Copy number variant calling on a 176-disease expanded carrier screening panel

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Disclosure

All authors are employees of Counsyl, Inc.

Objective

Expanded carrier screening (ECS) identifies couples whose future children are at increased risk of Mendelian conditions. Historically, ECS has been performed with limited or no copy number variant (CNV) calling, often restricted to a handful of founder deletions. The lack of broad CNV calling may reduce the detection rate of ECS. We performed panel-wide copy number deletion calling on a large ECS patient cohort to determine its impact on detecting at-risk couples.

Study Design

For 24,316 anonymized patient samples tested on a CLIA-certified 176-disease ECS panel, we performed CNV deletion calling on 161 autosomal-recessive disease genes and 10 genes associated with X-linked conditions (calls for several conditions such as *SMN1*, are treated as special cases and are not included in calculations of CNV prevalence).[1] Copy number calling was performed using a Hidden Markov Model on next generation sequencing depth data, and CNVs were identified down to single-exon resolution. Positive and low-confidence CNV calls emitted by the bioinformatics pipeline were reviewed manually by certified experts prior to being curated and reported to patients if found to be deleterious.

Figure 1: Panel expansion guided by disease severity and impact on MFDR.

A)

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 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

B)

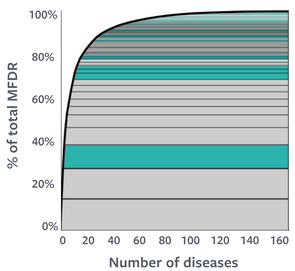

Figure 1: A) The sensitivity of ECS panels can be quantified in multiple ways, with the MFDR[2,3] permitting straightforward comparison of autosomal recessive conditions, X-linked diseases, or conditions with complicated inheritance (e.g., alpha thalassemia). **B)** Relative to the previous 94-gene panel (gray), the 176-disease Foresight™ panel screens for 82 additional genes (teal) that range in MFDR[1].

REFERENCES

1. Hogan, GJ et al. Development and validation of an expanded carrier screen that optimizes sensitivity via full-exon sequencing and panel-wide copy-number-variant identification. bioRxiv, 2017. **2.** Haque, IS et al. Modeled Fetal Risk of Genetic Diseases Identified by Expanded Carrier Screening. JAMA. 2016;316(7):734-742. **3.** Beauchamp, KA et al. Systematic Design and Comparison of Expanded Carrier Screening Panels. GIM (2017; in Press).

Figure 2: Role of CNV detection in identification of at-risk couples.

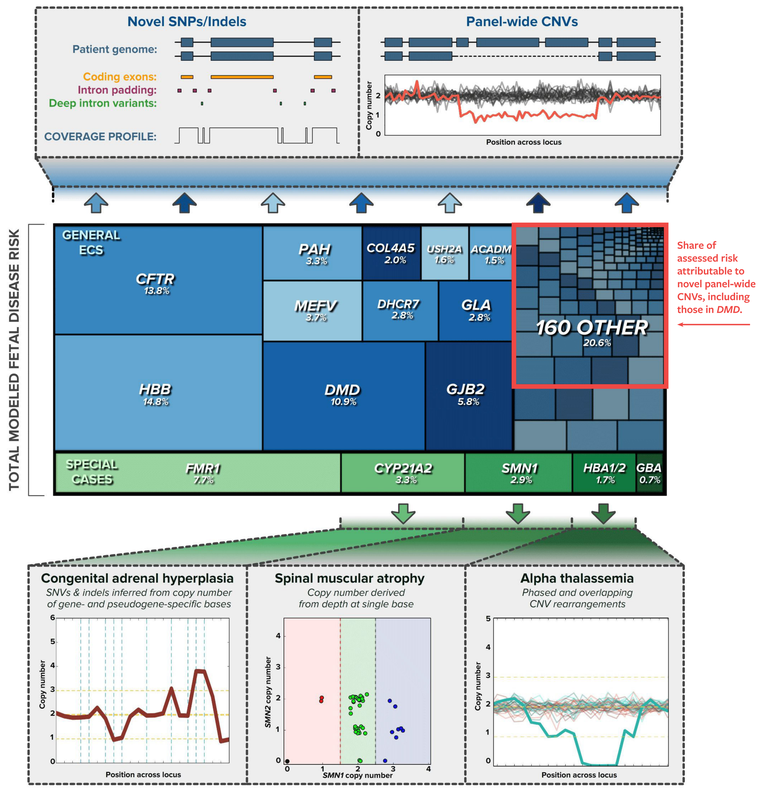

Figure 2: In addition to having NGS coverage for the detection of single nucleotide variants (upper left), novel CNV calling (upper right) and custom assays relying heavily on CNV calling (bottom row) contribute to the modeled fetal disease risk of a 176-disease expanded carrier screen (middle). The modeled fetal disease risk is shown for each disease gene, estimated from carrier rates of 24,316 patients (percent indicates share of total panel disease risk). Five conditions require special-case treatment. For 21-OH deficient congenital adrenal hyperplasia (CAH; bottom left) and alpha thalassemia (bottom right), copy-number profiles are plotted from 5' to 3' across the gene. Dashed yellow lines in the CAH profile indicate copy-number levels, and dashed vertical blue lines show sites where pseudogene-derived bases are pathogenic. For spinal muscular atrophy (bottom middle), each spot represents the copy number of *SMN1* and *SMN2* for a single sample; carriers are shown in red.

Figure 3: CNVs range in size and increase ECS sensitivity.

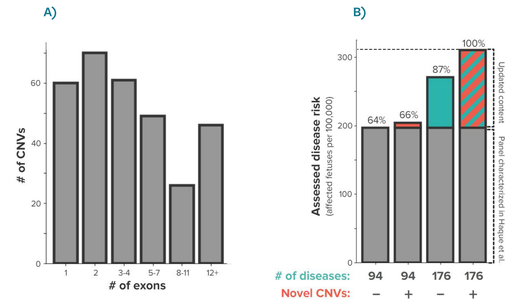

Figure 3: Support for novel CNVs accounts for substantial assessed disease risk. **A)** The size distribution, expressed in exons, of observed deletions. **B)** Disease risk is plotted as a function of both panel size and support for novel CNVs.

Figure 4: Novel CNVs are collectively more common than founder CNVs.

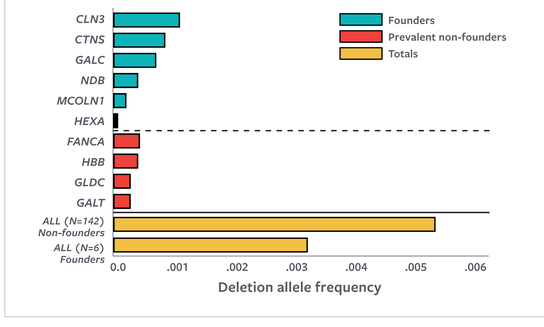

Figure 4: Novel CNVs are collectively more common than the six founder CNVs typically screened on ECS panels. Founder variants, shown in teal and named by the gene in which they appear, have high allele frequency, but some non-founder variants (red)—discovered on the 176 gene panel—are also common. Summing frequencies over all novel variants (many not shown in the plot) indicates the collective allele frequency due to novel CNV detection is greater than the aggregate frequency of founder variants.

Conclusions

An ECS that detects both novel exon-level CNVs and complex CNVs boosts ECS sensitivity relative to panels that lack these features. The MFDR contribution of novel CNVs exceeds that of founder CNVs and is comparable to that of the 150 least-common diseases on the panel. This observation suggests that the manner in which genes are screened on an ECS (e.g., NGS with novel CNVs) has arguably higher impact on detection of at-risk couples than the number of genes on the panel.