Diagnostic Exome Sequencing with Inheritance Model-Based Analysis: Results from a Cohort of 81 Probands Referred with Cardiac Indications as Compared to the Group of 500 Unselected Families with Undiagnosed Genetic Conditions

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BACKGROUND

- Cardiac defects are a broad class of issues that can be both congenital or acquired as well as structural or rhythmic.
- Defining the type of abnormality can be challenging as defects may have a variety of etiologies and present in a multitude of ways.
- Expanded use of next generation sequencing (NGS) has permitted transition of sequential analysis to simultaneous testing of disease-specific genes.
- Although detection rates of multi-gene panels are improved over single-gene testing methods, there remain many cases of strong familial cardiovascular disease yielding inconclusive or negative genetic testing results.
- Diagnostic exome sequencing (DES) serves as a potential next step in cases of undiagnosed or familial cardiovascular disease.
- DES is one of the most comprehensive tests available for identifying an underlying genetic cause of a patient or family's medical condition in a time-effective and cost-effective manner.
- DES has been shown to have definite utility in cases of undiagnosed genetic conditions. This paper presents data from 81 referrals with a cardiac indication, as compared to the entire population of 500 unselected referrals.

METHODS

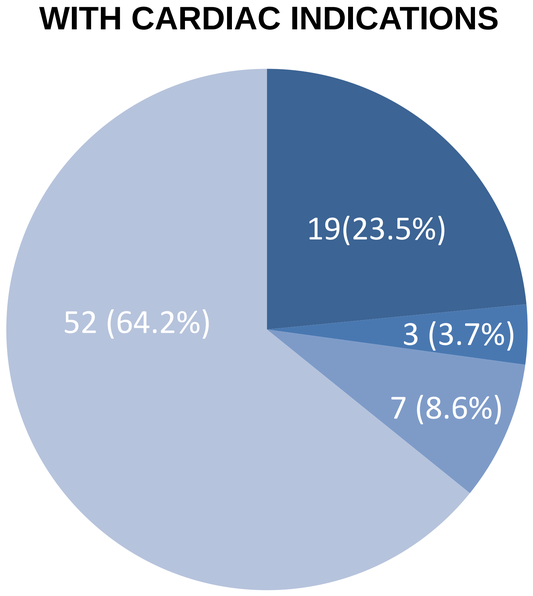
- **Patients/study population**: Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from probands and relatives referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES). Informed consent was obtained from all family members involved in the testing process.
- Exome library preparation, sequencing, bioinformatics, and data analysis were performed as previously described (Farwell et al. 2014)
- **Patient demographics** (age, gender, referral indication) were collected from required testing documents supplied to the laboratory with the requisition form and the biospecimens. Patient identifiers were removed.
- **Data curation** included the primary exome test option ordered, patient age, diagnosis and/or clinical description, and exome sequencing results, including gene(s), alteration(s), gene category (novel, characterized), alteration interpretation (pathogenic, likely pathogenic, uncertain, likely benign), and clinical overlap of gene-association and patient phenotype (positive, uncertain, partial).

POSITIVE FINDINGS: CASES REFERRED WITH A CARDIAC INDICATON

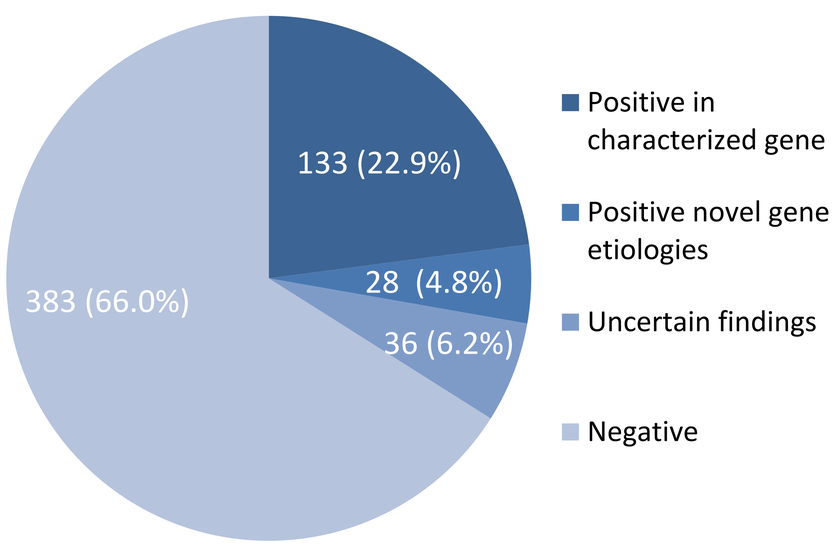
Gene	Mutation	Associated Clinical Syndrome(s)	Inheritance Pattern
CACNA1C	p.I66T	Timothy Syndrome	AD, de novo
CHD7	p.F1722Sfs*15	CHARGE Syndrome	AD, de novo
TUBB4A	p.R262H	Hypomyelinating Leukodystrophy 6	AD, de novo
***SLC52A2* (*GPR172A*)**	p.S218L & p.L339P	Brown-Vialetto-Van Laere Syndrome	AR, inherited
CAV1	p.F160*	Lipodystrophy, congenital generalized, type 3	AD, de novo
PACS1	p.R203W	Mental retardation, autosomal dominant 17	AD, de novo
***COL6A3* (*COL3A1*)**	c.2114_2121+4del12insCT	Ehlers-Danlos Syndrome, type IV	AD, de novo
***MLL* (*KMT2A*)**	p.H753fs*11	Wiedemann-Steiner Syndrome	AD, de novo
MLL2	p.3695Lfs*54	Kabuki Syndrome	AD, de novo
CHD8	p.C944Yfs*3	Autism	AD, de novo
NPHP3	p.D640E & p.E145Vfs*3	Nephronophthisis Syndrome	AD, inherited
SATB2	p.A328D	Cleft palate and mental retardation	AD, de novo
NTRK1	c.851-33T>A	Congenital Insensitivity to Pain with Anhidrosis (CIPA)	AR, unknown
SRI	c.206-1G>A	Cardiomyopathy, hypertrophic	AD, inherited
CASK	p.Q824R	Mental retardation and microcephaly with pontine and cerebellar hypoplasia	XLR, inherited
ATRX	p.N2475	Alpha-thalassemia/mental retardation syndrome, X-linked	XLR, inherited
SMC1A	p.I1185Gfs*23	Cornelia de Lange syndrome 2, CDLS2	XLD, de novo
***HNRNPU* & SMC1A**	p.E236Tfs*6	HNRNPU-related epileptic encephalopathy	AD, de novo
HNRNPU* & *SMC1A	p.F1193C	Cornelia de Lange syndrome 2, CDLS2	XLR, inherited
ITGA11*	p.S1148I	Novel genetic etiology	Unknown
EMILIN1*	p.A22T	Novel genetic etiology	Complex (XLR or XLD)
LAMA1*	p.E236Tfs*6	Novel genetic etiology	AD, de novo

DIAGNOSTIC EXOME SEQUENCING RESULTS

WITH CARDIAC INDICATIONS



WITHOUT CARDIAC INDICATIONS



CLINICAL SPECIFICS OF CARDIAC REFERRALS*

CLINICAL SPECIFICS	CARDIAC REFERRALS WITH POS/NOVEL RESULT (total = 22)	CARDIAC REFERRALS W/ NEG/UNC RESULT (total= 59)
Intellectual disability and/or developmental delay	14 (63.6%)	29 (49.2%)
Brain MRI positive	5 (22.7%)	9 (15.3%)
Multiple congenital anomalies	10 (45.5%)	27 (45.8%)
Seizures/epilepsy	3 (13.6%)	9 (15.3%)
Progressive phenotype	1 (4.5%)	9 (15.3%)
Ataxia	1 (4.5%)	2 (3.4%)
Autism spectrum disorder	1 (4.5%)	5 (8.5%)
Psychiatric	1 (4.5%)	11 (18.6%)
Healthy	4 (18.2%)	9 (15.3%)

- 68/81 (84.0%) of referrals with cardiac indication had at least one additional clinical specific finding
- Within a group of 22 referrals with a positive/likely positive or novel finding, intellectual disability (14 individuals) +/- developmental delay and multiple congenital anomalies (10 individuals) were the most common additional clinical specifics.
- Within the group of cardiac referrals with a negative or uncertain result (59 individuals), 29 had a clinical specific of intellectual disability and/or developmental delay and 27 individuals had a clinical specific of multiple congenital anomalies.

* Some individuals had multiple clinical specifics for referral.

GENES POSITIVE IN CARDIAC VERSUS NON-CARDIAC CASES

Inheritance	# Genes Positive Cardiac Indications	# Genes Positive Non-cardiac Indications
Autosomal Dominant	14 (66.6%)	72 (50.7%)
Autosomal Recessive	3 (4.3%)	48 (33.8%)
X-linked	4 (19.0%)	22(15.5%)

- Among the 19 positive/likely positive cases, a total of 21 molecular defects were identified in 21 unique genes, including 14 AD (66.6%), 3 AR (14.3%), and 4 (19%) X-linked conditions.
- The % of AD mutations identified in patients with cardiac indications is not significantly different than the 50.7% found in patients without cardiac indications (72/142) (p=0.1713).
- De novo alterations accounted for approximately half of all the identified gene defects: at least 47.6% (10/21) and up to 61.9% (13/21) when including likely de novo (2 cases) and possible de novo (1 case) results. This is not significantly different from the number of de novo results found in patients without a cardiac indication (49.3%, 70/142, p=0.8863)

RESULTS

- Referrals made with a cardiac indication comprised 16.2% (81/500) of the total referrals.
- A positive or likely positive DES result in a characterized gene was identified in 23.5% (19/81) of patients with a cardiac indication. This rate is not significantly different from the group of referrals made without a cardiac indication. 133/419 (31.7%) (p= 0.1378).
- Uncertain findings in characterized genes were found in 8.6% (7/81) of patients with a cardiac indication. This is the same rate as was observed in the population of patients without a cardiac indication. (8.6%, 36/419)
- A novel gene finding was identified in 4.3% of patients (3/70) which is not statistically different from the 8.1% (28/346) in patients with a novel finding in the non-cardiac group.
- The novel findings within the cardiac indication cohort all occur in genes associated with cell adhesion protein production.
- Negative results were reported in 61.4% (43/70) patients with a cardiac indication versus 54.5% (198/364) (p=0.2784) in the non-cardiac group.

TAKE-HOME POINTS

- Diagnostic exome sequencing establishes a molecular diagnosis in a quarter of patients with a cardiac indication for referral.
- Having a cardiac indication for referral was not significantly associated with obtaining a positive result with DES.
- Novel findings in cardiac genes (n=3) were genes associated with cell adhesion protein production. This is a class of genes and pathways that have been associated with cardiac abnormalities (Gupta, 2013).
- A limitation of this study was the inability to reliably sort cases referred for cardiac indication by class of abnormality such as structural versus rhythmic abnormalities.

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