The novel qNIPT concept:
Detection of a fetal trisomy 21 based on a quantitative real-time PCR

Objectives

Current non-invasive prenatal testing (NIPT) methods for the detection of fetal trisomy 21 (T21) are primarily based on next generation sequencing (NGS) strategies which are quite costly in clinical application and hence are limited to patients who can afford the testing. Here, we describe the results of a blinded study with respect to the test accuracy of a newly developed NIPT assay based on quantitative real-time PCR (qPCR) for prenatal testing of fetal trisomy 21 (qNIPT).

Method

The novel qNIPT determines the differences in methylation patterns of specific gene regions of the maternal and fetal DNA. Certain gene regions in maternal DNA are hypo-methylated whereas the same gene regions are hyper-methylated in fetal DNA. These methylation specific gene regions are used as DNA biomarkers for the determination of fetal trisomy 21 (fig. 1 and 2).

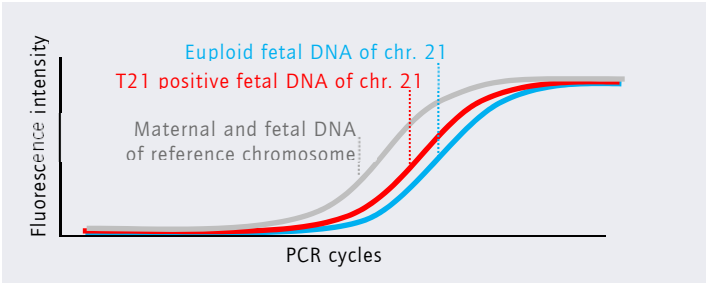

Fig. 1: Methylation specific qPCR on reference and target

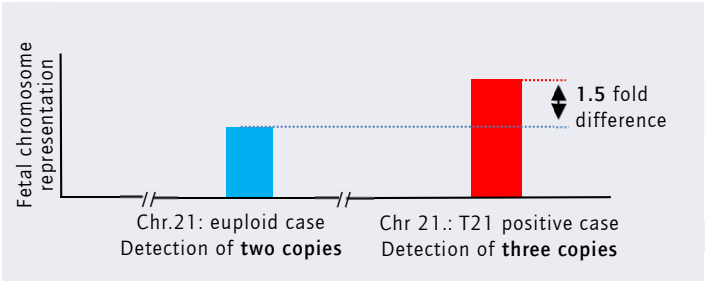

Fig. 2: Relative quantification

Following proof-of-principle and feasibility studies with around 1.500 samples, a recent blinded study was performed to assess the clinical performance in maternal blood samples from singleton pregnancies. The samples were blinded by an independent Contract Research Organization. After extraction of cell-free DNA using a QIAsymphony instrument and methylation-specific digestion of DNA samples, a multiplex qPCR was performed. The primary qPCR data were finally evaluated with the CE marked PrenaTest® DAP.plus analysis software.

Results from the analysis and from confirmatory NGS testing were compared with PrenaTest® results using NGS.[1]

Results

The study results of the maternal plasma samples (n=966) demonstrated a positive percentage agreement (PPA; equates to sensitivity) of 100 % (lower 1-sided 95% confidence interval of 91.8 %; n=35/35) and a negative percentage agreement (NPA; equates to specificity; n=931/931) of 100% compared to NGS-based PrenaTest®. The negative predictive value (NPV) for the novel qNIPT and confirmatory NGS testing was 100 % (lower 1-sided 95 % confidence interval of 99.68 %). The average fetal fraction of all examined blood samples was 8.1%. The qNIPT provided reliable test results in 54 blood samples with a fetal fraction below 4% and as low as 2.4%.

	Study 2016
Correctly classified samples	966/966 (100%)
Trisomy 21 positive	35/35 (100%)
Trisomy 21 negative	931/931 (100%)
Sensitivity (lower 1-sided 95% CI)	100% (91.88%)
Specificity (lower 1-sided 95% CI)	100% (-)
NPV (lower 1-sided 95% CI)	100% (99.68%)

Conclusion

Our results suggest that the proprietary qNIPT is a very reliable and robust method suitable for clinical routine in accordance with international medical associations.[2]

The assay represents a more cost-efficient solution over NGS testing and will also be able to provide results in the shortest possible time. While current NIPT methods require a minimum fetal fraction of 4% in blood samples from singleton pregnancies, we could demonstrate in the study that our smart qNIPT assay can be employed on blood samples with a fetal fraction of as low as 2.4%.

In summary, the smart qNIPT concept could have the potential to become a NIPT solution on a global scale for pregnant women of all ages and risk groups. Further studies which aim to include the determination of trisomy 13 and trisomy 18 are currently underway.

1 LifeCodexx AG internal data from laboratory routine (August 2012 to September 2016)
2 ACOG 2015; ESHG/ASHG 2015; ACMG 2016; ISPD 2015; Austrian-German-Swiss Recommendations for NIPT 2016; available at www.lifecodexx.com/en/for physicians/publications

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