The impact of copy number analysis in expanded carrier screening

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Introduction

Expanded carrier screening (ECS) identifies carriers of recessive diseases and may be performed using either targeted genotyping (TG) or next generation sequencing (NGS). Historically, ECS panels have focused on deleterious SNPs and indels but have been performed with limited or no copy number variant (CNV) calling. Using the Modeled Fetal Disease Risk[1,2], here we evaluate the performance of hypothetical TG and NGS panels. We also evaluate the impact of CNVs on two ECS panels with 94 conditions and 176 conditions, respectively.

Modeled Fetal Disease Risk

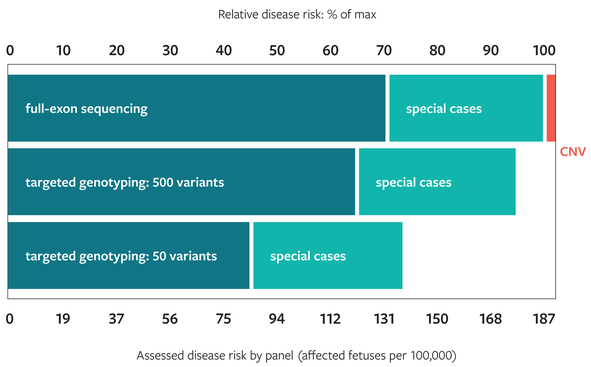
Previously, carrier frequency (CF) and carrier couple frequency (CCF) have been used to quantify the sensitivity of ECS panels. However, to serve this purpose more effectively, especially for diseases with complex inheritance (e.g., Fragile X, alpha thalassemia, and 21-hydroxylase-deficient congenital adrenal hyperplasia), we recently introduced[1,2] the "modeled fetal disease risk", which is the probability that a random fetus will be affected by one of the panel diseases. The modeled fetal disease risk allows comparisons of ECS panel sensitivity even for diseases with complex inheritance.

Method	Carrier Frequency	Carrier Couple Frequency	Modeled Fetal Disease Risk
			
Meaning	400 in 10,000 persons are carriers of this disease	16 in 10,000 couples are carrier couples of this disease	4 in 10,000 fetuses will be affected by this disease
Limitations	Cannot compare autosomal and X-linked diseases	Cannot compare diseases with complex inheritance	Challenging to compute for diseases with complex inheritance

Lessons from hypothetical panels

To assess the sensitivity of various ECS approaches, we compared the modeled fetal disease risk captured by hypothetical panels containing up to 94 "Severe" and "Profound" conditions[3]. We first considered an NGS panel that excludes several "special case" diseases (fragile X syndrome, 21-hydroxylase-deficient congenital adrenal hyperplasia, alpha thalassemia, and spinal muscular atrophy) that are technically challenging to probe. We then considered the effect of adding special cases and panel-wide (i.e., non-founder) copy number deletion (CNV) calling. We finally considered "best-possible" TG panels with a fixed number of optimally-selected variants, both with and without the special cases. The disease risk of each hypothetical panel shows that neglecting special cases and exon-wide coverage overlooks 10% to 55% of affected fetuses. Furthermore, non-founder CNVs contribute approximately 4 affected fetuses per 100,000—roughly equivalent to the contribution of the 50 least-prevalent diseases on the 94 condition panel.

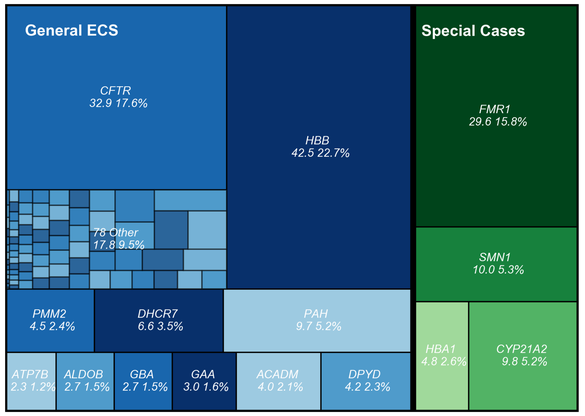
Modeled fetal disease risk (per 100,000 births) and percent of total risk is shown for hypothetical TG and NGS versions of the 94 condition ECS panel



High-prevalence genes dominate disease risk

A common question is whether an ECS panel would benefit from the addition of more genes. While adding more genes always improves the disease risk, typically the most prevalent diseases contribute over half of the disease risk. Thus, improving ECS panels will likely require both increasing detection rate for existing diseases (such as via panel-wide CNV calling) and adding additional conditions.

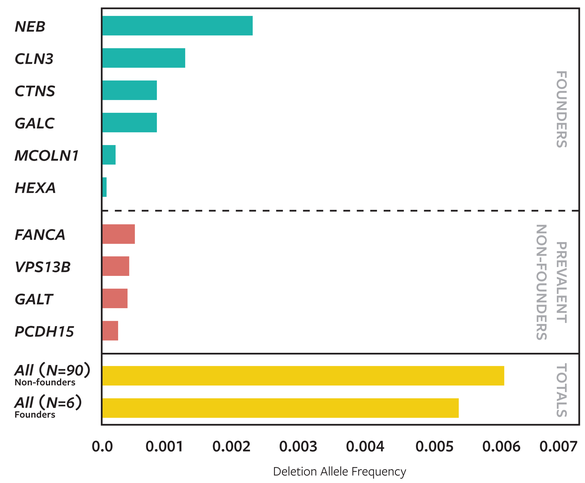
Modeled fetal disease risk (per 100,000 births) and percent of total risk is shown for each condition, with rectangle areas proportional to the disease risk. Analysis is performed on the 94 Severe/Profound condition panel.



Panel-wide CNV Calling on a 176 disease panel

Based on the previous lessons, we developed an expanded ECS panel with 176 diseases and panel-wide deletion calling. Here we report CNV deletion statistics for the autosomal genes on this panel. Although the list of genes with the most observed deletions contains known founder mutations, 53% of deletions are located outside of the six genes for which we previously called deletions (CLN3, CTNS, GALC, HEXA, MCOLN1, and NEB), highlighting the importance of not restricting CNV analysis to a handful of founder variants.

Autosomal genes with prevalent or founders deletions



Conclusions

Modeled fetal disease risk allows systematic comparison of ECS panels and identified non-founder CNVs as a potential avenue for improving sensitivity. We therefore developed an expanded ECS panel with 176 conditions and panel-wide deletion calling. On this new panel, panel-wide deletion calling is expected to identify more than twice as many variants as deletion calling that is limited to six founder variants.

Methods

405,195 patients seeking ECS (Counsyl *Family Prep Screen*) between January 2012 and December 2016 for reason of "Carrier Testing" were anonymized and included in the disease risk analysis on the 94 disease panel; 56,267 of these samples were used for panel-wide deletion CNV analysis. TG and NGS based allele counts were combined to reduce statistical uncertainty[1]. Results for self-reported ethnicities were reweighted based on US census data. For the 176-disease panel, we performed deletion calling using 10,352 anonymized patient samples processed between October 2016 and December 2016. Due to limited data, no US census re-weighting was done on the 10,352 patient analysis. 161 autosomal genes were considered for this analysis; this includes all autosomal genes that do not involve special case calling.

REFERENCES:
1. Haque, IS et al. Modeled Fetal Risk of Genetic Diseases Identified by Expanded Carrier Screening. JAMA. 2016;316(7):734-742
2. Beauchamp, KA et al. Systematic Design and Comparison of Expanded Carrier Screening Panels. BioXriv (2016).
3. Lazarin, GA. et al. Systematic classification of disease severity for evaluation of expanded carrier screening panels. PLoS ONE 2014; 9(12): e114391.

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