LETTER TO THE EDITOR

# Origins of iAMP21 in children who later developed pediatric acute lymphoblastic leukemia: an investigation of neonatal blood spots

## Authors

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# Supplementary Material

**for**

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## Supplementary Table 1

| Patient ID | Year at Diagnosis | Age, years /Sex | Karyo-type | Leucocyte count (x10⁹/L) | Platelet count (x10⁹/L) | NOPHO protocol | Relapse? Yes=1, No=0 | Second Third Treat-ment | Alive =0 Dead =1 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Pre-B-ALL 2016 | 8.4/ M | See below * | 14.6 | 36 | IR, high MRD day29 Block Therapy | 1, BM and CNS relapse | IntReALL 2010 protocol<br><br>HCT | 0 |
| 2 | Pre-B-ALL 2009 | 14.4/ F | See below ** | 8.5 | 109 | IR, high MRD day 29 Block Therapy | 0 | HCT | 1 |

*NOPHO, Nordic Pediatric Hematology Oncology; IR, Intermediate Risk Protocol; MRD, Minimal Residual Disease; BM, bone marrow; CNS, Central nervous system;HCT, Hematopoietic cell transplantation*

**Patient 1**

*46,XY,del(7)(p12.1p14.2),del(7)(q32.1q32.3),del(7)(q33q34),del(8)(p21.2p21.3),del(12)(p13.2p13.2),del(13)(q14.2q14.2),del(17)(q11.2q12),del(21)(q22.3q22.3),qdp(21)(q11.2q22.3

*FISH analysis did not show t(1;19), t(9;22), t(12;21), 11q23 (MLL) rearrangement, or del(9p21). There were also no signs of dic(9;20). Multiple extra RUNX1 signals were observed, consistent with iAMP21, and array analysis confirmed amplification of the long arm of chromosome 21. The result is thus consistent with iAMP21, indicating that the patient should be treated according to the intermediate-risk protocol.

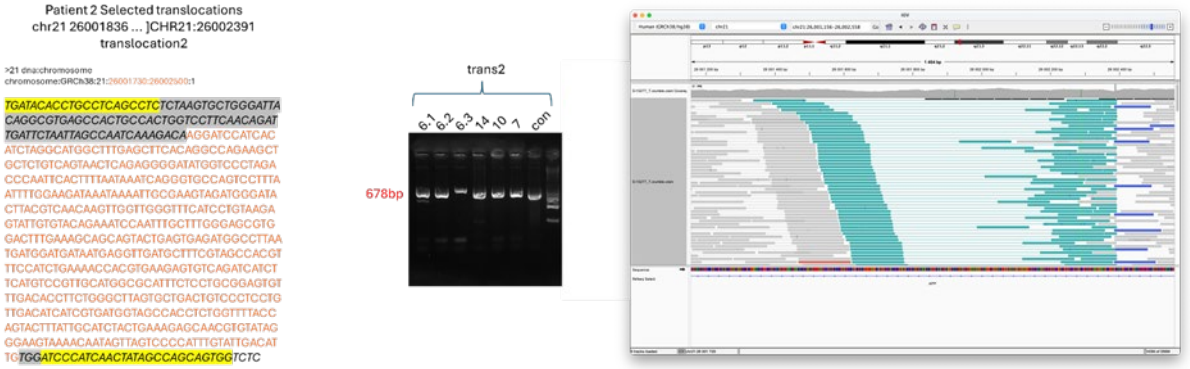
**Patient 2**

**46,X,t(X;7)(p2?1;q2?1),der(21)i(21)(q10)invdup(21)(q11q22)[13].ish.iamp(21)(q22)/46,XX[6]

**Chromosome analysis from bone marrow showed a karyotype with additional material of unclear origin on the short arm of one chromosome 7. In addition, an isochromosome 21 with amplified material was identified (ic21amp). FISH analysis also demonstrated ic(21) amplification containing the RUNX1 gene. Initially classified as intermediate risk due to ic21 amplification.

# Supplementary Figure 1

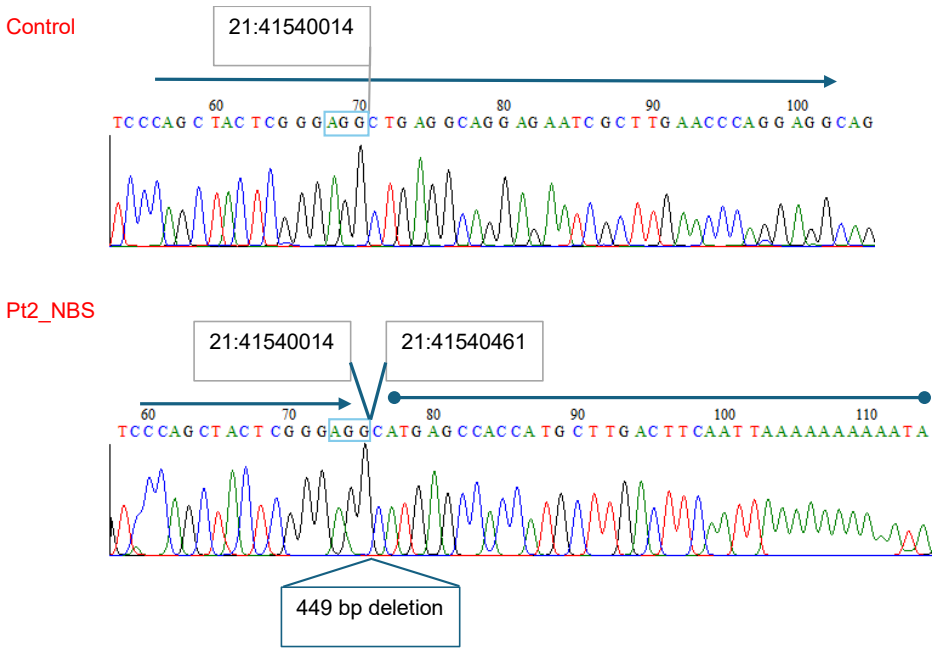
## A




**A selected translocation in iAMP-ALL patient 2 (inversion, see Figure 2A) is present at birth.**

Top: PCR primers are shown in yellow blocks, prospective inverted sequence is shown in brown, flanking regions in grey. Middle: gel picture after PCR. Lower panel: IGV of selected region at diagnosis of patient 2.

## B




**The small deletion within chromosome 21 identified at diagnosis for patient 2 (chr21:41540011–41540460) is already present in the NBS at birth.**

Control and NBS DNA sequence of PCR products. Boxed DNA sequences show possible microhomology

# Supplementary Figure 2

Patient 2 Selected duplication
chr21:43292455:43292524
+73 bp

GCTGGGGTAGAGCCTTTTAGCTATGGCCAGACCTGCTCTGCAGATTCCAACAAGGCCTCATGTC
CCAGCAACGGTGGCGTCCCTGCCAAAGGGGACGGGAGCTGAGCAAGGGTCTTCACTCCCTC
GTGCCACCCCTCCGGCCTCTAGGAAAGTGCTGATAGCAAGGCCGAGGACAGCAAAGCCGAGACA
GCAAGGCCGAAAACGGCAAGGCCGAGGACAGCAAGGCCGAGACAGCAAGGCCGAGGACAGCAAA
GCCGAGACAGCAAGGCCGAGACAGCAAGGCCGAGGACAGCGCTCCGGGAAGAA
AGCGAAGCTCCAGTGGTTGCTCCAAGACGTGGGTCTGGTGGGAGCAGAGTGGGCTGTTTTTGG
AGCTTCTCCCCTGCGATGTGAAGGCAGGAGCAAGGGCAGCTGTCCTGCTCAGCTGAGGCATG
GGATGGCCACAGCATCCCTGCAGAGCCCACCAGGATGCACTCAGAACTGACTTGT

>21 dna:chromosome: GRCh38:21:43292455-43292524

*GGCCGAGGACAGCAAAGCCGAGACAGCAAGGCCGAGACAGCAAGGCCGAGACAGCAAGGCCGAGGAC*

Pt2_NBS small deletion

```
Query  1         CCCTCCGGCCTCTAGGAAAGTGCTGATAGCAAGGCCGAGGACA----------------  43
                 |||||||||||||||||||||||||||||||||||||||||||
Sbjct  43292429  CCCTCCGGCCTCTAGGAAAGTGCTGATAGCAAGGCCGAGGACAGCAAAGCCGAGACAGCA  43292488

Query  44        -----------GCAAGGCCGAGACAGCAAGGCCGAGGACAGCGCTCCGGGAATGAAAGCG  92
                           ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  43292489  AGGCCGAGACAGCAAGGCCGAGACAGCAAGGCCGAGGACAGCGCTCCGGGAATGAAAGCG  43292548

Query  93        AAGCTCCAGTGGTTGCTCCAAGACGTGGGTCTGGTGGGAGCAGAGTGGGCTGTTTTTGGA  152
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  43292549  AAGCTCCAGTGGTTGCTCCAAGACGTGGGTCTGGTGGGAGCAGAGTGGGCTGTTTTTGGA  43292608

Query  153       GCTTCTCCCCTGCGATGTGAAGGCAGGAGCAAGGGCAGCTGTCCTGNTCAGCTGAGGC  210
                 |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  43292609  GCTTCTCCCCTGCGATGTGAAGGCAGGAGCAAGGGCAGCTGTCCTGCTCAGCTGAGGC  43292666
```

Pt2_diagnosis duplication

```
Query  77        AGGGTCTTCACTCCCTCGTGCCACCCCTCCGGCCTCTAGGAAAGTGCTGATAGCAAGGCC  136
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  43292405  AGGGTCTTCACTCCCTCGTGCCACCCCTCCGGCCTCTAGGAAAGTGCTGATAGCAA----  43292460

Query  137       GAGGACAGCAAAGCCGAGACAGCAAGGCCGAAAACGGCAAGGCCGAGGACAGCAAGGCCG  196
                                                 ||||||||||
Sbjct  43292461  ----------------------------------------GGCCGAGGAC----------  43292470

Query  197       AGACAGCAAGGCCGAGGACAGCAAAGCCGAGACAGCAAGGCCGAGACAGCAAGGCCGAGG  256
                                  ||||||||||||||||||||||||||||||||||||||||| |
Sbjct  43292471  ------------------AGCAAAGCCGAGACAGCAAGGCCGAGACAGCAAGGCCGA-G  43292510

Query  257       ACAGCAAGGCCGAGGACAGCGCTCCGGGAATGAAAGCGAAGCTCCAGTGGTTGCTCCAAG  316
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  43292511  ACAGCAAGGCCGAGGACAGCGCTCCGGGAATGAAAGCGAAGCTCCAGTGGTTGCTCCAAG  43292570
```

**A deletion in patient 2 at birth evolves to a postnatal duplication**

Top: Primer sequences in yellow blocks, potential duplicated region in red.

Bottom: Standard nucleotide BLAST comparison (Altschul SF et al., Basic local alignment search tool. J Mol Biol. 1990;215(3):403-10) between PCR amplicons derived around chr21:43292455-43292524 in patient 2. Sequence alignment shows that a small deletion present in the NBS had likely progressed to a duplication event at diagnosis of iAMP21-ALL.