Received: 23 November 2022 | Accepted: 8 December 2022

DOI: 10.1111/jop.13394

ORIGINAL ARTICLE

Journal of Oral Pathology & Medicine WILEY

Analysis of *KRAS*, *BRAF*, and *EGFR* mutational status in respiratory epithelial adenomatoid hamartoma (REAH)

Letícia Martins Guimarães[1] | Tamara da Silva Vieira[1] | Luiz Armando De Marco[2] | Lester D. R. Thompson[3] | Carolina Cavalieri Gomes[1]

[1]Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[2]Department of Surgery, Faculty of Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[3]Head and Neck Pathology Consultations, Woodland Hills, California, USA

Correspondence
Carolina Cavalieri Gomes, Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, CEP 31270-901, Brazil.
Email: gomes.carolinac@gmail.com; carolinacgomes@ufmg.br

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico; Fundação de Amparo à Pesquisa do Estado de Minas Gerais

Abstract

Background: Respiratory epithelial adenomatoid hamartoma (REAH) is a sinonasal glandular overgrowth arising from the surface respiratory epithelium and invaginating into the stroma. Clinically, it appears as a polypoid mass that may cause nasal obstruction, anosmia, and epistaxis. The presence of cartilaginous and/or osseous areas move the lesion to a chondro-osseous respiratory epithelial (CORE) hamartoma subtype. Scattered small seromucinous glands may be observed between typical REAH glands and when it is the only feature, it represents seromucinous hamartoma (SH). The molecular pathogenesis of REAH has been poorly explored and remains unclear. Given that *KRAS*, *BRAF*, and *EGFR* mutations have been detected in a variety of sinonasal tumors, we aimed to assess these mutations in REAH and SH.

Methods: Ten REAH (including one CORE subtype), in addition to two SH cases, were Sanger sequenced by standard techniques. The targeted regions included *KRAS* exons 2–4 (encompassing hotspots codons 12, 13, 61, and 146), *BRAF* exons 11 and 15 (spanning the V600 codon), and *EGFR* exons 19 and 20.

Results: All REAH and SH samples showed wild-type sequences for *KRAS*, *BRAF*, and *EGFR* genes.

Conclusion: Our results demonstrate a lack of *KRAS*, *BRAF*, or *EGFR* pathogenic variants with further evaluation of REAH and SH needed to elucidate driver genetic events.

KEYWORDS
genetics, KRAS, mutations, respiratory epithelial adenomatoid hamartoma, seromucinous hamartoma

1 | INTRODUCTION

Respiratory epithelial adenomatoid hamartoma (REAH) is a benign polypoid overgrowth of medium-sized ciliated glands that arise from the surface epithelium of the sinonasal tract, invaginate into the stroma, and are surrounded by a thickened basement membrane.[1] The presence of an admixture of cartilaginous and/or osseous trabeculae characterizes the chondro-osseous respiratory epithelial (CORE) hamartoma subtype.[1] Notably, REAH often arises in the context of allergy and sinonasal inflammatory disorders.[1] Seromucinous hamartoma (SH) is a benign proliferation of small eosinophilic glands and ducts lined by cuboidal epithelial cells arising in the sinonasal tract.[2] REAH and SH features may be combined, giving rise to a mixed lesion.[1,2]

REAH shows significant clinicopathologic overlap with other benign and malignant sinonasal entities. The differential diagnosis

Letícia Martins Guimarães and Tamara da Silva Vieira contributed equally to this study.

TABLE 1 Clinical and molecular findings of the respiratory epithelial adenomatoid hamartoma (REAH) and seromucinous hamartoma (SH) samples included in the study.

				Genes		
Sample#	Sex	Age (years)	Location	*KRAS* exons 2–4	*BRAF* exons 11 and 15	*EGFR* exons 19 and 20
Respiratory epithelial adenomatoid hamartoma						
1A[a]	M	74	Right upper nasal cavity	WT	WT	WT
B[a]			Left upper nasal cavity	WT	WT	WT
2A[a]	M	72	Left upper nasal septum	WT	WT	WT
B[a]			Right upper nasal septum	WT	WT	WT
3	M	54	Left nasal cavity	WT	WT	WT
4	F	60	Left posterior nasal cavity	WT	WT	WT
5	M	30	Right nasal cavity	WT	WT	WT
6	M	64	Right upper nasal septum	WT	WT	WT
7	F	47	Right maxillary sinus and nasal cavity	WT	WT	WT
8[b]	M	42	Right nasal cavity (lateral wall)	WT	WT	WT
Seromucinous hamartoma						
1	M	49	Right upper nasal septum	WT	WT	WT
2	F	69	Bilateral upper nasal cavity	WT	WT	WT

Abbreviations: F, female; M, male; WT, wild-type.
[a]These cases have two sites in the same patient.
[b]This sample corresponds to a chondro-osseous respiratory epithelial (CORE) hamartoma subtype.

includes inflammatory polyp, inverted papilloma, biphenotypic sinonasal sarcoma, and low-grade non-intestinal-type sinonasal adenocarcinoma.[1] The differential diagnosis between these entities may be challenging and misdiagnosis of biologically aggressive sinonasal lesions such as inverted papillomas or sinonasal adenocarcinomas may potentially result in overtreatment.[1,3,4]

KRAS, *BRAF*, and *EGFR* are proto-oncogenes frequently mutated in cancer.[5–7] With advances in molecular pathology, it is recognized that genetic alterations in cancer-related genes are also identified in benign lesions,[8,9] including inflammatory conditions,[10] hamartomas,[11,12] as well as normal tissues.[13–16]

The mutational status of *KRAS*, *BRAF*, and *EGFR* genes has been investigated in sinonasal lesions that are in the differential diagnosis of REAH.[17–20] While inflammatory polyps do not harbor *KRAS*, *BRAF*, or *EGFR* mutations,[20] recurrent somatic activating *EGFR* mutations have been reported in inverted sinonasal papilloma and sinonasal squamous cells carcinoma associated with inverted sinonasal papilloma.[18,19] The low-grade non-intestinal-type sinonasal adenocarcinoma harbors *BRAF* mutations at a low prevalence, whereas *KRAS* mutations are not reported.[17] Concerning other sinonasal lesions, *KRAS* mutations have been detected in intestinal-type sinonasal adenocarcinoma at a low frequency,[21] while recurrent somatic *KRAS* mutations have been reported in oncocytic sinonasal papillomas and sinonasal squamous cell carcinoma associated with oncocytic sinonasal papilloma.[19,22]

The genetic underpinnings of REAH are incompletely explored and its molecular pathogenesis still remains unclear. An exploratory study described a fractional allelic loss of 31% at tumor suppressor genes loci in REAH.[23] Recently, a NGS panel has been used to investigate fusion transcripts in 53 genes in REAH ($n = 5$) and SH ($n = 5$).[24] No fusion gene was detected in REAH, while a *EGFR::ZNF267* fusion was detected in a single case of SH.[24]

Herein, we aimed to investigate *KRAS*, *BRAF*, and *EGFR* mutational status in REAH and SH to elucidate if hotspot mutations in these genes are involved in their pathogenesis and if they share molecular features with other sinonasal tumors.

2 | MATERIAL AND METHODS

2.1 | Case selection

This study was approved by the Research Ethics Committee of Universidade Federal de Minas Gerais (protocol number CAAE/approval: 58148722.5.1001.5149/5.497.297) and followed the Declaration of Helsinki principles. Appropriate samples of 10 formalin-fixed paraffin-embedded (FFPE) REAH (including 1 CORE hamartoma subtype) and 2 SH from 10 patients was selected. For two patients, two different REAH samples occurring at different sites were evaluated. Hematoxylin and eosin stained slides were reviewed for diagnosis confirmation, following the 2022 World Health Organization Classification of Head and Neck Tumours criteria.[1,2]

2.2 | DNA isolation, polymerase chain reaction, and Sanger sequencing

Genomic DNA was isolated from the FFPE samples using QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's

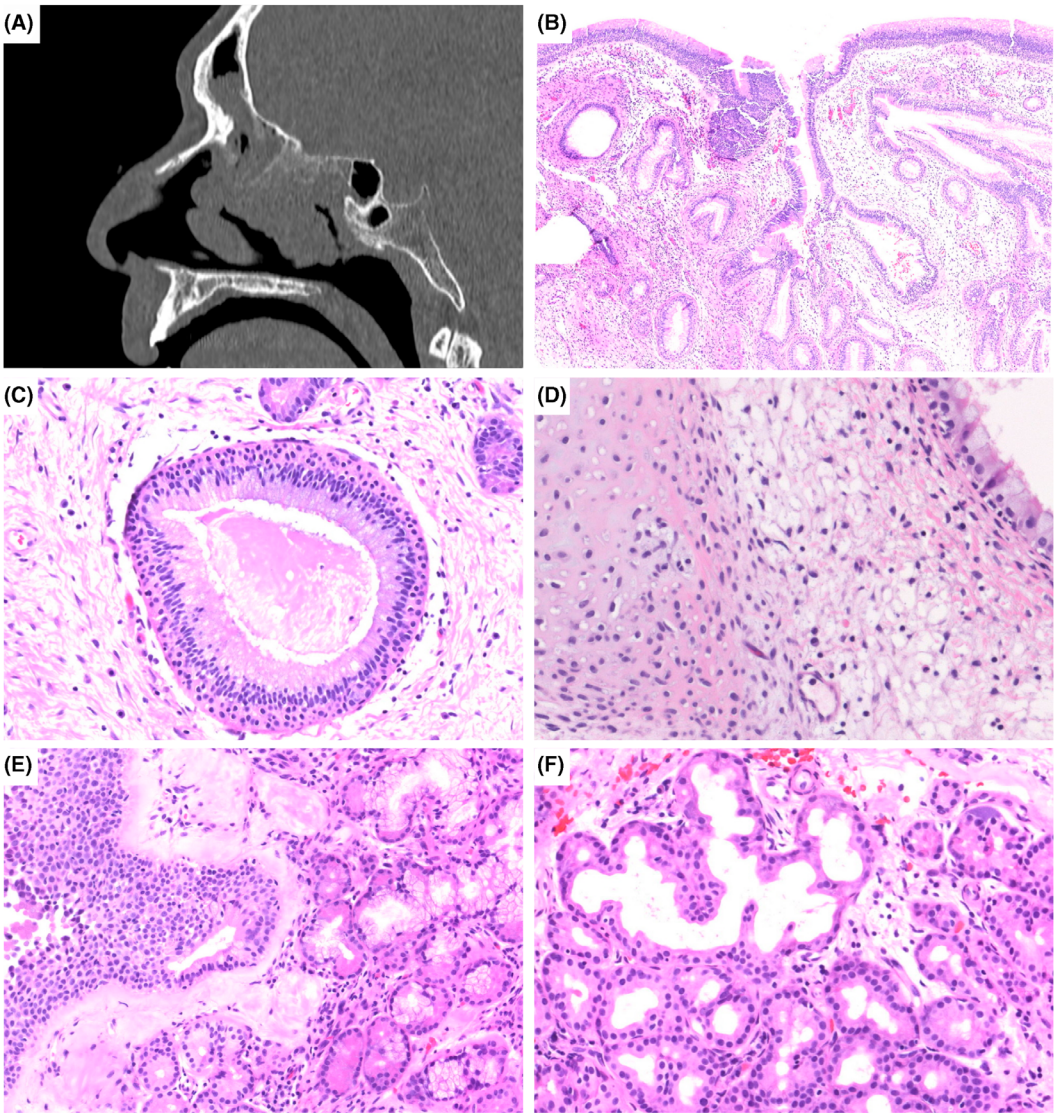

FIGURE 1 Imaging and histological features of respiratory epithelial adenomatoid hamartomas (REAH), including chondro-osseous subtype (CORE), and seromucinous hamartomas (SH). (A) Sagittal computed tomography of the nasal cavity depicting a homogeneous soft tissue mass involving the right upper nasal septum (REAH histologically). (B) REAH glandular proliferation arising from the surface respiratory epithelium, expanding downward into the stroma, and surrounded by thickened eosinophilic basement membrane. Chronic inflammatory infiltrate is observed separating the glandular structures. (C) REAH round gland composed of pseudostratified ciliated epithelium with mucin-secreting cells. (D) CORE marked by cartilaginous matrix (left) intimately associated with the glandular component (upper right). (E) SH lined by respiratory ciliated epithelium with small seromucinous glands. (F) SH at higher magnification showing the small tubules lined by epithelial cuboidal cells with small nuclei, without mitoses, and sometimes exhibiting amorphous eosinophilic material.

instructions. A spectrophotometer (Nano-Drop 2000 instrument; Thermo Fisher Scientific) was used to evaluate both DNA concentration and purity.

KRAS (exons 2–4), *BRAF* (exons 11 and 15), and *EGFR* (exons 19 and 20) mutations were investigated using conventional polymerase chain reaction (PCR) followed by Sanger sequencing (specific primers available upon request). The PCR reactions were carried out using MyTaq HS Red Mix, 2x (Bioline Reagents), following the manufacturer's recommendations. Positive and negative controls were included. The PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Life Technologies). Bidirectional Sanger sequencing was performed using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and capillary electrophoresis was carried out in the SeqStudio Genetic Analyzer (Applied Biosystems by Thermo Fisher Scientific).

The chromatograms were manually inspected using the SnapGene software (GSL Biotech; available at snapgene.com) and using the reference sequences of each gene for comparison (*KRAS*: NM_004985.5; *EGFR*: NM_005228.5; *BRAF*: NM_001354609.2).

3 | RESULTS

3.1 | Sample characterization

For REAH cases, the male:female ratio was 3:1, with a median age of 57 (average, 55.4; range of 30–74 years). The nasal cavity was the most frequently affected site (100%), with the upper nasal cavity and nasal septum most common subsites affected. Two cases had bilateral disease, with four cases affecting the right and two cases affecting the left exclusively. The individuals affected by SH were a 49-year-old male and 69-year-old female, with the right upper nasal septum and bilateral nasal cavities affected, respectively (Table 1). All cases demonstrated characteristic imaging and histological findings for REAH and SH (Figure 1).

3.2 | Molecular findings

All REAH, including the CORE subtype, and SH samples showed wild-type sequences for the regions investigated in *KRAS*, *BRAF*, and *EGFR* genes. The Sanger sequencing results are illustrated in Figure 2 and summarized in Table 1.

4 | DISCUSSION

The molecular basis of REAH has been incompletely evaluated and there is an ongoing debate surrounding its pathogenesis. Although some authors have suggested a neoplastic nature for REAH based on the presence of allelic loss at tumor suppressor genes loci,[23] the mere presence of genetic alterations is not sufficient to classify a given lesion as neoplastic.[9] Given that REAH may arise in an inflammatory context and that *KRAS*, *BRAF*, and *EGFR* mutations have been reported in non-neoplastic conditions and/or in sinonasal lesions, we interrogated the mutational status of these three genes in REAH and two additional SH for comparison.

KRAS is one of the most frequently mutated genes in cancer, with hotspot mutations mainly occurring at codons 12, 13, and 61.[5] *KRAS* codon 12 mutations have also been reported in inflammatory conditions and normal tissue.[9] Moreover, activating mutations in *KRAS* exons 2, 3, and/or 4 have been identified in several head and neck lesions, such as gnathic giant cell granuloma,[25] adenomatoid odontogenic tumor,[26] and squamous papilloma.[27] Considering the lesions of the sinonasal tract, *KRAS* codons 12 or 61 mutations have been reported in 100% (51/51) of oncocytic sinonasal papillomas and in 5 of 5 sinonasal squamous cell carcinoma associated with oncocytic sinonasal papillomas.[22] Moreover, *KRAS* codons 12 and 13 mutations have been detected in intestinal-type sinonasal adenocarcinoma, but at a lower frequency (12%, 7/58).[21] Furthermore, 14 nasal polyps all showed wild-type *KRAS*.[20] In this study, we demonstrated a lack of *KRAS* hotspot mutations in REAH or SH, showing this gene is not involved in the pathogenesis of these lesions.

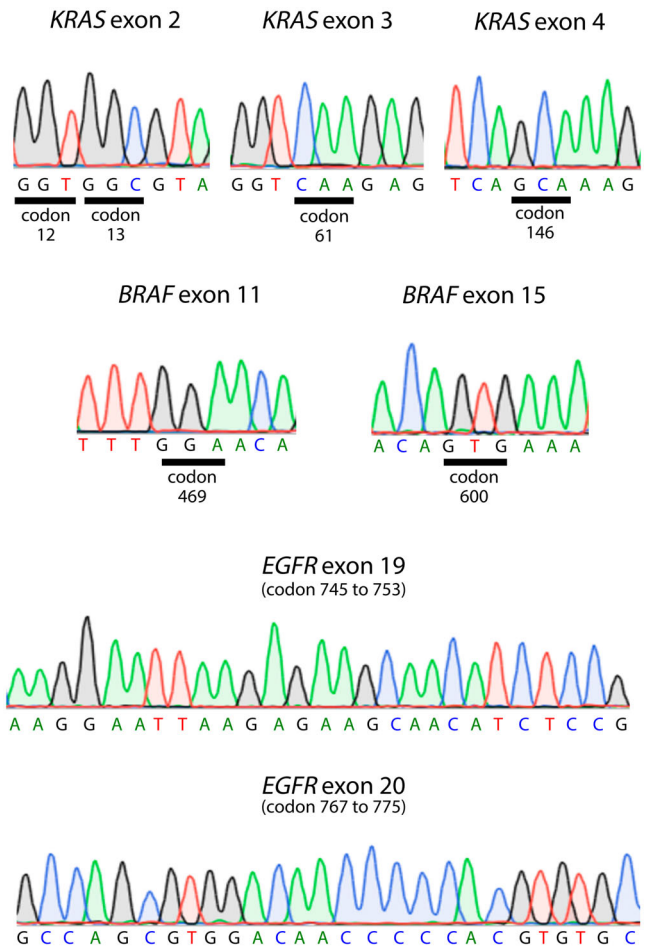

FIGURE 2 Gene regions assessed by Sanger sequencing in respiratory epithelial adenomatoid hamartomas, the chondro-osseous subtype, and seromucinous hamartomas. Sequencing chromatograms showing wild-type sequences for the analyzed regions of *KRAS* (exons 2–4), *BRAF* (exons 11 and 15), and *EGFR* (exons 19 and 20).

Several human tumors, including melanomas and colorectal cancer, harbor *BRAF* mutations, predominantly affecting exons 15 (p.V600E) followed by exon 11 (codon 469).[6,28] In addition to malignant neoplasms, *BRAF* oncogenic mutations have been reported in the context of benign lesions, including head and neck neoplasms.[9] In the context of sinonasal lesions, mutations in *BRAF* have rarely been reported. Notably, p.V600E was only detected in 2 of 12 non-intestinal-type sinonasal adenocarcinoma,[17] while nasal polyps have been shown to be *BRAF* wild-type.[20] In line with that, we showed that REAH and SH are *BRAF* wild-type, suggesting that mutations in this gene are not part of their pathogenesis.

Mutations in *EGFR* occur at high frequencies in certain cancer types, mainly lung adenocarcinomas but also in squamous cell carcinomas affecting the head and neck region.[7,18] In the sinonasal context, recurrent activating *EGFR* mutations, specifically exon 20 insertions and exon 19 deletions, have been reported in 88% (44/50) of inverted sinonasal papilloma and 77% (17/22) of sinonasal squamous cell carcinoma associated with inverted sinonasal papilloma.[18] Conversely, nasal polyps are *EGFR* wild-type.[20] Investigation by Sanger sequencing

of *EGFR* exons 19 and 20 in REAH and SH showed that they are *EGFR* wild-type, and thus do not play a role in pathogenesis.

Microscopic characterization remains the gold standard for diagnosis. However, the use of molecular techniques has been incorporated as an important auxiliary diagnostic tool. The recognition and histologic characterization of REAH is important to avoid unnecessary and aggressive surgery because it may be confused with other sinonasal tumors, such as inverted papilloma and sinonasal adenocarcinoma.[29] Future studies may help elucidating the molecular underpinnings of REAH and SH.

In conclusion, *KRAS*, *BRAF*, and *EGFR* mutations have not been detected in the evaluated samples of REAH and SH, and if these lesions harbor genetic alterations, they remain to be elucidated.

AUTHOR CONTRIBUTIONS

Carolina Cavalieri Gomes contributed to the study conception, design, and supervision. Material preparation and data collection were performed by Lester D. R. Thompson. Experiments and result analysis were performed by Letícia Martins Guimarães, Tamara da Silva Vieira, Luiz Armando De Marco, and Carolina Cavalieri Gomes. The first draft of the manuscript was written by Letícia Martins Guimarães and Tamara da Silva Vieira. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

Luiz Armando De Marco and Carolina Cavalieri Gomes are research fellows at The National Council of Scientific and Technological Development (CNPq)/Brazil. Letícia Martins Guimarães and Tamara da Silva Vieira receive Coordination for the Improvement of Higher-Level Education Personnel (CAPES)/Brazil scholarships. The authors thank the Image Acquisition and Processing Center (CAPI-ICB/UFMG) for the slide scanning service. The study was funded by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)/Brazil, and The National Council of Scientific and Technological Development (CNPq)/Brazil.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS STATEMENT

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Research Ethics Committee of Universidade Federal de Minas Gerais (protocol number CAAE/approval: 58148722.5.1001.5149/5.497.297). Consent to participate was waived by the IRB due to the retrospective nature of the work without therapeutic alterations. Consent for publication was obtained from all individual participants for whom identifying information is uniquely included in this manuscript. Availability of data and material is possible upon reasonable request, deidentified for maintenance of anonymity and compliance with IRB approval.

ORCID

Carolina Cavalieri Gomes https://orcid.org/0000-0003-1580-4995

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How to cite this article: Guimarães LM, Vieira TS, De Marco LA, Thompson LDR, Gomes CC. Analysis of *KRAS*, *BRAF*, and *EGFR* mutational status in respiratory epithelial adenomatoid hamartoma (REAH). *J Oral Pathol Med*. 2022;1-6. doi:10.1111/jop.13394