Exome Sequencing Points to Differences in Genenetic Instability Level in MGUS Compared to MM

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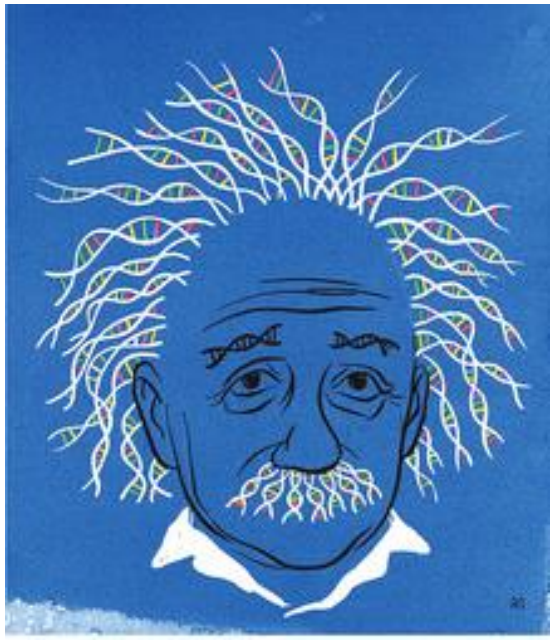

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Disclosure

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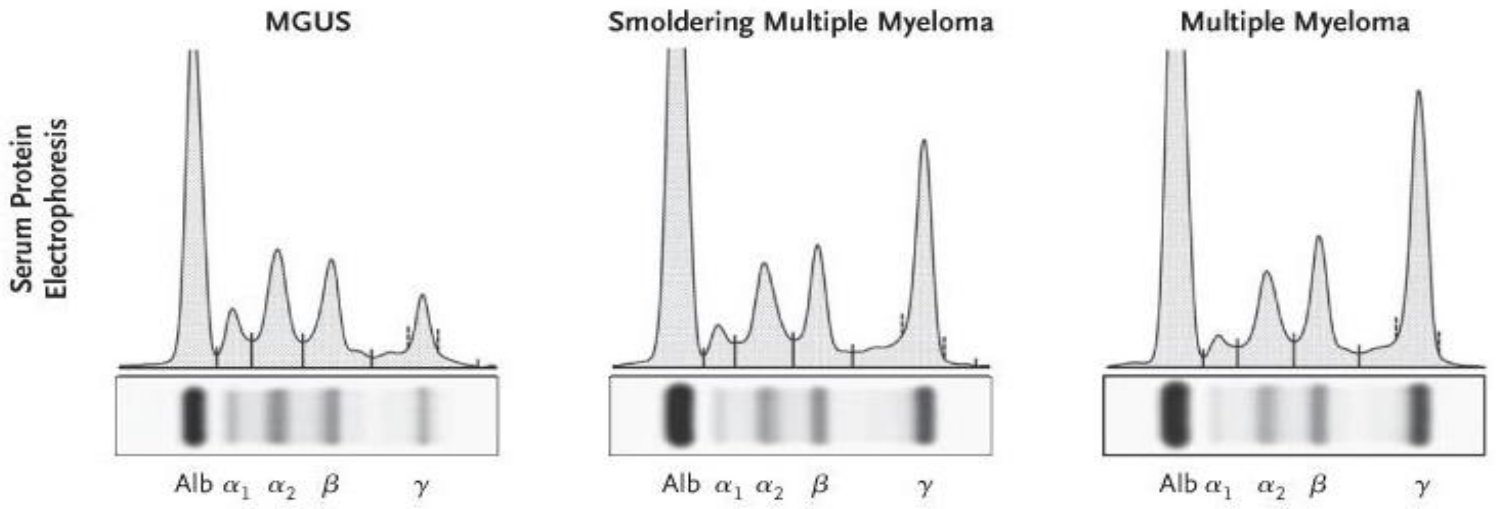
no relevant conflicts of interest to disclose

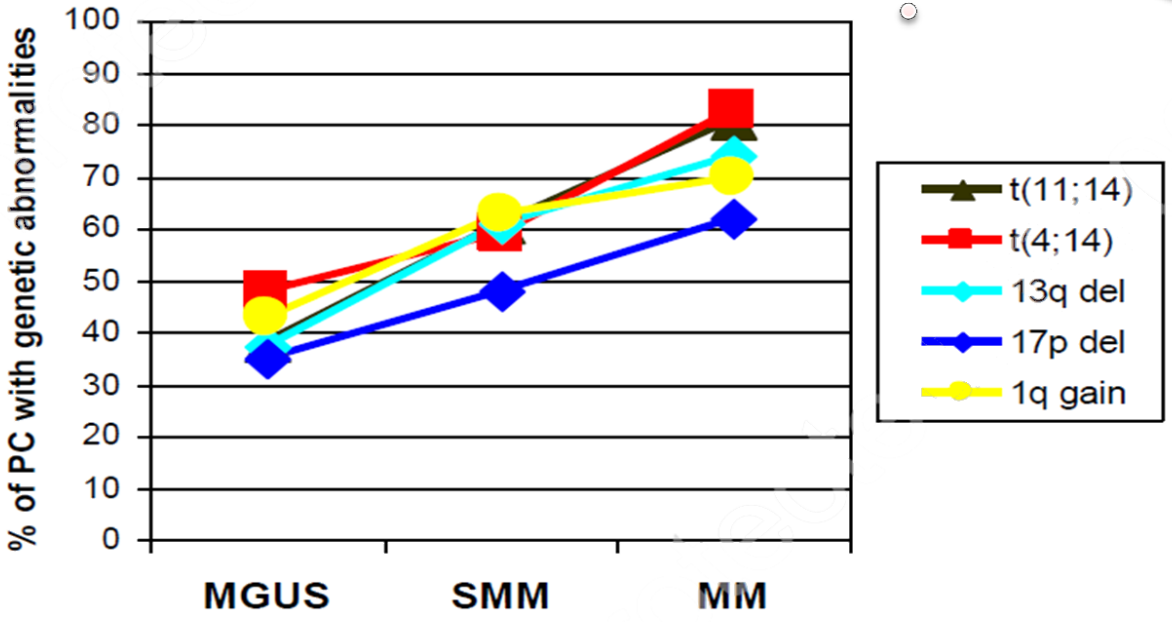
Monoclonal Gammopathies (MG)

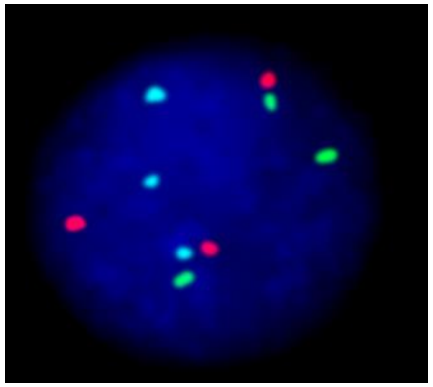


Feature	MGUS	SMM	MM
BMPC (%)	<10	≥10	≥10
Serum M-protein (g/dL)	<3	≥3	≥3
Clinical manifestation	Absent	Absent	Present

Evolution of Methods in MG

Cytogenetics **FISH** aCGH NGS





Clonal expansion of genetic abnormal PCs during malignant transformation of MGUS to MM

Lopéz-Corral L. *et al.* 2011. Clin. Cancer Res. 17(7): 1692-1700.

Evolution of Methods in MG

Cytogenetics FISH **aCGH** NGS

MGUS
90% (18/20)

SMM
85% (17/20)

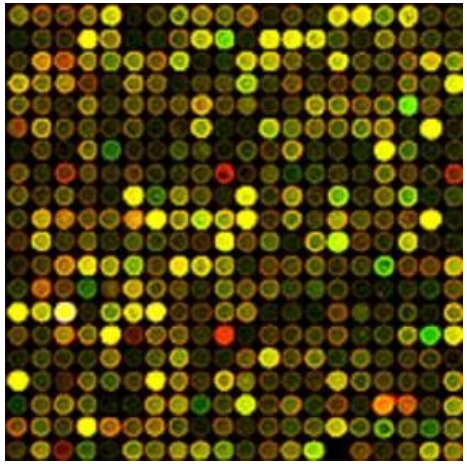
MM
100% (34/34)

5 (0-12)

7.5 (0-23)

12 (1-32)

CNAs median (range)

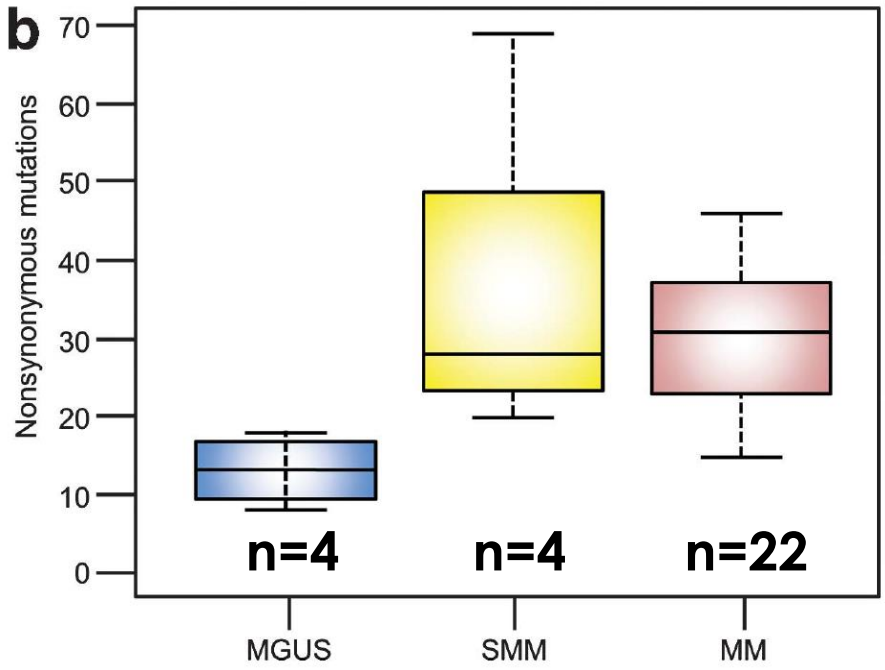


CNAs number increasing from MGUS to MM

López-Corral L. *et al.* 2012. Leukemia 26(12): 2521-2529.

Evolution of Methods in MG

Cytogenetics FISH aCGH **NGS**



	MGUS	SMM	MM
Median (range)	**13 (8-18)**	**28 (20-69)**	**31 (15-46)**

SNVs number increasing from MGUS to MM

Walker B. et al. 2014. Leukemia 28(2): 384-390.

Aim of Study

Analysis of somatic gene mutations (SNVs) by exome sequencing together with copy-number alterations (CNAs) by CGH+SNP arrays in MGUS patients to describe comprehensive genetic instability at both gene and chromosome level.

MM cohorts for comparison:

33 MM cohort analysed by aCGH

463 MM cohort analysed by NGS
(Walker *et al.* 2015)

Methods

Peripheral Blood

DNA isolation

Bone Marrow

Abnormal PCs separation $CD138^{+}CD19^{-}CD56^{+/-}$

FACSAria (BD Biosciences)

DNA isolation, **whole genome amplification**

n=33

Exome Sequencing

SureSelect Human All Exom V5 (Agilent) capture enriched by *IGH*, *IGK*, *IGL* and *MYC*
HiSeq 2000 (Illumina), 4 pooled samples per one lane, 76-bp paired ends reads

n=97

CGH+SNP arrays

SurePrint G3 CGH+SNP, 4x180K (Agilent)
Microarray Scanner (Agilent)

Data Analysis

SNP array data analysis: Feature Extraction Software, Genomic Workbench (both Agilent)
Exom data viewer: Integrative Genomics Viewer (Broad Institute)
Exom data processing tools and algorithms: FastQC, BWA/Stampy, GATK, Picard (Broad Institute)
Exom data analysis: MuTect, Indelocator, SnpEff, MutSigCV (Broad Institute)
Basic statistics: Statistica (StatSoft), MedCalc (MedCalc Software)

Differences in Whole Genome Profiling of CNAs

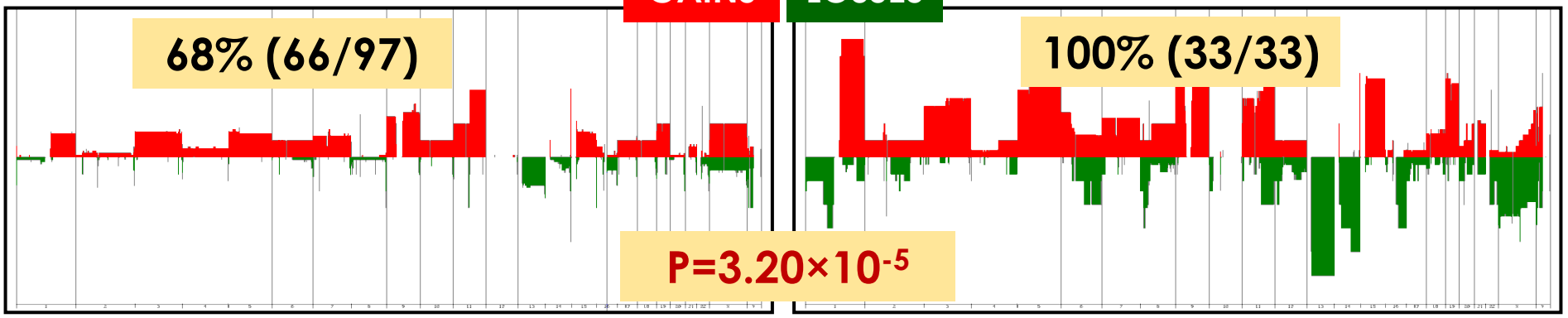
Genome-wide profiles of CNAs

MGUS (n=97)

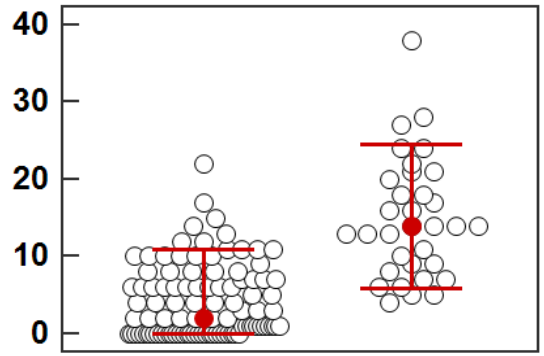
MM (n=33)

GAINS

LOSSES

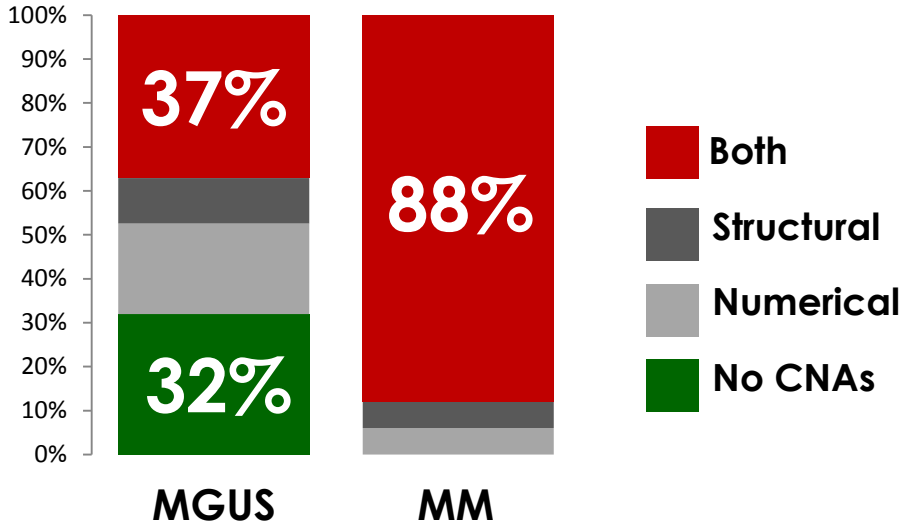


Number of CNAs per patient



P=1.53×10-11

Numerical and structural CNAs distribution



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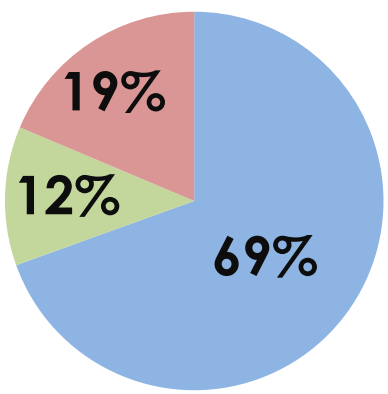
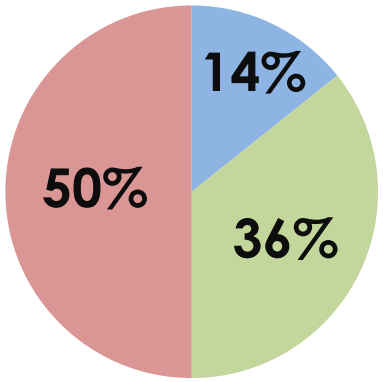
Numerical CNAs

Non-hyperdiploidy

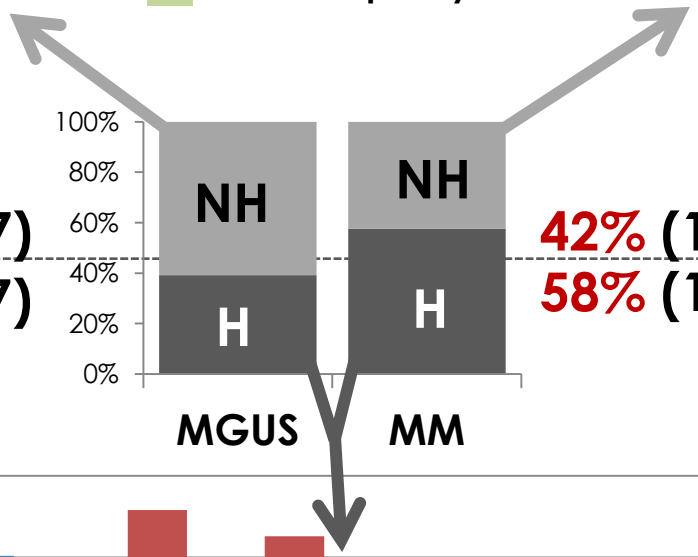
Non-hyperdiploid MGUS is usually diploid, while MM hypo/pseudodiploid.



Diploidy
Hypodiploidy
Pseudodiploidy



61% (59/97)
39% (38/97)

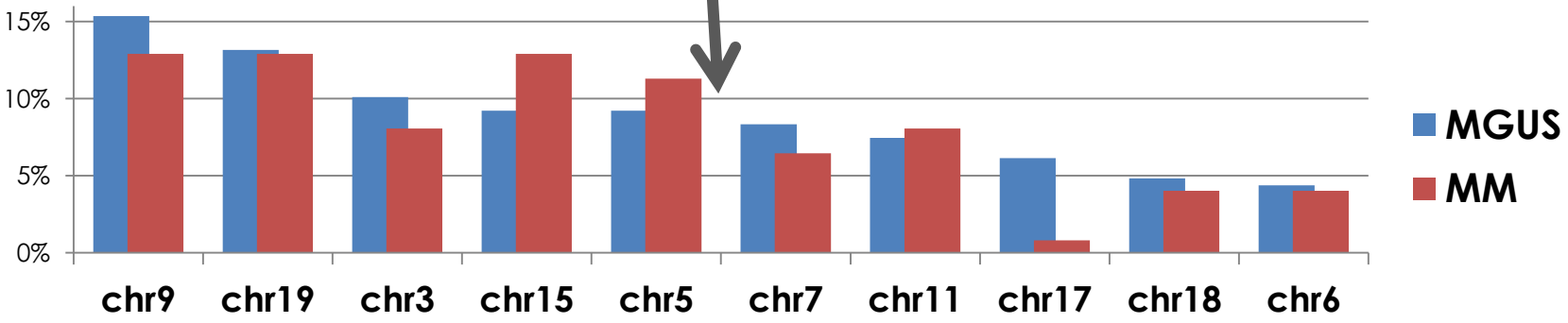


42% (14/33)
58% (19/33)

P=0.071

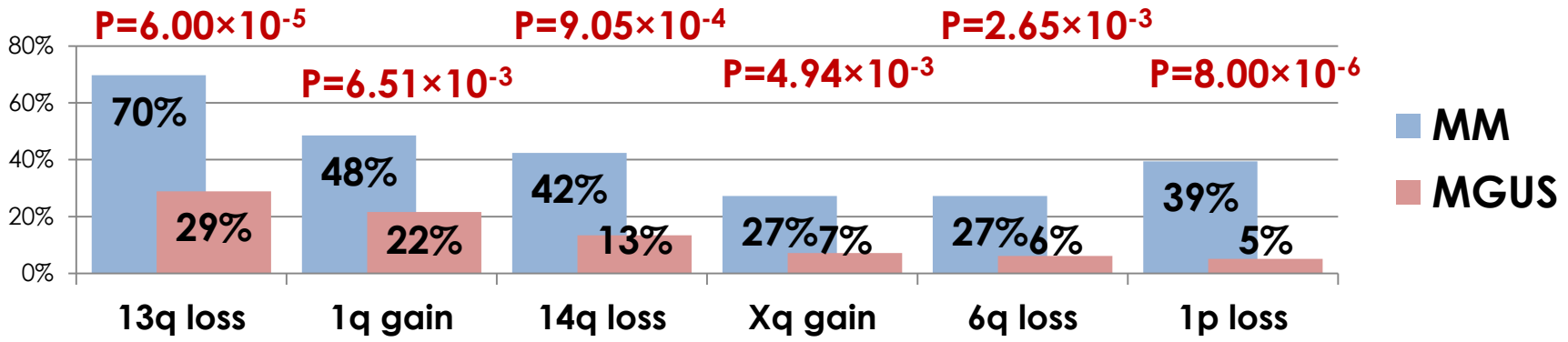
=> PRIMARY EVENT

Hyperdiploidy



Distribution of whole chromosome gains is similar to MM.

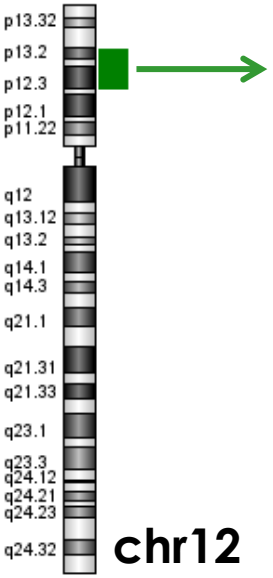
Structural CNAs



Frequency of structural CNAs is significantly lower in MGUS compared to MM.

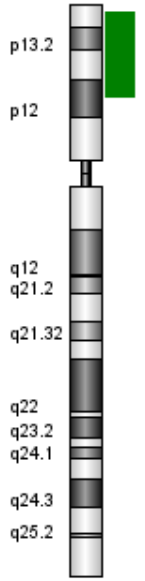
Potential risk chromosome areas:

- 12p loss
- 17p loss
- 10p loss
- 20q loss
- 6p loss



chr12

ETV6
CDKN1B
BCL2L14
GPRC5A



chr17

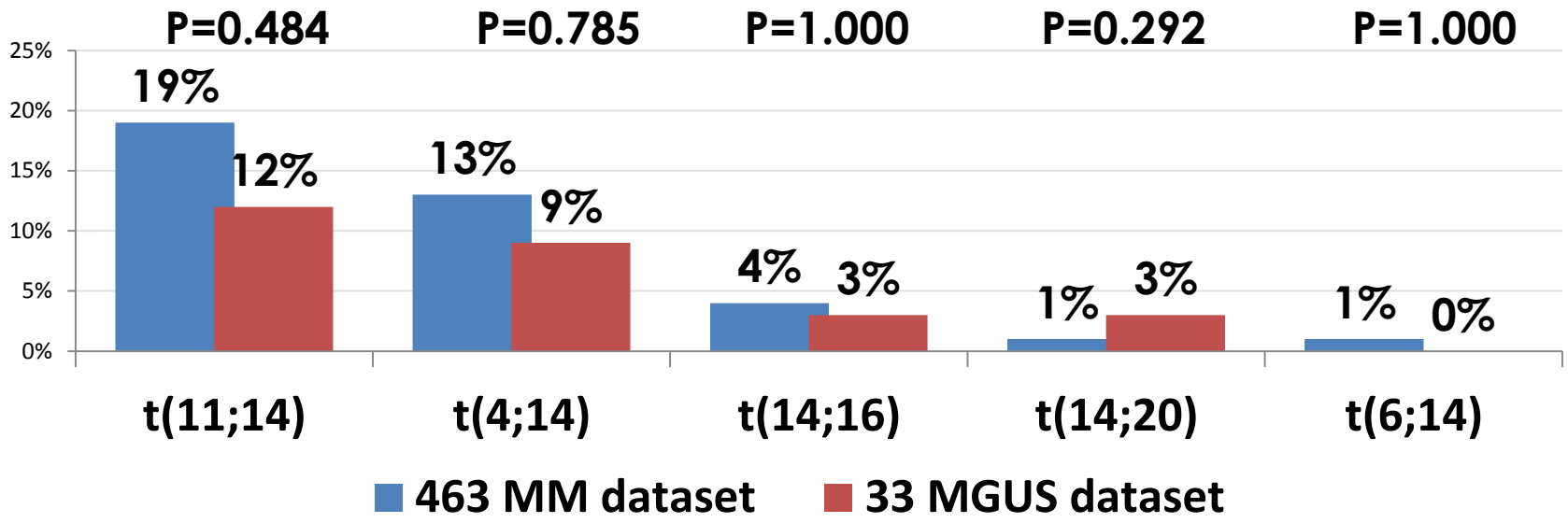
TP53
HIC1
MYBBP1A

Chromosome Translocations

33 MGUS compared to 463 MM analysed by NGS (Walker *et al.* 2015)

IGH gene (14q32.33) translocations are present in similar frequence in MGUS compared to MM.

=> PRIMARY EVENT



No MGUS case with _MYC_ (8q24.21), _IGK_ (2p12) and _IGL_ (22q11.2) gene translocations

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Walker *et al.* 2015. In print.

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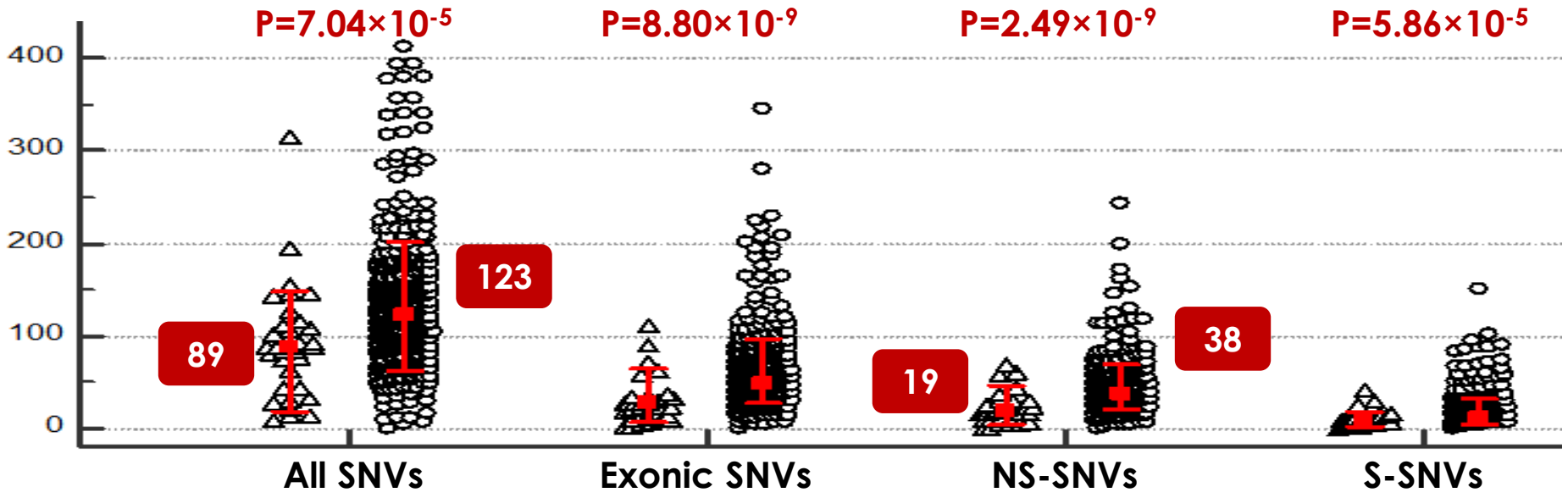
Somatic Gene Mutations

33 MGUS compared to 463 MM analysed by NGS (Walker *et al.* 2015)

	MGUS	MM
All SNVs	100% (33/33)	100% (463/463)
NS-SNVs	97.0% (32/33)	99.8% (462/463)



Somatic mutations are present in all MGUS patients, but in significantly lower level compared to MM.

Walker *et al.* 2015. In print.

Somatic Gene Mutations

33 MGUS compared to 463 MM analysed by NGS (Walker *et al.* 2015)

6 myeloma-significantly mutated genes:

MGUS	Gene	Mutation
1	***EGR1***	c.85A>C (p.M29L)
	LTB	c.167C>T (p.T56M)
2	***KRAS***	c.182A>T (p.Q61)
	NRAS	c.37G>C (p.G13R)
3	***HIST1H1E***	c.169G>C (p.V57L)
	HIST1H1E	c.266G>C (p.S89T)
	PRKD2	c.1697A>G (p.Y566C)
4	***KRAS***	c.436G>A (p.A146T)
5	***HIST1H1E***	c.193G>C (p.A65P)
	DIS3	c.1462G>A (p.D488N)

KRAS
NRAS
HIST1H1E
DIS3
EGR1
LTB

Genes with a negative impact on MM survival:

TP53, ATM, ATR, ZFHX4 genes involved in DNA repair pathway – **none**
CCND1 gene - **MGUS case** with two mutations (p.K50T, p.E51D)

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Walker *et al.* 2015. In print.

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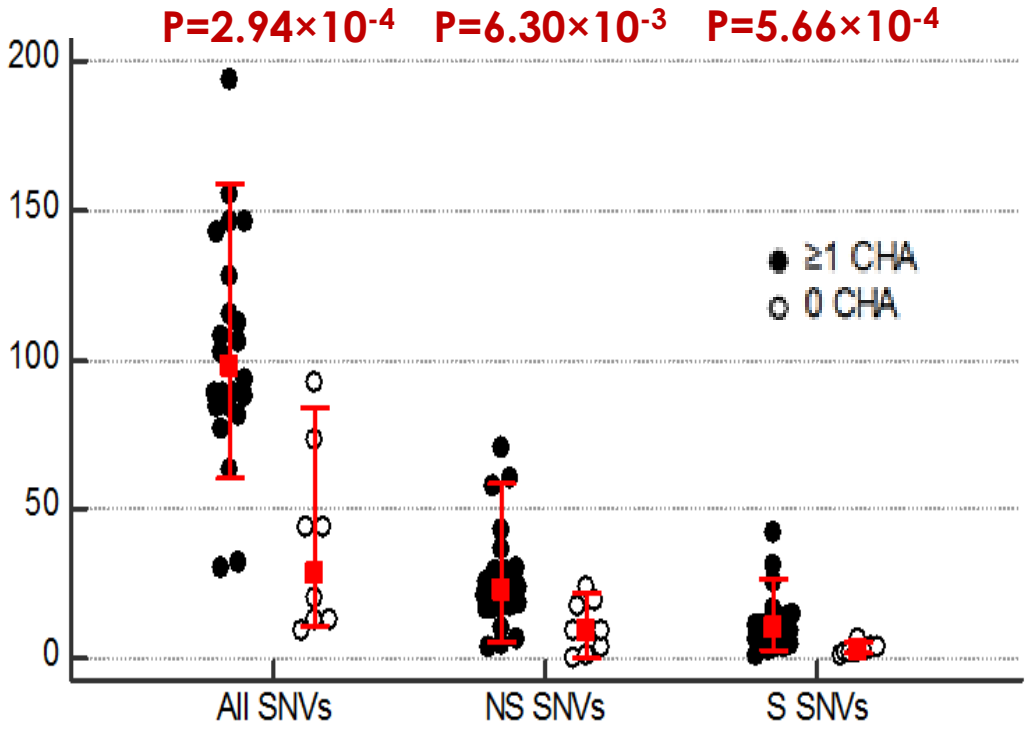
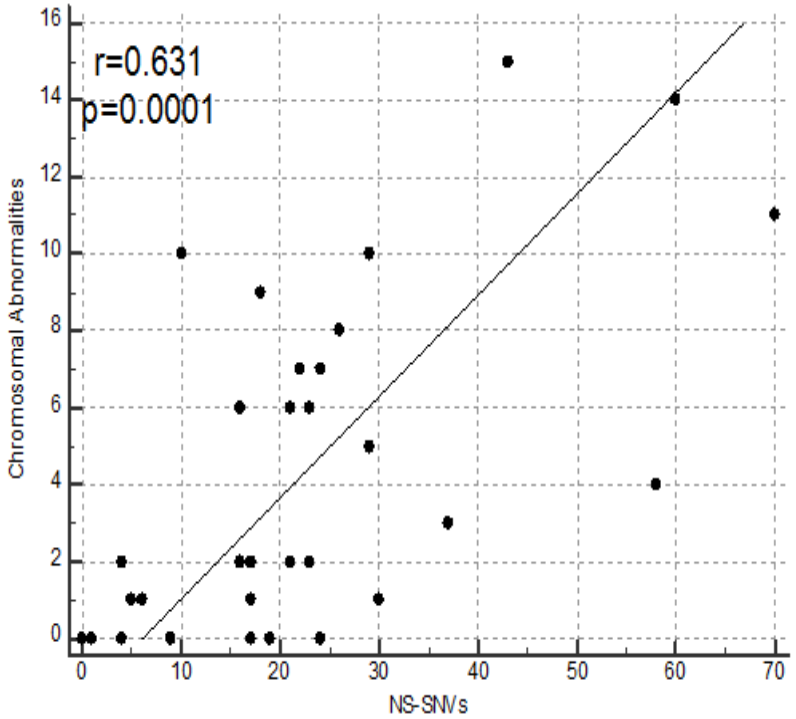
SNVs in Context to CHAs





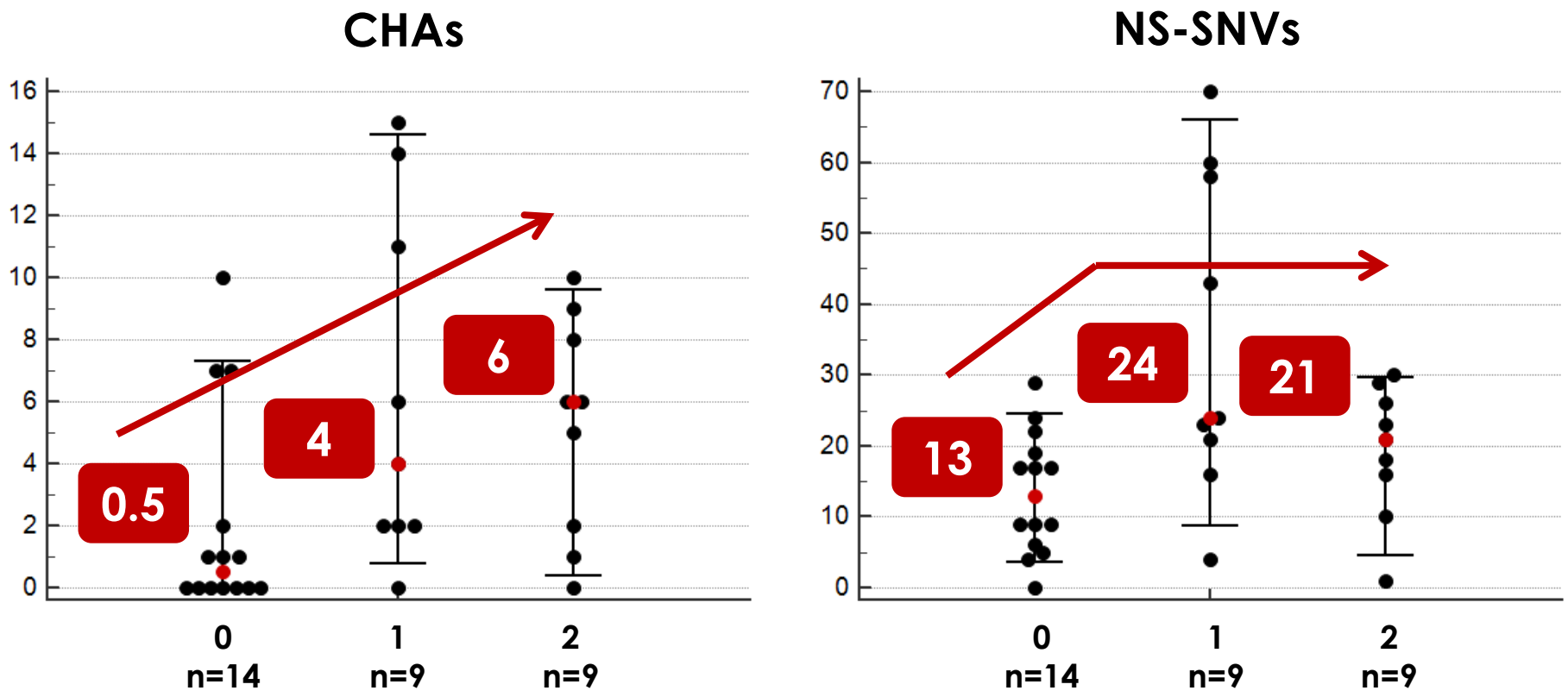
Genetic instability is formed at chromosome and gene level.

Positive correlation of increasing CNAs and SNVs.

Genetic Instability in Context to Clinical Data

Risk-stratification model by Rajkumar *et al.* 2005:

serum MIG ≥15 g/l, non-IgG subtype, abnormal FLC ratio



Median number of CHAs per patient is increasing across the risk groups.

Rajkumar *et al.* 2005. Blood 106(3): 812-817.

Summary

- Exome sequencing and SNP arrays genome-wide analysis showed **genetic instability formation precedes clinical manifestation** and at both gene and chromosome level.

- Gene level changes precede chromosome level changes.
- We confirmed *IGH* translocation and hyperdiploidy are primary genetic events.
- Overall **genetic instability is very heterogenous** in both MGUS and MM, but more complex in MM.
- **Frequency and level of CNAs and SNVs are significantly lower in MGUS.**

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