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Original Article

## **A pilot study of next generation sequencing–liquid biopsy on cell-free DNA as a novel noninvasive diagnostic tool for Klippel–Trenaunay syndrome**

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### **Abstract**

**Objectives:** Somatic mosaicism of PIK3CA gene is currently recognized as the molecular driver of Klippel–Trenaunay syndrome. However, given the limitation of the current technologies, PIK3CA somatic mutations are detected only in a limited proportion of Klippel–Trenaunay syndrome cases and tissue biopsy remains an invasive high risky, sometimes lifethreatening, diagnostic procedure. Next generation sequencing liquid biopsy using cell-free DNA has emerged as an innovative non-invasive approach for early detection and monitoring of cancer. This approach, overcoming the spacetime profile constraint of tissue biopsies, opens a new scenario also for others diseases caused by somatic mutations.

**Methods:** In the present study, we performed a comprehensive analysis of seven patients (four females and three males) with Klippel–Trenaunay syndrome. Blood samples from both peripheral and efferent vein from malformation were collected and cell-free DNA was extracted from plasma. Tissue biopsies from vascular lesions were also collected when available. Cell-free DNA libraries were performed using Oncomine™ Pan-Cancer Cell-Free Assay. Ion Proton for sequencing and Ion Reporter Software for analysis were used (Life Technologies, Carlsbad, CA, USA).

**Results:** Cell-free circulating DNA analysis revealed pathogenic mutations in PIK3CA gene in all patients. The mutational load was higher in plasma obtained from the efferent vein at lesional site (0.81%) than in the peripheral vein (0.64%) leading to conclude for a causative role of the identified variants. Tissue analysis, available for one amputated patient, confirmed the presence of the mutation at the malformation site at a high molecular frequency (14–25%), confirming its causative role.

**Conclusions:** Our data prove for the first time that the cell-free DNA-next generation sequencing–liquid biopsy, which is currently used exclusively in an oncologic setting, is indeed the most effective tool for Klippel–Trenaunay syndrome diagnosis and tailored personalized treatment.

**Keywords**

