The mutational landscape of 18 investigated genes clearly separates four subtypes of myelodysplastic/myeloproliferative neoplasms

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SUPPLEMENTAL INFORMATION

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1. Patients and methods

1.1 Patient cohorts

A total of 177 patients were analyzed. The clinical data sex, age, white blood cell count (WBC), hemoglobin levels (Hb), platelet counts (PLT) and karyotype are given in table S1. Samples were referred to our laboratory between October 2005 and November 2014 for diagnostic assessment. Diagnosis was performed on bone marrow smears according to standard World Health Organization (WHO) criteria.[1]

All patients gave their written informed consent for scientific evaluations. The study design adhered to the tenets of the Declaration of Helsinki and was approved by our institutional review board before its initiation.

Table S1. Clinical information.

	Total cohort	aCML	CMML	MDS/MPN, U	MDS/MPN-RS-T
Case number	177	35	58	39	45
Clinical variables					
Ratio female: male	72:105	14:21	14:44	19:20	25:20
Median age in years (range)	75 (22-89)	76 (45-89)	73 (22-86)	73 (32-89)	76 (55-89)
Median WBC 10^9/L (range)	13.1 (1.3-206.5)	33.9 (16.3-206.5)	13.5 (2.4-160)	12.1 (1.3-78.7)	7.5 (2.9-20)
Median Hb g/dL (range)	10.1 (5.7-19.7)	10.2 (5.7-14.6)	11.0 (5.9-17.0)	10.0 (6.9-19.7)	9.6 (6.9-13.1)
Median PLT 10^9/L (range)	323.5 (6-1,474)	150 (6-1,455)	80 (16-933)	555 (7-1,474)	572 (454-1,416)
Karyotype					
Normal number (%)	127 (74%)	22 (63%)	41 (75%)	27 (71%)	37 (86%)
Aberrant number (%)	44 (26%)	13 (37%)	14 (25%)	11 (29%)	6 (14%)
Investigated material, n					
Cytomorphology	BM: 75 BM + PB: 78 PB: 24	BM: 20 BM + PB: 11 PB: 4	BM: 26 BM + PB: 16 PB: 16	BM + PB: 35 PB: 4	BM: 29 BM + PB: 16
Genetics	BM: 153 PB: 24	BM: 31 PB: 4	BM: 42 PB: 16	BM: 35 PB: 4	BM: 45

BM: Bone marrow, PB: peripheral blood.

1.2 Cytomorphology

In all cases bone marrow/peripheral blood smears underwent May Giemsa Gruenwald staining. For cytomorphology, at least 200 nucleated cells were counted in the bone marrow/peripheral blood. Cytochemistry was performed for myeloperoxidase (MPO) and non-specific esterase (NSE), and iron staining was done for detection of ring sideroblasts in cases with increased erythropoiesis or anemia.[2] Classification of the disease entities and dysplasia was rated according to WHO criteria.[1]

1.3 Cytogenetics

Chromosome banding analysis was performed in 171/177 cases after short-term culture according to standard methods.[3] Karyotypes were analyzed after G-banding and described according to the International System for Human Cytogenetic Nomenclature.[4]

1.4 Next generation sequencing

All patients were analyzed by a myeloid gene panel containing *ASXL1*, *BRAF*, *CALR*, *CBL*, *CSF3R, DNMT3A*, *ETNK1*, *JAK2*, *MPL*, *NPM1, NRAS*, *KRAS*, *RUNX1, SETBP1*, *SF3B1*, *SRSF2*, *TET2* and *U2AF1*. The library of 18 genes was generated either with the ThunderStorm (RainDance Technologies, Billerica, MA) or with the Access Array System (Fluidigm, San Francisco, CA). Both libraries were sequenced and demultiplexed on a MiSeq instrument (Illumina, San Diego, CA) as described previously.[5] The FASTQ files were further processed using the Sequence Pilot software version 4.1.1 Build 510 (JSI Medical Systems, Ettenheim, Germany) for alignment and variant calling. Analysis parameters were set according to manufacturer's default recommendation. Validity of the somatic mutations was checked against the publicly accessible COSMIC v69 database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic) and functional interpretation was performed using SIFT 1.03 (http://sift.jcvi.org), PolyPhen 2.0 (http://genetics.bwh.harvard.edu/pph2) and MutationTaster 1.0 algorithms (http://www.mutationtaster.org).[6] Single-nucleotide polymorphisms (SNP) were annotated according to the NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp; Build 137) database. The detection limit for single nucleotide variants was 3% variant allele frequency, for GC-rich sequences the detection limit was set to 5% variant allele frequency (e.g. the homopolymeric region in *ASXL1*).

1.5 Statistical analyses

Dichotomous variables were compared between different groups using the χ^2-test or Fisher's exact test. Results were considered significant at $p<0.05$; the reported p-values are two-sided.

Adjustment for multiple testing was not done. Statistical analyses were performed using SPSS version 19.0 (IBM Corporation, Armonk, NY).

2. Results

2.1 Mutation frequencies

The mutation frequencies of all 18 analyzed genes and cytogenetic information are given for all four entities separately as well as the total cohort in table S2.

Table S2. Genetic characterization.

	Total cohort			**aCML**			**CMML**			**MDS/MPN, U**			**MDS/MPN-RS-T**		
Gene	n mutated/ aberrant	n analyzed	%	n mutated/ aberrant	n analyzed	%	n mutated/ aberrant	n analyzed	%	n mutated/ aberrant	n analyzed	%	n mutated/ aberrant	n analyzed	%
ASXL1	74	177	42%	21	35	60%	30	58	52%	14	39	36%	9	45	20%
TET2	70	177	40%	15	35	43%	31	58	53%	10	39	26%	14	45	31%
DNMT3A	15	177	8%	3	35	9%	1	58	2%	3	39	8%	8	45	18%
SRSF2	53	177	30%	12	35	34%	31	58	53%	6	39	15%	4	45	9%
SF3B1	53	177	30%	4	35	11%	3	58	5%	5	39	13%	41	45	91%
U2AF1	15	177	8%	3	35	9%	3	58	5%	7	39	18%	2	45	4%
JAK2	37	177	21%	1	35	3%	4	58	7%	9	39	23%	23	45	51%
CALR	3	177	2%	1	35	3%	0	58	0%	2	39	5%	0	45	0%
MPL	4	177	2%	1	35	3%	0	58	0%	2	39	5%	1	45	2%
NRAS	26	177	15%	11	35	31%	15	58	26%	0	39	0%	0	45	0%
KRAS	12	177	7%	3	35	9%	8	58	14%	0	39	0%	1	45	2%
CBL	21	177	12%	2	35	6%	14	58	24%	2	39	5%	3	45	7%
RUNX1	22	177	12%	7	35	20%	12	58	21%	2	39	5%	1	45	2%
SETBP1	17	177	10%	8	35	23%	5	58	9%	4	39	10%	0	45	0%
BRAF	2	177	1%	1	35	3%	1	58	2%	0	39	0%	0	45	0%
CSF3R	3	177	2%	2	35	6%	0	58	0%	1	39	3%	0	45	0%
ETNK1	5	177	3%	1	35	3%	2	58	3%	1	39	3%	1	45	2%
NPM1	0	177	0%	0	35	0%	0	58	0%	0	39	0%	0	45	0%
Karyotype	44	171	26%	13	35	37%	14	55	25%	11	38	29%	6	43	14%
Epigenetic	122	177	69%	27	35	77%	49	58	84%	20	39	51%	26	45	58%
Splicing	116	177	66%	18	35	51%	36	58	62%	17	39	44%	45	45	100%
JAK-STAT pathway	44	177	25%	3	35	9%	4	58	7%	13	39	33%	24	45	53%
RAS pathway	49	177	28%	13	35	37%	30	58	52%	2	39	5%	4	45	9%

2.2 Variant allele frequencies of gene mutations within the different affected pathways

To get more insights in the different affected pathways and their input to clonal complexity, we addressed the mean variant allele frequencies (VAF) of gene mutations within one affected pathway. The mean VAF of gene mutations of the epigenetic regulation (*ASXL1*, *DNMT3A* and *TET2*), the splicing machinery (*SRSF2*, *SF3B1* and *U2AF1*), the JAK-STAT pathway (*JAK2*, *CALR* and *MPL*) and the RAS pathway (*KRAS*, *NRAS* and *CBL*) were addressed (Fig. S1).

The mean VAF were similar in all four entities for all four pathways, pointing to driver mutations in the main cell clone of the neoplasms.

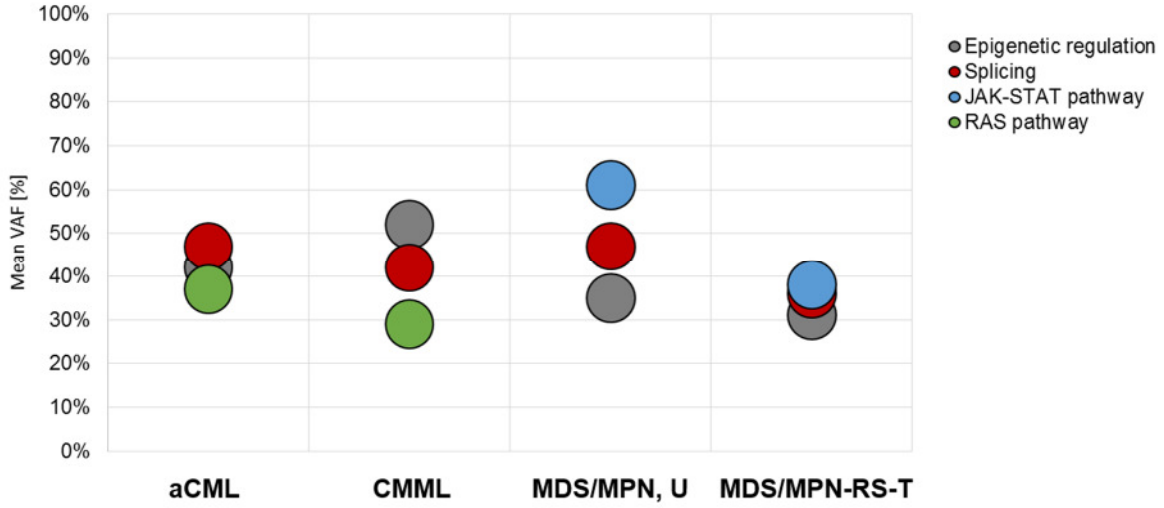

Figure S1. Mean variant allele frequencies (VAF) of gene mutations grouped by different affected pathways are given for all four entities. The four affected pathways are colored in grey: epigenetic regulation, red: splicing, blue: JAK-STAT pathway and green: RAS pathway.

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