UAB MEDICAL GENOMICS LABORATORY

Capillary Malformation-Arteriovenous Malformation Syndrome Panel: Next-Gen Sequencing of *RASA1* and *EPHB4* and Deletion/Duplication Analysis of *RASA1* (RASA-NG)

Ordering Information

Acceptable specimen types:

- Fresh blood sample (3-6 ml EDTA; no time limitations associated with receipt)
- Saliva (OGR-575 DNA Genotek; kits are provided upon request)
- DNA (extracted from lymphocyte cells; a minimum volume of 25µL at 3µg; O.D. of 260:280nm ≥1.8; must be extracted in a CLIA or equivalent certified lab)

Turnaround time:

30 working days

Price, CPT codes, and Z code:

$1000 (USD – institutional/self-pay);

CPT: 81479 (x2)

Z code: ZB6AB

Candidates for this test:

Patients with features suggestive for Capillary Malformation Arteriovenous Malformation or Parkes Weber syndrome

Specimen shipping and handling:

- Please find acceptable specimen type above.

- All submitted specimens must be sent at room temperature. DO NOT ship on ice.
- Specimens must be packaged to prevent breakage and absorbent material must be included in the package to absorb liquids in the event that breakage occurs. Also, the package must be shipped in double watertight containers (e.g. a specimen pouch + the shipping company's diagnostic envelope).
- To request a sample collection kit, please visit the website or email medgenomics@uabmc.edu to complete the specimen request form.
- Please contact the MGL (via email at medgenomics@uabmc.edu, or via phone at 205-934-5562) prior to sample shipment and provide us with the date of shipment and tracking number of the package so that we can better ensure receipt of the samples.

Required forms:

- Test Requisition Form
- Form for Customs (for international shipments)

Note: Detailed and accurate completion of this document is necessary for reporting purposes. The Medical Genomics Laboratory issues its clinical reports based on the demographic data provided by the referring institution on the lab requisition form. It is the responsibility of the referring institution to provide accurate information. If an amended report is necessary due to inaccurate or illegible documentation, additional reports will be drafted with charge.

Requests for testing may not be accepted for the following reasons:

- No label (patients full name and date of collection) on the specimens
- No referring physician's or genetic counselor's names and addresses
- No billing information
- DNA samples must be extracted in a CLIA or equivalent certified lab

For more information, test requisition forms, or sample collection and mailing kits, please call: 205-934-5562.

Disorder Background

Capillary malformation-arteriovenous malformation syndrome (CM-AVM) is a disorder of the vascular system. Parkes Weber syndrome is characterized by congenital vascular abnormalities known as capillary malformations and arteriovenous fistulas (AVFs). Some vascular abnormalities seen in Parkes Weber syndrome are similar to those that occur in capillary malformation-arteriovenous malformation syndrome (CM-AVM). CM-AVM and some cases of Parkes Weber syndrome are caused by variants in the *RASA1* gene. (Erola I et al, Am J Hum Genet. 2003;73:1240–9). In addition, loss-of-function variants in the *EPHB4* gene have been associated with similar phenotypic presentations (Amyere M et al, Circulation. 2017;136(11):1037-48). *EPHB4* interacts with *RASA1* and indicates the EPHB4-RAS-ERK signaling pathway as a major cause for AVMs. Therefore, the CM-AVM Syndrome panel includes both *RASA1* and *EPHB4* sequencing coverage and deletion/duplication analysis of *RASA1*.

Test Description

The ***RASA1* and *EPHB4* by NGS** involves sequencing for *RASA1* and *EPHB4* as well as **deletion/duplication analysis** of the entire coding *RASA1* region. The test uses an extensively customized and optimized set of Agilent HaloPlex capture probes, followed by sequencing of overlapping amplicons within the regions of interest using 300bp paired-end Illumina sequencing chemistry. Each coding exon plus ~50bp of flanking intronic sequence are simultaneously sequenced. 5' and 3' untranslated sequences are not included.

The average coverage is ~1500x with 93% of the coding regions ≥350x and 97% ≥200x. This allows for detection of very low-level mosaicism by sequencing (as low as 3-5% of the alleles). Variant and copy number calls are made using a unique bioinformatics pipeline detecting all types of variants including single nucleotide substitutions, indels, and frameshifts caused by deletion/ duplication up to 112bp.

REFERENCES available on website.

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