Clonal megakaryocyte dysplasia with normal blood values: a covert, thrombosis-prone, early myeloproliferative neoplasm

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Supplemental material

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Supplemental Methods

Genomic profiling

Screening for myeloid cancer associated mutations was performed using oncoReveal Myeloid Panel (Pillar Biosciences, MA, USA) an amplicon-based library preparation chemistry that interrogates genes recurrently mutated in myeloid neoplasms. We focused on a core panel of 45 genes (ANKRD26 (exon 1), ASXL1 (exons 12-13), BCOR (exons 2-15), BCORL1 (exons 1-12), CALR (chr 19: g.13054521-13054710), CBL (exons 1-3, 5, 8-10, 12-13, 16), CEBPA (exon 1), CSF3R (exons 14-16), CUX1 (exons 1-5, 9-10, 12, 17, 20-21, 24), DDX41 (exons 1, 3, 5-6, 8, 10-11, 14-15), DNMT3A (exons 1-23), ETNK1 (exon 3), ETV6 (exons 1-8), EZH2 (exons 2-20), FLT3 (exons 14, 20), GATA2 (exons 3-7), GNAS (exons 8-9), HRAS (exons 2-3), IDH1 (exon 4), IDH2 (exons 4, 6), JAK2 (exons 12-15), KIT (exons 2, 8-11, 13-15, 17-18), KMT2A (exons 1-11, 27, 31, 35), KRAS (exons 2-4), MPL (exon 10), NF1 (exons 1-4, 6, 12, 30, 37-39, 41, 45, 49, 52, 58), NPM1 (exon 11), NRAS (exons 2-4), PDGFRA (exons 12, 14-15, 18), PHF6 (exons 2-10), PIGA (exons 2-6), PPM1D (exon 6), PTEN (exons 5, 7), PTPN11 (exons 3, 13), RAD21 (exons 2-14), RUNX1 (exons 2-9), SETBP1 (exon 4), SF3B1 (exons 13-16), SRSF2 (exon 1), STAG2 (exons 3-35), TET2 (exons 3-11), TP53 (exons 2-11), U2AF1 (exons 1-3, 5-6, 8), WT1 (exons 1-10), ZRSR2 (exons 1-11)). Briefly, pairs of DNA oligos targeting each region of interest were used in the first round of gene-specific PCR and the products subsequently purified via size selection. After purification, a second round of PCR adds index adaptors and P5 & P7 sequences to each library for sample tracking and sequencing. The resulting libraries were further purified and 2 x 250 bp paired-end sequenced on an Illumina MiSeq-system platform.

NGS Data Analysis

The analysis of NGS data performed all the typical steps of secondary analysis from reads mapping and refinement [1, 2] to the identification of single nucleotide variants (SNVs) and insertions/deletions (Indels). The variant calling step integrated four variant calling algorithms [3, 4, 5, 6] since such approach allows identifying variants with higher sensitivity and accuracy than using any single algorithm [7, 8]. Detected variants were annotated with information about their effects on the structure or function of proteins, their frequency in populations (e.g. dbSNP, ESP6500, ExAC, gnomAD) or their presence in cancer databases (e.g. COSMIC, cBioPortal for Cancer Genomics, ClinVar). Synonymous

variants, variants located outside protein coding regions and variants with VAF <2% were filtered. The remaining variants, were tagged using different criteria based on information retrieved from literature, sequence conservation and in silico prediction effect. Classification of germline variants was performed using Franklin (https://franklin.genoox.com) a curation tools based on the American College of Medical Genetics (ACMG) guidelines.

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Supplemental Table 1. Circumstance of CMD-NBV diagnosis.

		Precipitating event	**Diagnostic strategy**
UPN1	M/25	Idiopathic mesenteric, portal, splenic vein thrombosis	Screen for MPN
UPN2	M/52	Portal cavernoma, oesophageal varices, splenomegaly	Screen for MPN
UPN3	F/49	Unexplained popliteal arterial thrombosis	Screen for MPN
UPN4	M/57	Idiopathic sagittal cerebral sinus venous thrombosis	Screen for MPN
UPN5	F/44	Recurrent idiopathic mesenteric and portal vein thromboses	Screen for MPN
UPN6	M/23	Hemoglobin = 16 g/dL, familial sclerosing cholangitis	Evaluate polycythemia
UPN7	M/49	Idiopathic portal and spleen vein thromboses	Screen for MPN
UPN8	M/49	Popliteal arterial portal vein thromboses	Screen for MPN
UPN9	F/38	Idiopathic portal vein thrombosis	Screen for MPN
UPN10	M/44	Vertebral bone alteration	Screen for MPN
UPN11	F/42	Portal cavernoma, splenomegaly	Screen for MPN

UPN12	F/32	Portal vein thrombosis	Screen for MPN
UPN13	M/52	Splenomegaly	Evaluate splenomegaly
UPN14	F/46	Portal cavernoma, splenomegaly	Screen for MPN
UPN15	M/49	Renal vein thrombosis	Screen for MPN
UPN16	F/37	Platelets = 343 x10E+9/L	Evaluate thrombocytosis
UPN17	M/71	Splenomegaly	Evaluate splenomegaly
UPN18	F/46	Portal, mesenteric vein thromboses	Screen for MPN
UPN19	F/54	Portal vein thrombosis, Budd-Chiari syndrome	Screen for MPN
UPN20	M/29	Platelets = 343 x 10E+9/L	Evaluate thrombocytosis
UPN21	M/52	Idiopathic mesenteric vein thrombosis	Screen for MPN
UPN22	F/53	Eosinophilia, WBC = 10 x E+9/L)	Evaluate eosinophilia
UPN23	F/70	Hematocrit = 47,6%	Evaluate polycythaemia
UPN24	F/43	Idiopathic spleen vein thrombosis	Screen for MPN
UPN25	M/20	Idiopathic portal vein thrombosis, Budd-Chiari syndrome	Screen for MPN
UPN26	M/49	Portal cavernoma	Screen for MPN
UPN27	F/70	Pulmonary post-embolic hypertension	Screen for MPN
UPN28	M/39	Portal vein thrombosis	Screen for MPN
UPN29	F/55a	Portal cavernoma	Screen for MPN

UPN30	M/75	Splenomegaly	Evaluate splenomegaly

Supplemental Table 2. European Health Interview Survey (EHIS)-defined co-morbidities present in CMD-NBV subjects at the first referral at our Center

Co-morbidity present	**Subjects number (%)** **Total =30**
Asthma (allergic asthma included)	0
Chronic bromchitis, chronic obstructive pulmonary disease, emphysema	1 (3)
Myocardial infarction (heart attack) or chronic consequences of myocardial infarction	2 (7)
Coronary heart disease or angina pectoris	2 (7)
High blood pressure (hypertension)	10 (33)
Stroke (cerebral haemorrhage, cerebral thrombosis) or chronic consequences of stroke	3 (10)
Arthrosis (arthritis excluded)	0
Low back disorder or other chronic back defect	0
Neck disorder or other chronic neck defect	0
Diabetes	1 (3)
Allergy, such as rhinitis, hay fever, eye inflammation, dermatitis, food allergy or other allergy (allergic asthma excluded)	1 (3)
Cirrhosis of the liver	0
Urinary incontinence, problems in controlling the bladder	0
Kidney problems	1 (3)
Depression	1 (3)

Note: The 15 illnesses diagnosed before or in conjunction with CMD-NBV included hypertension, coronary heart disease and angina pectoris, myocardial infarction, stroke, diabetes, asthma, chronic bronchitis, chronic obstructive pulmonary disease and emphysema, urinary incontinence, kidney problems, spinal and back pain, neck spine pain, allergies, cirrhosis of the liver, arthrosis.

Supplemental Table 3. Horvat-defined comorbidities present in subjects with CMD-NBV. Data obtained at the first referral at our Center

	Subjects number (%) Total=30
Cardiovascular/metabolic	12 (40)
Autoimmune	10 (33)
Inflammatory	0
Malignancy	4 (13)

Supplemental Table 4. Putative germline variations in subjects with CMD-NBV

Patient ID	Gene	Nucleotide change	Aminoacid change	Transcript ID	Consequence	VAF (%)	ExAC Frequency	ClinVar Status	Franklin Status
UPN4	*ASXL1*	c.3306G>T	p.Glu1102Asp	NM_015338	missense	52	0.01	benign	polymorphism
UPN5	*DNMT3A*	c.1502A>G	p.Asn501Serqì	NM_022552	missense	42	0.0003	likely benign	likely benign
UPN8	*RUNX1*	c.167C>T	p.Leu56Ser	NM_001754.5	missense	51	0.01629	benign	benign
UPN10	CUX1	c.2371G>A	p.Ala791Thr	NM_181552R	missense	47	0.000017	NR	uncertain signficance
UPN10	*ABL1*	c.589G>A	p.Glu197Lys	NM_005157.6	missense	54	0.00027	uncertain significante/benign	uncertain signficance
UPN12	*CSF3R*	c.2422G>A	p.Glu808Lys	NM_000760.4	missense	50	0.00622	benign/likely benign	benign
UPN18	*NF1*	c.-22G>C	Null	NM_001042492	5'UTR variant	35	0.00358	benign/likely benign	benign
UPN20	*ASXL1*	c.*87A>G	Null	NM_015338	3'UTR variant	49	NR	NR	benign
UPN25	*TET2*	c.1018A>G	p.Ile340Val	NM_0011127208	missense	51	0.000025	NR	likely benign
UPN27	*NF1*	c.6790A>T	p.Ile2264Leu	NM_001042492	missense	50.6	0.000025	uncertain significante/likely benign	likely benign
UPN28	*TET2*	c.521C>A	p.Pro174His	NM_001127208	missense	44.6	0.0019	uncertain significance/likely benign	likely benign
UPN29	*KIT*	*c.101C>T*	p.Pro34Leu	NM_000222	missense	52	0.00006	Uncertain	uncertain significance

Patient ID	Gene	Nucleotide change	Aminoacid change	Transcript ID	Consequence	VAF (%)	ExAC Frequency	ClinVar Status	Franklin Status
								significance/likely benign	

Abbreviations: VAF, variant allele frequency; NR, not reported.