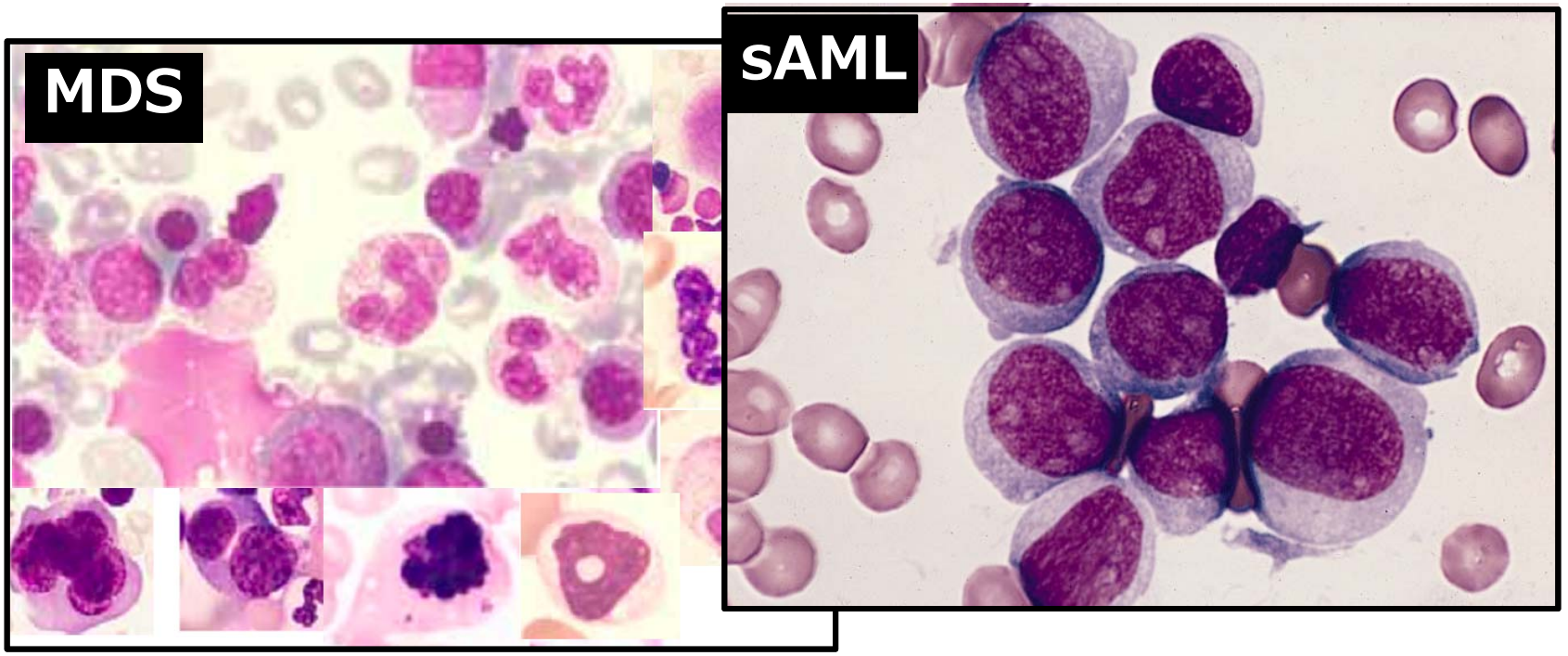
Illumina Webinar

骨髄異形成症候群の分子プロファイリング

京都大学
KYOTO UNIVERSITY

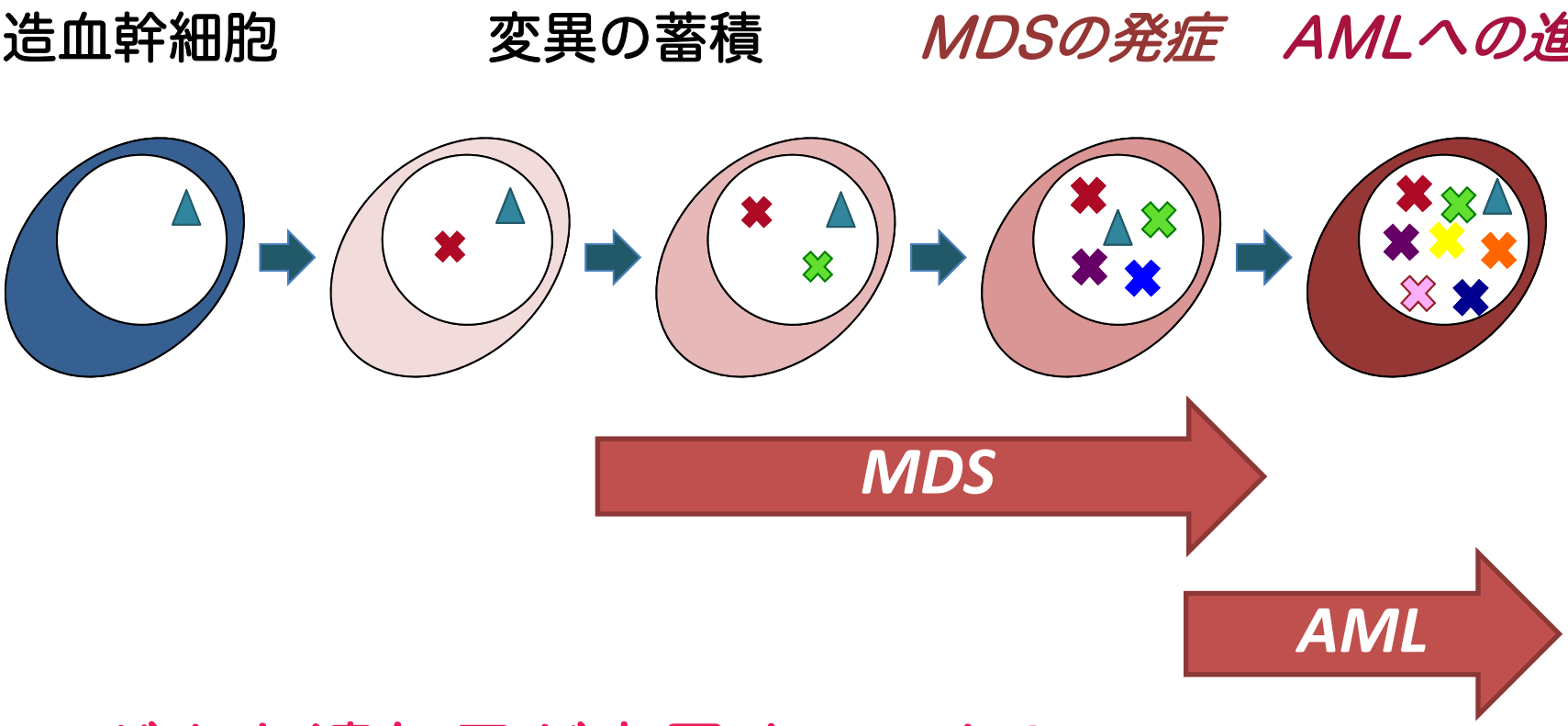
腫瘍生物学講座
小川誠司

骨髄異形成症候群(MDS)



- 代表的な造血器腫瘍(血液のがん)のひとつ。骨髄の異形成を伴った血球減少と急性骨髄性白血病(AML)への移行が特徴。
- 日本国内だけで数万人が罹患、年間5千～1万人以上が新規に発症。
- 高齢者がほとんどを占め(90%以上が60歳以上!)
- 人口の高齢化に伴い近年増加傾向。
- 骨髄移植(60歳以下)以外に根治的治療法がない!!

MDSは遺伝子変異によって発症する



1. どんな遺伝子が変異するのか?
2. それらの遺伝子異常によってなぜMDSになるか?

MDSにおける遺伝子変異

A point mutation at codon 13 of the N-*ras* oncogene in myelodysplastic syndrome

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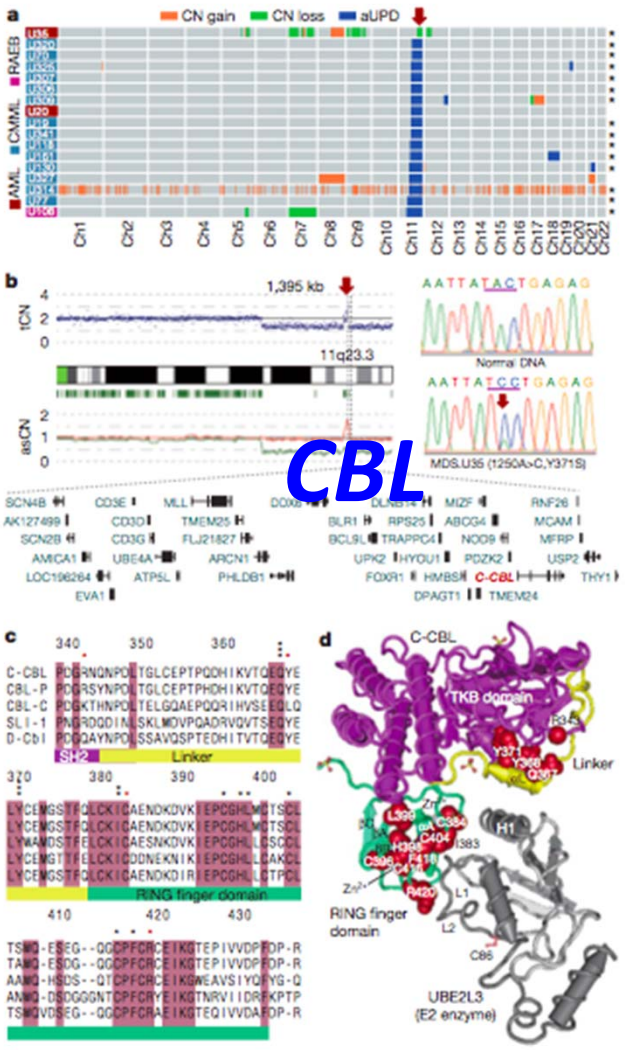
Nature 327:430-432, 1987

Mutations of the p53 Gene in Myelodysplastic Syndrome (MDS) and MDS-Derived Leukemia

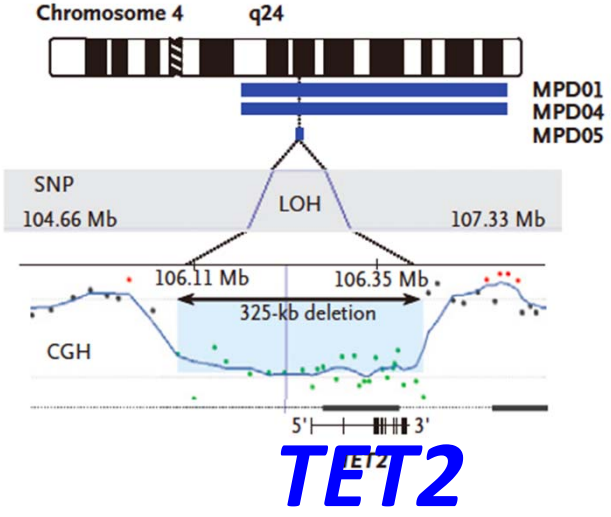
By Koichi Sugimoto, Naoto Hirano, Hideo Toyoshima, Shigeru Chiba, Hiroyuki Mano, Fumimaro Takaku, Yoshio Yazaki, and Hisamaru Hirai

Blood 81: 3022-3026, 1993

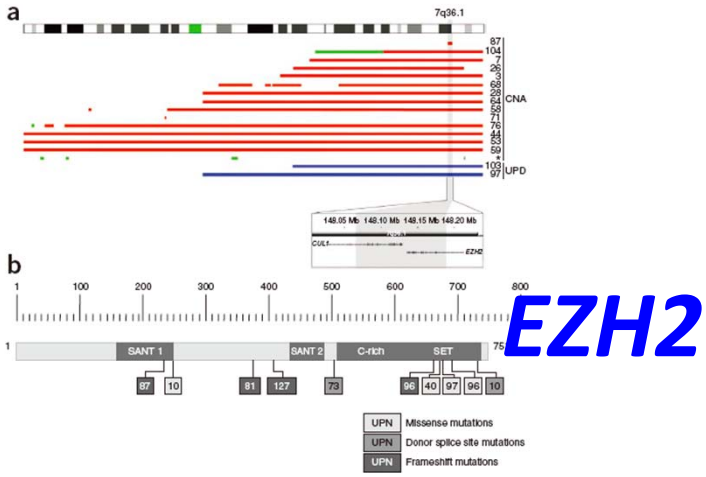
ゲノムワイドなコピー数解析による新規変異の同定



Sanada M, et al., Nature, 2009

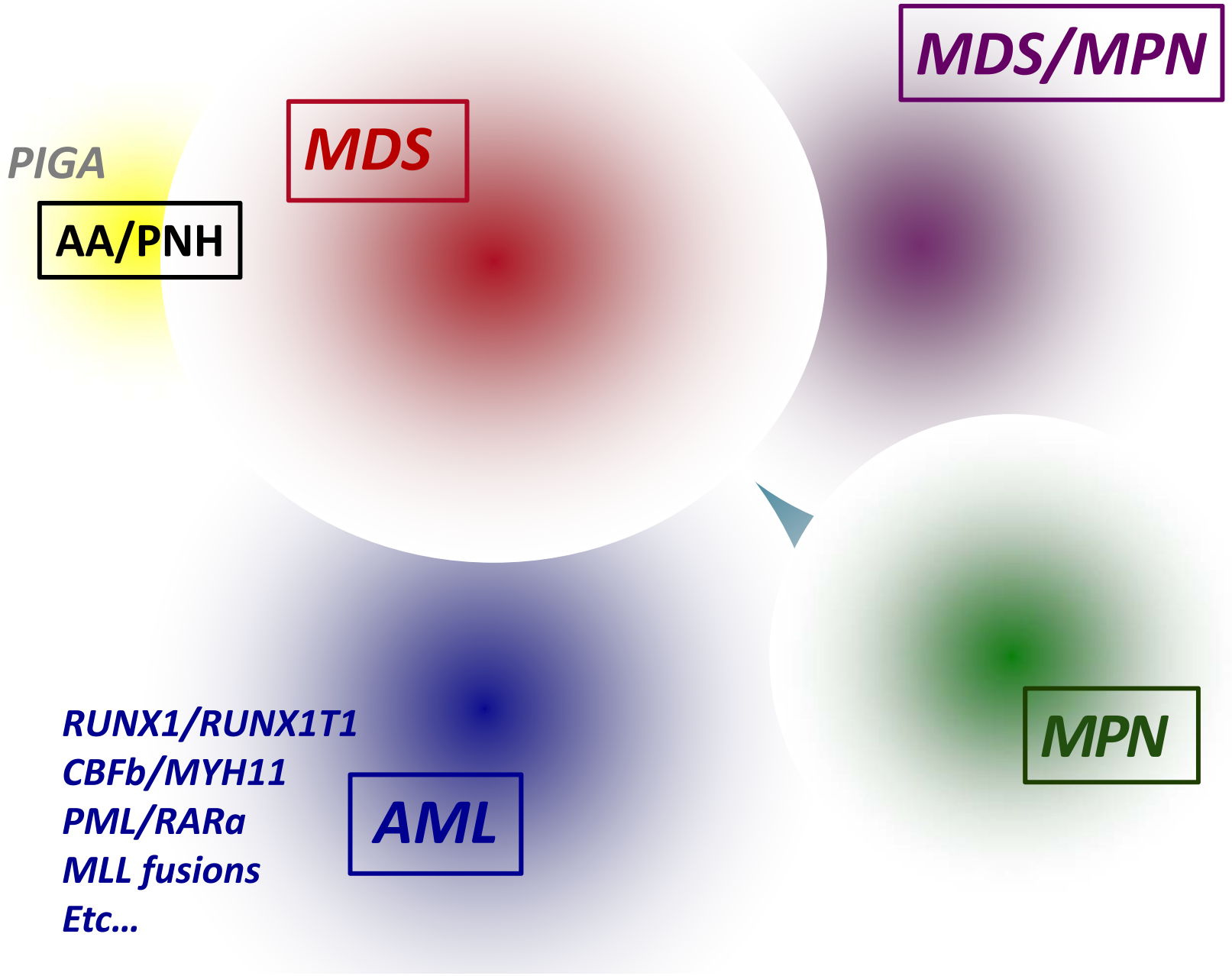


Delhommeau F, NEJM, 2009



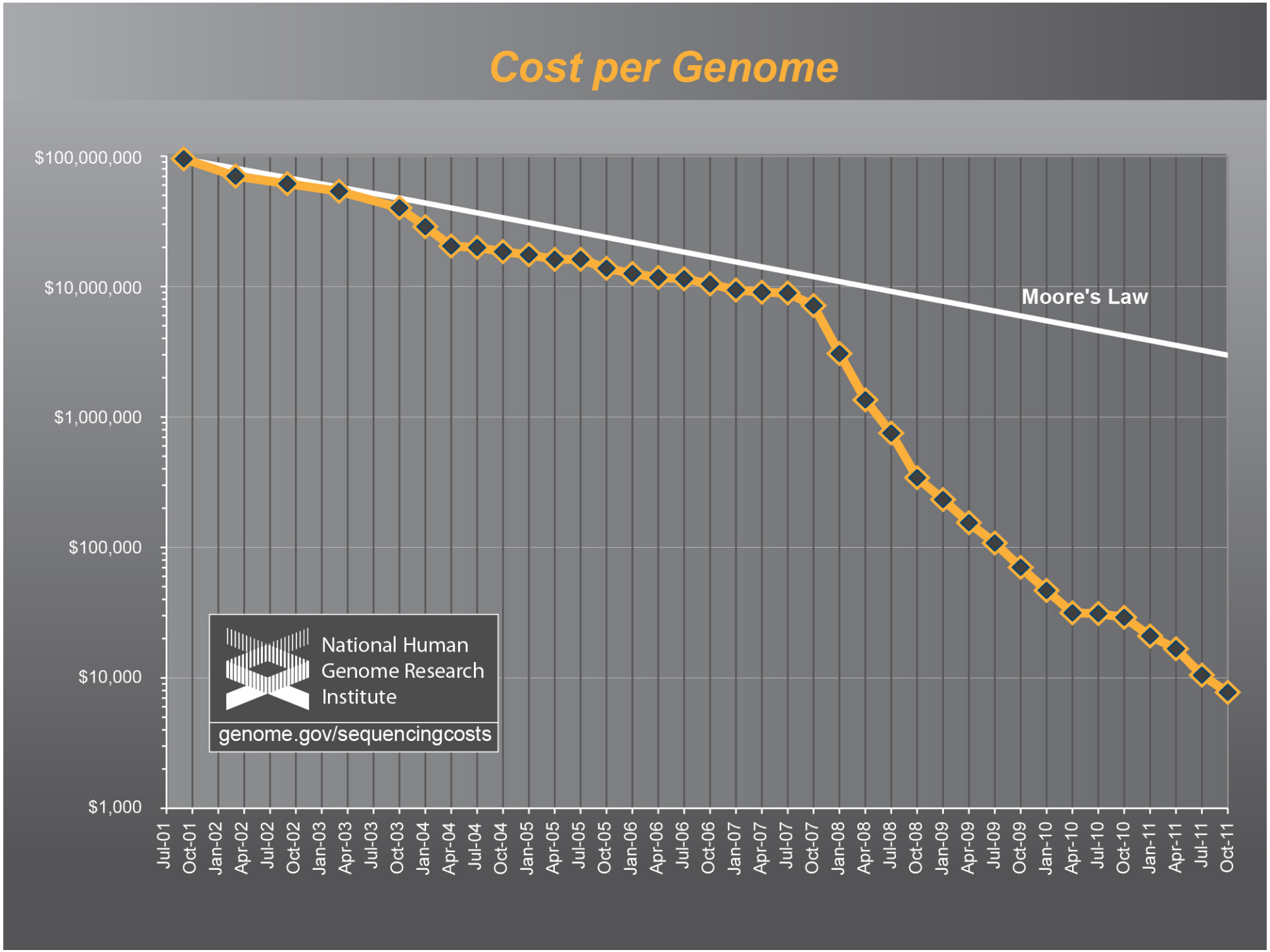
Nikoloski G, et al., Nat Genet, 2010

Landscape of gene mutations in MDS 2010

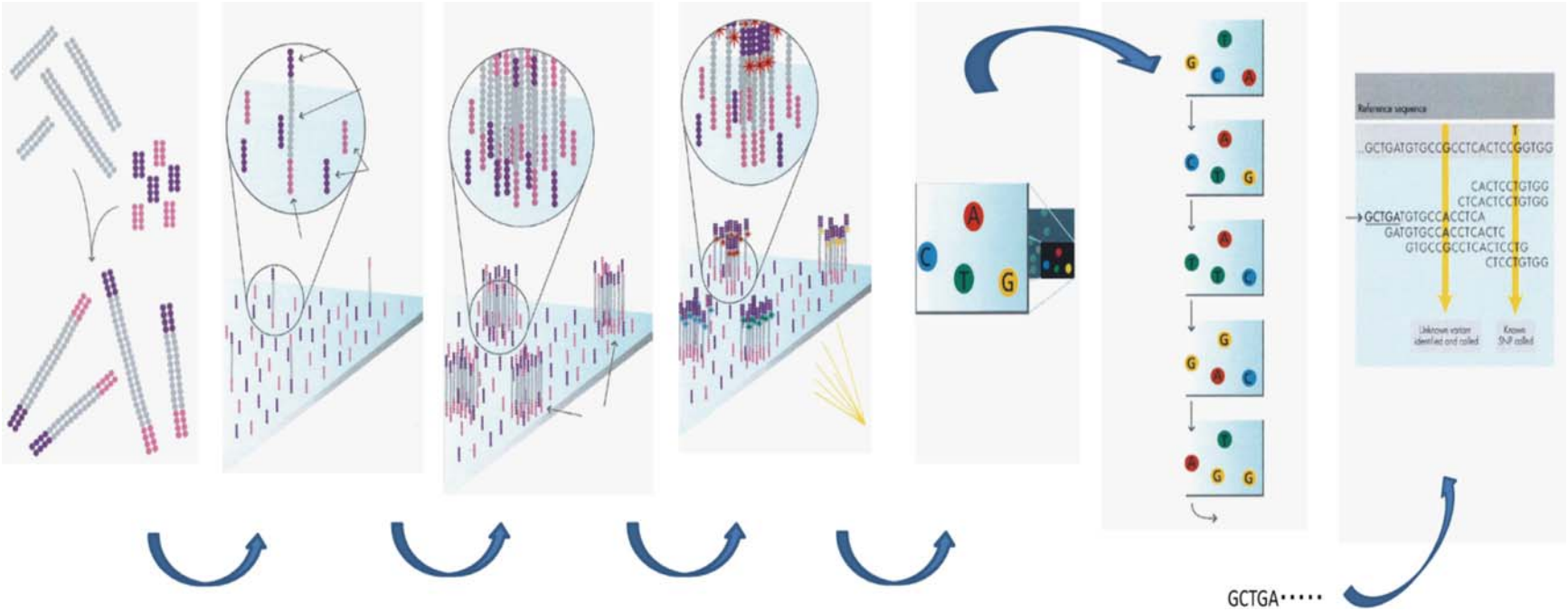


Genomic Profile of 222 cases with myelodysplasia

Cost per Genome

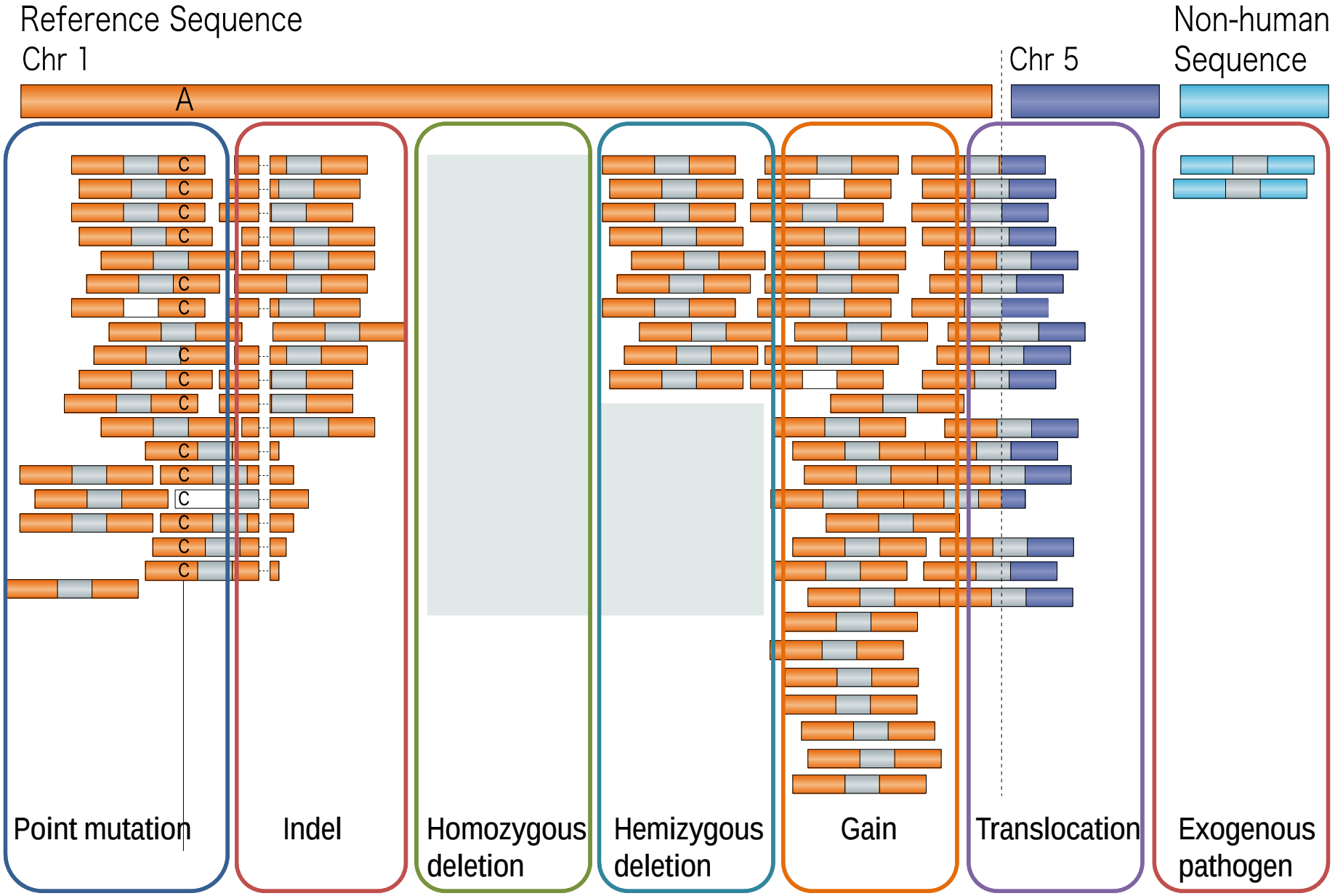


Massively parallel sequencing



	GAIIx	HiSeq 2000
Cluster density	800K	750K~850K
Area	200	2188.8mm2
Read length	100~150 x2	100 x 2
Total reads	~72Gbp	>600Gbp ->～ 1Tbp

全ゲノムシーケンスによるがんゲノム解析



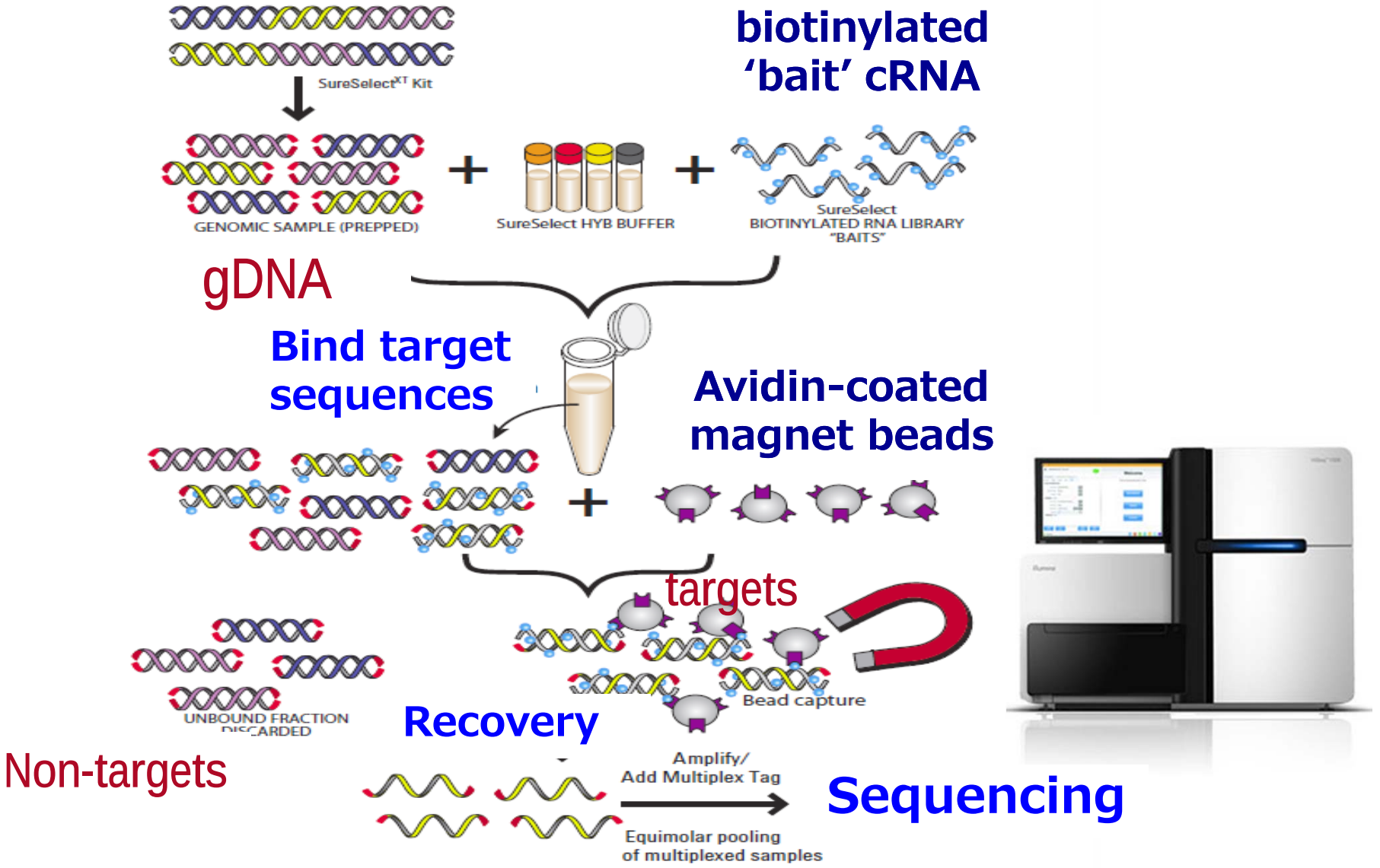
NATURE REVIEWS | GENETICS 2010

(M. Meyerson, Nat Genet, 2010)

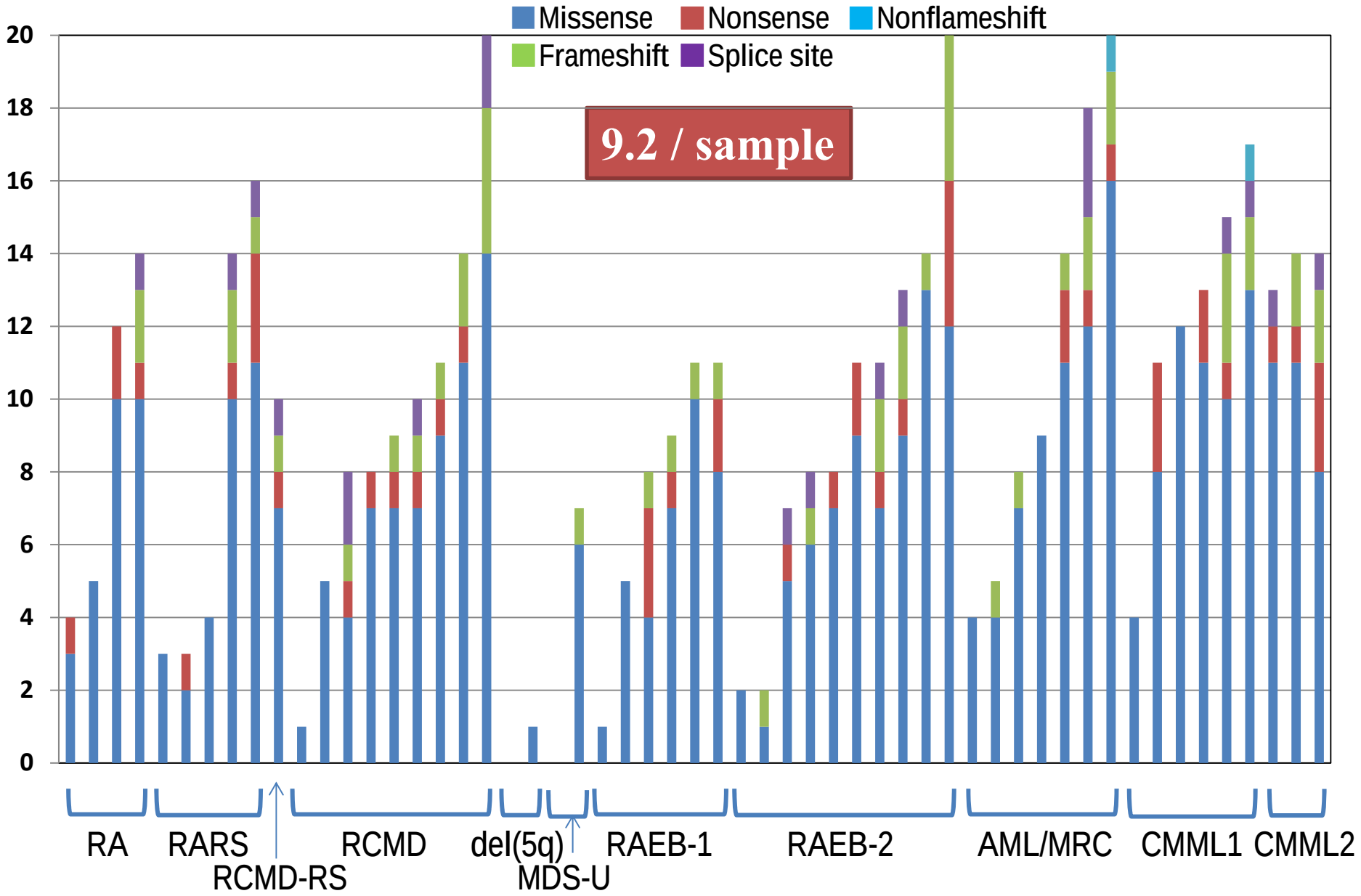
MDSの全エクソン解析

WHO Classification	N
RA	5
RARS	5
RCMD	9
RCMD-RS	1
del(5q)	2
MDS-U	1
RAEB-1	7
RAEB-2	10
AML/MRC	7
CMML1	6
CMML2	3
Total	56

Whole exome capture and sequencing

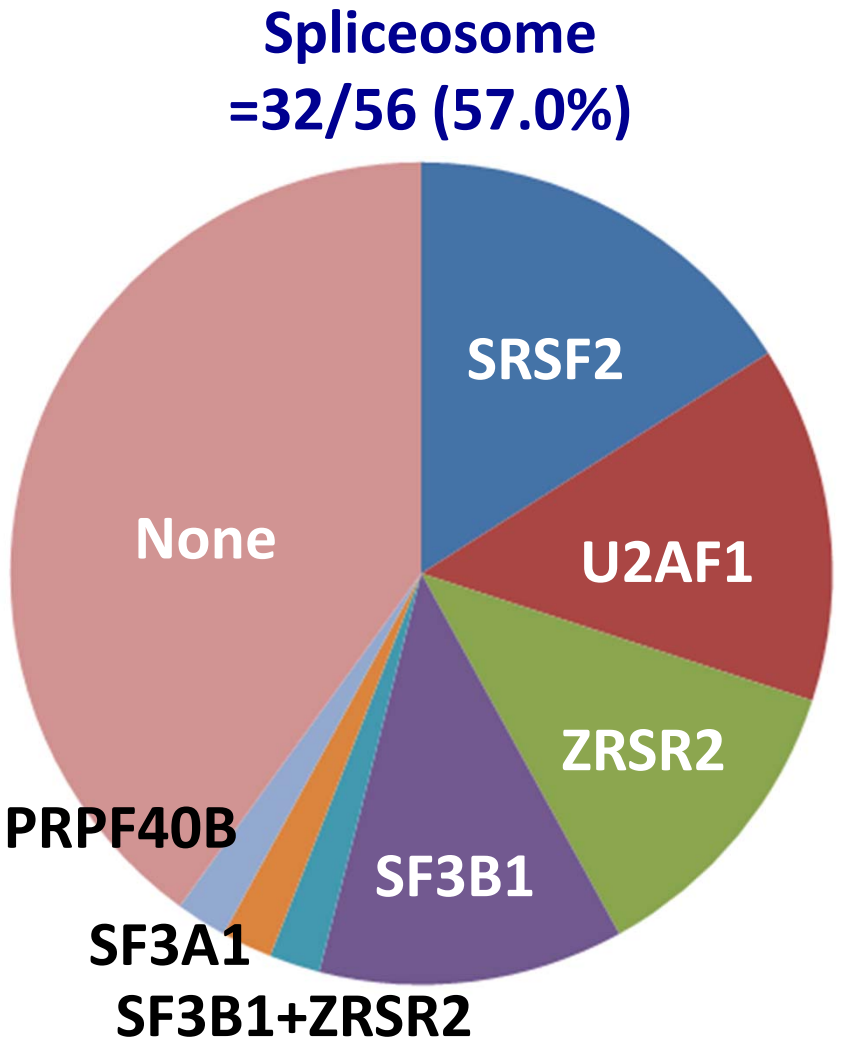


Number of somatic mutations / sample

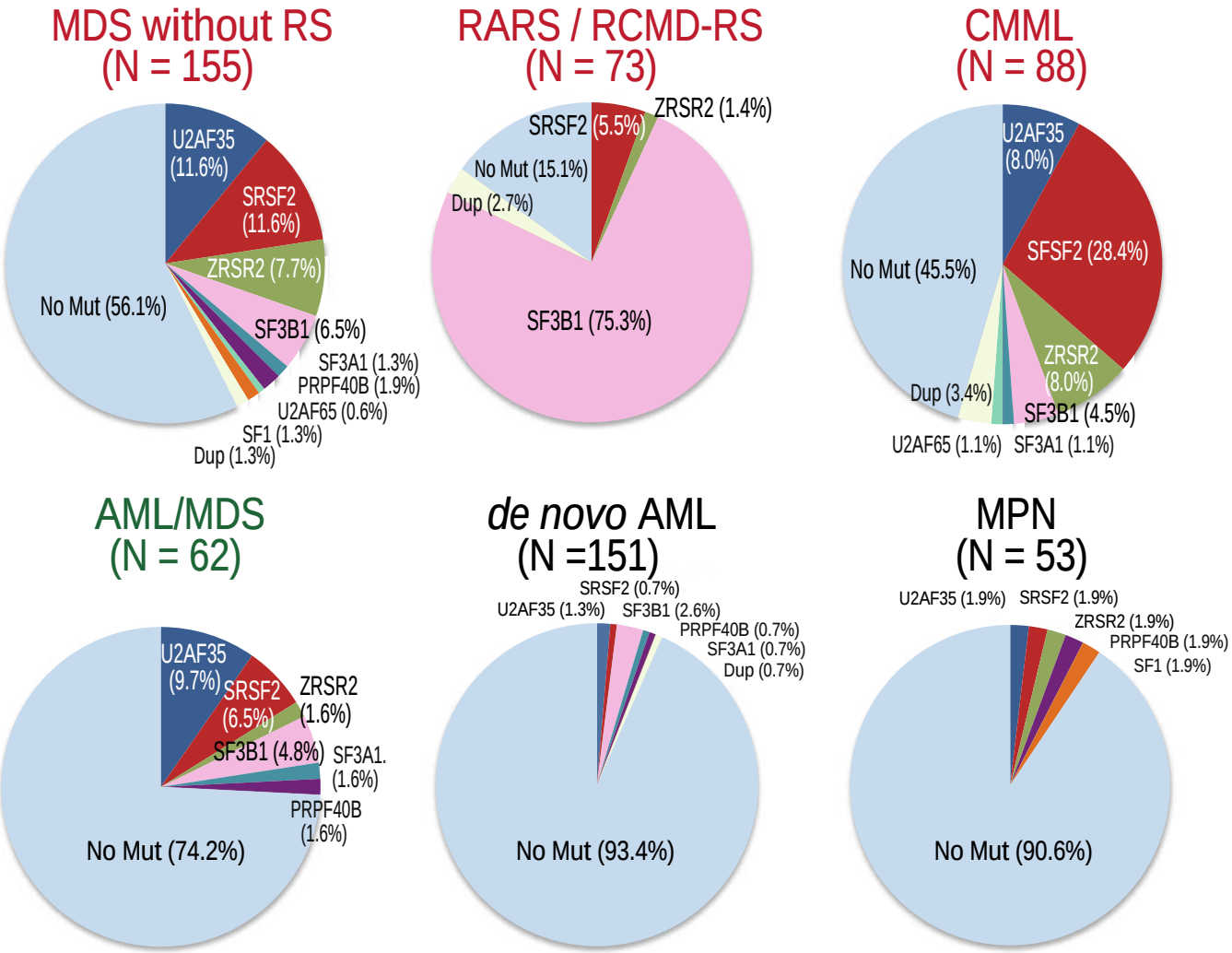


MDSにおけるドライバー変異

	Gene			Gene	
1	*TET2*	13	14	*STAG2*	3
2	*SRSF2*	9	15	*IDH1/2*	2
3	*U2AF1*	7	16	*PHF6*	2
4	*ZRSR2*	7	17	*BCOR*	2
5	*SF3B1*	9	18	*SETBP1*	2
6	*EZH2*	5	19	*HTR1A*	2
7	*ASXL1*	4	20	*LUC7L2*	2
8	*NRAS*	4	21	*PCDHAC1*	2
9	*KRAS*	4	22	*TCF4*	2
10	*CBL*	4	23	*CACNA1E*	2
11	*DNMT3A*	3	24	*TTN*	2
12	*RUNX1*	3	25	*ETNK1*	2
13	*TP53*	3	26	*GNB1*	2



RNAスプライシング変異とMDS



***SF3B1* mutations**

RARS (64~83%)

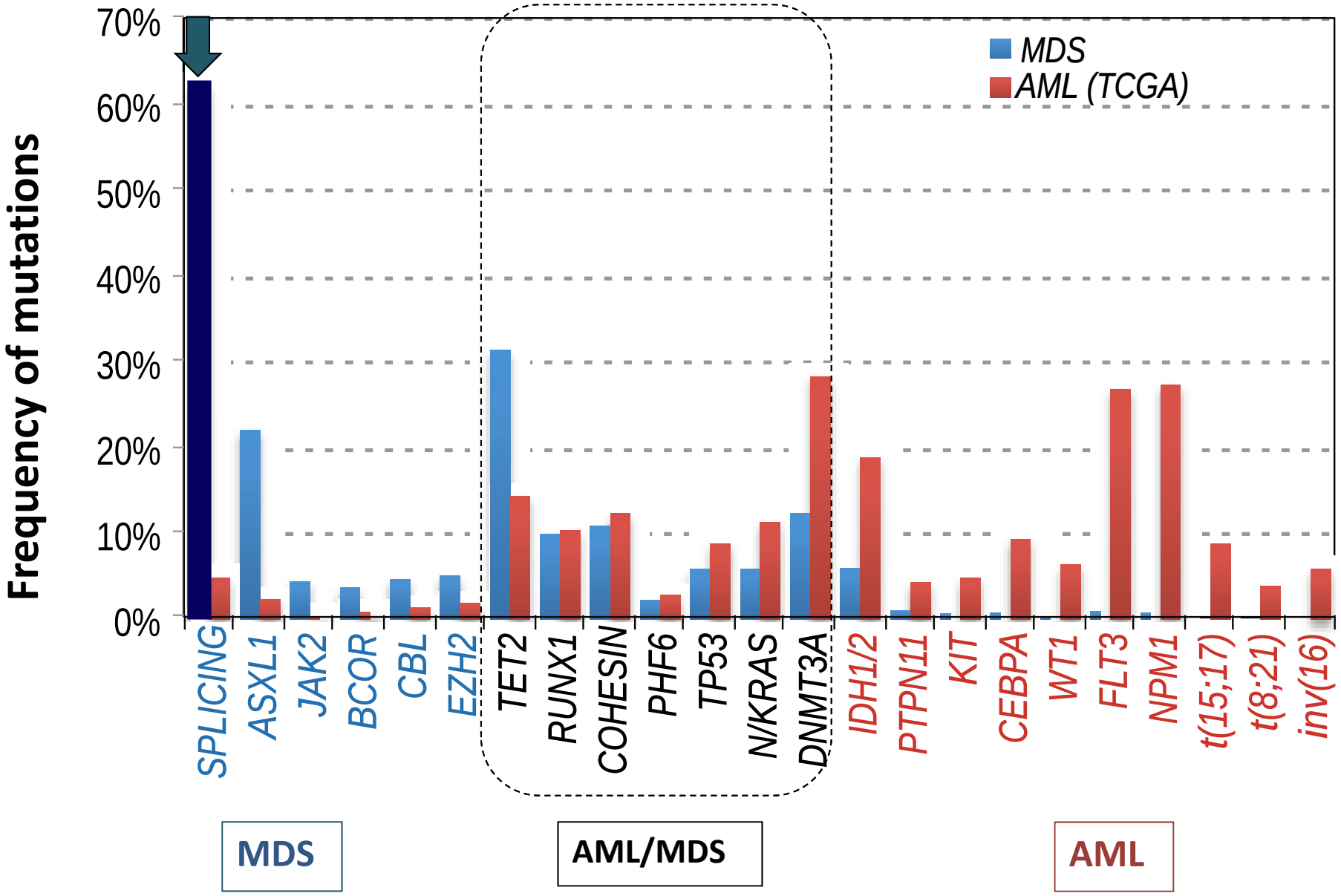
RCMD-RS (57~76%)

RARS-T (68~73%)

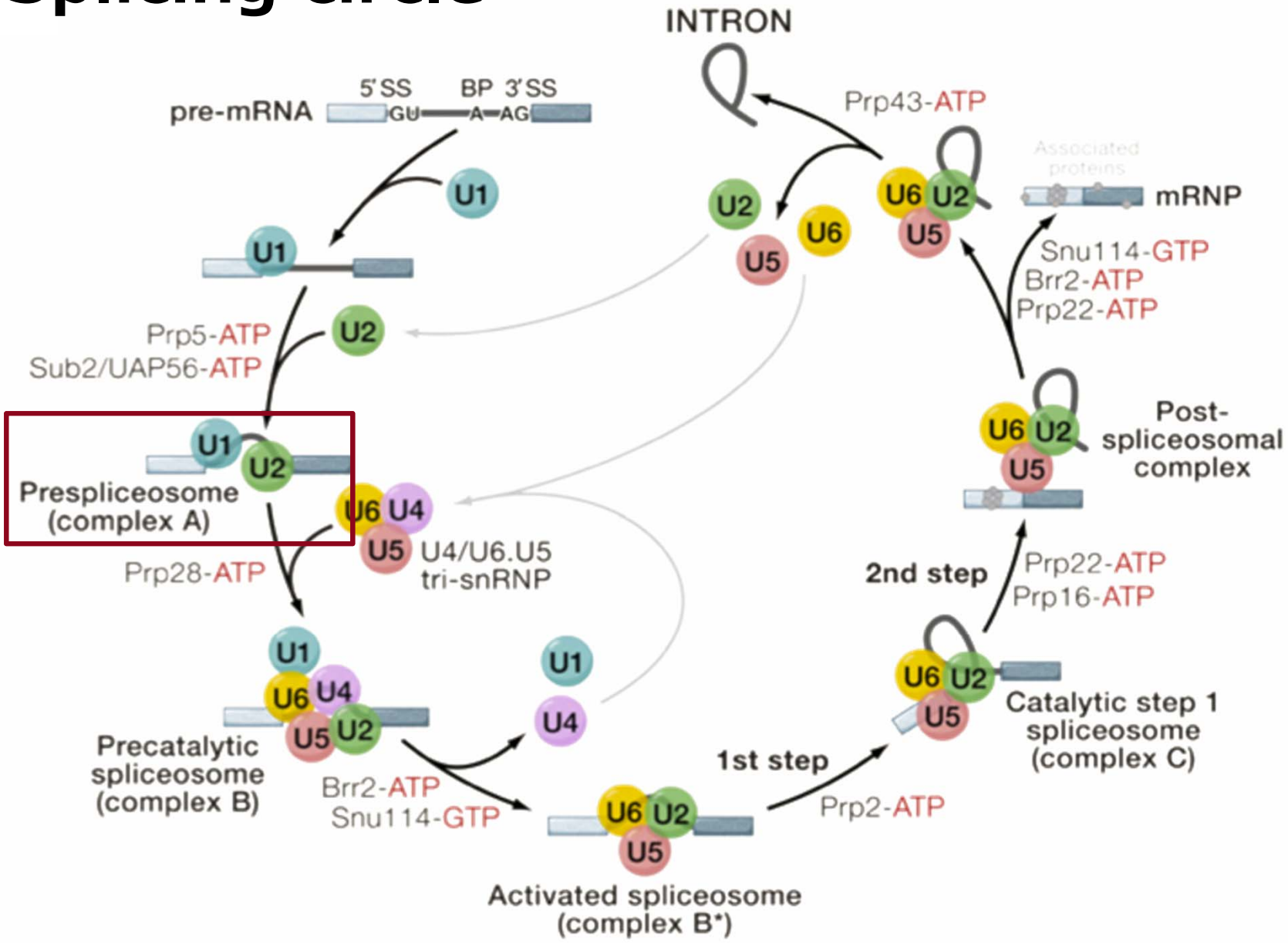
PPV = 97.7%,
NPV = 98.7%
(Malcovati et al, *Blood*, 2011)

(Yoshida et al., Nature, 2011)

Gene mutations in MDS vs AML

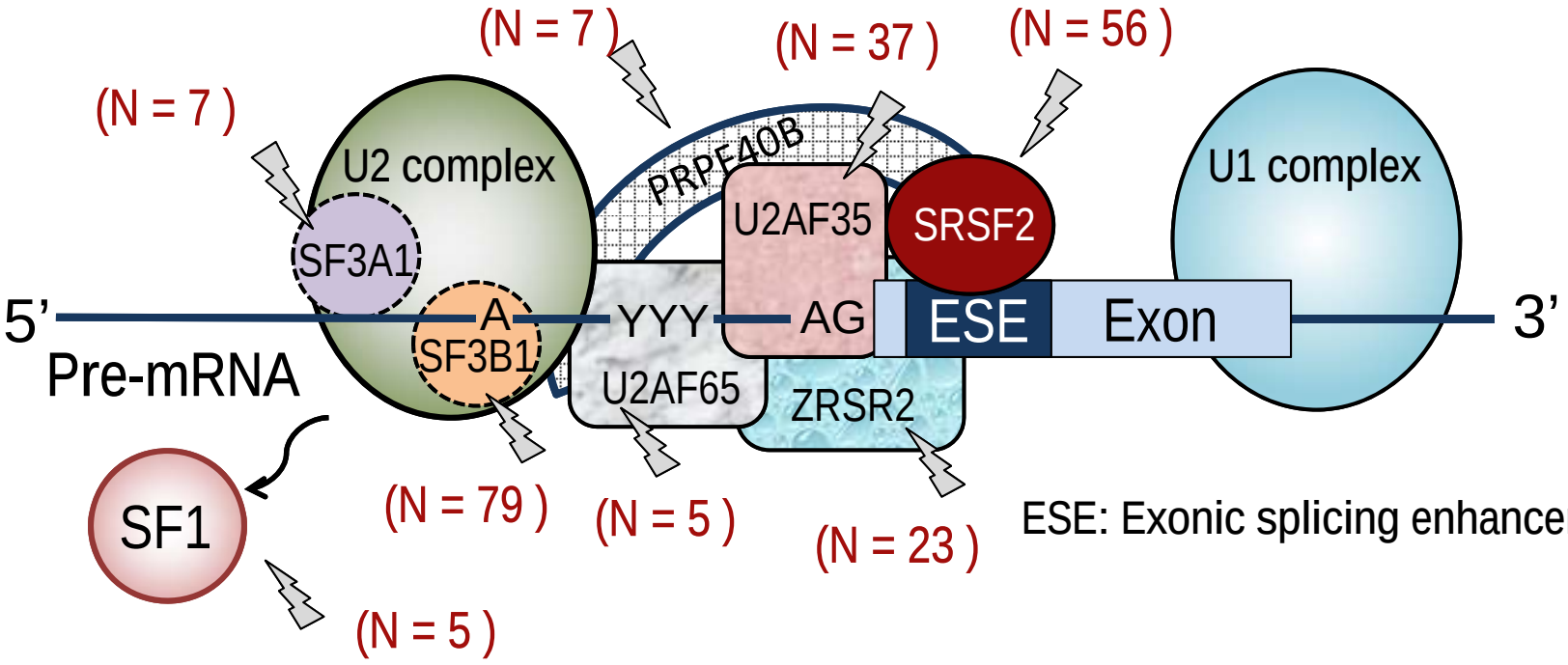


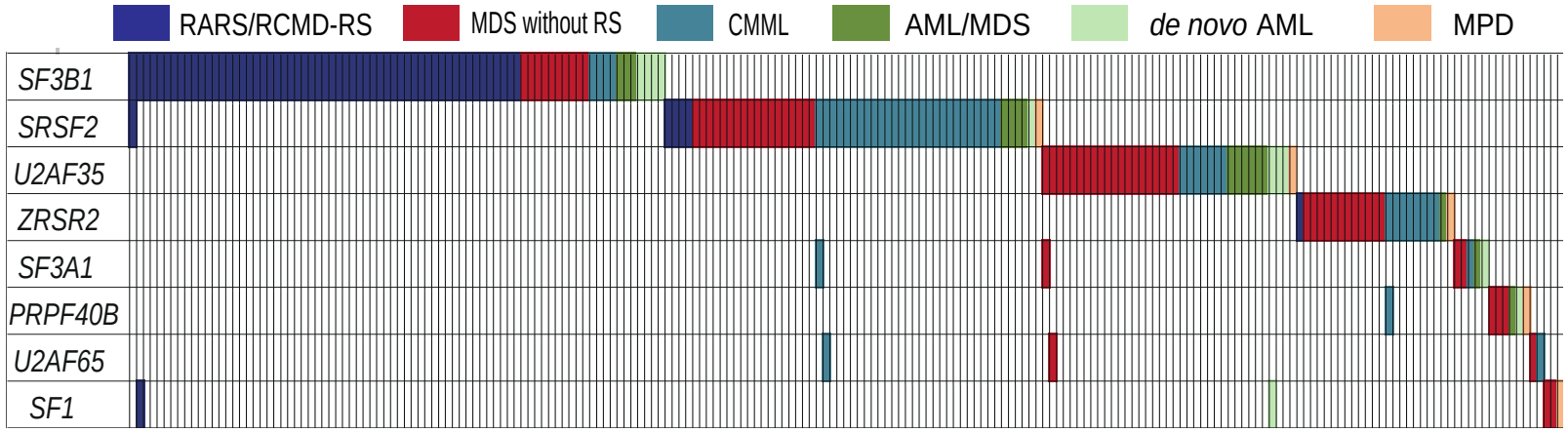
“Splicing circle”



(Wahl MC, et al. Cell 136:701, 2009)

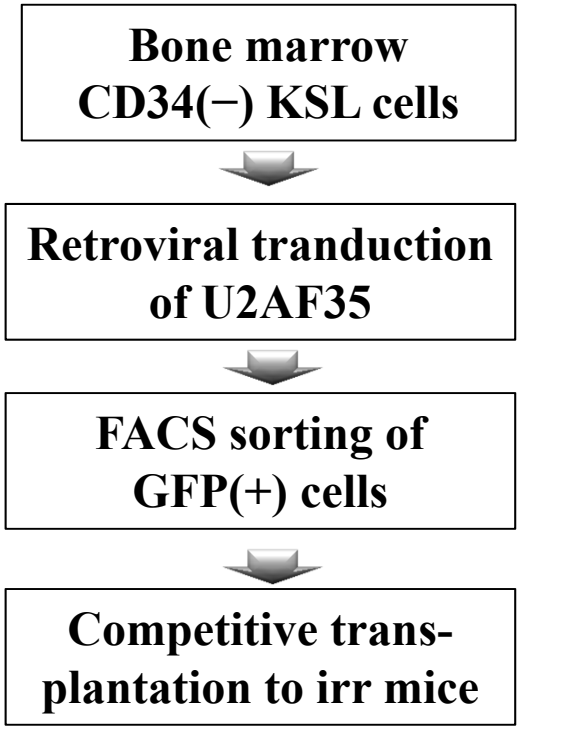
Pathway mutations of splicing machinery

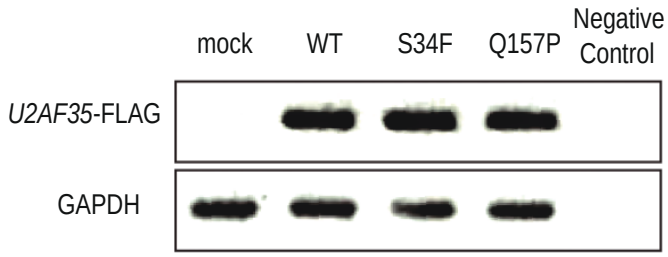


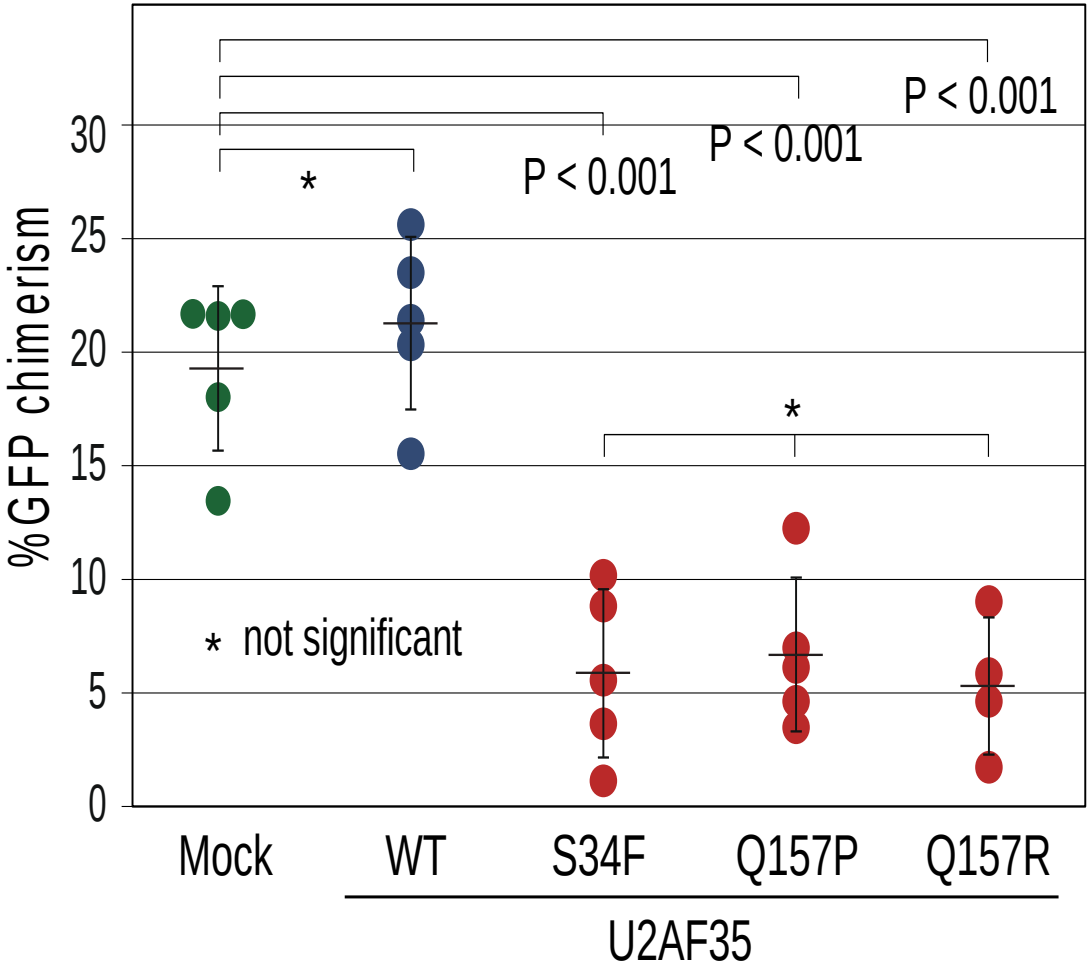


(Yoshida K, et al., Nature, 2011)

Reduced chimerism after transplants with *U2AF35*^{mut}

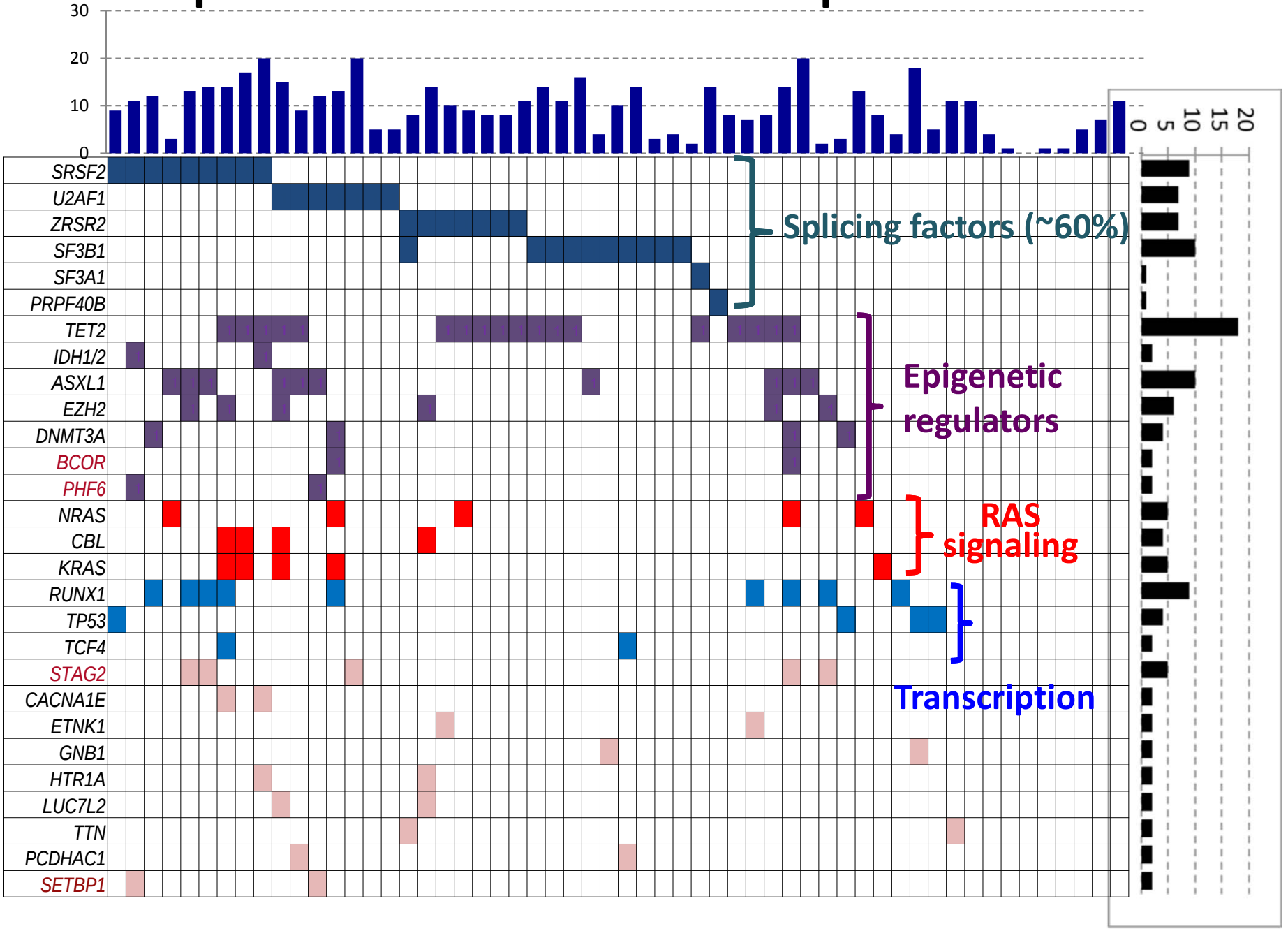




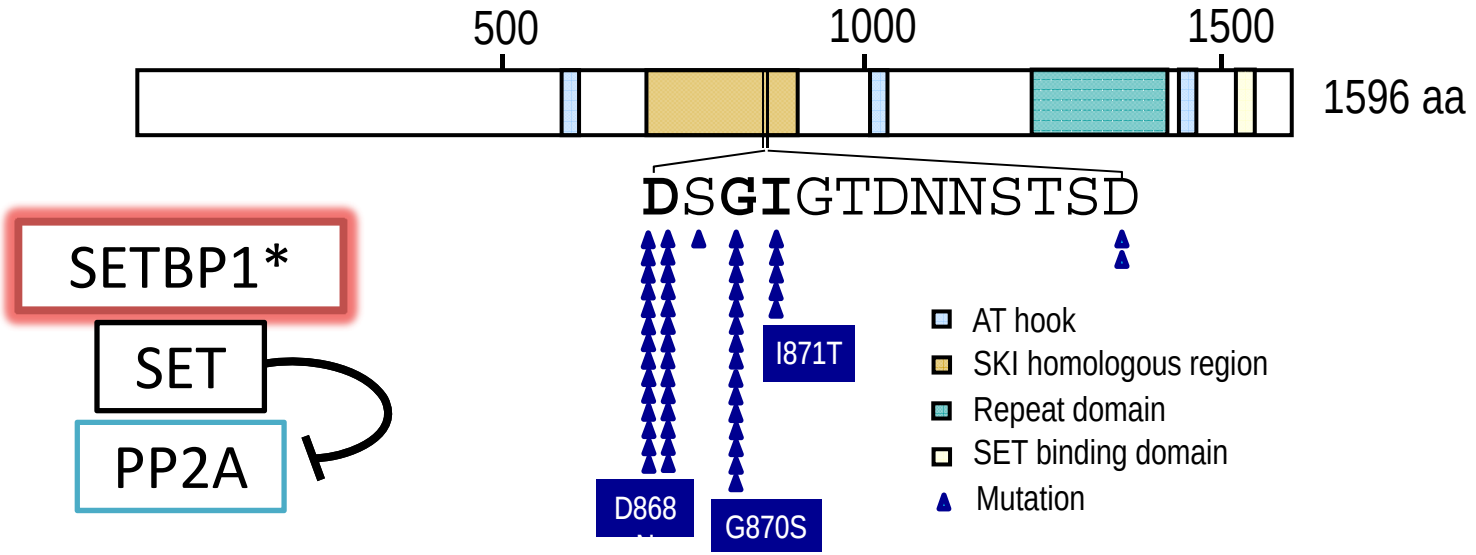


(Otsu M, Yamamoto R, Nakauchi H)

Landscape of mutations in exome seq in 56 MDS cases



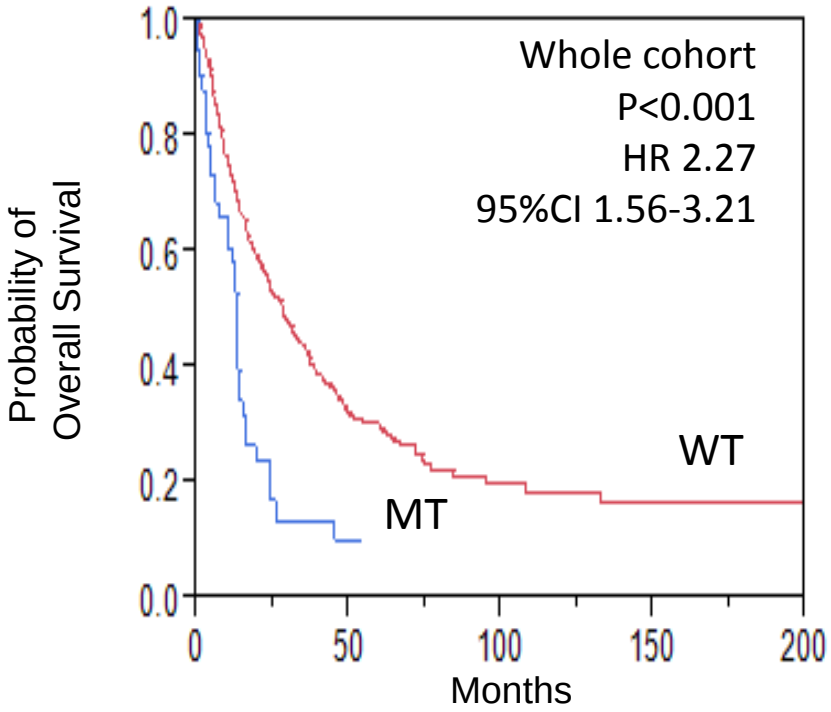
SETBP1

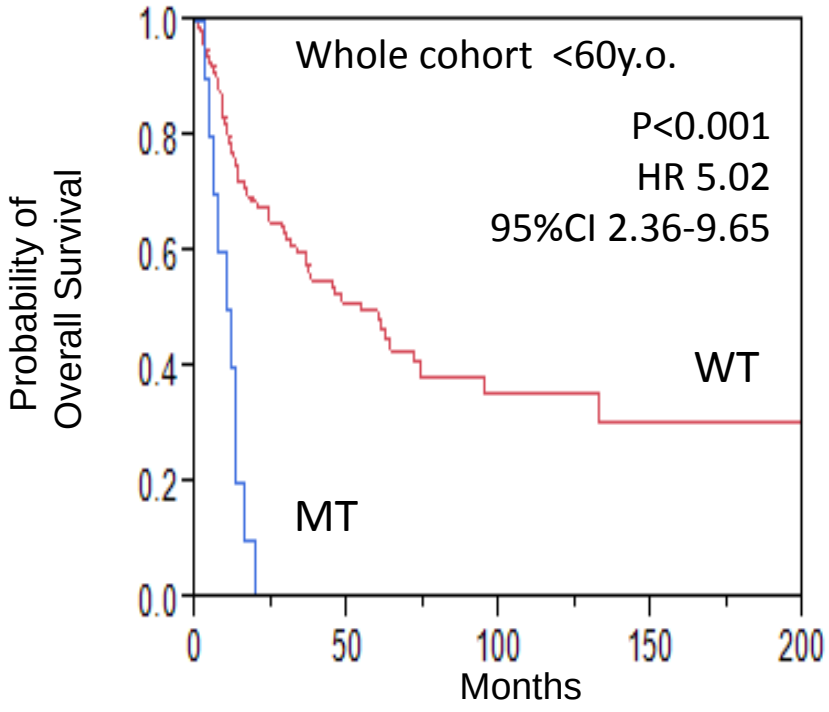


- ✓ 170kD protein associated with SET protein, which are thought to inhibit PP2A tumor suppressor
- ✓ Translocated/overexpressed in leukemia
- ✓ Major downstream target of Evi-1 oncogene
- ✓ Mutated in 10-25% of MDS/MPN subtypes and sAML
- ✓ Hotspot mutations involving D868, G870, and I871 identical to germline mutations in Schinzel-Giedeon syndrome

(Makishima, et al. Nature genet, 2013)

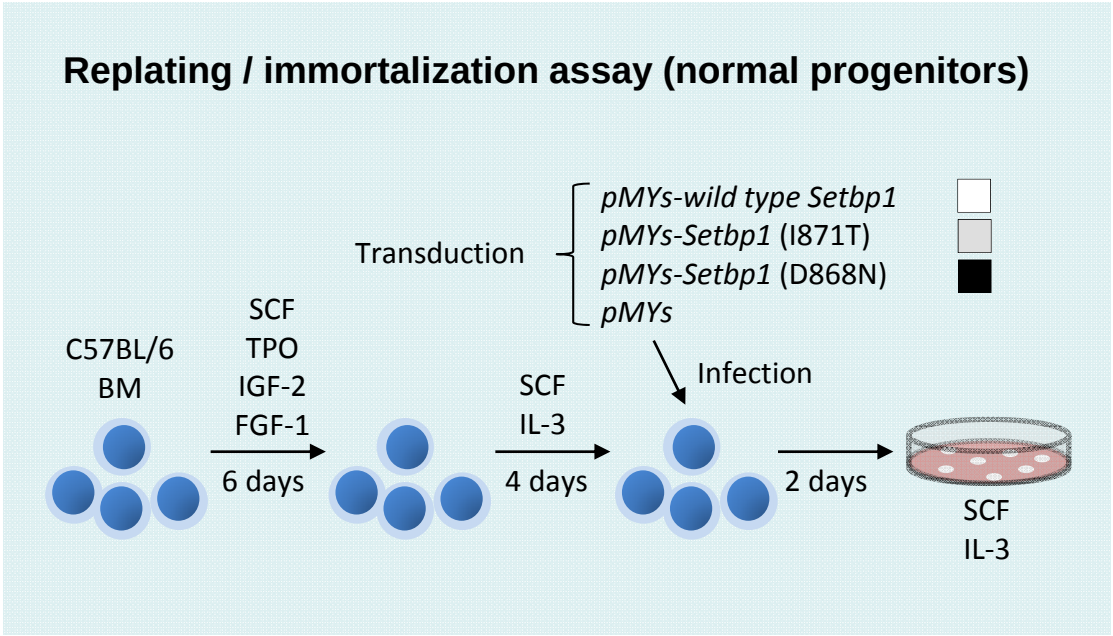
SETBP1 mutations are associated with poor survival

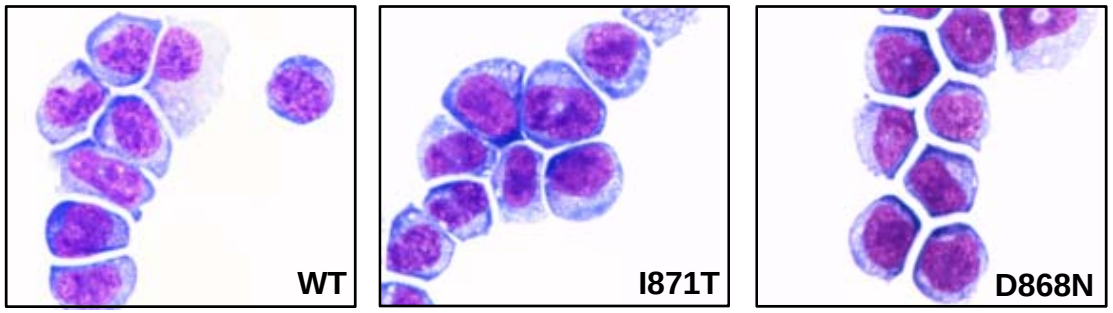


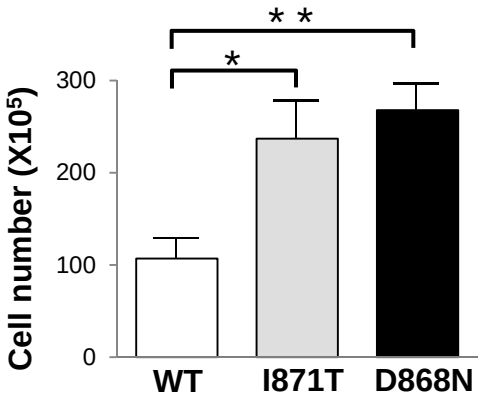


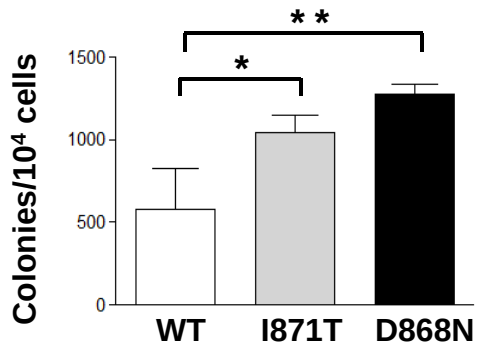
(Makishima, et al. Nature genet, 2013)

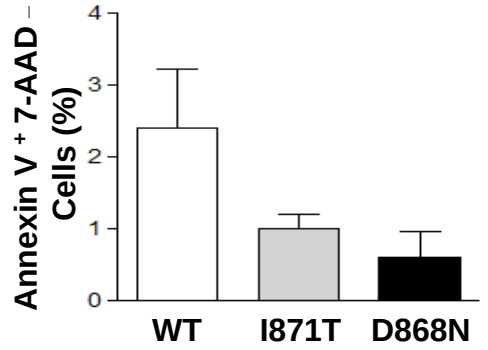
Leukemogenic potential of *SETBP1* mutants





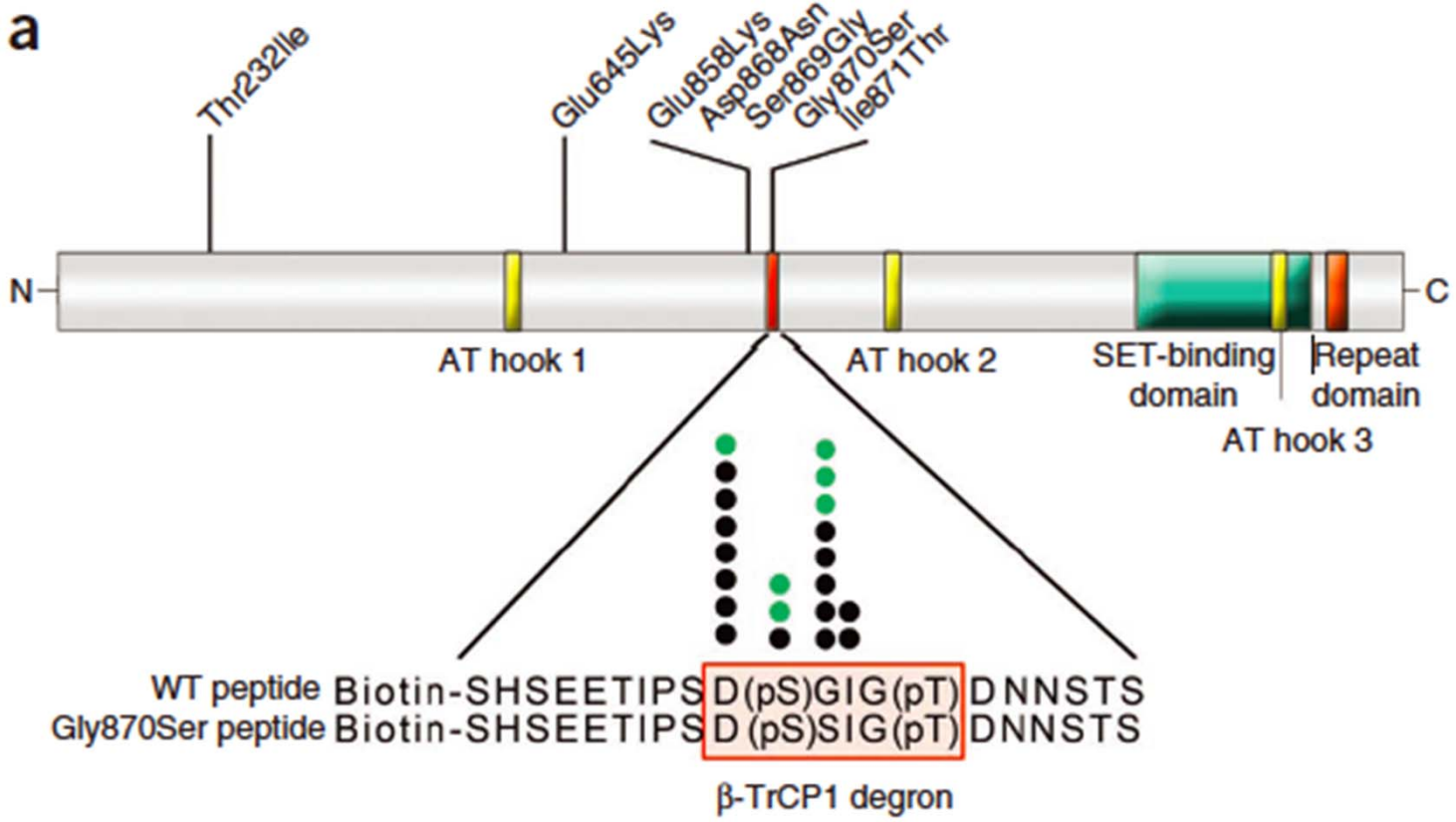






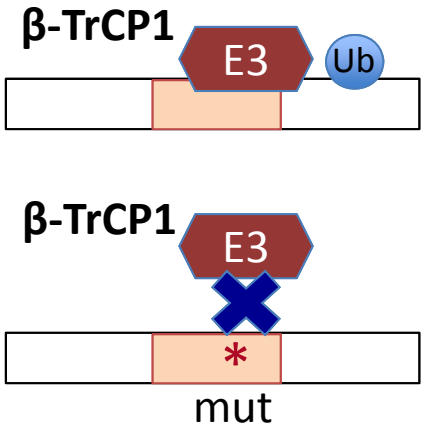
(Makishima, et al. Nature genet, 2013)

Origin of gain-of-function

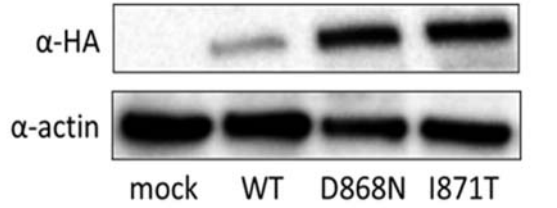


(Piazza R et al., Nat Genet, 2013)

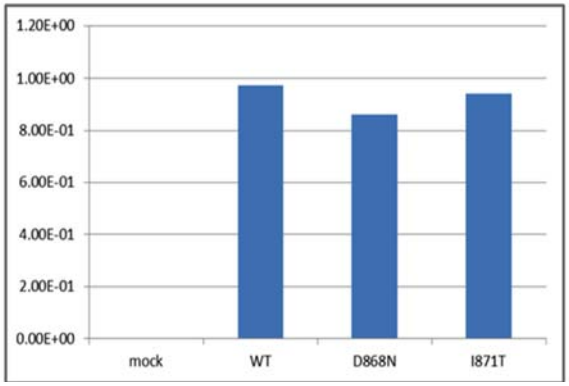
SETBP1 escapes from proteosome degradation



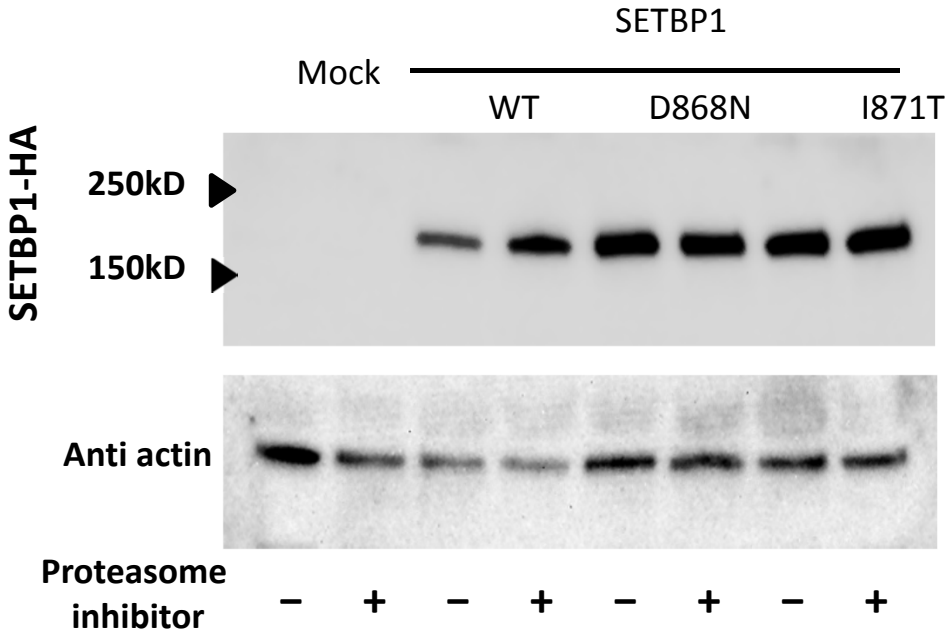
A

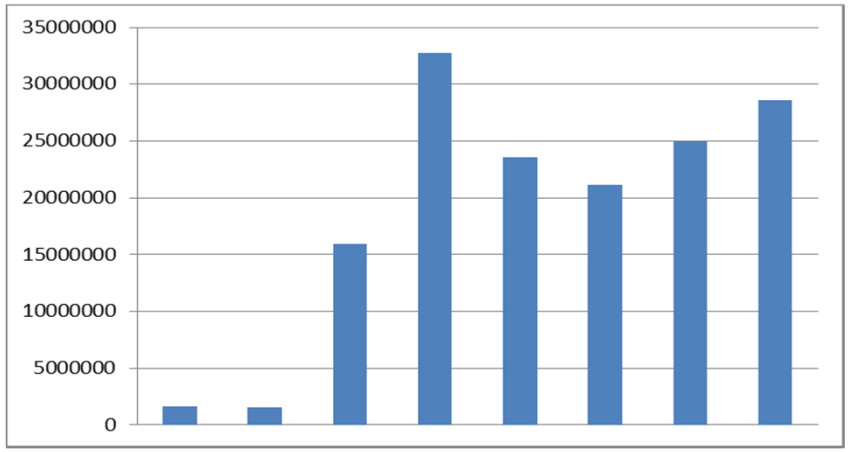


Protein
SETBP1-HA



mRNA
SETBP1-HA



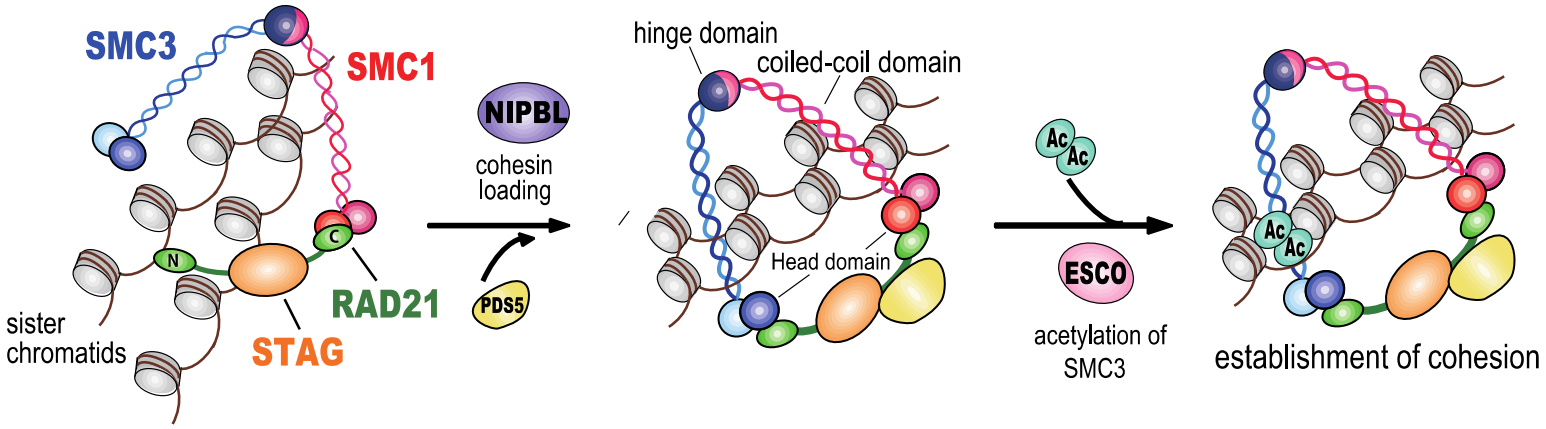


(Makishima, et al. Nature genet, 2013)

nature genetics

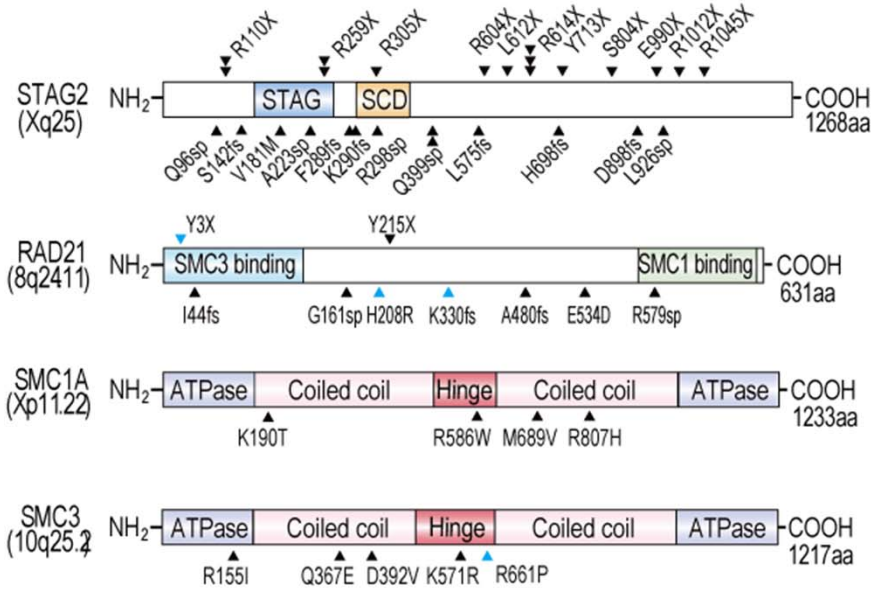
Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms

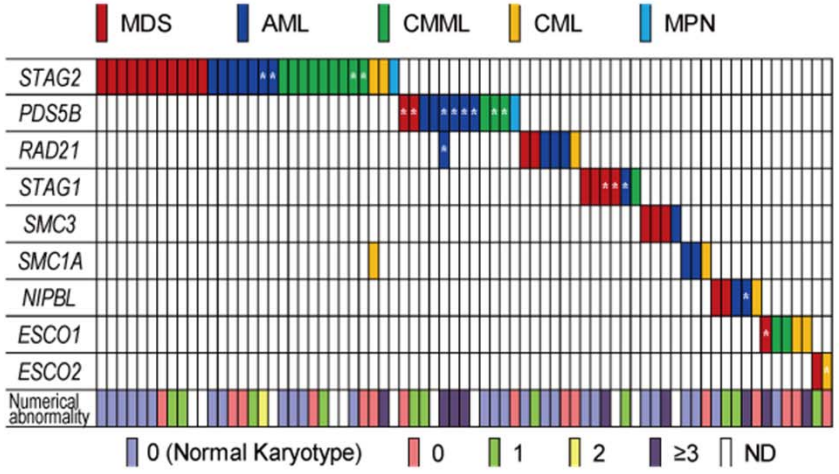
Ayana Kon[1], Lee-Yung Shih[2], Masashi Minamino[3], Masashi Sanada[1,4], Yuichi Shiraishi[5], Yasunobu Nagata[1], Kenichi Yoshida[1], Yusuke Okuno[1], Masashige Bando[3], Ryuichiro Nakato[3], Shumpei Ishikawa[6,7], Aiko Sato-Otsubo[1], Genta Nagae[8], Aiko Nishimoto[6], Claudia Haferlach[9], Daniel Nowak[10], Yusuke Sato[1], Tamara Alpermann[9], Masao Nagasaki[11], Teppei Shimamura[5], Hiroko Tanaka[12], Kenichi Chiba[5], Ryo Yamamoto[13], Tomoyuki Yamaguchi[13,14], Makoto Otsu[15], Naoshi Obara[16], Mamiko Sakata-Yanagimoto[16], Tsuyoshi Nakamaki[17], Ken Ishiyama[18], Florian Nolte[10], Wolf-Karsten Hofmann[10], Shuichi Miyawaki[18], Shigeru Chiba[16], Hiraku Mori[17], Hiromitsu Nakauchi[13,14], H Phillip Koeffler[19,20], Hiroyuki Aburatani[8], Torsten Haferlach[9], Katsuhiko Shirahige[3], Satoru Miyano[5,12] & Seishi Ogawa[1,4]



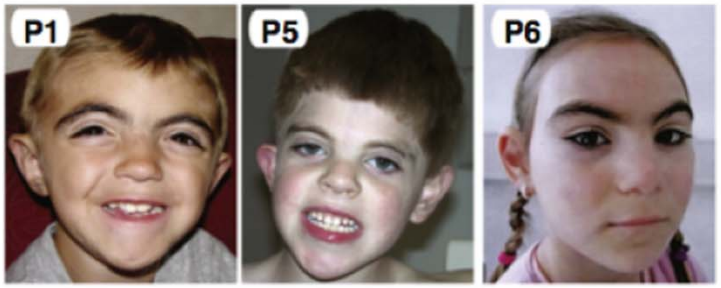
(Kon A., et al., Nature gent, 2013)

Cohesin mutations in myeloid neoplasms



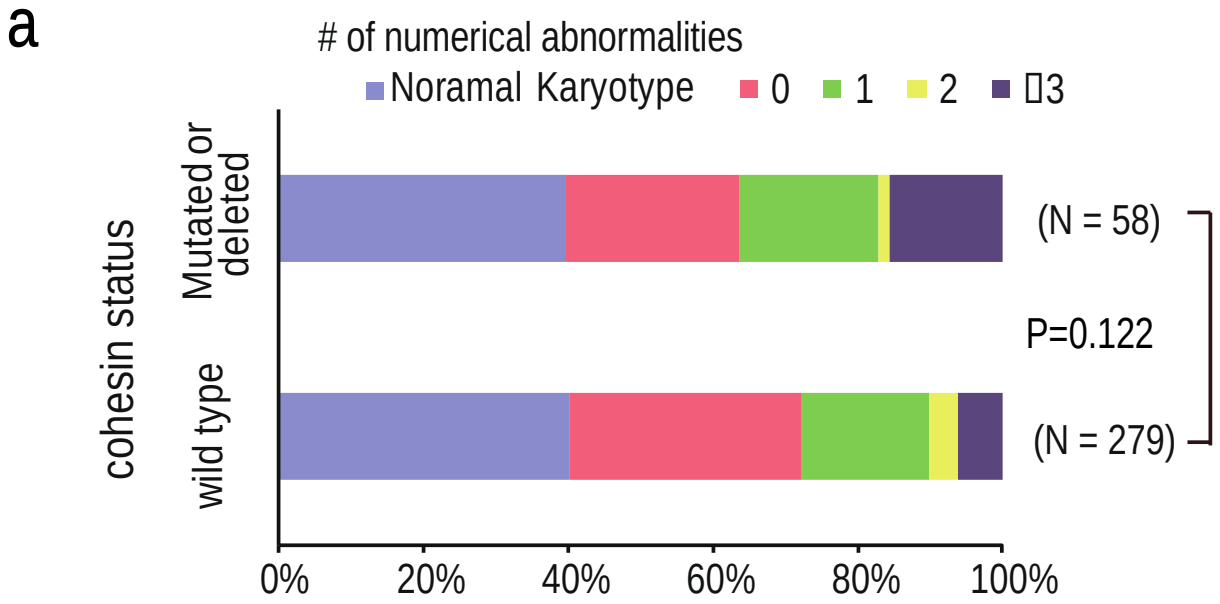


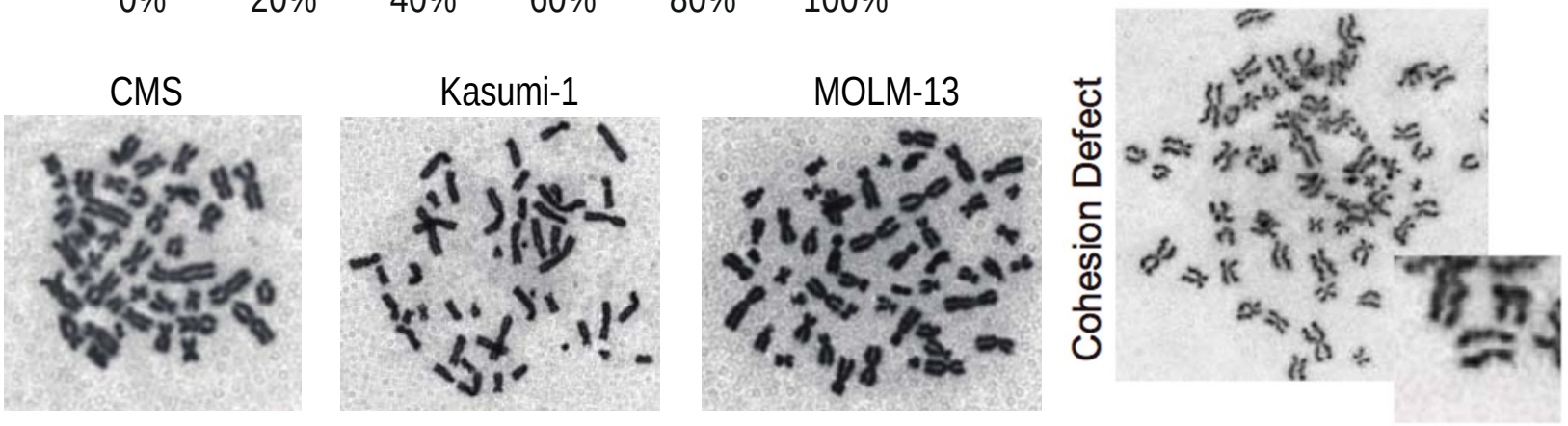
- Cohesion of sister chromatids
- Long-range regulation of gene expression
- Post-replicative DNA repair
- Responsible gene for congenital disorders (Cornelia de Lange syndrome and Roberts syndrome)



(A Kon, et al. Nature genet, 2013)

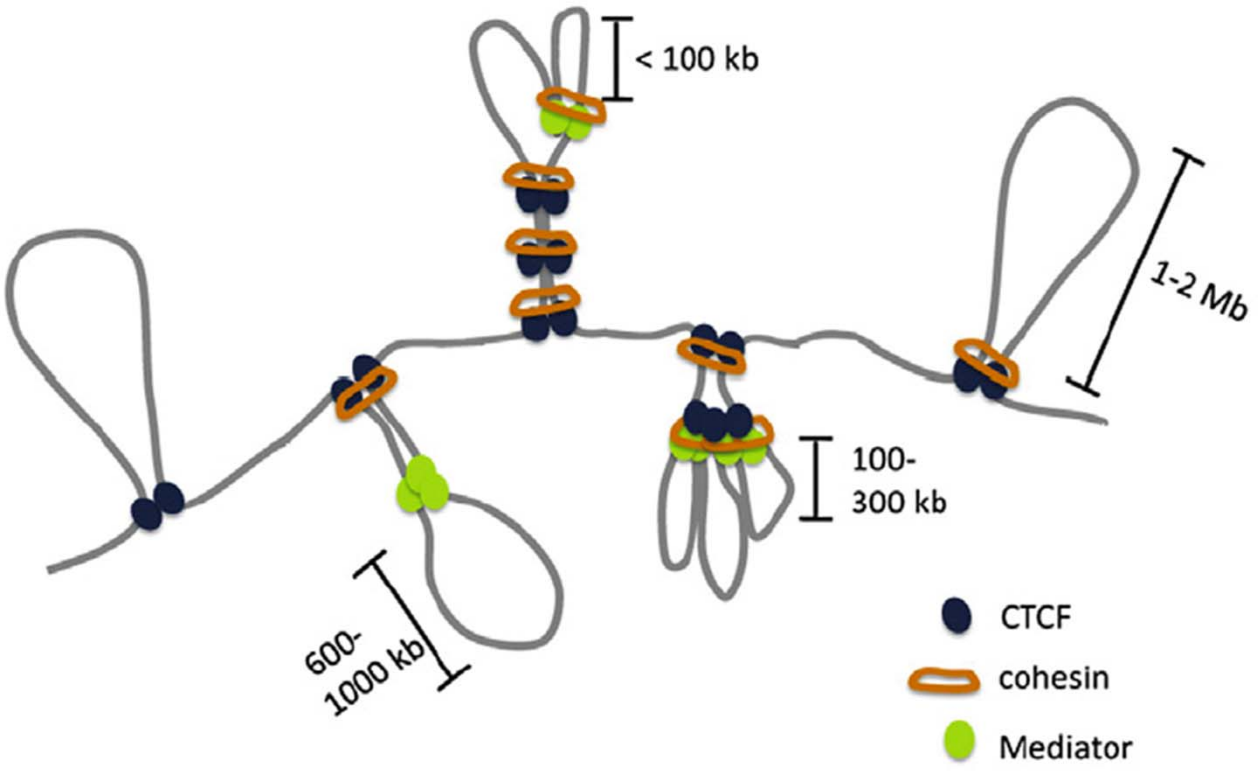
Karyotypes of cohesin mutated cases





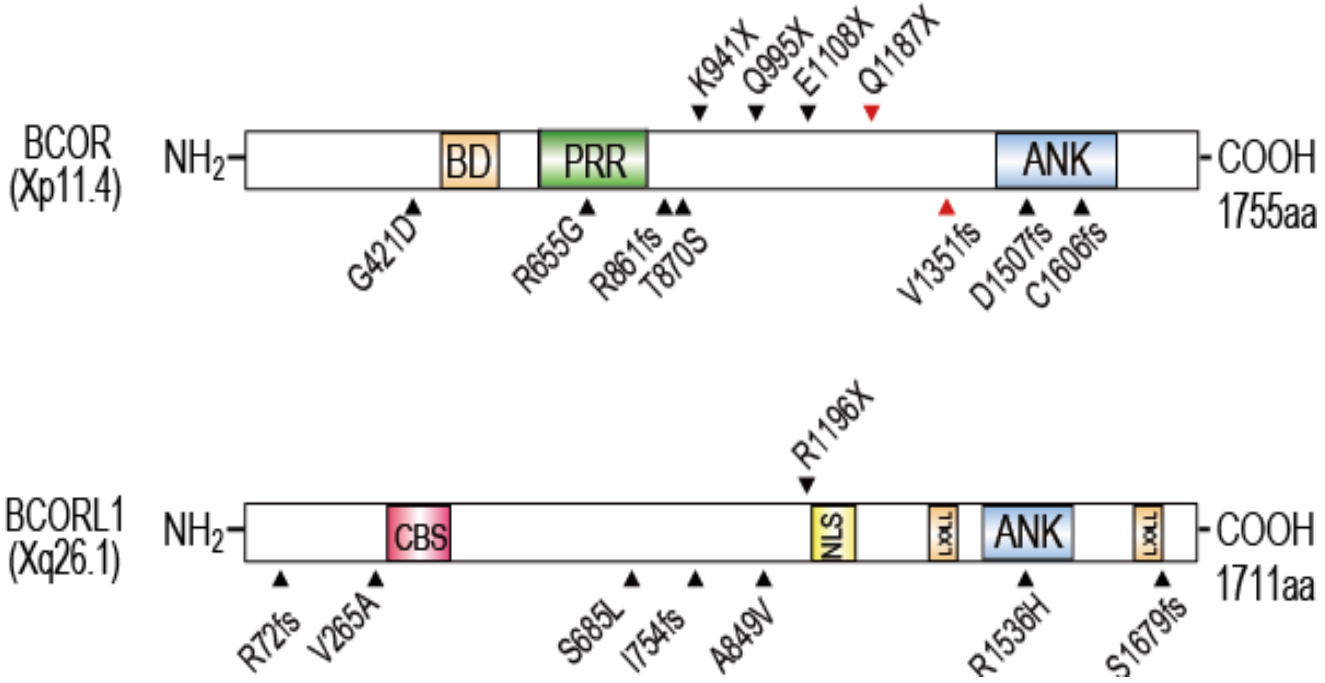
(A Kon, et al. Nature genet, 2013)

Cohesin for long-range regulation of gene expression



Phillips-Cremins et al, Cell, 2013

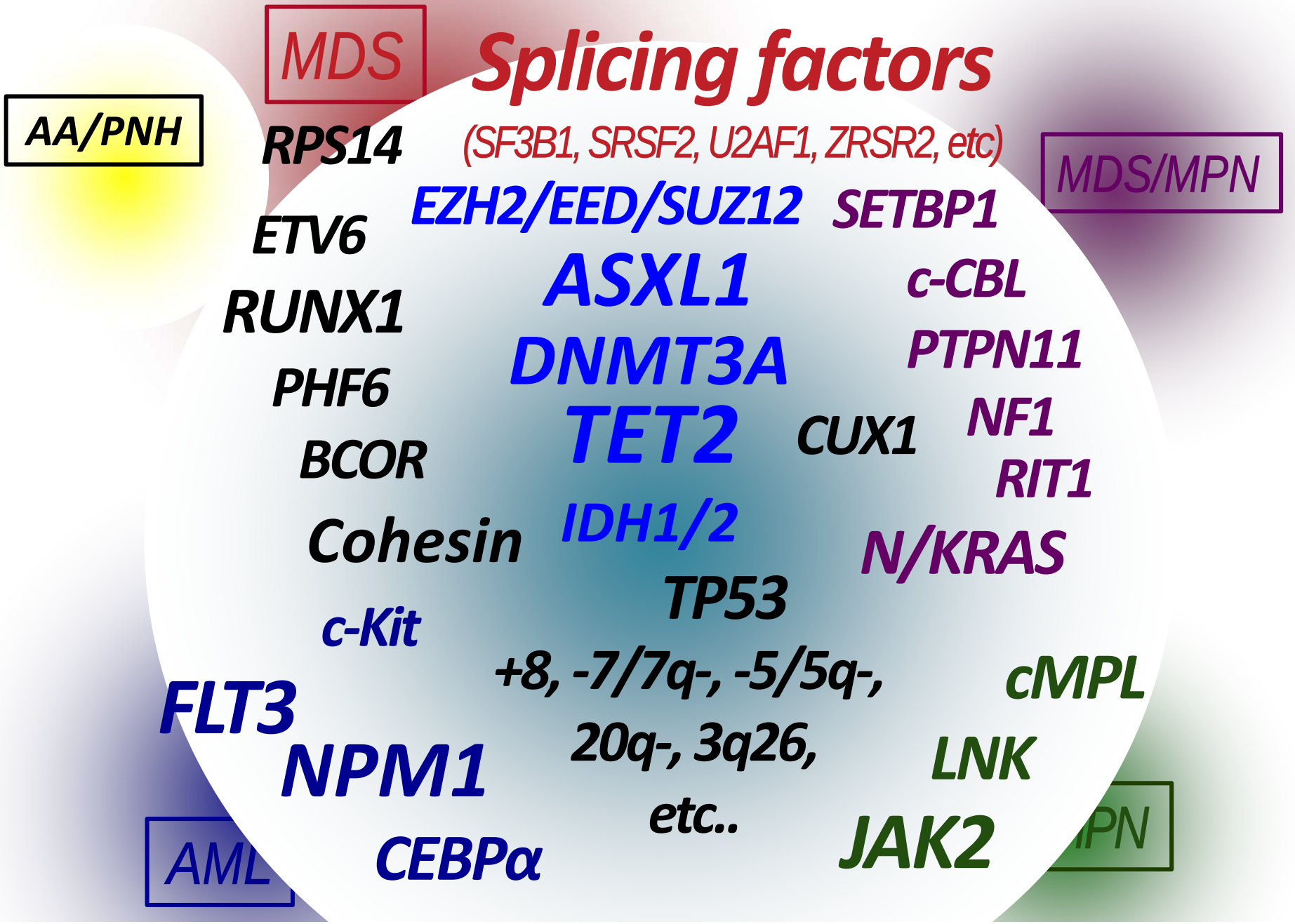
BCOR/BCORL1 mutations in myeloid malignancies



- Responsible for Oculofaciocardiodental (OFCD) syndrome
- Involved in polycomb regulation
- Mutated in 19/208 myeloid malignancies (9.1%)
- 18/19(95%) were mutated in advanced disease types (RAEB,CMML,AML/MRC)
- 12/19(63%) were inactivating mutations
- Associated with poor clinical outcomes

(F. Damm et al., Blood, 2013)

Spectrum of somatic mutation in MDS in 2014



Characteristics of 944 patients with MDS

Parameter		Total Cohort	Training Cohort	Validation Cohort
Patient Numbers		944	730	214
Median age	(years)	72.8 (23.3 – 90.8)	73.0 (24.3–90.8)	72.6 (23.3–90.4)
Patients with Follow-up data		786	611	175
Median overall survival	(months)	54.5	54.2	61.6
Median follow-up	(months)	32.3	34.2	15.9
Treatment	Supportive care only	592 (75.3%)	463 (75.8%)	129 (73.7%)
MDS Subtypes	(WHO, 2008)			
	RA	41 (4.3%)	32 (4.4%)	9 (4.2%)
	RARS	81 (8.6%)	63 (8.6%)	18 (8.4%)
	RARS-T	28 (3.0%)	22 (3.0%)	6 (2.8%)
	RCMD	195 (20.7%)	150 (20.5%)	45 (21.0%)
	RCMD-RS	183 (19.4%)	141 (19.3%)	42 (19.6%)
	RAEB-1	191 (20.7%)	147 (20.1%)	44 (20.6%)
	RAEB-2	188 (19.9%)	144 (19.7%)	44 (20.6%)
	MDS with isolated 5q-deletion	37 (3.9%)	31 (4.2%)	6 (2.8%)

Summary of targeted deep sequencing and somatic mutations calling

104 known or putative mutational genes captured using SureSelect

HiSeq 2000 (100bp, paired-end)@mean depth of 1000x

Align to the human genome reference (hg19) using BWA

Discard low-quality reads and low-quality bases

- Reads with 5 or more mismatches
- Reads with < 25 mapping quality
- Bases with < 30 base quality

Remove SNPs

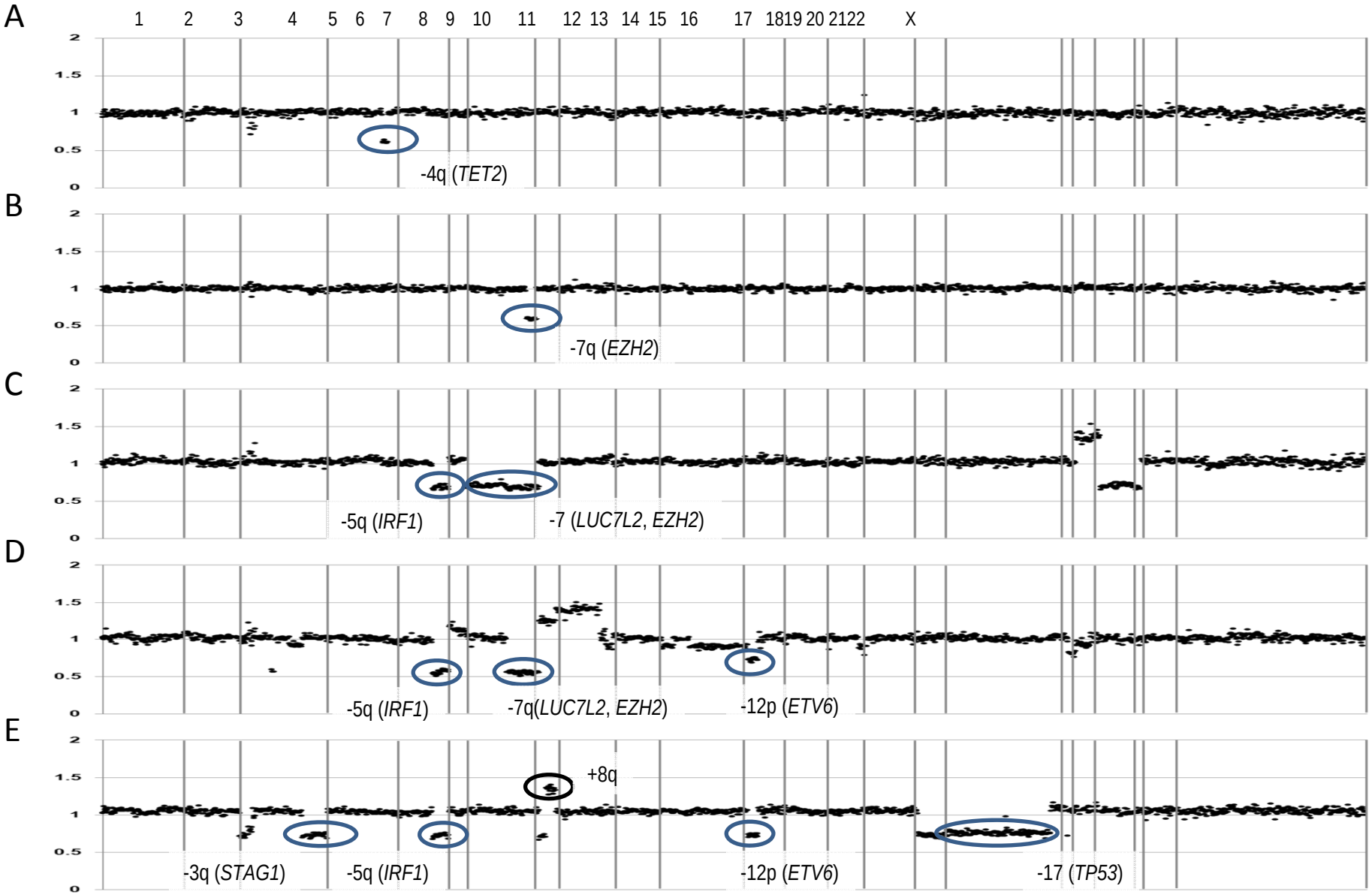
- dbSNP131
- 1000 genomes as of 2011/05/21
- ESP 6500
- Missense SNVs with 0.45 ~ 0.55 allele frequency or on copy number change without registered in COSMIC V60.

Remove Sequence errors

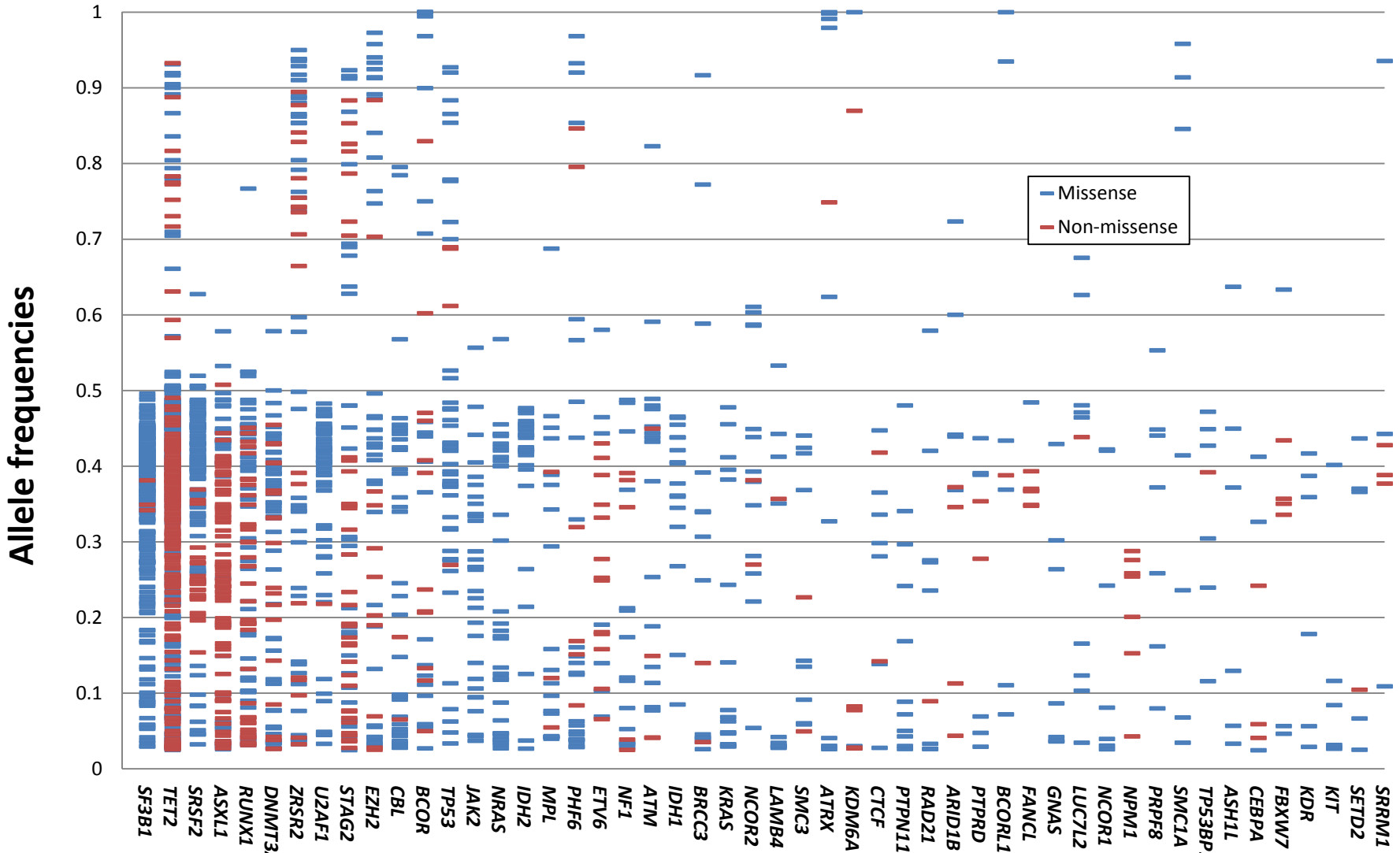
- Remove candidates in 53 normal samples
- Mapping errors by visual inspection with IGV

A total of 2,764 single nucleotide variants (SNVs) and short indels were identified.

高深度シーケンスを用いたゲノムコピー数解析

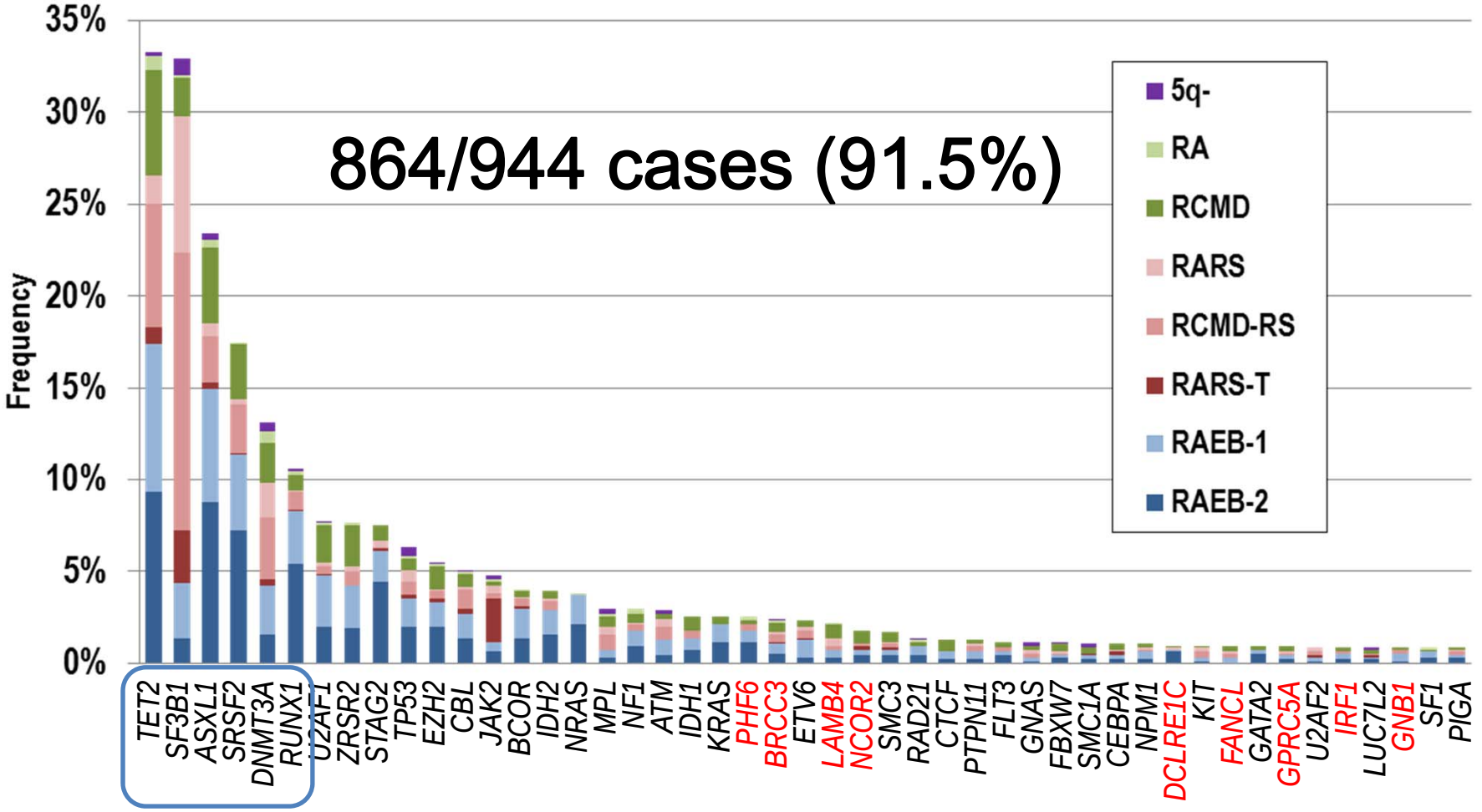


Distribution of allele frequencies



A total of 2,764 single nucleotide variants (SNVs) and short indels were identified.

MDSにおけるドライバー変異/欠失の頻度

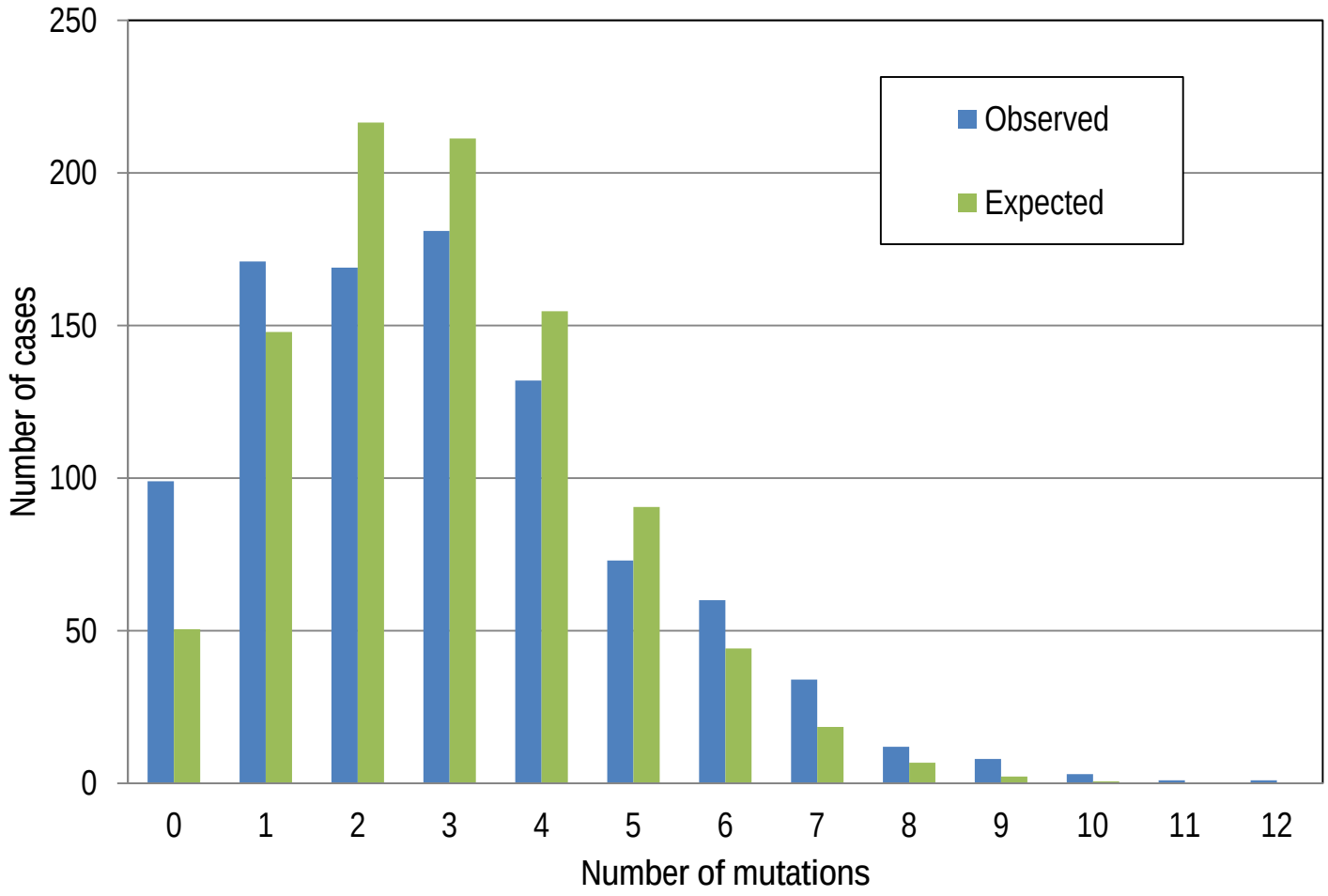


(Haferlach et al., Leukemia, 2014)

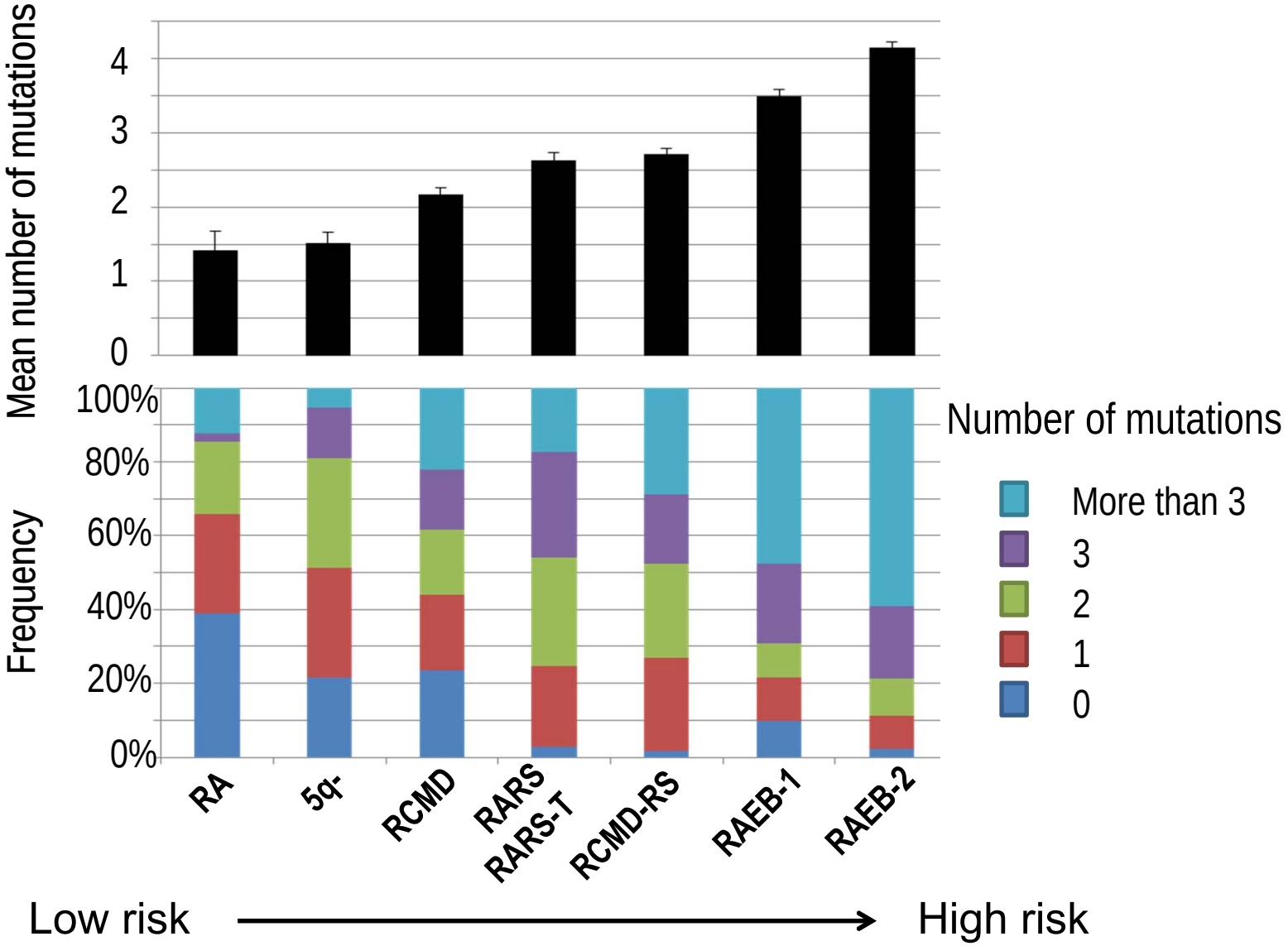
Concordance between two large studies

Top 12 Genes in our results	Top12 Genes in Papaemmanuil et al
TET2	*SF3B1*
SF3B1	*TET2*
ASXL1	*SRSF2*
SRSF2	*ASXL1*
DNMT3A	*DNMT3A*
RUNX1	*RUNX1*
U2AF1	*U2AF1*
ZRSR2	*TP53*
STAG2	*EZH2*
TP53	*IDH2*
EZH2	*STAG2*
CBL	*ZRSR2*
N = 944	**N = 738**
104 genes tested	**111 genes tested**
Torsten Haferlach et al, Leukemia, 2013	Elli Papaemmanuil et al, Blood, 2013

Median of 3 driver gene mutations per semple



Number of gene mutations correlate blast counts MDS subtypes



Landscape of gene mutations in MDS (N=944)

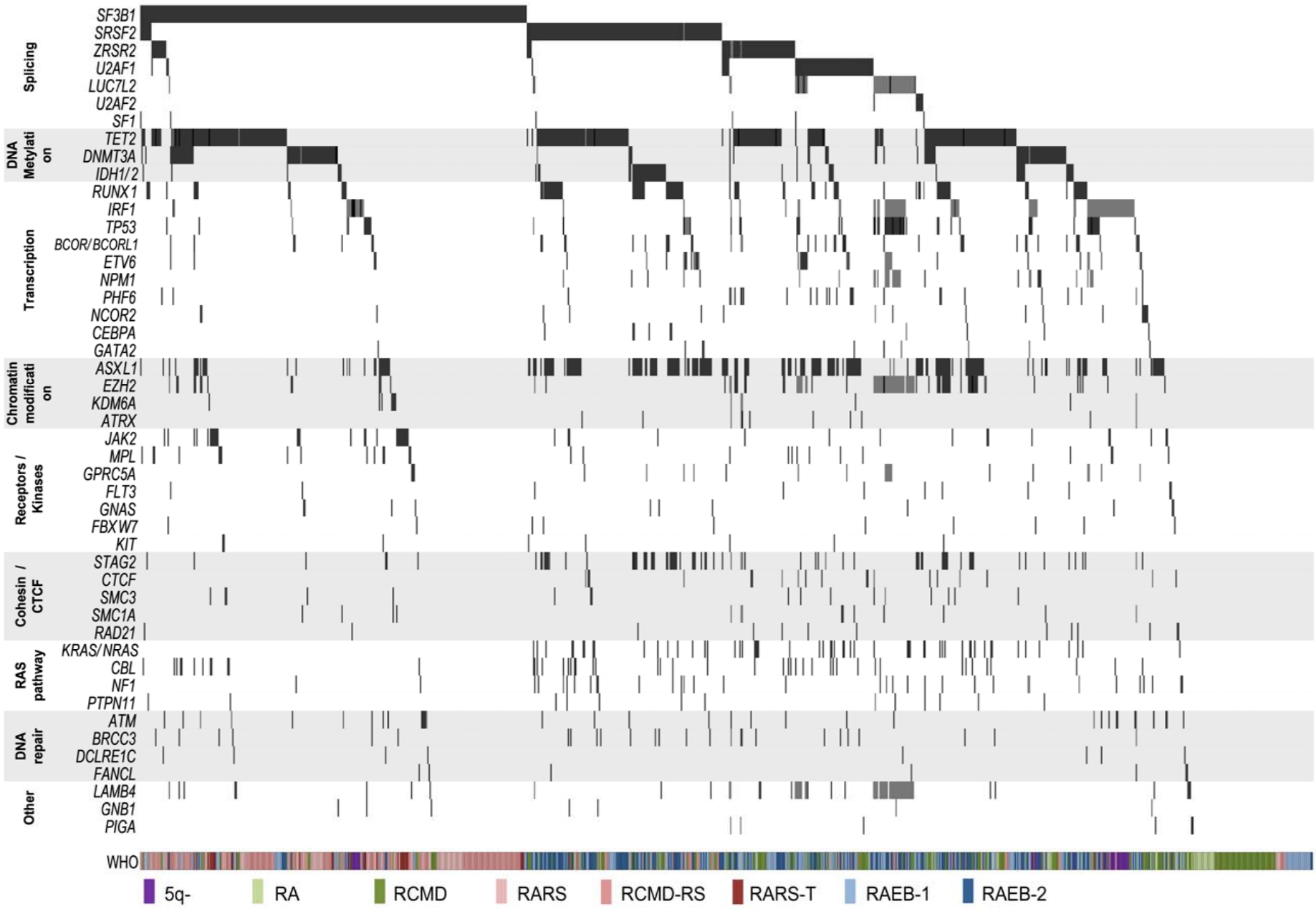
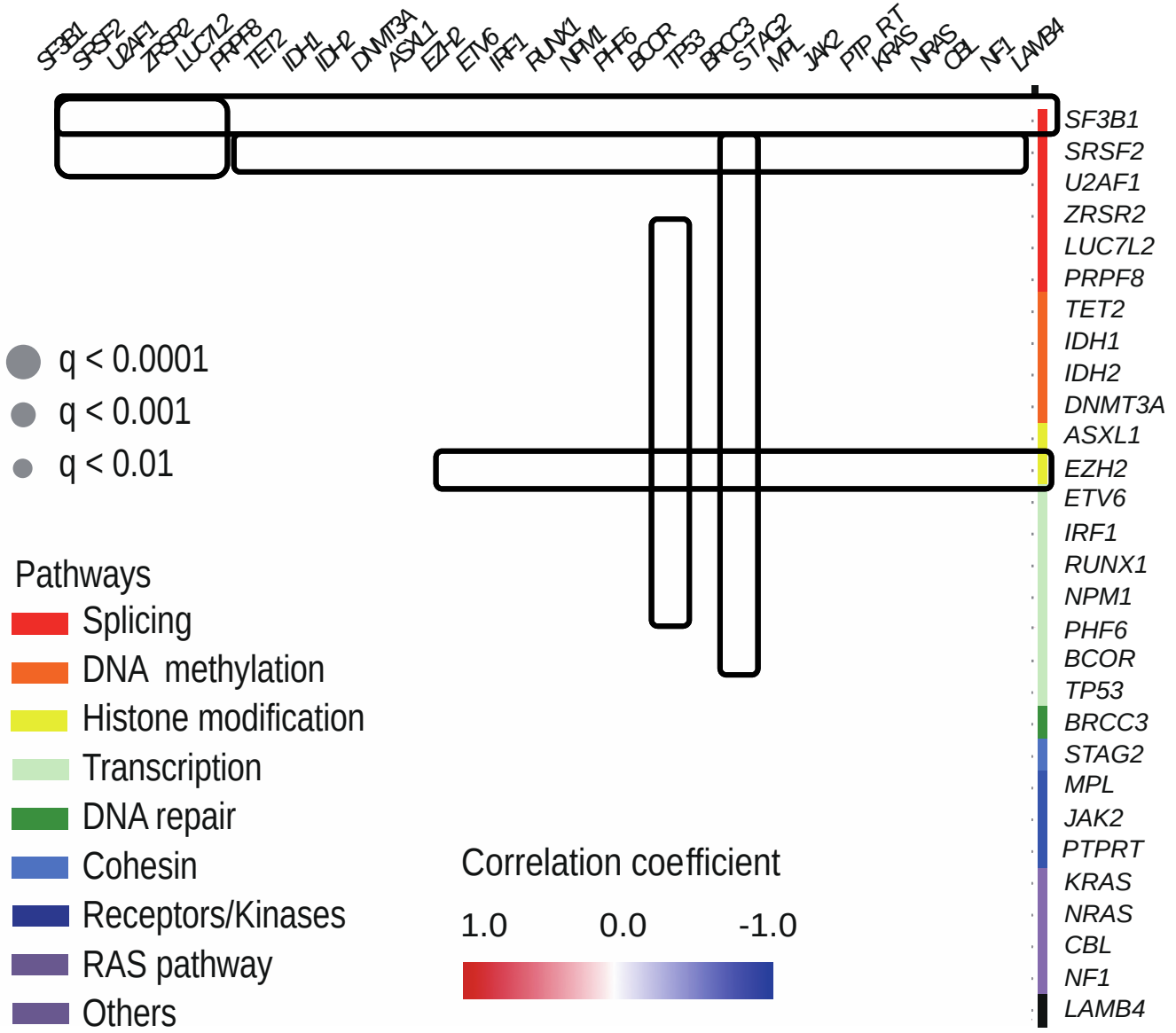


Fig 1D

Correlation among different mutations

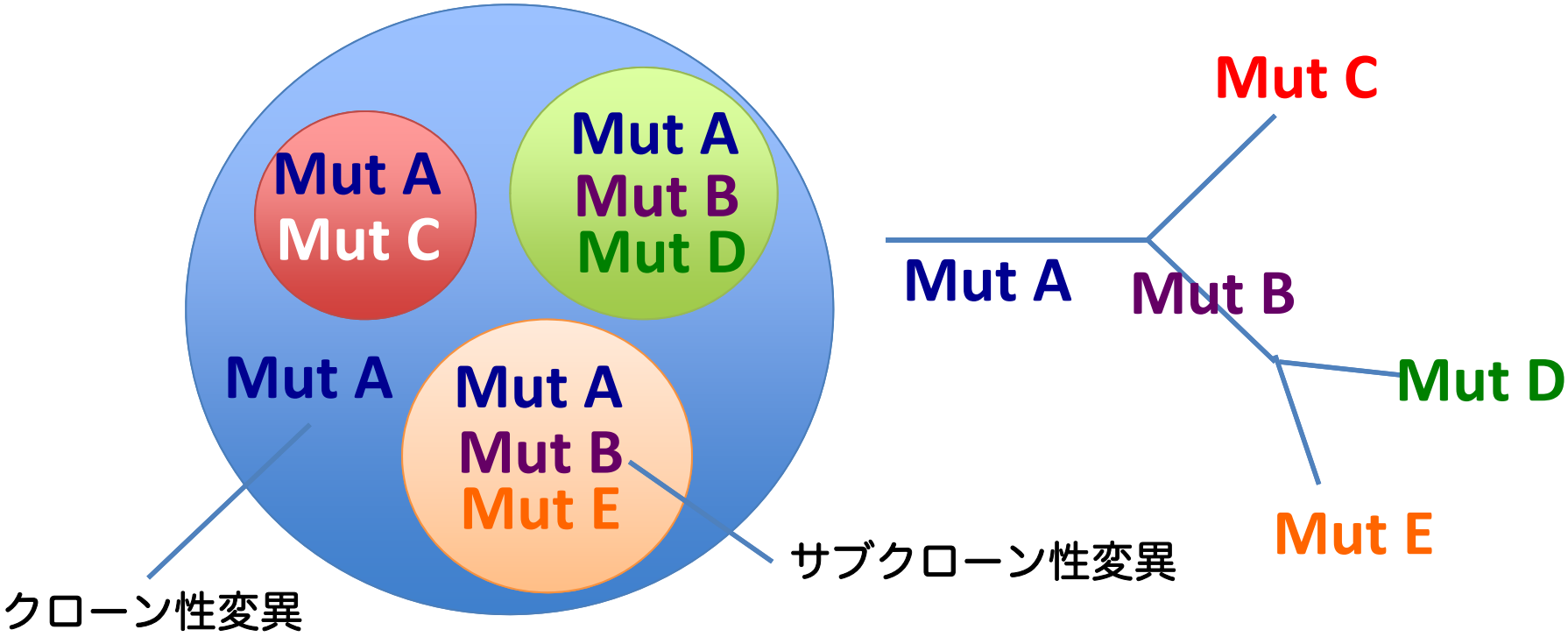


(Torsten, et al., Leukemia, 2014)

Concordance of coexisting mutations between two studies

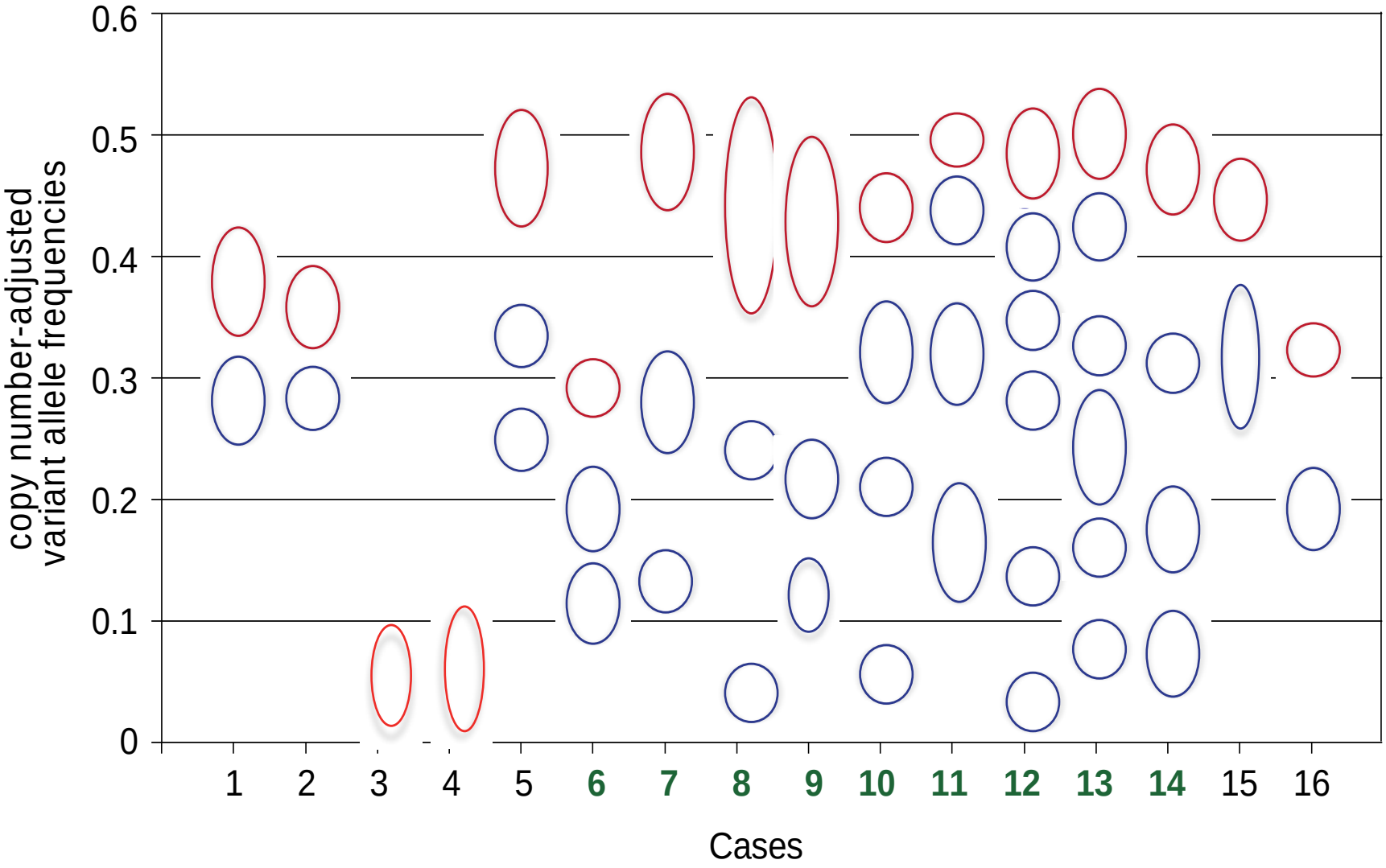
	Haferlach et al, Leukemia 2013 (N= 944)	*Papaemmanuil et al, Blood 2013 (N= 738)*
TET2	***ZRSR2*** *(q<0.0001),* ***SRSF2*** *, CBL (q<0.001)*	***SRSF2, ZRSR2*** *(q<0.01)*
SF3B1	***DNMT3A****, JAK2 (q<0.01)*	***DNMT3A*** *(q<0.01)*
SRSF2	***STAG2****,* ***ASXL1****,* ***RUNX1****,* ***IDH2*** *(q<0.0001)* ***TET2*** *(q<0.001), CBL (q<0.01)*	***STAG2, IDH2,*** *CUX1* ***ASXL1, TET2, RUNX1*** *(q<0.01)*
U2AF1	***ASXL1*** *(q<0.0001), PHF6, ETV6 (q<0.01)*	***ASXL1*** *(q<0.05)*
ZRSR2	***TET2*** *(q<0.0001), PHF6 (q<0.01)*	***TET2*** *(q<0.01)*
STAG2	***ASXL1 , RUNX1 , SRSF2,*** *NRAS, BCOR (q<0.0001)* ***IDH2*** *(q<0.001)* ***EZH2*** *(q<0.01)*	***SRSF2****,* ***RUNX1*** *(q<0.01)* ***ASXL1*** *(q<0.05)* ***IDH2,EZH2*** *(q<0.1)*
DNMT3A	***SF3B1*** *(q<0.001)*	***SF3B1*** *(q<0.01), BCOR (q<0.05)*
RUNX1	***SRSF2, ASXL1 , EZH2, STAG2****, BCOR (q<0.0001)*	***STAG2, ASXL1, SRSF2*** *(q<0.01)* ***EZH2*** *(q<0.05),* *NRAS (q<0.1)*
ASXL1	***SRSF2, U2AF1 , STAG2 , IDH2****, EZH2,* ***RUNX1, NRAS*** *(q<0.0001) CBL (q<0.01)*	***SRSF2, RUNX1*** *(q<0.01) JAK2,* ***U2AF1, STAG2*** *(q<0.05)* ***IDH2, NRAS,*** *CUX1 (q<0.1)*
EZH2	***RUNX1,*** *ASXL1, TP53, LAMB4, LUC7L2, NPM1, ETV6 (q<0.0001) NRAS (q<0.001),* ***STAG2*** *(q<0.01)*	***RUNX1*** *(q<0.05)* ***STAG2*** *(q<0.1)*

腫瘍内多様性

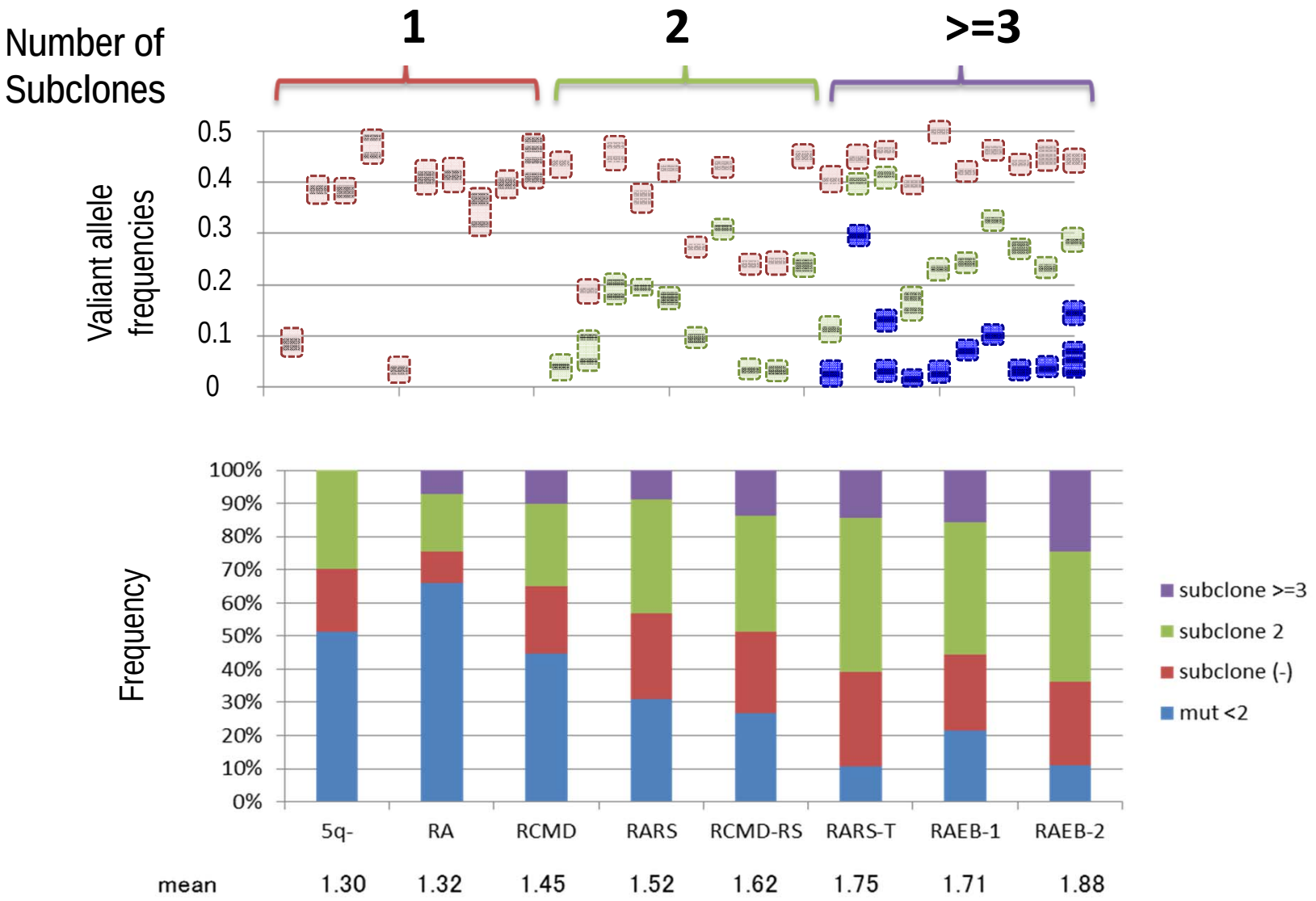


- ✓ **Mechanism of cancer development**
- ✓ **Relapse**
- ✓ **Resistance to anti-tumor therapy**

MDSにおける亜集団構造

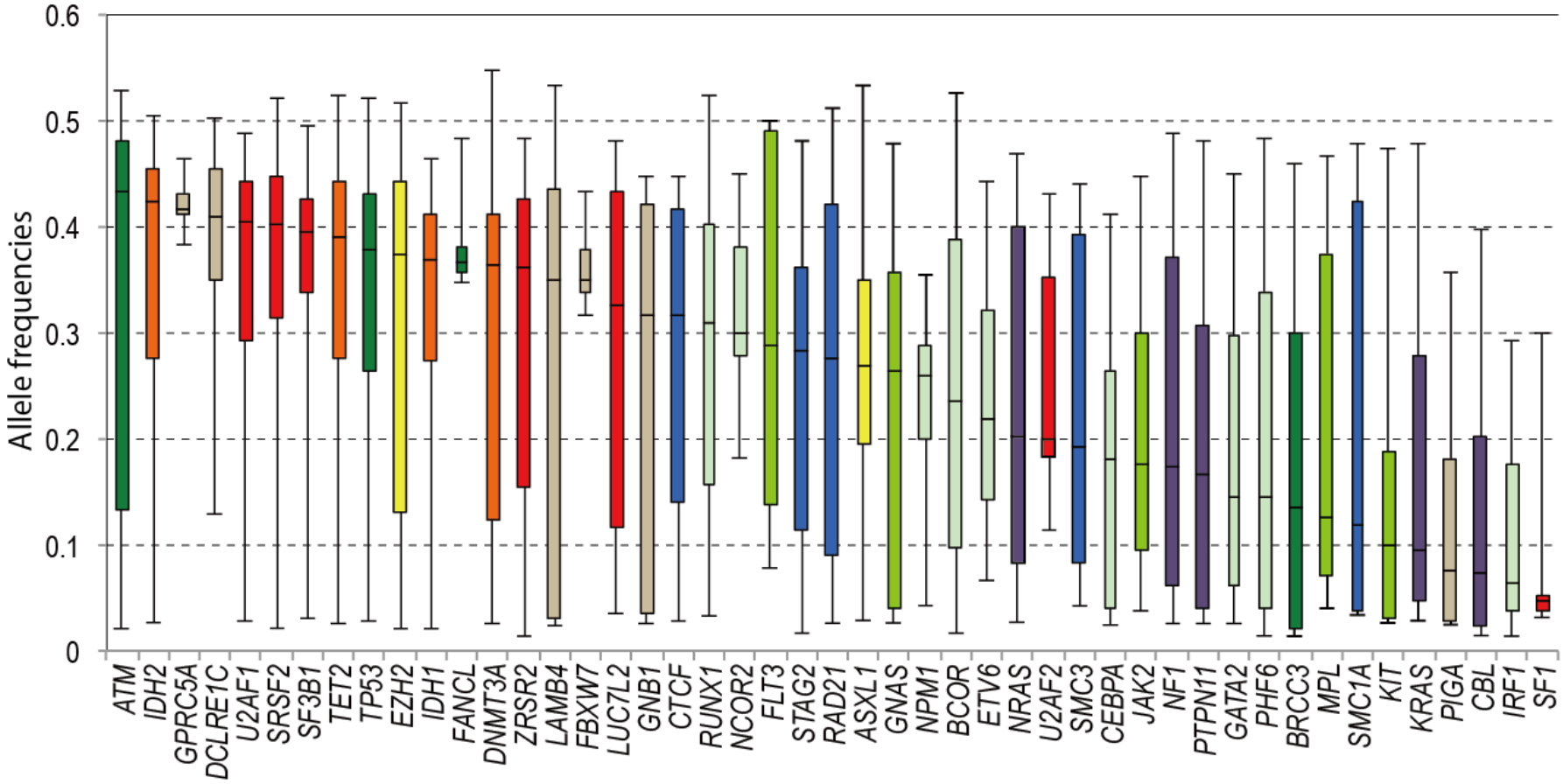


MDSにおける腫瘍内多様性

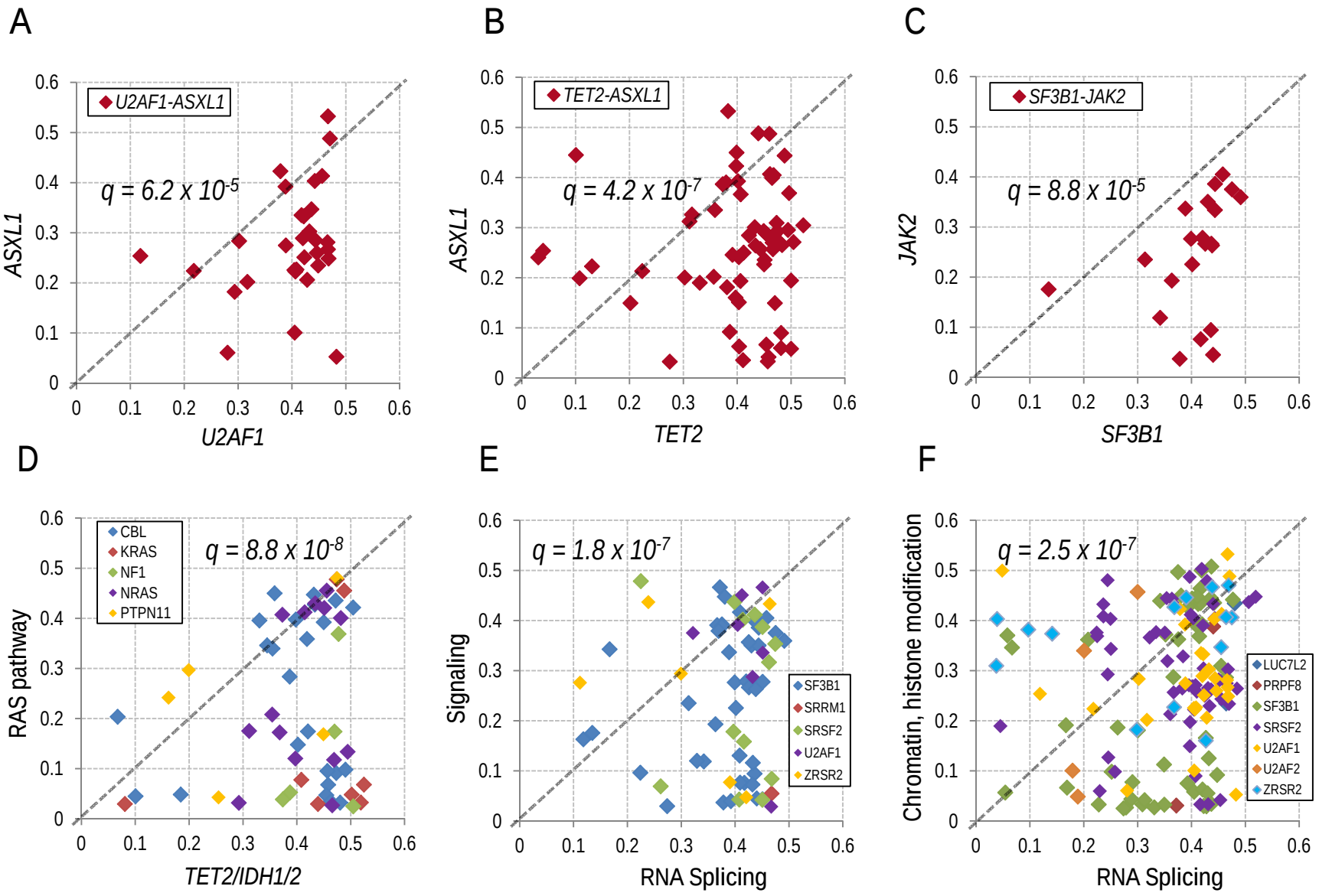


Intratumor heterogeneity was evident in 456 cases. (48.3%)

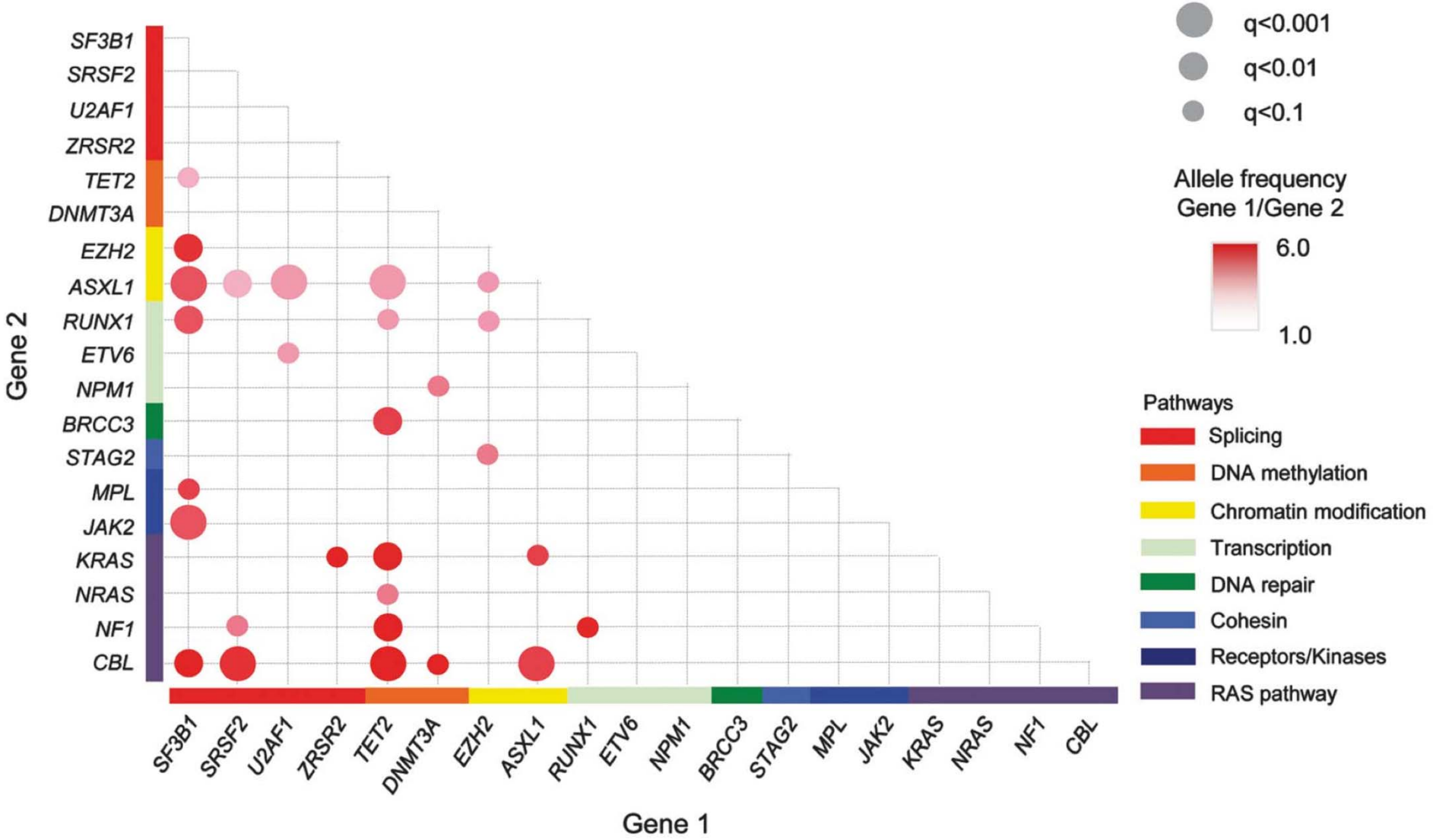
Variations among variant allele frequencies



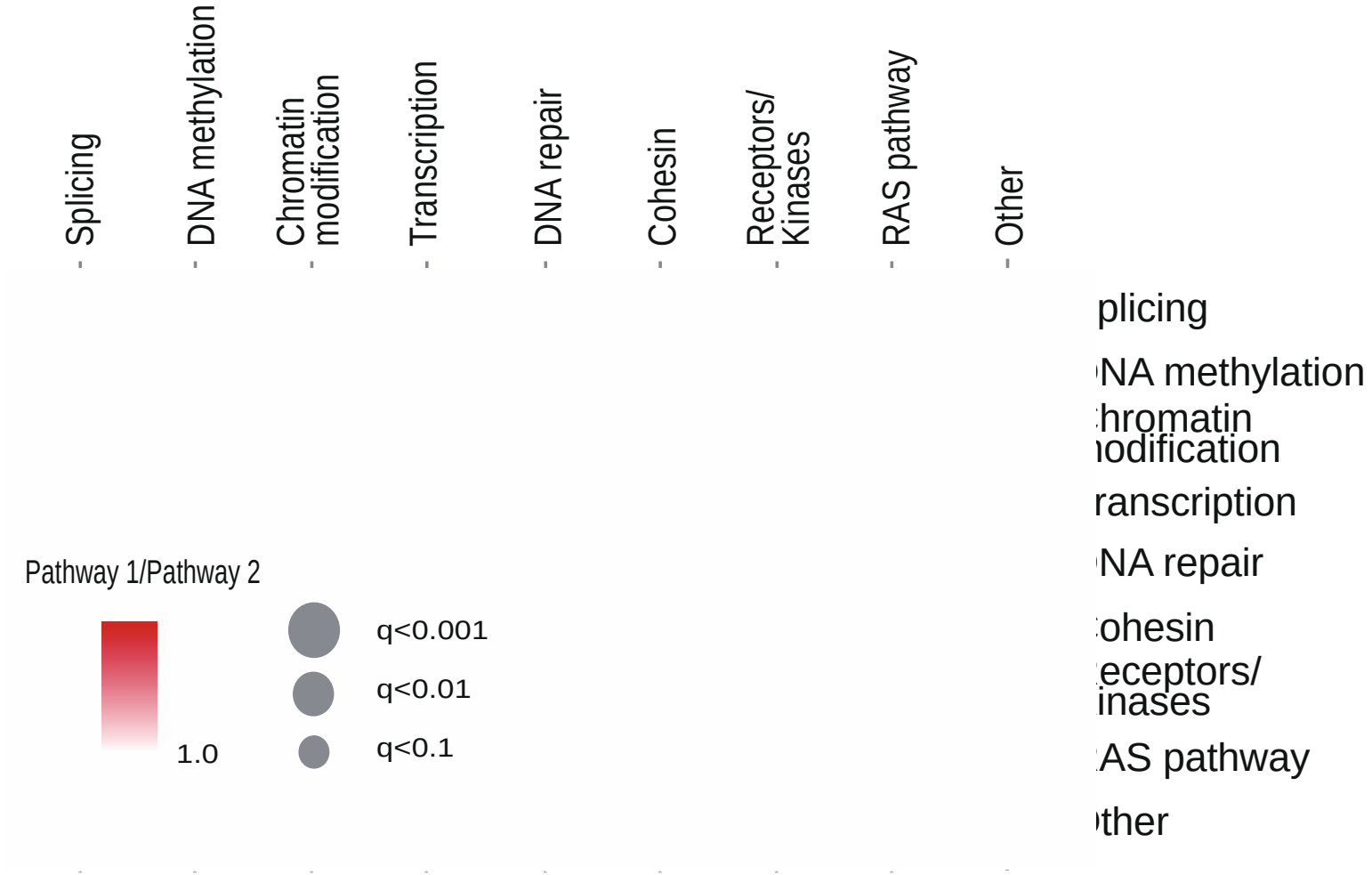
Significant difference in allele freq between common mutations



変異アレル頻度の比較

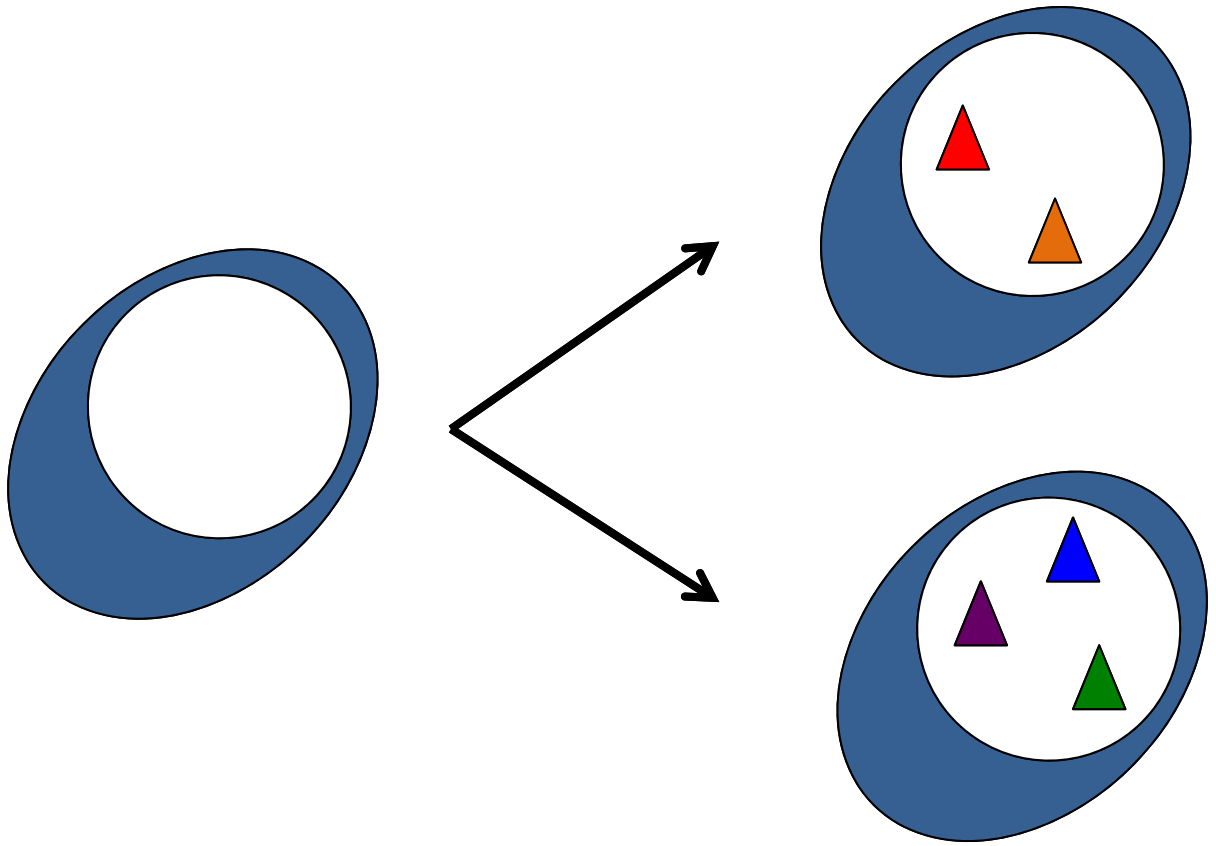

Gene 2
SF3B1
SRSF2
U2AF1
ZRSR2
TET2
DNMT3A
EZH2
ASXL1
RUNX1
ETV6
NPM1
BRCC3
STAG2
MPL
JAK2
KRAS
NRAS
NF1
CBL
Gene 1
q<0.001
q<0.01
q<0.1
Allele frequency
Gene 1/Gene 2
6.0
1.0
Pathways
Splicing
DNA methylation
Chromatin modification
Transcription
DNA repair
Cohesin
Receptors/Kinases
RAS pathway

MDSにおける変異のヒエラルキー



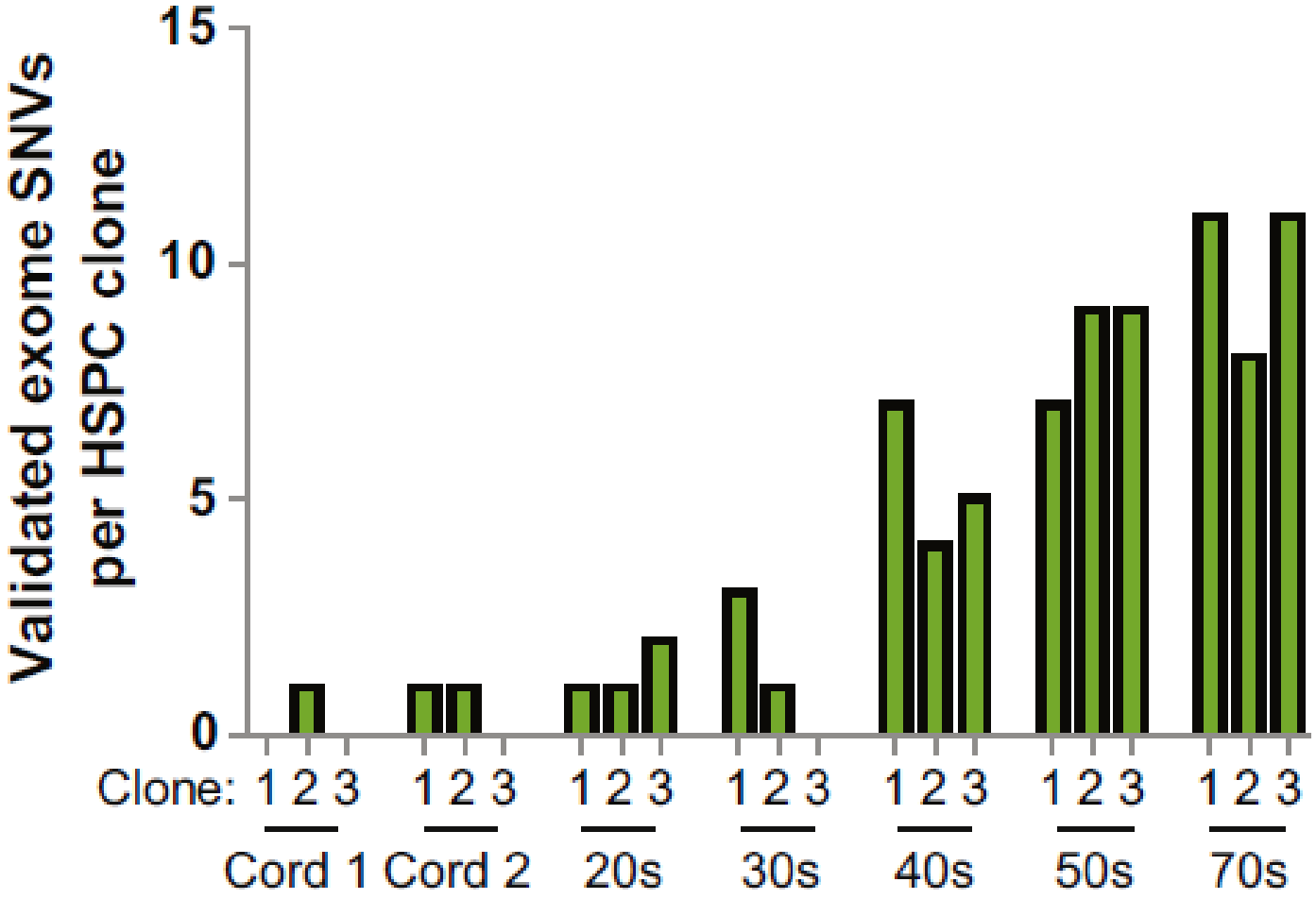
(Torsten, et al., under review)

変異の起源



～0.2-4.5 per haploid genome/devision (Gundry and Vijg, 2012; Lynch, 2010).

健常人造血前駆細胞における変異数



(Welch, J et al. Cell, 2012)

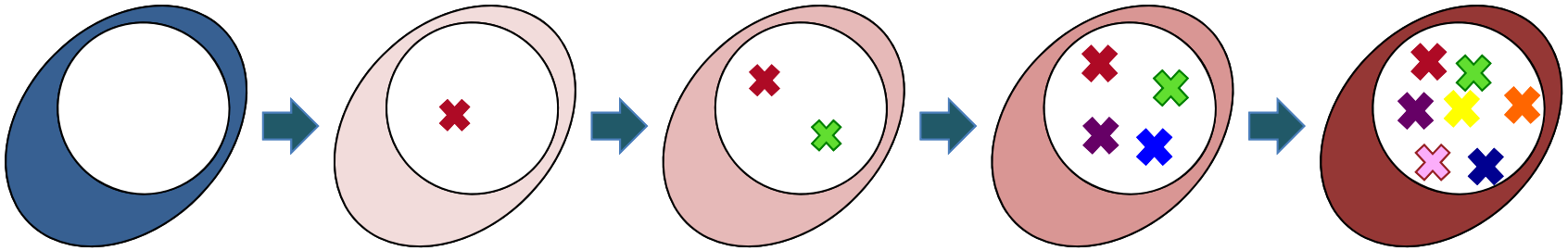
Origin of mutations and MDS clones

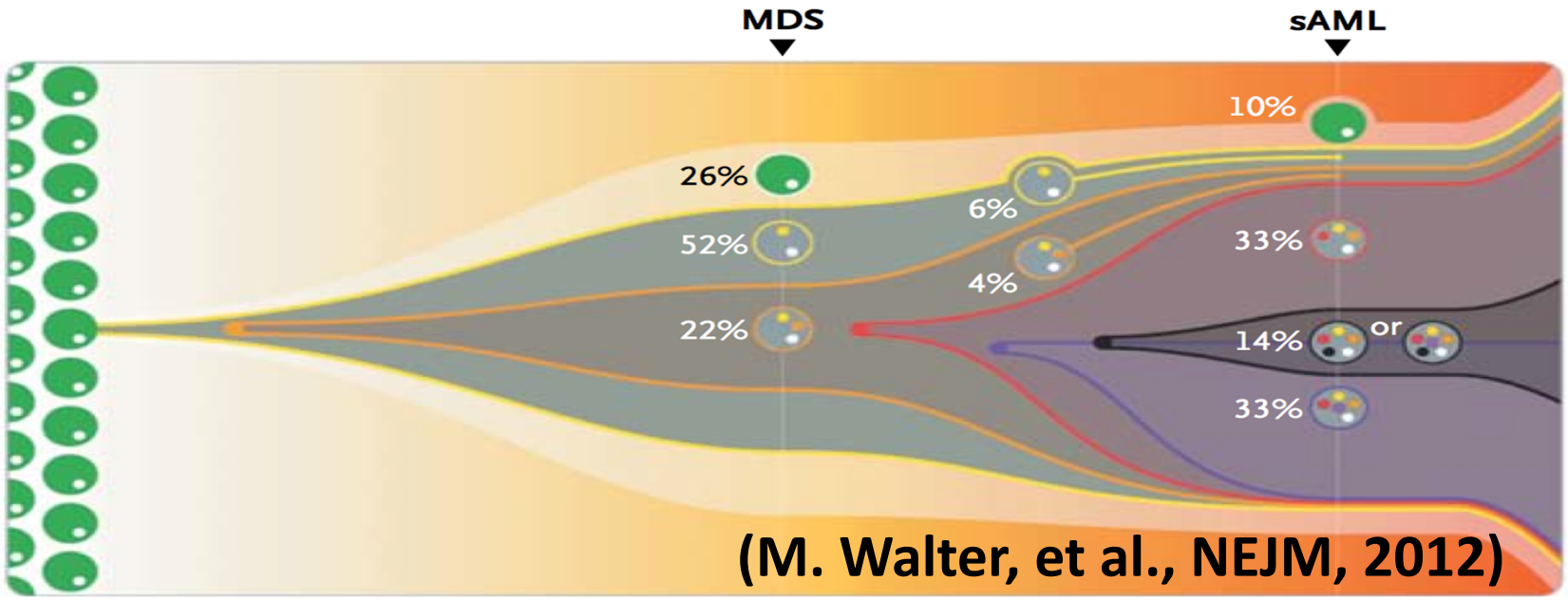
HSC
Progenitor cells

Splicing factors
TET2/IDH1/2

EZH2/ASXL1
RUNX11,
***ETV6*, etc..**

SETBP1
RAS pathway
***JAK2/cMPL* etc**





(M. Walter, et al., NEJM, 2012)

健常人におけるTET2 変異とクローン性造血

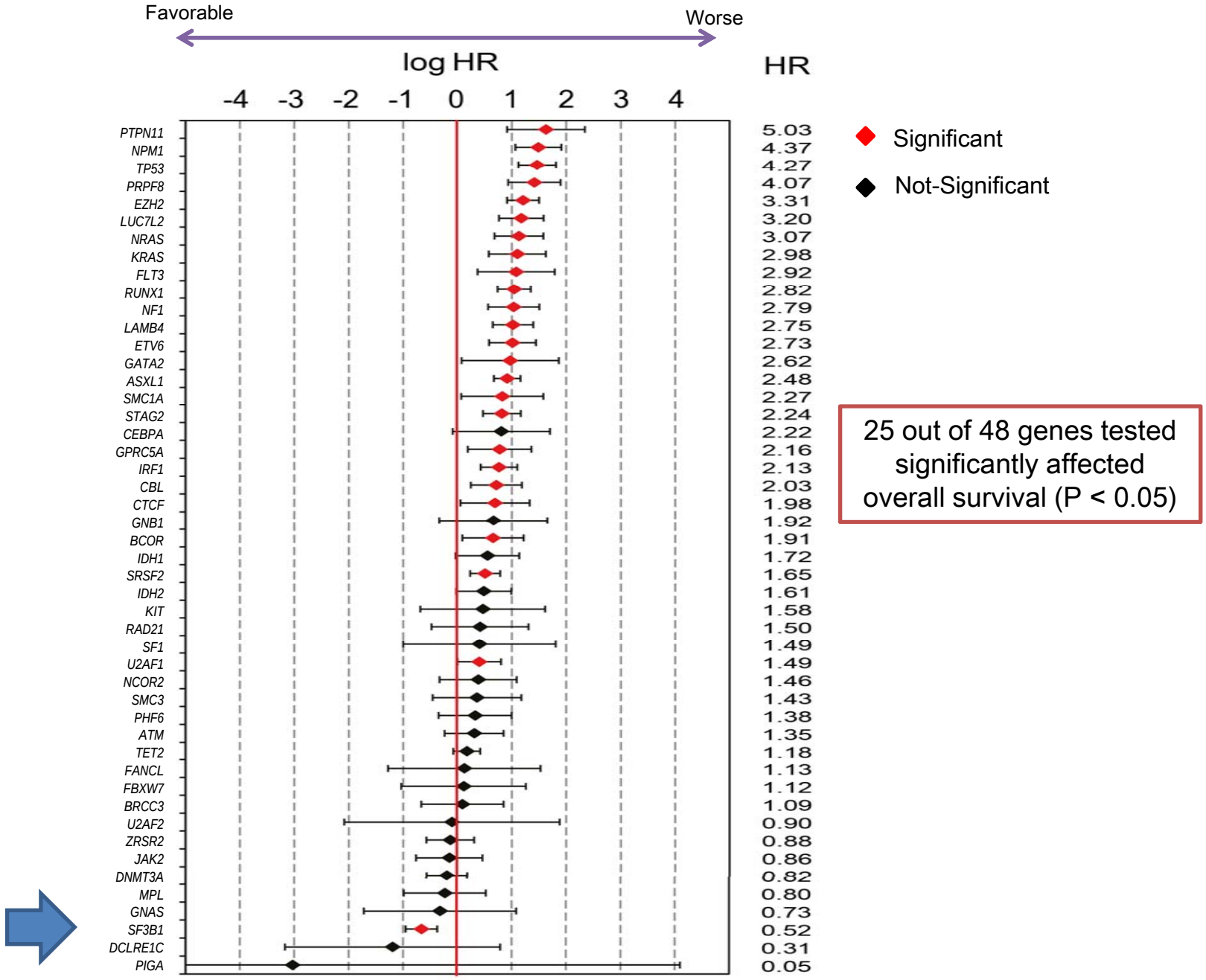
Recurrent somatic *TET2* mutations in normal elderly individuals with clonal hematopoiesis

Lambert Busque[1–3,16], Jay P Patel[4,16], Maria E Figueroa[5], Aparna Vasanthakumar[6], Sylvie Provost[7], Zineb Hamilou[2,3], Luigina Mollica[1–3], Juan Li[8], Agnes Viale[8], Adriana Heguy[4], Maryam Hassimi[9], Nicholas Socci[9], Parva K Bhatt[4], Mithat Gonen[10], Christopher E Mason[11,12], Ari Melnick[12,13], Lucy A Godley[6], Cameron W Brennan[4,14], Omar Abdel-Wahab[4,15,17] & Ross L Levine[4,12,15,17]

Aging is characterized by clonal expansion of myeloid-biased hematopoietic stem cells and by increased risk of myeloid malignancies. Exome sequencing of three elderly females with clonal hematopoiesis, demonstrated by X-inactivation analysis, identified somatic *TET2* mutations. Recurrence testing identified *TET2* mutations in 10 out of 182 individuals with X-inactivation skewing. *TET2* mutations were specific to individuals with clonal hematopoiesis without hematological malignancies and were associated with alterations in DNA methylation.

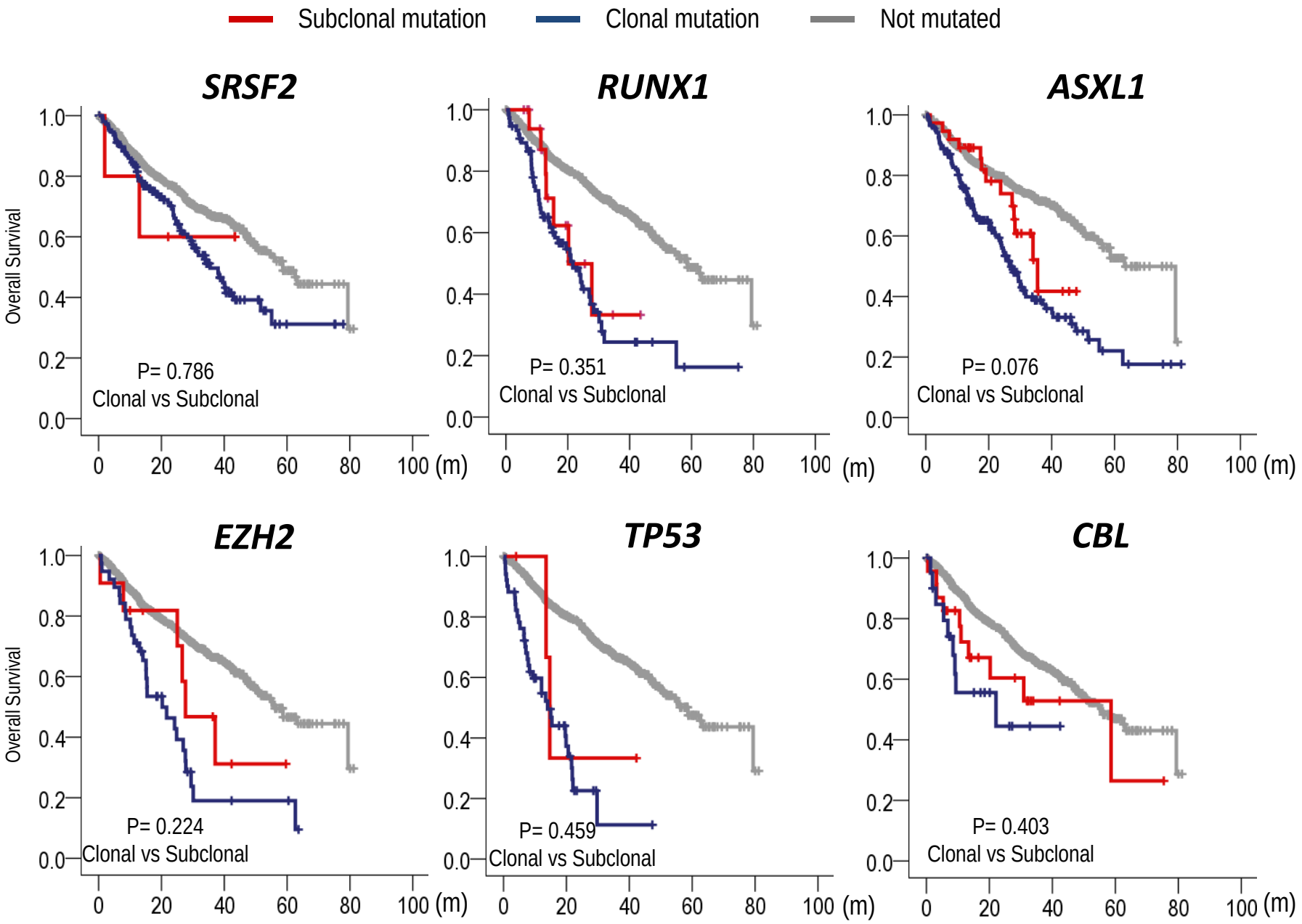
-Y is also observed in older individuals : clonal hematopoiesis

Hazard ratios of mutated/deleted genes in univariate analysis



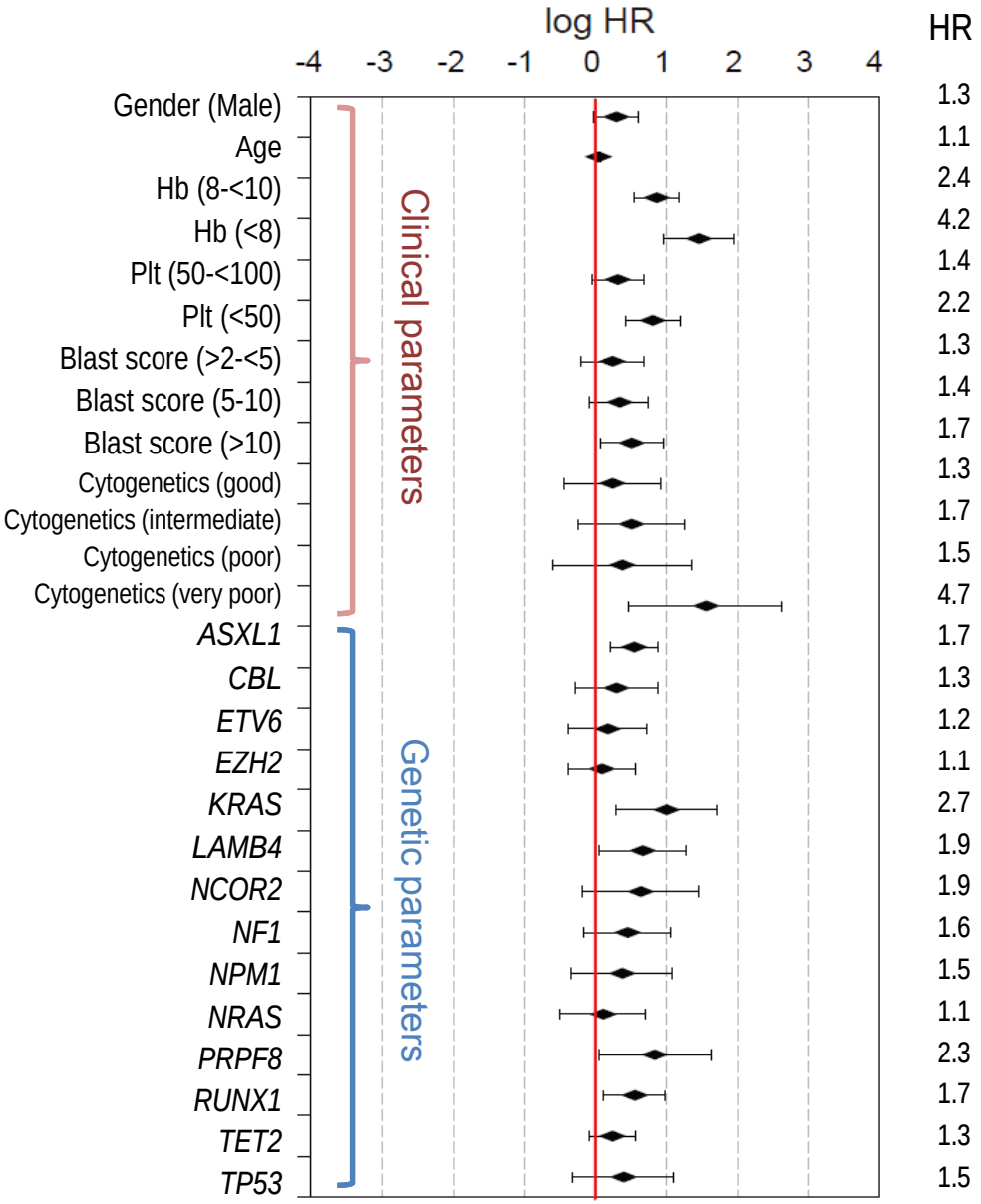
25 out of 48 genes tested significantly affected overall survival (P < 0.05)

サブクローン性変異が予後に及ぼす影響

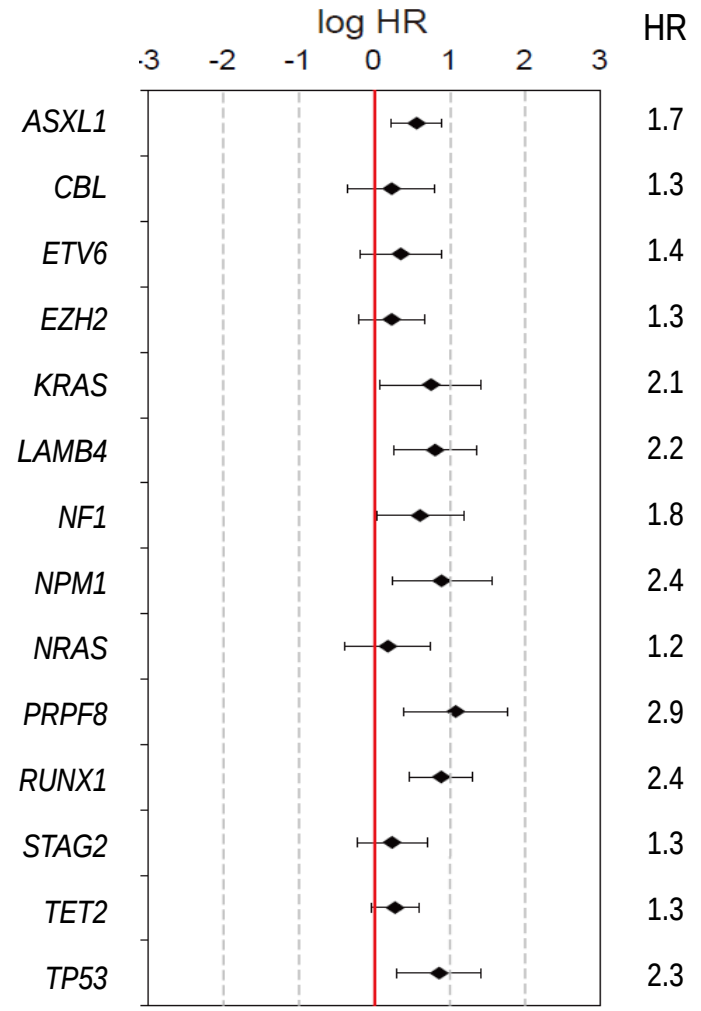


Hazard ratios in multivariate analysis

(A) clinical and genetic parameters (Model-1)



(B) Only genetic parameters (Model-2)



Variables were selected by LASSO

Development of a novel prognostic risk classification

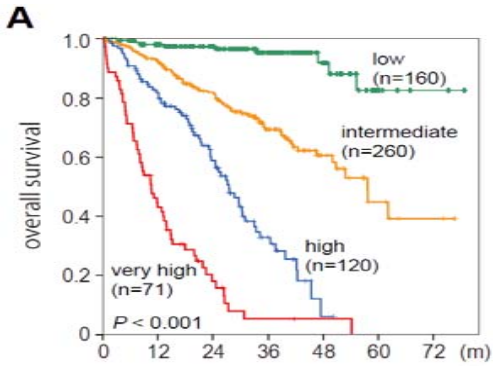
(Training cohort, N=611)

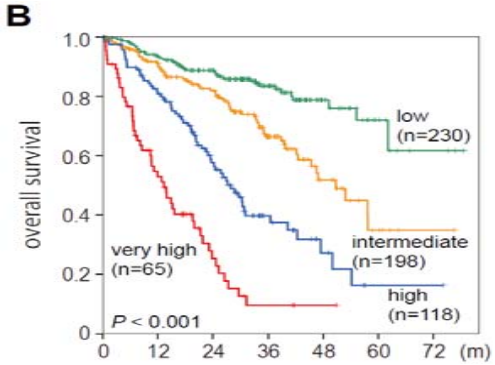
(Validation cohort , N=175)

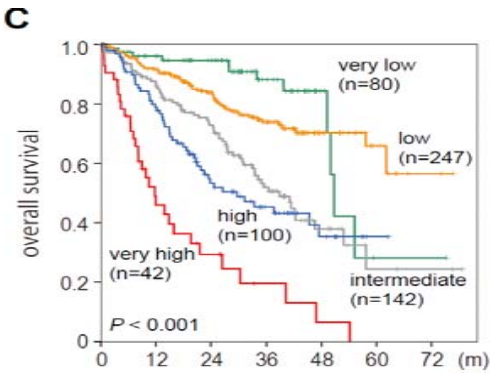
Clinical and genetic parameters (Model-1)

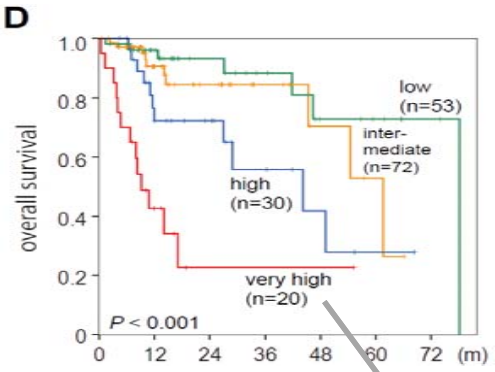
Only genetic parameters (Model-2)

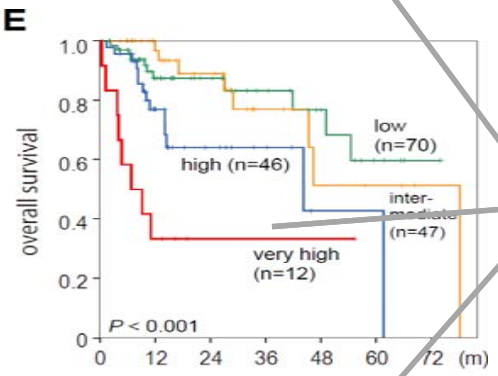
Conventional model (IPSS-R)

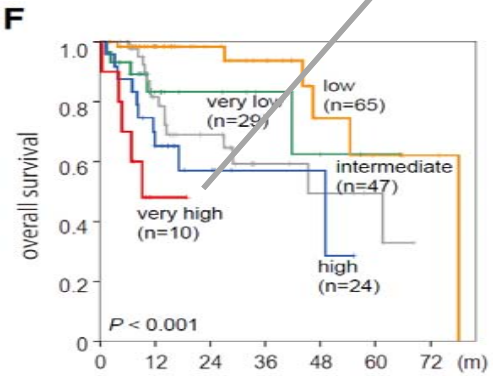




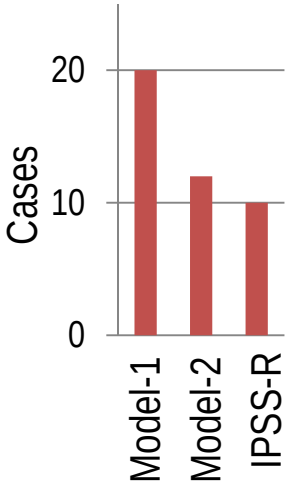






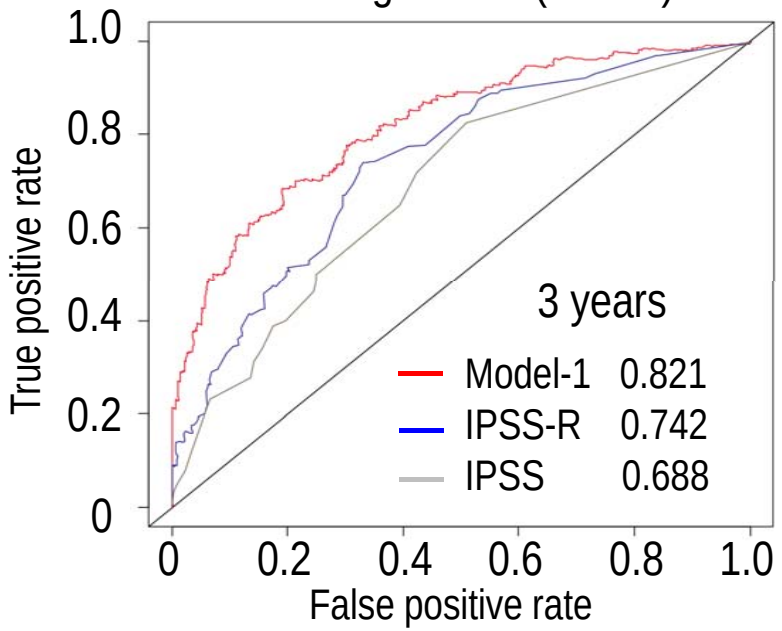


The number of very high risk cases

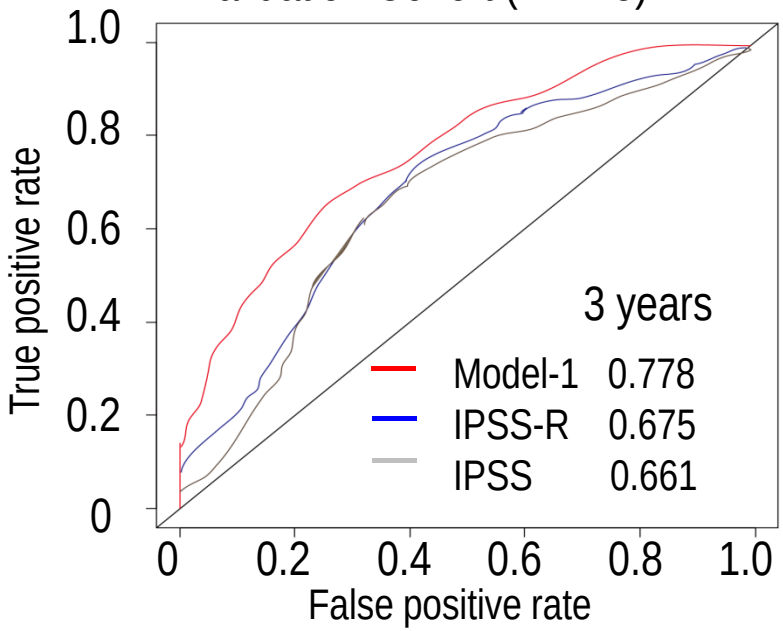


Model-1 was expected to outperform the IPSS-R

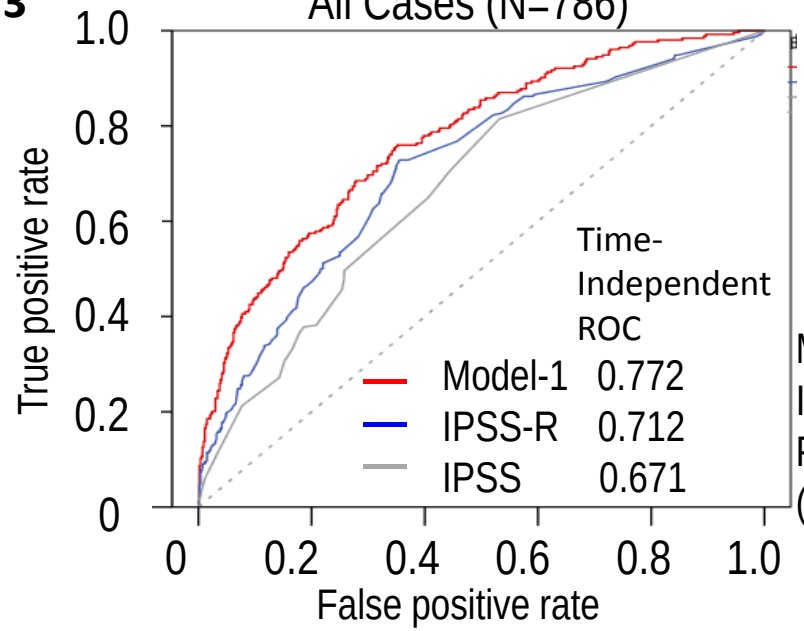
1 Training Cohort (N=611)



2 Validation Cohort (N=175)



3 All Cases (N=786)



Model1 vs IPSS-R P=0.0004 (Delong Test)

4 Comparison between Model-1 and IPSS-R by statistical procedure

Model	Validation cohort (N=175)		
	AIC	J test	
		Add Model1 to IPSS-R	Add IPSS-R to Model1
Model-1	327.2	P<0.001	P=0.07
Model-2	337.5	-	-
IPSS-R	334.8	-	-

AIC (Akaike's Information Criterion)

Summary

1. Massively parallel sequencing technologies enabled identification of a full spectrum of gene mutations in MDS.
2. Major mutational targets in MDS included splicing factor genes (*SF3B1, SRSF2, U2AF1 and ZRSR2),* epigenetic regulators, and other novel gene targets*, such as BCOR, SETBP1, PPRF8, and* multiple components of the cohesin complex.
3. Intratumoral heterogeneity is common in MDS, indicating multiple round of acquisition of new mutations and clonal selections shapes the development and progression of MDS, in which multiple gene mutations occurred in a hierarchical fashion.
4. Molecular profiling of these mutations in a large MDS cohort clarified their impacts on clinical outcomes and disease phenotypes, enabling to construct a powerful prognostic model that outperforms current models*.*

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