COLD SPRING HARBOR
Molecular Case Studies

RESEARCH REPORT

Perinatal-lethal nonimmune fetal hydrops attributed to *MECOM*-associated bone marrow failure

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Abstract Pathogenic variants in *MECOM*, a gene critical to the self-renewal and proliferation of hematopoietic stem cells, are known to cause a rare bone marrow failure syndrome associated with amegakaryocytic thrombocytopenia and bilateral radioulnar synostosis known as RUSAT2. However, the spectrum of disease seen with causal variants in *MECOM* is broad, ranging from mildly affected adults to fetal loss. We report two cases of infants born preterm who presented at birth with symptoms of bone marrow failure including severe anemia, hydrops, and petechial hemorrhages; radioulnar synostosis was not observed in either patient, and, unfortunately, neither infant survived. In both cases, genomic sequencing revealed de novo variants in *MECOM* considered to be responsible for their severe presentations. These cases add to the growing body of literature that describe *MECOM*-associated disease, particularly *MECOM* as a cause of fetal hydrops due to bone marrow failure in utero. Furthermore, they support the use of a broad sequencing approach for perinatal diagnosis, as *MECOM* is absent from available targeted gene panels for hydrops, and highlight the importance of postmortem genomic investigation.

[Supplemental material is available for this article.]

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INTRODUCTION

Bone marrow failure syndromes are rare conditions that, when responsible for congenital anemia, may manifest as nonimmune fetal hydrops (Adam et al. 2017; Quinn et al. 2021). A specific combination of bone marrow failure and bilateral radioulnar synostosis, RUSAT2 (radioulnar synostosis with amegakaryocytic thrombocytopenia 2, MIM #616378), has been linked to damaging variants in *MECOM*, comprised of complex loci *MDS1* (myelodysplastic syndrome 1) and *EV11* (ecotropic viral integration site 1) (Niihori et al. 2015; Bluteau et al. 2018; Germeshausen et al. 2018; Lord et al. 2018; Loganathan et al. 2019; Lozano Chinga et al. 2023). *MECOM* encodes multiple protein isoforms through the alternative splicing of these loci, is expressed in hematopoietic stem cells (HSCs), and plays a role in regulating HSC self-renewal (Bard-Chapeau et al. 2012; Kataoka and Kurokawa 2012). Although

the RUSAT phenotype was first associated with variants in *HOXA11* (Thompson and Nguyen 2000), subsequent genomic analyzes of individuals with RUSAT without evidence of aberration in *HOXA11* identified damaging variants in *MECOM*, leading to the delineation of RUSAT2 as a distinct condition (Niihori et al. 2015). Variations in the classical presentation of RUSAT2 have been reported, with certain individuals harboring damaging *MECOM* variants manifesting with bone marrow failure without radioulnar synostosis; other features such as hand abnormalities, heart defects, renal malformations, and sensorineural deafness have also been reported (Germeshausen et al. 2018; Lozano Chinga et al. 2023). Thus, disease-causing variants in *MECOM*, which are transmitted in an autosomal dominant pattern, are associated with a phenotypic spectrum encompassing RUSAT2 in addition to variable degrees of bone marrow failure without radioulnar synostosis (Lozano Chinga et al. 2023).

Although *MECOM*-associated syndromes have previously been associated with nonlethal fetal hydrops (Niihori et al. 2015), in addition to other severe manifestations leading to early neonatal death (van der Veken et al. 2018), reports of fetal hydrops attributed to pathogenic variants in *MECOM* are rare. We therefore describe two cases of congenital bone marrow failure for whom genomic sequencing identified disease-causing variants in *MECOM*. The first neonate (P1) was delivered preterm with significant hydrops, anemia, hypotension, and acidosis that proved lethal at <12 h of life. Postmortem trio exome sequencing (ES) revealed a de novo splice impacting variant in *MECOM*. The second patient (P2) presented on the first day of life with severe anemia, thrombocytopenia, significant hydrops, hypotension, and metabolic acidosis. In this case, trio rapid genome sequencing (GS) revealed a de novo missense variant in *MECOM*. Both variants have been previously reported in people with *MECOM*-associated syndromes (Yoshida et al. 2010; Niihori et al. 2015, 2022). Notably, neither infant was reported to have radioulnar synostosis, nor was a *MECOM*-associated syndrome suspected clinically prior to the molecular diagnosis.

RESULTS

Clinical Presentation

P1 was born at 29 6/7 wk of gestation via emergent cesarean section because of sinusoidal fetal heart tracing. She was born to a 35-yr-old gravida 2, para 1 mother with an unremarkable prenatal history. Family history was noncontributory. The infant emerged pale, limp, apneic, and with significant bruising all over her body. Pitting skin edema was observed around the head and the neck, notable as a sign of early hydrops. She was ultimately intubated, with initial point-of-care blood gas analysis revealing severe metabolic acidosis (arterial pH of 6.9) as well as severe anemia, with undetectable hemoglobin. A complete blood count obtained after admission to the neonatal intensive care unit (NICU) revealed leukopenia with a white blood cell count of 4000 cells/μL, significant anemia with hemoglobin of 1.4 g/dL and hematocrit of 5.0%, and thrombocytopenia with platelets of 5000 cells/μL. Serum bicarbonate was measured at 3 mmol/L with anion gap of 38.0 mmol/L. The infant received normal saline boluses and bicarbonate boluses for her acidosis and packed red blood cell transfusions for her anemia. She was also started on prophylactic antibiotics.

In the NICU, the infant was noted to be hydropic, hypotensive, coagulopathic, and extremely pale with extensive bruising of the lower extremities and diffuse petechiae over the chest, in addition to diffuse, blue-appearing small macules along the chest and back. A detailed examination for possible dysmorphic features was limited by the infant's critical illness and edema. A head ultrasound revealed neither intracranial hemorrhage nor intraventricular hemorrhage and no calcifications were present, but gray matter heterotopia were noted in the right occipital lobe. Additionally, slight simplification and dysmorphia of the

sulci was noted. These findings were interpreted as suggesting a genetic etiology to her illness or as representative of infectious sequelae.

The infant continued to experience worsening hypotension, disseminated intravascular coagulation, and profound hypoxia with subsequent bradycardia. Because of the low likelihood of meaningful recovery in light of her multisystem disease, resuscitation was halted and the infant was compassionately extubated. Postmortem examination revealed skin, pleural, and peritoneal surfaces with confluent petechial hemorrhages, consistent with disseminated intravascular coagulation. Pale organs were noted, consistent with anemia. The spleen displayed evidence of extramedullary hematopoiesis, while the liver demonstrated diminished extramedullary hematopoiesis with myeloid predominance. Right atrial and ventricular dilation of the heart was seen, consistent with heart failure. Of note, the musculoskeletal system and extremities, including the forearms, had no observed anomalies. An infectious etiology was thought to be less likely because of negative lung, spleen, and placental cultures, in addition to negative TORCH (toxoplasmosis, rubella, cytomegalovirus, and herpes/HIV) titers in the mother. Rather, given the diminished red blood cell hematopoiesis in the liver, a disorder affecting red blood cell turnover was suspected. The family was enrolled in a research study for postmortem genomic sequencing.

P2 was also born at 29 6/7 wk of gestation via emergent cesarean section because of sinusoidal fetal heart tracing shortly after attempted induction of labor for nonreassuring fetal heart tracing. He was born to a 36-yr-old gravida 7, para 4 → 5 mother (two prior losses, one was spontaneous). This was the first pregnancy with her current partner. The prenatal course was complicated by pre-eclampsia, smoking, maternal obesity with impaired glucose tolerance, and macrosomia. Family history was unremarkable. The infant emerged pale and limp, with poor respiratory effort and associated bradycardia, as well as with progressive abdominal distension and significant bruising all over his body. He received bag-mask ventilation in the delivery room with improvement in heart rate and oxygen saturation, but not color. He was therefore admitted to the NICU at his birth hospital on continuous positive airway pressure (CPAP). Progressive dependent edema was noted over the first hour of life. His initial complete blood count revealed hemoglobin of 2.3 g/dL and platelet count of 5000 cells/μL. Multiple blood products were transfused emergently and empiric antibiotics were started. He became progressively more edematous, ultimately requiring intubation. The patient was then transported to a level IV NICU for further evaluation and management, where capillary blood gas on arrival at 2 h of life was notable for pH 6.9, pCO_2 65 mmHg, bicarbonate 15 mmol/L, and hemoglobin of 9.5 g/dL. The lactate was measured at 10.7 mmol/L. He became coagulopathic, with spontaneous bleeding from venipuncture sites, and hypotensive, requiring inotropic support. Postnatal ultrasound demonstrated bilateral pleural effusions and four-quadrant abdominal ascites. Although he was initially difficult to oxygenate and ventilate, despite surfactant administration, he was ultimately stabilized on the ventilator.

On postnatal exam, the infant was noted to have a large anterior fontanelle and a left clubfoot but was otherwise felt to be nondysmorphic in the setting of a limited exam. There was no evidence of hemolysis on his peripheral blood smear. Extensive infectious evaluation was negative. His total bilirubin rose to 18.8 mg/dL (direct 2.5 mg/dL) at 4 d of life despite maximal phototherapy and empiric intravenous immunoglobulin, and he subsequently received a single volume exchange transfusion. Initial head ultrasound was normal, but repeat imaging at 1 wk of life revealed left grade IV intraventricular hemorrhage as well as ipsilateral venous hemorrhagic infarction with mass effect. Neurosurgical intervention could not be pursued because of his continued thrombocytopenia. Thereafter, the child settled into a chronic course of daily platelet transfusions and weekly red cell transfusions. At 1 mo of life, the infant was noted to be profoundly neutropenic and granulocyte colony-stimulating factor was trialed without benefit. He subsequently developed necrotizing

enterocolitis and bacteremia with a rapidly progressive decline. Profiling of trihexoside, sulfatide, glycosaminoglycan, and oligosaccharides; peripheral blood cell telomere length; a 130-gene nonimmune fetal hydrops panel; and chromosomal microarray were nondiagnostic. A diagnosis was ultimately identified via research rapid GS (see below) and confirmed on clinical rapid trio ES. In the setting of his molecular diagnosis and anticipated prognosis, the family elected for palliative extubation and the infant died shortly thereafter. Autopsy was declined. Of note, although radiographs of forearms were not obtained, there were no noted limitations in their range of motion despite manipulation for multiple procedures.

Genomic Analyses

P1

Trio research ES performed postmortem revealed a de novo splice impacting variant (see Table 1). This finding was validated by Sanger sequencing and has been previously reported using a different transcript (NM_001105078:c.2208-4A > G), with functional evaluation in this report demonstrating an in-frame single amino acid insertion (Niihori et al. 2022). It was interpreted as likely pathogenic via American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines (PM2, PP1, PP3, PS2, and PS4) (Richards et al. 2015).

P2

Trio research ultra-rapid GS revealed a revealed a de novo missense variant within the highly conserved eighth zinc finger motif of *MECOM* (see Table 1). The finding was confirmed by Sanger sequencing and later by clinical rapid ES and has been previously reported (Yoshida et al. 2010; Niihori et al. 2015). This variant was interpreted as pathogenic via ACMG/AMP guidelines (PM2, PP5, and PS2).

DISCUSSION

We describe two infants born preterm in the setting of severe fetal anemia leading to hydrops with clinical features of a bone marrow failure syndrome noted after birth. In both cases, genomic sequencing (ES or GS) revealed variants in *MECOM* to which these severe and lethal presentations were attributed. Disease-causing variants in *MECOM* are associated with a bone marrow failure syndrome typically characterized by early-onset thrombocytopenia that progresses into pancytopenia and is accompanied by radioulnar synostosis, though variable expressivity is well-described (Niihori et al. 2015; Lozano Chinga et al. 2023). Although mildly affected individuals with pathogenic variants in *MECOM* have been

Table 1. Variants identified

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP ID	ClinVar ID	Genotype	Parent of origin
MECOM	3	c.2772-4A>G NM_004991.4	N/A	Splice region variant	Creation of new splice acceptor leading to 3-bp insertion	N/A	2500844 SCV003922107.1	Heterozygous	De novo
MECOM	3	c.2812C>T NM_004991.4	p.Arg938Trp NP_004982.2	Missense		rs864309724	218953 SCV001523713.1	Heterozygous	De novo

reported, including those with the radial findings only and no bone marrow failure (Germeshausen et al. 2018), our cases highlight the lethal nature of this disorder as well as its possible manifestation as fetal hydrops.

MECOM is located at chromosomal position 3q26 and encodes a zinc finger transcription factor (Germeshausen et al. 2018); alternative splicing after transcription yields proteins containing only *EVI1* exons, only *MDS1* exons, or a combination product (MDS1–EVI1) (Métais and Dunbar 2008). EVI1 is believed to play an essential part in hemopoietic stem cell activity, as supported by murine experiments (Yuasa et al. 2005). *Mecom* expression has also been identified in high levels to the urinary system, lungs, heart, and limb buds of mice embryos (Perkins et al. 1991), suggesting that pathogenic variants in this gene may lead to multiorgan anomalies (Lozano Chinga et al. 2023). Variants in *MECOM* leading to a bone marrow failure syndrome were first identified in three individuals with radioulnar synostosis and thrombocytopenia, two of whom presented at birth with severe anemia, similar to our cases (Niihori et al. 2015). Although this report initially emphasized the RUSAT2 phenotype and its association with *MECOM* variants, additional cases identified have revealed more phenotypic variation. In a study of 179 people with suspected bone marrow failure syndromes, six were found to have *MECOM* variants, with all displaying severe neonatal aplastic anemia, but only one with radioulnar synostosis. Other phenotypes reported in this cohort included different skeletal abnormalities (clubfoot, thumb anomalies), cardiac anomalies (tetralogy of Fallot, myocardial atrophy, and pulmonary stenosis), renal anomalies (hypoplasia), and facial dysmorphism (Bluteau et al. 2018). Another description of 12 individuals with *MECOM*-associated bone marrow failure syndromes reports seven with no observable radioulnar anomalies, although additional anomalies included hand malformations in six, congenital heart disease in three, renal malformations in two, sensorineural deafness in three, and hormonal disturbances (precocious puberty) in one (Germeshausen et al. 2018). A more recent cohort of eight individuals with *MECOM*-associated syndrome similarly reported a broad spectrum of both hematologic manifestations as well as other anomalies (Lozano Chinga et al. 2023). Genotype–phenotype correlations remain unclear, with variable manifestations seen both in missense variants and those presumed to result in haploinsufficiency (Niihori et al. 2015; Germeshausen et al. 2018; Lozano Chinga et al. 2023). A prior report has demonstrated mosaic variants in *MECOM* to manifest without radioulnarsynostosis (Osumi et al. 2018), although there was no evidence of mosaicism in the cases that we present. We also acknowledge the difficulty in discerning the radioulnar findings in these cases involving preterm birth and early death.

Correlation between *MECOM* genotype and severity of disease is similarly not apparent, and intrafamilial variability has also been observed. In a recent report, an infant presented with macrocytic anemia, thrombocytopenia, and bilateral clubfeet noted immediately after birth and became transfusion-dependent. Her mother, who shared the same pathogenic *MECOM* splice-site variant, only had mild radioulnar synostosis, leukopenia, and thrombocytopenia (Niihori et al. 2022). In another family, although one child had anemia and thrombocytopenia noted after birth, her father and brother, who shared a *MECOM* variant with her, solely displayed skeletal abnormalities (radioulnar synostosis in the father and clinodactyly in the brother) (Niihori et al. 2022). A possible explanation put forth in the report is that this phenotypic variation was due to differing variant allele frequencies and copy neutral loss of heterozygosity (Niihori et al. 2022). However, explanations for phenotypic differences between individuals sharing the same *MECOM* variant remain speculative, and more research is needed into the cause of variable expressivity seen with *MECOM*-related disease.

Given these challenges in relying on the presence of radioulnar synostosis to raise suspicion for *MECOM*-associated bone marrow failure, our cases in particular highlight the value in a broad sequencing approach to investigate nonimmune hydrops fetalis (NIHF). Potential etiologies underlying NIHF are broad and involve multiple possible organ systems and

classes of disorders, from RASopathies to lysosomal storage diseases to congenial aplastic anemia, as others have described (Al-Kouatly et al. 2021; Quinn et al. 2021; Wagner et al. 2022). A recent study of a cohort of 127 neonates with NIHF and molecular diagnoses identified by ES found that only about half of the 29 pathogenic variants would have been identified on NIHF-specific gene panels (Norton et al. 2022). Of note, none of the pathogenic variants in this cohort were in *MECOM* (Sparks et al. 2020). Additionally, a recent systematic review listing genes associated with NIHF also does not include *MECOM* (Quinn et al. 2021), highlighting the diversity of bone marrow failure syndromes that might lead to NIHF and the drawbacks of relying upon a selective sequencing approach for diagnosis. Our own review of current commercially available gene panels for NIHF (conducted in March, 2023) did not identify a single test that includes *MECOM*, although as noted in a prior systematic review, several of these panels include genes never reported in the literature to cause NIHF (Quinn et al. 2021).

Importantly, although the extremely early presentation of bone marrow failure in both of our cases, coupled with the challenges of managing this condition in an infant born at 29 wk of gestation, limited their chances at survival, *MECOM*-related bone marrow failure has been successfully treated with transfusions or hemopoietic stem cell transplant when identified in older infants, even those presenting with congenital anemia and hydrops (Irie et al. 2022). Thus, it is possible that future neonates with a history of NIHF may benefit from early identification of this diagnosis. Nonetheless, genetic verification of the causative variant observed in our cases, even postmortem as in P1, was beneficial for numerous reasons. First, it confirmed that both variants were de novo in the probands: valuable information for their families' reproductive counseling and assessment of recurrence risks. In P2, understanding of the prognosis for this condition aided in the decision to redirect goals of care. Finally, recognizing these cases and their shared features will ideally inform future approaches to diagnosis in the setting of NIHF and provide further justification for the use of ES or GS, rather than gene panels, in order to identify a molecular explanation for this phenotype.

METHODS

P1 and both parents were enrolled into the Manton Center for Orphan Disease Research at Boston Children's Hospital. Trio ES for P1 was performed by the Genomics Platform at the Broad Institute of MIT and Harvard. Libraries from DNA samples were created with an Illumina exome capture (37 Mb target) and sequenced (150-bp paired reads) to cover >85% of targets at >20×, comparable to ~55× mean coverage (see Supplemental Table). Sample identity quality assurance checks were performed on each sample. The ES data was demultiplexed and each sample's sequence data was aggregated into a single Picard CRAM file. Variants were called using Genome Analysis Toolkit (GATK) and uploaded to seqr for analysis (Pais et al. 2022). The *MECOM* variant was orthogonally confirmed by Sanger sequencing.

P2 received clinical rapid ES from Baylor Genetics and concurrent research ultra-rapid GS at the Steve and Cindy Rasmussen Institute for Genomic Medicine. Libraries from DNA samples were generated using the NEB Ultra FS kit per manufacturer protocol and sequenced on a NovaSeq 6000 S1 flow cell (150 bp paired-end reads). Genome-wide mean coverage of 79.6× was achieved, with >97% of 232,175 coding exons covered at >20×. Parental samples were also sequenced, achieving mean coverages of 38.5× and 40.0×. Secondary analysis was performed using Churchill (Kelly et al. 2015), which includes alignment to GRCh38 reference genome was performed via bwa mem (v0.7.17), deduplication QC including sample identity and provenance, and SNV, INDEL, and CNV calling via GATK (v4.1.6). Variant calls

were annotated, filtered, and interpreted using an in-house variant and annotation warehouse.

ADDITIONAL INFORMATION

Data Deposition and Access

For P1, The Broad CMG shares raw sample genotype data (crams), phenotype data (high level and HPO terms), and causal and candidate gene/variant information. Raw sequence data is uploaded to AnVIL (https://anvilproject.org/data/). Data access applications are through dbGaP (http://www.ncbi.nlm.nih.gov/gap) Study Accession phs001272.v1.p1. Data in this manuscript can be found in project AnVIL_CMG_Broad_Orphan_Manton_WES. This variant may be found on ClinVar (SCV003922107.1): https://www.ncbi.nlm.nih.gov/clinvar/variation/2500844.

For P2, pursuant to the IRB governing generation of the research ultra-rapid GS data, sequencing, phenotypic, and diagnostic variant data is retained by the Steve and Cindy Rasmussen Institute for Genomic Medicine and may be shared, in a deidentified manner, with investigators subject to appropriate IRB approvals. This variant may be found on ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/variation/218953/.

Ethics Statement

P1 was enrolled in an Institutional Review Board (IRB)-approved protocol for the Manton Center for Orphan Disease Research at Boston Children's Hospital, with written, informed consent obtained from both parents. P2 was enrolled in an IRB-approved protocol at the Nationwide Children's Hospital Steve and Cindy Rasmussen Institute for Genomic Medicine, with written, informed consent obtained from both parents.

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Author Contributions

C.A.D., M.H.W., and B.P.C. conceptualized the study. C.A.D., M.H.W., B.P.C., P.B.A., and J.A.M. were involved in data collection and analysis. C.A.D. and M.H.W. drafted the initial manuscript. M.M., M.R., and S.D.W. provided additional clinical data and critically reviewed the manuscript. All authors have approved the current version of the manuscript and its submission to *CSH Molecular Case Studies*.

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Competing Interest Statement

The authors have declared no competing interest.

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REFERENCES

Adam S, Melguizo Sanchis D, El-Kamah G, Samarasinghe S, Alharthi S, Armstrong L, Lako M. 2017. Concise review: getting to the core of inherited bone marrow failures. *Stem Cells* **35:** 284–298. doi:10.1002/stem.2543

Al-Kouatly HB, Makhamreh MM, Rice SM, Smith K, Harman C, Quinn A, Valcarcel BN, Firman B, Liu R, Hegde M, et al. 2021. High diagnosis rate for nonimmune hydrops fetalis with prenatal clinical exome from the hydrops-yielding diagnostic results of prenatal sequencing (HYDROPS) study. *Genet Med* **23:** 1325–1333. doi:10.1038/s41436-021-01121-0

Bard-Chapeau EA, Jeyakani J, Kok CH, Muller J, Chua BQ, Gunaratne J, Batagov A, Jenjaroenpun P, Kuznetsov VA, Wei CL, et al. 2012. Ecotopic viral integration site 1 (EVI1) regulates multiple cellular processes important for cancer and is a synergistic partner for FOS protein in invasive tumors. *Proc Natl Acad Sci* **109:** 2168–2173. doi:10.1073/pnas.1119229109

Bluteau O, Sebert M, Leblanc T, Peffault de Latour R, Quentin S, Lainey E, Hernandez L, Dalle JH, Sicre de Fontbrune F, Lengline E, et al. 2018. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood* **131:** 717–732. doi:10.1182/blood-2017-09-806489

Germeshausen M, Ancliff P, Estrada J, Metzler M, Ponstingl E, Rutschle H, Schwabe D, Scott RH, Unal S, Wawer A, et al. 2018. *MECOM*-associated syndrome: a heterogeneous inherited bone marrow failure syndrome with amegakaryocytic thrombocytopenia. *Blood Adv* **2:** 586–596. doi:10.1182/bloodadvances.2018016501

Irie M, Niihori T, Nakano T, Suzuki T, Katayama S, Moriya K, Niizuma H, Suzuki N, Saito-Nanjo Y, Onuma M, et al. 2022. Reduced-intensity conditioning is effective for allogeneic hematopoietic stem cell transplantation in infants with *MECOM*-associated syndrome. *Int J Hematol* **17:** 598–606. doi:10.1007/s12185-022-03505-7

Kataoka K, Kurokawa M. 2012. Ecotropic viral integration site 1, stem cell self-renewal and leukemogenesis. *Cancer Sci* **103:** 1371–1377. doi:10.1111/j.1349-7006.2012.02303.x

Kelly BJ, Fitch JR, Hu Y, Corsmeier DJ, Zhong H, Wetzel AN, Nordquist RD, Newsom DL, White P. 2015. Churchill: an ultra-fast, deterministic, highly scalable and balanced parallelization strategy for the discovery of human genetic variation in clinical and population-scale genomics. *Genome Biol* **16:** 6. doi:10.1186/s13059-014-0577-x

Loganathan A, Munirathnam D, Ravikumar T. 2019. A novel mutation in the *MECOM* gene causing radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT-2) in an infant. *Pediatr Blood Cancer* **66:** e27574. doi:10.1002/pbc.27574

Lord SV, Jimenez JE, Kroeger ZA, Patrick CS, Sanchez-Pena I, Ziga E, Bademci G, Tekin M. 2018. A *MECOM* variant in an African American child with radioulnar synostosis and thrombocytopenia. *Clin Dysmorphol* **27:** 9–11. doi:10.1097/MCD.0000000000000200

Lozano Chinga MM, Bertuch AA, Afify Z, Dollerschell K, Hsu JI, John TD, Rao ES, Rowe RG, Sankaran VG, Shimamura A, et al. 2023. Expanded phenotypic and hematologic abnormalities beyond bone marrow failure in *MECOM*-associated syndromes. *Am J Med Genet A* **191:** 1826–1835. doi:10.1002/ajmg.a.63208

Métais JY, Dunbar CE. 2008. The MDS1-EVI1 gene complex as a retrovirus integration site: impact on behavior of hematopoietic cells and implications for gene therapy. *Mol Ther* **16:** 439–449. doi:10.1038/sj.mt.6300372

Niihori T, Ouchi-Uchiyama M, Sasahara Y, Kaneko T, Hashii Y, Irie M, Sato A, Saito-Nanjo Y, Funayama R, Nagashima T, et al. 2015. Mutations in MECOM, encoding oncoprotein EVI1, cause radioulnar synostosis with amegakaryocytic thrombocytopenia. *Am J Hum Genet* **97:** 848–854. doi:10.1016/j.ajhg.2015.10.010

Niihori T, Tanoshima R, Sasahara Y, Sato A, Irie M, Saito-Nanjo Y, Funayama R, Shirota M, Abe T, Okuyama Y, et al. 2022. Phenotypic heterogeneity in individuals with *MECOM* variants in 2 families. *Blood Adv* **6:** 5257–5261. doi:10.1182/bloodadvances.2020003812

Norton ME, Ziffle JV, Lianoglou BR, Hodoglugil U, Devine WP, Sparks TN. 2022. Exome sequencing vs targeted gene panels for the evaluation of nonimmune hydrops fetalis. *Am J Obstet Gynecol* **226:** 128.e1–128.e11. doi:10.1016/j.ajog.2021.07.014

Osumi T, Tsujimoto SI, Nakabayashi K, Taniguchi M, Shirai R, Yoshida M, Uchiyama T, Nagasawa J, Goyama S, Yoshioka T, et al. 2018. Somatic MECOM mosaicism in a patient with congenital bone marrow failure without a radial abnormality. *Pediatr Blood Cancer* **65:** e26959. doi:10.1002/pbc.26959

Pais LS, Snow H, Weisburd B, Zhang S, Baxter SM, DiTroia S, O'Heir E, England E, Chao KR, Lemire G, et al. 2022. seqr: a web-based analysis and collaboration tool for rare disease genomics. *Hum Mutat* **43:** 698–707. doi:10.1002/humu.24366

Perkins AS, Mercer JA, Jenkins NA, Copeland NG. 1991. Patterns of Evi-1 expression in embryonic and adult tissues suggest that Evi-1 plays an important regulatory role in mouse development. *Development* **111:** 479–487. doi:10.1242/dev.111.2.479

Quinn AM, Valcarcel BN, Makhamreh MM, Al-Kouatly HB, Berger SI. 2021. A systematic review of monogenic etiologies of nonimmune hydrops fetalis. *Genet Med* **23:** 3–12. doi:10.1038/s41436-020-00967-0

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* **17:** 405–424. doi:10.1038/gim.2015.30

Sparks TN, Lianoglou BR, Adami RR, Pluym ID, Holliman K, Duffy J, Downum SL, Patel S, Faubel A, Boe NM, et al. 2020. Exome sequencing for prenatal diagnosis in nonimmune hydrops fetalis. *N Engl J Med* **383:** 1746–1756. doi:10.1056/NEJMoa2023643

Thompson AA, Nguyen LT. 2000. Amegakaryocytic thrombocytopenia and radio-ulnar synostosis are associated with *HOXA11* mutation. *Nat Genet* **26:** 397–398. doi:10.1038/82511

van der Veken LT, Maiburg MC, Groenendaal F, van Gijn ME, Bloem AC, Erpelinck C, Groschel S, Sanders MA, Delwel R, Bierings MB, et al. 2018. Lethal neonatal bone marrow failure syndrome with multiple congenital abnormalities, including limb defects, due to a constitutional deletion of 3′ MECOM. *Haematologica* **103:** e173–e176. doi:10.3324/haematol.2017.185033

Wagner T, Fahham D, Frumkin A, Shaag A, Yagel S, Yanai N, Porat S, Mor-Shaked H, Meiner V, Daum H. 2022. The many etiologies of nonimmune hydrops fetalis diagnosed by exome sequencing. *Prenat Diagn* **42:** 881–889. doi:10.1002/pd.5977

Yoshida H, Hashii Y, Okuda T, Kusuki S, Sato E, Inoue A, Kawakami C, Yabe M, Ohta H, Ozono K. 2010. A case of congenital bone marrow failure with radio-ulnar synostosis. *Int J Hematol* **91:** 331–332. doi:10.1007/s12185-010-0494-z

Yuasa H, Oike Y, Iwama A, Nishikata I, Sugiyama D, Perkins A, Mucenski ML, Suda T, Morishita K. 2005. Oncogenic transcription factor *Evi1* regulates hematopoietic stem cell proliferation through *GATA-2* expression. *EMBO J* **24:** 1976–1987. doi:10.1038/sj.emboj.7600679