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PRELIMINARY REPORT

Copy Number Alterations Associated with Acute Lymphoblastic Leukemia in Mexican Children. A report from The Mexican Inter-Institutional Group for the identification of the causes of childhood leukemia

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B-cell precursor acute lymphocytic leukemia (B-ALL) represents a worldwide public health issue. Particularly, Mexico is one of the countries with the highest incidence of ALL in children. Between the multiple factors involved in ALL etiology, genetic alterations are clearly one of the most relevant features. In this work, a group of 24 B-ALL patients, all negative for the four most frequent gene fusions (*ETV6-RUNX1, BCR-ABL1, TCF3-PBX1* and *MLL-AF4*), were included in a high-resolution microarray analysis in order to evaluate genomic copy-number alterations (CNAs). The results of this preliminary report showed a broad genomic heterogeneity among the studied samples; 58% of the patients were hyperdiploid and 33% displayed a chromosome 9p deletion of variable length affecting genes *CDKN2A/B*, two patients displayed genomic instability with a high number of focal CNAs, three patients presented unique duplications affecting 2q, 12p and 1q, respectively, and one patient displayed no copy number imbalances. The copy-number profile of 44 genes previously related to B-ALL was heterogeneous as well. Overall results highlight the need for a detailed description of the genetic alterations in ALL cancer cells in order to understand the molecular pathogenesis of the disease and

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to identify any prognostic markers with clinical significance.

Key Words: Acute lymphoblastic leukemia, Copy number alterations, Molecular cytogenetics, genomic instability, Copy number imbalance.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common cancer in children worldwide. Mexico is one of the countries with the highest incidence and mortality rate (1). Multiple environmental, socioeconomic and biological factors are involved in the etiology and course of this disease; however, as for any other neoplasm, there is a significant genetic component revealed through multiple studies that encompass evidences from ethnicity disparities (2) to the characterization of specific variants linked to an increased risk. One of the most common approaches for the study of leukemia is cytogenetic analysis, which enables the detection of gross chromosomal alterations and, more recently, the introduction of genomic technologies; the analysis with genomic microarrays has revealed the presence of copy number alterations (CNAs), either gains or losses, with higher resolution.

Previous studies revealed that aneuploidies are a common feature in B-cell precursor ALL in children (B-ALL) (3,4), including hyperdiploidy (>50 chromosomes) as well as hypodiploidy (<44 chromosomes). It should be pointed out that the presence of common translocations resulting from gene fusions and finally the results from GWAs studies that show the association of genetic variants linked to a higher risk, which reflects the role of the involved genes in the carcinogenesis process as is the case of *ARID5B*, *IKZF1*, *CEBPE*, and *CDKN2A* (5,6). In Mexico, multiple studies have been performed under a classical cytogenetic approach with samples from children with ALL as well as the directed detection of the most common rearrangement through PCR (7). Interestingly, children with ALL in Mexico had a low frequency of identification of the main rearrangement (19%) compared to the findings in the U.S. (32%) (7). In this preliminary report we describe the findings from the analysis of 24 Mexican children with B-ALL by high-density genomic microarrays. As in other populations we coincide with the finding of hyperdiploidy in most cases, but we also detected particularly large as well as focal alterations, which contain genes with possible clinical significance to be further analyzed.

Materials and Methods

Patients

The Mexican Inter-Institutional Group for the Identification of the Causes of Childhood Leukemia (MIGICCL) is carrying out a prospective study of newly diagnosed ALL patients <19 years of age between August 1, 2015 and December 31, 2016 in eight public hospitals in Mexico City: *Hospital de Pediatría*, *Centro Médico Nacional (CMN) Siglo XXI*, *Hospital General Gaudencio González Garza, CMN La Raza* and *Hospital General Regional Carlos McGregor Sánchez Navarro* of the *Instituto Mexicano del Seguro Social (IMSS)*; *Hospital Infantil de México Federico Gómez*, *Hospital General de México* and *Hospital Juárez de México* (*Secretaría de Salud*, SSA); *Hospital Pediátrico de Moctezuma* (*Secretaria de Salud del Distrito Federal*, SSDF); and *Hospital CMN 20 de Noviembre* (*Instituto de Seguridad Social al Servicio de los Trabajadores del Estado, ISSSTE)*. A total of 24 patients included by group were selected in this study. These patients met the following conditions: acute lymphoblastic leukemia diagnosed by bone marrow morphology and immunophenotyping, a standard risk classification (8) according to the National Cancer Institute (ages ranging from 1 to 9.99 years and initial white blood cell count <50 000/µl) and negative for the gene fusions *ETV6-RUNX1, BCR-ABL1, TCF3-PBX*. All parents of patients signed informed consent and the Ethics Board of the Instituto Mexicano del Seguro Social and of each institution approved this study.

Affymetrix CytoScan HD Array and Copy Number Analysis

Genomic DNA was extracted from the mononuclear cell fraction isolated from bone marrow aspirate samples to perform high-resolution genome-wide DNA copy number analysis, with Affymetrix CytoScan HD Arrays (Santa Clara, CA), according to the manufacturer's protocol. CNAs were classified as large-scale genome imbalances including whole chromosome, chromosome arm, and partial aneuploidies (>10 Mb), which were analyzed separately; or focal CNAs (>100 Kb <10 Mb) that are not located in polymorphic CNAs regions (<80% overlap).

Copy-number Profiling of ALL-associated Genes

Genes previously associated with ALL were compiled from the literature and their genomic coordinates were used as overlap mapping in ChAS to determine their copy-number status. Heatmaps were generated using Cluster 3.0 (9) and JavaTreeView (10).

Results

A total of 24 patients diagnosed with B-ALL were included in this study. The patient samples displayed genomic imbalances affecting widely variable extents of the genome (Figure 1). The mean of detected focal CNAs per patient