

# A paediatric patient with AML M1 and a t(11;19)(q23;p13.1) rearrangement

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## Abstract

The t(11;19)(q23;p13) translocation is found in a variety of haematological malignancies, but most are present as either acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) with an M4 or M5 subtype. The t(11;19)(q23;p13.1) translocation results in a MLL/ELL(MEN) chimeric fusion gene and has only been reported in AMLs to date, with the majority occurring in adults. Few paediatric t(11;19)(q23;p13.1) cases have been reported. We present the case of a ten year old patient with AML, WHO subtype AML without maturation (FAB classification M1), who was found to have a t(11;19)(q23;p13.1) translocation on conventional cytogenetics, and an 11q23 MLL gene rearrangement on FISH. Although molecular studies to confirm the MLL/ELL gene fusion were not performed, the paucity of information about paediatric cases, and the uncertain contribution of MLL/ELL to leukemogenesis, makes this case of clinical interest.

## Introduction

The t(11;19)(q23;p13) translocation is found in a variety of haematological malignancies, but most are reported as either acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) with an M4 or M5 subtype under French-American-British (FAB) criteria [1]. AML cases with the t(11;19) are usually confined to the myeloid lineage only, and are rarer than t(11;19) ALLs, which may be biphenotypic [1]. The t(11;19)(q23;p13.1) typically occurs as the sole abnormality in AML [1], although it can also occur as a secondary therapy-related change [2].

All patients with the t(11;19) share a common 11q23 (*MLL* gene) breakpoint, but two cytogenetically different 19p breakpoints have been observed, one at 19p13.1 (*ELL* gene) and the other at 19p13.3 (*ENL* gene) [3], although they may be indistinguishable when G-banding is less than 400 bands per haploid set (bph) [4]. The 19p13.1 *ELL* (*eleven lysine-rich leukaemia*) gene breakpoint is exclusive to AML patients, but generally occurs only in adult AMLs (over 40 years of age), whereas the 19p13.3 *ENL* (*eleven nineteen leukaemia*) gene breakpoint is thought to occur in younger patients affecting myeloid or lymphoid lineages [1,3,5], particularly in congenital myeloid cases [6].

AML is primarily a disease of adults [3], and only ten cases of the t(11;19)(q23;p13.1) have been reported in paediatric AMLs (Table 1), with molecular confirmation performed in only two cases. Here we report a 10 year old patient with AML, WHO subtype AML without maturation (FAB classification M1).

The *ENL* gene has variable breakpoints: *ENL*<sup>1</sup> fusion is detected using the nucleotide 148-167 RT-PCR primer, *ENL*<sup>2</sup> uses the nucleotide 1262-1280 RT-PCR primer [5] and *ENL*<sup>3</sup> uses nucleotide 1239-1260 and 1262-1280 RT-PCR primer set [7]. WCC refers to white cell count (10<sup>9</sup>/L) FAB refers to French-American-British morphological classification; and EFS refers to event-free survival probability.

## Methods

Two 24 hour cultures were set up according to routine protocols [8] for each bone marrow aspirate specimen (3ml in 100µL heparin). Cytogenetic G-banding was performed on 350bph metaphase spreads obtained from each culture [9].

In the case of fluorescence *in situ* hybridisation (FISH), slides were placed in a 0.01% pepsin/HCl solution pre warmed to 37°C for 13 minutes, then rinsed in 1xPBS and dehydrated in an ethanol series at room temperature (2 minutes in each of 70%, 80% then 100%). The slides were air dried, and 2 µl of probe added to each hybridization site, before being covered with a 13mm diameter round cover slip and rubber cement. Slides were denatured for 2 min at 87°C on a thermal cycler and then hybridized in a humidity box at 37°C overnight (approximately 16 hours). A post hybridization wash was performed by immersing the slides in a coplin jar of 0.4xSSC/0.03% NP40 pre heated to 72°C for 2 min, then transferring them to a coplin jar of 2xSSC/0.01% NP40 for 30 sec at room temperature. Slides were drained briefly then mounted with 8 µl of DAPI counterstain (Vectashield) and a coverslip.

## Results and discussion

Cytogenetic G-banding showed a 46,XX,t(11;19)(q23;p13.1) karyotype in 25 cells at the patient's initial presentation (Figure 1); the translocation was detected in 19/20 metaphase cells at her second presentation.

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Interphase FISH studies using the 11q23 *MLL* (mixed lineage leukaemia) dual colour break apart probe (Vysis) confirmed an *MLL* gene rearrangement in 92.3% of 209 nuclei examined in the patient's initial presentation (Figure 2), and was reported as nuc ish(MLLx2) (5'*MLL* sep 3'*MLLx1*)[193/209]. The presence of a high level *MLL* gene rearrangement in her second presentation (92% of 213 nuclei) indicated that the more mature cells harboured the rearrangement; however, as the sole abnormality at both diagnosis and relapse, the high level *MLL* gene rearrangement at diagnosis would have to be explained by loss of blast cells in culture.

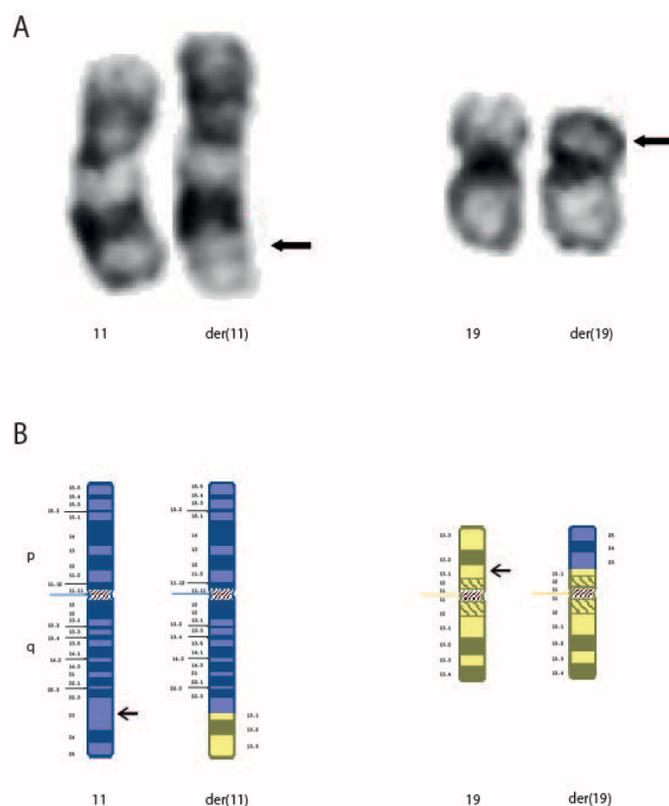
Further studies were undertaken of the patient's bone marrow aspirate at her second presentation in order to establish whether additional treatment-related mutations had occurred, leading to a myeloproliferative syndrome. FISH analysis to detect a *BCR/ABL1* gene rearrangement using the *BCR/ABL1* dual fusion probe (Vysis) was negative, excluding an atypical CML. In addition, molecular-based studies excluded the presence of the *FIP1L1-PDGFR*A gene fusion [10], and mutations in the *JAK2* and *c-MPL* genes [11-13].

The t(11;19) rearrangement has been reported in approximately 12% of paediatric *MLL*+ AML patients [3], but only ten cases of the t(11;19)(q23;p13.1) have been reported in childhood AML, and only two cases of *MLL/ELL* gene rearrangement have been confirmed by molecular analysis. A third case with a variant *MLL/ELL* breakpoint was reported as negative on RT-PCR [14], which may be due to two other partner genes (*EEN* and *MYOF1*) that have been mapped to the 19p13 region [1,7].

In this patient the t(11;19) appears as the sole abnormality, which is characteristic of the t(11;19) rearrangement [1], and the myeloid sarcoma reported in the patient is a common finding in acute paediatric cases [3]. The 19p13.1 and 19p13.3 breakpoints are not always cytogenetically distinguishable [4]; however, the 19p13.2 band was observed on the der(11) in this case (Figure 1), meaning the proband is likely to have the t(11;19)(q23;p13.1) rearrangement (11q+, 19p-) which results in an *MLL/ELL* gene fusion [7]. As the banding resolution was only 350bph, confirmation of the rearrangement by FISH [4] or PCR-based testing would be desirable, as the literature suggests the *MLL/ELL* (MEN) fusion protein does not have a direct leukemogenic effect whereas the *MLL/ENL* (MLLT1) protein does [3,15].

The proband is described as an AML with recurrent genetic abnormalities under the World Health Organisation (WHO) classification [16] due to the presence of an 11q23 *MLL* gene rearrangement (Figure 2). Such AMLs are characteristically heterogeneous [15], with the *MLL* gene rearrangement thought to drive the disease, as the der(11) fusion transcript is always expressed, but the der(19) transcript may not be formed [17], as breaks in the breakpoint cluster of the *MLL* gene are often accompanied by downstream deletions [3]. It is currently assumed that the der(19) does not contribute to leukemogenesis for this reason [3,15], and also because the t(11;19) fusion gene and the chimeric protein that is formed is thought to be insufficient to cause leukemogenesis on its own [3,16].

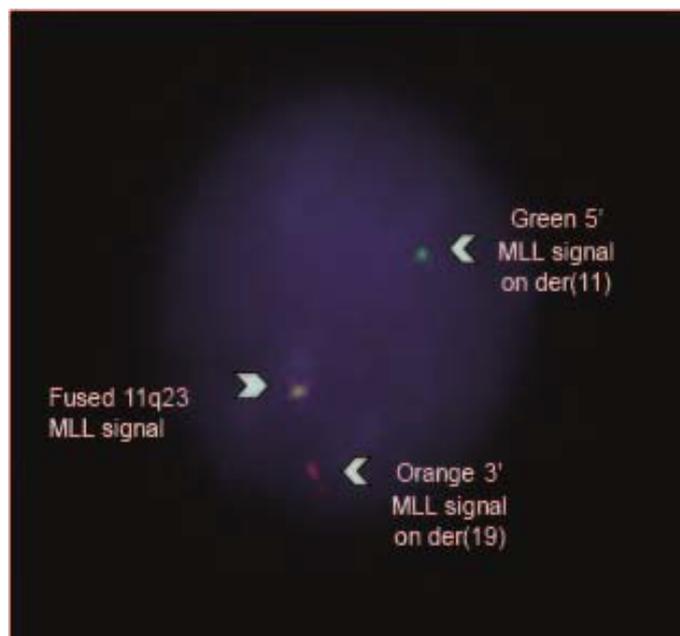
Critically, the current theory of *MLL* being the driver of disease does not seem to fit with literature describing disease development in AML. Classical AML theory holds that development of AML is a multi-step process requiring at least two genetic abnormalities to occur for disease development [18]. This may explain the paucity of paediatric AML cases compared to ALLs. If two mutations are required for the development of AML then another abnormality must be present, and may be linked to the unusual clinical presentation of the proband.



**Figure 1.** G-banded karyotype and ideogram of the t(11;19)(q23;p13.1) translocation in the proband.

Panel A: Karyotype of the normal and derivative chromosomes 11 and 19.

Panel B: Ideogram of the normal and derivative chromosomes 11 and 19. The presence of the 19p12 band on the end of the der(11) indicates that the breakpoint must be at 19p13.1. The arrows indicate the approximate locations of the translocation breakpoints.



**Figure 2.** FISH analysis.

The *MLL* break apart probe (Vysis) showed a rearrangement of 11q23 in interphase cells. The normal 11q homologue is indicated by the orange/green fusion signal, with the single green signal on the der(11) and the single orange signal on the der(19).

The diagnosis of a paediatric AML (M1 subtype) with the t(11;19)(q23;p13.1) has been reported only twice before, in patients of 17 and 18 years of age [1], and is at odds with the report by Moorman *et al.* [1] that the AML FAB M4/5 subtype is typical of 11q23 abnormalities as a whole.

*MLL* gene rearrangements are more frequent in younger subjects with de novo AML [19] such as reported here. Although the *MLL* gene rearrangement is traditionally considered to be the driver of the disease, it is not clear whether the 11q23 rearrangement alone can explain differences in clinical presentation between ALLs and AMLs [20]. ALLs are reported to have a higher white cell count (median 102 vs 25.9×10<sup>9</sup>/L) and shorter survival than AML patients due to an increased incidence of relapse [20], and *MLL* gene rearrangements occur in 66% of ALLs but only 35% of AMLs [20]. *MLL* gene rearrangements are thought to arise by multiple mechanisms, such as recombination mediated by VDJ, Alu elements, and DNA topoisomerase II, or by non-homologous end joining [3], and breaks in the *MLL* gene generally occur closer to the centromeric end of the *MLL* gene breakpoint cluster region (bcr) in *de novo* AMLs, compared to the telomeric end observed in infant leukaemia and t-AML [15]. It is unknown, however, if *MLL* gene breakpoint variation can contribute to differences in clinical presentation between ALL and AML patients with the t(11;19), as the incidence of the t(11;19) in ALL is 18% compared to 15% in AML [20]

Several scenarios therefore seem plausible to explain the clinical presentation of the proband. Aside from variation in the *MLL* breakpoint, other possibilities include the co-involvement of the 19p13 *ELL* gene in disease development, the presence of a treatment-

related mutation in addition to the t(11;19), or the presence of an underlying mutation causing an overlapping AML/MDS syndrome. The fact that the alternate 19p13.3 *MLL/ENL* (*MLLT1*) fusion protein is known to have a direct leukemogenic effect [3,15], suggests that the 19p13 breakpoint does in fact play a role in disease development, so the contribution of the *ELL* gene to the clinical presentation of the disease must therefore be considered. *ELL* is an RNA polymerase II elongation factor and promotes transcription, and overexpression of *ELL* is reported to be toxic, so the gene may in fact be critical for cell growth regulation and survival [21]. A review of the literature shows that although Rubnitz *et al.* [14] reported that *MLL/ENL* fusions predominate in paediatric AML cases; half of the published paediatric AML cases have been reported cytogenetically as carrying the 19p13.1 *ELL* breakpoint (Table 1). This suggests that it may in fact play a more definitive role than previously reported.

The 19p13.1 *ELL* gene breakpoint is exclusive to AML patients and generally only found in adult AMLs, whereas the 19p13.3 *ENL* gene breakpoint occurs in younger patients of both myeloid or lymphoid lineages [1,3,5]. Fusion of the 5'*MLL* gene sequence on the der(11) to the 3'*ELL* gene sequence causes disruption of the normal *MLL* protein function and methyltransferase activity [22]. The formation of the *MLL/ELL* oncogene is thought to express a novel chimeric transcription factor that leads to AML [14]. Molecular confirmation of the breakpoints of 19p13 rearrangements is therefore desirable, as the prognostic information is currently based on that of the *MLL* gene rearrangement alone.

The presence of a treatment-related change seems an unlikely

**Table 1.** Paediatric AML cases with the 19p13 breakpoint (19p13.1 = *ELL* gene, 19p13.3=*ENL* gene) and associated clinical information.

Patient	Sex	Age	WCC	FAB	19p breakpoint	Fusion transcript	Survival/EFS (months)	Reference
1	F	<1m	304	M4	19p13.1	Not known	0.03	1
2	M	1.1	16	Not known	19p13.3	<i>MLL/ENL</i> <sup>2</sup>	Not known	7
3	F	1.2	111	Not known	19p13.1	Negative	Not known	7
4	F	1.3	16	Not known	19p13.1	<i>MLL/ELL</i>	Not known	7
5	F	1.3	133	Not known	19p13.3	<i>MLL/ENL</i> <sup>2</sup>	Not known	7
6	F	4m	520	M4	19p13.1	Not known	0.8	1
7	M	7m	184.9	M5a	Not known	<i>MLL/ENL</i> <sup>3</sup>	18.7 (EFS)	8
8	F	7m	22	M5a	19p13.1	Not known	5	1
9	M	8m	231	M5a	19p13.3	Not known	0.1	1
10	F	3	26	M7	Not known	<i>MLL/ENL</i> <sup>2</sup>	13.8 (EFS)	8
11	F	8	3	M2	19p13.1	Not known	32+	1
<b>12</b>	<b>F</b>	<b>10</b>	<b>1.99</b>	<b>M1</b>	<b>19p13.1</b>	<b>Not known</b>	<b>10+ with BMT</b>	<b>This paper</b>
13	M	11	61	Not known	19p13.3	<i>MLL/ENL</i> <sup>1</sup>	Not known	7
14	F	12	95	Not known	19p13.1	<i>MLL/ELL</i>	Not known	7
15	M	12	11	Not known	19p13.3	<i>MLL/ENL</i> <sup>1</sup>	Not known	7
16	F	16	16	Not known	19p13.3	<i>MLL/ENL</i> <sup>1</sup>	Not known	7
17	M	17	56	M1	19p13.1	Not known	5.4 with BMT	1
18	F	17		M4	19p13.3		9.8 with BMT	1
19	M	18	2	Not known	19p13.3	<i>MLL/ENL</i> <sup>1</sup> – lineage switch from ALL to AML	Not known	7
20	M	18	7	M1	19p13.1	Not known	6.6 with BMT	1

explanation for the clinical presentation of the proband, as the t(11;19) was the sole abnormality at both diagnosis and relapse, and the bone marrow sample was negative for *FIP1L1-PDGFR*A, *BCR/ABL1*, *JAK2* and *c-MPL* at relapse. Furthermore t-AMLs or AMLs which arise from a pre-existing MDS/MPD generally have a worse prognosis [3], which was not observed in the proband, who had survival of more than 22 months. The AML FAB M1 subtype (no differentiation) observed in the proband has been reported only twice in paediatric cases, and only in cases with the 19p13.1 breakpoint (Table 1), thus suggesting that either ELL does in fact contribute to the clinical presentation of the disease, or a second underlying mutation was present in the proband, leading to a rare overlapping AML/MDS syndrome. Two such cases have been recently reported by Hahn *et al.* [22] and Gao *et al.* [23], and were shown to have underlying *GATA2* gene mutations.

Without combined clinical, cytogenetic and molecular studies, it is difficult to establish whether the heterogeneity of the 19p13 gene locus translates into differences in patient survival; however, as the rearrangement generally occurs as the sole cytogenetic abnormality, it may provide an excellent basis for comparative studies. It is unclear whether this patient had an underlying MLL-positive myeloproliferative disorder that first presented as AML or whether she acquired new mutations after initial treatment which led to a myeloproliferative disorder rather than AML. We attempted to clarify this by testing for *BCR-ABL1* and *FIP1L1-PDGFR*A gene rearrangements, and *JAK2* and *c-MPL* gene mutations at the time of relapse, but the negative results did not help us form an hypothesis regarding the underlying pathogenesis. Ideally, further analysis such as gene array studies comparing DNA at initial and subsequent presentations could provide answers. This would potentially add to available knowledge regarding leukemogenesis in MLL-rearranged leukaemia.

## Conclusions

The current cure rate for paediatric AMLs is approximately 65%, with a 5-year event-free survival probability ranging from 32-54% for MLL-rearranged AML patients [15]. MLL-rearranged AMLs are heterogeneous in nature, so the prognostic outcome is dependent on the translocation partner, white cell count, additional cytogenetic aberrations and early response to treatment [24]. The prognosis of childhood AMLs with 11q23 rearrangements differs between studies, but is generally thought to be intermediate in both prognosis and outcome following optimised treatment regimens [2,15]. Although the survival of patients with the t(11;19) is generally poor (Table 1), there is some evidence that older children with the t(11;19) have a good prognosis and increased survival [1]. The patient reported here had a t(11;19) rearrangement with no additional abnormalities, and responded well initially to the Children's Oncology Group (COG) AAML1031 chemotherapy treatment protocol after diagnosis. Her minimum residual disease (MRD) result after induction put her into a low risk classification, although she later relapsed, undergoing chemotherapy and a subsequent stem cell transplant before achieving remission 10 months post procedure. The patient showed the t(11;19) rearrangement at both diagnosis and relapse, despite different bone marrow morphologies, suggesting an AML with an overlapping myeloproliferative disorder/myelodysplasia syndrome.

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## Conflict of Interest

The authors declare no conflict of interest.

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