the $^{A}\gamma$ chain (Figure 1A). This mutation results in the lack of the Eco RI restriction site, normally occurring at this codon (Figure 2A). Familial analysis revealed that the mother was also a carrier. This is the third description of an alteration at codon 121 of the $^{A}\gamma$ gene; the other two previously described variants are Hb F-Siena ($^{A}\gamma^{T}$ 121 Glu→Lys) and Hb F-Hull ($^{A}\gamma$121 Glu→Lys), detected in Italians and English babies, respectively. Both these variations are clinically silent.[5,6] Hemoglobin F-Campinas is the $^{A}\gamma$ counterpart of Hb D-Punjab (β121 Glu→Gln), which co-polymerizes with Hb S but has no clinical consequences in the presence of Hb A[1].

Hemoglobin F-Paulinia is due to a base substitution at codon 80 (<u>G</u>AT→<u>T</u>AT) of the $^{G}\gamma$ gene (Figure 1B), causing the replacement of aspartic acid by tyrosine. This substitution was confirmed by MboI digestion (the site normally present was abolished) (Figure 2B). The carrier's father was also a heterozygote. This is the second description of mutation at codon 80 of the $^{G}\gamma$ gene; the previously described variant, Hb F-Marietta ($^{G}\gamma$ 80 Asp→Asn), was identified in a healthy Caucasian neonate.[7] In the β-globin, there is only one described variant with a replacement at this position, normally occupied by asparagine, Hb Szuhu (β80 Asn→Lys). Although the replacement is located on the 2,3 DPG binding site, functional studies and the clinical presentation of the heterozygotes were normal.[8]

Hemoglobin F-Joanópolis is the result of an alteration at codon 73 (G<u>A</u>T→G<u>C</u>T) of the $^{G}\gamma$ gene (Figure 1C), causing the replacement of aspartic acid by alanine in the corresponding chain; the mutation was confirmed by analysis of the family (the mother was also a carrier). This is the first description of mutation at codon 73 of the $^{G}\gamma$ gene. Other substitutions at residue 73 that have been described in the $^{A}\gamma$ chain are Hb F-Xin-Su [$^{A}\gamma^{I}$ 73 (E17) Asp→His] and Hb F-Forest Park [$^{A}\gamma^{T}$ (E17) Asp→Asn], found in a healthy Chinese neonate and in normal Caucasian babies, respectively.[9,10] Hemoglobin F-Joanopolis has four analogous mutations in the β-globin gene: Hb Korle-Bu (β 73 Asp→Asn), Hb Vancouver (β 73 Asp→Tyr), Hb Tilburg (β 73 Asp→Gly) and Hb Mobile (β 73 Asp→Val), all with reduced O_2 affinity, but none associated with clinical manifestations in heterozygotes.[8]

The carriers of the three variants described here did not show any clinical abnormalities, and their hematologic data were all normal, suggesting that the residue replacements did not compromise the stability or the function of the molecule. However, as functional studies could not be performed, the apparent normality may also be because the low proportion of the variant in the total Hb was insufficient to cause significant alterations.

Regarding globin-chain synthesis, these mutations seem not modify the expression of the affected γ-genes, since Hb F levels below were below 1% in all the adult family carriers.

Denise Faustino Duarte, Dulcinéia Martins Albuquerque,° Vitoria Regia Pereira Pinheiro ,# Fernando Ferreira Costa,° Maria de Fátima Sonati**

**Dept. of Clinical Pathology and °Dept. of Clinical Medicine, School of Medical Sciences, State University of Campinas, UNICAMP; #Integrated Center for Childhood Oncology-Hematology Investigation, CIPOI, State University of Campinas, UNICAMP, Brazil*

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Correspondence: Dr. Maria de Fátima Sonati, Department of Clinical Pathology, School of Medical Sciences, State University of Campinas, UNICAMP, P.O.Box 6111, Zip Code 13083-970 , Campinas, São Paulo, Brazil. Phone: international +55.19.3788-9453. Fax: international +55.19.37889434. E-mail: sonati@fcm.unicamp.br

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Cell lineage assignment of cytogenetic findings in acute lymphoblastic leukemia using combined immunomagnetic cell separation and chromosome preparation

In acute lymphoblastic leukemia (ALL) abnormal karyotypes frequently constitute a minor part of the dividing cells, and the origin of metaphases in normal diploid cases remains obscure. We used a combination of immunomagnetic cell separation and chromosome preparation (ICSCP) to focus on the metaphases of interest and to assign the chromosome findings to CD19+ or CD7+ leukemia cells.

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In order to select CD19+ cells, we followed the manufacturers' instructions to prepare immunomagnetic microspheres coated with a monoclonal anti-CD19 antibody (clone AB-1; Dynabeads®, M-450 CD19, DYNAL, Hamburg, Germany). For

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Table 1. Overall results of the chromosome banding analyses of leukemia cells which were positively selected for CD19 and/or CD7 expression by immunomagnetic beads after short-term culture.

			Leukemia cell antigen expression (%)					*Number of metaphases showing the respective karyotype*			
No./ age	*Stage of disease&*	*Sub-type§*	*CD19+*	*CD7+*	*Karyotype(s) [n. of metaphases]*	*Sample#*	*Cultivation time (h)*	*CD19+ fraction*	*CD7+ fraction*	*Super-natant*	*Unselected culture*
1/46	PD	pro-B	nd	nd	46,XX [10] /46,XX,t(4;11)(q21;q23) [2]	BM	24	–	–	6/0	4/2
2/48	PD	pro-B	97	3	46,XY [2] /46,XY,t(4;11)(q21;q23) [6]	BM	24	–	–	–	2/6
3/24	PD	pro-B	86	10	46,XY [1] /46,XY,t(4;11)(q21;q23) [16]/ 47,XY,t(4;11)(q21;q23), +der(4)t(4;11) [32]	BM	24 0/2/0	nd	1/4/17	0/10/15	
4/27	PD	pro-B	90	nd	46,XY [26]/46,XY,t(12;22)(p13;q11) [50]	BM	24	1/19	nd	14/9	11/22
5/27	PD	C	96	nd	46,XX [28]	BM	24	–	nd	20	8
6/63	PD	C	93	6	46,XX [2]	BM	24 48	– –	– –	– –	1 1
7/49	PD	C	92	3	46,XX [17] /46,XX,t(9;22)(q34;q11) [6]	BM	4	0/1	nd	9/2	8/3
8/23	R	C	90	4	46,XX [7] /46,X,del(X)(q24), del(9)(p21), -9, +mar [14]	BM	24	0/9	–	4/2	3/3
9/61	PD	C	93	2	47,XX,+4,+5,dic(8;9)(p11;p13), t(9;22)(q34;q11)[7]	BM	24	2	–	3	2
10/62	R	C	70	20	46,XY [16] /46,XY,del(1)(q32),i(8)(q10),-9, -10, der(22)t(9;22)(q34;q11),+2mar [17]	BM	24 48	– 0/2	– –	1/4 1/1	6/7 8/3
11/43	R	C	97	4	46,XY [17] /47,XY,+1,del(1)(p13),-9, add(15)(q24), der(22) t(9;22) (q34;q11), +der(22)t(9;22) [26]	BM	24 48	0/9 2/7	– –	6/0 6/1	1/6 2/3
12/58	PD	C	nd	nd	46,XX [2] /55,XX,+X,+4,+8,+10, +11,+14, +18,+21,+21 [73]	BM	24 48	0/4 1/10	– –	– 0/10	0/28 1/21
13/44	PD	pre-B	97	1	46,XY,t(4;11)(q21;q23) [14]	BM	24	3	nd	–	11
14/43	PD	pre-B	89	3	46,XX [3]/47,XX,+8,t(9;22)(q34;q11), del(9)(p21) [18]/50,XX,+X,+8, t(9;22)(q34;q11), +22, der(22)t(9;22) [8]	BM	24 48	– 0/11/5	– –	2/0/0 0/1/1	– 1/6/2
15/58	PD	pre-B	93	5	45,XX,-7,t(9;22)(q34;q11) [12]/45, XX,-7, t(9;22)(q34;q11),add(6)(q23-25) [15]	BM	24	6/5	–	5/8	1/2
16/20	PD	B	70	20	46,XY [21]	BM	24	–	–	10	11
17/49	PD	pre-T	2	98	46,XX [11]	BM	24	–	3	1	7
18/28	R	T	nd	45	46,XX [49]	pB	48	nd	27	nd	22
19/16	PD	T	0	97	46,XY [65]	BM	24	–	25	15	25
20/40	PD	T	1	85	46,XX [3] /50,XX,+mar [inc] [11]	BM	24	–	0/2	1/3	2/6
21/66	PD	T	2	96	45,XY,t(1;7)(q23;p15),BM dic(8;12)(p11;p11), del(20)(p11) [12]	24	–	4	–	8	
22/34	PD	AML, M0	1	88	46,XY [5] /46,Y,t(X;9)(p11;p24), t(1;12)(p34;p11), del(12)(p11-12) [19]	BM	24 48	– –	– 0/5	– 0/5	4/7 1/9
23/35	PD	AML, M1	0	0	97,XXYY,+5,+13,+13,+19,+19 [28]	BM	24 48	– –	– –	17 4	11 5
24/20	PD	AML, M4Eo	2	5	46,XY [22] /46,XY,inv(16)(p13q22) [46]	BM	24 48	– –	– –	1/8 11/14	1/8 9/16

&PD: primary diagnosis; R: relapse; §pro-B: pro-B-ALL; C: common-ALL; pre-B: pre-B-ALL; B: mature B-ALL; pre-T: pre-T-ALL; T : T-ALL; #BM: bone marrow; pB: peripheral blood; $-: no metaphases; nd; not done; numbers are separated by a slash and in the same order as written in the column of the karyotypes.

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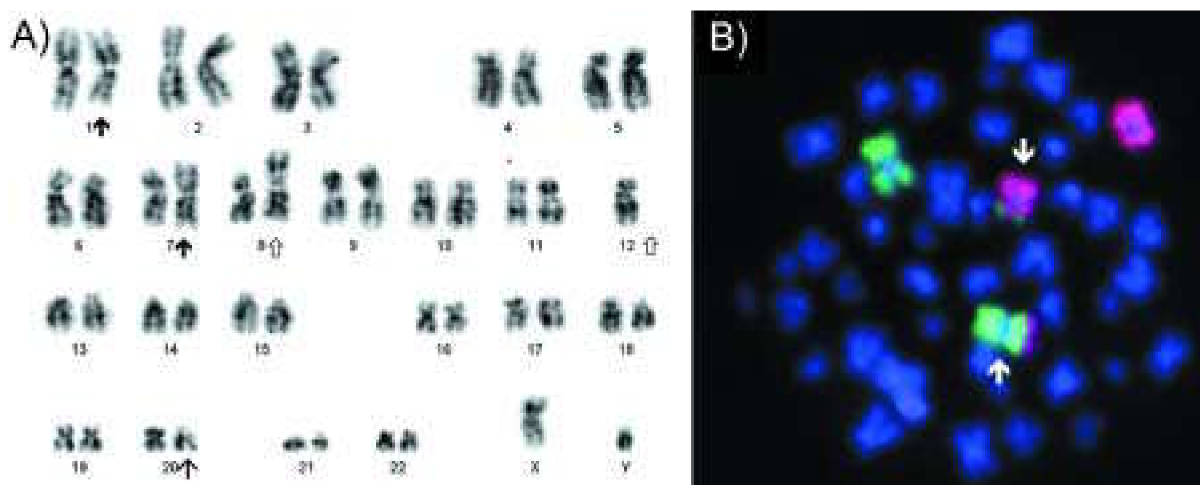

Figure 1. Cytogenetic findings using combined immunomagnetic cell separation and chromosome preparation. A) G-banded karyotype of a bone marrow metaphase of a CD7$^+$ selected cell from case n. 21 with T-ALL showing a translocation t(1;7)(q23;p15) (bold arrows), a dicentric translocation dic(8;12)(p11;p11) (outlined arrows), and a deletion of the short arm of a chromosome 20 (arrow). B) Bone marrow metaphase of case n. 19 with seemingly normal karyotypes in CD7$^+$ selected cells by G-banding analysis; molecular cytogenetic investigations including whole chromosome probes for chromosome 1 (green) and 7 (red) revealed a hidden translocation t(1;7)(p3?4;p1?5) (bold arrows).

the CD7$^+$ cell isolation we used Fc-specific human anti-pan mouse IgG coated immunomagnetic beads (CELLection™, Pan mouse IgG kit, DYNAL) coupled with anti-CD7 monoclonal antibody (clone 8H8.1, Beckman Coulter, Krefeld, Germany). Heparinized bone marrow or blood samples were cultured at a density of 0.5×10^6 nucleated cells/mL as described elsewhere.[1] Positive cell selection using a concentration of 3.2×10^6 beads/mL was performed essentially as recommended by the manufacturers with the exception that 1h prior to start of the separation procedure 0.02 μg/mL colchemide was added to the cultures. Immunomagnetic particles were removed from CD19$^+$ cells using DETACHaBEAD® (DYNAL), and from the CD7$^+$ cells by DNase. Unselected cultures were used as controls. Chromosome preparations, G-banding, fluorescence *in situ* hybridization (FISH), multicolor karyotyping and immunophenotyping were performed as previously described.[2-4] Chromosome findings from unselected cultures were not known prior to the analysis of the chromosomes of the positive selected fractions.

Twenty-four patients with acute leukemia were investigated, 20 at primary diagnosis and 4 at relapse (Table 1). The abnormal metaphases were enriched from 68.9% (84/122 metaphases) in unselected cultures to 95.2% (80/84 metaphases) in the positive selected cell fractions. Of 11 cases with a mosaicism of abnormal and normal karyotypes in unselected cultures, 8 showed only abnormal metaphases in the positive selected fractions, and 3 cases had 78-91% abnormal metaphases (cases 4, 11, 12). This demonstrates that ICSCP helps to focus cytogenetic analysis in ALL blast chromosomes by enriching the leukemia cell metaphases (Figure 1A). In none of three cases with B-lineage ALL and normal karyotypes were metaphases present in the CD19$^+$ or CD7$^+$ selected fraction (cases 5, 6, and 16) which suggests that the normal karyotypes were not derived from the leukemic cell population. This is in accordance with previous findings in a case with ALL using the morphology-antibody-chromosomes (MAC) technique, in which normal bone marrow metaphases belonged to the granulocytic and/or erythroid cell compartment.[5] However, it cannot be ruled out that normal diploid leukemic metaphase cells were missed by the separation procedure. In contrast, only normal diploid cells were found in CD7$^+$ selected fractions of 3 patients with T-lineage ALL suggesting that the metaphases represented the leukemic clone. Multicolor karyotyping was performed in all three cases and revealed a hidden translocation t(1;7)(p3?4;p1?5) in case n. 19 which was subsequently confirmed by FISH with subtelomeric and whole chromosome probes in a total of 9 metaphases (Figure 1B). Thus, ICSCP may help to focus on leukemic metaphase cells for well directed use of molecular cytogenetic methods for the detection of new cryptic chromosome rearrangements. Chromosome abnormalities were missed in 3 out of 22 cultures used for cell selection. In one of these cases the abnormal metaphases were detected in the 48h selection culture (case n. 10). Both remaining cases showed a t(4;11)(q21;q23) and normal diploid metaphases in unselected cultures. In the cultures used for cell separation, one case lacked metaphases completely (case n. 2) and the other showed only normal chromosomes in the supernatant (case n. 1). The presence of interphase nuclei in the positive selected fractions in both cases indicated that reduced mitotic activity of the blast cells and/or loss of the leukemic metaphase cells during the separation procedure may have caused the failure to detect t(4;11). Prolongation of the colchemide exposure may help to improve the yield of metaphases in the positively selected cells. Whereas all CD19$^+$ cases were diagnosed as B-lineage ALL, the CD7$^+$ cases were T-lineage ALL except in one patient with AML, M0, and 88% CD7 expression as revealed by flow cytometry. CD7 expression is found in 16-25% of cases of AML so that ICSCP could also be used to identify leukemic metaphase cells in CD7$^+$ AML.[6-9] In summary, the ICSCP described here for the first time combines chromosome banding and immunophenotype in one simple procedure which is suitable for routine cytogenetic analysis in ALL.

Harald Rieder, Ina Bachmann,* Sylke Busch,* Stefan Schwartz,° Barbara Reckzeh,§ Dieter Hoelzer,&,#*

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*Institute of Clinical Genetics, Philipps-University, Marburg, Germany; °Department of Internal Medicine, Hematology, Oncology, and Immunology, Benjamin Franklin University Hospital, Free University, Berlin, Germany; §Department of Hematology, Oncology, and Immunology, Philipps-University, Marburg, Germany; &Department of Hematology, Oncology, and Immunology, Internal Medicine III, Johann Wolfgang Goethe-University, Frankfurt, Germany; #the German Multicenter Adult Acute Lymphoblastic Leukemia Study Group GMALL

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Correspondence: Harald Rieder, Institut fuer Klinische Genetik, Klinikum der Philipps-Universitaet, Bahnhofstr. 7, D-35033 Marburg, Germany. Phone: international +49.64212862986. Fax: international + 49.642128 63984. E-mail: riederh@mailer.uni-marburg.de

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