Clonal hematopoiesis in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis

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Supplementary Material

Clonal Hematopoiesis in Patients with ANCA-associated Vasculitis

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Supplementary Methods

Patients and Materials

We collected peripheral blood (PB) samples from patients with AAV (MPA and GPA), seen at the Charité/HELIOS outpatient departments and wards of Nephrology between 04/2005 and 10/2018. Demographical and clinical data including blood counts, creatinine values, disease activity and manifestations, previous treatment regimen, comorbidities and relapse data were extracted from patient records. The age- and sex-matched control cohort was constructed from the cohort of healthy stem cell donors described in Frick et al. 2018[1] and 33 younger healthy individuals screened for CHIP with identical sequencing technology and bioinformatic analysis as was used in the AAV cohort. Age- and sex-matching was performed randomly by an R-algorithm, allowing for a maximum age difference of 3 years (occurs in only three cases, see also Supplementary Figure 2). All patients gave their written informed consent. The study was conducted in accordance with the Declaration of Helsinki, and ethical approval was obtained from the local ethics committees.

Sequencing analysis

DNA from PB was extracted using the Qiagen DNA Mini Kit (Qiagen, Hilden, Germany). DNA samples were screened for CHIP using the Illumina TruSight Myeloid Sequencing Panel (n = 19) or a customized version thereof (n = 93, cf. Supplementary Table 1; Illumina, San Diego, California, USA). Libraries were prepared according to the manufacturer's protocol and paired-end sequenced on a MiSeq or NextSeq sequencer. Demultiplexing and further processing of the sequencing data was performed using the Illumina platform BaseSpace® sequencing HUB: reads were aligned to the human genome hg19 by the Smith-Waterman algorithm. Variant calling was performed using the somatic variant caller of the BaseSpace® TruSeq Amplicon app version 2.0.0 with a minimum variant allele frequency (VAF) of 1%. Variants were annotated with the RefSeq database, dbSNP database of known polymorphisms and the catalogue of somatic mutations in cancer (COSMIC) using Illumina Variant Studio 3.0 (BaseSpace® Annotation Engine version 1.4.2.60). Further filtering was performed in R

version 3.4.3 and RStudio version 1.1.442 with the following settings: Intronic and/or synonymous variants as well as variants with VAF < 2% or variant supporting reads < 20 were discarded. Variants with 45% < VAF < 55% or VAF > 95% annotated as polymorphisms in dbSNP were classified as polymorphisms and excluded. Variants among the following list of "hotspots" were rescued: *DNMT3A* R882C/H, *GNB1* K57E, *JAK2* V617F, *SF3B1* K666N and K700E, *SFRS2* P95L, *U2AF1* S34F and Q157P/R. All variants passing these filters were manually evaluated in the Integrative Genome Viewer (IGV, Broad Institute, version 2.3) and selected for validation.

DNMT3A R882H/C mutations were validated using digital droplet PCR as detailed elsewhere.[2] Variants with VAFs > 10% were validated using conventional Sanger Sequencing. For validation of all other variant candidates and longitudinal quantification of mutational burden in follow-up samples, targeted deep sequencing was performed.[2] Amplicons of length 150-200bp covering the mutations were created by conventional touchdown PCR. Amplicons were pooled without overlap, libraries were created using the NEBNext® library preparation kit (New England Biolabs, Massachusetts, USA) and paired-end sequenced on a MiSeq sequencer. Alignment was performed as described above, VAFs at known positions were extracted and manually revised using IGV.

Gene expression, autoantigen expression and activation assays

For mRNA expression in peripheral blood leukocytes, blood was collected in PAXgene Blood RNA Tubes and RNA was subsequently isolated according to the manufacturer's instructions using the PAXgene Blood RNA Kit (Qiagen, Hilden, Germany). 1 µg RNA was reverse transcribed using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Finally, cDNA was amplified by real-time quantitative PCR with specific primers for *CD177, PR3, MPO, RUNX3, JMJD3,* and *18S* (housekeeping gene) using either Fast SYBER® Green Master Mix (Applied Biosystems, Foster City, CA, USA) or Taqman Fast Universal PCR Master Mix (Applied Biosystems). Amplification was conducted using a QuantStudio3 Real-Time PCR System (Applied Biosystems). Gene expressions were normalized to *18S* and analyzed using the $2^{-\Delta\Delta Ct}$ method.

Healthy controls were defined as reference and assigned a mean of 1. Primers were synthesized by Biotez (Berlin, Germany).

ANCA autoantigen PR3 and CD177 expression was measured on isolated neutrophils by flow-cytometry using anti-NB1 Ab (clone MEM-166) from Biolegend (San Diego, CA, USA) or anti-PR3 (clone 12.8) from Sanquin (Amsterdam, The Netherlands) as described before.[3] Neutrophil membrane expression of CD177 and PR3 is reported as either expression index (EI, $MFI_{stimulated}$ cells- $MFI_{unstimulated}$ cells) /MFI_{unstim} cells) or by reporting the percentage with membrane CD177 and mPR3 expression.

Neutrophil reactive oxygen generation was quantified by flow-cytometry using the DHR assay for selected $CHIP^{positive}$, $CHIP^{negative}$ patients and healthy controls. In short, neutrophils (1×10^7/ml HBSS) were loaded with rhodamine (Sigma-Aldrich, Steinheim, Germany) followed by priming with 2 ng/ml recombinant human TNF-α (R&D Systems, Wiesbaden-Nordenstadt, Germany) and 5×10^5 cells were incubated with 10 □g/ml of the following monoclonal antibodies: either isotype control (clone 11711) from R&D Systems, anti-PR3 (clone 12.8) from Sanqui (Amsterdam, The Netherlands), or anti-MPO (clone 2C7) from Acris Antibodies GmbH (Herford, Germany) in a total assay volume of 100 µl in polypropylene tubes. Stimulation is expressed as Stimulation Index (SI), which is calculated as $MFI_{stimulated}$ cells- $MFI_{unstimulated}$ cells) /MFI_{unstim} cells. .All flow cytometry experiments were performed using a BD FACSCalibur (BD Biosciences, Heidelberg, Germany) and analyzed with the CellQuest™ software (BD Biosciences).

Statistical analysis

For exploratory data analysis, two-sided Mann-Whitney tests for numerical variables and two-sided Fisher tests for categorical variables were performed with the level of statistical significance set to $p<0.05$. We did not take into account corrections for multiple hypothesis testing. The relapse risk was assessed using the Kaplan-Meier method and a log rank test to test for differences between relapse curves with respect to CHIP status. The statistical analysis was carried out in R version 3.4.3 and RStudio version 1.1.442.

Supplementary Tables

Supplementary Table 1: List of genes and target regions included in the TruSight Myeloid panel (Illumina) and in a customized version of the panel used in screening for clonal hematopoiesis.

Gene	Target region (exon)	Gene	Target region (exon)
ABL1	4–6	*KDM6A*	full
ASXL1	12	*KIT*	2, 8–11, 13, 17
*ASXL2***	12, 13 (exon 13 CDS only)	*KLHDC8A***	full
*ATRX**	8-10, 17–31	*KRAS*	2, 3
BCOR	full	*MLL*	5–8
BCORL1	full	*MPL*	4**, 10, 12**
BRAF	15	*MYB***	full
*BRCC3***	full	*MYD88*	3–5
CALR	9	*NOTCH1*	26-28, 34
CBL	8, 9	*NPM1*	12
CBLB	9, 10	*NRAS*	2, 3
CBLC	9, 10	*PDGFRA*	12, 14, 18
CDKN2A	full	*PHF6*	full
*CEBPA**	full	*PPM1D***	5, 6 (exon 6 CDS only)
CSF3R	14–17	*PTEN*	5, 7
CUX1	full	*PTPN11*	3, 13
DNMT3A	full	*RAD21*	full
ETV6/TEL	full	*RUNX1*	full
EZH2	full	*SETBP1*	4 (partial)
FBXW7	9, 10, 11	*SF3B1*	13–16
FLT3	14, 15, 20	*SMC1A*	2, 11, 16, 17
GATA1	2	*SMC3*	10, 13, 19, 23, 25, 28
GATA2	2–6	*SRSF2*	1
GNAS	8, 9	*STAG2*	full
*GNB1***	2, 3, 4, 5	*STAT3***	20, 21
HRAS	2, 3	*TET2*	3–11
IDH1	4	*TP53*	2–11
IDH2	4	*U2AF1*	2, 6
IKZF1	full	*WT1*	7, 9
JAK2	12, 13**, 14	*ZRSR2*	full
JAK3	13	*ZBTB7A***	full

* excluded in Custom Myeloid panel
** in Custom Myeloid panel only

Supplementary Table 2: List of 46 somatic mutations identified in 34 AAV patients.

UPN ID	Gene	Ref	Alt	Chr	Coordinate	Type	VAF	Transcript	Consequence	CDS Position	Protein Position
UPN070	*ASXL1*	A	AG	20	31022441	ins	0.1720	NM_015338.5	frameshift_variant	c.1926-1927	p.G642fs
UPN098	*ASXL1*	A	AGG	20	31022441	ins	0.0410	NM_015338.5	frameshift_variant	c.1926-1927	p.G642fs
UPN110	*ASXL1*	GC	G	20	31023636	del	0.0710	NM_015338.5	frameshift_variant	c.3122	p.A1041fs
UPN057	*ASXL1*	GT	G	20	31022934	del	0.2440	NM_015338.5	frameshift_variant	c.2420	p.V807fs
UPN090	*ASXL2*	ATGGGGGAGATTCTG	A	2	25967320	del	0.2680	NM_018263.4	frameshift_variant	c.1872-1885	p.R624fs
UPN068	*BCOR*	G	A	X	39932102	snv	0.0370	NM_001123385.1	stop_gained	c.2497	p.Q833*
UPN054	*DNMT3A*	G	A	2	25467083	snv	0.1818	NM_022552.4	stop_gained	c.1792	p.R598*
UPN108	*DNMT3A*	A	C	2	25470587	snv	0.0830	NM_022552.4	missense_variant	c.887	p.V296G
UPN013	*DNMT3A*	A	G	2	25467473	snv	0.0260	NM_022552.4	missense_variant	c.1603	p.S535P
UPN021	*DNMT3A*	C	T	2	25457242	snv	0.3850	NM_022552.4	missense_variant	c.2645	p.R882H
UPN019	*DNMT3A*	GT	G	2	25467519	del	0.0940	NM_022552.4	frameshift_variant	c.1556	p.N519fs
UPN045	*DNMT3A*	CG	C	2	25469613	del	0.0550	NM_022552.4	frameshift_variant	c.1154	p.P385fs
UPN065	*DNMT3A*	GG	CA	2	25470916	mnv	0.0300	NM_022552.4	stop_gained	c.844-845	p.P282*
UPN068	*DNMT3A*	G	A	2	25457243	snv	0.0260	NM_022552.4	missense_variant	c.2644	p.R882C
UPN072	*DNMT3A*	T	C	2	25463289	snv	0.0180	NM_022552.4	missense_variant	c.2204	p.Y735C
UPN071	*DNMT3A*	G	A	2	25463265	snv	0.0210	NM_022552.4	missense_variant	c.2228	p.P743L
UPN006	*DNMT3A*	G	A	2	25457243	snv	0.0264	NM_022552.4	missense_variant	c.2644	p.R882C
UPN101	*DNMT3A*	C	T	2	25470597	snv	0.0230	NM_022552.4	missense_variant	c.877	p.G293R
UPN002	*DNMT3A*	A	G	2	25458595	snv	0.0511	NM_022552.4	missense_variant	c.2578	p.W860R
UPN037	*DNMT3A*	TGGTGGAGGTG	T	2	25469099	del	0.0417	NM_022552.4	frameshift_variant	c.1349-1358	p.P450fs
UPN001	*DNMT3A*	C	G	2	25463320	snv	0.0356	NM_022552.4	splice_acceptor_variant	c.2174-1	p.?
UPN003	*DNMT3A*	TTGGC	T	2	25462059	del	0.0298	NM_022552.4	frameshift_variant	c.2344-2347	p.A782fs
UPN036	*DNMT3A*	G	A	2	25469939	snv	0.3364	NM_022552.4	missense_variant	c.1103	p.A368V
UPN036	*DNMT3A*	T	G	2	25467496	snv	0.0516	NM_022552.4	missense_variant	c.1580	p.Q527P
UPN060	*DNMT3A*	T	C	2	25469647	snv	0.0920	NM_022552.4	splice_acceptor_variant	c.1123-2	p.?
UPN090	*ETV6*	C	T	12	12038876	snv	0.2470	NM_001987.4	missense_variant	c.1169	p.T390I
UPN090	*GNAS*	G	A	20	57484421	snv	0.1080	NM_080425.2	missense_variant	c.2531	p.R844H
UPN050	*GNB1*	C	A	1	1747227	snv	0.1780	NM_002074.3	missense_variant	c.171	p.K57N

UPN066	*KDM6A*	G	A	X	44913078	snv	0.0230	NM_021140.2	stop_gained	c.753	p.W251*
UPN091	*PPM1D*	CT	C	17	58740731	del	0.0580	NM_003620.3	frameshift_variant	c.1637	p.L546fs
UPN103	*PPM1D*	CA	C	17	58740653	del	0.0260	NM_003620.3	frameshift_variant	c.1559	p.Q520fs
UPN079	*RAD21*	T	C	8	117868939	snv	0.0390	NM_006265.2	missense_variant	c.760	p.M254V
UPN101	*RAD21*	C	G	8	117875458	snv	0.0430	NM_006265.2	missense_variant	c.185	p.G62A
UPN079	*SF3B1*	C	A	2	198267359	snv	0.0305	NM_012433.2	missense_variant	c.1998	p.K666N
UPN032	*SRSF2*	GGG	TGA	17	74732959	mnv	0.0500	NM_001195427.1	missense_variant	c.282-284	p.P95L
UPN090	*SRSF2*	G	A	17	74732959	snv	0.2180	NM_001195427.1	missense_variant	c.284	p.P95L
UPN098	*SRSF2*	G	T	17	74732959	snv	0.4480	NM_001195427.1	missense_variant	c.284	p.P95L
UPN075	*TET2*	AC	A	4	106194049	del	0.0334	NM_001127208.2	frameshift_variant	c.4512	p.N1504fs
UPN034	*TET2*	G	A	4	106180838	snv	0.0220	NM_001127208.2	missense_variant	c.3866	p.C1289Y
UPN026	*TET2*	A	T	4	106158380	snv	0.0210	NM_001127208.2	missense_variant	c.3281	p.K1094I
UPN045	*TET2*	AAAAT	A	4	106156074	del	0.0470	NM_001127208.2	frameshift_variant	c.976-979	p.K326fs
UPN090	*TET2*	G	A	4	106190843	snv	0.0450	NM_001127208.2	missense_variant	c.4121	p.C1374Y
UPN096	*TET2*	C	T	4	106197269	snv	0.5090	NM_001127208.2	missense_variant	c.5602	p.H1868Y
UPN057	*TET2*	C	T	4	106157845	snv	0.1770	NM_001127208.2	stop_gained	c.2746	p.Q916*
UPN105	*TP53*	C	G	17	7577099	snv	0.0280	NM_000546.5	missense_variant	c.839	p.R280T
UPN002	*TP53*	T	TGGCGCG	17	7578452	ins	0.0603	NM_000546.5	tandem_duplication	c.472_477	p.R158_A159dup

Supplementary Table 3: Comparison of CHIP prevalence in recent cohorts of comparable age and sequencing technology. In those cohorts with a VAF detection limit < 2%, only mutations with VAF ≥ 2% were taken into account.

Publication	Patients	Patient Cohort	N	Median age	VAF Cut-off*	Number of Genes in Panel	Sequencing depth	Prevalence of CHIP	95%-CI	p-value**
AAV patients	autoimmune	Patients with ANCA-associated vasculitis	112	64.5	0.02	54/60	3000	30.36%	8.52%	
Arends et al. 2018	cardiovascular	Patients from cardiology/nephrology ward, Charité University Medical Center, Berlin, Germany.	365	75.3	0.02	54/60	2400	27.12%	4.45%	0.547
Gibson et al. 2017	lymphoma	Lymphoma patients undergoing autologous stem cell transplantation	401	60 - 61	0.02	95	>1000	29.93%	4.48%	1.000
Coombs et al. 2017	solid cancer	MSK-IMPACT sequencing data of 8810 cancer patients	8810	58.3	0.02	341/410	>400	25.12%	0.91%	0.228
Savola et al. 2018	autoimmune	Rheumatoid arthritis patients	59	61	0.02	34	>5000***	17.00%	9.58%	0.067
Frick et al. 2018	healthy	Elderly donors for allogenic stem cell transplantation, multicenter study	500	64.5	0.02	60/66	2000	16.00%	3.21%	**0.001**
Abelson et al. 2018 *Discovery cohort*	healthy	Unselected controls, discovery cohort	414	56.8	0.02	264	4000	8.70%	2.71%	**<0.001**
Abelson et al. 2018 *Validation cohort*	healthy	Unselected controls, validation cohort	262	66.1	0.02	111	2000	10.69%	3.74%	**<0.001**
Buscarlet et al. 2017	healthy	Women of French-Canadian ancestry without any known hematological disorder	2530	69.1	0.02	19	4000	13.70%	1.34%	**<0.001**
Desai et al. 2018	healthy	Unselected controls	212	66.1	0.02	68	>2000***	18.40%	5.22%	**0.017**
All healthy controls	healthy	All control cohorts above taken together	3918		0.02	-	-	13.52%	1.07%	**< 0.001**

*in cohorts, where the VAF cutoff was lower than 0.02, prevalence was re-calculated on the basis of mutations with VAF > 0.02 only

**p-values were calculated with Fisher tests comparing the prevalence of CHIP in the respective publication with the prevalence in our AAV cohort.

***estimated from supplemental table of called mutations

Supplementary Table 4: List of 22 somatic mutations identified in 20 out of 112 age- and sex-matched healthy individuals.

ID	Gene	Ref	Alt	Chr	Coordinate	Type	VAF	Transcript	HGVS.c	HGVS.p	dbSNP/COSMIC
Ctrl011	*ASXL1*	G	T	20	31024308	missense_variant	0.480	ENST00000306058	c.3778G>T	p.(Asp1260Tyr)	COSM133584
Ctrl087	*ASXL1*	A	AG	20	31022441	frameshift_variant	0.043	ENST00000306061	c.1919dup	p.(Gly641Trpfs*12)	COSM1411076;COSM4170082
Ctrl082	*CBL*	T	C	11	119148891	missense_variant	0.053	ENST00000264033	c.1111T>C	p.(Tyr371His)	COSM34052
Ctrl048	*DNMT3A*	TGGTGGAGGTG	T	2	25469099	frameshift_truncation	0.031	ENST00000264709	c.1349_1358del	p.(Pro450Glnfs*198)	.
Ctrl079	*DNMT3A*	GC	G	2	25470556	frameshift_variant	0.150	ENST00000264709	c.918del	p.(Trp306Cysfs*10)	.
Ctrl108	*DNMT3A*	C	T	2	25470551	missense_variant	0.038	ENST00000264709	c.923G>A	p.(Gly308Asp)	COSM4169957;COSM4169958
Ctrl110	*DNMT3A*	T	C	2	25457164	missense_variant	0.397	ENST00000264709	c.2723A>G	p.(Tyr908Cys)	.
Ctrl008	*DNMT3A*	C	T	2	25457242	missense_variant	0.064	ENST00000264709	c.2645G>A	p.(Arg882His)	rs147001633;COSM442676; COSM52944
Ctrl027	*DNMT3A*	C	T	2	25469541	stop_gained	0.0539	ENST00000264709	c.1227G>A	p.(Trp409*)	.
Ctrl105	*DNMT3A*	G	A	2	25466800	nonsynonymous SNV	0.2028	NM_153759	c.C1336T	p.R446W	1500429;1582386;1606706; 1673543;1674321;1674333;1674353
Ctrl003	*DNMT3A*	CAA	C	2	25466783	frameshift_truncation	0.1823	ENST00000264709	c.1919_1920del	p.(Phe640fs)	.
Ctrl047	*JAK2*	G	T	9	5073770	missense_variant	0.028	ENST00000381652	c.1849G>T	p.(Val617Phe)	rs77375493;COSM12600
Ctrl037	*MYD88*	T	C	3	38182641	stop_lost	0.049	ENST00000443433	c.613T>C	p.(*205Argext*8)	COSM5416224;COSM85940
Ctrl110	*PPM1D*	C	T	17	58740806	stop_gained	0.362	ENST00000305921	c.1711C>T	p.(Gln571*)	.
Ctrl096	*SF3B1*	C	A	2	198267491	missense_variant	0.017	ENST00000335508	c.1866G>T	p.(Glu622Asp)	COSM110693
Ctrl057	*SRSF2*	G	C	17	74732959	missense_variant	0.438	ENST00000359995	c.284C>G	p.(Pro95Arg)	COSM146290;COSM211661; COSM4385016
Ctrl036	*SRSF2*	G	A	17	74732959	missense_variant	0.390	ENST00000359995	c.284C>T	p.(Pro95Leu)	COSM146288;COSM146290; COSM211028;COSM211506
Ctrl054	*TET2*	TG	T	4	106156936	frameshift_truncation	0.170	ENST00000305737	c.1842del	p.(Gly614Glyfs*25)	COSM2952744;COSM2952745
Ctrl040	*TET2*	GC	G	4	106190817	frameshift_truncation	0.049	ENST00000513237	c.4159del	p.(Arg1387Valfs*82)	.
Ctrl051	*TET2*	C	T	4	106157836	stop_gained	0.055	ENST00000265149	c.2737C>T	p.(Gln913*)	COSM1735762;COSM4171287
Ctrl087	*TET2*	C	T	4	106156339	stop_gained	0.043	ENST00000265149	c.1240C>T	p.(Gln414*)	COSM87085
Ctrl031	*TET2*	-	TT	4	106157970	frameshift insertion	0.051	NM_001127208	c.2871_2872insTT	p.L957fs	.

Supplementary Table 5: Demographic data and baseline characteristics of 112 AAV patients stratified according to CHIP status.

		CHIP negative n = 78	CHIP positive n = 34	Missing data	p-value*
Demographic data					
Age	Median	63.0	70.5	0	**0.017**
	Range	44 - 84	18 - 81		
Sex – no. (%)	male	46 (59.0)	18 (52.9)	0	0.678
	female	32 (41.0)	16 (47.1)		
Diagnosis – no. (%)					
AAV type	GPA	54 (69.2)	22 (66.7)	1	0.825
	MPA	24 (30.8)	11 (33.3)		
Disease activity	active	47 (66.2)	18 (60.0)	11	0.877
	PR	19 (26.8)	10 (33.3)		
	CR	5 (7.0)	2 (6.7)		
Prior Therapies – no. (%)					
Prior Treatment	yes	50 (68.5)	20 (62.5)	7	0.708
	no	23 (31.5)	12 (37.5)		
Steroids	yes	50 (68.5)	20 (62.5)	7	0.708
	no	23 (31.5)	12 (37.5)		
Cyclophosphamide	yes	45 (61.6)	18 (56.2)	7	0.762
	no	28 (38.4)	14 (43.8)		
Rituximab	yes	11 (15.1)	3 (9.4)	7	0.633
	no	62 (84.9)	29 (90.6)		
Azathioprine	yes	6 (8.2)	5 (15.6)	7	0.427
	no	67 (91.8)	27 (84.4)		
Methotrexate	yes	5 (6.8)	4 (12.5)	7	0.566
	no	68 (93.2)	28 (87.5)		
Cardiovascular comorbidities – no. (%)					
Arterial hypertension	yes	48 (80.0)	20 (69.0)	23	0.292
	no	12 (20.0)	9 (31.0)		
Diabetes mellitus	yes	18 (30.0)	7 (24.1)	23	0.623
	no	42 (70.0)	22 (75.9)		
Coronary heart disease	yes	12 (20.0)	6 (20.7)	23	1.000
	no	48 (80.0)	23 (79.3)		
Previous myocardial infarction	yes	3 (5.0)	0 (0.0)	23	0.548
	no	57 (95.0)	30 (100.0)		
Atrial fibrillation	yes	11 (18.3)	8 (27.6)	23	0.409
	no	49 (81.7)	21 (72.4)		
Previous stroke	yes	5 (8.3)	2 (6.9)	23	1.000
	no	55 (91.7)	27 (93.1)		
Peripheral artery occlusive disease	yes	3 (5.0)	1 (3.4)	23	1.000
	no	57 (95.0)	28 (96.6)		

Abbreviations: AAV = ANCA-associated vasculitis, GPA = Granulomatosis with polyangiitis, MPA = Microscopic polyangiitis, ENT = ear, nose and throat, PR = partial remission, CR = complete remission.

*p-values from two-sided Fisher tests for categorical variables and from two-sided Mann-Whitney tests for continuous variables.

Supplementary Table 6: Blood counts of the AAV cohort according to CHIP status. P-values were calculated with Mann-Whitney tests.

Blood counts	CHIP negative n = 78	CHIP positive n = 34	Missing data	p-value
Hemoglobin [g/dl]	11.30	11.70	2	0.749
Hematocrit	0.35	0.36	2	0.712
Erytrocytes [1000/nl]	3.91	3.90	2	0.744
Leukocytes [1/nl]	9.60	8.35	2	0.102
Thrombocytes [1/nl]	277	284	2	0.525
Mean Corpuscular Volume (MCV) [fl]	89.0	89.0	2	0.992
Mean Corpuscular Hemoglobin (MCH) [pg]	29.35	29.15	4	0.837
Mean Corpuscular Hemoglobin concentration (MCHC) [g/dl]	33.25	33.25	4	0.538
Red cell distribution width (RDW) [%]	14.40	14.50	4	0.770
Neutrophils [1/nl]	8.31	6.88	30	0.201
Lymphocytes [1/nl]	1.07	1.05	30	0.669
Monocytes [1/nl]	0.56	0.55	30	0.904
Eosinophils [1/nl]	0.08	0.04	30	0.276

Supplementary Table 7: Disease manifestation patterns in 35 patients with MPA and 76 patients with GPA according to CHIP status.

GPA Disease manifestation – no. (%)		CHIP negative n = 54	CHIP positive n = 22	Missing data	p-value*
General	yes	20 (39.2)	9 (40.9)	3	1.000
	no	31 (60.8)	13 (59.1)		
Skin	yes	15 (29.4)	2 (9.1)	3	0.077
	no	36 (70.6)	20 (90.9)		
Mucosa/Eye	yes	16 (31.4)	11 (50.0)	3	0.186
	no	35 (68.6)	11 (50.0)		
ENT	yes	30 (57.7)	17 (77.3)	3	0.123
	no	22 (42.3)	5 (22.7)		
Pulmonal	yes	33 (63.5)	15 (68.2)	3	0.793
	no	19 (36.5)	7 (31.8)		
Renal	yes	46 (88.5)	15 (68.2)	3	**0.049**
	no	6 (11.5)	7 (31.8)		
Neuronal	yes	10 (19.2)	0 (0.0)	3	**0.028**
	no	42 (80.8)	22 (100.0)		

MPA Disease manifestation – no. (%)		CHIP negative n = 24	CHIP positive n = 11	Missing data	p-value*
General	yes	6 (25.0)	3 (27.3)	0	1.000
	no	18 (75.0)	8 (72.7)		
Skin	yes	4 (16.7)	1 (9.1)	0	1.000
	no	20 (83.3)	10 (90.9)		
Mucosa/Eye	yes	3 (12.5)	0 (0.0)	0	0.536
	no	21 (87.5)	11 (100.0)		
ENT	yes	1 (4.2)	1 (9.1)	0	0.536
	no	23 (95.8)	10 (90.9)		
Pulmonal	yes	6 (25.0)	6 (54.5)	0	0.130
	no	18 (75.0)	5 (45.5)		
Renal	yes	23 (95.8)	10 (90.9)	0	0.536
	no	2 (4.2)	1 (9.1)		
Neuronal	yes	2 (8.3)	0 (0.0)	0	1.000
	no	22 (91.7)	11 (100.0)		

Abbreviations: GPA = Granulomatosis with Polyangiitis, MPA = Microscopic Polyangiitis, ENT = ear, throat, nose,
*p-values from two-sided Fisher tests for categorical variables and from two-sided Mann-Whitney tests for continuous variables.

Supplementary Table 8: Complications occurring during follow-up time with respect to CHIP status.

Complications during Follow-up – no. (%)		CHIP negative n = 78	CHIP positive n = 34	Missing data	p-value*
Median follow up time (months)		26.20	31.96	0	0.754
Death	yes	6 (7.8)	2 (6.1)	2	1.000
	no	71 (92.2)	31 (93.9)		
Malignoma	yes	11 (14.7)	6 (19.4)	6	0.569
	no	64 (85.3)	25 (80.6)		
Infection	yes	21 (28.4)	10 (31.2)	6	0.818
	no	53 (71.6)	22 (68.8)		
AAV Relapse	yes	42 (57.5)	17 (51.5)	6	0.674
	no	31 (42.5)	16 (48.5)		
Dialysis	yes	18 (24.7)	8 (24.2)	6	1.000
	no	55 (75.3)	25 (75.8)		
Cardiovascular Event	yes	10 (13.0)	6 (18.2)	6	0.557
	no	67 (87.0)	27 (81.8)		

*p-values from two-sided Fisher tests for categorical variables and from two-sided Mann-Whitney tests for continuous variables.

Supplementary Table 9: List of CHIP^{positive} AAV patients whose samples were used in neutrophil activation and gene expression assays.

UPN ID	Gene	Ref	Alt	Chr	Coordinate	Type	VAF	Transcript	Consequence	CDS Position	Protein Position
UPN045	*DNMT3A*	CG	C	2	25469613	del	0.0550	NM_022552.4	frameshift_variant	c.1154	p.P385fs
	TET2	AAAAT	A	4	106156074	del	0.0470	NM_001127208.2	frameshift_variant	c.976-979	p.K326fs
UPN050	*GNB1*	C	A	1	1747227	snv	0.1780	NM_002074.3	missense_variant	c.171	p.K57N
UPN054	*DNMT3A*	G	A	2	25467083	snv	0.1818	NM_022552.4	stop_gained	c.1792	p.R598*
UPN057	*ASXL1*	GT	G	20	31022934	del	0.2440	NM_015338.5	frameshift_variant	c.2420	p.V807fs
	TET2	C	T	4	106157845	snv	0.1770	NM_001127208.2	stop_gained	c.2746	p.Q916*
UPN065	*DNMT3A*	GG	CA	2	25470916	mnv	0.0300	NM_022552.4	stop_gained	c.844-845	p.P282*
UPN072	*DNMT3A*	T	C	2	25463289	snv	0.0180	NM_022552.4	missense_variant	c.2204	p.Y735C
UPN075	*TET2*	AC	A	4	106194049	del	0.0334	NM_001127208.2	frameshift_variant	c.4512	p.N1504fs
UPN079	*RAD21*	T	C	8	117868939	snv	0.0390	NM_006265.2	missense_variant	c.760	p.M254V
	SF3B1	C	A	2	198267359	snv	0.0305	NM_012433.2	missense_variant	c.1998	p.K666N
UPN090	*ASXL2*	ATGGGGGAGATTCTG	A	2	25967320	del	0.2680	NM_018263.4	frameshift_variant	c.1872-1885	p.R624fs
	ETV6	C	T	12	12038876	snv	0.2470	NM_001987.4	missense_variant	c.1169	p.T390I
	GNAS	G	A	20	57484421	snv	0.1080	NM_080425.2	missense_variant	c.2531	p.R844H
	SRSF2	G	A	17	74732959	snv	0.2180	NM_001195427.1	missense_variant	c.284	p.P95L
	TET2	G	A	4	106190843	snv	0.0450	NM_001127208.2	missense_variant	c.4121	p.C1374Y
UPN091	*PPM1D*	CT	C	17	58740731	del	0.0580	NM_003620.3	frameshift_variant	c.1637	p.L546fs
UPN096	*TET2*	C	T	4	106197269	snv	0.5090	NM_001127208.2	missense_variant	c.5602	p.H1868Y
UPN098	*ASXL1*	A	AGG	20	31022441	ins	0.0410	NM_015338.5	frameshift_variant	c.1926-1927	p.G642fs
	SRSF2	G	T	17	74732959	snv	0.4480	NM_001195427.1	missense_variant	c.284	p.P95L
UPN103	*PPM1D*	CA	C	17	58740653	del	0.0260	NM_003620.3	frameshift_variant	c.1559	p.Q520fs
UPN105	*TP53*	C	G	17	7577099	snv	0.0280	NM_000546.5	missense_variant	c.839	p.R280T
UPN108	*DNMT3A*	A	C	2	25470587	snv	0.0830	NM_022552.4	missense_variant	c.887	p.V296G
UPN110	*ASXL1*	GC	G	20	31023636	del	0.0710	NM_015338.5	frameshift_variant	c.3122	p.A1041fs

Supplementary Table 10: Characteristics of AAV patients and healthy controls whose samples were used in neutrophil activation assays and transcript analysis. P-values were calculated with Kruskal-Wallis tests for three groups for continuous variables and Fisher's test for categorical variables. No correction for multiple hypothesis testing was applied.

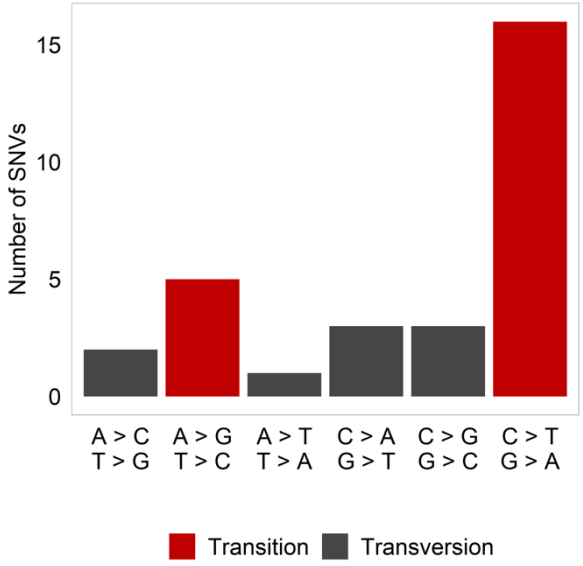
Differential blood counts		AAV, CHIP negative n = 31	AAV, CHIP positive n = 16	Healthy Controls n = 14	Missing data	p-value
Median age [yrs]		58	71	40	3	<0.001
Sex	female	15	9	9	3	0.174
	male	16	7	2		
AAV type	GPA	20	10	NA	0	1.000
	MPA	11	6	NA		
Hemoglobin [g/dl]		12.40	11.60	13.60	5	0.044
Hematocrit [1]		0.37	0.33	0.40	5	0.057
Erythrocytes [1000/nl]		4.09	3.90	4.50	5	0.059
Leukocytes [1/nl]		8.64	8.80	6.28	5	0.048
Thrombocytes [1/nl]		283.0	285.0	237.5	5	0.525
MCV [fl]		90.0	89.0	88.5	5	0.784
MCH [pg]		29.8	29.4	30.5	5	0.469
MCHC [g/dl]		33.80	33.70	33.95	5	0.575
Red cell distribution width [%]		14.30	14.50	12.85	7	0.010
Neutrophils [1/nl] Neutrophil fraction [1]		7.22 0.82	7.65 0.81	3.93 0.61	12	0.004 <0.001
Lymphocytes [1/nl] Lymphocyte fraction [1]		0.79 0.09	0.95 0.08	1.73 0.27	12	0.001 <0.001
Monocytes [1/nl] Monocyte fraction [1]		0.52 0.07	0.61 0.06	0.45 0.07	13	0.437 0.798
Eosinophils [1/nl] Eosinophil fraction [1]		0.08 0.01	0.04 0.00	0.20 0.03	12	0.041 0.014

Supplementary Table 11: Mutations in genes involved in the DNA damage response (*TP53, PPM1D, RAD21*) were not enriched in the group of patients which had received immunosuppressive treatment prior to sampling (4/70 vs 2/35, p=1).

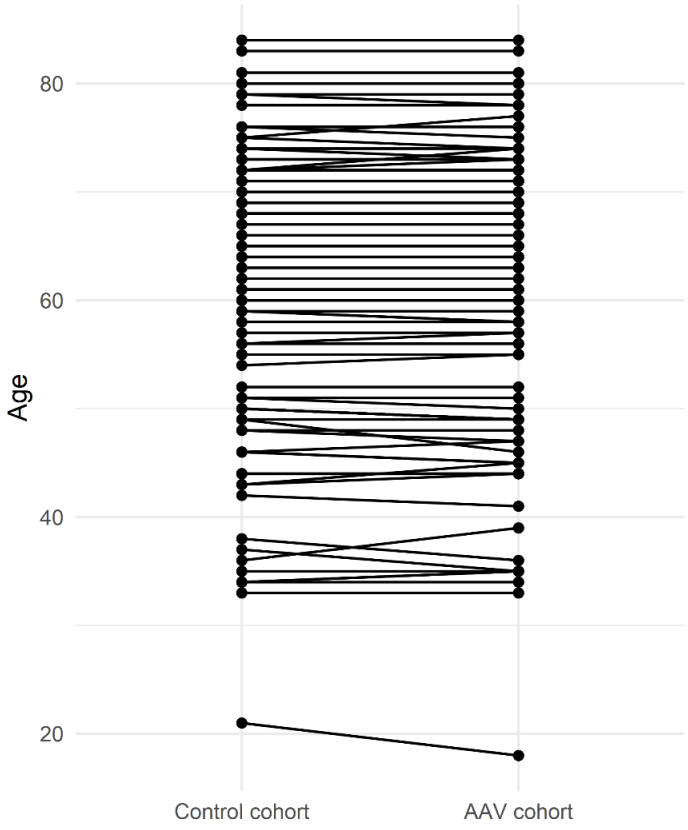
Patients with available treatment data (n=105)	Treated before first sampling	Not treated before first sampling	
PPM1D-, *TP53*- or *RAD21*-mutated	4	2	6
PPM1D-, *TP53*- and *RAD21*-wildtype	66	33	99
	70	35	105

Supplementary Figures

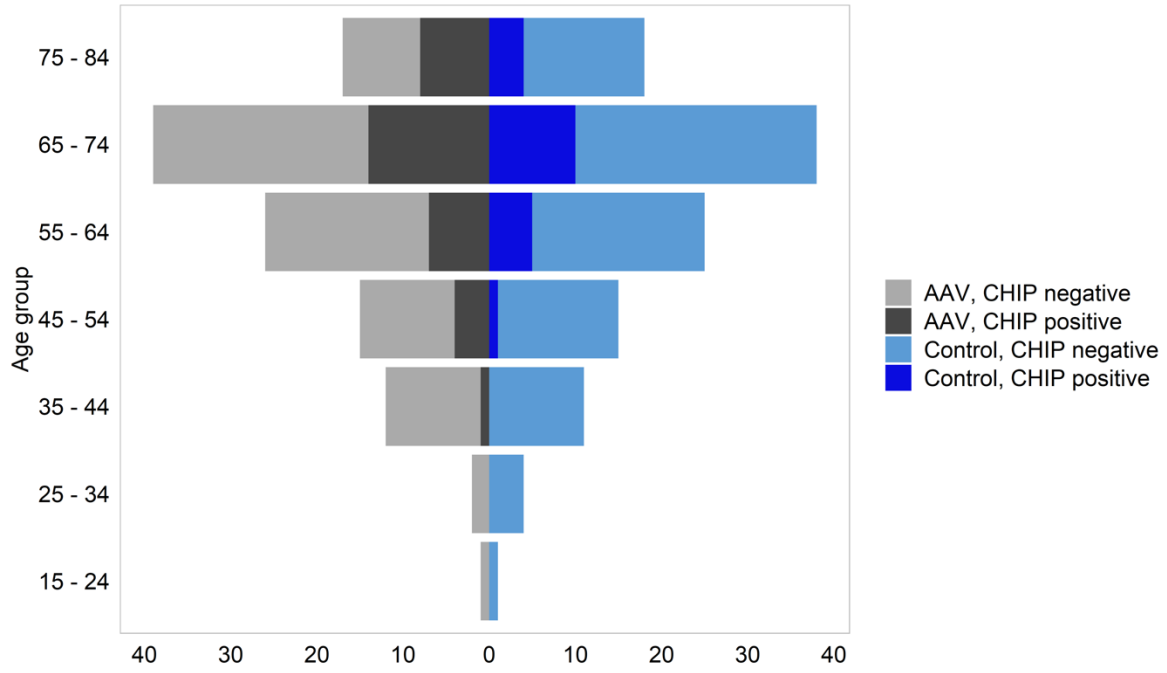
Supplementary Figure 1: Among the 30 single nucleotide variants (SNVs) identified, transitions (purine to purine or pyrimidine to pyrimidine) were the most frequent base changes. C to T substitutions have been shown to represent a mutational signature characteristic of aging.[4]



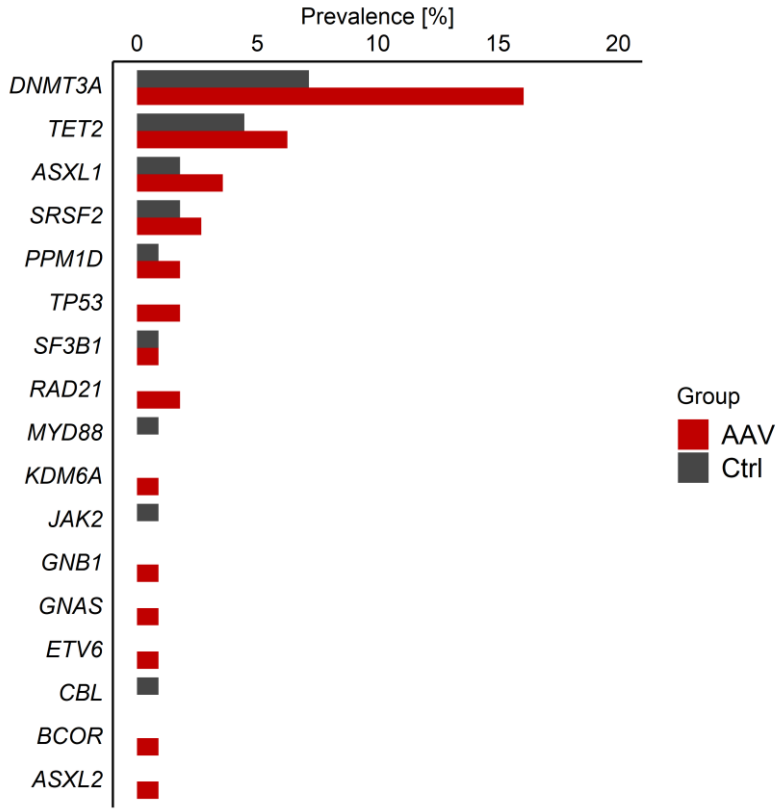
Supplementary Figure 2: Line plot depicting the age matching of the AAV cohort and control cohort.



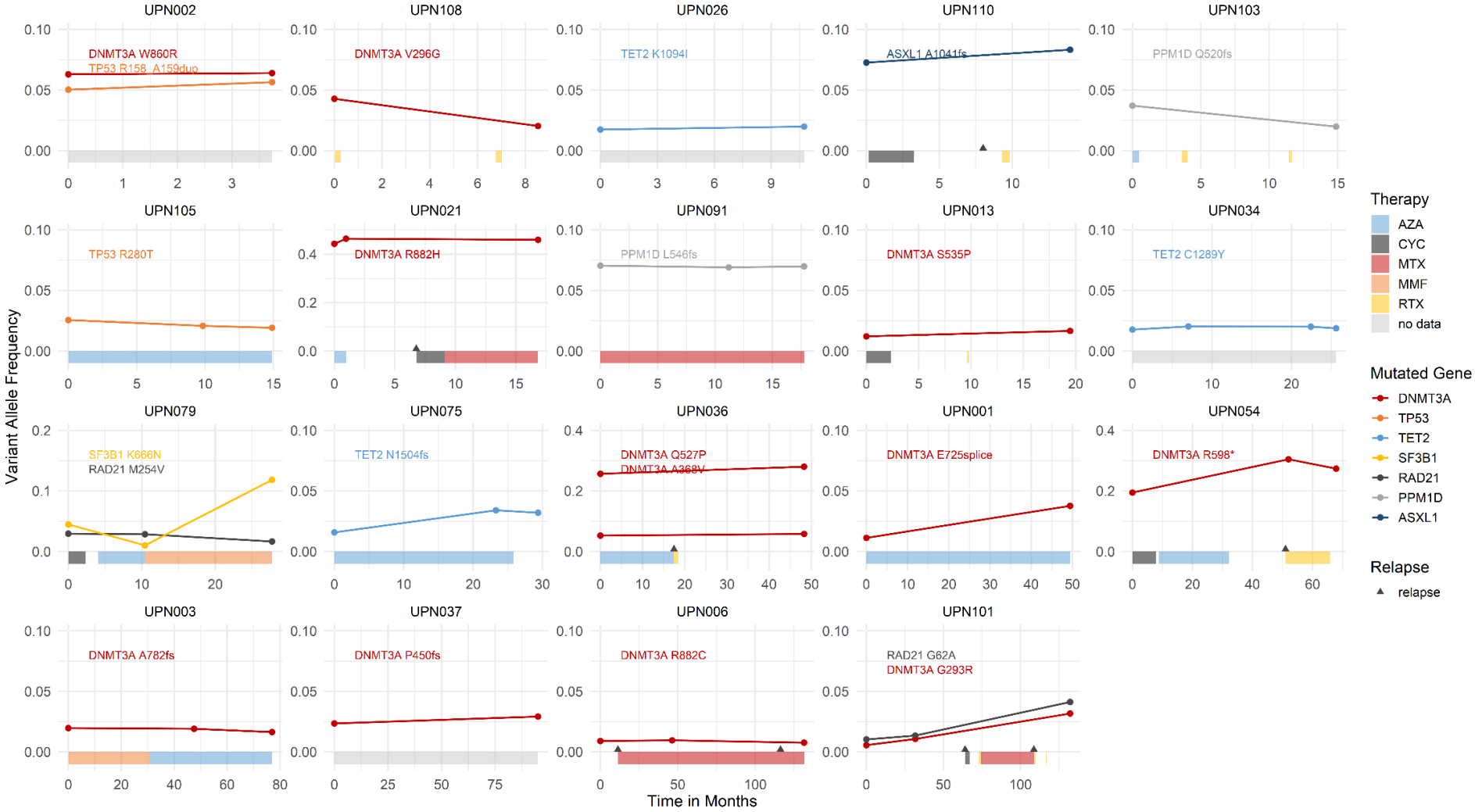
Supplementary Figure 3: Age distribution and prevalence of CHIP in different age groups in the AAV cohort and control cohort.



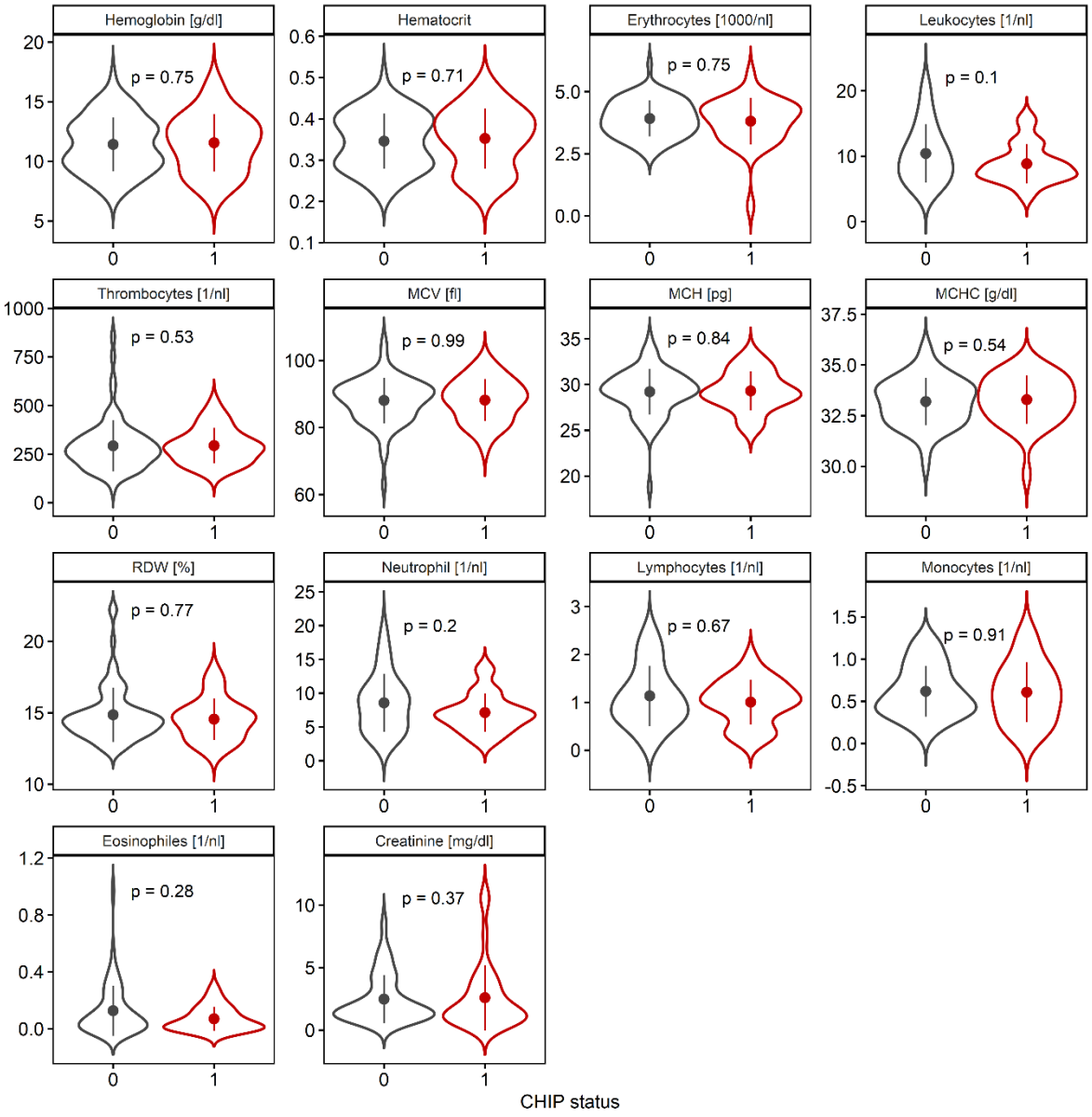
Supplementary Figure 4: Prevalence of gene mutations in AAV and age- and gender-matched control cohorts



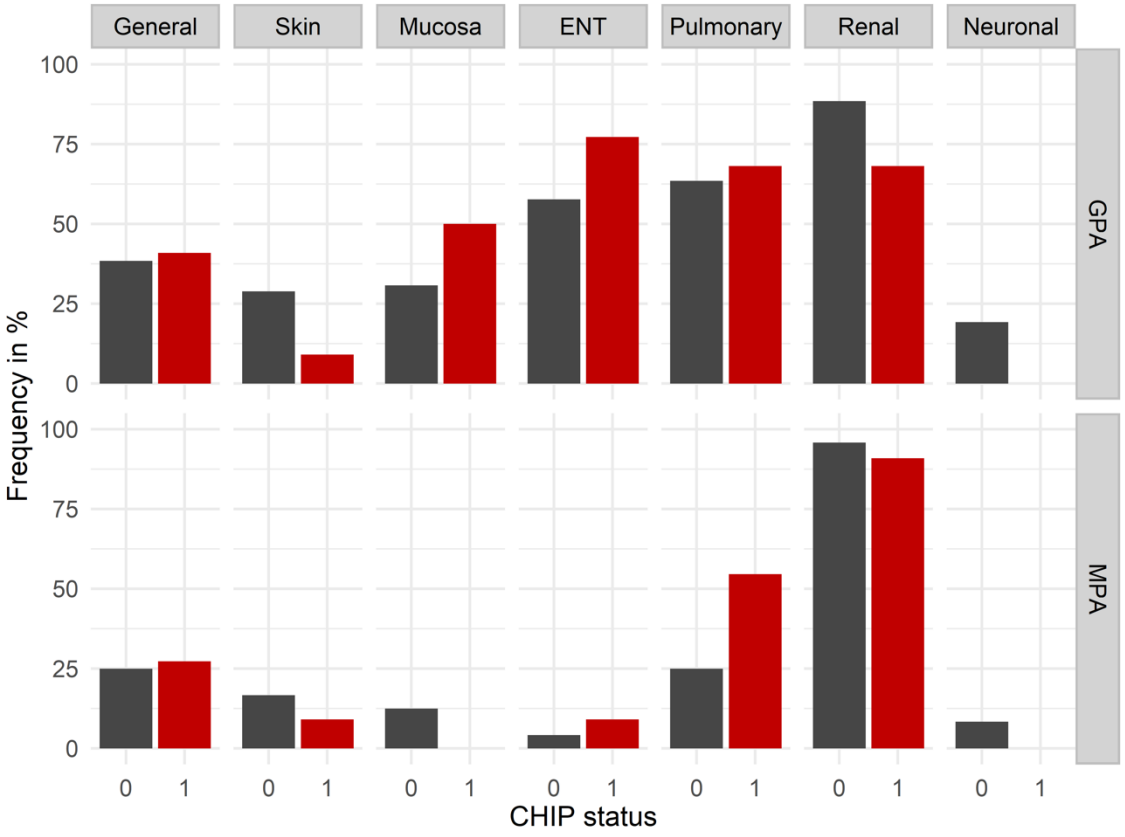
Supplementary Figure 5: Longitudinal quantification of mutational burden for patients with available follow-up samples. Administered therapy is depicted at the x-axis of each graph. Relapses are plotted as triangles. Abbreviations: AZA = azathioprine, CYC = cyclophosphamide, MTX = methotrexate, MMF = mycophenolate-mofetil, RTX = rituximab.



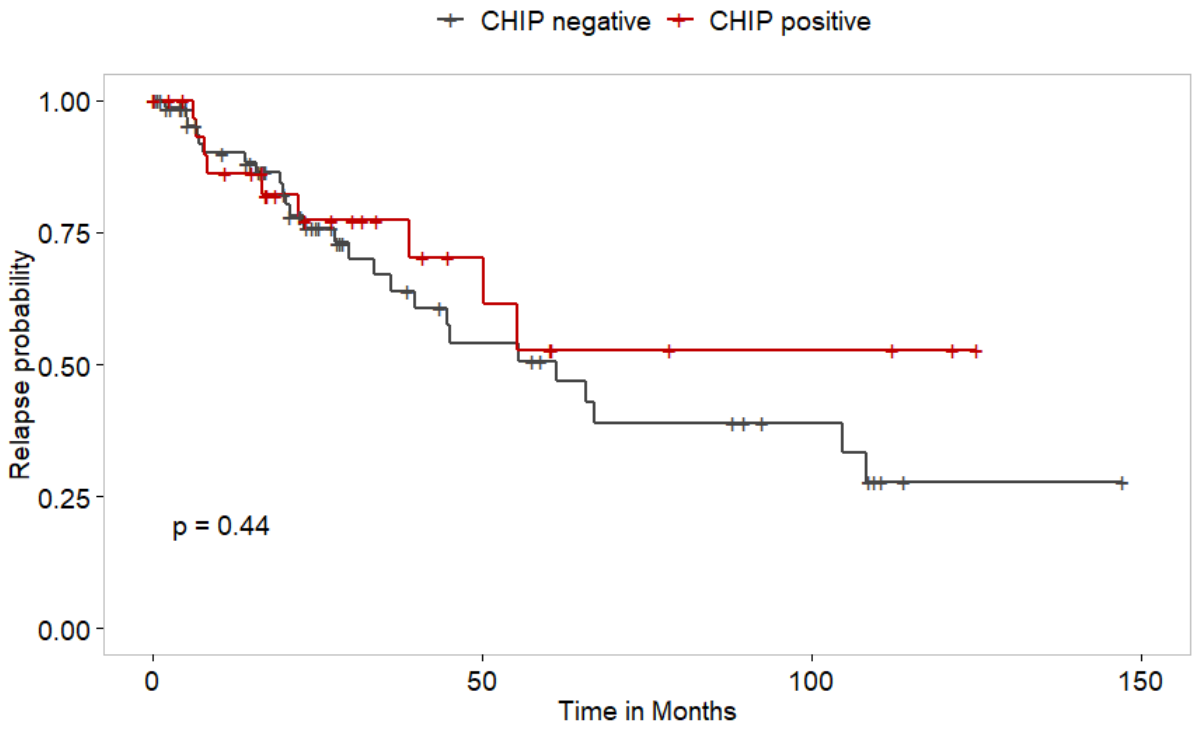
Supplementary Figure 6: Violin plots of blood counts and creatinine levels of CHIP^{positive} (red) and CHIP^{negative} AAV patients (grey). P-values were calculated with two-sided Mann-Whitney tests.



Supplementary Figure 7: Disease manifestation patterns of MPA and GPA patients according to CHIP status. 1 = CHIP positive, 0 = CHIP negative.



Supplementary Figure 8: Relapse risk according to CHIP status assessed with the Kaplan-Meier method and log rank test.



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