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# Acquisition of monosomy 7 and a *RUNX1* mutation in Pearson syndrome

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**Running head:** Monosomy 7 and *RUNX1* mutation in PS

| Abbreviations |                                         |
|---------------|-----------------------------------------|
| BM            | bone marrow                             |
| HSCT          | hematopoietic stem cell transplantation |
| IBMFS         | inherited bone marrow failure syndrome  |
| MDS           | myelodysplastic syndrome                |
| mtDNA         | mitochondrial DNA                       |
| PS            | Pearson syndrome                        |
| WES           | whole exome sequencing                  |

## ABSTRACT

Pearson syndrome (PS) is a very rare and often fatal multisystem disease caused by deletions in mitochondrial DNA that result in sideroblastic anemia, vacuolization of marrow precursors, and pancreatic dysfunction. Spontaneous recovery from anemia is often observed within several years of diagnosis. We present the case of a 4-month-old male diagnosed with PS who experienced prolonged severe pancytopenia preceding the emergence of monosomy 7. Whole-exome sequencing identified two somatic mutations including *RUNX1* p.S100F that was previously reported as associated with myeloid malignancies. The molecular defects associated with PS may have the potential to progress to advanced MDS.

## 44 INTRODUCTION

45 Pearson syndrome (PS) is a multi-organ system disorder characterized by refractory  
46 sideroblastic anemia with vacuolization of bone marrow (BM) precursors, lactic  
47 acidosis and exocrine pancreatic dysfunction that result from the deletion of  
48 mitochondrial DNA (mtDNA) sequences. Pancreatic dysfunction frequently  
49 accompanies PS but it is not critical for the diagnosis.<sup>1,2</sup> The incidence of PS is very  
50 low, at approximately one case per million individuals.<sup>3</sup> PS is one of the disorders to  
51 be considered in the differential diagnosis of hypocellular BM in young children.<sup>4,5</sup> It is  
52 not clear whether PS is associated with malignant transformation; the long-term  
53 prognosis of PS is generally poor, as children often succumb to fatal lactic acidosis.<sup>6</sup>

54 Monosomy 7 is a common cytogenetic abnormality identified in inherited BM failure  
55 syndromes (IBMFS) and pediatric myelodysplastic syndrome (MDS).<sup>7</sup> The basic  
56 mechanisms underlying the acquisition of monosomy 7, including haplo-insufficiency  
57 and related somatic events, have been explored previously.<sup>8</sup> Here we describe a case  
58 of a patient diagnosed with PS who experienced prolonged and severe pancytopenia  
59 followed by the emergence of monosomy 7 and a somatic mutation in *RUNX1*  
60 underwent hematopoietic stem cell transplantation (HSCT).

61

## 62 **METHODS**

63 Written informed consent from the guardians of the patient was obtained for sample  
64 storage and analyses. The analyses were conducted in accordance with the  
65 Declaration of Helsinki. DNA from BM cells obtained from the patient at diagnosis and  
66 again upon development of pancytopenia were subjected to whole-exome sequencing  
67 (WES); DNA from buccal cells was used as a germline control. Whole-exome capture  
68 was performed using SureSelect Human All Exon Kit V6 (Agilent Technology, Santa  
69 Clara, CA, USA). Captured targets were subjected to sequencing using a HiSeq 2000  
70 (Illumina, San Diego, CA, USA). With mean depths of 114-143x, sequence alignments  
71 and mutation identifications were performed using our in-house Genomon program,  
72 as previously described.<sup>9</sup> Candidate mutations were identified with the following filters:  
73 (i)  $P$ -value  $< 10^{-1.3}$  (by Fisher's test); (ii) EB call (Empirical Bayesian mutation calling)<sup>10</sup>  
74  $P$ -value  $< 10^{-4}$ ; (iii) variant allele frequency in normal sample  $< 0.02$ . Copy number  
75 analysis was performed using in-house program known as CNACS<sup>11</sup>  
76 ([https://github.com/papaemmelab/toil\\_cnacs](https://github.com/papaemmelab/toil_cnacs)). Frequency of deletion in mtDNA was

calculated as mean depth of mtDNA with deletion detected (MT:8,469–13,446) divided by those without (MT:1–8,468).

## **RESULTS AND DISCUSSION**

A 4-month-old boy was admitted for treatment of respiratory syncytial virus infection and mild pancytopenia. There was no past medical history or any notable family history. Examination of the BM was notable for vacuolated myeloid and erythroid precursors with ring sideroblasts. Chromosomal analysis revealed 46,XY in 20 out of 20 metaphase spreads. Blood levels of lactic acid were elevated; as such, genetic testing was performed. A large deletion of mtDNA was detected, which indicates a diagnosis of PS. The deleted mtDNA allele was detected at a frequency 81% in BM as a consequence of heteroplasmy that was identified by WES with off-target sequencing reads on mtDNA (Figure 1a). No somatic mutations were detected in BM cells at diagnosis.

The patient began a series of regular red blood cell transfusions to treat his anemia. At the age of 22 months, BM examination revealed significant hemophagocytosis after a respiratory tract infection that resolved in response to prednisolone. Profound

94 thrombocytopenia and neutropenia emerged at the age of 30 months. Platelets counts  
95 were fluctuating with regular transfusion. Absolute neutrophil counts was stable  
96 around 300 / $\mu$ L. BM examination revealed hypo-cellular marrow with 2 % of  
97 myeloblasts and minimal dysplasia. A repeat chromosomal analysis revealed a 45,XY,  
98 -7 [12]/ 45, idem, t(4;21)(p11;q22) [4]/ 46, XY [4] aberration; monosomy 7 was also in  
99 38% of the cells by fluorescent *in situ* hybridization and WES (Figure 1b). Furthermore,  
100 WES revealed two somatic mutations of *RUNX1* and *LINGO4* in addition to monosomy  
101 7 (Table 1). *RUNX1* p.S100F mutation was previously reported in myeloid  
102 malignancies.<sup>12</sup> The frequency of mtDNA deletion was 78%. The patient received an  
103 unrelated cord blood transplantation at the age of 42 months to treat prolonged  
104 pancytopenia. The conditioning regimen included anti-thymocyte globulin, fludarabine,  
105 and melphalan from HLA fully matched (8/8) unrelated cord blood. Lactic acidosis  
106 deteriorated with infusion reaction by anti-thymocyte globulin. Neutrophil engraftment  
107 was obtained on day 20. Acute GVHD of skin (stage 3) and liver (stage 1) were  
108 resolved with prednisolone. He was discharged on day 66. His hematological status  
109 and acid-base balance are stable 20 months after HSCT.

110 The proportion of deleted mtDNA in hematopoietic cells of patients diagnosed with  
111 PS varies due to heteroplasmy; the severity of hematologic manifestations is directly  
112 related to this phenomenon. Cytopenia associated with PS may resolve  
113 spontaneously with a decreasing frequency of mitochondria with deleted mtDNA.<sup>13,14</sup>  
114 Our patient did not experience any resolution of his anemia; the ratio of deleted to  
115 intact mtDNA did not change over time as assessed by WES. In addition to persistent  
116 BM failure, monosomy 7 appeared two years after initial diagnosis. PS is important as  
117 a differential diagnosis of IBMFS, however it is considered to be a non-hematological  
118 disorder. Actually, development of cytogenetic abnormalities was previously reported  
119 in three cases of PS; all cases had chromosome 7 related abnormalities. The clinical  
120 course varied from transient abnormalities to progression AML.<sup>4, 15,16</sup>

121 Monosomy 7 occurs during the clonal evolution to MDS/leukemia in a variety of  
122 IBMFSs.<sup>7</sup> MDS with monosomy 7 in children has been associated with a high risk of  
123 disease progression.<sup>17</sup> Among those cohorts, patients with germline *SAMD9/9L*  
124 mutations also frequently developed monosomy 7 as a consequence of an adaptation-  
125 by-aneuploidy mechanism.<sup>18</sup> *SAMD9/9L* locate on chromosome 7, and their mutations  
126 have growth-restricting activity. WES confirmed that our patient harbored no known

127 germline abnormalities including *SAMD9/9L*, *GATA2* and *FANC* genes that would  
128 suggest a predisposition to MDS. Acquisition and selection of monosomy 7 clones  
129 may be caused with a similar mechanism in patients with BM failure<sup>7</sup> where the  
130 hematopoietic milieu is exposed to cytopenia-induced stress. Of note, our patient did  
131 not undergo treatment with G-CSF, which is known to be associated with the  
132 development of monosomy 7 in patients with BM failure.<sup>19</sup>

133 Acquisition of additional genetic abnormalities predicts disease progression in adult  
134 MDS.<sup>20</sup> One study showed that MDS in children was often associated with Ras/MAPK  
135 pathway mutations; by contrast, children with germline *SAMD9/9L* mutations rarely  
136 acquired additional gene or chromosomal alterations.<sup>21</sup> Monosomy 7 itself results in  
137 haplo-insufficiency of tumor suppressor genes on chromosome 7, which could  
138 cooperate with other driver events in modulating the pathogenesis of myeloid  
139 malignancies. For example, loss of *EZH2* (located on 7q36) has been shown to  
140 interact with *RUNX1* mutations and to generate myeloid tumors in mice.<sup>22</sup> Somatic  
141 mutations in *RUNX1* are reported frequently in association with childhood MDS with  
142 monosomy 7.<sup>23</sup> In IBMFSSs, including Fanconi anemia and severe congenital  
143 neutropenia, the combination of *RUNX1* mutations and monosomy 7 also contribute

to myeloid leukemogenesis.<sup>24,25</sup> Of note, a *RUNX1* mutation that has been associated with myeloid malignancies was also identified in our patient. This clone might have had the potential to progress to advanced MDS.

The cases of two patients with PS who received unrelated HSCT were previously described in the literatures.<sup>15,16</sup> In one patient, both hematological and non-hematological manifestations resolved in response to this intervention,<sup>16</sup> similar to that observed in our case. PS has features of fatal multisystem dysfunction and spontaneous recovery from anemia within several years of diagnosis. Although HSCT can result in serious complications, it may be a feasible option for patients with severe PS who acquired cytogenetically abnormalities in BM and should be considered in a future prospective clinical trial.

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### **Authorship Contributions**

Conception and design: A.N, S.H., D.H., and A.M. Data analysis and interpretation: all  
authors. Manuscript writing and final approval: all authors.

### **Disclosure of Conflicts of Interest**

All authors declare that there are no conflicts of interest.

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254 **TABLE AND FIGURE LEGENDS**

255

256 **Table 1.**

257 Mutations identified by whole-exome sequencing.

258

259 **Figure 1.**

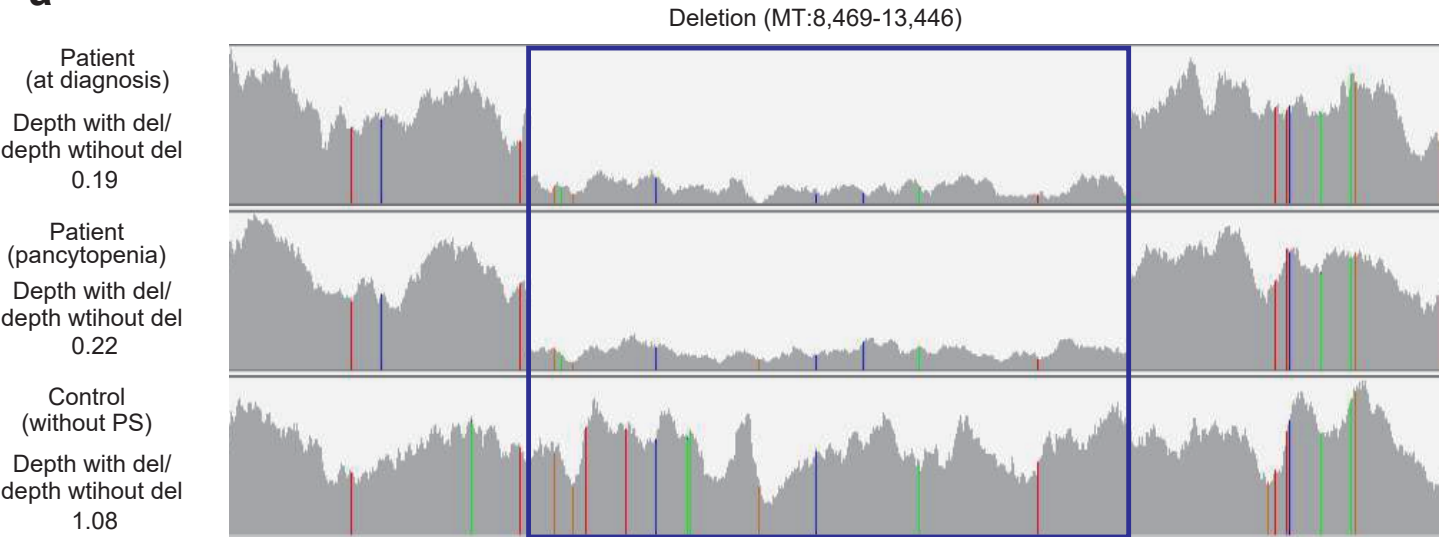
260 Deletion in mtDNA and monosomy 7 detected by whole-exome sequencing.

261 a) Deletion in mtDNA. Sequencing depth on mtDNA are displayed in gray using IGV  
262 for samples at diagnosis and after the development of pancytopenia as well as a  
263 control sample. Colored positions mean the positions where alleles different from the  
264 reference sequence were called.

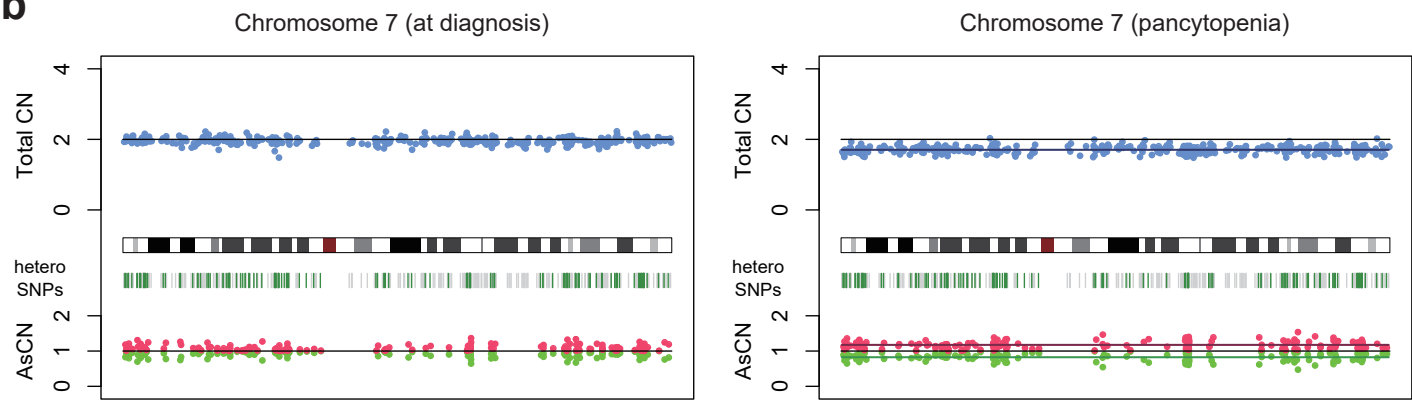
265 b) Monosomy 7 after the development of pancytopenia. Total copy number (CN) and  
266 allele specific (AS) CN of chromosome 7 are shown for samples at diagnosis and after  
267 the development of pancytopenia.

# Figure 1

**a**



**b**



**Table 1. Mutations identified by whole exome sequencing**

| Chr | Start     | End       | Ref | Alt | Gene          | Exonic function | Amino acid change                 | VAF_tumor | VAF_normal |
|-----|-----------|-----------|-----|-----|---------------|-----------------|-----------------------------------|-----------|------------|
| 21  | 36259192  | 36259192  | G   | A   | <i>RUNX1</i>  | Missense SNV    | NM_001754:exon4:c.C299T:p.S100F   | 0.086     | 0          |
| 1   | 151774970 | 151774970 | G   | A   | <i>LINGO4</i> | Missense SNV    | NM_001004432:exon2:c.C211T:p.R71C | 0.032     | 0          |