Non-invasive prenatal diagnosis (NIPD) of cystic fibrosis by quantitative real time mutant enrichment with 3′-modified oligonucleotides (MEMO) PCR

C. Guissart1, V. Debat1, C. Bareil2, V. Viat1, M. des Georges1, M. Claustres1, M. C. Vincent1, 2, IURC, Montpellier, France; 3 INSERM, Laboratoire des Maladies Rares, Montpellier, France

In the recent years, NIPD has found new applications in monogenic diseases diagnostic for paternally inherited mutations. Such an approach remains very challenging because of the inherent limitations of molecular testing methods, the low concentration of circulating free fetal DNA (cfDNA) in maternal plasma and the “maternal contamination”.

We developed and evaluated an NIPD test for Cystic Fibrosis for the most common French mutation. It consists in searching the paternal mutation in compound heterozygous families using MEMO-PCR method (Lee et al 2011) associated with real-time PCR. The analytical validation step was made on chimeric DNA control samples that contain 0%, 2%, 5%, 10%, 50% and 100% of mutant DNA at a final concentration of 100 ng/μL. In addition, we have assessed a mini STR kit as quality control to confirm the presence of cfDNA in the maternal plasma. We obtained the first proofs of concept of our approach by the accuracy for the detection of the p.Gly542* mutation. Real-time MEMO PCR demonstrated the blocking efficiencies of normal allele with an enrichment of mutant allele and reveals differences in melting curve shape that correlate with the nature of each chimeric sample with a detection threshold of 2%. A tri-allelic profile was found for all tested maternal plasma.

Our new approach offers numerous advantages: it is simple, cost and time efficient, and applicable to different type of variants: point mutations or small insertion/deletions. Therefore, this method does not require complex equipment or bioinformatics setting and can be easily applicable in routine. Nevertheless validation studies are necessary to be manageable in clinical practice.