Identifying and managing changes to clinically reported variants in the rapidly evolving field of genomics

Sarah King-Smith
PathUpdate 2019
Overview

• Why variant classifications sometimes change & how often it happens
• Overview of the Australian reclassification landscape
• Highlight key recommendations from the Australian Genomics Reclassification working group
• Preview of the Australian Genomics variant sharing platform ‘Shariant’
The Genomic Interpretation Pipeline

1. vcf filtered
2. Variant(s) Identified
3. Evidence weighed
4. Variant classified (ACMG)
5. Report issued

Review

Reclassification
Variant classification is complex

- Variant interpretation is probabilistic & subjective
- >28 lines of evidence
- There is a lot that we still don’t understand
  - Reference genome is a work in progress
  - Sequencing dead zones
  - Function of most genes poorly described
  - Complex genetic interactions
  - Reversion
  - Mosaicism
  - Environmental impact
- Dependent on test selection – we won’t find it if we don’t know to look for it
- Phenotype
- **Classification is dependent on current knowledge**
Rapid Expansion of Genomics Data

2010 = 1.51M
2018 = 2.54M

PubMed

2014 = 203 gene discoveries

Major genomics data releases: 1000 genomes (2,500), ESP (6,500), ExAC (60,706), gnomAD (141,456)...
National Programmes: US, China, Japan, UK, Aus, Saudi Arabia, Dubai, Estonia, France, Turkey...
What's the problem?

A reclassification, particularly an unexpected reclassification, may have serious clinical and legal consequences.
An example of BRCA2 reclassification

1997

Family first seen

BRCA1 pathogenic

1998

Predictive testing

BRCA2 pathogenic

2001

2014

BRCA2 paused

2015

Variant Review

BRCA1 - no change

BRCA2 ⇒ ??

RNA analysis requested

ENIGMA review requested

2016

ENIGMA: BRCA2 ⇒ 3

Reports amended

SLS incident report

DOH review

Notification approved:

Family members

Clinical contacts

Head of FCCs notified

2.5 yrs for review and DOH approval for notification

BRCA2 c.426-12_426-8delGTTTT (BIC IVS4-12del5)
A Decade Later, a Patient Finds Out Her Genetic Test Was Wrong

Should scientists give results to participants in research studies if they haven’t been validated in a clinical lab?

SARAH ZHANG  MAY 11, 2017

When Genetic Screening Goes Very, Very Wrong

Based on genetic testing, 20 members of the same family were misdiagnosed with a potentially fatal heart condition.

Quest Diagnostics Wrongful Death Lawsuit Ordered to Move to Discovery

Oct 23, 2018 | Tuna Ray

NEW YORK (GenomeWeb) – In a move that surprised legal experts, a South Carolina federal district court has denied Quest Diagnostics’ motion to dismiss several allegations it is facing in a wrongful death lawsuit, allowing the case to move to discovery.

In Williams v Quest/Athena, the US District Court for the District of South Carolina last week determined that the majority of plaintiff Amy Williams’ claims can move to discovery, including the wrongful death of her son Christian, a survivorship action, negligent misrepresentation, constructive fraud, and violation of the state’s Uniform Trade Practices Act. The only claim the court dismissed was the civil conspiracy Williams’ alleged against Quest and subsidiary Athena Diagnostics.
# Measuring uncertainty in historical variant classifications

## Over-classification

<table>
<thead>
<tr>
<th>Study</th>
<th>Date</th>
<th>Description</th>
<th>Over-classification Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shah et al 2018</td>
<td>ns</td>
<td>3/26 ACMG 59 conditions inflated &gt;10% using ‘high quality’ pathogenic ClinVar assertions</td>
<td></td>
</tr>
<tr>
<td>Bell et al 2011</td>
<td>&lt;2011</td>
<td>460 HGMD variants detected in carrier testing cohort</td>
<td>27%</td>
</tr>
<tr>
<td>Andreasen 2013</td>
<td>&lt;2013</td>
<td>HCM assoc. variants in HGMD &amp; ARVD/C db</td>
<td>14-18%</td>
</tr>
<tr>
<td>Clemens et al 2018</td>
<td>&lt;2018</td>
<td>KCNQ1 expert publications</td>
<td>13%</td>
</tr>
</tbody>
</table>

## Discordance

<table>
<thead>
<tr>
<th>Study</th>
<th>Date</th>
<th>Database/Initiative</th>
<th>Total classifications</th>
<th>Discordance Rate</th>
<th>P/LP discrepancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rehm et al, NEJM 2015</td>
<td>ClinVar</td>
<td>12,895</td>
<td>17%</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Amendola et al, AJHG 2016</td>
<td>ACMG pilot (9 labs)</td>
<td>97</td>
<td>41%</td>
<td>22% ⇒ 5%</td>
<td></td>
</tr>
<tr>
<td>Pepin et al, Gen Med, 2016</td>
<td>Collagen diagnostic lab</td>
<td>38</td>
<td>60%</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Harrison et al, Gen Med, 2017</td>
<td>ClinGen initiative (4 labs)</td>
<td>6,169</td>
<td>12% ⇒ 8%</td>
<td>3.5% ⇒ 2%</td>
<td></td>
</tr>
<tr>
<td>Yang et al, Gen Med 2017</td>
<td>ClinVar</td>
<td>27,224</td>
<td>60%</td>
<td>NS</td>
<td>10.7%</td>
</tr>
</tbody>
</table>

## Reclassification

<table>
<thead>
<tr>
<th>Study</th>
<th>Date</th>
<th>Condition/Database</th>
<th>Total classifications (Amended reports)</th>
<th>Reclassification Rate</th>
<th>P/LP downgrade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mersch et al 2018</td>
<td>2006-2016</td>
<td>Hereditary Cancer, Myriad</td>
<td>44,777 (3.6%)</td>
<td>6.7%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Slavin et al 2018</td>
<td>1996-2016</td>
<td>Cancer (excl. benign variants)</td>
<td>1,483</td>
<td>18%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Das et al 2013</td>
<td>2000-2012</td>
<td>Hypertrophic cardiomyopathy</td>
<td>54</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Macklin et al 2018</td>
<td>2013-2017</td>
<td>Hereditary Cancer</td>
<td>1,103</td>
<td>4%</td>
<td></td>
</tr>
</tbody>
</table>
ClinVar 2016 - 2017

7.6% reclassification

Sharing mitigates risk to labs

(Amendola 2016) See also: Garber 2017, Harrison 2017
Australian Genomics Variant Reclassification Working Group

Chair: Prof Hamish Scott

Sub-group chairs: A/Prof Jodie Ingles, Dr Nicola Poplawski, Dr Damien Bruno

Objectives

• Map the existing reclassification landscape in Australia
• Develop and publish recommendations for standard practice to enable harmonised national reclassification.
• Determine feasibility of recommendations in pilot study
Reclassification in Australia

- Missing data
- Reclassification not currently recorded
- Ad hoc & via expert committees
Australian Laboratory Experiences
Preliminary data

- 22 reclassification occurrences reported from: VIC, NSW, SA & QLD

Recurring drivers of reclassification: updated classification criteria, population frequency, curation of variants in a GUS, classification in absence of phenotype or absence of functional evidence, simple error.

Clinical impact: predictive testing, clinical screening, PGD. Family implications.
Challenges identified in the management of variant review in Australia

1. No current standards or recommendations in Australia
2. Challenging to communicate across borders and multiple laboratories and clinical services
3. Variant review is unfunded and under-resourced
4. Databases are limited and often out of date
   - e.g. some labs only deposit into ClinVar on an annual basis (or not at all)
   - Enormous expansion of available population data
5. Increasing number of genes analysed
   - obtaining expert opinion or review panel not always feasible
6. Requires cooperation with private & international laboratories
7. Rapid changes to classification standards and terminology
8. Patient expectations to reclassification
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Variant Management ‘Toolkit’

- Summary of the current guidelines & statements
- Terminology
- Report waivers & terms for patient letters
- Recommendations for variant management
- Flowcharts for discordance resolution & notification of reclassification
Australian Genomics Program 2 Project 1:
Shariant
National Variant Sharing Platform

Amanda Spurdle (Lead)
Emma Tudini (Project Co-ordinator)
David Lawrence (Developer)
James Andrews (Developer)
Sarah King-Smith (Program 1 Reclassification Co-ordinator)
Shariant: National Variant Sharing Platform

A controlled access variant hub and communication platform for real-time sharing of expertise and detailed scientific evidence about clinically curated variants between Australian healthcare providers.

Shariant: share variants

Australian Genomics Variant Classification Sharing Platform

Share clinically curated variants with structured supporting evidence and phenotypes between Australian labs
Allow collaborative monitoring & review of curated variants
Act as a central administrative node for submission to international databases such as ClinVar
Key Shariant Functionalities - Phase 1

- **Automated submission and retrieval** of variant information (via an API)
- **Structured evidence** supporting the classification (based on ACMG guidelines)
- **Semi-automated submission to international databases** upon laboratory approval (such as ClinVar)
- **Automatic notification** upon differences in classification and a communication platform for resolution (collaboration with Australian Genomics Program 1)
Structured evidence

ACMG criteria (strongly recommend)

Shariant does/will not:
- Store genomic data files, e.g. BAM, VCF files
- Store personally identifiable information (e.g. patient DOB, address)
- Replace a lab’s existing curation tool
Discordance Resolution

1. Internal review (completed within 10 working days)
2. Contact external lab(s) to request variant review and notify relevant labs/clinics that process has been initiated.
3. Acknowledge receipt of request for variant review.
4. Review audit trail and interpretation evidence.
5. Record and report discordance. Notify relevant stakeholders.
6. Detailed discussion between laboratories and relevant clinics.
### Compare curations

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>BRCA1</th>
<th>BRCA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome Build</td>
<td>hg19 (GRCh37)</td>
<td>hg19 (GRCh37)</td>
</tr>
<tr>
<td>Transcript ID</td>
<td>NM_007298</td>
<td>NM_007298</td>
</tr>
<tr>
<td>UniProt ID</td>
<td>P38398</td>
<td>P38398</td>
</tr>
<tr>
<td>g.HGVS</td>
<td>NC_000017.10:g.41197752G&gt;C</td>
<td>NC_000017.10:g.41197752G&gt;C</td>
</tr>
<tr>
<td>c.HGVS</td>
<td>NM_007298:3(BRCA1):c.2223C&gt;G</td>
<td>NM_007298:3(BRCA1):c.2223C&gt;G</td>
</tr>
<tr>
<td>p.HGVS</td>
<td>p.Tyr741'</td>
<td>p.Tyr741'</td>
</tr>
<tr>
<td>Variant type</td>
<td>Nonsense (stop gained)</td>
<td>Stop gained</td>
</tr>
<tr>
<td>Molecular consequence</td>
<td>Stop gained</td>
<td>Stop gained</td>
</tr>
<tr>
<td>Zygosity</td>
<td>Not provided</td>
<td>Not provided</td>
</tr>
<tr>
<td>Allele origin</td>
<td>Not provided</td>
<td>Not provided</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Uncertain Significance (3)</td>
<td>Likely Pathogenic (4)</td>
</tr>
</tbody>
</table>

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**Australian Genomics Health Alliance**
Chat & resolve differences

Resolution Report
Variant Flagged in Shariant
Lab notification by email

Live Chat
Take home messages

• Reclassification is an inherent property of genomic testing.

• Reclassification and discordance is likely to increase with improvements in databases and release of large datasets from population studies.

• Processes & guidelines are being developed to help laboratories manage variant review, reclassification, discordance and notification.

• Imperative that expertise & variant classification evidence is shared – no one group can do it alone.

• A centralised national software solution is in development & testing to determine the feasibility of implementing recommendations.
Acknowledgements

Australian Genomics P1 Variant Reclassification (Hamish Scott) & P2 Variant Curation (Amanda Spurdle) working groups.

Hamish Scott
Nicola Poplawski
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Damien Bruno

Amanda Spurdle
Emma Tudini
David Lawrence
James Andrews

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Cliff Meldrum
Paul James
Bruce Bennett
Edwin Kirk
Julie McGaughran
Anne Ronan
Chris Semsarian
Sebastian Lunke
Zornitza Stark

Australian Genomics
Health Alliance
Thank you.

For more information on Shariant: shariant@australiangenomics.org.au
Abstract

The interpretation of the pathogenicity of a genetic variant is a complex and challenging process wholly dependent on the standards and knowledge available at the time of reporting. Variant interpretations are used to inform treatment regimens, surgical decisions and reproductive choices. Yet interpretations are also inherently subject to change with data from international studies suggesting reclassification rates between 12–53%. Such changes occur due to differences in the implementation of guidelines, inequity in access to supporting evidence, the evolution of curation standards and the emergence of new scientific data. In a recent Australian survey of reclassification by the Australian Genomics Health Alliance Variant Reclassification working group, 22 events were identified on an ad hoc basis by clinical recollection as there is currently no system to monitor or evaluate variants curation. Of these events, 32% were clinically beneficial, assigning clinical significance to a variant that was originally of uncertain significance. A further 59% of the identified variants represented downgrades of likely pathogenic or pathogenic variants, some of which had important clinical implications. This talk will describe the recommendations from the working group for improving the management of the process of reclassification, and also introduce a prototype web-based variant sharing platform ‘Shariant’ that is being developed to assist laboratories to address this enormous challenge.
Current Guidelines & Literature

- National and international guidelines mostly silent on variant review, but some preliminary recommendations for:
  - Consent for re-testing (silent on consent for reanalysis or review)
  - Who is responsible for initiating the review process – primarily the patient
  - Sharing of clinically curated variants – uniformly encouraged
  - Systematic review - most recommendations specifically advise against systematic review, NPAAC position:
    - The curation of an internal laboratory and platform-specific list of common benign variants can assist with the interpretation process. This could aid with the process of systematic review of variant interpretations.

- Little guidance on when variants should be reviewed, or how any changes to the classification should be communicated to patients and other stakeholders.
Recommendations: Curation

Considerations:
- Curation is complex and we’re currently dealing with legacy data
- Identified multiple cases where reclassification may have been avoided e.g. GUS
- The need for reclassification can be reduced by improved curation standards & expert panels
- Data sharing will decrease VUS’ & improve curation standards

Recommendations:
- Clear phenotypic information should be provided at test request. Ideally using a structured ontology.
- Variants should preferably be curated in a setting with gene and/or syndrome specific expertise.
- Information describing the public databases, including the database version, used in curation should be recorded by the laboratory and included in the report.
Recommendations: Review

Considerations:
- Regular systematic review of VUS increases diagnostic yield
- But... review is resource intensive.
- Most Australian laboratories currently reinterpret variants on request.

Recommendation:
It is not feasible to recommend regular systematic review of all variants. Review of variant classifications should occur on:
- Clinician request (if sufficient time has elapsed/new evidence available)
- Variants re-encountered in testing if classification is older than 12 mths
- The laboratory or clinic becoming aware of new information relevant to the classification of the variant

**Blue Sky:** Semi-automated review of VUS’. Variants flagged & prioritised for review.
Recommendations: Notification

Considerations:

• Changes in reported results can seriously impact patients & alter clinical management.
• There is (currently) no way to identify which labs/clinics have reported a variant.

Recommendation:

• The potential for reclassification of variants should be discussed in advance with patients and their preferences for recontact recorded.
• Patients should be encouraged to contact clinics after an appropriate period of time to determine if new information is available.
• The minimum requirement for notification includes:
  • description of information leading to the revision
  • amended reports for all individuals tested by that lab
• It is recommended that an Australia wide variant review and notification system be considered for development to lessen the burden for laboratories and clinical service.