Cerebral Cavernous Malformation Panel, Sequencing and Deletion/Duplication

Cerebral cavernous malformations (CCMs) are vascular malformations occurring in the brain or other central nervous system locations, which involve closely clustered, enlarged capillary channels without normal intervening brain parenchyma. CCMs do not always cause clinical symptoms but may result in intracranial hemorrhage, seizures, headaches, or focal neurological deficits without intracranial bleed. Familial CCM (FCCM) is defined by the presence of multiple CCMs, a single CCM and at least one family member with one or more CCMs, or a pathogenic heterozygous variant in one of the associated genes (CCM2, KRIT1, or PDCD10).

Disease Overview

Symptoms

- CCM may result in intracranial hemorrhage (25-32%), and symptoms such as seizures (40-70%), headaches (10-30%), or focal neurological deficits without intracranial bleed (25-50%).
- CCMs may increase in number over time; lesions may also decrease or increase in size over time.
- Cutaneous vascular lesions (9%) or retinal vascular lesions (5%) may be present in FCCM.
- CCM disease presentation often first occurs in the second to fifth decade of life, but may occur at any age.
- FCCM resulting from PDCD10 variants may result in a more severe disease course and manifest at younger ages, compared to causative KRIT1 or CCM2 variants.

Genetics

See Genes Tested table

Etiology

Pathogenic germline variant in CCM2, KRIT1, or PDCD10 genes

Penetrance

Up to 50% of individuals with a molecular diagnosis of FCCM remain clinically asymptomatic.
Epidemiology

Based on autopsy studies, CCMs occur in approximately 0.4-0.5% of the general population. FCCM is estimated to occur in 1:2,000 to 1:10,000 individuals and up to 20% of all CCMs are familial.²

Inheritance

Autosomal dominant with reduced penetrance; the frequency of de novo variants is unknown.

Pathogenic Founder Variants

- **KRIT1** c.1363C>T; p.Gln455Ter: common in individuals with ancestry from northern Mexico and the Southwestern United States¹
- **CCM2** deletion of exons 2-10: identified in up to 22% of affected individuals in U.S. populations¹
- **CCM2** c.30+5_30+6delGCinsTT: identified in unrelated Ashkenazi Jewish kindreds¹

Test Interpretation

Methodology

This test is performed using the following sequence of steps:

- Selected genomic regions, primarily coding exons and exon-intron boundaries, from the targeted genes are isolated from extracted genomic DNA using a probe-based hybrid capture enrichment workflow.
- Enriched DNA is sequenced by massively parallel sequencing (MPS; also known as NGS) followed by paired-end read alignment and variant calling using a custom bioinformatics pipeline. The pipeline includes an algorithm for detection of large (single exon-level or larger) deletions and duplications.
- Sanger sequencing is performed as necessary to fill in regions of low coverage and in certain situations, to confirm variant calls.
- Large deletion/duplication calls made using MPS are confirmed by an orthogonal exon-level microarray when sample quality and technical conditions allow.

Clinical Sensitivity

- 85-95% for FCCM¹
- The majority of identifiable pathogenic variants in **CCM2**, **KRIT1**, and **PDCD10** are sequence variants. Large deletions and duplications account for 20-25% of identifiable pathogenic variants in these genes.

Analytic Sensitivity
<table>
<thead>
<tr>
<th>Variant Class</th>
<th>Analytic Sensitivity (PPA) Estimatea (%) and 95% Credibility Region (%)</th>
<th>Analytic Specificity (NPA) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNVs</td>
<td>&gt;99 (96.9-99.4)</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Deletions 1-10 bpb</td>
<td>93.8 (84.3-98.2)</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Insertions 1-10 bpb</td>
<td>94.8 (86.8-98.5)</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Exon-levelc deletions</td>
<td>97.8 (90.3-99.8) [2 exons or larger]</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td></td>
<td>62.5 (38.3-82.6) [Single exon]</td>
<td></td>
</tr>
<tr>
<td>Exon-levelc duplications</td>
<td>83.3 (56.4-96.4) [3 exons or larger]</td>
<td>&gt;99.9</td>
</tr>
</tbody>
</table>

aGenes included on this test are a subset of a larger methods-based validation from which the PPA values are derived. These values do not apply to testing performed by multiplex ligation-dependent probe amplification (MLPA).
bVariants greater than 10 bp may be detected, but the analytic sensitivity may be reduced.
cIn most cases, a single exon deletion or duplication is less than 450 bp and 3 exons span a genomic region larger than 700 bp.

bp, base pairs; PPA, positive percent agreement; NPA, negative percent agreement; SNVs, single nucleotide variants

Results

<table>
<thead>
<tr>
<th>Result</th>
<th>Variant(s) Detected</th>
<th>Clinical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>One CCM2, KRIT1, or PDCD10 pathogenic variant detected</td>
<td>Consistent with a diagnosis of FCCM; individual may or may not develop clinical symptoms</td>
</tr>
<tr>
<td>Negative</td>
<td>No CCM2, KRIT1, or PDCD10 pathogenic variants detected</td>
<td>Diagnosis of FCCM is unlikely but not excluded</td>
</tr>
<tr>
<td>Uncertain</td>
<td>CCM2, KRIT1, or PDCD10 variant of unknown clinical significance detected</td>
<td>It is unknown whether variant is benign or pathogenic</td>
</tr>
</tbody>
</table>

Limitations

- A negative result does not exclude a diagnosis of FCCM.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- The following will not be evaluated:
  - Variants outside the coding regions and intron-exon boundaries of targeted genes
  - Regulatory region and deep intronic variants
  - Breakpoints of large deletions/duplications
- The following may not be detected:
  - Deletions/duplications/insertions of any size by massively parallel sequencing
  - Large duplications less than 3 exons in size
  - Single exon deletions/duplications in the following exons:
- *CCM2* (NM_001363458) 7
- *CCM2* (NM_001363459) 6
  - Noncoding transcripts
  - Some variants due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions
  - Low-level somatic variants

### Genes Tested

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Aliases</th>
<th>MIM #</th>
<th>Disorders</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCM2</td>
<td>C7orf22, MGC4607, OSM</td>
<td>607929</td>
<td>CCM2</td>
<td>AD</td>
</tr>
<tr>
<td>KRIT1</td>
<td>CCM1, CAM</td>
<td>604214</td>
<td>CCM1</td>
<td>AD</td>
</tr>
<tr>
<td>PDCD10</td>
<td>CCM3, TFAR15</td>
<td>609118</td>
<td>CCM3</td>
<td>AD</td>
</tr>
</tbody>
</table>

AD, autosomal dominant

### References
