A Retrospective Analysis of Diagnostic Exome Sequencing Results Among Neonatal Patients

Layla Shahmirzadi[1], Kelly Gonzalez[1], Tehila Ness[1], Stephanie Gandomi[1], Dima El-Khechen[1], Zöe Powis[1], Christina Alamillo[1], Kelly Radtke[1], Deepali Shinde[1], Christian Gund[1], Vruti Mehta[1], Marilyn Tsang[1], Katherine Helbig[1], Ira Lu[1], Cameron Mroske[1], Ruth Baxter[1], Brigette Tippin[1], Elizabeth C. Chao[1,2], Sha Tang[1]

[1]Ambry Genetics, Aliso Viejo, CA, 92656

[2]Department of Pediatrics, University of California, Irvine, Irvine, CA 92697

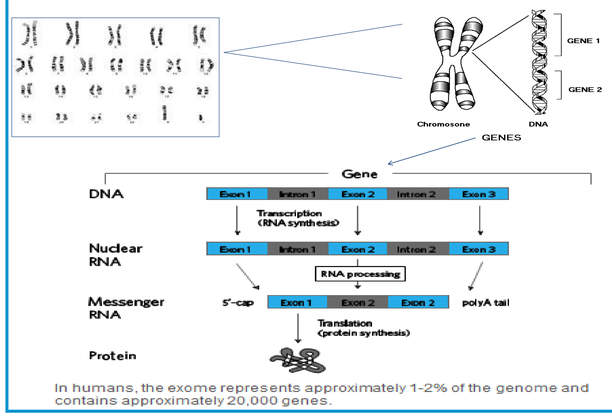
BACKGROUND

- Exome sequencing is the sequencing and analysis of the protein coding regions of the genome **(Figure 1).**
- Since 2011, diagnostic exome sequencing (DES) has been instrumental in establishing a molecular diagnosis in patients with previously undiagnosed disease, enhancing genetic counseling and aiding in clinical management.
- DES has been used in combination with or after inconclusive genetic testing strategies, such as karyotype, single gene testing and microarray, to help establish a diagnosis among a wide range of ages and indications.
- The aim of this study was to observe the application of DES in a neonatal population.
- "Neonatal" is defined in this study as any patient presenting with symptoms prenatally or within the first 4 weeks of life and whose sample was submitted for diagnostic exome sequencing at 4 months of age or less.

METHODS

- **Patients/study population:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from probands and relatives referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES). Informed consent was obtained from all family members involved in the testing process.
- **Whole exome sequencing:** 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).
- **Characterized and Disease-causing (ChaD) and novel gene databases**: The ChAD gene database was curated on a weekly basis to include genes currently known to be responsible for causing human disease. The ChAD database included genes which are associated with syndromes listed in the Human Gene Mutation Database (HGMD) (Stenson, 2009) and the Online Mendelian Inheritance in Man (OMIM) database. Novel genes were defined as those not known to underlie a Mendelian condition at the time of data analysis. Any RefSeq gene not included in the ChAD database was included in the novel gene database.
- **Bioinformatics annotation, filtering of variants, and Family history Inheritance-based Detection (FIND):** HGMD, OMIM, the Single Nucleotide Polymorphism database (dbSNP) (Sherry, 2001), 1000 Genomes, HapMap data (International HapMap, 2003) and online search engines (e.g., PubMed) were used to search for previously described gene mutations and polymorphisms. Stepwise filtering included the removal of common SNPs, intergenic and 3'/5' UTR variants, non-splice-related intronic variants, and lastly synonymous variants. Variants were then filtered further based on family history and possible inheritance models using the informatics program "FIND" (Family history Inheritance-based Detection).
- **Personalized Medical Review with Enhanced and Comprehensive Assessment (PRECISE) of potentially causal variants:** Each candidate mutation was assessed by a molecular geneticist to identify the most likely causative mutation(s) using the "PRECISE" (Personalized Medical Review with Enhanced and Comprehensive Assessment) analysis method. In brief, interpretive filtering was based on the deleterious nature of the candidate alterations, literature search, and analysis of the relevance of the candidate genes' function in relation to the patient's phenotype. Most candidate alterations undergo Sanger sequencing confirmation and familial co-segregation analysis.
- **Data curation:** Data regarding the primary exome test ordered, patient age, diagnosis and/or clinical description, and exome sequencing results were also curated. Data on results included gene(s), alteration(s), gene category (novel, characterized), alteration interpretation (pathogenic, likely pathogenic, uncertain, likely benign), and clinical overlap of gene-association and patient phenotype (positive, uncertain, partial).
- **Statistical analyses** were computed by chi^2 goodness of fit tests and Fisher's Exact Probability.

FIGURE 1. DIAGNOSTIC EXOME SEQUENCING



In humans, the exome represents approximately 1-2% of the genome and contains approximately 20,000 genes.

FIGURE 2. PATIENT POPULATION

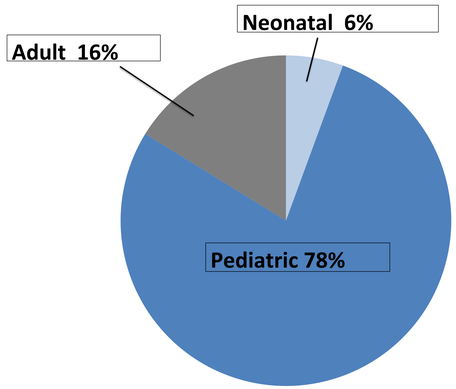


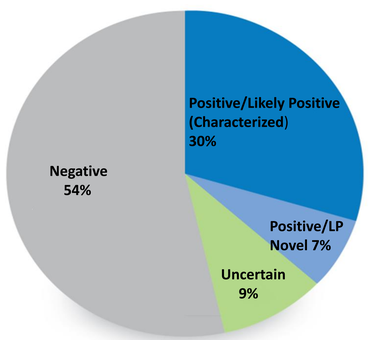
FIGURE 3. COMMON INDICATIONS FOR TESTING

Note: Majority of patients presented with clinical features involving 1+ body system.

RESULTS

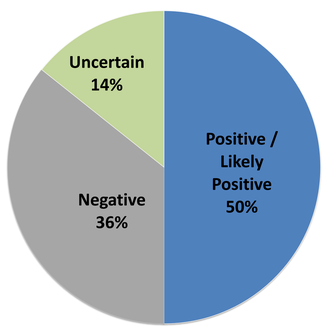
- A retrospective analysis of the first 500 patients submitted for DES at one laboratory revealed that the majority of cases were pediatric patients under the age of 18 (78%) **(Figure 2).**
- 28 of the 500 cases (6%) included patients from the neonatal period, including 2 cases of fetal demise.
- Among this neonatal cohort, the most common clinical indications for testing (observed in >15% of patients) among this neonatal cohort included musculoskeletal/structural symptoms (50%), while the most common indication for testing overall involved neurologic findings (65%) **(Figure 3).**
- Among patients for whom a positive finding was identified, the most common indication for testing was a musculoskeletal/structural involvement (57%).
- Of the patients with negative DES results, 50% presented with musculoskeletal/structural or pulmonary involvement.
- In patients with uncertain findings the most common indications for testing included craniofacial, gastrointestinal, or neurologic, where 75% of the patients had at least one of these findings among other features.
- Overall, detection rates were higher among the NICU patients (50%) than among all patients undergoing exome sequencing (37%) **(Figures 4 & 5)**.

FIGURE 4. OVERALL DETECTION RATES



N=500

FIGURE 5. NICU DETECTION RATES



N=28

TAKE-HOME POINTS

- When compared to previously reported diagnostic rates among unselected patients of all ages undergoing whole exome sequencing, neonatal patients had up to 35% higher detection rates.
- This data suggests that detection rates may not be predictable based on indication for testing, as musculoskeletal/structural findings were the most common indication among both positive and negative DES results.
- These findings indicate an increased detection of Mendelian disease among neonatal patients, likely correlating with congenital or early-onset severe phenotypes
- This data highlights the power of DES, particularly during the neonatal period, to help provide a diagnosis for patients with complex or nonspecific findings. Additionally, we suggest the use of this data to help guide clinical management and eliminate unnecessary treatments and testing in the early neonatal period.

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15 Argonaut, Aliso Viejo, CA 92656 Toll Free 866 262 7943 Fax 949 900 5501 ambrygen.com