Identification of the NUP98-PHF23 fusion gene in pediatric cytogenetically normal acute myeloid leukemia by whole-transcriptome sequencing

Marco Togni1, Riccardo Masetti1*, Martina Pigazzi2, Annalisa Astolfi2, Daniele Zama1, Valentina Indio3, Salvatore Serravalle1, Elena Manara2, Valeria Bisio2, Carmelo Rizzari4, Giuseppe Basso2, Andrea Pession1 and Franco Locatelli5

Abstract
The genomic landscape of children with acute myeloid leukemia (AML) who do not carry any cytogenetic abnormality (CN-AML) is particularly heterogeneous and challenging, being characterized by different clinical outcomes. To provide new genetic insights into this AML subset, we analyzed through RNA-seq 13 pediatric CN-AML cases, corroborating our findings in an independent cohort of 168 AML patients enrolled in the AIEOP AML 2002/01 study. We identified a chimeric transcript involving NUP98 and PHF23, resulting from a cryptic t(11;17)(p15;p13) translocation, demonstrating, for the first time, that NUP98-PHF23 is a novel recurrent (2.6 %) abnormality in pediatric CN-AML.

Keywords: NUP98 gene fusions, Pediatric acute myeloid leukemia, PHD domain

Findings
Childhood acute myeloid leukemia (AML) is a heterogeneous disease with current survival rates of approximately 60–70 %. Cytogenetics, recurrent molecular abnormalities, and early response to treatment are the main factors influencing outcome [1]. However, around 20 % of pediatric AML do not carry any known cytogenetic abnormality (cytogenetically normal-AML or CN-AML). In order to shed light on this subgroup we performed whole-transcriptome sequencing (WTS) in 13 pediatric CN-AML cases, corroborating relevant findings in an independent cohort of 168 cases.

Sequencing was performed on a HiScanSQ sequencer (Illumina), and bioinformatic analysis was performed as previously described [2]. In 2 (CN-AML_54, CN-AML_66) out of 13 cases analyzed, we identified a chimeric transcript involving nucleoporin 98 kDa (NUP98) and PHD finger protein 23 (PHF23) genes, resulting from a cryptic translocation t(11;17)(p15;p13) (Fig. 1a and Table 1). In both cases, we identified an in-frame fusion between NUP98 exon 13 and PHF23 exon 4 (Fig. 1b). To date, the cryptic translocation t(11;17)(p15;p13) has been described only once in an adult AML patient [3], but never in a pediatric AML cohort. Different from what was previously reported by Reader and colleagues [3], in this study the recurrent breakpoint in PHF23 was always identified at the beginning of exon 4 and not within it (Fig. 1a and b).

To assess the incidence of NUP98-PHF23 fusion in pediatric CN-AML, we examined through RT-PCR analysis and Sanger sequencing a validation cohort of 168 AML children enrolled in the AIEOP AML 2002/01 study [4]; one-hundred thirty-nine patients (76 males and 63 females, median age at diagnosis 7.7 years, range 17 days to 17.9 years) were negative for known recurrent genetic abnormalities involving MLL, CBFB, and FLT3, while the remaining 29 patients (15 males and 14 females, median age at diagnosis 11.8 years, range 3 to 17.4 years) harbored internal tandem duplication of FLT3 (FLT3-ITD), this latter abnormality being chosen because we previously reported a strong association between NUP98-NSD1 rearrangement and FLT3-ITD [5]. With the exception of FAB M3 (acute promyelocytic leukemia), all the FAB types were represented in the validation cohort. RNA was extracted from fresh bone marrow at diagnosis, and multiplex RT-PCR was used. Sequencing by Sanger method was applied to all cases
positive by PCR to NUP98-PHF23 fusion gene. Overall, 2 out of 139 CN-AML cases were found to harbor NUP98-PHF23 (Table 1). NUP98-PHF23 was not found in any patient harboring FLT3-ITD. Fluorescence in-situ hybridization confirmed the cryptic chromosomal translocation t(7;11)(p15;p13) leading to the fusion between NUP98 and PHF23 in all cases (Fig. 1c).

So far, many NUP98-rearrangements have been found to be associated with both de novo and therapy-related AML but also with T-cell acute lymphoblastic leukemia with over 28 different partner genes [6]. Recently, the fusion NUP98-JARID1A has been described to be a recurrent event in pediatric acute megakaryoblastic leukemia (11%), with a distinct HOX gene-expression pattern [7]. Conversely, chromosomal rearrangements and/or mutations of PHF23 have never been previously described in children with AML. Located on the reverse strand of 17p13.1, PHF23 encodes for a protein containing a plant homeodomain (PHD) finger [8] involved in chromatin remodeling [3]. Expression of NUP98-PHF23 has been demonstrated to impair the differentiation of myeloid progenitor cells and promote leukemia development in vitro.

Table 1 Clinical features of pediatric CN-AML patients harboring the NUP98-PHF23 fusion gene

| Id     | Age, years | Gender | WBC, x 10^9/L | FAB | BM blast, % at diagnosis | Extramedullary involvement | HSCT (type) | CR after induction therapy | Relapse (site) | Disease-free duration (months) | Survival duration (months) |
|--------|------------|--------|---------------|-----|--------------------------|----------------------------|-------------|--------------------------|---------------|-------------------------------|--------------------------|
| CN-AML_54* | 2.9        | M      | 187           | M1  | 90                       | No                         | Yes (AUTO)  | Yes                      | Yes (BM)      | 5                             | 30          |
| CN-AML_66a | 9.0        | M      | 1.2           | M0  | 70                       | No                         | Yes (MUD)   | Yes                     | –             | 65                            | 66          |
| CN-AML_3  | 9.7        | M      | 6.9           | M4  | 40                       | No                         | Yes (MUD)   | Yes                     | –             | 40                            | 41          |
| CN-AML_4  | 7.0        | M      | 1.8           | M5A | 54                       | No                         | Yes (AUTO)  | Yes                     | –             | 103                           | 104         |

AUTO autologous, HSCT hematopoietic stem cell transplantation, MUD matched unrelated donor, BM bone marrow, WBC white blood cells
*patients identified by RNA-seq
a dead patient
and in vivo [8–10]. Cells expressing NUP98-PHF23 are sensitive to disulfiram, an FDA-approved drug, demonstrating the feasibility of targeting this oncoprotein [9].

In summary, we identified, for the first time in childhood AML, a NUP98-PHF23 fusion, demonstrating that this genomic aberrancy is not exceptional (tentative frequency of 2.6 %) in pediatric CN-AML. These findings enforce the role of epigenetic regulators in pediatric AML and suggest novel therapeutic targets for this disease.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MT performed the research, coordinated the work, analyzed data, and wrote the paper. RM coordinated the work, analyzed data, and wrote the paper. MT and AA performed the whole-transcriptome massively parallel sequencing. VI performed bioinformatics analyses. MP, EM, and VB performed the screening in the validation cohort. DZ collected and analyzed clinical data. SS performed the cytogenetic analyses. GB, AP, and FL designed and supervised the research. CR and FL equally contributed to the critical revision and writing of the manuscript. All authors read and approved the final version of the manuscript.

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Author details
1Department of Pediatrics, “Lalla Selagno” Hematology-Oncology Unit, University of Bologna, Bologna, Italy. 2Department of Paediatric Haematology, University of Padova, Padova, Italy. 3Giorgio Prodi Cancer Research Centre, University of Bologna, Bologna, Italy. 4Department of Pediatrics, San Gerardo Hospital, University of Milano-Bicocca, Monza, Italy. 5Department of Pediatric Hematology-Oncology, IRCCS Ospedale Bambino Gesù, Roma - University of Pavia, Pavia, Italy.

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