

Down syndrome-like acute megakaryoblastic leukemia in a patient with Cornelia de Lange syndrome

Cornelia de Lange Syndrome (CdLS) is a rare autosomal dominant developmental disorder characterized by a distinctive facial dysmorphism and variable developmental anomalies including prenatal and postnatal growth delay, microcephaly, intellectual disability, upper limb anomalies and hirsutism. CdLS is caused by heterozygous loss of function mutations in genes encoding the core components of the cohesin complex, *SMC1A*, *SMC3*, *RAD21* or its regulators, *NIPBL* and *HDAC8*.¹ The cohesin complex is a key player in sister chromatid cohesion, ensuring correct chromosome segregation during mitosis but it also plays an essential role in transcriptional regulation, DNA repair and maintenance of genome stability.² More recently, somatically acquired mutations compromising functions of cohesin complex have been recurrently reported in patients with myeloid malignancies³⁻⁶ and to a less extent in other cancers⁷ supporting a tumor suppressor function of cohesins. However, CdLS is usually not considered as a cancer prone syndrome.

Our patient is the first child of healthy Polynesian parents. At birth (34 weeks of gestation), he presented with intrauterine growth retardation, atrial septal defect, unilateral palpebral ptosis, digital anomalies, hypertrichosis, gastroesophageal reflux and facial features (Figure 1A) leading to a clinical diagnosis of CdLS. However, Sanger sequencing of *NIPBL* and *SMC1A* performed on lymphocyte DNA, 15 days after birth (D15), did not reveal any mutation in these genes. The clinical course was marked by a significant psychomotor delay as the patient had no speech at the age of 3 and did not achieve walking until the age of 2.5 years. As he was hospitalized for febrile seizures at the age of 3, a systematic blood count revealed 3.7 g/dL hemoglobin and 45 x 10⁹/L platelets. Bone marrow cytology reported discrete erythro-

lastopenia and dysmegakaryopoiesis, with the presence of 7% blast cells. Valproate treatment was introduced for seizures. Hematological disorder completely recovered and was assigned to concomitant infection. He developed thrombocytopenia (42x10⁹/L) and anemia (hemoglobin: 8.8 g/dL) again 4 months later. Blood smear showed 36% blasts. Bone marrow examination and flow cytometry analyses evidenced 75% blast cells that displayed the typical morphology and immunophenotypic profile of acute megakaryoblastic leukemia observed in Down syndrome (DS-AMKL) (Figure 1B). Bone marrow karyotype identified trisomy 8, 21 and 22 in all metaphases. FISH analysis evidenced a del(7)(q22) with additional chromosomal material on chromosome 7 and 15. Furthermore, a 1 bp insertion in exon 2 of GATA1 (NM_002049: c.47dup, p.Gln17ProfsX23) was detected by Sanger sequencing of bone marrow cells. Considering the blasts immunophenotype and the presence of a GATA1 mutation predicted to result in the shorter, truncated form called GATA1s typical of DS-AMKL, the patient received dose-reduced chemotherapy according to the I-BFM protocol ML DS 2006. Ten days post induction, he succumbed to a severe sepsis due to *Candida krusei* infection.

Because the causal mutation for the CdLS phenotype was still unidentified, a trio-based exome sequencing (SureSelect Human all exon V5, Agilent) was undertaken on the blood sample obtained at D15. A *de novo* mutation was found in the acceptor splice site of intron 36 of *NIPBL* (NM_015384.4 :c.6344-2A>G, p.?), which is predicted to result in premature truncation leading to loss of cohesin function (Online Supplementary Table S1). The frequency of the mutated *NIPBL* allele was consistent with a mosaic heterozygous mutation in 62% of hematopoietic cells. Indeed, a high and unexplained rate of mosaicism (23%) has been reported in individuals with CdLS which is not associated with any clinical differences or outcome in patients harboring constitutional mosaic mutations.⁸ As *de novo* loss of function mutation in *NIPBL* is the

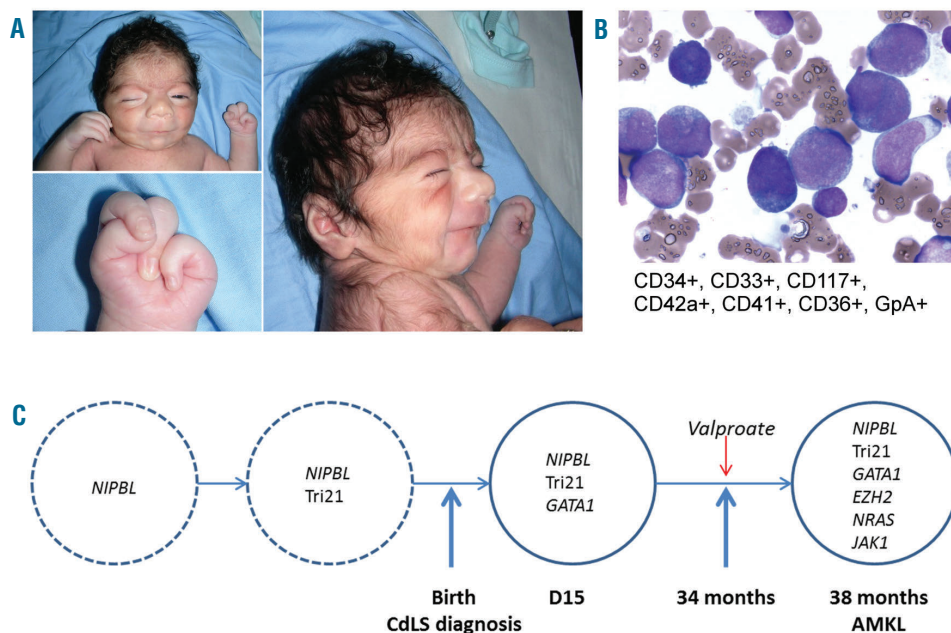


Figure 1. Clinical features, phenotypic aspects of blast cells and natural history of AMKL. (A) Phenotypic characteristics of CdLS in the patient: low anterior hairline, arched eyebrows, synophrys, long philtrum, thin lips, “carp mouth” and ectrodactyly of the left hand. (B) Cytomorphologic and immunophenotypic examination of bone marrow collected at the time of AMKL. The photomicrograph shows a picture compatible with AMKL including both undifferentiated myeloid blasts and megakaryoblasts. Immunophenotyping shows expression of both megakaryocyte and erythroid markers. (C) Proposed model of clonal evolution in this patient. Each circle represents a clone. Dashed lines indicate clones whose presence were not directly assessed but was deduced from experimental data.

most common identifiable cause of CdLS1, we assumed that this *de novo* NIPBL splice mutation was responsible for the CdLS in our patient.

Exome sequencing also revealed that the GATA1 mutation found in the leukemic sample collected at the age of 3 was already present at D15 in 38% of peripheral blood cells (VAF: 38%). Surprisingly, trisomy 21 was also evidenced which was confirmed by CGH array (CGH+SNP 4x180K, Agilent) in 44% of cells, whereas other cytogenetic abnormalities were not present at that stage (*Online Supplementary Table S2*).

To better understand the clonal evolution in our patient, a panel of 148 genes known to be involved in acute leukemia was screened on the D15 and the leukemic samples by next generation sequencing after Custom SureSelectQXT enrichment (Agilent). This analysis confirmed the presence of NIPBL and GATA1 mutations in both samples and revealed 3 additional missense mutations at the stage of overt leukemia: EZH2 (NM_004456 : c.46_48del, p.R16del; VAF: 64 %), NRAS (NM_002524.4:c.436G>A, p.A146T; VAF: 37 %) and JAK1 (NM_002227.2:c.2879C>G, p.P960R; VAF: 30%) in 78%, 74% and 60 % of cells, respectively (*Online Supplementary Table S4*). These specific EZH2 and NRAS mutations have never been reported before but are consistent with recurrent mutations found in DS-AMKL. These mutations were neither found in exome, sequenced with a mean depth of 97X, nor targeted NGS performed on the D15 sample suggesting that AMKL evolved from a pre-existing clone harboring the trisomy 21 and the GATA1 mutation through the acquisition of these additional mutations. Mutation frequencies were consistent with trisomy 21, GATA1, EZH2 and NRAS mutations being present in all megakaryoblasts from which a subclone evolved through the acquisition of JAK1 mutation (Figure 1C).

To our knowledge this is the first report of leukemia in a CdLS patient with a germline cohesin mutation. However, loss of function somatic mutations have frequently been reported in patients with hematopoietic malignancies.^{3,4} Reviewing causes of death in a large cohort of patients with CdLS, Schrier *et al.* showed that cancer accounted for 2% of deaths, highlighting a slightly increased cancer risk in these children.⁹ In this respect, the occurrence of a leukemia and a germline mutation in cohesin could constitute a predisposing factor to leukemia in CdLS patients.

The mechanism by which the loss of cohesin functions supports oncogenesis remains unclear. Some studies have suggested a major role for aneuploidy. In our patient, the NIPBL mutation may have favored the somatic occurrence of trisomy 21 via sister chromatid cohesion defect. However, we cannot rule out the possibility that NIPBL mutation and trisomy 21 are coincidental. Indeed, although cell lines derived from CdLS patients show both spontaneous and induced chromosome aberrations, abnormal karyotypes were not reported so far in CdLS patients. Furthermore, normal karyotypes were found in patients with myeloid neoplasms harboring somatic cohesin mutations, suggesting that cohesin-mutated cells are not clonally selected due to aneuploidy.⁵ If we assume that trisomy 21 is coincidental in our patient, the NIPBL germline mutation could have acted just as an additional oncogenic event, promoting the progression of a pre-leukemic state induced by trisomy 21 and GATA1 mutation to overt AMKL.

With cohesin mutations found in up to 53% of cases,¹⁰ DS-AMKL appears as the leukemia subset showing the more preferential involvement of the cohesins in leuke-

mogenesis. Such an association suggests a strong benefit of the loss of cohesin function in this setting. Several lines of evidence suggest a specific role of cohesins in megakaryopoiesis. Cohesins regulate the expression of genes involved in hematopoiesis and megakaryocytic lineage commitment such as RUNX1^{11,12} and thrombocytopenia was recurrently reported in CdLS patients, with progression to pancytopenia and death in 2 patients.¹³ Perturbation of megakaryocytic development by germline cohesin mutation may thus render progenitor cells more susceptible to leukaemic transformation. In this respect, it is interesting to note that the clinical and biological presentation of the leukemia in our CdLS patient harbors all the characteristics of DS-AMKL. Children with DS have a 500-fold increased risk of developing acute AMKL¹⁴ preceded by a transient pre-leukemic syndrome in the neonatal period; Transient Abnormal Myelopoiesis (TAM) caused by cooperation between constitutional trisomy 21 and somatic truncating mutations in GATA1 acquired during fetal development. Our patient also presented with a transient disorder, although in our case we cannot exclude the possibility that the treatment of seizures with valproate, an inhibitor of histone deacetylase shown to improve myelodysplastic syndrome,¹⁵ did not contribute to remission. Recent studies suggest that trisomy 21 and GATA1 mutation are sufficient for clonal expansion of DS-associated TAM but additional driver mutations are necessary for DS-AMKL progression with a high frequency of mutations in cohesins, but also in the epigenetic regulator EZH2 and activating mutations in the RAS and JAK signaling pathways.^{10,16} Strikingly, our patient developed a pre-leukemic clone combining the germline NIPBL mutation with somatically acquired trisomy 21 and GATA1 mutation, a combination which is typical of DS-AMKL although in this latter situation, trisomy 21 is the germline event and NIPBL mutation is somatically acquired. The finding of trisomy 21 and GATA1 mutations as soon as D15 is consistent with early events acquired during fetal life, as previously demonstrated for DS-AMKL. Furthermore, with mutations targeting EZH2, RAS and JAK, CdLS-AMKL and DS-AMKL share the same spectrum of driver mutations underscoring close leukemogenesis, although in a different temporal hierarchy. Our observation reinforces the idea that unique mutational combinations lead to specific AML sub-types, and further suggests that this is independent of their order of acquisition.

There are two implications of this observation in terms of clinical management. Firstly, DS-AMKL is distinct from other subtypes of AMKL with respect to genetics and outcome. Therefore, given that the genetics of CdLS-AMKL is similar to DS-AMKL, one may wonder if it would respond to the same chemotherapy regimen. Secondly, it raises awareness about the risk of leukemia in patients possibly associated with CdLS. Waiting for further epidemiologic studies in large cohort to better evaluate the cancer risk of these patients, we suggest a regular clinical and biological management based on systematic blood analyses as previously recommended for monitoring on thrombocytopenia in these patients.¹³

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