159

Prevention of Inherited Genetic Disease Using Next Generation Sequencing

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Introduction/Scientific Premise

- "An ounce of prevention is worth a pound of cure"
- Advances in genome sequencing methods have completely revolutionized our approach to **diagnosis in children** with suspected genetic neurological disorders.
- We have entered the era of genetic therapies – but very expensive

Background

- Preconception carrier screening has evolved over the last fifty years from a single-genes in high-risk ethnic groups to pan-ethnic screening using large gene panels.
- The American College of Medical Genetics has recommended including 133 genes linked to autosomal recessive (AR) and X-linked disorders in panels offered to anyone in the general population who is pregnant or considering a future pregnancy (Gregg et al., 2021).

Goals

- The goal of this study is to access the **feasibility of using next generation sequencing in guiding preconception screening for couples** planning to have a child and to create a workflow for identifying AR and X-linked disorders without constraints of a predetermined gene panel.

Research Design

- Enrollment: Families (trios) were enrolled in a research study approved by the Western Institutional Review Board (WIRB protocol #20120789).

- Patient cohort: Retrospective analysis of deidentified whole exome (WES) and whole genome sequence (WGS) data of 150 couples, derived from previously enrolled trios. For the purpose of this study, the child was excluded and the parental genomic data was analyzed.

Detailed Methods

- We used the VarSeq software (Golden Helix, Inc) to generate gene variant annotation files.
- We combined the variant call format (VCF) files from the female and male parents to generate data for a "synthetic proband". Data from this trio was analyzed using our standard trio template in VarSeq.
- Variant filtering was done as described in table 1.
- This generated variants for autosomal recessive and x-linked disorders that were manually reviewed.

Table 1

01	CADD Score < 15	Variants with a Combined Annotation Dependent Depletion (CADD) score of less than 15 were excluded.
02	VUS in 3'-UTR and 5'-UTR	Variants of unknown significance in 3'-UTR and 5'-UTR were excluded.
03	> 5 hemizygotes or homozygotes in gnomAD	Variants with more than 5 hemizygotes or homozygotes in the Genome Aggregation Database (gnomAD) were excluded.
04	Benign or likely-benign in ClinVar	Variants scored as benign or likely-benign in the ClinVar database were excluded.
05	No OMIM description	Variants without an OMIM disorder description were excluded.

Results

- 17 couples would receive counseling based on carrier screening (Table 2)
- 19 genes were identified with pathogenic or likely-pathogenic variants
- In 13 couples both parents were carriers and at risk for transmitting an AR disorder
- 4 females were at risk for transmitting an X-linked disorder
- Disorders include intellectual disability (*TMCO1, L1CAM, HEXA, SMS, GALC, TAF8, GEMIN5*), skeletal problems (*COL27A1, SMS, CYP27B1*), degenerative conditions (*HEXA, GALC*), peripheral neuropathy (*PDK3*), eye conditions (*ABCA4*), deafness (*OTOA*), endocrine (*SLC5A5*), skin conditions (*LAMA3*), kidney disorders (*OCRL*), ciliary dyskinesias (DNAH11).

Table 2

Couple Number	Gene Name	Disorder	Mode of Inheritance	ACMG Panel
1	*TMCO1*	Cerebro-facio-thoracic dysplasia	AR	No
2	*COL27A1*	Steel syndrome	AR	No
2	*L1CAM*	L1 syndrome	X-linked	Yes
3	*HEXA*	Tay-Sachs disease	AR	Yes
4	*PDK3*	Charcot-Marie-Tooth disease	X-linked	No
5	*ABCA4*	Retinal dystrophy early-onset, severe	AR	No
6	*SMS*	Snyder-Robinson syndrome	X-linked	No
7	*OTOA*	Deafness	AR	No
8	*GALC*	Krabbe disease	AR	No
9	*SLC5A5*	Thyroid dyshormonogenesis 1	AR	No
10	*TNFRSF13B*	Common variable immunodeficiency (CVID)	AR	No
11	*OTOA*	Deafness	AR	No
12	*LAMA3*	Epidermolysis bullosa	AR	No
12	*OTOA*	Deafness	AR	No
13	*CYP27B1*	Vitamin D-dependent rickets	AR	Yes
14	*OCRL*	Dent disease-2	X-linked	No
15	*TAF8*	Neurodevelopmental disorder with severe motor impairment, absent language, cerebral hypomyelination, and brain atrophy (NEDMLHB)	AR	No
16	*GEMIN5*	Neurodevelopmental disorder with cerebellar atrophy and motor dysfunction (NEDCAM)	AR	No
17	*DNAH11*	Primary ciliary dyskinesia-7 (CILD7)	AR	No

Limitations/Challenges

- Carrier status for variants in AR genes not reported as this study is focused on couples.
- Technical Challenges
  - Certain diseases (SMA, hemoglobinopathies, Fragile X syndrome, congenital adrenal hyperplasia, Gaucher's disease) pose technical challenges with NGS and require alternative methods (Chen et al., 2020).
  - Current next generation sequencing (NGS) does not pick up expansion repeats (will not detect Fragile X syndrome >200 CGG repeats) (Beauchamp et al., 2018). New tools like "Expansion Hunter".
  - Mitochondrial DNA requires deep sequencing to determine heteroplasmy.
  - Structural variants (copy number changes, translocations, mosaicism) may be missed
  - Difficult to interpret non-coding variants in WGS.
  - Difficult to differentiate pseudogenes.

- Determining which variants to report by phenotype; Disease severity?
- Not reporting back VUS or residual risk – important in counseling couples
- May not have a good genotype-phenotype correlation for all variants
- Does reporting on over 6,335 disorders create too large a burden on a couple?

Conclusions

- It is feasible to use WGS technologies to develop a pre-conception genetic test for guiding couples who are contemplating having a child.
- Special tools may have to be developed for conditions caused by technically challenging classes of mutations (including fragile X and other repeat expansion disorders, SMA, deletions/insertions, and others).
- Additional studies are required to determine the emotional impact on couples of receiving results of extremely rare conditions and disorders with a mild phenotype, variable phenotype, late onset, variable penetrance, deafness, and sex determination.
- Counseling of couples should begin before testing, should include a thorough discussion of the limitations of testing, and review reproductive options available to the couple.

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