Diagnostic Exome Sequencing detection rates in patients with prior uninformative microarray results

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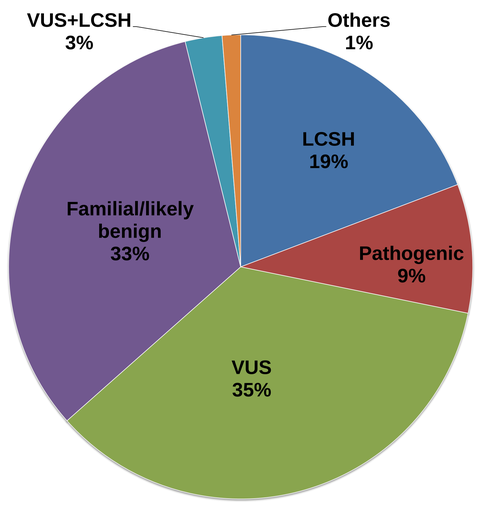
BACKGROUND

- Chromosomal microarray analysis (CMA), which has become standard of care, is now routinely performed as a first tier test in pediatric settings. Although CMA has significantly improved diagnostic yield, clinicians often receive results that are clinically uninformative.
- Clinically uninformative results are those that do not explain the patient's clinical phenotype because 1) they are variants of uncertain significance (VUS), 2) they are clinically significant but unrelated to the clinical indication of primary concern, or 3) they constitute long contiguous stretches of homozygosity (LCSH) and thereby indicate an increased risk for autosomal recessive disease.
- Until recently, CMA was the only whole genome diagnostic testing option; however, in recent years diagnostic exome sequencing (DES) has improved the diagnostic yield even further, especially in patients with undiagnosed genetic conditions and/or in those who have had uninformative genetic testing in the past.
- Herein, we performed a retrospective analysis of the first 800 cases received at Ambry Genetics for DES in order to determine the detection rate in patients who had prior clinically uninformative CMA results.

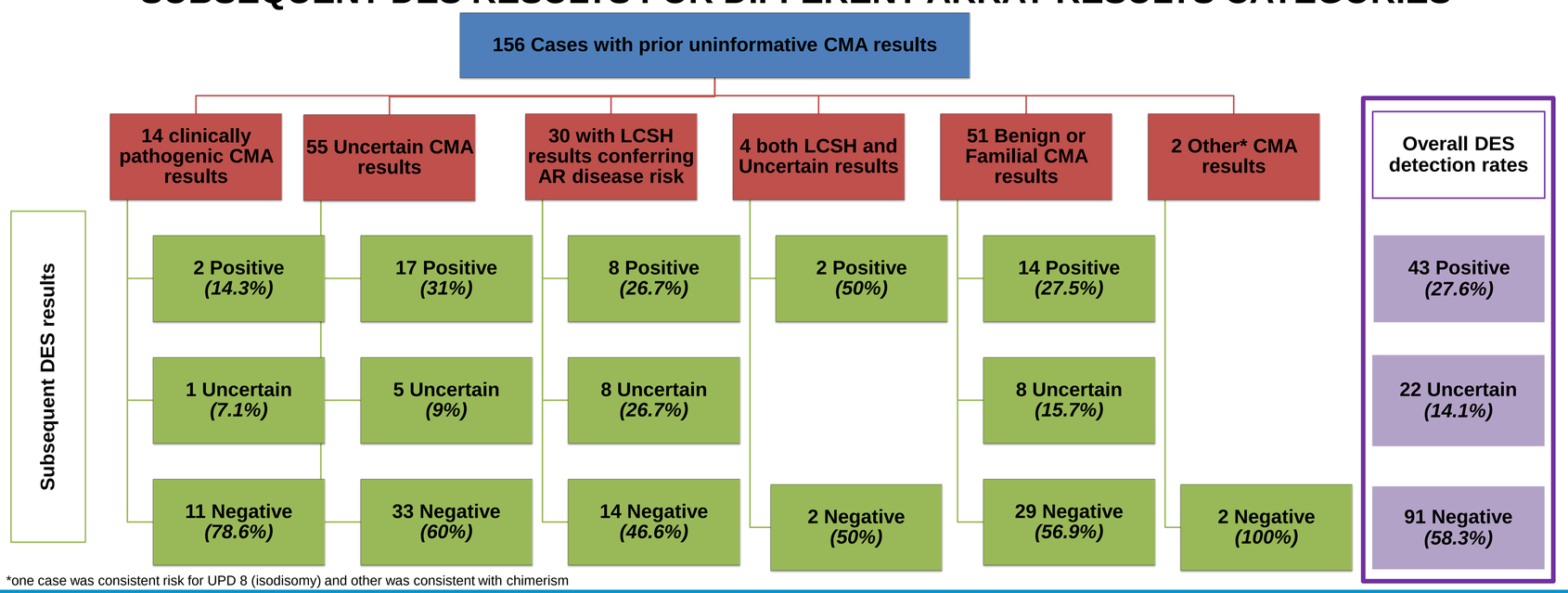
METHODS

- **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).
- Clinical information and prior test history submitted at the time od DES testing were retrospectively reviewed to gather CMA results, if performed in the past.

TYPES OF PRIOR UNINFORMATIVE CMA RESULTS



SUBSEQUENT DES RESULTS FOR DIFFERENT ARRAY RESULTS CATEGORIES



*one case was consistent risk for UPD 8 (isodisomy) and other was consistent with chimerism

RESULTS

- Of these 800 cases, 592 (74%) had undergone CMA testing prior to DES.
- Of these 592 cases, 156 (26.4%), had a clinically uninformative prior CMA result, while the remaining had a normal CMA result.
- Approximately 28% (43/156) of patients with clinically uninformative CMA results subsequently had a positive, clinically informative DES result, while ~14% had a subsequent uncertain DES result.
- Of particular interest was a patient (#1) who had a clinically pathogenic contiguous deletion on 16p11.2 including *TBX6* gene and DES detected a mutation in the other *TBX6* allele; both the test results in combination provided complete clinical diagnosis.
- 80% of the positive DES cases with prior LCSH results on CMA had homozygous mutations consistent with autosomal recessive disease. For all the cases where the breakpoints for the LCSH regions were provided, the candidate gene was within the detected LCSH region.
- Of the positive DES cases, five had novel gene findings while the remaining positive findings were in characterized genes.

EXAMPLES OF POSITIVE DES CASES

		Exome Results					
Patient	CMA Alteration	Gene (Location)	Inheritance	Alteration	Zygosity	Diagnosis	Clinical Details Provided for DES
1	550 kb deletion on 16p11.2 including *TBX6* Clinically pathogenic	*TBX6* (16p11.2)	AD/AR	c.512G>A	Heterozygous, Inherited	Spondylocostal dysostosis 5	Spondylocostal dysostosis, imperforate anus, respiratory insufficiency, gastrointestinal anomalies, normal development, dysmorphic craniofacial features, broad chest, wide spaced nipples, and short trunk with scoliosis.
2	Interstitial duplication at 2p24.3 Variant of uncertain signifiance	*TUBB4A* (19p13.3)	AD	c.785G>A	Heterozygous, *de novo*	Hypomyelinating Leukodystrophy with Atrophy of the Basal Ganglia and Cerebellum	Developmental delay, ID, absent speech, hypotonia, leukoencephalopathy, nystagmus, microcephaly, feeding difficulties, cardiac heart defects, gastrointestinal problems, scoliosis, non-ambulatory, hypomyelination and cerebellar hypoplasia/atrophy on MRI.
3	Interstitial deletion at 7q31.1 Familial/likely benign	*SCN2A* (2q24.3)	AD	c.2645G>A	Heterozygous, *de novo*	Epileptic encephalopathy, early infantile	Intractable epilepsy, dystonia, ID, cortical visual impairment, dysautonomia, and sleep dysfunction. Neonatal onset of seizures, multiple seizure types, mild diffuse atrophy and hypoplasia of both cerebral hemispheres and corpus callosum on MRI.
4	LCSH spanning 10.23% of the genome including 11q13.2 and two CNVs at 5q31.3 and 15q15.3 of likely benign significance	*SPTBN2* (11q13.2)	AR	c.1915G>T	Homozygous, Inherited	Spinocerebellar ataxia	Developmental delay, seizures, hypotonia, hypothyroidism, dysmorphic features, ocular anomalies, and disproportional vision loss with high myopia, possibly cerebral in origin.

TAKE-HOME POINTS

- Approximately 1 in 4 patients with uninformative CMA results had a subsequent informative DES result.
- DES is a valuable clinical testing option that enables clinicians to provide a definitive diagnosis in a significant proportion of patients.
- Even though the detection rate in this cohort is not significantly different from the overall published detection rates for DES (25-30%), these data suggest that DES is a valuable testing option for patients with clinically uninformative CMA results.
- CMA and DES are complementary diagnostic testing options; for some patients it is the combination of the two technologies that makes a comprehensive diagnosis possible.

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