Interpreting Mutations In Colorectal Cancer

Dr Ian M Frayling
Consultant in Genetic Pathology, C&VUHB
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Member of Council, InSiGHT
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The International Society for Gastrointestinal Hereditary Tumours (InSiGHT) Variant Interpretation Initiative

Ian M Frayling
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Honorary Medical Advisor to *Lynch Syndrome UK*
Institute of Medical Genetics, Cardiff
fraylingim@cardiff.ac.uk
The consequences of mutations can be severe …

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

http://www.portsdowntunnels.org.uk/palmerston_forts/fort_southwick/2_ughq_wwii_p1.html
“Any idiot can find a mutation, but only wise men can interpret them.”

Anon.
“Any idiot can find a mutation, but only wise men can interpret them.”

Anon.

So the consequences of misinterpreting mutations can also be severe …
THE 40/70 RULE

‘Don’t take action if you only have enough information to give you less than 40% chance of being right. But don’t wait until you have enough facts to be 100% sure, because by then it is almost always too late. Once the information is in the 40 to 70 range, go with your gut.’

Gen (Ret) Colin Powell, Secretary of State
Colorectal Cancer: Familial Risk

**HIGH RISK:** >1:2 (FAP, LS, PJS, JPS)

**HIGH-MODERATE RISK:** ~1:6

**LOW-MODERATE RISK:** ~1:12

**AVERAGE RISK:** ~1:20

**BELOW AVERAGE RISK:** <1:20
Colorectal Cancer: Genetic Risk

- Familial Adenomatous Polyposis (FAP)
- Peutz-Jeghers Syndrome (PJS)
- Juvenile Polyposis Syndrome (JPS)
- Lynch Syndrome (LS) ~ “Hereditary Non-Polyposis Colorectal Cancer” (HNPCC)
Colorectal Cancer: Genetic Risk

Familial Adenomatous Polyposis (FAP)
Attenuated FAP (AFAP)
MUTYH-Associated Polyposis (MAP)

Serrated Polyposis Syndrome (SPS)
Peutz-Jeghers Syndrome (PJS)
Juvenile Polyposis Syndrome (JPS)
Hereditary Mixed Polyposis Syndrome (HMPS)

Familial Colorectal Cancer / Syndrome X (FCCX)
Lynch-like Syndrome (LLS)

Lynch Syndrome (LS)
Colorectal Cancer: Genetic Risk

- Familial Adenomatous Polyposis (FAP)
- Attenuated FAP (AFAP)
- MUTYH-Associated Polyposis (MAP)
- NTHL1-Associated Polyposis (NAP)
- Polymerase-Associated Polyposis (PPAP)
- Serrated Polyposis Syndrome (SPS)
- Peutz-Jeghers Syndrome (PJS)
- Juvenile Polyposis Syndrome (JPS)
- Hereditary Mixed Polyposis Syndrome (HMPS)
- Familial Colorectal Cancer / Syndrome X (FCCX)
- Lynch-like Syndrome (LLS)

~1.5%

~3.3% Lynch Syndrome (LS)

MSH2, MLH1, MSH6, PMS2, EPCAM
Colorectal Cancer: Genetic Risk

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~1.5%

~3.3% Lynch Syndrome (LS)

MSH2, MLH1, MSH6, PMS2, EPCAM
Lynch Syndrome: 1895

- Warthin: Family ‘G’

Warthin, A. S. (1913). Heredity with reference to carcinoma: As shown by the study of the cases examined in the pathological laboratory of the University of Michigan, 1895-1913. Archives of Internal Medicine, 12(5), 546-555.
Lynch Syndrome: 1966

• “Cancer family syndrome”
• Hereditary Non-Polyposis Colorectal Cancer Syndrome

Picture courtesy of Dr Patrick Lynch, MD Anderson Cancer Centre, Houston, TX
‘Textbook’ LS Family History

Bowel "young"

Womb 40’s
Bowel 72y

F+W 85y

Caecum 45y

Endometrial 38y

TCC Ureter 35y

85y
Lynch Syndrome: 2016

- Genetics:
  - Autosomal Dominant
  - Partially penetrant, variably expressed, sex limited and phenocopied
  - CRC risks start 20s – 40s; average age of CRC ~42y
  - Due to constitutional ("germline") pathogenic mutations in a DNA mismatch repair (MMR) gene
    - **MSH2, MLH1, MSH6, PMS2**
    - **EPCAM**
      - Risks vary with each gene …
      - Common, as rare diseases go - ~1:1000 x 4 = 1:250
      - Biallelic / recessive (two mutations in the same gene) = Constitutional MisMatch Repair-Disorder (CMMR-D)
Lynch syndrome: mutations

- Constitutional (‘germline’) mutations come in all sorts
  - Methylation: *MLH1, MSH2 (EPCAM)*
  - Point
    - Truncating
    - Splice
    - Missense
  - Indels
  - Large del/dup
  - Chromosomal
    - 46,XX,inv(2)(p21.1p22.2).arr(1-22,X)x2
    - Undetectable on seq, MLPA, aCGH …

Courtesy Dr Peter Thompson
Institute of Medical Genetics, Cardiff
                      :::::::::::::::::
:C:::C:::C:::C:::C:::C:::C:::C:::C:::C:::C:::C:

:C:::C:::C:::C:::C:::C:::C:::C:::C:::C:::C:::C:
<table>
<thead>
<tr>
<th>MSH2</th>
<th>MSH6</th>
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<tr>
<th>MSH2</th>
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</tr>
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</table>
Lynch Syndrome Risks

- **Cancers**
  - *principal*: colon & rectum
  - *major*: endometrium (lower segment)
  - *minor*:
    - Ovary (non-serous)
    - Stomach
    - Small intestine
    - Pancreas
    - Hepato-biliary tract
    - Urinary pelvis/ureter; bladder (TCC)
    - Skin (sebaceous adenoma/carcinoma & keratoacanthoma - *Muir-Torre*)
    - CNS glioblastoma
    - Prostate
    - Breast
    - ...
  
- Gene dependent
Lynch Syndrome Risks

- **Cancers**
  - **principal**: colon & rectum
  - **major**: endometrium (lower segment)
  - **minor**:
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    - CNS glioblastoma
    - Prostate
    - Breast
    - ...
  
- Gene dependent
Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database

Pål Møller,1 Toni Seppälä,2 Inge Bernstein,3,4 Elke Holinski-Feder,5,6 Paola Sala,7 D Gareth Evans,8,9 Annika Lindblom,10 Finlay Macrae,11,12 Ignacio Blanco,13 Rolf Sijmons,14 Jacqueline Jeffries,15 Hans Vasen,16 John Burn,17 Sigve Nakken,18,19 Eivind Hovig,18,19,20 Einar Andreas Rødland,18 Kukatharmini Tharmaratnam,21 Wouter H de Vos tot Nederveen Cappel,22 James Hill,23 Juul Wijnen,24 Kate Green,8 Fiona Laloo,8 Lone Sunde,3,25,26 Miriam Mints,27 Lucio Bertario,7 Marta Pineda,13 Matilde Navarro,13 Monika Morak,5,6 Laura Renkonen-Sinisalo,28,29 Ian M Frayling,15 John-Paul Plazzer,11 Kirsti Pylvanainen,30 Julian R Sampson,15 Gabriel Capella,13 Jukka-Pekka Mecklin,30,31 Gabriela Möslin,32 in collaboration with The Mallorca Group (http://mallorca-group.eu)

http://www.lscarisk.org

LS Risks

All cancers, males

All cancers, females

LS Risks

Endometrial

Ovarian

Management of LS

- **Colonoscopic surveillance, 3 yrly**
  - Reduces CRC mortality by ~50%

**FIGURE 25** Cumulative risks of CRC for people with and without MMR mutations.

**FIGURE 26** Impact of colonoscopy (age 25–75 years) in reducing cumulative risk of CRC for people with LS.

Management of LS

**TABLE 78** Stage distribution of CRCs for individuals undergoing colonoscopic surveillance, from Mecklin and colleagues (2007)\(^{188}\)

<table>
<thead>
<tr>
<th>Dukes’ stage</th>
<th>Number of carcinomas (simple proportion)</th>
<th>Dirichlet posterior hyperparameters (prior distribution of Jeffreys prior)</th>
<th>Expected proportion used in model</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29 (0.707)</td>
<td>29.5</td>
<td>0.686</td>
</tr>
<tr>
<td>B</td>
<td>4 (0.098)</td>
<td>4.5</td>
<td>0.105</td>
</tr>
<tr>
<td>C</td>
<td>5 (0.122)</td>
<td>5.5</td>
<td>0.128</td>
</tr>
<tr>
<td>D</td>
<td>3 (0.073)</td>
<td>3.5</td>
<td>0.081</td>
</tr>
</tbody>
</table>

*Source: Mecklin and colleagues.\(^{188}\)*

**FIGURE 33** Stage distribution of CRCs for people with and without LS surveillance.

---

Future Management of LS

• Aspirin
  – CAPP2: “600 mg aspirin/day for 2 yrs reduced all Lynch syndrome cancers at 5 yrs by over 60%”

• Vaccines
  – Micoryx http://clinicaltrials.gov/show/NCT01461148
  – Monocyte-derived dendritic cells https://clinicaltrials.gov/ct2/show/NCT01885702
Diagnosis of LS

- Multidisciplinary …
- **Pathogenic mutation in a DNA MMR gene**

- Microsatellite instability in a LS-associated tumour
  - MSI in 12% of sporadic colon cancers – not due to LS
  - MSI in 4% of colon cancers – due to LS (or somatic MMR mutations etc.)
  - MSI in ?1-2% of rectal cancers – due to LS

- Loss of MMR protein on immunohistochemistry
  - MLH1-neg PMS2-neg in sporadic colon cancers with MSI

- **BRAF** V600E mutation in a CRC
  - Present in most sporadic CRC with MSI; absent in LS

- Methylation of the **MLH1** gene in sporadic MSI+ colon cancers
  - Some cases are constitutional
Microsatellite Instability (MSI)

Immunohistochemistry

Dr Mark Arends, University of Cambridge Department of Pathology
If …

It takes five consultants to change a light bulb:

One to change the actual bulb and four to tell him or her how much better they could have done it.

How many geneticists does it take to interpret a mutation?
**InSiGHT Variant Interpretation Committee (VIC)**

Established Yokohama, 2007

<table>
<thead>
<tr>
<th>Bryony A. Thompson</th>
<th>Chris Heinen</th>
<th>Rajkumar Ramesar</th>
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<tbody>
<tr>
<td>Amanda B. Spurdle</td>
<td>Elke Holinski-Feder</td>
<td>Brigitte Royer-Pokora</td>
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<td>Maija Kohonen-Corish</td>
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<td>Annika Lindblom</td>
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<td>Kristina Lagerstedt</td>
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<td>Michael Woods</td>
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<td>Monika Morak</td>
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<td>Desiree du Sart</td>
<td>Minna Nystrom</td>
<td>David Goldgar</td>
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<tr>
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<td>Aurelie Fabre</td>
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<tr>
<td>Thierry Frebourg</td>
<td>Ming Qi</td>
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</tbody>
</table>

**Logos:**
- Cancer Council Queensland
- Cancer Council Victoria
- Istituto Toscano Tumori
- Australian Government Cancer Australia
A Systematic Approach to Clinical Classification of DNA Sequence Variants in Mismatch Repair Genes:

The InSiGHT Variant Interpretation Committee

Established Yokohama, 2007
InSiGHT Variant Interpretation Committee (VIC)
Clinical consequences of DNA variants identified by genetic tests
Clinical consequences of DNA variants identified by genetic tests

• Not pathogenic
Clinical consequences of DNA variants identified by genetic tests

- Not pathogenic
- Pathogenic
Clinical consequences of DNA variants identified by genetic tests

- Not pathogenic
- Uncertain significance
- Pathogenic
Clinical consequences of DNA variants identified by genetic tests

- Not pathogenic
- Uncertain significance
- Pathogenic
The Uncertain Significance dilemma

• No clinical use

• Uncertainty / Indecision

• Worry

• Risk of inappropriate use
InSiGHT Variant Interpretation Committee (VIC)

- Safety, in numbers and variety
  - Multidisciplinary
  - Liability (USA; IARC)
- Consistent
- 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
Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results

Sharon E. Plon\textsuperscript{1,\textdagger}, Diana M. Eccles\textsuperscript{2,\textdagger}, Douglas Easton\textsuperscript{3}, William D. Foulkes\textsuperscript{4}, Maurizio Genuardi\textsuperscript{5}, Marc S. Greenblatt\textsuperscript{6}, Frans B.L. Hogervorst\textsuperscript{7}, Nicoline Hoogerbrugge\textsuperscript{8}, Amanda B. Spurdle\textsuperscript{9}, and Sean Tavitian\textsuperscript{10} for the IARC Unclassified Genetic Variant Group\textsuperscript{†}

Proposed Classification System for Sequence Variants Identified by Genetic Testing

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Probability of being Pathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Definitely Pathogenic</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>4</td>
<td>Likely Pathogenic</td>
<td>0.95–0.99</td>
</tr>
<tr>
<td>3</td>
<td>Uncertain</td>
<td>0.05–0.949</td>
</tr>
<tr>
<td>2</td>
<td>Likely Not Pathogenic or of Little Clinical Significance</td>
<td>0.001–0.049</td>
</tr>
<tr>
<td>1</td>
<td>Not Pathogenic or of No Clinical Significance</td>
<td>&lt;0.001</td>
</tr>
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Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results

Sharon E. Plon, Diana M. Eccles, Douglas Easton, William D. Foulkes, Maurizio Genuardi, Marc S. Greenblatt, Frans B.L. Hogervorst, Nicoline Hoogerbrugge, Amanda B. Spurdle, and Sean Tavtigian for the IARC Unclassified Genetic Variants Working Group

Testing Recommendations Associated with Each Class of Variant

<table>
<thead>
<tr>
<th>Class</th>
<th>Clinical Testing</th>
<th>Surveillance Recommendations if At-Risk Relative is Positive</th>
<th>Research Testing of Family Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Test at-risk relatives for variant</td>
<td>Full high-risk surveillance guidelines</td>
<td>Not indicated</td>
</tr>
<tr>
<td>4</td>
<td>Test at-risk relatives for variant*</td>
<td>Full high-risk surveillance guidelines</td>
<td>May be helpful to further classify variant</td>
</tr>
<tr>
<td>3</td>
<td>Do not use for predictive testing in at-risk relatives*</td>
<td>Based on family history (and other risk factors)</td>
<td>May be helpful to further classify variant</td>
</tr>
<tr>
<td>2</td>
<td>Do not use for predictive testing in at-risk relatives*</td>
<td>Treat as “no mutation detected” for this disorder</td>
<td>May be helpful to further classify variant</td>
</tr>
<tr>
<td>1</td>
<td>Do not use for predictive testing in at-risk relatives*</td>
<td>Treat as “no mutation detected” for this disorder</td>
<td>Not indicated</td>
</tr>
</tbody>
</table>
5-tiered Classification System:
www.insight-group.org/criteria/

- Class 5: Pathogenic
- Class 4: Likely pathogenic
- Class 3: Uncertain
- Class 2: Likely not pathogenic
- Class 1: Not pathogenic

Clinically Actionable

Clinically Actionable
The InSiGHT Variant Database

Original ICG-HNPCC database established in 1994 (curator Paivi Peltomäki)

⇒ InSiGHT Mutation Database (2001)

Incorporated two other databases in 2008:

• Mismatch Repair Genes Variant Database, Michael Woods, University of Newfoundland (literature)

• MMR Gene Unclassified Variant Database, Rolf Sijmons, University of Groningen (functional)
InSiGHT Mutation Database:
data cleaning, nomenclature standardization and systematic data review

12,645 MMR gene submissions

3,468 nomenclature alterations
(12 not resolvable)
Duplicate entries resolved

2,360 unique constitutional MMR gene variants
(MSH2, MLH1, MSH6, PMS2)

7 EPCAM

10 Source unknown / non-existent

230 Somatic

132 Synthetic
Criteria development process

- Used modified Delphi approach

- Evolution of criteria
  - Started with the 117 most commonly cited variants with discordant interpretation
  - Iterative amendments & clarifications over 12 meetings

- Quantitative (multifactorial) or qualitative evidence considered

- Work towards fully quantitative (Bayesian), recognising limits
  - Calibration of in silico algorithms
Bayes’ Rule

Prior probability

Updated by

*Likelihood ratios*  -or-  *odds ratios of causality*

→ Posterior probability
Prior probability

What is the prior probability any given mutation is pathogenic?
What is the prior probability any given mutation is pathogenic?
Classification of MMR gene UVs: A modified BIC probability model

Prior probability: $LR = \frac{Pr(Data_i | HR)}{Pr(Data_i | N)}$

Empirical:
- in silico missense analysis
- in silico splicing analysis

Current LRs ($Data$):
- LR1: Co-segregation
- LR2: Microsatellite instability
- LR3: BRAF V600E

$tba$: Summary family history
$tba$: MLH1 promoter methylation
$tba$: in vitro MMR assays

Classification: IARC 5-class model with posterior probability cutoffs

Thompson et al., Human Mutation 34: 200-209, 2012.
• Prior probability of pathogenicity of all possible missense nucleotide substitutions in all 4 MMR genes. http://hci-lovd.hci.utah.edu/home.php
• Cut-offs <0.10 & >0.90
• Clinical actionability therefore only possible with additional data
In Silico Missense Analysis

- Getting ‘Prior Probabilities’ in the first place.
- Training algorithms – The Calibration Problem
- Deciding what algorithm/s is/are best and what’s not needed.
• Exceed an average of 3 substitutions per position
• Reach SIFT’s optimum sequence diversity criterion

Courtesy: Sean Tavtigian
Grantham Variation (GV) (tolerated range of variation)
Grantham Deviation (GD) (relative severity)

The Calibration Problem

How can you train your *in silico* algorithms to find pathogenic mutations?
The Calibration Problem

How do you train a pet to sniff out truffles?
The Calibration Problem

Set of missense substitutions
- Scorable with one or more *in silico* algorithms
- Labeled with data that are logically independent to the *in silico* evaluation but truly related to pathogenicity

Courtesy: Sean Tavtigian
<table>
<thead>
<tr>
<th>Class</th>
<th>Pathogenic/likely not pathogenic</th>
<th>Criteria</th>
</tr>
</thead>
</table>
| Class 5 – Pathogenic | All of the following characteristics: | - Deficient protein function in *in vitro/*ex vivo functional assays in mammalian system (cannot be in yeast only)  
- Co-segregation with disease in at least oneAMS family with ≥4 affected carriers, or ≥2 families with ≥3 affected non-proband carriers  
- Not present in the general population (<160 individuals=320 alleles)  
- MSI-H in ≥2 independent tumors with no contradictory IHC results or immunoloss of MMR protein consistent with the variant location in ≥2 independent tumors for MLH1 or ≥1 tumor for MSH2, MSH6, PMS2 |
| Class 4 – Likely pathogenic | Deficient protein function in one or more *in vitro/*ex vivo assays in any eukaryote, plus one of the following: | - Deficient protein function in one or more *in vitro/*ex vivo assays in any eukaryote, plus one of the following:  
- Co-segregation with disease in at least oneAMS family with ≥3 affected carriers  
- MSI-H in ≥2 independent tumors with no contradictory IHC results or immunoloss of MMR protein consistent with the variant location in ≥2 independent tumors for MLH1 or ≥1 tumor for MSH2, MSH6, PMS2 |
| Class 3 – Uncertain | Insufficient evidence to classify i.e., not Class 1, 2, 4 or 5 |
| Class 2 – Likely not pathogenic | Variants reported to occur in the general ethnic group at frequency ≥1%, and that have not yet been excluded as founder mutations  
Or variants reported to occur in the general population at frequency <1%, with normal protein function and *in vitro/*ex vivo functional assays in any eukaryote and no aberrant splicing |
| Class 1 – Not pathogenic | Variants reported to occur in the general population at frequency ≥1%  
Or present in the general population at frequency 0.1-1% and determined by large case-control studies to be associated with estimated risk <1.5, with upper bound 95% confidence limit of 1.5 |

* Courtesy: Sean Tavtigian
Table 2. Qualitative classification of 143 Missense substitutions evaluated for use in calibration of in silico ... to the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.

Class 1 – Not pathogenic (n=20)
MLH1 p.(Ile32Val)
MLH1 p.(Val213Met)
MLH1 p.(Ile219Val)
MLH1 p.(Ile219Leu)
MLH1 p.(Gln689Arg)
MLH1 p.(Val716Met)
MLH1 p.(His718Tyr)
MSH2 p.(Ile207Ser)
MSH2 p.(Arg475Gln)
MSH2 p.(Thr1919Val)
MSH6 p.(Gly39Glu)
MSH6 p.(Leu396Val)
MSH6 p.(Val878Ala)
MSH6 p.(Ile886Val)
PMS2 p.(Thr277Lys)
PMS2 p.(Pro470Ser)
PMS2 p.(Thr485Lys)
PMS2 p.(Thr597Ser)
PMS2 p.(Met622Ile)

Class 2 – Likely not pathogenic (n=9)
MLH1 p.(Val326Ala)
MLH1 p.(Ile384Asp)
MLH1 p.(Gly88Asn)
MLH1 p.(Ile661Arg)
MSH2 p.(Sp167His)
MSH2 p.(Gln629His)
MSH6 p.(Met688Ile)
MSH6 p.(Val509Ala)

Class 4 – Likely pathogenic (n=37)
MLH1 p.(Pro28Leu)
MLH1 p.(Asp63Glu)
MLH1 p.(Gly67Trp)
MLH1 p.(Gly67Glu)
MLH1 p.(Cys77Arg)
MLH1 p.(Cys77Tyr)
MLH1 p.(Phe80Val)
MLH1 p.(Lys84Glu)
MLH1 p.(Ile107Arg)
MLH1 p.(Leu155Arg)
MLH1 p.(Val185Gly)
MLH1 p.(Gly244Asp)
MLH1 p.(Ser247Pro)
MLH1 p.(Leu550Pro)
MLH1 p.(Asn551Thr)
MLH1 p.(Leu559Arg)
MLH1 p.(Leu582Phe)
MLH1 p.(Ala589Phe)
MLH1 p.(Pro648Asp)
MLH1 p.(Pro648Leu)
MLH1 p.(Pro654Leu)
MLH1 p.(Arg687Trp)
MLH1 p.(Leu749Pro)
MLH1 p.(Arg755Ser)
MSH2 p.(Val161Asp)
MSH2 p.(Gly162Arg)
MSH2 p.(Gly164Arg)
MSH2 p.(Leu173Pro)
MSH2 p.(Leu187Pro)
MSH2 p.(Cys333Tyr)
MSH2 p.(Asp603Asp)
MSH2 p.(Gly692Asn)
MSH2 p.(Cys697Arg)
MSH2 p.(Cys697Arg)
MSH2 p.(Glu749Phe)
MSH2 p.(Glu751Arg)
PMS2 p.(Ser46Ile)

Class 5 – Pathogenic (n=8)
MLH1 p.(Met35Arg)
MLH1 p.(Leu38His)
MLH1 p.(Gly99Phe)
MLH1 p.(Asp103Phe)
MLH1 p.(Leu109Arg)
MLH1 p.(Pro302Ile)
MLH1 p.(Glu302His)
MLH1 p.(Pro622Leu)
MLH1 p.(Ala636Pro)

MMR genes: The Classified Substitutions

0.1% 5% 95% 99%

Courtesy: Sean Tavitgian
The Calibration Problem

Set of missense substitutions
- Scorable with one or more *in silico* algorithms
- Labeled with data that are logically independent to the *in silico* evaluation but truly related to pathogenicity
- Make some simplifying assumptions
- Execute regressions
- Evaluate internal consistency
- If appropriate, codify a recalibration

Courtesy: Sean Tavtigian
The Calibration Problem

Innocuous
Severe

Native \textit{in silico} algorithm score

Low probability
High probability

External pathogenicity estimate

Innocuous \hspace{1cm} Native \textit{in silico} algorithm score \hspace{1cm} Severe

Courtesy: Sean Tavtigian
The Calibration Problem

- Plan for verification, refined recalibration

Calibrated probability of pathogenicity

Calibrated probability of pathogenicity

Native *in silico* algorithm score

Innocuous

Severe

Courtesy: Sean Tavtigian
## MMR genes: Scoring the substitutions

<table>
<thead>
<tr>
<th>Analysis Program</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPP&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PolyPhen-2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SIFT&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Align-GVGD&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Xvar&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>MutPred&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> MAPP: This method uses a combination of sequence and structural information to predict the functional impact of amino acid substitutions.

<sup>b</sup> PolyPhen-2.1: A program that predicts the functional effects of single nucleotide polymorphisms (SNPs).

<sup>c</sup> SIFT: A software tool that predicts whether amino acid substitutions are deleterious or not based on evolutionary conservation.

<sup>d</sup> Align-GVGD: This method uses alignment information and conservation scores to predict the functional impact of amino acid changes.

<sup>e</sup> Xvar: A tool that predicts the functional impact of amino acid substitutions using multiple sequence alignment.

<sup>f</sup> MutPred: A program that predicts the functional impact of mutations based on a combination of sequence and structural features.

---

*Courtesy: Sean Tavtigian*
MMR genes: Regression results

<table>
<thead>
<tr>
<th>Analysis Program</th>
<th>Least squares regression</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted $R^2$</td>
<td>P-value</td>
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<tr>
<td>MAPP$^a$</td>
<td>0.586</td>
<td>1.15 x 10$^{-15}$</td>
</tr>
<tr>
<td>PolyPhen-2.1$^b$</td>
<td>0.575</td>
<td>3.15 x 10$^{-15}$</td>
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<tr>
<td>SIFT$^c$</td>
<td>0.541</td>
<td>5.07 x 10$^{-14}$</td>
</tr>
<tr>
<td>Align-GVGD$^d$</td>
<td>0.505</td>
<td>7.73 x 10$^{-13}$</td>
</tr>
<tr>
<td>Xvar$^e$</td>
<td>0.449</td>
<td>4.01 x 10$^{-11}$</td>
</tr>
<tr>
<td>MutPred$^f$</td>
<td>0.396</td>
<td>1.12 x 10$^{-9}$</td>
</tr>
</tbody>
</table>

Courtesy: Sean Tavitgian
MMR genes: Data before regressions (MAPP)

Pathogenicity estimates

ln(MAPP score)

Courtesy: Sean Tavtigian
MMR genes: Calibration of MAPP

Logit $R^2 = 0.586$

- Class 5
- Class 4
- Class 2
- Class 1

MLH1 V326A
MLH1 V185G
MSH6 V509A
MLH1 H718Y
MLH1 L582F

Probability in favor of pathogenicity vs. $\ln$(MAPP score)

Courtesy: Sean Tavtigian
<table>
<thead>
<tr>
<th>Program combination</th>
<th>Ordered logistic regression</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pseudo R²</td>
<td>First program P value</td>
<td>Second program P value</td>
<td>Model P value</td>
<td>ROC area</td>
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<tr>
<td>MAPP</td>
<td>0.2753</td>
<td>0.038</td>
<td>0.048</td>
<td>$2.57 \times 10^{-11}$</td>
<td>0.933</td>
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<td>MAPP</td>
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<td>0.108</td>
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<td>MAPP</td>
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<td>MAPP</td>
<td>0.2622</td>
<td>0.001</td>
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<td>MAPP</td>
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<td>&lt;0.001</td>
<td>0.122</td>
<td>$6.07 \times 10^{-11}$</td>
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<tr>
<td>PolyPhen-2.1</td>
<td>0.2635</td>
<td>0.123</td>
<td>0.132</td>
<td>$7.34 \times 10^{-11}$</td>
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<tr>
<td>PolyPhen-2.1</td>
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<td>0.006</td>
<td>0.073</td>
<td>$4.66 \times 10^{-11}$</td>
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<tr>
<td>PolyPhen-2.1</td>
<td>0.2515</td>
<td>0.002</td>
<td>0.610</td>
<td>$2.12 \times 10^{-10}$</td>
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<tr>
<td>PolyPhen-2.1</td>
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<td>0.078</td>
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<tr>
<td>SIFT</td>
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<td>0.072</td>
<td>$4.43 \times 10^{-11}$</td>
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<tr>
<td>SIFT</td>
<td>0.2529</td>
<td>0.002</td>
<td>0.496</td>
<td>$1.87 \times 10^{-10}$</td>
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<tr>
<td>SIFT</td>
<td>0.2649</td>
<td>&lt;0.001</td>
<td>0.103</td>
<td>$6.45 \times 10^{-11}$</td>
<td></td>
</tr>
</tbody>
</table>

*aComputational tool operating conditions were as defined in Table 4.

Pseudo $R^2$ ordered logistic regression

Courtesy: Sean Tavtigian
## MMR genes: Multiple regression results

<table>
<thead>
<tr>
<th>Program combination</th>
<th>Ordered logistic regression</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First program $P$ value</td>
<td>Second program $P$ value</td>
</tr>
<tr>
<td>MAPP PolyPhen-2.1</td>
<td>0.038</td>
<td>0.048</td>
</tr>
</tbody>
</table>

**Answer:**

The best performing pets, used together, are MAPP and custom-PP2.1.

Both having been trained specifically to sniff out pathogenic missense MMR mutations.

*Courtesy: Sean Tavitgian*
Qualitative points of evidence

Sequence-based, e.g. nonsense & frameshifts

5’...TCT CAA AAA TTT ACG...3’

S Q K F T

5’...TCT CAA TAA TTT ACG...3’

S Q *

Clinical/molecular

Functional

Segregation data

Tumour data

Frequency data

Co-occurrence in trans

In vitro data

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

Controls
Major issue in classification process was conflicting data from functional assays

Functional assay subcommittee formed to tackle the issue

Flowchart developed to assist assay interpretation:
Functional assay interpretation

Gene transcript assays

Figure 1

Heinen & Rasmussen 2012, Hered Cancer Clin Pract
Functional assay interpretation

Mammalian MMR activity assays (x2)

Figure 1

Heinen & Rasmussen 2012, Hered Cancer Clin Pract
Functional assay interpretation

Protein expression/stability (x2)
Subcellular localization
Review of functional assay controls

**MLH1 p.Gly67Arg**

**MLH1 p.Thr117Met**

**MLH1 p.Val326Ala**

**MLH1 p.Ile219Val**
Outcomes

- Model developed over 15 months
- 117 most-cited unclassified mutations classified
- Agreed model then used to classify the remaining 2,243 ...

www.insight-group.org/classifications
Outcomes

- Model developed over 15 months
- 117 most-cited unclassified mutations classified
- Agreed model then used to classify the remaining 2,243 … in one evening

www.insight-group.org/classifications
# Transparent classification data

http://chromium.lovd.nl/LOVD2/colon_cancer/home.php

## InSiGHT Classification

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Protein</th>
<th>Consensus InSIGHT Classification</th>
<th>Classification Data</th>
<th>Summary Justification</th>
<th>Posterior Probability of Pathogenicity</th>
<th>Splicing/Transcript expression</th>
<th>Co-occurrence &amp; co-observation</th>
<th>MSI Status</th>
<th>IHC MLH1</th>
<th>IHC MSH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>c.190G&gt;A</td>
<td>p.Gly67Arg</td>
<td>Class S. pathogenic</td>
<td>30/04/2013</td>
<td>Abrogated function, &gt;2 MLH1 tumours, cosegregation with disease &amp; MAF 0.00, PP 0.89</td>
<td>1.00 (Thompson et al., 2012)</td>
<td>No effect on splicing (diploid-haplod conversion analysis). Casey et al., 2005; RT-PCR of patient MR; Auerbach et al., 2005; 100% clone inclusion with aDPLS minigene Landeira et al., 2006; aCAS minigene Tsunoda et al., 2008; balanced variant allele expression in primer-extension assay Pastelie et al., 2011)</td>
<td>18 MSI-H tumours (1 CRC Haraf et al., 1997, 1 CRC Ramil-Gómez et al., 2006, 1 CRC Paciò et al., 2006, 1 CRC Ciechaniz et al., 2004, 1 adenoma Hovav et al., 2006, 1 CRC Rasavara et al., 2005, 1 CRC Blou et al., 2006, 1 CRC Schaeffer et al., 2004, 1 CRC Oester et al., 2011, 1 CRC Pastelie et al., 2011, 6 CRC &amp; 1 ileal Hardi et al., 2011, 1 CRC CRC/UCDi Mengesgård &amp; Løkenæs, 1 CRC CRC/UCDi Simons)</td>
<td>Absent in 3 tumours (1 CRC, MSI reported in Rasavara et al., 2005, Young et al., 2002, Casey et al., 2005, 1 adenoma Hovav et al., 2006, 1 CRC Rasavara et al., 2005, 1 CRC Blou et al., 2006, 1 CRC Oester et al., 2011, 1 CRC CRC/UCDi Mengesgård &amp; Løkenæs, 1 CRC CRC/UCDi Simons)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
LOVD submission classifications vs InSiGHT VIC variant classifications

Class 5a = assumed pathogenic nonsense mutations, small frameshift indels, and large deletions.

Class 5b = not-obviously truncating variants considered to be pathogenic on the basis of combined evidence.
LOVD submission classifications vs InSiGHT VIC variant classifications

![Diagram showing classification percentages for different classes:](chart)

- Class 1
- Class 2
- Class 3
- Class 4
- Class 5a
- Class 5b

Legend:
- Orange: Class 5b
- Dark Teal: Class 5a
- Purple: Class 4
- Green: Class 3
- Blue: Class 1

Categories:
- No known pathogenicity
- Probably no pathogenicity
- Unknown
- Probably pathogenic
- Pathogenic
LOVD submission classifications vs InSiGHT VIC variant classifications

- No known pathogenicity
- Probably no pathogenicity
- Unknown
- Probably pathogenic
- Pathogenic

Classifications:
- Class 5b
- Class 5a
- Class 4
- Class 3
- Class 2
- Class 1
LOVD submission classifications vs InSiGHT VIC variant classifications
LOVD submission classifications vs InSiGHT VIC variant classifications

- No known pathogenicity
- Probably no pathogenicity
- Unknown
- Probably pathogenic
- Pathogenic

Classifications:
- Class 5b
- Class 5a
- Class 4
- Class 3
- Class 2
- Class 1
Outcomes and Significance

• Standardized & normalized MMR gene variant classification scheme
• 2,479* classifications now available
• Transparent - summary information used to assign variant pathogenicity is there
• Microattribution process set up for MMR & other InSiGHT genes
• Provides a model for other conditions
• Publication ...
Outcomes and Significance

- Now there is an established list of pathogenic mutations, for standardised comparison
- Types and sites of mutation x ascertainment
  - subdivision of LS by gene
- People with >1 mutation in different genes
- What does Class 3 mean?
  - Odds of pathogenicity ≠ Degree of pathogenicity
  - Clinical actionability
- Ends
  - Same missense, different mutation
  - $P$(amino acid missense) v. $P$(splice ‘missense’)
Outcomes and Significance

• Submission of unpublished data for variant classification
  – PLEASE
  – bulk uploading now possible
  – “not on the InSiGHT database” is no longer a valid statement on a report!

• Put data in and you will get an output:
• Research to validate functional assays
• Splicing
• XRC data instead of GVGD
• APC, MUTYH, POLD1, POLE …
Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database

Bryony A Thompson¹,²,⁴,⁶, Amanda B Spurdle¹,⁴,⁶, John-Paul Plazzer³, Marc S Greenblatt⁴, Kiwamu Akagi⁵, Fahd Al-Mulla⁶, Bharati Bapat⁷, Inge Bernstein⁸,⁹, Gabriel Capellà¹⁰, Johan T den Dunnen¹¹, Desirée du Sart¹², Aurelie Fabre¹³, Michael P Farrell¹⁴, Susan M Farrington¹⁵, Ian M Frayling¹⁶, Thierry Frebourg¹⁷,¹⁸, David Goldgar¹⁹,²⁰, Christopher D Heinen²¹,²², Elke Holinski-Feder²³,²⁴, Maija Kohonen-Corish²⁵,²⁷, Kristina Lagerstedt Robinson²⁸, Suet Yi Leung²⁹, Alexandra Martins³⁰, Pal Moller³⁰, Monika Morak³³,²⁴, Minna Nystrom³¹, Paivi Peltonen³², Marta Pineda³⁰, Ming Qi³³,³⁴, Rajkumar Ramesar³⁵, Lene Juel Rasmussen³⁶, Brigitte Royer-Pokora³⁷, Rodney J Scott³⁸,³⁹, Rolf Sijmons⁴⁰, Sean V Tavitgian³⁰, Carli M Tops¹¹, Thomas Weber⁴¹, Juul Wijnen¹¹, Michael O Woods⁴², Finlay Macrae³ & Maurizio Genuardi³³,⁴⁴ on behalf of InSiGHT⁴⁵

Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database

Standards for clinical use of genetic variants
23rd Dec 2013

From the highest rated international genetics journal to the UK newspaper with the lowest required reading age in <48 h

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**CANCER’S DNA HOPE**

**SCIENTISTS** have found a more accurate test for bowel and womb cancer which they say will save lives.

Their research identified the genes that cause Lynch Syndrome – the most common form of hereditary bowel cancer. Sufferers also have an increased risk of developing other cancers, including in the womb.

Patients could now have more idea if the cancers run in their family – meaning they can be screened earlier.

Bowel cancer kills 16,000 people each year and womb cancer 2,000. Dr Ian Frayling, from Cardiff University, said: “This study will help to save lives.”
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td><strong>MLH1</strong></td>
<td>60</td>
<td>27</td>
<td>284</td>
<td>94</td>
<td>506</td>
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<td><strong>MSH2</strong></td>
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<td><strong>MSH6</strong></td>
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<td><strong>PMS2</strong></td>
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</tr>
</tbody>
</table>

p = <0.001 0.001~0.049 0.05~0.949 0.95~0.99 >0.99
Custom target exome-panel: **123 genes**

- ~ 25 HBOC genes
- ~ 20 Hereditary CRC genes
- 14 genes of RASopathies (NF1, Noonan, Leopard,…)
- 5 genes of Multiple familial meningioma
- 2 (+1) genes of NF2-Schwannomatosis
- ~ 3 genes of Phakomatoses (VHL, TSCs…)
- ~ 40 sample identification SNPs
- 46 risk associated SNPs
- ~ 60 genes of other hereditary cancers

Courtesy of Dr Conxi Lazaro
## Cancer Gene Panel

<table>
<thead>
<tr>
<th>GENES</th>
<th>Breast/ovary</th>
<th>Polyposis</th>
<th>Colon/Endometrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>1</td>
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</tr>
<tr>
<td>BRCA2</td>
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<tr>
<td>TP53</td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>MLH1</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>MSH2</td>
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<td></td>
<td>1</td>
</tr>
<tr>
<td>MSH6</td>
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<td>1</td>
</tr>
<tr>
<td>PMS2</td>
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<tr>
<td>MUTYH</td>
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<td>POLE</td>
<td>1*</td>
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</tr>
<tr>
<td>POLD1</td>
<td>1*</td>
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<td>BMPR1A</td>
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<td>BRIP1</td>
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<tr>
<td>CHEK2</td>
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<td>PALB2</td>
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</tr>
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<tr>
<td>RAD51C</td>
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<td>RAD51D</td>
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<td>SMAD4</td>
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<tr>
<td>STK11</td>
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</tr>
</tbody>
</table>

Courtesy of Dr Conxi Lazaro
“Sixteen pathogenic variants were detected in women who tested negative for \textit{BRCA1/2} mutations … 15 met our criteria for being potentially actionable.

A total of 428 VUS were identified in 39 genes among 175 participants. Most of the VUS were novel (n = 380; 88.8%); the PolyPhen program predicted that 151 were benign (35.3%), 65 were probably damaging (15.2%), and 50 were possibly damaging (11.7%).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{VUS count per gene.}
\end{figure}

\textbf{Allison W. Kurian et al. JCO 2014;32:2001-2009}
### Gene Transcript/cDNA change Predicted protein change

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Variant calling</th>
<th>Splicing</th>
<th>Protein function</th>
<th>Protein stability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MUTYH</strong></td>
<td>NM_001128425.1:c.1227_1228dup</td>
<td>p.Glu410GlyfsX43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>105</td>
<td>SETD2 NM_014159.6:c.1204C&gt;T</td>
<td>p.Arg402Trp</td>
<td>Loss of acceptor splicing site</td>
<td>Damaging</td>
</tr>
<tr>
<td>115</td>
<td>MLH3 NM_001040108.1:c.1870G&gt;C</td>
<td>p.Glu624Gln</td>
<td>Inconclusive</td>
<td>Inconclusive</td>
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<tr>
<td>111</td>
<td>BUB1 NM_004336.4:c.3005C&gt;G</td>
<td>p.Thr1002Ser</td>
<td>Loss of acceptor splicing site</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>MUTYH</strong></td>
<td>NM_001128425.1:c.1187G&gt;A</td>
<td>p.Gly396Asp</td>
<td>Loss of donor splicing site</td>
<td>Damaging</td>
</tr>
<tr>
<td>117</td>
<td>BUB3 NM_004725.3:c.*1124G&gt;A</td>
<td>p.?</td>
<td>Inconclusive</td>
<td>Benign</td>
</tr>
<tr>
<td>111</td>
<td>MLH3 NM_001040108.1:c.*2058G&gt;T</td>
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<td>Inconclusive</td>
<td>Benign</td>
</tr>
<tr>
<td>107</td>
<td>SETD2 NM_014159.6:c.2798G&gt;T</td>
<td>p.Gly933Val</td>
<td>Loss of donor splicing site</td>
<td>Benign</td>
</tr>
<tr>
<td>110</td>
<td>ENG NM_000118.3:c.1712G&gt;A</td>
<td>p.Arg571His</td>
<td>Inconclusive</td>
<td>Benign</td>
</tr>
<tr>
<td>111</td>
<td>EPCAM NM_002354.2:c.-280G&gt;C</td>
<td>p.?</td>
<td>Inconclusive</td>
<td>Benign</td>
</tr>
<tr>
<td>109</td>
<td>MLH3 NM_001040108.1:c.*2485G&gt;C</td>
<td>p.?</td>
<td>Inconclusive</td>
<td>Benign</td>
</tr>
<tr>
<td>107</td>
<td>SETD2 NM_014159.6:c.2508T&gt;G</td>
<td>p.Cys836Trp</td>
<td>No change</td>
<td>Damaging</td>
</tr>
<tr>
<td>110</td>
<td>MSH2 NM_000251.2:c.211G&gt;C</td>
<td>p.Gly71Arg</td>
<td>Loss of donor splicing site</td>
<td>Inconclusive</td>
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<tr>
<td>109</td>
<td>PMS1 NM_000534.4:c.2186A&gt;G</td>
<td>p.Asns690Ser</td>
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<tr>
<td>109</td>
<td>TP53 NM_000546.5:c.*1175A&gt;C</td>
<td>p.?</td>
<td>Benign</td>
<td></td>
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<tr>
<td>109</td>
<td>APC NM_000038.4:c.*1684A&gt;G</td>
<td>p.?</td>
<td>Benign</td>
<td></td>
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<tr>
<td>109</td>
<td>ENG NM_000118.3:c.*704delAGTT</td>
<td>p.?</td>
<td>Benign</td>
<td></td>
</tr>
</tbody>
</table>

**Germline variants in CRC-associated genes**

18 MSH2 deficient LLS

6 carriers of predicted pathogenic variants in other genes

**Possible oligogenic effect**

Custom NGS panels help in the identification of other possibly associated genes

Courtesy of Dr Gardenia Vargas
Clinico-pathological features of biallelic MUTYH carriers

N = 225 Lynch-like syndrome patients

15 MUTYH mutation carriers

- 8 monoallelic
- 7 biallelic

So MUTYH is a bona fide Lynch-like syndrome gene

[and as these cases had <10 adenomas, so they didn’t have MAP …]
### Table: Gene Transcript/cDNA change Predicted protein change Splicing Protein function

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transcript/cDNA change</th>
<th>Predicted protein change</th>
<th>Splicing</th>
<th>Protein function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BUB1B</strong></td>
<td>NM_001211.5:c.1738G&gt;T</td>
<td>p.Glu580*</td>
<td>Inconclusive</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>MLH1</strong></td>
<td>NM_001167618.1:c.1253G&gt;A</td>
<td>p.Arg418Gln</td>
<td>Inconclusive</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>MSH6</strong></td>
<td>NM_000179.2:c.2625G&gt;T</td>
<td>p.Met875lle</td>
<td>No change</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>BMPR1A</strong></td>
<td>NM_004329.2:c.878C&gt;T</td>
<td>p.Ala293Val</td>
<td>No change</td>
<td>Damaging</td>
</tr>
<tr>
<td><strong>POLE</strong></td>
<td>NM_006231.2:c.2284C&gt;T</td>
<td>p.Arg762Trp</td>
<td>No change</td>
<td>Damaging</td>
</tr>
<tr>
<td><strong>SETD1B</strong></td>
<td>NM_015048.1.c.22del</td>
<td>p.His8Thrfs*27</td>
<td>No change</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>MSH3</strong></td>
<td>NM_002439.4:c.1114delAA</td>
<td>p.Lys383Argfs*32</td>
<td>Inconclusive</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>PMS2</strong></td>
<td>NM_000535.5:c.1501G&gt;A</td>
<td>p.Val501Met</td>
<td>No change</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>MLH1</strong></td>
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<td>p.Arg233Trp</td>
<td>No change</td>
<td>Damaging</td>
</tr>
<tr>
<td><strong>POLE</strong></td>
<td>NM_006231.2:c.2284C&gt;T</td>
<td>p.Arg762Trp</td>
<td>No change</td>
<td>Damaging</td>
</tr>
<tr>
<td><strong>SETD1B</strong></td>
<td>NM_0015048.1.c.22del</td>
<td>p.H8fs*27</td>
<td>No change</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>PMS2</strong></td>
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<td>p.Glu109Lysfs*3</td>
<td>No change</td>
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<tr>
<td><strong>PTEN</strong></td>
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<td><strong>SETD2</strong></td>
<td>NM_014159.6:c.3165T&gt;A</td>
<td>p.Asp1055Glu</td>
<td>No change</td>
<td>Inconclusive</td>
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<tr>
<td><strong>MSH6</strong></td>
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<td>p.Arg361His</td>
<td>No change</td>
<td>Benign</td>
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<tr>
<td><strong>POLD1</strong></td>
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<td>p.Arg444Trp</td>
<td>Inconclusive</td>
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<tr>
<td><strong>MLH3</strong></td>
<td>NM_001040108.1:c.1755delA</td>
<td>p.E586fs*24</td>
<td>No change</td>
<td>Damaging</td>
</tr>
<tr>
<td><strong>MLH3</strong></td>
<td>NM_001007793.2:c.973T&gt;C</td>
<td>p.His8Thrfs*27</td>
<td>No change</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>BUB3</strong></td>
<td>NM_001007793.2:c.973T&gt;C</td>
<td>p.His8Thrfs*27</td>
<td>No change</td>
<td>Benign</td>
</tr>
<tr>
<td><strong>CHEK2</strong></td>
<td>NM_007194.3:c.880G&gt;A</td>
<td>p.Lys792Arg</td>
<td>Gain of donor</td>
<td>Damaging</td>
</tr>
</tbody>
</table>

### Patient IDs and Tumor Tested

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Tumor tested</th>
<th>Variant calling</th>
</tr>
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<tbody>
<tr>
<td>108_C1</td>
<td>108_C2</td>
<td>Possible</td>
</tr>
<tr>
<td>114_C1</td>
<td></td>
<td>No apparent</td>
</tr>
<tr>
<td>121_C1</td>
<td></td>
<td>No apparent</td>
</tr>
<tr>
<td>111_C3</td>
<td></td>
<td>Not analyzed</td>
</tr>
</tbody>
</table>
Panels: what does it all mean?

- Mutations in >1 gene

**Review**

*Multilocus Inherited Neoplasia Alleles Syndrome: A Case Series and Review*

James Whitworth, MRCP; Anne-Bine Skytte, MD; Lone Sunde, PhD, MD; Derek H. Lim, MRCGP; Mark J. Arends, PhD, FRCPath; Lisa Happerfield, FIBMS, MSc; Ian M. Frayling, PhD, FRCPath; Rick van Minkelen, PhD; Emma R. Woodward, PhD, MRCP; Marc D. Tischkowitz, PhD, FRCP; Eamonn R. Maher, FRCP, FMedSci

**JAMA Oncology**  Published online December 10, 2015

**At a Glance**

- The identification of a constitutional deleterious variant at more than 1 locus associated with inherited neoplasia has substantial potential implications for cancer risks, but these are not readily predictable.
- We propose the term *multilocus inherited neoplasia alleles syndrome* (MINAS) to describe such cases, which are expected to become more frequently reported with increased clinical

http://databases.lovd.nl/shared/diseases/04296

Panels: what does it all mean?

• Implications for how we test in the future …

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polyps</th>
<th>No polyps</th>
<th>MSS</th>
<th>MSI (?)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>APC (FAP/AFAP)</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ (?)</td>
</tr>
<tr>
<td><em>AXIN2</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ (?)</td>
</tr>
<tr>
<td><em>BUB1</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><em>MUTYH (MAP)</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><em>NTHL1 (NAP)</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><em>POLD1 (PPAP)</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><em>POLE (PPAP)</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
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<td>✓</td>
<td>✓</td>
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<td>✓</td>
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<tr>
<td><em>MSH6</em></td>
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<td>✓</td>
</tr>
<tr>
<td><em>PMS2</em></td>
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<td>✓</td>
</tr>
<tr>
<td>_ _ _ _ 1</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><em>SETD2</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Panels: what does it all mean?

- Implications for how we test in the future …
- We [used to] use polyps and MSI/IHC testing to guide which gene/s to test, but better now to test all cases with a panel and use tumour tests to interpret genetic findings - not to limit which genes to test.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polyps</th>
<th>No polyps</th>
<th>MSS</th>
<th>MSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC (FAP/AFAP)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ (?)</td>
</tr>
<tr>
<td>AXIN2</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ (?)</td>
</tr>
<tr>
<td>BUB1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MUTYH (MAP)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>NTHL1 (NAP)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>POLD1 (PPAP)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>POLE (PPAP)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MSH2</td>
<td>✓ (✓)</td>
<td>✓</td>
<td>✓ (✓)</td>
<td>✓</td>
</tr>
<tr>
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<td>✓</td>
<td>✓ (✓)</td>
<td>✓</td>
</tr>
<tr>
<td>MSH6</td>
<td>✓ (✓)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>PMS2</td>
<td>✓ (✓)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>SETD2</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Panels: what does it all mean?

- Implications for how we test in the future …
- And, logically, if we are **screening** for hereditary causes of CRC by systematic testing of incident cases, then better to test first with a gene panel, and not exclude those without LS by testing first with MSI and IHC.

<table>
<thead>
<tr>
<th></th>
<th>Polyps</th>
<th>No polyps</th>
<th>MSS</th>
<th>MSI</th>
</tr>
</thead>
</table>
| APC (FAP/AFAP) | ✓ | ✓ | ✓ | ✓ (?)
| AXIN2 | ✓ | ✓ | ✓ | ✓ (?)
| BUB1 | ✓ | ✓ | ✓ | ✓
| MUTYH (MAP) | ✓ | ✓ | ✓ | ✓
| NTHL1 (NAP) | ✓ | ✓ | ✓ | ✓
| POLD1 (PPAP) | ✓ | ✓ | ✓ | ✓
| POLE (PPAP) | ✓ | ✓ | ✓ | ✓
| MSH2 | (✓) | ✓ | (✓) | ✓
| MLH1 | (✓) | ✓ | (✓) | ✓
| MSH6 | (✓) | ✓ | ✓ | ✓
| PMS2 | (✓) | ✓ | ✓ | ✓
| SETD2 | ✓ | ✓ | ✓ | ✓

Summary

• Through teamwork, a rare genetic syndrome amenable to multidisciplinary testing, has enabled an objective probabilistic method to determine the pathogenicity of mutations

• Safer, less time consuming – automated input to testing

• Example for others – only 20,996 genes to go!

• Helps addresses the challenges of panel testing to maximise benefits

• Importance of phenotype – other branches of pathology

• We start to explain the wonderful complexity of biology

• http://chromium.lovd.nl/LOVD2/colon_cancer
1st Meeting of the European Hereditary Tumour Group (EHTG) Palma, Mallorca

A two and a half day symposium hosted by the Mallorca Group

Early deadline date for registration is 15 April 2016

Call for Abstracts

Deadline for submission: 28 March 2016
Visit www.mallorca-group.org for further details about how to submit.

www.mallorca-group.org
Meet Mallorca Group
EUROPEAN HEREDITARY TUMOUR GROUP (EHTG)

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