S Tang[1], KD Gonzalez[1], W Zeng[1], L Shahmirzadi[1], BT Davis[1], S Nahas[1], JJ Wei[1], X Li[1], EC Chao[1, 2]
1) Ambry Genetics, Aliso Viejo, CA, 92656; 2) Department of Pediatrics, University of California, Irvine, Irvine, CA 92697

Utilization of Diagnostic Exome Sequencing for the Molecular Diagnosis of Mitochondrial Disorders and Therapeutic Implications

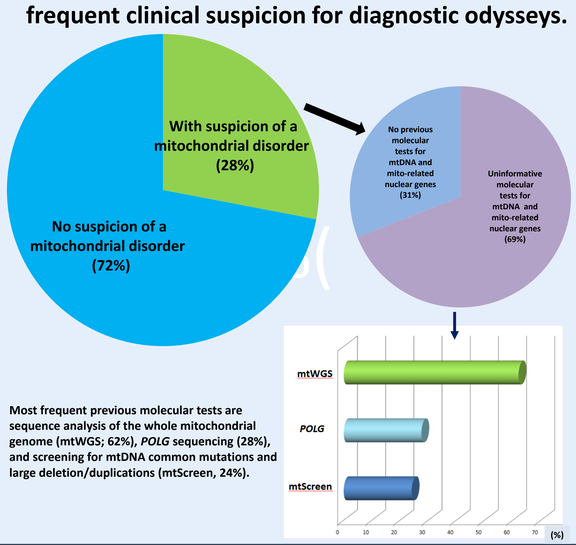
BACKGROUND

- The last two years have demonstrated the clinical utility of Diagnostic Exome Sequencing (DES) in establishing a molecular diagnosis, enhancing genetic counseling, and aiding in clinical managements.
- The diagnosis of mitochondrial disorders is challenging due to clinical variability and genetic heterogeneity. The searching for causative genetic variants requires interrogation of both the mitochondrial (mtDNA) and nuclear genomes.
- For mtDNA mutation analysis, NextGen-based testing is undoubtedly the method of choice (Wong, 2013). However, it is still unclear whether a targeted gene panel or whole exome sequencing is more time/cost-effective in analyzing suspected mitochondrial disease in patients without recognizable syndromes.

METHODS

- Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from patient samples provided to the laboratory for DES. Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) and sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).
- Data is annotated with the Ambry Variant Analyzer tool (AVA), including nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, predicted functional impact, and clinical disease associations (Human Gene Mutation Database, OMIM, and several other databases). A molecular geneticist performed interpretive filtering 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. Each candidate variant was analyzed by Sanger sequencing for mutation confirmation and co-segregation analysis.
- Positive findings were curated into a database along with patient referral indications and clinical/test history. A retrospective study was carried out to identify cases with a suspicion of mitochondrial disorders among the first 150 unselected cases submitted for DES and categorize the positive findings.

Figure 1. Mitochondrial disorders represent a frequent clinical suspicion for diagnostic odysseys.



Most frequent previous molecular tests are sequence analysis of the whole mitochondrial genome (mtWGS; 62%), *POLG* sequencing (28%), and screening for mtDNA common mutations and large deletion/duplications (mtScreen, 24%).

Figure 2. DES provided a definitive molecular diagnosis in 33% of patients with a suspicion of a mitochondrial disorder.

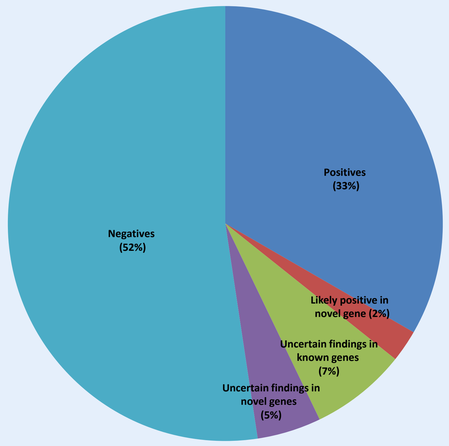


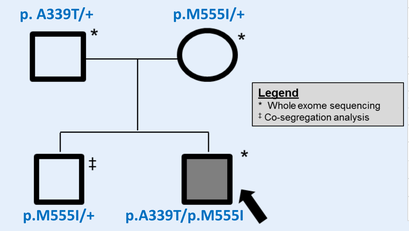
Table 1. The 14 positive cases in DES patients with a clinical or differential diagnosis of mitochondrial disease.
Six cases are due to primary mitochondrial cytopathy while the other eight are associated with disorders with overlapping clinical features.

Case	Primary mito?		Age	Previous mito-related Molecular Tests	Genetic Model	*Gene*	Diagnosis based on DES findings	Mutant Allele 1	Mutant Allele 2
1	YES	F	0.5 yr	None	AR	*ACAT1*	Beta-ketothiolase deficiency	p.D253N	p.D253N
2	YES	F	2 yr	mtDNA depletion panel	AR	*SLC52A2*	Brown-Vialetto-Van Laere syndrome	p.S128L	p.L339P
3	YES	M	10 yr	mtWGS, mitoNuclear 448-gene panel	AR	*ADCK3*	Coenzyme Q10 deficiency	p.A339T	p.M555I
4	YES	F	14 yr	None	AR	*NUBPL*	Mitochondrial Complex I deficiency	p.L104P	c.815-27T>C
5	YES	F	1 yr	None	AR	*C20orf7*	Mitochondrial Complex I Deficiency	p.K52T	p.M279R
6	YES	M	1 yr	None	AR	*RARS2*	Pontocerebellar hypoplasia, type 6	p.M1?	p.F140C
7	NO	M	5 yr	mtWGS, mitoNuclear 448-gene panel	AD	*SHANK3*	Autism Spectrum Disorder	**p.A1243GfsX69 (*de novo*)**	
8	NO	F	3 yr	None	AR	*PANK2*	PKAN	p.G521R	p.G521R
9	NO	M	4 yr	mtScreen	XLR	*IQSEC2*	Mental Retardation, X-linked 18	**p.C684X (*de novo*)**	
10	NO	F	5 yr	mtWGS	AR	*TPP1*	Ceroid lipofuscinosis, neuronal, 2	p.Q422H	c.509-1G>C
11	NO	M	2 yr	mtWGS	AD	*ANKRD11*	KBG Syndrome	**p.Q2609X (*de novo*)**	
12	NO	M	13 yr	mtWGS and mtDNA del/dup	AD	*PRNP*	Prion Disease	**p.P105L (*de novo*)**	
13	NO	M	2 yr	None	AD	*STXBP1*	Early infantile epileptic encephalopathy-4	**p.R292L (*de novo*)**	
14	NO	F	26 yr	None	XLD	*MTM1*	Myotubular myopathy, X-linked	**p.P453YfsX4 (*de novo*)**	

Abbreviations: **mito (mitochondrial), mtWGS (whole mitochondrial genome sequencing analysis, mtScreen (screening for common mtDNA point mutations and large deletion/duplications), AR (autosomal recessive), AD (autosomal dominat), XLR (X-linked recessive), XLD (X-linked dominant).**

CASE EXAMPLES: The molecular diagnosis made by DES enables more efficient targeted therapy in patients with mitochondrial disorders

Case Example 1: *ADCK3*

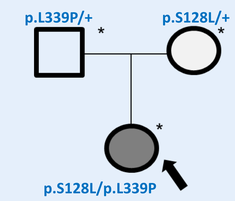


NUMBER OF GENES & ALTERATIONS IDENTIFIED BASED ON BIOINFORMATICS & INTERPRETATION						
	Post-Inheritance Model Filtering	Post-Medical Review*				Notable Candidate Genes**
		Post-alteration review	Post-clinical association review			
		TOTAL	Characterized‡	Clinically novel‡	TOTAL	
Autosomal Dominant Genes (Alterations)	16(17)	9(9)	0(0)	8(8)	8(8)	0(0)
Autosomal Recessive Genes (Alterations)	17(42)	13(27)	1(2)	9(17)	10(19)	1(2)
X-linked Recessive Genes (Alterations)	3(3)	3(3)	0(0)	2(2)	2(2)	0(0)
X-linked Dominant Genes (Alterations)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Y-linked Genes (Alterations)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
TOTAL GENES (Alterations)	37(64)	25(40)	1(2)	19(27)	20(29)	1(2)

*Post-medical review filtering involves the manual removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign. Please inquire with the laboratory for more information about post-medical review variant filtered genes and alterations. ‡Characterized Genes: Genes known to be associated with a clinical phenotype based on the HGMD or OMIM-Morbid databases or the medical literature; A clinically novel gene is a gene which is not currently known to underlie a genetic condition (the alterations within these genes are located in parenthases). **Notable Candidate Genes: Gene alterations selected for co-segregation.

- Proband is a 10-yr boy with developmental delay, hypotonia, encephalopathy, cerebellar atrophy, ataxic gait, and abnormal reflexes. Differential diagnoses were Neiman-Pick Type C, Mitochondrial disorder, and Joubert Syndrome. He had extensive uninformative molecular tests including CMA/SNP array, fragile X, X-linked intellectual disability panel, mitoNuclear gene panel, SCA panel, mtWGS, and sequencing analysis of *NPC1*, *NPC2*, *CEP290*, *TMEM67*, and *POLG*.
- DES performed on the proband-parents trio prioritized two heterozygous alterations, c.1015G>A (p.A339T) and c.1665G>A (p.M555I), in the *ADCK3* gene. Both changes have been confirmed by Sanger sequencing and co-segregation analysis indicated that the healthy brother is a carrier for the p.M555I allele. The c.1665G>A (p.M555I) change was actually picked up by the mitoNuclear gene panel test but c.1015G>A (p.A339T) was missed.
- **DES made a diagnosis of Coenzyme Q10 deficiency (MIM_607426) in this patient and high-dose CoQ10 administration may be beneficial** (DiMauro *et al*., 2007).

Case Example 2: *SLC52A2 (GPR172A)*



NUMBER OF GENES & ALTERATIONS IDENTIFIED BASED ON BIOINFORMATICS & INTERPRETATION						
	Post-Inheritance Model Filtering	Post-Medical Review*				Notable Candidate Genes**
		Post-alteration review	Post-clinical association review			
		TOTAL	Characterized‡	Clinically novel‡	TOTAL	
Autosomal Dominant Genes (Alterations)	15(16)	8(8)	0(0)	6(6)	6(6)	0(0)
Autosomal Recessive Genes (Alterations)	21(46)	17(33)	2(4)	13(26)	15(30)	1(2)
X-linked Recessive Genes (Alterations)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
X-linked Dominant Genes (Alterations)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Y-linked Genes (Alterations)						
TOTAL GENES (Alterations)	36(62)	25(41)	2(4)	19(32)	21(36)	1(2)

*Post-medical review filtering involves the manual removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign. Please inquire with the laboratory for more information about post-medical review variant filtered genes and alterations. ‡Characterized Genes: Genes known to be associated with a clinical phenotype based on the HGMD or OMIM-Morbid databases or the medical literature; A clinically novel gene is a gene which is not currently known to underlie a genetic condition (the alterations within these genes are located in parenthases). **Notable Candidate Genes: Gene alterations selected for co-segregation.

- Proband is a 2.5-yr girl with developmental delay, hypotonia, bilateral optic atrophy, fine nystagmus, wasting in upper extremities, progressive muscle weakness, tachycardia, regression of milestones, peripheral polyneuropathy, and mitochondrial complex III deficiency.
- DES performed on the proband-parents trio prioritized two heterozygous alterations, c.383C>T (p.S128L) and c.1016T>C (p.L339P), in the *SLC52A2* gene. *SLC52A2* is a most recently identified disease-causing locus (Bosch *et al*., 2012; Haack *et al*., 2012) and for which there is no current single gene or panel test clinically available.
- **DES made a diagnosis of Brown-Vialetto-Van Laere Syndrome (MIM_614707) with riboflavin transporter dysfunction in this patient and high-dose riboflavin supplements may be beneficial** (Bosch *et al*., 2011).

RESULTS/ DISCUSSION

- Mitochondrial disorder represents a frequent clinical suspicion for diagnostic odysseys. In the first 150 probands for DES, 42 (28%) had a clinical or differential diagnosis of mitochondrial disease according to the clinical notes provided (**Figure 1**). However, among these 42 patients, only two (5%) had Electron Transport Chain (ETC) deficiency meeting the modified Walker's criteria. A majority of patients (29, 69%) with a suspected mitochondrial disorder had previous uninformative results including analysis of the mtDNA or mitochondrial disease-related nuclear gene(s); these molecular tests varied from single gene tests to large gene panels targeting more than 400 genes.
- DES identified the causative mutations in 14 families (33%), including biallelic mutations in *ACAT1*, *SLC52A2*, *ADCK3*, *PANK2*, *NUBPL*, *C20orf7*, *TPP1*, and *RARS2*, as well as *de novo* mutations in *SHANK3*, *IQSEC2*, *ANKRD11*, *PRNP*, *STXBP1*, and *MTM1* (**Figure 2** and **Table 1**). In addition, variations in "novel' genes not currently known to underlie Mendelian conditions but with mouse model/pathway information implicating their role in the etiology have been interpreted as "likely positive" in 1 family and "uncertain" in 2 families (**Figure 2**).
- Among the 14 positive cases with mutations identified in known genes, 6 can be considered as primary mitochondrial disorders and all of them are associated with inherited biallelic mutations. The remaining 8 positives were caused by mutations in genes presenting with overlapping clinical features to mitochondrial defects. A majority of the mutations in the latter are *de novo* changes (6/8, 75%) (**Table 1**).
- DES picked up a heterozygous mutation missed in a previous gene panel test. This mutation was confirmed by Sanger sequencing (**Case example 1**).
- DES could be the sole possible clinical approach to pin down the causative mutations in newly identified disease-causing loci (**Case example 2**).
- The molecular diagnosis made through DES enables more efficient targeted therapy for mitochondrial disorders. For example, supplementation of high-dose CoQ10 and riboflavin may benefit the patients with coenzyme Q10 deficiency caused by *ADCK3* mutations (**Case example 1**) and riboflavin transporter dysfunction due to SLC52A2 defects (**Case example 2**), respectively.

CONCLUSIONS

- Mitochondrial disorders are clinically and genetically heterogenous and represent a frequent clinical suspicion for diagnostic odysseys.
- Our experience highlights the clinical utility of DES as an efficient and comprehensive scheme for the diagnosis of mitochondrial disorders caused by nuclear gene mutations.
- These data demonstrate the clinical utility of DES for mitochondrial disease where traditional testing was uninformative, likely due to the phenotypic overlaps of mitochondrial dysfunction and various neuromuscular disorders. Differential diagnosis genes should be included in mitoNuclear panel for a higher diagnosis successful rate.
- More than half of the patients with a suspicion of mitochondrial disease remain undiagnosed, accentuating the great potential and continuing challenges in the molecular diagnosis of mitochondrial diseases. Mutations in non-coding regions, epigenetic changes, oligenic inheritance, and yet-to-be-discovered novel genes may contribute to the phenotypes in patients with a negative results for Mendelian disorder based on whole exome sequencing.

References

- Bosch AM, *et al.* (2011) *J Inherit Metab Dis* **34**:156-164.
- Bosch AM, *et al.* (2012) *Orphanet J Rare Dis* **7**:83.
- DiMauro S, *et al.* (2007) *J Clin Invest* **117**:587-589.
- Haack TB, *et al.* (2012) *J Inherit Metab Dis* **35**:943-948.
- Wong L. (2013) Mitochondrion Mar 6. [Epub ahead of print]