KRIT1, CCM2 and PDCD10 Analysis in Familial Cerebral Cavernous Malformations

PANEL GENE LIST
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[7,3] and skin, as hyperkeratotic cutaneous capillary-venous malformations [2,5]. 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 pathogenic variant 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[9]. Penetrance is incomplete, which may account for unrecognized familial forms initially thought to be sporadic.

GENETICS
Autosomal dominant with incomplete clinical and neuroradiological penetrance.

Cerebral cavernous malformations are known to be caused by pathogenic variants in three genes at this time: KRIT1, CCM2, and PDCD10. Studies have suggested that 40-65% of variants 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 mutationin 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 variants 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[6]. The CCM phenotype is hypothesized to occur as the result of Knudson's two-hit hypothesis, where individuals are born with one variant and develop lesions only after a second somatic variant ("hit") is acquired.

TEST METHODS
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).

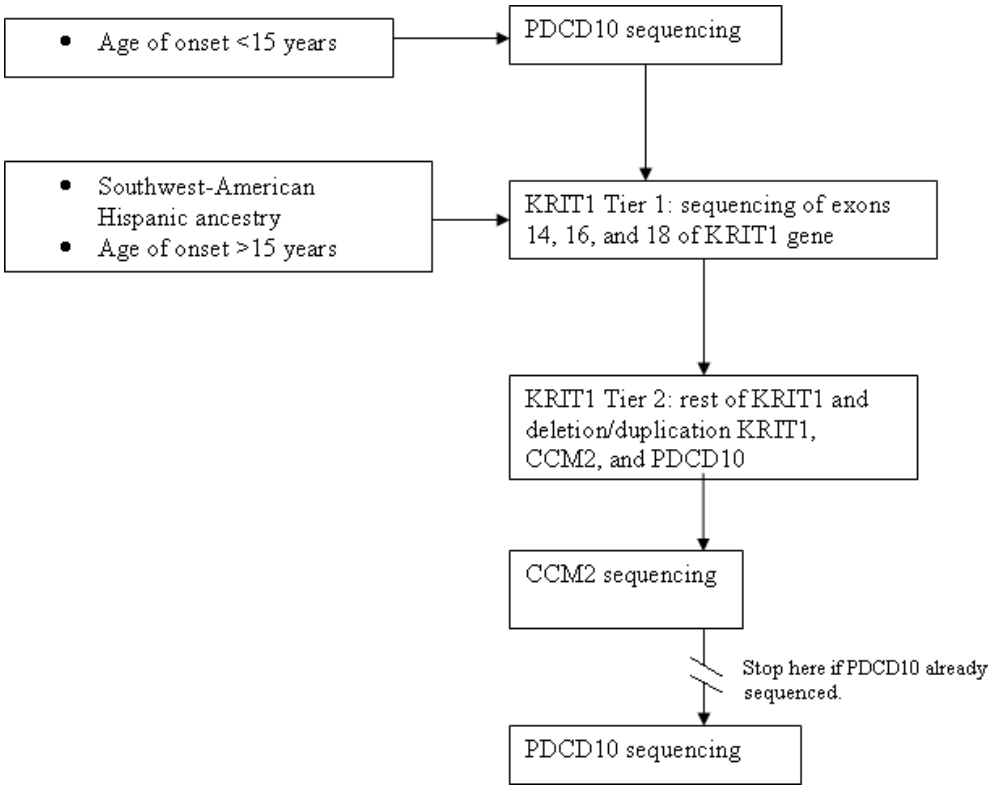
For KRIT1, sequence analysis is offered in two tiers. Tier 1 includes analysis of exons 14, 16, and 18 (previously published as exons 13, 15, and 17 using alternate nomenclature), in which 56% of the variants in KRIT1 have

been identified1. Tier 1 includes testing for the common Southwest-American Hispanic Q455X nonsense variant. 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 variant 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). Variants 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.

TEST SENSITIVITY

The likelihood of an individual with a clinical diagnosis of familial CCM having an identified pathogenic variant in KRIT1, CCM2, or PDCD10 is 78%[4]; the sensitivity increases to 94% in families with at least two affected individuals [11,4]. 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 variants are in KRIT1, 18% in CCM2, and 10% in PDCD10[9]. In isolated cases, when affected individuals have multiple lesions, the variant detection rate ranges between45% and 67%9. Individuals with symptoms presenting before 15 years of age are more likely to have variants in PDCD10 than KRIT1 or CCM24. In one study, large deletions in either KRIT1, CCM2, or PDCD10 were detected in 60% of individuals that had no identifiable variant by gene sequencing[8]. Overall, deletions have been detected in 9-24% of individuals with familial CCM[11,8].

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



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