Clinical interpretation of genomic variants

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• I will not discuss off label use and/or investigational use in my presentation.
Learning objectives

• Distinguish between the interpretation of germline and somatic variants
• Determine the elements involved in interpretation of germline and somatic variants
• Understand the process of clinical characterization of variants in the context of tissue or disease site
Genomics in Medicine

- Pharmacogenomics: Precision dosing and avoidance of life threatening drug toxicity
- Early detection of cancer: Circulating tumour DNA (ct DNA) in blood to detect recurrence early. Trials underway of using ctDNA for screening
- Molecular oncology: Management decisions and targeted drugs based on tumour genomics (see box 2)
- Sequencing of pathogen genomes: Rapid detection, delineation of species taxonomy, antimicrobial resistance
- Rare diseases: Attaining a specific genetic diagnosis to improve patient management (see box 1)
- Newborn screening and carrier testing: For severe inherited diseases
- Non-invasive prenatal testing: Using cell free circulating DNA from mother eg, for Down's syndrome
- Identification of people with inherited high risk of cancer: To direct intensive screening, preventive drugs, and risk reducing surgery

Taken from BMJ 2018 361:k1687
Variant (mutation) types- not all variants are the same

https://useast.ensembl.org/info/genome/variation/prediction/predicted_data.html
Germline vs somatic variants

- Initiates in germ cell- therefore every cell in the body
- Often contribute to monogenic disease
- May contribute to risk of developing disease
- When linked to disease often involved in disease pathogenesis
- Concept of polymorphism
- Concept of benign, pathogenic

-Originates in a non-germline cell- therefore, not every cell in the body
- Most often in the context of cancer
- When linked to tumours may be diagnostic, predictive, prognostic
- Interpretation must include the tissue type
- Concept of drivers and passengers
- Concept of actionable or not
Generic Massively Parallel Sequencing

Library Preparation

Clonal Amplification

Sequencing

TUMOUR DNA/RNA

GERMLINE DNA/RNA
Germline vs somatic variants

- Tumor
  - "Normal" (non-neoplastic tissue, blood, fibroblast culture, saliva, etc.)
    - Subtract variant calls in the normal from variant calls in the tumor
      - Somatic variants
        - Germline variants for selected cancer susceptibility genes
          - Pathogenic
            - Likely pathogenic
              - VUS
                - Likely benign
                  - Benign
                    - Return results to patient

https://gatkforums.broadinstitute.org/gatk/discussion/11127/somatic-calling-is-not-simply-a-difference-between-two-callsets
Pathway to variant calling

**Annotation** = identification of variant characteristics

**Interpretation** = synthesis of characteristics and relation to clinical context

Taken from Kamps et al 2017 Int J Mol Sci 18:308
| Coverage | Ref | Alt | Alt#(F,R) | Alt% | Ins% | De% | Overall Score | Mutation Call: HGVS Coding | Function | Zygosity | Gene | Strand | Exon | SNP db_xref | Amino Acid Change |
|----------|-----|-----|-----------|------|------|-----|---------------|----------------------------|----------|----------|------|--------|------|--------|----------------|------------------|
| 504      | C   | G   | 1:1       | 99.6 | 0.0  | 0.0 | 21.5         | NM_000251.2:c.[211+9C>G]  | Noncoding| Homozygous| MSH2| +     | rs2303426 |                |
| 1152     | A   | T   | 325:248   | 50.2 | 0.0  | 0.0 | 24.3         | NM_000251.2:c.[1511-9A>T] | Noncoding| Heterozygous| MSH2| +     | rs12998837 |                |
| 822      | G   | A   | 0:1       | 99.7 | 0.0  | 0.0 | 23.1         | NM_000251.2:c.[1661+12G>A] | Noncoding| Homozygous| MSH2| +     | rs3732183  |                |
| 857      | T   | C   | 240:187   | 50.1 | 0.0  | 0.0 | 23.3         | NM_000251.2:c.[2006-6T>A] | Noncoding| Homozygous| MSH2| +     | rs2303426  |                |
| 689      | G   | A   | 199:184   | 44.4 | 0.0  | 0.0 | 23.6         | NM_000179.2:c.[1163>A]   | Missense| Heterozygous| MSH6| +     | rs1042821 | p.G39EG          |
| 448      | A   | G   | 139:95    | 47.7 | 0.0  | 0.0 | 23.1         | NM_000465.2:c.[1568+14C>T] | Noncoding| Homozygous| BRD1| -5    | rs5031011  |                |
| 832      | CA  | TG  | 236:197   | 47.8 | 0.0  | 0.0 | 24.5         | NM_000465.2:c.[1518_1519delTGinsCA] | Missense| Heterozygous| BRD1| -5    | rs229571   | p.R373S          |
| 1211     | C   | G   | 632:579   | 98.8 | 0.0  | 0.0 | 23.4         | NM_000465.2:c.[1134G>C]  | Missense| Heterozygous| BRD1| -5    | rs1048108  | p.P24SP          |
| 1144     | A   | G   | 309:303   | 46.2 | 0.0  | 0.0 | 22.6         | NM_000465.2:c.[70C>T]    | Noncoding| Homozygous| MLH1| +     | rs1799977  | p.I219IV         |
| 896      | C   | T   | 278:208   | 45.7 | 0.0  | 0.0 | 23.5         | NM_000535.5:c.[2006+6G>A] | Noncoding| Homozygous| PMS2| -8    | rs11190575 |                |
| 1376     | T   | C   | 1:2       | 99.8 | 0.0  | 0.0 | 24.5         | NM_000535.5:c.[1621A>G]  | Missense| Heterozygous| PMS2| -8    | rs228206    | p.K541E          |
| 1185     | G   | T   | 323:310   | 46.2 | 0.0  | 0.0 | 24.5         | NM_000535.5:c.[1454C>A]  | Missense| Heterozygous| PMS2| -8    | rs180523    | p.T485K          |
| 796      | G   | C   | 0:1       | 100.0| 0.0  | 0.0 | 23.0         | NM_000535.5:c.[780C>G]  | Synonymous| Homozygous| PMS2| -8    | rs180531    | p.S260S          |
| 764      | A   | G   | 0:0       | 99.8 | 0.0  | 0.0 | 22.9         | NM_000051.3:c.[5948A>G]  | Missense| Heterozygous| ATM| +40   | rs659243   | p.N1983S         |
| 1230     | A   | C   | 324:303   | 42.0 | 0.0  | 0.0 | 24.7         | NM_000059.3:c.[1114C>A]  | Missense| Heterozygous| BRCA2| -10   | rs144848   | p.N372GH        |
| 1354     | A   | G   | 0:1       | 99.7 | 0.0  | 0.0 | 24.9         | NM_000059.3:c.[4563A>G]  | Missense| Heterozygous| BRCA2| +11   | rs206075   | p.L1521L        |
| 1081     | G   | C   | 0:0       | 100.0| 0.0  | 0.0 | 24.1         | NM_000059.3:c.[6513G>A]  | Missense| Heterozygous| BRCA2| +11   | rs206076   | p.V2171V        |
| 935      | T   | C   | 0:0       | 100.0| 0.0  | 0.0 | 23.6         | NM_000059.3:c.[7397T>C]  | Missense| Heterozygous| BRCA2| +14   | rs169547    | p.V2466A        |
| 644      | T   | C   | 207:129   | 47.3 | 0.0  | 0.0 | 22.4         | NM_000059.3:c.[7806-14T>C] | Missense| Heterozygous| BRCA2| -11   | rs934282   |                |
| 437      | C   | T   | 125:119   | 43.4 | 0.0  | 0.0 | 21.0         | NM_000436.0:c.[48+6C>T]  | Noncoding| Heterozygous| CDH1| +13   | rs3743674  |                |
| 961      | T   | C   | 226:233   | 52.3 | 0.0  | 0.0 | 23.7         | NM_000436.0:c.[2076T>A]  | Noncoding| Heterozygous| CDH1| +13   | rs8101552  | p.A692AA         |
| 1078     | G   | C   | 264:293   | 51.9 | 0.0  | 0.0 | 24.1         | NM_000056.5:c.[215C>G]   | Missense| Heterozygous| TP53| -4    | rs1042522  | p.P72RP         |
| 1072     | A   | G   | 253:265   | 51.5 | 0.0  | 0.0 | 24.1         | NM_000243.2:c.[3411T>C]  | Missense| Heterozygous| BRIP1| -20   | rs4986763  | p.Y1137Y        |
| 1164     | A   | G   | 276:302   | 50.3 | 0.0  | 0.0 | 24.4         | NM_000243.2:c.[2755T>C]  | Missense| Heterozygous| BRIP1| -19   | rs4986764  | p.S919SP        |
| 1244     | T   | C   | 316:333   | 47.8 | 0.0  | 0.0 | 24.6         | NM_000243.2:c.[2637A>G]  | Missense| Heterozygous| BRIP1| -19   | rs4986765  | p.E879KE        |
| 1769     | C   | T   | 513:447   | 46.0 | 0.0  | 0.0 | 25.8         | NM_000243.2:c.[89A>G]    | Missense| Heterozygous| BRIP1| -2    | rs45458966 | p.P23PP         |
| 1369     | G   | C   | 341:399   | 45.9 | 0.0  | 0.0 | 25.0         | NM_000055.4:c.[920+7G>C] | Noncoding| Homozygous| STK11| +7    | rs20756703 |                |
Example from somatic cancer
Variant Interpretation Classifications
Germline Variants

Allele frequency in large studies of control chromosomes— is the allele frequency too high? Has the allele been seen in homozygous state? Is it particular to some ethnic group? Is the prevalence in disease increased over controls? Computational predictions are just that— predictions. Generally not assigned a high impact and can only be used if all tools point in the same direction, which becomes more unlikely the more tools one tries. However some can be very strong— ie null variants. Functional data requires actually reading the papers and assessing the work. Requires the interpreter is expert in all fields of physiology, biochemistry, metabolomics, genomics, statistics, analytics. Segregation data can be highly impactful if sufficient meiosis are examined— the more meiosis where segregation works out, the stronger the evidence. De novo— did the variant appear in the affected individual for the first time when parents are unaffected and this is confirmed? Has the variant been seen in trans or in cis with another variant? Depending on inheritance mode, this will have different impact.

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NM_005228.3(EGFR):c.2361G>A(p.=)

- Synonymous variant - does not change amino acid
- No indication that it could affect splicing either
- Population data says it is found across all tested populations at a frequency of 40%, reaching 53% in some populations
- This is sufficient to know that this is not a pathogenic germline variant
- Falls clearly into the neutral or benign polymorphism category
- No need to do additional work
NM_000059.2(BRCA2):9976A>T(p.Lys3326*)

- Truncating variant- in a disease where this is a known mechanism - but the variant is in the last exon, towards the end- the last amino acid should be 3419
- Population data shows that the variant has been identified across all populations at a frequency of ~0.6%- much higher than the frequency of hereditary breast/ovarian cancer syndrome
- In some populations, frequency >1%, defining this as a polymorphism
- More recently, literature shows this variant impacts HR ability, although does not abolish it
- May be associated with specific cancers (i.e. not necessarily breast or ovarian)
- Large study of ~400,000 cases and controls suggests it is associated with cancers with environmental genotoxic risk factors
Somatic genomics is different

• The goal of interpretation of somatic genomic variants is to guide/change patient management
• Unlike germline variants, won’t necessarily see variant allele fractions of 50% or 100%, although that’s possible
• Have issues of sensitivity that are different from germline analysis
• Issues of tumour heterogeneity as well
Rationale for developing a new scheme for somatic variant interpretation
### Table 3: Categories of Clinical and/or Experimental Evidence

<table>
<thead>
<tr>
<th>Category</th>
<th>Therapeutic</th>
<th>Diagnosis</th>
<th>Prognosis</th>
</tr>
</thead>
</table>
| Level A    | 1. Biomarkers that predict response or resistance to FDA-approved therapies for a specific type of tumor  
             2. Biomarkers included in professional guidelines that predict response or resistance to therapies for a specific type of tumor | Biomarkers included in professional guidelines as diagnostic for a specific type of tumor             | Biomarkers included in professional guidelines as prognostic for a specific type of tumor             |
| Level B    | Biomarkers that predict response or resistance to therapies for a specific type of tumor based on well-powered studies with consensus from experts in the field | Biomarkers of diagnostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field | Biomarkers of prognostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field |
| Level C    | 1. Biomarkers that predict response or resistance to therapies approved by the FDA or professional societies for a different type of tumor  
             2. Biomarkers that serve as inclusion criteria for clinical trials | Biomarkers of diagnostic significance based on the results of multiple small studies                   | Biomarkers of prognostic significance based on the results of multiple small studies                   |
| Level D    | Biomarkers that show plausible therapeutic significance based on preclinical studies | Biomarkers that may assist disease diagnosis themselves or along with other biomarkers based on small studies or a few case reports | Biomarkers that may assist disease prognosis themselves or along with other biomarkers based on small studies or a few case reports |

Taken from Li et al 2017 JMD 19:4
AMP/ASCO/CAP Interpretation scheme

**Tier I: Variants of Strong Clinical Significance**
Therapeutic, prognostic & diagnostic

- **Level A Evidence**
  - FDA-approved therapy
  - Included in professional guidelines

- **Level B Evidence**
  - Well-powered studies with consensus from experts in the field

**Tier II: Variants of Potential Clinical Significance**
Therapeutic, prognostic & diagnostic

- **Level C Evidence**
  - FDA-approved therapies for different tumor types or investigational therapies
  - Multiple small published studies with some consensus

- **Level D Evidence**
  - Preclinical trials or a few case reports without consensus

**Tier III: Variants of Unknown Clinical Significance**
Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases

- **Level E Evidence**
  - No convincing published evidence of cancer association

**Tier IV: Benign or Likely Benign Variants**
Observed at significant allele frequency in the general or specific subpopulation databases

- **Level F Evidence**
  - No existing published evidence of cancer association

*Taken from Li et al 2017 JMD 19:4*
<table>
<thead>
<tr>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>Class 4</th>
<th>Class 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant previously reported:</td>
<td>Yes, pathogenic</td>
<td>Yes, pathogenic</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Specific variant is actionable:</td>
<td>In same site/histology</td>
<td>In different site/histology</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Other variants in same gene are actionable:</td>
<td></td>
<td></td>
<td>In same site/histology</td>
<td>In different site/histology</td>
</tr>
<tr>
<td>Variant effect from prediction tools:</td>
<td></td>
<td></td>
<td>3A: pathogenic</td>
<td>4A: pathogenic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3B: unknown</td>
<td>4B: unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3C: benign</td>
<td>4C: benign</td>
</tr>
</tbody>
</table>

Taken from Sukhai et al 2016 Genet Med 18:128
NM_004333.4(BRAF):c.1799T>A(p.Val600Glu)

Obvious clinically relevant call in the context of melanoma

But what about other tumours?

Also need to consider the proportion of the case that was tumour cells versus the variant allele fraction seen in the analysis
Beyond variant interpretation

10% tumour cells
All are heterozygous for variant of interest
**Variant is present at 5%**

25% tumour cells
40% of them are heterozygous for variant of interest
**Variant is present at 5%**

Established the assay with a limit of detection for an SNV of 5% at a depth of 400

**Identify an EGFR L858R variant present at 5% VAF**

Both patients eligible for targeted therapy, but will both respond equally well? If information not conveyed, missing opportunity to look for other strategies? If tumour % estimate way off, is this predictive information less useful?
When two worlds collide

Comparing germline and somatic variants

**Germline**
1. Pathogenic
2. Likely Pathogenic
3. VUS
4. Likely Benign
5. Benign

**Somatic**
- Diagnostic
- Prognostic
- Predictive

**“Category”**

**“Evidence”**

- Evidence of Pathogenicity
  - Very Strong
  - Strong
  - Moderate
  - Supporting

- Evidence of Benign
  - Stand-alone
  - Strong
  - Supporting

- Level of Evidence
  - Prospective trials
  - Retrospective trials
  - FDA-approval / guideline indication
  - Expert opinion
  - Case reports
  - Preclinical
  - Inferential
Summary

Interpretation of germline variants generally uses a 5 category schema.

All variants are assessed using a combination of population, computational, literature and physical data.

Variants are generally associated with diagnosis or prediction of risk - to that extent variants are interpreted in the context of disease.

Interpretation of somatic variants is more complex in the sense that they may be more difficult to identify.

They require interpretation in the context of clinical management.

They require interpretation in the context of disease.