Copy number variation analysis by next-generation sequencing enhances molecular diagnostic yield of lysosomal storage disorders

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Disclosure Information
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I am an employee and stockholder of Invitae.

I will not discuss off-label use or investigational use in my presentation.

Introduction

- It is important to confirm the diagnosis of a lysosomal storage disease quickly to initiate management and treatment.

- Enzyme analysis may yield false positive results (i.e., pseudodeficiency alleles) or false negative results, which may not exclude a diagnosis. (PMID: 27293520)

Introduction

- Molecular analysis for diagnosis of lysosomal storage disorders is well established and often used as first-line testing.
    - Read-through sequence analysis alone is insufficient as diagnoses involving CNVs may be missed.
    - Historical methods of copy number variant (CNV) analysis (such as aCGH and MLPA) for LSDs are well established, but use of a second method may delay diagnostic results and treatment. (PMIDs: 22704718, 22848519).
    - Next-generation sequencing (NGS) for CNV detection has not been well characterized in the literature.

Objectives

- Identify molecular causes of disease in individuals with suspected lysosomal storage disorders.
- Assess the contribution of CNVs to positive molecular diagnoses of lysosomal storage disorders.

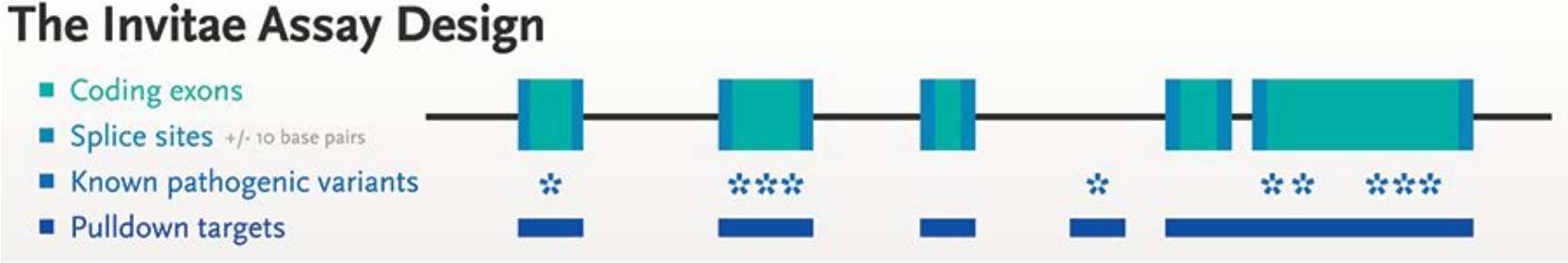
Methods

- Samples: Genomic DNA samples (n = 444) from blood or saliva

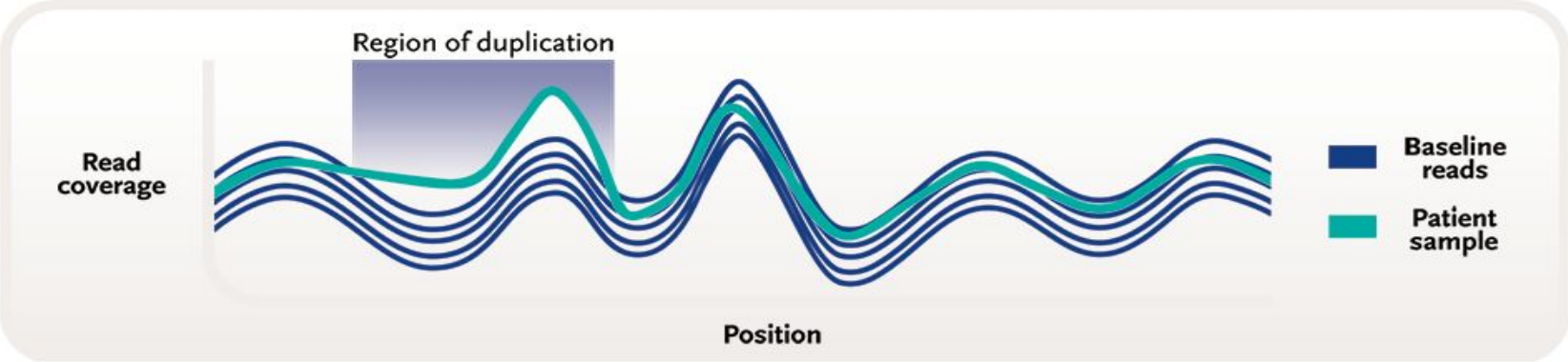
- Next-generation sequencing:
  - Coverage: 350x avg, 50x min
  - + CNV analyses
  - Confirmation of LP/P variants:
    - Sanger or Pacific Biosciences Sequencing
    - Array CGH

- Variant interpretation: ACMG guidelines-based system, SHERLOC (Nykamp *et al*., *Genet Med*. 2017)

Methods

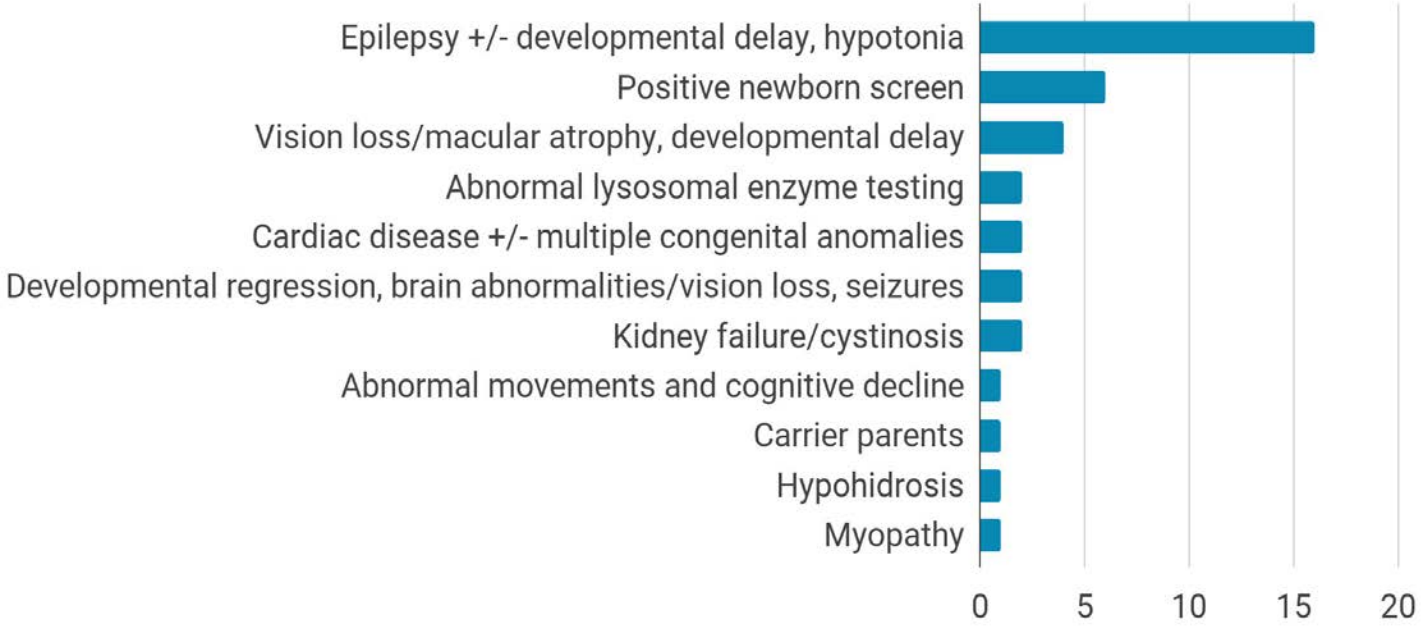


Copy number analysis (deletion/duplication detection)



Results: Demographics of patients with CNVs identified

- 38 patients identified
- Gender: 20 males, 18 females
- Age range: 0-55 years
- Indications:



Results: Spectrum of CNVs (38/444 patients)

Gene	Classification	Variant details	N	Results outcome
ATP13A2	Pathogenic	Deletion (Exons 17-20)*	1	1 molecular diagnosis
CLN3	Pathogenic	Deletion (Exons 8-9)	9	4 molecular diagnoses, 5 carrier statuses
CLN8	VUS	Duplication (Entire coding sequence)*	1	1 uncertain
CLN8	Pathogenic	Deletion (Entire coding sequence)	1	1 carrier status
CTNS	Pathogenic	Deletion (Exons 1-10)	2	2 molecular diagnoses
DNAJC5	VUS	Duplication (Entire coding sequence)	1	1 uncertain
GAA	Pathogenic	Deletion (Exon 18)	1	carrier status
GALC	Pathogenic	Deletion (Exons 11-17)	8	1 molecular diagnosis, 7 carrier statuses
GLA	Pathogenic	Deletion (Entire coding sequence)	1	1 molecular diagnosis
GLA	VUS	Duplication (Entire coding sequence)*	1	1 uncertain
HEXA	Likely Pathogenic	Deletion (Exons 11-13)*	1	1 molecular diagnosis
IDS	Pathogenic	Deletion (Entire coding sequence)	1	1 molecular diagnosis
KCTD7	VUS	Duplication (Exons 3-4)*	7	7 uncertain
LAMP2	Pathogenic	Deletion (Exon 1)	1	1 molecular diagnosis
LAMP2	VUS	Duplication (Entire coding sequence)*	1	1 uncertain
MFSD8	VUS	Duplication (Exons 2-10)*	1	1 uncertain

*** = novel variant**

Positive molecular diagnoses: 12 cases with CNVs

Disease	Gene	N	Demographics	CNV	Co-occurring variant
Kufor-Rakeb syndrome	*ATP13A2*	1	17y male	Deletion (Exons 17-20)	c.1903C>T (p.Q635*)
Neuronal ceroid lipofuscinosis	*CLN3*	4	5y male, 7y female, 10y female, 13y male	Deletion (Exons 8-9)	c.944dupA (p.H315Qfs*67) c.569dupG (p.A191Sfs*45) 2 homozygotes
Cystinosis	*CTNS*	2	4y female, 30y female	Deletion (Exons 1-10)	c.206_210delTCCTT (p.I69Rfs*5), 1 homozygote
Krabbe disease	*GALC*	1	9m male	Deletion (Exons 11-17)	c.1541T>C (p.F514S)
Fabry disease	*GLA*	1	6y female	Deletion (Entire sequence)	N/A (mosaic Turner syndrome)
Tay-Sachs disease	*HEXA*	1	1y female	Deletion (Exons 11-13)	homozygote
Hunter syndrome	*IDS*	1	1y male	Deletion (Entire sequence)	hemizygote
Danon disease	*LAMP2*	1	16y female	Deletion (Exon 1)	N/A (symptomatic)

Conclusions

- NGS-based CNV analysis is a rapid, efficient, and comprehensive method for diagnosing LSDs.
  - Can shorten turnaround time and reduce cost

- CNV analysis significantly expands the diagnostic yield of LSDs
  - CNVs found in 8.6% (38/444) of patients
  - One-third (12/38) of detected CNVs were clinically actionable

- NGS CNV analysis should be part of first-tier testing for individuals with suspected LSDs.

Acknowledgements

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