Optimized Detection of 22q11.2 Deletions Using Whole Genome Sequencing

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OBJECTIVE

Genome-wide sequencing of cell free DNA (cfDNA) in maternal plasma now offers the possibility of noninvasive prenatal testing to detect genome wide copy number variants (CNVs) ≥7 Mb. Detection of smaller CNVs is more challenging and typically results in lower sensitivity and/or specificity than for larger CNVs typically detected by routine karyotyping. One such small CNV includes the loss of 22q11.2 and typically comprises 2 to 3 Mb. Our objective was to improve sensitivity to detect 22q11 deletions while maintaining high specificity >99.9%

STUDY DESIGN

An analytical validation comprising *in silico* models verified by genomic DNA (gDNA) mixture models was used to determine sensitivity. Genomic DNAs comprised 15 samples obtained from cell lines or individuals with 22q11.2 deletions ranging from 1.3 to over 3 Mb. Libraries prepared from these gDNAs were mixed with cell free plasma DNA libraries from non-pregnant women to model a 4-24% range of fetal fractions. *In silico* models used over 7000 maternal plasma sequencing results as a background to create 22q11.2 deletion events at the genomic coordinates from 206 deletions within the 22q11.2 region that are listed in the International Standards of Cytogenomic Arrays database (ISCA). Sensitivity was assessed utilizing sample specific features along with a hybrid method of focal and dynamic break-point detection to detect CNVs in 22q11.2 region.

RESULTS

The 22q11.2 deletion events in ISCA closely approximate the size and genomic positions of 22q11.2 deletions in patients with DiGeorge syndrome. Deletion events in gDNA samples were less evenly distributed in size and position compared to ISCA events. Detection of 22q11.2 deletions using a combined analysis method shown here enabled sensitivity of 74% across fetal fractions in maternal plasma samples.

Sensitivity to detect 22q11.2 deletions in gDNA samples at mixture ratios mimicking 9-11% fetal fraction was over 97%.

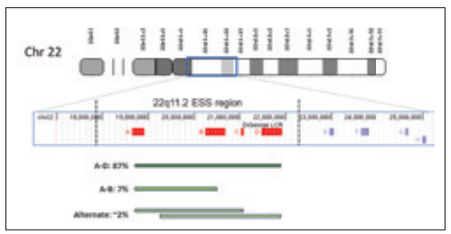
CONCLUSION

The hybrid method of focal and dynamic break-point detection can improve sensitivity to detect 22q11.2 deletions. With the availability of genome wide sequencing data, such analyses may extend microdeletion content in maternal plasma.

RESULTS

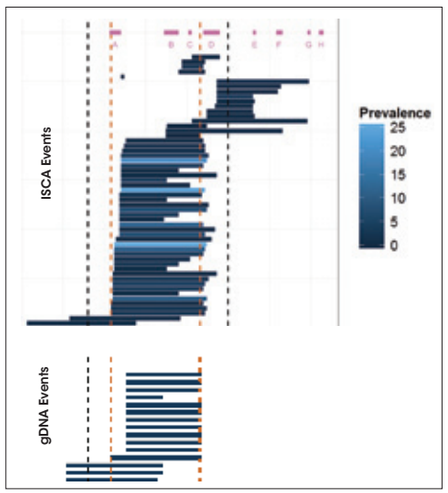
Chromosome 22q11.2 deletion regions associated with DiGeorge syndrome

- Regions A thru D comprise the primary recombination sites involved in deletion of 22q11.2 (Saitta SC et al., and Burnside RD)
- Current analysis of 22q11.2 deletion includes the regions indicated by the vertical dotted lines



Chromosome 22q11.2 deletions reported in the ISCA database and in gDNAs used to determine sensitivity

- ISCA 22q11.2 deletion size and coordinate frequencies correspond well with those reported in the literature and range from <1 Mb - >3 Mb in size
- 22q11.2 gDNA model samples cover the core 22q11.2 deletion region but are limited in diversity compared to ISCA events
- Black dotted vertical lines represent an analysis window for 22q11.2 deletions using our genome wide analysis algorithms
- Red vertical dotted lines represents a 'focal' analysis window for 22q11.2 deletion analysis optimized around the primary 22q11.2 deletion region



Methods to detect 22q11.2 deletions

Schematic depiction of 22q11.2 deletions by whole genome sequencing

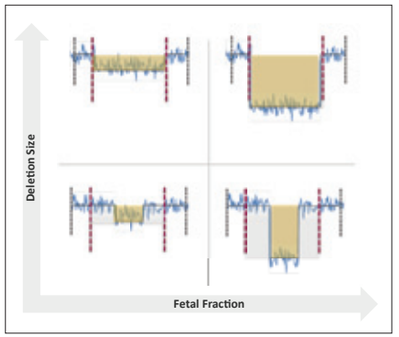
- Simulated signal, noise, and event sizes are shown to depict 22q11.2 deletions

Genome wide analysis

- Uses a circular binary segmentation (CBS) method to find event edges within a genomic window (black dotted lines) encompassing 22q11.2 (Zhao et al.)
- Improves genome-wide specificity by avoiding multiple hypothesis testing
- Shows best performance at higher fetal fractions and adapts to varying sizes of events

Focal analysis

- Focal analysis uses a fixed region (red dotted lines) within 22q11.2 to maximize detection of 22q11.2 deletions at low fetal fractions



- Maximizes detection of deletion sizes that cover entire region of analysis across a broader range of fetal fractions

Combined analysis

- Combined analysis maximizes sensitivity by using the edge detection capabilities of CBS to identify small deletions and the improved sensitivity at lower fetal fractions with a focused analysis window while maintaining current specificity >99.9%

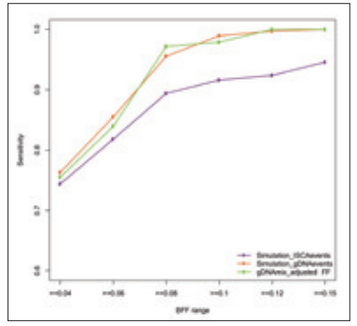
Combined analysis sensitivity to detect 22q11.2 deletions

***In silico* sensitivity of ISCA events**

- *In silico* modeling of ISCA 22q11.2 events indicates a sensitivity of 74% in the distribution of fetal fractions (BFF range) seen in maternal plasma DNA
- A sensitivity of over 90% at the median fetal fraction of ~10% is observed – however, this would be an overestimate of performance across the range of maternal cfDNA fetal fractions

gDNA model system sensitivity

- gDNA model systems support the *in silico* model results with sensitivity of ~75% after correction for mixture ratio inaccuracy
- At mixture ratios mimicking fetal fractions greater than 10%, sensitivities over 97% are observed – however, this would be an overestimate of performance across the range of maternal cfDNA fetal fractions as well as the range of 22q11.2 deletion sizes identified in the literature



CONCLUSION

- With combined analysis methods to detect 22q11.2 deletions, a 74% detection rate is expected
- Inclusion of a range of 22q11.2 deletion sizes and genomic coordinates from the ISCA database provides a more stringent and realistic estimate of performance, as exclusion of smaller deletions may artificially enhance the appearance of test performance
- When considering sensitivity in the context of cfDNA, the fetal fraction of the population must be considered as opposed to merely taking the median fetal fraction value of the population
- Utilization of the approach described here may enable analysis for a range of microdeletions screening assays using genome wide sequencing

REFERENCES

Saitta SC et al., *Hum Mol Genet* 2004; 13:417-428 (DOI:10.1093/hmg/ddh041).

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Zhao et al., *Clinical Chemistry* 2015; 61:608-616 (DOI:10.1373/clinchem. 2014.233312).