The Royal College of Pathologists
Pathology: the science behind the cure

InSiGHT

Interpreting Genetic Variants Enables Fine-scale Genome Mapping & Precision Medicine

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CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

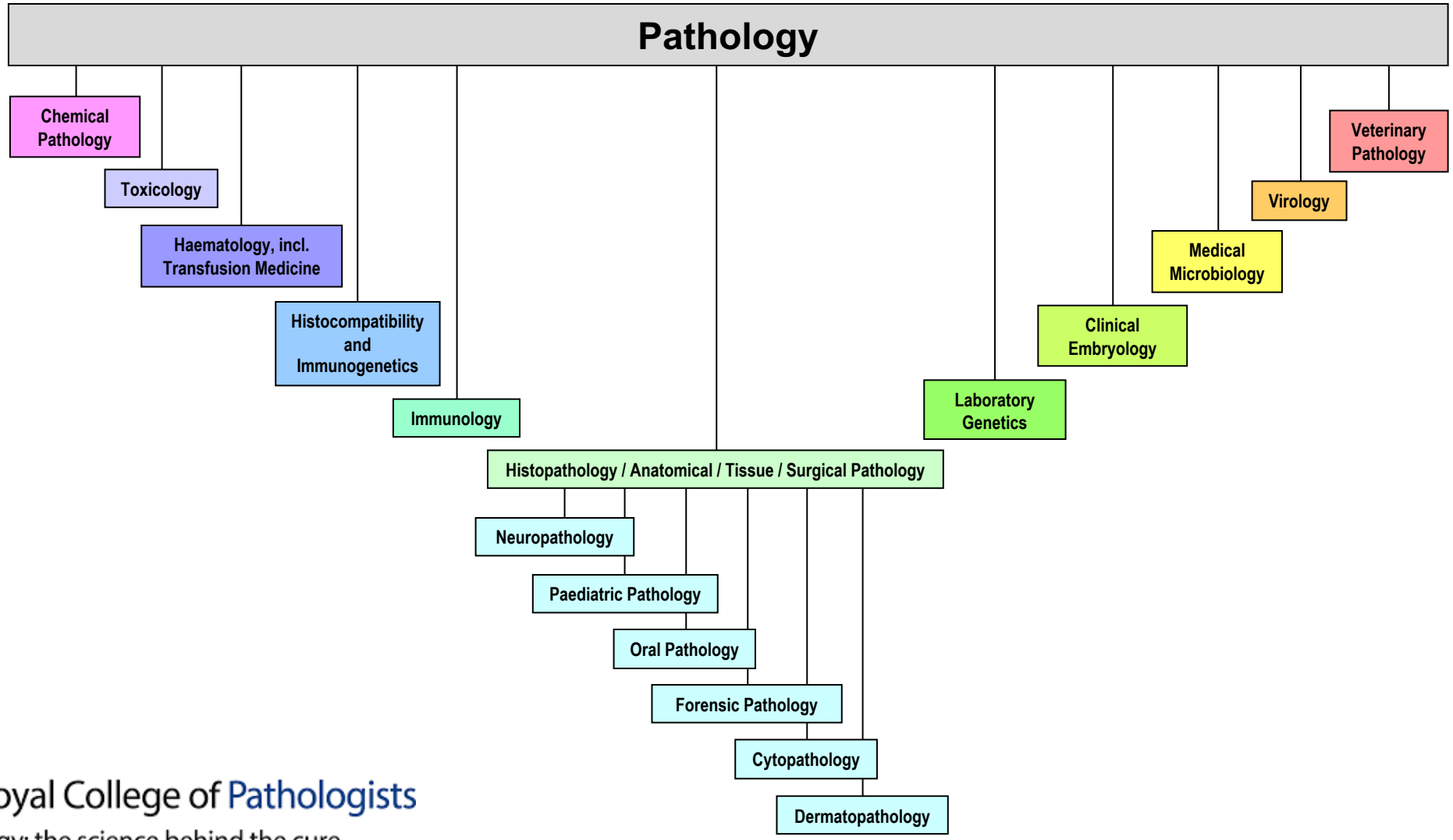
INHERITED TUMOUR SYNDROMES RESEARCH GROUP
ITSR

Cancer Genetics Group

Lynch Syndrome UK

The Association of Clinical Pathologists

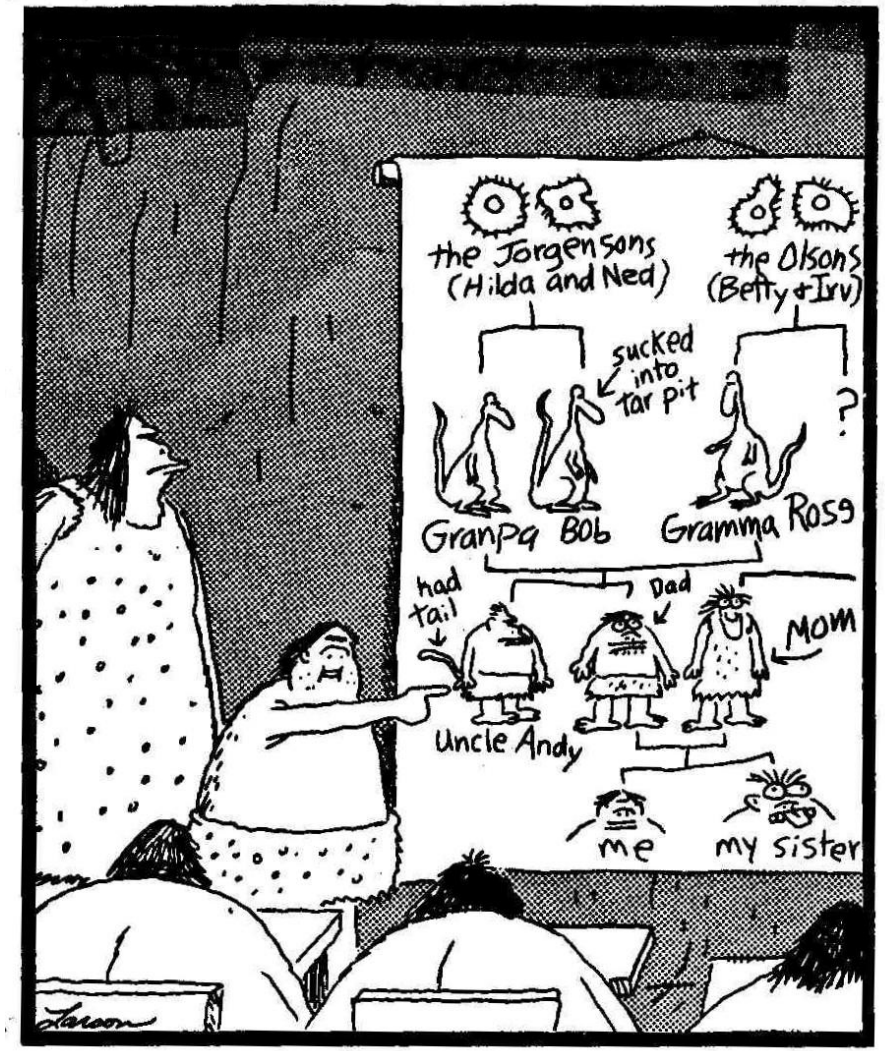
UK Pathology Specialties



The Royal College of Pathologists

Pathology: the science behind the cure

Genetics



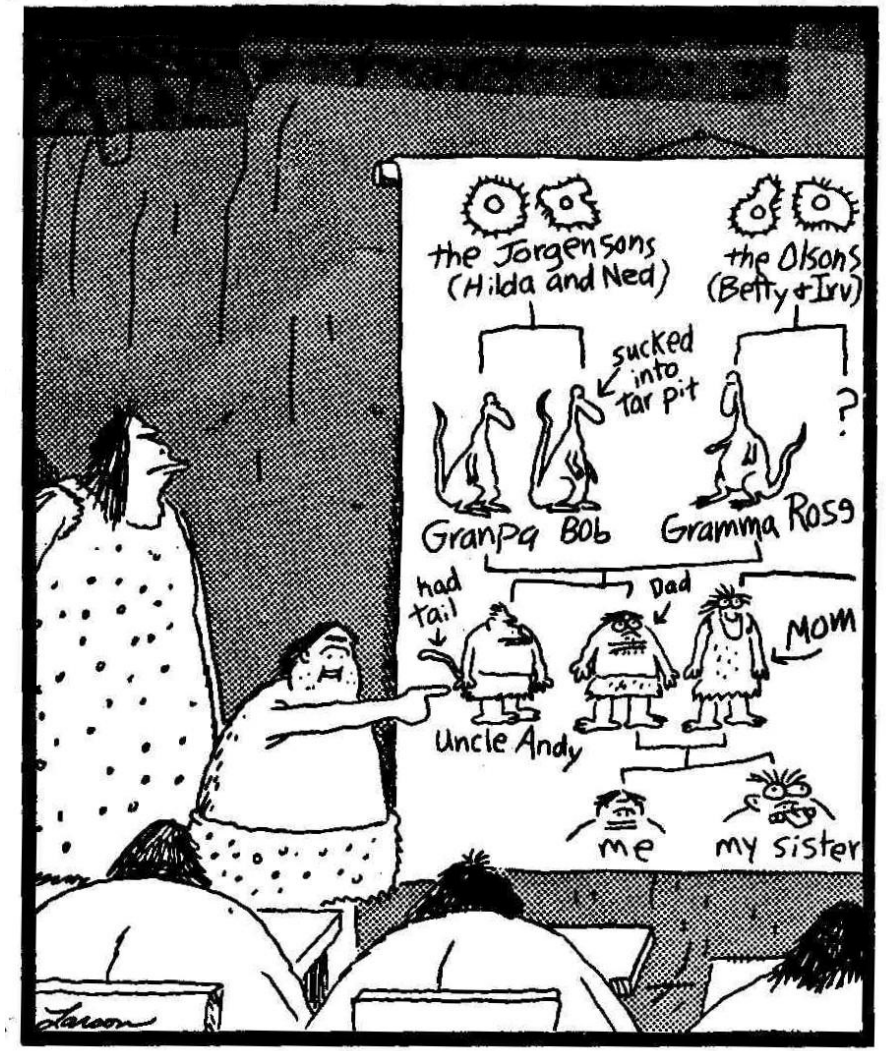
Dirk brings his family tree to class

Genetics

Family histories allow gene identification …

Science:
Genes are messages for proteins → pathways → pathogenesis → diagnosis → treatment → cure

Plus, *evolutionary conservation* is a critical component of gene variant interpretation.

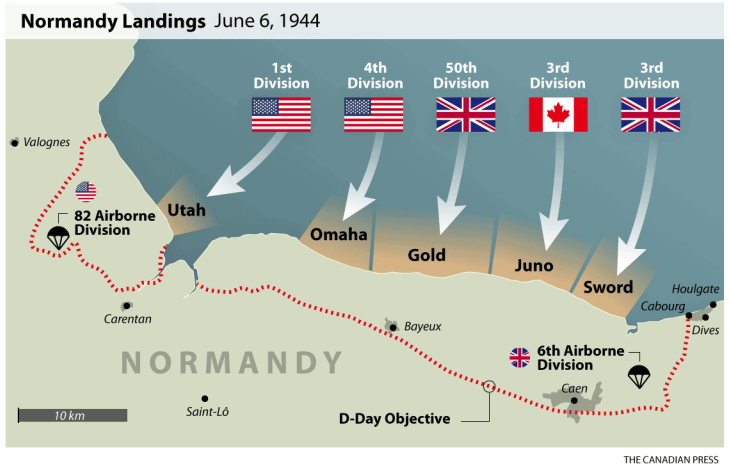


Dirk brings his family tree to class

A family story

The consequences of wrong messages can be severe …

“Type a number or letter wrong and men will die.”



http://www.portsdown-tunnels.org.uk/palmerston_forts/fort_southwick/2_ughq_wwii_p1.html

Gene Variants

Mutations cause changes in genes, resulting in *variants* (ultimately in proteins)

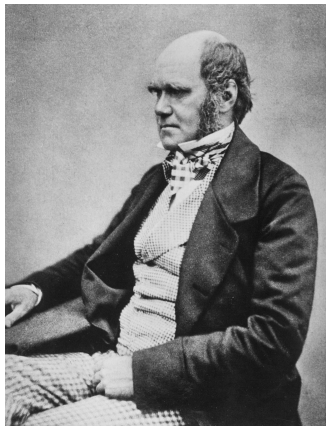
One variant or the sum total of all your variants = *genotype*

Variants in combination with the *environment* cause variety: skin colour, height, susceptibility to disease …

What you are, your *phenotype*, is a function of *genotype* **and** *environment*

The environment of any one gene includes the other 20,000 and their variants = *genomics*

Gene Variants & Evolution



1st Law of Biology:

Variation by mutation is acted upon by selective pressure in the fight for survival.

- Darwinian evolution
- Perpetual “work in progress”

A *population* is defined by its *frequency of genetic variants*

- Favourable variants will increase in frequency & vice versa

When variants occur in the germline they may be passed on to offspring: heritable

When variants occur in cells of the body they are passed on when those cells divide, potentially causing tumours and eventually cancers: somatic

Gene Variants

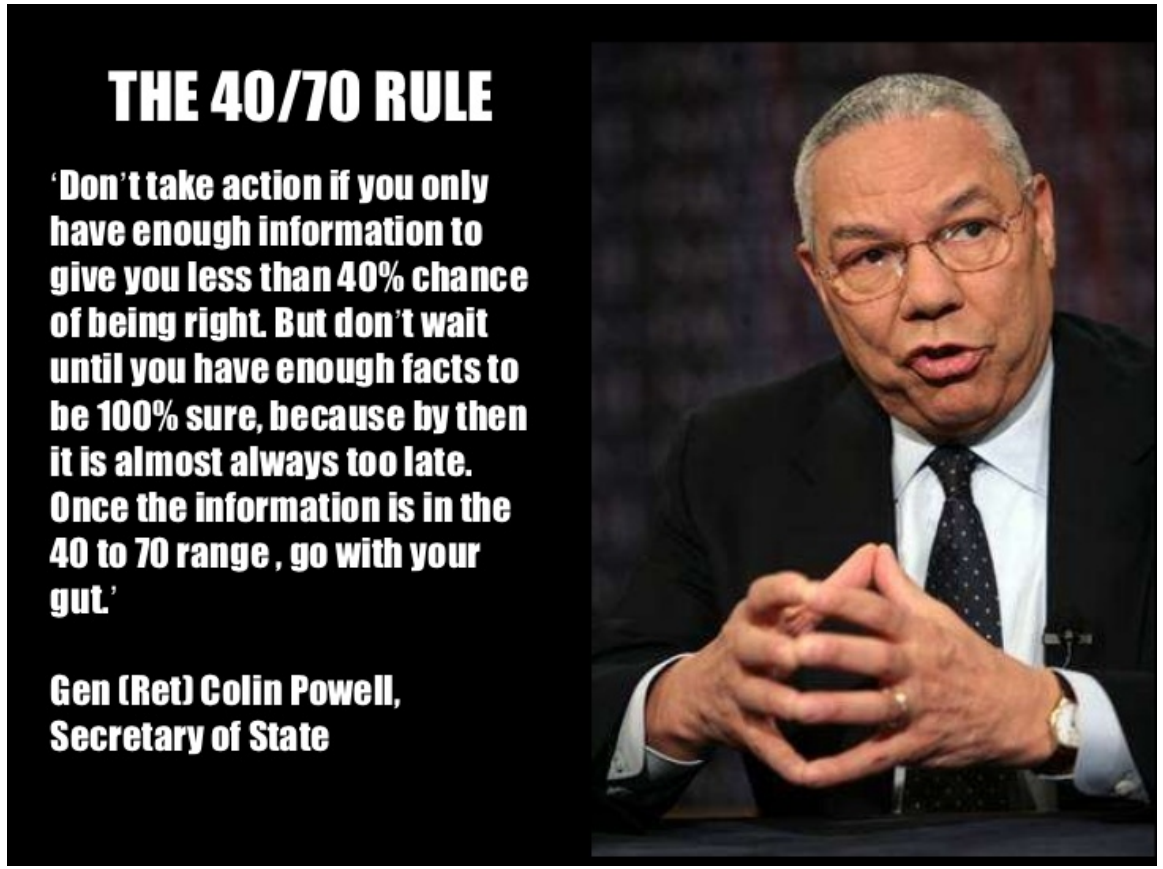
“Any idiot can find a mutation,

but only wise men can interpret them.”

Anon.

So the consequences of misinterpreting variants can also be severe …

… as can indecision or delay.



Clinical consequences of gene variants identified by genetic tests

- Not pathogenic

- Uncertain significance

- Pathogenic

The Uncertain Significance dilemma

- No clinical use
- Uncertainty / Indecision
- Worry
- Risk of inappropriate use

Example 1: Lynch Syndrome

- Common(est) cancer predisposition syndrome
  - ? 1/140 … 1/100
- Causes cancers of the large bowel, womb, ovaries, stomach, small bowel, gall bladder, pancreas, urinary tract, prostate, brain, etc, often at young ages
- Colonoscopy, prophylactic surgery, aspirin … vaccination
- Due to variants in a family of DNA repair genes
- **https://www.insight-group.org/**
- Molecular testing strategies for Lynch syndrome in people with colorectal cancer

  Diagnostics guidance
  Published: 22 February 2017
  nice.org.uk/guidance/dg27

Lynch Syndrome UK

https://www.lynch-syndrome-uk.org/

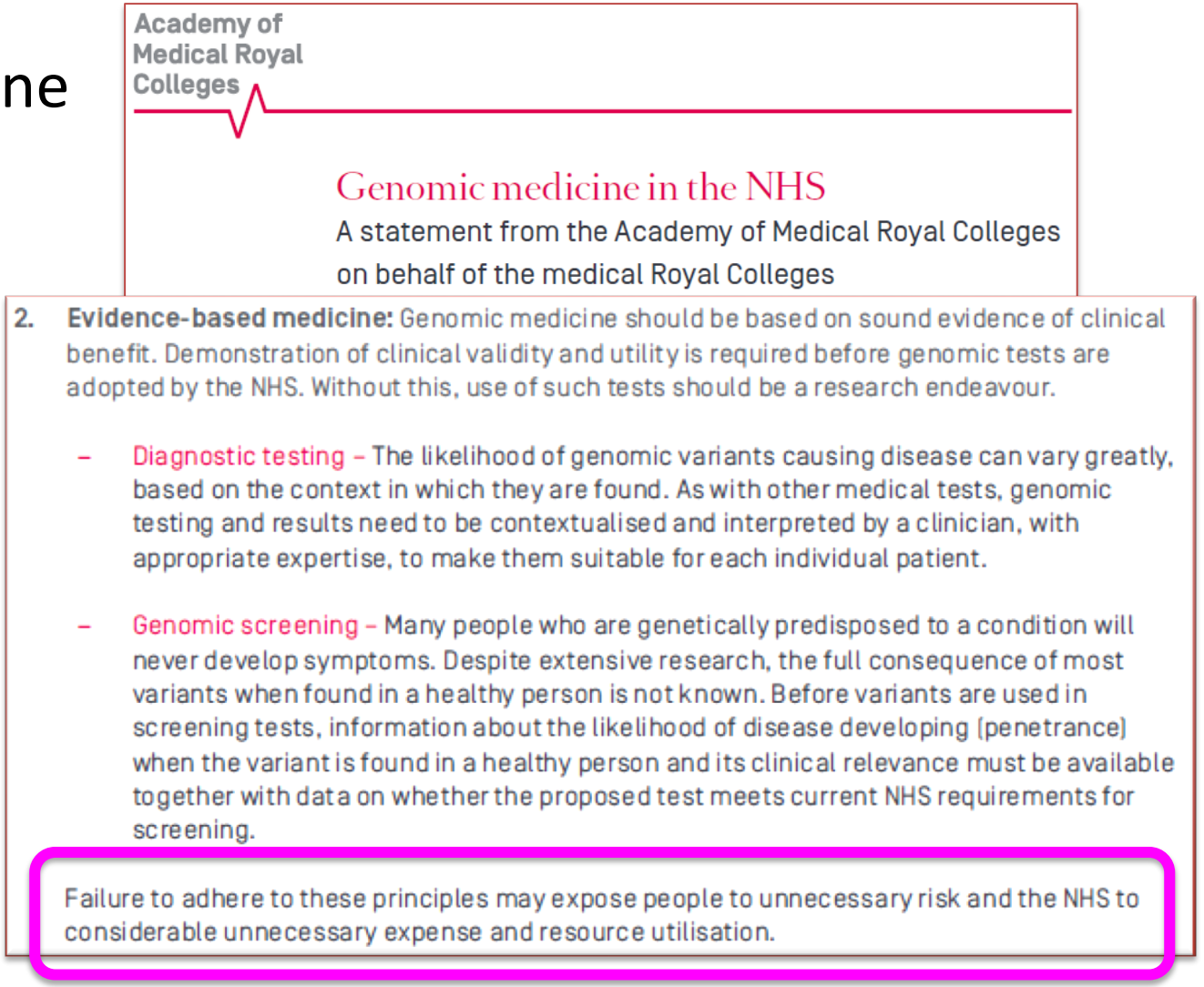
http://clinicaltrials.gov/show/NCT01461148

How Do We Interpret Variants?

- Define and determine the phenotype
- Gather evidence from as many sources as possible
- Gather a multidisciplinary team
- Put it all together using scientific principles, including *probability*
- https://www.insight-group.org/variants/

How Do We Interpret Variants?

- Define and determine the phenotype



Academy of Medical Royal Colleges

Genomic medicine in the NHS

A statement from the Academy of Medical Royal Colleges on behalf of the medical Royal Colleges

2. **Evidence-based medicine:** Genomic medicine should be based on sound evidence of clinical benefit. Demonstration of clinical validity and utility is required before genomic tests are adopted by the NHS. Without this, use of such tests should be a research endeavour.

   - Diagnostic testing – The likelihood of genomic variants causing disease can vary greatly, based on the context in which they are found. As with other medical tests, genomic testing and results need to be contextualised and interpreted by a clinician, with appropriate expertise, to make them suitable for each individual patient.

   - Genomic screening – Many people who are genetically predisposed to a condition will never develop symptoms. Despite extensive research, the full consequence of most variants when found in a healthy person is not known. Before variants are used in screening tests, information about the likelihood of disease developing (penetrance) when the variant is found in a healthy person and its clinical relevance must be available together with data on whether the proposed test meets current NHS requirements for screening.

Failure to adhere to these principles may expose people to unnecessary risk and the NHS to considerable unnecessary expense and resource utilisation.

Courtesy: Dr Helen Firth, Chair, Joint Committee on Genomic Medicine of the Royal College of Physicians, Royal College of Pathologists and British Society of Genetic Medicine

http://www.aomrc.org.uk/statements/genomic-medicine-in-the-nhs/

A Systematic Approach to Clinical Classification of DNA Sequence Variants in Mismatch Repair Genes:

The InSiGHT Variant Interpretation Committee

Established Yokohama, 2007

San Antonio, Mar 2011

UNITED NATIONS
Educational, Scientific, Cultural Organization

THE HUMAN VARIOME PROJECT

Global Alliance for Genomics & Health

Genomics england

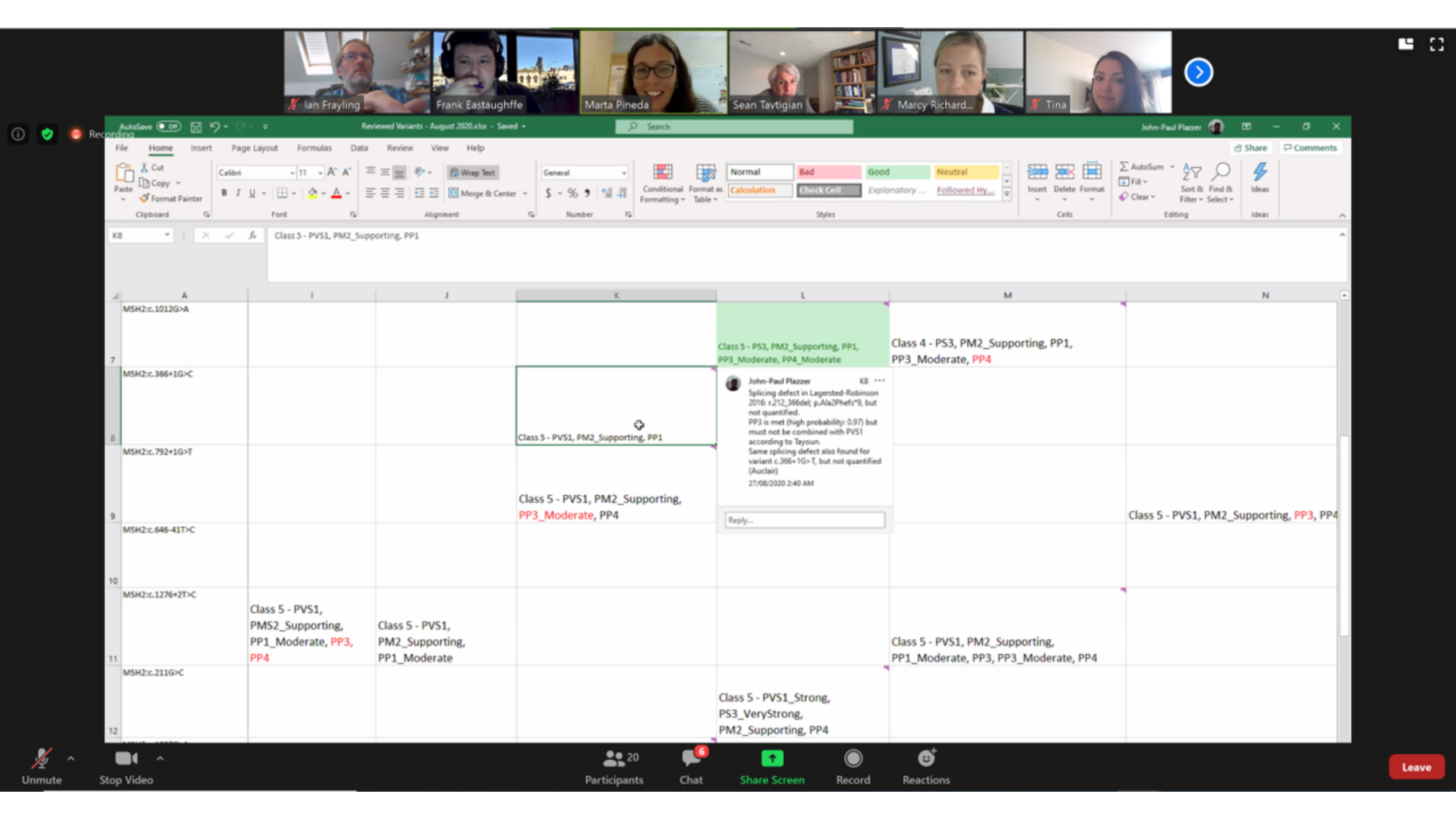
https://www.insight-group.org/variants/

ClinVar

InSiGHT Variant Interpretation Committee (VIC)

- Safety, in numbers and variety
  - Multidisciplinary
  - Liability (USA; IARC)
- Consistent – inconsistency is very harmful
- Definitive = Clinically Actionable
- Peer reviewed & publically available: Transparent
- Large set of curated mutations
- Availability of *in vitro* and *in vivo* clinical data
- Disease with an effective intervention
- Credit for such data: clinical labs (quality)
- Model for others

https://www.insight-group.org/variants/


Ian Frayling
Frank Eastaughffe
Marta Pineda
Sean Tavtigian
Marcy Richard...
Tina
Recording
AutoSave
Reviewed Variants - August 2020.xlsx - Saved
Search
John-Paul Plazzer
File
Home
Insert
Page Layout
Formulas
Data
Review
View
Help
Share
Comments
Cut
Copy
Format Painter
Paste
Calibri
11
Wrap Text
Merge & Center
General
Conditional Formatting
Format as Table
Normal
Bad
Good
Neutral
Calculation
Check Cell
Explanatory ...
Followed Hy...
Insert
Delete
Format
AutoSum
Fill
Clear
Sort & Filter
Find & Select
Ideas
Clipboard
Font
Alignment
Number
Styles
Cells
Editing
KB
Class 5 - PVS1, PM2_Supporting, PP1
A
I
J
K
L
M
N
MSH2:c.1012G>A
Class 5 - PS3, PM2_Supporting, PP1, PP3_Moderate, PP4_Moderate
Class 4 - PS3, PM2_Supporting, PP1, PP3_Moderate, PP4
MSH2:c.366+1G>C
John-Paul Plazzer KB
Splicing defect in Lagersted-Robinson 2016: r.212_366del; p.Ala2Phefs*9, but not quantified.
PP3 is met (high probability: 0.97) but must not be combined with PVS1 according to Tayoun.
Same splicing defect also found for variant c.366+1G>T, but not quantified (Auclair)
27/08/2020 2:40 AM
Reply...
Class 5 - PVS1, PM2_Supporting, PP1
MSH2:c.792+1G>T
Class 5 - PVS1, PM2_Supporting, PP3_Moderate, PP4
Class 5 - PVS1, PM2_Supporting, PP3, PP4
MSH2:c.646-41T>C
MSH2:c.1276+2T>C
Class 5 - PVS1, PMS2_Supporting, PP1_Moderate, PP3, PP4
Class 5 - PVS1, PM2_Supporting, PP1_Moderate
Class 5 - PVS1, PM2_Supporting, PP1_Moderate, PP3, PP3_Moderate, PP4
MSH2:c.211G>C
Class 5 - PVS1_Strong, PS3_VeryStrong, PM2_Supporting, PP4
Unmute
Stop Video
20
Participants
6
Chat
Share Screen
Record
Reactions
Leave

Interpreting Variants in Lynch Syndrome

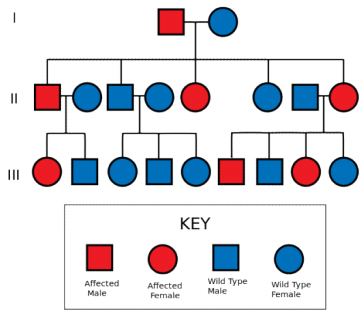
Sequence-based, e.g. nonsense & frameshifts

5'…TCT CAA **A**AA TTT ACG…3' 5'…TCT CAA **T**AA TTT ACG…3'

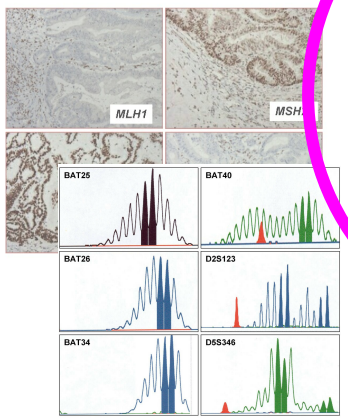
S Q **K** F T S Q *****

Clinical/molecular

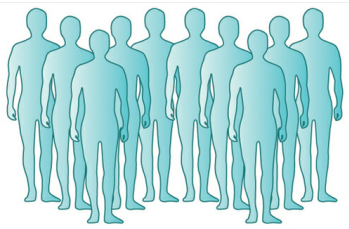
Segregation data



Tumour data



Frequency data



Co-occurrence *in trans*

Presence/absence of haematological malignancies, childhood cancers – CMMR-D phenotype

Functional

In vitro data

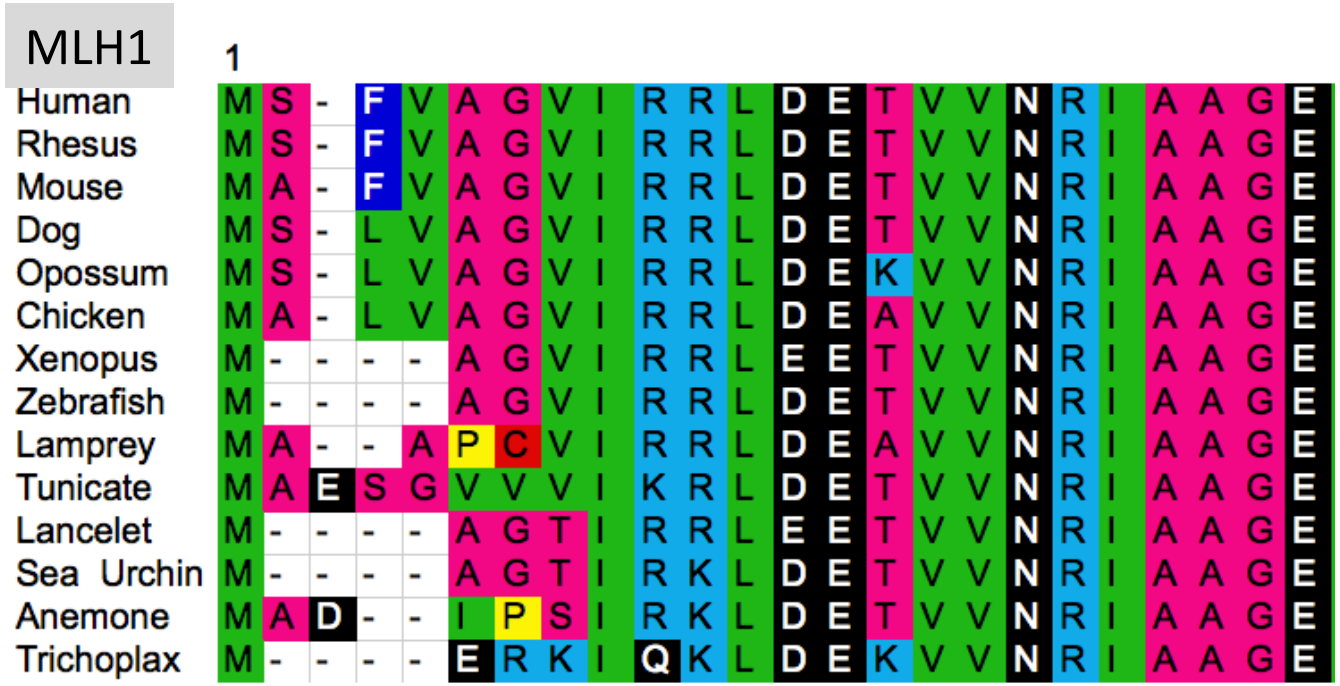
https://www.insight-group.org/variants/databases/

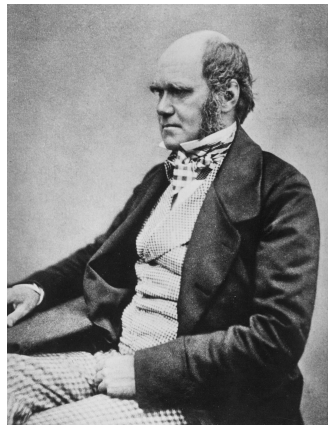
Example 1: Lynch Syndrome

- *MLH1* c.306G>T p.(E102D)
- Query from UK Genetic Counsellor
- 17 entries on the InSiGHT database, but conflicting interpretations 🤔
- What does evolution tell us?

Example 1: Lynch Syndrome

From evolutionary conservation we can derive prior probabilities of pathogenicity.





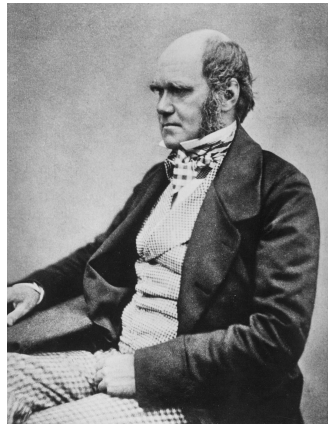
Thompson, Bryony A., et al. (2013) "Calibration of multiple in silico tools for predicting pathogenicity of mismatch repair gene missense substitutions." *Human Mutation* **34**: 255-265.

http://hci-lovd.hci.utah.edu/home.php?action=switch_db

Example 1: Lynch Syndrome

From evolutionary conservation we can derive prior probabilities of pathogenicity.

Protein variant	Prior probability
p.E102K	87%
p.E102Q	71%
p.E102A	91%
p.E102G	93%
p.E102V	95%
p.E102D	72%



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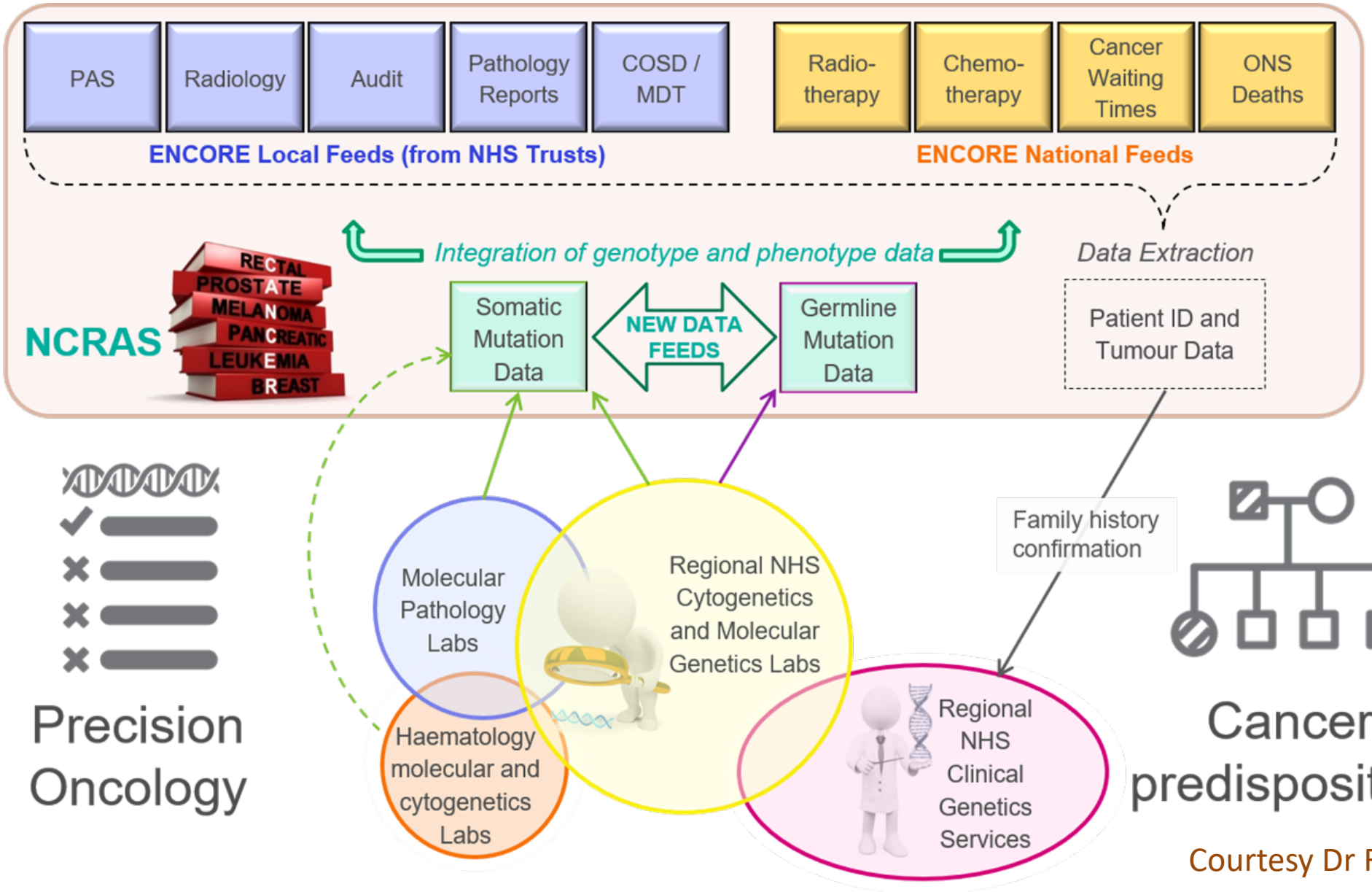
Example 1: Lynch Syndrome

- *MLH1* c.306G>T p.(E102D)
- Query from UK Genetic Counsellor
- 17 entries on the InSiGHT database, but conflicting interpretations
- What does evolution tell us?
- 72% chance of pathogenicity, but we need >95% to be sure enough 🤔

- 💡 Newly released Public Health England data on Lynch variant frequency in the UK, email Dr Fiona McRonald …

Public Health England



Courtesy Dr Fiona McRonald

Public Health England

Section 251, *NHS* Act 2006

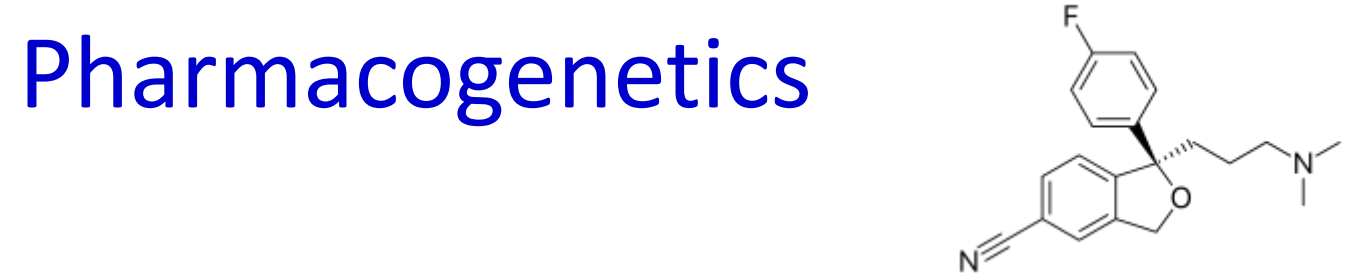
- The **National Cancer Registration & Analysis Service** (NCRAS) has legal permission to collect information about all confirmed or suspected cancer patients without consent under Section 251 of the NHS Act 2006 (*Control of Patient Information*)
    - Activity must have a medical purpose
    - Activity must be in the public interest or in the interests of improving patient care
    - Must be compliant with DPA/GDPR
    - Impracticable to obtain consent and anonymised information cannot be used
- Undergo yearly review with Confidentiality Advisory Group (CAG) of the Health Research Authority (HRA)
- Patient can opt out at any time and their wishes are respected

Courtesy Dr Fiona McRonald

Example 1: Lynch Syndrome

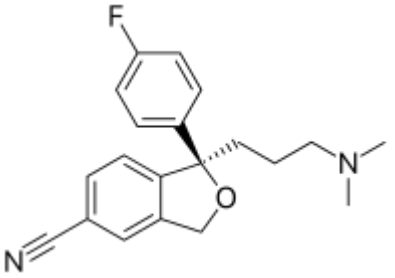
- *MLH1* c.306G>T p.(E102D)
- Population frequencies:
- 0 / 113654 non-Finnish white northern Europeans unaffected by cancer (gnomAD)
- 6 / 2041 white British NHS patients affected with Lynch-type cancers (PHE/CanVIG)
- Hazard Ratio = 57 (Confidence intervals: 1.1 – 2800)
- Probability if random = 0.000 000 003%
- Combined with 72% - the chance this is not random = 99.999 999 998 8%
  - 83 billion: 1 against
- **Conclusion**: this variant is **pathogenic**
- **6 + 1 NHS patients & families, plus the 17 on the InSiGHT database, plus … will now benefit from NHS data gathered in the UK by PHE**

Example 2: Pharmacogenetics



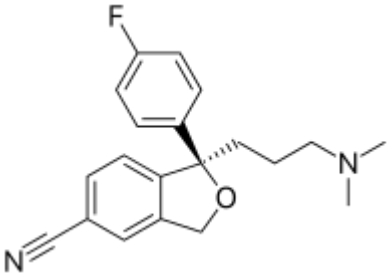
- 2011: NHS Consultant, male, 51 y
- Treated with Citalopram, a commonly used SSRI antidepressant
- 1st Dose (20 mg): immediate severe adverse drug reaction – serotonin syndrome
- Disabled for 2 weeks with Parkinsonian movement disorder, muscle twitching, unable to focus, tachycardia, vomiting, diarrhoea, severe flushing, confusion, drowsiness
- Toxicity even with 0.3 mg
- Unable to tolerate any SSRI, obliged to take 18 months off work
- Considerable cost to him, his family and the NHS

Example 2: Pharmacogenetics



- SSRIs are metabolised by two liver enzymes
  - Primary: 2C19
  - Secondary: 2D6
- 2% of non-Finnish Northern Europeans (NFE) are 2C19 deficient
- 10% of NFE are 2D6 deficient
- 2% of 10%, so 0.2% (1/500) NFE are deficient in both
- Testing not available in UK, but testing in The Netherlands (2012) showed:
- *"2D6 del/del, but a high activity variant of 2C19 (*17)"* 🤔

Example 2: Pharmacogenetics



- 2012: *"2D6 del/del, but a high activity variant of 2C19 (*17)…"*
- 2018: Professor at the Karolinska Institute: *"Ah, but we now know some people with 2C19*17 also have *4, an inactivating variant in the same gene … so the ignition is always on, but there is no starter motor!"*

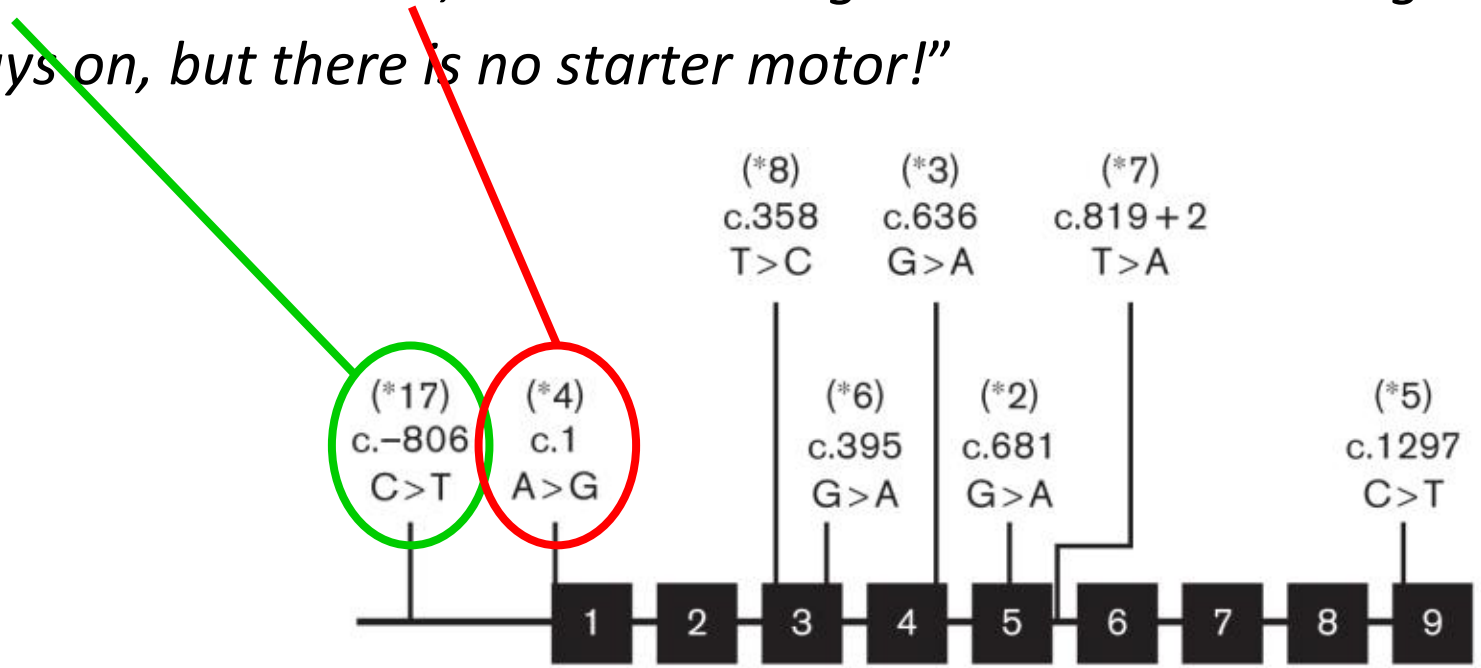
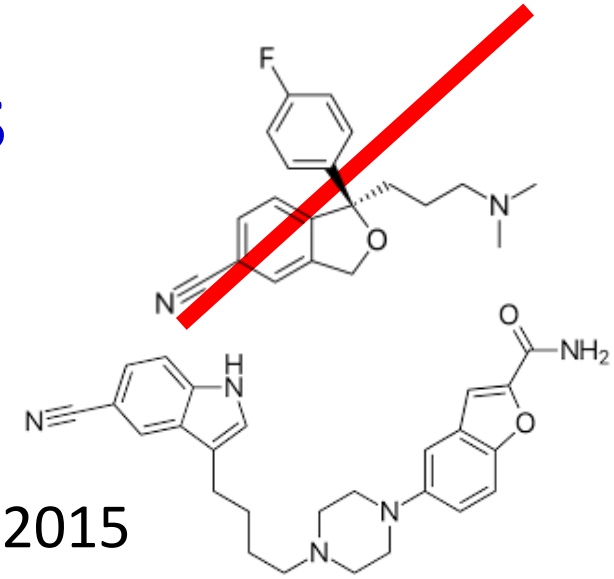


Illustration of the *CYP2C19* gene highlighting the location of selected loss-of-function (**2–*8*) and gain-of-function (**17*) variant alleles. Exons are represented by numbered black boxes (not to scale).

Example 2: Pharmacogenetics



Implications

- 20 – 40% of BAME are 2C19 deficient
- Alternative SSRI not metabolised by 2C19: *Vilazodone*, introduced 2015
- 2C19 also activates an anticoagulant commonly prescribed after cardiac stenting: 2C19 deficiency associated with clotting – "Black boxed" by FDA:-

WARNING: DIMINISHED ANTIPLATELET EFFECT IN PATIENTS WITH TWO LOSS-OF-FUNCTION ALLELES OF THE CYP2C19 GENE

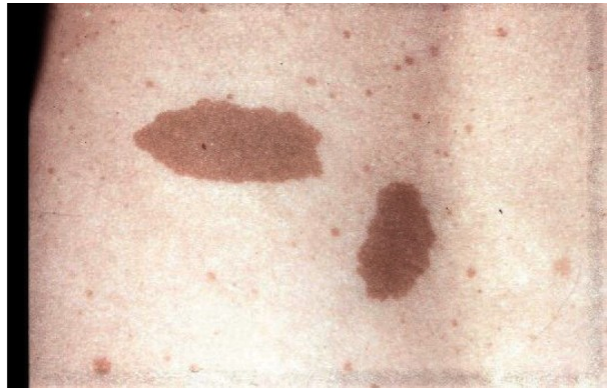
See full prescribing information for complete boxed warning.

- **Effectiveness of Plavix depends on conversion to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19. (5.1, 12.3)**
- **Tests are available to identify patients who are CYP2C19 poor metabolizers. (12.5)**
- **Consider use of another platelet $P2Y_{12}$ inhibitor in patients identified as CYP2C19 poor metabolizers. (5.1)**

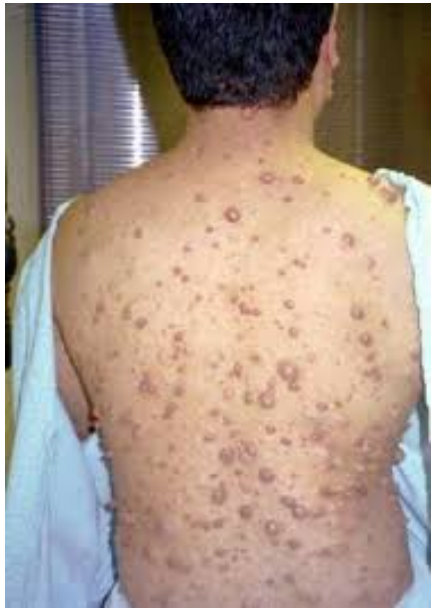
- 2D6 metabolizes opiates and PPIs:
  - lower doses needed if deficient
- But many BAME have high activity 2D6
  - risk of insufficient analgesia

Example 3: Neurofibromatosis type 1 & Breast Cancer risk

- Neurofibromatosis type 1
- Disfiguring & disabling: 1/3000
- Café-au-lait spots
- Multiple skin tumours: neurofibromas
- Internal nerve, spinal cord and brain tumours:
  - benign & malignant
- Scoliosis
- Learning difficulties
- Cancers, including Breast cancer
- Leukaemia



ghr.nlm.nih.gov/condition/neurofibromatosis-type-1



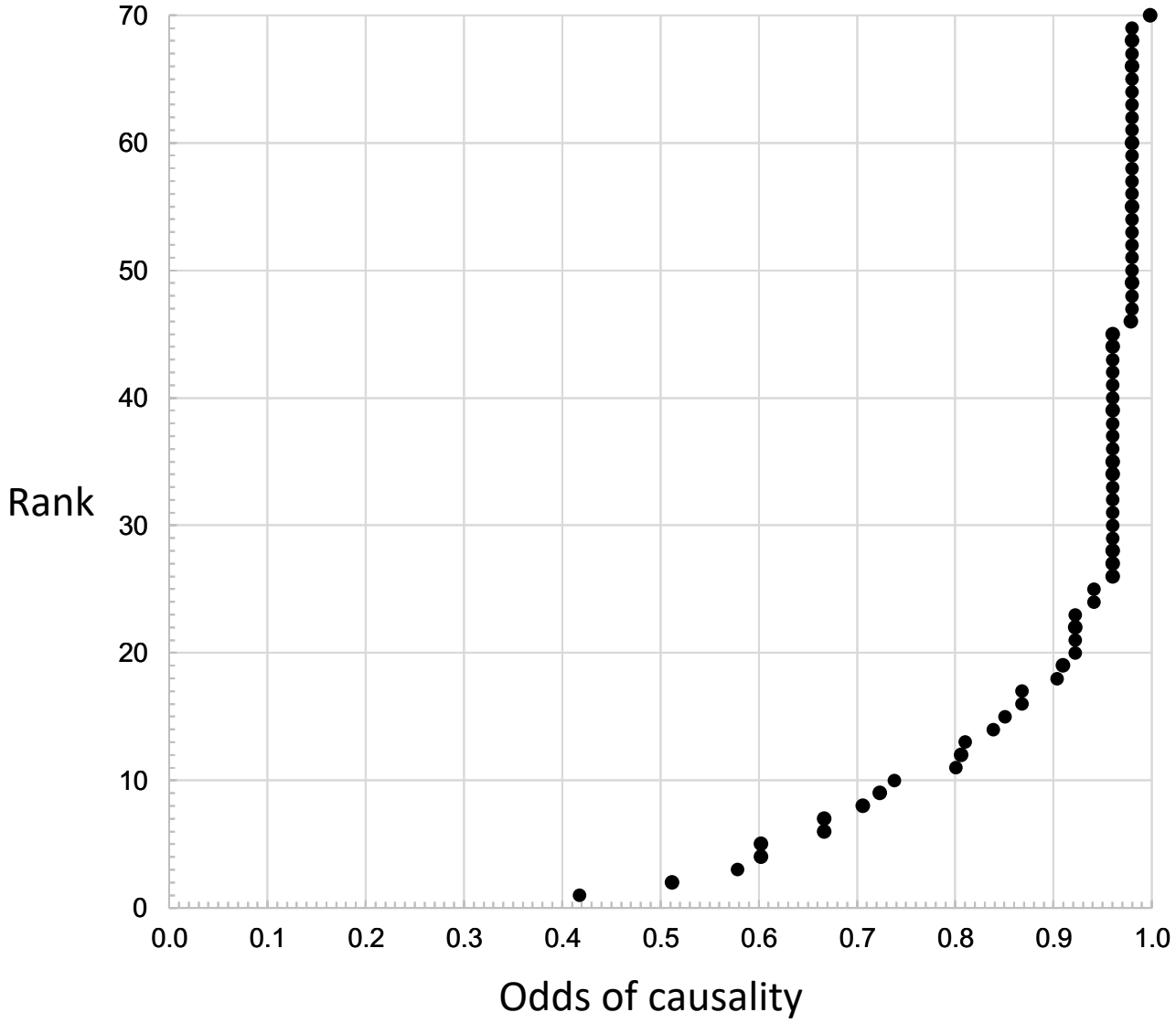
emedicine.medscape.com

Example 3: Neurofibromatosis type 1 & Breast Cancer risk

- Breast cancer in NF1 (NF1-BC)
- A clinical problem
- Males & females
- Risk x4 ~ 8 to age 50 y, x2 ~ 2.8 age >50 y
- More malignant
- ? Surveillance, prophylaxis, treatment
- ? NF1-BC genotype-phenotype correlation

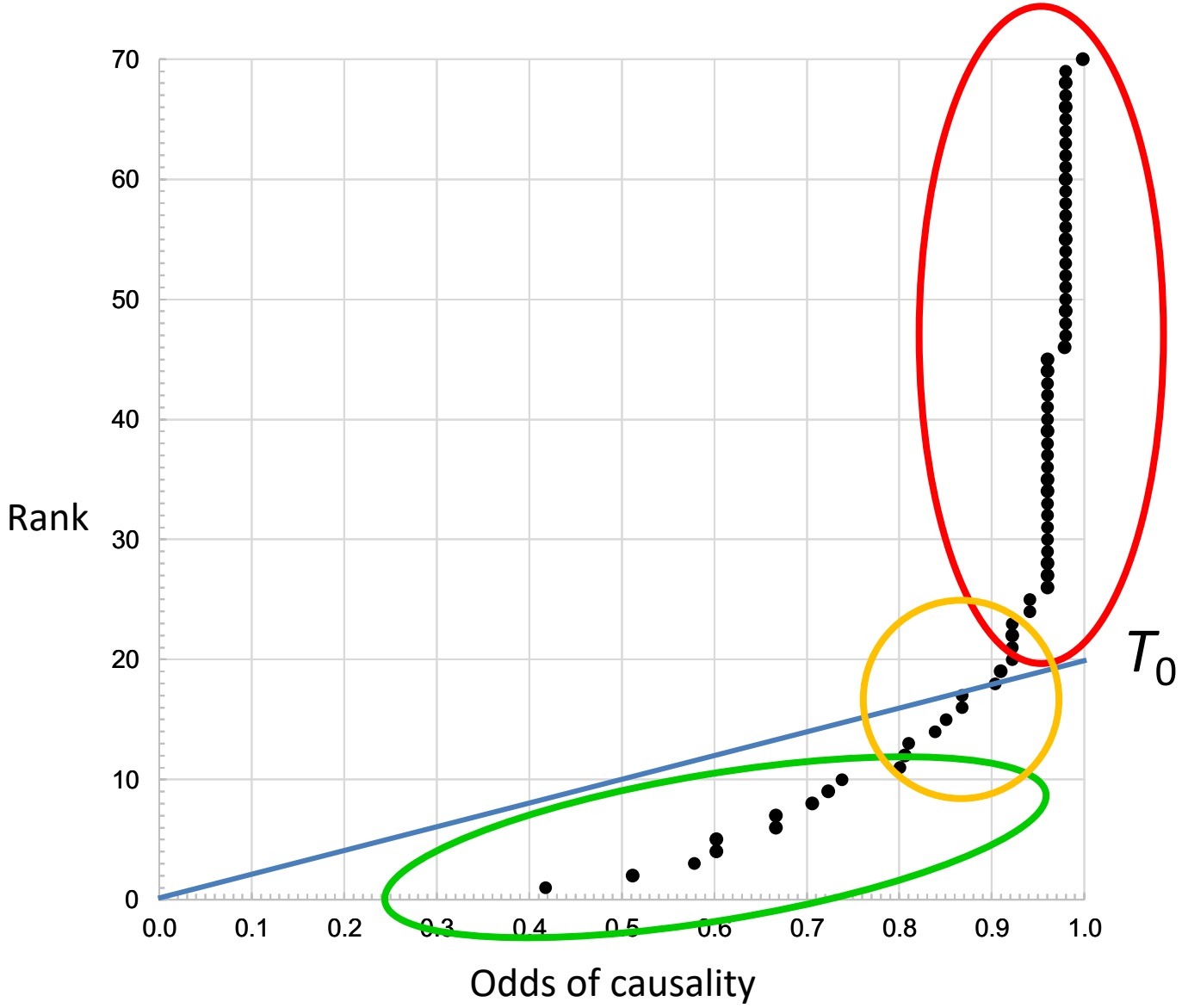
Professor Meena Upadhyaya OBE PhD FRCPath

NF1-BC: individual variants



Frayling, Ian M., et al. (2019) "Breast cancer risk in neurofibromatosis type 1 is a function of the type of NF1 gene mutation: a new genotype-phenotype correlation." *Journal of Medical Genetics* 56: 209-219.

NF1-BC: individual variants



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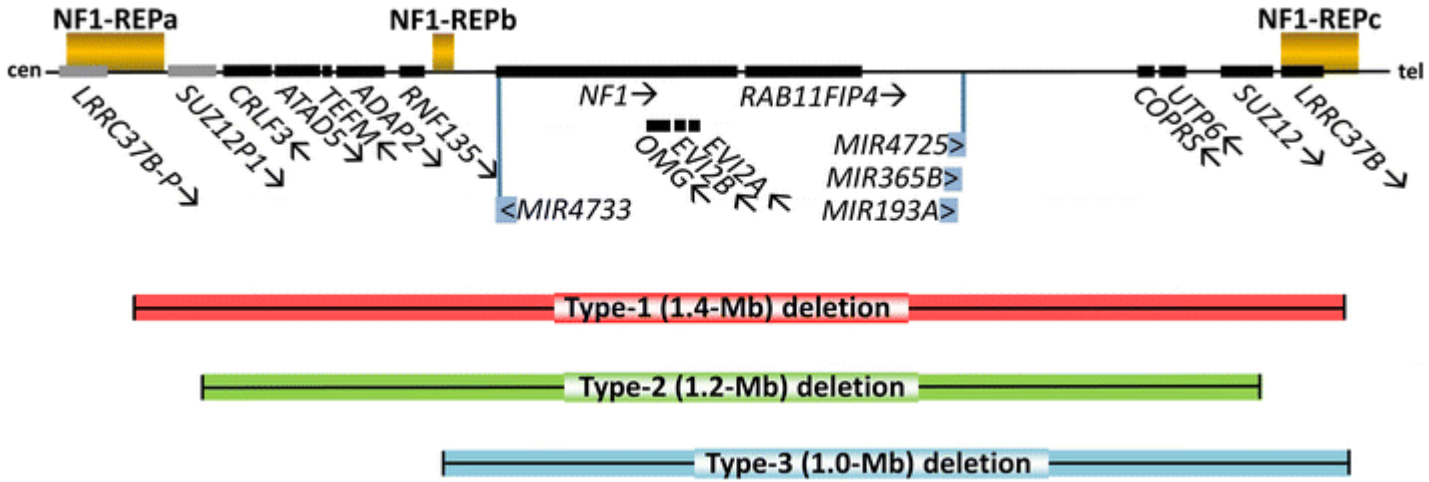
NF1-BC: individual variants

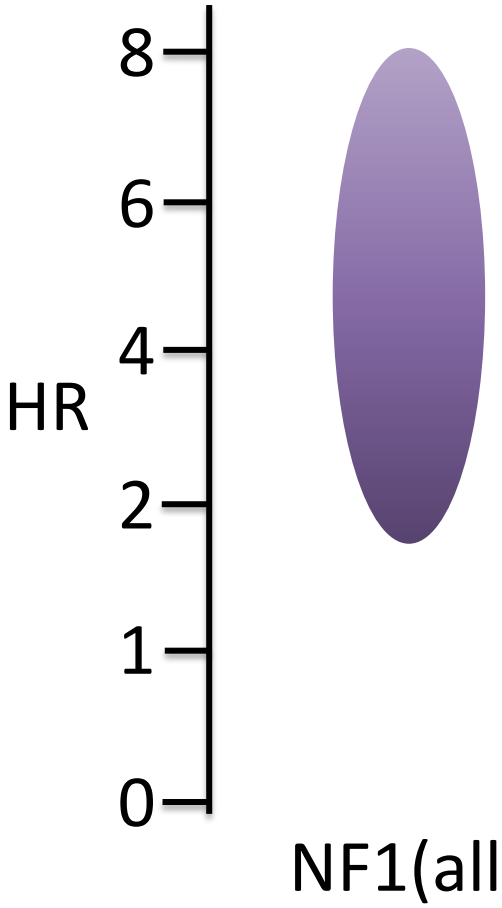


- Whole *NF1* gene deletions probably *not* associated with NF1-BC (2018)
  - Found in ~1/7 NF1 patients, but 0/217 NF1-BC cases (expect ~30)
  - HR 0.10 (CI: 0.006 – 1.63) ...
    - 2020: 1/249, so HR 0.16 (CI: 0.03 – 0.80)
  - Perhaps even protective?
- Variant heterogeneity: can no longer generalise advice

Frayling, Ian M., et al. (2019) "Breast cancer risk in neurofibromatosis type 1 is a function of the type of NF1 gene mutation: a new genotype-phenotype correlation." *Journal of Medical Genetics* 56: 209-219.
Kehrer-Sawatzki, Hildegard, Victor-Felix Mautner, and David N. Cooper. (2017) "Emerging genotype–phenotype relationships in patients with large NF1 deletions." *Human Genetics* 136: 349-376.

NF1-BC: individual variants

- Tells us risks vary with the variant, and advice likewise



NF1-BC: individual variants

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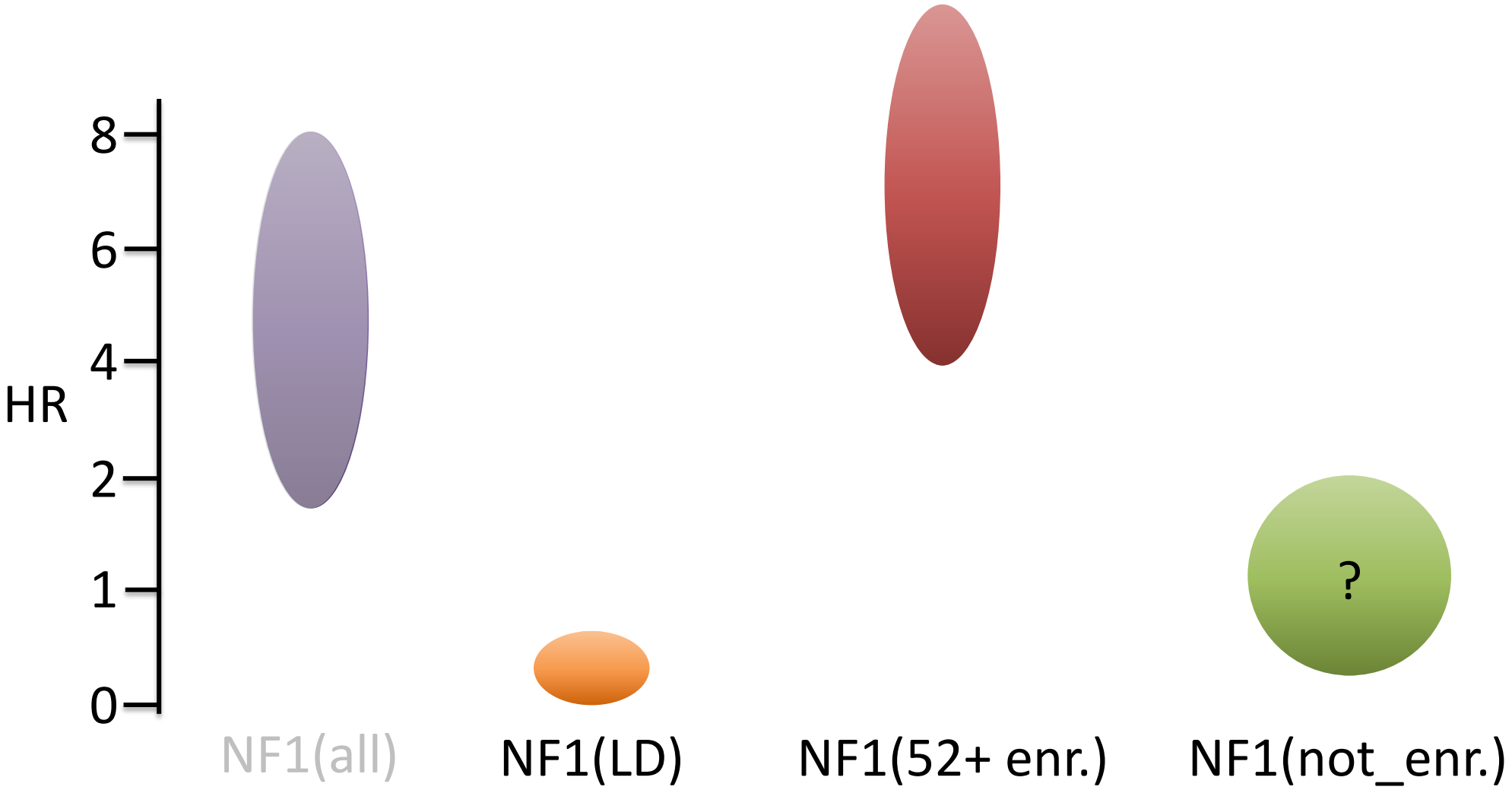
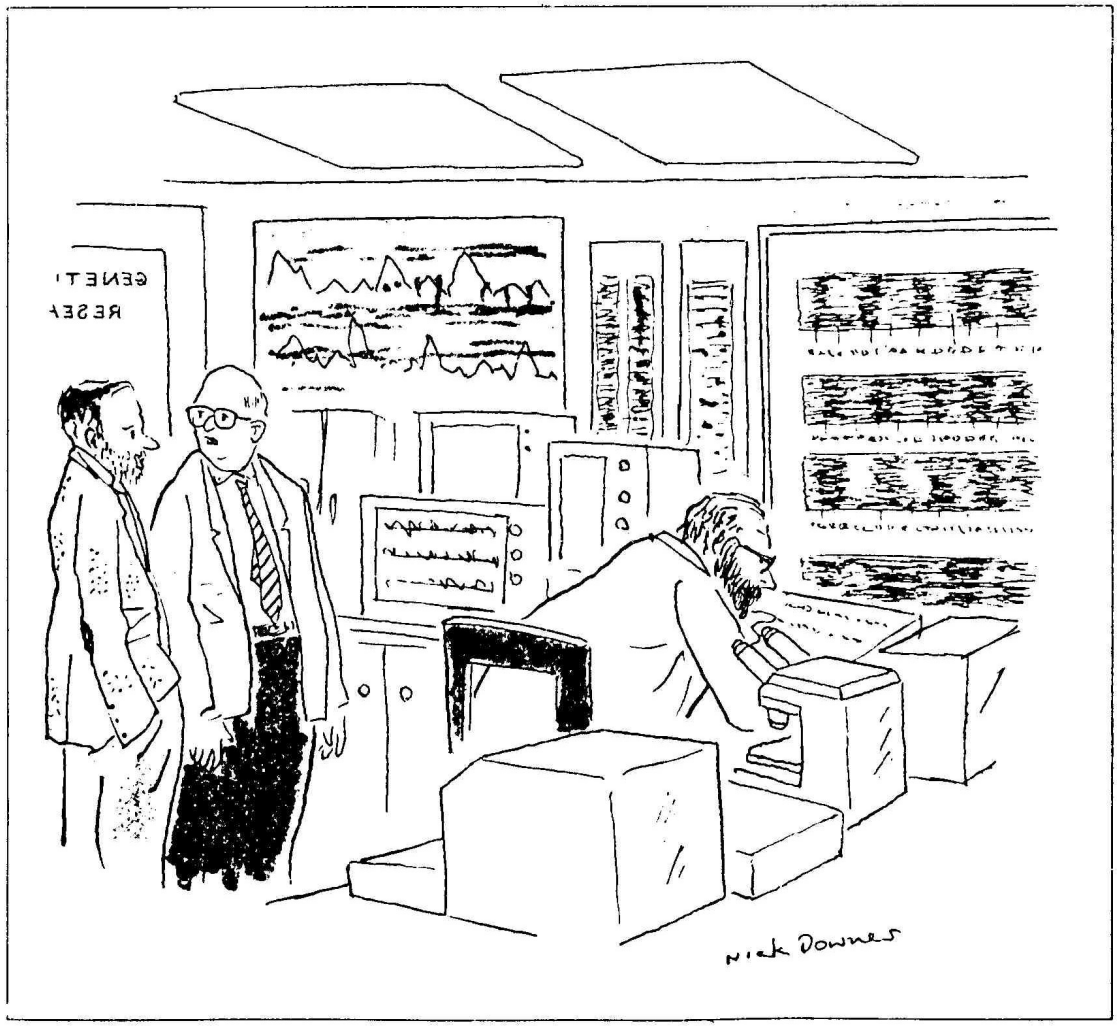


Interpreting Genetic Variants

- **Science:** Genes → proteins → pathways → diagnosis → treatment → cure
- Not possible to generalise – the devil is in the detail, of individual variants & combinations
- "*You can get your genome for <$1000, but you need a $100M Institute to interpret it*."
- Like any test, interpretation and actionability depends on the clinical context
- Determining phenotype is critical – medical task requiring clinical skilling (AoMRC)
- Large inter/national multidisciplinary expert groups
- Data collection and database curation *in perpetuum*
- Open access to data
- May change as knowledge increases
- Education
- Investment

http://www.aomrc.org.uk/statements/genomic-medicine-in-the-nhs/



Dr Farnsworth is attempting to isolate the gene that makes people do this sort of thing for a living."

https://www.newscientist.com/