Genetic heterogeneity highlighted by differential FDG-PET response in diffuse large B-cell lymphoma

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Supplementary Information

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Supplementary Methods

Study oversight

Consent followed Swiss national law and ethical approval was obtained; (London Research Ethics Committee (LREC) of the East London and the City Health authority (10/H0704/65 and 06/Q0605/69).

Immunohistochemistry and fluorescent *in situ* hybridization (FISH)

Initial diagnosis and confirmatory histologic review of all biopsies were performed by expert haemato-pathologists. Immunohistochemistry (IHC) was performed on 1.5 µm formalin-fixed paraffin-embedded (FFPE) tissue sections using the Leica BOND III staining machine with the following primary antibodies: anti-CD20 (RTU, clone L26, DAKO), anti-CD10 (RTU, clone 56C6; Leica), anti-BCL6 (RTU, clone PG B6p; DAKO), anti-MUM1(IRF4) (RTU, clone MUM1p; DAKO). Interphase FISH analysis for *MYC*-, *BCL6*- and *BCL2*-translocations was performed on 2µm thick FFPE tissue sections according to standard validated protocols using Abbott Vysis probes SPEC MYC Dual Color Break Apart for *MYC* (8q24)-split, SPEC MYC/IGH Dual Color Dual Fusion for t(8;14)(q24;q32) *MYC-IGH,* SPEC BCL2/IGH Dual Color Dual Fusion for t(14;18)(q32;q21) *BCL2-IGH* and the SPEC BCL6 Dual Color Break Apart for *BCL6* (3q27)-split detection.

Whole exome sequencing (WES) and analysis.

Genomic DNA was extracted from 2-4x10µm sections of formalin-fixed paraffin embedded (FFPE) tissue using the Generead FFPE kit (QIAGEN) according to the manufacturer's instructions with an overnight 56°C incubation step. Whole exome capture libraries were constructed from 200ng of DNA. Library preparation included shearing, end repair, phosphorylation and ligation to a barcoding sequencing adaptor, using the Accel-NGS 2S Hyb DNA Library Kit with the 2S SureSelectXT MID Compatibility Module (Swift Biosciences) and KAPA HiFi HotStart PCR Kit (Kapa Biosystems) and

NEBNext Index Primers for Illumina (New England Biolabs). PCR amplified libraries were used in the hybrid capture reaction using the SureSelect XT Reagent Kit (Agilent Technologies) and SureSelect Human All Exon V6 60MB biotinylated baits. Enriched exome libraries were multiplexed and sequenced on the Illumina Hiseq 2500 platform to generate 100bp paired end reads. Sequencing metrics are provided in Table S1.

The bioinformatic analysis of WES data was performed using our previously described pipeline [1,2]. After de-multiplexing, sequencing reads were aligned to the reference genome hg19 using the Burrows-Wheeler Aligner (BWA) [3]. Further Indel realignments and base quality scores were amended using the Genome Analysis Toolkit (GATK) version 2.5.2[4]. The Strelka pipeline[5] was used to identify somatic single nucleotide variants and insertion-deletions. The VarScan2 tool[6] was used to verify variants across all tumors and matched normal samples based on high quality reads with a mapping quality > 20 and minimum base quality of 15 at the targeted sites. To reduce the number of false positive calls, all variants also required a variant allele frequency (VAF) of > 2% and the number of supporting reads >= 2. Additional criteria were used to call mutations only observed in one biopsy, with site specific variants requiring a coverage >20 reads and a VAF > 10%. Somatic variants were annotated using the SNPnexus tool[7] and all non-synonymous somatic variants identified are listed in Table S2. WES data has been deposited into the European Genome-phenome Archive (accession number EGAS00001003833).

Copy number analysis

Copy number analysis using WES data was performed as previously described[8]. Briefly, two different approaches, (ASCAT[9] and VarScan2 ‘copynumber’ and ‘copyCaller’ modules followed by the DNAcopy R Bioconductor package version 1.50.1), were used to analyze tumor and germline DNA and identify areas with copy number aberrations and copy neutral loss-of-heterozygosity (cnLOH).

Tumor content estimation and clonality analysis

The tumor content for each sample was derived from the VAFs of variants using the ‘mclust’ R package[10] (Table S1). Samples were then analyzed in pairs to infer the mutational cellular prevalence of somatic variants (shared and unique) using PyClone (v0.13.0)[11] as previously described[8].

Immunoglobulin heavy-chain variable (IGHV) gene sequencing and analysis

The European BIOMED-2 protocol was followed to detect clonal IGHV sequences[12]. IGH VDJ sequencing was unsuccessful in the ING biopsy but demonstrated clonality between the three thoracic biopsies (M1, T2, PL3) (IGH V3-7*01, D3-10*01, J4*02). Clonality was inferred by the shared synonymous and non-synonymous (32) mutations between all four biopsies.

Phylogenetic tree reconstruction

The R package Rphylip was utilised to generate a phylogenetic tree based on a distance matrix of the synonymous and non-synonymous variants found in each sample. A rooted tree was then redrawn, with the branch lengths proportional to the number of somatic variants shared by all nodes on that branch.

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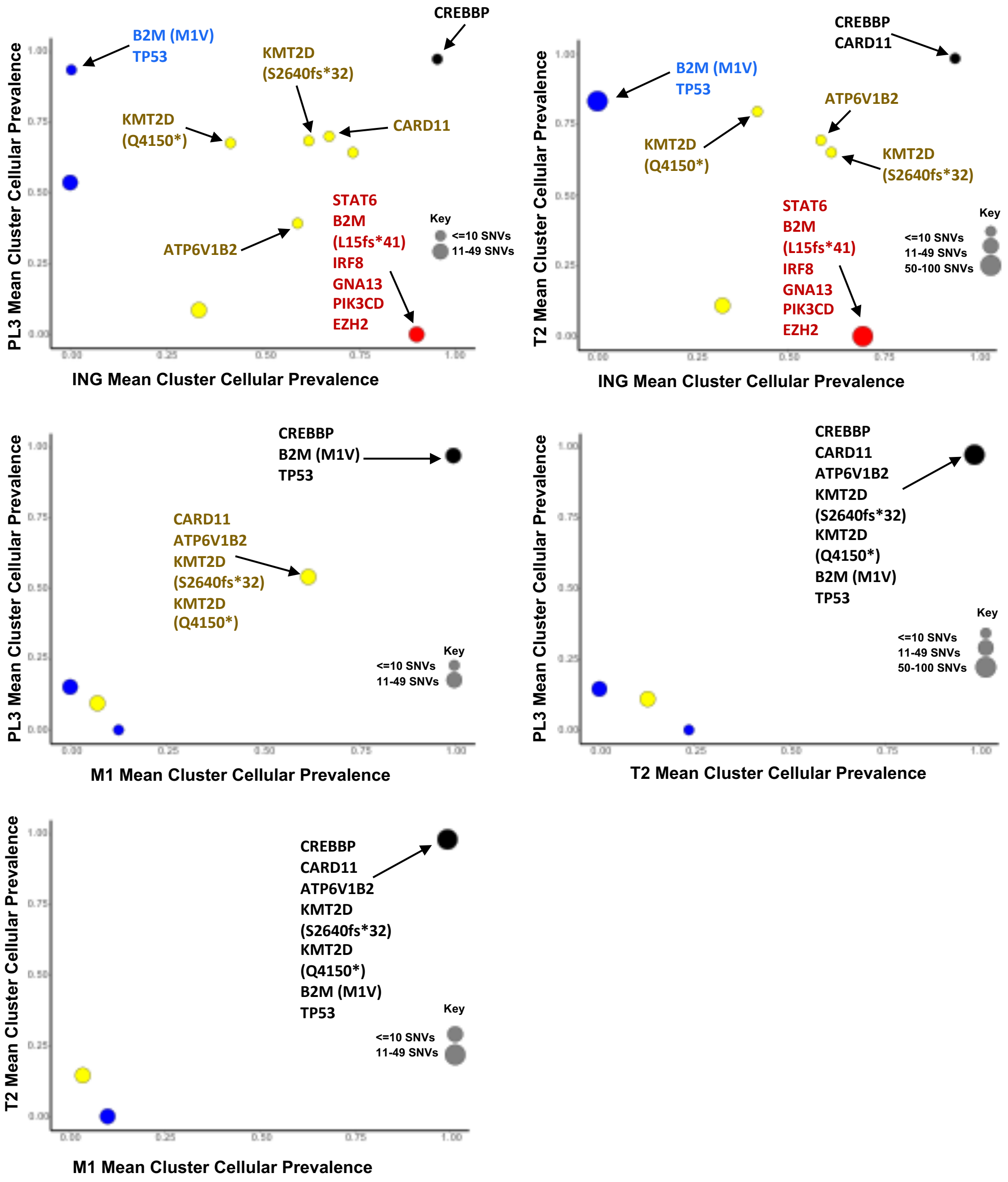
Supplementary Figure

Figure S1: Pairwise mean cluster cellular prevalence (MCCP) plots between the four biopsies (ING, M1, T2 and PL3). The greatest heterogeneity is observed between the inguinal tumor (ING) and supra-diaphragmatic sties (M1, T2, PL3) of disease. Derived mutation clusters represent the mean cellular prevalence of all mutations within a cluster. Each cluster is denoted by a circle with the size of the circle equivalent to the number of mutations within the cluster. The major clone is shown in black, shared subclones in yellow and site-specific clones in red (ING) and blue (M1, T2, PL3). Mutations in known DLBCL-associated genes are highlighted to show their locations within clusters.



Supplementary Tables

Table S1: Details of sequencing coverage and somatic variants

Sample	Mean Agilent exon coverage	% bases >= 10X coverage	Mclust estimated tumor purity	Non-synonymous variants	Total variants
ING	76.49	99.70%	26%	67	124
M1	65.77	99.50%	73%	59	121
T2	50.61	99.20%	52%	59	129
PL3	60.61	99.40%	77%	64	120

Table S2: List of all non-synonymous mutations in the 4 tumor samples

Chromosome	Position	Ref	Alt	Symbol	Gene	Transcript	aa_pos	aa_change	ING: Total number of reads	ING: VAF (%)	ING: Tumor purity corrected VAF (%)	M1: Total number of reads	M1: VAF (%)	M1: Tumor purity corrected VAF (%)	T2: Total number of reads	T2: VAF (%)	T2: Tumor purity corrected VAF (%)	PL3: Total number of reads	PL3: VAF (%)	PL3: Tumor purity corrected VAF (%)
chr14	21543659	G	A	ARHGEF40	ENSG00000165801	ENST00000555038	540	G>D	83	4.82%	18.54%	109	36.70%	50.27%	82	29.27%	56.29%	78	41.03%	53.29%
chr8	20074768	G	A	ATP6V1B2	ENSG00000147416	ENST00000276390	400	R>Q	49	10.20%	39.23%	63	33.33%	45.66%	64	32.81%	63.10%	53	16.98%	22.05%
chr7	2977606	T	C	CARD11	ENSG00000198286	ENST00000396946	360	M>V	64	14.06%	54.08%	92	60.87%	83.38%	75	44.00%	84.62%	82	48.78%	63.35%
chr7	148080821	C	A	CNTNAP2	ENSG00000174469	ENST00000361727	1186	Q>K	70	17.14%	65.92%	68	25.00%	34.25%	66	25.76%	49.54%	84	30.95%	40.19%
chr7	51132910	C	T	COBL	ENSG00000106078	ENST00000395540	445	A>T	53	13.21%	50.81%	76	2.63%	3.60%	47	10.64%	20.46%	65	3.08%	4.00%
chr16	3788618	G	A	CREBBP	ENSG00000005339	ENST00000262367	1446	R>C	41	51.22%	100.00%	52	86.54%	100.00%	34	76.47%	100.00%	43	86.05%	100.00%
chr8	88886005	A	T	DCAF4L2	ENSG00000176566	ENST00000319675	65	D>E	70	2.86%	11.00%	86	55.81%	76.45%	49	18.37%	35.33%	85	36.47%	47.36%
chr8	1626690	G	A	DLGAP2	ENSG00000198010	ENST00000520901	790	D>N	70	17.14%	65.92%	81	33.33%	45.66%	63	31.75%	61.06%	62	41.94%	54.47%
chr12	49432408	CACTT	C	KMT2D	ENSG00000167548	ENST00000301067	2911	TPA>TSA|TP	63	11.11%	42.73%	69	18.84%	25.81%	51	23.53%	45.25%	57	40.35%	52.40%
chr12	49426040	G	A	KMT2D	ENSG00000167548	ENST00000301067	4150	Q>*	56	5.36%	20.62%	69	47.83%	65.52%	55	41.82%	80.42%	44	36.36%	47.22%
chr14	75508332	C	A	MLH3	ENSG00000119684	ENST00000238662	1151	A>S	57	15.79%	60.73%	69	42.03%	57.58%	45	20.00%	38.46%	52	44.23%	57.44%
chr3	195506156	G	C	MUC4	ENSG00000145113	ENST00000478156	4099	L>V	144	7.64%	29.38%	140	2.86%	3.92%	111	4.50%	8.65%	167	5.39%	7.00%
chr1	66458731	C	T	PDE4B	ENSG00000184588	ENST00000423207	48	R>C	48	8.33%	32.04%	67	80.60%	100.00%	45	68.89%	100.00%	59	74.58%	96.86%
chr13	50054407	T	A	SETDB2	ENSG00000136169	ENST00000317257	320	L>M	71	30.99%	100.00%	68	76.47%	100.00%	45	62.22%	100.00%	43	76.74%	99.66%
chr22	31495073	G	A	SMTN	ENSG00000183963	ENST00000358743	787	V>M	100	12.00%	46.15%	55	32.73%	44.84%	50	32.00%	61.54%	43	48.84%	63.43%
chr20	46292275	T	C	SULF2	ENSG00000196562	ENST00000359930	717	N>D	70	15.71%	60.42%	63	39.68%	54.36%	32	25.00%	48.08%	53	39.62%	51.45%
chr19	55858734	G	A	SUV420H2	ENSG00000133247	ENST00000255613	436	A>T	246	15.45%	59.42%	81	38.27%	52.42%	62	16.13%	31.02%	99	29.29%	38.04%
chr1	152082539	C	T	TCHH	ENSG00000159450	ENST00000368804	1052	E>K	292	5.48%	21.08%	158	2.53%	3.47%	169	0.00%	0.00%	205	4.88%	6.34%
chr12	21995321	C	G	ABCC9	ENSG00000069431	ENST00000261200	1134	V>L	78	0.00%	0.00%	72	41.67%	57.08%	69	28.99%	55.75%	89	34.83%	45.23%
chr2	241907591	G	A	AC104809.3	ENSG00000226321	ENST00000443866	27	R>H	136	0.00%	0.00%	39	35.90%	49.18%	46	34.78%	66.88%	49	46.94%	60.96%
chr5	139876719	G	A	ANKHD1	ENSG00000131503	ENST00000421134	973	V>I	42	0.00%	0.00%	51	31.37%	42.97%	27	48.15%	92.60%	31	29.03%	37.70%
chr7	91977272	G	A	ANKIB1	ENSG00000001629	ENST00000413588	66	S>N	30	0.00%	0.00%	44	56.82%	77.84%	36	58.33%	100.00%	42	51.22%	66.52%
chr15	45003745	A	G	B2M	ENSG00000166710	ENST00000558401	1	M>V	31	0.00%	0.00%	50	76.00%	100.00%	33	54.55%	100.00%	30	73.33%	95.23%
chr2	36668555	A	C	CRIM1	ENSG00000150938	ENST00000280527	220	K>N	44	0.00%	0.00%	45	35.56%	48.71%	41	31.71%	60.98%	45	33.33%	43.29%
chr1	62958466	G	A	DOCK7	ENSG00000116641	ENST00000251157	1748	S>F	14	0.00%	0.00%	27	33.33%	45.66%	19	47.37%	91.10%	27	44.44%	57.71%
chr20	23966743	G	A	GGTLC1	ENSG00000149435	ENST00000286890	92	P>S	73	0.00%	0.00%	48	41.67%	57.08%	46	15.22%	29.27%	46	36.96%	48.00%
chr7	819622	G	A	HEATR2	ENSG00000164818	ENST00000297440	758	D>N	50	0.00%	0.00%	89	33.71%	46.18%	62	11.29%	21.71%	94	24.47%	31.78%
chr3	120393784	G	A	HGD	ENSG00000113924	ENST00000283871	47	S>L	34	0.00%	0.00%	50	38.00%	52.05%	33	30.30%	58.27%	34	32.35%	42.01%
chr16	47347718	C	G	ITFG1	ENSG00000129636	ENST00000320640	273	M>I	24	0.00%	0.00%	48	25.00%	34.25%	28	35.71%	68.67%	36	38.89%	50.51%
chr9	24544231	G	T	IZUMO3	ENSG00000205442	ENST00000543880	153	T>N	102	0.00%	0.00%	89	34.83%	47.71%	67	26.87%	51.67%	93	43.01%	55.86%
chr9	12821596	A	G	LURAP1L	ENSG00000153714	ENST00000319264	175	D>G	33	0.00%	0.00%	27	25.93%	35.52%	32	25.00%	48.08%	25	40.00%	51.95%
chr5	162945286	C	T	MAT2B	ENSG00000038274	ENST00000321757	308	R>*	52	0.00%	0.00%	28	35.71%	48.92%	29	44.83%	86.21%	48	27.08%	35.17%
chr13	29600513	G	A	MTUS2	ENSG00000132938	ENST00000431530	570	V>M	70	0.00%	0.00%	82	46.34%	63.48%	64	32.81%	63.10%	66	42.42%	55.09%
chr12	55846005	A	C	OR6C2	ENSG00000179695	ENST00000322678	3	N>T	44	0.00%	0.00%	56	35.71%	48.92%	50	38.00%	73.08%	43	30.23%	39.26%
chr7	24856263	C	T	OSBPL3	ENSG00000070882	ENST00000313367	665	V>I	57	0.00%	0.00%	89	21.35%	29.25%	66	16.67%	32.06%	89	28.09%	36.48%
chr11	66636387	C	G	PC	ENSG00000173599	ENST00000393955	318	G>R	26	0.00%	0.00%	52	25.00%	34.25%	50	4.00%	7.69%	52	15.38%	19.97%
chr12	20806966	T	C	PDE3A	ENSG00000172572	ENST00000359062	1004	F>S	67	0.00%	0.00%	34	44.12%	60.44%	36	27.78%	53.42%	49	44.90%	58.31%
chr4	55155279	A	G	PDGFRA	ENSG00000134853	ENST00000257290	960	K>E	40	0.00%	0.00%	55	40.00%	54.79%	34	35.29%	67.87%	46	50.00%	64.94%
chr12	41967356	G	A	PDZRN4	ENSG00000165966	ENST00000402685	925	M>I	74	0.00%	0.00%	60	33.33%	45.66%	52	23.08%	44.38%	86	38.37%	49.83%
chr6	4032953	G	A	PRPF4B	ENSG00000112739	ENST00000480058	401	R>Q	78	0.00%	0.00%	132	17.42%	23.86%	96	21.88%	42.08%	110	30.00%	38.96%
chr3	138762937	C	T	PRR23C	ENSG00000233701	ENST00000413199	176	A>T	115	0.00%	0.00%	46	34.78%	47.64%	68	47.06%	90.50%	69	30.43%	39.52%
chr12	15262141	C	T	RERG	ENSG00000134533	ENST00000256953	168	R>H	39	0.00%	0.00%	58	44.83%	61.41%	36	44.44%	85.46%	51	54.90%	71.30%
chr8	10584126	C	T	SOX7	ENSG00000171056	ENST00000553390	149	E>K	150	0.00%	0.00%	67	56.72%	77.70%	51	31.37%	60.33%	72	26.39%	34.27%
chr17	36705377	C	A	SRCIN1	ENSG00000017373	ENST00000578925	1045	R>L	54	0.00%	0.00%	42	42.86%	58.71%	45	44.44%	85.46%	56	37.50%	48.70%
chr16	77246055	T	C	SYCE1L	ENSG00000205078	ENST00000378644	151	I>T	114	0.00%	0.00%	52	36.54%	50.05%	45	24.44%	47.00%	44	29.55%	38.38%
chr9	27213585	A	G	TEK	ENSG00000120156	ENST00000380036	994	K>R	37	0.00%	0.00%	51	39.22%	53.73%	30	30.00%	57.69%	43	27.91%	36.25%
chr17	7578290	CTAAGAG	C	TP53	ENSG00000141510	ENST00000413465	0		113	0.00%	0.00%	43	41.86%	57.34%	32	18.75%	36.06%	38	60.53%	78.61%
chr10	105923866	C	T	WDR96	ENSG00000197748	ENST00000428666	1079	V>M	54	0.00%	0.00%	53	47.17%	64.62%	33	24.24%	46.62%	54	40.74%	52.91%
chr5	167891759	C	G	WWC1	ENSG00000113645	ENST00000521089	987	S>C	46	0.00%	0.00%	47	31.91%	43.71%	43	32.56%	62.62%	41	41.46%	53.84%
chr7	148767732	T	C	ZNF786	ENSG00000197362	ENST00000491431	711	K>R	37	0.00%	0.00%	85	61.18%	83.81%	64	48.44%	93.15%	67	56.72%	73.66%
chr18	9887212	A	G	TXNDC2	ENSG00000168454	ENST00000306084	246	T>A	94	7.45%	28.65%	177	2.26%	3.10%	115	3.48%	6.69%	166	0.00%	0.00%
chr18	9887185	A	G	TXNDC2	ENSG00000168454	ENST00000306084	237	S>G	95	7.37%	28.35%	175	4.00%	5.48%	115	3.48%	6.69%	165	0.00%	0.00%
chr1	152327620	T	G	FLG2	ENSG00000143520	ENST00000388718	881	Y>S	56	7.14%	27.46%	80	10.00%	13.70%	49	0.00%	0.00%	46	4.35%	5.65%
chr16	88800398	G	C	PIEZO1	ENSG00000103335	ENST00000301015	749	Q>E	179	4.47%	17.19%	84	2.38%	3.26%	60	0.00%	0.00%	78	3.85%	5.00%
chr17	74288502	T	C	QRICH2	ENSG00000129646	ENST00000262765	603	H>R	52	3.85%	14.81%	68	4.41%	6.04%	39	0.00%	0.00%	48	14.58%	18.94%
chr7	141920186	G	A	RP11-1220K	ENSG00000257743	ENST00000477922	1959	D>N	93	3.23%	12.42%	140	4.29%	5.88%	103	0.00%	0.00%	140	7.14%	9.27%
chr19	21991811	G	T	ZNF43	ENSG00000198521	ENST00000354959	343	T>K	104	6.73%	25.88%	103	2.91%	3.99%	78	0.00%	0.00%	100	2.00%	2.60%

chr8	10466095	G	T	RP1L1	ENSG00000183638	ENST00000382483	1838	P>Q	138	3.62%	13.92%	105	0.00%	0.00%	115	2.61%	5.02%	121	7.44%	9.66%
chr7	100350456	C	A	ZAN	ENSG00000146839	ENST00000349350	910	P>T	97	4.12%	15.85%	132	0.00%	0.00%	73	8.22%	15.81%	107	10.28%	13.35%
chr7	100350298	C	T	ZAN	ENSG00000146839	ENST00000349350	857	P>L	148	6.76%	26.00%	118	0.00%	0.00%	96	4.17%	8.02%	161	4.35%	5.65%
chr9	97063263	A	G	ZNF169	ENSG00000175787	ENST00000395395	475	R>G	69	2.90%	11.15%	71	0.00%	0.00%	55	5.45%	10.48%	66	9.09%	11.81%
chrX	49126548	TGGC	T	PPP1R3F	ENSG00000049769	ENST00000055335	72	DG>DG	144	2.78%	10.69%	27	0.00%	0.00%	35	0.00%	0.00%	28	10.71%	13.91%
chr1	152082565	A	C	TCHH	ENSG00000159450	ENST00000368804	1043	L>R	270	4.81%	18.50%	157	0.00%	0.00%	161	0.00%	0.00%	199	4.52%	5.87%
chr1	152082544	T	C	TCHH	ENSG00000159450	ENST00000368804	1050	Y>C	292	0.00%	0.00%	162	3.09%	4.23%	164	4.88%	9.38%	202	0.00%	0.00%
chr9	133759773	C	T	ABL1	ENSG00000097007	ENST00000372348	718	A>V	157	0.00%	0.00%	54	0.00%	0.00%	53	0.00%	0.00%	91	0.00%	0.00%
chr4	73186481	T	C	ADAMTS3	ENSG00000156140	ENST00000286657	351	D>G	43	13.95%	53.65%	64	0.00%	0.00%	40	0.00%	0.00%	64	0.00%	0.00%
chr15	45003780	ACT	A	B2M	ENSG00000166710	ENST00000558401	12	LL>LL	66	22.39%	86.12%	72	0.00%	0.00%	58	0.00%	0.00%	64	0.00%	0.00%
chr14	99642124	C	T	BCL11B	ENSG00000127152	ENST00000357195	350	R>H	206	14.56%	56.00%	51	0.00%	0.00%	48	0.00%	0.00%	66	0.00%	0.00%
chr21	40568609	T	C	BRWD1	ENSG00000185658	ENST00000333229	2129	K>R	125	17.60%	67.69%	205	0.00%	0.00%	151	0.00%	0.00%	172	0.00%	0.00%
chr3	49701092	C	T	BSN	ENSG00000164061	ENST00000296452	3834	A>V	51	0.00%	0.00%	20	0.00%	0.00%	20	0.00%	0.00%	25	0.00%	0.00%
chr17	34190040	G	A	C17orf66	ENSG00000172653	ENST00000311880	239	R>*	53	20.75%	79.81%	58	0.00%	0.00%	52	0.00%	0.00%	68	0.00%	0.00%
chr8	113267633	T	C	CSMD3	ENSG00000164796	ENST00000297405	3296	I>V	57	14.04%	54.00%	65	0.00%	0.00%	59	0.00%	0.00%	83	0.00%	0.00%
chr17	76778289	C	A	CYTH1	ENSG00000108669	ENST00000591455	6	S>I	87	26.44%	100.00%	18	0.00%	0.00%	20	0.00%	0.00%	34	0.00%	0.00%
chr1	162748502	C	T	DDR2	ENSG00000162733	ENST00000367922	806	R>*	30	16.67%	64.12%	34	0.00%	0.00%	31	0.00%	0.00%	26	0.00%	0.00%
chr19	19038786	GC	G	DDX49	ENSG00000105671	ENST00000247003	402	A>A	220	14.09%	54.19%	167	0.00%	0.00%	109	0.00%	0.00%	148	0.00%	0.00%
chr7	21583012	C	T	DNAH11	ENSG00000105877	ENST00000409508	50	A>V	269	20.07%	77.19%	200	0.00%	0.00%	160	0.00%	0.00%	230	0.00%	0.00%
chr16	67698949	G	A	ENKD1	ENSG00000124074	ENST00000243878	135	R>C	83	25.30%	97.31%	53	0.00%	0.00%	55	0.00%	0.00%	54	0.00%	0.00%
chr7	148508728	A	T	EZH2	ENSG00000106462	ENST00000320356	646	Y>N	49	14.29%	54.96%	88	0.00%	0.00%	55	0.00%	0.00%	74	0.00%	0.00%
chr17	63010778	A	G	GNA13	ENSG00000120063	ENST00000439174	244	L>P	51	25.49%	98.04%	95	0.00%	0.00%	51	0.00%	0.00%	80	0.00%	0.00%
chr16	85954874	C	T	IRF8	ENSG00000140968	ENST00000268638	423	Q>*	47	14.89%	57.27%	89	0.00%	0.00%	41	0.00%	0.00%	65	0.00%	0.00%
chr17	38936037	G	A	KRT27	ENSG00000171446	ENST00000301656	254	T>M	52	19.23%	73.96%	45	0.00%	0.00%	48	0.00%	0.00%	66	0.00%	0.00%
chr7	141776550	C	T	MGAM	ENSG00000257335	ENST00000475668	1806	P>L	38	18.42%	70.85%	81	0.00%	0.00%	57	0.00%	0.00%	78	0.00%	0.00%
chr7	100683623	A	G	MUC17	ENSG00000169876	ENST00000306151	2976	S>G	67	11.94%	45.92%	121	0.00%	0.00%	81	0.00%	0.00%	86	0.00%	0.00%
chr3	195511262	C	G	MUC4	ENSG00000145113	ENST00000478156	2397	D>H	258	0.00%	0.00%	328	0.00%	0.00%	295	0.00%	0.00%	321	0.00%	0.00%
chr4	164271649	C	T	NPY5R	ENSG00000164129	ENST00000338566	75	T>M	48	14.58%	56.08%	52	0.00%	0.00%	44	0.00%	0.00%	47	0.00%	0.00%
chr12	106461671	C	G	NUAK1	ENSG00000074590	ENST00000261402	299	D>H	31	16.13%	62.04%	47	0.00%	0.00%	25	0.00%	0.00%	38	0.00%	0.00%
chr17	45885704	G	A	OSBPL7	ENSG00000006025	ENST00000007414	828	R>W	38	15.79%	60.73%	40	0.00%	0.00%	27	0.00%	0.00%	24	0.00%	0.00%
chr1	9780849	A	C	PIK3CD	ENSG00000171608	ENST00000361110	548	Y>S	163	15.34%	59.00%	92	0.00%	0.00%	73	0.00%	0.00%	95	0.00%	0.00%
chr1	2433816	C	T	PLCH2	ENSG00000149527	ENST00000419816	976	A>V	140	12.86%	49.46%	35	0.00%	0.00%	33	0.00%	0.00%	50	0.00%	0.00%
chr12	106853132	A	T	POLR3B	ENSG00000013503	ENST00000228347	852	K>N	56	10.71%	41.19%	50	0.00%	0.00%	40	0.00%	0.00%	57	0.00%	0.00%
chr5	149213146	G	T	PPARGC1B	ENSG00000155846	ENST00000394320	504	G>C	73	0.00%	0.00%	61	0.00%	0.00%	49	0.00%	0.00%	56	0.00%	0.00%
chr9	123949229	A	G	RAB14	ENSG00000119396	ENST00000373840	0		36	19.44%	74.77%	57	0.00%	0.00%	47	0.00%	0.00%	46	0.00%	0.00%
chr13	48919300	T	A	RB1	ENSG00000139687	ENST00000267163	155	Y>*	54	24.07%	92.58%	48	0.00%	0.00%	33	0.00%	0.00%	46	0.00%	0.00%
chr3	16475380	T	C	RFTN1	ENSG00000131378	ENST00000334133	104	I>V	63	19.05%	73.27%	70	0.00%	0.00%	42	0.00%	0.00%	40	0.00%	0.00%
chr13	37394095	G	C	RFXAP	ENSG00000133111	ENST00000255476	0		68	27.94%	100.00%	37	0.00%	0.00%	42	0.00%	0.00%	32	0.00%	0.00%
chr1	237870406	G	T	RYR2	ENSG00000198626	ENST00000366574	3246	M>I	23	26.09%	100.00%	15	0.00%	0.00%	30	0.00%	0.00%	32	0.00%	0.00%
chr6	25665246	G	A	SCGN	ENSG00000079689	ENST00000377961	108	V>M	53	13.21%	50.81%	87	0.00%	0.00%	55	0.00%	0.00%	91	0.00%	0.00%
chr5	54945062	A	T	SLC38A9	ENSG00000177058	ENST00000396865	345	F>I	47	19.15%	73.65%	47	0.00%	0.00%	27	0.00%	0.00%	48	0.00%	0.00%
chr17	74732959	G	T	SRSF2	ENSG00000161547	ENST00000392485	95	P>H	123	15.45%	59.42%	53	0.00%	0.00%	56	0.00%	0.00%	68	0.00%	0.00%
chr12	57498345	C	T	STAT6	ENSG00000166888	ENST00000300134	372	E>K	84	25.00%	96.15%	79	0.00%	0.00%	63	0.00%	0.00%	70	0.00%	0.00%
chr1	43903336	C	T	SZT2	ENSG00000198198	ENST00000562955	2064	R>*	73	0.00%	0.00%	86	0.00%	0.00%	83	0.00%	0.00%	57	0.00%	0.00%
chr4	949650	G	A	TMEM175	ENSG00000127419	ENST00000264771	272	V>M	71	11.27%	43.35%	53	0.00%	0.00%	33	0.00%	0.00%	49	0.00%	0.00%
chr9	74360268	C	T	TMEM2	ENSG00000135048	ENST00000377044	234	G>R	53	13.21%	50.81%	57	0.00%	0.00%	47	0.00%	0.00%	58	0.00%	0.00%
chr22	37494541	C	T	TMPRSS6	ENSG00000187045	ENST00000346753	93	R>H	55	16.36%	62.92%	65	0.00%	0.00%	42	0.00%	0.00%	45	0.00%	0.00%
chr4	6302546	G	A	WFS1	ENSG00000109501	ENST00000503569	342	A>T	135	0.00%	0.00%	106	0.00%	0.00%	118	0.00%	0.00%	120	0.00%	0.00%
chr7	64167004	G	C	ZNF107	ENSG00000196247	ENST00000344930	108	E>Q	45	15.56%	59.85%	92	0.00%	0.00%	88	0.00%	0.00%	86	0.00%	0.00%
chr19	24310331	T	G	ZNF254	ENSG00000213096	ENST00000357002	510	I>R	97	0.00%	0.00%	105	0.00%	0.00%	92	0.00%	0.00%	97	0.00%	0.00%
chr1	227842961	G	C	ZNF678	ENSG00000181450	ENST00000397097	392	S>T	119	0.00%	0.00%	144	0.00%	0.00%	110	0.00%	0.00%	113	0.00%	0.00%
chr1	233750026	G	A	KCNK1	ENSG00000135750	ENST00000366621	37	G>S	87	0.00%	0.00%	36	13.89%	19.03%	49	0.00%	0.00%	65	0.00%	0.00%
chr2	98834332	G	T	VWA3B	ENSG00000168658	ENST00000477737	620	Q>H	93	0.00%	0.00%	96	0.00%	0.00%	70	0.00%	0.00%	70	0.00%	0.00%
chr7	4830463	G	A	AP5Z1	ENSG00000242802	ENST00000348624	700	V>M	28	0.00%	0.00%	38	0.00%	0.00%	40	12.50%	24.04%	42	0.00%	0.00%
chrX	114468376	C	T	LRCH2	ENSG00000130224	ENST00000317135	77	V>M	110	0.00%	0.00%	34	0.00%	0.00%	37	13.51%	25.98%	55	0.00%	0.00%
chr4	100570909	G	A	RP11-766F14	ENSG00000248713	ENST00000511828	1633	R>W	35	0.00%	0.00%	37	0.00%	0.00%	34	11.76%	22.62%	23	0.00%	0.00%
chr12	88532963	GA	G	CEP290	ENSG00000198707	ENST00000552810	86	K*>NQ|NK	93	0.00%	0.00%	90	0.00%	0.00%	60	0.00%	0.00%	71	15.49%	20.12%
chr3	37340806	C	CAT	GOLGA4	ENSG00000144674	ENST00000356847	366	L>H	59	0.00%	0.00%	36	0.00%	0.00%	28	0.00%	0.00%	27	22.22%	28.86%
chr17	48823233	AAGGGCT	A	LUC7L3	ENSG00000108848	ENST00000505658	282	ERA>ERD	200	0.00%	0.00%	145	0.00%	0.00%	128	0.00%	0.00%	155	0.00%	0.00%
chr4	41748010	CGCCGCC	C	PHOX2B	ENSG00000109132	ENST00000226382	253	LPTSPVW>LA	151	0.00%	0.00%	48	0.00%	0.00%	38	0.00%	0.00%	71	0.00%	0.00%
chr17	20910297	A	G	USP22	ENSG00000124422	ENST00000261497	412	F>L	72	0.00%	0.00%	36	0.00%	0.00%	56	0.00%	0.00%	39	15.38%	19.97%
chr3	42955888	TCATGGT	T	ZNF662	ENSG00000182983	ENST00000328199	134	LMV>LYQ	78	0.00%	0.00%	57	0.00%	0.00%	51	0.00%	0.00%	43	0.00%	0.00%