# Association of prognostic parameters with cytogenetic and molecular markers at presentation in Indian children with high risk acute lymphoblastic leukemia

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## Introduction

- **Acute lymphoblastic leukemia is the commonest leukemia in children. The relapse rate continues to be high in developing countries.**
- Common genetic alterations in ALL include the genes with key roles in
- lymphoid development and differentiation (eg, PAX5, IZKF1, EBF1, and LMO2)
- cell-cycle regulation and tumor suppression (CDKN2A/CDKN2B, PTEN, and RB1)
- lymphoid signaling (BTLA, CD200, TOX, and the glucocorticoid receptor NR3C1)
- transcriptional regulation and co-activation (TBL1XR1, ETV6, and ERG). (mullighan 2012)
- **There are very few studies suggesting the role of above genes in ALL from developing countries.**

**Table 1: prevalence of gene deletions (adult ALL)**

| Gene | Frequency (UK study;Moorman et al) | Frequency (Indian study (SK Gupta et al) |
|---|---|---|
| IKZF1 | 14% | 26.5% |
| CDKN2A | 28% | 34% |
| PAX5 | 19% | 31.5% |
| ETV6 | 22% | 14.8% |
| EBF1 | 2% | 3.1% |
| RB1 | 6% | 9.3% |
| BTG1 | 6% | 11.7% |
| PAR1 | 4% | 4.3% |

**Table 2: Prevalence gene deletion in India (adult ALL)**

## Aim

- **To find out the prevalence and association of cytogenetic and molecular markers.**

## Materials & Methods

### Patient selection

**Inclusion criteria:**

- **Children of 1-14 years of age**

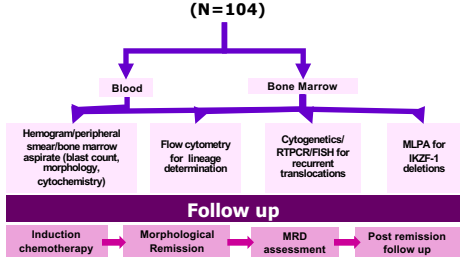
**Exclusion criteria:**

- **ALL patients with cardiac, endocrine, autoimmune, neurological disease, infections or any other malignancy**

**Place of study:**

- **Patient recruitment- Pediatric oncology clinic and Pediatric Medical Oncology Clinic, Dr BRAIRCH, AIIMS, New Delhi**
- **Ethical clearance- Ethics subcommittee, AIIMS, New Delhi**

### Clinical history and Examination




### Methods

- **Multiplex ligation dependent probe amplification (MLPA)**
- **SALSA MLPA probemix P-335-B2 ALL-IKZF-1, MRC-Holland, Amsterdam, NL**
- **Capillary electrophoresis - Done on ABI3130, Applied Biosystems**
- **Fragment analysis – Genemapper ver 4.0/ coffalyser software**
- **Gene expression analysis of CRLF2 gene was done by relative quantification using Taqman gene expression assay**

## Results

### MLPA Ratio chart

Fig 1: Normal (control)

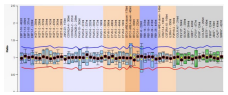


Fig 2: IKZF1, CDKN2A/B and PAX5 deletions

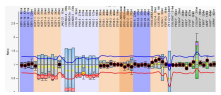


Fig 3: IKZF1, PAX5 and ETV6 deletions

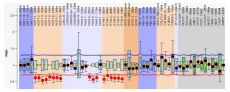


Fig 4: CDKN2A/B deletions

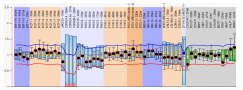
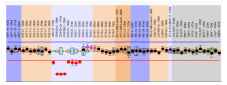


Fig 5: CDKN2A/B and PAX5 deletions



### Table 4: Association of genes with clinical and hematological parameters

| | IKZF1 deletions + N(%) | IKZF1 deletions- N(%) | P value | CDKN2A deletions + N(%) | CDKN2A deletions- N(%) | P value N(%) | PAX5 deletions + N(%) | PAX5 deletions – N(%) | P value N(%) | ETV 6+ N(%) | ETV6 – N(%) | P value N(%) | EBF1- N(%) | EBF+ N(%) | P value N(%) | RB+ N(%) | RB- N(%) | P value N(%) | PAR gene del + N(%) | PAR gene del – N(%) | p value N(%) | BTG gene del + N(%) | BTG gene del – N(%) | p value N(%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age (years)** | | | 0.2 | | | | | | 1.0 | | | 1.0 | | | 0.38 | | | 0.3 | | | 1.0 | | | |
| **1-10** | 6(60) | 76 (80.9) | | 12 (14.6) | 70 (85.4) | **0.001** | 10(12.2) | 72(87.8) | | 9(11) | 73(89) | | 81 (98.7) | 1(1.2) | | 3(3.7) | 79 (96.3) | | 13(15.9) | 69 (84.2) | | 2(2.4) | 80(97.6) | 0.2 |
| **10-14** | 4(40) | 18 (19.2) | | 11 (50.0) | 11 (50.0) | | 2(9.1) | 20(90.9) | | 2(9.1) | 20(90.9) | | 21 (95.4) | 1(4.5) | | 2(9.1) | 20(90.9) | | 3(13.6) | 19 (86.4) | | 2 (9.1) | 20(90.9) | |
| **Gender** | | | 0.72 | | | 0.6 | | | 0.87 | | | 0.48 | | | | | | 1.0 | | | | | | 1.0 |
| **F** | 2(20) | 26 (27.7) | | 5 (17.9) | 23 (82.1) | | 3(10.7) | 25(89.3) | | 4(14.3) | 24(85.7) | | 27 (96.4) | 1 (3.5) | | 1(3.6) | 27 (96.4) | | 6(21.4) | 22 (78.6) | 0.3 | 1 (3.5) | 27 (96.4) | |
| **M** | 8 (80) | 68 (72.3) | | 18 (23.7) | 58 (76.3) | | 9 (11.8) | 67 (88.2) | | 7(9.21) | 69(90.8) | | 75 (98.6) | 1(1.3) | | 4 (5.3) | 72(94.4) | | 10 (13.2) | 66 (86.8) | | 3 (3.9) | 73 (96.1) | |
| **NCI** | | | 0.09 | | | | | | 0.96 | | | 0.8 | | | 0.17 | | | 0.16 | | | 0.4 | | | 0.3 |
| **HR** | 7 (70) | 37(39.4) | | 17 (38.6) | 27 (61.4) | **0.001** | 5(11.4) | 39 (88.6) | | 5(11.4) | 39 (88.6) | | 42(41.1) | 2(4.5) | | 4 (9.1) | 40(90.9) | | 5(11.4) | 39 (88.6) | | 3 (6.8) | 41 (93.2) | |
| **SR** | 3(30) | 57 (60.6) | | 6 (10.0) | 54 (90.0) | | 7 (11.7) | 53 (88.3) | | 6(10) | 54(90) | | 60(100) | 0 | | 1(1.7) | 59(98.3) | | 11 (18.3) | 49 (81.7) | | 1 (1.7) | 59 (98.3) | |
| **Immunophenotype** | | | | | | 0.16 | | | 1.0 | | | | | | **0.015** | | | 0.5 | | | | | | 1.0 |
| **B-ALL** | 10 (100) | 81(86.2) | 0.36 | 18 (19.8) | 73 (80.2) | | 11(12.1) | 80 (87.9) | | 10(11) | 81(89) | 1.0 | 91(100) | 0(0.0) | | 4(4.4) | 87(95.6) | | 15 (16.5) | 76 (83.5) | 0.7 | 4 (4.4) | 87(95.6) | |
| **T-ALL** | 0 | 13 (13.8) | | 5 (38.5) | 8 (61.5) | | 1(7.7) | 12(92.5) | | 1(7.7) | 12(92.3) | | 11(84.6) | 2(15.4) | | 1(7.7) | 12 (92.3) | | 1(7.7) | 12 (92.3) | | 0 | 13 (100) | |
| **TLC** | | | **0.03** | | | **0.036** | | | | | | | | | 0.5 | | | 0.6 | | | 0.4 | | | 0.6 |
| **<50,000** | 4 (40) | 70 (74.5) | | 12 (16.2) | 62 (83.8) | | 9(12.2) | 65 (87.8) | 1.0 | 7(9.5) | 67(90.5) | 0.7 | 73 (98.7) | 1(1.3) | | 3(4.1) | 71 (95.9) | | 13 (17.6) | 61 (82.4) | | 2(2.7) | 72(97.3) | |
| **>50,000** | 6 (60) | 24 (25.5) | | 11 (36.7) | 19 (63.3) | | 3(10) | 27(90) | | 4(13.3) | 26(86.7) | | 29(96.7) | 1(3.3) | | 2(6.7) | 28(93.3) | | 3 (10.0) | 27 (90) | | 2(6.7) | 28(93.3) | |
| **HB** | | | | | | | | | | | | | | | 1.0 | | | 0.36 | | | | | | **0.05** |
| **>10** | 2 (22.2) | 36 (39.1) | 0.48 | 12 (31.6) | 26 (68.4) | 0.046 | 5(13.2) | 33(86.8) | 0.7 | 5(13.2) | 33(86.8) | 0.7 | 37 (97.4) | 1 (2.6) | | 3(7.9) | 35(92.1) | | 8 (21.0) | 30 (79.0) | 0.28 | 3(7.9) | 35 (92.1) | |
| **<10** | 7 (77.8) | 56 (60.9) | | 9 (14.29) | 54 (85.7) | | 6 (9.5) | 57 (90.5) | | 6(9.5) | 57(90.5) | | 62(98.4) | 1 (1.6) | | 2(3.1) | 61(96.8) | | 8 (12.7) | 55 (87.3) | | 0.0 | 63 (100) | |
| **PLatlet count** | | | | | | 0.77 | | | **0.04** | | | | | | 1.0 | | | 0.3 | | | 0.7 | | | 1.0 |
| **>1,00,000** | 3 (33.3) | 19 (20.6) | 0.4 | 5 (22.7) | 17 (77.3) | | 5(22.7) | 17 (77.3) | | 1(4.6) | 21(95.5) | 0.45 | 22 (100) | 0 (0.0) | | 2(9.1) | 20(90.9) | | 4 (18.2) | 18 (81.8) | | 0(0.0) | 22(100) | |
| **<1,00,000** | 6 (66.7) | 73 (79.4) | | 16 (20.3) | 63 (79.8) | | 6(7.6) | 73(92.4) | | 10(12.7) | 69(87.3) | | 77 (97.5) | 2 (2.5) | | 3(3.8) | 76(96.2) | | 12 (15.2) | 67 (84.8) | | 3(3.8) | 76 (96.2) | |
| **Integrated cyto** | | | 0 **.01** | | | 0.07 | | | 0.62 | | | 0 **.01** | | | 0.5 | | | **0.008** | | | 0.5 | | | **0.004** |
| **Good** | 0 | 19 (21.1) | | 1 (5.3) | 18 (94.7) | | 2 (10.5) | 17 (89.5) | | 6 (31.6) | 13(68.4) | | 18 (94.7) | 1 (5.3) | | 4(21.1) | 15 (78.9) | | 3 (15.8) | 16 (84.2) | | 2 (10.5) | 17 (89.5) | |
| **Intermediate** | 6(60) | 65(72.2) | | 16 (22.5) | 55 (77.5) | | 10 (14.1) | 61 (85.9) | | 5 (7.0) | 66 (92.9) | | 70(98.6) | 1 (1.4) | | 1(1.4) | 70(98.6) | | 13 (18.3) | 58 (81.7) | | 0 | 71 (100) | |
| **Poor** | 4(40) | 6(6.7) | | 4 (40.0) | 6 (60.0) | | 0 (0.0) | 10 (100) | | 0 | 10 (100) | | 10 (100) | 0.0 | | 0(0.0) | 10(100) | | 0 (0.0) | 10 (100) | | 2 (20.0) | 8 (80.0) | |

### Table 5: Association of genes with clinical outcome

| | zzz | IKZF1 deletions- | P value | CDKN2A deletions + | CDKN2A deletions- | P value | PAX5 deletions+ | PAX5 deletions - | P value | ETV6+ | ETV6- | P value | EBF- | EBF+ | P value | RB+ | RB- | P value | PAR gene del + | PAR gene del - | p value | BTG gene del + | BTG gene del - | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Respnse to prednisolone (n=98)** | | | 0.49 | | | | | | 1.0 | | | 0.49 | | | 0.09 | | | 1.0 | | | 0.7 | | | 1.0 |
| **Good** | 6 (60) | 62 (70.5) | | 14 (20.6) | 54 (79.4) | 0.6 | 8(11.8) | 60(88.2) | | 6(8.8) | 62(91.2) | | 68 (100) | 0 (0.0) | | 4(5.9) | 64(94.1) | | 10 (14.7) | 58 (85.3) | | 3 (4.4) | 65 (95.6) | |
| **Poor** | 4 (40) | 26 (29.6) | | 8 (26.7) | 22 (73.3) | | 4(13.3) | 26(86.7) | | 4(13.3) | 26(86.7) | | 28 (93.3) | 2 (6.6) | | 1(3.3) | 29(96.7) | | 5(16.7) | 25 (83.3) | | 1(3.3) | 29 (96.7) | |
| **MRD (n=64)** | | | **0.015** | | | 0.7 | | | 0.09 | | | 0.3 | | | 1.0 | | | 1.0 | | | 0.5 | | | 0.3 |
| **Absent** | 1 (20) | 46 (78.0) | | 9 (19.2) | 38 (80.9) | | 8(17.1) | 39(82.9) | | 5(10.6) | 42(89.4) | | 45 (95.7) | 2 (4.2) | | 1(2.1) | 46 (97.9) | | 10(21.3) | 37(78.7) | | 2(4.2) | 45(95.7) | |
| **Present** | 4 (80) | 13 (22.0) | | 4 (23.5) | 13 (76.5) | | 0 | 17(100) | | 0(0.0) | 17(100) | | 17 (100) | 0 (0.0) | | 0(0.0) | 17(100) | | 12 (18.7) | 52 (81.2) | | 2(11.7) | 15 (88.2) | |
| **CR (n=92)** | | | 0.1 | | | 0.6 | | | 1.0 | | | 1.0 | | | 1.0 | | | 1.0 | | | | | | 0.2 |
| **Achieved** | 7 (77.8) | 79 (95.2) | | 17 (19.8) | 69 (80.2) | | 12(14.0) | 74(86.1) | | 10 (11.6) | 76(88.4) | | 84 (97.6) | 2(2.3) | | 4(4.6) | 82(95.4) | | 15 (17.4) | 71(82.6) | 0.5 | 3(3.5) | 83(96.5) | |
| **Not achieved** | 2 (22.2) | 4 (4.8) | | 2 (33.3) | 4 (66.7) | | 0(0.0) | 6(100) | | 0 | 6(100) | | 6 (100) | 0 | | 0(0.0) | 6 (100) | | 0(0.0) | 6(100) | | 1(16.7) | 5(83.3) | |

**Table 3: Prevalence gene deletion in India (childhood ALL)**

| Variable (n=104) | Freqency | % | 95% CI |
|---|---|---|---|
| IKZF1 | 10 | 9.6 | 3.8-15.3 |
| CDKN2A /B | 23 | 22.1 | 14-30 |
| PAX5 | 12 | 11.5 | 5.29-17.7 |
| ETV6 | 11 | 10.6 | 4.5-16.5 |
| EBF1 | 2 | 1.9 | .7-4.47 |
| RB1 | 5 | 4.8 | 0.6-8.9 |
| PAR gene | 16 | 15.4 | 8.3-22.4 |
| BTG1 | 4 | 3.9 | .08-7.6 |

**Table 6: Hazard ratio of the genes in total cohort, High risk cohort and B-ALL cohort**

| | Total cohort | | High risk cohort | | B-ALL | |
|---|---|---|---|---|---|---|
| | Hazard ratio | P value | Hazard ratio | P value | Hazard ratio | P value |
| **IKZF1** | 1.6 | 0.4 | 0.9 | 0.8 | 1.8 | 0.3 |
| **CDKN2A** | **2.72** | **0.019** | 2.2 | 0.1 | 1.8 | 0.2 |
| **PAX5** | 0.78 | 0.7 | 1.2 | 0.7 | 0.4 | 0.4 |
| **ETV6** | 0.76 | 0.7 | 0.4 | 0.4 | 0.9 | 0.9 |
| **EBF1** | No death | | - | | - | - |
| **RB code** | **3.6** | **0.03** | 3.2 | 0.069 | 2.9 | 0.1 |
| **PAR gene** | 0.22 | 0.14 | No death | | 0.2 | 0.2 |
| **BTG1** | 1.48 | 0.7 | 1.27 | 0.8 | 1.7 | 0.5 |

## Conclusions

**Significant associations:**

- **IKZF1 deletions-**
  - **higher total leukocyte count**
  - **integrated cytogenetics**
  - **presence of minimal residual disease**
- **CDKN2A/B deletions**
  - **Higher age at presentation**
  - **High risk group**
- **Integrated cytogenetics**
  - **IKZF1 gene deletions**
  - **ETV6 gene deletions**
  - **RB1 gene deletions**
  - **BTG gene deletions**
- **CDKN2A and RB1 gene deletion with higher Hazard ratio in total cohort but not in high risk cohort or B-ALL cohort**