]

Having established the spectrum of variants across the siblings, we next sought to derive the chronological sequence of chromosomal and
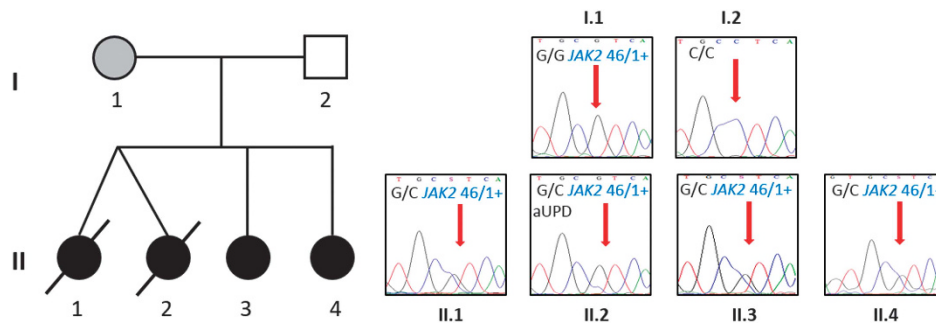
a (i) / **(ii)**

II.1: *SH2B3*
p.(Arg392Gln)
VAF 67%

II.2: *JAK2*
p.(Val617Phe)
VAF 68%

b (i) II.1 / **(ii)** II.1

c (i) II.2 / **(ii)** II.2

d

II.1 — RUNX1 (100%) — SH2B3 (100%) — 12q aUPD (100%) — +21q (69%)

II.2 — RUNX1 (100%) — Mono 7 (100%) — JAK2 (100%) — 9p aUPD (88%) — -9q (81%) — CDC27 (50%) VAF 17%

II.4 — RUNX1 (100%) — U2AF2 (100%) VAF 41% — JAK2 (50%) VAF 20%

Predisposing event | Co-operating genetic events

genetic aberrations by calculating their respective cancer cell fractions based on the 'Battenberg' algorithm[12] (Figures 2b and c; Supplementary Data). In II.1, *SH2B3* c.1175G > A (p.(Arg392Gln)) and 12q aUPD preceded gain of 21q; while in II.2, monosomy 7, *JAK2* c.1849G > T (p.(Val617Phe)) and 12q aUPD were followed by 9q

deletion (Figure 2d). This pattern of disease evolution revealed the selective and sequential amplification of *RUNX1*, *JAK2* and *SH2B3* variants, affirming their key roles as leukemic driver lesions.

The rarity of *JAK2* and *SH2B3* variants in sporadic AML cases,[13] suggested these siblings may share an inherited genotype predisposing

Figure 3 Identification of *JAK2* 46/1 haplotype in mother and children. Sequencing of *JAK2* rs12340895 in LD with the 46/1 haplotype, revealed the mother (I.1) was homozygous (GG) for the 46/1 haplotype and the father was homozygous for the non-46/1 allele (CC). All four children were heterozygous for the 46/1 haplotype (GC), with II.2 demonstrating prominence of the 46/1 allele due to 9p aUPD, confirming *JAK2* c.1849G>T (p.(Val617Phe)) (also subject to dosage amplification) was acquired on the 46/1 allele. LD, linkage disequilibrium.

to the specific acquisition of JAK-STAT pathway variants. The *JAK2* 46/1 haplotype is associated with an increased population risk of developing *JAK2* p.(Val617Phe)-positive myeloproliferative neoplasms (MPNs) and sequencing of rs12340895 (in linkage disequilibrium with this haplotype) revealed heterozygosity in all four children (GC). Notably, 9p aUPD in II.2 amplified both V617F and rs12340895 variants, showing that p.(Val617Phe) was acquired on the 46/1 allele, as in sporadic neoplasms.[14–16] The mother was homozygous for the 46/1 haplotype (GG) and the father was homozygous for the non-46/1 allele (CC) (Figure 3). Subclonal *JAK2* and *SH2B3* variants were excluded in sibling II.3 and maternal DNA (I.1) by deep sequencing to a depth of ×10 000, suggesting that these lesions were specifically acquired upon leukemic transformation, potentially influenced by additional, yet unknown, genetic variants.

All three siblings with AML and JAK2 upregulation demonstrated an aggressive disease course, characterized by relapse and resistance to chemotherapy. Following combination chemotherapy, II.1 achieved complete remission (CR) but died 1 year later with AML relapse. II.2 failed to achieve CR and succumbed to chemotherapy–refractory disease after 8 months. Sibling II.4 received induction chemotherapy with idarubicin, cytarabine and etoposide, and underwent unrelated double cord blood stem cell transplantation (HSCT) following a delay in hematopoietic reconstitution. AML recurred after 17 months requiring a second volunteer unrelated donor HSCT and she continues in remission with full donor chimerism 30 months post transplant.

DISCUSSION

In this study, we report a novel *RUNX1* pedigree with recurrence of somatic JAK-STAT pathway variants in three siblings with aggressive myelomonocytic AML. Our data suggest that inherited genetic traits may govern leukemic transformation and disease behavior by influencing somatic variant acquisition and molecular co-operation. Concurrent disruption of JAK2 and RUNX1 has been reported in sporadic AML,

where *JAK2* variants occur in 5–8% of core binding factor leukemia with the t(8;21)(q22;q22) *RUNX1-RUNX1T1* translocation[17,18] and in a study of 200 AML cases, solitary *SH2B3* and *JAK2* c.1849G>T (p.(Val617Phe)) variants both coincided with *RUNX1* variants.[13] Conversely, *RUNX1* variants have also been reported during leukemic transformation of *JAK2*-positive MPNs.[19,20] The lack of recurrent JAK-STAT variants in a recent study of *RUNX1*-mutated AML[1] suggests that this co-operation is rare and likely to be governed by additional features, including haplotype. Clinically, all three siblings with somatic JAK-STAT pathway variants demonstrated an adverse disease profile, highlighting the importance of observing phenotypic patterns of disease across family members to improve investigation, management and counseling of these rare pedigrees.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

KT, JW and CB wrote the paper. KT, JW, JF, JC and CB designed research and provided materials. KT, PK, AAS and AR-M performed research. KT, JW and CC performed data analysis. KK, GB, MZ, AM and JC provided clinical data.

1 Gaidzik VI, Teleanu V, Papaemmanuil E *et al*: RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. *Leukemia* 2016; **30**: 2282.

2 Song WJ, Sullivan MG, Legare RD *et al*: Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet* 1999; **23**: 166–175.

3 Antony-Debre I, Duployez N, Bucci M *et al*: Somatic mutations associated with leukemic progression of familial platelet disorder with predisposition to acute myeloid leukemia. *Leukemia* 2016; **30**: 999–1002.

4 Preudhomme C, Renneville A, Bourdon V *et al*: High frequency of RUNX1 biallelic alteration in acute myeloid leukemia secondary to familial platelet disorder. *Blood* 2009; **113**: 5583–5587.

5 Yoshimi A, Toya T, Kawazu M *et al*: Recurrent CDC25C mutations drive malignant transformation in FPD/AML. *Nat Commun* 2014; **5**: 4770.

6 Churpek JE, Pyrtel K, Kanchi KL *et al*: Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood* 2015; **126**: 2484–2490.

7 Haslam K, Langabeer SE, Hayat A, Conneally E, Vandenberghe E: Targeted next-generation sequencing of familial platelet disorder with predisposition to acute myeloid leukaemia. *Br J Haematol* 2016; **175**: 161–163.

8 Bersenev A, Wu C, Balcerek J, Tong W: Lnk controls mouse hematopoietic stem cell self-renewal and quiescence through direct interactions with JAK2. *J Clin Invest* 2008; **118**: 2832–2844.

9 Hu J, Hubbard SR: Structural basis for phosphotyrosine recognition by the Src homology-2 domains of the adapter proteins SH2-B and APS. *J Mol Biol* 2006; **361**: 69–79.

10 Auer PL, Teumer A, Schick U *et al*: Rare and low-frequency coding variants in CXCR2 and other genes are associated with hematological traits. *Nat Genet* 2014; **46**: 629–634.

11 Sakurai M, Kasahara H, Yoshida K *et al*: Genetic basis of myeloid transformation in familial platelet disorder/acute myeloid leukemia patients with haploinsufficient RUNX1 allele. *Blood Cancer J* 2016; **6**: e392.

12 Nik-Zainal S, Van Loo P, Wedge DC *et al*: The life history of 21 breast cancers. *Cell* 2012; **149**(5): 994–1007.

13 Cancer Genome Atlas Research N: Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. *N Engl J Med* 2013; **368**: 2059–2074.

14 Jones AV, Chase A, Silver RT *et al*: JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat Genet* 2009; **41**: 446–449.

15 Kilpivaara O, Mukherjee S, Schram AM *et al*: A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nat Genet* 2009; **41**: 455–459.

16 Olcaydu D, Harutyunyan A, Jager R *et al*: A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nat Genet* 2009; **41**: 450–454.

17 Krauth MT, Eder C, Alpermann T *et al*: High number of additional genetic lesions in acute myeloid leukemia with t(8;21)/RUNX1-RUNX1T1: frequency and impact on clinical outcome. *Leukemia* 2014; **28**: 1449–1458.

18 Schnittger S, Bacher U, Kern W, Haferlach C, Haferlach T: JAK2 seems to be a typical cooperating mutation in therapy-related t(8;21)/ AML1-ETO-positive AML. *Leukemia* 2007; **21**: 183–184.

19 Beer PA, Delhommeau F, LeCouedic JP *et al*: Two routes to leukemic transformation after a JAK2 mutation-positive myeloproliferative neoplasm. *Blood* 2010; **115**: 2891–2900.

20 Engle EK, Fisher DA, Miller CA *et al*: Clonal evolution revealed by whole genome sequencing in a case of primary myelofibrosis transformed to secondary acute myeloid leukemia. *Leukemia* 2015; **29**: 869–876.

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