Clinical Validity of Expanded Carrier Screening: Evaluating the Gene-Disease Relationship in more than 200 Conditions

Marie Balzotti
Clinical Genomics Scientist, Myriad Genetics

Presented at ACMG on March 18, 2020
Financial Disclosure

All authors are current or former employees of Myriad Genetics or Baylor Genetics.
Introduction

• The purpose of carrier screening is to determine whether couples are at high risk of having children affected with serious genetic conditions.

• Expanded carrier screening (ECS) is an acceptable testing strategy for pre-pregnancy and prenatal screening.

• Broader guideline support and payer adoption requires evidence of gene-disease association.
Objective

Apply a standardized framework for evaluation of gene-disease association to assess the clinical validity of conditions screened by ECS panels.
Methods

- The Clinical Genome Resource (ClinGen) gene curation framework was used to assess 208 genes and conditions:
  - Twenty-one conditions were previously classified by ClinGen
  - The remaining 187 were evaluated by curation teams at Myriad and Baylor.
- Concordance was evaluated on a subset of conditions.
- Myriad also evaluated nine rare recessive conditions not typically screened for ECS.
Methods
The Gene Curation Process

Gene-disease curation

Curate Genetic Evidence

Curate Experimental Evidence

Curate Contradictory Evidence (if any)

Lab Director Review

ClinGen Expert Panel Review

Manuscript

GenCC

Data available to public

Summarize Evidence and Assign Classification
# Methods

## Evidence types

| Case-Level Data<sup>A</sup> | Evidence Type | Case Information | Suggested Points/Case | Points Given | Max Score |
|---------------------------|---------------|------------------|-----------------------|-------------|-----------|
|                           | Autosomal Dominant OR X-Linked Disorder<sup>B</sup> | Variant is de novo<sup>C</sup> | 2 | 0-3 | 12 |
|                           |                | Proband with predicted or proven null variant<sup>D</sup> | 1.5 | 0-2 | 10 |
|                           |                | Proband with other variant type with some evidence of gene impact<sup>E</sup> | 0.5 | 0-1.5 | 7 |
|                           | Autosomal Recessive | Two variants in trans and at least one de novo<sup>F</sup> or a predicted/proven null variant<sup>G</sup> | 2 | 0-3 | 12 |
|                           |                | Two variants (not predicted/proven null) with some evidence of gene impact<sup>H</sup> in trans | 1 | 0-1.5 | 7 |
|                           | Segregation Evidence | Evidence of segregation in one or more families | LOD Score Examples | 3 | 0-7 | 7 |

| Case-Control Study Type<sup>I</sup> | Case-Control Quality Criteria<sup>J</sup> | Suggested Points/Study | Points Given | Max Score |
|-------------------------------------|------------------------------------------|------------------------|-------------|-----------|
| Single Variant Analysis<sup>K</sup> | - Variant Detection Methodology<sup>L</sup> | 0-6 | 12 |
|                                     | - Power<sup>M</sup>                       |                        |             |           |
| Aggregate Variant Analysis<sup>N</sup> | - Bias and Confounding Factors<sup>O</sup> | 0-6 | 12 |
|                                     | - Statistical Significance<sup>P</sup> |                        |             |           |

**Total Allowable Points for Genetic Evidence** 12

## Evidence Type

| Evidence Category | Evidence Type | Suggested Points/Case | Points Given | Max Score |
|-------------------|---------------|-----------------------|-------------|-----------|
| Function          | Biochemical Function | 0.5 | 0-2 | 2 |
|                   | Protein Interaction | 0.5 | 0-2 | 2 |
|                   | Expression | 0.5 | 0-2 | 2 |
| Functional Alteration | Cells from affected individual | 1 | 0-2 | 2 |
|                   | Engineered cells | 0.5 | 0-1 | 2 |
| Models & Rescue   | Animal model | 2 | 0-4 | 4 |
|                   | Cell culture model system | 1 | 0-2 | 4 |
|                   | Rescue in animal model | 2 | 0-4 | 4 |
|                   | Rescue in engineered equivalent | 1 | 0-2 | 4 |

**Total Allowable Points for Experimental Evidence** 6

---

Strande et. al., AJHG 100(6), 895-906 (2017)
# Methods

## Evidence types

| Evidence Type | Case Information | Suggested Points/Case | Points Given | Max Score |
|---------------|-------------------|-----------------------|--------------|-----------|
| **Autosomal Dominant OR X-Linked Disorder**<sup>A</sup> | Variant is *de novo*<sup>C</sup> | 2 | 0-3 | 12 |
| | Proband with predicted or proven null variant<sup>A</sup> | 1.5 | 0-2 | 10 |
| | Proband with other variant type with some evidence of gene impact<sup>A</sup> | 0.5 | 0-1.5 | 7 |
| **Autosomal Recessive** | Two variants in *trans* and at least one *de novo*<sup>D</sup> or a predicted/proven null variant<sup>D</sup> | 2 | 0-3 | 12 |
| | Two variants (not predicted/proven null) with some evidence of gene impact<sup>E</sup> in *trans* | 1 | 0-1.5 | |
| **Segregation**<sup>F</sup> Evidence | Evidence of segregation in one or more families | LOD Score Examples | 3 2 1.5 1 | 5 4 3 1.5 | 0.7 | 7 |
| **Case-Control Study Type**<sup>H</sup> | Case-Control Quality Criteria<sup>I</sup> | Suggested Points/Study | Points Given | Max Score |
| **Single Variant Analysis**<sup>Ab</sup> | Variant Detection Methodology<sup>a</sup> | 0-6 | | 12 |
| | Power<sup>b</sup> | | | |
| **Aggregate Variant Analysis**<sup>Ab</sup> | Bias and Confounding Factors<sup>c</sup> | 0-6 | | |
| | Statistical Significance<sup>g</sup> | | | |

**TOTAL ALLOWABLE POINTS for Genetic Evidence** 12

| Evidence Category | Evidence Type | Suggested Points | Points Given | Max Score |
|-------------------|---------------|------------------|--------------|-----------|
| **Function** | Biochemical Function | 0.5 | 0-2 | 2 |
| | Protein Interaction | | | |
| | Expression | | 0-2 | |
| **Functional Alteration** | Cells from affected individual | 1 | 0-2 | 2 |
| | Engineered cells | 0.5 | 0-1 | |
| | Animal model | 2 | 0-4 | |
| | Cell culture model system | 1 | 0-2 | |
| | Rescue in animal model | 2 | 0-4 | |
| | Rescue in engineered equivalent | 1 | 0-2 | |

**Total Allowable Points for Experimental Evidence** 6

*Strande et. al., AJHG 100(6), 895-906 (2017)*
# Methods

## Evidence types

| Case-Level Data<sup>a</sup> | Evidence Type | Case Information | Suggested Points/Case | Points Given | Max Score |
|-----------------------------|---------------|------------------|-----------------------|--------------|-----------|
| Autosomal Dominant OR X-Linked Disorder<sup>b</sup> | Variant Evidence | Variant is de novo<sup>c</sup> | 2 | 0-3 | 12 |
| Autosomal Recessive | Proband with predicted or proven null variant<sup>d</sup> | 1.5 | 0-2 | 10 |
| | Proband with other variant type with some evidence of gene impact<sup>e</sup> | 0.5 | 0-1.5 | 7 |
| Segregation Evidence<sup>f</sup> | Two variants in trans and at least one de novo<sup>g</sup> or a predicted/proven null variant<sup>h</sup> | 2 | 0-3 | 12 |
| | Two variants (not predicted/proven null) with some evidence of gene impact<sup>i</sup> in trans | 1 | 0-1.5 | 7 |
| Case-Control Study Type<sup>h</sup> | Evidence of segregation in one or more families | LOD Score Examples | 3 | 5 | 0.7 |
| | 2 | 4 |
| | 1.5 | 3 |
| | 1 | 1.5 |

### Evidence Category

| Evidence Type | Suggested Points/Case | Points Given | Max Score |
|---------------|-----------------------|--------------|-----------|
| Function      | Biochemical Function  | 0.5          | 0-2       | 2         |
|               | Protein Interaction   | 0.5          | 0-2       |
|               | Expression            | 0.5          | 0-1       |
| Functional Alteration | Cells from affected individual | 1 | 0-2 | 2 |
| Models & Rescue | Engineered cells | 0.5 | 0-1 |
|                | Animal model          | 2            | 0-4       |
|                | Cell culture model system | 1 | 0-2 |
|                | Rescue in animal model | 2            | 0-4       |
|                | Rescue in engineered equivalent | 1 | 0-2 |

### Case-Control Data

| Case-Control Quality Criteria<sup>i</sup> | Suggested Points/Study | Points Given | Max Score |
|------------------------------------------|------------------------|--------------|-----------|
| Single Variant Analysis<sup>ja</sup>     | Variant Detection Methodology<sup>ja</sup> | 0-6 | 12 |
| Aggregate Variant Analysis<sup>jb</sup>  | Power<sup>jb</sup>     | 0-6 | 12 |
|                                          | Bias and Confounding Factors<sup>jc</sup> |
|                                          | Statistical Significance<sup>jd</sup> |

TOTAL ALLOWABLE POINTS for Genetic Evidence 12

---

*Strande et. al., AJHG 100(6), 895-906 (2017)*
## Methods

### Evidence types

| Evidence Type                      | Case Information                                           | Suggested Points/Case | Points Given | Max Score |
|------------------------------------|------------------------------------------------------------|-----------------------|-------------|-----------|
| Autosomal Dominant OR X-Linked Disorder | Variant is de novo<sup>a</sup>                             | 2                     | 0-3         | 12        |
|                                     | Proband with predicted or proven null variant<sup>b</sup>  | 1.5                   | 0-2         | 10        |
|                                     | Proband with other variant type with some evidence of gene impact<sup>c</sup> | 0.5                   | 0-1.5       | 7         |
| Autosomal Recessive                | Two variants in trans and at least one de novo<sup>d</sup> or a predicted/proven null variant<sup>e</sup> | 2                     | 0-3         | 12        |
|                                     | Two variants (not predicted/proven null) with some evidence of gene impact<sup>f</sup> in trans | 1                     | 0-1.5       | 7         |
| Segregation Evidence               | Evidence of segregation in one or more families            | LOD Score Examples    |             |           |
|                                     |                                                                | 3                     | 5           |           |
|                                     |                                                                | 2                     | 4           |           |
|                                     |                                                                | 1.5                   | 3           |           |
|                                     |                                                                | 1                     | 1.5         |           |
|                                     | Evidence of segregation in one or more families            | 0.7                   |             |           |
|                                      |                                                               | LOD Score Examples    |             |           |
|                                      |                                                               | 3                     | 5           |           |
|                                      |                                                               | 2                     | 4           |           |
|                                      |                                                               | 1.5                   | 3           |           |
|                                      |                                                               | 1                     | 1.5         |           |

### Evidence Category

| Evidence Type                          | Suggested Points | Points Given | Max Score |
|----------------------------------------|------------------|--------------|-----------|
| Function                               | 0.5              | 0-2          | 2         |
| Protein Interaction                    | 0.5              | 0-2          | 2         |
| Expression                             | 0.5              | 0-2          | 2         |
| Cells from affected individual         | 1                | 0-2          | 2         |
| Engineered cells                       | 0.5              | 0-1          | 2         |
| Animal model                           | 2                | 0-4          | 4         |
| Cell culture model system              | 1                | 0-2          | 2         |
| Rescue in animal model                 | 2                | 0-4          | 4         |
| Rescue in engineered equivalent        | 1                | 0-2          | 2         |

### Case-Control Data

| Case-Control Study Type | Case-Control Quality Criteria | Suggested Points/Study | Points Given | Max Score |
|-------------------------|-------------------------------|------------------------|-------------|-----------|
| Single Variant Analysis | • Variant Detection Methodology<sup>a</sup> | 0-6                    |             | 12        |
|                         | • Power<sup>b</sup>            |                        |             |           |
| Aggregate Variant Analysis | • Bias and Confounding Factors<sup>c</sup> | 0-6                    |             |           |
|                         | • Statistical Significance<sup>d</sup> |                        |             |           |

**TOTAL ALLOWABLE POINTS for Genetic Evidence**: 12

---

Strande et. al., AJHG 100(6), 895-906 (2017)
## Methods

### Evidence types

| Evidence Type | Case Information | Suggested Points/Case | Points Given | Max Score |
|---------------|------------------|-----------------------|--------------|-----------|
| Autosomal Dominant OR X-Linked Disorder | Variant is de novo<sup>C</sup> | 2 | 0-3 | 12 |
| | Proband with predicted or proven null variant<sup>D</sup> | 1.5 | 0-2 | 10 |
| | Proband with other variant type with some evidence of gene impact<sup>F</sup> | 0.5 | 0-1.5 | 7 |
| Autosomal Recessive | Two variants in trans and at least one de novo<sup>G</sup> or a predicted/proven null variant<sup>H</sup> | 2 | 0-3 | 12 |
| | Two variants (not predicted/proven null) with some evidence of gene impact<sup>I</sup> in trans | 1 | 0-1.5 | 7 |
| Segregation Evidence | Evidence of segregation in one or more families LOD Score Examples | 3 | 5 | 0-7 |
| Case-Control Study Type | Case-Control Quality Criteria<sup>J</sup> | Suggested Points/Study | Points Given | Max Score |
| Single Variant Analysis<sup>K</sup> | - Variant Detection Methodology<sup>L</sup> | 0-6 | | 12 |
| | - Power<sup>M</sup> | | | |
| Aggregate Variant Analysis<sup>L</sup> | - Bias and Confounding Factors<sup>L</sup> | 0-8 | | |
| | - Statistical Significance<sup>L</sup> | | | |

**Total Allowable Points for Genetic Evidence:** 12

---

*Strande et. al., AJHG 100(6), 895-906 (2017)*
Methods

Clinical Validity Classifications

- Definitive
- Strong
- Moderate
- Limited
- No reported evidence
- Disputed
- Refuted

Supportive evidence

| Assertion criteria | Genetic Evidence (0-12 points) | Experimental Evidence (0-6 points) | Total Points (0-18) | Replication Over Time (Y/N) |
|--------------------|--------------------------------|-----------------------------------|---------------------|-----------------------------|
| Description        | Case-level, family segregation, or case-control data that support the gene-disease association | Gene-level experimental evidence that support the gene-disease association | Sum of Genetic & Experimental Evidence | > 2 publications with convincing evidence over time (>3 yrs) |
| Assigned Points    |                                  |                                   |                     |                             |

**CALCULATED CLASSIFICATION**

- LIMITED 1-6
- MODERATE 7-11
- STRONG 12-18
- DEFINITIVE 12-18 & Replicated Over Time

List references and describe evidence:

**CURATOR CLASSIFICATION**

**FINAL CLASSIFICATION**

Strande et. al., AJHG 100(6), 895-906 (2017)
An Example:

NEB – Nemaline myopathy

Evidence Type | Case Information | Suggested Default | Range | Points Given | Max Score | PMIDs/Notes
---|---|---|---|---|---|---
Autosomal dominant disease, OR X-linked disease, affected males | Variant is de novo | 2 | 0-3 | 0 | 12 | 12
| Proband with predicted or proven null variant | 1.5 | 0-2 | 0 | 10 | 10
| proband with other variant type with some evidence of gene impact | 0.5 | 0-1.5 | 0 | 7 | 7

Autosomal recessive disease, OR X-linked disease, affected females | Two variants in trans, at least one is LOF or de novo | 2 | 0-3 | 0 | 12 | 12
| Two non-LOF variants in trans | 1 | 0-1.5 | 0 | 7 | 7

Segregation Evidence

| Total LOD Score | Candidate Gene Sequencing | Exon/Gene name or all genes sequenced in linkage region | Total Cases | Points Given | Max Score |
|---|---|---|---|---|---|
| 2-2.99 | 0.5 | 1 | 0-3 | 0 | 3 |
| 3-4.99 | 1 | 2 | 0-3 | 0 | 3 |

Total Genetic Evidence Points (Maximum 12): 12

Case-Control Study Type | Case-Control Quality Criteria | Suggested points/study | Points Given | Max Score |
---|---|---|---|---|
Single Variant Analysis | Variant Detection Methodology | 0-6 | 0 | 12 |
| Power | | | | |
Aggregate Variant Analysis | Bias and Confounding Factors | 0-6 | 0 | 12 |
| Statistical Significance | | | | |

Total Genetic Evidence Points (Maximum 12): 12

Experimental Evidence Summary

| Evidence Category | Evidence Type | Suggested Default | Range | Points Given | Max Score | PMIDs/Notes |
|---|---|---|---|---|---|---|
Function | Biochemical Function | 0.5 | 0-2 | 0 | 2 | 25110572, 15206903, 22941678, 19944167 |
| Protein Interaction | 0.5 | 0-2 | 0.5 | 2 | |
| Expression | 0.5 | 0-2 | 1 | 2 | |
Functional Alteration | Patient Cells | 1 | 0-2 | 1 | 2 | 22159874, 27215641, 16802413 |
| Non-Patient Cells | 0.5 | 0-1 | 0 | 2 | |
Models | Non-human model organism | 2 | 0-4 | 5 | 6 | |
| Cell culture model | 1 | 0-2 | 0 | 2 | |
Rescue | Rescue in human | 2 | 0-4 | 0 | 4 | |
| Rescue in non-human model organism | 2 | 0-4 | 0 | 4 | |
| Rescue in cell culture model | 1 | 0-2 | 0 | 2 | |
| Rescue in Patient Cells | 1 | 0-2 | 0 | 2 | |

Total Experimental Evidence Points (Maximum 6): 6

Strande et. al., AJHG 100(6), 895-906 (2017)
| **Summary Matrix** |
|-------------------|
| **Assertion Criteria** | Genetic Evidence (0-12 points) | Experimental Evidence (0-6 points) | Total Points (0-18) | Replication over time (Y/N) |
| **Description** | Case-level, family segregation, or case-control data that support the gene-disease association | Gene-level experimental evidence that supports the gene-disease association | Sum of Genetic & Experimental Evidence | >2 publications with convincing evidence over time (>3 years) |
| **Assigned Points** | 12 | 6 | 18 | Y |
| **Calculated Classification** | Limited | 1-6 | |
| | Moderate | 7-11 | |
| | Strong | 12-18 | |
| | Definitive | 12-18 AND replication over time | |
| **Valid Contradictory Evidence (Y/N)** | List PMIDs and describe evidence: | |
| **Calculated Curator Classification:** | Definitive | Date: | 10/8/2018 |
| **Comments:** | | | |
| **LD Classification:** | Definitive | Date: | 11/15/18 |
| **Final Expert Classification:** | | Date: | |
| Assertion Criteria | Genetic Evidence (0-12 points) | Experimental Evidence (0-6 points) | Total Points (0-18) | Replication over time (Y/N) |
|-------------------|-------------------------------|-----------------------------------|-------------------|--------------------------|
| Description       | Case-level, family segregation, or case-control data that support the gene-disease association | Gene-level experimental evidence that supports the gene-disease association | Sum of Genetic & Experimental Evidence | >2 publications with convincing evidence over time (>3 years) |
| Assigned Points   | 12                            |                                   | 6                 | 18 Y                     |
| Calculated Classification | Limited | 1-6                            | Moderate         | 7-11                    |
|                    | Strong                         | 12-18                           | Definitive       | 12-18 AND replication over time |
| Valid Contradictory Evidence (Y/N) | List PMIDs and describe evidence: |                                  |                   |                          |
| Calculated Curator Classification: | Definitive | Date: | 10/8/2018 |
| Comments: | | | | |
| LD Classification: | Definitive | Date: | 11/15/18 |
| Final Expert Classification: | | Date: | | |

Strande et. al., AJHG 100(6), 895-906 (2017)
Results

- All 208 evaluated conditions met the evidence threshold for supporting a gene-disease association.
- 203 of 208 (98%) achieved the strongest ('Definitive') level of gene-disease association.
- Rare conditions predominantly showed 'Moderate' evidence.

|                  | Definitive | Strong | Moderate | Limited | No Evidence | Disputed | Refuted | Total |
|------------------|------------|--------|----------|---------|-------------|----------|---------|-------|
| ECS Panel        | 203        | 0      | 4        | 1       | 0           | 0        | 0       | 208   |
| Rare Conditions  | 1          | 2      | 4        | 2       | 0           | 0        | 0       | 9     |
Results

• Conditions evaluated by both commercial laboratories were similarly classified.
Results

- Conditions evaluated by both commercial laboratories were similarly classified.
Results

- Conditions evaluated by both commercial laboratories were similarly classified.
Results

Genetic evidence
- 2 non-LOF variants in *trans* or de novo variant
- 2 variants in *trans*; ≥1 LOF or de novo
- case-control data
- proband w/ variant

Experimental evidence
- Functional data
- Functional alteration
- Models & Rescue

| Gene   | X-linked Severe Combined Immunodeficiency | Hb Beta Chain-Related Hemoglobinopathy | Familial Mediterranean Fever | Joubert Syndrome 2 |
|--------|-----------------------------------------|---------------------------------------|-----------------------------|---------------------|
| IL2RG  | LIMITED                                 | MODERATE                              | STRONG / DEFINITIVE         |                     |
| HBB    | LIMITED                                 | MODERATE                              | STRONG / DEFINITIVE         |                     |
| MEFV   | LIMITED                                 | MODERATE                              | STRONG / DEFINITIVE         |                     |
| TMEM216| LIMITED                                 | MODERATE                              | STRONG / DEFINITIVE         |                     |

# of ECS gene-disease pairs vs Evidence points
Results

‘Limited’ Gene-disease associations

HYLS1 – hydrolethalus syndrome (HLS)

- Borderline between 'Moderate' and 'Limited'
- Conservatively downgraded to 'Limited'
Conclusions

• Strong evidence shown for gene-disease association on two ECS panels.

• Established disease-level clinical validity of these panels.

• Clinical validity of gene-disease association is just one of many factors that influence the selection of conditions included on ECS panels.

• All classifications have been submitted to ClinGen for public availability.
Acknowledgements

- Krista Moyer
- Katie Johansen Taber
- Dale Muzzey
- Jenny Goldstein
- Becca Mar-Heyming
- Bethany Buckley
- Linyan Meng
- Jim Goldberg
- Anna Gardiner
- Myriad and Baylor Curation Teams
References

- Strande, N. T., Riggs, E. R., Buchanan, A. H., Ceyhan-Birsoy, O., DiStefano, M., Dwight, S. S., . . . Berg, J. S. (2017). Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. Am J Hum Genet, 100(6), 895-906. doi:10.1016/j.ajhg.2017.04.015

- The Clinical Genome Resource Gene Curation Working Group. (2017). Gene Clinical Validity Curation Process Standard Operating Procedure, Version 5. Retrieved from https://www.clinicalgenome.org/site/assets/files/2169/gene_curation_sop_2016_version_5_11_6_17.pdf

- The Clinical Genome Resource. Gene Validity Curations. Retrieved from https://www.clinicalgenome.org/site/assets/files/2169/gene_curation_sop_2016_version_5_11_6_17.pdf

- Bean, L. J. H., Funke, B., Carlston, C. M., Gannon, J. L., Kantarci, S., Krock, B. L., . . . Bayrak-Toydemir, P. (n.d.). Diagnostic gene sequencing panels: from design to report - a technical standard of the American College of Medical Genetics and Genomics (ACMG). GENETICS in MEDICINE. https://doi.org/10.1038/s41436-019

- McGlaughon, J. L., Goldstein, J. L., Thaxton, C., Hemphill, S. E., & Berg, J. S. (2018). The progression of the ClinGen gene clinical validity classification over time. Hum Mutat, 39(11), 1494-1504. doi:10.1002/humu.23604