MDS1 and *EVI1* complex locus (MECOM): a novel candidate gene for hereditary hematological malignancies

Tim Ripperger,[1] Winfried Hofmann,[1] Jan C. Koch,[2] Katayoon Shirneshan,[1,3] Detlef Haase,[3] Gerald Wulf,[3] Peter R. Issing,[4] Matthias Karnebogen,[5] Gunnar Schmidt,[1] Bernd Auber,[1] Brigitte Schlegelberger,[1] Thomas Illig,[1,6] Birgit Zirn[7] and Doris Steinemann[1]

[1]Department of Human Genetics, Hannover Medical School; [2]Department of Neurology, University Medical Centre, Göttingen; [3]Department of Hematology and Oncology, Georg-August University, Göttingen; [4]Department of Otorhinolaryngology, Head, Neck and Facial Plastic Surgery, Klinikum Bad Hersfeld; [5]Division of Surgery and Orthopedics, Healthpark Lenglern, Bovenden; [6]Hannover Unified Biobank, Hannover Medical School and [7]Genetic Counseling and Diagnostics, Genetikum, Stuttgart, Germany

Correspondence: ripperger.tim@mh-hannover.de
doi:10.3324/haematol.2017.178723

Supplemental Information

Supplemental Table 1. Phenotypic findings in affected individuals.

	I:1	II:3	III:2	III:3
limb dysmorphisms *radioulnar synostosis; brachy-, campto- and clinodactyly ; patella hypoplasia, metatarsus adductus and hallux valgus*	+	+	+	+
dysplastic middle ear & impaired hearing	+	+	+	+
congenital thrombocytopenia	+	–	–	–
myeloid malignancy	+	+	–	–
ischemic insult *	–	–	–	+

Person identifiers are given with respect to the pedigree in Figure 1. * Individual III:2 had multifocal ischemic insults at age 18 that were caused by bilateral stenosis of internal carotid arteries requiring neurosurgical intervention. Regarding these insults, it is remarkable that angiogenic defects were reported in *Evi*$^{-/-}$ mice.[1]

Supplemental Table 2. Overview of myeloid malignancies

	I:1	II:3
disease	**MDS-EB-2** (73 years)	**MDS/MPN*-U** (48 years)
PB	cong. thrombocytopenia, progressive neutropenia, anemia full blood cell count: erythrocytes 3.17 x 10^{12}/l, leukocytes 2.95 x 10^{9}/l, thrombocytes 14.7 x 10^{9}/l	bicytopenia; leucocytosis with 'left-shift' full blood cell count: hemoglobin concentration 83 g/l, leukocytes 18.7 x 10^{12}/l, thrombocytes 41 x 10^{9}/l
BM	**hypercellularity**; **dysplastic erythropoiesis** and granulopoiesis; **megakaryocytopenia** with micromegakaryocytes; 10-15% blasts	moderate **hypercellularity**; **dysplastic erythropoiesis**; granulopoiesis with dysplasia and with terminal maturation; mastocytosis; **megakaryocytopenia** ; 6-8% blasts
chr.	46,XY, **del(9)(q13q32)**[4]/46,XY[18]. nuc ish 5p15.2(D5S23/D5S721x2), 5q31(EGR1x2),7p11.1q11.1(CEP7x2), 7q31(D7S522x2),8p11.1q11.1(D8Z2x2), 9q34.1(LSI9q34x2),17p13.1(TP53x2), 20q12(D20S108x2)	46,XX,**t(1;14)(q44;q32)**[3]/46,XX[21]. ish t(1;14)(q44;q32)(IGH+;IGH+)[11/24]. nuc ish 14q32(3'IGHx2,5'IGHx3)(3'IGH sep 5'IGHx1)[200/232] (analyses of peripheral blood cells due to *punctio sicca*)
therapy	**chemotherapy** including 5-azacytidine; deceased during first treatment cycle	myeloablative conditioning: BuCyATG; **allo-PBSCTx**, MUD; 5 years post-Tx: no recurrence, no GvHD

Person identifiers are given with respect to the pedigree in Figure 1. allo-PBSCTx, allogenic peripheral blood stem cell transplantation BM, bone marrow; BUCy,ATG, busulfan, cyclophosphamide, ATG; chr, cytogenetic results; MDS-EB-2, myelodysplastic syndrome with excess blasts; MDS/MPN-U, MDS/myeloproliferative neoplasm-unclassifiable; MUD, HLA-matched unrelated donor; PB, peripheral blood. *, an association of *MECOM* single nucleotide polymorphisms and myeloproliferative neoplasms was reported by Tapper *et al.*, Chiang *et al.* and Trifa *et al.* [2-4].

Supplemental Table 3. Whole exome sequencing results.

A – filtering

	II:3	III:2	III:3
DNA extracted from	buccal swab	peripheral blood	peripheral blood
variants identified	41481	41596	43922
(i) variants identified in all individuals		30377	
(ii) predicted to be damaging (SIFT,[5] Polyphen,[6] and MetaLR[7])		29	
(iii) allele frequency of ≤ 0.1% (1000G,[8] ESP6500, ExAc[9])		8	
(iv) not listed in our in-house database		8	

Person identifiers are given with respect to the pedigree in Figure 1. ESP6500, Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), http://evs.gs.washington.edu/EVS/.

B – list of candidate variants

gene	HGNC	HGVS
COL14A1	2191	NM_021110.2:c.4975C>G p.(Pro1659Ala)
GLRA1	4326	NM_000171.3:c.1292A>G p.(Asn431Ser)
MECOM	3498	NM_004991.3:c.2860T>G p.(Cys954Gly)
OR11H4	15347	NM_001004479.1:c.394C>G p.(Arg132Gly)
SH2D6	30439	NM_201594.2:c.428C>T p.(Pro143Leu)
TMPRSS3	11877	NM_024022.2:c.756C>G p.(Ile252Met)
TRMU	25481	NM_018006.4:c.985T>A p.(Cys329Ser)
WNT10B	12775	NM_003394.3:c.943C>T p.(Pro315Ser)

HGNC, gene identifier with respect to the HUGO gene nomenclature committee; HGVS, variant description follows recommendations of the Human Genome variation Society.

Supplemental Table 4. Variants in familial MDS/AL syndromes genes in II:3.

gene	HGNC	HGVS	AF
ANKRD26	29186	NM_014915.2:c.2373-16A>G	0.8041
		NM_014915.2:c.59A>G p.(Gln20Arg)	0.8616
ETV6	3495	NM_001987.4:c.34-632T>C	0.3495
		NM_001987.4:c.34-617C>T	0.2857
		NM_001987.4:c.34-614A>T	0.3485
GATA2	4171	NM_001145661.1:c.1018-19C>T	0.1565
		NM_001145661.1:c.[15C>G];[15C>G] p.[(Pro5=)];[(Pro5=)]	0.5946
RUNX1	10471	NM_001754.4:c.805+186C>T	0.0350
SRP72	11303	NM_006947.3:c.21G>T p.(Gly7Gly)	0.2068
		NM_006947.3:c.826-23A>G	0.3859
TERT	11730	NM_198253.2:c.2843+17G>A	0.0001

With respect to genes known to be associated with familial MDS/AL syndromes (*ANKRD26*, *CEBPA*, *DDX41*, *ETV6*, *GATA2*, *RUNX1*, *SRP72, TERC*, and *TERT*),[10] the following tables states all variants identified in these genes and gives their allele frequency (AF) with respect to KAVIAR[11] database. No *CEBPA* and *TERC* variants were identified. The person identifier refers to the pedigree in Figure 1. HGNC, gene identifier with respect to the HUGO gene nomenclature committee; HGVS, variant description follows recommendations of the Human Genome variation Society.

Supplemental Table 5. *In silico* prediction regarding the functional consequences of MECOM:c.2296T>G p.(Cys766Gly).

in silico tool	predicted result	information
Align GVGD[12]	most likely interfere with function	GV score 0.00; GD score 158.23, class C65
MutationTaster[13]	disease causing	simple_aae model; probability 0.999999999882093, PhyloP score 5.089, phastCons score 1
PolyPhen-2 v2.2.2r398[6]	possibly damaging	HumDiv score 0.845 (sensitivity 0.83, specificity 0.93); HumVar score 0.846 (sensitivity 0.73, specificity 0.88)
SIFT[5]	affect protein function	score 0.00, median sequence conservation 3.82, 11 sequences represented at this position, there is low confidence in this prediction

To assess the functional impact of the missense mutation segregating with the RUSAT phenotype in our family, four individual *in silico* tools were applied. For SIFT *in silico* prediction, MECOM reference protein sequences obtained from UniProtKB database were aligned using ClustalW2 with default settings and 'fasta' as output format. Web Resources: Align GVGD, http://agvgd.hci.utah.edu; ClustalW2 align, https://www.ebi.ac.uk/Tools/msa/clustalw2; MutationTaster, http://www.mutationtaster.org; PolyPhen-2 v2.2.2r398, http://genetics.bwh.harvard.edu/pph2; SIFT, http://sift.bii.a-star.edu.sg/index.html; UnitProtKB protein knowledgebase, http://www.uniprot.org/.

Web Resources

- 1000 Genome Project, http://www.internationalgenome.org/
- ClustalW2 align, https://www.ebi.ac.uk/Tools/msa/clustalw2
- ESP6500, Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), http://evs.gs.washington.edu/EVS/
- Exome Aggregation Consortium (ExAc), http://exac.broadinstitute.org/
- UnitProtKB protein knowledgebase, http://www.uniprot.org/

References

1. Yuasa H, Oike Y, Iwama A, et al. Oncogenic transcription factor Evi1 regulates hematopoietic stem cell proliferation through GATA-2 expression. EMBO J. 2005;24(11):1976-1987.
2. Tapper W, Jones AV, Kralovics R, et al. Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. Nat Commun. 2015;6(6691.
3. Chiang YH, Chang YC, Lin HC, et al. Germline variations at JAK2, TERT, HBS1L-MYB and MECOM and the risk of myeloproliferative neoplasms in Taiwanese population. Oncotarget. 2017; Oct 19. doi: 10.1002/ajh.24946. [Epub ahead of print]
4. Trifa AP, Banescu C, Bojan AS, et al. MECOM, HBS1L-MYB, THRB-RARB, JAK2 and TERT polymorphisms defining the genetic predisposition to myeloproliferative neoplasms - a study on 939 patients. Am J Hematol. 2017;
5. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res. 2012;40(Web Server issue):W452-457.
6. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7(4):248-249.
7. Dong C, Wei P, Jian X, et al. Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. Hum Mol Genet. 2015;24(8):2125-2137.
8. Genomes Project C, Auton A, Brooks LD, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68-74.
9. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-291.
10. Churpek JE, Godley LA. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood. 2016;128(14):1800-1813.
11. Glusman G, Caballero J, Mauldin DE, Hood L, Roach JC. Kaviar: an accessible system for testing SNV novelty. Bioinformatics. 2011;27(22):3216-3217.
12. Tavtigian SV, Deffenbaugh AM, Yin L, et al. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J Med Genet. 2006;43(4):295-305.
13. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11(4):361-362.