Copy number variant calling on a 176 condition expanded carrier screening panel including *DMD*

Kyle A. Beauchamp PhD, Peter Grauman BS, Kevin R. Haas PhD, Gregory J. Hogan PhD, Genevieve M. Gould PhD, Gabriel A. Lazarin MS CGC, Kenny Wong MS CGC, Eric A. Evans PhD, James D. Goldberg MD, Imran S. Haque PhD, Dale Muzzey PhD

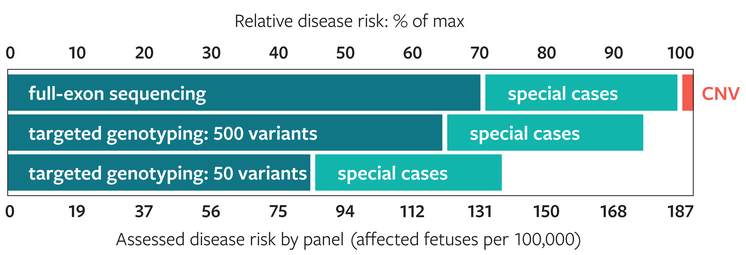
South San Francisco, California

Introduction

Expanded carrier screening (ECS) identifies carriers of recessive and X-linked 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.

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.

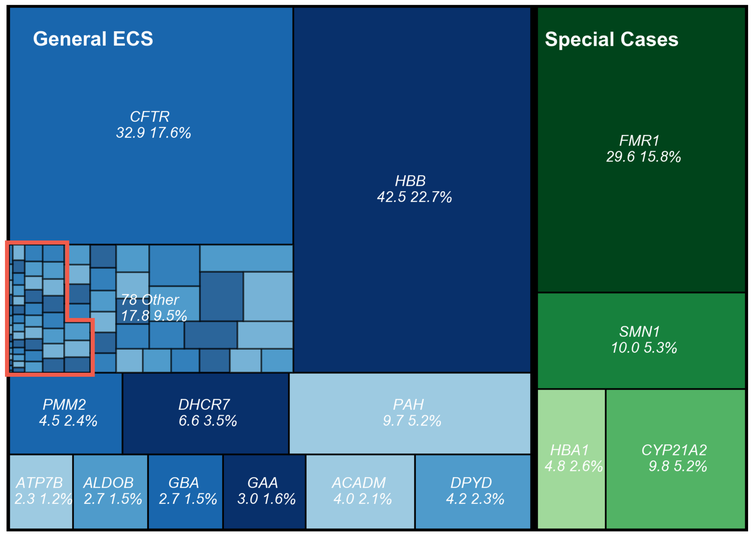


Modeled fetal disease risk (per 100,000 births) and percent of total risk is shown for hypothetical TG and NGS versions of the 94 condition ECS panel. Non-founder deletion CNVs contribute an additional 4 affecteds per 100,000.

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). https://doi.org/10.1101/080713 | 3. Lazarin, GA. et al. Systematic classification of disease severity for evaluation of expanded carrier screening panels. PLoS ONE 2014; 9(12): e114391. | 4. Tayeb M. Saudi J Biol Sci. 2010 Jul; 17(3): 237–240. | 5. http://www.dmd.nl/DMD_deldup_USA.html

High-Prevalence Genes Dominate Disease Risk

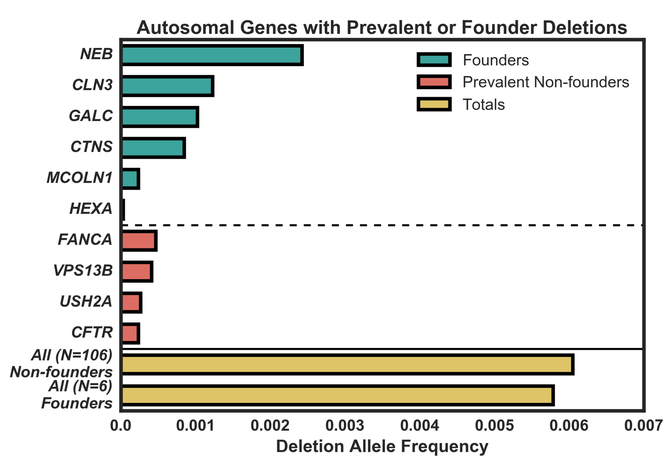
A common question is whether an ECS panel would benefit from the addition of more genes. While adding more genes always improves the disease risk, typically the most prevalent diseases contribute over half of the disease risk. Thus, improving ECS panels will likely require both increasing detection rate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



Modeled fetal disease risk (per 100,000 births) and percent of total risk is shown for each condition on the 94 condition panel. The red box shows the approximate number of single-gene conditions required to achieve a disease risk comparable to panel-wide deletion CNVs.

Panel-wide CNV calling on a 176 disease panel

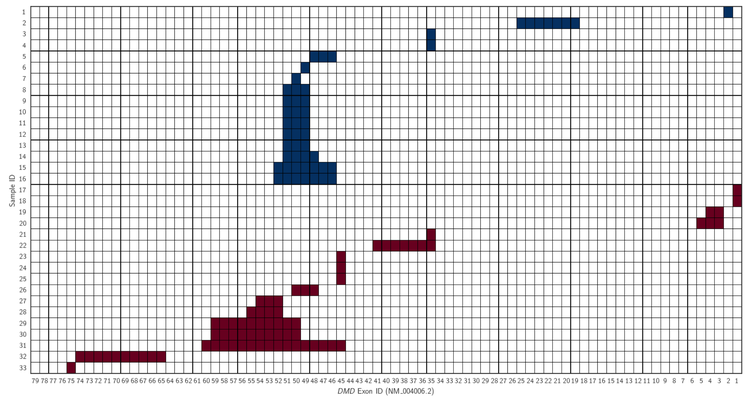
Based on the previous lessons, we developed an expanded ECS panel with 176 diseases and panel-wide deletion calling. Here we report CNV deletion statistics for the autosomal genes on this panel. We observed 420 deletions in 112 autosomal genes with observed CNVs. Although the list of genes with the most observed deletions contained known founder mutations, 51% 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.



Allele frequency is shown for deletion CNVs in the 176 disease panel.

A preliminary map of *DMD* CNVs in ECS patients

We next explored the exon-level consequences of CNV deletions and duplications in *DMD*. We observed 23 deletions and 22 duplications in *DMD*, 33 of which were found to intersect the exons of *DMD*. Although variant curation is ongoing, the most commonly observed CNV is a deletion of exons 49-51 — known hotspot exons[4,5]. With additional samples, this analysis could provide unbiased estimates of *DMD* CNV rates in a carrier screening population.



CNVs in *DMD* that intersect exons are shown, with deletions in blue and duplications in red.

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 between Jan. 2012 and Dec. 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 17,114 anonymized patient samples processed between Oct. 2016 and Dec. 2016. Due to limited data, no US census re-weighting was done on the 17,114 patient analysis. 161 autosomal genes were considered for this analysis; this includes all autosomal genes that do not involve special case calling.

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