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Rare *KMT2A-ELL* and Novel *ZNF56-KMT2A* Fusion Genes in Pediatric T-cell Acute Lymphoblastic Leukemia

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Abstract. *Background/Aim: Previous reports have associated the KMT2A-ELL fusion gene, generated by t(11;19)(q23;p13.1), with acute myeloid leukemia (AML). We herein report a KMT2A-ELL and a novel ZNF56-KMT2A fusion genes in a pediatric T-lineage acute lymphoblastic leukemia (T-ALL). Materials and Methods: Genetic investigations were performed on bone marrow of a 13-year-old boy diagnosed with T-ALL. Results: A KMT2A-ELL and a novel ZNF56-KMT2A fusion genes were generated on der(11)t(11;19)(q23;p13.1) and der(19)t(11;19)(q23;p13.1), respectively. Exon 20 of KMT2A fused to exon 2 of ELL in KMT2A-ELL chimeric transcript whereas exon 1 of ZNF56 fused to exon 21 of KMT2A in ZNF56-KMT2A transcript. A literature search revealed four more T-ALL patients carrying a KMT2A-ELL fusion. All of them were males aged 11, 11, 17, and 20 years. Conclusion: KMT2A-ELL fusion is a rare recurrent genetic event in T-ALL with uncertain prognostic implications. The frequency and impact of ZNF56-KMT2A in T-ALL are unknown.*

The chromosomal translocation t(11;19)(q23;p13) has been reported in both acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (1). Early cytogenetic studies did not discriminate between different breakpoints within band 19p13 in cases with t(11;19)(q23;p13), but it later became clear that two breakpoint clusters existed within band 19p13 which could be distinguished by fluorescence *in situ* hybridization (FISH) (2, 3). Breakpoints within sub-band 19p13.3 have been found in both ALL (primarily in infants and children) and AML. The translocation t(11;19)(q23;p13.3) leads to fusion of the histone-lysine N-methyltransferase 2A (*KMT2A;* also known as myeloid/lymphoid or mixed lineage leukemia, *MLL*) gene in 11q23 with the MLLT1 super elongation complex subunit *MLLT1* gene (also known as *ENL, LTG19*, and *YEATS1*) in 19p13.3 generating a *KMT2A-MLLT1* fusion (1, 4-6). Two more *KMT2A*-fusion genes have been reported with relevance to sub-band 19p13.3: a fusion of *KMT2A* with the SH3 domain containing GRB2 like 1, endophilin A2 (*SH3GL1*) gene [translocation t(11;19)(q23;p13.3)] in a case of childhood AML (7), and fusion of *KMT2A* with the vav guanine nucleotide exchange factor 1 (*VAV1*) gene at 19p13.3 in a pediatric AML (8). In sub-band 19p13.2, a recurrent fusion of *KMT2A* with the myosin IF (*MYO1F*) gene [translocation t(11;19)(q23;p13.2)] has been detected in infant and pediatric AML (8-10).

Breakpoints within sub-band 19p13.1 were believed to be exclusively found in AML, where the translocation t(11;19)(q23;p13.1) resulted in fusion of *KMT2A* with the elongation factor for RNA polymerase II (*ELL*) gene (11). The overall frequency of *ELL* as *KMT2A*'s translocation partner was found to be 4.1% and *KMT2A-ELL* fusion was found in 15% of infant AML, 7% of pediatric AML, and 12% of adult AML (8). Recently, *KMT2A-ELL* fusion gene was detected also in two bi-phenotypic leukemias and in four pediatric T-ALL patients (12-15). Because of the rarity of T-ALL carrying a *KMT2A-ELL* fusion gene, we report here the genetic and clinical features of a pediatric T-ALL with an unbalanced chromosome translocation between the chromosome bands 11q23 and 19p13 resulting in two fusion genes: a *KMT2A-ELL* in which the 5'-part of *KMT2A* is fused to *ELL,* and a novel fusion gene in which the 5'-part of the zinc finger protein 56 gene *(ZNF56)* is fused to the 3'-part of *KMT2A*.

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Key Words: T-cell acute lymphoblastic leukemia, pediatric, fusion gene, *KMT2A, ELL, ZNF56, KMT2A-ELL ZNF56-KMT2A,* RNA-sequencing.

Table I. *BAC probes used for fluorescence in situ hybridization (FISH) experiments.*

BAC clones	Accession number	Chromosome mapping	Targeted gene	Position on GRCh38/hg38 assembly	Labelling
RP11-770J1	AP001267.4	11q23.3	*KMT2A*	chr11:118374563-118524770	Green
RP11-861M13	AP000941.6	11q23.3	*KMT2A*	chr11:118524771-118608821	Green
CH17-258D2	Not avaliable	19p13.11	*ELL*	chr19:18213782-18431838	Red
CH17-343F16	Not avaliable	19p13.11	*ELL*	chr19:18318183-18514614	Red
CH17-413G9	Not avaliable	19p13.11	*ELL*	chr19:18568795-18764997	Red
CH17-338M17	Not avaliable	19p13.11	*ELL*	chr19:18611693-18826326	Red

Materials and Methods

Ethics statement. The study was approved by the regional ethics committee (Regional komité for medisinsk forskningsetikk Sør-Øst, Norge, http://helseforskning.etikkom.no; 2010/1389/REK sør-øst A), and written informed consent was obtained from the patient's parents. All patient information has been anonymized.

Case report. A previously healthy thirteen-year-old boy presented with lethargy, pan-cytopenia, hepatosplenomegaly, and pathological glandules on both sides of the neck. He was diagnosed with T-ALL and treated according to the Nordic Society for Pediatric Hematology and Oncology Protocol ALL2008 (16). Because of T-lineage ALL, he started high risk induction therapy. Due to cytogenetic detection of the *KMT2A* rearrangement (see below) and inadequate therapy response, he was stratified to first to high intensity treatment and then stem cell transplantation in first remission. His pre-transplantation treatment was complicated with severe toxicities. A stem cell transplantation with a 10/10 matched unrelated donor was performed after five blocks and conditioning with total body irradiation and etoposide. He had a skin graft vs host disease grad 1-2, otherwise it was an uncomplicated transplantation. Bone marrow evaluation before conditioning and on day 28 post transplantation showed detectable residual disease but below quantifiable level. Bone marrow 3 months post transplantation showed no residual disease, and he is still in remission 20 months post transplantation.

G-banding and fluorescence in situ hybridization (FISH) analyses. Bone marrow cells were short-term cultured and analyzed cytogenetically as previously described (17). FISH analyses of bone marrow interphase nuclei and metaphase spreads were performed with the Cytocell *KMT2A* (*MLL*) break-apart probe (Cytocell, Oxford Gene Technology, Begbroke, Oxfordshire, UK).
For the detection of *KMT2A-ELL* fusion gene a home-made double fusion FISH probe was used. The BAC probes were purchased from BACPAC Resource Center which is operated by BACPAC Genomics, Emeryville, CA (https://bacpacresources.org/) (Table I). The probes for *KMT2A* were labelled with fluorescein-12-dCTP (PerkinElmer, Boston, MA, USA) in order to obtain green signals. The probes for *ELL* were labelled with Texas Red-5-dCTP (PerkinElmer, Boston, MA, USA) in order to obtain a red signal. Detailed information on the FISH procedure was given elsewhere (18, 19). Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems, Newcastle, UK).

RNA isolation and complementary DNA (cDNA) synthesis. Total RNA was extracted from the patient's bone marrow at diagnosis using the miRNeasy Mini Kit (Qiagen, Hilden, Germany). The concentration and purity of RNA were measured with the QIAxpert microfluidic UV/VIS spectrophotometer (Qiagen). The quality of RNA, in terms of RNA Integrity Number (RIN), was assessed using the Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA, USA) (20). The RIN was found to be 8.9. For the synthesis of complementary DNA (cDNA), one μg of total RNA was reverse transcribed using iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA).

RNA sequencing. High-throughput paired-end RNA-sequencing was performed at the Genomics Core Facility, Norwegian Radium Hospital, Oslo University Hospital (http://genomics.no/oslo/) and 106 million 75 bp-reads were generated. The FASTQC software was used for quality control of the raw sequence data (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The software FusionCatcher was used to find fusion transcripts (21, 22).

PCR analyses. The primers used for PCR amplification and Sanger sequencing are listed in Table II. For reverse transcription-polymerase chain reaction (RT-PCR) and cycle Sanger sequencing, the BigDye Direct Cycle Sequencing Kit was used (ThermoFisher Scientific, Waltham, MA, USA) according to the company's recommendations. As template, cDNA corresponding to 20 ng total RNA was used. For the detection of *KMT2A-ELL* chimeric cDNA fragments, the primer sets were M13ForMLL5580F1/M13RevELL438R1 and M13For-MLL-5589F1/M13Rev-ELL-415R1. For the detection of *ZNF56-KMT2A* chimeric cDNA fragments, the primer sets were M13ForZNF56-249F1/M13RevMLL5867R1 and M13ForZNF56-277F1/M13RevMLL5815R1.

Sequence analyses were performed on the Applied Biosystems SeqStudio Genetic Analyzer system (ThermoFisher Scientific). The basic local alignment search tool (BLAST) software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for computer analysis of sequence data (23). The BLAT alignment tool and the human genome browser at UCSC were also used to map the sequences on the Human GRCh37/hg19 assembly (24, 25).

Results

G-banding analysis of bone marrow cells at diagnosis yielded the karyotype 46,XY,der(11)t(11;19)(q23;p13),del(12)(p11), der(15)?t(15;19)(q26;q11),der(19) t(11;19)(q23;p13)del(19) (q11) [10]/46,XY[2] (Figure 1).

Interphase FISH with the *KMT2A* break-apart probe showed a normal (yellow) as well as split (separated red and

Table II. *Primers used for PCR amplification and Sanger sequencing analyses. The M13 forward and reverse primer sequences are in bold and italics.*

Name	Sequence (5'->3')	Position	Reference sequence	Gene
M13For-MLL-5580F1	***TGTAAAACGACGGCCAGT***-AGGAGTCGAGAAGACAGTCCAGAGC	5580-5604	NM_005933.3	*KMT2A*
M13For-MLL-5589F1	***TGTAAAACGACGGCCAGT***-GAAGACAGTCCAGAGCTGAACCCA	5589-5612	NM_005933.3	*KMT2A*
M13Rev-MLL-5815R1	***CAGGAAACAGCTATGACC***-AGCTGCTTGCCCCTGATCACAG	5815-5794	NM_005933.3	*KMT2A*
M13Rev-MLL-5867R1	***CAGGAAACAGCTATGACC***-TGTGAGACAGCAACCCACGGTG	5867-5846	NM_005933.3	*KMT2A*
M13Rev-ELL-438R1	***CAGGAAACAGCTATGACC***-CCTTCTGGTAGGAGTCGTCGGTG	460-438	NM_006532.3	*ELL*
M13Rev-ELL-415R1	***CAGGAAACAGCTATGACC***-GCACACACCGTGATCTTGTCCTG	437-415	NM_006532.3	*ELL*
M13For-ZNF56-277F1	***TGTAAAACGACGGCCAGT***-AGAGCTGTTCCGCCATGCAGAC	277-298	NM_001355194.1	*ZNF56*
M13For-ZNF56-249F1	***TGTAAAACGACGGCCAGT***-ACCTTCAGCCTCGCTCCTCCAT	249-270	NM_001355194.1	*ZNF56*

green) signals of the probe in 183 out of 200 examined nuclei. On metaphase spreads the proximal part of the *KMT2A*-probe (green signal) was located on der(11)t(11;19)(q23;p13), while the distal part (red signal) was seen on der(19)del(19)(q11)t(11;19)(23;p13) (data not shown).

Interphase FISH with the home-made *KMT2A-ELL* double fusion probe (Figures 2A-D) showed a yellow fusion signal, a red *ELL* signal and two *KMT2A* signals in interphase nuclei (Figure 2E). On metaphase spreads the yellow signal was detected on der(11)t(11;19)(q23;p13), the red signal on normal chromosome 19, a green signal on normal chromosome 11, and a green signal on der(19)del(19)(q11)t(11;19)(23;p13) (Figure 2F).

The FusionCatcher software detected an in-frame *KMT2A-ELL* fusion transcript in which exon 20 of *KMT2A* (nt 5678 in sequence with accession numbers NM_005933.3) fused to exon 2 of *ELL* (nt 208 in sequence with accession number NM_006532.3) (ACAGTGTGCGTTATGTTTGACTTATG GTGATGACAGTGCTAAT*GATTCTGTTTCACTGAGGCC ATCTATCCGATTTCAAGGAAGCC). A *ZNF56-KMT2A* fusion transcript was also found in which the untranslated exon 1 of the *ZNF56* gene from 19p13.11 (nt 428 in sequence with accession number NM_001355194.1) fused to exon 21 of *KMT2A* (nt 5679 in sequence with accession numbers NM_005933.3) (CCGCATCCCCCAACGTGCTGGCTTCCT GACTTCCAAAGTTGCG*GATGCTGGTCGTTTACTATAT ATTGGCCAAAATGAGTGGACAC).

RT-PCR and cycle Sanger sequencing confirmed the results obtained by RNA sequencing/FusionCatcher analysis (Figure 3).

Discussion

The *KMT2A* gene fuses in acute leukemias with more than 100 different genes coding for structurally heterogeneous proteins (8). Most breakpoints occur in the major breakpoint cluster region of *KMT2A* which spans from exon 7 to exon 13 (8, 26) (or exon 8 to 14 based on the sequence reported by Nilson *et al*. (27)). The exact breakpoint position in the major breakpoint cluster region of *KMT2A* influences the structure of plant homeodomains (PHD) 1-3 and correlates with clinical outcome in acute leukemias (8, 26, 28, 29). Breakpoints in intron 10 [listed as intron 11 in the references (8, 26, 28, 29)] are associated with worse prognosis whereas breakpoints within *KMT2A* introns 8 and 9 [introns 9 and 10 in references (8, 26, 28, 29)] are associated with better clinical outcomes (8, 26, 28-30). Recently, a minor breakpoint cluster region (less than 1% incidence) was detected between intron 18 and exon 23 of *KMT2A*, which is what we detected in our patient (8, 30) [in the study of (30)], the minor breakpoint cluster region is given between intron 19 and exon 24). The minor breakpoint cluster region is usually associated with *KMT2A-USP2* and *KMT2A-USP8* fusions (30), but it has also been reported in four T-ALL patients with *KMT2A-AFDN* fusion gene (*AFDN* maps in 6q27 and is also known as *AF6* and *MLLT4*) (8).

The fusion genes with breakpoints in the minor breakpoint cluster region of *KMT2A* code for KMT2A-fusion proteins which contain intact PHD1-3 and bromodomain (BD) regions of KMT2A (30). Although the exact role of an intact PHD1-3 region in the fusion proteins is still unclear, it may be important (30). PHD1 was shown to play a role in the stability of the N-terminal part of KMT2A through interaction of PHD1 with PHD4 (31, 32). PHD2 was found to be an E3 ubiquitin ligase in the presence of the E2-conjugating enzyme CDC34, and mutation of PHD2 was shown to stabilize the KMT2A protein and increase its transactivation ability (33). PHD3 was reported to interact with peptidylprolyl isomerase E (cyclophilin E) and binds to histone H3K4me3 (31, 34). Furthermore, *in vitro* experiments showed that the presence of the PHD regions in KMT2A-MLLT3 fusion proteins influenced their function and inhibited KMT2A-MLLT3 transformation of mouse bone marrow cells (35). Loss of PHD3 was necessary for KMT2A-MLLT1-induced hematopoietic stem cell immortalization (36).

The *ELL* gene (19p13.1) encodes a nuclear protein (accession number NP_006523) which regulates the activity of the RNA

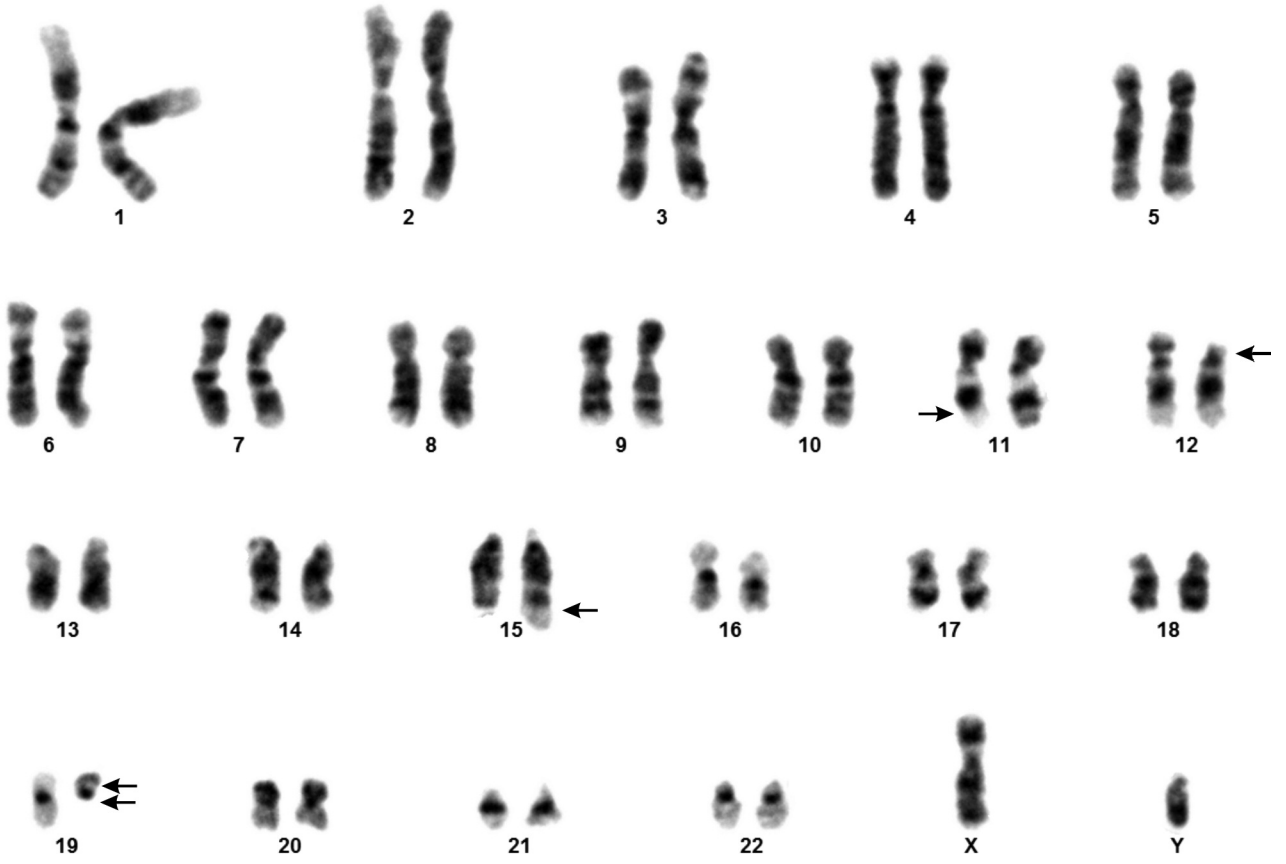

Figure 1. *Cytogenetic analyses of the pediatric T-ALL. Karyogram showing the der(11)t(11;19)(q23;p13), del(12)(p11), der(15)?t(15;19)(q26;q11), and der(19)del(19)(q11)t(11;19)(q23;p13). Breakpoint positions are indicated by arrows.*

polymerase II elongation complex (37, 38). The ELL protein modulates gene expression (39), plays an important role in embryogenesis (40), and is a partner of steroid receptors, TP53, hypoxia-inducible factor 1α, and elongation-associated factors 1 and 2. (41-45). However, the exact mechanism of ELL activity in normal and neoplastic cells is still unclear.

The ELL protein has various functional domains. At the N-terminal, between amino acid residues 5 to 293, it has the RNA polymerase II elongation factor ELL domain (pfam10390). This is bound stably to elongation-associated factors 1 and 2, and together these act as a strong regulator of transcription activity (37, 38, 43, 44, 46). This N-terminal domain is encoded by exons 1-6 of *ELL* (NM_006532.3). In addition to its elongation activation domain, ELL contains a RNA polymerase II interaction domain (37). This domain of ELL negatively regulates polymerase activity in promoter-specific transcription, is within the first 60 amino acids and is encoded by exons 1 and 2 of *ELL* (37, 47). A nuclear localization signal is found between amino acid residues 445-459 (encoded by exon 8). At the C-terminal part of the protein, there is an occludin homology domain (pfam07303). Occludin is an integral membrane protein that localizes to tight junctions (48). This domain represents a conserved region approximately 100 residues long between amino acid residues 513-614 thought to mediate protein interactions (48, 49). This C-terminal domain is encoded by exons 9-12 of *ELL* (NM_006532.3). Between amino acid residues 534-619 (encoded by exons 9-12) one also finds a SMC_prok_A domain (chromosome segregation protein SMC, primarily archaeal type TIGR02169). SMC (structural maintenance of chromosomes) proteins bind DNA and act to organize and segregate chromosomes for partition (50).

Three types of *KMT2A-ELL* fusion transcripts have been reported (51). In type 1 fusion transcripts, found in the majority of cases, exons 9, 10, 11 or 12 of *KMT2A* (within the major breakpoint cluster region) fuse to exon 2 of *ELL* (11, 30, 52-57). In type 2, *KMT2A* exon 9 [exon 10 according to Nilson *et al*. (27)] fuses to *ELL* exon 3 (58, 59). Thus, types 1 and 2 of KMT2A-ELL proteins both maintain the ability to interact with RNA polymerase II and be active in transcription elongation, whereas the functional domain required for inhibition of promoter-specific initiation by ELL is absent (60). In type 3, exon 8 or 9 of *KMT2A* fuses to exon 6 of *ELL* (51, 61-63).

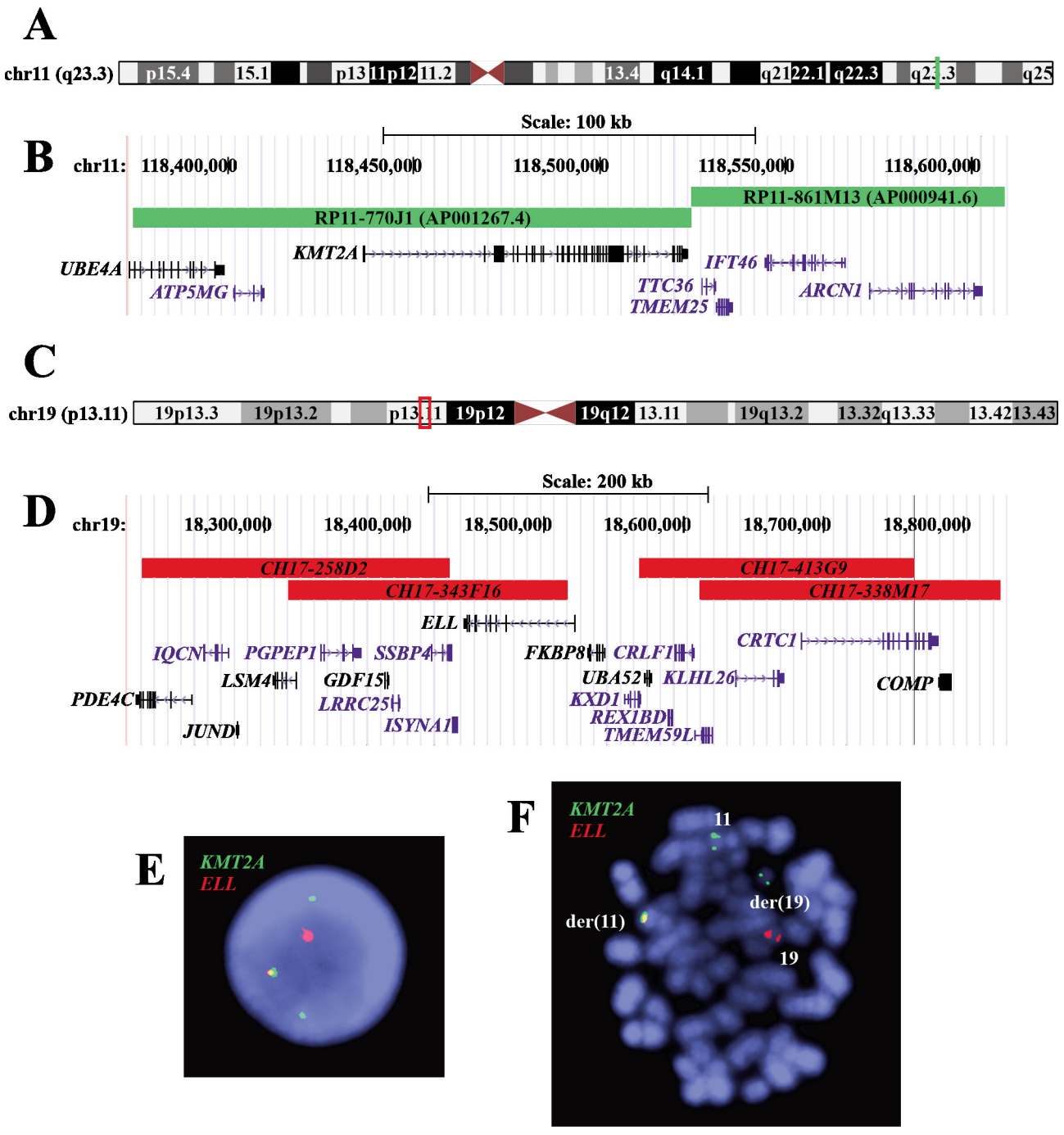

Figure 2. *Fluorescence in situ hybridization (FISH) analysis of the pediatric T-ALL using a home-made, dual color fusion probe for the detection of the chimeric gene histone-lysine N-methyltransferase 2A - elongation factor for RNA polymerase II (KMT2A-ELL) gene. (A) Ideogram of the chromosome 11 showing the mapping position of the KMT2A gene at 11q23.3 (vertical green line). (B) Diagram showing the FISH probes RP11-770J1 and RP11-861M13 for KMT2A. The neighboring genes are also shown. (C) Ideogram of chromosome 19 showing the mapping position of the ELL gene at 19p13.11 (red box). (D) Diagram showing the FISH probes CH17-258D2, CH17-343F16, CH17-413G9, and CH17-338M17 for ELL. The neighboring genes in this region are also shown. (E) FISH results with the KMT2A (green signal) and ELL (red signal) probes on interphase nuclei. A nucleus with a yellow signal for the KMT2A-ELL fusion, a red signal for normal ELL, a green signal for normal KMT2A, and a green signal corresponding to the 3'end of the KMT2A which was moved to der(19). (F) FISH results with the KMT2A (green signal) and ELL (red signal) probes on a metaphase spread. A KMT2A-ELL fusion yellow signal on der(11), a normal KMT2A green signal on chromosome 11, a KMT2A green signal on der(19), and a normal ELL red signal on chromosome 19 are shown.*

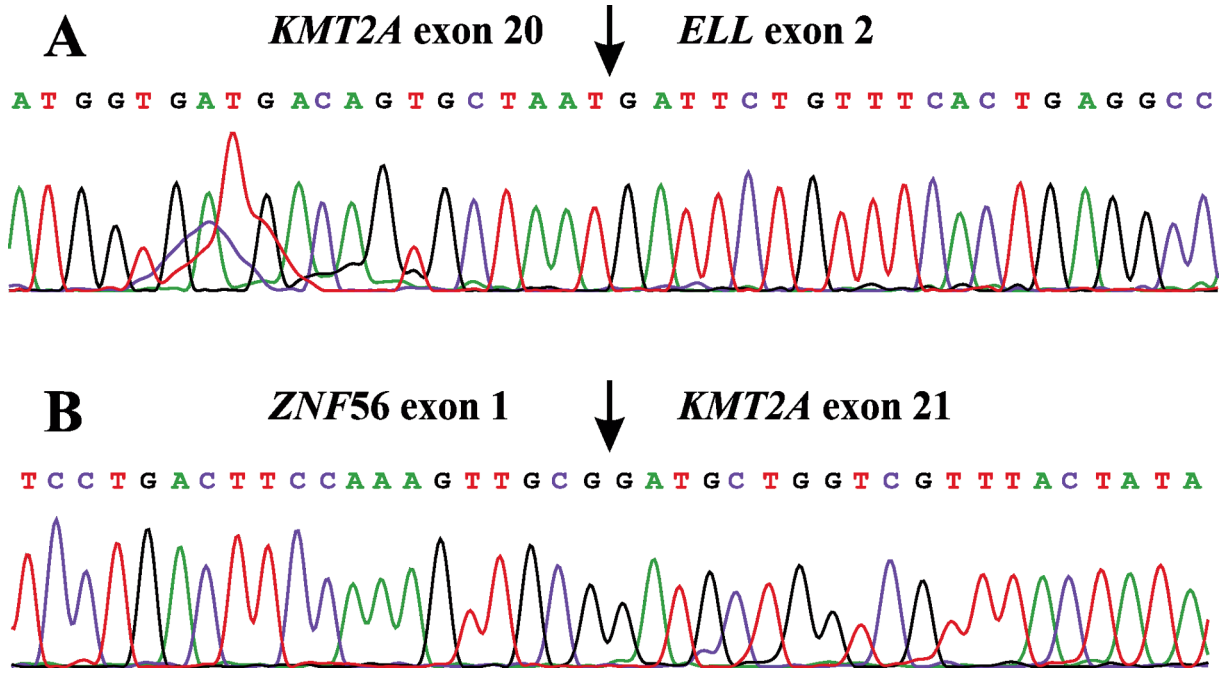

Figure 3. *Molecular genetic analyses of the pediatric T-ALL. l(A) Partial sequence chromatogram showing the junction position of exon 20 of KMT2A with exon 2 of ELL in the chimeric transcript. (B) Partial sequence chromatogram showing the junction position of exon 1 of ZNF56 with exon 21 of KTM2A.*

This transcript codes for a KMT2A-ELL protein which abolishes the function of the RNA polymerase II elongation factor ELL domain, *i.e.*, it cannot interact with RNA polymerase II to active transcription elongation, nor can it mediate negative regulation of promoter-specific transcription initiation (47, 60). The KMT2A-ELL proteins which are encoded by the above-mentioned three fusion transcripts retain from the KMT2A protein the amino-terminal region which interacts with menin, the AT hooks which bind to the minor grove of DNA, the nuclear localization signal, and the CXXC domain which binds to unmethylated CpGs (64, 65). From the ELL protein they retain the nuclear localization signal, the three phosphorylation sites, the occludin homology domain, and the SMC_prok_A domain.

Our patient had a *KMT2A-ELL* chimeric transcript in which exon 20 of *KMT2A* fused with exon 2 of *ELL* (we have called it type 4 *KMT2A-ELL* chimeric transcript). Thus, the breakpoint is within the minor breakpoint cluster region of *KMT2A* (8, 30). To the best of our knowledge, this chimera was previously reported only in a 20-year-old male patient who also had T-ALL, but with a seemingly normal karyotype (13). Based on the reference sequences of *KMT2A* (NM_005933.3/NP_005924.2) and *ELL* (NM_006532.3/NP_006523.1), the chimeric *KMT2A-ELL* transcript codes for a 2461-amino acid residue protein and retains, in addition to the above-mentioned domains of KMT2A, the three PHD domains and the bromodomain together with ELL functional domains encoded by *KMT2A-ELL* transcripts 1-3. The presence of KMT2A PHD3 and bromodomain might be of importance in the role of the transcript 4 in leukemogenesis. PHD3 domain together with bromodomain are involved in a highly complex epigenetic mechanism (66).

Although more studies are required to address possible differences in the leukemogenesis of the different types of *KMT2A-ELL* fusion transcripts, previous reports showed that the C-terminal region of ELL (occludin homology domain and SMC_prok_A domain) was necessary and sufficient for immortalization of myeloid progenitors by KMT2A-ELL, whereas the transcriptional elongation domain of ELL was nonessential (60, 67).

In addition to the *KMT2A-ELL* fusion gene/transcript, our patient carried a novel reciprocal *ZNF56-KMT2A* fusion gene which, based on FISH results, was generated on the chromosome der(19)del(19)(q11)t(11;19)(q23;p13). *ZNF56* maps on 19p13.11, codes for a zinc finger protein and RNA sequencing of total RNA from 20 human tissues showed it is expressed on all of them (https://www.ncbi.nlm.nih.gov/gene/7608). The *ZNF56* gene maps on chromosome position chr19:19,887,383-19,946,990, whereas the *ELL* gene on position chr19:18,553,473-18,632,937. Therefore, in our case, it is likely that the area between these two genes has been deleted on der(19) in order to fuse 5'of *ZNF56* and 3'of

Table III. *The published T-lineage acute lymphoblastic leukemias carrying a KMT2A-ELL fusion.*

Gender/Age	Reported karyotype	*KMT2A-ELL* fusion transcript	Reference (patient)
Male/20	46,XY	*KMT2A* exon *20-ELL* exon 2	(13) (patient A51)
Male/11	46,XY,ins(19;11)(p13.3;q23q23),del(12)(p13)	*KMT2A* exon *10-ELL* exon 2	(14) (patient USI: PAUAJA)
Male/11	46,XY	Not determined	(15) (patient 18)
Male/17	46,XY,t(11;19)(q23;p13.1),t(12;14)(p11.2;q24)	Not determined	(15) (patient 19)
Male/13	46,XY,der(11)t(11;19)(q23;p13),del(12)(p11),der(15)? t(15;19)(q26;q11),der(19)del(19)(q11)t(11;19)(q23;p13)	*KMT2A* exon *20-ELL* exon 2	Present study

KMT2A. This interpretation is supported by the fact that the FISH-analysis only showed signal for *KMT2A* (green), but no red signal for *ELL*, on der(19) (Figure 2E and F).

In the detected *ZNF56-KMT2A* fusion transcript the untranslated exon 1 of the *ZNF56* gene (from 19p13.11) fused to exon 21 of *KMT2A*. In exon 21 of *KMT2A* there is an ATG which could act as a starting codon (NM_005933.3; position 5790-5793). Thus, in *ZNF56-KMT2A* the part of *KMT2A* coding for the last 2047 amino acids (positions 1923-3969 in the sequence with accession number NP_005924.2) is under control of the *ZNF56* promoter. This part of the KMT2A protein contains PHD4, phenylalanine-tyrosine-rich N-terminal domain (FYRN), threonine aspartase 1 (TASP1) cleavage site 1, TASP1 cleavage site 2, transactivation domain, phenylalanine-tyrosine-rich C-terminal domain (FYRC), WD repeat-containing protein 5 (WDR5) interaction motif, Su(Var)3-9, enhancer-of-zeste, trithorax domain (SET), and post-SET domain (68).

Fusion genes in which the *KMT2A* is the 3'-end partner gene (reciprocal-*KMT2A*) were shown to have oncogenetic properties (69). In a mouse model, expression of *AFF1-KMT2A* (also known as *AF4-MLL*), the reciprocal-*KMT2A* product of the t(4;11)(q21;q23), was found to induce ALL (70). In another study, expression of *AFF1-KMT2A* in cord blood CD34-positive cells transiently enhanced long-term hematopoietic reconstitution in immunodeficient mice, but it was not sufficient for leukemia development (71). *AFF1-KMT2A* was also shown to alter transcription and epigenetic signatures, to mediate transcriptional elongation of 5-lipoxygenase mRNA, and to interact with the SIAH ubiquitin ligases (72-74). Oncogenic properties were also found for the reciprocal *KMT2A* fusions *NEBL-KMT2A*, *LASP1-KMT2A*, *MAML2-KMT2A*, and *SMAP1-KMT2A* generated by the chromosome aberrations t(10;11)(p12;q23), t(11;17)(q23;q12) inv(11)(q21q23), and t(6;11)(q13;q23), respectively (69, 75, 76). Recently, *YAP1-KMT2A* and *VIM-KMT2A* fusion genes were found in sarcomas (77-80) and an *MN1-KMT2A* fusion in a case of dural-based spindle cell neoplasm (81).

The Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (82) contains information on 428 cases of various hematological malignancies with the chromosome translocation t(11;19)(q23;p13) (database last updated on October 15, 2020). Only 44 of them (10.3 %) are T-lineage ALL/lymphoblastic lymphoma. However, the *KMT2A-ELL* fusion (detected by FISH, RT-PCR, or high throughput sequencing) was found in five male patients with T-ALL, the present case included (Table III) (13-15). The fusion transcript junction was between exon 10 of *KMT2A* and exon 2 of *ELL* in one case (14), between exon 20 of *KMT2A* and exon 2 of *ELL* in two cases [(13), present case], whereas two cases were reported without information on the junction of the fusion transcript (15). The current data are very limited in order to draw any conclusion on the prognosis of *KMT2A-ELL* fusion in T-ALL but in an international study with pediatric AML patients, the 5-year overall survival and the 5-year event-free survival of patients with t(11;19)(q23;p13.1) (corresponding to *KMT2A-ELL*) were 61% and 46%, respectively (83).

In conclusion, we describe herein a pediatric T-ALL with a *KMT2A-ELL* and a novel *ZNF56-KMT2A* fusion genes generated on the derivative chromosomes 11 and 19, respectively. A very rare *KMT2A-ELL* fusion transcript was found in which exon 20 of *KMT2A* fused to exon 2 of *ELL*. This fusion transcript was previously reported only in a 20-year-old male patient who also had T-ALL. In the novel *ZNF56-KMT2A* fusion transcript the untranslated exon 1 of the *ZNF56* gene fused to exon 21 of *KMT2A*.

Conflicts of Interest

The Authors declare that they have no potential conflicts of interest in regard to this study.

Authors' Contributions

IP designed and supervised the experiments, performed bioinformatics analysis, molecular genetic experiments, evaluated the data, and drafted the manuscript. KA performed cytogenetic, FISH, and molecular experiments, and evaluated the data. ME-O evaluated the cytogenetic and FISH data. AGR made clinical evaluations and treated the patient. MCM-K made clinical evaluations and treated the patient. FM evaluated the cytogenetic and FISH data. SH evaluated the data and assisted with writing of the manuscript. All Authors read and approved the final manuscript.

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