Genomic Pathology for Surgical Pathologists

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Genomic Medicine

Patient ➔ Specimen ➔ DNA Testing of Many Genes ➔ Diagnosis ➔ Treatment/Management
Pathologist

‘Genomic Pathologist’
Test Selection

Global Evaluation (Large deletions)  CGH Microarray

Targeted Evaluation (Mutation in a gene)  Sanger Sequencing

Specific Rearrangement (Translocations)  FISH

Genomic - Multiple Gene Panels ( Syndromic)  Next Generation Sequencing
DNA Variants

- Single nucleotide variants (SNV)
  - REFERENCE: GGCCTTAACCCCGGATTATCAG
  - PATIENT: GGCCTTAACCTCCGATTATCAG

- Small insertions/deletions (INDEL)
  - REFERENCE: GGCCTTAACC CCC GATTATCAG
  - PATIENT: GGCCTTAACC - - - GATTATCAG

- Structural variants (Chromosomal)
  - Large insertions/deletions
  - Copy number variants
  - Translocations
Nucleotide change resulting in NO change in amino acid:

• Synonymous
• Silent

Nucleotide change resulting in CHANGE in amino acid:

• Non-Synonymous
• Missense
Question:
Is a non-synonymous variant (change in amino acid) a mutation?

Answer:
Only some are pathogenic (mutation)

Each non-synonymous variant is interpreted by using databases and literature
Each ‘disease-free’ person has:

• **Thousands** of rare or unique DNA variants that change the amino acids at a single position

• **Hundreds** of rare frameshift DNA variants that result in truncated proteins
Clinical Case 1

- 13 month-old female presented with nystagmus; imaging showed an optic-hypothalamic mass
- Surgically debulked, pathology showed *pilocytic astrocytoma*
- 4 chemotherapy regimens over 3 years
- No response

Drobysheva et al 2017 J Natl Compr Canc Netw
Further Progression

- Radiation therapy recommended
- Family declined radiation due to long-term morbidity concerns
- BRAF molecular testing
  - \textit{BRAF-KIAA1549} fusion \textbf{NEGATIVE (FISH)}
  - \textit{BRAF V600E} \textbf{NEGATIVE (IHC)}
Identified: **BRAF** c.1799_1800delinsAT, p.Val600Asp

- V600D, rare variant not previously described in LGG (2015)
- Melanoma cell line data indicated V600D inhibited similarly as V600E (Gentilcore 2013)
- Subsequent studies show V600D in infantile ganglioglioma/astrocytoma

Frequent *BRAF* gene alterations in pediatric high and low grade gliomas

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>BRAF-KIAA1549 Fusion (%)</th>
<th>BRAF V600E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilocytic astrocytoma</td>
<td>78%</td>
<td>6%</td>
</tr>
<tr>
<td>Pilomyxoid astrocytoma</td>
<td>63%</td>
<td>5%</td>
</tr>
<tr>
<td>Pleomorphic xanthoastrocytoma</td>
<td>50%</td>
<td>50%</td>
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<tr>
<td>Ganglioglioma</td>
<td>26%</td>
<td>21%</td>
</tr>
<tr>
<td>Diffuse fibrillary astrocytoma</td>
<td>3%</td>
<td>8%</td>
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<tr>
<td>Anaplastic astrocytoma</td>
<td>0%</td>
<td>16%</td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>0%</td>
<td>9%</td>
</tr>
</tbody>
</table>
BRAF signaling

- RAF kinase which effects:
  - growth factor signaling
  - cell cycle progression
  - proliferation

- Part of the mitogen activated protein kinase (MAPK) pathway

https://www.mycancergenome.org/content/disease/melanoma/braf/54/
Question: How should the \textit{BRAF} variant be interpreted?

A. Variant of Strong Clinical Significance
B. Variant of Potential Clinical Significance
C. Variant of Unknown Clinical Significance
D. Benign or Likely Benign Variant
## Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

**AMP, ASCO, CAP (2017)**

### 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

- 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

- No existing published evidence of cancer association

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Li et al 2017 J Mol Diagn 19:4-23
**Question:** How should the *BRAF* variant be interpreted?

A. Tier 1: Strong Clinical Significance  
B. Tier 2: Potential Clinical Significance  
C. Tier 3: Unknown Clinical Significance  
D. Tier 4: Benign or Likely Benign Variant

**Answer:** B, Tier 2 Potential Clinical Significance

- *BRAF* V600D variant is biologically significant
- FDA approved therapies in other cancers
- No data on *BRAF* or MEK inhibitors for V600D in LGG (2015)
Question: Should off-label therapy be recommended?

**PROS**

- No other therapy options (family resistant to radiation therapy)
- BRAF V600E responds to targeted inhibition

**CONS**

- Limited data on V600E (no data on V600D)
- BRAF inhibitors have high rate of toxicity in adult studies of melanoma
Question: Should off-label therapy be recommended?

Answer:

• Off-label dabrafenib was initiated

- 3 mo: dabrafenib
- 6 mo: dabrafenib
- 9 mo: dabrafenib/trametinib

• >40 months stable disease on combination dabrafenib/trametinib
Clinical Case Summary

- Patient with low grade glioma and novel BRAF variant (BRAF V600D)
- 2017 somatic interpretation guideline (CAP/ASCO/AMP) is a useful framework
- Uncertain whether V600D response will be broadly applicable
- LGG routine testing of BRAF fusions and V600 variants is recommended
Clinical Case 2

- 60 year-old man with colon adenocarcinoma identified as mismatch repair deficient (MSH2/MSH6 loss by immunohistochemistry)
- *MSH2* gene sequencing identifies: c.1A>C (p.Met1_Gly25del)

Truta et al 2008 Familial Cancer 7:267-74
**MSH2** c.1A>C (p.Met1_Gly25del)

MutS homolog 2
Patient’s *MSH2* c.1A>C reported as **likely pathogenic**

*MSH2* is one of the DNA mismatch repair genes (*MLH1*, *MSH6*, and *PMS2*) associated with Lynch Syndrome

Lynch syndrome is an autosomal dominant cancer syndrome associated with early colorectal, endometrial and ovarian cancers

Patient’s children tested
Two Years Later...Revised Classification:
c.1A>C is no longer considered “likely pathogenic”

- Clinical genetics laboratories noted new evidence
  - Multiple cases of same variant seen
  - Alternative start codon is proven
  - Low penetrance with an inheritance pattern inconsistent with pathogenicity

- New interpretation: variant of uncertain significance

Question: If all children are tested and two are positive, which of the following are important next steps?

Previous Answer: Counsel/screen only gene test positive
Revised Answer: Counsel/screen all gene test positive and negative
Genomic Test Results are *Dynamic*

• New discoveries require corrections
• Laboratory needs to provide dynamic reporting
• Pre-genomic Example:
  – Harvard Partners noted 214 changes over 7 years
  – >100 corrected reports

Aronson 2012 Genet Med
Medical Literature is Constantly Growing

• 27% of mutations cited in literature (2011) were found to be common population variants or incorrectly reported

• Traditional human genetic studies (pre-2010) used 100s of control genomes to define a novel or rare disease variant
  – High rate of calling benign variants as mutations

• Recent studies use >100,000 control genomes (gnomAD, 1000 Genomes)

Bell et al 2011 Sci Trans Med
• UT Southwestern study of 1.45 mil patients tested at Myriad Genetics (2006-2016)
• Hereditary cancer genetic testing (56.6% with personal history)
• 59,955 amended reports because of variant reclassification
• Overall, **6.4% of variants were reclassified**
• 24.9% of Uncertain (VUS) variants were changed
• UT Southwestern study of 309 patients tested at GeneDx (2012-2015)
• Genomic pediatric epilepsy testing
• Overall, 36.2% of patients had variants reclassified
• *31.3% of reclassified variant changed the diagnosis*
Emerging Diagnostic Split Decisions

- Clinical labs interpret variants differently
- Example: ClinVar database for Hereditary Cancer-Predisposing Syndrome Genetics

### Diagnoses from one laboratory

<table>
<thead>
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<th></th>
<th>pathogenic</th>
<th>likely pathogenic</th>
<th>uncertain significance</th>
<th>likely benign</th>
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<tr>
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<td>1</td>
<td>411</td>
<td>1012</td>
<td>0</td>
</tr>
</tbody>
</table>

https://clinvarminer.genetics.utah.edu/
Final Question: How do you manage patients with the same genetic change, but tested by different labs with different interpretations?

A. Create internal consensus opinion and treat the patients the same
B. Treat patients based on the different lab interpretations
C. Share discordant information with patients and let them decide
D. I don’t know
Summary

• Genomic testing and interpretation are critical for advanced malignancies
• Genomic test interpretations are dynamic over time
• The practice of genomic medicine requires pragmatic judgment despite diagnostic uncertainty (i.e., surgical pathologist)