Implementation of a targeted enrichment method for the detection of microdeletion and microduplication syndromes for NIPT

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1. Introduction

The discovery of fetal specific cell free DNA (cfDNA) in maternal plasma has revolutionized the field of prenatal diagnosis. cfDNA enables the non-invasive prenatal screening of abnormalities like fetal aneuploidies and submicroscopic genomic alterations without the invasive procedure abortion risk [1].

Genomic alterations such as microdeletion and microdublication syndromes have high prevalence in population (Fig1) and have long been associated with severe intellectual disabilities, high morbidity and mortality rates[2-3]. Despite the technological advances sub-chromosomal copy variations detection remain challenging using current NIPT methodologies [1-3]. In this study we demonstrate the feasibility of a novel targeted enrichment method, in combination with next generation sequencing (NGS) to detect copy number variations (CNVs) using cell free DNA from plasma. This approach can effectively be implemented for the non-invasive screening of microdeletion and microduplication syndromes that occur during the first trimester of pregnancy.

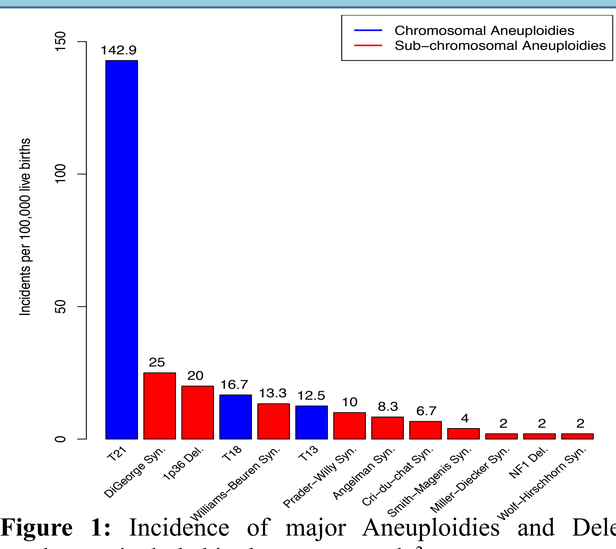

Figure 1: Incidence of major Aneuploidies and Deletion/Duplication syndromes included in the current study[3].

2. Methods

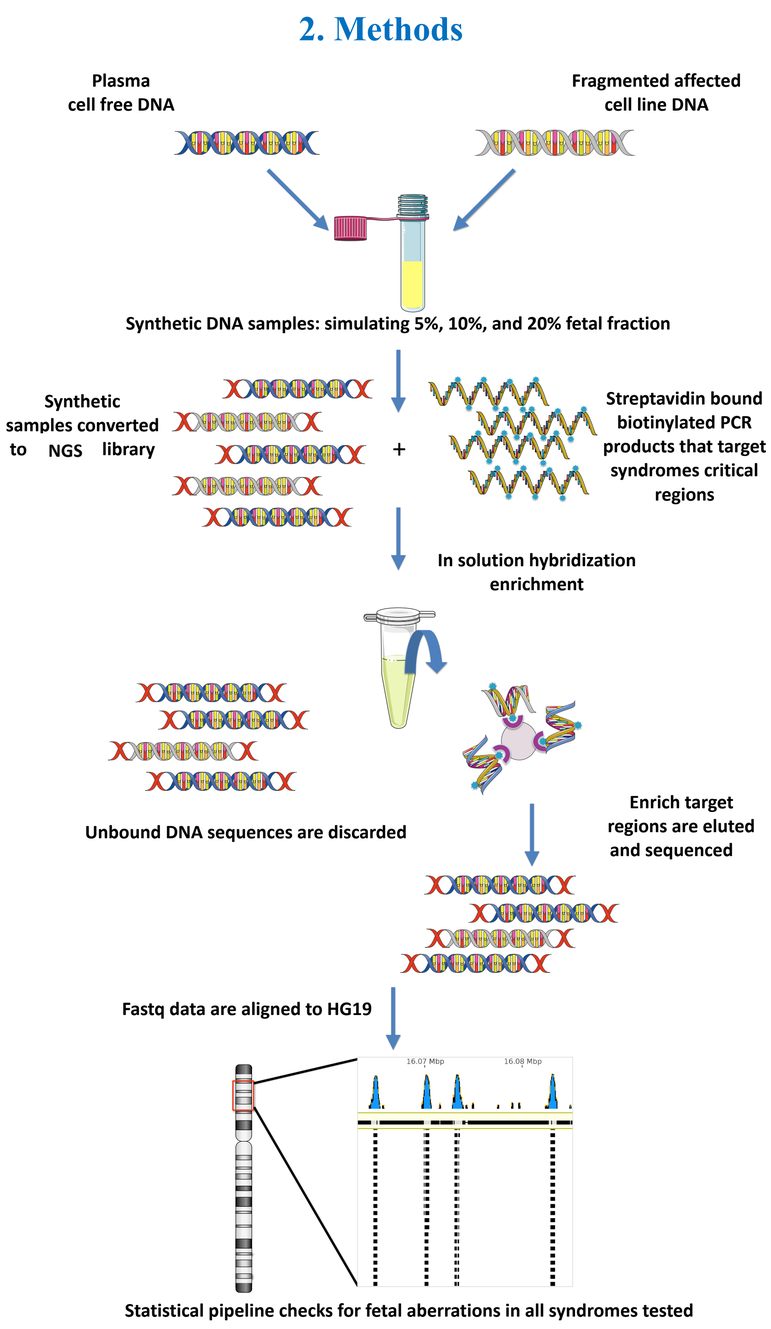

Figure 2: Plasma DNA samples and affected cell lines were used to prepare artificial spike in samples of ten syndromes, simulating 5%, 10% and 20% fetal fraction. cfDNA obtained from 10 normal pregnancy plasma samples were also used as negative controls. All samples were converted into NGS libraries and enriched in solution using biotinylated PCR products that cover the critical region of all ten syndromes . Eluted samples were then sequenced using NGS followed by a bioinformatics and statistical pipeline to determine the presence of any aberrations.

3. Results

Figure 3: Detection of Smith-Magenis (SMS) and Potocki-Lupski syndromes (PLS)

- Detection of a 3Mb deletion underlying Smith-Magenis syndrome (z-scores above 3.09)
- Detection of a 3.45Mb duplication underlying Potocki-Lupski syndrome (z-scores below 3.09)
- The aberration was detected successfully in 6 synthetic samples (red dots) with the deletion or duplication in 20% 10% and 5% of the total cfDNA
- All other synthetic (black dots) , and normal plasma samples (blue dots) show a normal representation of reads for the tested two syndromes

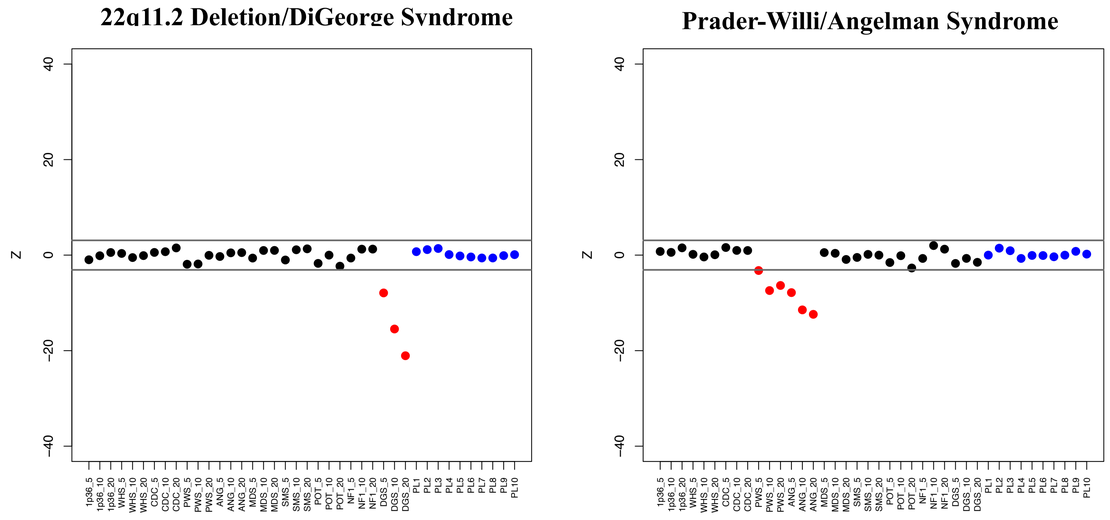

Figure 4: Detection of 22q11.2 deletion (DiGeorge) and Prader-Willi/Angelman Syndromes
Affected synthetic plasma samples with the deletion (red dots) were successfully detected. Z score exceed the defined threshold of 3.09 in all 5% 10% and 20% synthetic spikes with the deletion. In addition, 10 plasma sample (blue dots) showed a normal representation of reads for the assessed region (PL1-PL10).

Syndrome	Overlapping Syndrome	True Positives	False Negatives	True Negatives	False Positives	Sensitivity %	Specificity %
1p36 deletion		3	0	26	1	100	96.3
Cri-Du Chat		3	0	24	3	100	88.9
DiGeorge		3	0	27	0	100	100
Miller-Dieker		3	0	27	0	100	100
Prader-Willi	Angelman	6	0	24	0	100	100
Smith-Magenis	Potocki-Lupski	6	0	23	1	100	95.8
Wolf Hirschhorn		3	0	26	1	100	96.3
Neurofibromatosis1 (NF1)		3	0	27	0	100	100
TOTAL		30	0	204	6	**100**	**97.1**

Table 1: Performance of the proposed NIPT methodology for the detection of 10 different deletion and duplication syndromes using synthetic spiked samples

4. Conclusions

- Novel targeted approach using NGS for the detection of sub-chromosomal CNVs in plasma samples was developed.
- The method was applied accurately for the non invasive detection of the ten most prevalent syndromes with high clinical severity.
- Novel targeted approach proposed is a cost effective technique as it allows the screening of ten different syndromes simultaneously in multiple samples.
- The developed targeted assay can detect aberrations as low as 541 kb (e.g. Wolf-Hirschhorn Syndrome).
- Larger validation study is needed using real maternal plasma and plasma affected samples to assess the methods performance.
- Targeted methodology proposed can also be applied to for non invasive screening of fetal pathogenic point mutations, as well as cancer tumor DNA related aberrations.

5. References

[1]Wapner, R. J., et al. (2015) Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *Am. J. Obstet. Gynecol.* 212.3, 332-e1.
[2] Watson, C.T. et al. (2014). The genetics of microdeletion and microduplication syndromes: an update. Annu. Rev. Genomics Hum. Genet. 15, 215–244.
[3] Yin, A. et al. (2015). Noninvasive detection of fetal subchromosomal abnormalities by semiconductor sequencing of maternal plasma DNA. *PNAS* 112.47 (2015): 14670-14675.