MECOM associated syndrome - a heterogeneous inherited bone marrow failure syndrome with amegakaryocytic thrombocytopenia

RUNNING HEAD: MECOM associated syndrome

Manuela Germeshausen, Phil Ancliff, Jaime Estrada, Markus Metzler, Eva Ponstingl, Horst Rütschle, Dirk Schwabe, Richard H. Scott, Sule Unal, Angela Wawer, Bernward Zeller, Matthias Ballmaier

Supplemental Information

Reference	Patient ID	Genomic (GRCh38.p7) Variation	Transcript variant 3 (NM_001105078.3)			Phenotype			
			E/I no.	CDS/protein	Type	BMF	RUS	other organ abnormalities	familial/sporadic
Bluteau et al., 2017[1]	UB100	chr3:g.169116002_ 169116006del	E7	c.1302_1306del p.Lys434fs	frameshift	Y	N	-	S
Bluteau et al., 2017[1]	UB093	chr3:g.169112870C>A	E8	c.1930G>T p.Glu644Ter	nonsense	Y	N	clubfoot, pulmonary stenosis, facial dysmorphia	S
Bluteau et al., 2017[1]	UB153	chr3:169100963C>T	I10	c.2208-1G>A loss of splice site	splice site mutation	Y	N	thumb abnormalities, renal hypoplasia	S
Niihori et al., 2015;[2]	TRS3	chr3:g.169100922G>A (rs864309724)	E11	c.2248C>T p.Arg750Trp	missense	Y	Y	deafness, hydrocele testicle	S
Lord et al., 2017[3]	–	chr3:g.169100922G>A (rs864309724)	E11	c.2248C>T p.Arg750Trp	missense	Y	Y	finger anomalies, facial dysmorphia, hip dysplasia, patent foramen ovale, nephrocalcinosis	S
Bluteau et al., 2017[1]	UB004	chr3:g.169100922G>A (rs864309724)	E11	c.2248C>T p.Arg750Trp	missense	Y	Y	Tetralogy of Fallot	S
Niihori et al., 2015[2]	TRS2	chr3:g.169100918T>C (rs864309723)	E11	c.2252A>G p.His751Arg	missense	Y	Y	finger abnormalities, deafness, cleft palate, dysarthria	S
Niihori et al., 2015[2]	TRS1	chr3:g.169100904T>C (rs864309722)	E11	c.2266A>G p.Thr756Ala	missense	Y	Y	finger abnormalities	S
Ripperger et al., 2017[4]	I:1, II:3, III:2, III:3	chr3:g.169095235A>C	E12	c.2296T>G p.Cys766Gly	missense	Y/N	Y	finger abnormalities, deafness, MDS in 2 patients	F
Bluteau et al., 2017[1]	UB036	chr3:g.169095197C>A	E12	c.2334G>T p.Arg778Ser	missense	Y	N	thumb abnormalities, myocardial atrophy	S
Bluteau et al., 2017[1]	UB104	chr3:169089116_ 169089119del	E15	c.2900_2903del	frameshift	Y	N	-	S

Supplemental Table 1: Previously reported patients/families with germ line mutations in MECOM

		Type of Evidence							
Patient ID	**Genomic Variation** (refSNP)	population data	computational and predictive data	functional data	segregation data	de novo data	other database	other data	**Pathogenicity**
P1	chr3:g.169100919G>A	PM2	PM5, PP3	PM1				PP4	**likely pathogenic (IV)**
P2	chr3:g.169100894T>A	PM2	PP3	PM1				PP4	**likely pathogenic (V)**
P3	chr3:g.169100892G>A	PM2	PP3	PM1		PM6		PP4	**likely pathogenic (IV)**
P4	chr3:g.169100962_ 169100963delTC	PM2	PVS1	PM1		PM6		PP4	**pathogenic (Ib)**
P5/P6	chr3:g.169100922G>A (rs864309724)	PM2	PS1	PM1		PM6	PP5		**pathogenic (IIIa)**
P7	chr3:g.169093016G>A	PM2	PVS1						**likely pathogenic (I)**
P8	chr3:g.169128041G>T	PM2	PVS1		BS4				**likely pathogenic (I)**
P9	chr3:g.169095075C>G	PM2	PVS1			PM6			**pathogenic (Ib)**
P10	chr3:g.169116194G>A	PM2	PVS1						**likely pathogenic (I)**
P11	chr3:g.169095111_ 169095112insT	PM2	PVS1						**likely pathogenic (I)**
P12	chr3:g.169116402G>C	PM2	PP3			PM6		PP4	**likely pathogenic (V)**
P13	chr3:g.169116402G>C	PM2			BS4				uncertain significance
P14/P15	chr3:g.169095082T>C (rs200049869)	PS4	BP4		BS4 (P15)				uncertain significance
P16/P17/P18	chr3:g.169143748C>A (rs116535717)	BS1			BS4 (P16)				benign (II)
P19	chr3:g.169122674T>C (rs34896995)	BS1	PP3						uncertain significance
P20	chr3:g.169145034T>C (rs370795924)	PM2							uncertain significance

Supplemental Table 2: Pathogenicity classification of variants according to the ACGM recommendations[5,6]

The used abbreviations are from the ACGM recommendations and are organized according to the type of evidence (B: benign, P: pathogenic), strength of the criteria (VS: very strong, S: strong, M: moderate, P: supporting) and a number code: BS1: Allele frequency greater than expected for disorder; BS4: Lack of segregation in affected members of a family; BP4: Multiple lines of computational evidence suggest no impact on gene or gene product; PM1: Located in a mutational hot spot and/or critical and well-established; PM2: Absent from controls in Exome Sequencing Project, 1000 Genomes or ExAc; PM4: Protein length change; PM5: Novel missense mutation at AA position where a different missense change determined to be pathogenic has been seen before; PM6: Assumed *de novo*, but without confirmation of paternity and maternity; PP1: Co-segregation in multiple affected family members; PP3: Predicted deleterious effect; PP4: Phenotype highly specific for gene; PS1: Same amino acid change as a previously established pathogenic variant; PS4: Prevalence of variant in affected individuals significantly increased compared to controls; PVS1: Null variant, when loss of function is a known mechanism of disease; (for detailed explanation see Richards et al.[5]). Nucleotide numbering according to GRCh38.p7

Pat ID	sex	hematological course	HSCT (age in months)	RUS	other skeletal malformations	other malformations	hearing	B cell lymphopenia	other / remarks	family history	MECOM mutation (transcript variant 3)
P13	f	congenital TP with with reduced MKs, amelioration of TP in the first months of life	N	N	N	N	ND	ND			c.906C>G p.Ser302Arg
P14	m	TP since age 5y, leukopenia since age 17y	N	N	N	sinus bradycardia	ND	ND	fatigue		c.2449A>G p.Met817Val
P15	m	severe congenital TP, spontaneous normalization of platelet counts at age 10m	N	N	N	pelvy-calyceal dilation left	normal	ND		N	c.2449A>G p.Met817Val
P16	m	TP reported since age 10y, hypocellular BM, progressive BMF at age 12y	(192)	N	"stubby fingers"	N	ND	N	Café-au-lait spots on trunk, increased chromosomal breakage (borderline), FANCC mutation		5'UTR (c.-105) *Transcript Variant 1: c.88G>T p.Ala30Ser*
P17	m	diagnosis of AA at age 5y, successful treatment with immunosuppressive therapy	N	N	N	N	ND	ND	accelerated bone maturation (precocious puberty)		5'UTR (c.-105) *Transcript Variant 1: c.88G>T p.Ala30Ser*
P18	f	development of pancytopenia development AML M0	MFD (25)	N	N	N	ND	N		N	5'UTR (c.-105) *Transcript Variant 1: c.88G>T p.Ala30Ser*
P19	f	isolated TP, normocellular bone marrow	N	N	N	N	normal	N		N	c.320A>G p.Gln107Arg
P20	m	congenital TP with reduced MKs, progressive BMF	UCB (48)	N	N	ASD II	normal	ND		N	*Transcript Variant 1: 5'UTR (c.-35A>G)*

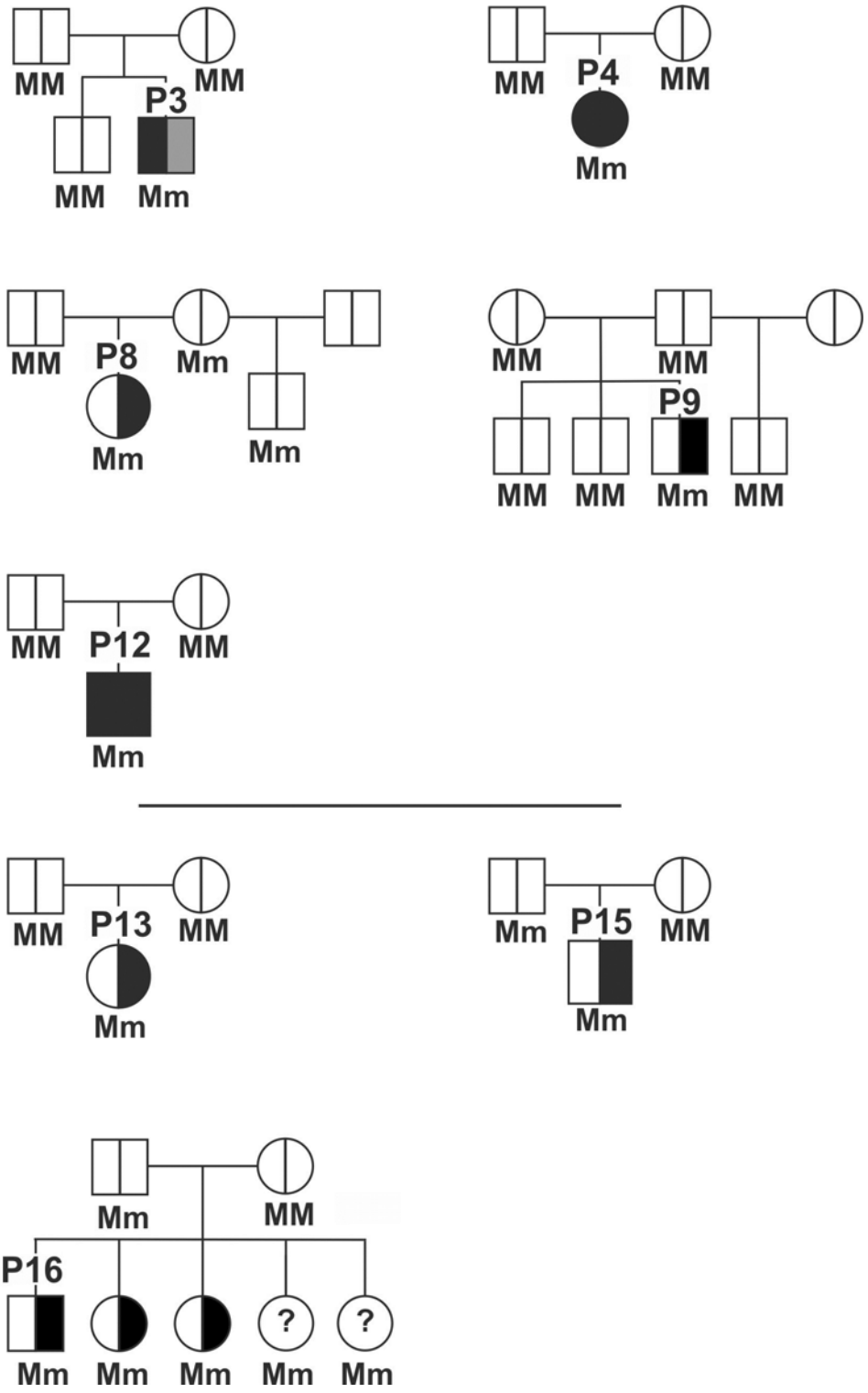
Supplemental Table 3: Clinical characteristics of patients with MECOM variations of uncertain significance or predicted to be benign

Abbreviations: AA: aplastic anemia; ASD: atrial septal defect; BMF: bone marrow failure; HSCT: hematopoietic stem cell transplantation; MFD: matched family donor; ND: no data; RUS: radio-ulnar synostosis; TP: thrombocytopenia; UCB: unrelated cord blood; HSCT outcome was positive in P16, P18 and P20.

Patient ID	age [y]	THPO [pg/mL]
P1	0 2/12	3342 ±168
P4	0 2/12	>3500
P4	0 5/12	>3500
P8	0 1/12	156 ±18
P11	0 8/12	1126 ±58
P11	0 9/12	1815 ±3
P16	14 10/12	585 ±322
P19	5 4/12	58 ±6
P20	3 0/12	734 ±31

Supplemental Table 4: THPO plasma levels

as determined by means of ELISA; reference value: median <32 pg/mL, range ND - 196 pg/ml



Supplemental Figure 1: Additional pedigrees of analyzed families

Phenotypes: left area black: RUS; right area black: congenital amegakaryocytic thrombocytopenia / congenital aplastic anemia (with amelioration: gray); ?: unknown phenotype; Genotypes: M - wildtype allele, m - mutated allele

References

1. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germline mutations in a cohort of inherited bone marrow failure patients. *Blood*. 2017.
2. Niihori T, Ouchi-Uchiyama M, Sasahara Y, et al. Mutations in MECOM, Encoding Oncoprotein EVI1, Cause Radioulnar Synostosis with Amegakaryocytic Thrombocytopenia. *Am J Hum Genet*. 2015;97(6):848-854.
3. Lord SV, Jimenez JE, Kroeger ZA, et al. A MECOM variant in an African American child with radioulnar synostosis and thrombocytopenia. *Clinical Dysmorphology*. 2017.
4. Ripperger T, Hofmann W, Koch JC, et al. MDS1 and EVI1 complex locus (MECOM): a novel candidate gene for hereditary hematological malignancies. *Haematologica*. 2017.
5. Richards S, Aziz N, Bale S, et al. 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*. 2015;17(5):405-424.
6. Kleinberger J, Maloney KA, Pollin TI, Jeng LJ. An openly available online tool for implementing the ACMG/AMP standards and guidelines for the interpretation of sequence variants. *Genet Med*. 2016;18(11):1165.