# Multiple Molecular Diagnoses Identified through Diagnostic Exome Sequencing

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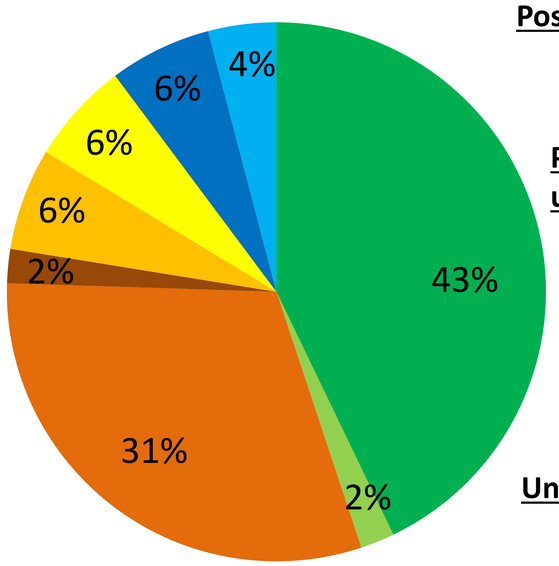
## BACKGROUND

- Diagnostic exome sequencing is often indicated when patients have symptoms of genetic disease but do not fit the criteria for any one specific syndrome or condition.
- Here we present a retrospective analysis of patients who have reportable variants (positive, likely positive, and uncertain) in 2 or 3 genes within our institution's first 880 cases.
- We reported variants in multiple genes in 49 patients (5.6% of total exomes analyzed, 12.9% of positive and uncertain exomes). Of those 3 patients had reportable findings in 3 genes (0.3% total, 0.8% positive/uncertain exomes).

## METHODS

- **Patients/study population:** The first 750 consecutive probands referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES) are included in this study. Informed consent was obtained from all family members involved in the testing process.
- **Whole exome sequencing and analysis:** Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) or SeqCap EZ VCRome 2.0 (Roche NimbleGen, Madison, WI). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA). DNA isolation, sequencing, data analysis, and interpretation were performed as previously described (Farwell et al. 2014).
- **Evaluation of the Molecular Basis of Disease (EMBoDy):** From the start of DES testing in Oct, 2011, we extensively evaluated variants in genes with novel genetic etoilogies in proband-family trios based on critical and highly stringent assessments at both the gene and variant(s) level. Please see **Poster # 651** for details on criteria used for analysis and interpretation of novel genetic etoilogy findings.
- Medical Findings and Organ System involvement was determined based on clinician reports of the patient's phenotype.
- Contribution of individual genes to patient's symptoms was determined from contribution of mutations in characterized genes causing similar phenotypes as those reported in literature, HGMD, and OMIM and, in cases of novel genes, hypothesized symptoms based on animal studies, microdeletion patients, and gene function. In cases in which the novel gene's contribution was unable to be hypothesized, then contribution is unknown. We cannot rule-out oligogenic affects of alterations in multiple genes leading to a unpredictable clinical phenotypes.
- Origin of alterations are only reported for patients in which both parents were available for co-segregation analysis. Consanguinity was self-reported to clinicians.
- **Statistical analyses** were computed by chi$^2$ goodness of fit tests and Fisher's Exact Probability.

## CLASSIFICATION OF FINDINGS IN MULTIPLE DIAGNOSIS CASES




**Positive findings in 2 or more genes**

- 2 Characterized genes (n=21)
- 2 Novel genetic etiology findings (n=1)

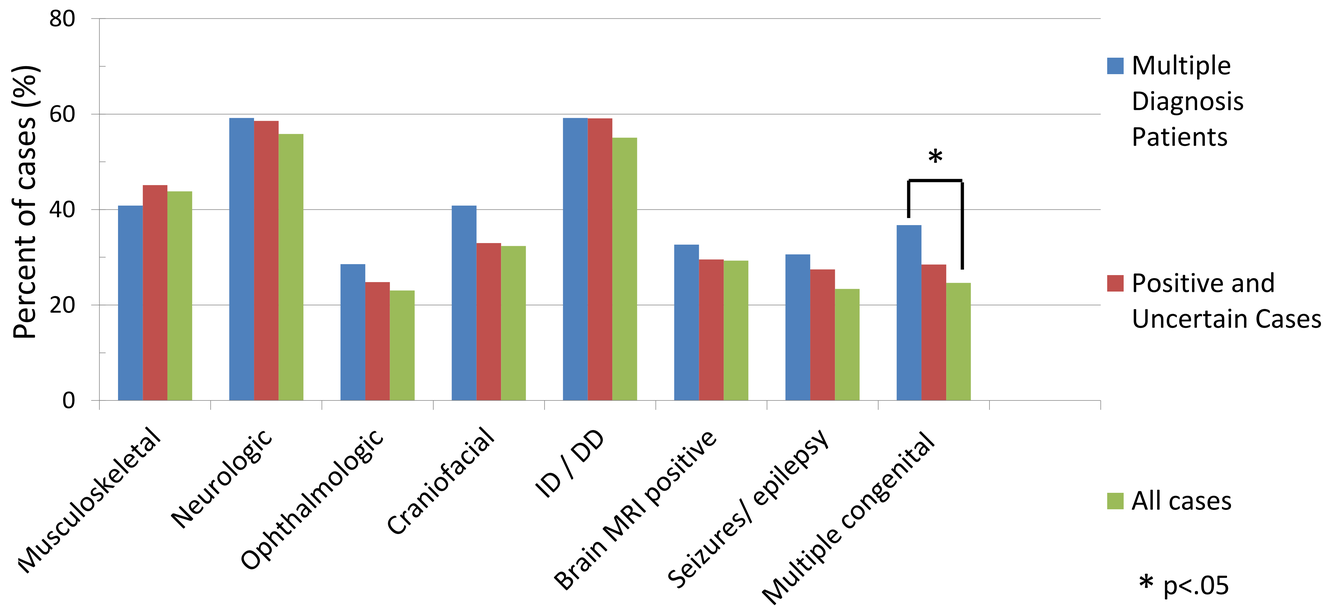
**Positive finding in one gene, uncertain finding additional genes**

- Positive Characterized, Uncertain Characterized (n=15)
- Positive Characterized, Uncertain Novel (n=1)
- Positive Novel, Uncertain Characterized (n=3)
- Positive Novel, Uncertain Novel (n=3)

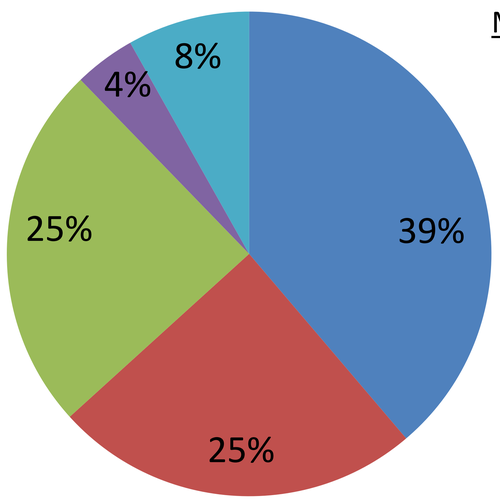
**Uncertain findings in 2 or more genes**

- 2 Characterized genes (n=3)
- 2 Novel findings (n=2)

## MEDICAL FINDINGS AND ORGAN SYSTEM INVOLVEMENT



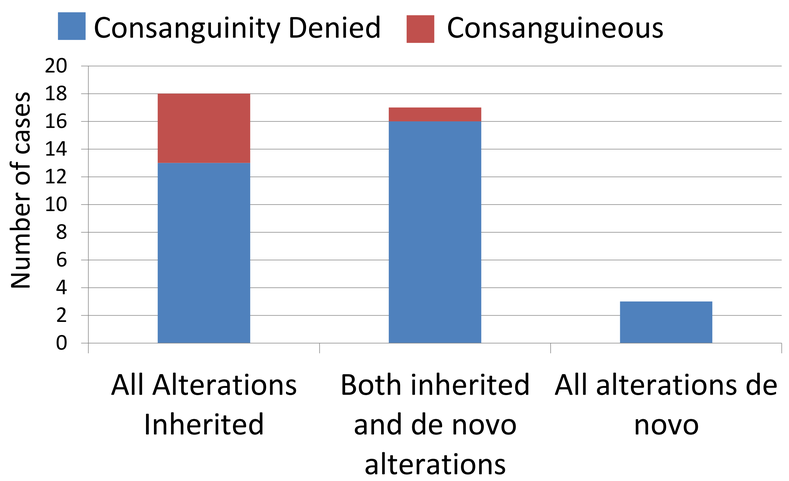

## CONTRIBUTION OF INDIVIDUAL GENES TO PATIENT'S SYMPTOMS




Multiple genes contribute to:

- Same symptoms (n=19)
- Unique and overlapping symptoms (n=12)
- Different symptoms (n=12)
- Proposed digenic (n=2)
- Unknown (n=4)

## BOTH INHERITED AND *DE NOVO* ALTERATIONS CONTRIBUTE TO MULTIPLE MOLECULAR DIAGNOSIS




Includes only cases in which inheritance can be determined

## TAKE-HOME POINTS

- Diagnostic exome sequencing has ability to diagnose complex medical conditions that result from mutations in two or more genes.
- 45% of multiple molecular diagnoses reported positive findings in 2 or more genes. An additional 45% reported positive findings in one gene and uncertain findings in additional genes .
- When compared to the 880 total patients tested, the patients who had reported variants in two or more genes have similar medical findings and organ system involvement but were significantly more likely to have multiple congenital abnormalities (36.7% compared to 24.6%, p<0.05).
- 39% of genes reported in multiple molecular diagnoses cases contribute to the same symptoms, while 50% of genes contribute to differing symptoms in patients' presentations.

## REFERENCES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