Fetal fraction amplification within NIPS enables detection of **clinically relevant genome-wide copy number variants** to **1Mb resolution**

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All authors were employed by Myriad Genetics, Inc at the time of this study

Objective

While **clinically relevant copy number variant (CNVs) occur in 6-8% of pregnancies with fetal structural anomalies,**[1-3] they are typically difficult to detect via non-invasive prenatal screening (NIPS) because of the low fetal fraction in maternal cell-free DNA (cfDNA).

By **leveraging fetal fraction amplification**, a method to preferentially sequence shorter fragments that are enriched for fetal-derived cfDNA, **we can detect small CNVs with high sensitivity**.

Here we describe an analytical validation of a NIPS that can detect clinically relevant CNVs across the genome.

Study Design

We developed and analytically validated a whole-genome sequencing-based NIPS assay that identifies novel fetal CNVs.

We then retrospectively analyzed >200k NIPS patient samples to evaluate the incidence of CNVs expected to have clinical impact because they either exceeded a length threshold (≥5Mb) or encompassed a region from our systematically curated list of 66 microdeletion and microduplication syndromes (nearly all <5Mb).

Conclusions

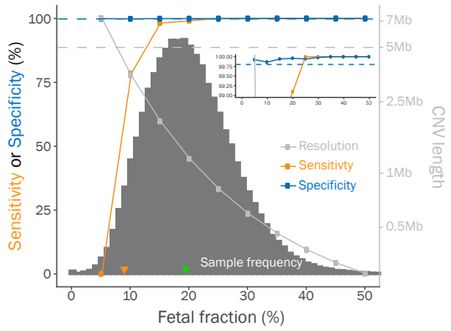
Fetal fraction amplification improves the resolution of novel fetal CNV detection in NIPS, permitting enhanced identification of genetic underpinnings for structural abnormalities.

Pairing this improved detection with carefully curated microdeletion and microduplication syndromes supports clinical actionability of reported CNVs.

Results

CNV calling performance

The genome-wide CNV caller detects simulated fetal CNVs ≥5Mb with **>95% aggregate sensitivity** and **>99.8% specificity**. To improve the resolution of CNV detection while maintaining high specificity, we imposed a dynamic CNV size threshold (*Resolution*, below) that takes into account the fetal fraction of each sample. For >99% of samples, we obtain ≥5Mb resolution, with **>40% of samples reaching 1Mb resolution without compromising specificity**.

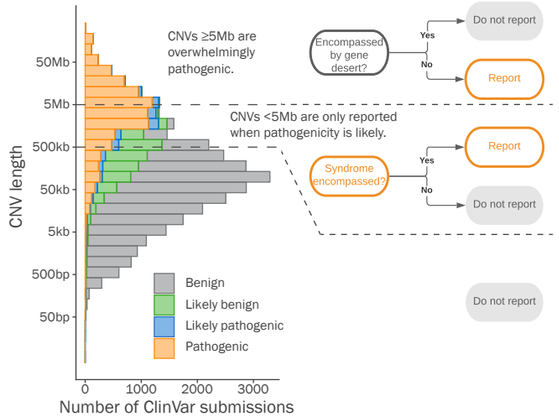


▼ Median FF without AMPLIFY™: 8.6%

▼ Median FF with AMPLIFY™: **19.5%**

Strategy for reporting actionable findings

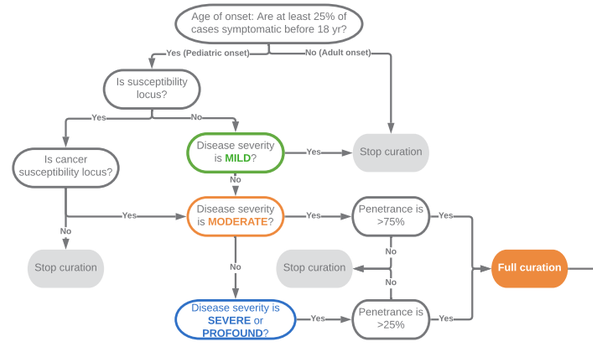
We will report only CNVs with strong evidence for pathogenicity, those ≥5Mb or fully encompassing the critical region of a curated syndrome.



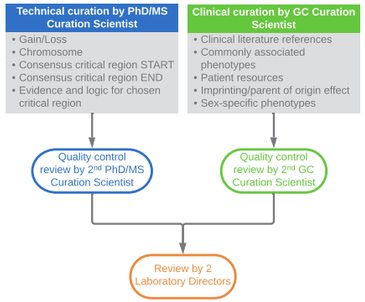
Systematic curation of clinically relevant syndromes

Our team of curators and laboratory directors evaluated >80 microdeletion and microduplication syndromes resulting in **66 clinically relevant syndromes**,

Evaluation workflow

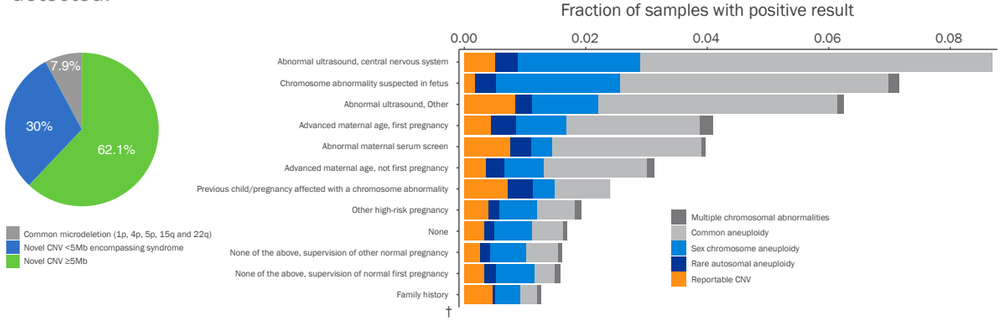


Data collection and review process



Retrospective analysis of >200,000 NIPS samples

The caller emitted positive CNV calls that were either ≥5Mb or that encompassed an established CNV-related syndrome in approxmately **1 in 339 NIPS samples** tested. In samples with fetal structural anomalies, such CNVs accounted for approximately 12% of all chromosomal abnormalities detected.



1. Lin, Y. et al. Acta Obstet Gynecol Scand (2020) 99:775-782.
2. Cai, M. et al. Sci Rep (2020) 10:15094.
3. Levy, B. and Wapner, R. Fertil Steril (2018) 109(2):201-212.

† Samples can be included in more than one testing indication.