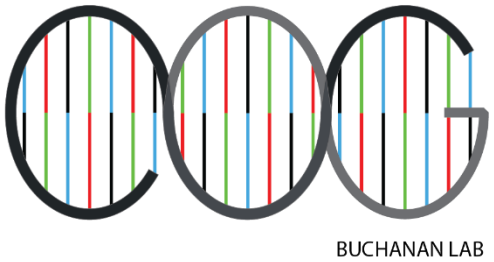


Melbourne Genomics Health Alliance

The role of heterozygous *NTHL1* gene mutations and Colorectal Cancer risk

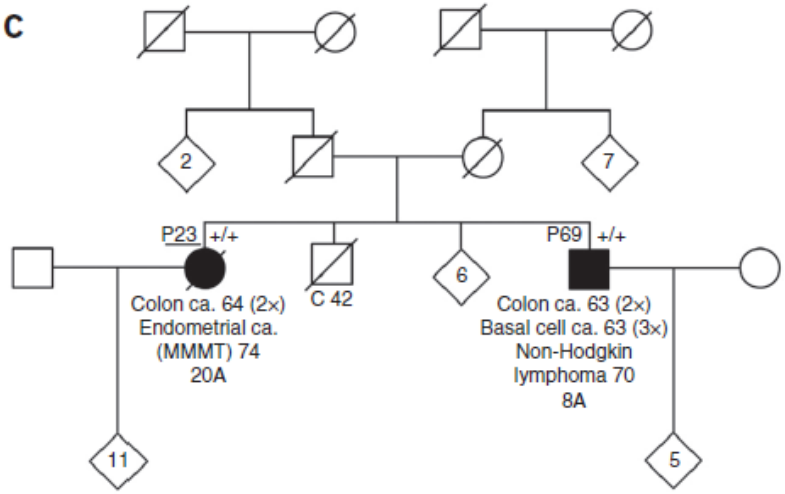
Dr Abi Ragunathan

InSiGHT conference

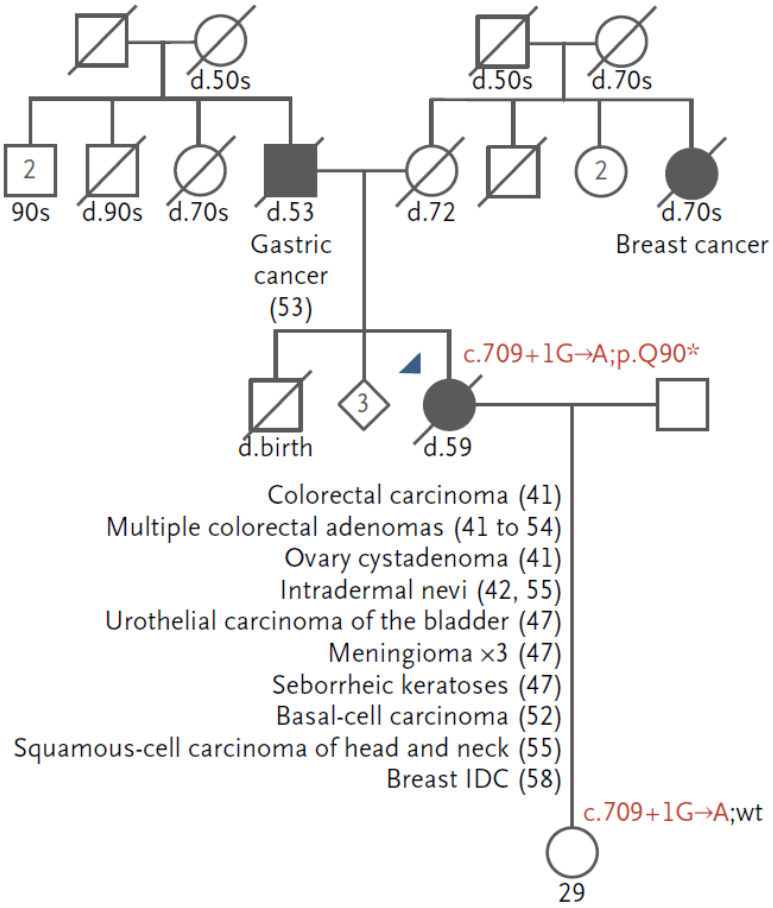
22nd March 2019

NTHL1-associated polyposis (NAP)

- Netherlands – 7 individuals homozygous for *NTHL1* p.Gln90* from 3 families



Weren et al, Nature Genetics 2015.



Rivera et al, NEJM. 2015

Study population - CCFR

Australian cohort
Population-based recruitment
- Incident CRC dx between 18-59 yrs (independent of FHx)

Controls: 18-59 yo from electoral role

Cases = 861
Controls = 247

Ontario cohort
Population-based recruitment
- Incident CRC dx between 20-74 yrs (weighted to FHx)

Controls: Random digit dialling or Ministry of Finance file

Cases = 633
Controls = 575

Seattle cohort
Population based recruitment
- Incident CRC dx between 18-74 yrs (independent of FHx)

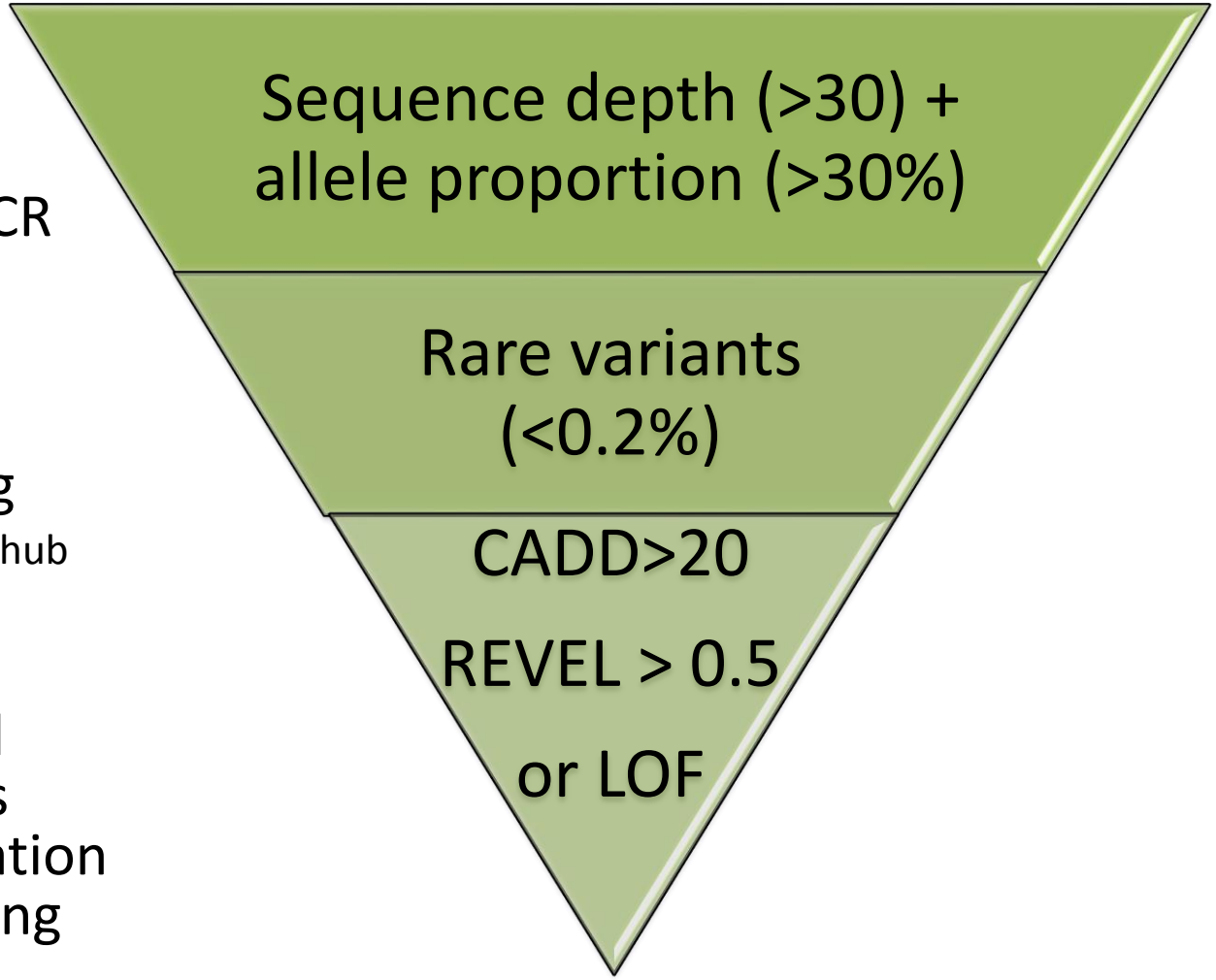
Controls: Unaffected individuals from the Washington State Department of Licencing

Cases = 459
Controls = 385

All CRC excluding appendiceal cancers

Methods

- Hi-Plex multiplex PCR based sequence screening
- Variant calling using Hiplexpipe (refer to github website)
- Subset of predicted pathogenic variants selected for verification by Sanger sequencing



Patient characteristics

	Cases (1953)	Controls (1207)
Gender		
Male	999 (51%)	636 (53%)
Female	954 (49%)	571 (47%)
Age (years)	CRC diagnosis	Baseline
Mean (SD)	51 +/- 10	57 +/- 12
<40	234 (12%)	76 (6.3%)
40-49	781 (40%)	273 (22.62%)
50-69	838 (42.9%)	662 (54.85%)
>= 70	100 (5.12%)	196 (16.24%)
Ethnicity		
Caucasian	1810 (92.68%)	1167 (96.69%)
Other	143 (7.32%)	40 (3.31%)
Site		
R-sided	538 (27.5%)	0
L-sided	508 (26.0%)	0
Rectum	653 (33.4%)	0
Mixed	66 (3.4%)	0
Unknown	188 (9.6%)	18 (1.5%)
MMR status		
Proficient	1562 (80%)	0
Deficient	196 (10%)	0
Unknown	195 (10%)	18 (1.5%)

Case-Control *NTHL1* results

	Cases (1953) n (%)	Controls (1207) n (%)	Cases-controls OR (95%CI)	p-value
All predicted pathogenic mutations	22 (1.13)	17 (1.41)	0.79 (0.42-1.48)	0.51
Total LOF mutations	5 (0.26)	5 (0.41)	0.62 (0.18-2.14)	0.52
p.Gln90Ter	4 (0.20)	3 (0.25)	0.82 (0.18-3.69)	0.80
p.Gln287Ter	1 (0.05)	1 (0.08)	0.62 (0.039-9.89)	0.73
c.139+1G>A	0 (0)	1 (0.08)	0	--
Predicted pathogenic missense mutations	17 (0.87)	12 (0.99)	0.87 (0.42-1.84)	0.72

Case vs gnomAD

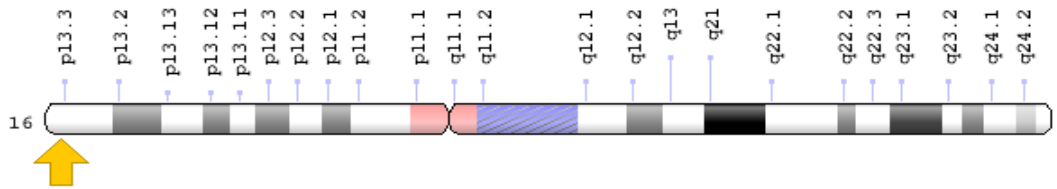
	Cases AF (%)	Controls AF (%)	gnomAD AF (%)	Cases-gnomAD AF OR (95% CI)	p-value
All predicted pathogenic mutations	0.563	0.704	0.54	1.04 (0.54-1.98)	0.82
Total LOF mutations	0.128	0.207	0.22	0.58 (0.18-1.86)	0.35
p.Gln90Ter	0.102	0.124	0.14	0.73 (0.24-2.22)	0.58
p.Gln287Ter	0.026	0.041	0.016	1.60 (0.21-12.07)	0.65
c.139+1G>A	0	0.041	0.0065	--	--
Predicted pathogenic missense mutations	0.435	0.497	0.32	1.36 (0.61-3.01)	0.24

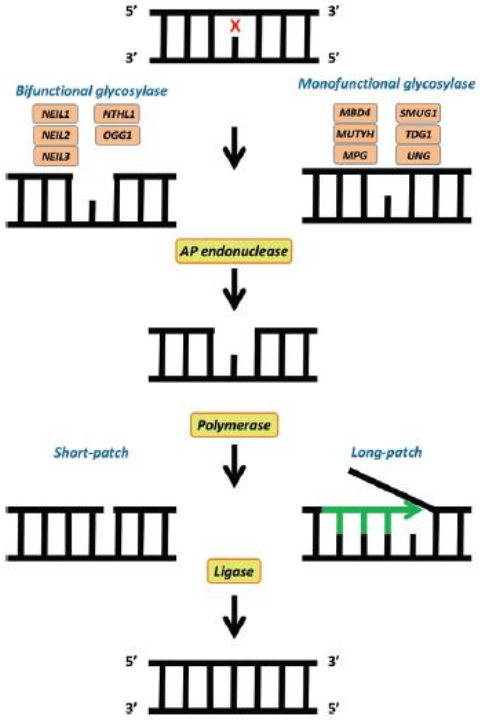
Case-case analysis

	NTHL1 variant carriers (n=20)	Non-carriers (n=1808)	p-value
Gender	n=20	n=1808	
Male	11 (55%)	926 (51%)	0.82
Female	9 (45%)	882 (49%)	
Age at first CRC dx	n=20	n=1808	
Mean age with SD	51 +/- 9	51 +/- 10	0.85
CRC site	n=16	n=1581	
R-sided	7 (44%)	476 (30%)	0.27
L-sided & rectum	9 (56%)	1105 (70%)	
MMR status	n=18	n=1629	
proficient	18 (100%)	1500 (92%)	--
deficient	0	129 (8%)	
BRAF status	n=17	n=1563	
Wildtype	16 (94%)	1396 (89%)	1.0
Mutant	1 (6%)	167 (11%)	
KRAS status	n=17	n=1500	
Wildtype	8 (47%)	978 (65%)	0.13
Mutant	9 (53%)	522 (35%)	

NTHL1 gene

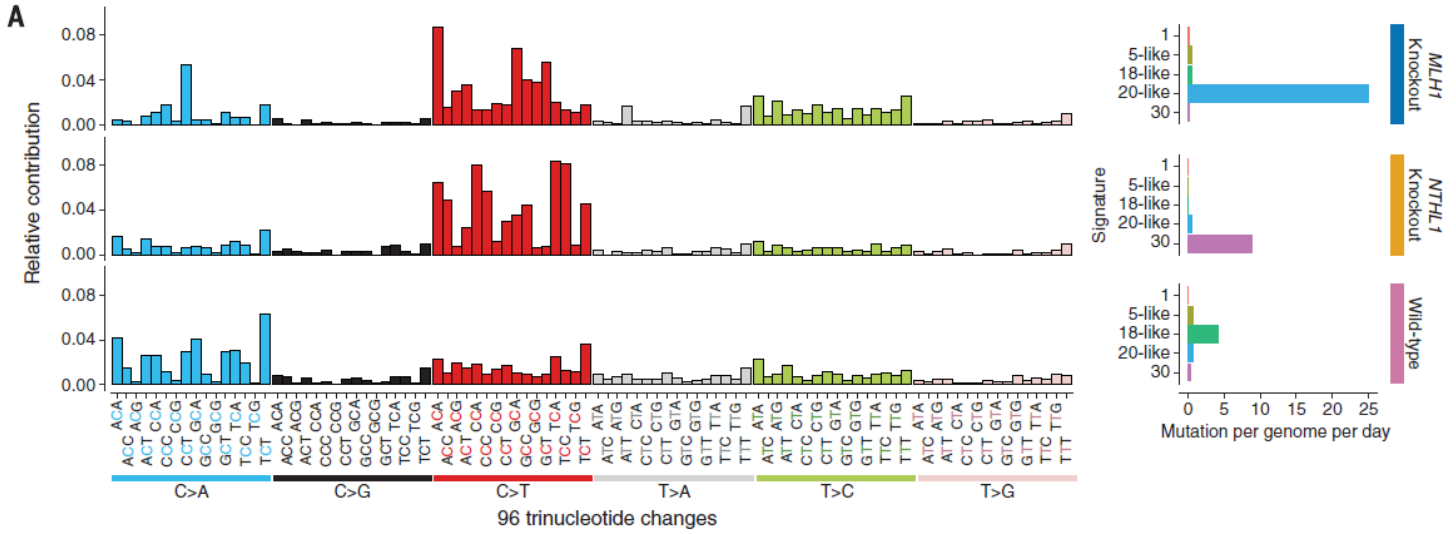
- Part of the base excision repair pathway
- Bifunctional glycosylase (glycosylase and AP lyase activity)





Weren et al. J Pathol, 2018.

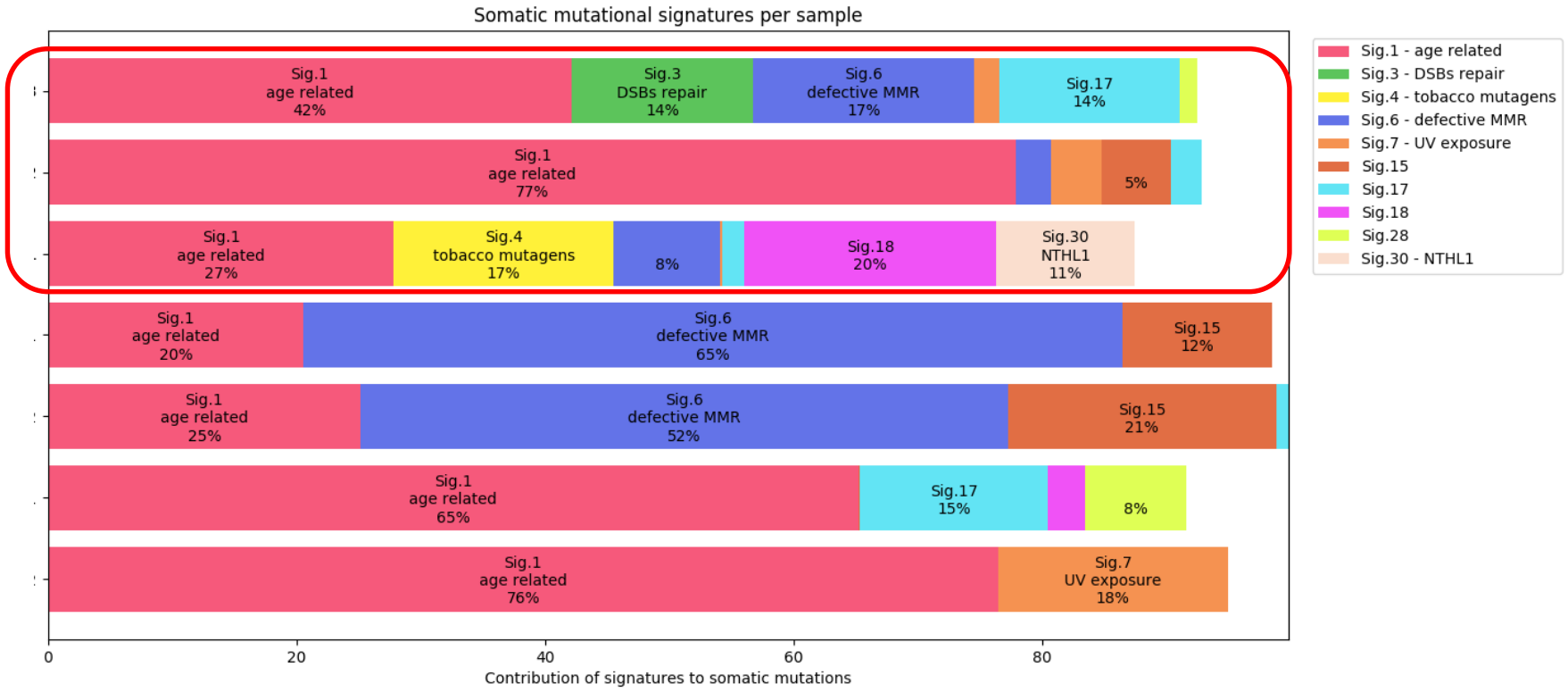
Mutational signature 30



- Identify oxidized cytosine products:
  - 5-hydroxycytosine
  - 5-hydroxyuracil
- Oxidized cytosine products have an increased propensity to pair with adenine resulting in a C>T transition

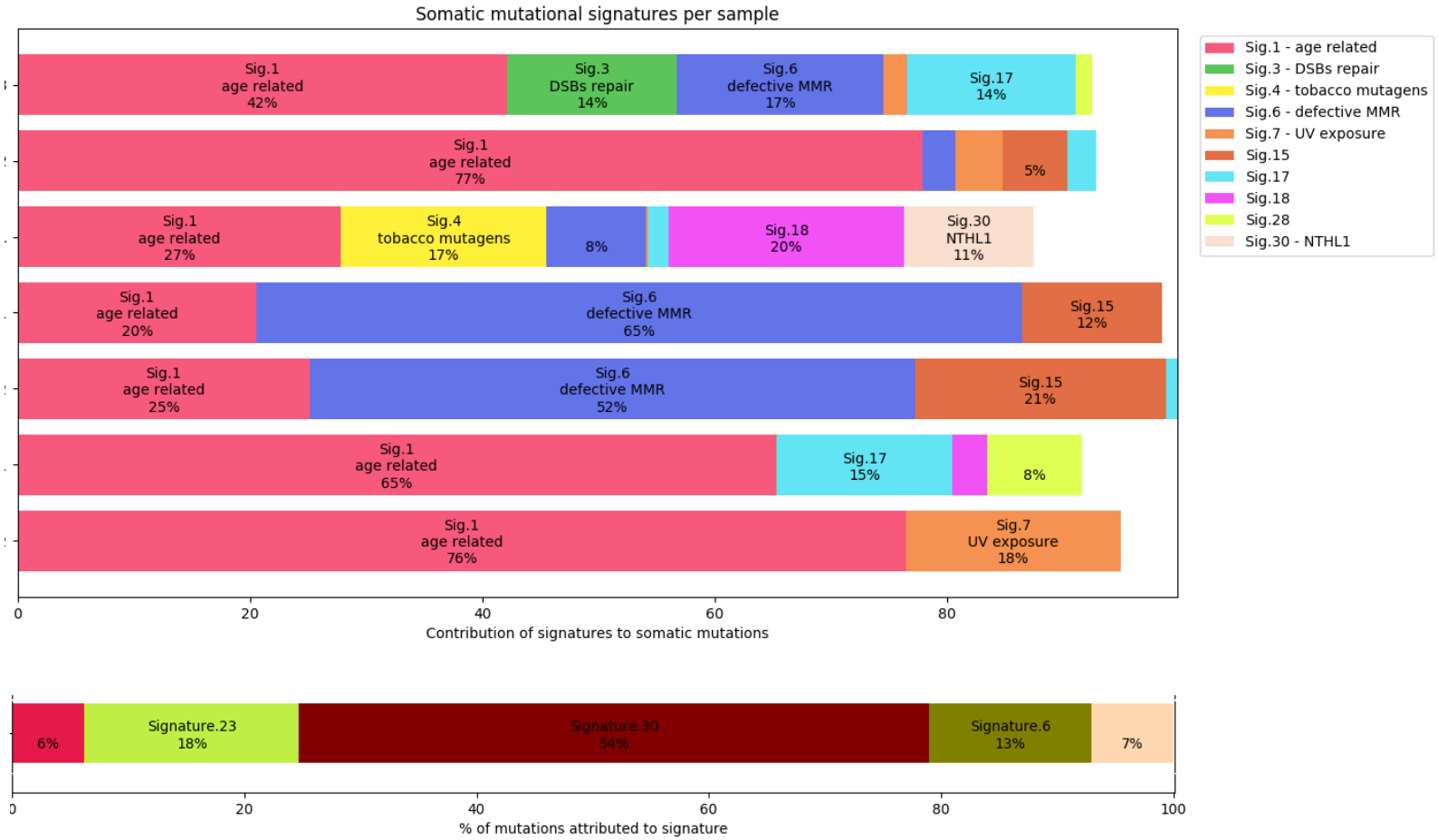
Drost et al. Science, 2017

Tumour mutational signature



NTHL1 p.Q90* heterozygote; pMMR (MSI-low) rectal ca dx at 43

Tumour mutational signature



Conclusions

Performed large case-control study of well characterised CRC-affected probands

- No statistical or aetiological evidence to suggest that heterozygous germline *NTHL1* mutations are associated with an increased CRC risk
- No evidence for implementation of risk mitigation strategies or cascade testing in those found to carry a heterozygous *NTHL1* gene mutation

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Melbourne Genomics Health Alliance

Global knowledge. Individual care.

http://www.buchananlab.org/

Colon Cancer Family Registry Cohort

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