

Frequency of the *ETV6-RUNX1*, *BCR-ABL1*, *TCF3-PBX1*, and *MLL-AFF1* fusion genes in Guatemalan pediatric acute lymphoblastic leukemia patients and their ethnic associations

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Fusion genes involved in acute lymphoblastic leukemia (ALL) occur mostly due to genetic and environmental factors, and only a limited number of studies have reported any ethnic influence. This study assesses whether an ethnic influence has an effect on the frequency of any of the four fusion genes: *BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, and *MLL-AFF1* found in ALL. To study this ethnic influence, mononuclear cells were obtained from bone marrow samples from 143 patients with ALL. We performed RNA extraction and reverse transcription, then assessed the quality of the cDNA by amplifying the *ABL1* control gene, and finally evaluated the presence of the four transcripts by multiplex polymerase chain reaction. We found 10 patients who had the *BCR-ABL1* fusion gene (7%); 3 patients (2%) were *TCF3-PBX1* positive; and 6 patients (4.5%) were *ETV6-RUNX1* positive. The incidence of this last fusion gene is quite low when compared to the values reported in most countries. The low incidence of the *ETV6-RUNX1* fusion gene found in Guatemala matches the incidence rates that have been reported in Spain and Indian Romani. Since it is known that an ethnic resemblance exists among these three populations, as shown by ancestral marker studies, the ALL data suggests an ethnic influence on the occurrence and frequency of this particular fusion gene.

Keywords Fusion genes, Guatemalan, populations, acute lymphoblastic leukemia, pediatric leukemia

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Chromosomal translocations in most leukemias result in fusion genes, which produce mRNAs that encode chimeric proteins with different structural and functional properties than the normal constitutional proteins. These chimeric proteins may activate transcription directly, without requiring the specific interaction of other proteins (1–3). Furthermore, the presence of such an alteration influences the type of therapy that the patient may receive.

The best-known case is that of the chimeric gene *BCR-ABL1* (Philadelphia chromosome), which results from the translocation t(9;22)(q34;q11.2). The genes involved in this translocation are the *ABL1* gene on chromosome 9 and the *BCR* gene on chromosome 22. The 5'portion of the *BCR* gene is fused to the 3'portion of the *ABL1* gene, resulting in the chimeric gene *BCR-ABL1* (4–9). Depending on the point of breakage of the translocation, four different transcripts may result, thus producing PCR products of somewhat different lengths. These transcripts are present in acute lymphoblastic leukemia (ALL), and historically this translocation has been associated with poor prognoses at all ages; however, the advent of tyrosine kinase inhibitors (e.g., imatinib, nilotinib, and dasatinib) has revolutionized the treatment of ALL *BCR-ABL1*-positive diseases. The

Received November 26, 2012; received in revised form May 21, 2013; accepted May 22, 2013.

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incidence of *BCR-ABL1* increases with age from 2% in children to 20% in adults (20–39 years) (4–10).

The translocation t(12;21)(p13;q22) results in the fusion gene *ETV6-RUNX1* (*TEL-AML1*, *ETV6-AML1*). This translocation is difficult to identify by conventional cytogenetics due to bone marrow chromosome morphology and resolution, and because the breakpoints are within light bands of similar size, but it is detectable by fluorescence in situ hybridization and reverse transcriptase-polymerase chain reaction (RT-PCR). These patients show an excellent response to chemotherapy and rapidly achieve a relapse-free remission. The *ETV6-RUNX1* fusion gene is the most prevalent translocation in pediatric ALL and has been reported at a 25% incidence in children 1–4 years old. This incidence decreases as age increases, reaching about 13% in children by 18 years of age (10–15).

The *MLL* gene is located on chromosome 11 in the q23 band. The incidence of the t(4;11) translocation involving the *MLL* gene is clearly influenced by age. In infants (0–1 years), it has been reported at a 50% frequency, whereas in children older than 1 year, the incidence is very low (2–3%). Patients with this genomic alteration (*MLL-AFF1* or *MLL-AF4*) have more aggressive disease and a higher probability of failure of standard chemotherapy protocols (10,16–19).

The t(1;19)(q23;p13.3) translocation forms a chimeric gene between the *TCF3* (*E2A*) gene located on chromosome 19 and the *PBX1* gene located on chromosome 1. Numerous studies have concluded that this alteration confers a poor prognosis, which can be mitigated by very aggressive chemotherapy. Interestingly, it is one of the few genetic abnormalities that does not appear to vary in frequency with age and is present in 3–5% of cases at all ages (10,20–23).

Generally, it has been found that these fusion genes are produced by genetic and environmental factors, and very little association between their presence and ethnicity has been reported. One of the few studies of ALL, in which an influence according to ethnicity was reported, was conducted in two populations: Hispanic and non-Hispanic Caucasians, in which Hispanics had an *ETV6-RUNX1* gene incidence of 13%, whereas in the non-Hispanic population the incidence was significantly greater at 24%. This study showed for the first time that an ethnic variation affected the presence of this particular marker (24).

The present study aimed to evaluate whether ethnicity influences the frequency of any of the four fusion genes studied in the Guatemalan ALL population. The incidence of fusion genes in these patients in Guatemala was determined and compared with incidence rates reported in other countries.

Materials and methods

The methodology used in this study was based on studies conducted by Cerveira et al., Pallisgaard et al., Scurto et al., Marin et al., and Carranza (25–30).

Patient samples

We collected 143 bone marrow samples from pediatric patients (0–18 years old) at diagnosis of ALL before any

therapeutic intervention. The samples were collected from patients referred from different national hospitals. Every patient signed the informed consent form approved by the local ethics committee. The technique used for extracting blasts from bone marrow was via the use of Ficoll (Ficoll-paque; GE Health Carre, Buckinghamshire, England), which allowed cell separation according to a density gradient. Blasts obtained by this technique were stored at -20°C .

RNA extraction and reverse transcription

The RNA was extracted with the RNAqueous kit (Life Technologies, Grand Island, NY), according to the manufacturer's instructions. For the synthesis of cDNA, 1 μg of RNA was used with the cDNA high capacity transcription kit (Life Technologies), with random hexamers in a 20 μl reaction volume under standard conditions.

cDNA quality

To verify the quality of the cDNA obtained, the *ABL1* control gene was amplified. The PCR conditions were standardized according to directions for the thermal cycler used. The reagents for the PCR procedure were obtained from Novagen (EMD Millipore, Darmstadt, Germany).

Multiplex PCR

Multiplex PCR was used to evaluate the transcripts *BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, and *MLL-AFF1*. The reaction was standardized according to the thermal cycler used. The primers used were described by Cerveira (29).

The PCR conditions included 6 minutes at 94°C , 40 cycles each of 1 minute at 94°C , 1 minute at 60°C , 1 minute at 72°C , and a final extension of 5 minutes at 72°C .

For the standardization of PCR, we used the K562 (*BCR-ABL1* positive), MV4–11 (*MLL-AF4* positive), and REH (*ETV6-RUNX1* positive) cell lines (ATCC, Manassas, VA). These cell lines were cultured and RNA was extracted. This RNA was used as a positive control to standardize the PCR conditions. To validate the technique, eight positive patients were sequenced by MacroGen (Rockville, MD).

Results

Initially, to evaluate cDNA quality and extraction efficiency, a PCR of the control gene *ABL1* was run on all analyzed samples (Figure 1A). A total of 143 samples were analyzed for the 4 transcripts: *BCR-ABL1*, *MLL-AFF1*, *TCF3-PBX1*, and *ETV6-RUNX1*.

As observed in Table 1, the *BCR-ABL1* transcript had the highest incidence, 7% of samples, which corresponds to the incidence rates reported in other countries. The *TCF3-PBX1* transcript was found in three patients, an incidence consistent with that reported in most countries (2–5%). The *MLL-AFF1* transcript incidence was 0%, which was similar to that described in other countries where the incidence in children was <2%.

On the other hand, we observed only a 4.5% incidence of the *ETV6-RUNX1* transcript. This is a low incidence, since in

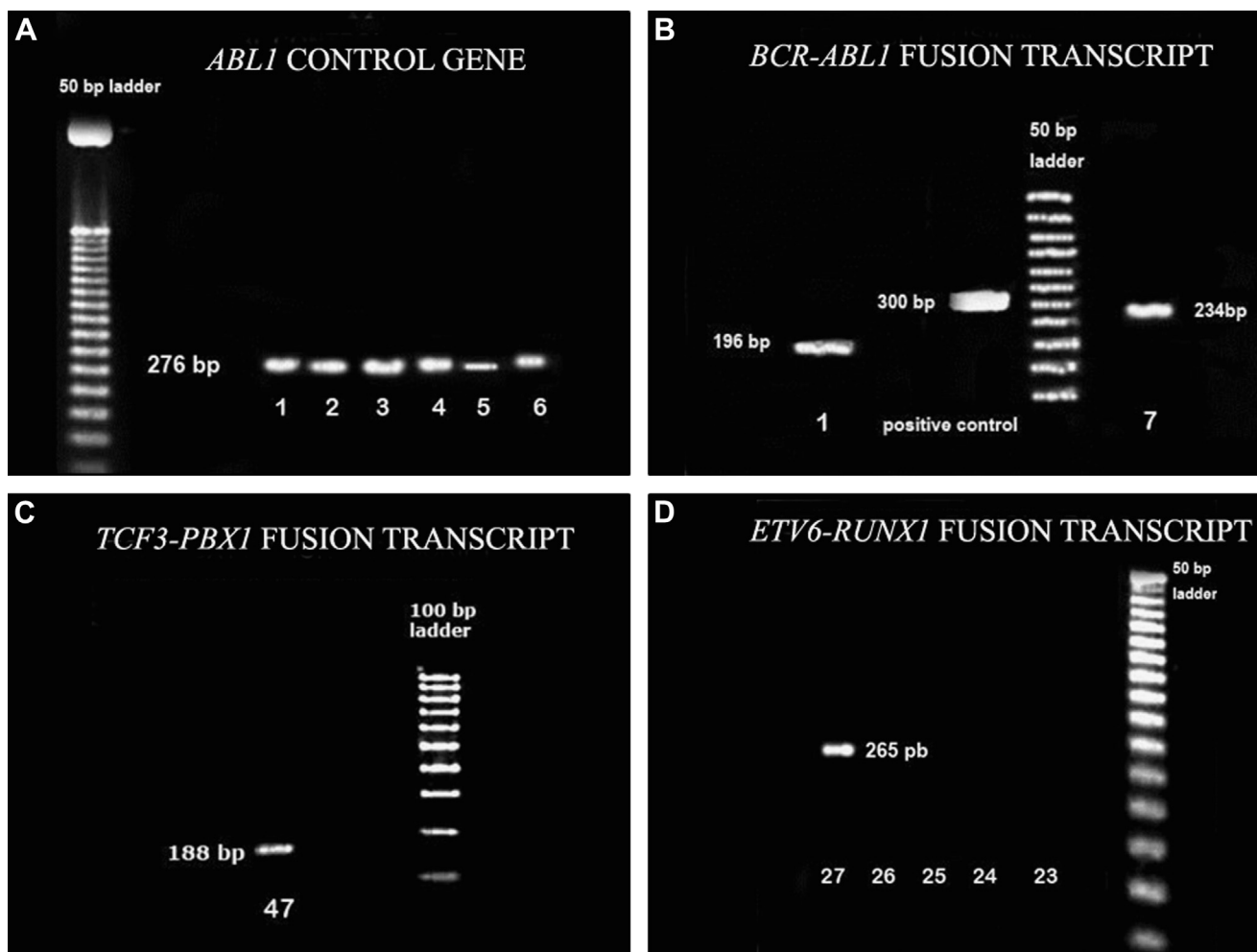


Figure 1 (A) The band at 276 bp confirms the amplification of the *ABL1* control gene and assures a good quality of cDNA. (B) Two patients positive for the *BCR-ABL1* transcript and the positive control. (C) A patient positive for the fusion gene *ETV6-RUNX1*. (D) A patient positive for the *TCF3-PBX1* fusion gene.

most countries the reported incidence is about 20%. This transcript is the only one for which the incidence differs from those reported internationally. Figure 1 shows examples of positive gene fusion patients.

To validate the standardized technique, eight samples from positive patients (one for *ETV6-RUNX1*, one for *TCF3-PBX1*, and six for the *BCR-ABL1* transcript) were sequenced and verified the presence of the fusion gene in question.

Because the incidence of three fusion genes included in the study (*ETV6-RUNX1*, *BCR-ABL1*, and *MLL-AFF1*) varies with age, Table 1 shows a distribution of the presence of the transcripts according to the different ages of children included in this study.

Discussion

Of 143 ALL patients analyzed, 10 patients were detected as positive for the *BCR-ABL1* transcript (7%), 6 patients were positive for the *ETV6-RUNX1* transcript (4.5%), and 3 patients were positive for the *TCF3-PBX1* transcript (2%). No patient had the *MLL-AFF1* transcript (Figure 1 and Table 1).

It has been reported that, in most countries, the incidence of the *ETV6-RUNX1* transcript decreases progressively (from approximately 25% to 13%) as age increases; however, in the present study, we found an overall low incidence of *ETV6-RUNX1* uninfluenced by age. The reported

Table 1 Age distributions of the patients and the relationships with the presence of transcripts

Age (yr)	Frequency	<i>ETV6-RUNX1</i>	<i>BCR-ABL1</i>	<i>TFC3-PBX1</i>	<i>MLL-AFF1</i>
0	9	0	0	0	0
1–4	33	1	2	0	0
5–9	35	2	4	2	0
10–14	38	1	1	1	0
15–19	28	1	3	0	0
Total	143	6	10	3	0

BCR-ABL1 incidence increases with age, with this increase being more marked among adults and older children. In the pediatric patients included in this study, no progressive age difference was found. For *MLL-AFF1*, the reported incidence is approximately 50% in patients younger than 1 year. Although the number of infants enrolled in this study is low (nine patients), no patient presented this alteration. Possibly, another *MLL* translocation could have been present. It should be noted that the population included in this study is small (143 patients), and this limits conclusions about the frequency of each of the studied translocations and the age influence (10).

In the present study, 10 patients were positive for the *BCR-ABL1* transcript. The percentage found in Guatemala (7%) was very similar to the incidence found in other developed and developing countries, where the childhood incidence was usually around 5% (4–10).

The *TCF3-PBX1* transcript, which comes from the translocation t(1;19), is associated with a poor prognosis, and more aggressive chemotherapy is highly recommended. The incidence of this transcript found in Guatemala was 2%, which does not differ significantly from the incidence rates reported in most developed and developing countries (up to 5%).

The *ETV6-RUNX1* transcript, which comes from the t(12;21), is an alteration associated with a good prognosis, and patients respond well to standard chemotherapy and have very low relapse rates. This transcript was found to have a 4.5% incidence in the Guatemalan population.

A number of countries have reported a high incidence of the fusion gene *ETV6-RUNX1*; among these are England, Australia, the United States, China, Taiwan, Hungary, Germany, Italy, and Brazil. On the other hand, a few countries have reported a low incidence of this fusion gene, including India and Spain (see Table 2) (31–48).

Our institution has participated in a series of research projects, where ancestry markers for different ethnic populations have been studied (coordinated by M. Seldin from the University of California) (24–26). In these studies, we identified a strong association between Guatemalan Mayans and East Asians (India), with a mean allele frequency

difference of 0.27. We also found that a strong genetic association existed between some European populations and Guatemalan Mayans, with a mean allele frequency difference of 0.30. Finally, it was found that a greater genetic association exists between the European and East Asian (i.e., India) populations. We found that the allele frequency median between the populations of Europe and India differed by only 0.08 (49–51).

Although the Mayan population has its own ancestral markers, it was found that it shares some ancestral markers with the populations of Europe and India. Historically, the Indian population (Romani) migrated to a large number of countries. The groups called Romani (or “Gypsies”) arrived in Spain in 1415. This may explain the association between markers shared by some European and Indian populations. In addition, the association among some Indian, Spanish, and Guatemalan populations may have its roots in the Spanish conquest (49–51).

This data was consistent with the incidence of *ETV6-RUNX1* among patients with ALL in the present study, in which the Guatemalan, Indian, and Spanish populations have significantly lower incidence rates than those reported in other countries (31–48).

Although ALL is considered to be up to 90% non-inherited but genomically acquired, there seems to be a pattern of genetic predisposition because the Spanish, Indian, Romani, and Guatemalan populations, which are known to share genomic similarities, share an extremely low *ETV6-RUNX1* fusion gene incidence.

Only one other study shows a possible difference in the presence of the fusion gene *ETV6-RUNX1* due to ethnicity (24). Two types of populations were studied (Hispanics and Caucasian non-Hispanics), and it was observed that the frequency of *ETV6-RUNX1* in Hispanics was 13%, whereas in non-Hispanics it was significantly higher at 24%. Although this study was conducted in the United States, it suggests that a lower incidence of this fusion gene exists in people with a Hispanic lineage, which would be consistent with what we found in Guatemalan patients.

In conclusion, the incidence rates for the fusion genes *BCR-ABL1* (7%), *TCF3-PBX1* (2%), and *MLL-AFF1* (0%) was similar to those reported in most countries. The low incidence of the *ETV6-RUNX1* gene (4.5%) found in Guatemala coincided with that of countries such as Spain and India, with which the Guatemalan Mayan population shares ancestral markers, suggesting an ethnic influence on the frequency of this fusion gene.

Acknowledgment

Support for this research was provided by The National Fund for Science and Technology (FODECYT), National Secretary of Science and Technology (SENACYT) (grant FODECYT 48-2009), and Rozas-Botran Foundation, Guatemala.

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Table 2 Incidence of the *ETV6-RUNX1* transcript in all patients from various countries

Country	No. of patients	Incidence (%)	Reference
Spain	41	2	(52)
Guatemala	143	4.5	This study
India	42	4.8	(32)
India	259	7	(33)
India	46	9	(34)
Japan	74	9.5	(35)
Scotland	36	11.1	(36)
Switzerland	72	15	(37)
Hong Kong	75	16	(38)
Taiwan	41	17	(53)
United States	86	17	(40)
Brazil	67	17.9	(41)
Germany, Italy	334	18.9	(42)
Australia	66	33	(43)
England	56	39	(44)

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