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ICR

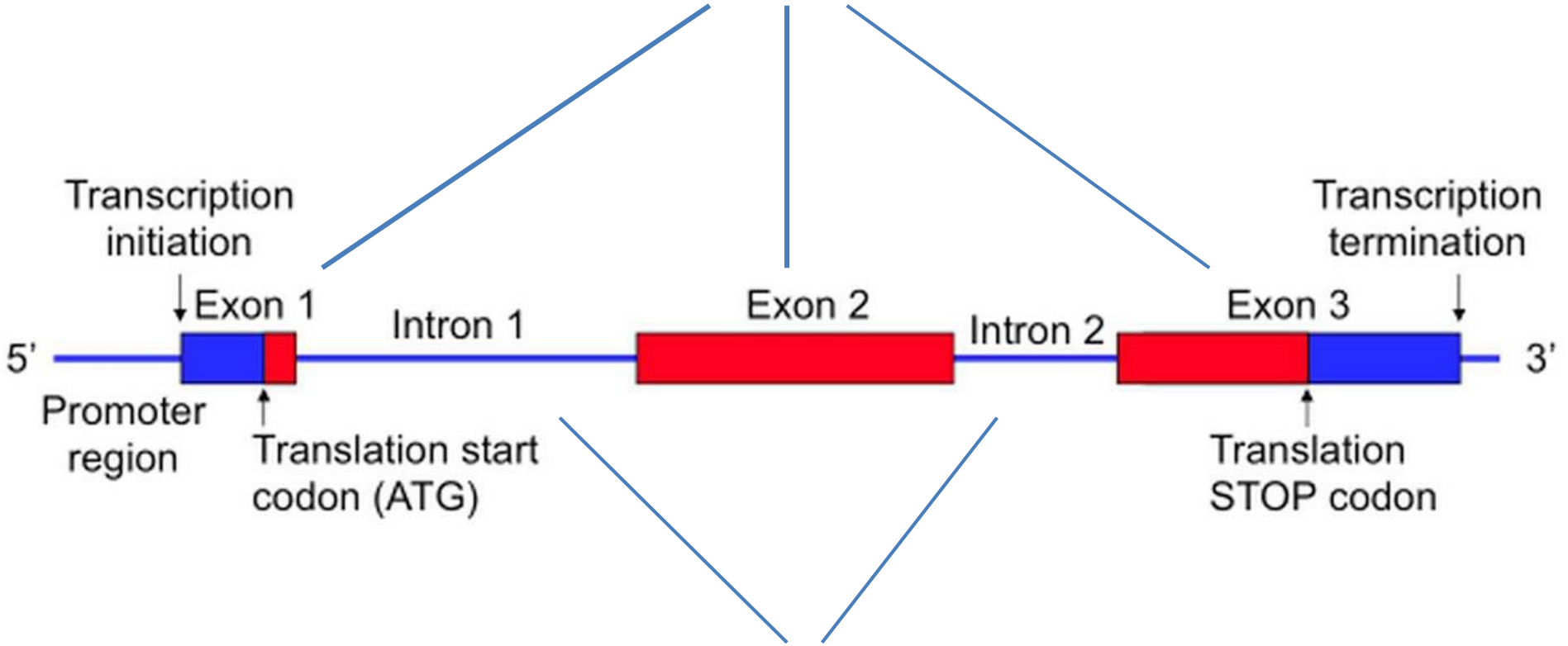
The International Sarcoma Kindred Study (ISKS)

Prof Ian Judson MD, FRCP

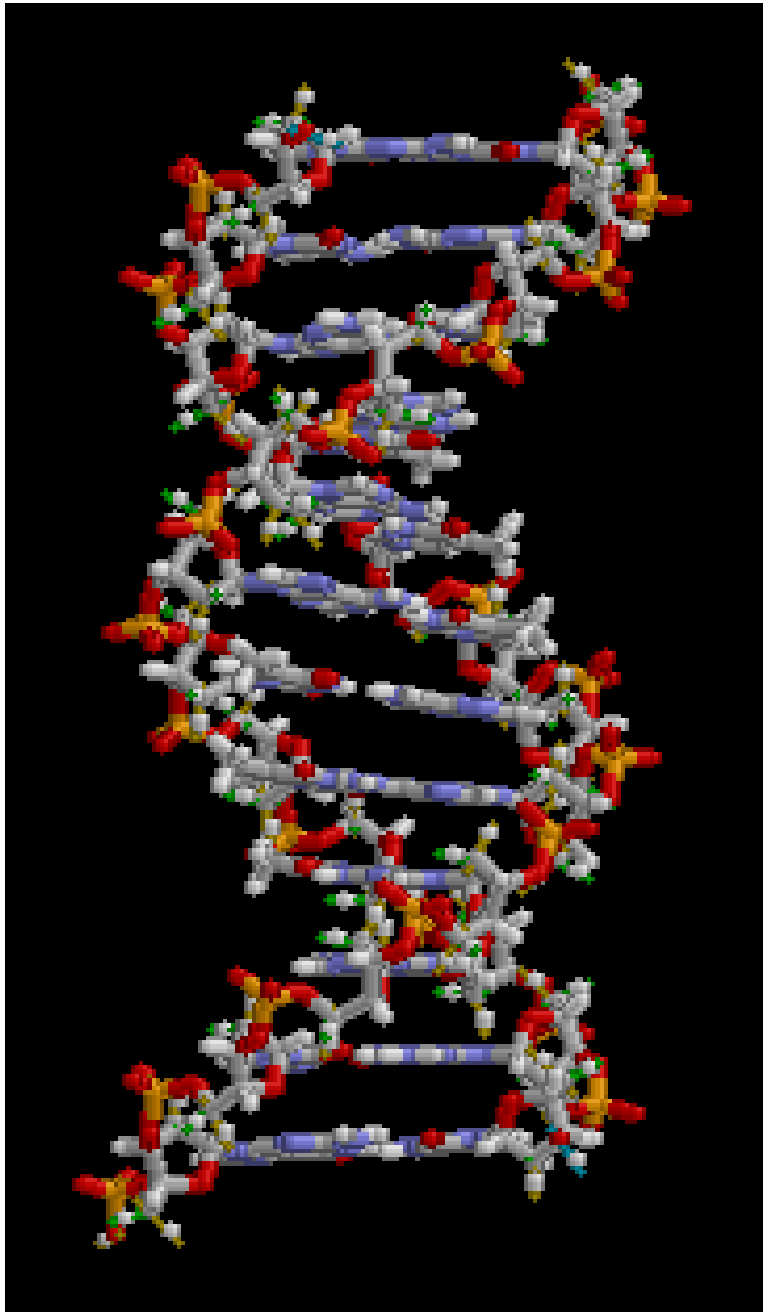
The Institute of Cancer Research

Structure of a gene

Exons: Transcribed and spliced together to form messenger RNA (mRNA) – codes for protein



Introns: are transcribed but then removed from mRNA – in part code for regulatory RNA

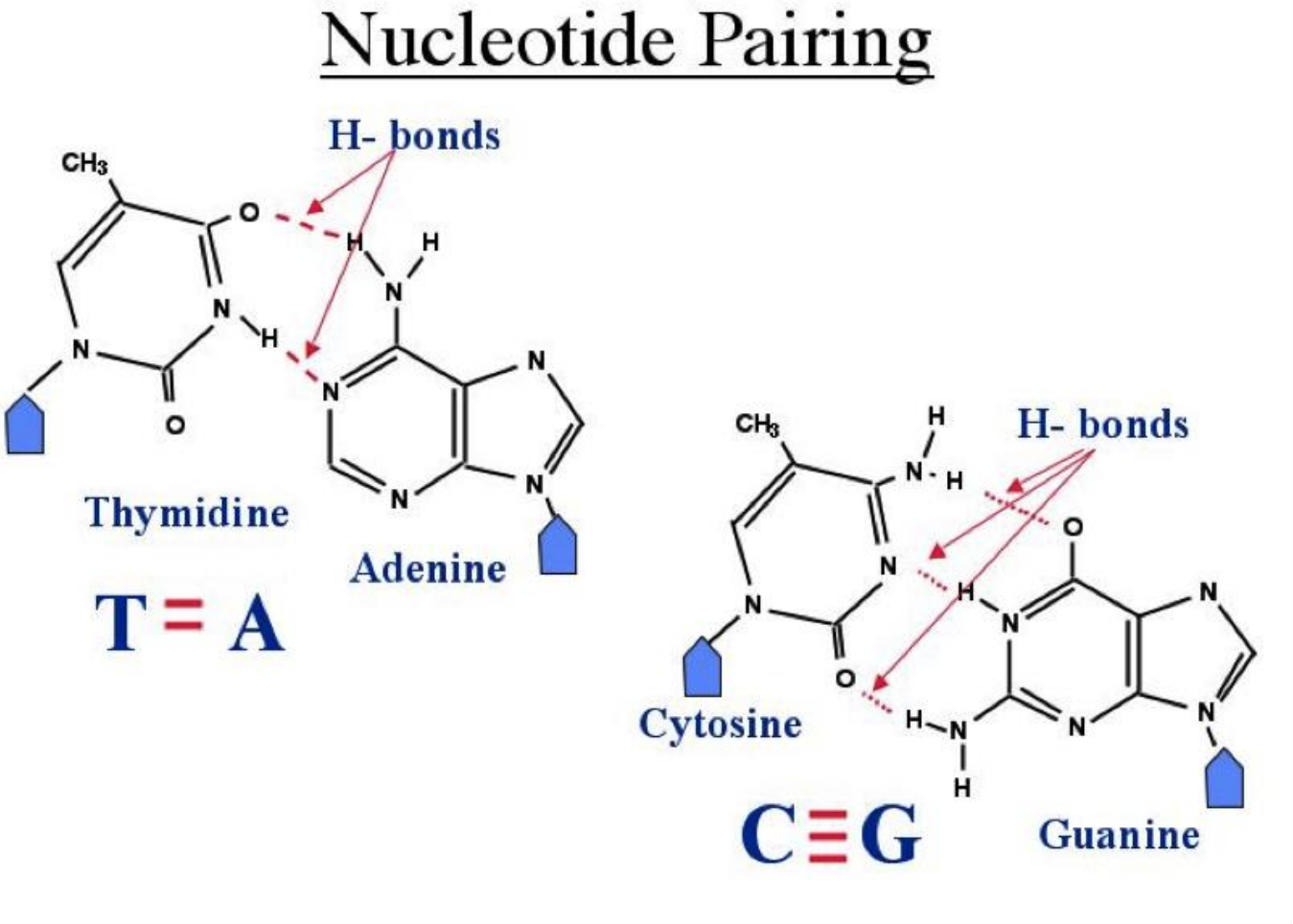


Base pair matching DNA-DNA; DNA-RNA

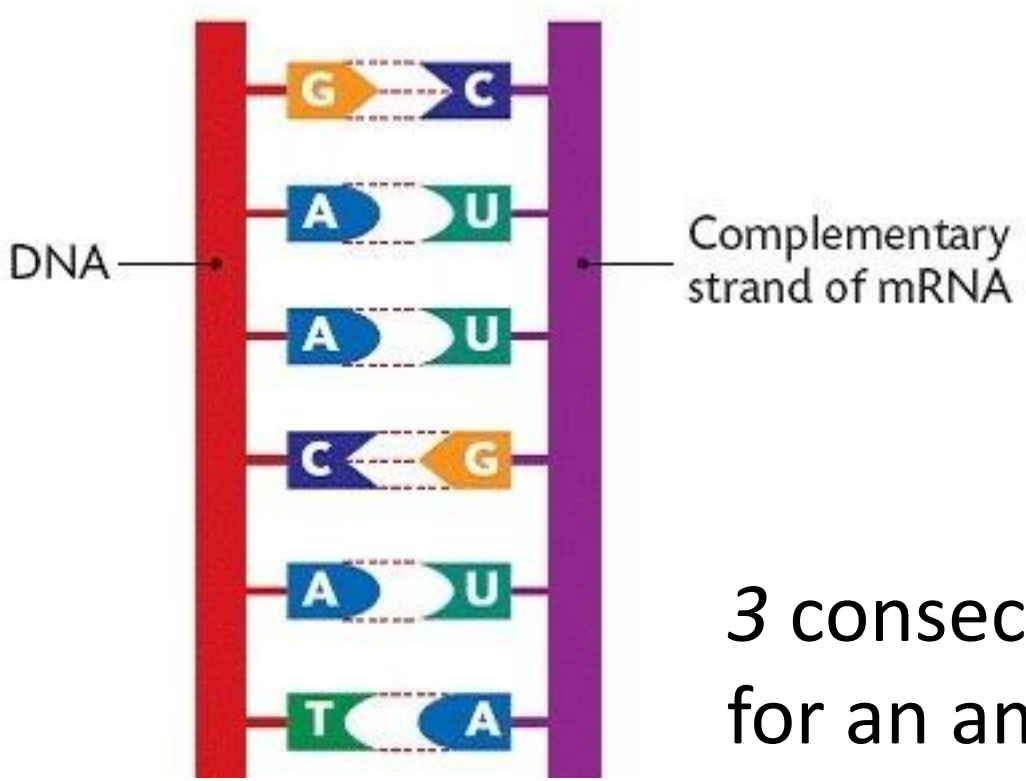
- DNA has 4 “letters” or bases & is double-stranded
  - TAGC: Thymine, Adenine, Cytosine, Guanine
- DNA bases pair as T-A & C-G, permitting the two complementary strands of the double helix to replicate precisely

- RNA has 4 bases and is single stranded
  - UACG: Uracil, Adenine, Cytosine, Guanine
- DNA-RNA bases pair as T-A, A-U, & C-G

How base pairing works



DNA → RNA: making mRNA

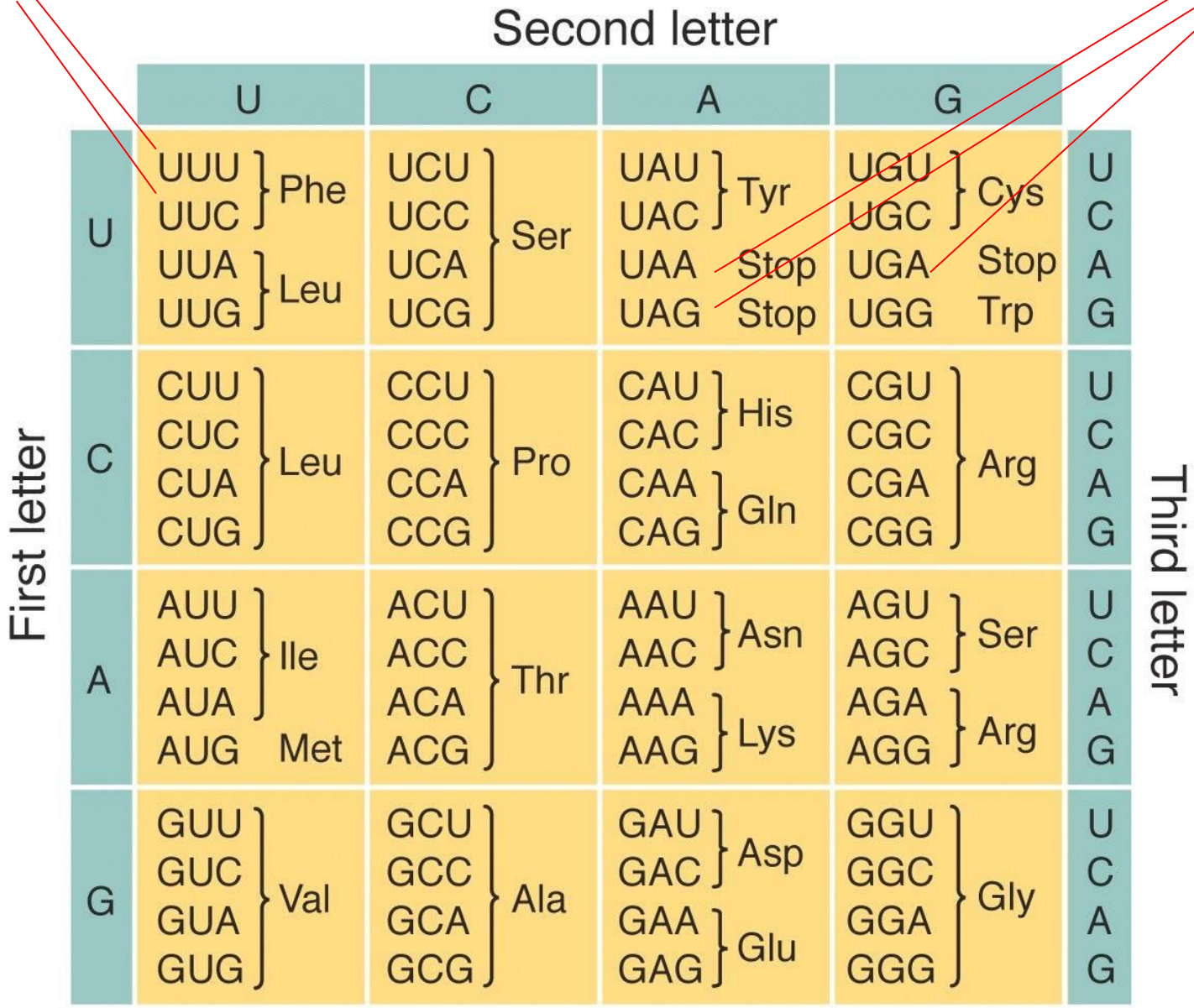


3 consecutive bases code for an amino acid – the building blocks of protein – *this is the real genetic code*

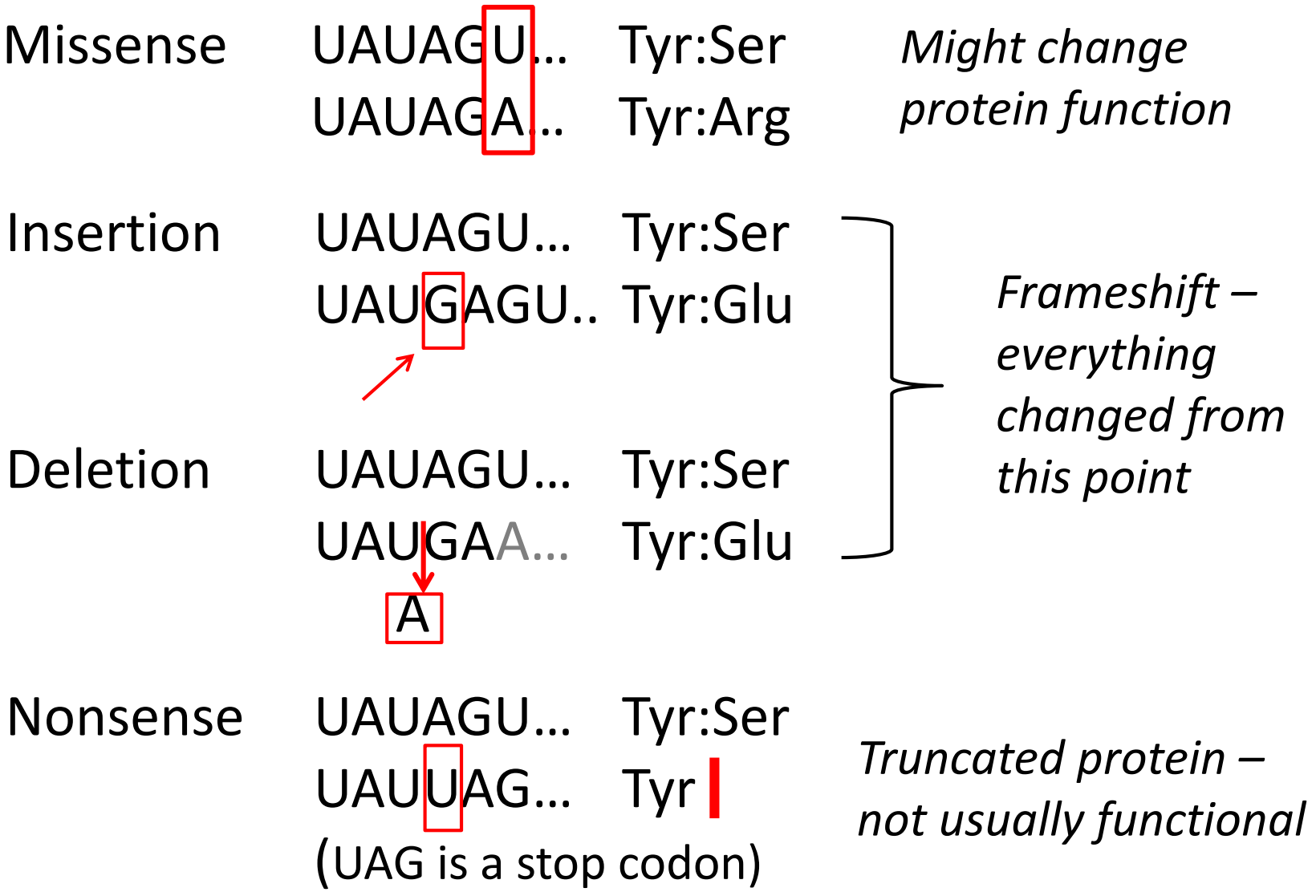
The genetic code - RNA

Note redundancy

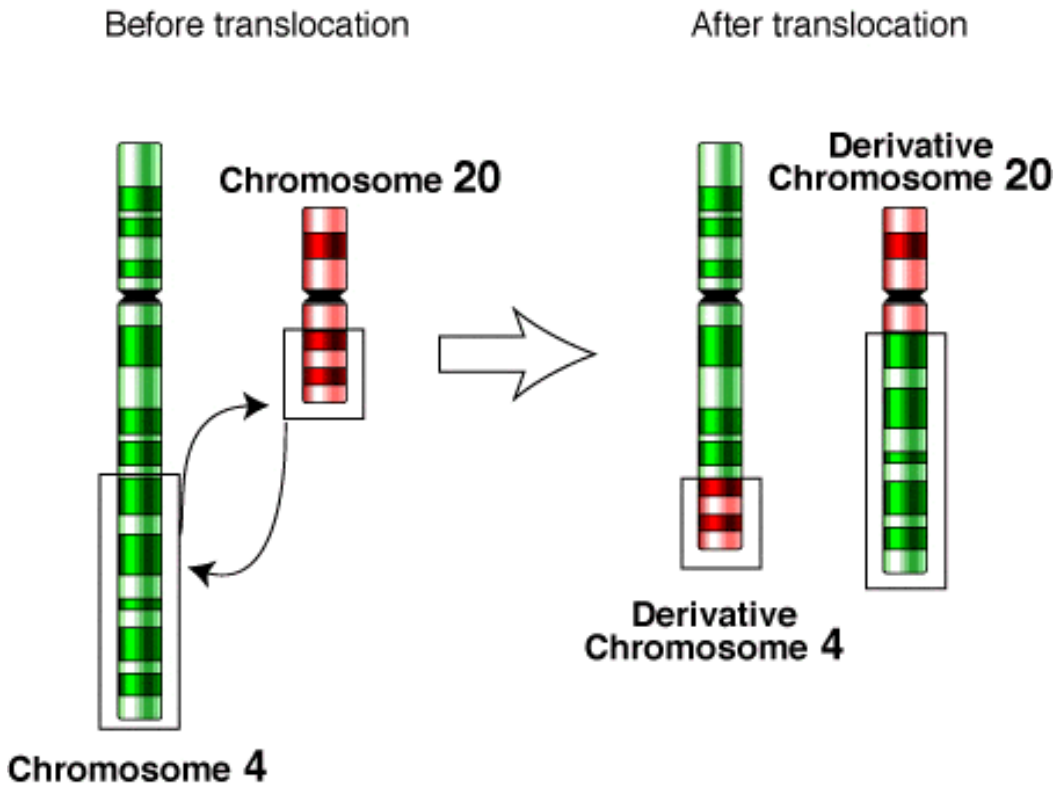
Stop codons



Some types of mutation



Chromosomal translocation – e.g. synovial sarcoma, Ewing sarcoma, etc



Result can be gain of function or loss of function

Complex karyotype sarcoma – e.g. LMS, UPS: multiple duplications, deletions, translocations, etc. – due to failure of DNA repair?

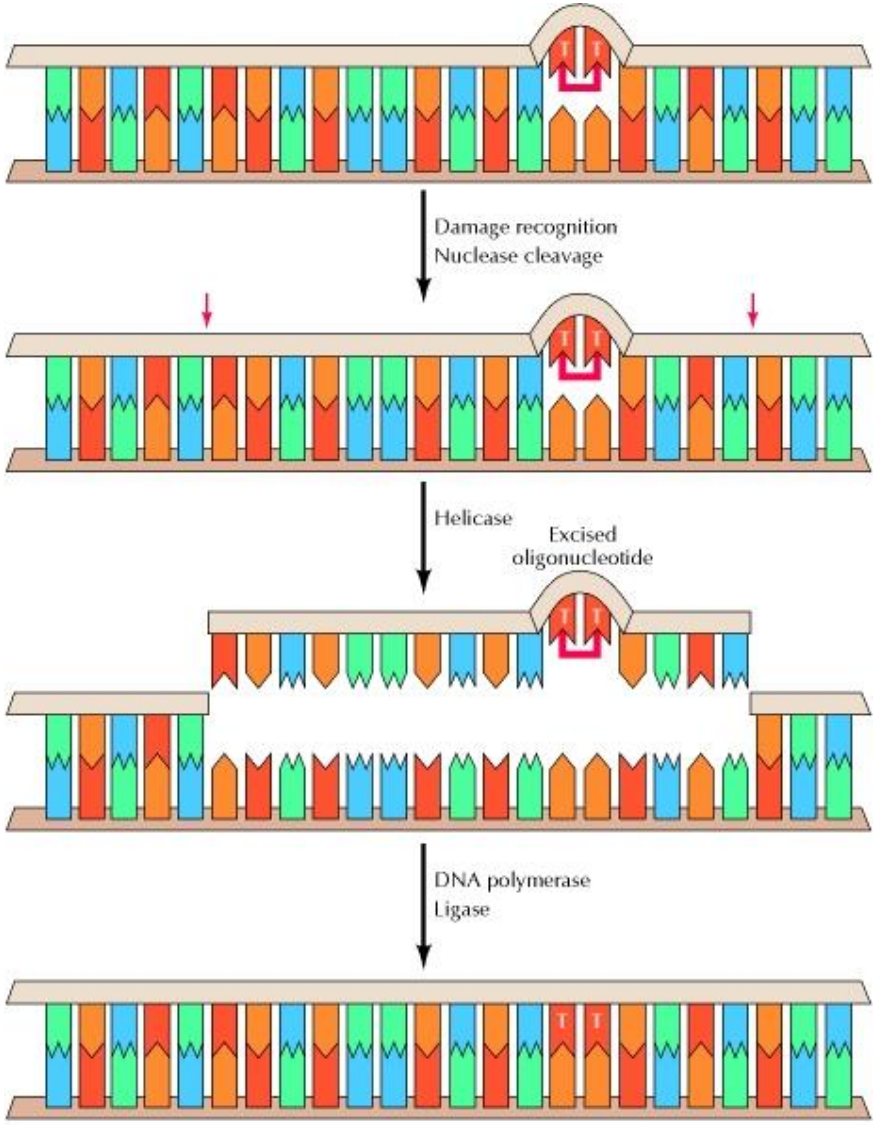
Types of DNA repair

- Base Excision Repair (BER)
- Mismatch Repair (MMR)
- Single-Strand Breaks (SSBs)
- Double-Strand Breaks (DSBs)

Example: Nucleotide Excision Repair (NER)

- removing a thymidine dimer from DNA

- Repairing a DSB *much* more complicated



Genomics - DNA sequencing in sarcoma

- Few sarcomas are driven by specific mutations
  - Activated *KIT* in GIST is an exception
- Gene sequencing studies have as yet had a relatively low yield for identifying targetable drivers
  - Some new targets discovered, e.g. NTRK2
- Understanding inherited predisposition may improve our understanding of the underlying molecular mechanisms involving sarcomas

Molecular targets in sarcomas

- Tumour-specific mutations
  - activating receptor tyrosine kinase
    - e.g. KIT in GIST
  - loss of function of tumour suppressor genes
    - e.g. TSC1/2 in PEComa activates mTOR pathway
- Translocation-related targets
  - activate key gene or lose suppression
    - e.g. COL1A1/PDGFB in DFSP; loss of SMARCB1 in synovial
- Gene amplification
  - usually activation
    - e.g. CDK4/MDM2 amplification in dediff liposarcoma
- Angiogenesis / tumour – stromal interactions

ASCO 2017 **N**ext **G**eneration **S**equencing (NGS) in 4900 sarcoma pts

Gounder et al, J Clin Oncol 2017;35(15_suppl) 11001

- 62,000 mutations, 1200 fusions
- 8% - abnormalities "*actionable*" by approved drugs – *not necessarily proven activity*
- 9% - possibly actionable by drugs approved for other indications
- 40% - a biomarker or possible driver linked to investigational agents
- **9% - germline abnormalities (incl *BRCA1/2*, *ARID1, FANCX*)**
- Potentially "actionable" mutations included: *AKT, ESR1, BRCA, NTRK, PTCH1, SMARCB1 & others*

ASCO 2017 NGS in sarcomas

Gounder et al, J Clin Oncol 2017;35(15_suppl) 11001

- Partial /Complete responses seen with inhibitors of NTRK, IDH1, BRAF, PI3K/mTOR, MDM, SMARCB1
- NGS changed diagnosis and treatment in 5% and avoided futile therapy in 5%
- NGS could have major impact in future, but requires further validation

ASCO 2017 NGS in sarcomas

Italiano et al J Clin Oncol 2017;35(15_suppl)11002

- AACR GENIE consortium 587 pts
  - 10 most frequently mutated genes: *TP53 (35%), ATRX, KMT2D, NF1, ATM, PI3KCA, ERBB4, PTEN, ARID1A*
  - Most frequently amplified genes: *MDM2, CDK4, MAP2KA, TERT*
  - Most frequently deleted genes: *RB1, CDKN2A, TP53, PTEN*
  - High percentage of *potentially* actionable mutations

Cancer predisposition syndromes associated with sarcoma

- **Li-Fraumeni** *(TP53)* – all sarcomas
- **Hereditary retinoblastoma** *(RB1)* – osteosarcoma, LMS, others
- **Neurofibromatosis** *(NF1)* – MPNST
- **Familial adenomatous polyposis** *(FAP)* – desmoid tumour
- **Familial, syndromic GIST** – (*KIT) SDH* – in Carney-Stratakis syndrome
- **Tuberous sclerosis complex** *(TSC1/2)* – PEComa
- **Hereditary leiomyomatosis** – *(FH)* - mainly benign leiomyomata (rarely malignant) and renal cancer

COMPLEX GENOTYPE SARCOMAS DISPLAY FAMILIAL INHERITANCE INDEPENDENT OF KNOWN CANCER PREDISPOSITION SYNDROMES

Kevin B. Jones, Josh Schiffman, Wendy Kohlmann, R. Lor Randall, Stephen L. Lessnick, and Lisa A. Cannon-Albright

Cancer Epidemiol Biomarkers Prev 2011 May ; 20(5): 751–757.

- Utah Cancer Registry and **Utah Population Database (2.3 million people)** interrogated for sarcomas – split into complex genotype and balanced translocation
- **Geneological Index for Familiality (GIF)** calculated and relative risk (RR) for 1st, 2nd , 3rd degree relatives estimated
- 229 balanced and 1161 complex genotype sarcomas identified with at least 3 generations of ancestral information
- No evidence inherited risk for balanced translocation group, but **significant GIF (p=0.03) in complex genotype group**
- 20 high risk pedigrees: >5 sarcomas and other cancers, didn't fit known syndromes

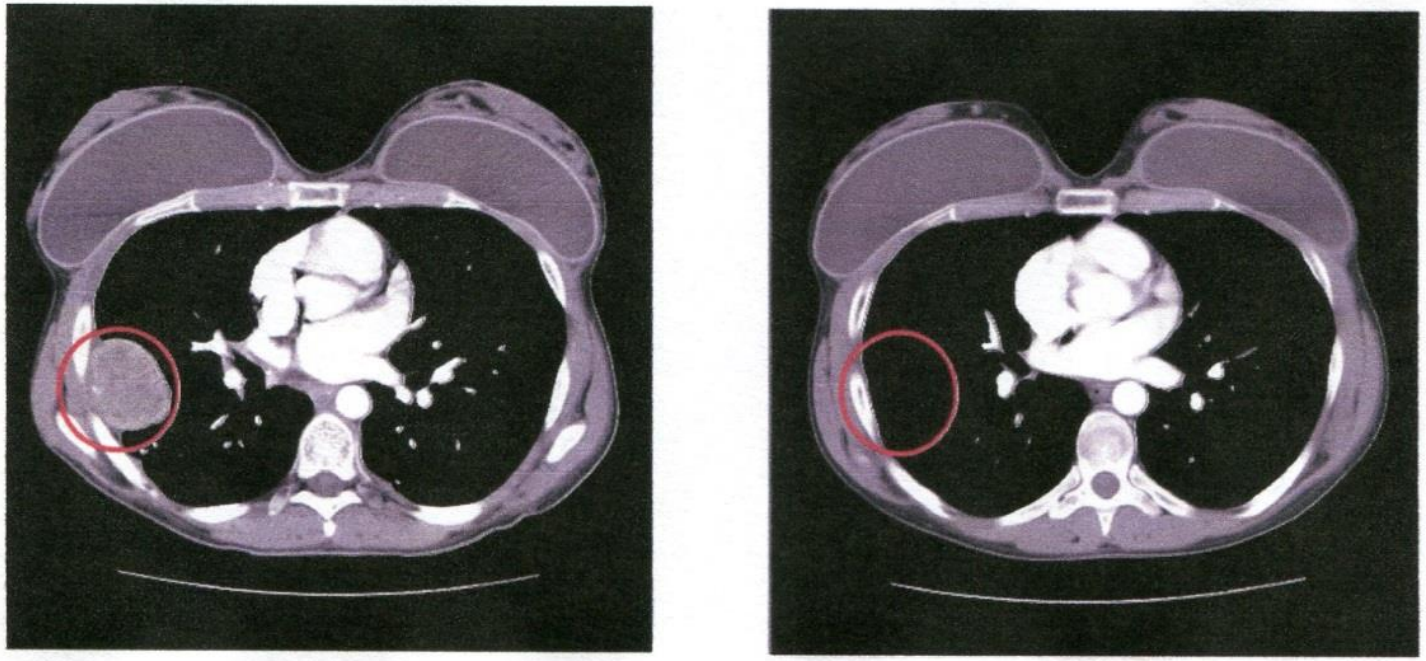
Germline, i.e. inherited, *PTPRD* Mutations in Ewing Sarcoma: Biologic and Clinical Implications

Yunyun Jiang, Filip Janku, Vivek Subbiah, Laura S. Angelo, Aung Naing, Peter M. Anderson, Cynthia E. Herzog, Siqing Fu, Robert S. Benjamin, Razelle Kurzrock

Oncotarget 2013;4(6):884-889

- Novel germline mutation in tumour suppressor gene *Protein tyrosine phosphatase delta (PTPRD)* in 3/8 pts with metastatic Ewing sarcoma (37.5%)
- Impact expected to be loss of STAT3 dephosphorylation, a function of PTPRD
- STAT3 phosphorylated after recruitment to IGF-1R so mutation could lead to constitutive activation of IGF-1R
- 2/3 pts with germline PTPRD mutatoins achieved durable responses following treatment with IGF-1R MAb – based therapy

Response to IGF-1R inhibition in patient with *PTPRD* mutant Ewing sarcoma



Prior to treatment

15 months after treatment

Patient 1 demonstrated a durable complete response to therapy with an IGF-1R inhibitor.

Jiang et al Oncotarget 2013;4(6):884-889

Frequent inactivating germline mutations in DNA repair genes in patients with Ewing sarcoma Germline mutations in Ewing sarcoma

Andrew Brohl, Rajesh Patidar, Clesson Turner, Xinyu Wen, Young Song, Jun Wei, Kathleen Calzone, Javed Khan.

Genet Med 2017;19:955-958

- Germline sequencing 175 pts with Ewing sarcoma
- 51 tier 1 variants, 23 likely pathogenic - including *APC, BLM, BRCA1, ERCC3, FANCC, FANCM, MITF, PTCH2, RAD51, RET, TP53*
- Genes involved in double-strand DNA repair enriched
- Number of potentially actionable mutations, e.g. *BRCA1/2:* PARP inhibitors, *PTCH1/2:* Hedgehog pathway inhibitors

Different approaches to studying cancer predisposition

GeMCaS
Genetics of Multiple Cancers Study

The ROYAL MARSDEN
NHS Foundation Trust

ICR

Molecular investigations into the genetic causes of multiple primary cancers: Pilot Phase

Short Title: Genetics of Multiple Cancers Study (GeMCaS)

Chief Investigator : Clare Turnbull

The International Sarcoma Kindred Study:
A global multi-site prospective cancer genetics study

Chief investigator David Thomas, Sydney

International Sarcoma Kindred Study

- Recruit sarcoma patients and their families
- Obtain germline DNA (from blood) from both patient and relatives (1st and 2nd degree)
- Construct family tree, or pedigree, of cancer history
  - Particular interest in patients with multiple primary tumours
- Study genetics – initially by sequencing known or likely cancer genes, later whole genome

Monogenic and polygenic determinants of sarcoma risk: an international genetic study

Mandy L Ballinger*, David L Goode*, Isabelle Ray-Coquard, Paul A James, Gillian Mitchell, Eveline Niedermayr, Ajay Puri, Joshua D Schiffman, Gillian S Dite, Arcadi Cipponi, Robert G Maki, Andrew S Brohl, Ola Myklebost, Eva W Stratford, Susanne Lorenz, Sung-Min Ahn, Jin-Hee Ahn, Jeong Eun Kim, Sue Shanley, Victoria Beshay, Robert Lor Randall, Ian Judson, Beatrice Seddon, Ian G Campbell, Mary-Anne Young, Rajiv Sarin, Jean-Yves Blay, Seán I O'Donoghue, David M Thomas, for the International Sarcoma Kindred Study†

Ballinger et al.
Lancet Oncology
2016;17(9):1261-71

International Sarcoma Kindred Study

		Proband
Participants		
	Male	586
	Female	576
Mean age at diagnosis (yrs±SD)		
	First cancer	44.1±18.5
	Sarcoma	45.2±18.9
Number with multiple primary cancers		170 **(15%)**
	2 primary cancers	128
	3 primary cancers	32
	≥4 primary cancers	10

Pedigree classification	Number	Risks to FDR (95% CI)
No syndrome	669	0.79 (0.71-0.88)
Classic/Chompret Li Fraumeni Syndrome	116	2.36 (1.95-2.87)
Hereditary breast/ovarian cancer	6	2.64 (1.32-5.28)
Hereditary colorectal cancer	14	2.29 (1.38-3.79)
Clinically suspicious*	87	1.83 (1.55-2.15)
Other	14	1.2 (0.89-1.61)
Uninformative	256	-

16% recognisable syndromes

Clinically actionable mutations

(American College of Genetics and Genomics reporting guidelines)

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ACMG POLICY STATEMENT | Genetics inMedicine

ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing

Robert C. Green, MD, MPH[1,2], Jonathan S. Berg, MD, PhD[3], Wayne W. Grody, MD, PhD[4–6], Sarah S. Kalia, ScM, CGC[1], Bruce R. Korf, MD, PhD[7], Christa L. Martin, PhD, FACMG[8], Amy L. McGuire, JD, PhD[9], Robert L. Nussbaum, MD[10], Julianne M. O'Daniel, MS, CGC[3], Kelly E. Ormond, MS, CGC[11], Heidi L. Rehm, PhD, FACMG[2,12], Michael S. Watson, PhD, FACMG[13], Marc S. Williams, MD, FACMG[14] and Leslie G. Biesecker, MD[15]

Genet Med 2013;15(7):565-74

Some key recommended reportable findings relating to cancer (C4/5)

	Gene	Number
Colorectal cancer		
	APC	6
	MMR	11
Breast/ovarian cancer		
	BRCA1	9
	BRCA2	19
	PALB2	5
Gastric cancer		
	CDH1	6
Chompret LFS		
	TP53	12
Neurofibromatosis		
	NF1	4
Gorlin syndrome		
	PTCH1	3
Paraganglioma		
	SDHB	2
Other		
	TSC2/RB1/PTEN	3
Total		80

Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results

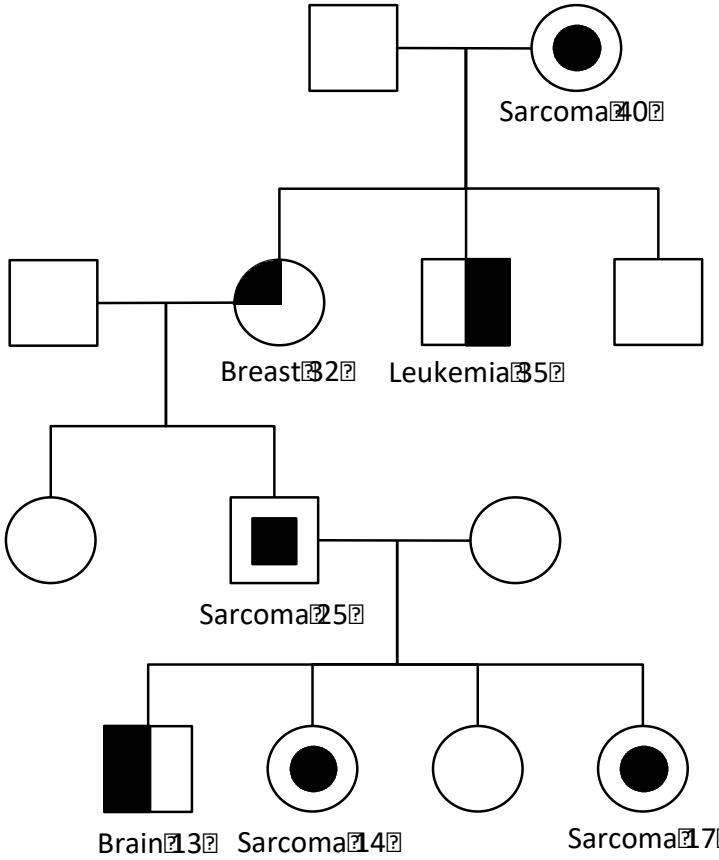
Sharon E. Plon1,*,#, **Diana M. Eccles**2,*, **Douglas Easton**3, **William D. Foulkes**4, **Maurizio Genuardi**5, **Marc S. Greenblatt**6, **Frans B.L. Hogervorst**7, **Nicoline Hoogerbrugge**8, **Amanda B. Spurdle**9, and **Sean Tavtigian**10 **for the IARC Unclassified Genetic Variants Working Group†**

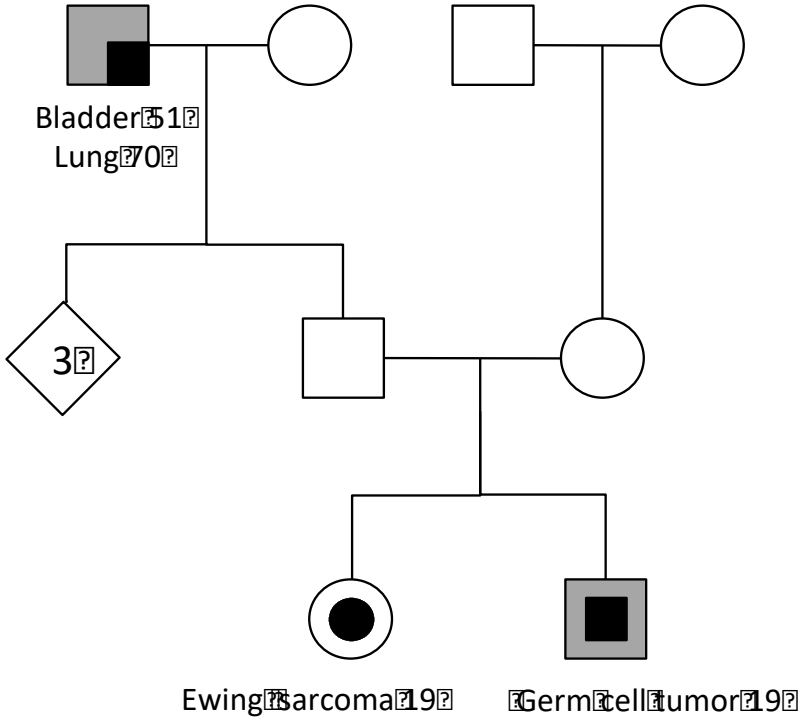
Hum Mutat. 2008 November ; 29(11): 1282–1291

Classification System for Sequence Variants Identified by Genetic Testing

Class	Description	Probability of being Pathogenic
5	Definitely Pathogenic	>0.99
4	Likely Pathogenic	0.95–0.99
3	Uncertain	0.05–0.949
2	Likely Not Pathogenic or of Little Clinical Significance	0.001–0.049
1	Not Pathogenic or of No Clinical Significance	<0.001

Diverse patterns of inheritance

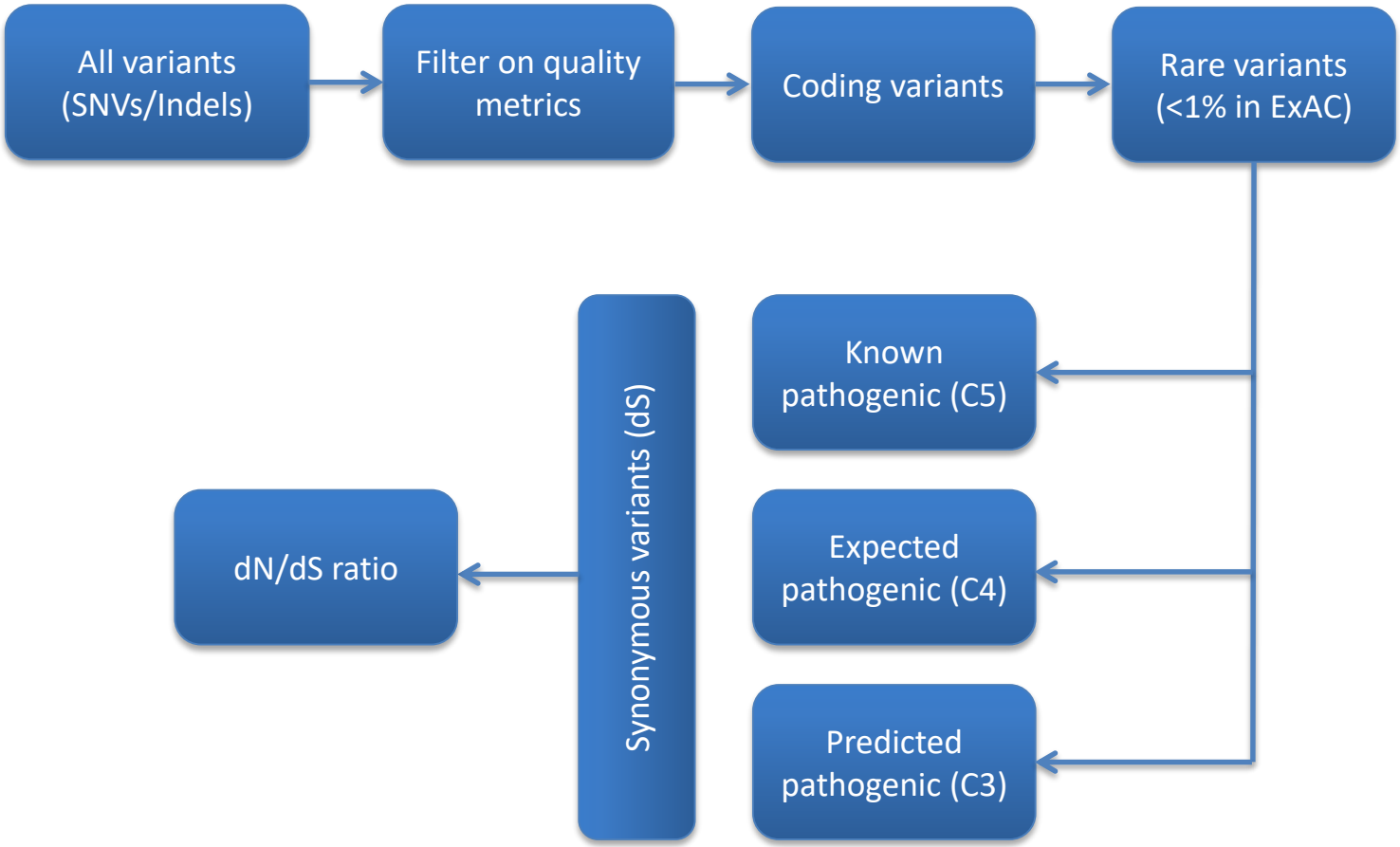




dN/dS ratio & other abbreviations

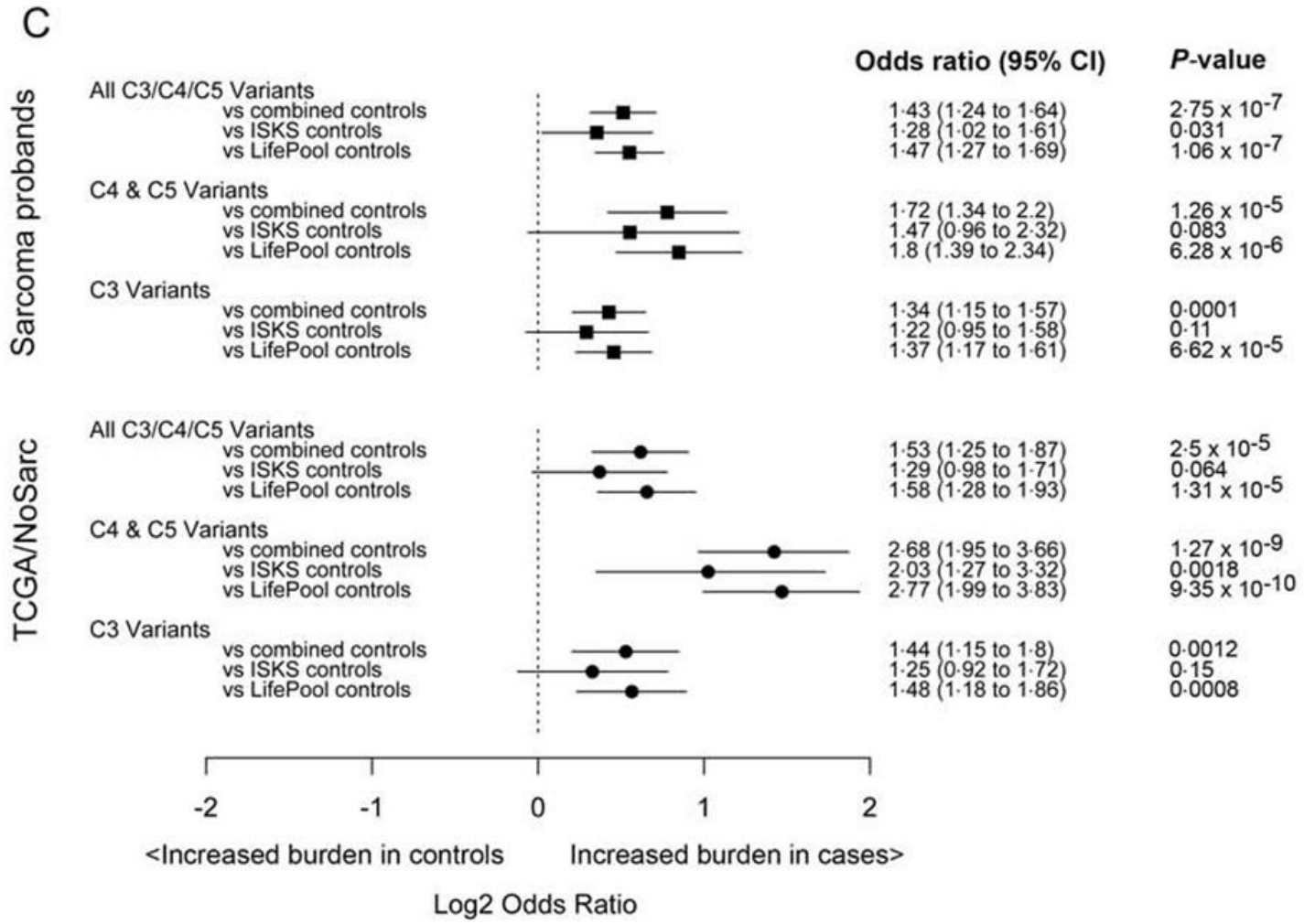
- dS: synonymous variants - nucleotide substitutions that don't change the amino acid
- dN: non-synonymous variants - nucleotide substitutions that change the amino acid, i.e. potentially meaningful mutations
- The dN/dS ratio indicates the amount of alteration from the norm, in normal or in cancer evolution
- SNV- single nucleotide variation
- Indel – insertion or deletion
- ExAc – Exome Aggregation Consortium - browser

Rare variant calling algorithm

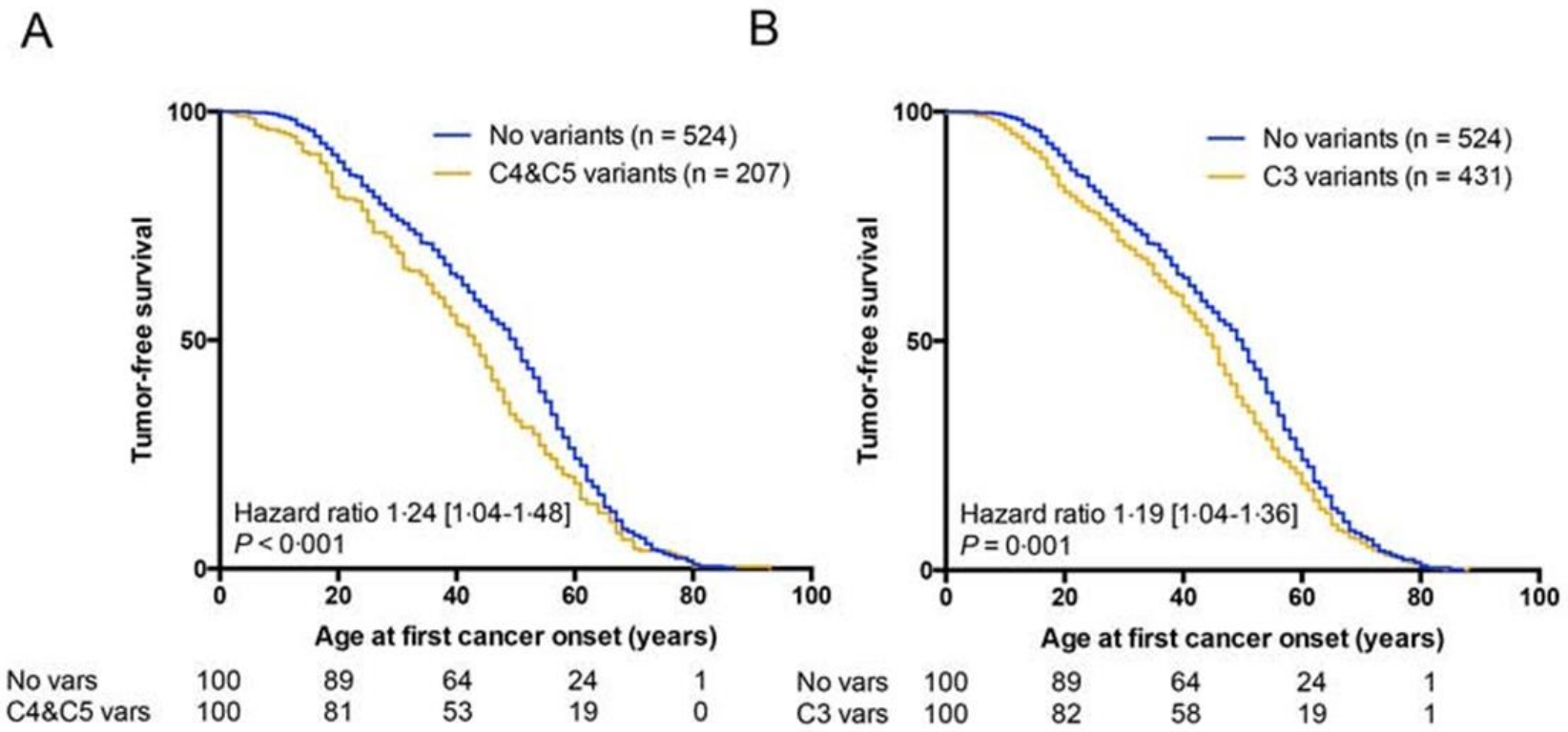


David Goode

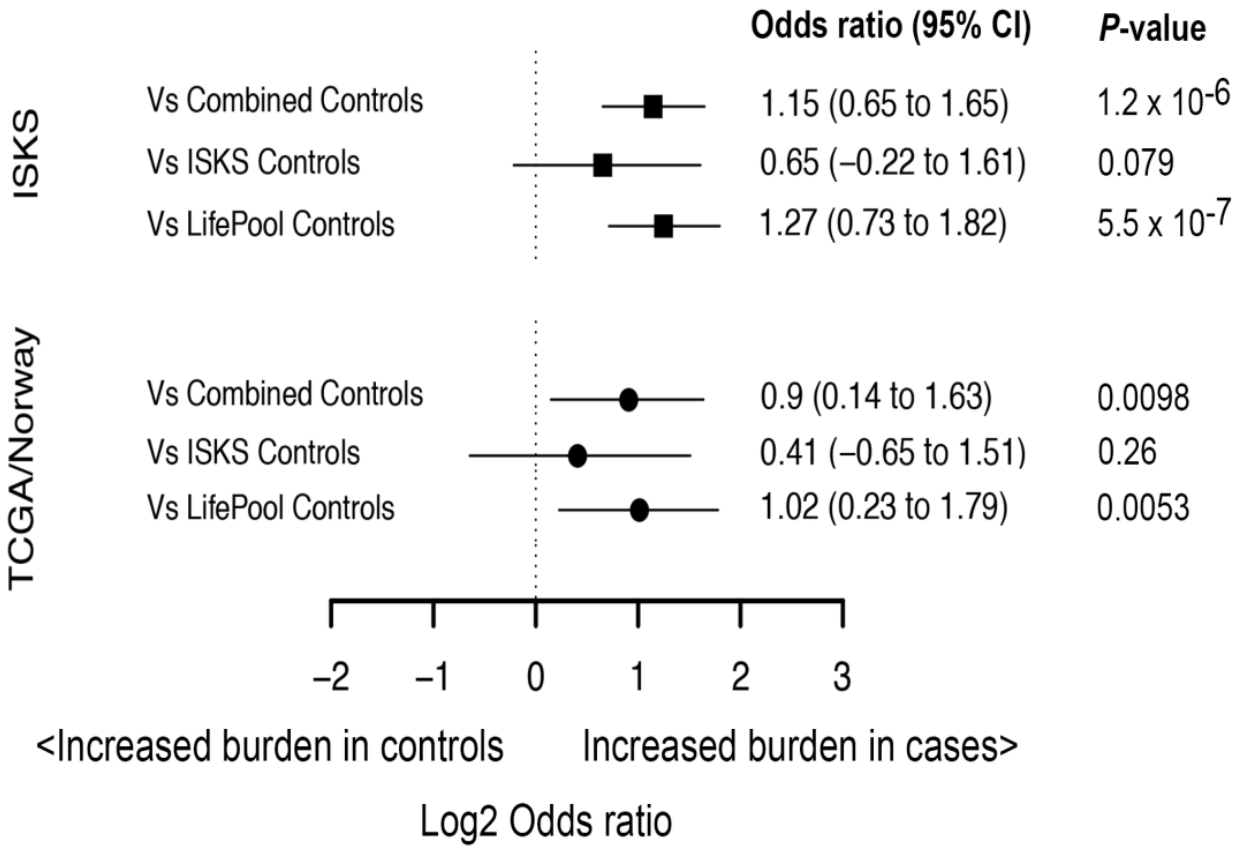
Between cohort analyses: case-control design, number of different series



Within cohort analyses: age at first onset as index of risk



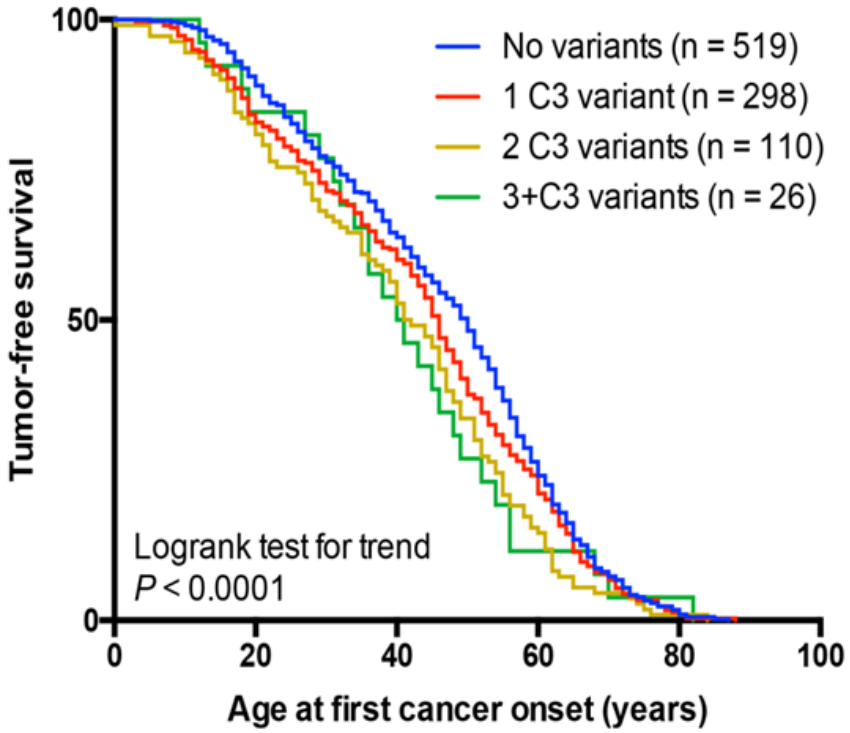
Polygenic inheritance and cancer burden



Slide courtesy of David Thomas

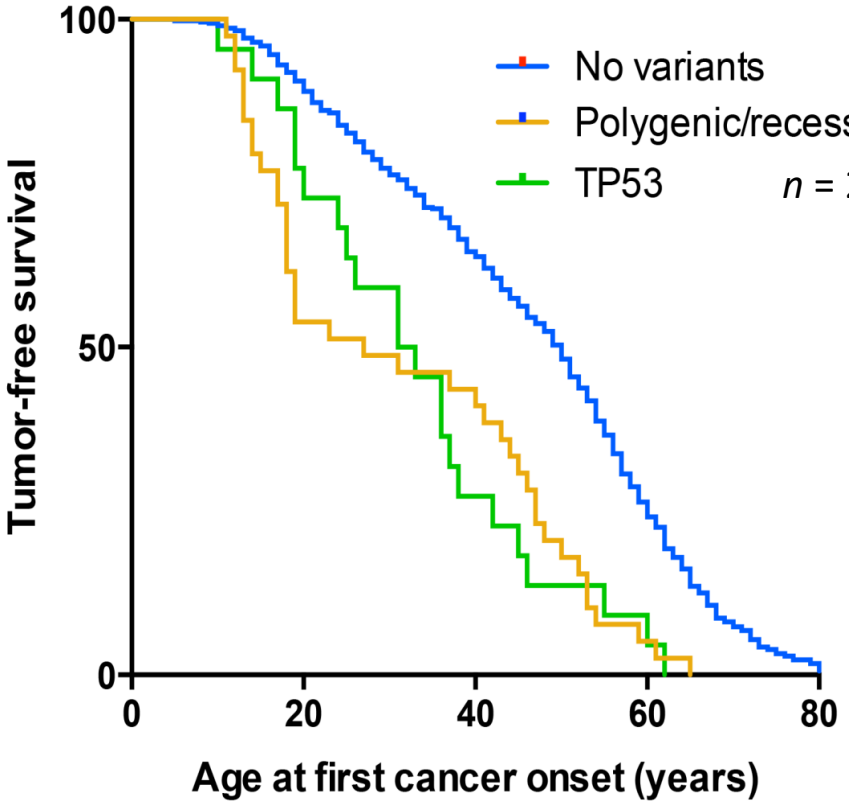
Polygenic inheritance and age at first cancer as measure of impact

A



No vars	100	89	64	24	1
1 C3 var	100	82	60	21	1
2 C3 vars	100	81	53	15	0
3+ C3 vars	100	84	50	12	0

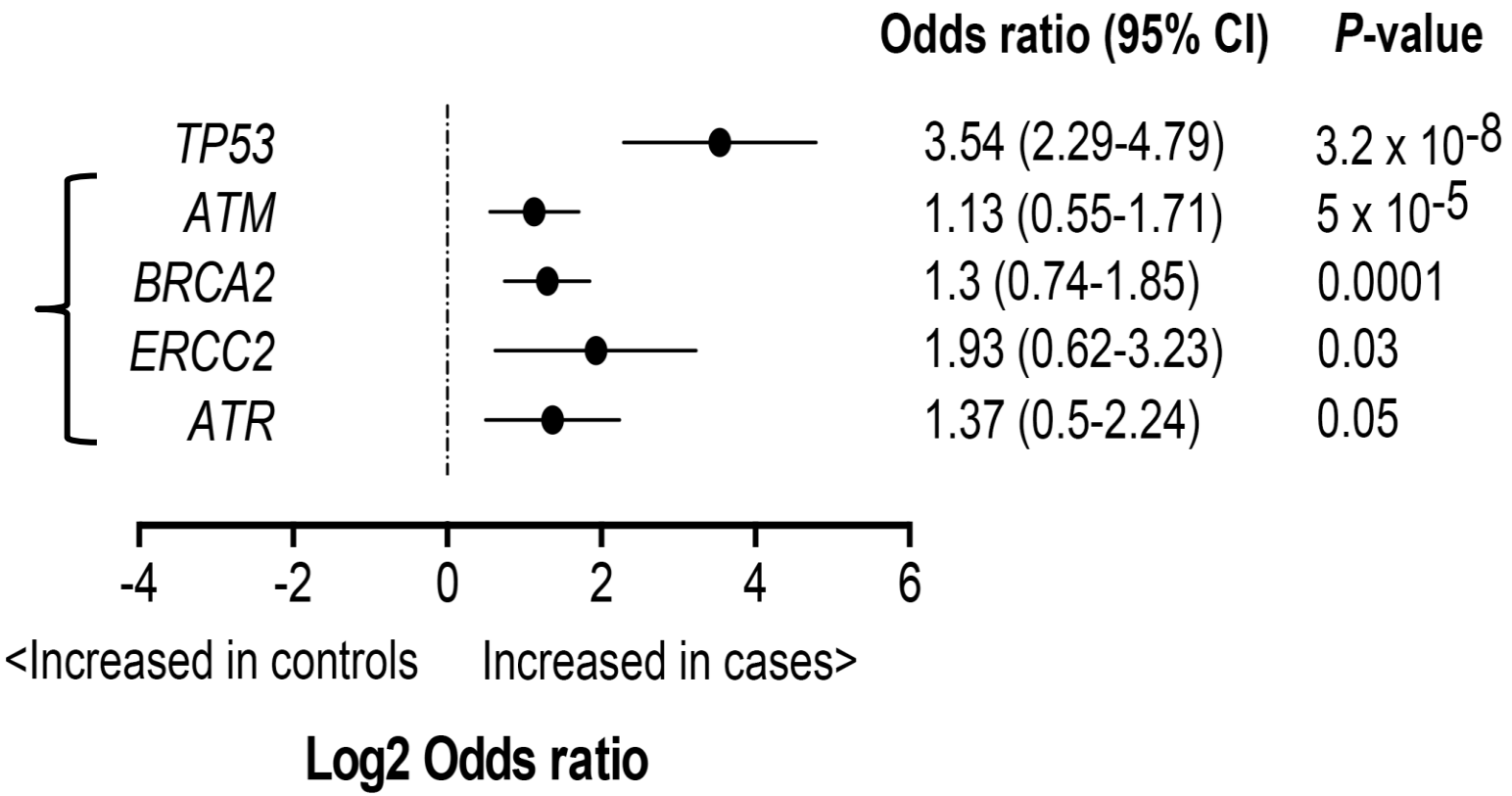
Age at first onset of cancer: polygenic disease *vs TP53 mutation*



	HR	[95% CI]	P-value	Median ACO
P/R	3.95	[2.42-6.46]	<0.0001	27 years
TP53	4.14	[2.15-8]	<0.0001	32 years

Slide courtesy of David Thomas

Alterations in four genes not previously associated with sarcoma



Slide courtesy of David Thomas

David Goode

Normal function of these “sarcoma genes” – recognising DNA damage and DNA repair

- *ATM* – MRN complex recognises DNA double strand breaks and activates ATM, which recruits repair processes
- *ATR* – sensing DNA damage - single strand breaks, activate CHK1, initiate cell cycle arrest
- *BRCA2* – involved in homologous recombination
- *ERCC2* - transcription-coupled nucleotide excision repair

Does ISKS suggest targets for therapy?

- DNA repair gene mutations: *BRCA1/2, ATR, ATM, ERCC2, FANCG, FANCM* suggest role for DNA repair inhibitors, e.g. PARP & ATR inhibitors
- *MLH1/2, MSH6* mutations – may predict response to immune checkpoint inhibitors
- *PTCH1* mutations - Hedgehog inhibitors
- *TSC1/2* mutations - mTOR inhibitors (PEComa)
- *IDH1/2* – specific inhibitors in development (chondrosarcoma?)

Conclusions from the ISKS to date

- 1 in 6 families affected by sarcoma conform to a recognisable heritable cancer predisposition syndrome
- One in 2 individuals carry biologically pathogenic ‘pan-sarcoma’ variants
- One in 15 individuals carry clinically pathogenic variants in actionable genes, mostly without an associated syndromic pattern
- Expanded ‘pan-sarcoma’ risk genes: ERCC2, BRCA2, ATM, ATR
- One in 4 individuals carry variants with possible therapeutic significance
- The frequency and biological impact of rare polygenic causes is at least comparable to monogenic causes

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PLOS | ONE

High Frequency of Germline *TP53* Mutations in a Prospective Adult-Onset Sarcoma Cohort

Gillian Mitchell[1,4☯], Mandy L. Ballinger[2,4☯], Stephen Wong[3], Chelsee Hewitt[3], Paul James[1,4], Mary-Anne Young[1,4], Arcadi Cipponi[2,4], Tiffany Pang[2,4], David L. Goode[2,4], Alex Dobrovic[3,4,5¶], David M. Thomas[1,2,4*¶], on behalf of the International Sarcoma Kindred Study[2]

3.6% incidence of germline *TP53* mutation in ISKS

Table 2. Proband cancers and clinical classification.

Case	Sex	Proband primary cancers, age at diagnosis (yrs)	Clinical classification
Putative germline			
1	M	**rhabdomyosarcoma 33**	LFS
2	M	**osteosarcoma 20**	LFS
3	M	**chondrosarcoma 24; liposarcoma 39**	LFS
4	M	**sarcoma NOS 37; liposarcoma 44**	LFS
5	F	**angiosarcoma 25**	Chomp LFL
6	F	breast 33; **leiomyosarcoma 48**	Chomp LFL
7	F	breast 38; **leiomyosarcoma 45**; thyroid 46	Chomp LFL
8	F	ALL 10; **Ewing sarcoma 16**	Chomp LFL
9	F	breast 26; **sarcoma NOS 36**; pheochromocytoma 37	Chomp LFL
10	M	Hodgkin's lymphoma 34; melanoma 47; **sarcoma NOS 60**	Chomp LFL
11	M	**DSRCT 21**	Negative
12	M	testis 36; rectum 69; **leiomyosarcoma 69**	Negative
13	F	**chondrosarcoma 57**	Negative
14	M	**osteosarcoma 19**	Negative
15	M	**osteosarcoma 31**	Negative
16	F	**leiomyosarcoma 58**	Negative
17	F	**liposarcoma 62**	Negative
Putative somatic			
18	M	**mediastinal GCT with rhabdomyosarcomatous differentiation 19**	Chomp LFL
19	M	**GIST 65;** melanoma 69; **sarcoma NOS 76;** mycosis fungoides 76	Negative
20	F	**sarcoma NOS 80**	Negative

ALL, acute lymphoblastic leukemia; DSRCT, desmoplastic small round cell tumour; GCT, germ cell tumour; GIST, gastrointestinal stromal tumour; Chomp, Chompret; M, male; F, female.

doi:10.1371/journal.pone.0069026.t002

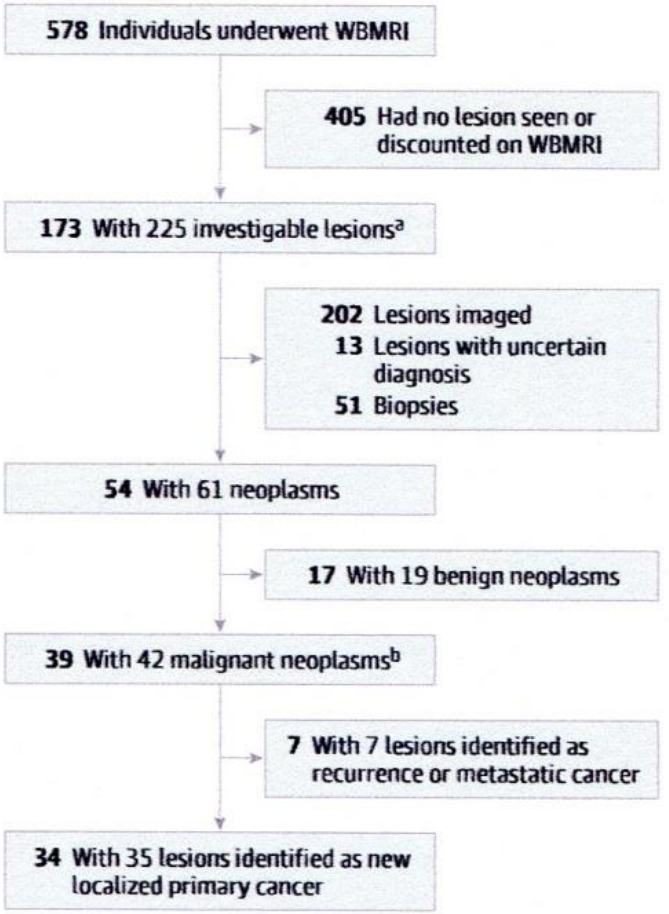
Screening in *TP53* mutation carriers

Ballinger et al – meta-analysis of 13 prospective cohorts of *TP53* mutation carriers screened by whole body MRI

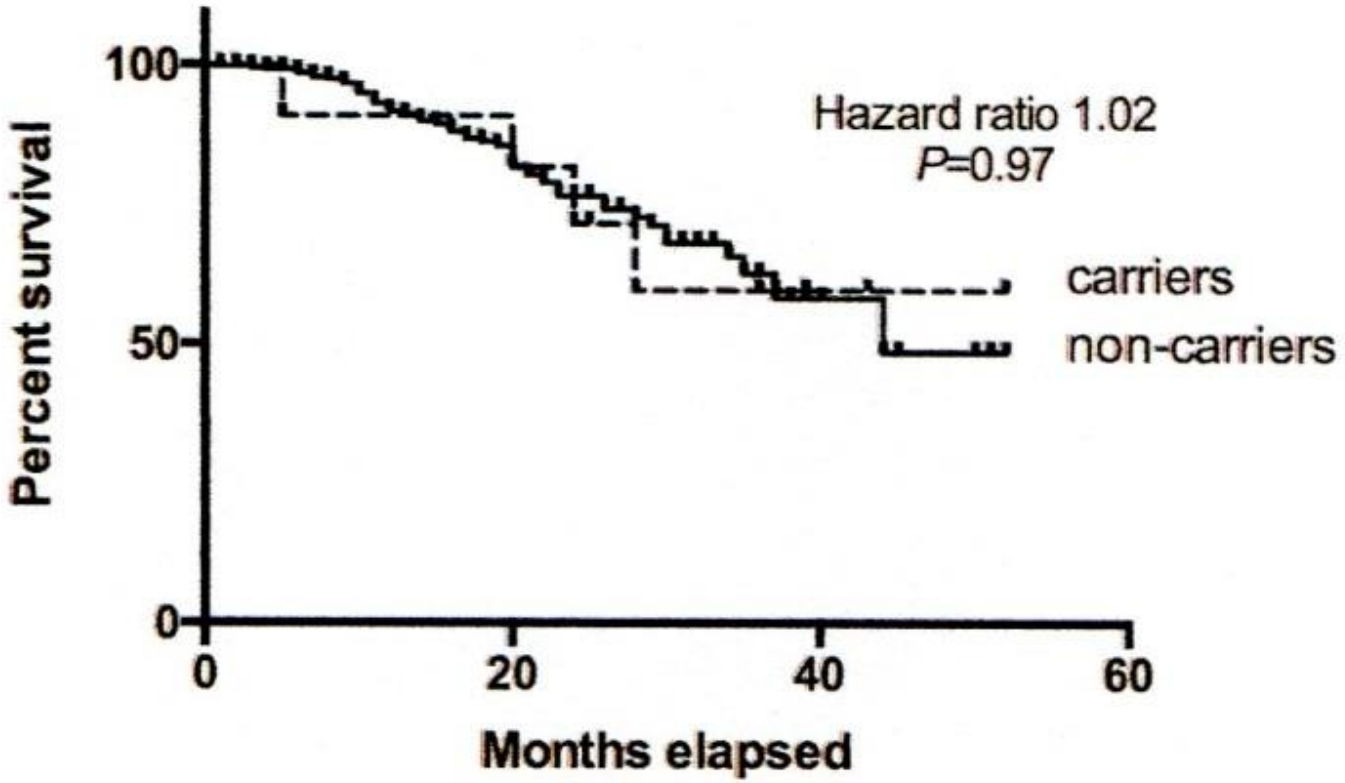
31% needed investigation

34/578 individuals found to have new primary cancer(s) – 6%

Figure. Flowchart of Disposition of Participants Undergoing Whole-Body Magnetic Resonance Imaging (WBMRI)



Prognosis of cancers in *TP53* carriers



Screening regimen for *TP53* mutation carriers at NCI

Mai *et al* JAMA Oncol 2017

Children (Aged 3-16 Years)

- Annual complete history and physical examination
- Blood tests every 4 months: FBC, LDH, ESR, β-HCG, α-FP, 17-hydroxyprogesterone, testosterone, dehydroepiandrosterone sulfate, androstenedione
- Abdominal ultrasonography every 4 months
- Annual brain MRI
- Annual rapid whole-body MRI

Children Older Than 16 Years and Adults

- Annual history and physical examination
- Blood tests every 4 months: FBC, LDH, ESR
- Annual brain MRI
- Annual rapid whole-body MRI
- Colonoscopy every 3 years, starting at 25 years

Females 20-40 years

annual breast MRI, mammography optional

- >40 years: annual breast MRI and mammography

Efficacy of NCI *TP53* screening schema

- 116 participants
- Baseline screening found cancer in 8
  - 2 lung adenocarcinomas, 1 osteosarcoma, 1 astrocytoma, 1 low grade glioma, 2 pre-invasive breast cancers
- 40 participants required additional tests
- Non-MRI techniques did not lead to diagnosis of cancer in this cohort
- Prospective screening now underway

LiFe-Guard Study – surveillance programme in the Netherlands

- *TP53* mutation carriers screened by annual whole body MRI, + breast MRI in females, & brain MRI or colonoscopy according to FH
- 56 pts
  - 32 abnormal findings
  - 4 cancers: 2 breast primaries, 1 metastatic breast, 1 CLL – *7% detection rate*
  - 28 false positives

Ruijs *et al* JAMA Oncol 2017

ISKS: next steps, next questions

- Whole genome sequencing completed on 1160 individuals and 2840 controls – results awaited
- Outstanding questions we hope to answer:
  - Extent of missing heritability?
  - Fraction of families with clinically recognisable syndromes carry mutation that can't currently be detected – non-coding DNA?, mitochondrial DNA??
  - New genes?
  - Are some variations subtype-specific?
  - Can we explain ethnic variation?

Acknowledgements

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