Concordant B-cell precursor acute lymphoblastic leukemia in non-twinned siblings

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Abstract

Associating the risk of childhood acute lymphoblastic leukemia (ALL) with genetic predisposition is still a challenge. Here, we discuss two non-twinned siblings (girl and boy) diagnosed with B-cell precursor (BCP-ALL) and ETV6-RUNX1. BCP-ALL clinical onset occurred 10 months apart from each diagnosis. One child is alive in complete continuous remission, whereas, the other relapsed and evolved to death with resistance to ALL treatment. Despite the fact that BCP-ALL with ETV6-RUNX1 usually results in a very good prognosis, the sibling experienced divergent outcomes; a remarkable difference in one child that presented a more aggressive disease was higher leukocytosis associated with IKZF1 deletion. The familial history of cancer and genetic susceptibility was explored. The siblings were absolutely identical in all 17 loci of genes tested: GSTM1, GSTT1, NQO1, TP53, and TP63 were wild-type, whereas at least one copy of the variant allele for IKZF1 was present. The familial pattern of ETV6 was tested by the 12p microsatellite analysis and demonstrated that deletions occurred in one child but not the other, while heterozygous patterns were found in the parents. Altogether, our data suggest that genetic predisposition aligned with chance has an additive effect in BCP-ALL outcome.

ARTICLE INFO

Article history:
Submitted 30 May 2014
Accepted 21 July 2014
Available online 20 August 2014

Keywords:
B-cell precursor ALL
ETV6-RUNX1
Siblings
Genetic-loci

Introduction

There is clinical evidence that specific subtypes of acute lymphoblastic leukemia (ALL) have distinct molecular abnormalities that might be linked with specific causal mechanisms. A model of the possible causal factors, including genetic alterations, environmental factors and lifestyle acquired modifications has been proposed for common-ALL [1]. In this model, some surrogate markers for a low burden of early childhood infections has been associated with increased risk for developing childhood B-cell precursor ALL (BCP-ALL), mainly common-ALL with ETV6-RUNX1 fusion genes [1,2]. Since this first proposal, a prenatal origin of somatic mutations that drive the leukemic clone has been demonstrated in MLL-AFF1, ETV6-RUNX1 and high-hyperdiploidy BCP-ALL cases, as well as, in other less frequent subsets of childhood acute myeloid leukemia [3–6]. The ALL initiating event that arises from mutations in a single cell is acquired rather than inherited and is passed on to its descendants.

It is assumed that genetic susceptibility contributes to leukemogenesis risk, through a variety of single nucleotide polymorphisms (SNPs) in genes that encode enzymes involved in carcinogen metabolism, cell detoxification, immune response to infection, DNA repair system and in cell cycle regulations, and act as modulators to increase the risk of childhood leukemia occurrence [7–9]. More recently, genome-wide association studies (GWAS) have linked other SNPs that are associated with growth regulation, hematopoiesis, and lymphocyte development, e.g. ARID5B, CEPBE, IKZF, CDN2A as candidate genes that are also able to modify the risk of developing BCP-ALL [11–13]. Although these findings demonstrated about two-three-fold rate of risk, the SNPs are not sufficient per se, and shared environmental factors that cannot be ruled out. The concordance of childhood leukemia among siblings is a rare event [14]. In a recent publication Schmiegelow et al. have reviewed data from an international collaborative series of ALL that identified 54 sibships with two or more cases of childhood ALL proposing that strong genetic risk factors for childhood ALL might be restricted to specific subtypes [15]. Besides, the familial history of cancer has been associated with increased risk of childhood leukemia [16].
We describe herein a pair of sibs with BCP-ALL ETV6-RUNX1 positive, diagnosed 10 months apart from each other. IKZF1 deletion and BTG1 amplification were molecular lesions that differed between their leukemia molecular profiles at diagnosis. Considering that both had the same BCP-ALL phenotype, but completely different outcomes, we performed a detailed cytogenetic–molecular investigation based on the latest key notions of leukemia etiology in order to add more data to the understanding of BCP-ALL.

Material and methods

Patients

**Case 1.** A 14 month old, girl, was admitted to the Hospital Joana Gusmão, Florianópolis, with one week history of fever, runny nose and flu. On physical examination only pallor was remarkable; lymph node and hepatosplenomegaly were absent. Laboratorial analysis disclosed a white blood cell count (WBC) of 8.0 × 10^9/L with 97.0% lymphoid cells and NK cells. Serum creatinine, AST and ALT levels were normal. Radiographic thorax and abdominal ultrasonography were negative. A detailed cytogenetic-molecular investigation based on the latest key findings was performed by immunophenotyping and cytogenetic analysis. The diagnosis of common-ALL (c-ALL) was made. The girl received one week oral prednisone treatment with good response. She entered a complete remission after treatment following the Berlin-Frankfurt-Münster (BFM) ALL-2004 protocol for standard risk. During the treatment she had two severe episodes of infections: urinary Escherichia coli infection and a sepsis by *Pseudomonas* with a positive anti-ETV6 signal in a peripheral blood sample. She is presently out of treatment for more than 36 months with disease free-survival.

**Case 2.** A 5 year-old-boy, was hospitalized in June 2010, [10 months later than his sister’s diagnosis of c-ALL] due to intermittent fever and claudicating, for two weeks. Physical examinations demonstrated small cervical inguinal lymph nodes. Laboratorial analysis showed WBC of 17.7 × 10^9/L and 51.0% lymphoblast cells; Hg, 7.6 and platelet count, 5000/L. Central nervous system fluid (CSF) examination was normal. Bone marrow (BM) aspiration showed an infiltration by lymphoblasts of uniform size and high nuclear to cytoplasmic ratio (ALL-L1). Immunophenotyping and cytogenetic analysis was performed and a diagnosis of common-ALL (c-ALL) was made. The patient received one week oral prednisone treatment with good response. She entered a complete remission after treatment following the Berlin-Frankfurt-Münster (BFM) ALL-2004 protocol for standard risk. During the treatment she had two severe episodes of infections: urinary Escherichia coli infections and a sepsis by *Serratia liquefaciens*. She is presently out of treatment for more than 36 months with disease free-survival.

Treatment protocol

Both patients received the BFM-2004 ALL backbone protocol, in which, the patient receives oral prednisone (60 mg/m^2) on days 1–7 and, one dose of methotrexate (MTX) intrathecally (IT) on day 1. Both patients received vincristine and daunorubicin hydrochloride once weekly in weeks 2 and 5; asparaginase on days 12 and 33; cyclophosphamide on days 36 and 64; cytarabine in weeks 6 and 5; mercaptopurine on days 36 and 63; and MTX-IT on days 1, 12, 33, 45, and 59. Patients were tested whether they are good responders to prednisone according to the presence or absence of lymphoblasts in the peripheral blood count on day 8 of the treatment. Minimal residual disease (MRD) was tested by flow cytometry at days 15, 33, 78 and in the final week of the protocol treatment.

Leukemia cell characterization

Leukemia classification of BCP-ALL was based on criteria previously published by the European Group for the Immunological Characterization of Leukemias — EGIL by immunophenotyping [17]. IGH/TCR rearrangements were detected according to the BIOMED2 protocol [18]. The karyotype was tested in BM aspirates before any treatment. Chromosomes were identified and analyzed in accordance with the International System of Human Cytogenetic Nomenclature 2005 [19], ETV6-RUNX1 fusion gene was identified by interphase FISH dual color probes and by reverse transcriptase-polymerase chain reaction [20]. Histocompatibility leukocyte antigen (HLA) haplotype was assessed as described [21]. Copy number alterations of SHOX, CRFL2, IL3RA, IKZF1, CDKN2A/CDKN2B, PAX5, ETV6, BTG1 and RBL were analyzed by multiple ligation-dependent probe amplification (MLPA) with P335–A4 ALL–IKZF1 probe mixx [22]. The resulting IKZF1Δ4–7 cDNA PCR product was subjected to direct sequencing for confirmation.

PB samples from the parents and the two siblings at the time of the diagnosis were tested for SNPs in gene of the xenobiotic system (CYP1B1 rs1056836, CYP2A4 rs2740574, CYP2D6 rs776746, and GSTT1 and GSTM1 homozygous deletion, NAT1 rs15561, NQO1 rs1800566), folate metabolism (RFC1 rs1051266, MTHFR rs1801133, MTHFR rs1801131), as well as cell differentiation and suppressor genes (IKZF1 rs11978267, ARID5B rs10821936, ARID5B rs10594982, CEBPA rs2239633, TET3 rs17505102, PTPRJ rs33942852, CDKN2A/B rs17756311 and CDKN2A/B rs17964672) by different techniques [23–26,10–13].

The microsatellite mapping on 12p of all family members was performed by PCR using primers that flank the region of interest, (available upon request to A.M.F.) [27].

Complete details of all methodological protocols and conditions are described in Supplemental material.

Familial health, occupation and cancer history

Both parents are lawyers working full-time in court-offices. Regarding the health history on the mothers’ side of the family: Great-grandmother died of breast cancer aged 50 years-old; great-granduncle died of lung cancer at age 50 years-old; great-grandaunt had long standing Sézary syndrome. The grand-mother died of CNS tumor aged 70 years-old; on the fathers’ side of the family: one daughter from his first marriage had Down syndrome; one sister had melanoma, and a niece had an aggressive gastrointestinal tumor at age 38 years-old.

Ethics

Clinical and demography data collection was obtained with informed consent from the parents and in accordance with the Declaration of Helsinki. The study design and laboratory procedures were approved by the Research Ethics Committee, Instituto Nacional de Câncer (studies CEP #005/06 and #024/10).

Results

A summary of clinical and laboratorial main findings in both children is shown in Table 1. The time frame of diagnosis was 10 months apart from each other. They completed a four drug induction with prednisone as steroid of choice. In both children the lymphoblast count was less than 1000 lymphocyte/mm^3 at D8 of treatment; therefore they were classified as good responders. The MRD was performed by flow cytometry at D15 and D33 of the treatment and in Case 1 there was 0.02% of CD10/TdT^+ cells (2/10,000) whereas in Case 2 there was a persistence of 13% of blast cells at D15 characterized by CD34/CD19/CD79a/TdT+. ETV6-RUNX1 fusion gene has been detected in both children; The FISH results are shown in Fig. 1 (A and B). ETV6-RUNX1 positive signal was found in 16 and 21% of interphase nuclei for Cases 1 and 2, respectively. ETV6-RUNX1 positive signal was still present (15%) at relapse in Case 2. RT-PCR confirmed the presence of the ETV6-RUNX1 transcripts. Submicroscopic genetic alterations demonstrated by MLPA in samples at diagnosis, remission or relapse in both children are shown in Fig. 1. The results show that samples from Case 1 (at diagnosis and remission) were wild-type for all the evaluated loci. Case 2 at diagnosis presented...
BTG1 gene amplification. At relapse additional alterations were found: IKZF1, CSF2RA and IL3RA deletions and PAX5 amplification. Both samples of Case 2 (diagnosis and relapse) were subsequently validated by direct sequencing regarding IKZF1 deletion demonstrating that IKZF1 deletion 4–7 was found in both samples.

In order to test whether parental pattern of ETV6 was balanced, the microsatellite analysis on chromosome 12p was performed in the family settings. As shown in Fig. 2, the microsatellite analysis on 12p where ETV6 is located demonstrated that, despite being heterozygous for D12s1697, D12s89, D12s358 and D12s269, no deletions were found in Case 2 across the three samples (diagnosis, D33 and relapse). Deletions were observed in Case 1, D12s98, D12s358 and D12s269 being D12s89 as heterozygous. The mother is heterozygous for these loci while for the father the majority of the analysis was not informative. We cannot rule out micro-deletions between the chosen satellite markers in any case.

Due to the history of cancer in first-degree relatives, samples from the mother and father, and their children were also genotyped in order to evaluate the inherited genetic susceptibility according to SNPs in xenobiotics/detoxification genes, in folate metabolism genes, as well as, in cell cycle-differentiation and developmental genes that confer risk of BCP-ALL. The analyzed genes are listed in Fig. 3, showing that the sibs are absolutely identical in all 17 loci. They are wild-type for GSTM1, GSTT1, NQO1, TP53 and TP63; heterozygous or homozygous for the known genetic variants that confer increased risk of BCP-ALL.

Table 1
Main clinical and laboratorial features of both siblings at diagnosis.

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
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</thead>
<tbody>
<tr>
<td>Demography</td>
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<td></td>
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<tr>
<td>Skin color</td>
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<td>White</td>
</tr>
<tr>
<td>Gender</td>
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<td>Male</td>
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<tr>
<td>Birth order</td>
<td>2nd child</td>
<td>1st child</td>
</tr>
<tr>
<td>Date of birth</td>
<td>June 1st 2008</td>
<td>April 15th 2003</td>
</tr>
<tr>
<td>Date of diagnosis</td>
<td>August 4th 2009</td>
<td>June 17th 2010</td>
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<tr>
<td>Age at diagnosis (months)</td>
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<td>86 months</td>
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<tr>
<td>Clinical features</td>
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<td></td>
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<tr>
<td>WBC Count</td>
<td>$8.0 \times 10^3/$mL</td>
<td>$17.7 \times 10^3/$mL</td>
</tr>
<tr>
<td>MRD at Day 33</td>
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<td>Positive</td>
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<td>Protocol Treatment</td>
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<td>BFM ALL-2004</td>
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<td>TdT, CD22,CD79a, CD34, HLA DR, CD19, CD10</td>
</tr>
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<tr>
<td>Haplotype</td>
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<td>B/D; HLA-A<em>29.58; HLA-B</em>38.58; DRB1*13.13</td>
</tr>
<tr>
<td>RT-PCR (fusion genes)</td>
<td>ETV6-RUNX1</td>
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<tr>
<td>MLL-AFF1</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>FISH</td>
<td>ETV6-RUNXI σ</td>
<td>ETV6-RUNXI</td>
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<tr>
<td>TCR Clonality</td>
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<td>igh-Fr3, LJH</td>
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<tr>
<td>FLT3/ITK/PTPN11</td>
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<td>WT</td>
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<tr>
<td>Follow-up Status</td>
<td>Alive without disease 26 months from end of treatment</td>
<td>Relapsed at 18 months of treatment and died with leukemia</td>
</tr>
</tbody>
</table>

Abbreviations; MRD, minimal residual disease by flow cytometry; nt, not tested; WT, wild-type.

σ FISH performed in the 2nd sample after 4 weeks of treatment.

Fig. 1. ETV6-RUNXI fusion gene identified by interphase FISH dual color probes at diagnosis (Dx) and at relapse samples of (A) case 1 and (B) case 2; Multiplex ligation-dependent probe amplification (P335-A4 ALL-8KZ1 probemix) analysis of (A) case 1 and (B) case 2.
IKZF1, ARID5B, PTPRJ and CEBPE). The parents and the children harbored at least one copy of the variant allele in more than 60% of the genes.

Discussion

The present sibships with c-ALL ETV6-RUNX1 gene fusion can provide important findings into the natural history of childhood ALL. One cannot exclude that this event occurred by chance in these sibs. However, based on a registry of double-ALL siblings, only 11% of them would harbor the same driving leukemia aberration by chance assuming an overall frequency of 20% for ETV6-RUNX1, (calculated as, 0.2 * 0.2/(0.2 * 0.8 + 0.8 * 0.2 + 0.2 * 0.2)), as previously discussed by Schmiegelow et al. [15,28]. The age of the children at the diagnosis being less than one-year apart, and the differences on time to the clinical onset of the BCP-ALL with ETV6/RUNX1 fusion gene are also of biological interest. The ETV6/RUNX1 fusion has been demonstrated to occur during prenatal period of life and the latency period to leukemia development is usually with a typical peak between 2 and 5 years [1,2]. In the present setting, the first born sib (Case 2) was diagnosed with BCP-ALL after the birth of Case 1. We cannot rule out the possibility that the initiating leukemic cells have been transferred to the mother during pregnancy and later transferred to the fetus. Although we could obtain the Case 1 blood spot at birth, unfortunately, it was not possible to clone the ETV6-RUNX1 breakpoint in any of the samples.

Therefore, according to results obtained herein, important points deserve to be discussed: i) BCP-ALL cases with shared environment exposures and presenting with identical genetic susceptibility background; ii) the same disease (c-ALL ETV6-RUNX1) with the same treatment and health care but different outcomes.

Regarding the first point, some speculations about the combined effect of both environmental and genetic susceptibility factors have been investigated and consensual results in the epidemiologic associations are reported [29]. Because the siblings described herein have lived together since birth, whatever the environmental exposures have been, it is very likely that they were the same. We assume therefore that ETV6-RUNX1 translocations have been acquired in utero, however, with different time-points to the clinical onset of disease.

Genomic imprinting is important in the cancer predisposition syndrome, as has already been demonstrated in some pediatric malignancies (for instance, Wilms' tumor and retinoblastoma), in which there are evident deletion either due to uniparental disomy inheritance or to gene deletion during gametogenesis [30]. Therefore, we have hypothesized that chromosome 12p could be a fragile site in these siblings and involved in the susceptibility to acquire the ETV6-RUNX1 fusion gene. It is plausible to think that a predisposing event could facilitate the leukemia appearance in these cases. The 12p loss of heterozygosity (LOH) found in three different markers of Case 1 suggests that predisposition could exist. Unfortunately, our data do not provide such evidence toward a predisposition phenomenon. Similarly, the microsatellite association analysis was mainly unfavorable because the majority of the father’s markers were not informative and the maternal pattern was heterozygous without deletion.

Nevertheless, both mother and father share a family history of cancer with several cases of malignant disease. This familial history of cancer...
cancer is remarkable and a cancer predisposition syndrome could be hypothesized. We have previously shown that familial history of cancer in second-degree relatives (grandparents, uncles, cousins) conferred an increased risk (OR = 1.66; 95% CI 1.12–2.45) for BCP-ALL [16]. Also, recent studies regarding syndrome associated with childhood ALL support an increased susceptibility within families. For instance, a heterozygous germ line variant of PAOX has been found to be associated with the occurrence of BCP-ALL in families [31]. Another study concerning the genetic basis of hypodiploid BCP-ALL, showed that 91.2% of low-hypodiploid cases present alterations in TCF3 that are commonly found in non-tumor cells, suggesting that this is a predisposing event [32]. In the same context of predisposition, a striking finding in these patients is that they share the exact same SNP genotypes that have been associated with susceptibility risks. The calculated probability of occurrence of this absolute concordance is ~15 in 1,000,000 events. This low probability could in part explain the reasons for leukemia in siblings being so rare, but also constitutes evidence that inherited susceptibility has affected the risk of the BCP-ALL children studied here. Gene variants will have additive effects, including common allelic variants in IKZF1, ARID5B, CEBPE, and CDKN2A that have been repeatedly and significantly associated with BCP-ALL [10–13]. An individual inheriting one copy of a variant allele will have an approximately 2-fold increased risk, whereas someone who inherits all four variants in homozygous form would have an ~10-fold increased risk. Therefore, it seems that these sibs were at a remarkably increased risk compared to the twofold increased risk for developing ALL indicated by previously published cohort studies [15,33]. Regarding the second point, despite the fact that the sibs seemed to be typical ETV6-RUNX1 (considering their age, WBC, immunophenotype, response to prednisone), they have experienced divergent treatment outcomes; whereas the girl is still alive in complete remission, the boy in which additional somatic mutations in IKZF1, CSF2RA, IL3RA, PAOX and BGT1, quickly relapsed and died. Although some patients suffer very late relapses, this is not common in current ALL treatment protocols, which usually result in a very good outcome for ETV6-RUNX1 cases [34]. Therefore, Case 2 seemed to present a more aggressive disease (higher WBC and delIKZF1). The IKZF1 deletion has been consistently associated with increased risk of treatment failure and relapse in BCP-ALL [35]. Analysis of the biological material of such settings by genome-wide next generation sequencing tools would certainly add more informative findings in the dissection of the clonal composition.

Conclusion

This present study reports the unusual observation of two non-twin siblings, both diagnosed with BCP-ALL and ETV6-RUNX1 fusion genes and different clinical outcomes. A selected number of genetic loci were examined and some variations on somatic mutations in IKZF1, CSF2RA, IL3RA, BGT1 and PAOX were found in one child. Both children shared the exact same SNP genotypes associated with substance detoxification and susceptibility risks. Analysis of the biological material of these patients by genome-wide next generation sequencing tools would certainly add more informative findings in the dissection of the clonal composition of these siblings.

Acknowledgments

We are grateful to Alessandra Faro, Bruno Almeida Lopes, Bruno Alves Aguiar Gonçalves, Caroline Barbieri Bluncck, Ellen Oliveira, Juliana Montpellier, Tállita Meciany, and Thayana Conceição Barbosa for technical diagnostic support. We thank Prof Kjeld Schmiegelow and Prof. Joseph Wiemels for the helpful comments. Authors’ contributions: A.P.F.W. and I.C. provided biological material and clinical data of the patients, M.E., M.B.M., and A.F. performed genomic analysis. M.E., M.B.M., and A.F. contributed to drafting of the manuscript. M.S.P.O. contributed to the study design, wrote the manuscript with the critical analysis of the data.

M.S.P.O has been supported by CNPq Research Scholarships #302423/2010-9 and FAPEJ #E026/101.562/2010. M.E has been supported by the Brazilian Ministry of Health #201426451 through the Institutional Development Program Scholarship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bcmd.2014.07.011.

References


