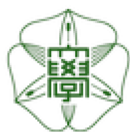

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1 **Acquisition of monosomy 7 and a *RUNX1* mutation in Pearson syndrome**

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26 **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

27 **ABSTRACT**

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

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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.[1,2] The incidence of PS is very

50 low, at approximately one case per million individuals.[3] PS is one of the disorders to

51 be considered in the differential diagnosis of hypocellular BM in young children.[4,5] 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.[6]

54 Monosomy 7 is a common cytogenetic abnormality identified in inherited BM failure

55 syndromes (IBMFS) and pediatric myelodysplastic syndrome (MDS).[7] The basic

56 mechanisms underlying the acquisition of monosomy 7, including haplo-insufficiency

57 and related somatic events, have been explored previously.[8] 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).

METHODS

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

77 calculated as mean depth of mtDNA with deletion detected (MT:8,469–13,446) divided
78 by those without (MT:1–8,468).
79

80 **RESULTS AND DISCUSSION**

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

91 The patient began a series of regular red blood cell transfusions to treat his anemia.
92 At the age of 22 months, BM examination revealed significant hemophagocytosis after
93 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 /μL. 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.[12] 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.[13,14]
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.[4, 15,16]
121 Monosomy 7 occurs during the clonal evolution to MDS/leukemia in a variety of
122 IBMFSs.[7] MDS with monosomy 7 in children has been associated with a high risk of
123 disease progression.[17] 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.[18] *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[7] 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.[19]

133 Acquisition of additional genetic abnormalities predicts disease progression in adult

134 MDS.[20] 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.[21] 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.[22] Somatic

141 mutations in *RUNX1* are reported frequently in association with childhood MDS with

142 monosomy 7.[23] In IBMFSs, including Fanconi anemia and severe congenital

143 neutropenia, the combination of *RUNX1* mutations and monosomy 7 also contribute

144 to myeloid leukemogenesis.[24,25] Of note, a *RUNX1* mutation that has been associated

145 with myeloid malignancies was also identified in our patient. This clone might have

146 had the potential to progress to advanced MDS.

147 The cases of two patients with PS who received unrelated HSCT were previously

148 described in the literatures.[15,16] In one patient, both hematological and non-

149 hematological manifestations resolved in response to this intervention,[16] similar to that

150 observed in our case. PS has features of fatal multisystem dysfunction and

151 spontaneous recovery from anemia within several years of diagnosis. Although HSCT

152 can result in serious complications, it may be a feasible option for patients with severe

153 PS who acquired cytogenetically abnormalities in BM and should be considered in a

154 future prospective clinical trial.

155

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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

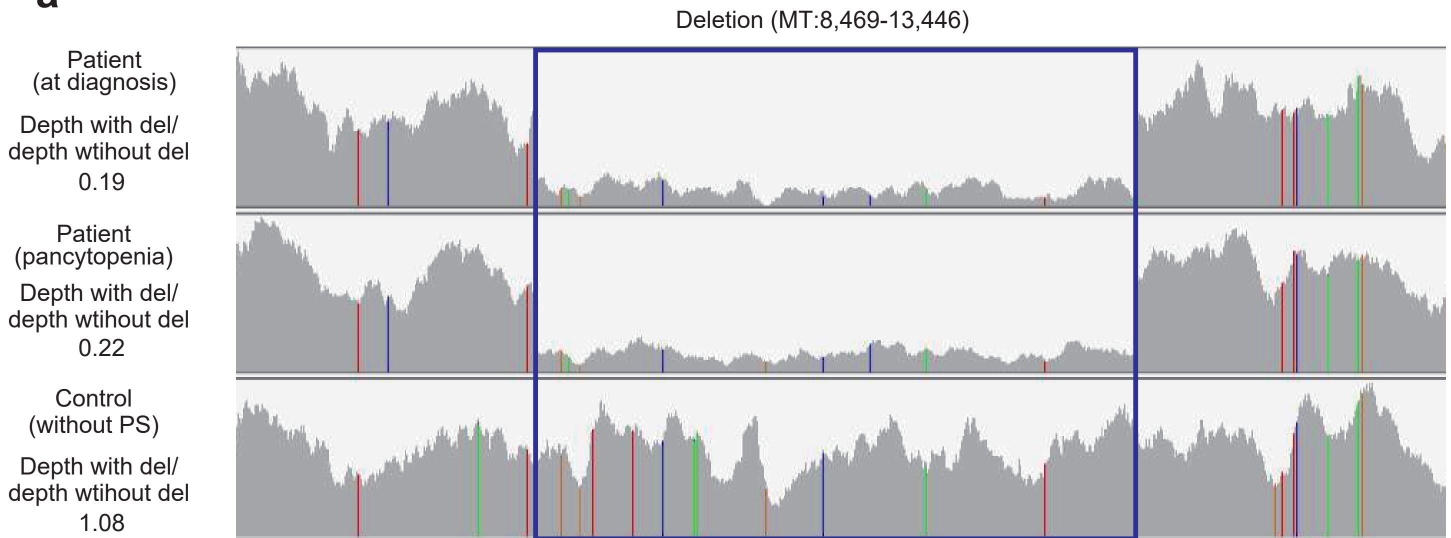
264 reference sequence were called.

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



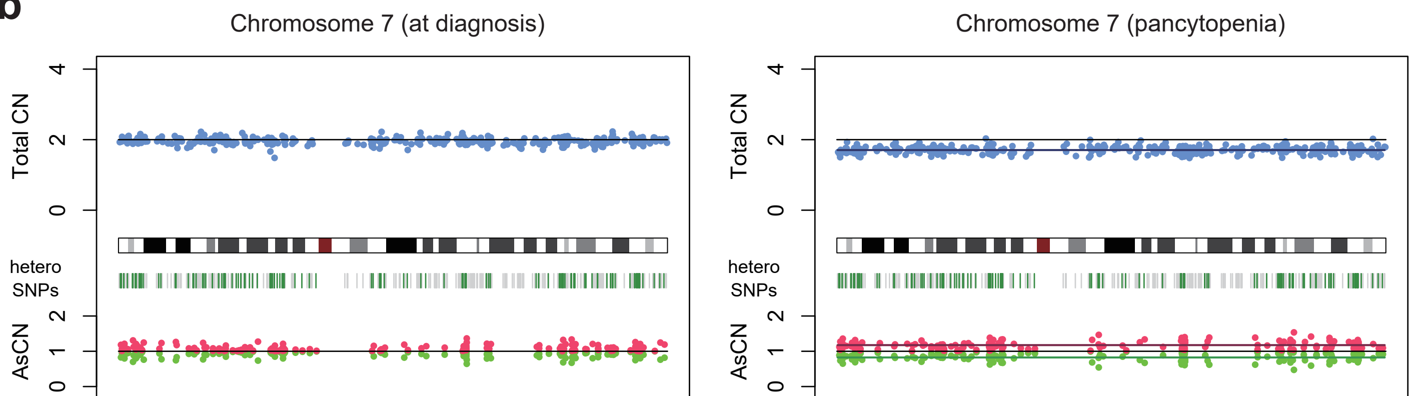


Table 1. Mutations identified by whole exome sequencing

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