Diagnostic Exome Sequencing: Diagnostic Yield, Novel Gene Discovery, Expected and Unexpected Results

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BACKGROUND

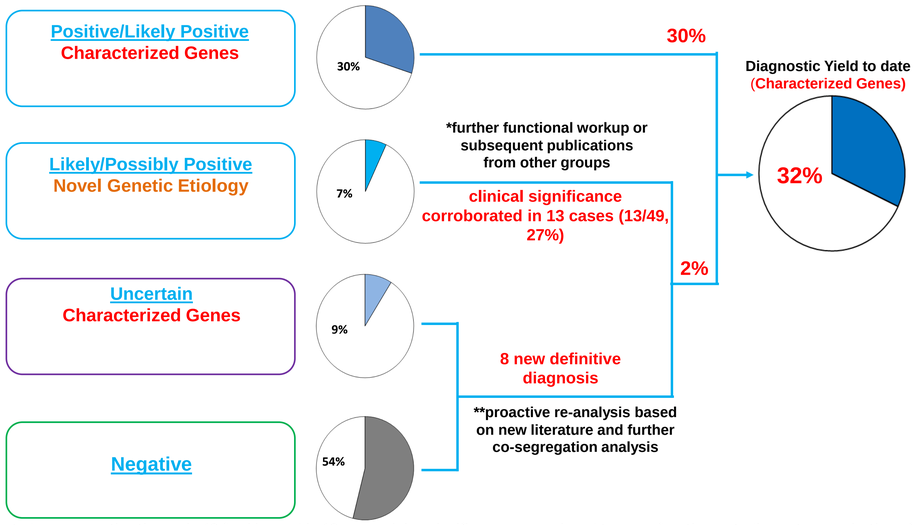
- Over the last three years, the application of whole exome sequencing in a clinical diagnostic setting (DES) has transformed the diagnosis and management of patients with genetic disease.
- DES, for the first time in molecular diagnosis, simultaneously interrogates virtually all genes, including those most recently discovered, as well as genes that are both 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 classification.

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 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). DNA isolation, sequencing, data analysis, and interpretation were performed as previously described (Farwell *et al.* 2014).
- **Novel gene analysis:** From the start 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).

DIAGNOSTIC YIELD AND NOVEL GENE RESULTS

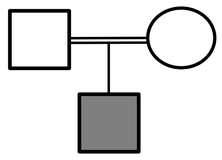
DES yielded a positive finding in characterized genes in 227 of 750 consecutive cases.



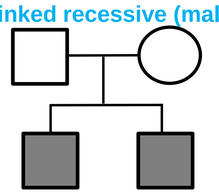
*We reported novel genetic etiology in 49 families and in 13 cases clinical significance was assigned through functional studies or the publication of new pertinent information. Novel genetic etiologies with confirmed clinical significance in the probands include *ACTG2* (3 cases), *PURA* (2 cases), *ZBTB20*, *DNMT3A*, *ETV6*, *MTOR*, and *DNM1* (Poster #529). In addition, novel genes published from our cohort are *SNAP25 (*Rohena *et al.,* 2013), *MYH10* (Tuzovic *et al.,* 2013), and *LAS1L* (Butterfield *et al.,* 2014). **Proactive re-analysis based on new publications and further familial co-segregation analysis provided a definitive molecular diagnosis for 8 more cases: *GNAO1* (2 cases), *TUBB4A*, *MED13L*, *ARID1B*, *POGZ*, and *PDGFRB*.

ASSUMED INHERITANCE PATTERN COULD BE MISLEADING

Consanguinity: higher diagnostic yield with homozygous mutations due to identity-by-descent?



Multiple affected children of same or opposite genders: Autosomal or X-linked recessive (males) conditions?



- **Diagnostic Yield**: 30% (20/67) families with a reported consanguineous 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).

DUAL/TRIPLE DIAGNOSIS: 8% OF THE POSITIVE FINDINGS

Multiple significant molecular etiologies contributing to the complete clinical picture – importance of considering synergistic effects in profound/atypical phenotypes.

Patient	Inheritance	Gene	Disease	Origin of mutations	Alteration(s)	Classification
1	AR	*SLC27A4*	Ichthyosis prematurity syndrome	Inherited	c.556+2T>G p.G592R	Positive
	AD	*FLG*	Ichthyosis vulgaris	not known	p.S2080*	Likely positive
2	AD	*GNAO1*	Epileptic encephalopathy, early infantile, 17	*de novo*	p.E246K	Positive
	XLR	*ATP2B3*	Spinocerebellar ataxia, X-linked 1	inherited	p.T1113M	Likely positive
3	XLR	*SMS*	Mental retardation, X-linked, Snyder-Robinson type	inherited	p.G56S	Positive
	XLR	*AFF2*	X-linked mental retardation	inherited	p.G482A	Likely positive
4	AD	*ANK2*	Autism spectrum disorder (Loss of function)	*de novo*	p.D2894Afs*20	Positive
	AD	*PKD1*	Polycystic kidney disease,	inherited[a]	p.N3188del	Positive
5	AR	*NALCN*	Neuroaxonal neurodegeneration with facial dysmophism	inherited	c.4197+1G>A p.R735*	Positive
	AD	*ANO3*	Dystonia 24	*de novo*	p.I308L	Likely positive
6	AD	*ARID1A*	Mental retardation, autosomal dominant 14/Coffin-Siris syndrome	*de novo*	p.E2078K	Positive
	AR	*ORC1*	Meier-Gorlin syndrome 1	inherited	c.721+1G>C c.2392-1G>T	Uncertain[b]
7	AR	*POLG*	DNA polymerase gamma mitochondrial deficiency	Likely inherited	p.G737R p.R597W	Positive
	AD	*DNA2*	Progressive external ophthalmoplegia with mt DNA deletions	not known	pF603S	Uncertain
8	AR	*SPAST*	Spastic paraplegia 4	Likely inherited	p.S545*	Positive
	AD	*GABRA1*	Epilepsy	not known	p.I389V	Uncertain
9	AD	*HNRNPU*	HNRNPU-related epileptic encephalopathy	*de novo*	p.E236Tfs*6	Likely positive
	XLR	*SMC1A*	Cornelia de Lange syndrome 2	inherited	p.F1193C	Likely positive
10	AD	*FOXG1*	Rett syndrome, congenital variant	*de novo*	p.P259R	Positive
	AD	*KAT6B*	SBBYSS syndrome	*de novo*	p.E1089Rfs*3	Positive
11	AR[c]	*CACNA1A*	Spinocerebellar ataxia 6	inherited[c]	p.E1015K p.E1015K	Likely positive
	AD	*TGM6*	Spinocerebellar ataxia 35	inherited[a]	p.S39C	Likely positive
12	AR	*CLU7*	3-M Syndrome	inherited	p.R1573* p.L1014R	Positive
	AR	*ACAN*	Spondyloepimetaphyseal dysplasia	inherited	p.T985A p.A2105T	Uncertain
13	AR	*SBF2*	Charcot-Marie-Tooth disease, type 4B2	inherited	p.V1286A p.H1201R	Uncertain
	AD	*SMARC4*	Mental retardation, autosomal dominant 16	*de novo*	p.L783P	Positive
14	AD	*LDLR*	Familial hypercholesterolemia	inherited[a]	c.190+4A>T	Positive
	AD	*RYR2*	Arrhythmogenic right ventricular dysplasia 2	inherited[a]	p.V3219M	Likely Positive
15	AR	*ETFDH*	Glutaric acidemia IIC	inherited	p.L377P p.L377P	Positive
	AR	*HEXA*	Tay-Sachs disease	inherited	p.R499H p.R499H	Likely Positive
16	AD	*JPH2*	JPH2-related hypertrophic cardiomyopathy	*de novo*	p.R231Q	Positive
	AD	*MYBPC3*	MYBP3-related hypertrophic cardiomyopathy	inherited[a]	c.3330+2T>G	Positive
17	XLD	*WDR45*	Neurodegeneration with brain iron accumulation 5	*de novo*	p.R234*	Positive
	AD	*NOTCH1*	Congenital heart defect	inherited[a]	p.C1018Afs*16 1	Likely Positive
18	AD	*ANKRD11*	KBG syndrome	*de novo*	p.H542Afs*34	Positive
	AR	*GAA*	Glycogen storage disease II	inherited	c.-32-13T>G p.P285R	Positive
	XLR	*MAOA*	Brunner syndrome	inherited	p.D46G	Likely Positive
19 (Poster #634)	AD	*SCN8A*	Cognitive impairment with or without cerebellar ataxia	*de novo*	p.R1620L	Positive
	AR	*CYP21A2*	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency	inherited	p.I173N p.V282L	Positive
	AD	*PTEN*	PTEN-related macrocephaly/autism syndrome	inherited	p.P354Q	Uncertain

a - Inherited from an affected parent; b - Compound heterozygosity for canonical splice donor site and splice acceptor site alterations detected; c- Generally inherited in an autosomal dominant manner, proband here is homozygous.

POSITIVE CASES WITH UNEXPECTED GERMLINE MOSAICISM

Family	Clinical Information	Multiple affected	Gene	Alteration	Trio Sequenced
1	Megacystis and echogenic bowel	Proband (Male) Sister (Female)	*ACTG2*	p.R257H	Proband Father Mother
2	Severe movement disorder and hypotonia	Proband (Male) Brother (Male)	*GNAO1*	p.R209H	Proband Affected brother Father
3	Macrocephaly, autism spectrum disorder, and hypotonia	Proband (Male) Brother (Male)	*MTOR*	p.E1799K	Proband Affected brother Mother

TAKE-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.

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