Detection of Microdeletion 22q11.2 in a Fetus by Next-Generation Sequencing of Maternal Plasma

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BACKGROUND: Efforts have been undertaken recently to assess the fetal genome through analysis of circulating cell-free (ccf) fetal DNA obtained from maternal plasma. Sequencing analysis of such ccf DNA has been shown to enable accurate prenatal detection of fetal aneuploidies, including trisomies of chromosomes 21, 18, and 13. We sought to extend these analyses to examine subchromosomal copy number variants through the sequencing of ccf DNA. We examined a clinically relevant genomic region, chromosome 22q11.2, the location of a series of well-characterized deletion anomalies that cause 22q11.2 deletion syndrome.

METHODS: We sequenced ccf DNA isolated from maternal plasma samples obtained from 2 patients with confirmed 22q11.2 deletion syndrome and from 14 women at low risk for fetal chromosomal abnormalities. The latter samples were used as controls, and the mean genomic coverage was 3.83-fold. Data were aligned to the human genome, repetitive regions were removed, the remaining data were normalized for GC content, and z scores were calculated for the affected region.

RESULTS: The median fetal DNA contribution for all samples was 18%, with the affected samples containing 17%–18% fetal DNA. Using a technique similar to that used for sequencing-based fetal aneuploidy detection from maternal plasma, we detected a statistically significant loss of representation of a portion of chromosome 22q11.2 in both of the affected fetal samples. No such loss was detected in any of the control samples.

CONCLUSIONS: Noninvasive prenatal diagnosis of subchromosomal fetal genomic anomalies is feasible with next-generation sequencing.

The field of prenatal diagnostics has advanced with the implementation of techniques that enable the characterization of circulating cell-free (ccf) fetal DNA isolated from maternal plasma. Using next-generation sequencing, multiple groups have shown that chromosomal aberrations can be detected reliably (1–6). Although the detection of trisomy 21 has been the most thoroughly validated to date—both analytically and in large-scale clinical studies—trisomies 13 and 18, sex aneuploidies, and other genetic aberrations will likely be similarly validated in the near future. One facet of genetic anomalies detected in other types of samples that has not yet been thoroughly addressed with ccf fetal DNA is subchromosomal copy number variation (CNV) (7). Previous reports have shown that approximately 12% of individuals with unexplained developmental delay/intellectual disability, autism spectrum disorder, or multiple congenital anomalies are diagnosed with clinically relevant CNV (7, 8).

One example of such a genetic condition is 22q11.2 deletion syndrome, a disorder responsible for multiple conditions, including DiGeorge syndrome, velocardiofacial syndrome, and conotruncal anomaly face syndrome. These syndromes are linked to a heterozygous deletion of approximately 3 × 106 bp on chromosome 22. Chromosome 22q11.2 deletion syndrome affects approximately 1 in 4000 live births and is characterized by frequent heart defects, cleft palate, developmental delays, and learning disabilities (9, 10).

We designed this study to determine the technical feasibility of detecting subchromosomal CNV by sequencing ccf DNA obtained from maternal plasma. We examined maternal plasma samples from 2 women, each carrying a fetus confirmed by karyotype analysis to be affected by 22q11.2 deletion syndrome; as controls, we analyzed samples from 14 women at low risk for fetal aneuploidies. The ccf DNA from each sample was sequenced for a genomic coverage of approximately 4-fold. We were able to detect a statistically significant decrease in the representation of a region of 3 × 106 bp on chromosome 22 that corresponds to the area known to be affected in the 2 verified cases.

Samples were collected under 2 separate investigational review board–approved clinical protocols (Western Institutional Review Board ID 20091396 and Compass Institutional Review Board 00462). The 2 blood samples from the affected patients were collected before an invasive procedure. The presence of a 22q11.2 microdeletion in these samples was confirmed by karyotype analysis of material obtained by nontransplacental amniocentesis. The 14 control samples were collected without a subsequent invasive proce-

3 Nonstandard abbreviations: ccf, circulating cell-free; CNV, copy number variation/variant.

We sequenced each sample with 2 lanes of an Illumina HiSeq2000 flow cell, for a genomic coverage of 3.1-fold to 4.4-fold (see Table 1 in the online Data Supplement). Reads were binned at a bin size of 50 kb, and bins were visualized across chromosome 22 for the affected samples to confirm the location of the microdeletion. Both samples that carried the 22q11.2 microdeletion exhibited decreased representation in this genomic area (see Fig. 1 in the online Data Supplement). For statistical testing, we used a consensus region of 3 × 10^6 bp located between Chr22:19000000 and Chr22:22000000 (see Fig. 1 and Table 1 in the online Data Supplement). We calculated the fraction and the z score for all autosomal reads that mapped to the target region. The control samples contained 0.075% of the reads mapping to 22q11, whereas the affected samples showed only 0.073% of reads in this region. Both affected samples had z scores lower than −3 (−5.4 and −7.1), whereas all samples from the low-risk control individuals had z scores greater than −3 (Fig. 1). Only one of the low-risk samples had a z score higher than +3. Analogous to methods used for aneuploidy detection, in which z scores less than −3 are not evaluated, the clinical relevance of this finding is unclear. Repeated analysis of the 2 affected samples at increased sequencing depth (15.87-fold to 16.77-fold)
confirmed the results (see Fig. 2 in the online Data Supplement).

Recent advances in the field of noninvasive prenatal diagnostics have facilitated the detection of fetal aneuploidies by sequencing of ccf DNA present in maternal plasma. Using a similar approach, the present study has demonstrated the feasibility of detecting subchromosome-level CNV in a developing fetus non-invasively via sequencing of the corresponding ccf DNA in maternal plasma. Albeit the number of cases is small, our data show that regions smaller than a single chromosome, in this case a deletion of 22q11.2, can be detected reliably in maternal plasma. The inclusion of additional affected and control samples would be beneficial for increasing the power of future studies. A recently published similar proof-of-concept study detected a 4.2-Mb deletion on chromosome 12 (14). This study evaluated a single case of a fetal microdeletion and detected it in a sample containing 5.7% fetal DNA obtained at a late gestational age (35 weeks). The investigators compared the results with those obtained for 7 samples known to be diploid for chromosomes 12 and 14. In contrast, the present study examined affected samples obtained at an earlier gestational age (19 and 20 weeks), used twice the number of affected and un-affected samples, and detected a microdeletion 28% smaller (3 Mb) than that of the previous study, although in a sample with a higher fetal DNA contribution (17%–18%). To detect this 3-Mb fetal deletion in our study, we used a genomic coverage of 4-fold—an increase in coverage of approximately 20-fold over that of current standard aneuploidy detection. Smaller deletions (potentially down to 0.5 Mb) or samples containing less fetal ccf DNA will require even higher coverage (15). Consequently, massively parallel shotgun sequencing will have to provide substantially higher throughput, or sequencing will have to be performed with a cost-effective targeted sequencing methodology before this method can be considered for routine clinical use.

When specific genetic aberrations are suspected, invasive procedures such as amniocentesis and chorionic villus sampling are used to provide genetic material for targeted cytogenetic analysis by well-established techniques, including G-banding, fluorescence in situ hybridization, quantitative fluorescence PCR, and microarrays (8, 16). The transition from these methodologies to nontargeted, genome-wide analysis via massively parallel shotgun sequencing will first face several technical challenges, including the need for increased data-processing capacity and sufficient genome-wide sequencing coverage. Once these challenges are overcome, the medical community will be relied on to determine the most appropriate strategies for handling the increased information provided by these genome-wide analyses, including CNVs of unknown significance. Although analyses will be performed and results will be obtained for the entire genome, these analyses should be indicated only in circumstances of a suspected fetal genetic anomaly, similar to the protocol currently used with invasive procedures. In contrast to methodologies that analyze fetal genetic material exclusively, ccf DNA analysis surely will also uncover CNVs present in the maternal genome. Before the implementation of such technology in the clinic, the medical community will need to evaluate the proper procedure for conveying such information. Despite the associated technical, medical, and ethical challenges, this novel extension of noninvasive prenatal aneuploidy detection has the potential to further advance recent progress in fetal–maternal medicine.

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References


