Whole genome sequencing as a diagnostic tool for Lynch syndrome

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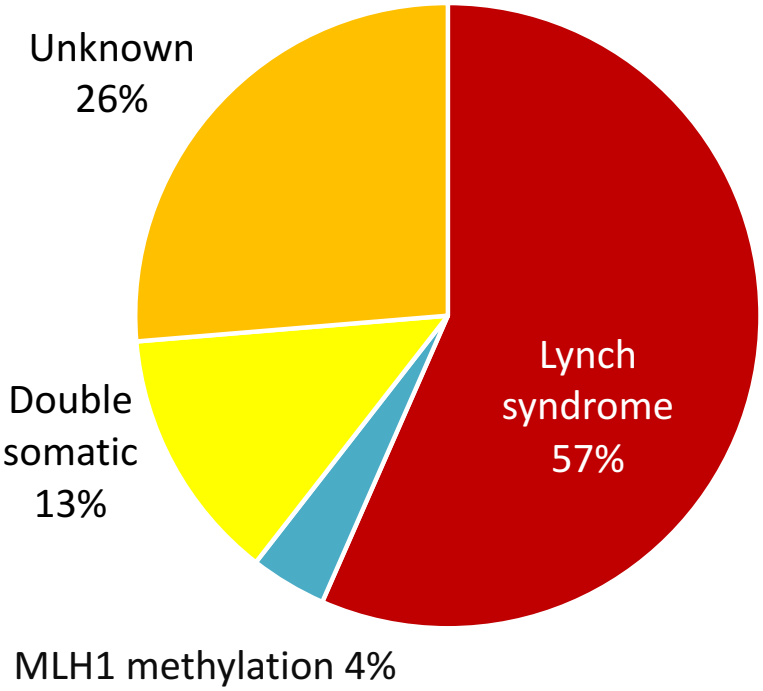
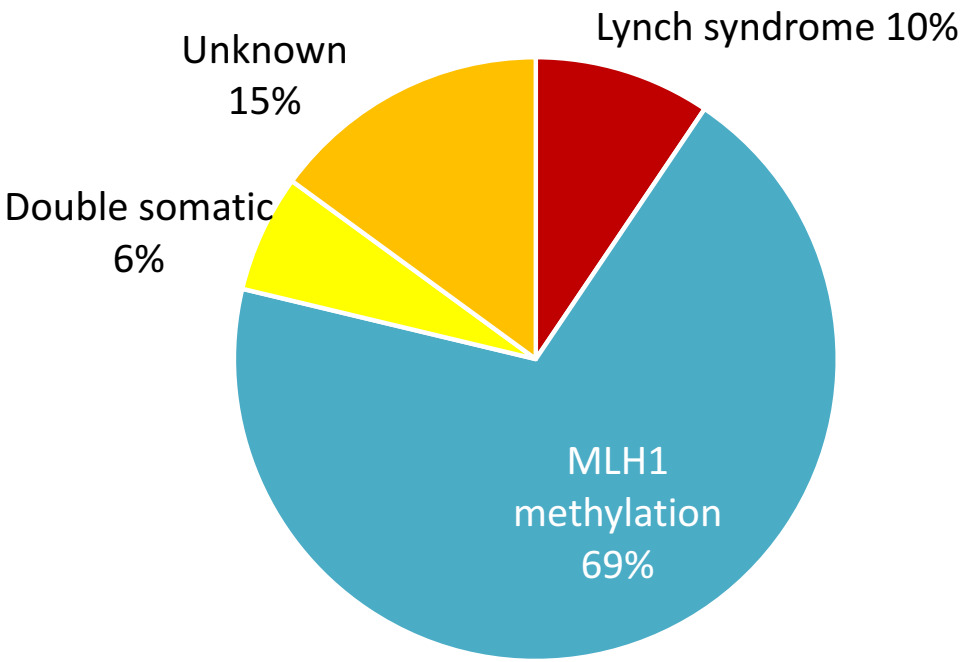
Bernard Pope
Lead Bioinformatician (Cancer Genomics, Clinical Genomics)
Melbourne Bioinformatics
The University of Melbourne, Australia
bjpope@unimelb.edu.au

MMR deficient CRC

MMR deficient CRC <50 years



MMR deficient CRC ≥50 years



Colon Cancer Family Registry

Aim

Investigate whole genome sequencing (WGS) for identifying novel germline causes of tumour MMR-deficiency.

Causes considered:

- Single nucleotide variants (SNVs)
- Short insertions and deletions (INDELS)
- Structural variants (SVs)

Methods

WGS for 16 suspected Lynch syndrome patients and 2 positive controls.

Group included 2 relative pairs.

Selection criteria:

- MMR deficient tumour (MMR IHC and MSI-H)
- Family history of CRC and/or young age at diagnosis.
- Negative for *MLH1* promoter methylation.
- No identified MMR gene germline mutation.
- No double somatic mutation in tumour.

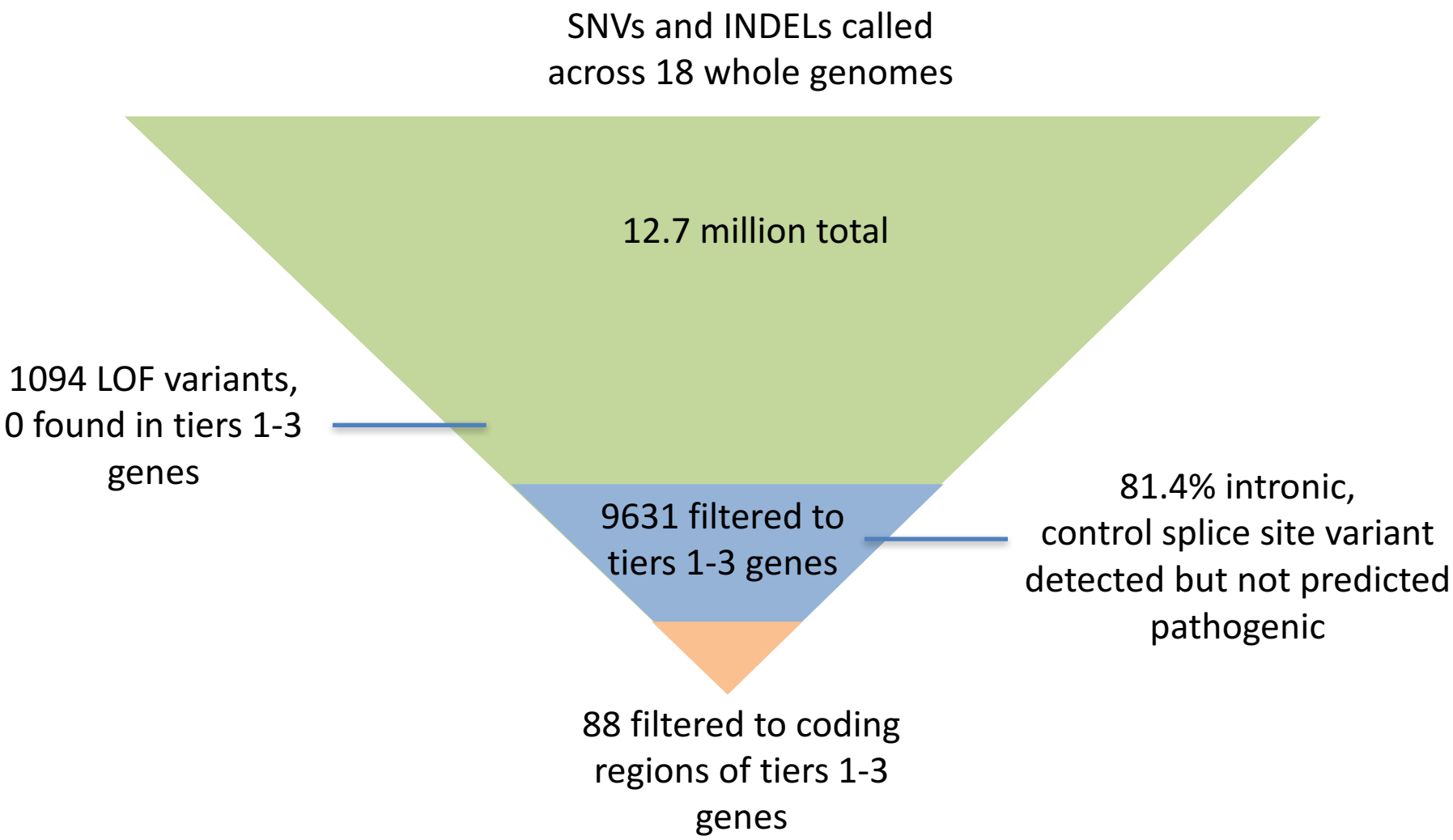
Positive controls:

1. *MSH2* deletion exon 6 identified by MLPA
2. Intronic splice site *MSH2* mutation (Clendenning et al, 2011).

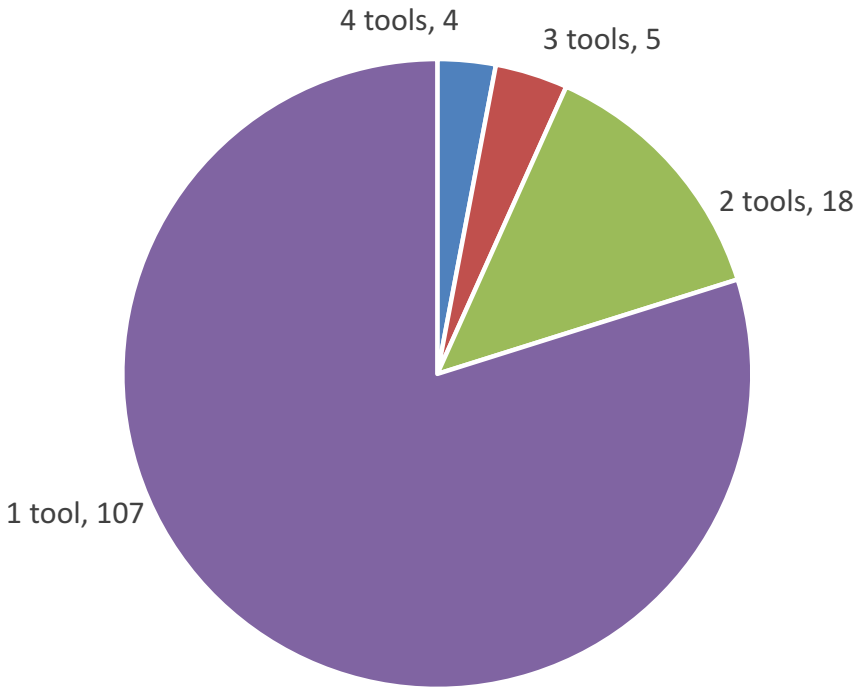
Methods

- SNVs and INDELs were called using the GATK Best Practices Pipeline.
- SVs were called by DELLY, LUMPY, Socrates, and GRIDSS.
- Variants filtered to those intersecting 4 tiers of genes:
  1. MMR genes (MLH1, MSH2, MSH6, PMS2, EPCAM).
  2. MMR-related genes from literature (MUTYH, POLE, POLD1, MSH3, MLH3, EXO1).
  3. DNA repair genes from literature.
  4. Genes with any rare loss of function variant identified.

SNVs and INDELs summary



SVs summary



SVs intersecting tier 1-3 genes, called by at most N tools

All 4 tools called:

- 9.5 Mb inversion affecting exons 1-7 of *MSH2* in mother-daughter pair (validated).
- 1927 base deletion of exon 6 in woman with endometrial cancer (positive control).
- 2 other variants which may be artefacts.

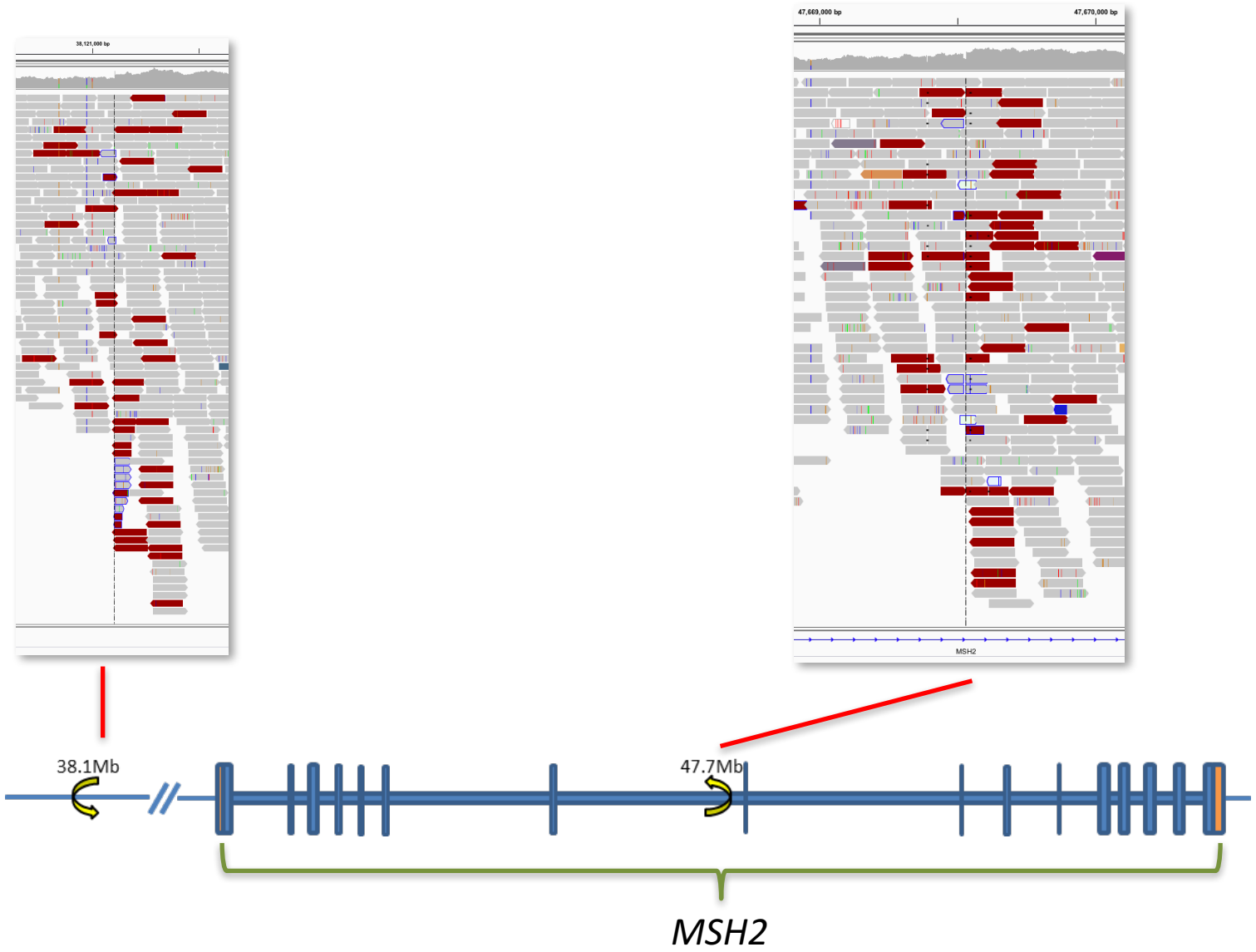
Highlighted germline variants

ID	Age	Cancer	MMR IHC	MSI	Gene	Fam History	Tier	Germline Variant	CADD	REVEL	ExAC
9	47	CRC	MSH2/MSH6 loss	MSI-H	MSH2	CRC FDR	2	POLE p.Arg680Cys	34	0.638	8.7E-6
11	40	CRC breast	MSH2/MSH6 loss (CRC)	NT	MSH2	CRC FDR	1	MSH2 inversion ex1-7			
12	35	CRC	MSH2/MSH6 loss	MSI-H	MSH2	CRC FDR	1	MSH2 inversion ex1-7			

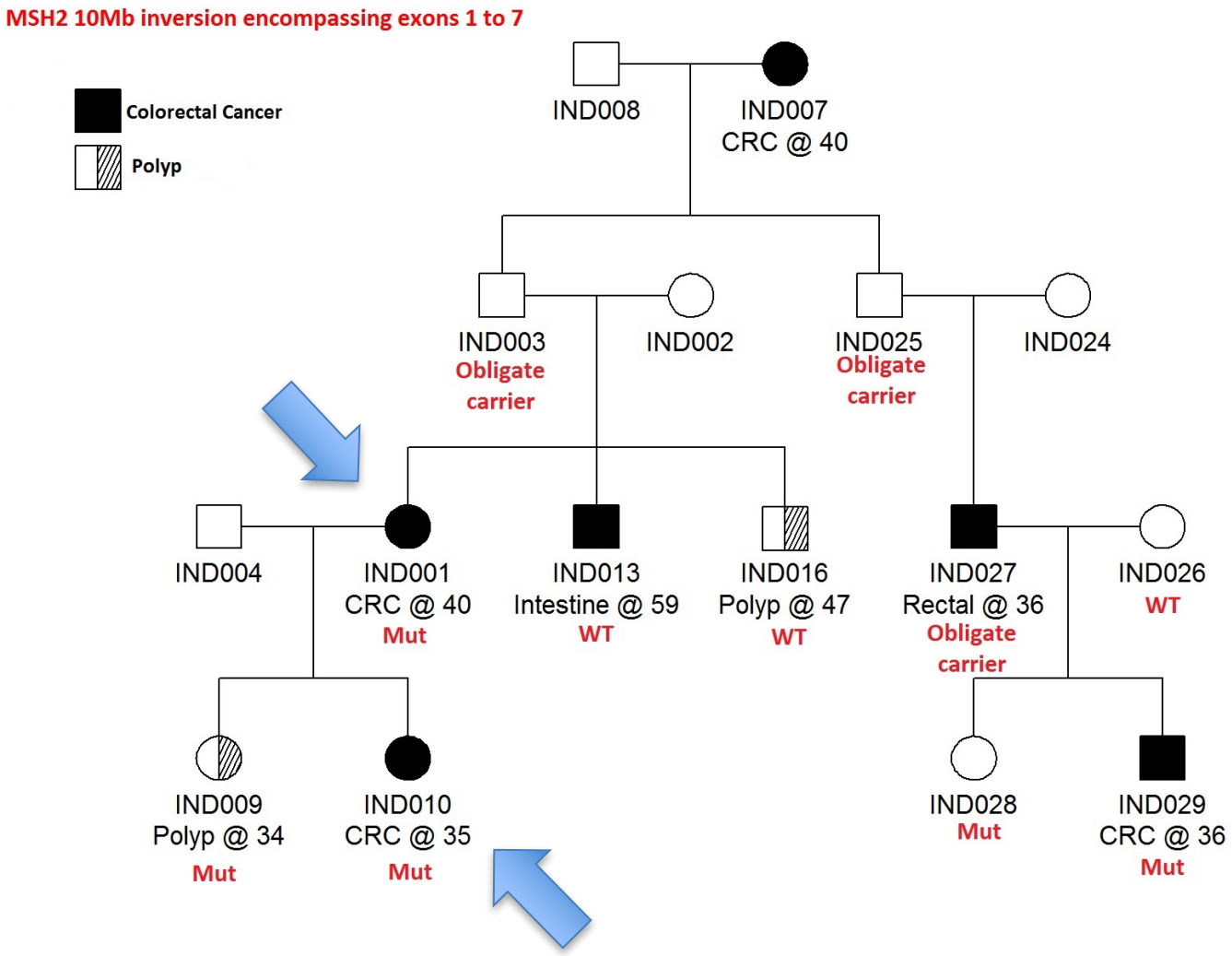
relatives

9.5 Mb MSH2 inversion reported in: Wagner *et al*, *Genes Chromosomes Cancer* (2002).
See also Rhees *et al*, *Fam Cancer* (2014), and Mork *et al*, *Fam Cancer* (2017).

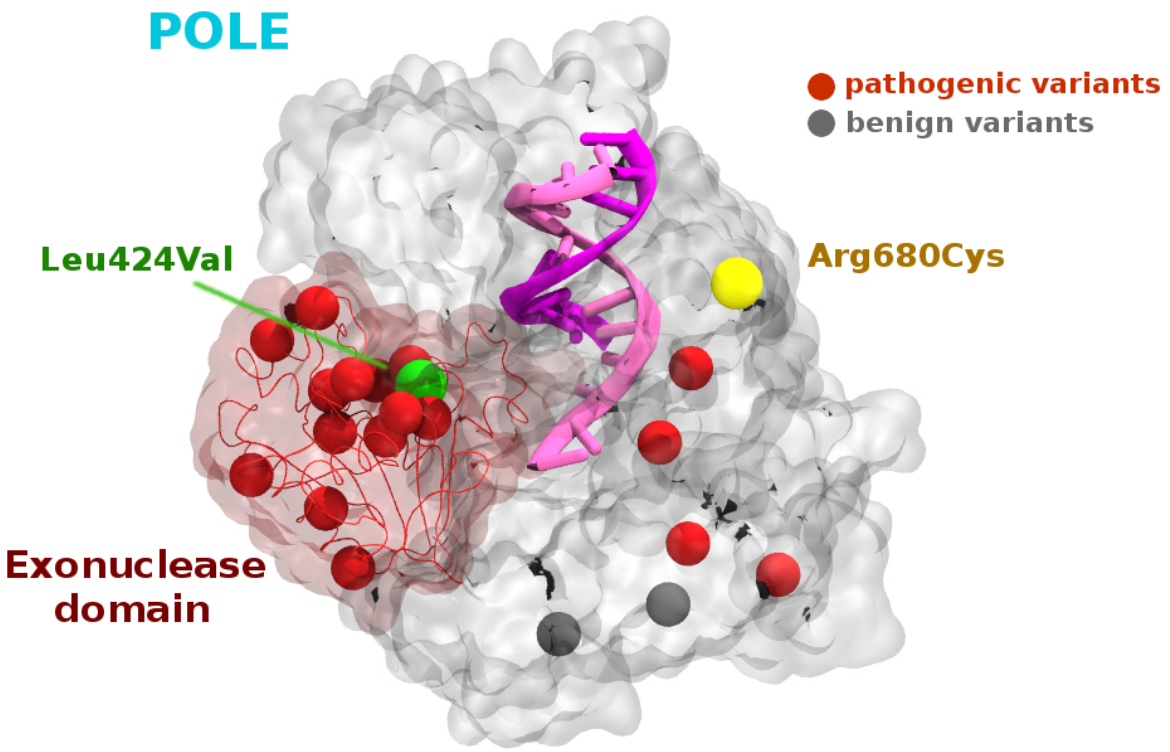
9.5 Mb inversion exons 1-7 of *MSH2*



9.5 Mb inversion exons 1-7 of *MSH2*

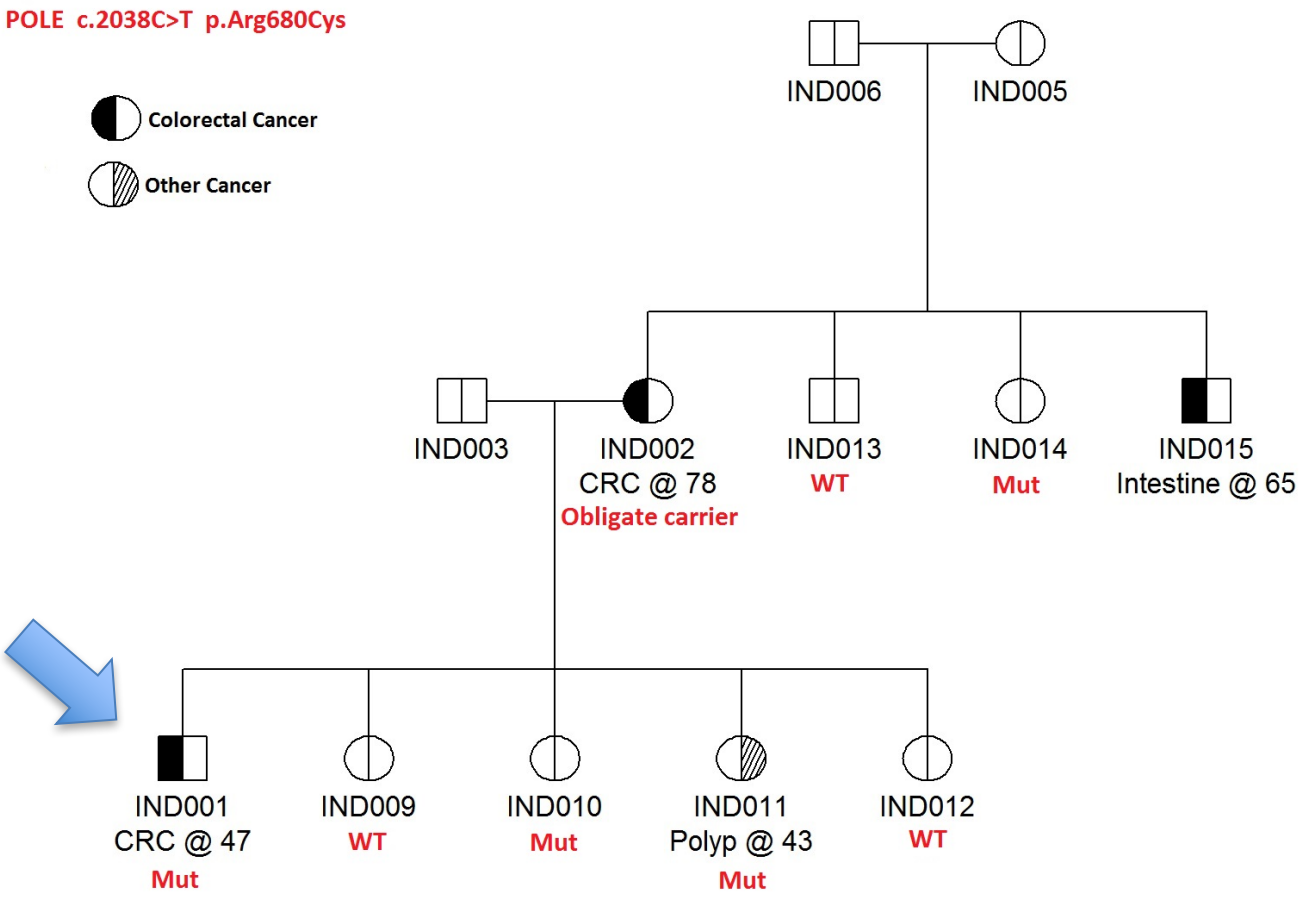


POLE p.Arg680Cys



Hypothesis: germline POLE mutations can result in two somatic mutations in one of the DNA MMR genes causing loss of protein expression (Elsayed *et al*, Eur J Hum Genet, 2015).

POLE p.Arg680Cys



Conclusions

- WGS can identify novel germline causes of tumour MMR-deficiency.
- As cost continues to fall, may replace existing biochemical diagnostic assays.
- Can detect complex structural variants
  - Use of multiple tools and concordance reduces false positive rate.
- Can detect likely pathogenic mutations outside the current gene screening paradigm.
- Variant interpretation in non-coding regions and novel genes remains challenging.

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