Cell-free DNA as a diagnostic analyte for molecular diagnosis of vascular malformations

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Objectives
- Determine the feasibility of cell-free DNA (cfDNA) as an analyte for molecular diagnosis of vascular malformations (VM).
- Describe a multiplex droplet digital PCR (ddPCR) assay used to detect PIK3CA mutations.
- Report the use of cyst fluid cfDNA as an analyte for genetic testing.

Methods
- Plasma and cyst fluid samples obtained from frozen biorepository or prospectively collected.
  - Prospective samples collected in Streck tubes: designed for cfDNA, decrease genomic DNA (gDNA) contamination.
  - Plasma separated from whole blood or cyst fluid supernatant from pellet by centrifugation.
  - cfDNA and gDNA isolated from plasma/cyst fluid supernatant and cyst fluid pellet, respectively.
  - Droplet digital PCR (ddPCR) used to determine mutation status.
  - Samples with known mutations from prior testing: ddPCR singleplex screening
  - Samples with unknown mutation status: ddPCR multiplex screening

Results

Mutations detected in plasma cfDNA from patients with AVM, VeM, and LM
Plasma samples obtained for 38 individuals: 8 with AVM (15 samples), 3 with VeM (4 samples), 27 with LM (36 samples). All were screened for known mutations with singleplex ddPCR (Fig. 1). Only 4/38 (10.5%) of the individuals had a detectable mutation on ddPCR (Table 1, only patients with detectable mutations shown).

Mutations detected in cyst fluid cfDNA from patients with LM and known mutations
Seven individuals with known mutations from prior testing had cyst fluid available. Mutations were detected in cyst fluid cfDNA in 7/7 individuals (100%) (Table 2). 5/7 had higher variant allele fractions (VAF) in cyst fluid cfDNA compared to tissue. Only 3/7 pellet gDNA samples tested positive.

Multiplex ddPCR used for prospective mutation detection from cyst fluid cfDNA
A multiplex ddPCR previously developed for PIK3CA mutation detection in cancer10 (Fig. 2) was used to screen cyst fluid cfDNA in individuals who had not had surgery previously. 4/5 cyst fluid cfDNA samples had mutations detected on multiplex ddPCR and confirmed on singleplex ddPCR (Table 3).

Discussion
We detected somatic activating variants in cfDNA from plasma and cyst fluid in individuals with AVM, VeM, and LM. Multiplex ddPCR allowed for prospective molecular diagnosis from LM cyst fluid cfDNA in individuals without LM tissue resection. This represents an opportunity to initiate targeted medical therapy in VM patients prior to surgery. Furthermore, our results support future exploration of the use of cfDNA in diagnosis of non-neoplastic congenital disorders due to post-zygotic activating variants.

References