Introduction

Whole exome sequencing (WES) is a tool that is increasingly used in the clinical setting for the diagnosis of genetic disorders.

As technology and bioinformatic pipelines improve and new disease genes are published, it is essential to continuously re-evaluate previously generated WES data.

Reanalysis of WES data may be initiated by the clinical genetics provider as the patient’s phenotype changes and new features emerge, or by the laboratory as new disease genes are published or discovered through ongoing re-evaluation of accumulated data.

346 WES cases were reanalyzed at an average of 14 months (+/- 5 months) after initial WES report issued.

Methods

Exome sequencing was performed on exon targets isolated by capture using the Agilent SureSelect Human All Exon V4 (50 Mb) or Clinical Research Exome kit.

The sequencing methodology and variant interpretation protocol has been previously described (Tanaka et al., 2015).

WES data for all sequenced family members was reanalyzed using a custom-developed, proprietary bioinformatics tool (XomeAnalyzer) for variant annotation, filtering, viewing of WES data, and variant evaluation.

Variants were filtered based on inheritance patterns, variant type, custom-developed gene lists of interest, phenotype, and allele frequency in the general population, following a standardized analysis checklist. Resources including the HGMD, 1000 Genomes database, NHLBI GO Exome Sequencing Project (ESP), OMIM, PubMed and ClinVar were used to evaluate genes and detected sequence changes of interest.

Identified sequence changes of interest were confirmed in all members of the family on whom biospecimens were available by di-deoxy DNA sequence analysis.

The primary results were classified into the following categories.

- **Category 1** (definitive result): Pathogenic or likely pathogenic variant(s) in a known disease gene associated with the reported phenotype provided for the proband.
- **Category 2** (possible/probable diagnosis): Variant(s) in a known disease gene possibly associated with the reported phenotype. This category includes novel variants, including missense variants or in-frame insertions/deletions in disease genes, which overlap the phenotype provided for the proband. This category also includes single pathogenic or likely pathogenic variants identified in a gene associated with an autosomal recessive disorder consistent or overlapping with the provided phenotype.
- **Category 3** (novel candidate gene): Variant(s) predicted to be deleterious in a novel candidate gene not previously implicated in human disease, or with an uncertain pathogenic role, in the presence of additional supporting data. Such data may include animal models, copy number variant data, tolerance of the gene to sequence variation, tissue or developmental timing of expression or knowledge of the gene function and pathway analysis. Further research is required to evaluate and confirm any of the suggested candidate genes.
- **Category 4** (negative result): No variants in genes associated with the reported phenotype were identified.

Reanalysis of WES did not reveal any new findings in half of all cases (n=171). A definitive answer was identified in 11.2% (n=39), a finding related to a possible/probable diagnosis was identified in 16.3% (n=56) and a new candidate was reported in 23.1% (n=80).

Reanalysis with a Definitive Answer

64.1% (25/39) of reanalysis cases were initiated by the referring providers, 35.9% (14/39) were recommended by the laboratory.

Determining factors leading to a definitive result:

- Newly available clinical and/or genetic evidence allowed an upgrade of previously reported variants (25.6%, n=10)
- Identification of new disease genes published after initial report (23.1%, n=9)
- Disease gene identified by ongoing research studies (7.7%, n=3)
- Improvements to laboratory bioinformatics pipeline and analysis workflow (10.2%, n=4)
Results Cont.

Reanalysis with a New Candidate Gene Finding
- 47.5% (38/80) stem from continuous analysis and systematic mining of the laboratory's internal WES data
- 16 new candidate genes identified in collaboration with clinicians and researchers (see references)
- Since 2012, 39/939 novel candidate genes reported by our laboratory have since been published as disease-causing genes (4.2%), impacting 236/6214 total WES cases (3.8%)

Conclusions
Progressive improvements in laboratory WES data analysis and data mining, identification of new disease genes, newly available clinical information and expanding collaborations between laboratory, clinicians and researchers are the main factors contributing to new insights by reanalysis of WES data in 34% of cases. Therefore, WES reanalysis can be expected to benefit a growing number of patients with previous WES testing, and is an important consideration for both the clinical team as part of routine medical care of patients who have had WES, as well as for the laboratory as a contributor in the discovery and characterization of new gene-phenotype correlations.

Reference