Hematopoietic alterations in chronic heart failure patients by somatic mutations leading to clonal hematopoiesis

Lena Dorsheimer,[1] Birgit Assmus,[2*] Tina Rasper,[3] Christina A. Ortmann,[1] Khalil Abou-El-Ardat,[1,4] Katharina C. Kiefer,[1] Jedrzej Hoffmann,[2,5] Florian Seeger,[2] Halvard Bonig,[6] Stefanie Dimmeler,[3,5,#] Andreas M. Zeiher[2,5,#] and Michael A. Rieger[1,4,7,#]*

[*]LD and BA contribed equally as co-first authors

[#]SD, AMZ and MAR contribed equally as co-senior authors

[1]Department of Medicine, Hematology/Oncology, Goethe University Hospital, Frankfurt; [2]Department of Medicine, Cardiology, Goethe University Hospital, Frankfurt; [3]Institute for Cardiovascular Regeneration, Goethe University Frankfurt, Frankfurt; [4]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg; [5]German Center for Cardiovascular Research DZHK, Berlin, partner site Frankfurt Rhine-Main; [6]Institute for Transfusion Medicine, Goethe University Hospital, Frankfurt and [7]Frankfurt Cancer Institute, Frankfurt, Germany

Correspondence: MICHAEL A. RIEGER - m.rieger@em.uni-frankfurt.de

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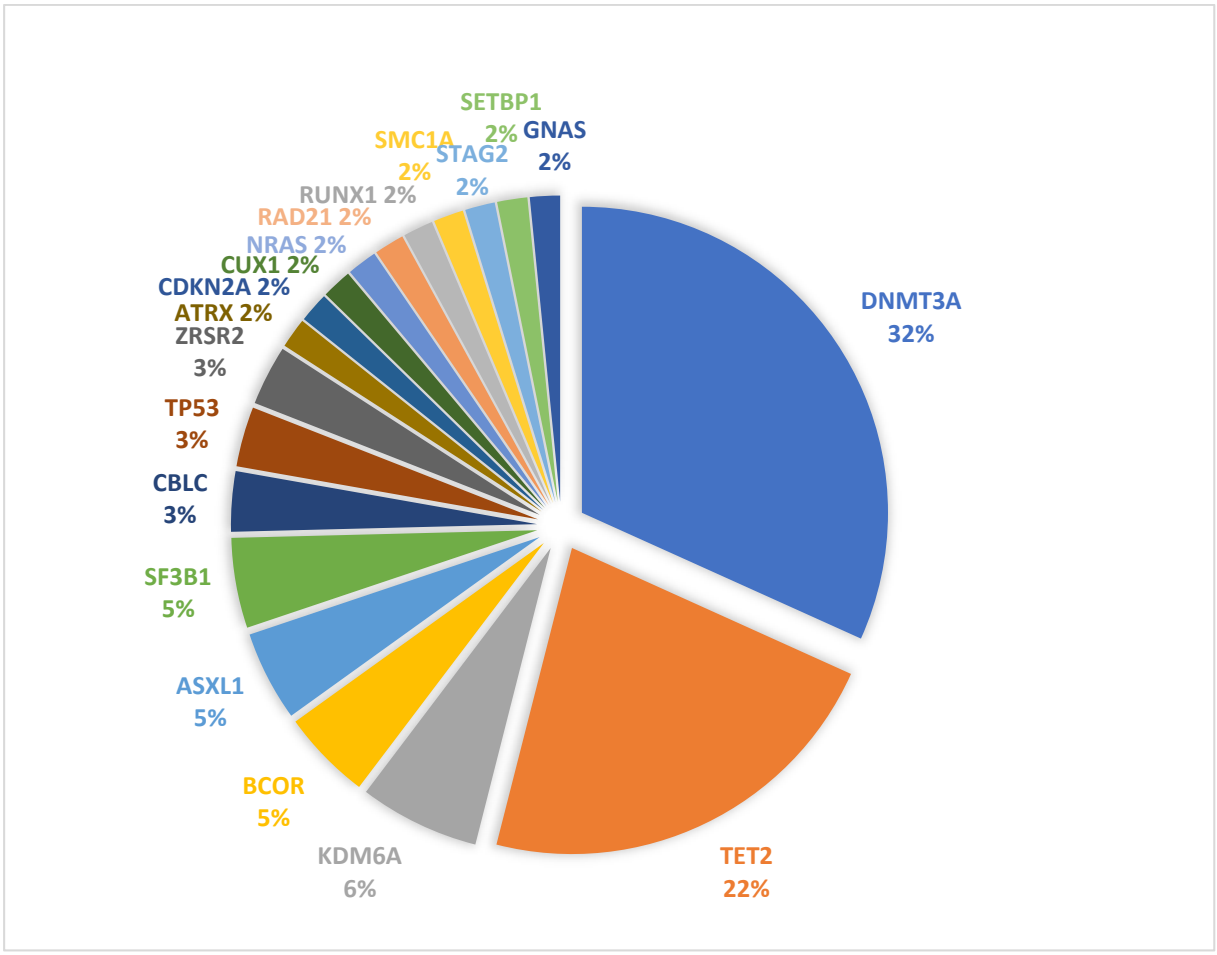

Figure S1. Somatic mutations with a variant allele fraction higher than 0.02 in our CHF patient cohort. 56 CHIP-associated genes were analyzed by error-corrected targeted amplicon sequencing. The distribution of identified mutated genes is displayed.

Unique Patient Number	Mutated gene 1	Mutated gene 2	Mutated gene 3
UPN1	TET2		
UPN2	RAD21		
UPN3	DNMT3A		
UPN4	SF3B1	TET2	
UPN5	CUX1		
UPN6	TP53		
UPN7	BCOR		
UPN8	DNMT3A		
UPN9	DNMT3A		
UPN10	KDM6A		
UPN11	ASXL1		
UPN12	DNMT3A		
UPN13	STAG2		
UPN14	KDM6A		
UPN15	RUNX1		
UPN16	BCOR		
UPN17	TET2		
UPN18	DNMT3A	KDM6A	
UPN19	DNMT3A	CDKN2A	
UPN20	TET2		
UPN21	DNMT3A		
UPN22	ZRSR2		
UPN23	DNMT3A		
UPN24	DNMT3A	CBLC	
UPN25	TP53		
UPN26	TET2		
UPN27	TET2		
UPN28	TET2	TET2	
UPN29	BCOR		
UPN30	DNMT3A	ASXL1	DNMT3A
UPN31	SF3B1	DNMT3A	
UPN32	TET2		
UPN33	DNMT3A	SMC1A	
UPN34	DNMT3A		
UPN35	CBLC		
UPN36	ATRX		
UPN37	KDM6A		
UPN38	NRAS		
UPN39	SETBP1	DNMT3A	
UPN40	DNMT3A		
UPN41	DNMT3A		
UPN42	TET2	SF3B1	
UPN43	ASXL1		
UPN44	DNMT3A		
UPN45	GNAS		
UPN46	TET2		
UPN47	DNMT3A		
UPN48	TET2		
UPN49	TET2		
UPN50	DNMT3A		
UPN51	ZRSR2		
UPN52	TET2		

Table S1. CHF patients with CHIP-associated mutations of a VAF ≥ 0.02.

When more than one mutation was identified, then these mutations were listed according to their VAF, beginning with the highest.

Table S2. List of CHIP-associated somatic variants identified in CHF patients

Hugo Symbol	Chr	Position	Variant Classification	Variant Type	Ref. Allele	Alt. Allele	DNA Change	RefSeq	Protein Change	Ref. Reads (CAT A)	Alt. Reads (CAT A)	Ref. Reads (CAT B)	Alt. Reads (CAT B)	VAF
ASXL1	20	31022263	stop_gained	SNP	G	A	c.1748G>A	NM_015338.5	p.W583*	882	64	700	81	0.0677
ASXL1	20	31024387	missense_variant	SNP	T	C	c.3872T>C	NM_015338.5	p.L1291P	121	4	632	4	0.0320
ASXL1	20	31022370	frameshift_variant	INS	G	GC	c.1858dupC	NM_015338.5	p.R620fs	1180	18	549	20	0.025
ATRX	X	76872185	missense_variant	SNP	G	A	c.5462C>T	NM_000489.4	p.T1821I	95	2	186	2	0.0206
BCOR	X	39921637	missense_variant	SNP	G	C	c.4183C>G	NM_001123385.1	p.R1395G	448	74	30	7	0.1418
BCOR	X	39930318	missense_variant	SNP	G	A	c.3146C>T	NM_001123385.1	p.A1049V	122	3	162	2	0.0240
BCOR	X	39933926	stop_gained	SNP	G	A	c.673C>T	NM_001123385.1	p.Q225*	627	31	390	29	0.0471
CBLC	19	45297479	missense_variant	SNP	C	T	c.1303C>T	NM_012116.3	p.P435S	34539	1527	123	2	0.0423
CBLC	19	45297479	missense_variant	SNP	C	T	c.1303C>T	NM_012116.3	p.P435S	745	32	100	2	0.0412
CDKN2A	9	21974878	5_prime_UTR_variant	SNP	C	T	c.-52G>A	NM_001195132.1		146	3	295	3	0.0201
CUX1	7	101839979	missense_variant	SNP	C	G	c.1321C>G	NM_001202543.1	p.P441A	121	3	62	2	0.0242
DNMT3A	2	25464430	splice_donor_variant	SNP	C	T	c.2082+1G>A	NM_022552.4		277	16	250	8	0.0546
DNMT3A	2	25457249	missense_variant	SNP	T	C	c.2638A>G	NM_022552.4	p.M880V	388	21	1107	58	0.0513
DNMT3A	2	25457217	frameshift_variant	DEL	GC	G	c.2669delG	NM_022552.4	p.G890fs	316	19	873	42	0.0567
DNMT3A	2	25457243	missense_variant	SNP	G	A	c.2644C>T	NM_022552.4	p.R882C	483	17	1143	83	0.0340
DNMT3A	2	25471084	missense_variant	SNP	G	A	c.677C>T	NM_022552.4	p.A226V	93	2	179	2	0.0211
DNMT3A	2	25471013	missense_variant	SNP	G	A	c.748C>T	NM_022552.4	p.P250S	142	4	249	2	0.0274
DNMT3A	2	25469936	missense_variant	SNP	A	C	c.1106T>G	NM_022552.4	p.I369S	397	91	92	19	0.1865
DNMT3A	2	25458696	splice_acceptor_variant	SNP	T	G	c.2479-2A>C	NM_022552.4		1260	28	572	5	0.0217
DNMT3A	2	25462077	missense_variant	SNP	G	T	c.2330C>A	NM_022552.4	p.P777H	1639	108	587	40	0.0618
DNMT3A	2	25464487	missense_variant	SNP	G	A	c.2026C>T	NM_022552.4	p.R676W	580	13	142	15	0.0218
DNMT3A	2	25469945	missense_variant	SNP	C	T	c.1097G>A	NM_022552.4	p.R366H	191	8	22	3	0.0402
DNMT3A	2	25467156	disruptive_inframe_deletion	DEL	CTGGGCA	C	c.1713_1718delTGCCCA	NM_022552.4	p.A572_Q573del	745	28	414	9	0.0362
DNMT3A	2	25464547	stop_gained; protein_protein_contact	SNP	G	A	c.1966C>T	#	p.Q656*	469	14	131	4	0.0290
DNMT3A	2	25462077	missense_variant	SNP	G	C	c.2330C>G	NM_022552.4	p.P777R	1724	207	644	73	0.1072
DNMT3A	2	25462017	missense_variant	SNP	T	C	c.2390A>G	NM_022552.4	p.N797S	847	99	557	65	0.1046
DNMT3A	2	25470920	stop_gained	SNP	C	A	c.841G>T	NM_022552.4	p.E281*	182	8	245	16	0.0517

DNMT3A	2	25462085	splice_acceptor_variant&intron_variant	SNP	C	T	c.2323-1G>A	NM_022552.4	.	1495	73	958	28	0.0375
DNMT3A	2	25458637	stop_gained	SNP	G	A	c.2536C>T	NM_022552.4	p.Q846*	1230	84	825	52	0.0616
DNMT3A	2	25457242	missense_variant	SNP	C	G	c.2645G>C	NM_022552.4	p.R882P	741	415	1113	661	0.3654
DNMT3A	2	25464445	protein_protein_contact; protein_protein_contact; missense_variant	SNP	C	A	c.2068G>T; c.2068G>T; c.2068G>T	NM_022552.4:4U7T_A:690_736;NM_022552.4:4U7T_C:690_736;NM_022552.4	.;.;p.V690F	431	6	313	9	0.0208
GNAS	20	57484420	missense_variant	SNP	C	A	c.2530C>A	NM_080425.3	p.R844S	512	157	307	81	0.2214
KDM6A	X	44732924	missense_variant	SNP	G	C	c.127G>C	NM_001291415.1	p.A43P	194	5	113	3	0.0251
KDM6A	X	44913149	frameshift_variant & missense_variant	COMPLEX	AG	T	c.824_825delAGinsT	NM_001291415.1	p.K275fs	231	48	138	44	0.1720
KDM6A	X	44941858	frameshift_variant	DEL	CAG	C	c.3340_3341delGA	NM_001291415.1	p.D1114fs	89	2	157	2	0.0220
KDM6A	X	44732828	missense_variant	SNP	G	A	c.31G>A	NM_001291415.1	p.A11T	134	3	180	3	0.0219
NRAS	1	115256464	missense_variant	SNP	C	T	c.247G>A	NM_002524.4	p.A83T	98	2	2032	2	0.0200
RAD21	8	117866614	frameshift_variant&stop_gained	INS	T	TAATC	c.1027_1030dupGATT	NM_006265.2	p.Y344fs	461	77	216	51	0.1431
RUNX1	21	36206842	missense_variant	SNP	G	A	c.670C>T	NM_001754.4	p.R224W	200	5	301	2	0.0244
SETBP1	18	42531925	disruptive_inframe_deletion	DEL	GACA	G	c.2627_2629delACA	NM_015559.2	p.N876del	528	396	183	108	0.3976
SF3B1	2	198266834	missense_variant	SNP	T	C	c.2098A>G	NM_012433.3	p.K700E	1310	328	477	114	0.2002
SF3B1	2	198266834	missense_variant	SNP	T	C	c.2098A>G	NM_012433.3	p.K700E	1600	190	595	83	0.1061
SF3B1	2	198266834	missense_variant	SNP	T	C	c.2098A>G	NM_012433.3	p.K700E	1788	45	446	13	0.0264
SMC1A	X	53432495	missense_variant	SNP	C	T	c.1841G>A	NM_006306.3	p.G614D	179	4	244	2	0.0219
STAG2	X	123227876	missense_variant	SNP	G	A	c.3587G>A	NM_001042749.2	p.G1196D	125	3	68	2	0.0234
TET2	4	106156963	stop_gained	SNP	C	T	c.1864C>T	NM_001127208.2	p.Q622*	986	211	268	68	0.1763
TET2	4	106197104	stop_gained	SNP	C	T	c.5437C>T	NM_001127208.2	p.Q1813*	1325	143	342	45	0.0974
TET2	4	106190804	missense_variant	SNP	G	A	c.4082G>A	NM_001127208.2	p.G1361D	256	6	151	3	0.0229
TET2	4	106193995	stop_gained	SNP	C	G	c.4457C>G	NM_001127208.2	p.S1486*	859	44	142	10	0.0487
TET2	4	106158479	frameshift_variant	DEL	AATATG	A	c.3383_3387delTGAT	NM_001127208	p.Y1128fs	1063	241	311	37	0.1848
TET2	4	106155920	frameshift_variant	DEL	TC	T	c.822delC	NM_001127208.2	p.N275fs	880	52	322	26	0.0558
TET2	4	106164775	stop_gained; protein_protein_contact	SNP	G	T	c.3643G>T; c.3643G>T	NM_001127208.2; NM_001127208.2:4NM6_A:1179_1215	p.E1215*	724	46	111	6	0.0597

TET2	4	106196850	frameshift_variant	INS	A	AGATG	c.5189_5192dupATGG	NM_001127208.2	p.H1732fs	339	17	323	19	0.0476
TET2	4	106164085	splice_donor_variant&intron_variant	SNP	G	C	c.3594+1G>C	NM_001127208.2		1126	351	590	182	0.2373
TET2	4	106156053	frameshift_variant	DEL	AC	A	c.956delC	NM_001127208.2	p.P319fs	911	30	674	22	0.0317
TET2	4	106157245	frameshift_variant	DEL	TCA	T	c.2150_2151delAC	NM_001127208.2	p.H717fs	1463	84	1189	79	0.0582
TET2	4	106190855	missense_variant	SNP	G	A	c.4133G>A	NM_001127208.2	p.C1378Y	1139	35	811	30	0.0327
TET2	4	106164933	frameshift_variant&splice_donor_variant&splice_region_variant&intron_variant	DEL	AGAGT	A	c.3802_3803+2delGAGT	NM_001127208.2	p.R1269fs	775	192	104	62	0.2857
TET2	4	106164866	missense_variant	SNP	A	G	c.3734A>G	NM_001127208.2	p.Y1245C	821	67	116	8	0.07
TP53	17	7578442	missense_variant	SNP	T	C	c.488A>G	NM_000546.5	p.Y163C	543	16	395	12	0.0286
TP53	17	7578394	missense_variant	SNP	T	C	c.536A>G	NM_000546.5	p.H179R	380	45	175	25	0.1059
ZRSR2	X	15821875	stop_gained	SNP	A	T	c.268A>T	NM_005089.3	p.K90*	94	2	330	2	0.0208
ZRSR2	X	15841230	disruptive_inframe_insertion;downstream_gene_variant	INS	C	CAGCCGGAGCCGG	c.1332_1343dupGAGCCGGAGCCG	NM_005089.3	p.R448_R449insSRSR	239	195	230	163	0.417

NM_022552.4;NM_022552.4:4U7T_A:634_656;NM_022552.4:4U7T_A:656_910;NM_022552.4:4U7T_A:656_911;NM_022552.4:4U7T_C:634_656;NM_022552.4:4U7T_C:656_910;NM_022552.4:4U7T_C:656_911

INS Insertion
DEL Deletion
SNP single nucleotide polymorphism
VAF Variant allele fraction
Ref. reference
Alt. alternate

	Total cohort (n=268)	CHIP (n=52)	Non-CHIP (n=216)	P-value (CHIP versus Non-CHIP)
Age (years; n=268)	63 ± 11 64 (55;71)	67 ± 9 68 (62;73)	62 ± 11 62 (54;71)	**<0.01**
Gender male (%; n=268)	83	77	84	0.21
Hypertension (%; n=266)	77	90	74	**0.01**
Hyperlipidemia (%; n=264)	77	82	76	0.38
Diabetes mellitus (%; n=268)	34	39	33	0.67
Current or former smoking (%; n=265)	66	69	65	0.59
Family history of coronary artery disease (%; n=243)	55	50	56	0.50
NYHA class (n=268)	2.2 ± 0.8 2 (2;3)	2.2 ± 0.7 2 (2;3)	2.3 ± 0.8 2 (2;3)	0.74
Seattle Heart Failure Score; (n=214)	0.3 ± 1.0 0.2 (-0.3;0.9)	0.3 ± 0.9 0.3 (-0.1;0.9)	0.3 ± 1.0 0.2 (-0.3;0.9)	0.99
Left ventricular ejection fraction (LVEF; %; n=253)	32.3 ± 11.7 31 (25;40)	33.8 ± 11.3 35 (25;42)	32.0 ± 11.8 30 (24;40)	0.33
NT-proBNP serum levels (pg/ml); (n=268)	1954 ± 3613 912 (401;2014)	2060 ± 2783 971 (419;2785)	1924 ± 3786 911 (354;1910)	0.83
High sensitivity C-reactive protein levels (mg/dl; n=264)	1.14 ± 4.69 0.28 (0.13;0.75)	1.02 ± 1.94 0.33 (0.17;0.97)	1.17 ± 5.12 0.27 (0.13;0.72)	0.83
PB Hemoglobin (g/dl); (n=267)	14 ± 2 14 (13;15)	14 ± 1 14 (13;15)	14 ± 2 14 (13;15)	0.09
PB Hematocrit (%); (n=266)	41 ± 5 41 (38;44)	40 ± 4 40 (36;42)	41 ± 5 41 (38;44)	0.11
PB Thrombocytes ($x10^3/\mu l$); (n=204)	213 ± 64 209 (167;252)	216 ± 74 200 (163;248)	213 ± 61 210 (168;253)	0.79
PB Leukocytes ($x10^3/\mu l$); (n=267)	7.8 ± 2.6 7.4 (6.2;8.8)	7.9 ± 3.0 7.5 (6.2;8.8)	7.8 ± 2.5 7.4 (6.1;8.8)	0.76
BM Erythrocytes ($x10^6/\mu l$); (n=251)	4.1 ± 0.9 4.1 (3.6;4.5)	4.0 ± 0.7 3.9 (3.6;4.4)	4.1 ± 1.0 4.1 (3.6;4.6)	0.38
BM Platelets ($x10^3/\mu l$); (n=252)	100 ± 42 96 (72;125)	103 ± 41 104 (71;129)	99 ± 43 95 (74;125)	0.53
BM Leukocytes ($x10^3/\mu l$); (n=243)	13.8 ± 5.9 12.5 (9.5;17.3)	15.7 ± 7.3 15.2 (9.5;18.7)	13.4 ± 5.4 12.2 (9.5;16.6)	**0.02**
BM Neutrophils (% of Leukocytes); (n=185)	61.1 ± 6.5 61.3 (56.3;65.7)	59.3 ± 6.1 59.2 (55.0;64.4)	61.6 ± 6.5 62.0 (56.9;66.5)	0.06
BM Lymphocytes (% of Leukocytes); (n=189)	24.6 ± 6.0 23.9 (20.0;28.7)	25.3 ± 5.7 25.5 (20.3;28.9)	24.4 ± 6.0 23.6 (19.9;28.6)	0.38
BM Monocytes (% of Leukocytes); (n=190)	8.0 ± 2.1 7.7 (6.4;9.6)	8.6 ± 1.9 8.4 (7.1;10.3)	7.9 ± 2.1 7.6 (6.3;9.5)	0.07
BM CD45+/CD34+ (% of CD45+) (n=231)	0.58 ± 0.32 0.54 (0.38;0.75)	0.64 ± 0.43 0.59 (0.39;0.81)	0.57 ± 0.28 0.53 (0.38;0.74)	0.14
BM CD45+/CD34+/CD133+ (% of CD45+/CD34+) (n=231)	62.1 ± 19.6 66.4 (55.8;75.8)	66.3 ± 17.9 70.3 (59.3;77.5)	61.0 ± 19.9 66.0 (53.5;75.7)	0.08
BM CD45+/CD34+ (cells/µl) (n=241)	84 ± 67 69 (35;120)	108 ± 90 86 (42;159)	78 ± 59 65 (34;110)	**<0.01**
BM CD45+/CD34+/CD133+ (cells/µl) (n=241)	53 ± 46 41 (16;76)	72 ± 64 48 (25;99)	48 ± 39 39 (15;72)	**<0.01**

Table S3. Baseline characteristics of CHF patients with and without CHIP-associated mutations

Continuous variables are shown as mean ± standard deviation; median (interquartile range), categorical variables are shown as frequency (%). The P value for continuous variables were determined by ANOVA testing and for categorical variables by Chi-Square test (two-sided).