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Intramuscular Fast-Flow Vascular Anomaly Contains Somatic *MAP2K1* and *KRAS* Mutations

Jeremy A. Goss, MD¹, Dennis J. Konczyk, BS¹, Patrick J. Smits, PhD¹, Harry P.W. Kozakewich, MD², Ahmad Alomari, MD³, Alyaa Al-Ibraheemi, MD², Amir H. Taghinia, MD¹, Belinda Dickie, MD⁵, Denise M. Adams, MD⁵, Steven Fishman, MD⁵, John B. Mulliken, MD¹, Matthew L. Warman, MD^{4,6}, Arin K. Greene, MD MMSc¹

¹Department of Plastic & Oral Surgery, Boston Children's Hospital, Harvard Medical School

²Department of Pathology, Boston Children's Hospital, Harvard Medical School

³Department of Radiology, Boston Children's Hospital, Harvard Medical School

⁴Department of Orthopedic Surgery, Boston Children's Hospital, Harvard Medical School

⁵Department of Surgery, Boston Children's Hospital, Harvard Medical School

⁶Department of Genetics, Harvard Medical School

Abstract

Background—The term “intramuscular hemangioma capillary type” (IHCT) refers to a fast-flow vascular lesion that is classified as a tumor, although its phenotype overlaps with arteriovenous malformation (AVM). The purpose of this study was to identify somatic mutations in IHCT.

Methods—Affected tissue specimens were obtained during a clinically indicated procedure. The diagnosis of IHCT was based on history, physical examination, imaging, and histopathology. Because somatic mutations in cancer-associated genes can cause vascular malformations, we sequenced exons from 446 cancer-related genes in DNA from 7 IHCT specimens. We then performed mutation-specific droplet digital PCR (ddPCR) to independently test for the presence of a somatic mutation found by sequencing and to screen one additional IHCT sample.

Results—We detected somatic mutations in 6 of 8 IHCT specimens. Four specimens had a mutation in *MAP2K1* (p.Q58_E62del, p.P105_I107delinsL, p.Q56P) and 2 specimens had mutations in *KRAS* (p.K5E and p.G12D, p.G12D and p.Q22R). Mutant allele frequencies detected by sequencing and confirmed by ddPCR ranged from 2 to 15%.

Conclusions—IHCT lesions are phenotypically similar to AVMs and contain the same somatic *MAP2K1* or *KRAS* mutations, suggesting that IHCT is on the AVM spectrum. We propose calling this lesion “intramuscular fast-flow vascular anomaly”.

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Corresponding Author Arin K. Greene MD, MMSc, Department of Plastic & Oral Surgery, Boston Children's Hospital, 300 Longwood Ave, Boston, MA 02115, Phone: 617.355.2306, Fax: 617.738.1657, arin.greene@childrens.harvard.edu.

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Keywords

arteriovenous malformation; capillary type; hemangioma; intramuscular; KRAS; malformation; MAP2K1; vascular

INTRODUCTION

In 1972, Allen and Enzinger categorized types of intramuscular vascular anomalies.¹ One of these lesions caused nosologic confusion and has been called: “intramuscular hemangioma capillary type”, “intramuscular angioma capillary type”, “intramuscular hemangioma small vessel type”, and “infiltrating angiolipoma of skeletal muscle”.^{1–5} Our group used the term “intramuscular hemangioma capillary type” (IHCT) and considered it to be a benign, locally aggressive tumor.^{1–6} The lesion, however, also was recognized to have overlapping features with arteriovenous malformation (AVM) and has been considered in the same differential diagnosis.^{4–6} Somatic mutations in *MAP2K1*, *KRAS*, and *BRAF* previously have been identified in sporadic extracranial AVMs.^{7,8} The purpose of this study was to identify somatic mutations in IHCT.

METHODS

The Committee on Clinical Investigation at Boston Children’s Hospital approved this study. A diagnosis of IHCT was made based on clinical, imaging, and histopathological review.⁴ Seven affected tissue specimens were collected during a clinically-indicated procedure, flash-frozen, and stored at –80°C. Genomic DNA was extracted from the frozen specimens using standard methods and used to sequence exons from 447 cancer-related genes (OncoPanel, Dana Farber Cancer Institute, Boston, MA).¹⁰ Putative somatic mutations were identified using MuTect v1.1.4 as previously described,^{9,10} with pairing of each patient specimen to the project normal specimen, CEPH1408-H1. Briefly, non-coding sequence variants were excluded, as were variants having a population frequency of > 0.1% in the gnomAD database. Conversely, any variant that was present in the “COSMIC” database at least twice was flagged for manual review. Translocations were detected using CCGD in-house software called Breakmer. Likely pathogenic mutations were independently tested using mutation-specific droplet digital PCR (ddPCR) assays on a separate DNA aliquot from the original specimen⁷.

RESULTS

Each sample achieved at least 30-fold coverage for 80% of the targeted bases; the mean target coverage was 217x (range 91–312x). Nine putative somatic mutations suggested by the MuTect software were manually reviewed. Among these only 5 mutations appeared to be true positives and were independently tested and confirmed using ddPCR. An additional IHCT sample was screened only by ddPCR and found to have a somatic p.Q56P mutation (Table 1). The two IHCT specimens that tested negative for *MAP2K1* and *KRAS* mutations had similar imaging and histopathological features as those specimens with identifiable mutations. One lesion involved the musculature of the vulva, perineum, and anus with extension into the subcutis; the second was contained within the lumbar paraspinous muscles.

Among the 6 IHCT specimens in which we detected a somatic mutation in either *MAP2K1* or *KRAS*, all were solitary with well-defined borders by MRI (n=6), ultrasonography (n=6), and angiography (n=2) (Figure 1). Three had intramuscular location with extension into the subcutis (Table, patient 2, 3, 5); 1 was contained within the subfascial part of the deltoid muscle (Table, patient 4); in 2 locations with small muscles (nose, scrotum) the relationship between the lesion and muscle could not be ascertained on imaging with certainty (Table patient 1, 6). The lesions exhibited homogenous hyperintense soft tissue signal on T2-weighted MR with strong contrast enhancement. Sonographic images demonstrated heterogeneously hyperechoic soft tissue lesions with hypervascularity. Both the feeding arteries and draining veins were proportionately mildly to moderately dilated. Well-defined tissue blush was noted on angiography with supply from mildly-moderately enlarged orthotopic arteries and proportionately dilated draining veins. No direct arteriovenous shunting was seen.

Histopathologically, all lesions exhibited aggregates, lobules, and/or anastomosing cords of capillary-like vessels. Sometimes vessels were two or three times larger, infiltrating among muscle fibers accompanied by a minimal amount of adipose tissue. Lesions were encapsulated and some extended into adjacent adipose tissue and dermis. The capillaries ranged from relatively inconspicuous to moderately plump with channels separated by pale-stained stroma. In several specimens, the capillary network was anastomotic. Occasional mitoses were present but there was no endothelial atypia. Most veins were enlarged, although some were thin-walled relative to their diameter. The majority of veins had focally or diffusely irregularly thickened walls from an excess of smooth muscle, myofibroblasts, collagen, and ground substance involving the intima and sometimes the entire wall. In some lesions, arteries were commensurate for the amount of mass. In other specimens, arteries were enlarged with fragmentation of the internal elastic lamina, minor neointimal and mural thickening or cushions, and medial smooth muscle hypertrophy. With some thick-walled vessels, it was uncertain whether they were modified arteries or veins, specifically whether they were arteries that had transformed into veins, or veins that had undergone “arterialization” Large caliber arteriovenous communications were not observed but connections between small vessels could not be excluded.

The specimens with *KRAS* mutations had massive capillary lobules, some with a focally anastomotic motif. There were unusually large veins with markedly thickened walls by some combination of smooth muscle, myofibroblasts, collagen, and ground substance, sometimes including capillarization (Figure 2). The lesions with *MAP2K1* mutations exhibited smaller lobules and veins with less dilation and mural thickening (Figure 3), with the exception of one specimen (patient 6 in Table) whose lesion had enlarged arteries and veins with alterations, as described above, but with many small channels with thin walls of indeterminate type and only a few capillary lobules.

DISCUSSION

Arteriovenous malformation (AVM) is a broad term used to describe a congenital anomaly with direct connections between arteries and veins through abnormal vessels instead of a normal capillary bed. AVMs are warm and pulsatile on physical examination, have fast-flow

on handheld Doppler, and exhibit arteriovenous shunting on imaging. There are familial forms of AVM that have a genotype-phenotype correlation. AVMs associated with hereditary hemorrhagic telangiectasia (HHT), caused by mutations in *ENG*, *ACVRL1*, *SMAD4*, or *GDF*, are multifocal, small, and affect the pulmonary, gastrointestinal, and nervous systems.¹¹ *RASA1* or *EPHB4* mutations cause capillary malformation-arteriovenous malformation (CM-AVM). This syndrome is characterized by multiple, small, fast-flow cutaneous lesions often surrounded by a pale halo; some patients also have an intracranial or extracranial AVM.^{12–14} Individuals with phosphatase and tensin homolog (PTEN) hamartoma-tumor syndrome can exhibit multiple AVMs that typically affect muscle, contain ectopic adipose tissue, and exhibit disproportionate segmental dilation of draining veins.^{15–16} Unlike germline mutations associated with AVMs, the more common sporadic AVMs have not been shown to have a genotype-phenotype correlation. Extracranial AVMs most often contain mutations in *MAP2K1*,⁷ but mutations in *KRAS* and *BRAF* also have been described.⁸ Intracranial somatic AVMs have mutations in *KRAS*.¹⁷

Although AVM and IHCT have been considered different lesions, often it is difficult to distinguish them clinically, radiographically, and histopathologically. AVM and IHCT are on the same differential diagnostic list because they share several features. Both lesions enlarge, cause pain and deformity, and are treated with embolization or resection. Radiographically each exhibits increased arterial flow (fast-flow) and enhancement with contrast. Common histopathologic findings include: enlarged arteries and veins with modified mural morphology, a lobular capillarous component, and small caliber, indeterminate type vessels. IHCT has been thought to be different than an AVM because it is more well-defined, typically involves skeletal muscle, and exhibits smaller vessels without obvious arteriovenous shunting.^{1–5,16}

Our finding that IHCT contains the same somatic mutations as AVM suggests that the lesions are on the same spectrum. Some of the mutations have been previously reported (*MAP2K1* Q58_E62del, *MAP2K1* Q56P) while others have not (*MAP2K1* P105_I107delinsL, *KRAS* G12D, *KRAS* K5E, *KRAS* Q22R). The accepted biological classification of vascular anomalies restricts the term “hemangioma” to describe a tumor.⁶ IHCT is not a “hemangioma” and does not exhibit significant cellular proliferation. A more accurate term for this lesion would be “intramuscular fast-flow vascular anomaly (IFVA)”. This description includes its most common anatomical location and its major vascular characteristic of fast-flow. It also differentiates the lesion from a “typical” AVM which is more diffuse and does not exhibit shunting between large vessels. This study provides further insight into gene-causing mutations for vascular anomalies and possible Targets for developing pharmacotherapy.

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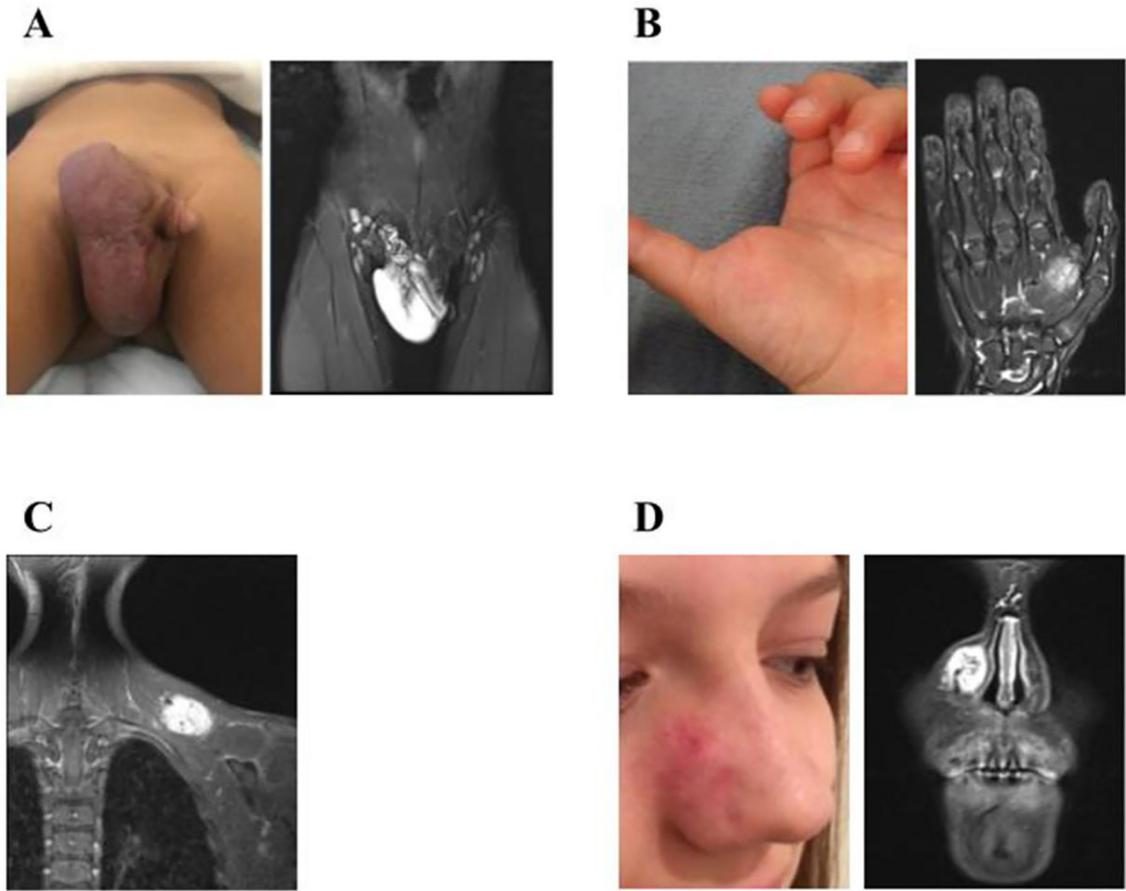


Figure 1. Study cohort with intramuscular hemangioma capillary type listed in Table. (A) Patient 1, (B) Patient 2, (C) Patient 3, (D) Patient 6.

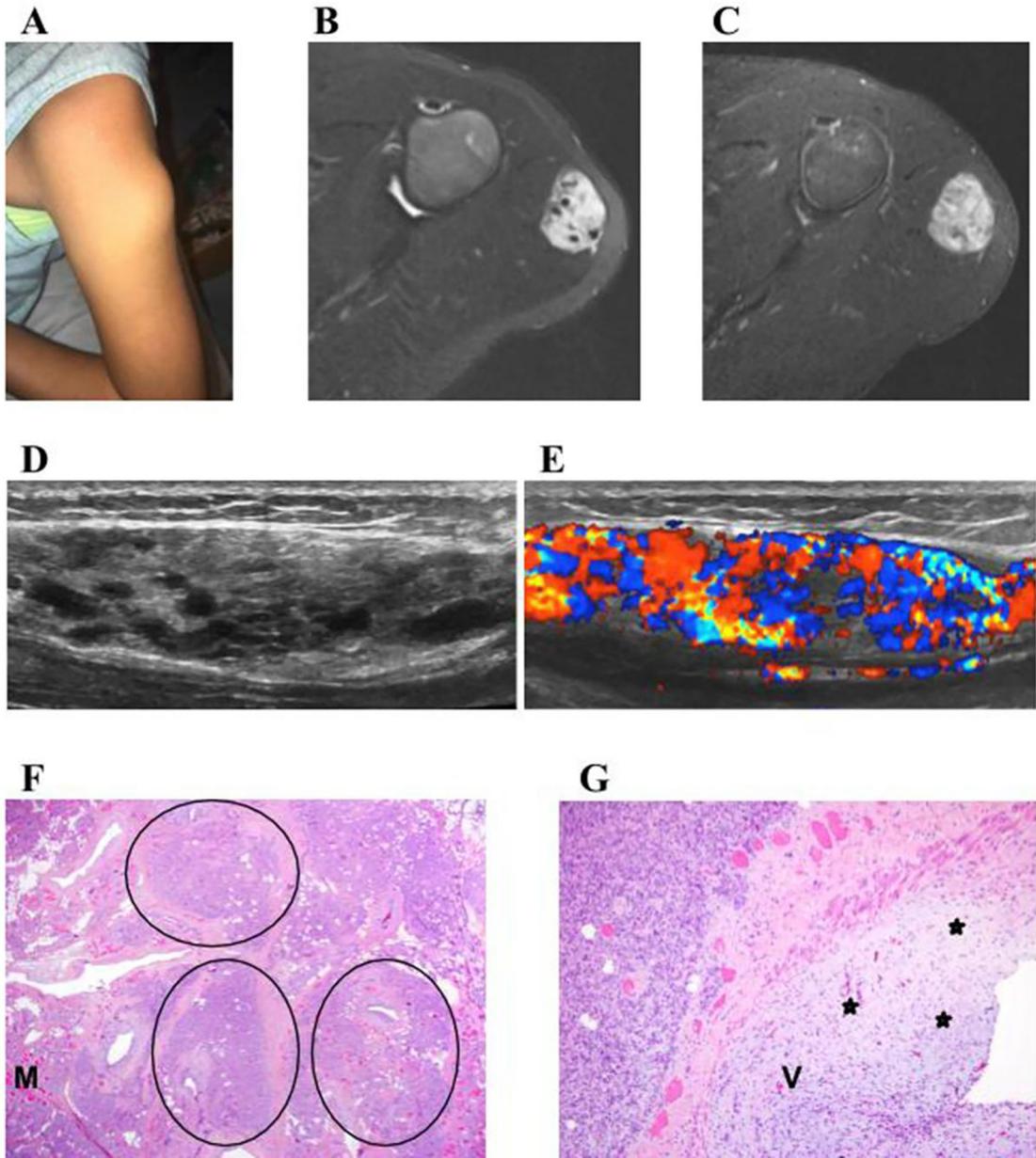


Figure 2. Clinical, radiological, and histopathological findings of Patient 4 (Table) with a *KRAS* mutation. (A) Clinical appearance showing a subcutaneous mass of the left arm. (B) Axial fat saturated T2- weighted and (C) post-contrast, T1-weighted MR images demonstrating focal well-defined T2-hyperintense lesion with enlarged vessels in the left deltoid muscle. Note strong enhancement post-contrast. (D) Gray-scale (E) and color Doppler ultrasound longitudinal images showed heterogeneously hyperechoic subfascial lesion containing large vessels. (F) Skeletal muscle (M) involved by large capillary lobules (circles), abnormal veins, and a small amount of adipose tissue (H & E, x40). (G) Portion of capillary lobule on left adjacent to vein (V) with focal loss of smooth muscle and mural expansion by vascularized neointimal hyperplasia (stars) (H & E, x 100).

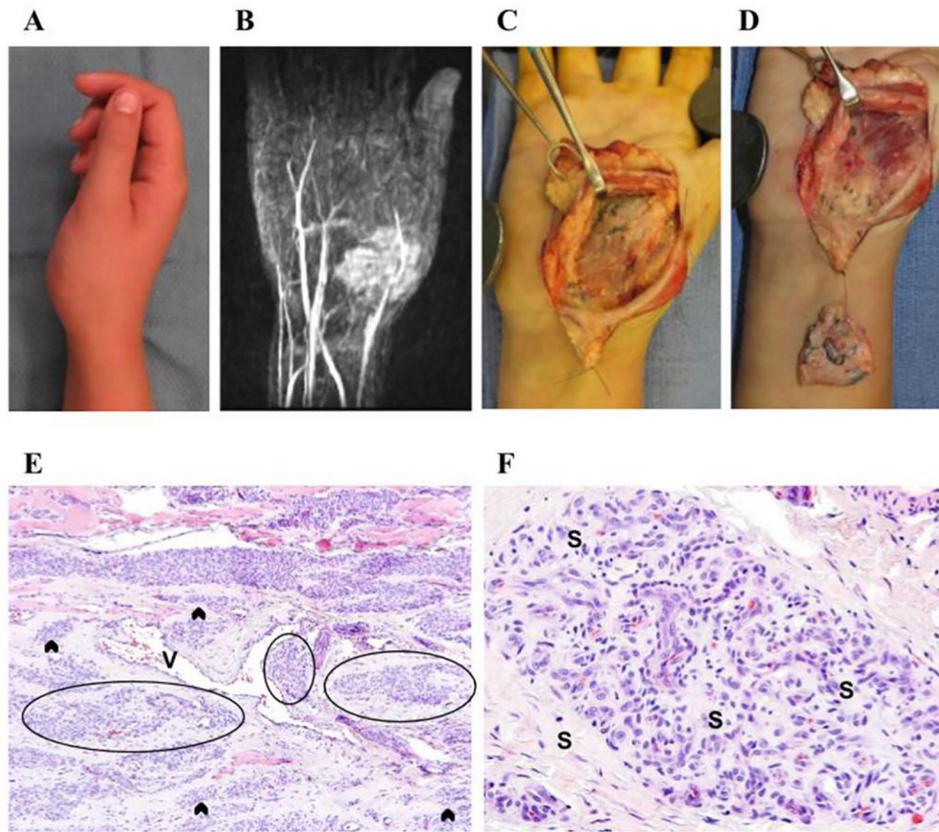


Figure 3. Clinical, radiological, and histopathological findings of Patient 5 (Table) with a *MAP2K1* mutation. (A) Clinical and (B) MRI appearance. (C) Intraoperative view of lesion prior to resection. (D) Excised lesion illustrates adipose component. (E) Skeletal muscle infiltrated by capillarous strands, clusters (arrow heads), lobules (circles), and anastomosing bands. Thin-walled irregularly muscularized vein present (V) (H & E, x100). (F) Typical lobule with branching capillaries with small lumens, plump endothelial cells with slight orientational disarray, relatively inconspicuous pericytes, and delicate palely stained lobular stroma (S) (H & E, x 400).

Table.

Intramuscular Hemangioma Capillary Type Mutation Detection

Participant	Age	Sex	Location	Mutation	Oncopanel*	ddPCR*
1	7	M	Genitalia	<i>MAP2K1</i> p.Q58_E62del	13%	15%
2	7	M	Hand	<i>MAP2K1</i> p.P105_I107delinsL	5%	2%
3	11	M	Shoulder	<i>KRAS</i> p.G12D	9%	10%
				<i>KRAS</i> p. K5E	9%	15%
4	11	F	Shoulder	<i>KRAS</i> p.G12D	9%	9%
				<i>KRAS</i> p.Q22R	9%	11%
5	11	M	Hand	<i>MAP2K1</i> p. Q56P	10%	10%
6	15	F	Nose [#]	<i>MAP2K1</i> p.Q56P	-	3%

(-) not performed

* mutant allele frequency

[#] non-affected tissue (white blood cell DNA) did not contain the lesional *MAP2K1* mutation