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BRIEF REPORT

WILEY

A novel *PPP2R2A::PRKD1* fusion in a cribriform adenocarcinoma of salivary gland

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Abstract

Cribriform adenocarcinoma of salivary gland (CASG) is a rare, salivary gland tumor. In this report, we describe a case of CASG harboring a novel *PPP2R2A::PRKD1* fusion. A 58-year-old female presented with an intraoral mass adjacent to the lower left third molar region. Morphological features at histological examination, immunohistochemical staining (p63+, p40−), and tumor location were indicative of CASG. However, due to the potential focal presence of a biphasic component within the tumor, RNA sequencing was performed to confirm the diagnosis. The subsequently found novel *PPP2R2A::PRKD1* fusion adds to the rapidly evolving molecular landscape of salivary gland tumors. Additionally, we report that CASG may show some entrapment of pre-existent salivary gland ducts, which may be misinterpreted as tumor cells with myoepithelial differentiation.

KEYWORDS
cribriform adenocarcinoma of salivary gland, cribriform subtype of polymorphous adenocarcinoma, *PPP2R2A*, *PRKD1*

1 | INTRODUCTION

To our knowledge, within salivary gland tumors, molecular aberrations within the *PRKD* gene family have only been described in polymorphous adenocarcinoma (PAC) and cribriform adenocarcinoma of salivary gland (CASG).[1] Though the concept of CASG as a separate entity has been initially described by Michal et al. in 1999, there is currently no consensus whether CASG constitutes its own entity or should be considered a subtype of PAC.[2,3] Though *PRKD1* hotspot mutations are found in approximately 56%–73% of PACs and *PRKD1/2/3* fusions are found in approximately 43%–80% of CASGs, these molecular aberrations are not exclusively found in either PAC or CASG.[4–6] *PRKD1* hotspot mutations are found in 20% of CASGs and *PRKD1/2/3* fusions are found in 13% of PACs and therefore these aberrations cannot be considered pathognomonic for either entity.[4–6] The 5th edition of the WHO Classification of Head and Neck tumors describes CASG as a cribriform subtype of PAC, stating that some authors consider CASG as a separate entity, while others consider it a subtype within the morphological spectrum of PAC.[3] In this report, we describe a case of CASG harboring a novel *PPP2R2A::PRKD1* fusion.

1.1 | Case presentation

A 58-year-old female was referred to the Oral and Maxillofacial surgery department of the University Medical Center Groningen (UMCG) for further diagnostic work up of an intraoral mass adjacent to the lower left third molar region. In the months prior to her referral, she noticed episodes of varying swelling of the intraoral mass causing difficulties in swallowing and interfering with mouth closure. Therefore,

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a diagnostic biopsy was taken under local anesthesia in a local hospital. The surgeon performing the procedure described a solid mass of approximately 15 mm with intact cortical layers of the mandible (region 38). Her lower left wisdom tooth (38) had been removed 30 years earlier in the same hospital without any particularities. Histological examination of the current specimen showed a salivary gland tumor not further specified, warranting a referral to our tertiary hospital specialized in head and neck oncology.

Revision of the original biopsy was performed at the UMCG. The specimen consisted entirely of nonencapsulated tumor tissue with no overlying epithelium. The tumor was composed of fields, nests and rows of tumor cells with varying growth patterns, including trabecular and cribriform areas, and the formation of pseudo-glandular structures (Figure 1A). The tumor was predominantly composed of luminal cells with round to round-oval shaped nuclei with smooth nuclear membranes and fine granular pattern, nonconspicuous nuclei and moderate cytoplasm (Figure 1B). Nuclear grooves and vacuolization were observed occasionally. The intercellular stroma was hyalinized and hypocellular (Figure 1C). Focally, a biphasic cellular component was suspected, containing both luminal and basal/myoepithelial cells (Figure 1D showing H&E staining and Figure 1E showing calponin staining in the same region). These potential basal/myoepithelial cells were hyperchromatic, spindle-shaped, lacking prominent nucleoli or atypia and contained scarce cytoplasm. Neither of these cell types showed pronounced atypia and mitotic figures were rarely seen, and therefore, these cells may represent pre-existent salivary gland ducts rather than a biphasic tumor component. Squamous or mucinous differentiation or chondromyxoid stroma was not observed. Perineural invasion was seen in several areas in H&E staining and was confirmed by S-100 staining (Figure 1F,G). Evident invasive growth could not be confirmed, as surrounding pre-existent salivary gland tissue was not present in the biopsy.

To rule out a potential biphasic component, immunohistochemical staining was performed. Initially performed immunohistochemical staining showed that tumor cells were positive for CK7 (Figure 2A) and S-100 (Figure 2B), partially positive for p63 (Figure 2C), SMMS-1 was positive in a great portion of cells, beta-catenin had membranous but no nuclear staining, and CD117 was focally positive (Figure 2D). The Ki-67 proliferation index was low (<5%) (Figure 2E). Though the p63 staining was partially positive, additional p40 staining was negative in tumor cells in the same tumor region (Figure 2F). In other additional immunohistochemical staining, tumor cells were focally positive for mammaglobin (Figure 2G), α-SMA (Figure 2H), BCL-2 (cytoplasmatic). DOG-1, EMA (Figure 2I), and GFAP staining were negative.

A convincing biphasic tumor component was not found and the remaining morphological features, tumor location, and immunohistochemical staining (positive S-100 staining, partially positive p63

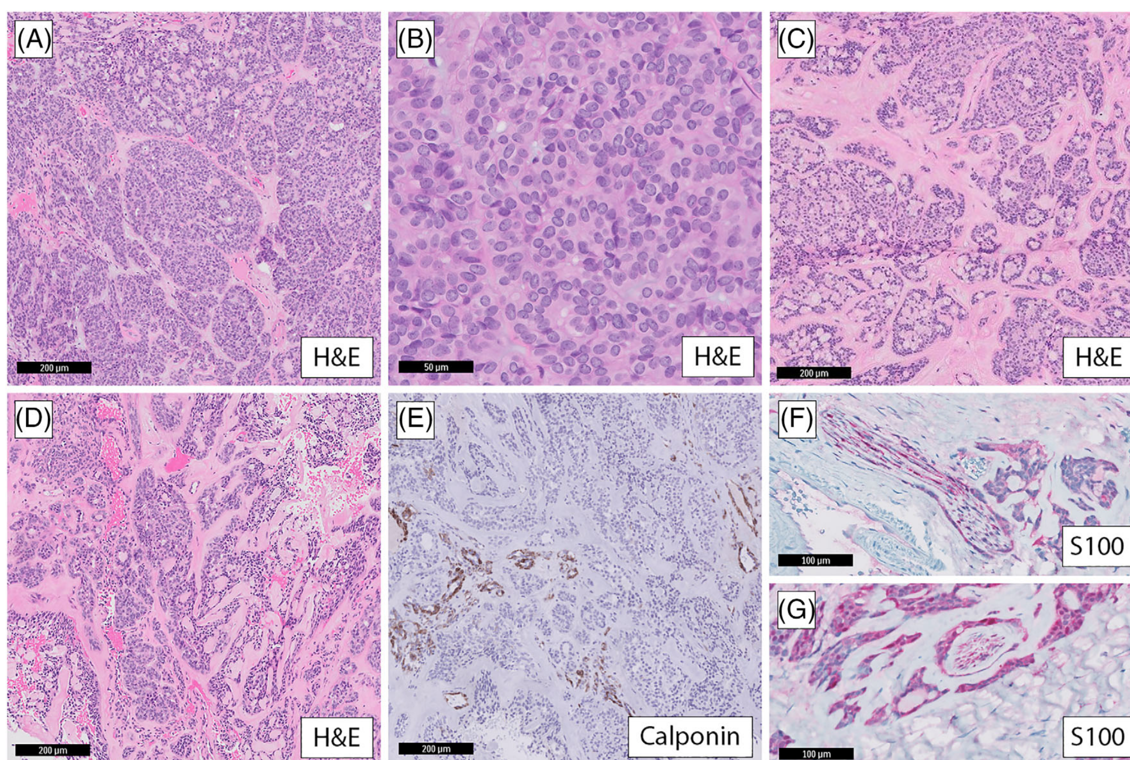

FIGURE 1 Histological findings of the biopsy. Hematoxylin and eosin-stained (H&E) sections demonstrated (A) fields, nests and rows of tumor cells with varying growth patterns, including trabecular and cribriform areas, and the formation of pseudo-glandular structures, (B) luminal cells with round to round-oval shaped nuclei with smooth nuclear membranes and fine granular pattern, nonconspicuous nuclei and moderate cytoplasm, and (C) hyalinized and hypocellular intercellular stroma. A potential biphasic cellular component was observed by H&E staining (D) and calponin immunohistochemistry (E). Perineural invasion was indicated by S-100 staining (F, G)

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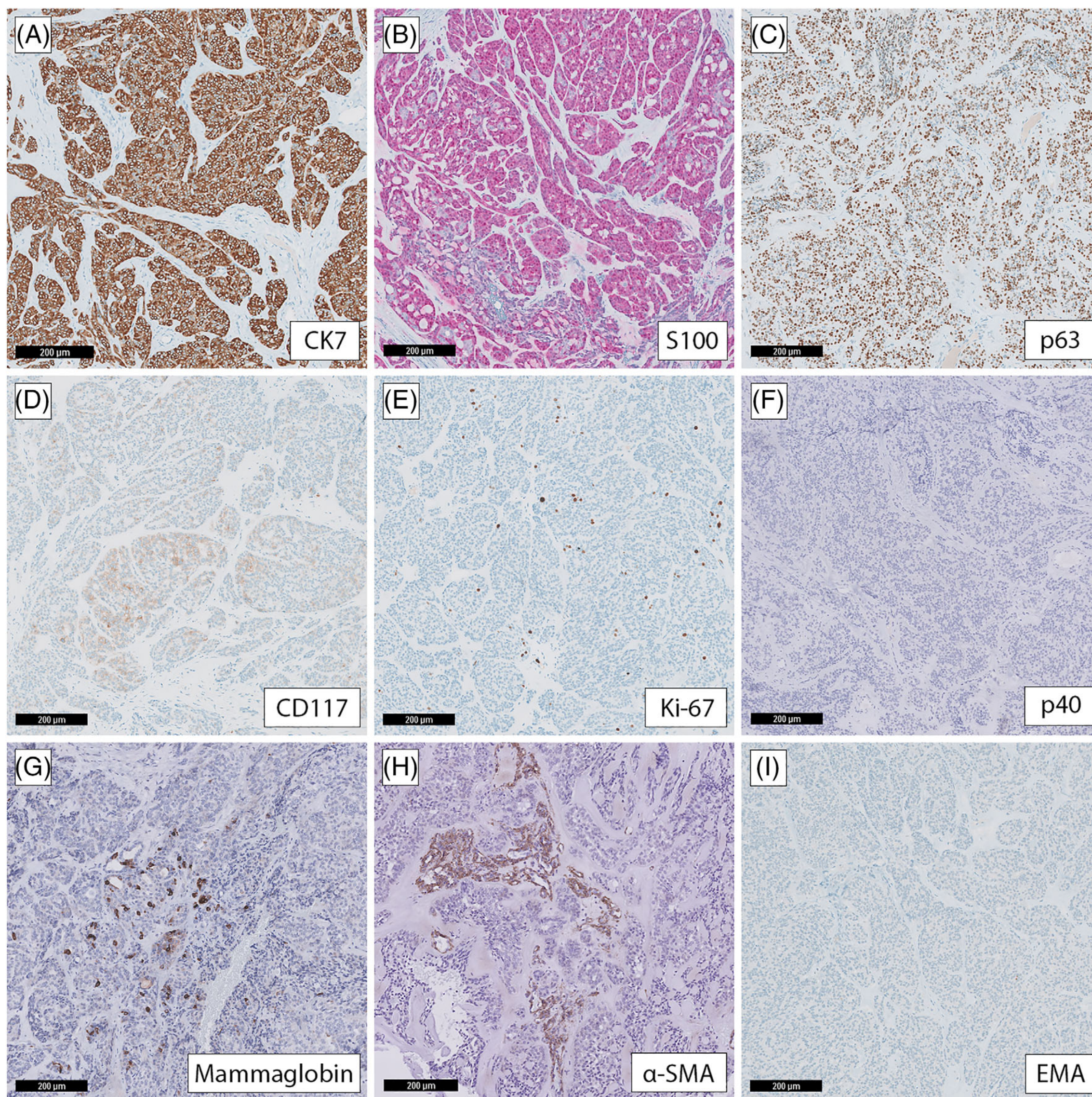

FIGURE 2 Immunohistochemical staining of the biopsy. Tumor cells were positive for CK7 (A) and S-100 (B), and partially positive for p63 (C). CD117 was focally positive (D). The Ki-67 proliferation index was low (<5%) (E). In the same region partially positivity for p63 was observed, p40 staining was negative (F). Tumor cells were focally positive for mammaglobin (G) and α-SMA (H). EMA staining was negative (I)

staining, negative p40) were indicative of CASG. Fusion gene analysis (Archer FusionPlex custom panel, including the *PRKD1/2/3*, *MYB*, *MYBL1* genes) was performed and revealed the expression of a novel *PPP2R2A* [NM_002717.3] exon 2::*PRKD1* [NM_002742.2] exon 11 fusion transcript, confirming a diagnosis of CASG.

Further diagnostic work-up and treatment were performed according to the relevant guidelines on head and neck cancer care. Magnetic resonance imaging (MRI) revealed a space-occupying lesion from the left retromolar pad to region 37 of the mandible. Computed tomography (CT) revealed no signs of nodal metastases of the neck or distant metastases of the lungs. Treatment consisted of local resection of the lesion by a partial mandible resection (marginal mandibulectomy) and primary closure of the wound. The recovery was uneventful. Histological examination of the resection specimen showed an irradical resection. Molecular diagnostics was not repeated. Postoperative radiotherapy with curative intent was started 4 weeks later. The patient provided written informed consent for publication.

2 | DISCUSSION

This report describes a case of CASG, harboring a novel *PPP2R2A*::*PRKD1* fusion. In this case, molecular testing was performed to definitively rule out a biphasic tumor component. Previously, some studies have shown that most PAC exhibit negative staining for the myoepithelial markers SMA and calponin, but that some of these tumors may show limited or even strong positivity for these markers.[7–9] One study described no expression of myoepithelial markers in a series of 26 PAC, except for patchy immunoreactivity in a subset of these

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tumors due to the involvement of entrapped non-neoplastic salivary acini and stromal myofibroblasts.[8] In retrospect, the biphasic component that was observed in the current case is more fitting for preexistent salivary gland ducts, due to its focality, its lack of cellular atypia, and the presence of morphological features and immunohistochemical staining which are fitting for CASG.

In this novel *PPP2R2A::PRKD1* rearrangement, *PPP2R2A* [NM_002717.3] exon 2 was fused to *PRKD1* [NM_002742.2] exon 11. The PPP2R2A protein is a regulatory subunit of the enzyme protein phosphatase 2 (PP2A), which is one of four major serine/threonine phosphatases and is involved in the negative control of cell growth and differentiation.[10] *PPP2R2A* has not been described previously as a fusion partner of *PRKD1*, but has been described as a fusion partner of the tumor-suppressor gene *CHEK2* in an intrathoracic mature teratoma and as a fusion partner of *PLAG1* in lipoblastoma.[11,12] In the described case of a *PPP2R2A::CHEK2* rearrangement, the fusion transcripts were out-of-frame, but the open reading frame of *CHEK2* was maintained, leading to the hypothesis of transcriptional deregulation of *CHEK2* through promoter swapping as the underlying oncogenic mechanism.[11] In the case of *PPP2R2A::PLAG1* rearrangement in lipoblastoma, the entire *PLAG1* coding sequence was placed under transcriptional control of the *PPP2R2A* fusion partner. Importantly, increased RNA expression of *PLAG1* was observed when compared to fusion-negative tumors.[12] Similar to our case, the involved *PPP2R2A* fusion partner concerned the *PPP2R2A* promoter region up to exon 2.

The *PRKD1* gene encodes the enzyme protein kinase D1 (PKD1), which has been implicated in mechanisms of tumorigenesis, including cell motility, proliferation, and apoptosis.[13] Importantly, both oncogenic and antioncogenic roles of PKD1 have been suggested in varying cancer types, including melanoma, gastrointestinal tract cancer, prostate cancer, and breast cancer.[13,14] The common molecular pathway of PAC and CASG may comprise of respectively increased activity and overexpression of *PRKD1*, which is implied by the previously reported gain-of-function *PRKD1* E710D hotspot mutation (present in 75% of PAC) and the *PRKD1* overexpression in *ARID1A::PRKD1* rearrangement (present in 24% of CASG).[15] The *PPP2R2A::PRKD1* fusion was an in-frame fusion between the *PPP2R2A* exon 2, containing the *PPR2R2A* gene promoter and transcription start site, and *PRKD1* exon 11, resulting in PPP2R2A regulated expression of an amino terminally truncated PRKD1 protein that still contained the kinase domain. Therefore, we hypothesize that the found *PPP2R2A::PRKD1* rearrangement may lead to overexpression of a functional PRKD1 kinase domain.

In summary, the current report describes a case of CASG, harboring a *PRKD1* fusion with a novel fusion partner (*PPP2R2A*). The presence of this *PRKD1* fusion supports the diagnosis of CASG. In addition, we confirm that CASG may show focal staining in myoepithelial markers (SMA and calponin) and some entrapment of preexistent salivary gland ducts, which may be misinterpreted as tumor cells with myoepithelial differentiation.

CONFLICT OF INTEREST

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

INFORMED CONSENT

The patient has provided written informed consent for publication.

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