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SHORT REPORT

TP53 variants underlying pediatric low-hypodiploidy B-cell acute lymphoblastic leukemia demonstrate diverse origins and may persist as a hematopoietic clone in remission

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TP53 variants underlying pediatric LH-ALL demonstrate diverse origins and may persist as hematopoietic clones in remission.

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Abstract

Pediatric low-hypodiploidy B-cell acute lymphoblastic leukemia (LH-ALL) with *TP53* variants has been proposed to be considered a manifestation of Li-Fraumeni syndrome (LFS). However, our study demonstrates that of the majority the pathogenic variants in the TP53 gene are somatic (70.5%), and only 12.5% of patients with germline fulfilled the criteria of LFS. We also describe the first case of hypodiploid BCP-ALL with a mosaic pathogenic mutation in *TP53* and the first case of the persistence of clonal hematopoiesis with the *TP53* gene mutation in the child during 3-year minimal residual disease-negative remission, similar to what has been described in adults.

KEYWORDS
clonal hematopoiesis, LH-ALL, Li-Fraumeni syndrome, *TP53*

1 | INTRODUCTION

Mutations in *TP53* are considered a driving event in the development of low hypodiploid acute lymphoblastic leukemia arising from B-cell precursor (LH-ALL), which still has a dismal prognosis irrespective of treatment modalities [1, 2]. In recent observations of adults with low hypodiploid BCP-ALL somatic pathogenic *TP53* variants were evidenced as a preleukemic clone resembling age-related clonal hematopoiesis [3–5]. Pediatric LH-ALL with *TP53* variants has been proposed to be considered a manifestation of Li-Fraumeni syndrome (LFS) based on the discovery of a mutation in an isolated population of T-cells and several cases of LH-ALL in Li-Fraumeni families [6, 7]. However, comprehensive data on the germline origin of *TP53* mutations in the pediatric low hypodiploid ALL cohort are scarce.

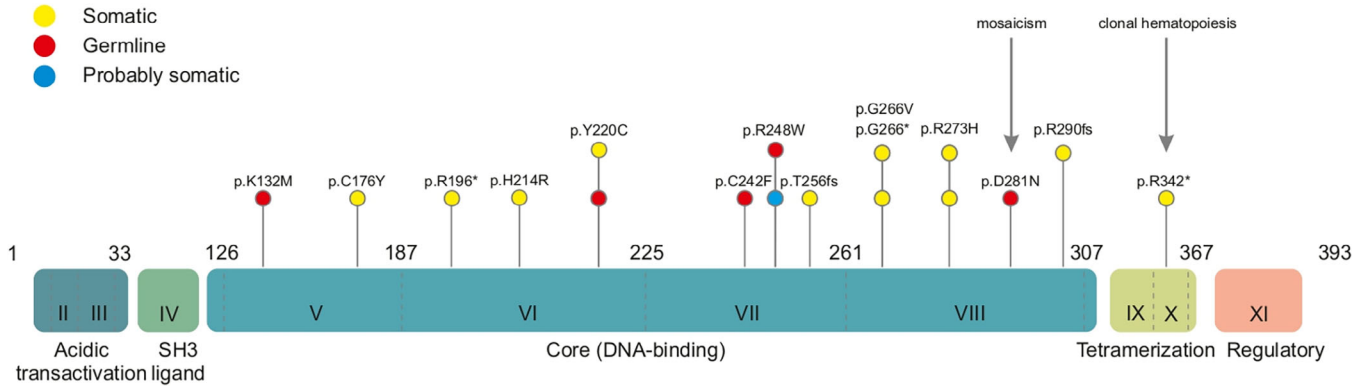

FIGURE 1 Deleterious *TP53* variants in pediatric hypodiploid B-cell precursor acute lymphoblastic leukemia.TP53 protein domains and their composing exons (in Roman numerals) are depicted on a ribbon. Deleterious variants identified by next-generation sequencing (NGS) are as follows: somatic ($n = 16$, yellow), germline ($n = 4$, red), and probably somatic ($n = 1$, blue). Variants associated with postzygotic origin and clonal hematopoiesis are marked with arrows.

Aiming to elucidate the somatic or germline nature of *TP53* variants we evaluated our cohort of pediatric patients with hypodiploid ALL.

2 | MATERIALS AND METHODS

We analyzed different tissue samples from 17 patients aged 9–17.3 years with hypodiploid BCP-ALL (Table S1). All patients underwent genetic testing at the Laboratory of Cytogenetics and Molecular Genetics of Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology, and Immunology between January 2010 and December 2023 and were registered in Moscow-Berlin Study group database (NCT01953770 and NCT03390387). The study was approved by the Institutional Review Board and ethics committee of the Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology, and Immunology, conducted by the Declaration of Helsinki. All patients or their parents/legal guardians provided written informed consent. Karyotyping and fluorescence in situ hybridization (FISH) analysis with commercially available DNA probes for t(12;21), t(4;11), t(9;22), and t(1;19) were performed for all patients. Hypodiploidy was recognized in the case of a clone with ≤44 chromosomes. In the case of failed karyotyping, when two or more chromosomes were supposed to be absent during routine FISH analysis, additional FISH for monosomy 7 and *TP53* deletion/monosomy 17 were performed to confirm the hypodiploidy.

To identify pathogenic variants in the *TP53* gene, bone marrow (BM) samples were analyzed at the onset of the disease using Sanger sequencing (list of primers in Table S2) followed by high throughput sequencing (QIASeg HMNP panel; Qiagen). The germline status of the identified variants was determined by analyzing BM and peripheral blood (PB) in remission, buccal epithelium, nail plates, flow-sorted T-cells from diagnostic BM and PB samples, and flow-sorted (BD FACSAria III) cell populations at remission-based on sample availability using the same techniques. One patient underwent evaluation using mesenchymal stromal cells from the BM.

3 | RESULTS AND DISCUSSION

Eight patients in the studied cohort had LH-ALL according to the karyotyping, and in nine patients hypodiploidy was recognized based on FISH results only (the details are described in Table S1).

TP53 variants were defined in all 17 cases. They were pathogenic in 16 cases and likely pathogenic in one patient, and mostly allocated in the DNA-binding domain. All the variants were previously described in patients with LFS. Several cancer hot spots were presented. *TP53* variant allelic frequency (VAF) varied from 11% to 90% on leukemia onset (Table S1).

TP53 variants were considered somatic in 12 patients (70.5%) and germline in four (23.5%) cases (Figure 1). Two of four patients with germline variants fulfilled the classic LFS diagnostic criteria and Chompret criteria [8, 9]. One patient (43,939) presented with LH-ALL four years after treatment of nasopharyngeal rhabdomyosarcoma. Evaluation of blood sample in remission confirmed germline status of *TP53* (VAF = 50%). Another patient (16,606) had a first-degree family member with breast cancer, diagnosed before age 45. In this patient, the *TP53* variant was found in isolated T-cells from the diagnostic sample with VAF = 50% thus indicating its germline origin with a high probability. The remaining two patients with the *TP53* variant detected in the buccal epithelium (VAF = 50%) have not demonstrated any additional criteria of LFS by the moment of manuscript preparation.

The mosaic form of LFS was suspected in the 15-year-old female patient (55,635) with *TP53* p.D281N pathogenic variant, found at leukemia onset with VAF = 45%. In remission (9 months after diagnosis), BM analysis revealed a *TP53* variant with VAF = 22% while FISH analysis for hypodiploidy was negative, and no flow minimal residual disease (MRD) was detected. Sequencing of flow-sorted BM cell populations in remission revealed the presence of *TP53* in T-cells, monocytes, granulocytes, and erythroid precursors (VAF = 13%, 33%, 29%, and 35%, respectively). Further analysis of buccal epithelium and nails showed a *TP53* variant with VAF of 11% and 15%, respectively. The patient's BM mesenchymal stromal cells showed of *TP53* variant with

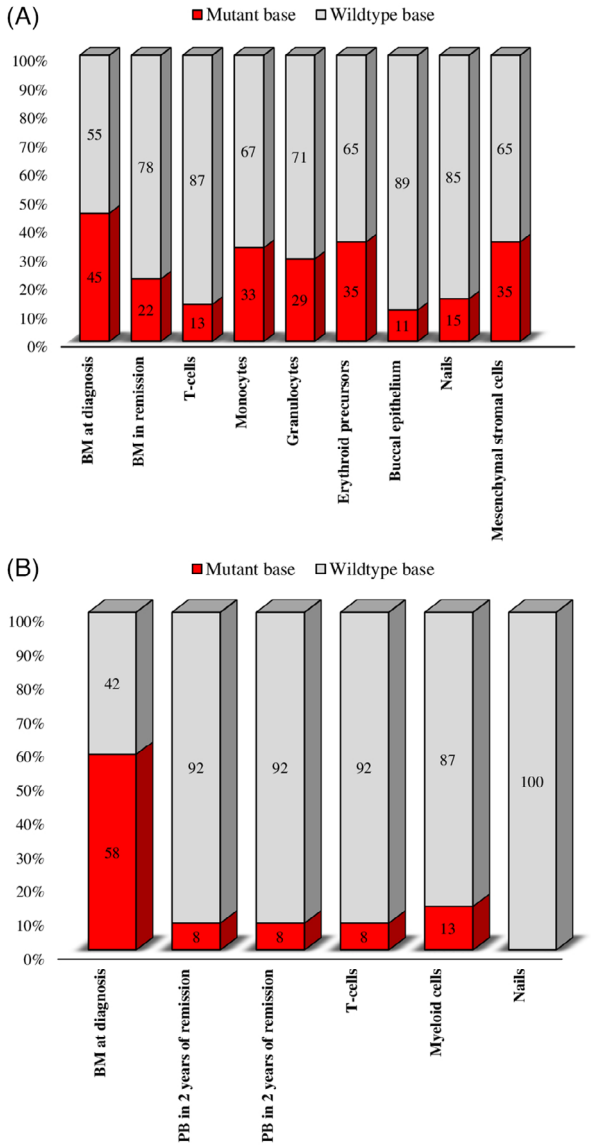

FIGURE 2 (A) Somatic mosaicism of *TP53* p.D281N variant in patient 55,635 detected by next-generation sequencing (NGS) in both mesoderm-derived (hematopoietic cells and mesenchymal stromal cells) and ectoderm-derived tissues (buccal epithelium and nails). (B) Clonal hematopoiesis in patient 44,225. *TP53* p.R342* variant restricted to hematopoietic lineage only. T-cells and myeloid precursors were flow-sorted from peripheral blood 30 months after remission. The nails were obtained at the same time. Samples were analyzed by NGS. BM, bone marrow; PB, peripheral blood.

VAF = 35%. Being presented in ectoderm and mesoderm-derived tissues, the *TP53* variant probably emerged as a result of postzygotic constitutional mutation, resulting in somatic tissue mosaicism (Figure 2A). Somatic mosaicism was previously described in rare cases of solid tumors in LFS patients [10–12]. Our observation demonstrates for the first time the mosaic form of *TP53* mutation giving rise to the LH-ALL.

We also could trace the kinetics of the *TP53* mutated clone in the 14-year-old male patient (44,225) with pathogenic somatic variant p.R342*. The somatic origin was confirmed as no mutation was detected in the nail plate sample. This variant was initially detected in the BM with VAF = 58% and later was found in the PB sample in flow MRD negative remission 24 and 30 months later with the identical allelic frequency (VAF~8%). No primary cytogenetic clone was detected in 30 months according to the FISH evaluation of the blood sample. In isolated T-cell and myeloid populations flow-sorted from PB (time point 30 months after diagnosis) the variant was presented also with VAF = 8% and 11%, respectively (Figure 2B). In recent studies of adult patients [5], the presence of *TP53* mutation in different hematopoietic lineages in remission was also demonstrated in flow-isolated myeloid cells, indicating the *TP53* positive clonal hematopoiesis, derived from the early hematopoietic precursor. Performing simultaneous genotyping and immunotyping at a single-cell level in remission samples, containing *TP53*-mutant clones, Rathana et al. [3] and Saiki et al. [4] have shown similar patterns of *TP53*-mutant clones distribution among hematopoietic lineage and suggested the presence of *TP53*-mutated hematopoietic stem clones before chemotherapy that can partly repopulate normal hematopoiesis after chemotherapy.

In one patient (17,112) isolated T-cells from a diagnostic BM sample had *TP53* initial variant occurring with VAF = 17% (Table S1), which may be a manifestation of clonal hematopoiesis from early hematopoietic precursors as well as the sign of constitutional somatic tissue mosaicism.

4 | CONCLUSIONS

Our results revealed that in the pediatric cohort, the majority of *TP53* pathogenic variants in LH-ALL were somatic (12 of 17, 70.5%). We describe for the first time the case of *TP53* somatic mosaicism in hypodiploid BCP-ALL. Only one-third of our cohort were considered germline (four of 17, 23.5%). Of those, only 2 patients (12.5%) with germline *TP53* mutation fulfilled the criteria of LFS. The present study offers novel insights into the molecular genetic mechanisms underlying the pathogenesis of low-hypodiploid pediatric acute lymphoblastic leukemia with *TP53* gene mutations. Our findings indicate that, in addition to germinal and somatic cases, *TP53* may be present in mosaic forms and variants of clonal hematopoiesis. The persistence of clonal hematopoiesis with the *TP53* gene mutation in the child during 3-year MRD-negative remission demonstrates that LH-ALL in adults and adolescents may have the same molecular mechanism of development.

AUTHOR CONTRIBUTIONS

Albert Itov: Conceptualization; methodology; data verification; study conduct; visualization; writing original draft, and writing—review and editing. **Karina Ilyasova, Olga Soldatkina, Anna Kazakova, and Vladimir Kozeev**: Cytogenetic and molecular genetic studies; played an important role in interpreting the results, and presentation. **Alexandra Semchenkova**: Immunophenotypic studies and cell sorting; played an important role in interpreting the results, and presentation. **Elena Osipova**: cultivation of mesenchymal cells; played an important role in interpreting the results, and presentation. **Elmira Boichenko and**

Egor Volchkov: Resources and played an important role in interpreting the results. **Elena Zerkalenkova and Alexander Popov**: Visualization; played an important role in interpreting the results; writing-review, and editing. **Yulia Olshanskaya**: Conceptualization; methodology; visualization; writing-review and editing, and study management. **Galina Novichkova, Alexander Karachunskiy, and Julia Roumiantseva**: Project administration and writing-review.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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For original data, please contact albertitov03@gmail.com

ETHICS STATEMENT

The authors have confirmed ethical approval statement is not needed for this submission.

PATIENT CONSENT STATEMENT

The authors have confirmed patient consent statement is not needed for this submission.

CLINICAL TRIAL REGISTRATION

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SUPPORTING INFORMATION

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