Supplementary materials and methods

Multigene panel testing

Library preparation. DNA libraries from FFPE samples were prepared using a commercially available kit (Integrated DNA Technologies, Iowa, USA), according to manufacturer's instructions. Briefly, 60-250 ng of DNA were subjected to enzymatic fragmentation, at 32˚C for 7 minutes followed by adaptor ligation at 20˚C for 20 minutes and clean up using magnetic beads. Next, samples were further subjected to indexing PCR and final beads-based clean up. Evaluation of library samples was performed using the 4150 Agilent Tapestation system (D1000 ScreenTape, Agilent, Waldbronn, Germany).

Library enrichment. DNA enrichment for the genomic regions of interest was carried out using an in solution-hybridization based method using TACS (TArget Capture Sequences) specifically designed to capture selected loci in the genes of interest. TACS were then immobilized on streptavidin-coated magnetic beads for subsequent hybridization with the DNA libraries based on previously established protocol (1). A custom NIPD Genetics tumour profile gene assay was used for the identification of single nucleotide variants (SNVs), small insertions and deletions (InDels), copy number alterations (CNAs) and rearrangements (Table SI). Eluted samples were amplified using outer-bound adaptor primers. Enriched DNA libraries were then normalized and subjected to sequencing on NextSeq (Illumina, San Diego, USA).

Reference

1. Koumbaris G, Kypri E, Tsangaras K, Achilleos A, Mina P, Neofytou M, Velissariou V, Christopoulou G, Kallikas I, González-Liñán A, *et al*: Cell-free DNA analysis of targeted genomic regions in maternal plasma for non-invasive prenatal testing of trisomy 21, trisomy 18, trisomy 13, and fetal sex. Clin Chem 62: 848-855, 2016.

Figure S1. Summary of structural findings. (A) Distribution of different types of structural variants identified in the cohort. (B) Frequency of structural variants per gene. (C) Frequency of single CNAs and concurrent events. CNAs, copy number alterations; FGFR3, fibroblast growth factor receptor 3; TACC3, transforming acidic coiled-coil containing protein 3.