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Two Approaches to Reclassifying Results from Previously Reported Diagnostic Exome Sequencing

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BACKGROUND

- New gene disease relationships are published at an increasing rate and these findings could result in genetic diagnosis for patients with previously negative diagnostic exome sequencing.
- Our diagnostic laboratory has offered clinician request DES reanalysis since February, 2013.
- More recently we began to issue laboratory-Initiated reclassification reports for patients with relevant findings in newly reported gene-disease relationships.
- Overall, 2.6% of DES results have been reclassified.
- Reclassification efforts have increased positive diagnostic rate 1.5% (from 23.7% to 25.3%).

METHODS

- DES was performed as published (Farwell et al, 2015)

59 Reports with Changed Overall Conclusions Were Issued in 2016

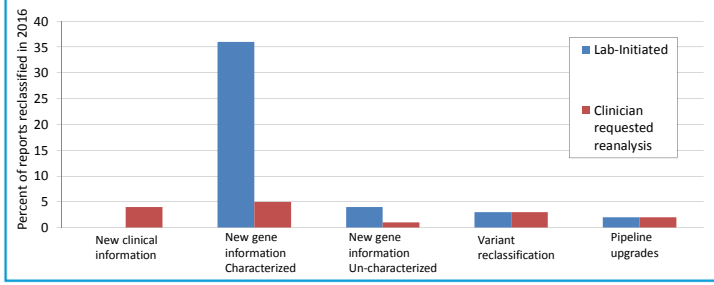
Clinician Requested Reanalysis
13/108= 12%

Overall result change	#
Negative to positive	7
Negative to uncertain	4
Uncertain to negative	2
Candidate to characterized	0

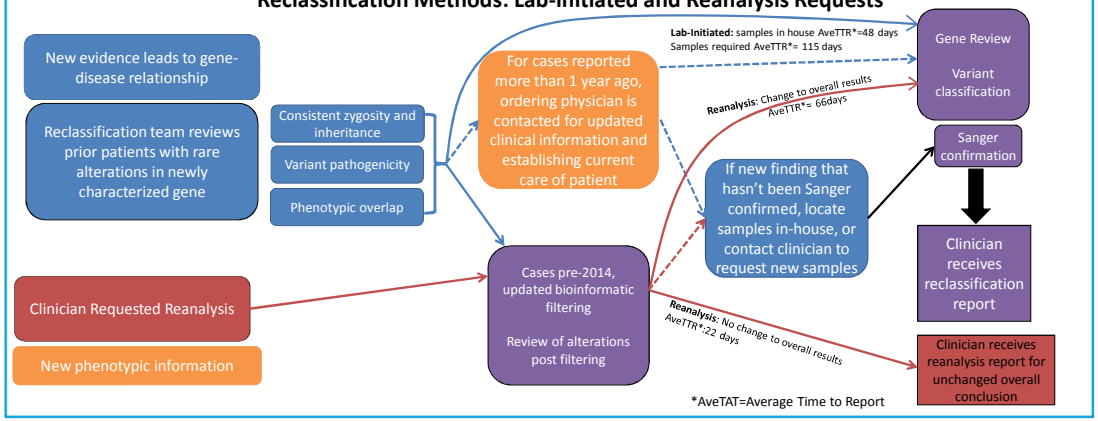
46 Laboratory Initiated

Overall result change	#
Negative to positive	15
Negative to uncertain	15
Uncertain to negative	4
Candidate to characterized	12

Lab-initiated Reclassification Mostly Due to New Gene Information, while Reclassification of Reanalysis Requests Is for Varied Reasons



Reclassification Methods: Lab-initiated and Reanalysis Requests

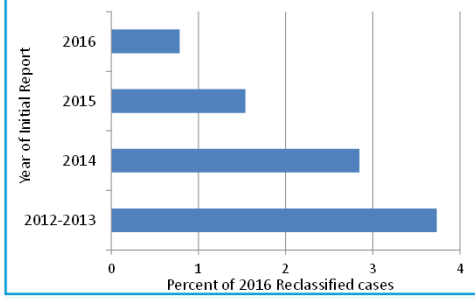


Laboratory-Initiated Reclassification Reports Follow Gene Characterization

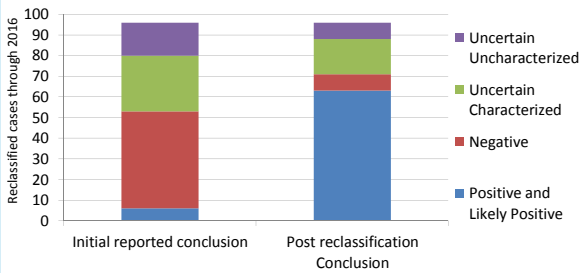
Gene	Associated syndrome/phenotypic findings
TAF6	Cornelia de Lange like syndrome
HECW2	Neurodevelopmental disorder
USP9X	XLD Neurodevelopmental disorder
EMC1	Cerebellar atrophy, visual impairment, and psychomotor retardation
*RERE**	Neurodevelopmental disorder
*GNB1**	Neurodevelopmental disorder
*ZBTB18**	Neurodevelopmental disorder
KAT6A	Neurodevelopmental disorder
PIGT	Multiple congenital abnormalities, hypotonia, and seizures
SAMD9	MIRAGE
*ARV1**	Epileptic encephalopathy
PPP1CB	Neurodevelopmental disorder
ECHS1	Exercise induced paroxysmal attacks without metabolic abnormalities
IARS	Neurodevelopmental disorder with hepatopathy
*SIN3A**	Neurodevelopmental disorder
CREBBP	Neurodevelopmental disorder without typical RTS gestalt
*RORB**	Neurodevelopmental disorder with epilepsy
CHD4	Sifrim-Hitz-Weiss syndrome
*HNRNPH2**	XLD Neurodevelopmental disorder
*TIMM50**	Mitochondrial epileptic encephalopathy
*SON**	Neurodevelopmental disorder
SLC25A4	AD Early-onset mitocondrial disorder
PIK3R1	Immunodeficiency

*Previously reported as candidate gene finding

Cases Reported Earlier have Higher Reclassification Rate



Reclassification Increases Patients with Positive Genetic Diagnoses



Overall, 7.2% of Positive and likely positive reports are due to reclassification

TAKE-HOME POINTS

- Clinician requested reanalysis, especially when patient has developed new clinical symptoms, and laboratory-initiated reclassification based on new literature compliment each other and may offer genetic diagnoses for previously undiagnosed patients.
- Clinician requested reanalysis has 12% change in overall DES results conclusion and cases are reclassified for a variety of reasons including new phenotypic information and change in variant classification.
- Laboratory-initiated reclassifications can provide genetic diagnoses for patients in an unbiased way by evaluating all previously sequenced patients for relevant findings in newly characterized genes.
- Laboratory-initiated reclassifications require the help of current clinicians for patients to receive accurate and timely reports. Laboratory-initiated reclassifications require significant effort locating current clinicians and maintaining HIPPA compliance.

REFERENCES

1. Farwell, K.D. *et al*. (2015) *Genet Med* **17(7)**:578-586

The Benefits and Drawbacks of Two Approaches to Reclassifying Results from Previously Reported Diagnostic Exome Sequencing

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Increased exome and genome sequencing results have led to an increase in publications reporting novel gene-disease relationships. These new publications can provide a genetic diagnosis for patients that had previously received negative diagnostic exome sequencing (DES) results. Here we present the results of one lab's DES reclassification efforts and the benefits and drawbacks of two differing approaches toward reclassification. Overall, 68% of reclassification reports issued are for positive or likely positive findings in a characterized gene, with additional 19% report uncertain findings in a characterized gene. The issuing of reclassification reports increases the overall diagnostic rate from26% to 29% of patients receiving positive or likely positive DES reports.

The traditional approach for a patient to receive a reclassification report is for a clinician to request reanalysis. This method allows for any changes in the patient's symptoms to be considered in addition to consideration of gene-disease relationships that have been recently established. This method also allows for re-interpretation and potential reclassification of variants previously detected and identified on the initial report with the latest population frequency data and literature. This method is very efficient owing to the clinician and patient being prepared for the reanalysis process with the average turn-around-time (TAT) from clinician request to report of 30 days and 70 days for cases with new relevant finding (2016 requests). This method has a low yield of overall changes in conclusion with only 7 of 82 requests resulting in changed conclusions (8.5%, 2016).

A second approach for reclassification is "gene-based" which is prompted by characterization of a gene-disease relationship that previously lacked evidence for clinical reporting. Characterization of gene-disease relationships mainly occurs following publication or due to internal patient data. All rare alterations in newly characterized genes that were detected in previously reported patients are reviewed for consistent inheritance, clinical overlap with reported patients, and pathogenicity of alteration. This method leads to 50proactive reclassification reports issued in 2016. One major challenge of this approach is potential difficulty coordinating with a physician currently treating the patient and obtaining new DNA samples for Sanger confirmation of the NGS finding(s). These complications are evidenced by an average 40 day TAT (n=30) for cases not requiring additional DNA compared to 111 days (n=9) for cases requiring additional sample with 8 cases pending for an average of 124 days, with an additional 3 cases reported without Sanger confirmation due to extenuating circumstances (2016 cases). This process requires that we evaluate the patient's phenotype based on clinical information provided when DES was ordered and follow-up with the clinician for updated information can be time-intensive.

These results confirm that considering both approaches for DES reclassification is ideal. Clinician submitted reanalysis requests are best in situations in which the patient's phenotype has changed over time and when questions remain regarding previously identified variants. Gene-based reclassification can report newly identified genetic diagnoses for patients who otherwise would still be seeking diagnosis. Diagnosis can provide possible new treatments, end a diagnostic odyssey for the family, and allow clinicians of patients with rare diseases to collaborate.

Differentiate between case-based and gene-based reclassification approaches

Select patients who would benefit from clinician requested DES reanalysis

Understand that DES reclassification can occur even years after DES test was completed