## Lethal neonatal bone marrow failure syndrome with multiple congenital abnormalities, including limb defects, due to a constitutional deletion of 3' *MECOM*

EVI1 (3q26.2) is a member of the SET/PR domain family of transcription factors and contains two domains of zinc finger (ZF) DNA binding motifs, one at the N-terminus (ZF1) and one at the C-terminus (ZF2), and a C-terminal acidic amino acid cluster domain (AD). The *MDS1/EVI1* locus gives rise to alternatively spliced variants, including intergenic *MDS1 EVI1* complex (*MECOM*) splicing. EVI1 is pivotal in leukemogenesis and mammalian development. *EVI1* over-expression is associated with the development and progression of high-risk acute myeloid leukemia (AML). EVI1/MECOM is a transcriptional regulator essential for maintaining embryonic and adult hematopoietic stem cells (HSCs) by directly regulating transcription of GATA2. In mouse models, homozygous disruption of *Evi1* results in embryonic lethality, with hypocellular bone marrow, reduced body size, small or absent limb buds, and abnormal development of the nervous system. *Evi1*$^{+/-}$ mice showed an intermediate phenotype compared to *Evi1*$^{-/-}$ mice both in fetal liver (FL) hematopoiesis as well as in adult hematopoiesis, suggesting a *Evi1* gene dosage requirement in the regulation of HSCs.[1]

To date, two case reports have been published on heterozygous copy number abnormalities (CNA) affecting *MECOM*. One patient, presenting with congenital thrombocytopenia, had a deletion which included the entire *MECOM* locus.[2] A second newborn presenting with respiratory problems, low birthweight, congenital thrombocytopenia, and slight facial dysmorphisms, had a deletion of the first two exons of MDS1.[3] Furthermore, heterozygous missense single nucleotide variations (SNVs) within the eighth zinc finger motif of ZF2 of *MECOM* were published in three simplex cases with radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT [Online Mendelian Inheritance in Man: 065432]), an inherited bone marrow failure (BMF) syndrome with skeletal anomalies.[4] Very recently, Ripperger *et al*. reported on a three generation familial SNV affecting the ninth zinc finger motif of ZF2 in four members with limb dysmorphisms, and thrombocytopenia in one member. Interestingly, two affected family members developed myeloid neoplasia later in life.[5] Furthermore, of late Bluteau *et al*. described six simplex cases; three with mutations affecting ZF2 and three with frameshift mutations, all associated with hypocellular bone marrow,

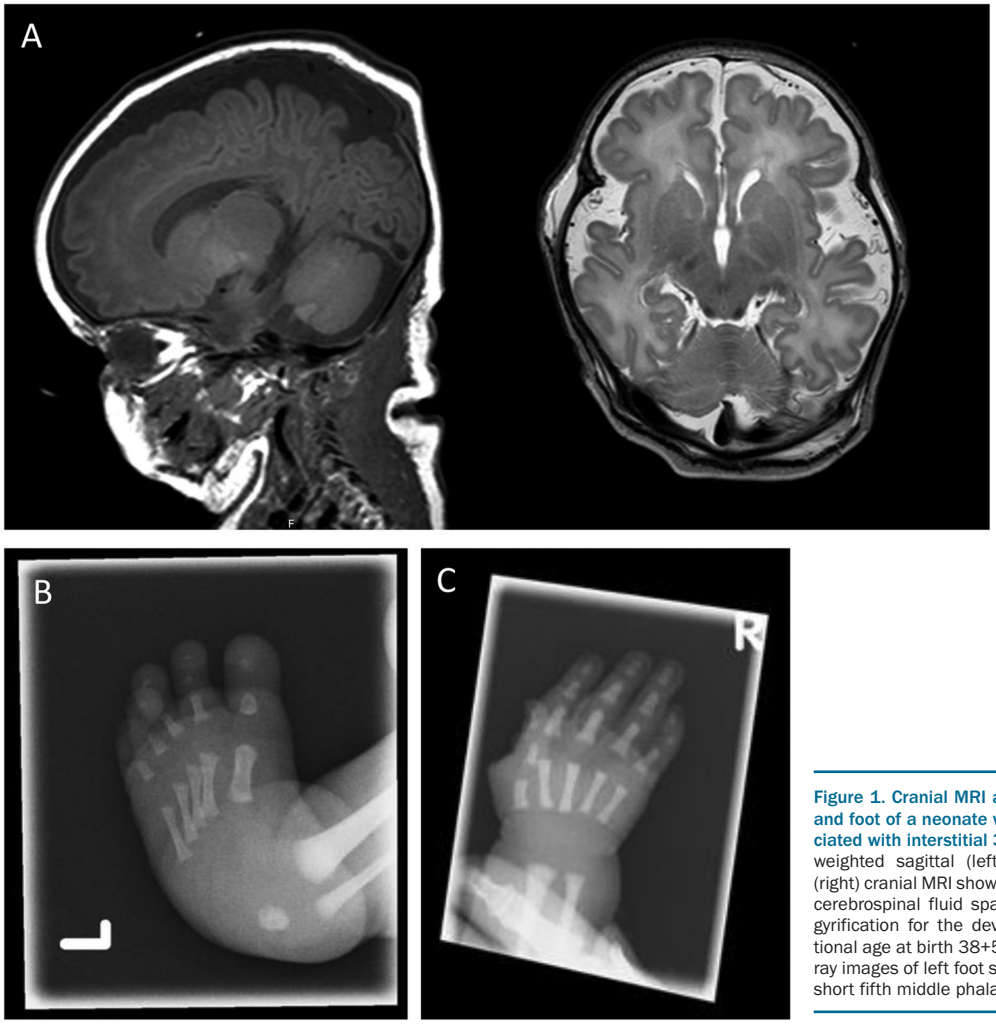


**Figure 1. Cranial MRI and X-ray images of hand and foot of a neonate with syndromic BMF associated with interstitial 3'*MECOM* deletion.** (A) T1-weighted sagittal (left) and T2-weighted axial (right) cranial MRI showing enlarged extracerebral cerebrospinal fluid spaces, and reduced frontal gyrification for the developmental stage (gestational age at birth 38+5 weeks, MRI day 6). (B) X-ray images of left foot showing clubfeet, and (C) a short fifth middle phalanx of the right hand.

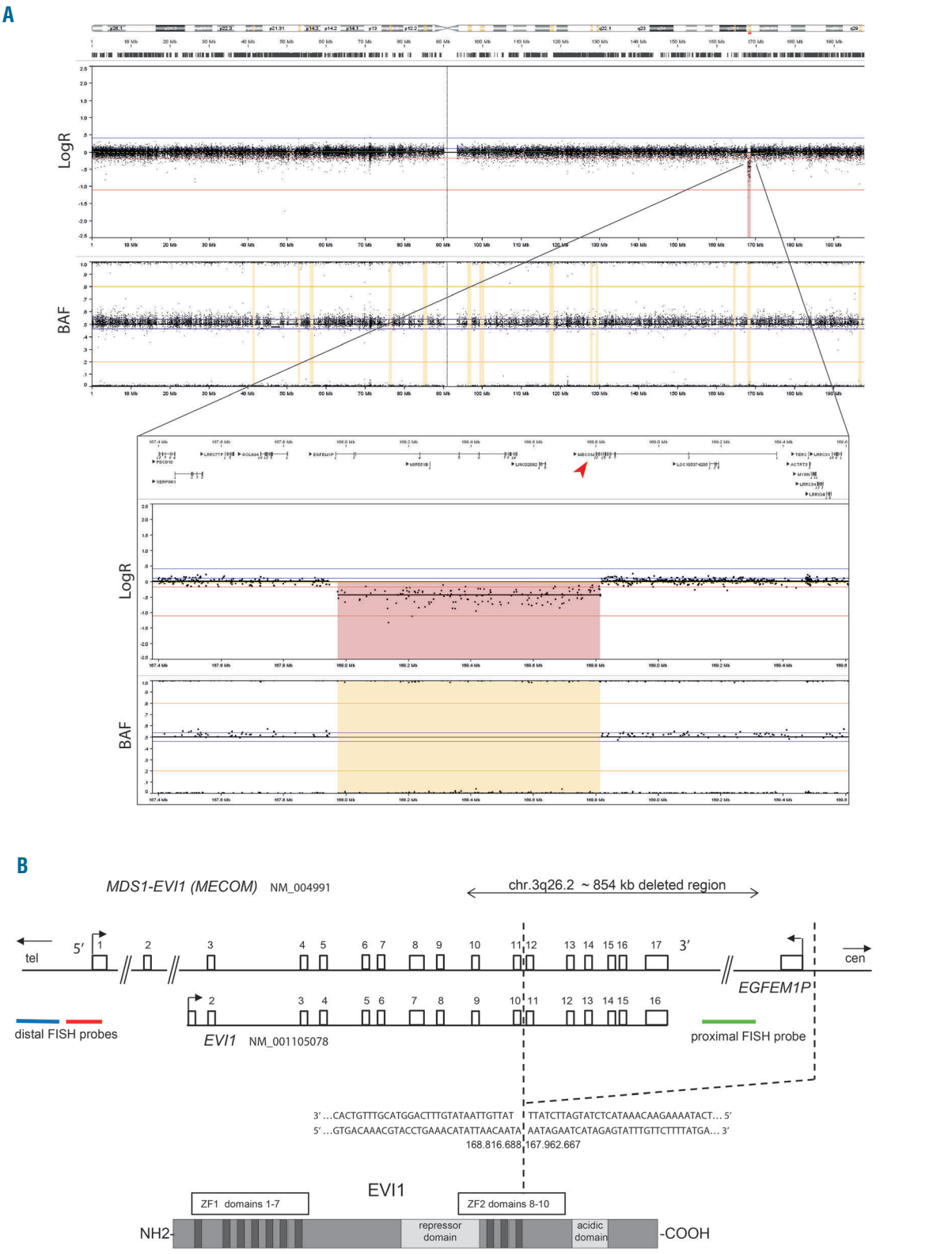


**Figure 2. Heterozygous copy number loss of 3′*MECOM* as a result of an interstitial deletion in 3q26.2 in a neonate with syndromic BMF.** (A) Chromosome 3 is shown, and detailed depiction of the affected area, demonstrating heterozygous 3q26.2 3′*MECOM* copy number loss (in red). SNP-array copy number profiling and analysis of regions of homozygosity were performed on DNA isolated from peripheral blood according to standard procedures using the Infinium Human CytoSNP-850K v1.1 BeadChip (Illumina, San Diego, CA, USA). Subsequently, visualizations of SNP-array results and data analysis were carried out using NxClinical software v3.0 (BioDiscovery, Los Angeles, CA, USA). Human genome build Feb. 2009 GRCh37/hg19 was used. Results were classified with BENCH Lab CNV software (Agilent, Santa Clara, CA, USA). (B) Schematic representation of the chromosome 3q26.2 *MECOM* region. The bar with whiskers represents the 854 kb deletion encompassing the *EGFEM1P* and *EVI1* (partially) genes. Highlighted is the breakpoint at chromosomal position 167.962.667 upstream of the gene *EGFEM1P* and at chromosomal position 168.816.688 between exons 11 and 12 of *MECOM* (hg19/GRCh37) by captured sequencing (as described in[10]). Breakpoint sequence post-deletion is provided. Localization of the *MECOM* triple color FISH probe. Representation of EVI1 protein. Map not drawn to scale. Chr: chrosome; FISH: fluorescence *in situ* hybridization; ZF: zinc finger; BAF: B allele frequency.

but some also had congenital limb defects.[6]

We report on the third patient, a neonate born with skeletal and neurological abnormalities and hypocellular bone marrow, with a constitutional copy number alteration in *MECOM*, particularly the first one with a deletion affecting only the 3' part of *MECOM*. The ~854 kb deletion resulted in loss of 3' *MECOM* exons, encoding the ZF2 and AD. We will discuss the genetic and clinical heterogeneity of these cases with *MECOM* abnormalities associated with BMF with or without additional congenital anomalies.

A male neonate was born with severe congenital abnormalities and apparent BMF. He was the fourth child of non-consanguineous parents. The couple had three healthy daughters and no history of miscarriages. Prenatal ultrasounds from 20 weeks gestation onward had shown club feet and polyhydramnios, but normal growth. The patient was born, with respiratory failure, at a gestational age of 38+5 weeks. The placental examination was unremarkable. Clinical examination showed macrocephaly, but otherwise only minor facial dysmorphisms. There was an ulnar deviation of the right hand (the left hand could not be evaluated). The lower extremities showed no abnormalities other than club feet. His hair and nails were normal. Laboratory evaluation showed pancytopenia. Flow cytometry only showed naïve $CD4^+$ and $CD8^+$ T cells with normal T-cell receptor Vβ chain distribution. Therefore, there was no sign of malignant monoclonal hematopoiesis. Bone marrow was hypocellular. A cranial ultrasound performed directly after birth showed subnormal gyrification for the gestational age, wide peripheral cerebrospinal fluid spaces and fissures and a large cavum of the septum pellucidum (Figure 1A). A skeletal evaluation showed club feet and a short fifth midphalanx of the right hand (Figure 1B,C). The cardiac ultrasound examination was normal. The patient died of infections and anemia at 25 days after birth. There was no consent for postmortem evaluation (see *Online Supplementary Table S1* for detailed clinical and laboratory characteristics).

Chromosome analysis on phytohaemaglutin-stimulated T-lymphocytes from peripheral blood resulted in a 46,XY karyotype. Single nucleotide polymorphism (SNP)-array analysis on DNA isolated from peripheral blood revealed a male array profile with heterozygous copy number loss within chromosome 3 band q26.2 of a minimal size of 841 kb, deleting 3' terminal exons 12 to 17 of *MECOM* (arr[GRCh37] 3q26.2[167974061_168815099]x1 [International System for Human Cytogenomic Nomenclature [ISCN] 2016]) (Figure 2A). Targeted next-generation sequencing (NGS) of the 3q26.2 region located the exact breakpoints of the 854 kb deletion at chromosomal position 167.962.667 (proximal breakpoint), and at chromosomal position 168.816.688 within intron 11 of *MECOM*, resulting in the loss of coding exons 12 to 17, affecting ZF2 and AD domains (Figure 2B). *Via* fluorescence *in situ* hybridization (FISH) analyses on peripheral blood the deletion was confirmed and its constitutional nature was demonstrated on skin fibroblasts and buccal swabs (46,XY.ish 3q26.2[3'MECOM-,5'MECOM+].nuc ish[3'MECOMx1,5'MECOMx2] [ISCN 2016]) (Figure 3). Neither targeted NGS, nor Sanger sequencing of the coding region of *MECOM* using intronic primers, indicated any additional circulating nucleic acids or sequence variants within *MECOM*. The coding regions of *GATA2* and *RUNX1* genes were investigated by Sanger sequencing using intronic primers, revealing no SNV or insertion/deletion. The intronic enhancer of *GATA2* was not investigated. Cultured lymphocytes from peripheral blood did not show increased sensitivity for mitomycin C, and as such did not positively identify the patient with Fanconi anemia. Karyotyping and FISH analyses of peripheral blood of both parents did not reveal a structural or numerical abnormality of *MECOM*, indicating the deletion to be *de novo*.

Of note are the resemblances of a RUSAT-like phenotypic presentation in the cases as described by Niihori *et al.*, Ripperger *et al.*, Bluteau *et al.*[4-6] and the present case. The constitutional nature of the published SNVs within

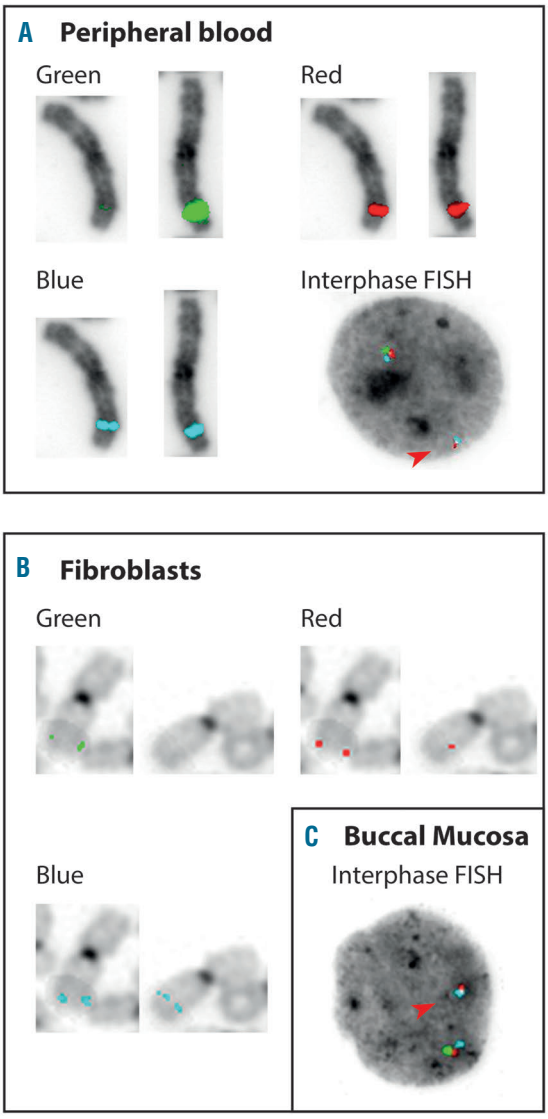


**Figure 3. FISH analyses demonstrating constitutional 3'MECOM deletion in syndromic BMF.** (A) Triple color FISH analyses demonstrating loss of proximal 3'MECOM signal (green) on one of two paired metaphase chromosome 3 homologues, and one interphase nucleus (phytohaemaglutin-stimulated peripheral blood T-lymphocytes), and (B) on one of two paired metaphase chromosome 3 homologues (cultured skin fibroblasts), and (C) on one interphase nucleus (buccal mucosa), using 3q26.2 *MECOM* probe on EVI t(3;3);inv(3)(q26) Break, TC (Kreatech, Rijswijk, The Netherlands). For metaphases the individual signals of the triple color probe combination are shown separately. Arrows in interphases point to loss of one green proximal 3'*MECOM* signal. Images represent all analyzed cells (10 metaphases and 200 interphase nuclei of T-lymphocytes, 10 metaphases of cultured skin fibroblasts, and 200 interphase nuclei of buccal mucosa). FISH: fluorescence *in situ* hybridization.

*MECOM*, and the deletion in the present case, affecting the ZF2 domain encoding exons of *MECOM*, are in strong support of their pathogenicity. Of similar distinction is the resemblance in the phenotypic presentation of our case and *Evi1*-deficit mouse models. *Evi1*$^{-/-}$ mice die during embryogenesis due to bone marrow hypocellularity. Embryos show defects in the heart, cranial ganglia and the peripheral nervous system with small dorsally positioned forelimb buds.[7,8]

Notably, the two previously reported cases with a deletion show some differences in the severity of clinical manifestation. The first presented with isolated thrombocytopenia at birth, progressing to pancytopenia with aplastic bone marrow at two months of age, requiring allogeneic stem cell transplantation, but without overt congenital malformations.[2] As one entire copy of *MECOM* was lost, the phenotype can be due to MECOM haploinsufficiency, resembling *Mecom+/-* mice models in some instantances. The second newborn presented with low birthweight, facial anomalies and a submucosal cleft palate, a small ventricular septal defect and thrombocytopenia.[3] There was no information on malformation of the extremities. The patient died 28 days after birth due to respiratory insufficiency. Bouman *et al*. hypothesized that the hematological phenotype could also be due to haploinsufficiency of MECOM. Nevertheless, as only the first two exons of *MDS1* were deleted, the authors stated that this could still allow *EVI1* expression because of alternative transcription starting sites. Therefore, one cannot rule out negative interference with normal MECOM function.

Available data do not allow for a conclusion as to whether in the present case the 3'*MECOM* deletion would result in MECOM haploinsufficiency by messenger ribonucleic acid (mRNA) decay, or in dominant interference by a structurally aberrant protein. It has been reported that EVI1 oligomerization is not impaired by C-terminal truncated EVI1 protein *in vitro*.[9] One could thus hypothesize that in the present case a C-terminally-affected EVI1 oligomerizes with EVI1, thereby tethering EVI1 protein-mediated transcriptional regulation of *GATA2* even below the level of 50%, as can be expected in the case of a loss of one *MECOM* allele as described by Nielsen *et al*., resulting in a more dramatic perturbation of HSC homeostasis (threshold model). This would be a plausible explanation for the difference in severity of the phenotype of our case, the embryonic lethality in *Evi1*- or *Gata2*-deficient mouse models, and the phenotype of *Evi1+/-* mice or the case with a heterozygous deletion spanning *MECOM*. One also has to consider the possibility of mosaicism, which could reflect divergence in phenotypic presentation. As *EVI1* mRNA expression is not restricted to the hematopoietic system, a direct deleterious effect of the EVI1 anomaly in other tissues seems likely. The data on the cases with *MECOM* SNV's as well as the present case indicate that the mutation was present at conception, or occurred during (early) postzygotic isolation. In the two other cases with a deletion affecting *MECOM*, no data were available on its status in non-hematopoietic cells. Given their phenotypic presentations one could also argue mosaicism in these latter cases.

In conclusion, we report on the third case of BMF syndrome associated with a constitutional CNA in *MECOM*, particularly the first one with a deletion affecting the 3' part of *MECOM*, presenting, however, with multiple congenital anomalies, including limb defects. Together with all reported cases on CNA and SNV of *MECOM* it clearly establishes *MECOM* as a recurrent causative gene in (syndromic) BMF. Therefore, one should consider, in case of neonatal thrombocytopenia with or without other dysmorphisms, investigating the *MECOM* locus for CNAs and SNVs. The syndrome appears to present with variable severity of phenotypic manifestations that might be due to the nature of the *MECOM* abnormality. Furthermore, investigation of tissues other than the hematopoietic system, together with the consequences both at the transcriptional and post-transcriptional level would greatly contribute to our understanding of the pathogenic mechanism(s).

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