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Somatic JAK-STAT variants in familial AML

RECURRENT SOMATIC JAK-STAT PATHWAY VARIANTS WITHIN A RUNX1-MUTATED PEDIGREE**SHORT REPORT**

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Short report

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Conflict of interest

The authors declare no conflict of interest.

ABSTRACT

Germline **variants** within the transcription factor *RUNX1* are associated with familial platelet disorder (FPD) 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 3 sisters harboring a germline nonsense *RUNX1* **variant**, **c.601C>T (p.(Arg201*))**, developed AML at 5 years of age. Whole exome sequencing of tumor samples revealed all 3 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 (MPNs). In-depth chromosomal characterization of tumors revealed acquired copy number gains and uniparental disomy amplifying *RUNX1*, *JAK2* and *SH2B3* **variants**, highlighting the significance of cooperation between these disrupted pathways. One sibling, presenting with MDS 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 3 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.

Keywords: *JAK2*, *RUNX1*, **variant**, Familial, MDS, AML, pedigree

INTRODUCTION

Acquired *RUNX1* variants occur in approximately 10% of sporadic 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 (RHD), predisposing to familial platelet disorder (FPD) and myelodysplasia (MDS) or acute leukemia (2). Leukemic transformation occurs in over 40% of variant carriers, coinciding 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 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) 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 96x (Table S1) using the HiSeq 2000 (Illumina) 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 (LOH), 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, 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) 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, (Figure 1A, B; 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 acute myelomonocytic leukemia (FAB AML M4). After 6 years, sibling II.4 presented with thrombocytopenia aged 1 year, followed by acute myelomonocytic leukemia (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 peripheral blood 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, 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 3 siblings with AML (II.2, II.3 and II.4) acquired variants upregulating JAK2 signalling, 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 4d.p., <http://www.mutationtaster.org>) and absent from both ExAC (<http://exac.broadinstitute.org>) and Thousand Genome databases (<http://www.internationalgenome.org/data/>). We hypothesise 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 remodelling 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 1-2 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 genetic aberrations by calculating their respective cancer cell fractions (CCFs) based on the “Battenberg” algorithm (12) (Figures 2B-C and 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 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 MPNs and sequencing of rs12340895 (in linkage disequilibrium [LD] with this haplotype) revealed heterozygosity in all 4 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 10,000x, suggesting that these lesions were specifically acquired upon leukemic transformation, potentially influenced by additional, yet unknown, genetic variants.

All 3 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 one 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 3 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 3 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 counselling of these rare pedigrees.

Authorship contributions

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

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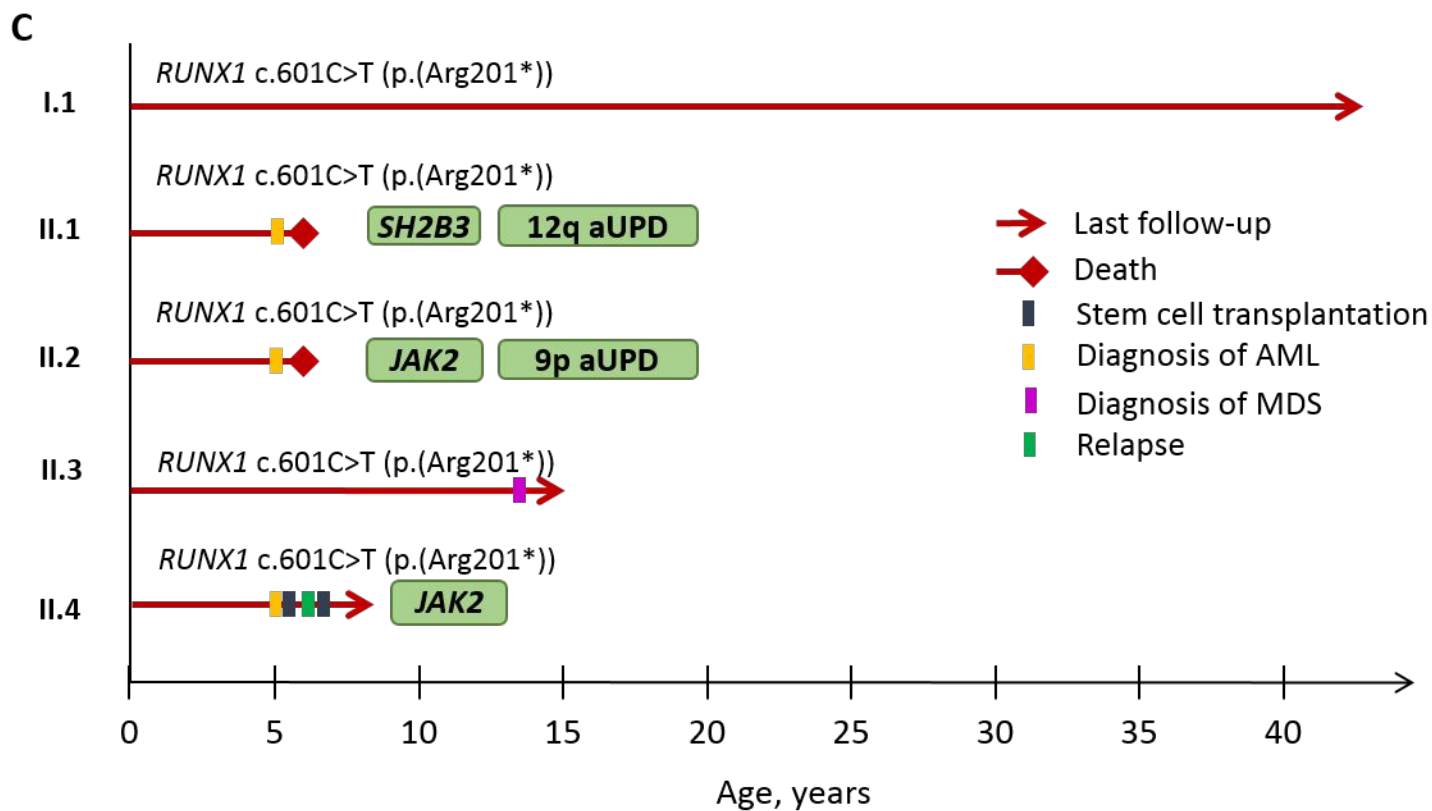
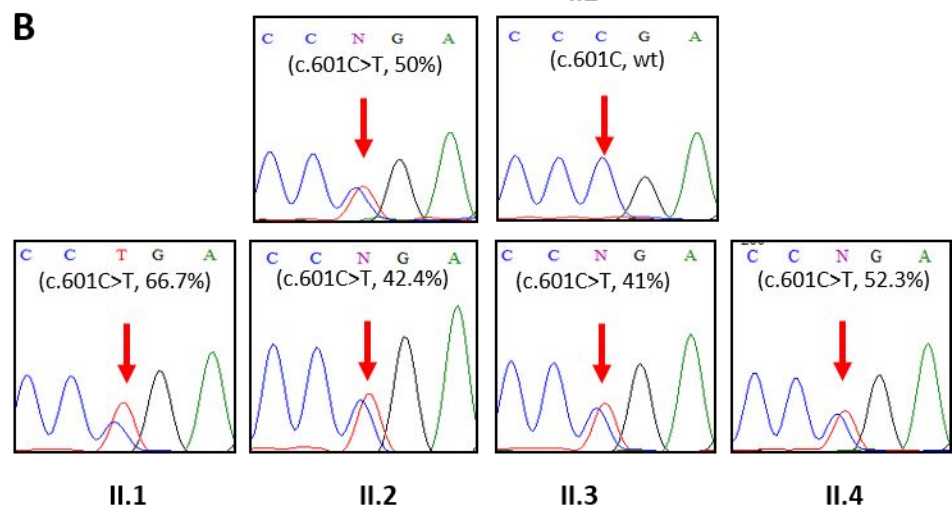
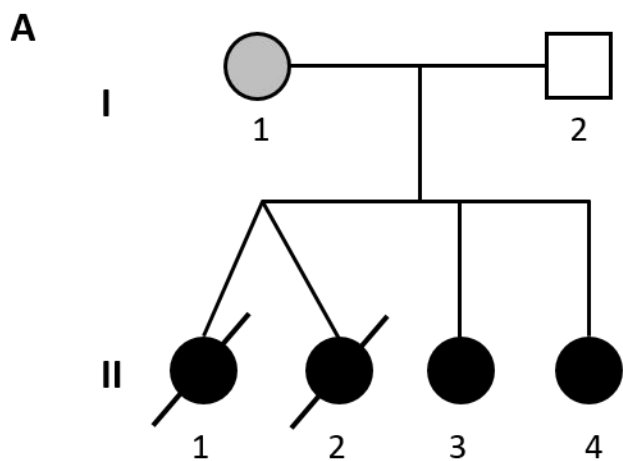
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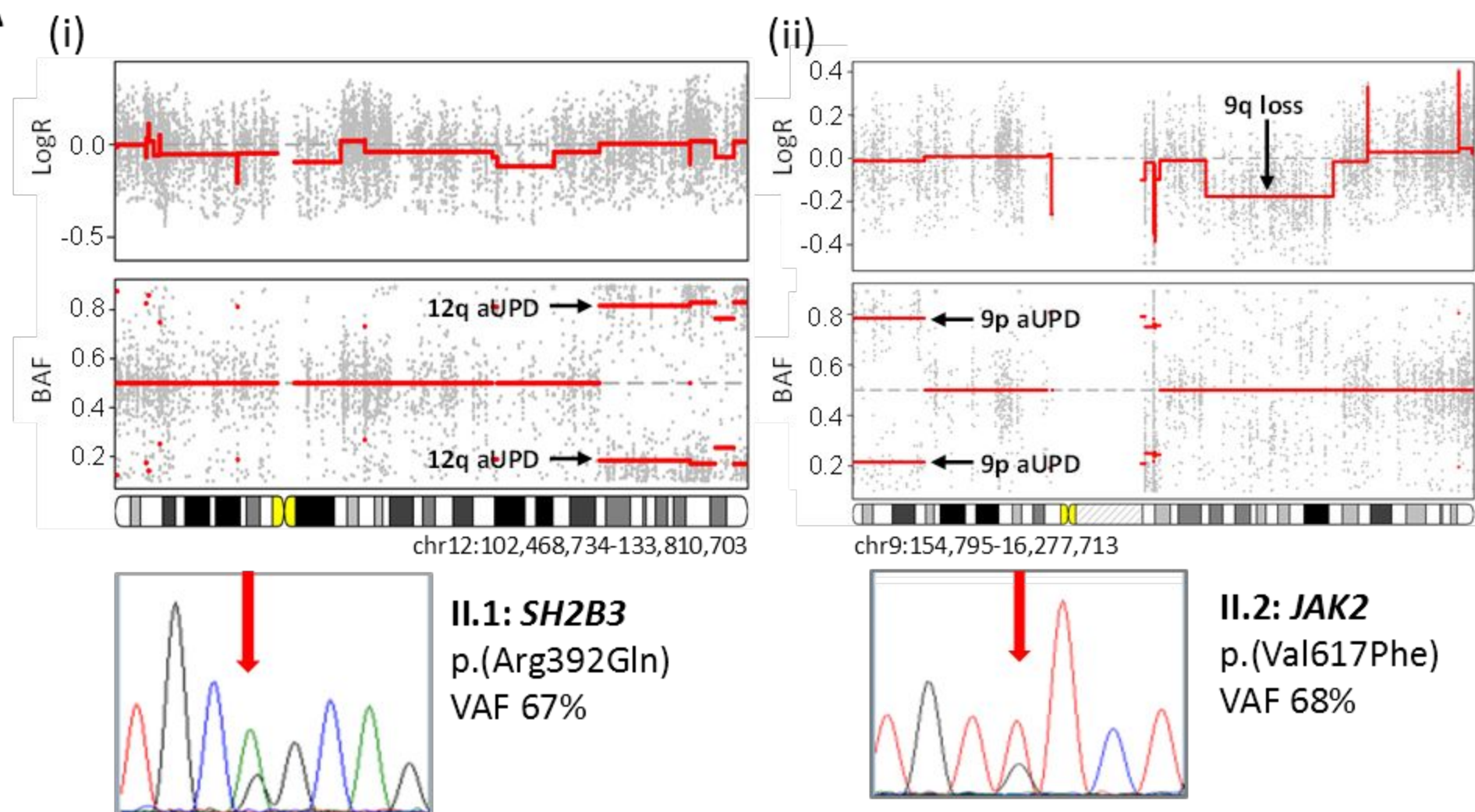
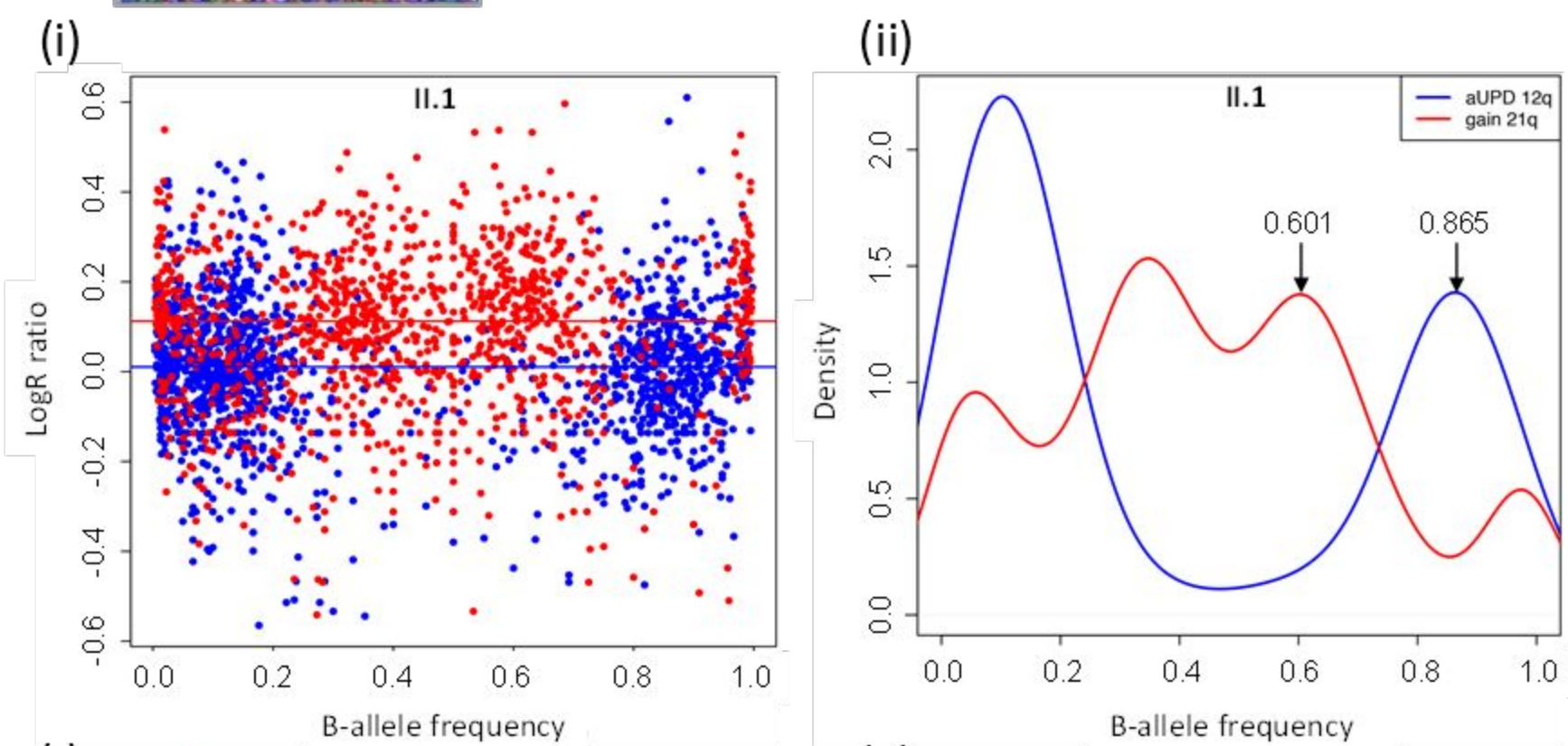
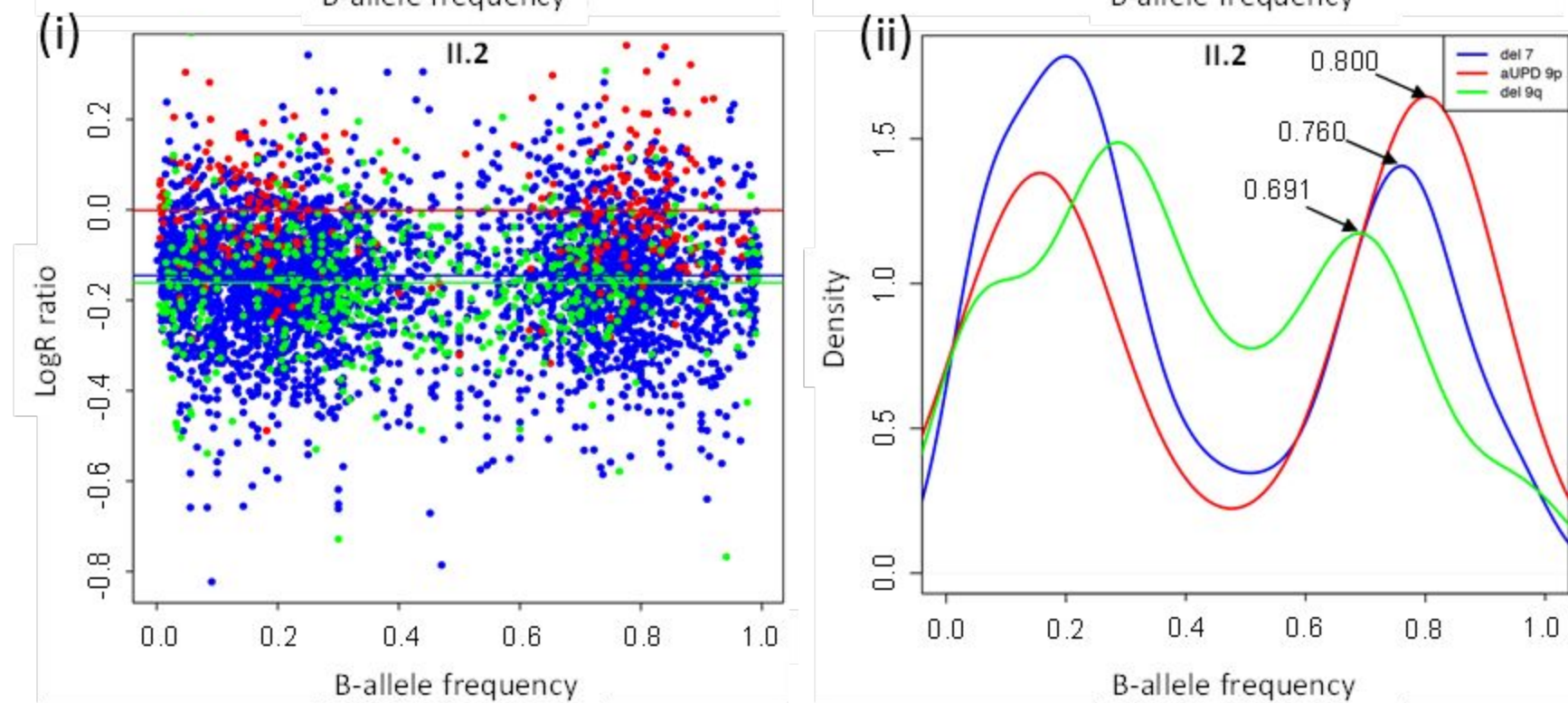
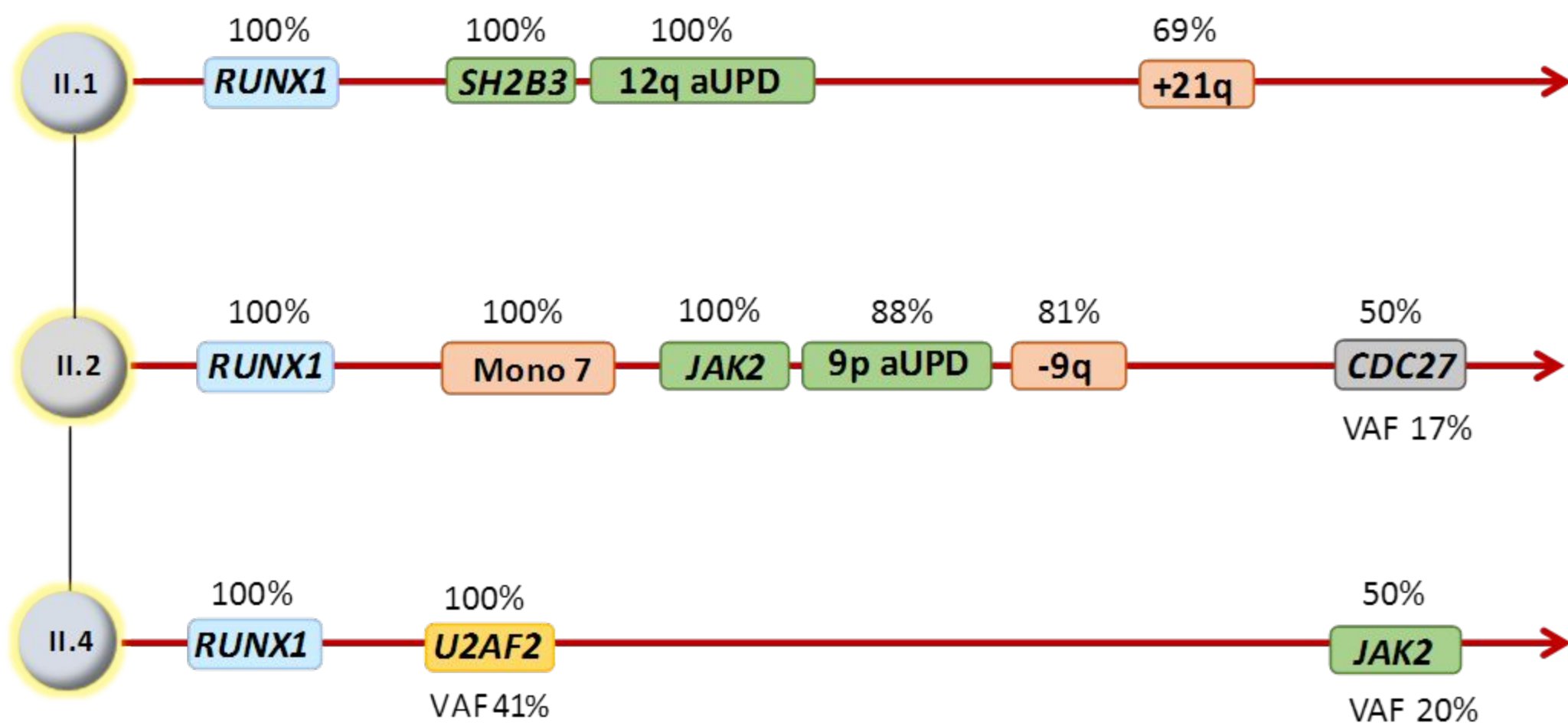
TITLES AND LEGENDS TO FIGURES

Figure 1. Profile of a novel *RUNX1*-mutated pedigree. (A) Novel *RUNX1* pedigree showing affected individuals (black shading) and asymptomatic carriage of germline variant (grey shading). (B) *RUNX1* c.601C>T (p.(Arg201*)) variant identified in 4 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 3 siblings with AML acquired variants upregulating JAK-STAT signalling, while sibling II.3 remains under expectant follow up with MDS. (*RUNX1* reference sequences: ENSG00000159216 / ENST00000300305).

Figure 2. Detailed analysis of chromosomal LOH to decipher sequence of genetic lesions. (A) i) WES B-allele frequencies (BAFs) demonstrated 12q aUPD (hg19 coordinates: chr12:102,468,734-133,810,703), causing homozygosity of the *SH2B3* c.1175G>A (p.(Arg392Gln)) variant in II.1. In II.2, 9p aUPD (hg19 coordinates: chr9:154,795-16,277,713), led to homozygosity of *JAK2* c.1849G>T (p.(Val617Phe)). 9q loss was also detected in II.2, characterized by reduced LogR ratios (LRR). (B) i) Plot of LRR and BAF for variants within 12q (blue) and 21q (red) in II.1. ii) Density plots showing the BAF of variants within regions of LOH, the peak density was used to calculate the CCF of each chromosomal lesion. (C) i) Plot of LRR and BAF for variants on chromosome 7 (blue), 9p (red) and 9q (green) in II.2. ii) Density plots showing the BAF of variants within the corresponding chromosomal regions, with the peak density used to calculate CCF. (D) From the calculations in (B) and (C), the CCF for each LOH segment was calculated allowing the evolutionary sequence to be determined in II.1 and II.2. In II.4, somatic LOH was not detected at AML diagnosis and variant CCFs were estimated using VAFs.

Figure3. 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.



A**B****C****D**

Predisposing event

Co-operating genetic events

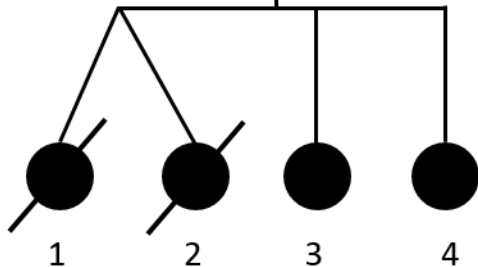
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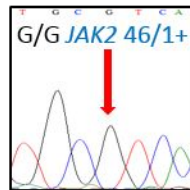
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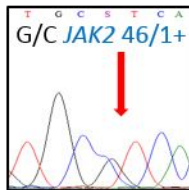
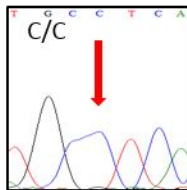
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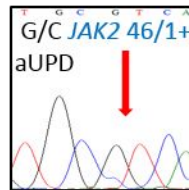
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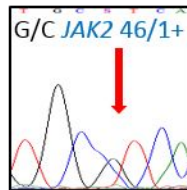
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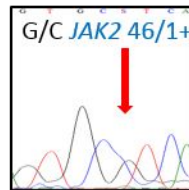
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