So far, 11 deletions, varying from 260bp-67kb in length, of the β-globin gene promoter and its flanking sequence have been reported.[5] In an Indian population, a deletion of 10.3 kb that extends from 3011 bp 5' to the mRNA cap site to an L1 repeat element present downstream of the β-globin gene has been found to cause elevated levels of HbA2 (7.1-7.8%) in a heterozygote state.[6] The novel mutation that we identified is similar to the 4237 bp deletion reported in a Czechoslovakian family which caused elevated levels of $HbA_2$ (8.1%-9.0%)[7] in a heterozygous state and DNA sequence analysis showed that the 5' and 3' breakpoints are a few bases apart (Figure 2C). We used robust, reliable and easier assays to identify the deletion in the β-globin cluster that are useful for appropriate genetic counseling and diagnosis of β-thalassemia, and help predict genotype-phenotype prediction. The exact mechanism for unusual levels of $HbA_2$ in these deletions is not known and it may be hypothesized that it is due to increased availability of transcription factors at the δ-globin promoter when the β-globin gene promoter is deleted, and this promoter competition is not evident between the β- and γ-globin genes.

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## Molecular characterization of identical, novel *MLL-EPS15* translocation and individual genomic copy number alterations in monozygotic infant twins with acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) occurring in the first year of life is rare, accounting for 2-5% of pediatric ALL cases. Infant ALL is distinguished by unique clinical and biological characteristics with an aggressive course following a short latency period. The mixed lineage leukemia (*MLL*) gene, located on chromosome 11q23, is involved in 80% of cases. Over 70 different *MLL*-fusion partner genes have been molecularly characterized,[1] with t(4;11), t(9;11) and t(11;19) occurring most frequently in infant ALL.

The outcome of *MLL*-rearranged infant ALL remains poor with up to 50% 5-year survival. The uncommon nature of ALL in infancy limits the rate of accrual for clinical trials and evidence for the best therapeutic approach has been conflicting, particularly regarding the benefit of hematopoietic stem cell transplantation. The clinical heterogeneity of ALL in infancy, such as poorer outcome under 90 days of age,[2] underlies the need for stratification on these trials. The age-related difference in outcome may be a reflection of underlying molecular characteristics with differences in gene expression profiles according to age.[3]

Risk stratification according to *MLL* translocation partners has not been undertaken due to small patient numbers and as the same biological process was thought to occur irrespective of the partner gene, for *MLL*-mediated leukemogenesis. However, recent identification of distinct molecular differences among the *MLL* subtypes, including variations in epigenetic[4] and gene expression profiles,[3] has given rise to the notion that the many fusion partners of *MLL* should be considered as distinct entities.[1] The complex interplay between *MLL* translocation and these additional molecular differences is not fully understood but further characterization of cases will contribute to the current understanding of the clinical heterogeneity of the disease.

The t(1;11) (*MLL-EPS15*) translocation is rare with only a small number of clinical cases published and three reports of molecular characterization at the transcriptional level.[5-7] The epidermal growth factor receptor pathway substrate 15 (*EPS15*) gene encodes a protein that is involved in receptor-mediated endocytosis of epidermal growth factor. We present an *MLL-EPS15* rearrangement with novel breakpoints at the transcriptional and DNA level in monozygotic infant twins with ALL and characterization of the molecular changes in their leukemic cells. The methods are described in the *Online Supplementary Appendix*. Both twins were diagnosed at seven weeks of age, with a peripheral blood blast population of $99.96 \times 10^9$/L in Twin One and $154.87 \times 10^9$/L in Twin Two. $CD19^+$, $CD24^+$, $CD10^-$, B-precursor ALL was confirmed on immunophenotyping. Banded chromosomal analysis revealed a 46,XX,t(1;11)(p32;q23)[13]/46,XX[7] karyotype in Twin One and a 46,XX,t(1;11)(p32;q23)[8]/ibid+X[4]/46,XX[8] karyotype in Twin Two, with fluorescence *in situ* hybridization confirming MLL involvement.[8]

The *MLL*-fusion transcripts were sequenced and novel, identical breakpoints were identified in both twins (Figure 1A). For the *MLL-EPS15* transcript, the breakpoint was located at the end of exon 8 of *MLL* which was fused to exon 10 of *EPS15*. For the *EPS15-MLL* transcript,

**Table 1.** List of tumor-associated genomic aberrations identified using whole-genome cytogenetic arrays in monozygotic twins with t(1;11)(p32;q23) infant acute lymphoblastic leukemia.

| Twin | Region | Type* (CN state) | Start | End | Size (Kb) | Confidence (%) | Relevant genes | sno/micro RNA |
|---|---|---|---|---|---|---|---|---|
| One | (6)(p12.1) | Gain (3) | 55,308,049 | 55,355,423 | 47.4 | 87.5 | *GFRAL* | |
| One | (X)(p21.1) | Gain (3) | 36,640,213 | 36,706,268 | 66.1 | 93.1 | No genes | |
| One | (11)(p11.2p11.12) | LOH | 47,847,646 | 49,645,413 | 1797.8 | 99.9 | *PTPRJ, FOLH1* | |
| One | (20)(q11.22q11.23) | LOH | 32,377,194 | 34,166,072 | 1788.9 | 99.9 | *ITCH, DYNLRB1, PHF20, TP53INP2, GGT7, MMP24, ROMO1, RBM39*, | mir-644, mir-499, mir-1289-1 |
| One | (X)(q28) | LOH | 152,270,145 | 154,849,094 | 2578.9 | 99.9 | *BRCC3, H2AFB3, AVPR2, L1CAM, MTCP1NB, LCAP, CTAG* family, *DUSP9* | mir-1184, ACA36, ACA56 |
| Two | (9)(q31.3) | Loss (1) | 112,486,139 | 112,514,070 | 27.9 | 86.8 | *MUSK* | |
| Two | (X)(p21.1) | Gain (3) | 36,627,235 | 36,736,354 | 109.1 | 92.5 | No genes | |
| Two | (16)(q12.2q21) | LOH | 54,430,930 | 56,753,662 | 2322.7 | 99.9 | *GNAO1, AMFR, CCL17, CCL22, DOK4, GPR56, CIAPIN1, MMP15* | mir-138-2 |
| Two | (20)(q11.22q11.23) | LOH | 32,377,194 | 34,434,692 | 2057.5 | 99.9 | *ITCH, DYNLRB1, PHF20, TP53INP2, GGT7, MMP24, ROMO1, RBM39* | mir-644, mir-499, mir-1289-1 |
| Two | (X)(q28) | LOH | 152,263,301 | 154,849,094 | 2585.8 | 77.4 | *BRCC3, H2AFB3, AVPR2, L1CAM, MTCP1NB, LCAP, CTAG* family, *DUSP9* | mir-1184, ACA36, ACA56 |

**Gain: gain of one copy number (CN); Loss: loss of one CN.*

the breakpoint was located at the end of exon 9 of *EPS15* which was fused to exon 10 of *MLL*. Skipping of *MLL* exon 9 was, therefore, evident at the transcriptional level. A novel, identical breakpoint was also identified at the DNA level (Figure 1B). The genomic breakpoint occurred within exon 9 of *MLL* and intron 9 of *EPS15*. A deletion of 10bp from exon 9 of *MLL* coincided with a duplicated 10bp segment from intron 9 of *EPS15*. These findings are consistent with and provide the first known evidence for the non-homologous end joining mechanism (NHEJ) of DNA repair occurring in the leukemic cells of infants with the t(1;11) translocation. NHEJ has previously been shown in infants with the t(4;11) translocation.[9]

In order to detect molecular aberrations that may be unique to each twin, the Affymetrix Cytogenetics Whole-Genome 2.7M Array technology was applied. Each twin had two copy-number alterations (CNAs) (Table 1) that were considered tumor-associated genomic aberrations. One CNA was common in both twins, namely amplification at Xp21.1, which is a gene poor region. This region has previously been identified with a 50kbp deletion in one infant with t(4;11) *MLL*-rearranged ALL.[10] The other CNAs were different for each twin, namely a 47kbp amplification at 6p12.1 which contains the GDNF family receptor alpha like gene (*GFRAL*), for Twin One and a 28kbp deletion at 9q31.3, containing the muscle, skeletal, receptor tyrosine kinase (*MUSK*) gene, for Twin Two. Each twin had three regions of tumor-associated copy-number neutral loss of heterozygosity (Table 1), two of which were common between the twins. In addition, Twin Two had Trisomy X occurring in a subpopulation of leukemic cells, evident from both the banded chromosomal and cytogenetic array analyses.

Studies using high-resolution SNP and cytogenetic arrays in singletons with infant t(4;11) *MLL*-rearranged ALL[10,11] revealed an exceptionally low frequency of CNAs. Our results corroborate these findings in a different cytogenetic subtype of *MLL*-rearranged infant ALL. Together with the concordance shown at the molecular level for the t(1;11) translocation, the identification of additional common aberrations provides further support for the concept of *in utero* development of a pre-leukemic clone in one twin with transfer to the other by means of the shared placental circulation.[12] However, as far as the low frequency of additional aberrations points towards the t(1;11) translocation as the major driver for leukemogenesis, further work is required to establish whether these additional aberrations have a co-operating role. CNAs of the Xp21.1 site have now been demonstrated in 3 infants with *MLL*-rearranged ALL and so this region merits particular attention in future research. Importantly, this study has shown that several of the additional aberrations present in the leukemic cells of the twins are unique and differ in each patient, providing evidence for molecular heterogeneity for cells that have originated from a common cell of origin. The distinct molecular differences between the twins can be explained by clonal evolution of the leukemic cells from a common precursor or by selection of pre-existing clones. Further clarification of the molecular differences among different *MLL*-subgroups, and indeed among patients of the same subgroup, will help explain the clinical heterogeneity of the disease.

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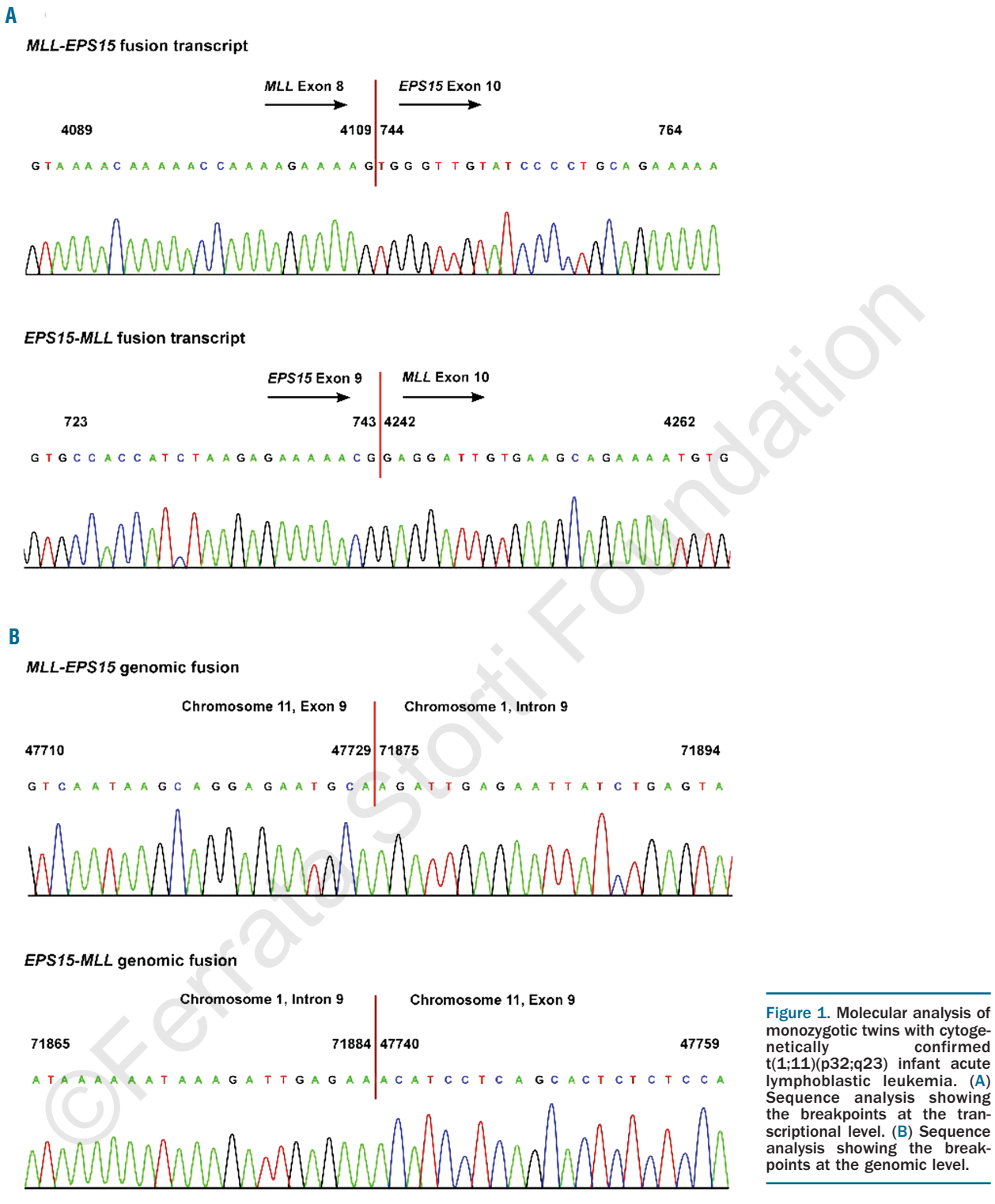


**Figure 1.** Molecular analysis of monozygotic twins with cytogenetically confirmed t(1;11)(p32;q23) infant acute lymphoblastic leukemia. (A) Sequence analysis showing the breakpoints at the transcriptional level. (B) Sequence analysis showing the breakpoints at the genomic level.

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