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Unravelling Facets of MECOM-Associated Syndrome: Somatic Genetic Rescue, Clonal Hematopoiesis and Phenotype Expansion

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Abstract:

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Unravelling Facets of MECOM-Associated Syndrome: Somatic Genetic Rescue, Clonal Hematopoiesis and Phenotype Expansion

Running title: Novel aspects of MECOM-Associated Syndrome

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Abstract: In this MECOM-associated syndrome cohort, we describe a notable prevalence of somatic genetic rescue events and clonal hematopoiesis in carriers. We also observe a higher rate of pregnancy loss than the general population.

Pathogenic germline heterozygous variants in *MECOM* (MDS1 and EVI1 complex locus) are associated with an autosomal dominant bone marrow failure (BMF) disorder characterized by radioulnar synostosis (RUS) often accompanied by amegakaryocytic thrombocytopenia (RUSAT2; MIM#616738). MECOM is a transcription factor which is essential for hematopoietic stem cell self-renewal, and the loss of MECOM decreases absolute long term hematopoietic stem cell numbers[1]. Several differentially spliced transcripts are encoded by the *MECOM* locus resulting in MDS1, MDS1-EVI1 and EVI1 isoforms. It has been demonstrated that these isoforms are involved in their own transcriptional regulation through distinct promoter regions and have an impact on the maintenance and transformation of hematopoietic stem and progenitor cell populations[2].

Individuals with MECOM-associated syndrome display variable clinical presentations ranging from no hematological manifestations to severe BMF with or without skeletal abnormalities[1,3] [3]. Other features include clinodactyly, cardiac and renal malformations, hearing loss and B cell deficiency. Most individuals eventually progress to pancytopenia and require hematopoietic stem cell transplants at relatively young ages[3–5] . Skeletal abnormalities particularly RUS are seen predominantly in individuals with missense variants within the eighth and ninth zinc finger motifs of MECOM[4,6,7] . However, there are reports of affected individuals with premature termination variants or constitutional deletions with skeletal involvement[8,9].

Here we report fifteen (3 families, 5 *de novo*) cases of MECOM-associated syndrome with onset of symptoms varying from *in utero* to late adulthood (Figure 1A-F). Our cohort represents the spectrum of this syndrome with individuals presenting with a) classical RUSAT, b) BMF (ranging from mild to severe) without RUS, c) RUS without hematological manifestations. Detailed information on clinical history and classification of germline *MECOM* variants as per the American College of Medical Genetics (ACMG) guidelines are included in Supplemental Information, Table 1 and Supplemental Table 1.

Herein we show that part of the variability in hematological presentation may be attributable to spontaneous reversion of germline variants observed in some affected individuals. Our study identifies 7/15 affected individuals who show spontaneous resolution, alleviation of hematological symptoms, or late onset of hematological manifestation of MECOM-associated syndrome. For 4/6 individuals (3-II-4, 4-II-4, 5-II-1 and Patient 11), amelioration of symptoms appears associated with somatic genetic rescue, in the form of copy neutral loss of heterozygosity of chromosome 3q encompassing *MECOM* (Figure 1H, Supplemental Figure 2, 3, 4, Supplemental Information) thereby duplicating the residual wildtype allele in an expanding clone. A chromosomal rearrangement involving the MECOM locus was detected by FISH in a small subset of cells in 7-II-1. However, the consequence of this event at the cell differentiation/fitness level remains to be established. For 2/6 individuals, an explanation for mild presentation or symptom resolution remains enigmatic: 2-II-6 displayed spontaneous resolution of hematopoietic symptoms while 4-II-1 has been free of hematological symptoms for most of her life. Longitudinal analysis of variant allele fractions in both these individuals showed no evidence of allelic imbalance (Supplemental Figure 5,6). Somatic genetic rescue has been reported in several genetic diseases including skin disorders

(*e.g.* Ichthyosis with Confetti[10], Epidermolysis bullosa[11]) and BMF syndromes (*e.g.* Fanconi Anemia[12], Diamond Blackfan Anemia[13], Wiskott Aldrich Syndrome[14], Dyskeratosis Congenita[15]); with only one report in MECOM-associated syndrome[16]. Spontaneous normalization of blood counts and absent/mild hematopoietic involvement in carriers have been described in the literature; however there has been limited information/follow-up as to the mechanism[3,6,17,18] .

Most reported *MECOM* cases have had allogeneic bone marrow transplants at relatively young ages, which could explain relatively low frequency of progression to myeloid malignancy in 5% of patients (3 adult MDS cases, 1 pediatric AML out of 80 individuals[19])[3,5,9]. The findings of aplasia with dysplastic features in 1-II-5 and MDS in 5-II-1 add two more cases of myeloid dysplasia in MECOM-associated syndrome. Notably, all three older individuals within our cohort (4-II-1, 4-II-4, 5-II-1) displayed somatic variants in known age-related clonal hematopoiesis genes (Supplemental Figure 7). Acquisition of somatic variants in genes such as *ASXL1*, *DNMT3A* and *TET2* have been linked with improved HSC fitness and self-renewal[20]. Both 3-II-4 and 4-II-4 also had transient 20q loss events (a common karyotypic abnormality observed in myeloid disorders and aging population[21]) in their surveillance marrows. Intriguingly, 4-II-1 and 5-II-1 also have somatic *ETV6* variants which is not usually reported in an age-related context. Though the presence of such somatic alterations likely improves hematopoietic output, it may also signify an elevated risk of myeloid malignancy development particularly with advancing age. Consistent with this, in addition to clonal haematopoiesis, 4-II-4 is beginning to exhibit dysplastic features in more than one lineage (Supplemental Figure 2).

In the dynamic hematopoietic environment, demand-adapted hematopoiesis can drive mosaicism down two roads: clonal evolution through the acquisition of deleterious variants leading to cancer or alternatively, revertant mosaicism resulting in partial/complete rescue of phenotype. Somatic genetic rescue is an important factor to consider in scenarios such as carriers with mild phenotype, selection of tissue sources for identification of causative lesion in individuals in remission and use of patient-derived cell lines for drug screening. Understanding the mechanisms of revertant clonal selection *in vivo* and *in vitro* will open windows for rational correction and selection protocols for effective therapeutic intervention in inherited BMF disorders such as MECOM-associated syndrome.

Strikingly, there were 12 pregnancy losses out of a total of 16 pregnancies in 5 mothers where detailed information regarding pregnancies was available (Supplemental Table 2) within our cohort. This is a higher rate of loss (75%) than expected pregnancy outcomes in the general population (15-25%) as well as other inherited BMF syndromes (12-20%)[22,23]. We observed recurrent pregnancy losses (including late losses) in 1-I-2, 4-II-1 and 5-II-1, all of whom are carriers of *MECOM* variants (Figure 1). 4-I-2, who tested negative for the MECOM variant and whose husband (4-I-1) displayed MECOM-associated phenotype, experienced stillbirth at 8.5 months of gestation (4-II-3) raising the possibility that pregnancy loss can also occur from MECOM-associated complications intrinsic to the developing fetus. Her remaining three children displayed symptoms of MECOM-associated syndrome with two testing positive for the variant while the third could not be assessed due to unavailability of

samples. However, we were unable to ascertain the *MECOM* status for the fetus. It is also worth noting that 2-I-2 and 3-I-2 (both wildtype for *MECOM)* have also experienced pregnancy losses. We cannot comment on whether one or more individuals are gonadal mosaics for the *MECOM* variants as we are unable to determine the *MECOM* status of the fetuses due to unavailability of material for genetic testing.

Cardiac and vascular abnormalities including atrial septal defect, ventricular septal defect and patent ductus arteriosis have been reported in MECOM-associated syndrome. However, there has only been one report each of aortic coarcatation and aortic root dilatation[3,24]. We have observed three cases of aortic dilatation with one progressing to an aortic aneurysm reaching the threshold for surgical correction in our cohort of 15 cases.

Overall, this report adds to the breadth of disease presentations in MECOM-associated syndrome and expands age of onset varying from *in utero* to late adulthood with at least one individual being in relatively good health well into their sixties. It is becoming increasingly clear that the complex disease presentations of MECOM-associated syndrome are primarily driven by genomic location and nature of the germline variants, and further complicated by mechanisms such as somatic genetic rescue and possibly also somatic compensation by other genes. The clinical presentation and threshold of somatic genetic rescue required for phenotypic improvement/reversion is likely dictated by how severe the impact on protein function/output is, in each affected individual. The prevalence of somatic genetic rescue provides a rationale for gene-corrected autologous transplantation or direct gene editing approaches as potential treatments for the hematopoietic phenotype of MECOM-associated syndrome in the absence of matched donors. The presence of CHIP in the older individuals and the finding of additional cases of myeloid dysplasia in our cohort warrant consideration of surveillance particularly in older carriers. Given the high rate of pregnancy losses in these families, they should be considered for counselling for reproductive planning and employment of preimplantation genetic diagnosis to reduce the risk of future pregnancy losses. Moreover, the variability of presentation can make accurate genetic diagnosis challenging, and the notable prevalence of somatic genetic rescue reiterates the importance of using DNA from non-hematopoietic tissue such as hair follicles or skin fibroblasts for genetic testing.

Clinical and genomics data from germline MECOM variant carriers were collected from Centre for Cancer Biology (Australia), Peter MacCallum Cancer Centre (Australia), Radboud University Medical Center (Netherlands). All procedures in this study involving human participants were performed in accordance with the Declaration of Helsinki. Studies were approved by institutional human research ethics committees and/or institutional research boards. All participants signed an informed consent form to share genomics and protected health information.

Figure Legends

Figure 1. Pedigrees, phenotypes and genotypes of families and individuals carrying rare germline *MECOM* variants. A-F. Pedigrees with germline *MECOM* variants. Affected individuals (grey), *MECOM* mutation carriers (+), *MECOM* WT (-). G. X-ray images from 3-II-4 demonstrating radioulnar synostosis and absent patella. H. Copy neutral loss of heterozygosity in 3-II-4 across chromosome 3q encompassing the *MECOM* germline variant and leading to somatic genetic rescue. I. Distribution of germline *MECOM* variants (NM_004991.4) visualized using ProteinPaint web application. Aplastic anemia (AA), fetal death *in utero* (FDIU), Spontaneous Abortion (SAB), termination of pregnancy (TOP), ectopic pregnancy (ECT).

Table 1. Genotypes and phenotypes of affected individuals carrying rare germline *MECOM* variants.

Author Contribution Statement: PV wrote the manuscript and was involved in all aspects of the project including designing the research, manuscript preparation, collecting/analyzing experimental and clinical data, American College of Medical Genetics and Genomics (ACMG)-variant classification; CNH, HSS and ALB were involved in research design, data analysis, manuscript preparation and providing scientific insight; PA, LCF, ASi, DKH were involved in different aspects of design and analysis of clinical and experimental data and manuscript preparation; MSBF, AB, STC, TTH, KA, SG, KSK, RKe, KH, MiB, AMG, LR, CV, LD, RF, DL carried out different aspects of experimental design and/or data analysis; DKH, PGB, ASw, DMR, LFDV, AB, BG, EF, TC, IK, MK, AAK, FAK, SM, PE, YB, MaB, SMR, NM, MJ, RKu, PBa, PBl, KP and NKP carried out collection and analysis of clinical patient information; L.A.M. performed bioinformatic analysis; All authors critically reviewed and approved the manuscript.

Conflict-of-interest disclosure: S.T.C has no paid advisory roles to declare. S.T.C is volunteer member of ClinGen Expert Panels: Muscular Dystrophies and Myopathies GCEP and Limb Girdle Muscular Dystrophy VCEP. S.T.C is named inventor of Intellectual Property (IP) relating to novel methods to identify splicing variants (PCT/AU2019/000141; PCT/AU2020/050234) owned jointly by The University of Sydney and Sydney Children's Hospitals Network. S.T.C is Director of Frontier Genomics Pty (Australia) which has licenced this IP. S.T.C performs this Director role outside of her UniSydney role and currently receives no consultancy fees or other remuneration for this role. Frontier Genomics has not traded and has no existing financial relationships that will benefit from publication of these data.

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Table 1. Genotypes and phenotypes of affected individuals carrying rare germline *MECOM* variants.

Patient ID	cDNA (NM_004991.4)	Protein (NP_004982.2)	Sex	Age at presentation	HSCT (Age)	Somatic Genetic Rescue	Additional Somatic genetic changes	Hematopoietic abnormalities (age at diagnosis)	Skeletal abnormalities	Cardiac/ vascular abnormalities	Other abnormalities
1-I-2	c.2577+4A>T	p.(Arg830Serfs*21) p.(Val831Cysfs*11)	F	15 y	Haploidentical HSCT (36y)	not detected	none detected	Pancytopenia and anemia (15 y).	Short stature, Brachydactyly, Short toe, proximal placement of hallux, short proximal phalanx of hallux, short proximal phalanx of 5th finger and cholelithiasis.	Ventricular septal defect	
1-II-5	c.2577+4A>T	p.(Arg830Serfs*21) p.(Val831Cysfs*11)	F	*in utero*	NA	N	not analysed	Aplasia with dysplastic features (in utero).	Preaxial polydactyly, supernumerary ribs and coronal cleft vertebrae.	none reported	Fetal Hydrops Splenic hemosiderin deposition Small placenta
2-II-6	c. 1174delT	p.(Cys392Alafs*29)	F	9 mo	N	not detected	none detected	• Thrombocytopenia and transient low relative B cell numbers (9 months). • Hypocellular bone marrow with complete absence of megakaryocytes, dyserythropoiesis and left shifted granulopoiesis with abnormal granulation. • Spontaneous recovery (3 y).	not present	Mild aortic root dilatation	Congenital hearing loss
3-II-4	c.2873_2875delTTA	p.(Phe958_Ser959delinsCys)	M	Birth	N	cnLOH chr3q	transient del(20q)	• Neonatal thrombocytopenia managed with multiple platelet transfusions followed by spontaneous recovery. • Subsequent mild pancytopenia and hypocellular bone marrow.	Proximal radioulnar synostosis, hypoplastic thumbs, short, broad fingers, short 5th digits and coalition of right capitate and hamate and bilateral absent patellae.	Mild mitral valve prolapse	
4-II-4	c.816dupT	p.(Pro273Serfs*2)	M	Birth	N	cnLOH chr3q	transient del(20q) ASXL1 p.(Arg860Glufs*7) ASXL1 p.(Leu775*)	• Pancytopenia • Aplastic anaemia (11 y).	Club foot, small patellae.	Aortic root dilatation progressing to aortic aneurysm, Mitral valve defect	

4-II-1	c.816dupT	p.(Pro273Serfs*2)	F	59 y	N	not detected	DNMT3A p.(Leu504Trpfs*147) ETV6 p.(Ser139Tyrfs*14) TET2 p.(Cys973*) TP53 p.(Ala276Asp)	• Mild intermittent thrombocytopenia and neutropenia • Hypocellular bone marrow.	Small patellae.	Aortic root dilatation	Bicornuate uterus, mild sensorineural hearing loss, cataract (50 y), bilateral scarring of kidneys with normal function
5-II-1	c.2889C>G	p.(Asn963Lys)	F	childhood (exact age unknown)	N	cnLOH chr3q	ASXL1 p.(Gly646Trpfs*12) SETBP1 p.(Ser869Asn) EZH2 p.(Tyr733Phe) EZH2 p.(Cys609_Ser610delinsTyr) ETV6 p.(Arg369Trp)	• Aplastic anemia in childhood. • Diagnosed with myelodysplastic syndrome (40 y).	Clinodactyly in fingers and toes.	none reported	Hearing impairment, locally recurrent anal squamous cell carcinoma,gynaeological warts. cataracts, glaucoma
6-I-1	c.2905C>T	p.(Arg969Cys)	M	4 y	N	not detected	not analysed	No abnormalities	Bilateral radioulnar synostosis - surgically corrected at age 4	none reported	
6-II-1	c.2905C>T	p.(Arg969Cys)	M	2 y	N	not detected	not analysed	No abnormalities	Bilateral radioulnar synostosis, clubfeet	Small patent ductus arteriosus/patent foramen ovale, hemodynamically not significant.	Slightly cupped ears borderline normal hearing
Patient 7	c.2813G>A	p.(Arg938Gln)	M	34 y	N	Y	none detected	• Thrombocytopenia diagnosed at birth • Mild thrombocytopenia and macrocytosis without anemia (33y).	Presumed bilateral radioulnar synostosis (limited ability to pronotate arms bilaterally), bilateral club foot, perthes-like hip disease, endochondromata and ecchondromata	none	Small kidneys without structural deficits, non-specific punctate foci of T2/FLAIR hyperintensity in the peripheral/subcortical white matter slightly greater than expected for patient's age
Patient 8	c.2776T>C	p.(Cys926Arg)	F	27 y	N	not detected	not analysed	Severe thrombocytopenia and mild leukopenia	Clinodactyly of the thumb, short toe, unfused vertebral arch L5, coxa valga and cam deformity	none reported	
Patient 9	c.3106C>T	p.(Arg1036*)	M	19 y	N	not detected	not analysed	Thrombocytopenia	Marfanoid habitus tall spindy fingers hypoplasic thumbs adducted toes	none reported	

Patient 10	c.1696G>T	p.(Glu566*)	F	Birth	MUD (11 mo)	not detected	not analysed	• Thrombocytopenia diagnosed at birth • Progressive pancytopenia (9 mo) • Profoundly hypocellular marrow (9 mo)	Retrognathia	Patent foramen ovale, pulmonary branch stenosis (resolved without intervention at 2 y)	Congenital conductive hearing loss both ears, cleft palate (corrected at 7 mo)
Patient 11	c.2813G>A	p.(Arg938Gln)	F	17 y	N	Y	not analysed	• Mild leukopenia and thrombocytopenia • Hypocellular marrow with absence of megakaryocytes	Right-sided radioulnar synostosis, camptodactyly of the 5th fingers, brachydactyly of the first toes, scoliosis	Bicuspid aortic valve	Congenital mixed hearing loss, bilateral relatively small kidneys without structural defects, from age 18 onwards chronic mild renal insufficiency (stage 2)
Patient 12	Complete loss of MECOM gene		F	*in utero*	N	N	not analysed	Hypocellular bone marrow	Micrognathia	At autopsy: ductus arteriosus type II with VSD. Pericardial fluid, ascites.	generalised edema hygroma colli nuchal translucency cleft palate, simple ears Lung hypoplasia due to pleural fluid. Unilateral renal agenesis.

MUD – matched unrelated donor

Figure 1