Two Pairs of Monozygotic Twins With Concordant Acute Lymphoblastic Leukemia (ALL): Case Report

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Summary: The occurrence of leukemia in twins is rare but has a crucial implication in the genetic research of leukemia. This report presents 2 pairs of monozygotic twins with precursor B-cell acute lymphoblastic leukemia. Mixed lineage leukemia (*MLL*)-*AF4* fusion genes were found in the twin sisters. This study is the first to report on infant ALL harboring the 46,XY, −4, + 10, −13, del(14)(q24), −15, + 2mar[4 cells] complex chromosome abnormality. Our report showed that the unified cytogenetic features in monozygotic twins and *MLL-AF4* fusion gene may be necessary but insufficient for the clinical development and prognosis of identical twins with leukemia.

Key Words: acute lymphoblastic leukemia, monozygotic twins, cytogenetic, fusion gene, chromosome translocation

(*J Pediatr Hematol Oncol* 2014;36:e299–e303)

Acute lymphoblastic leukemia (ALL), the most common type of leukemia in children, has achieved clinical success, with >80% cure rate in a particular phenotype. However, infant ALL, including infant twins, is a biologically and clinically distinct disease from childhood ALL. The prognosis of infant ALL remains poor and most cured children face long-term sequel.[1] Thus, prevention and early detection of this disease is of great concern. With the deep understanding of cancer cytogenetics and the current progress in biotechnology, a great diversity of chimeric fusion genes has been identified in childhood ALL; most of these genes are involved in prenatal, early genetic events in the evolutionary trajectory of this cancer.[2]

The common inheritance of twins has an important implication in medical research. In particular, it provides an opportunity to explore the vulnerability of twins to diseases. In this report, we present 2 cases of concordant ALL in monozygotic twins through cytogenetic studies. These cases are used as baseline to define the sequence of genetic events underlying the development of leukemia and infer the contribution of these mutations to clonal progression and adverse prognosis.

Received for publication March 25, 2013; accepted November 25, 2013.
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Supported by Grants of Shandong Province Natural Science Foundation (ZR2011HM007), National Natural Science Foundation of China (81102710), and Shandong Province Scientific and Technological Development Project (2010G0020213).
The authors declare no conflict of interest.
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CASE REPORT

Case 1

Twin sisters were born by spontaneous delivery in 2010 at 4 weeks before term. They shared a single placenta. Twin A, the younger one, was examined for a history of anorexia and intermittent fever for over 1 month at the age of 14 months. The hemogram showed moderate anemia, high total white blood cell (WBC) count with 66% blasts, and low platelet count. The bone marrow aspirate showed 90% of blasts, and the immunophenotyping of abnormal cells confirmed the diagnosis of pro-B-ALL, that is, CD10 negative without surface or cytoplasmic immunoglobulin (Ig). The G-banded karyotype was 46,XX[7 cells]. Examination showed that the cerebrospinal fluid was normal.

One year and 3 months after twin A was diagnosed, twin B was hospitalized for sallow complexion, fever, and abdominal distension. Physical examination revealed hepatosplenomegaly. The laboratory data upon admission showed 67.5% blasts in WBC. The hemoglobin concentration was declining, but the platelet count was within the normal range. Twin B was diagnosed with pro-B-ALL by bone marrow aspiration and immunophenotyping. Cell surface marker analysis exhibited B-cell lymphoblastic proliferation. The G-banded karyotype was 46,XX,t(4;11)(a21; q23)[20 cells]. RT-PCR was also carried out on the cytogenetic preparation of peripheral blood samples to analyze the fusion genes in leukemic cells. We tested the following 15 fusion genes: *MLL-AFX, MLL-AF1P, MLL-AF4, MLL-AF6, dupMLL, MLL-ENL, E2A-PBX1, BCR-ABL (p210), E2A-HLF, BCR-ABL (p190), SIL-TAL1, TEL-AML1, TLS-ERG, TEL-ABL*, and *HOX11*. The twins had demonstrable *MLL-AF4* fusion genes and *IgH* rearrangement (Fig. 1, Table 1).

The twins were treated for high-risk ALL with chemotherapy according to the Chinese Children's Leukemia Group 2008 (CCLG-2008) protocol.[3] The minimal residual disease in the twins reached complete remission on the 33rd day of chemotherapy.

Case 2

Twin brothers were delivered by cesarean section at 38 weeks of gestation. They shared a single placenta. Twin C, the younger one, presented skin petechiae at 7 months. Twin D was hospitalized for the same reason at 10 months. The peripheral blood counts of the twins were as follows: decreased hemoglobin levels, high WBC count with blasts, and low platelet counts. The bone marrow aspirate findings of the brothers and the immunophenotype of the leukemic cells were consistent with the diagnosis of common precursor B-cell ALL, that is, CD10 positive without surface or cytoplasmic Ig. The fusion gene test of the leukemic cells presented normal without *MLL-AF4* rearrangement. G-banding chromosomal analysis showed 46, XY, −4, + 10, −13, del(14)(q24), −15, + 2mar[4 cells]/46, XY[6 cells] for twin C and 46,XY[20 cells] for twin D. The twin brother had *IgH* rearrangement (Fig. 2, Table 1) The twins were treated by the CCLG-2008 protocol for high-risk ALL. Twin C achieved complete remission with induction chemotherapy. However, twin D failed with the chemotherapy, with an minimal residual disease of 3.12% on the 33rd day of the VDLP protocol. Twin C was again treated with induction chemotherapy, after which he successfully entered into the consolidation stage of the therapy.

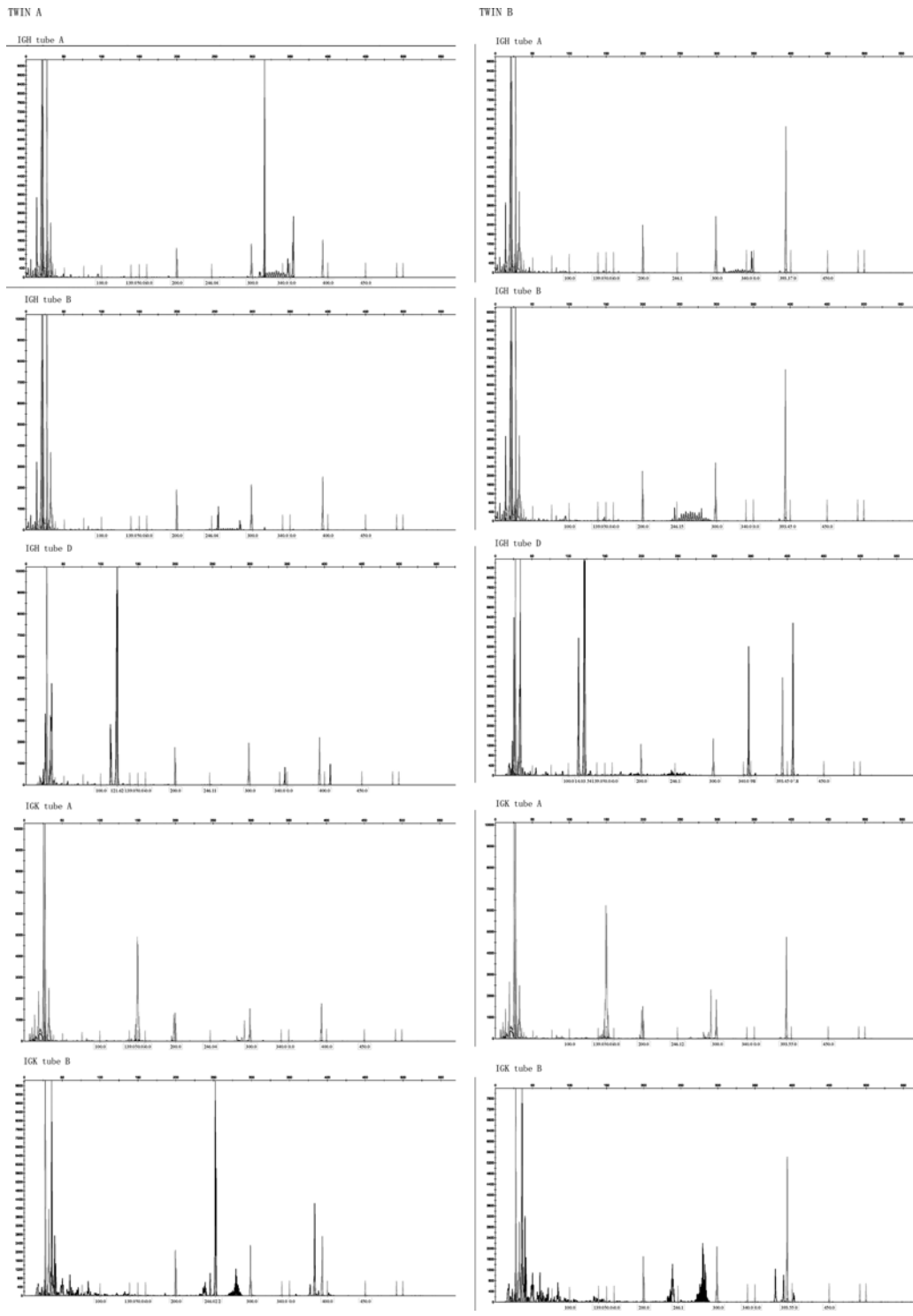

FIGURE 1. *IgH* rearrangement in twin sisters with acute lymphoblastic leukemia.

DISCUSSION

Research has shown that the concordance rate in monozygotic twins with ALL, at least for infants, is extraordinarily high. The pathogenetic mechanism underlying this phenomenon has been studied for years. In 1962, Wolman[4] suspected that this disease may have originated in one twin within the uterus and may have been transmitted to the other through the conjoined circulation. This idea was resurrected and developed more fully in 1971 by Clarkson and Boyse.[5] Current research favored the view that the in utero single clonal origin hypothesis of leukemia and intraplacental metastasis is the basis for concordant ALL. Rich innate genetics and less external environmental disturbances are crucial in such research. Molecular studies

TABLE 1. *IgH* and *IgK* Rearrangement in Patients With Acute Lymphoblastic Leukemia as Validated by RT-qPCR

		1	2	3	4	5	6	
	Master Mix	Control Gene Tube	*IgH* Tube A	*IgH* Tube B	*IgH* Tube D	*IgK* Tube A	*IgK* Tube B	
	Target	House-keeping Gene	FR1-JH	FR2-JH	DH-JH	Vk-Jk	Vk-Kde + intron-Kde	
Sample Number	Effective Detection Range	101, 200, 300, 395	310-360	250-295	110-290, 390-420	120-160, 190-210, 260-300	210-250, 270-300, 350-390	Clonal Gene Rearrangement Results
Twin A		√	+ + +	−	+ + +	−	+ + +	Positive
Twin B		√	+ +	−	+ + +	−	+ +	Positive
Twin C		√	+ + +	+ + +	−	+ + +	+ + +	Positive
Twin D		√	+ + +	+ + +	−	+ +	+ + +	Positive

+ + + : monoclonal peak is significantly higher than polyclonal peak, fluorescence signal is higher than the signals of 4 control gene tubes.
+ + : monoclonal peak is significantly higher than polyclonal peak. Moreover, fluorescent signal is higher than the lowest fluorescent signals of control gene tubes but lower than the highest signals of control gene tubes.
+ : monoclonal peak is higher than polyclonal peak but not significantly higher than polyclonal peak. Fluorescence signal is above the lowest fluorescent signal of control gene tubes but lower than the highest signal of control gene tubes.
√: the quality and quantity of DNA samples meet the requirements.

on several pairs of monozygotic twins have provided strong insights into the natural history and pathogenesis of pediatric leukemia. Nevertheless, ALL remains a relatively rare disorder with distinctive clinical and diagnostic features. Therefore, more clinical cases are needed to be pursed and studied.

The outcome of ALL in infants, especially those below 90 days of age, remains poor. The age-related difference in outcome may reflect the underlying molecular characteristics with differences in gene expression profiles according to age.[1] The most common chromosome alterations in leukemia in infants, including identical twins, results from the rearrangement of the *MLL* gene at 11q23 with various partner genes, principally *AF4*, in ALL.[6]

MLL translocations are common in 2 groups of patients: infants and those who have been previously treated for other cancers with topoisomerase II-inhibiting drugs. Recent identification of in utero rearrangements has implicated the *MLL* gene in the leukemogenesis of infant ALL.[7] The *AF4* gene encodes a protein with serine and proline-rich regions located near the *AF4* breakpoint and a nuclear localization signal domain located to the breakpoint (4;11)(q21;q23). The *MLL-AF4* fusion transcript is reportedly the most important prognostic factor in infant ALL.[8]

In our report, twins A and B tested positive for the *MLL-AF4* fusion gene without or with the chromosomal t(4; 11) translocation. Rubnitz et al[9] have shown the presence of *MLL* gene rearrangements in infant ALL patients with or without cytogenetically detectable t(4;11) or other structural chromosomal aberrations involving 11q23. With the recent development in protocols in molecular biology, several reports have documented the occurrence of malignancy-associated fusion transcripts in normal or non-leukemic cells. Uckun et al[10] examined the expression of *MLL-AF4* fusion transcripts in pediatric and infant ALL patients with or without cytogenetically detectable t(4;11)(a21;q23) and in normal bone marrow and fetal tissues using standard and nested RT-PCR assays. They conjectured that the effects of *MLL-AF4* fusion genes restricted to blood-forming tissues in normal individuals or t(4;11)-negative leukemia patients may be much less dire.

The immunophenotype of infant ALL mainly represents the CD10-negative pro-B-ALL, often with coexpression of myeloid-associated antigens.[11] Twins C and D were consistent with the diagnosis of common B-ALL, an infrequent immunophenotype for infant ALL. This finding suggests that this genetic lesion may be acquired during fetal hematopoiesis in utero. In addition, the abnormal chromosomal complex (46, XY, −4, + 10, −13, del(14)(q24), −15, + 2mar[4 cells]) existed in twin C. Such a chromosomal change may not be harmful in consideration of the treatment effect. However, the function of this translocation in the pathogenesis and prognosis of leukemia remains unknown.

In conclusion, these cases illustrate a rare presentation of the unified cytogenetic features in monozygotic twins. However, it cannot be interpreted in accordance with the in utero single clonal origin hypothesis. We speculate that exogenous factors also contribute to the development of leukemia in the twins after in utero initiation. Such a mode of presentation, although distinctly rare, is important to recognize that the *MLL-AF4* fusion gene may be necessary but insufficient for the clinical development and prognosis of identical twins with leukemia. Thus, simultaneous

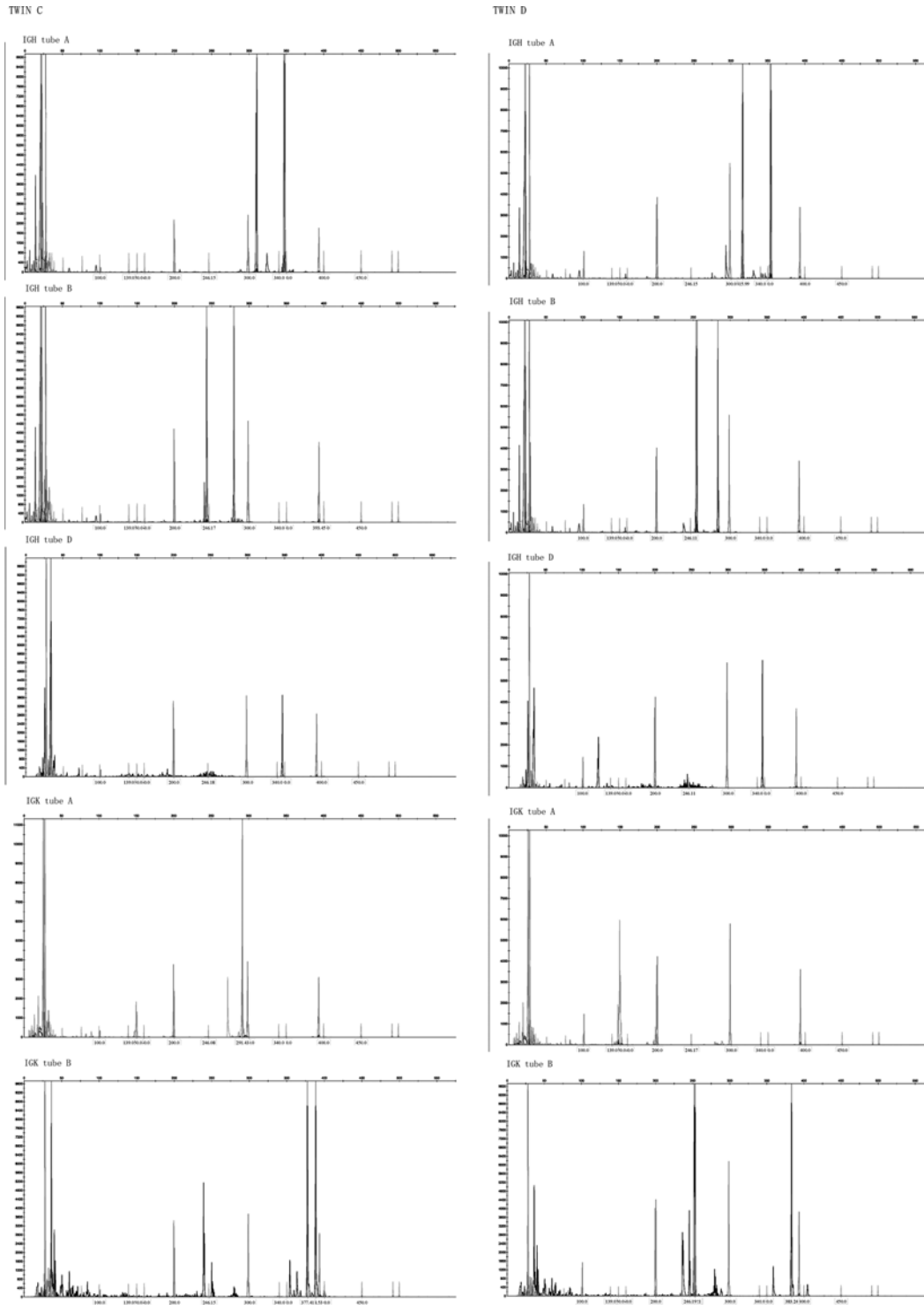

FIGURE 2. *IgH* rearrangement in twin brothers with acute lymphoblastic leukemia.

detection of a rearranged *MLL* gene and chromosomal translocations is clinically important for the therapy and prognosis of ALL. However, the mechanism underlying this phenomenon is yet to be elucidated by future studies.

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