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Original Contributions

Siblings with *ETV6/RUNX1*-positive B-lymphoblastic leukemia: A single site experience and review of the literature

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ABSTRACT

Siblings diagnosed with B-lymphoblastic leukemia (B-ALL) that share the same driver abnormality have been rarely described in the literature. Herein, we report three pairs of siblings (one non-identical pair, one maternal half-sibling pair, and one identical pair) all diagnosed with *ETV6/RUNX1*-positive B-ALL. Considering that *ETV6/RUNX1* fusion is thought to represent a prenatal event and necessitates additional genomic alterations to result in leukemia, siblings of patient's with known *ETV6/RUNX1*-positive B-ALL may be at increased risk of *ETV6/RUNX1*-positive B-ALL due to common exposures (environmental or infectious) or shared germline polymorphisms.

1. Introduction

Approximately 1 in 2000 children will develop acute lymphoblastic leukemia (ALL) with a peak incidence at age 2–5 years, making it one of the most common childhood malignancies [1,2]. Genomic studies have greatly advanced our understanding of the underlying somatic mutations that lead to the development of ALL and the varying prognosis in ALL based on the mutation(s) observed. One of the most common aberrations seen in childhood B-ALL is a cytogenetically "cryptic" translocation between the *ETV6* gene on chromosome 12p13.2 and the *RUNX1* gene on chromosome 21q22.12, resulting in a chimeric *ETV6/RUNX1* fusion. This translocation is identified in up to 25% of childhood B-ALL, most frequently between ages 1–10, but is very rare in infants and only rarely documented in adults [3]. Presenting clinical features of patients with this *ETV6/RUNX1* are comparable to other pediatric cases of ALL. The blast immunophenotype characteristically shows expression of CD19, CD10, and typically CD34, with frequent aberrant expression of myeloid markers, especially CD13 [4]. B-ALL cases with *ETV6/RUNX1* fusion overall have a favorable prognosis with cure rates generally > 90% [4].

To date, only rare reports of siblings have shown the *ETV6/RUNX1* translocation as a recurrent cytogenetic abnormality independently arising in both siblings with B-ALL [5-7]. Herein, we present a single institution experience with three pairs of siblings presenting with *ETV6/RUNX1*-positive B-ALL.

2. Sibling pair 1

The diagnostic bone marrow collection dates for patients 1A and 1B were separated by 8 days and occurred in August 2007.

2.1. Patient 1A

The first presentation of B-ALL for sibling pair 1 occurred in a 4-year-old-male. The bone marrow biopsy demonstrated 90% involvement by B-lymphoblasts. By flow cytometry, the blasts expressed CD45, CD19, CD10, HLA-DR, CD34, TdT, CD13, CD33 and CD15. Conventional chromosome analysis revealed a normal karyotype (46,XY) in all 20 metaphases, while B-ALL FISH studies demonstrated approximately 97% of nuclei with *ETV6/RUNX1* fusion. In addition, deletions of the *CDKN2A* (9p21) and *KMT2A* (11q23) gene regions were observed.

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2.2. Patient 1B

The second presentation of B-ALL for sibling pair 1 occurred in a 5-year old-male. The bone marrow biopsy demonstrated 95% involvement by B-lymphoblasts. By flow cytometry, the blasts expressed CD45, CD19, CD10, HLA-DR, CD34, TdT, CD13, CD33, and weakly, CD15. Conventional chromosome analysis revealed a normal karyotype (46,XY) in all 20 metaphases, while B-ALL FISH studies demonstrated approximately 85% of nuclei with *ETV6/RUNX1* fusion.

3. Sibling pair 2

The diagnostic bone marrow collection dates for patients 2A and 2B occurred in April 2015 and in April 2019, respectively. These two patients represent maternal half-siblings.

3.1. Patient 2A

The first presentation of B-ALL for sibling pair 2 occurred in a 5-year-old male. The bone marrow biopsy demonstrated 85% involvement by B-lymphoblasts. By flow cytometry, the blasts expressed HLA-DR, CD19, CD10, partial CD34, partial dim CD13, and bright CD38. Conventional chromosome analysis revealed a normal karyotype (46,XY) in all 20 metaphases, while B-ALL FISH studies demonstrated approximately 94% of nuclei with *ETV6/RUNX1* fusion and an equivocal result for the *IGH* gene region (loss of the *IGH* variable region).

3.2. Patient 2B

The second presentation of B-ALL for sibling pair 2 occurred in a 3-year-old male. The bone marrow biopsy demonstrated 60–70% involvement by B-lymphoblasts. By flow cytometry, the blasts expressed bright CD10, CD13, CD19, CD22 (surface, low density), partial CD33, partial bright CD34, HLA-DR, and TdT. Conventional chromosome analysis revealed an abnormal clone (51,XY, + 5, + 21, + 22, + 2mar) in 3 metaphases and a normal karyotype (46,XY) in 16 metaphases. B-ALL FISH studies revealed approximately 68% of nuclei with *ETV6/RUNX1* fusion and a gain of the *BCR* gene region (22q11.2), consistent with the trisomy 22 observed by chromosome studies.

4. Sibling pair 3

The diagnostic bone marrow collection dates for patient 3A and 3B were separated by approximately 5 weeks and occurred in October 2019 and in November 2019, respectively. These two patients are identical twins.

4.1. Patient 3A

The first presentation of B-ALL for sibling pair 3 occurred in a 2-year-old male. The bone marrow biopsy demonstrated 50% involvement by B-lymphoblasts. By flow cytometry, the blasts expressed dim CD45, CD19, CD10, dim CD38, subset CD34, HLA-DR, CD79a, dim CD22, dim TdT, small subset CD20, and small subset CD138. Conventional chromosome analysis revealed an abnormal clone (46,XY),t(12;21;13) (p13;q22;q12)inv(13)(q12q32),der(12)del(12)(p11.2p13)t(12;21)(p13;q22),der(21) t(12;21)(p13;q22) in 2 metaphases and a normal karyotype (46,XY) in 18 metaphases. B-ALL FISH studies revealed approximately 48% of nuclei with *ETV6/RUNX1* fusion.

4.2. Patient 3B

The second presentation of B-ALL for sibling pair 3 occurred in a 2-year-old male. The bone marrow biopsy demonstrated 90% involvement by B-lymphoblasts. By flow cytometry (peripheral blood), the blasts expressed CD19, heterogeneous CD34, CD10, dim CD38, HLA-DR, CD79a, dim CD22, and dim TdT. Conventional chromosome analysis revealed an abnormal clone (46,XY),der(12)t(12;21)(p13;q11.2)der(12)t(12;21) (p12;q22),-13, + 21, + der(21)t(12;21)(p13;q11.2)der(12)t(12;21)(p13;q22),der(21)t(12;21) (p13;q22) in 5 metaphases and a normal karyotype (46,XY) in 15 metaphases. B-ALL FISH studies revealed approximately 90% of nuclei with *ETV6/RUNX1* fusion.

5. Conventional chromosome analysis

White blood cells from the diagnostic bone marrow aspirate specimens were cultured (24 and 48 h, unstimulated), harvested, and G-banded slides were prepared utilizing standard cytogenetic techniques according to specimen-specific protocol.

6. Fluorescence *in situ* hybridization (FISH)

FISH studies targeting the *ETV6* and *RUNX1* gene regions (Abbott Molecular) using a dual-color dual-fusion probe strategy was performed on the diagnostic bone marrow aspirate specimens. Five hundred interphase nuclei were analyzed per specimen. Cells from the bone marrow aspirates were subjected to standard FISH pretreatment, hybridization, and fluorescence microscopy according to specimen-specific laboratory protocols.

7. Discussion

A search of the Mayo Clinic Cytogenetics database identified three sets of siblings who presented with *ETV6/RUNX1*-positive B-ALL (Table 1). The sibling pairs included one set of non-twin siblings (pair 1), one set of maternal half-siblings (pair 2), and one set of monozygotic twins (pair 3). The age range of the patients was 2–5 years and the time interval between diagnoses of the three sibling pairs included 8 days (sibling pair 1), 1458 days (sibling pair 2) and 38 days (sibling pair 3). Bone marrow biopsies from all cases showed significant involvement by B-ALL, ranging from the 50 to 95% of marrow cellularity. The blast cell immunophenotypes were concordant for two of the sibling pairs with the only discrepancies being variation in strength of expression of a single antigen (sibling pair 1) or expression of a single additional antigen (sibling pair 3). The most variation was observed in the maternal half-brothers (sibling pair 2) where four different antigens (CD22, CD33, CD38, TdT) were only present on the blasts of one sibling and an additional three antigens (CD10, CD13, CD34) showed variation in strength of expression. Similarly, chromosome studies showed the most variation the maternal half-siblings as sibling 2A presented with a normal karyotype, while sibling 2B demonstrated a several trisomies and marker chromosomes.

In a review of the literature, only three sibling pairs have been reported with *ETV6/RUNX1*-positive B-ALL [5-7]. Among these three sibling pairs, two sibling pairs had one male and one female and the ages at diagnosis ranged from 1.2 years to 8.6 years, while the third sibling pair consisted of identical female twins who were diagnosed at ages 3.5 years and 4.8 years. Additional information was available for two of the sibling pairs, one non-twin sibship and the identical twin sibship. For the non-twin sibling pair, the WBC counts at presentation were dissimilar (8.0×10^3/mL and 17.7×10^3/mL), as were the peripheral blood blast counts (25% and 51%). The outcomes of the two siblings were also different with one sibling being disease free 26 months after treatment and the other siblings succumbing to leukemia during a relapse 18 months after treatment. The identical twins were monochorionic twins that showed no congenital malformations at birth. Twin 1 presented with 2% peripheral blood blasts and 59% bone marrow blasts, while twin 2 presented with 27% peripheral blood blasts and 97% bone marrow blasts.

Multiple population based studies have shown that *ETV6/RUNX1* fusions are detected in healthy newborns [8-10]. An early study found

Table 1
Immunophenotypic and cytogenetic results for three pairs of siblings with *ETV6/RUNX1*-positive B-lymphoblastic leukemia.

Sibling pair	Patient	Gender	Age (yrs) at diagnosis	Immunophenotype	Karyotype	Additional FISH abnormalities
1 (non-identical siblings)	1A	M	4	CD45, CD19, CD10, HLA-DR, CD34, TdT, CD13, CD33 and CD15	46,XY[3]	Deletions of *CDKN2A* (9p21) and *KMT2A* (11q23)
	1B	M	5	CD45, CD19, CD10, HLA-DR, CD34, TdT, CD13, CD33 and weakly CD15	46,XY[30]	No other abnormalities detected
2 (maternal half siblings)	2A	M	5	HLA-DR, CD19, CD10, partial CD34, partial dim CD13 and bright CD38	46,XY[20]	Potential *IGH* rearrangement
	2B	M	3	Bright CD10, CD13, CD19, CD22 (surface, low density), partial CD33, partial bright CD34, HLA-DR and TdT	51,XY, + 5, + 21, + 22, + 2mar[cp3]/46,XY[16]	Gain of the *BCR* gene region (22q11.2)
3 (monozygotic twins)	3A	M	2	Dim CD45, CD19, CD10, dim CD38, subset CD34, HLA-DR, CD79a, dim CD22 and dim TdT	46,XY,t(12;21;13)(p13;q22;q12)inv(13)(q12q32),der(12)del(12)(p11.2p13)t(12;21)(p13;q22),der(21)t(12;21)(p13;q22)[2]/46,XY[18]	No other abnormalities detected
	3B	M	2	CD19, heterogeneous CD34, CD10, dim CD38, HLA-DR, CD79a, dim CD22 and dim TdT	46,XY,der(12)t(12;21)(p13;q11.2)der(12)t(12;21)(p12;q22),-13, + 21, + der(21)t(12;21)(p13;q11.2)der(12)t(12;21)(p13;q22),der(21)t(12;21)(p13;q22)[5]/46,XY[15]	No other abnormalities detected

FISH, fluorescence *in situ* hybridization.

that up to 1% of healthy newborns harbor detectable *ETV6/RUNX1*–-positive cells, while a later study suggested a much lower frequency. Regardless of the actual prevalence, it is understood that an *ETV6/RUNX1* fusion is necessary, but not sufficient to develop B-ALL. The *ETV6/RUNX1* fusion protein is proposed to cause a dominant negative effect on the non-translocated *RUNX1* gene product, thereby interfering with the normal function of RUNX1 and a lack of appropriate B-cell differentiation. The initial fusion event is thought to be an early, prenatal event that requires additional events to develop leukemia, the most common of which is a subsequent deletion of the non-rearranged *ETV6* allele [11,12]. Development of B-ALL has been linked to genetic polymorphisms in genes involved in detoxification and folate metabolism, as well as delayed exposure to common infections of childhood with associated abnormal immune response [13-16]. Further supporting this idea is an observed decrease in development of B-ALL in children who attend childcare in early life [17]. Given the multiple, heterogeneous factors that can contribute to the development of *ETV6/RUNX1* fusion B-ALL, it is not surprising that the concordance rate for B-ALL in identical twins is only 5% [10].

It is unclear if the sibling pairs presented herein may have inherited the same genotype and/or may have experienced similar environmental exposures that resulted in a progression of the prenatal "first hit" of *ETV6/RUNX1* fusion. Unfortunately, there is not sufficient clinical information or additional molecular studies available to further investigate these complex etiologies. While it is logical that both twin and non-twin siblings would have similar germline genomics and exposures to the same environmental factors, the maternal half-siblings described herein represent an intriguing sibship due to greater variation in both germline and environmental factors. Thus, while these sibling pairs with *ETV6/RUNX1*- positive B-ALL represent a rare co-occurrence, additional germline and environmental evaluation on these families could shed light into the complex and primarily unknown etiology of pediatric B-ALL.

Declaration of competing interest

Authors have no conflicts of interest to disclose.

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