Genetic Characterization of Adenoid Cystic Carcinoma of the Minor Salivary Glands: A Potential Familial Occurrence in First-Degree Relatives

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Abstract Adenoid cystic carcinoma (AdCC) is a malignant salivary gland tumor. To date, no cases of AdCC in first-degree relatives have been reported in the literature. We present a 50-year-old female (Case 1) and this patients' father (Case 2), both of whom were diagnosed with AdCC of the minor salivary glands. Histology of Case 1 demonstrated a tubulocribriform AdCC whereas Case 2 primarily was an AdCC of solid type. Both cases harbored the *MYB–NFIB* gene fusion as demonstrated by FISH and RNA-sequencing. After filtering and selection of putative deleterious variants, whole exome sequencing identified 18 germline variants in common between Case 1 and Case 2. However, none of the variants were associated with AdCC or other head and neck cancers. To our knowledge, we present the first potential case of familial AdCC. The presented genetic data may contribute to further investigations of the underlying genetic mechanisms for AdCC susceptibility.

Keywords Adenoid cystic carcinoma · Relatives · *MYB–NFIB* · Sequencing · Hereditary · Minor salivary glands

Introduction

Adenoid cystic carcinoma (AdCC) is the most frequent salivary gland malignancy in Denmark [1]. In the head and neck, AdCC can arise in the major salivary glands as well as in the minor salivary glands throughout the aerodigestive tract, of which the palatal site is the most frequent [1]. Clinically, AdCC can appear in any age-group, being rare in children and more frequent in middle-aged or older patients [2, 3]. AdCC has been called “the slow killer” due to its protracted but relentless clinical course marked by local- and distant recurrences for many years after initial treatment in up to 50% of patients [3, 4]. The solid type of AdCC is the most aggressive histological subtype and is associated with a poorer prognosis [3]. At the time of diagnosis, the spread of tumor tends to be widely extended beyond the primary tumor site, in part due to the propensity of AdCC to grow along nerves, and particularly in minor salivary gland sites due to submucosal extension. Radical primary surgery with or without radiotherapy (RT) is the current treatment of choice for localized disease, although the effect of RT is moderate at best [3, 5].

Genetically, it is well-established that AdCC is characterized by an, among salivary gland tumors, pathognomonic balanced translocation between chromosomes 6 and 9 (t(6;9)(q22–23;p23–24), leading to fusion of the *MYB* and *NFIB* genes [6, 7]. Conferring an overexpression of the transcriptionally active 5' of the *MYB* gene, this genetic event causes overexpression of *MYB* target genes which is considered a distinct feature of AdCC biology and therefore a potential therapeutic target [7, 8]. Approximately one-third of AdCCs do not harbor the *MYB–NFIB* gene fusion, but are instead driven by *MYBL1–NFIB* fusions or by the insertion of super-enhancer sequences upstream for *MYB* resulting in *MYB* overexpression [9, 10]. The distinct

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molecular mechanism of some diseases has been unraveled by the exploration of the genetics of families with an apparent disposition for the disease, i.e., the *BRCA* genes in breast cancer patients. This has dramatically increased understanding of the genetics of these cancers, and this approach continues to be relevant for more rare types of cancer.

To the best of our knowledge, no hereditary transmission of AdCC has been reported to date. We report a potential case of hereditary AdCC occurring in the minor salivary glands in first-degree relatives and present histological and genetic data on both cases.

Case Report

Case 1

A 50-year-old female presented with a 3-month history of swelling of the hard palate located left of the midline. The patient had a medical history of seven skin tumors including four skin adnexal tumors; one pilomatrixoma in 2007 (diagnosed at age 43) which was removed from her left underarm; one dermatofibroma in 2008 removed from her lower left leg, and two trichofolliculomas were removed in 2009 and were located under the nose and at the right nasolabial fold. Three epithelial inclusion cysts were removed in 2009 (unknown location), 2011 (located on the back) and 2013 (located on the abdomen) respectively. According to the patient and her mother, no other family members beside her father (Case 2) had a history of cancer (Fig. 1). The patient was a former occasional smoker in 16 years, had

no alcohol abuse and was not previously exposed to any work-related carcinogens. Clinical examination revealed a 15×25×10 mm mass in relation to the border of soft and hard palate with an intact overlying mucosa with no signs of inflammation. The swelling was firm, elastic and fixated to the underlying tissue. Fine-needle aspiration cytology showed cells from a salivary gland tumor. Magnetic resonance imaging (MRI) of the head and neck revealed a contrast enhancing mass of the hard palate on the left side extending posteriorly to the soft palate and laterally to the pterygoid muscles and left maxilla and no pathological lymph nodes (Fig. 2). Chest X-ray was normal. Core needle biopsies demonstrated infiltrative growth of a tubulocribriform AdCC with perineural invasion (Fig. 3a). The patient initially underwent a left-sided maxillary swing procedure which was, due to tumor extent, converted to a hemimaxillectomy through a facial Weber Ferguson incision. Computer-assisted functional endoscopic sinus surgery was used to perform a frontal sinus resection and a left-sided ethmoid- and sphenoidectomy. Frozen sections used perioperatively showed extensive spread of AdCC involving the entire sinonasal region extending to the retromaxillary area, the left palate and maxilla and nasopharynx. For this reason, a hemimaxillectomy was performed including sacrifice of V₂ and V₃ of the trigeminal nerve and a part of the orbital floor (Fig. 4). A left-sided neck dissection and reconstruction of the left maxilla was performed in a subsequent procedure with a free osteomyocutaneous fibula flap. Final histology of the resected maxilla confirmed the diagnosis of AdCC of tubulocribriform type with no lymph node involvement.

The patient received postoperative radiotherapy (RT) to the primary site after approximately 3 months from initial surgery. At follow-up 16 months after radiation and 21 months after surgery, the patient remains free of

Fig. 1 Pedigree of the family showing the two cases with AdCC (black-shaded) as well as the age at diagnosis. Circles indicate females and squares indicate males, while a diagonal slash indicates deceased. The proband is indicated with an arrow

Fig. 2 Case 1 Axial magnetic resonance imaging of the head and neck showing a contrast enhanced mass localized in the maxilla and the retromaxillary space

Fig. 3 **a** Biopsies from Case 1 showing adenoid cystic carcinoma of tubulocribriform type (Hematoxylin and eosin). **a**, *Inset* Perineural invasion was frequent with carcinoma cells within the perineurium (*arrow*). **b** *Case 1* Intense nuclear expression of MYB protein in primarily the peripheral carcinoma cells at the periphery of the tumour islands (*arrow*). The stromal cells were consistently negative (*arrowhead*). **c** Fluorescence in situ hybridization (FISH) demonstrating split signals for the *MYB* gene, as demonstrated by isolated red (*arrows*) and green (*arrowheads*) signals. **d** In the same tumor, the

NFIB gene displayed split signals whereas (*inset*) *MYBL1* showed intact, fused signals (*asterisks*). **e** Primary tumor tissue from Case 2 showing adenoid cystic carcinoma with predominantly solid growth and (*inset*) few but distinct tubulocribriform areas. **f** Case 2 showed the same pattern of MYB expression staining as Case 1 (*arrow*). **f**, *upper inset* FISH demonstrated split signals for *MYB* and (*lower inset*) *NFIB* genes. *MYBL1* showed intact, fused signals in Case 2 (data not shown). Scale bar = 100 μm

Case 2

The patients' father was diagnosed with AdCC in the right nasal cavity at age 42 (27 years before Case 1). He had no other history of cancer. The patient had smoked pipe tobacco occasionally through many years (the exact smoking history was not known), and had no alcohol abuse. From age 21, he had worked for seven years (1964–1970) as an industrial mechanic for a filament winding machine manufacturer and was exposed to organic compounds as well as polyurethane foam machines and polyester-glass fiber spraying machines which he maintained and repaired.

He was primarily treated with RT and later developed five local recurrences which were surgically resected until he died 16 years after the primary diagnosis. He did not develop any distant metastases. Formalin-fixed paraffin embedded tissue from the biopsy of the primary tumor was retrieved for analysis (Fig. 3e).

RNA- and DNA-Sequencing, Fluorescence In Situ Hybridization and Immunohistochemistry

To confirm AdCC diagnosis, RNA-sequencing was performed on Case 1 which demonstrated fusion of *MYB* exon 8 to *NFIB* exon 9 (Fig. 5). Fluorescence in situ hybridization (FISH) analysis was performed on both cases to

Fig. 4 Gross specimen of the left maxilla measuring 6.5×5×5 cm showing the large extent of tumor on the hard palate

recurrence. The patient reported that her father had died of AdCC at a young age, and genetic work-up was initiated due to the possibility of heritability and early clinical management of the patients' four children aged 18, 16, 15 and 12, respectively.

Fig. 5 *Case 1* Identification of a fusion transcript between *MYB* and *NFIB* by RNA-sequencing. RNA sequencing was performed on tumor RNA. A fusion transcript between *MYB* (exon 8) and *NFIB* (exon 9), resulting from a translocation between the *MYB* gene on chromosome 6 and the *NFIB* gene on chromosome 9, was identified using FusionMap (Array Suite Wiki). To validate the fusion junction, reads from the RNA-Seq data were aligned to a *MYB–NFIB* reference sequence in CLC Biomedical Workbench (Qiagen) as indicated in the figure. Reads spanning the *MYB–NFIB* junction in the patient sample are shown (*top*), while no reads spanning the junction are found in a control sample (*bottom*)

identify *MYB/NFIB* and *MYBL1/NFIB* rearrangements (Fig. 3c, d, f). In Case 1, 76% of cells had break apart signals for *MYB* and 65% of cells had *NFIB* break apart signals. In Case 2, 35% of cells had break apart signals for *MYB* and 51% of cells had *NFIB* break apart signals. Both cases were wild-type for *MYBL1*. With immunohistochemistry, both cases overexpressed MYB protein (Fig. 3b, f).

To investigate the possibility of heritability, whole exome sequencing (HighSeq, Illumina) of DNA purified from whole blood (Case 1) and normal FFPE tissue (Case 2) was performed after informed and written consent was obtained from Case 1 and the widow of Case 2.

After filtering, selection of putative deleterious variants and manual inspection (CLC Biomedical workbench and Ingenuity variant analysis, Qiagen), 18 germline variants in common between Case 1 and Case 2 were identified (Table 1). To our knowledge, none of the variants were associated with AdCC or other types of head and neck cancer.

The history of multiple skin tumors originating in the hair follicles of the head and neck region in Case 1 led us to suspect Brooke–Spiegler (BS) syndrome (familial cylindromatosis; OMIM 605 041) which is characterized by mutations in the tumor suppressor gene *CYLD* [11]. However, in exome data from Case 1, no germline mutations in *CYLD* were identified.

Discussion

AdCC is rare but enigmatic among human cancers due to its prolonged, but relentless, clinical course. As the achievement of complete surgical resection often is complicated by tumor extension and a poor response to radiotherapy and resistance to chemotherapy, identification of novel therapeutic targets are highly warranted. In recent years several genetic hallmarks of AdCC have been uncovered, namely translocations involving the *MYB*, *MYBL1* and *NFIB* genes

Table 1 Germline variants mutual between Case 1 and Case 2 after whole exome sequencing (WES) performed on DNA from blood (Case 1) and normal formalin-fixed paraffin-embedded (FFPE) tissue (Case 2)

Chr.	Position	Gene region	Gene symbol	Transcript ID	Transcript variant	Protein variant	Translation impact
1	117,146,504	Exonic	<i>IGSF3</i>	NM_001542.3	c.1426C>T	p.R476C	Missense
3	121,126,425	Exonic	<i>STXBP5L</i>	NM_014980.2	c.2995G>T	p.A999S	Missense
5	140,590,211	Exonic	<i>PCDHB12</i>	NM_018932.3	c.1732T>C	p.W578R	Missense
5	156,479,572	Exonic	<i>HAVCR1</i>	NM_012206.3	c.473T>C	p.M158T	Missense
6	109,763,464	Exonic	<i>SMPD2</i>	NM_003080.2	c.370C>T	p.H124Y	Missense
6	154,360,864	Exonic	<i>OPRM1</i>	NM_000914.4	c.186_188dupCGG	p.G63dup	In-frame
7	74,558,397	Exonic	<i>GTF2IRD2/GTF2IRD2B</i>	NM_173537.3	c.1142C>A	p.A381D	Missense
12	11,244,356	Exonic	<i>TAS2R43</i>	NM_176884.2	c.473T>A	p.F158Y	Missense
12	123,889,486	Exonic	<i>KMT5A</i>	NM_020382.3	c.713G>C	p.R238P	Missense
12	123,892,186	Exonic	<i>KMT5A</i>	NM_020382.3	c.995T>C	p.L332P	Missense
12	124,824,739	Exonic	<i>NCOR2</i>	NM_006312.5	c.5499_5500insC	p.S1834fs*193	Frameshift
14	105,716,854	Exonic	<i>BTBD6</i>	NM_033271.2	c.1303G>A	p.V435I	Missense
16	113,177	Exonic	<i>RHBDF1</i>	NM_022450.3	c.466A>G	p.I156V	Missense
16	2,138,507	Exonic	<i>TSC2</i>	NM_000548.4	c.5320A>G	p.S1774G	Missense
17	40,913,906	Exonic	<i>RAMP2</i>	NM_005854.2	c.152C>T	p.P51L	Missense
17	40,914,670	Exonic	<i>RAMP2</i>	NM_005854.2	c.328G>T	p.G110C	Missense
20	23,805,930	Exonic	<i>CST2</i>	NM_001322.2	c.259A>G	p.I87V	Missense
22	51,160,154	Exonic	<i>SHANK3</i>	NM_033517.1	c.3893G>A	p.R1298K	Missense

The variants are not filtered for cancer association

and the insertion of super-enhancer sequences upstream of the *MYB* gene, resulting in overexpression of MYB protein in 80–90% of AdCCs [6, 10, 12–15]. Also, *NFIB* seems to be involved in other salivary gland malignancies [16]. The molecular cause of these genetic aberrations of apparent fundamental importance remains obscure. So far, AdCC is not believed to be a hereditary disease as no first line relatives diagnosed with AdCC have been reported.

We present two first-degree relatives with AdCC of the minor salivary glands of the head and neck. AdCC is considered to occur sporadically with no known environmental causative agents, making the sheer rarity of these tumors suggestive of our two cases being caused by an inherited genetic disposition to AdCC. Both cases harbored the characteristic *MYB–NFIB* gene fusion and overexpressed MYB protein, unanimously confirming the diagnosis. In pursuit of identifying germline variants of possible significance for AdCC predisposition, whole exome sequencing of DNA from blood from Case 1 and DNA from normal FFPE tissue from Case 2 was performed and 18 germline variants were identified. None of these findings could assist us in the assessment of a hereditary component in our cases. Genetically, AdCC is characterized by a translocation and not a germline “first hit” mutation as is the case in most hereditary syndromes involving cancer. Identification of a susceptibility locus for AdCC disease is therefore more challenging, possibly

involving defects in genes responsible for repair of chromosomal breaks or inherited fragile regions at chromosomal sites in the *MYB* and/or *NFIB* genes not detectable by sequencing.

Of special interest is the history of numerous cutaneous tumors localized in the head and neck region in Case 1, which could be associated with the BS syndrome that is characterized by cutaneous adnexal tumors as well as tumors of the salivary glands [17–19]. Genetically, BS syndrome is defined by germline mutations in the tumor suppressor gene *CYLD*. However, sequencing of Case 1 identified no deleterious mutations in the *CYLD* gene.

To our knowledge, this is the first potential case of first-degree relatives diagnosed with AdCC of the salivary glands. Both cases harbored the characteristic *MYB–NFIB* translocation, however the two cases had no germline mutation associated with any known human cancer in common. This could imply that this disposition to AdCC occurs by yet unknown genes, by unknown functions of the 18 identified germline variants, or that these two cases are simply coincidental. With the advancement of knowledge on the heritability of cancer and molecular techniques, our results could aid in unraveling a possible mechanism for AdCC susceptibility.

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Compliance with Ethical Standards

Conflict of interest No conflict of interest to disclose.

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