**KRIT1, CCM2 and PDCD10 Analysis in Familial Cerebral Cavernous Malformations**

**Mendelian Inheritance in Man Number:** 116860 (cerebral cavernous malformations 1); 604214 (KRIT1 gene); 603284 (cerebral cavernous malformations 2); 607929 (CCM2 gene); 603285 (cerebral cavernous malformations 3); 609118 (PDCD10 gene, also known as TFAR15 gene)

**Includes:** CCM1, CCM2, and CCM3

**Clinical features:**
Cerebral cavernous malformations (CCM) are predominantly central nervous system (CNS) vascular lesions formed by a cluster of grossly dilated blood vessels. Each vessel is comprised of a single layer of epithelium without normal intervening brain parenchyma or vascular support cells. Symptoms typically present in the 2nd-5th decades and can include seizures, focal neurological deficits, chronic headaches, epilepsy, stroke, and cerebral hemorrhage. Interfamilial and intrafamilial variability of symptoms has been noted. Individuals may be clinically asymptomatic; MRI may be necessary to diagnose asymptomatic lesions in at-risk individuals. Although the vascular anomalies primarily affect the CNS, lesions also have been reported in the retina (Labauge et al, 2006; Davenport et al, 2001) and skin, as hyperkeratotic cutaneous capillary-venous malformations (Chen et al., 2002; Eerola et al, 2000). CCM occurs in 0.1-0.5% of the general population and can occur isolated or as a familial form. Familial CCM is defined as the presence of CCM in at least two family members, and/or the presence of an identified disease causing mutation in one of the three genes known to be associated with CCM (KRIT1, CCM2, PDCD10), and/or the presence of multiple CCMs in an individual. The prevalence of the familial form is estimated to be as high as 50% within the Southwest American Hispanic population (due to a founder mutation in the KRIT1 gene) and as high as 10-40% within the Caucasian population (Riant et al, 2010). Penetrance is incomplete, which may account for unrecognized familial forms initially thought to be sporadic.

**Inheritance pattern:**
Autosomal dominant with incomplete clinical and neuroradiological penetrance.

**Genetics:**
Cerebral cavernous malformations are known to be caused by mutations in three genes at this time: KRIT1, CCM2, and PDCD10. Studies have suggested that 40-65% of mutations would be identified in KRIT1, 15-20% in CCM2, and 10-15% in PDCD10. KRIT1 (Krev Interaction Trapped 1) is located on chromosome 7q11.2-q22 and has 16 exons coding for the KRIT1 protein. The KRIT1 protein is comprised of 736 amino acids containing four ankyrin domains and a FERM domain. Individuals of Southwest American Hispanic ancestry commonly have a founder mutation in KRIT1. CCM2, located on chromosome 7p13, is a 10 exon gene that codes for the MGC4607 protein, also known as malcaverin, which contains a phosphotyrosine binding domain. CCM3 is due to mutations in the PDCD10 (programmed cell death 10) gene located on chromosome 3q26.1 and has 7 exons. The CCM3 protein has no known conserved functional domain. KRIT1, CCM2, and PDCD10 form a protein complex, with CCM2 acting as a linker protein. This protein complex is connected to the plasma membrane and helps to regulate cell-cell adhesion, cell shape and polarity, and likely cell adhesion to the extracellular matrix (Faurobert and Rizo, 2010). The CCM phenotype is hypothesized to occur as the result of Knudson’s two-hit hypothesis, where individuals are born with one mutation and develop lesions only after a second somatic mutation (“hit”) is acquired.

**Reasons for referral:**
- Confirmation of a clinical diagnosis
- Identification of family members at-risk for cerebral cavernous malformations (CCMs)
- Genetic counseling and recurrence risk assessment
- Prenatal diagnosis in families with a known mutation

**Test method:**
Bi-directional sequence analysis of the complete coding region of the KRIT1 (exons 5-20), CCM2 (exons 1-10) and PDCD10 genes (exons 2-8) can be provided sequentially (reflex testing) or concurrently. An individual’s clinical history and ethnic background should be taken into consideration in determining the best testing approach (please refer to the flow chart below).
For **KRIT1**, sequence analysis is offered in two tiers. Tier 1 includes analysis of exons 14, 16, and 18 (previously published as exons 13, 15, and 17 using alternate nomenclature), in which 56% of the mutations in KRIT1 have been identified (Cavé-Riant et al, 2002). Tier 1 includes testing for the common Southwest-American Hispanic Q455X nonsense mutation. If negative, sequencing the remaining exons of **KRIT1** (Tier 2) analysis is performed along with deletion/duplication analysis using ExonArrayDx, a targeted array CGH with exon level resolution, to evaluate for a deletion or duplication in one or more exons of these genes (**KRIT1**, **CCM2**, and **PDCD10**). Approximately 81% of individuals with familial CCM are expected to have a mutation identified in KRIT1 Tier 1 or Tier 2 testing. If an individual presents with clinical symptoms in childhood (<15 years of age), sequencing of **PDCD10** should be considered first (Denier et al, 2006). As an alternative to sequential testing, sequence and deletion/duplication analysis for all three genes (KRIT1, CCM2, and PDCD10) can be ordered concurrently as one comprehensive test (test code 526). Mutations found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis, or other appropriate method.

**Mutation spectrum**

Nonsense, missense, splicing, and insertion/deletion mutations have been reported in the **KRIT1**, **CCM2**, and **PDCD10** genes. At this time, 150 unique mutations have been noted to cause CCM in these three genes (Riant et al, 2010). Nearly all mutations that have been identified in these genes lead to premature termination codons. These are loss of function mutations leading to mRNA decay or protein truncation (haploinsufficiency). A founder mutation for individuals of Southwest American Hispanic ancestry is reported in the **KRIT1** gene (Q455X). Deletion/duplication studies have been conducted and gross deletions have been noted in all three genes. Most notable is a common 77.6 kb deletion spanning exons 2-10 in the **CCM2** gene; deletions within **CCM2** have been reported more frequently compared to deletions in **KRIT1** and **PDCD10**.

**Test sensitivity**

The likelihood of an individual with a clinical diagnosis of familial CCM having an identified mutation in **KRIT1**, **CCM2**, or **PDCD10** is 78% (Denier et al, 2006); the sensitivity increases to 94% in families with at least two affected individuals (Stahl et al, 2008; Denier et al, 2006). Half of the familial cases in the Southwest-American Hispanic population are caused by the founder mutation Q455X in **KRIT1**. In Caucasian familial cases, 72% of the identified mutations are in **KRIT1**, 18% in **CCM2**, and 10% in **PDCD10** (Riant et al, 2010). In isolated cases, when affected individuals have multiple lesions, the mutation detection rate ranges between 45% and 67% (Riant et al, 2010). Individuals with symptoms presenting before 15 years of age are more likely to have mutations in **PDCD10** than **KRIT1** or **CCM2** (Denier et al, 2006). In one study, large deletions in either **KRIT1**, **CCM2**, or **PDCD10** were detected in 60% of individuals that had no identifiable mutation by gene sequencing (Liquori et al, 2007). Overall, deletions have been detected in 9-24% of individuals with familial CCM (Stahl et al, 2008; Liquori et al, 2007).

The following chart may be used as a guide to determine the best approach for sequential testing.

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  • Age of onset < 15 years  ➔ PDCD10 sequencing

  • Southwest-American Hispanic ancestry
    • Age of onset > 15 years
  ➔ KRIT1 Tier 1 sequencing of exons 14, 16, and 18 of **KRIT1** gene

  ➔ KRIT1 Tier 2: rest of **KRIT1** and deletion/duplication data
    **KRIT1**, **CCM2**, and **PDCD10**

  ➔ **CCM2** sequencing
  ➔ Stop here if **PDCD10** already sequenced.

  ➔ **PDCD10** sequencing
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Specimen Requirements and Shipping/Handling:

- **Blood**: A single tube with 1-5 mL whole blood in EDTA. Ship overnight at ambient temperature, using a cool pack in hot weather. Specimens may be refrigerated for 7 days prior to shipping.
- **Buccal Brushes**: NOT ACCEPTED FOR THIS TEST.
- **Prenatal Diagnosis**: For prenatal testing for a known mutation in the KRIT1, CCM2, or PDCD10 genes, please refer to the specimen requirements table on our website at: [http://www.genedx.com/test-catalog/prenatal/](http://www.genedx.com/test-catalog/prenatal/). Ship specimen overnight at ambient temperature, using a cool pack in hot weather.

Required Forms:

- Sample Submission (Requisition) Form – complete all pages
- Payment Options Form or Institutional Billing Instructions

For test codes, prices, CPT codes, and turn-around-times, please refer to the “Cerebral Cavernous Malformation” page on our website: [www.genedx.com](http://www.genedx.com)

ICD9 codes that might apply to new patients having this diagnostic test -

- 747.81 anomalies of cerebrovascular system; 228 Angioma NOS; 747.6 Other anomalies of peripheral vascular system;
- 325 Phlebitis and thrombophlebitis of intracranial venous sinuses

References cited: