

Supplemental Data

CHIP & HIPs: Clonal Hematopoiesis is Common in Hip Arthroplasty Patients and Associates with Autoimmune Disease

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Supplemental Methods:**Sample collection and storage**

Samples were collected at four independent university centers: Technical University of Munich (TUM), University Hospital (LMU) Munich, Technical University of Dresden (TUD) and University Hospital Leipzig (UHL). Patients were enrolled through the prospective BOHEME registry (Leipzig and Dresden sites, NCT02867085) or within local protocols at the Munich sites. Initially, only BM from femoral heads was collected as the studies were planned as biomaterial repositories. The protocols were amended to include peripheral blood samples and more comprehensive clinical information when the high prevalence of CH in this cohort emerged. Approval numbers of the respective ethics committees were TUM 538/16, LMU 19-220, TUD EK 393092016, UHL 137/19-Ik. After disintegrating and mincing the femoral head, BM fragments were transferred into tubes with PBS, and BM cells were harvested by shaking the BM suspension and filtered through a 70 μ m cell strainer. Mononuclear cells were isolated from the BM cell suspension or PB by density gradient centrifugation, frozen at -80°C as dry pellets and stored until further use.

Mutational analysis

Genomic DNA of ficoll-enriched mononuclear cells was analyzed by targeted sequencing of 68 genes recurrently altered in myeloid diseases (Haloplex, Agilent, Boeblingen, Germany; Miseq, Illumina, San Diego, CA, USA). Sequence alignment was performed using the Burrows-Wheeler aligner¹, and CH-associated variants (single nucleotide changes and small insertions/deletions) were called using VarScan2 and Pindel algorithms, as previously described². Sequence alterations were identified with a VAF threshold of $\geq 1\%$. Missense, nonsense, insertion/deletion and splice site variants, but not synonymous changes, were considered in downstream analyses. For correlative analyses of laboratory and clinical parameters such as comorbidities, only variants fulfilling the current definition of CHIP (i.e., VAF $\geq 2\%$) were included.

Statistical data analysis*Univariate analysis*

Categorical variables were compared using the chi-squared test or Fisher's exact test. Wilcoxon-Mann-Whitney test was used to compare continuous variables between two groups. Continuous variables are presented as median (ranges). We adjusted p values for multiple testing with the false discovery rate (FDR) method and using the Benjamini-Hochberg procedure (referred to in the manuscript as q -values). Correlations between two continuous variables were evaluated using the Spearman's rank correlation coefficient. Univariate analysis was performed with GraphPad Prism v6.0 and R, version v3.6.3.

Multivariate analysis

In addition, we studied clinical associations of CHIP using a multivariate logistic regression. Our model included covariates that had data available for at least 120 patients and were associated with presence of CHIP in the univariate analysis with a corrected p -value < 0.1 : age, hemoglobin level, cardiovascular disease, autoimmune and malignant disease status. Sex was additionally included as a covariable. We used the glm function in R. Computational methods and raw data

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Clonal Hematopoiesis in THA

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are provided in supplemental files “Rscript-BLD-2020-010163” and “RawData-BLD-2020-010163.csv”.

References

1. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2010;26(5):589–595.
2. Rothenberg-Thurley M, Amler S, Goerlich D, et al. Persistence of pre-leukemic clones during first remission and risk of relapse in acute myeloid leukemia. *Leukemia*. 2018;32(7):1598–1608.

Supplemental Tables:

Supplemental Table 1: Detected variants according to HGVS-Nomenclature (VAF $\geq 1\%$).

Gene	Unique patient number	Variant	Mutation type	VAF (%)
ASXL1	UPN_1	NM_015338.6:c.2036dup; p.(Gly680Argfs*38)	Indel	3.71
ASXL1	UPN_1	NM_015338.6:c.2385del; p.(Trp796Glyfs*22)	Indel	5.93
ASXL1	UPN_2	NM_015338.6:c.[2083C>T]; p.(Gln695*)	Nonsense	1.29
ASXL1	UPN_3	NM_015338.6:c.2954_2957del; p.(Ile985Thrfs*7)	Indel	4.73
ASXL1	UPN_4	NM_015338.6:c.1934dup; p.(Gly646Trpfs*12)	Indel	6.07
ASXL1	UPN_5	NM_015388.6:c.1934dup; p.(Gly646Trpfs*12)	Indel	7.01
ASXL1	UPN_6	NM_015338.5:c.2512_2537dup; p.(Ser846Argfs*5)	Indel	11.42
ASXL1	UPN_7	NM_015338.6:c.2644C>T; p.(Gln882*)	Nonsense	1.05
BCOR	UPN_8	NM_001123385.2:c.[4163C>T]; p.(Ala1388Val)	Missense	1.23
BCOR	UPN_9	NM_001123385.2:c.838G>A; p.(Val280Ile)	Missense	13.32
BCORL1	UPN_10	NM_021946.4:c.4013G>A; p.(Arg1338Gln)	Missense	6.39
BCORL1	UPN_11	NM_021946.4:c.4853+1G>C; p.?	Splice site variant	10.86
CXCR4	UPN_9	NM_001008540.2:c.1045dup; p.(Glu349Glyfs*13)	Indel	1.92
DNMT3A	UPN_12	NM_175629.2:c.[2567_2568delAG]; p.(Glu856Glyfs*7)	Indel	1.56
DNMT3A	UPN_13	NM_175629.2:c.[2393T>C]; p.(Leu798Pro)	Missense	1
DNMT3A	UPN_14	NM_175629.2:c.2339T>C; p.(Ile780Thr)	Missense	1.14
DNMT3A	UPN_15	NM_175629.2:c.[1123-2A>G]; p.?	Splice site variant	23.6
DNMT3A	UPN_16	NM_175629.2:c.[2106T>G]; p.(Asp702Glu)	Missense	3.59
DNMT3A	UPN_17	NM_175629.2:c.[1940T>G]; p.(Leu647Arg)	Missense	4.47
DNMT3A	UPN_18	NM_175629.2:c.2092T>G; p.(Trp698Gly)	Missense	5.77
DNMT3A	UPN_19	NM_175629.2:c.[2195T>C]; p.(Phe732Ser)	Missense	1.15
DNMT3A	UPN_20	NM_175629.2:c.2591T>A; p.(Met864Lys)	Missense	21.29
DNMT3A	UPN_21	NM_175629.2:c.2047_2056del; p.(Tyr683Thrfs*19)	Indel	3.41
DNMT3A	UPN_22	NM_175629.2:c.2711C>T; p.(Pro904Leu)	Missense	6.64
DNMT3A	UPN_23	NM_175629.2:c.1506delT; p.(Thr503Profs*148)	Indel	4.73
DNMT3A	UPN_24	NM_175629.2:c.1258A>T; p.(Lys420*)	Nonsense	3.99
DNMT3A	UPN_25	NM_175629.2:c.994G>A; p.(Gly332Arg)	Missense	2.3
DNMT3A	UPN_26	NM_175629.2:c.2332G>A; p.(Val778Met)	Missense	5.07
DNMT3A	UPN_27	NM_175629.2:c.1271del; p.(Pro424Hisfs*227)	Indel	1.49
DNMT3A	UPN_28	NM_175629.2:c.2711C>T; p.(Pro904Leu)	Missense	5.33
DNMT3A	UPN_29	NM_175629.2:c.[2322+2T>C]; p.?	Splice site variant	1.35
DNMT3A	UPN_30	NM_175629.2:c.[1948C>G]; p.(Leu650Val)	Missense	1.06
DNMT3A	UPN_31	NM_175629.2:c.[1904G>A]; p.(Arg635Gln)	Missense	6.54
DNMT3A	UPN_32	NM_175629.2:c.[2204A>G]; p.(Tyr735Cys)	Missense	2.65
DNMT3A	UPN_33	NM_175629.2:c.2330C>G; p.(Pro777Arg)	Missense	1.32
DNMT3A	UPN_34	NM_175629.2:c.2393T>A; p.(Leu798His)	Missense	4.37
DNMT3A	UPN_35	NM_175629.2:c.2401A>G; p.(Met801Val)	Missense	12.06
DNMT3A	UPN_36	NM_175629.2:c.1015-2A>G; p.?	Splice site variant	4.76
DNMT3A	UPN_37	NM_175629.2:c.[1532G>A]; p.(Gly511Glu)	Missense	1.49
DNMT3A	UPN_38	NM_175629.2:c.[2185C>T]; p.(Arg729Trp)	Missense	8.48
DNMT3A	UPN_39	NM_175629.2:c.[2644C>T]; p.(Arg882Cys)	Missense	1.06
DNMT3A	UPN_40	NM_175629.2:c.[1900A>T]; p.(Ile634Phe)	Missense	1.07
DNMT3A	UPN_41	NM_175629.2:c.[1430-3C>G]; p.?	Splice site variant	3.08
DNMT3A	UPN_42	NM_175629.2:c.2377T>C; p.(Tyr793His)	Missense	1.74
DNMT3A	UPN_43	NM_175629.2:c.2141C>G; p.(Ser714Cys)	Missense	2.14
DNMT3A	UPN_44	NM_175629.2:c.1924G>A; p.(Gly642Arg)	Missense	1.17
DNMT3A	UPN_45	NM_175629.2:c.2322+3A>G; p.?	Splice site variant	1.44
DNMT3A	UPN_46	NM_175629.2:c.2127del; p.(Cys710Alafs*69)	Indel	1.79
DNMT3A	UPN_47	NM_175629.2:c.1969G>A; p.(Val657Met)	Missense	5.62
DNMT3A	UPN_48	NM_175629.2:c.1903C>T; p.(Arg635Trp)	Missense	1
DNMT3A	UPN_49	NM_175629.2:c.2727T>A; p.(Phe909Leu)	Missense	1.85
DNMT3A	UPN_50	NM_175629.2:c.[2391C>A]; p.(Asn797Lys)	Missense	1.51

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Supplemental Table 1 (continued):

Gene	Unique patient number	Variant	Mutation type	VAF (%)
DNMT3A	UPN_51	NM_175629.2:c.[2494A>G]; p.(Thr832Ala)	Missense	6.42
DNMT3A	UPN_52	NM_175629.2:c.1949T>G; p.(Leu650Arg)	Missense	1.28
DNMT3A	UPN_53	NM_175629.2: c.2644C>T; p.(Arg882Cys)	Missense	27.42
DNMT3A	UPN_54	NM_175629.2:c.2332G>A; p.(Val778Met)	Missense	1.03
DNMT3A	UPN_55	NM_175629.2:c.1668-2A>G; p.?	Splice site variant	2.32
DNMT3A	UPN_56	NM_175629.2:c.1123-8C>G; p.?	Splice site variant	7.11
DNMT3A	UPN_57	NM_175629.2:c.[2096G>T]; p.(Gly699Val)	Missense	1.44
DNMT3A	UPN_57	NM_175629.2:c.[1911_1914delGTCT]; p.(Phe640Metfs*10)	Indel	3.33
DNMT3A	UPN_58	NM_175629.2:c.[2656C>T]; p.(Gln886*)	Nonsense	1.2
DNMT3A	UPN_58	NM_175629.2:c.[1936+1G>T]; p.?	Splice site variant	6.3
DNMT3A	UPN_59	NM_175629.2:c.1071_1093del; p.(Thr358Profs*27)	Indel	5.38
DNMT3A	UPN_59	NM_175629.2:c.2206C>T; p.(Arg736Cys)	Missense	31.7
DNMT3A	UPN_60	NM_175629.2:c.2106_2107insA; p.(Leu703Thrfs*10)	Indel	1.23
DNMT3A	UPN_60	NM_175629.2:c.1501_1508dup; p.(Leu504Metfs*150)	Indel	4.19
DNMT3A	UPN_61	NM_175629.2:c.2045T>G; p.(Met682Arg)	Missense	2.39
DNMT3A	UPN_61	NM_175629.2:c.2245C>T; p.(Arg749Cys)	Missense	3.29
DNMT3A	UPN_62	NM_175629.2:c.2339T>C; p.(Ile780Thr)	Missense	1.22
DNMT3A	UPN_62	NM_175629.2:c.1040T>C; p.(Leu347Pro)	Missense	32.7
DNMT3A	UPN_63	NM_175629.2:c.2411C>T; p.(Pro804Leu)	Missense	1.19
DNMT3A	UPN_63	NM_175629.2:c.2114T>C; p.(Ile705Thr)	Missense	1.36
DNMT3A	UPN_64	NM_175629.2:c.1154del; p.(Pro385Argfs*22)	Indel	4.88
DNMT3A	UPN_65	NM_175629.2:c.[2580G>A]; p.(Trp860*)	Nonsense	1.21
DNMT3A	UPN_66	NM_175629.2:c.[2644C>T]; p.(Arg882Cys)	Missense	1.96
DNMT3A	UPN_2	NM_175629.2:c.[2141C>G]; p.(Ser714Cys)	Missense	1.08
DNMT3A	UPN_67	NM_175629.2:c.2206C>T; p.(Arg736Cys)	Missense	2.77
DNMT3A	UPN_8	NM_175629.2:c.[886G>A]; p.(Val296Met)	Missense	3.21
DNMT3A	UPN_68	NM_175629.2:c.2609T>A; p.(Phe870Tyr)	Missense	9.83
DNMT3A	UPN_68	NM_175629.2:c.2612del; p.(Pro871Glnfs*10)	Indel	9.96
DNMT3A	UPN_65	NM_175629.2:c.1851+1G>A; p.?	Splice site variant	2.57
DNMT3A	UPN_65	NM_175629.2:c.2458G>T; p.(Glu820*)	Nonsense	2.2
DNMT3A	UPN_66	NM_175629.2:c.[1240_1245delTTCCAG]; p.(Phe414_Gln415del)	Indel	1.18
IDH2	UPN_16	NM_002168.3:c.[678+1G>T]; p.?	Splice site variant	1.07
IDH2	UPN_66	NM_002168.3:c.[419G>A]; p.(Arg140Gln)	Missense	7.43
JAK2	UPN_69	NM_004972.3:c.1849G>T p.(Val617Phe)	Missense	9.92
JAK2	UPN_2	NM_004972.3:c.1849G>T, p.(Val617Phe)	Missense	5.96
JAK2	UPN_70	NM_004972.3:c.1849G>T; p.(Val617Phe)	Missense	3.54
KRAS	UPN_71	NM_033360.3:c.[35G>A]; p.(Gly12Asp)	Missense	22.19
MYD88	UPN_55	NM_001172567.1:c.818T>C; p.(Leu273Pro)	Missense	27.86
MYD88	UPN_72	NM_001172567.1:c.818T>C; p.(Leu273Pro)	Missense	2.38
NFE2	UPN_50	NM_001136023.3:c.[705dup]; p.(Pro236Serfs*14)	Indel	3.09
NFE2	UPN_53	NM_001136023.3:c.578_581del; p.(Asn193Ilefs*12)	Indel	24.62
NOTCH1	UPN_73	NM_017617.3:c.7541_7542delCT;p.Pro2514fs	Indel	1.39
PHF6	UPN_74	NM_032458.3:c.[998A>T]; p.(Asp333Val)	Missense	1.43
PPM1D	UPN_75	NM_003620.3:c.1545_1546delGT; p.(Met515Ilefs*12)	Indel	1.82
PPM1D	UPN_76	NM_003620.3:c.1636delC; p.(Leu546*)	Indel	2.66
PPM1D	UPN_77	NM_003620.4:c.[1573G>T]; p.(Glu525*)	Nonsense	1.38
RAD21	UPN_10	NM_006265.3:c.65A>G; p.(His22Arg)	Missense	4.49
RUNX1	UPN_78	NM_001754.4:c.664delT; p.(Ser222Profs*15)	Indel	2.69
RUNX1	UPN_79	NM_001754.4:c.833C>A; p.(Pro278Gln)	Missense	1.76
SF3B1	UPN_49	NM_012433.3:c.1997A>C; p.(Lys666Thr)	Missense	9.01
SF3B1	UPN_80	NM_012433.3:c.2230G>C; p.(Ala744Pro)	Missense	2.61
SF3B1	UPN_81	NM_012433.3:c.1873C>T; p.(Arg625Cys)	Missense	14.97
SRSF2	UPN_82	NM_003016.4:c.[283C>G]; p.(Pro95Ala)	Missense	26.53

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Supplemental Table 1 (continued):

Gene	Unique patient number	Variant	Mutation type	VAF (%)
SRSF2	UPN_83	NM_003016.4:c.284C>T; p.(Pro95Leu)	Missense	1.06
SRSF2	UPN_84	NM_003016.4:c.284C>G; p.(Pro95Arg)	Missense	2.56
STAT3	UPN_1	NM_139276.2:c.1940A>T; p.(Asn647Ile)	Missense	1.79
TERT	UPN_79	NM_198253.3:c.1573+6G>T; p.?	Splice site variant	4.96
TET2	UPN_12	NM_001127208.2:c.[3954+5G>A]; p.?	Splice site variant	1.13
TET2	UPN_13	NM_001127208.2:c.[5582G>A]; p.(Gly1861Glu)	Missense	4.66
TET2	UPN_14	NM_001127208.2:c.5686A>G; p.(Arg1896Gly)	Missense	5.34
TET2	UPN_15	NM_001127208.2:c.[4079T>G]; p.(Leu1360Arg)	Missense	1.19
TET2	UPN_16	NM_001127208.2:c.[1842dup]; p.(Leu615Alafs*23)	Indel	1.18
TET2	UPN_17	NM_001127208.2:c.[4136C>A]; p.(Ala1379Asp)	Missense	2.42
TET2	UPN_17	NM_001127208.2:c.[3116C>A]; p.(Ser1039*)	Nonsense	2.81
TET2	UPN_67	NM_001127208.2:c.2053C>T; p.(Gln685*)	Nonsense	32.59
TET2	UPN_85	NM_001127208.2:c.3885delC; p.(Tyr1295*)	Indel	2.81
TET2	UPN_86	NM_001127208.2:c.[5127dup]; p.(Thr1710Tyrfs*3)	Indel	1.66
TET2	UPN_87	NM_001127208.2:c.[670G>T]; p.(Glu224*)	Nonsense	2.16
TET2	UPN_88	NM_001127208.2:c.[456_457dup]; p.(Ser153Phefs*3)	Indel	2.24
TET2	UPN_89	NM_001127208.2:c.3954+5G>A; p.?	Splice site variant	2.1
TET2	UPN_90	NM_001127208.2:c.4353del; p.(Arg1452Glnfs*6)	Indel	5.17
TET2	UPN_91	NM_001127208.2:c.5079_5082del; p.(Tyr1693*)	Indel	7.13
TET2	UPN_92	NM_001127208.2:c.1486del; p.(Met496*)	Indel	1.3
TET2	UPN_93	NM_001127208.2:c.2236C>T; p.(Gln746*)	Nonsense	1.97
TET2	UPN_94	NM_001127208.2:c.840dup; p.(Asn281*)	Indel	2.27
TET2	UPN_95	NM_001127208.2:c.822del; p.(N275Ilf*18)	Indel	2.68
TET2	UPN_71	NM_001127208.2:c.[4393C>T]; p.(Arg1465*)	Nonsense	3.02
TET2	UPN_70	NM_001127208.2:c.3789T>A; p.(Cys1263*)	Nonsense	2.6
TET2	UPN_69	NM_001127208.2:c.4045-2A>G; p.?	Splice site variant	3.56
TET2	UPN_96	NM_001127208.2:c.[3662G>A]; p.(Cys1221Tyr)	Missense	3.24
TET2	UPN_96	NM_001127208.2:c.[3482G>C]; p.(Arg1161Thr)	Missense	5
TET2	UPN_1	NM_001127208.2:c.5760A>T; p.(Lys1920Asn)	Missense	4.75
TET2	UPN_84	NM_001127208.2:c.1703del; p.(Lys568Argfs*12)	Indel	9.86
TET2	UPN_8	NM_001127208.2:c.[4525A>T]; p.(Lys1509*)	Nonsense	2.46
TET2	UPN_78	NM_001127208.2:c.1081C>T; p.(Gln361*)	Nonsense	1.37
TET2	UPN_97	NM_001127208.2:c.5650A>G; p.(Thr1884Ala)	Missense	1.11
TET2	UPN_97	NM_001127208.2:c.4161C>A; p.(Asn1387Lys)	Missense	15.24
TET2	UPN_98	NM_001127208.2:c.[663_666delACAT]; p.(His222Valfs*27)	Indel	2.14
TET2	UPN_98	NM_001127208.2:c.[1038_1039delAG]; p.(Ala347Valfs*3)	Indel	3.27
TET2	UPN_75	NM_001127208.2:c.3817T>C; p.(Cys1273Arg)	Missense	3.44
TET2	UPN_75	NM_001127208.2:c.980C>G; p.(Ser327*)	Nonsense	1.5
TET2	UPN_72	NM_001127208.2:c.2156del; p.(Leu719Cysfs*32)	Indel	1.21
TET2	UPN_72	NM_001127208.2:c.5629A>G; p.(Lys1877Glu)	Missense	1.78
TET2	UPN_72	NM_001127208.2:c.3647G>A; p.(Arg1216Gln)	Missense	2.42
TP53	UPN_67	NM_000546.5:c.584T>C; p.(Ile195Thr)	Missense	14.71
TP53	UPN_52	NM_000546.5:c.536A>G; p.(His179Arg)	Missense	4.48
TP53	UPN_56	NM_000546.5:c.574C>T; p.(Gln192*)	Nonsense	1.34
TP53	UPN_64	NM_000546.5:c.524G>A; p.(Arg175His)	Missense	1.99
U2AF1	UPN_1	NM_001025203.1:c.470A>C; p.(Gln157Pro)	Missense	9.73
U2AF2	UPN_54	NM_007279.2:c.977G>C; p.(Gly326Ala)	Missense	7.93
U2AF2	UPN_64	NM_007279.2:c.866A>G; p.(Asn289Ser)	Missense	1.58
U2AF2	UPN_99	NM_007279.2:c.[259_261delAAG]; p.(Lys87del)	Indel	1.08
ZBTB7A	UPN_7	NM_015898.4:c.1228T>A; p.(Tyr410Asn)	Missense	2.89
ZBTB7A	UPN_100	NM_015898.4:c.149C>T; p.(Ser50Leu)	Missense	3.18
ZRSR2	UPN_8	NM_005089.3:c.1338_1343dup; p.(Ser447_Arg448dup)	Indel	31.32
ZRSR2	UPN_51	NM_005089.3:c.370C>T; p.(Gln124*)	Nonsense	2.93

UPN, unique patient number; VAF, variant allele frequency.

Supplemental Data

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Supplemental Table 2: Overview of assumed causes of anemia per given anemic patient.

Unique patient number	Age (years)	Sex	Hemoglobin level (g/dL)	Severity of anemia	Mutations	Number of mutations	Gene	VAF (%)	CHIP (VAF ≥2%)	Prior or present malignant disease	Treatment of malignant disease	AID	Treatment of AID	Cardiovascular disease	Anticoagulant treatment
UPN_127	78	f	11.8	mild	no	-	-	-	no	no	-	no	-	yes	no
UPN_166	61	f	11.6	mild	no	-	-	-	no	no	-	no	-	yes	no
UPN_170	75	f	10.8	mild	no	-	-	-	no	no	-	no	-	yes	no
UPN_172	64	f	9.4	clinically significant	no	-	-	-	no	no	-	no	-	no	no
UPN_182	70	f	11.7	mild	no	-	-	-	no	no	-	no	-	no	no
UPN_138	80	m	11.7	mild	no	-	-	-	no	no	-	no	-	yes	no
UPN_198	74	m	11.7	mild	no	-	-	-	no	yes, prior prostate cancer	Surgery, radiotherapy	no	-	no	yes
UPN_37	82	f	11.9	mild	yes	1	DNMT3A	1.49	no	no	-	no	-	no	no
UPN_39	85	f	10.9	mild	yes	1	DNMT3A	1.06	no	no	-	no	-	yes	no
UPN_32	76	f	11.9	mild	yes	1	DNMT3A	2.65	yes	no	-	no	-	yes	yes
UPN_88	79	f	11.9	mild	yes	1	TET2	2.24	yes	no	-	no	-	yes	no
UPN_81	67	f	11.1	mild	yes	1	SF3B1	14.97	yes	no	-	yes, psoriasis vulgaris	no	yes	no
UPN_38	66	f	11.8	mild	yes	1	DNMT3A	8.48	yes	no	-	yes, pemphigus	yes, etoricoxib	yes	no
UPN_2	66	f	11.4	mild	yes	3	JAK2	5.96	yes	no	-	yes, psoriatic arthritis	yes, MTX weekly	no	no
							ASXL1	1.29	no						
							DNMT3A	1.08	no						
UPN_58	74	f	10	mild	yes	2	DNMT3A	6.3	yes	no	-	no	-	yes	no
							DNMT3A	1.20	no						
UPN_41	70	f	8	clinically significant	yes	1	DNMT3A	3.08	yes	yes, present breast cancer	Anastrozole, palbociclib	no	-	no	no
UPN_54	82	f	11.8	mild	yes	2	U2AF2	7.93	yes	no	-	no	-	yes	yes
							DNMT3A	1.03	no						
UPN_97	76	f	8.5	clinically significant	yes	2	TET2	15.24	yes	yes, prior colon cancer	Surgery	no	-	yes	no
							TET2	1.11	no						
UPN_4	86	f	11.4	mild	yes	1	ASXL1	6.07	yes	no	-	no	-	yes	yes
UPN_10	86	f	10.9	mild	yes	2	BCORL1	6.39	yes	no	-	no	-	yes	no
							RAD21	4.49	yes						
UPN_61	67	f	10	mild	yes	2	DNMT3A	3.29	yes	no	-	no	-	yes	no
							DNMT3A	2.39	yes						
UPN_49	80	m	12.7	mild	yes	2	SF3B1	9.01	yes	yes, prior urothelial carcinoma	Surgery	yes, CIDP	no	yes	yes
							DNMT3A	1.85	no						
UPN_50	64	m	11.1	mild	yes	2	NFE2	3.09	yes	no	-	yes, polymyalgia rheumatica	no	yes	yes
							DNMT3A	1.51	no						
UPN_64	74	m	12.5	mild	yes	3	DNMT3A	4.88	yes	no	-	no	-	no	no
							TP53	1.99	no						
UPN_43	82	m	9.9	clinically significant	yes	1	U2AF2	1.58	no	yes, prior colon cancer	Surgery	no	-	yes	no
							DNMT3A	2.14	yes						
UPN_95	77	m	9.7	clinically significant	yes	1	TET2	2.68	yes	yes, present prostate cancer	Surgery, radiotherapy, anti-hormonal therapy	no	-	yes	no
UPN_56	80	m	11.7	mild	yes	2	DNMT3A	7.11	yes	no	-	no	-	yes	yes
							TP53	1.34	no						

Mild anemia: hemoglobin levels below the lower limit of normal (<13 g/dL in men and <12 g/dL in women) but ≥10g/dL. Clinically significant anemia: hemoglobin levels of below 10 but ≥8 g/dL. AID, autoimmune disease; CHIP, clonal hematopoiesis of indeterminate potential; CIDP, chronic inflammatory demyelinating polyneuropathy; f, female; m, male; MTX, methotrexate. UPN, unique patient number; VAF, variant allele frequency.

Supplemental Data

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Supplemental Table 3: Characteristics of patients with malignant disease and CHIP.

Unique patient number	Malignant disease (years since initial diagnosis)	Treatment	Gene	Variant	VAF (%)
UPN_79	Prior breast cancer (4 years)	Tamoxifen	TERT	NM_198253.3:c.1573+6G>T; p.?	4.96
			RUNX1	NM_001754.4:c.833C>A; p.(Pro278Gln)	1.76
UPN_18	Prior breast cancer (18 years)	Surgery, radiotherapy, trastuzumab	DNMT3A	NM_175629.2:c.2092T>G; p.(Trp698Gly)	5.77
UPN_65	Prior breast cancer (31 years)	Unknown	DNMT3A	NM_175629.2:c.1851+1G>A; p.?	2.57
			DNMT3A	NM_175629.2:c.2458G>T; p.(Glu820*)	2.2
			DNMT3A	NM_175629.2:c.[2580G>A]; p.(Trp860*)	1.21
UPN_31	Prior breast cancer (2 years)	Surgery, radiotherapy, tamoxifen	DNMT3A	NM_175629.2:c.[1904G>A]; p.(Arg635Gln)	6.54
UPN_41	Present breast cancer	Anastrozole, palbociclib	DNMT3A	NM_175629.2:c.[1430-3C>G]; p.?	3.08
UPN_24	Prior breast cancer (1 year)	Surgery, radiotherapy, letrozol	DNMT3A	NM_175629.2:c.1258A>T; p.(Lys420*)	3.99
UPN_98	Prior prostate cancer	Surgery, goserelin	TET2	NM_001127208.2:c.[1038_1039delAG]; p.(Ala347Valfs*3)	3.27
			TET2	NM_001127208.2:c.[663_666delACAT]; p.(His222Valfs*27)	2.14
UPN_1	Prior prostate cancer (11 years)	Surgery	U2AF1	NM_001025203.1:c.470A>C; p.(Gln157Pro)	9.73
			ASXL1	NM_015338.6:c.2385del; p.(Trp796Glyfs*22)	5.93
			ASXL1	NM_015338.6:c.2036dup; p.(Gly680Argfs*38)	3.71
			TET2	NM_001127208.2:c.5760A>T; p.(Lys1920Asn)	4.75
			STAT3	NM_139276.2:c.1940A>T; p.(Asn647Ile)	1.79
UPN_68	Prior prostate cancer	Surgery, radiotherapy	DNMT3A	NM_175629.2:c.2612del; p.(Pro871Glnfs*10)	9.96
			DNMT3A	NM_175629.2:c.2609T>A; p.(Phe870Tyr)	9.83
UPN_95	Present prostate cancer (6 years)	Surgery, radiotherapy, anti-hormonal therapy	TET2	NM_001127208.2:c.822del; p.(N275Ilf*18)	2.68
UPN_97	Prior colon cancer (13 years)	Surgery	TET2	NM_001127208.2:c.4161C>A; p.(Asn1387Lys)	15.24
			TET2	NM_001127208.2:c.5650A>G; p.(Thr1884Ala)	1.11
UPN_43	Prior colon cancer (4 years)	Surgery	DNMT3A	NM_175629.2:c.2141C>G; p.(Ser714Cys)	2.14
UPN_100	Prior gastric cancer (2 years)	Surgery	ZBTB7A	NM_015898.4:c.149C>T; p.(Ser50Leu)	3.18
UPN_49	Prior urothelial carcinoma (3 years)	Surgery	SF3B1	NM_012433.3:c.1997A>C; p.(Lys666Thr)	9.01
			DNMT3A	NM_175629.2:c.2727T>A; p.(Phe909Leu)	1.85
UPN_7	Prior renal cell carcinoma	Surgery	ZBTB7A	NM_015898.4:c.1228T>A; p.(Tyr410Asn)	2.89
			ASXL1	NM_015338.6:c.2644C>T; p.(Gln882*)	1.05

UPN, unique patient number; VAF, variant allele frequency.

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Supplemental Table 4: Characteristics of patients with autoimmune disease and CHIP.

Unique patient number	AID	Treatment	Gene	Variant	VAF (%)
UPN_87	Hashimoto's thyroiditis	No	TET2	NM_001127208.2:c.[670G>T]; p.(Glu224*)	2.16
UPN_81	Psoriasis vulgaris	No	SF3B1	NM_012433.3:c.1873C>T; p.(Arg625Cys)	14.97
UPN_2	Psoriatic arthritis	Yes, MTX weekly	JAK2	NM_004972.3:c.1849G>T; p.(Val617Phe)	5.96
			ASXL1	NM_015338.6:c.[2083C>T]; p.(Gln695*)	1.29
			DNMT3A	NM_175629.2:c.[2141C>G]; p.(Ser714Cys)	1.08
UPN_68	Psoriatic arthritis	No	DNMT3A	NM_175629.2:c.2609T>A; p.(Phe870Tyr)	9.83
			DNMT3A	NM_175629.2:c.2612del; p.(Pro871Glnfs*10)	9.96
UPN_50	Polymyalgia rheumatica	No	NFE2	NM_001136023.3:c.[705dup]; p.(Pro236Serfs*14)	3.09
			DNMT3A	NM_175629.2:c.[2391C>A]; p.(Asn797Lys)	1.51
UPN_7	Polymyalgia rheumatica	No	ZBTB7A	NM_015898.4:c.1228T>A; p.(Tyr410Asn)	2.89
			ASXL1	NM_015338.6:c.2644C>T; p.(Gln882*)	1.05
UPN_91	Polyarthritis	Yes, MTX weekly, diclofenac (if necessary)	TET2	NM_001127208.2:c.5079_5082del; p.(Tyr1693*)	7.13
UPN_49	Chronic inflammatory demyelinating polyradiculoneuropathy	No	SF3B1	NM_012433.3:c.1997A>C; p.(Lys666Thr)	9.01
			DNMT3A	NM_175629.2:c.2727T>A; p.(Phe909Leu)	1.85
UPN_57	Graves' disease	No	DNMT3A	NM_175629.2:c.[1911_1914delGTCT]; p.(Phe640Metfs*10)	3.33
			DNMT3A	NM_175629.2:c.[2096G>T]; p.(Gly699Val)	1.44
UPN_24	Polymyalgia rheumatica	Yes, diclofenac (if necessary)	DNMT3A	NM_175629.2:c.1258A>T; p.(Lys420*)	3.99
UPN_38	Pemphigus	Yes, etoricoxib	DNMT3A	NM_175629.2:c.[2185C>T]; p.(Arg729Trp)	8.48
UPN_90	Rheumatoid polyarthritis	No	TET2	NM_001127208.2:c.4353del; p.(Arg1452Glnfs*6)	5.17

AID, autoimmune disease; MTX, methotrexate; UPN, unique patient number; VAF, variant allele frequency.

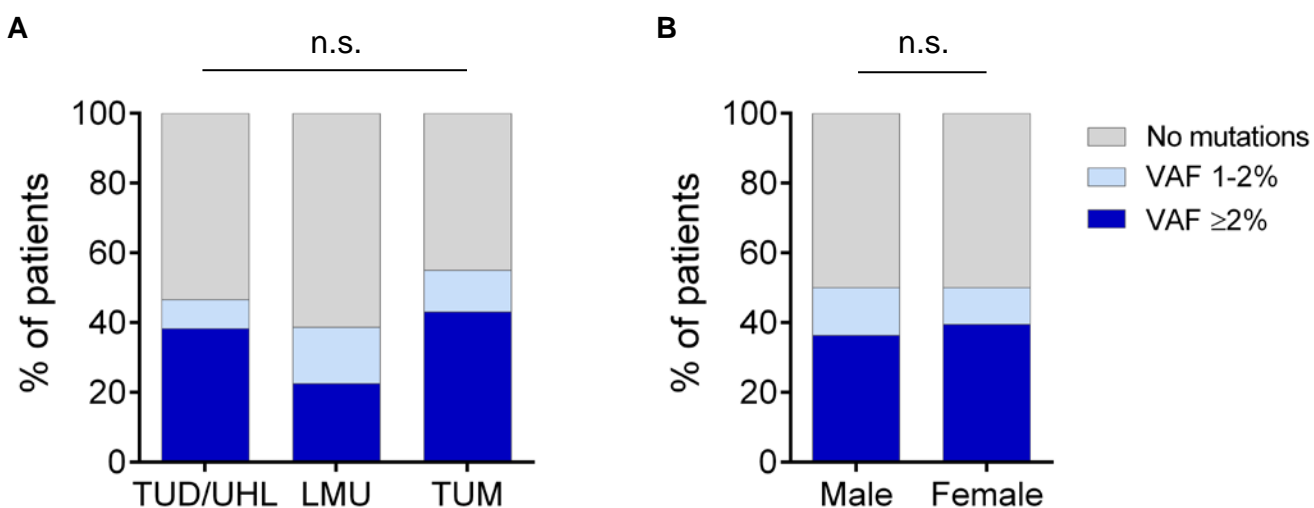
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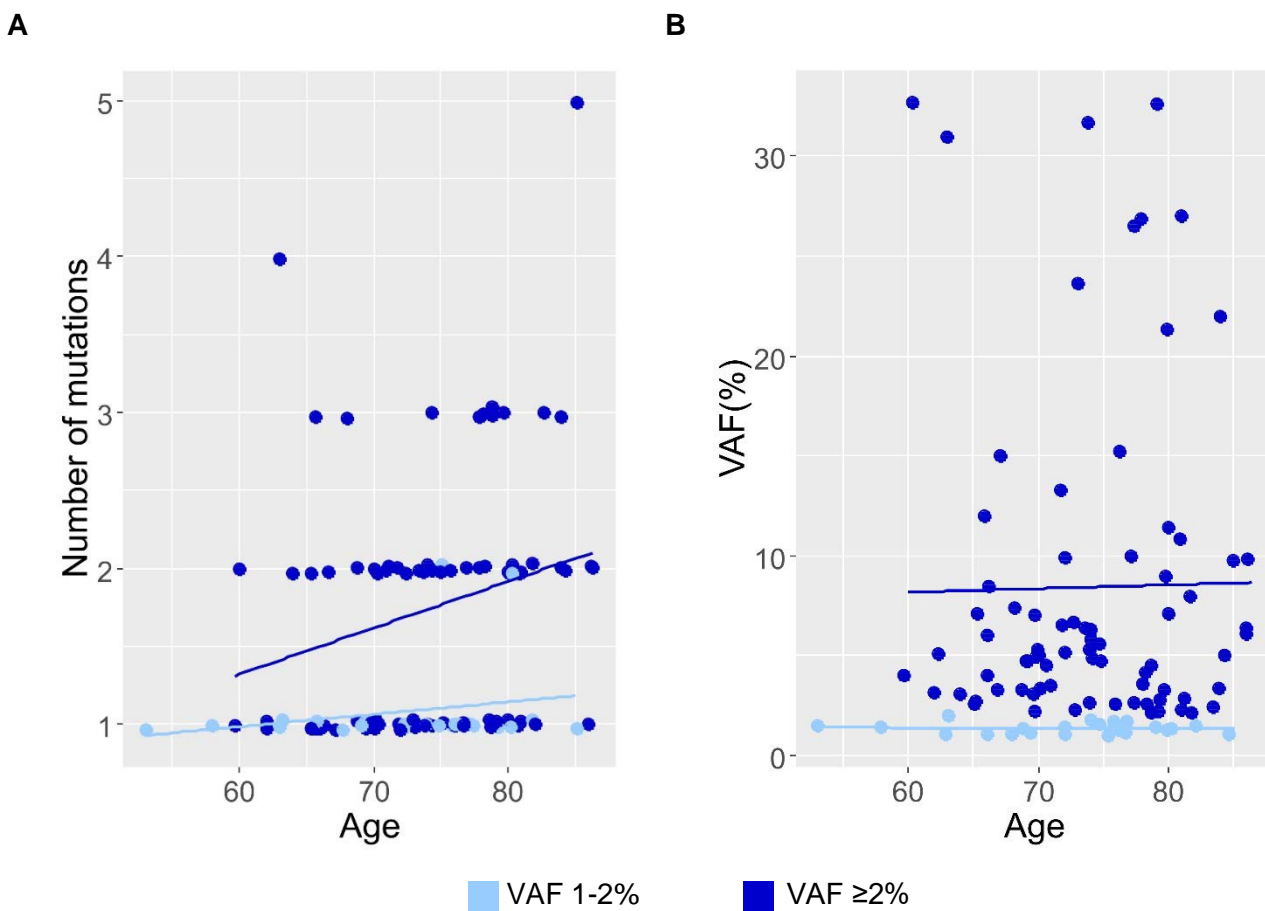
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Supplemental Figures:

Supplemental Figure 1



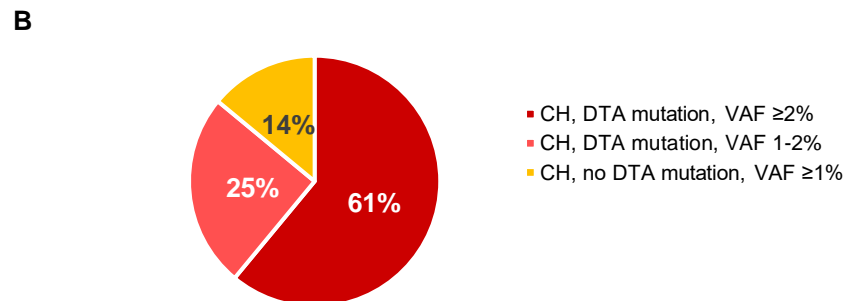
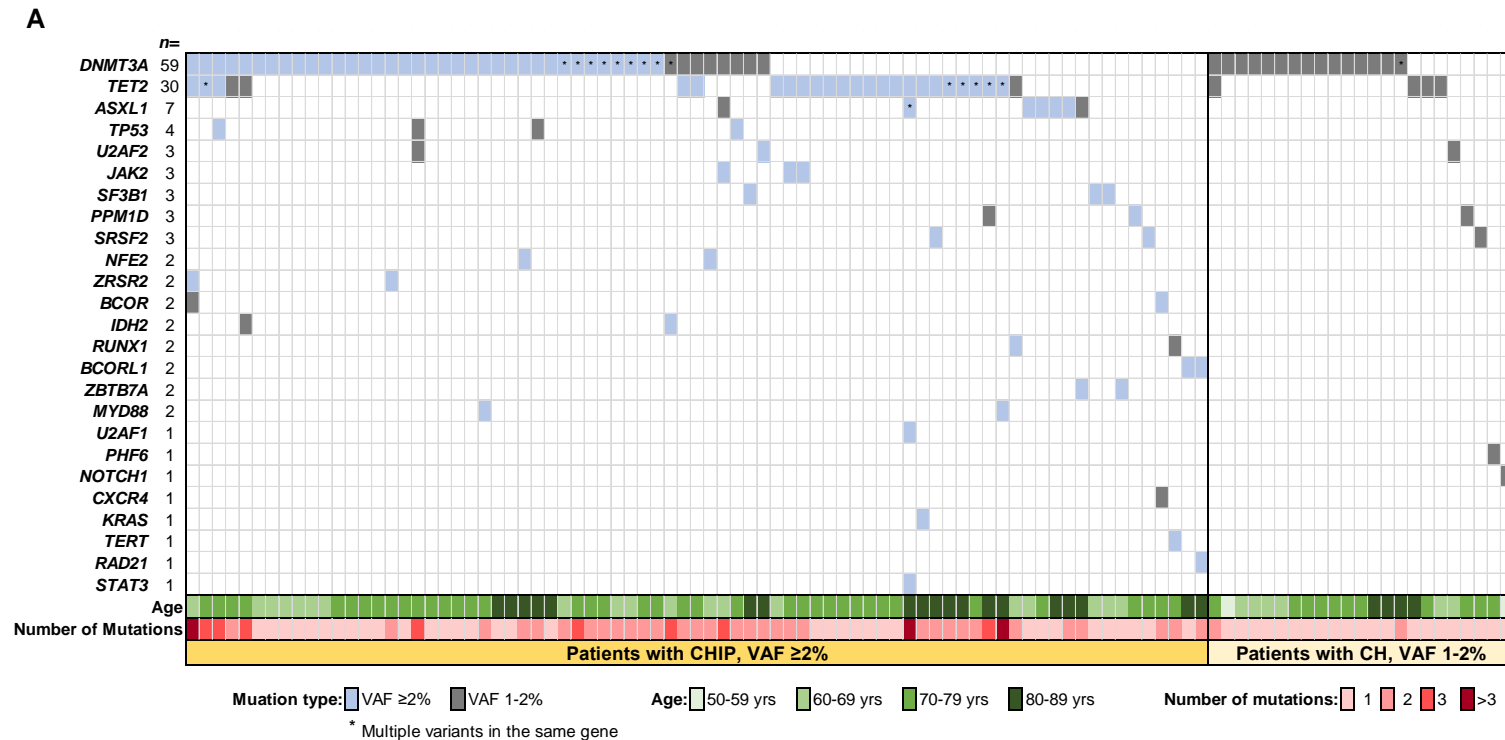
Supplemental Figure 1: Comparison of CH incidence among the four participating study centers and among males and females. (A) Frequency of CH in patients from TUD/UHL (n=60), LMU (n=31) and TUM (n=109), $p=0.26$. (B) Incidence of CH among males (n=66) and females (n=134), $p=0.78$. P -values were calculated using the chi-squared test. n.s., not significant; LMU, University Hospital of Munich; TUD, Technical University of Dresden; TUM, Technical University of Munich; UHL, University Hospital Leipzig.



Supplemental Figure 2: Correlation of mutations and age. (A) Correlation between age and number of mutations, VAF $\geq 2\%$: $p=0.031$. (B) Correlation between age and VAF, not significant ($p=0.73$). Correlations were evaluated using the Spearman's rank correlation coefficient.

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Supplemental Figure 3

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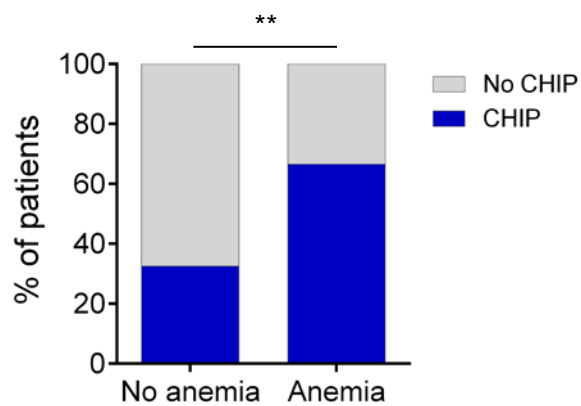
Supplemental Figure 3: Overview of the mutational landscape and frequency of *DNMT3A/TET2/ASXL1* (DTA) mutations. (A) Spectrum of all identified variants with VAF $\geq 1\%$. Each line represents one gene, each column represents one individual. (B) Frequency of DTA mutations in patients with CH (n=100).

Supplemental Data

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Supplemental Figure 4



Supplemental Figure 4: Overview of patients with anemia. Prevalence of CHIP detected in patients with anemia (n=18/27) compared to those without (n=32/98), ** $p=0.0014$. P -value was calculated using the chi-squared test.

```

# R-script: Statistical analysis CHIP and HIPs (BLD-2020-010163)-----
ChipHip <- read.csv2("C:/RawData-BLD-2020-010163.csv")

# Univariate analysis (each variable according to CHIP status)-----
# Univariate analysis for categorical variables (Chi-squared test)-----
# CHIP / Center
Univar_Center <- chisq.test(ChipHip$Center,ChipHip$CHIP,correct=FALSE)
Ctr <- Univar_Center$p.value
# CHIP / Indication
Univar_Indication <- chisq.test(ChipHip$Indication,ChipHip$CHIP,correct=FALSE)
Idct <- Univar_Indication$p.value
# CHIP / Sex
Univar_Sex <- chisq.test(ChipHip$Sex,ChipHip$CHIP,correct=FALSE)
Sx <- Univar_Sex$p.value
# CHIP / Cardiovascular disease
Univar_CVD <- chisq.test(ChipHip$CardioVascularDisease,ChipHip$CHIP,correct=FALSE)
CVD <- Univar_CVD$p.value
# CHIP / Hypercholesterolemia
Univar_Hypercholesterolemia <-
chisq.test(ChipHip$Hypercholesterolemia,ChipHip$CHIP,correct=FALSE)
Hpch <- Univar_Hypercholesterolemia$p.value
# CHIP / Type 2 Diabetes
Univar_Diabetes2 <- chisq.test(ChipHip$DiabetesType2,ChipHip$CHIP,correct=FALSE)
Dbt <- Univar_Diabetes2$p.value
# CHIP / Hypothyroidism
Univar_Hypothyroidism<- chisq.test(ChipHip$Hypothyroidism,ChipHip$CHIP,correct=FALSE)
Hpth <- Univar_Hypothyroidism$p.value
# CHIP / Autoimmune disease
Univar_AID <- chisq.test(ChipHip$AutoimmuneDisease,ChipHip$CHIP,correct=FALSE)
AID <- Univar_AID$p.value
# CHIP / Malignant disease
Univar_MalignantDisease <- chisq.test(ChipHip$MalignantDisease,ChipHip$CHIP,correct=FALSE)
MlgD <- Univar_MalignantDisease$p.value
# CHIP / Thrombosis-pulmonary embolism (Thrombosis)
Univar_Thrombosis<- chisq.test(ChipHip$Thrombosis,ChipHip$CHIP,correct=FALSE)
Thromb <- Univar_Thrombosis$p.value
# CHIP / Anti-inflammatory drug use (AntilInflammatoryDrug)
Univar_AntilInflammatoryDrug <-
chisq.test(ChipHip$AntilInflammatoryDrug,ChipHip$CHIP,correct=FALSE)
Antfld <- Univar_AntilInflammatoryDrug$p.value

# Univariate analysis for continuous variables (Wilcoxon-Mann-Whitney test)-----
# CHIP / Age
Univar_Age <- wilcox.test(ChipHip$Age~ChipHip$CHIP)
AGE <- Univar_Age$p.value
# CHIP / BMI
Univar_BMI <- wilcox.test(ChipHip$BMI~ChipHip$CHIP)
Bmi <- Univar_BMI$p.value
# CHIP / Leucocytes levels (Leucocytes)
Univar_Leucocytes <- wilcox.test(ChipHip$Leucocytes~ChipHip$CHIP)
Leuco <- Univar_Leucocytes$p.value
# CHIP / Hemoglobin levels (Hemoglobin)
Univar_Hemoglobin <- wilcox.test(ChipHip$Hemoglobin~ChipHip$CHIP)
Hbg <- Univar_Hemoglobin$p.value
# CHIP / MCV levels (MCV)
Univar_MCV <- wilcox.test(ChipHip$MCV~ChipHip$CHIP)
Mcv <- Univar_MCV$p.value
# CHIP / Platelets levels (Platelets)
Univar_Platelets <- wilcox.test(ChipHip$Platelets~ChipHip$CHIP)
Plt <- Univar_Platelets$p.value

```

```

# Multiple hypothesis testing -----
# Adjusted p-values (Benjamini-Hochberg method)
listP <- c(Ctr, Idct, AGE, Sx, Bmi, Leuco, Hbg, Mcv, Plt, CVD, Hpch, Dbt, Hpth, AID, MlgD, Thromb,
Antfld)
listP
p.adjust(listP, method = "BH")

# Multivariate logistic regression accounting for the CHIP phenotype -----
# Includes variables for which p-value < 0.1 in univariate analysis and at least 120 observations-----
# Definition of the variable types
CHIP <- as.factor(ChipHip$CHIP)
Age <- as.numeric(ChipHip$Age)
Sex <- as.factor(ChipHip$Sex)
Hb <- as.numeric(ChipHip$Hemoglobin)
Cvd <- as.factor(ChipHip$CardioVascularDisease)
Aid <- as.factor(ChipHip$AutoimmuneDisease)
MD <- as.factor(ChipHip$MalignantDisease)

# Multivariate logistic regression
MVA <- glm(CHIP~ Age + Sex + Hb + Cvd + Aid + MD, family=binomial(link="logit"), data=ChipHip)
summary(MVA)
library(questionr)
odds.ratio(MVA)

# Statistical analysis for supplemental Figure 1 (Chi-squared test) -----
# Supplemental Figure 1A: No mutations / VAF 1-2% / VAF >2% according to the center
chisq.test(ChipHip$Center,ChipHip$VAFlevel,correct=FALSE)
# Supplemental Figure 1B: No mutations / VAF 1-2% / VAF >2% according to the sex
chisq.test(ChipHip$Sex,ChipHip$VAFlevel,correct=FALSE)

# Statistical analysis for supplemental Figure 2 (Spearman's rank correlation coefficient) -----
# Supplemental Figure 2A
#VAF 1-2%
cor.test(ChipHip$Age[which(ChipHip$VAFlevel=="VAF 1-2%")],
ChipHip$NbOfMutation[which(ChipHip$VAFlevel=="VAF 1-2%")], method="spearman")
#VAF >2%
cor.test(ChipHip$Age[which(ChipHip$CHIP=="1")],
ChipHip$NbOfMutation[which(ChipHip$CHIP=="1")], method="spearman")
# Supplemental Figure 2B
#VAF 1-2%
cor.test(ChipHip$Age[which(ChipHip$VAFlevel=="VAF 1-2%")],
ChipHip$HighestVAF[which(ChipHip$VAFlevel=="VAF 1-2%")], method="spearman")
#VAF >2%
cor.test(ChipHip$Age[which(ChipHip$CHIP=="1")], ChipHip$HighestVAF[which(ChipHip$CHIP=="1")],
method="spearman")

# Statistical analysis for supplemental Figure 4 (Chi-squared test) -----
# CHIP / Anemia
chisq.test(ChipHip$Anemia,ChipHip$CHIP,correct=FALSE)

```