Human Genetics (2019) 138:1419–1421
https://doi.org/10.1007/s00439-019-02072-y

SHORT COMMUNICATION

# Arteriovenous malformation associated with a *HRAS* mutation

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Received: 14 September 2019 / Accepted: 4 October 2019 / Published online: 21 October 2019


**Abstract**
The majority of extracranial arteriovenous malformations (AVMs) are caused by somatic mutations in *MAP2K1*. We report a somatic *HRAS* mutation in a patient who has a facial AVM associated with subcutaneous adipose overgrowth. We performed whole exome sequencing on DNA from the affected tissue and found a *HRAS* mutation (p.Thr58_Ala59delinsValLeuAspVal). Mutant allelic frequency was 5% in whole tissue and 31% in isolated endothelial cells (ECs); the mutation was not present in blood DNA or non-ECs. Somatic mutations in *HRAS* can cause AVM.

**Abbreviations**

| | |
|---|---|
| AVM | Arteriovenous malformation |
| ECs | Endothelial cells |
| WES | Whole exome sequencing |
| ddPCR | Droplet digital polymerase chain reaction |
| pERK | Phosphorylated ERK |
| ECFCs | Endothelial colony forming cells |
| HUVECs | Human umbilical vein endothelial cells |

## Short report

Most extracranial arteriovenous malformations (AVMs) arise sporadically and are caused by somatic mutations in *MAP2K1*; mutations in *BRAF* and *KRAS* also have been identified (Couto et al. 2017; Al-Olabi et al. 2018). Sporadic AVMs have not exhibited a genotype–phenotype relationship (Couto et al. 2017; Al-Olabi et al. 2018). We report here that extracranial AVM also can be caused by a somatic mutation in *HRAS*.

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AVM tissue was collected during a clinically indicated procedure from an 11-year-old female with an AVM of her cheek and endothelial cells (ECs) were separated from non-ECs (Fig. 1). DNA was extracted from tissue, cells, and blood (unaffected tissue) using DNeasy blood and tissue kit. Whole exome sequencing (WES) was performed by Macrogen and analyzed for putative somatic disease-causing mutations. Single-nucleotide and indel mutations having ≥ 2% mutant allelic fraction and ≥ 5 reads were identified using MosaicHunter and MuTect (Cibulskis et al. 2013; Huang et al. 2017). Common variants reported in population polymorphism databases were excluded. Droplet digital polymerase chain reaction (ddPCR) was used to confirm the mutation (Table 1). Whole cell lysates were obtained from the AVM-derived ECs as well as wild-type adipose tissue-derived endothelial colony forming cells (ECFCs) from another patient. Fifteen micrograms of protein was separated by SDS-PAGE on 4–20% gradient gels. After transfer to a PVDF membrane and blocking in TBST, immune detection of ERK 1/2 (1/1000 dilution), p-ERK 1/2 (1/1000 dilution), and actin (1/15,000 dilution) was performed. Membranes were washed and incubated in anti-mouse or anti-rabbit secondary antibody (1/5000 dilution) in blocking buffer. Membranes were visualized with CDP-star substrate using autoradiography film.

WES achieved an average coverage of 316-fold and 256-fold for ECs and non-ECs, respectively. By comparing ECs with non-ECs of the same patient, we identified a mutation in *HRAS* (p.Thr58_Ala59delinsValLeuAspVal) exclusively in ECs with an allelic frequency of 31%. In addition to independently confirming the presence of the mutant allele in ECs, ddPCR also detected a mutant allelic frequency of 5%

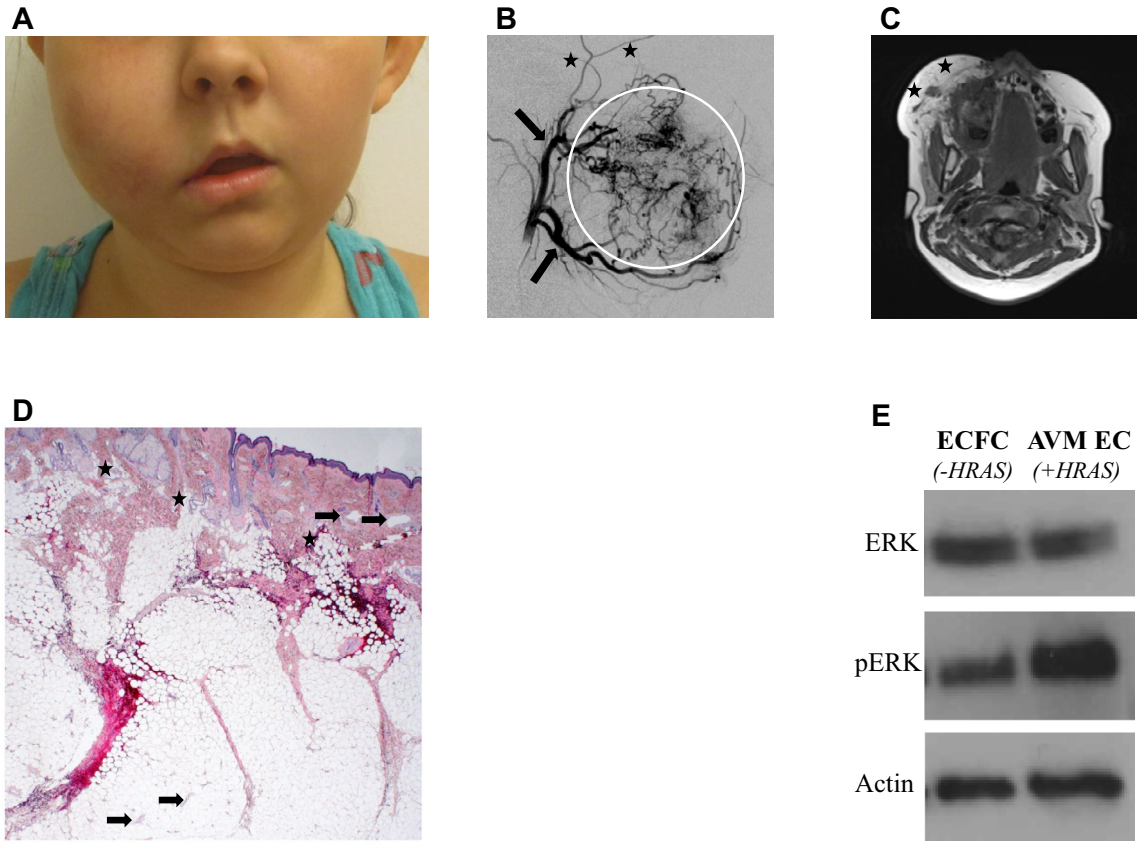


**Fig. 1** An 11-year-old female with a facial arteriovenous malformation (AVM). **a** Appearance of right cheek overgrowth. **b** Preoperative angiogram shows the AVM with tortuous arterial feeding vessels (arrows), a nidus abnormally connecting arteries and veins (circle), and early filling of draining veins (stars). **c** MRI illustrates significant adipose tissue of right cheek (stars). **d** Hematoxylin and eosin (H&E)-stained section (×20) of the excised lesion demonstrates skin and subcutis with abundant adipose tissue extending to the overlying dermis (stars). Dispersed through the dermis and subcutaneous adipose tissue are venular and capillary vascular channels (arrows). **e** Representative western blot showing increased phosphorylated ERK (pERK) relative to total ERK (ERK) in *HRAS* mutant endothelial cells (ECs) from the patient with the AVM *(right column)* compared to wild-type endothelial colony forming cells (ECFCs) (*left column*)

**Table 1** *HRAS* mutation-specific droplet digital PCR primers and probes

| | |
|---|---|
| Forward primer | 5′-CCTCACGGGGTTCACCTGTA-3′ |
| Reverse primer | 5′-GGATTCCTACCGGAAGCAGG-3′ |
| Wild-type probe | 5′-/5HEX/CCTGGCCGGCGGTAT CCA/3IABkGQ/-3′ |
| Mutant probe | 5′-/56-FAM/CCTGGCCAACATCCA GGACATCC/3IABkFQ/-3′ |

in the original sample. The mutation was not present in non-ECs or peripheral blood. Western blot analysis of cell lysates showed a higher ratio of phosphorylated ERK (pERK) to non-phosphorylated ERK in the patient's ECs compared to wild-type ECFCs (Fig. 1).

This report shows that *HRAS* is a new locus for causing AVM. Like *MAP2K1* AVM-associated mutations, we found that *HRAS* mutations were restricted to ECs, further supporting the role of the EC in the pathophysiology of AVM (Couto et al. 2017; Greene and Goss 2018). The *HRAS* mutation we identified affects amino acid residues 58 and 59 within the switch II region of the *HRAS* protein. This region is important for GTP hydrolysis (Hobbs et al. 2016). Somatic missense mutations affecting amino residue 60 are commonly found in cancer and lead to hyperactivation of *HRAS* signaling (Tate et al. 2019). Thus, it is likely the mutation we identified also increases *HRAS* signaling. Consistent with this interpretation is that ECs from our patient have an increased pERK–ERK ratio. Compared to most other individuals with AVM, our subject had significant adipose hypertrophy at the AVM site. This raises the possibility that adipocyte overgrowth may be a distinguishing feature of *HRAS*-associated AVMs. Inhibitors of targets in this pathway might prove to have efficacy for AVMs.

**Acknowledgements** Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health

under Award Number R01HD093735 (AKG), the Translational Research Program Boston Children's Hospital (AKG), and the National Institutes of Health under Award Number NIH R01AR064231 (MLW). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

**Data availability** The dataset generated during the current study is available in the ClinVar repository with accession number SCV000992589.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Ethical approval** All procedures performed in this study were in accordance with the ethical standards of the Committee on Clinical Investigation at Boston Children's Hospital and with the 1964 Helsinki Declaration and its later amendments.

**Informed consent** Consent was obtained for the participant in the study.

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