Germline POLE and POLD1 variation in persons with colorectal cancer from the Colon Cancer Family Registry Cohort

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Germline POLE and POLD1 in Colorectal Cancers (CRCs)

- Germline mutations in POLE and POLD1 are associated with CRCs
- Recurring germline exonuclease domain mutations (EDMs):
  - POLE: p.Leu424Val
  - POLD1: p.Leu474Pro and p.Ser478Asn

- EDMs result in defective proofreading function
  - structural proximity to DNA binding/active sites likely perturbs function
  - resulting in characteristic hypermutator phenotype
  - also shown by functional assays in yeast and T4 bacteriophage

Palles *et.al.* (2013), Valle et.al. (2014) and Bellido *et.al.* (2016), Kokoska RJ *et.al.* (2000)

Aims

- Identify new germline mutations is POLE and POLD1 using a large cohort of persons with CRC

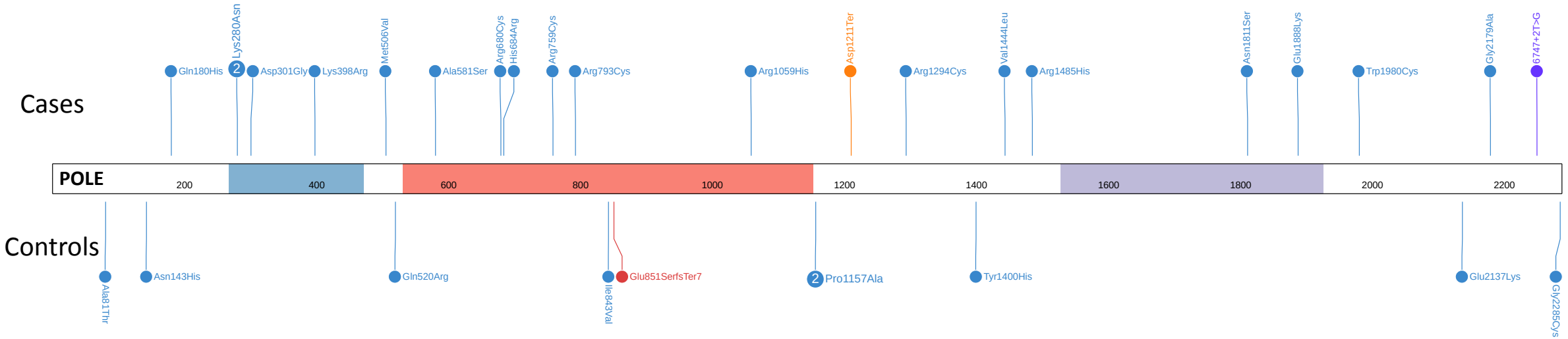
- Incorporate clinico-pathological and tumour molecular features to classify new POLE and POLD1 pathogenic variants

Study cohort: Colon Cancer Family Registry (CCFR)

Australian cohort	**Ontario cohort**	**Seattle cohort**
Cases: recruited as population based probands diagnosed with CRC		
Incident CRC dx between 18-59 yrs (independent of FHx)	Incident CRC dx between 20-74 yrs (weighted to FHx)	Incident CRC dx between 18-74 yrs (independent of FHx)
Controls: recruited by random sampling from population without prior CRC		
Cases = 861 Controls = 247	Cases = 633 Controls = 575	Cases = 459 Controls = 385

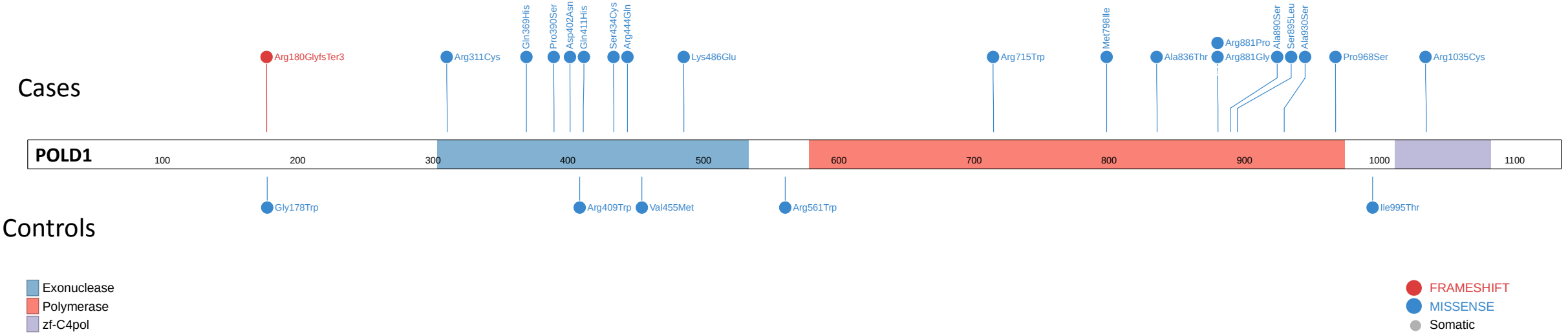
Cases = 1953
Controls = 1207

Results: germline POLE predicted pathogenic variants



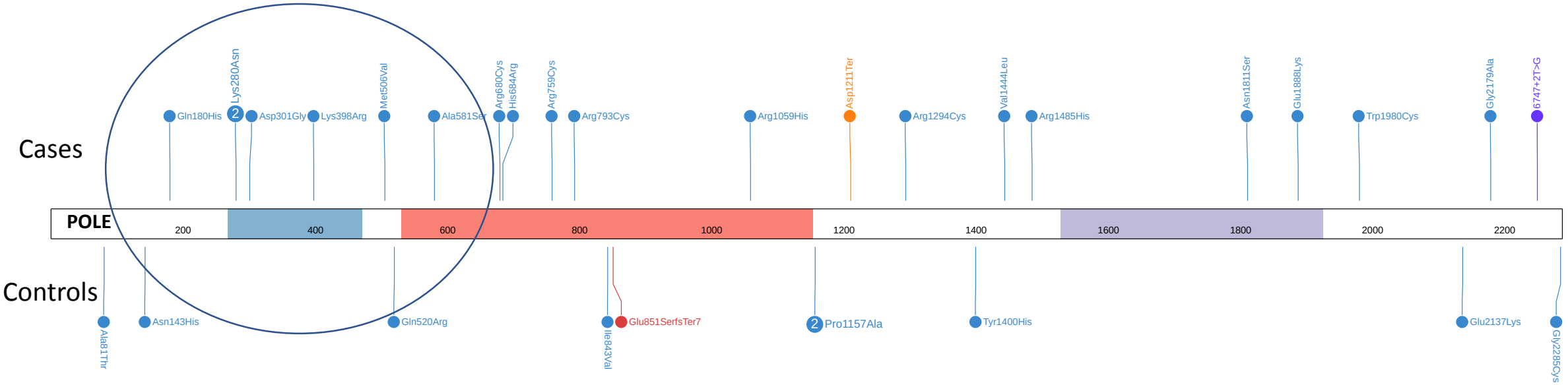
	POLE	POLE_exo
Cases (n=1953)	21 (1.09%)	4 (0.21%)
Controls (n=1207)	10 (0.84%) OR 1.3 [0.61-2.75] P=5.8E-01	0 (0%) OR - p=3.1E-01
gnomAD (n=123134)	1168 (0.96%) OR 1.1 [0.74-1.74] P=5.6E-01	111(0.1%) OR 2.27 [0.84-6.16] p=1.1E-01

Results: germline POLD1 predicted pathogenic variants



	POLD1	POLD1_exo
Cases (n=1953)	19 (0.98%)	8 (0.41%)
Controls (n=1207)	5 (0.42%) OR 2.35 [0.88-6.27] P=9.3E-02	2 (0.17%) OR 2.47 [0.53-11.62] p=3.4E-01
gnomAD (n=123134)	438 (0.36%) OR 2.73 [1.73-4.32] P=1.4E-04	84 (0.07%) OR 6.0 [2.91-12.38] p=7.6E-05

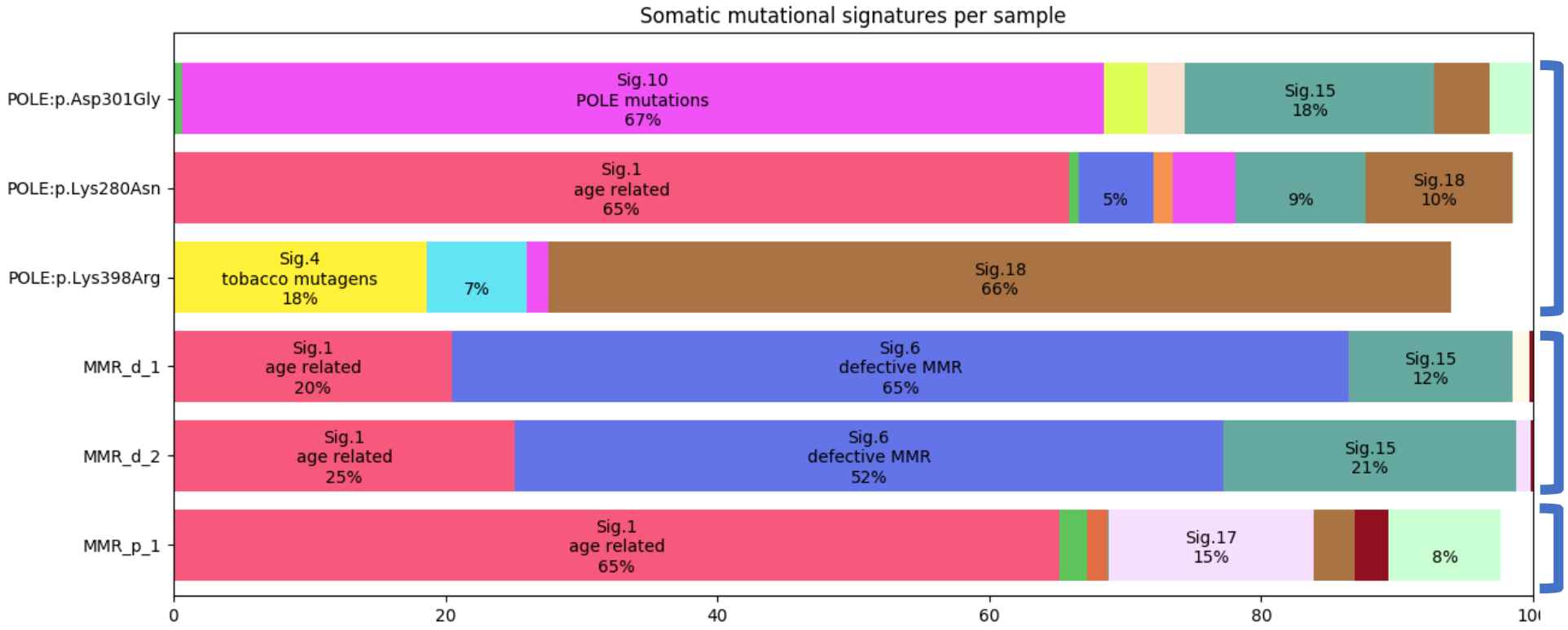
Characterising POLE exonuclease domain mutations (EDMs)

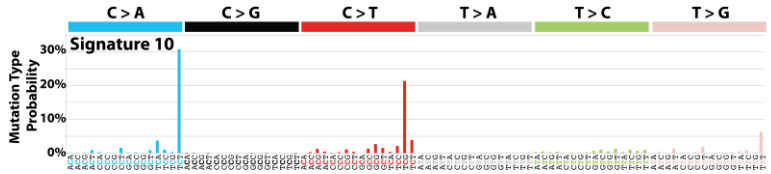


Use matching tumour molecular features to characterise POLE EDMs

POLE:p.Lys280Asn
POLE:p.Asp301Gly
POLE:p.Lys398Arg

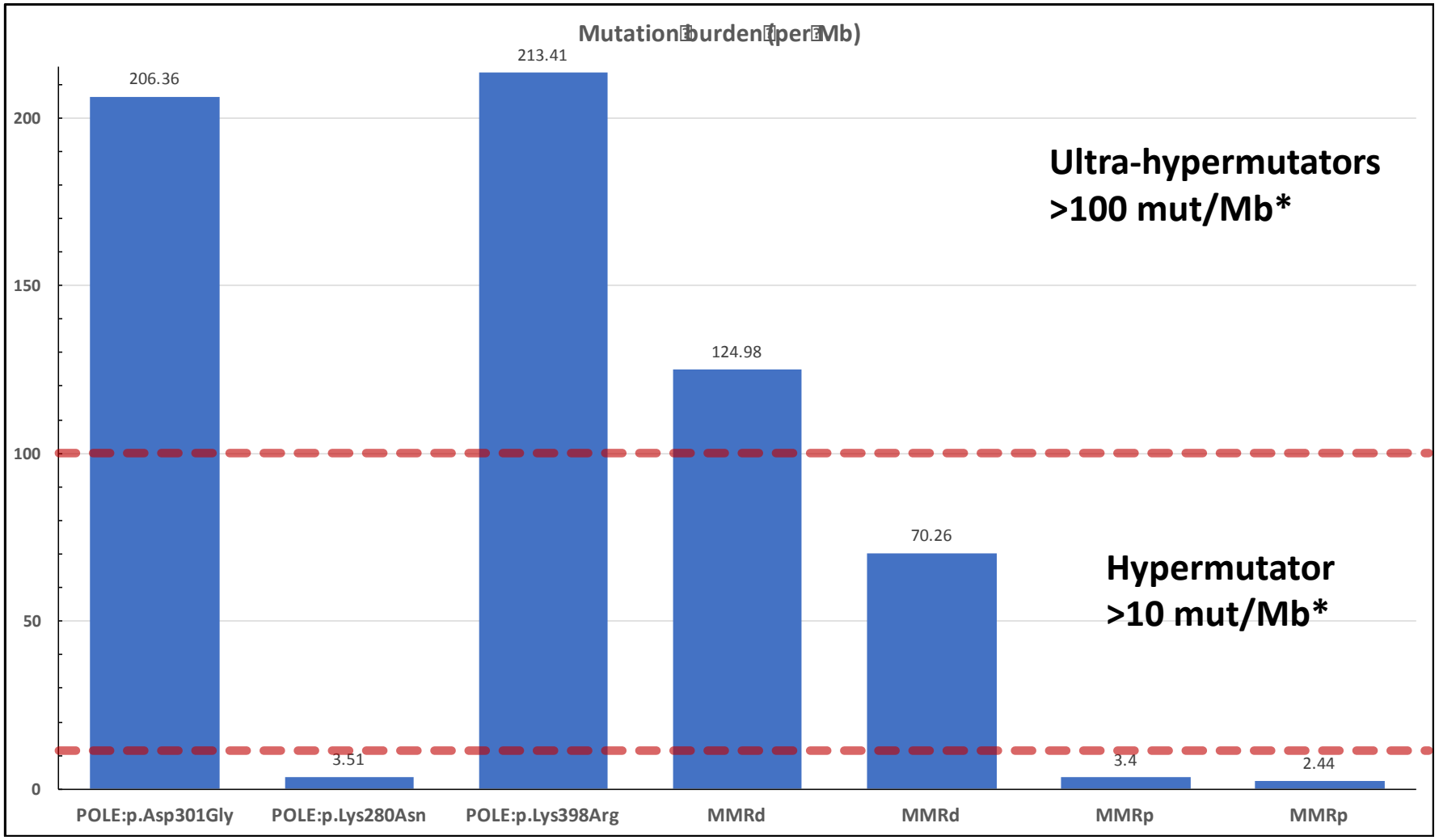
Results: Somatic mutational signature analysis





[TCT→A] and [TCG→T] mutations are enriched in tumours with POLE EDMs

Results: Tumour mutation burden analysis



*Campbell BB. et.al. Cell (2017)

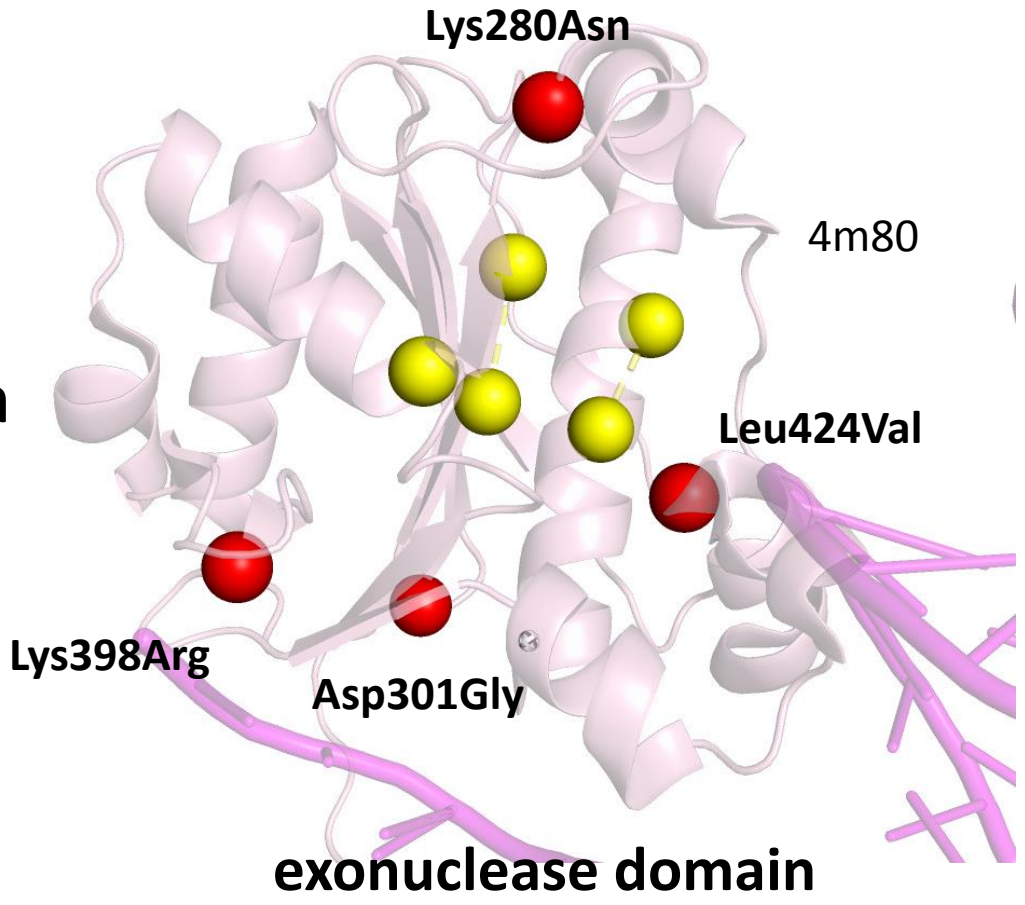
Summary

POLE EDMs	In silico prediction (CADD, REVEL)	gnomAD	Predicted structure stability (DynaMut)* yeast POL2 structure 4m8o	Mutational Signature.10	Mutation burden (mut/Mb)	ClinVar classification
p.Asp301Gly	31.0, 0.7	8.12E-06 (1 in 100k)	destabilising	High	Ultra-hypermutator	VUS
p.Lys280Asn	27.7, 0.28	ultra-rare	destabilising	Low	Low mutation burden	VUS
p.Lys398Arg	17.8, 0.15	ultra-rare	stabilising	Low	Ultra-hypermutator	VUS

*DynaMut, Rodrigues CHM et.al. NAR (2018)

Conclusions and Future directions

- Analysing matching tumour molecular features can help characterise EDMs
- Future directions for EDM characterisation
  - incorporate structural and functional data
  - perform segregation analysis for variant characterisation



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Australasian Colorectal Cancer Family Registry

Colon Cancer Family Registry Cohort

Genetics of Colonic Polyposis Study

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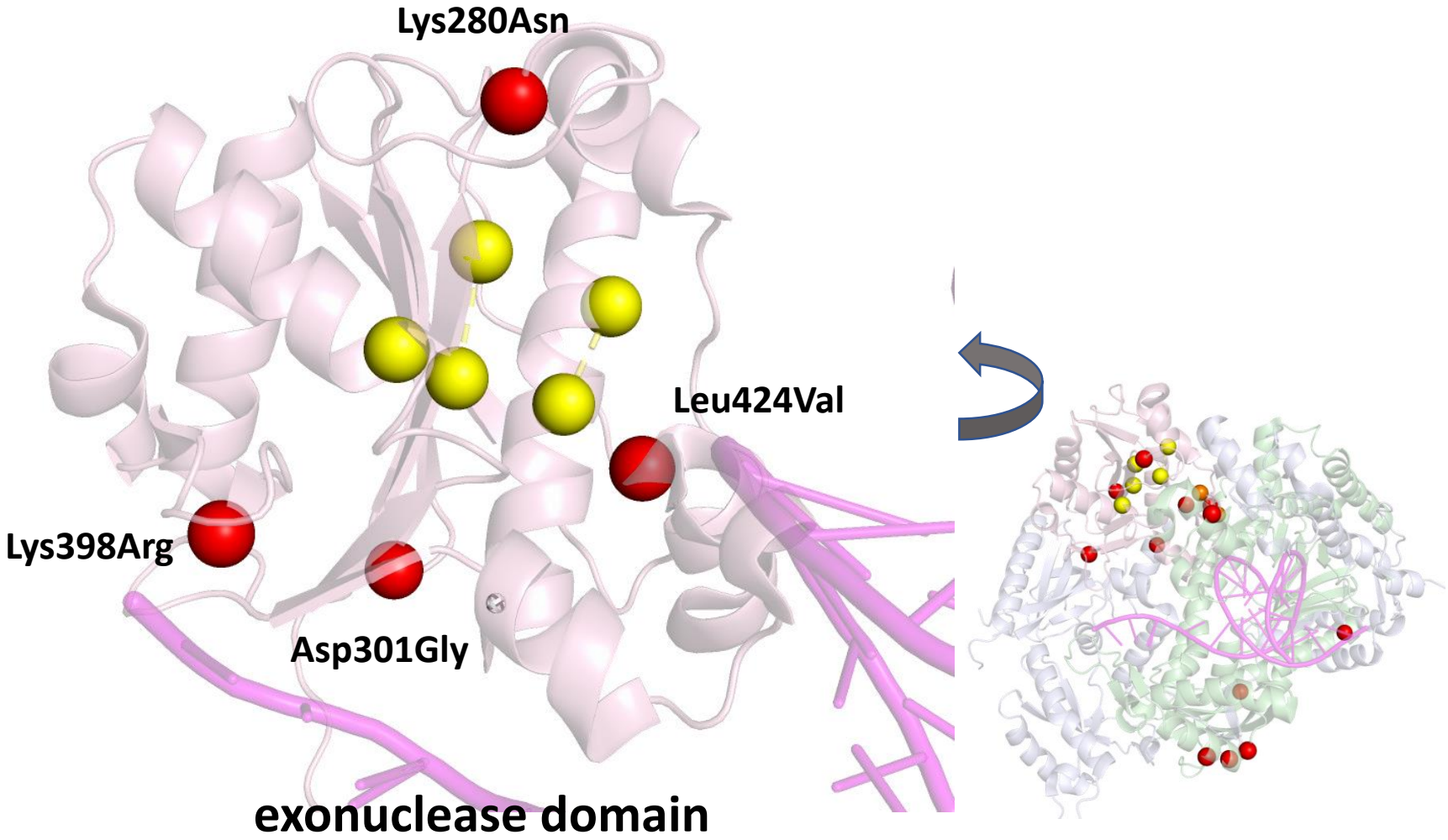
Structural analysis



predicted pathogenic variants

Somatic POLE and POLD1 mutations (COSMIC)