

Medulloblastoma intra-tumoural heterogeneity has potential to improve biomarker-driven therapeutic stratification.

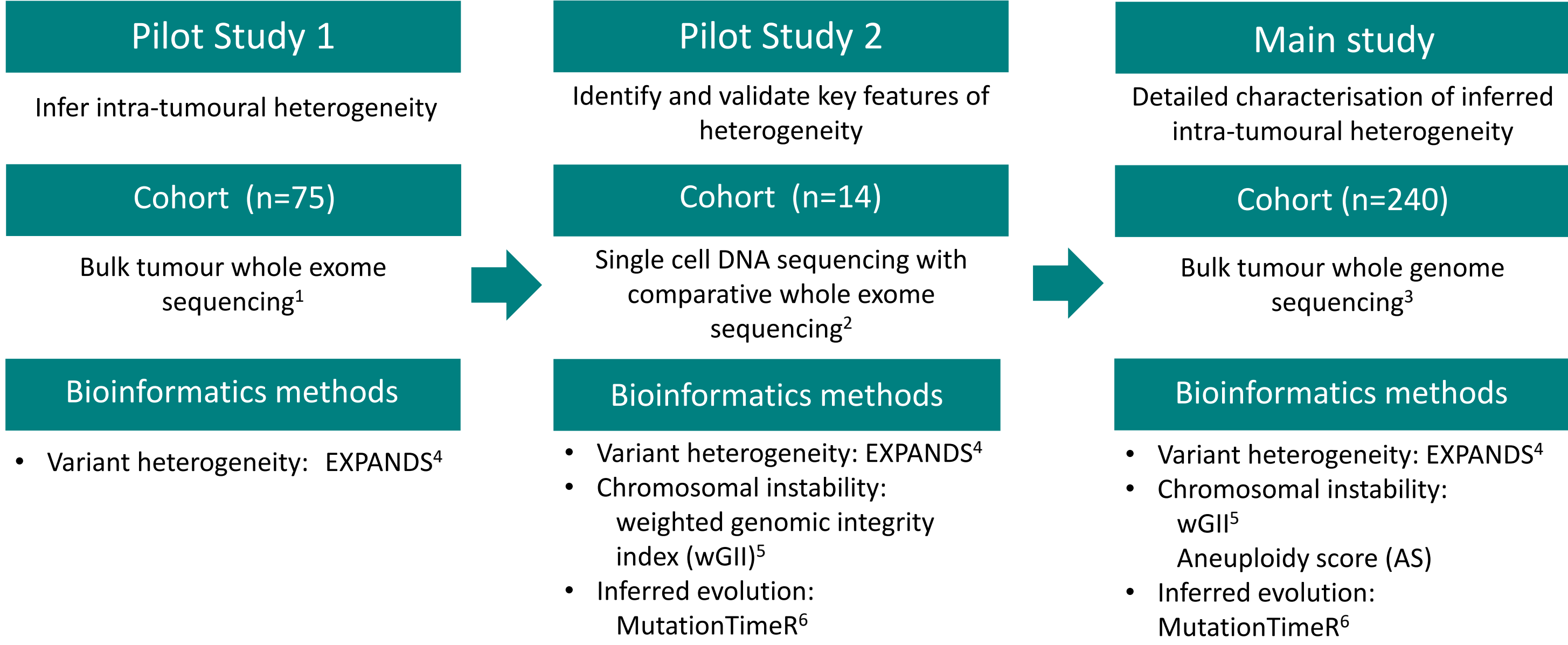
Claire Keeling¹, Marina Danilenko¹, Masood Zaka², Matthew Bashton¹, Louise Pease¹, Dean Thompson¹, Edward C Schwalbe^{1,3}, Rebecca M Hill¹, Daniel Williamson¹, Simon Bailey^{1,4}, Steven C Clifford¹
¹Newcastle University Centre for Cancer, Newcastle upon Tyne, ²National Horizons Centre, Darlington, ³Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, ⁴Great North Children's Hospital, Newcastle upon Tyne.

1. Introduction

- Medulloblastoma is a heterogeneous disease
- Its principal molecular groups (WNT, SHH, Group 3 and Group 4) impact treatment stratification and outcomes
- Survival rates have plateaued despite biological and clinical advances
- Understanding of medulloblastoma intra-tumoural heterogeneity, initiation and evolution is increasing
- Clinical relevance of medulloblastoma intra-tumoural heterogeneity features remains uncertain

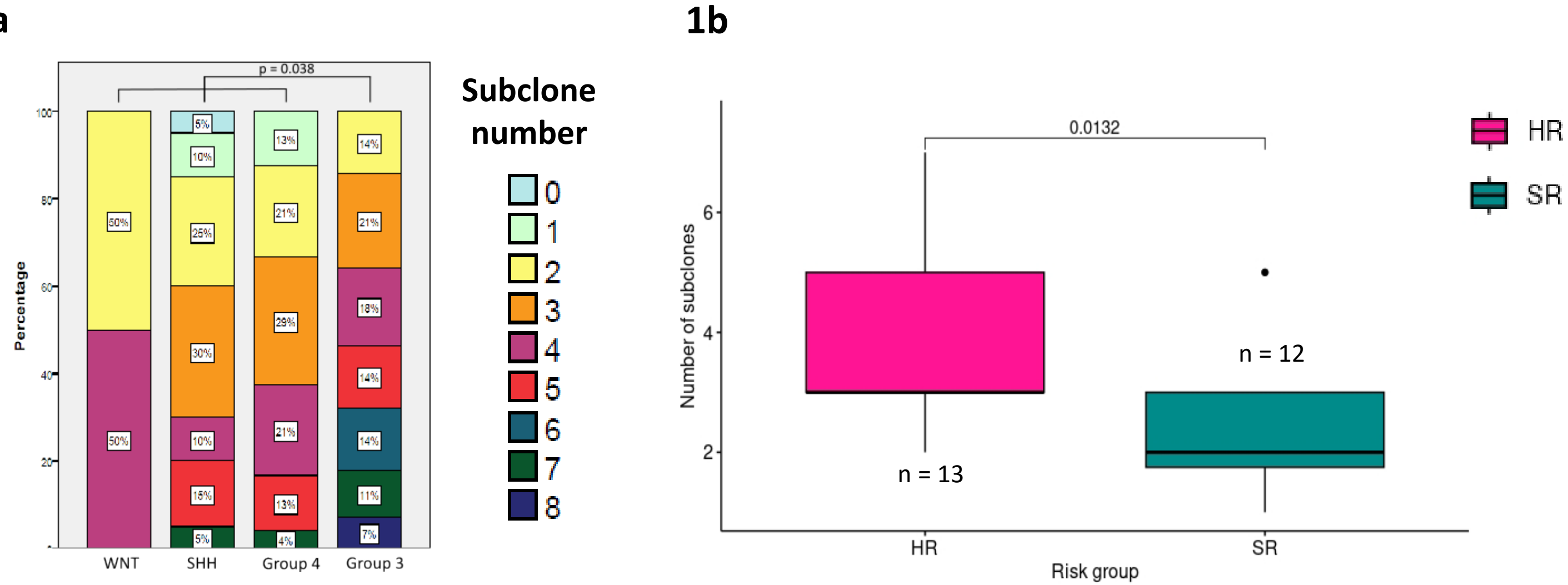
2. Methodology

Establish methods for inference of heterogeneity in genomic datasets from cohorts of bulk tumours

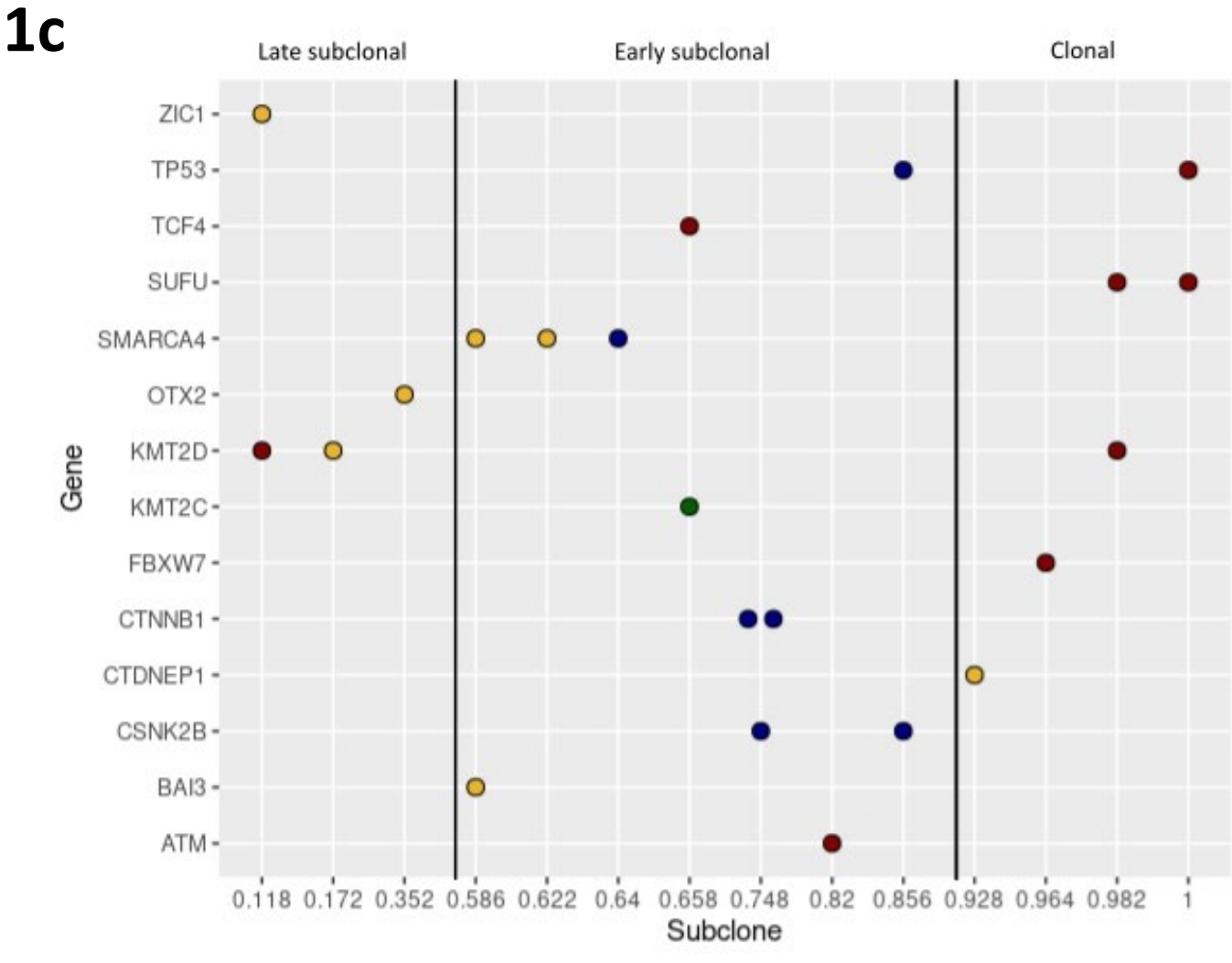


3. Results Pilot Study 1 (Whole exome sequences n=75)

Association between inferred heterogeneity and clinicopathological features



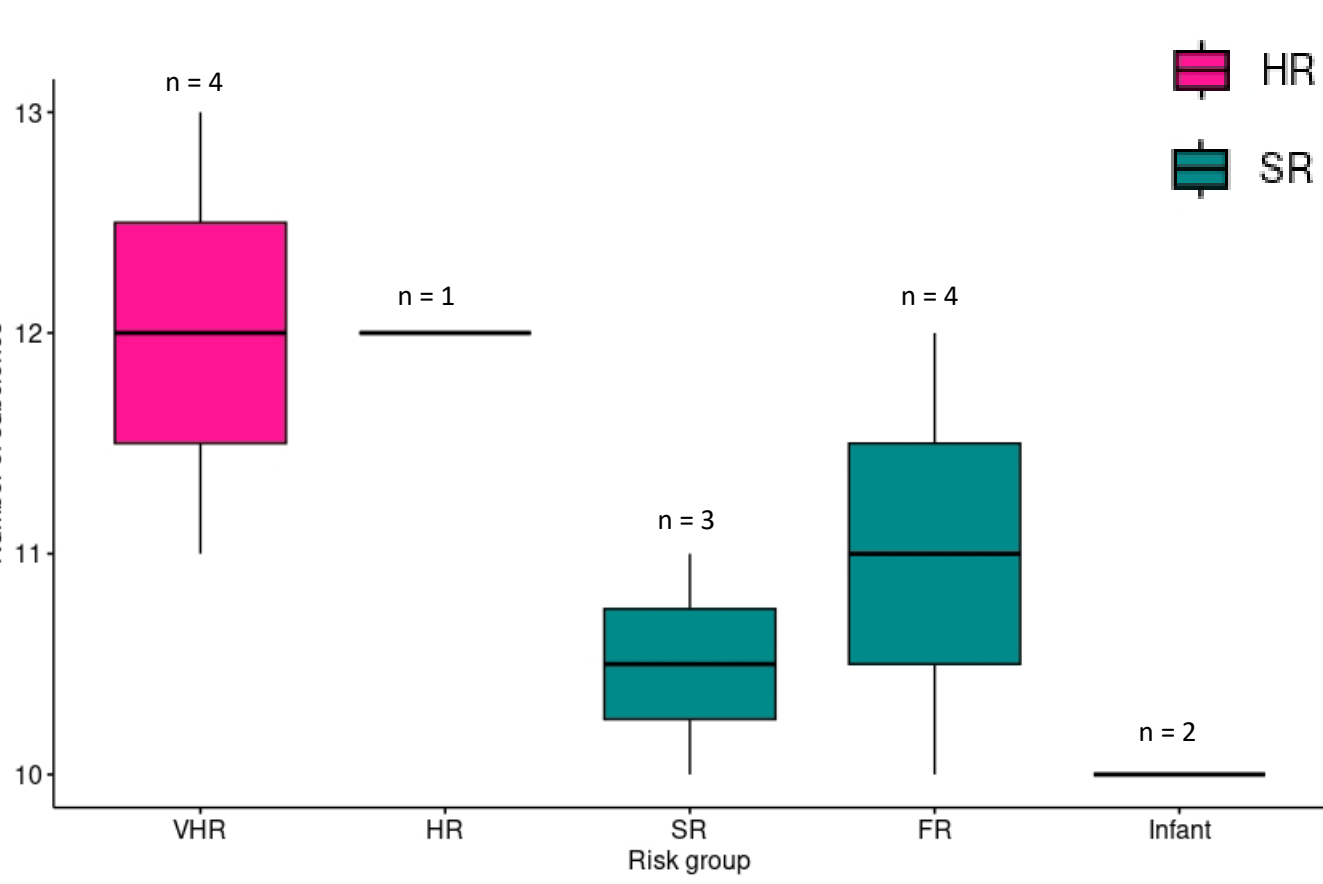
Clonal evolution of known driver mutations



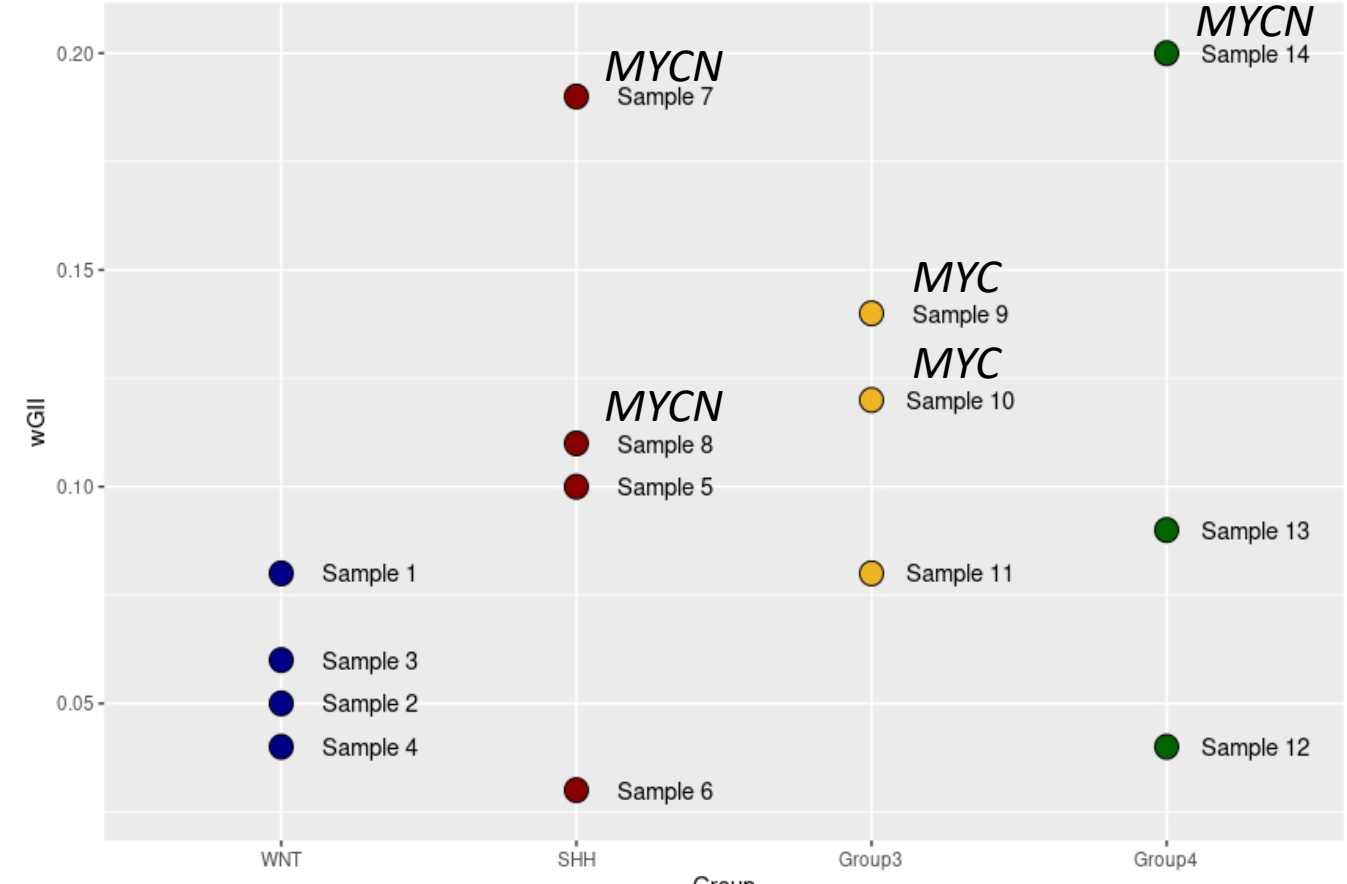
1a; Chi-squared test showed a significant difference between the number of subclones in Group 3 tumours compared to the other molecular groups. **1b;** Boxplot to show the association between the number of subclones predicted per tumour and the presence of high-risk features. HR=high-risk (metastatic disease, large-cell/anaplasia, *TP53*-mutation, *MYC*-amplification in any setting or *MYCN*-amplification in SHH tumours), SR=standard-risk (no established high-risk feature known). **1c;** Dot plot demonstrating the distribution of driver mutations according to their subclone size. The molecular colour is demonstrated by the colour of the dots. The separation between fully clonal (subclones 0.9-1.0), early subclonal (subclones 0.5-0.9) and late subclonal (subclones <0.5) are indicated by demarcation lines. G3 = Group 3, G4 = Group 4.

4. Results Pilot Study 2 (Single cell DNA sequencing with comparative whole exome sequences n=14)

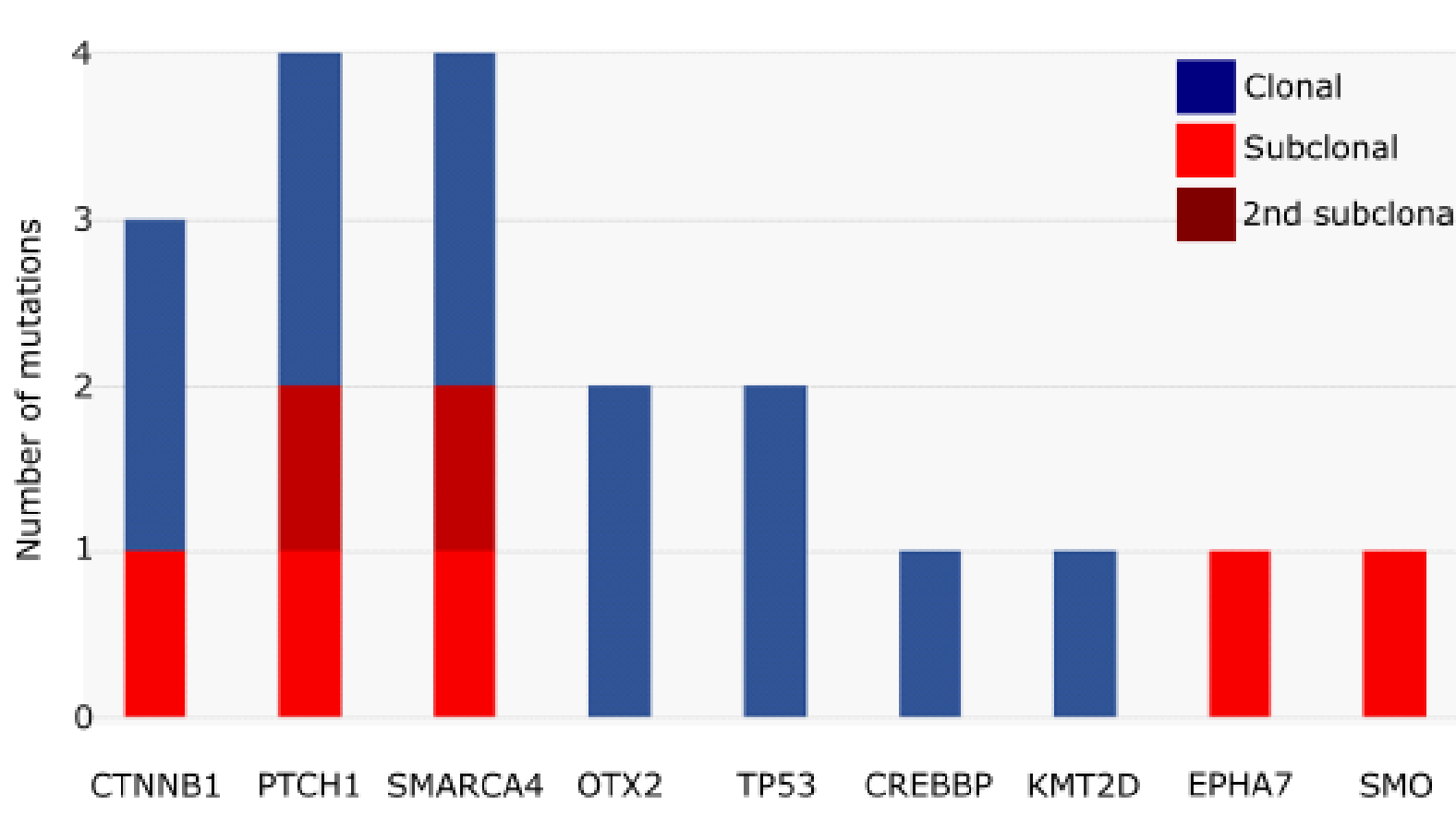
2a Higher subclone numbers with higher risk disease



2b Higher chromosomal instability values in *MYC*/*MYCN* amplified tumours



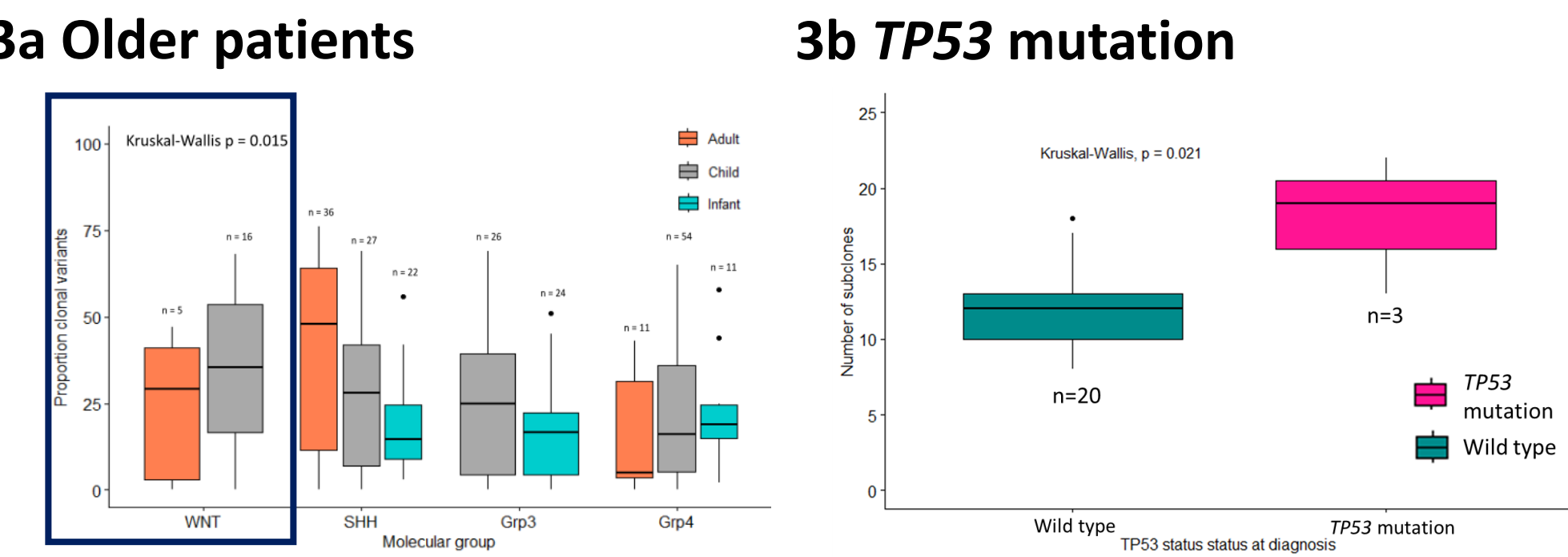
2c The majority of driver events are clonal with evidence of secondary subclonal events



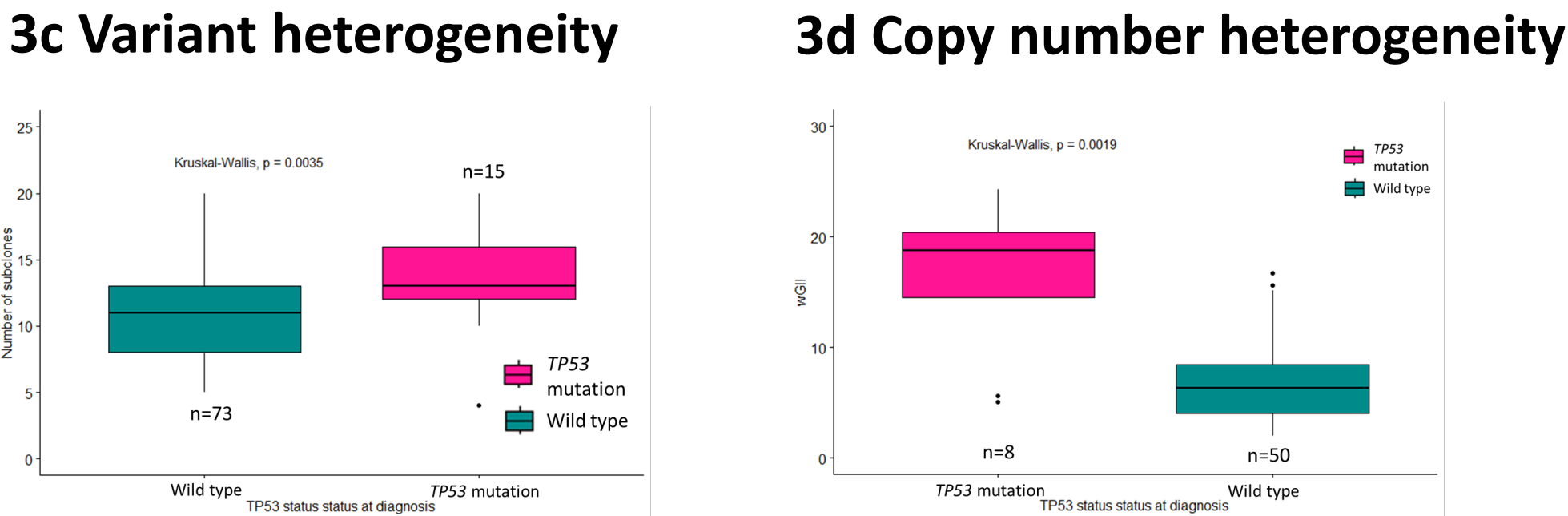
2a; Boxplot to show the association between the number of subclones predicted per tumour and the presence of high-risk features. FR=favourable-risk, SR=standard-risk, HR=high-risk, VHR=very high-risk. **2b;** Dotplot demonstrating the calculated wGII for each sample. Each dot represents a tumour and is coloured according to molecular group. **2c;** Barplot to show the predicted clonality of known driver genes within medulloblastoma based upon the MutationTimeR output. Blue; clonal, red; subclonal, maroon indicates a second variant within the gene in the tumour that was subclonal.

5. Results Main Study (Whole genome sequencing n=240)

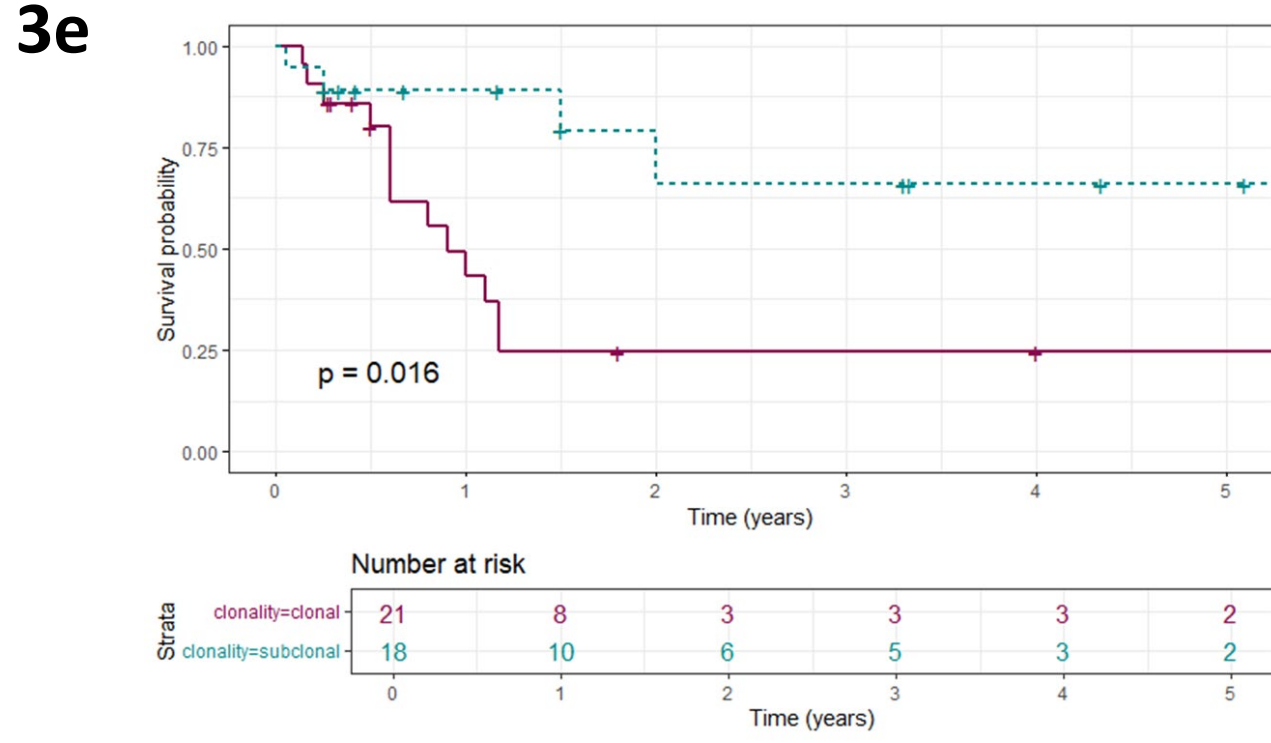
WNT tumours: More subclonal features in adult disease and with *TP53* mutation



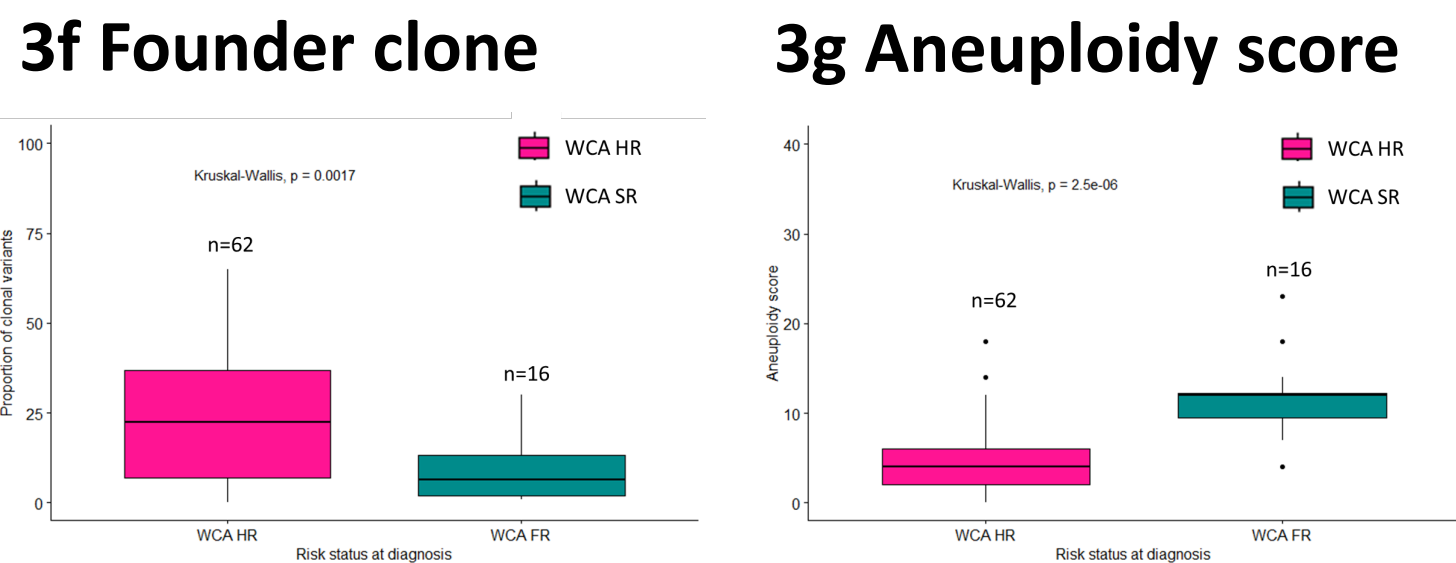
SHH *TP53* mutated tumours: High heterogeneity features



Group 3 tumours: Proportionally larger founder clone associated with clinical high-risk features

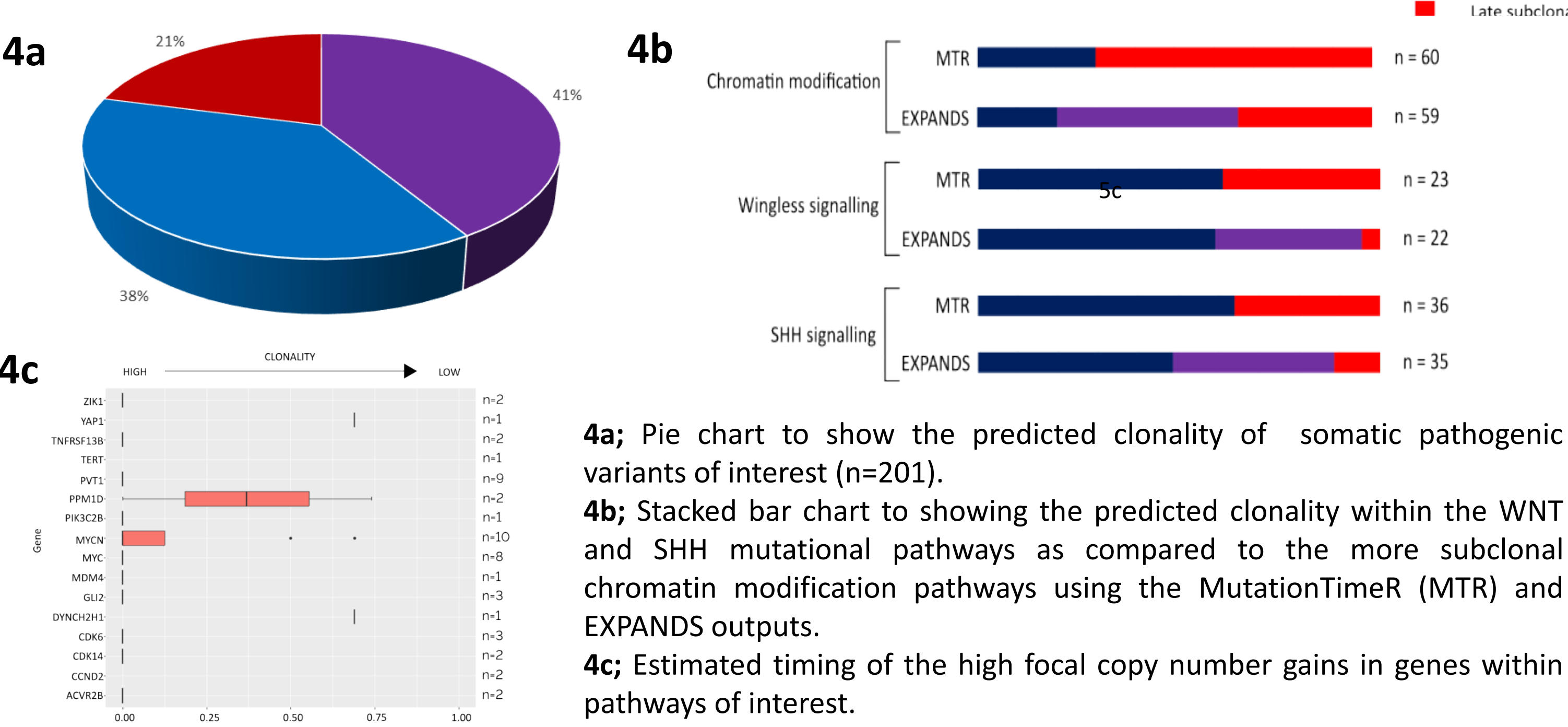


Group 4 tumours: High-risk disease association with proportionally larger founder clone and lower features copy number heterogeneity



6. Results Main Study (Whole genome sequencing n =240)

Clonal timing of majority driver variants and focal copy number gains



4a; Pie chart to show the predicted clonality of somatic pathogenic variants of interest (n=201). **4b;** Stacked bar chart to showing the predicted clonality within the WNT and SHH mutational pathways as compared to the more subclonal chromatin modification pathways using the MutationTimeR (MTR) and EXPANDS outputs. **4c;** Estimated timing of the high focal copy number gains in genes within pathways of interest.

7. Conclusions

- Driver events occur early across all medulloblastoma groups
- Molecular subgroups evolve genomically in distinctive ways
- Patterns of evolution are dependent on the molecular origin of the disease
- Differences in evolutionary patterns and timing are clinically significant
- Timing of variant events and features of heterogeneity may prospectively guide risk stratification or integration of targeted therapies

8. References

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