Exome Sequencing Identifies New Disease Phenotypes in Characterized Genes

Kelly D. Farwell Hagman[1], Kelly Radtke[1], Erica Smith[1], Sha Tang[1], Zöe Powis[1], Dima El-Khechen[1], Katherine Helbig[1], Kendra Webb[1], Cameron Mroske[1], Mari Rossi[1], Wendy Alcaraz[1], Samin A. Sajan[1], Jesse M. Hunter[1], and Deepali N. Shinde[1]

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

BACKGROUND

- Major benefits of diagnostic exome sequencing (DES):
  - *An unbiased and assumption-free approach*
  - *Simultaneous interrogation of virtually all genes both characterized and uncharacterized disease genes*
  - *Analysis of genes that are both related to and outside of the clinician's differential diagnoses*
  - *Opportunities for:*
    - *novel candidate gene discovery*
    - *finer delineation of genotype-phenotype correlations*
    - *new disease discovery among already characterized genes.*
- Overall, DES is successful in ending the diagnostic odyssey for 30% of undiagnosed patients with underlying Mendelian disorders in characterized genes and novel candidate gene-disease relationships are identified among ~8% of patients in whom uncharacterized genes are analyzed.
- DES has expanded phenotypes within genes with well-established disease associations and in a subset of cases. ***These broader phenotypes are distinct enough from the already-established diseases to be considered novel gene-disease associations.***

METHODS

- **Whole exome sequencing and data analysis:** Exome sequencing, bioinformatics pipeline and filtering, data analysis and results categories (positive, likely positive, uncertain, candidate gene, and negative) are as previously published (Farwell *et al*, 2015 and 2017).
- **Data analysis:** We retrospectively analyzed 128 patient results with novel candidate disease reports to determine what proportion of the findings were novel disease phenotypes in an already characterized gene.
- **Novel Candidate Criteria:** A novel candidate disease gene was reported based on an internally developed and recently published candidate gene scoring criteria (Farwell Hagman, 2017). Briefly, the criteria evaluates evidence including relevant human microdeletion/duplication syndrome, gene function/expression profiles, co-localization/interaction with gene products known to cause similar presentations, *in vivo* animal models, gene family/pathway information, and the possible mutational mechanism inferred from the distribution of variants in control populations.
- **Statistical analyses:** Fisher's Exact Test was utilized to compute statistical significance.

FIGURE 1: Proportion of Novel Candidate Phenotypes Among All Novel Candidate Findings

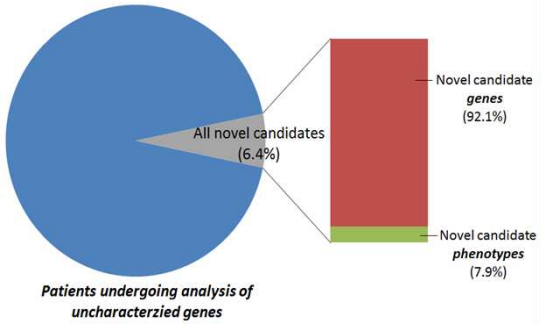

TABLE 1: Description of Novel Phenotypes in Characterized Genes

Gene	Established Associated Syndrome	Novel Phenotype	Possible Molecular Explanation for Novel Phenotype
ACTG2	Visceral myopathy	Berdon syndrome	New phenotype, same proposed mechanism of haploinsufficiency. Syndromes have overlapping features. ACTG2 is abundantly expressed in the stomach, intestine, colon, aorta, and bladder (Sun, 2009) and plays an important role in bladder smooth muscle formation in mice embryogenesis (Shiroyanagi, 2007).
BCL11B	Severe combined immunodeficiency (SCID)	Neurodevelopmental and craniofacial symptoms	New mutational mechanism - dominant negative for SCID, haploinsufficiency for novel. Knockout mice exhibit defective axon guidance during development of the corticospinal tract (Arlotta, 2005). Heterozygous null mice exhibit craniofacial defects possibly due to downregulation of *BCL11B* that causes an upregulation of *RUNX2* (Kytylkova, 2016).
DLL4	Adams-Oliver Syndrome	Alagille-like symptoms	New phenotype, same proposed mechanism of haploinsufficiency. Syndromes have some overlapping features. *DLL4* is an essential component of the Notch signaling pathway and mutations in two other genes in this pathway, JAG1 and NOTCH2 have been shown to cause Alagille syndrome (OMIM: 118450; and OMIM 610205).
KATNAL2	AD Autism	AR Developmental delay	New mutational mechanism - AD with reduced penetrance for autism, proposed AR loss of function - neither parent with heterozygous loss of function alterations was affected.
KCNA2	Epilepsy	Spastic paraplegia	New phenotype, proposed mechanism for epilepsy is gain-of-function whereas the observed alteration causes a dominant negative loss of channel function (Helbig, 2016).
KMT5B	Autism	Developmental delay	New inheritance pattern with more severe phenotype: Biallelic.
MYH6	Congenital heart defects	Hypoplastic left heart syndrome	New inheritance pattern with more severe phenotype: Biallelic.
NTF4	Glaucoma	Mitochondrial dysfunction	Mitochondrial membrane localization was found for TrkB isoforms in both brain and skeletal muscles with co-localization of NT-4 and cytochrome c oxidase (Wiedemann, 2006).
PDGFRB	Basal ganglia calcification	Overgrowth	New mutational mechanism. Loss-of-function alterations cause BGC (Sanchex-Conteras, 2014), whereas gain-of-function alterations have been shown to cause overgrowth (Takenouchi, 2015).
TBX6	AD Spondilocostal dysostoses	AR Spondilocostal dysostoses	New inheritance pattern with more severe phenotype: Biallelic.

Results

- Among 3388 patients undergoing DES, uncharacterized genes were analyzed in 1979 patients and we reported a novel candidate or suspected novel candidate gene-disease in 126 (6.4%) patients using our candidate gene scoring criteria (Farwell Hagman, 2017).
- Among these 126 candidate gene-disease reports, 10 (7.9%) described novel phenotypes and/or molecular mechanisms within characterized genes (**Figure 1**).
- New phenotypes were proposed for the *ACTG2*, *BCL11B*, *DLL4, KATNAL2, KCNA2, KMT5B, NTF4*, and *PDGFRB* genes (**Table 1**).
- For four genes, *KATNAL2*, *KCNA2, PGGFRB* and *BCL11B*, the mutational mechanism was different from the mutational mechanisms associated with the previously-described syndromes, offering a likely explanation for the new disease phenotypes (**Table 1**).
  - Additionally, the following *TBX6, MYH6*, and *KMT5B* genes showed a new inheritance pattern (AR vs AD) and a more severe phenotype.

TAKE-HOME POINTS

- Patients undergoing DES have a 6.4% and 0.5% likelihood of receiving a novel candidate or a novel candidate phenotype result, respectively.
- These data highlight the continual evolvement of our understanding of the molecular mechanisms of disease, even among already characterized genes.
- Further, the data emphasize the utility of DES in providing a comprehensive molecular diagnosis matched by no other clinical test available.
- These data have significant implications for genetic counseling and clinical management and may inspire potential diagnostic, preventive, and therapeutic opportunities.

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1. Ambry Genetics, Aliso Viejo, CA, 92656

A major benefit of diagnostic exome sequencing (DES) is the simultaneous interrogation of virtually all genes, both characterized and uncharacterized as well as genes that are both related to and outside of the clinician's differential diagnoses. This unbiased and assumption-free approach enables opportunities for novel candidate gene discovery, finer delineation of genotype-phenotype correlations, as well as new disease discovery among already characterized genes. Overall, DES is successful in ending the diagnostic odyssey for 30% of undiagnosed patients with underlying Mendelian disorders in characterized genes. Additionally, a novel candidate gene-disease relationship is identified among ~8% of patients in whom uncharacterized genes are analyzed. DES has expanded phenotypes within genes with well-established disease associations and in a subset of cases these broader phenotypes are distinct enough from the already-established diseases to be considered novel gene-disease associations.

Among 3388 DES patients, we analyzed uncharacterized genes in 1979 patients and reported a candidate or suspected candidate gene-disease in 128 (6.5%) patients using an internally developed and recently published candidate gene scoring criteria. Briefly, the criteria evaluates evidence including relevant human microdeletion/duplication syndrome, gene function/expression profiles, co-localization/interaction with gene products known to cause similar presentations, *in vivo* animal models, gene family/pathway information, and the possible mutational mechanism inferred from the distribution of variants in control populations. Among these 126 candidate gene-disease reports, 10 (8%) described novel phenotypes and/or molecular mechanisms within characterized genes. New phenotypes were proposed for the *ACTG2*, *BCL11B*, *DLL4, KATNAL2, KCNA2, KMT5B, NTF4*, and *PDGFRB* genes, which are characterized for visceral myopathy, severe combined immunodeficiency (SCID), Adams-Oliver Syndrome, autism, epilepsy, autism, glaucoma, and basal ganglia calcification, respectively. The new disease phenotypes were Berdon syndrome, neurodevelopmental symptoms, Alagille-like symptoms, developmental delay, spastic paraplegia, developmental delay, mitochondrial dysfunction, and overgrowth, respectively. For three of the five genes, *KATNAL2*, *KCNA2* and *BCL11B*, the mutational mechanism was different from the mutational mechanisms associated with the previously-described syndromes, offering a likely explanation for the new new disease phenotypes. Additionally, we propose a different molecular mechanism for two genes: *TBX6* (characterized as autosomal dominant; we propose autosomal recessive inheritance) and *MYH6* (characterized as autosomal dominant; we propose autosomal recessive inheritance and a more severe phenotype).

These data highlight the utility of DES in providing a comprehensive molecular diagnosis matched by no other clinical test available. These data have significant implications for genetic counseling and clinical management and may inspire potential diagnostic, preventive, and therapeutic opportunities.