Systemic Hemangiomatosis with a Deletion in the KRIT1 Gene: An Unusual Manifestation of Cerebral Cavernous Malformation

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Introduction

Cavernous hemangiomas are common hamartomatous vascular proliferations often seen as incidental solitary lesions. Rarely they occur as multifocal lesions, often in characteristic locations as part of a hereditary syndrome. We report a case of systemic hemangiomatosis with multifocal involvement of the CNS, spleen, liver, adrenal, and axial skeleton recognized at autopsy. Systemic hemangiomatosis involving solid organs, the central nervous system, and bone has been described in case reports(1), but this collection of findings is exceedingly rare. We hypothesized that the anomalies noted at autopsy in this case might represent sporadic cerebral cavernous malformation (CCM1), specifically *de novo* mutation of KRIT1.

Case Report

A 76 yo female with a complicated past medical history including paraplegia secondary to spinal surgery 40 years ago, died following a short hospitalization. She had multiple complications of her long-term paraplegia, including chronic decubitus ulcers and multiple urinary tract infections secondary to neurogenic bladder. She also had a sudden-onset cardiac event during her hospitalization. An autopsy was performed to clarify the cardiac and neurologic disorders.

Autopsy examination revealed that the patient died as a result of cardiorespiratory complications related to a partially thrombosed left anterior descending coronary artery aneurysm. Additionally, a unique constellation of cavernous hemangiomas involving the central nervous system, bone, and several solid organs was identified. Other findings included bilateral renal papillary adenomas, bilateral renal cortical cysts, and multiple seborrheic keratoses (see figures 1-3).

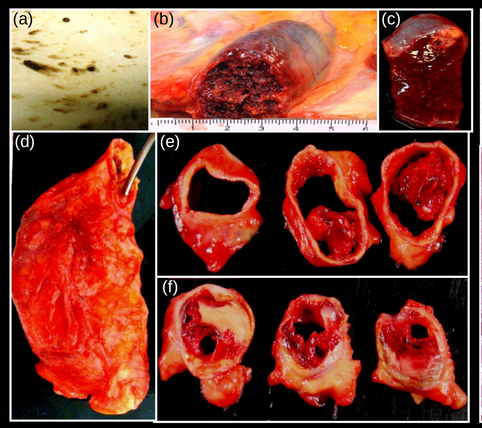


Figure 1: gross autopsy findings including (a) seborrheic keratoses, (b) rib hemangioma, (c) spleen hemangioma, and (d) LAD coronary artery aneurysm (e,f = cross sections of aneurysm highlight partially occluding thrombus)

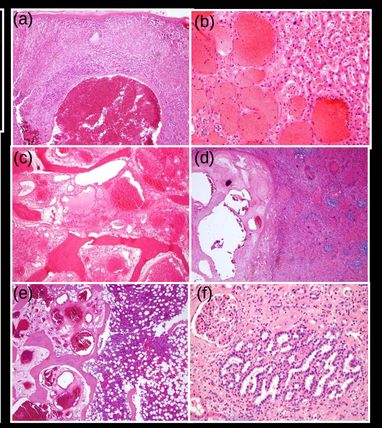


Figure 2: histologic images of (a) adrenal gland hemangioma, (b) liver hemangioma, (c) rib hemangioma, (d) spleen hemangioma, (e) vertebral body hemangioma, (f) renal papillary adenoma (H&E stain, 40-100x magnification)

Case Report (continued)

The unusual pattern of pathologic abnormalities prompted the consideration of an underlying genetic disorder. Cavernous hemangiomas of the CNS, termed cerebral cavernous malformations, characteristically occur in three clinically-similar genetic disorders: CCM1, CCM2, and CCM3. These disorders result from mutations in the KRIT1, CCM2, or PDCD10 gene, respectively. Although extremely rare, CCM1 is by far the most common of these disorders(2). DNA was extracted from formalin fixed, paraffin-embedded tissue, including the cerebral hemangiomas and normal brain, and amplified by polymerase chain reaction. Analysis of the DNA product using bidirectional sequencing was performed to identify potential abnormalities in the KRIT1 gene which is located on the long arm of chromosome seven, specifically 7q21.2.

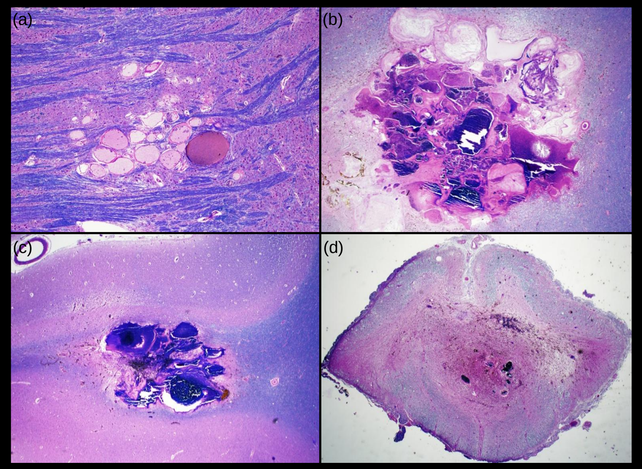


Figure 3: histologic images of CNS cavernous hemangiomas including (a) pons, (b) right frontal lobe, (c) right occipital lobe, and (d) thoracic spinal cord (luxol-fast blue H&E stain, 20-40x magnification)

Materials and Methods

Appropriate IRB approval was obtained for genetic study of the case. Approximately 50 mg of formalin-fixed tissue from lesional areas in the cerebrum and cerebellum, as well as uninvolved brain tissue, was washed in PBS overnight. Genomic DNA was extracted using DNeasy blood & tissue kit (Qiagen, Valencia, CA). DNA concentration and purity were determined by a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). Normal male and female genomic DNA from pooled human peripheral blood were purchased from Promega (Madison, WI). Exon 15 of the KRIT1 gene and its flanking intronic region were amplified by PCR using primers 5'- GGTGGGATTCTTAAGTACATCTGC-3' and 5'-GGCTTTTATACAGCTCCTGATGC-3'. The amplified fragments were purified with agarose gel electrophoresis and QIAquick gel extraction kit. Sequencing was performed at the University of Missouri DNA core facility on a 3730xl 96-capillary DNA Analyzer (Applied Biosystems). The data was analyzed with the software Sequence Scanner v1.0.

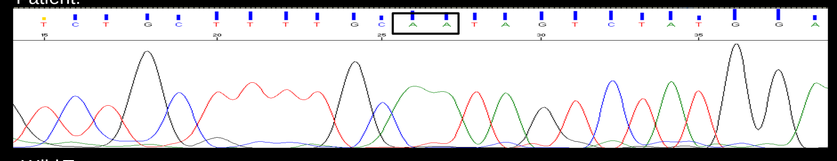
Results

A single nucleotide deletion (c2463 of exon 15, Krit1 transcript NM_194456.1) was identified from all 3 DNA samples isolated from the patient, while sequencing of normal genomic DNA (both male and female) revealed wild-type sequence (see figure 4).

GAT GAA GCC AGA TAT AAT TTA TTG AAG GGC TTT TAT ACA GCT CCT GAT GCT AAG CTG
D E A R Y N L L K G F Y T A P D A K L

ATA ACA TTG GCA AGT CTG CTT TTG CAA **TAG** TCTATGGAAATTATGAGAGT
I T L A S L L L Q stop

Patient:



Wild Type:

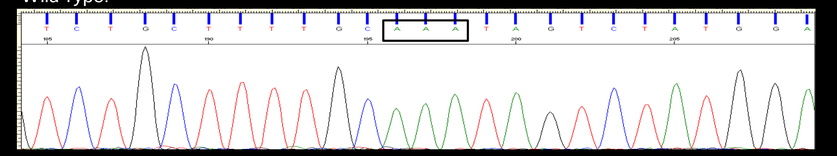


Figure 4: (top) patient KRIT1 exon 15 DNA sequence highlighting the amino acid sequence and premature stop codon, (middle) patient KRIT1 exon 15 sequence highlighting the single nucleotide deletion, (bottom) wild type KRIT1 exon 15 sequence

Discussion and Conclusion

CCM1 results from mutations in the Krev1 Interaction Trapped-1 (KRIT1) gene, which encodes a microtubule-associated protein and is thought to play a role in regulating endothelial cell shape and function as well as cell-cell and cell-matrix interactions(3). These abnormalities lead to cavernous hemangioma formation and increased vascular fragility, often resulting in hemorrhage. Most often, the syndrome is inherited in an autosomal dominant pattern, representing the familial form of the disorder. However, sporadic mutations in KRIT1 have been described (4).

This case highlights the potential clinical utility of PCR and DNA sequencing in the diagnosis of unique and clinically-significant genetic disorders. The current case was recognized at autopsy; however, similar techniques could be used in living patients with clinical signs and symptoms suggestive of this disorder or other similar underlying genetic disorders.

References

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2. http://ghr.nlm.nih.gov/condition/cerebral-cavernous-malformation
3. Gunel M, Laurans MS, Shin D, DiLuna, ML, Voorhees J, Choate K, Nelson-Williams C, Lifton RP. *KRIT1, a gene mutated in cerebral cavernous malformation, encodes a micrtubule-associated protein.* Proc Natl Acad Sci U S A; 99 (16): 10677-82. PMID: 12140362
4. Cave-Riant F, Denier C, Labauge P, Cecillon M, Maciazek J, Joutel A, Laberge-Le Couteulx S, Tournier-Lasserve E. *Spectrum and expression analysis of KRIT1 mutations in 121 consecutive and unrelated patients with cerebral cavernous malformations.* Eur J Hum Genet.; 10 (11): 733-40. PMID: 12404106