Over the last three years, the application of whole exome sequencing in a clinical diagnostic setting (DES) has transformed the diagnosis and management of patients with genetic disease.

DES, for the first time in molecular diagnosis, simultaneously interrogates virtually all genes, including those most recently discovered, as well as those that are related to and outside of the clinician's differential diagnoses.

With DES at the cutting edge of clinical and research sciences, novel genetic etiology (gene-disease association) discovery has also been an important component to enhance clinical sensitivity upon in-depth assessment of gene function and critical variant classifications.

**BACKGROUND**

DES yielded a positive finding in characterized genes in 227 of 750 consecutive cases.

**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 Version 2.0 (Roche). Whole exome libraries were sequenced using paired-end, 1-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).

**Novel gene analysis:** From analysis of DES testing in October 2011, we extensively evaluated variants in novel genetic etiology 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 gene findings.

**Proactive re-analysis:** We maintain an internal database of characterized genes, which we update on a weekly basis with the latest literature. When a new gene is added to the database, we review and perform clinical correlations on all previous cases that had rare variants detected within the gene (Farwell et al. 2014).

**ASSUMED INHERITANCE PATTERN COULD BE MISLEADING**

- **Diagnostic Yield:** 30% (20/67) families with a reported consanguaneous family history received a definite diagnosis in characterized genes.
- **Unexpected:** 25% (5/20) positive findings are unrelated to the generally assumed autosomal recessive inheritance pattern. These five cases are associated with autosomal dominant or X-linked de novo alterations.

**Germline Mosaicism:** Three of the 227 positive cases (1%) indicated a gonadal mosaic origin of mutations (Tuzovic et al., 2015; Kulkarni et al., 2015).

**TAKEN HOME POINTS**

- DES has paved its way and been proven to be a cost-effective tool to provide prompt (average 8-week turnaround time for ExomeNext™) and definitive answers for at least 32% of patients in our cohort who had evaded a diagnosis through extensive clinical evaluation and molecular testing.
- DES, in the form of family-centered trio sequencing, is well-suited to be considered as standard care early in the diagnosis of rare genetic disorders.
- Such an unbiased and assumption-free approach also enables unprecedented opportunities for novel gene discovery, oligogenic inheritance studies, and a finer delineation of genotype-phenotype correlations.

**REFERENCES**