Clinical Utility of Whole-Genome Sequencing to Aid Histologic Diagnosis and to Direct Personalized Medicine in Salivary Gland Cancer

Matt Church, BMBCh, MA, MRCP, MRes[1,2]; George Burghel, PhD, DClinSci, FRCPath[2,3]; Guy Betts, PhD, MBChB, FRCPath[3]; Steven Michael Churchill, MBChB, BMedSci, MRCGP[1]; Helene Barbara Schlecht, PhD, BSc[3]; Yatin Jain, MBChB, FRCR[1]; Kevin Harrington, PhD, MBBS, FRCP[4,5]; and Robert Metcalf, PhD, MBChB, MRCP[1,2]

DOI https://doi.org/10.1200/PO-25-00490

ABSTRACT

PURPOSE Salivary gland cancers (SGCs) are rare and comprise multiple histologic entities. In the recurrent or metastatic (R/M) setting, there is limited evidence for effective systemic anticancer treatment for most subtypes, affecting prognosis and quality of life. Molecular analysis of SGCs holds promise to more accurately classify SGC subtypes and to determine novel therapeutic targets.

MATERIALS AND METHODS Fifteen patients with R/M SGC underwent tumor biopsy and blood sampling to perform whole-genome sequencing (WGS) of tumor and germline as part of their standard-of-care management. Small somatic mutations, structural alterations, copy number variation, and mutational signatures were processed using WGS pipelines alongside germline testing. Alterations were correlated to clinical features and fed back to clinical team to inform treatment decisions.

RESULTS WGS quality control was acceptable in 14 of 15 patients (adenoid cystic carcinoma [AdCC, n = 10], salivary duct carcinoma ex pleomorphic adenoma [n = 1]; clear cell myoepithelial carcinoma [n = 1]; epithelial-myoepithelial carcinoma [n = 1]; and acinic cell carcinoma [n = 1]). Genomic rearrangements/fusions were present in 12 of 14. Rearrangements involving MYB and or NFIB were identified in 8 of 10 patients with AdCC. One patient harbored a clinically actionable *FGFR1-pleomorphic adenoma gene 1* fusion and responded to fibroblast growth factor receptor–targeted therapy, in addition to enabling histologic reclassification. Other fusions included *EWSR1-ATF1* and *CRTC1-MAML2*, which also aided definitive histologic classification. Small somatic alterations were identified in all but one patient. There were no pathogenic germline mutations.

CONCLUSION WGS in SGC is achievable in clinically relevant timeframes, providing genomic information for deeper understanding of disease pathophysiology, to clarify histologic subtype and can identify actionable genomic targets which may not be found through routine sequencing technologies. Further use of WGS has the potential to improve care for patients with SGC.

ACCOMPANYING CONTENT

Appendix

Accepted August 21, 2025
Published October 30, 2025

JCO Precis Oncol 9:e2500490

INTRODUCTION

Salivary gland cancers (SGCs) are rare, representing 0.5% of cancers and is subtyped into over 30 distinct histopathologic entities.[1,2] The most common subtype, mucoepidermoid carcinoma (MEC), has an estimated incidence of only 0.285 per 100,000.[3]

The mainstay of treatment for SGC is surgery and radiotherapy.[4-6] Despite optimal treatment, approximately 20% develop metastatic disease with a median overall survival (mOS) of 15 months (varying by subtype).[7,8] Cytotoxic chemotherapy has limited efficacy in SGC and is reserved for symptomatic patients.[4,9] Therapeutic developments are hampered by low incidence, given the challenges in recruitment to large-scale or randomized clinical trials. These difficulties have increased interest in precision medicine.[4] Multiple genomic alterations have been identified in SGC, including *MYB-NFIB* fusions in adenoid cystic carcinoma (AdCC).[10] Significant clinical benefits have been seen to precision approaches in *NTRK* fusion–positive secretory carcinomas, androgen receptor–positive, or HER2-positive salivary duct carcinoma (SDC) as well as in SGCs with *ALK* and *RET* alterations.[3,11-15]

CONTEXT

Key Objective

To evaluate how whole-genome sequencing (WGS) of a consecutive series of patients with recurrent or metastatic salivary gland carcinoma might influence clinical decisions.

Knowledge Generated

WGS identified somatic alterations in all but one patient, including variants in *NOTCH*, *TERT* promoter, and chromatin-modifying genes, and various germline variants of uncertain significance and uncovered novel *NFIB-QDPR* and *NFIB-PHIP* rearrangements while also reinforcing the prevalence of *MYB-NFIB* fusions in adenoid cystic carcinoma. WGS informed histologic diagnosis in at least two patients who were difficult to classify using histology alone despite expert salivary review, including a *CRTC1-MAML2* fusion enabling reclassification as a high-grade clear cell mucoepidermoid carcinoma and additionally a *FGFR1-pleomorphic adenoma gene 1* fusion assisted reclassification to a high-grade myoepithelial carcinoma and enabled treatment with pemigatinib with a durable clinical response.

Relevance

WGS can be performed in clinically relevant timescales, and resulting molecular information can identify precision oncology targets and support histopathologic classification of uncertain cases.

Low numbers and histologic heterogeneity impair deep understanding of the genomics of SGC, and more detailed profiling is of value.[16] Traditionally, tumor profiling has been limited to sequencing panels decided a priori.[17] Whole-genome sequencing (WGS) is now entering clinical care in the English National Health Service and has the potential to improve understanding of SGC biology and identify novel precision medicine targets.[18] We therefore sought to evaluate the potential role of WGS on clinical decision making for patients with SGC. In addition to further illuminating the genomic landscape of SGC, we report the use of WGS to aid histologic diagnosis and the finding of an *FGFR1* fusion with an excellent response to directed therapy.

MATERIALS AND METHODS

Patient Recruitment

Ethical approval was obtained via Manchester Cancer Research Centre Biobank (22/NW/0237). Fifteen consecutive patients with recurrent or metastatic (R/M) SGC at a single center were approached, counseled, and consented. EDTA blood samples and frozen core biopsies (under radiologic guidance from the safest anatomic location) were obtained. All patients had previous DNA next-generation sequencing (NGS) and RNA fusion testing panels as standard of care. All histopathology samples from diagnostic biopsy, surgery, and recurrence biopsy were reviewed by an experienced salivary pathologist. HPV status was not available for samples studied.

Whole-Genome Sequencing

WGS followed Genomics England protocols.[19] Briefly, somatic and germline DNA was extracted, followed by library preparation (TruSeq) and sequencing using HiSeqX (Illumina, San Diego, CA). Sequences were aligned (GRCh38 reference) using DRAGEN.[20] Sequence variants are reported using Human Genome Variation Society nomenclature.[21] Results were discussed at a genomic tumor advisory board (GTAB). Potentially clinically significant results were reported to clinicians and fed back to patients.

Small Somatic Variant Analysis

Small somatic variants (single-nucleotide variants and indels <50 bp), with tumor normal subtraction, were detected using Strelka2 (v2.9.9). Detected variants were reviewed following published guidelines.[22,23] Variants were annotated using Cellbase and classified as domain 1 if a variant in genes indicated by the National Genomic Test Directory for Cancer and 2 if a variant in one of 553 cancer-related genes defined by the Cancer Gene Census.[24-27] Domain 1 variants were assessed for clinical relevance and Association for Molecular Pathology (AMP) tier.[23] The potential impact of somatic alterations was estimated for missense changes using in silico tools PolyPhen2 and Ensembl variant effect predictor (VEP)[70] for Sorting Intolerant From Tolerant (SIFT),[71] Combined Annotation Dependent Depletion (CADD),[72] Rare Exome Variant Ensemble Learner (REVEL)[73] and AlphaMissense in line with evidence of accuracy in somatic variants.[28]

Copy Number and Somatic Structural Variant Analysis

Somatic structural variants and indel >50bp analysis was performed with Manta5, and copy number variants (CNVs) were detected with Canvas6.[18] For structural variants with the potential to result in gene fusion, a predictive algorithm was used to predict for in-frame and out-of-frame fusions.

TABLE 1. Summary of Clinical and Disease Characteristics of Patients With Recurrent/Metastatic Salivary Cancer With Successful WGS Analysis

Patient ID	Sex	Age	Primary Site	Histologic Subtype	Years Since Primary Surgery	Previous Radiotherapy to Primary	Metastatic Sites	First-Line SACT	Second-Line SACT
1	Female	76	Parotid	AdCC	NA (inoperable)	Palliative XRT	Liver	NA	NA
2	Male	72	Tongue base	AdCC	NA (inoperable)	Radical XRT	Lung, bone, Liver	NA	NA
3	Female	42	Submandibular	AdCC	5	Adjuvant XRT	Lung	NA	NA
4	Female	52	Maxillary sinus	AdCC	NA (inoperable)	Radical XRT	Lung, brain	NA	NA
5	Female	57	Buccal mucosa	AdCC	9	Adjuvant XRT	Lung, pleura	NA	NA
6	Female	48	Thyroid/ unknown	AdCC	4	Adjuvant XRT	Lung, pleura, bone	Carboplatin and vinorelbine	NA
7	Male	53	Parotid	AdCC	5	Adjuvant XRT	Liver, lung	Axitinib + avelumab	**Lenvatinib**
8	Female	63	Maxilla mucosa	AdCC	7	Adjuvant XRT	Lung, kidney	NA	NA
9	Female	57	Hard palate	AdCC	11	Adjuvant XRT	Lung, liver, nodes	Cisplatin + 5-FU	**Carboplatin and capecitabine**
10	Male	50	Parotid	AdCC/adenocarcinoma NOS; *Reclassified as high-grade myoepithelial carcinoma*	4	Adjuvant XRT	Lung, liver, nodes, pleura	Carboplatin and Capecitabine	**Pemigatinib**
11	Male	55	Parotid	Salivary duct carcinoma ex pleomorphic adenoma	2	Adjuvant XRT	Lung, pleural, nodes, chest wall, bone	Bicalutamide and triptorelin	Carboplatin and Capecitabine
12	Female	53	Eustachian tube	Clear cell myoepithelial carcinoma; *On reassessment, considered likely to represent a hyalinizing clear cell carcinoma (full reassessment pending)*	NA (inoperable)	Radical XRT	Lung, liver, kidney, nodes	Doxorubicin, cisplatin, cyclophosphamide	NA
13	Female	48	Parotid	Epithelial-myoepithelial carcinoma; *On reassessment, considered as likely to represent a high-grade clear cell mucoepidermoid carcinoma*	5	Adjuvant XRT	Nodes, liver, bone	NA	NA
14	Male	38	Maxillary sinus	Acinic cell	9	Adjuvant XRT	Lung, long, chest wall, bone	NA	NA

NOTE. Years since primary surgery defined from time of WGS. Palliative radiotherapy 30 Gy in 30#, radical/adjuvant radiotherapy ranged from 60 to 66 Gy in 30-33#. Treatment lines commenced after the WGS biopsy are listed in bold. Several patients histological subtype were reclassified following the additional molecular context from WGS on salivary pathologist re-review.
Abbreviations: 5-FU, fluorouracil; AdCC, adenoid cystic carcinoma; NA, not applicable; NOS, not otherwise specified; SACT, systemic anticancer therapy, WGS, whole-genome sequencing; XRT, radiotherapy.

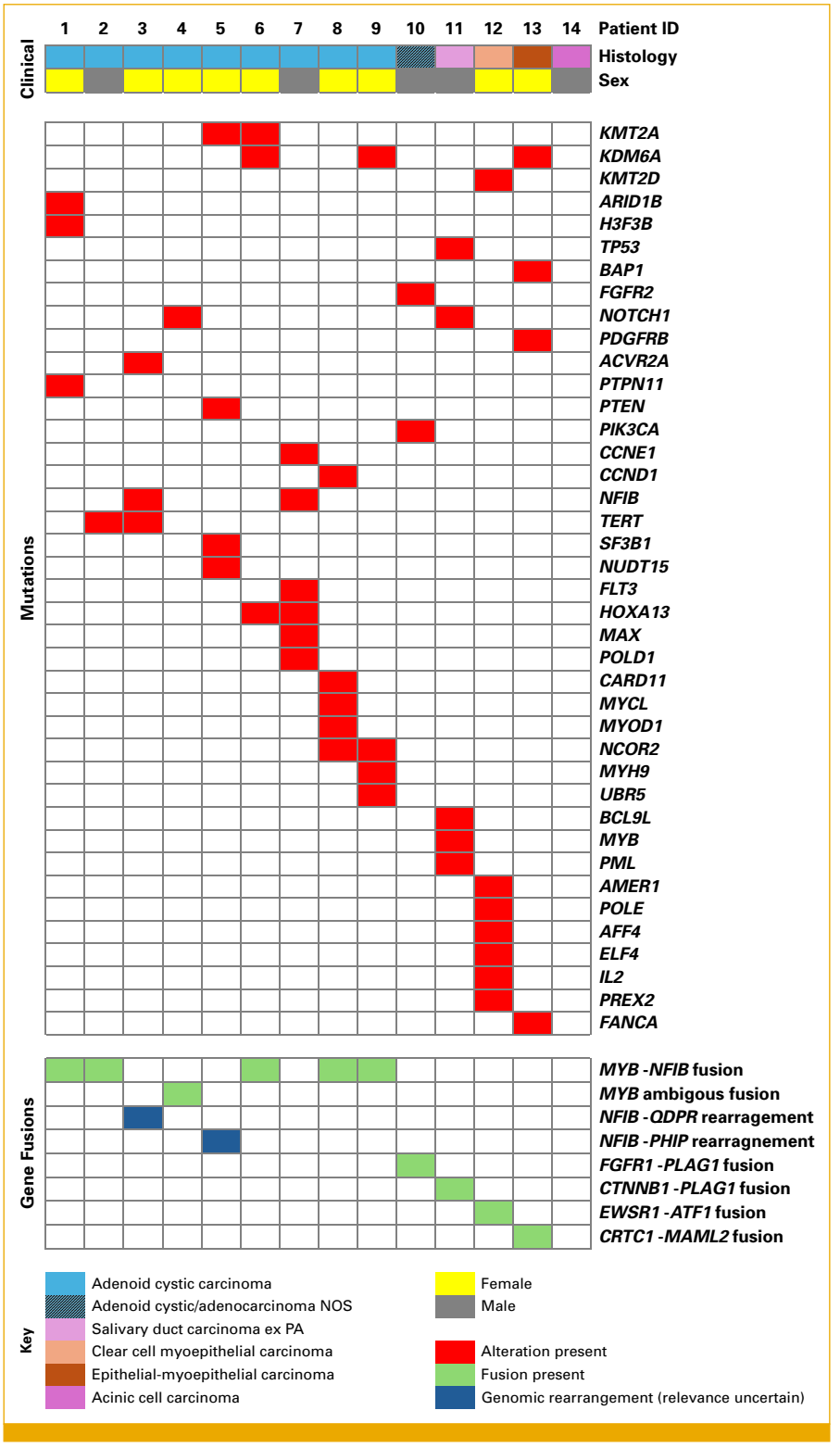

FIG 1. Tabular representation of tumor samples alongside histology (at the time of WGS), sex, small somatic variants, and somatic gene fusions. Key pathways related to somatic variants are displayed. After WGS-guided reassessment, patient 10 was reclassified as a myoepithelial carcinoma, patient 13 was reassessed as a high-grade clear cell mucoepidermoid carcinoma, and patient 12 was considered likely to represent a hyalinizing clear cell carcinoma (full reassessment pending). Specific HGVS of variants are described in Table 2. HGVS, Human Genome Variation Society; NOS, not otherwise specified; PA, pleomorphic adenoma; WGS, whole-genome sequencing.

TABLE 2. Summary of Genomic Results for Tumors Across Standard-of-Care NGS/RNA Fusion Panel Testing, IHC Along With Site of WGS Biopsy (neoplastic cell content in brackets) and WGS Results for Small Somatic Variants (variant allele frequency in brackets), Structural Variants (copy numbers in brackets), TMB, and Germline Variants (ACMG class in square brackets)

Patient ID	Standard-of-Care Genomic Panel	WGS Biopsy (NCC)	WGS Somatic Variants	WGS Structural	TMB	Germline Findings
1	DNA NGS Panel not available RNA Fusion Panel negative	Neck Node (30%)	*ARID1B* c.613T>G p.(Leu205Val) (11%) *H3F3B* c.316G>A p.(Glu106Lys) (20%) *PTPN11* c.80G>C p.(Gly27Ala) (39%)	*MDM2* GAIN (25), *MYB::NFIB* fusion	0.93	*ERCC4* c.1727G>C p.(Arg576Thr) [Class 3]
2	DNA NGS Panel negative RNA Fusion Panel negative	Liver (60%)	*TERT* c.-124C>T (18%)	*CDKN2A* Loss (1), *MYB:NFIB* fusion	0.81	*RTEL1* c.2345C>T p.(Ala782Val) [Class 3]
3	DNA NGS Panel negative RNA Fusion Panel negative	Lung (80%)	*TERT* c.-124C>T (39%) *ACVR2A* c.799_816+11del29 (21%) *NFIB* c.667_673del7 p.(Leu223GlufsTer7) (51%)	*NFIB::QDPR* ambiguous genomic rearrangement	0.75	*MSH6* c.866G>A p.(Gly289Asp) [Class 3] *TSC1* c.2626-3_2626-2insTA [Class 3] *TSC1* c.2626-3C>T [Class 3]
4	DNA NGS Panel negative RNA Fusion Panel negative	Lung (80%)	*NOTCH1* c.1039G>T p.(Gly347Cys) (8%)	*NFIB* Loss (1), *ERBB3* Loss (1), *CDK4* Loss (1), *MYB* ambiguous inversion and fusion	0.63	*POLD1* c.1666G>A p.(Val556Ile) [Class 3]
5	DNA NGS Panel failed testing RNA Fusion Panel negative	Lung (90%)	*PTEN* c.737dupC p.(Leu247ValfsTer6) (36%) *SF3B1* c.1873C>T p.(Arg625Cys) (14%) *KMT2A* c.25T>G p.(Phe9Val) (14%) *NUDT15* c.59T>C p.(Val20Ala) (15%)	*KDM6A* LOSS (0), *NFIB::PHIP* out of frame genomic rearrangement	1.24	*APC* c.8213T>C p.(Ile2738Thr) [Class 3] *CBL* c.1477C>T p.(Leu493Phe) [Class 3]
6	DNA NGS Panel negative RNA Fusion Panel negative	Lung (60%)	*KDM6A* c.1693C>T p.(Gln565Ter) (66%) *HOXA13* c.172T>G p.(Phe58Val) (17%) *KMT2A* c.1702C>T p.(Pro568Ser) (33%)	*NOTCH1* LOH (2), *KDM6A* LOSS (1), *MYB::NFIB* fusion	0.75	*FANCG* c.1538G>A p.(Arg513Gln) [Class 3] *MUTYH* c.536A>G p.(Tyr179Cys) [Class 5] *XPC* c.1984G>A p.(Glu662Lys) [Class 3]
7	DNA NGS Panel negative RNA Fusion Panel negative	Liver (80%)	*MAX* c.223C>T p.(Arg75Ter) (64%) *POLD1* c.952G>A p.(Glu318Lys) (60%) *CCNE1* c.737T>A p.(Ile246Asn) (34%) *FLT3* c.2198C>G p.(Pro733Arg) (35%) *HOXA13* c.172T>G p.(Phe58Val) (15%) *NFIB* c.6_7insGAAA p.(Tyr3GlufsTer*11) (39%)	*MAX* LOSS (1), *PTPRK* deletion of exons 20 to 31	0.93	*NTHL1* c.859C>T p.(Gln287Ter) [Class 5] *PMS2* c.916G>A p.(Val306Met) [Class 3] *TSC2* c.1839+6G>A [Class 3]
8	DNA NGS Panel negative RNA Fusion Panel negative	Kidney (100%)	*CCND1* c.860C>T p.(Pro287Leu) (30%) *CARD11* c.2533A>T p.(Lys845Ter) (38%) *MYCL* c.577A>C p.(Ser193Arg) (12%) *MYOD1* c.695A>G p.(Asn232Ser) (44%) *NCOR2* c.5734C>T p.(Pro1912Ser) (38%)	*MYB::NFIB* ambiguous fusion	1.3	Nil
9	DNA NGS: *TP53* loss (0.77 copies) RNA Fusion Panel negative	Liver (80%)	*KDM6A* c.995dup p.(Asn332LysfsTer32) (90%) *MYH9* c.1230_1247dup18 p.(Asp411_Ala416dup) (47%) *NCOR2* c.2787delC p.(Glu930ArgfsTer34) (53%) *UBR5* c.4843G>A p.(Asp1615Asn) (47%)	*KDM6A* LOSS (1), *MYB::NFIB* fusion	1.05	*ATM* c.7788+8G>T [Class 3] *FANCA* c.2982-8C>G [Class 3] *FANCG* c.1538G>A p.(Arg513Gln) [Class 3] *POLH* c.1253_1255delCTC p.(Pro418del) [Class 3] *SMAD4* c.1573A>G p.(Ile525Val) [Class 3]

(continued on following page)

TABLE 2. Summary of Genomic Results for Tumors Across Standard-of-Care NGS/RNA Fusion Panel Testing, IHC Along With Site of WGS Biopsy (neoplastic cell content in brackets) and WGS Results for Small Somatic Variants (variant allele frequency in brackets), Structural Variants (copy numbers in brackets), TMB, and Germline Variants (ACMG class in square brackets) (continued)

Patient ID	Standard-of-Care Genomic Panel	WGS Biopsy (NCC)	WGS Somatic Variants	WGS Structural	TMB	Germline Findings
10	DNA NGS: *PIK3CA* c1634A>C RNA Fusin Panel negative	Liver (80%)	*PIK3CA* c.1634A>C p.(Glu545Ala) (41%) *FGFR2* c.1171_1172insATA p.(Cys391delinsTyrSer) (40%)	*FGFR1::PLAG1* fusion, gain in 8q	0.9	*POLE* c.3718G>A p.(Glu1240Lys) [Class 3]
11	Androgen receptor positive on IHC DNA NGS: *TP53* c.673-AA>G RNA Fusion Panel negative	Chest wall (40%)	*TP53* c.673-2A>G (45%, LOH) *BCL9L* c.610_611delAG p.(Ser204GlnfsTer16) (14%) *MYB* c.1958C>T p.(Ser653Phe) (25%) *NOTCH1* c.4620C>G p.(Phe1540Leu) (52%, LOH) *PML* c.1909C>G p.(Arg637Gly) (22%)	*CTNNB1::PLAG1* fusion	2.14	*TSC2* c.1318G>A p.(Gly440Ser) [Class 3]
12	DNA NGS Panel negative RNA Fusion Panel negative	Liver (80%)	*AMER1* c.3367A>T p.(Ser1123Cys) (6%) *POLE* c.2934A>C p.(Glu978Asp) (10%) *AFF4* c.745A>T p.(Met249Leu) (54%) *ELF4* c.1187+1G>T (42%) *IL2* c.203A>T p.(Lys68Met) (6%) *KMT2D* c.11493G>T p.(Gln3831His) (20%) *PREX2* c.2326C>T p.(Pro776Ser) (42%)	*CDKN2A* Loss, *EWSR1::ATF1* fusion	2.86	*FANCG* c.1538G>A p.(Arg513Gln) [Class 3]
13	DNA NGS Panel negative RNA Fusion Panel failed testing	Parotid (38%)	*BAP1* c.757C>T p.(Gln253Ter) (48%) *FANCA* c.2602-9_2602-8delCT (35%) *KDM6A* c.907_909delGCC p.(Ala303del) (33%) *PDGFRB* c.236G>A p.(Gly79Asp) (24%)	*BAP1* loss (1) *CRTC1::MAML2* fusion	2.38	*FANCA* c.220C>G p.(Leu74Val) [Class 3]
14	DNA NGS Panel negative RNA Fusion Panel negative	Lung (90%)	Nil	Loss of 2q	0.51	*ERCC2* c.1238-7T>C [Class 3] *ERCC2* c.545C>T p.(Ala182Val) [Class 3] *FANCG* c.20C>T p.(Ser7Phe) [Class 3] *PMS2* c.1437C>G p.(His479Gln) [Class 3] *SDHA* c.436G>A p.(Ala146Thr) [Class 3]

NOTE. Genes with variants or alteration in italic.

Abbreviations: ACMG, American College of Medical Genetics and Genomics; IHC, immunohistochemistry; LOH, loss of heterozygosity; NCC, neoplastic cell content; NGS, next-generation sequencing; TMB, tumor mutational burden; WGS, whole genome sequencing.

Fusions were classified as ambiguous if the specific fusion was uncertain.

Mutation Signature Analysis

Mutational signatures were determined from frequencies of different small nucleotide variants (SNVs) as defined from Sanger Institute v2 signatures.[29] Decomposition by non-negative least squares was used to determine the contribution of each of these signatures.

Germline Variation Analysis

Germline samples were assessed for cross-patient contamination using the VerifyBamID algorithm (3% threshold). Germline small variants were detected using DRAGEN with Cellbase annotation.[18] Variants were interpreted for clinical relevance following CanVIG guidance.[22] Variants classified as benign and likely benign are not reported.

RESULTS

Clinical Characteristics

Fifteen patients with R/M SGC consented to WGS and underwent biopsy. One sample failed quality control. Ten had a histologic report in keeping with AdCC; patient 10 was initially classified as AdCC on surgical resection; however, contained features of myoepithelial carcinoma and on recurrence were considered as AdCC/adenocarcinoma not otherwise specified (NOS). There were patients with SDC ex pleomorphic adenoma (PA), clear cell myoepithelial carcinoma, epithelial-myoepithelial carcinoma and acinic cell carcinoma (patients 11-14; Table 1). The mean age was 56 (range, 38-76) years, and 64% were female. DNA NGS panel results were not available for one patient.

Whole-Genome Sequencing

The mean time from diagnosis to WGS biopsy was 62.3 months. All tumor cores contained over 30% neoplastic cell content. The mean time from biopsy to WGS report was 76 days (52-150). WGS revealed genomic changes across somatic variants, copy number changes, and structural variants (Appendix Fig A1). Tumor mutational burden was low across patients, particularly AdCC.

Small Somatic Alterations

WGS confirmed *TP53* and *PIK3CA* variants identified on previous testing. Small somatic variants were identified in all but one patient, including all patients with AdCC (Fig 1). Notably, many somatic variants were within genes of the histone modification and chromatin remodeling pathways (Fig 1, Table 2). Sixteen alterations were present in COSMIC, and *in silico* predictive tool-based calculations for missense variants predicted the majority to be potentially damaging (Appendix Table A1).

Somatic Structural Variants

Sixty percent of AdCC samples harbored *MYB* fusions, including four *MYB-NFIB* fusions (Fig 1, Table 2). Of patients classified as AdCC at the time of WGS without *MYB* fusion, one had a *FGFR1* fusion (subsequently reclassified as high-grade myoepithelial carcinoma) and two contained alternative *NFIB* genomic rearrangements (*NFIB-QDPR* ambiguous and *NFIB-PHIP* out-of-frame rearrangements), although it is uncertain whether these would functionally result in a fusion protein. This *FGFR1* fusion was included in the previous diagnostic panel; however, it was not detected on the previous analysis as part of this patient's standard-of-care clinical testing.

Within non-AdCC, all but one had a gene fusion event. A patient with clear cell morphology suggestive of an epithelial-myoepithelial carcinoma (patient 13) was found to contain a *CRTC1-MAML2* fusion, and on histologic review, it was considered a high-grade clear cell MEC (Fig 2). Additionally, a *EWSR1-ATF1* fusion was seen in a patient with clear cell predominant myoepithelial carcinoma. Although it was not possible to review historical slides, this raises the possibility that this tumor could be reclassified as hyalinizing clear cell carcinoma. A *CTNNB1-pleomorphic adenoma gene 1* (*PLAG1*) fusion was also seen in SDC ex PA. These fusions were not included in the prior panels used for standard-of-care testing.

Several copy number changes were found, including loss of *NFIB*, *ERRB3*, and *KDM6A* (Table 2). There was also a notable gain of *MDM2* (25 copies) in one patient with AdCC.

Mutational Signatures

Mutational signatures generally followed histopathologic categorizations (Fig 3).[29] Similar frequencies of signatures were found in the samples from patients with AdCC, with high relative proportion of signature 1 (age related, clock-like 5-meC demination). Signature 3 (homologous recombination deficiency) was seen in 12 of 14 patient samples.

Germline Variants

All but one patients had germline alterations (Table 2, Appendix Table A2). Most identified alterations were American College of Medical Genetics and Genomics (ACMG) class 3 (variant of uncertain significance).[30] Two class 5 (pathogenic) variants were identified, *MUTYH* c.536A>G p.(Tyr179Cys) and *NTHL1* c.859C>T p.(Gln287Ter), although these were monoallelic carrier mutations. Within the class 3 variants, clinical significance was uncertain or conflicting. There was a predominance of class 3 germline variants within DNA damage repair pathway genes.

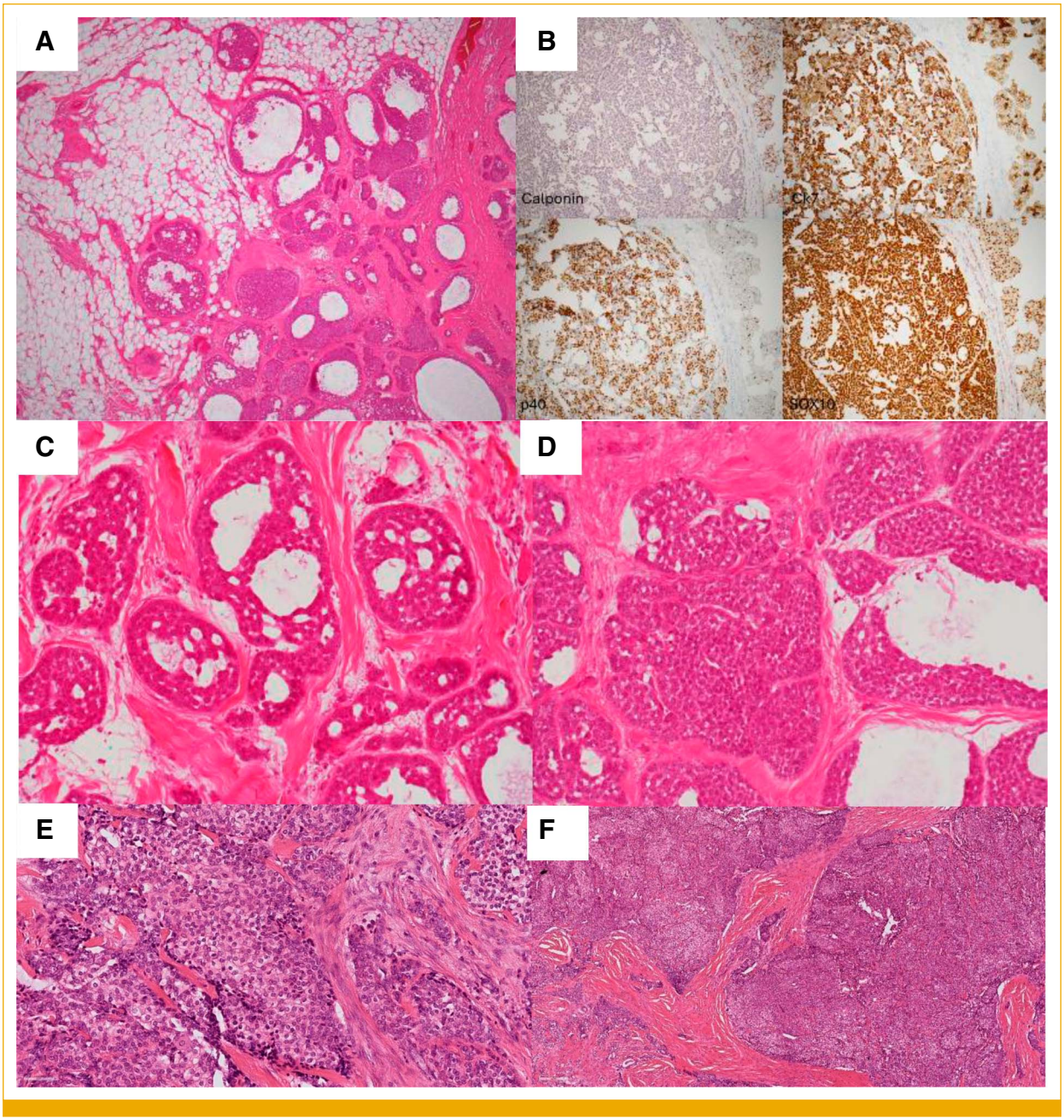

FIG 2. Histologic images of patients where WGS enabled tumor classification through additional molecular information from structural rearrangements. (A-D) A patient with high-grade SGC with an initial histologic differential diagnosis given between solid variant of adenoid cystic carcinoma, high-grade myoepithelial carcinoma, and adenocarcinoma not otherwise specified. After WGS and identification of a *FGFR1* fusion, the tumor was classified as a high-grade myoepithelial carcinoma. (A) Tumor forming discohesive nests with infiltration into periparotid fat, (B) immunohistochemistry findings with positivity for SOX10, Ck7, p40, and negative calponin, (C) tumor composed of solid nests with pseudo-cribriform areas, and (D) more solid tumor nodules with cells showing high nuclear:cytoplasmic ratios and small but prominent nucleoli. (E, F) A clear cell tumor initially favored as an epithelial-myoepithelial carcinoma but with a *CRTC1-MAML2* fusion on WGS, indicating a high-grade clear cell MEC on review. MEC, mucoepidermoid carcinoma; SGC, salivary gland cancer; WGS, whole-genome sequencing.

Case Report of Clinical Utilization of WGS

A 50-year-old male patient (patient 10) was offered WGS after progression on first-line systemic treatment. The patient was diagnosed at age 46 years with a left parotid tumor (pT3 N2b), histologically a high-grade/poorly differentiated carcinoma with a differential diagnosis between solid variant of AdCC, high-grade myoepithelial carcinoma,

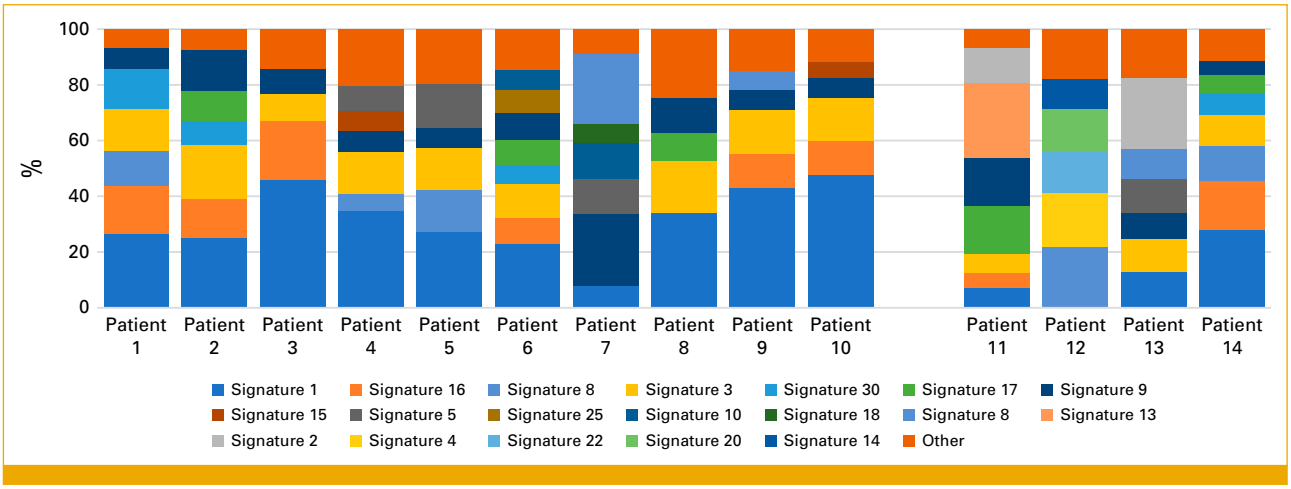

FIG 3. Bar plot demonstrating relative contributions of mutational signatures of tumor samples across patients. Mutational signatures as curated by COSMIC and Wellcome Sanger institute use unique mutational patterns to suggest etiologies of cancers.[29]

and adenocarcinoma NOS. NGS, *MYB*-FISH, and *EWSR1*-FISH at diagnosis failed to assist classification. He underwent neck dissection surgery, with positive tumor content in 5 of 39 lymph nodes excised, followed by postoperative radiotherapy (60Gy in 30 fractions). Metastatic disease was identified 3 years subsequently, with histology favoring solid variant

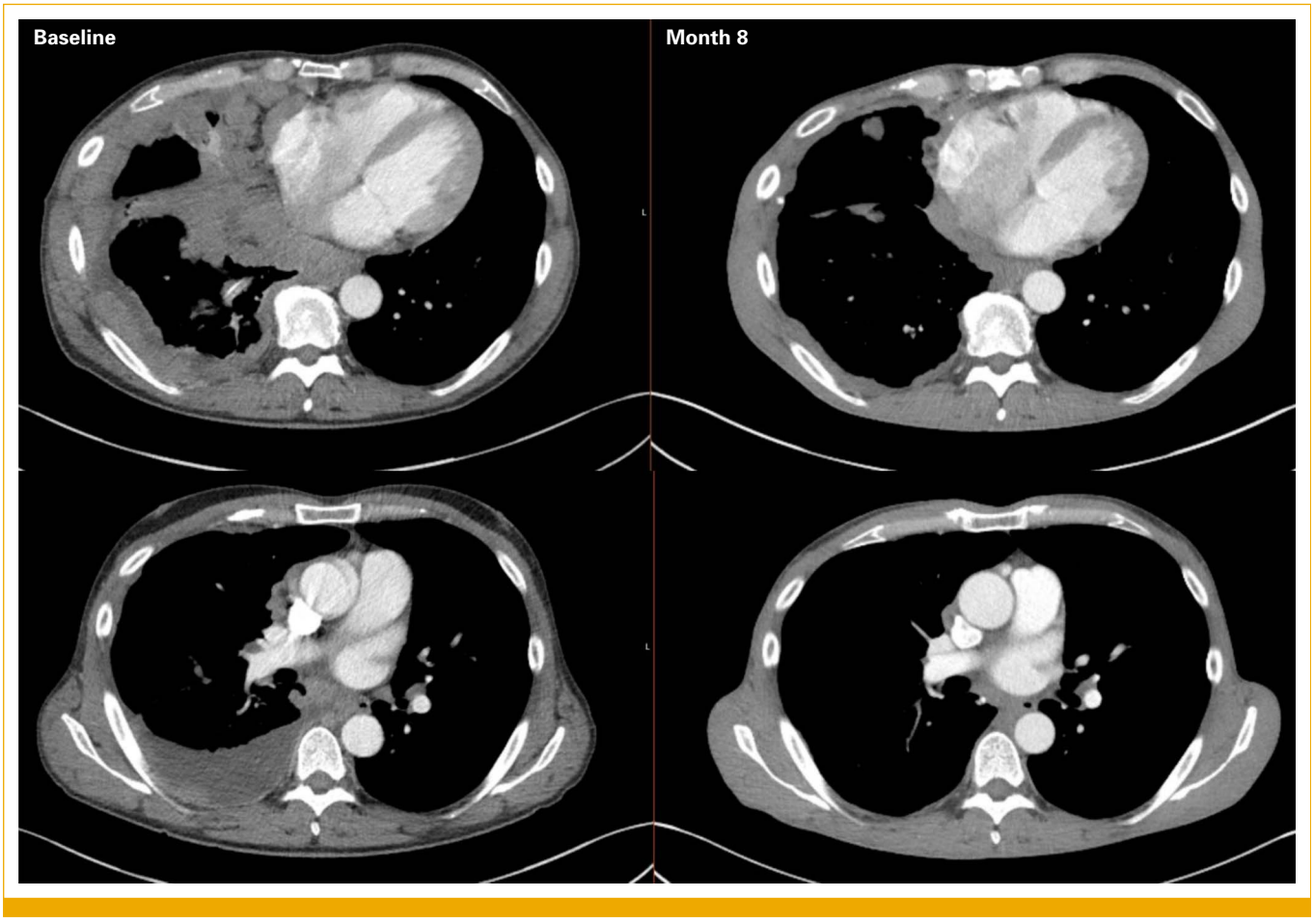

FIG 4. Radiologic results of R/M salivary cancer with *FGFR1* fusion identified with WGS with clinical biochemical and radiologic response to pemigatinib. R/M, recurrent or metastatic; WGS, whole-genome sequencing.

AdCC or adenocarcinoma NOS. A tunneled pleural drain was required for symptomatic pleural effusions. The patient was commenced on carboplatin AUC 5 once every three weeks and capecitabine 750 mg/m² (twice daily; day 1-14). A computed tomography (CT) scan following six cycles demonstrated progressive disease. A liver biopsy was performed for WGS.

WGS revealed a *FGFR1-PLAG1* gene fusion in addition to a *FGFR2* frame shift mutation and confirmation of a *PIK3CA* SNV. Fibroblast growth factor receptor (FGFR) alterations are recognized as oncogenic, with *FGFR1* fusions seen in a several cancers.[31,32] *PLAG1* is a common alteration in benign PA of the salivary gland.[33,34] *FGFR1-PLAG1* gene fusions have previously been identified in myoepithelial carcinoma, including in 18% of a cohort of 40 patients with myoepithelial carcinoma where 53% had rearrangements involving *PLAG1*.[35] In the clinical trial FIGHT-101, a patient with SGC (specific histology not reported) and an *FGFR1-PLAG1* fusion achieved stable disease with the reversible FGFR1-3 inhibitor pemigatinib and remained on treatment for over 1 year.[36] Separately, the FGFR1-4 inhibitor futibatinib in a patient with head and neck cancer and *FGFR1-PLAG1* fusion demonstrated 65% tumor shrinkage and progression-free survival of 6.9 months.[37] The *FGFR2* SNV represents another genomic hit to the FGFR signaling pathway and notably a *FGFR2* comutation was also seen in the FIGHT-101 patient.[36]

Following the WGS results, the patient's case was reassessed by a salivary pathologist (Figs 2A-2D). Given the additional molecular context, the tumor was considered to most appropriately represent a high-grade myoepithelial carcinoma. This highlights the role of WGS to aid histologic diagnosis in addition to identifying potential treatment. Initial molecular testing was negative, emphasizing the benefit from dedicated tissue sampling. Further structural variants included a large copy number gain in 8q, which is associated with increased MYC signaling.[38] 8q gains are also seen in salivary myoepithelial carcinoma, further supporting reclassification.[39] Notably, 8q12.1 is a known breakpoint for the *PLAG1* gene when fused with *FGFR1*.[33]

The patient was commenced on pemigatinib (13.5 mg once daily) through a compassionate access scheme.[40] At commencement, the patient had grade 2 AST elevation and was dependent on twice-weekly pleural drainage. A CT scan 2 months after initiation showed shrinkage of lesions. At 4 months, clinical improvement permitted removal of the pleural drain. Subsequent follow-up confirmed partial response (RECIST −33%) and improvement in AST derangement (Fig 4). The clinical course was complicated by raised phosphate (controlled with sevelamer), constipation, nail changes, and lethargy. Pemigatinib was paused and reintroduced at 9 mg once daily with improvement. The patient continued treatment for 17 months before clinical and radiologic progression.

DISCUSSION

This cohort represents one of the largest SGC cohorts to undergo WGS and complements other SGC WGS studies.[41-44] WGS was performed in a clinically meaningful timeframe, identified novel gene rearrangements, assisted histologic classification in several patients, and informed precision treatment in one individual.

A notable number of small somatic alterations were related to histone modification and chromatin remodeling pathways, confirming the role of these systems in SGC identified previously.[43,45,46] *KDM6A* and *KMT2C/D* variants for example have previously been reported in AdCC (*KMT2C/D* more frequently in recurrent/metastatic samples) and in SDC.[45-47] *TERT* promotor and *NOTCH1* alterations meanwhile have been proposed as being mutually exclusive, a finding also seen in this cohort.[46] CNV changes included loss of the cell cycle control genes *CDKN2A* (reported previously in SDC) and *CDK4*, as well as loss of the tumor suppressor *BAP1*.[44] There was also a notable gain of *MDM2* (25 copies) in one AdCC sample, which has been implicated in therapeutic resistance.[48]

MYB-NFIB fusions are a hallmark of AdCC, generating MYB overexpression and upregulation in cell proliferation genes such as *CCND1*.[10,49,50] In this study, *MYB-NFIB* fusions were frequent in AdCC (including one somatic *CCND1* comutation), although no *MYBL1*-fusions were seen.[51] In contrast to other studies, *TERT* promotor mutations and *MYB-NFIB* fusions were not mutually exclusive.[46] Significantly, WGS identified an *FGFR1* fusion which was not detected on previous testing, and two patients with AdCC were found to have non-*MYB NFIB* genomic rearrangements (*NFIB-QDPR* and *NFIB-PHIP*). These *NFIB* rearrangements have not been previously reported in SGC, although their functional relevance is uncertain. Previous WGS of AdCC has, however, identified alternative 5′-*NFIB* rearrangements in *MYB*/*MYBL1*-negative tumors, emphasizing the importance of WGS in identifying novel structural changes.[41,43]

High-grade and poorly differentiated SGC often show overlapping histologic features, and molecular testing on archival formalin-fixed paraffin-embedded (FFPE) tissue carries a risk of false negatives. Molecular information from WGS can produce an extensive morphomolecular classification to aid classification in uncertain cases. Using WGS, one patient initially favoring AdCC/adenocarcinoma NOS was reclassified as a high-grade myoepithelial carcinoma. *CRTC1-MAML2* fusions meanwhile are pathognomic in MEC, yet in this cohort was found in a patient originally classified as epithelial-myoepithelial carcinoma.[52,53] This notably co-occurred with a *BAP1* SNV, given the high rate of *BAP1* SNVs in *CRTC1-MAML2* fusion–positive MEC.[42] Histologic reassessment of samples alongside WGS information designated this tumor as likely to represent a high-grade clear cell MEC. These cases highlight the value in dedicated tissue sampling

and WGS in molecular analysis, as multiple previous tissue samples for these tumors were negative for translocations.

Additionally, a *EWSR1-ATF1* fusion was identified in a clear cell predominant myoepithelial carcinoma. Such fusions are common in clear cell cancers such as clear cell sarcoma and in the majority of hyalinizing clear cell carcinoma of the salivary gland.[54-57] *EWSR1-ATF1* fusions have been previously reported in soft-tissue myoepithelial tumors but, to our knowledge, have not been described in salivary gland myoepithelial tumors.[58,59] Although detailed pathologic reassessment was not possible for this patient, this also raises the possibility that this tumor may represent a hyalinizing clear cell carcinoma. This use of WGS to aid histologic diagnosis is remarkable, however is not intended to replace experienced salivary pathologists. Rather, its role could be in supporting the diagnosis when histopathology remains uncertain despite comprehensive multidisciplinary review. Although accurate classification of the histologic subtype in SGC often does not alter clinical management, subtypes such as AdCC are a particular case whereby histology determines recommendations for duration of follow-up and the recommendation for postoperative radiotherapy.[60] Specific histology in R/M disease may also determine the predicated efficacy of systemic therapy and biomarker testing requested.[60]

Several germline variants were identified, although none were of direct clinical relevance. Two ACMG class 5 monoallelic changes were detected. *MUTYH* and *NTHL1* are both DNA base excision repair genes and are causative of the autosomal recessive familial adenomatous polyposis 2 and NTHL1 tumor syndromes, respectively, when present biallelically.[61,62] However, there is evidence that germline monoallelic carriers of *MUTYH* variants are potentially at a higher risk of extraintestinal cancers including breast, endometrial, and hepatobiliary.[61,63] *MUTYH* alterations have also been reported previously in salivary gland secretory carcinomas.[64] Although these germline mutations are not clinically actionable in a SGC cohort, they highlight the ability of WGS to detect carrier variants that may become relevant as further data emerge.

High frequency of germline variants within DNA damage repair associated genes within SGC has been reported previously within AdCC.[46] In this study, most class 3 variants identified were associated with DNA damage repair, which may be biased by the germline testing design for such genes, but remains a worthwhile area of future confirmation in SGC profiling studies.[65] Indeed, even if not directly implicated in SGC, such results emphasize the challenge of interpreting clinical relevance of WGS results and underscores the need for GTAB systems and increased profiling of rare tumors and international collaboration to accumulate evidence in rare variants.

The targeted genes in traditional oncology panels relies on variant frequencies obtained from common cancer cohorts, whereas WGS permits identification of novel or rare alterations and thus has a unique role in rare tumors such as SGC. WGS delivers significant structural resolution and is increasingly accessible as costs decline and pathways mature in health systems.[18] WGS has demonstrated ability to detect clinically relevant alterations and aid selection to experimental medicine.[66,67] Additionally, concomitant germline testing during WGS allows for the same test to evaluate familial risks and pharmacogenomic markers of drug efficacy or tolerance.[18] These benefits of WGS must be weighed against the potential negatives which include the need for a fresh biopsy, handling of frozen tissue, germline ethical implications, and need for bioinformatic and GTAB infrastructure. Furthermore, as evidence for SGC genomic variants of clinical relevance accumulates (such as from WGS), these alterations could be included in expanded versions of traditional panels. This approach may have greater clinical applicability than widespread standard-of-care WGS because of ease of traditional testing, while acknowledging potential false-negative results from archival FFPE. Despite these challenges of WGS, this study serves to underscore the potential of WGS for research and in select clinical cases.

Several limitations exist within this study. First, participants were limited, which prevented statistical comparisons. Second, identified variants from WGS may not have direct clinical relevance or require further evidence accumulation.[68] However, this study adds to existing genomic knowledge in SGC and highlights the role of WGS in SGC research for informing underlying tumor biology and identifying novel targets. This study demonstrates that WGS can be performed in clinically relevant timescales, can direct personalized medicine, and aid histopathologic classification in uncertain cases. When considering the wider clinical role of WGS, it is important to consider the context that although this study is one of the largest series of patients globally with salivary cancers undergoing WGS as part of their standard-of-care management, and to our knowledge, the only study evaluating a consecutive series of patients to understand the impact on the clinical decision making, additional studies are required to increase the numbers of patients to provide additional confidence in the assessment of the clinical utility of this approach.[69]

AFFILIATIONS

[1]The Christie NHS Foundation Trust, Manchester, United Kingdom
[2]The University of Manchester, Manchester, United Kingdom
[3]Manchester University NHS Foundation Trust, Manchester, United Kingdom
[4]Royal Marsden NHS Foundation Trust, London, United Kingdom
[5]The Institute of Cancer Research, London, United Kingdom

CORRESPONDING AUTHOR

Robert Metcalf, PhD, MBChB, MRCP; e-mail: robert.metcalf1@nhs.net.

SUPPORT

Supported by The Christie Charity, Syncona Foundation and Infrastructure Industry Foundation.

AUTHOR CONTRIBUTIONS

Conception and design: Matt Church, Steven Michael Churchill, Kevin Harrington, Robert Metcalf
Provision of study materials or patients: Guy Betts, Kevin Harrington
Collection and assembly of data: Matt Church, Steven Michael Churchill, Helene Barbara Schlecht, Yatin Jain, Robert Metcalf
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.
Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Matt Church
Travel, Accommodations, Expenses: SERVIER

George Burghel
Honoraria: AstraZeneca, Menarini
Consulting or Advisory Role: Menarini

Guy Betts
Speakers' Bureau: Seagen (I)

Helene Barbara Schlecht
Honoraria: Roche
Speakers' Bureau: Bayer, Lilly, AstraZeneca

Kevin Harrington
Honoraria: AstraZeneca (Inst), BMS (Inst), Boehringer Ingelheim (Inst), Merck Serono (Inst), MSD (Inst), Replimune (Inst), Scenic Biotech, Johnson and Johnson (Inst), Nanobiotix (Inst), ALX Oncology (Inst), BeiGene (Inst), GlaxoSmithKline (Inst), Bicara Therapeutics (Inst), Merus (Inst), PDS Biotechnology (Inst), Exelixis (Inst), Flamingo Pharma (Inst), PsiVac Ltd (Inst), AbbVie (Inst)
Consulting or Advisory Role: AstraZeneca (Inst), BMS (Inst), Boehringer Ingelheim (Inst), Merck Serono (Inst), MSD (Inst), Replimune (Inst), Nanobiotix (Inst)
Speakers' Bureau: BMS (Inst), Merck Serono (Inst), MSD (Inst)
Research Funding: AstraZeneca (Inst), Replimune (Inst), Boehringer Ingelheim (Inst)

Robert Metcalf
Honoraria: Sanofi
Consulting or Advisory Role: Avacta Life Sciences, Rgenta

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

We would like to thank Incyte for access to pemigatinib for our patient through their compassionate access scheme.

REFERENCES

1. Ettl T, Schwarz-Furlan S, Gosau M, et al: Salivary gland carcinomas. Oral Maxillofac Surg 16:267-283, 2012
2. Skálová A, Hyrcza MD, Leivo I: Update from the 5th edition of the World Health Organization classification of head and neck tumors: Salivary glands. Head Neck Pathol 16:40-53, 2022
3. Skálová A, Vanecek T, Sima R, et al: Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: A hitherto undescribed salivary gland tumor entity. Am J Surg Pathol 34:599-608, 2010
4. van Herpen C, Vander Poorten V, Skalova A, et al: Salivary gland cancer: ESMO–European Reference Network on Rare Adult Solid Cancers (EURACAN) Clinical Practice Guideline for diagnosis, treatment and follow-up. ESMO Open 7:100602, 2022
5. Valstar MH, Van Den Brekel MWM, Smeele LE: Interpretation of treatment outcome in the clinically node-negative neck in primary parotid carcinoma: A systematic review of the literature. Head Neck 32:1402-1411, 2010
6. Lau VH, Aouad R, Farwell DG, et al: Patterns of nodal involvement for clinically N0 salivary gland carcinoma: Refining the role of elective neck irradiation. Head Neck 36:1435-1439, 2014
7. Nam SJ, Roh JL, Cho KJ, et al: Risk factors and survival associated with distant metastasis in patients with carcinoma of the salivary gland. Ann Surg Oncol 23:4376-4383, 2016
8. Girelli L, Locati L, Galeone C, et al: Lung metastasectomy in adenoid cystic cancer: Is it worth it? Oral Oncol 65:114-118, 2017
9. Laurie SA, Siu LL, Winquist E, et al: A phase 2 study of platinum and gemcitabine in patients with advanced salivary gland cancer: A trial of the NCIC Clinical Trials Group. Cancer 116:362-368, 2010
10. Wagner VP, Bingle CD, Bingle L: MYB-NFIB fusion transcript in adenoid cystic carcinoma: Current state of knowledge and future directions. Crit Rev Oncol Hematol 176:103745, 2022
11. Le X, Baik C, Bauman J, et al: Larotrectinib treatment for patients with TRK fusion-positive salivary gland cancers. Oncologist 29:e779-e788, 2022
12. Adashek JJ, Sapkota S, de Castro Luna R, et al: Complete response to alectinib in ALK-fusion metastatic salivary ductal carcinoma. NPJ Precis Oncol 7:36, 2023
13. Subbiah V, Wolf J, Konda B, et al: Tumour-agnostic efficacy and safety of selpercatinib in patients with RET fusion-positive solid tumours other than lung or thyroid tumours (LIBRETTO-001): A phase 1/2, open-label, basket trial. Lancet Oncol 23:1261-1273, 2022
14. Takahashi H, Tada Y, Saotome T, et al: Phase II trial of trastuzumab and docetaxel in patients with human epidermal growth factor receptor 2-positive salivary duct carcinoma. J Clin Oncol 37:125-134, 2019
15. Fushimi C, Tada Y, Takahashi H, et al: A prospective phase II study of combined androgen blockade in patients with androgen receptor-positive metastatic or locally advanced unresectable salivary gland carcinoma. Ann Oncol 29:979-984, 2018
16. Laurie SA, Ho AL, Fury MG, et al: Systemic therapy in the management of metastatic or locally recurrent adenoid cystic carcinoma of the salivary glands: A systematic review. Lancet Oncol 12:815-824, 2011
17. Rodon J, Soria JC, Berger R, et al: Genomic and transcriptomic profiling expands precision cancer medicine: The WINTHER trial. Nat Med 25:751-758, 2019
18. Sosinsky A, Ambrose J, Cross W, et al: Insights for precision oncology from the integration of genomic and clinical data of 13,880 tumors from the 100,000 genomes Cancer Programme. Nat Med 30:279-289, 2024
19. Pagnamenta AT, Yu J, Walker S, et al: The impact of inversions across 33,924 families with rare disease from a national genome sequencing project. Am J Hum Genet 111:1140-1164, 2024
20. Behera S, Catreux S, Rossi M, et al: Comprehensive genome analysis and variant detection at scale using DRAGEN. Nat Biotechnol 43:1177-1191, 2024
21. den Dunnen JT, Dalgleish R, Maglott DR, et al: HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat 37:564-569, 2016
22. Garrett A, Callaway A, Durkie M, et al: Cancer Variant Interpretation Group UK (CanVIG-UK): An exemplar national subspecialty multidisciplinary network. J Med Genet 57:829-834, 2020
23. Li MM, Datto M, Duncavage EJ, et al: Standards and guidelines for the interpretation and reporting of sequence variants in cancer: A joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 19:4-23, 2017
24. NHS England: National Genomic Test Directory. 2018. https://www.england.nhs.uk/publication/national-genomic-test-directories/
25. Barwell J, Snape K, Wedderburn S: The new genomic medicine service and implications for patients. Clin Med (Lond) 19:273-277, 2019
26. Sondka Z, Bamford S, Cole CG, et al: The COSMIC Cancer Gene Census: Describing genetic dysfunction across all human cancers. Nat Rev Cancer 18:696-705, 2018
27. Lopez J, Coll J, Haimel M, et al: HGVA: The human genome variation archive. Nucleic Acids Res 45:W189-W194, 2017
28. Suybeng V, Koeppel F, Harlé A, et al: Comparison of pathogenicity prediction tools on somatic variants. J Mol Diagn 22:1383-1392, 2020

29. COSMIC. Signatures of Mutational Processes in Human Cancer. 2015. https://cancer.sanger.ac.uk/signatures/signatures_v2/
30. Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405-424, 2015
31. Kendre G, Murugesan K, Brummer T, et al: Charting co-mutation patterns associated with actionable drivers in intrahepatic cholangiocarcinoma. J Hepatol 78:614-626, 2023
32. Chen L, Zhang Y, Yin L, et al: Fibroblast growth factor receptor fusions in cancer: Opportunities and challenges. J Exp Clin Cancer Res 40:345, 2021
33. Persson F, Winnes M, Andrén Y, et al: High-resolution array CGH analysis of salivary gland tumors reveals fusion and amplification of the FGFR1 and PLAG1 genes in ring chromosomes. Oncogene 27:3072-3080, 2008
34. Voz ML, Agten NS, Van de Ven WJM, et al: PLAG1, the main translocation target in pleomorphic adenoma of the salivary glands, is a positive regulator of IGF-II. Cancer Res 60:106-113, 2000
35. Dalin MG, Katabi N, Persson M, et al: Multi-dimensional genomic analysis of myoepithelial carcinoma identifies prevalent oncogenic gene fusions. Nat Commun 8:1197, 2017
36. Subbiah V, Iannotti NO, Gutierrez M, et al: FIGHT-101, a first-in-human study of potent and selective FGFR 1-3 inhibitor pemigatinib in pan-cancer patients with FGF/FGFR alterations and advanced malignancies. Ann Oncol 33:522-533, 2022
37. Meric-Bernstam F, Bahleda R, Hierro C, et al: Futibatinib, an irreversible FGFR1-4 inhibitor, in patients with advanced solid tumors harboring *FGF/FGFR* aberrations: A phase I dose-expansion study. Cancer Discov 12:402-415, 2022
38. Watson EV, Lee JJK, Gulhan DC, et al: Chromosome evolution screens recapitulate tissue-specific tumor aneuploidy patterns. Nat Genet 56:900-912, 2024
39. Vékony H, Röser K, Löning T, et al: Copy number gain at 8q12.1-q22.1 is associated with a malignant tumor phenotype in salivary gland myoepitheliomas. Genes Chromosomes Cancer 48:202-212, 2009
40. Rodón J, Damian S, Furqan M, et al: Pemigatinib in previously treated solid tumors with activating FGFR1-FGFR3 alterations: Phase 2 FIGHT-207 basket trial. Nat Med 30:1645-1654, 2024
41. Mitani Y, Liu B, Rao PH, et al: Novel MYBL1 gene rearrangements with recurrent MYBL1-NFIB fusions in salivary adenoid cystic carcinomas lacking t(6;9) translocations. Clin Cancer Res 22: 725-733, 2016
42. Karpinets TV, Mitani Y, Liu B, et al: Whole-genome sequencing of common salivary gland carcinomas: Subtype-restricted and shared genetic alterations. Clin Cancer Res 27:3960-3969, 2021
43. Rettig EM, Talbot CC, Sausen M, et al: Whole-genome sequencing of salivary gland adenoid cystic carcinoma. Cancer Prev Res 9:265-274, 2016
44. Kim Y, Song S, Lee M, et al: Integrative genomic analysis of salivary duct carcinoma. Sci Rep 10:14995, 2020
45. Ho AS, Kannan K, Roy DM, et al: The mutational landscape of adenoid cystic carcinoma. Nat Genet 45:791-798, 2013
46. Ho AS, Ochoa A, Jayakumaran G, et al: Genetic hallmarks of recurrent/metastatic adenoid cystic carcinoma. J Clin Invest 129:4276-4289, 2019
47. Kobayashi K, Saito Y, Kage H, et al: CDK12 alterations and ARID1A mutations are predictors of poor prognosis and therapeutic targets in high-grade salivary gland carcinoma: Analysis of the National Genomic Profiling Database. Jpn J Clin Oncol 53:798-807, 2023
48. Hou H, Sun D, Zhang X: The role of MDM2 amplification and overexpression in therapeutic resistance of malignant tumors. Cancer Cell Int 19:216, 2019
49. West RB, Kong C, Clarke N, et al: MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinicopathologic correlation. Am J Surg Pathol 35:92-99, 2011
50. Xu LH, Zhao F, Yang WW, et al: MYB promotes the growth and metastasis of salivary adenoid cystic carcinoma. Int J Oncol 54:1579-1590, 2019
51. Togashi Y, Dobashi A, Sakata S, et al: MYB and MYBL1 in adenoid cystic carcinoma: Diversity in the mode of genomic rearrangement and transcripts. Mod Pathol 31:934-946, 2018
52. Saade RE, Bell D, Garcia J, et al: Role of CRTC1/MAML2 translocation in the prognosis and clinical outcomes of mucoepidermoid carcinoma. JAMA Otolaryngol Head Neck Surg 142:234-240, 2016
53. Chen Z, Ni W, Li JL, et al: The CRTC1-MAML2 fusion is the major oncogenic driver in mucoepidermoid carcinoma. JCI Insight 6:e139497, 2021
54. Thway K, Fisher C: Tumors with EWSR1-CREB1 and EWSR1-ATF1 fusions: The current status. Am J Surg Pathol 36:e1-e11, 2012
55. Antonescu CR, Katabi N, Zhang L, et al: EWSR1-ATF1 fusion is a novel and consistent finding in hyalinizing clear-cell carcinoma of salivary gland. Genes Chromosomes Cancer 50:559-570, 2011
56. Möller E, Praz V, Rajendran S, et al: EWSR1-ATF1 dependent 3D connectivity regulates oncogenic and differentiation programs in clear cell sarcoma. Nat Commun 13:2267, 2022
57. Bilodeau EA, Weinreb I, Antonescu CR, et al: Clear cell odontogenic carcinomas show ewsr1 rearrangements: A novel finding and a biological link to salivary clear cell carcinomas. Am J Surg Pathol 37:1001-1005, 2013
58. Flucke U, Mentzel T, Verdijk MA, et al: EWSR1-ATF1 chimeric transcript in a myoepithelial tumor of soft tissue: A case report. Hum Pathol 43:764-768, 2012
59. LeckeyJr BD, Ivy J, Miguel R-M, et al: EWSR1-ATF1 fusion in a myoepithelial carcinoma of soft tissue with small round cell morphology: A potential diagnostic pitfall. Pediatr Dev Pathol 24:258-263, 2021
60. Geiger JL, Ismaila N, Beadle B, et al: Management of Salivary Gland Malignancy: ASCO Guideline. J Clin Oncol 39:1909-1941, 2021
61. Magrin L, Fanale D, Brando C, et al: MUTYH-associated tumor syndrome: The other face of MAP. Oncogene 41:2531-2539, 2022
62. Kuiper RP, Nielsen M, Voer RMD, et al: NTHL1 Tumor Syndrome. GeneReviews, 2020
63. Win AK, Reece JC, Dowty JG, et al: Risk of extracolonic cancers for people with biallelic and monoallelic mutations in MUTYH. Int J Cancer 139(7):1557-1563, 2016
64. Na K, Hernandez-Prera JC, Lim JY, et al: Characterization of novel genetic alterations in salivary gland secretory carcinoma. Mod Pathol 33:541-550, 2020
65. Brownlie J, Kulkarni S, Algethami M, et al: Targeting DNA damage repair precision medicine strategies in cancer. Curr Opin Pharmacol 70:102381, 2023
66. Pruis MA, Groenendijk FH, Badloe KS, et al: Personalised selection of experimental treatment in patients with advanced solid cancer is feasible using whole-genome sequencing. Br J Cancer 127: 776-783, 2022
67. Bagger FO, Borgwardt L, Jespersen AS, et al: Whole genome sequencing in clinical practice. BMC Med Genomics 17:1-16, 2024
68. Harrison SM, Biesecker LG, Rehm HL: Overview of specifications to the ACMG/AMP variant interpretation guidelines. Curr Protoc Hum Genet 103:e93, 2019
69. Akhoundova D, Rubin MA: The grand challenge of moving cancer whole-genome sequencing into the clinic. Nat Med 30:39-40, 2024
70. McLaren W, Gil L, Hunt SE, et al: The Ensembl variant effect predictor. Genome Biol 17:122, 2016
71. Ng PC, Henikoff S: Predicting deleterious amino acid substitutions. Genome Res 11:863-874, 2001
72. Rentzsch P, Witten D, Cooper GM, et al: CADD: Predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res 47:D886-D894, 2019
73. Ioannidis NM, Rothstein JH, Pejaver V, et al: REVEL: An ensemble method for predicting the pathogenicity of rare missense variants. Am J Hum Genet 99:877-885, 2016

APPENDIX

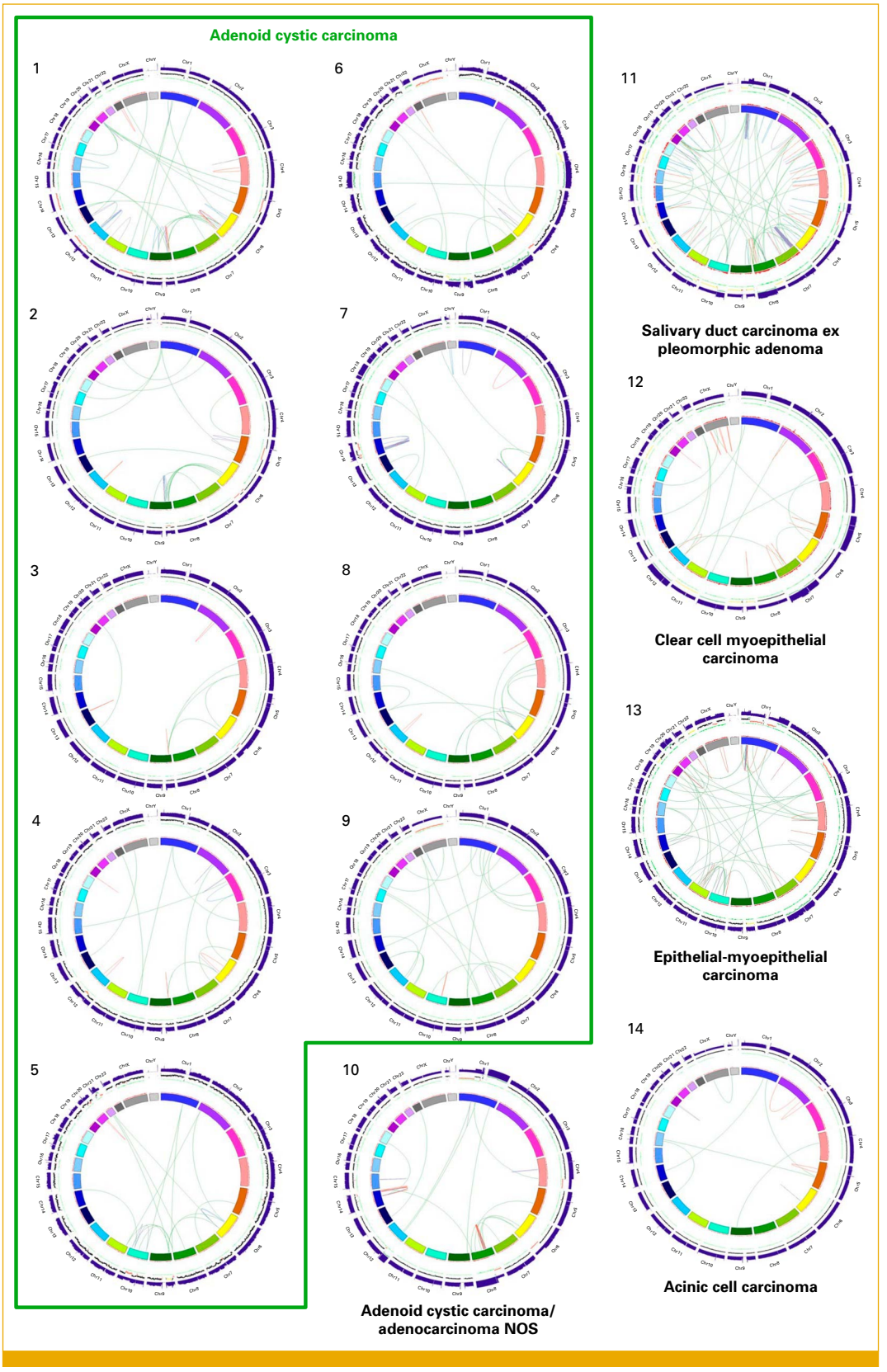

FIG A1. Circos plots from WGS analysis for each patient. Chromosomes are arranged sequentially around circumference as indicated, the inner most track corresponds to chromosomes, the second (red) track indicates the number of somatic small nucleotide variants in a 2Mb window (scale 0-100), the third (green) track indicates

FIG A1. (Continued). number of somatic indels in a 2Mb window (scale 0-35), the fourth track indicates the normalized depth of coverage with diploid regions a value of 0, the outermost track indicates absolute depth of coverage for tumor sample. Structural variants of over 100kbs are indicated by arcs inside the plot, translocations in green, inversions in purple, duplications in blue, deletions in red. Plots 1-10 represent adenoid cystic patients, plots 11-14 represent other histology (by original assigned histology at time of whole genome sequencing). NOS, not otherwise specified.

TABLE A1. Germline Variants With Alternative Allele Frequency, Population Frequency in gnomAD and 1,000 Genomes Project (1KGP), ClinVar Listings, and ACMG Classification

Patient	Variant	Alt allele	Population (1KGP|gnomAD)	ClinVar ID	Functional Evidence	ClinVar Classification	ACMG Class
1	*ERCC4* c.1727G>C p.(Arg576Thr) missense variant	27/51	0.0019|0.0007	134158	No functional evidence in ClinVar	Uncertain significance (8); likely benign (1)	Class 3
2	*RTEL1* c.2345C>T p.(Ala782Val) missense variant	14/29	0.0001|<0.00005	839112	No functional evidence in ClinVar	Uncertain significance	Class 3
3	*MSH6* c.866G>A p.(Gly289Asp) missense variant	38/79	0.0002|0.0001	627661	No functional evidence in ClinVar	Uncertain significance (2); likely benign (3)	Class 3
	TSC1 c.2626-3_2626-2insTA splice region variant, intron variant	17/54	—|—	365512	No functional evidence in ClinVar	Uncertain significance	Class 3
	TSC1 c.2626-3C>T splice region variant, intron variant	17/17	0.0004|—	411211	No functional evidence in ClinVar	Uncertain significance (3); benign (2); likely benign (3)	Class 3
4	*POLD1* c.1666G>A p.(Val556Ile) missense variant	20/42	—|<0.00005	239249	No functional evidence in ClinVar	Uncertain significance	Class 3
5	*APC* c.8213T>C p.(Ile2738Thr) missense variant(T)	29/50	—|<0.00005	216184	No functional evidence in ClinVar	Uncertain significance (4); likely benign (3)	Class 3
	CBL c.1477C>T p.(Leu493-Phe) missense variant	20/47	0.0002|0.0001	180819	No functional evidence in ClinVar	Uncertain significance (5); likely benign (2)	Class 3
6	*FANCG* c.1538G>A p.(Arg513Gln) missense variant	23/42	0.0103|0.0084	134361	No functional evidence in ClinVar	Uncertain significance (1); benign (6); likely benign (4)	Class 3
	MUTYH c.536A>G p.(Tyr179Cys) missense variant	19/38	0.0019|0.0015	5293	No functional evidence in ClinVar	Pathogenic/likely pathogenic *autosomal recessive*	Class 5
	XPC c.1984G>A p.(Glu662Lys) missense variant	20/43	—|0.0001	343567	No functional evidence in ClinVar	Uncertain significance	Class 3
7	*NTHL1* c.859C>T p.(Gln287Ter) stop gained(T)	26/46	—|0.0002	620182	No functional evidence in ClinVar	Pathogenic/likely pathogenic *autosomal recessive*	Class 5
	PMS2 c.916G>A p.(Val306-Met) missense variant	31/49	—|<0.00005	127801	No functional evidence in ClinVar	Uncertain significance (6); likely benign (1)	Class 3
	TSC2 c.1839+6G>A splice region n variant, intron variant	25/50	0.0006|0.0004	49658	No functional evidence in ClinVar	Uncertain significance (1); benign (6); likely benign (5)	Class 3
8	No Germline Variants Identified						
9	*ATM* c.7788+8G>T splice region variant, intron variant	21/39	0.0022|0.0014	127450	No functional evidence in ClinVar	Uncertain significance (2); benign (8); likely benign (9)	Class 3
	FANCA c.2982-8C>G splice region variant, intron variant	33/55	0.0003|<0.00005	1009909	No functional evidence in ClinVar	Uncertain significance (1); likely benign (1)	Class 3
	FANCG c.1538G>A p.(Arg513Gln) missense variant	17/30	0.0103|0.0084	134361	No functional evidence in ClinVar	Uncertain significance (1); benign (6); likely benign (4)	Class 3

(continued on following page)

TABLE A1. Germline Variants With Alternative Allele Frequency, Population Frequency in gnomAD and 1,000 Genomes Project (1KGP), ClinVar Listings, and ACMG Classification (continued)

Patient	Variant	Alt allele	Population (1KGP|gnomAD)	ClinVar ID	Functional Evidence	ClinVar Classification	ACMG Class
	POLH c.1253_1255delCTC p.(Pro418del) splice region variant, inframe deletion	23/50	0.0002|0.0006	356907	No functional evidence in ClinVar	Uncertain significance (2); likely benign (2)	Class 3
	SMAD4 c.1573A>G p.(Ile525Val) missense variant(T)	21/48	0.0017|0.0006	41788	No functional evidence in ClinVar	Uncertain significance (2); benign (7); likely benign (11)	Class 3
10	*POLE* c.3718G>A p.(Glu1240Lys) missense variant	22/44	0.0014|0.0008	240475	No functional evidence in ClinVar	Uncertain significance (6); benign (1); likely benign (3)	Class 3
11	*TSC2* c.1318G>A p.(Gly440Ser) missense variant	19/35	0.0009|0.0007	41726	No functional evidence in ClinVar	Uncertain significance (1); benign (3); likely benign (11)	Class 3
12	*FANCG* c.1538G>A p.(Arg513Gln) missense variant	20/48	0.0103|0.0084	134361	No functional evidence in ClinVar	Uncertain significance (1); benign (6); likely benign (4)	Class 3
13	*FANCA* c.220C>G p.(Leu74Val) missense variant	40/72	—|<0.00005	1336856	No functional evidence in ClinVar	Uncertain significance	Class 3
14	*ERCC2* c.545C>T p.(Ala182Val) missense variant	25/40	0.0007|0.0006	134114	No functional evidence in ClinVar	Uncertain significance (1); benign (1); likely benign (2)	Class 3
	FANCG c.20C>T p.(Ser7Phe) missense variant	21/49	0.0023|0.0034	134358	No functional evidence in ClinVar	Uncertain significance (1); benign (3); likely benign (2)	Class 3
	PMS2 c.1437C>G p.(His479Gln) missense variant	12/26	0.0025|0.0043	41701	No functional evidence in ClinVar	Uncertain significance (1); benign (12); likely benign (4)	Class 3
	SDHA c.436G>A p.(Ala146Thr) missense variant	20/40	—|<0.00005	2048783	No functional evidence in ClinVar	Uncertain significance	Class 3

Abbreviation: ACMG, American College of Medical Genetics and Genomics.

TABLE A2. Somatic Variants From Whole-Genome Sequencing With Classifications, COSMIC Details, Polyphen2 In-Silico Protein Change Prediction for Missense Variants

Patient	Domain	Variant	VAF (%)	Classification	ACMG Class	AMP Tier	COSMIC	Polyphen 2 (using HumDiv)	VEP PolyPhen2 (HumVar)	VEP SIFT	VEP CADD PHRED	VEP AlphaMissense	VEP REVEL
1	Domain 1	*ARID1B* c.613T>G p.(Leu205Val)	11.00	Missense		Tier III	Not present in COSMIC	Possibly damaging (0.557)	Benign (0.081)	Deleterious—low confidence (0)	22.4		
	Domain 2	*H3F3B* c.316G>A p.(Glu106Lys)	20.00	Missense			COSV54665444, seen previously in urinary tract and cervical carcinoma	Probably damaging (1.0)	Probably damaging (0.999)	Deleterious—low confidence (0)	31		
	Domain 2	*PTPN11* c.80G>C p.(Gly27Ala)	39.00	Missense			Not present in COSMIC	Probably damaging (0.995)	Probably damaging (0.939)	Deleterious—low confidence (0)	26.6	Likely pathogenic (0.8894)	0.923
2	Domain 1	*TERT* c.-124C>T	18.00	Promotor	Class 5	Tier I	Not present in COSMIC, present in PubMed articles—melanoma, breast, thyroid, GIST	—					
3	Domain 1	*TERT* c.-124C>T	39.00	Promotor		Tier IID	Not present in COSMIC, present in PubMed articles—melanoma, breast, thyroid, GIST	—					
	Domain 2	*ACVR2A* c.799_816+11del29	21.00	Splice variant			Not present in COSMIC, other ACVR2A variants present	—					
	Domain 2	*NFIB* c.667_673del7 p.(Leu223GlufsTer7)	51.00	Frame shift			Not present in COSMIC	—					
4	Domain 2	*NOTCH1* c.1039G>T p.(Gly347Cys)	8.0	Missense		Tier III	COSV53031159, seen previously in esophageal carcinoma	Probably damaging (1.0)	Probably damaging (1)	Deleterious—low confidence (0)	28.2	Likely pathogenic (0.972)	
5	Domain 1	*PTEN* c.737dupC p.(Leu247ValfsTer6)	36.00	Frame shift		Tier III	COSV64288549, seen previously in GOJ carcinoid tumor and acute lymphoblastic T-cell leukemia	—					
	Domain 1	*SF3B1* c.1873C>T p.(Arg625Cys)	14.00	Missense		Tier III	COSV59205859, seen previously in several cancer types includig AdCC (Ho)	Probably damaging (1.0)	Probably damaging (0.993)	Deleterious—low confidence (0)	33	Likely pathogenic (0.9996)	0.823
	Domain 2	*KMT2A* c.25T>G p.(Phe9Val)	14.00	Missense			Not present in COSMIC	Benign (0.414)	Unknown	Deleterious—low confidence (0)	24.6	Likely pathogenic (0.7249)	0.455
	Domain 2	*NUDT15* c.59T>C p.(Val20Ala)	15.00	Missense			Not present in COSMIC	Probably damaging (0.999)	Possibly damaging (0.829)	Deleterious (0.01)	25.6	Likely pathogenic (0.7325)	0.25
6	Domain 1	*KDM6A* c.1693C>T p.(Gln565Ter)	66.0	Nonsense	Class 5	Tier IID	Not present in COSMIC	—					
	Domain 2	*HOXA13* c.172T>G p.(Phe58Val)	17.0	Missense			Not present in COSMIC	Possibly damaging (0.462)	Unknown	Tolerated (0.12)	16.8	Ambiguous (0.4046)	
	Domain 2	*KMT2A* c.1702C>T p.(Pro568Ser)	33.0	Missense			Not present in COSMIC	Probably damaging (0.999)	Probably damaging (0.97)	Tolerated—low confidence (0.07)	21.9	Likely benign (0.2524)	0.267

(continued on following page)

TABLE A2. Somatic Variants From Whole-Genome Sequencing With Classifications, COSMIC Details, Polyphen2 In-Silico Protein Change Prediction for Missense Variants (continued)

Patient	Domain	Variant	VAF (%)	Classification	ACMG Class	AMP Tier	COSMIC	Polyphen 2 (using HumDiv)	VEP PolyPhen2 (HumVar)	VEP SIFT	VEP CADD PHRED	VEP AlphaMissense	VEP REVEL
7	Domain 1	*MAX* c.223C>T p.(Arg75Ter)	64.0	Nonsense	Class 5	Tier III	COSV52415613, seen previously in breast, lung, adrenal.	—			38		
	Domain 1	*POLD1* c.952G>A p.(Glu318Lys)	60.0	Missense	Class 3	Tier III	COSV101486838, seen previously in endometrium	Probably damaging (1.0)	Probably damaging (0.998)	Deleterious—low confidence (0)	29.7	Likely pathogenic (0.9977)	0.6
	Domain 2	*CCNE1* c.737T>A p.(Ile246Asn)	34.0	Missense			Not present in COSMIC	Probably damaging (1.0)	Probably damaging (0.99)	Deleterious (0.03)	26.9	Ambiguous (0.5207)	0.236
	Domain 2	*FLT3* c.2198C>G p.(Pro733Arg)	35.0	Missense			Not present in COSMIC	Benign (0.007)	Benign (0.003)	Tolerated (0.52)	20.5	Likely benign (0.869)	0.222
	Domain 2	*HOXA13* c.172T>G p.(Phe58Val)	15.0	Missense			Not present in COSMIC	Possibly damaging (0.462)	Unknown	Tolerated (0.12)	16.8	Ambiguous (0.4046)	
	Domain 2	*NFIB* c.6_7insGAAA p.(Tyr3GlufsTer11)	39.0	Frame shift			Not present in COSMIC	—					
8	Domain 1	*CCND1* c.860C>T p.(Pro287Leu)	30.0	Missense		Tier III	COSV57119569, seen previously in upper GI, lower GI, skin, and lung cancers	Probably damaging (1.0)	Probably damaging (1.0)	Deleterious (0)	28.4	Likely pathogenic (0.981)	0.418
	Domain 2	*CARD11* c.2533A>T p.(Lys845Ter)	38.0	Nonsense			Not present in COSMIC	Probably damaging (0.996)			42		
	Domain 2	*MYCL* c.577A>C p.(Ser193Arg)	12.0	Missense			Not present in COSMIC	—					
	Domain 2	*MYOD1* c.695A>G p.(Asn232Ser)	44.0	Missense			Not present in COSMIC	Benign (0.0)	Benign (0.0)	Tolerated (1)	15.2	Likely benign (0.0444)	0.216
	Domain 2	*NCOR2* c.5734C>T p.(Pro1912Ser)	38.0	Missense			Not present in COSMIC	Probably damaging (0.997)	Benign (0.165)	Deleterious—low confidence (0.01)	19.79	Likely benign (0.712)	0.033
9	Domain 1	*KDM6A* c.995dupA p.(Asn332LysfsTer32)	90.0	Frame shift	Class 4	Tier IID	COSV65037652 similar variant seen in SCG (c.994_995dup, p.N332Kfs*28)	—					
	Domain 2	*MYH9* c.1230_1247dup18 p.(Asp411_Ala416dup)	47.0	Frame shift			Not present in COSMIC	—					
	Domain 2	*NCOR2* c.2787delC p.(Glu930ArgfsTer34)	53.0	Frame shift			Not present in COSMIC	—					
	Domain 2	*UBR5* c.4843G>A p.(Asp1615Asn)	47.0	Missense			Not present in COSMIC	Probably Damaging (0.993)	Possibly Damaging (0.818)	Deleterious—low confidence (0)	29.3	Likely pathogenic (0.9737)	0.213
10	Domain 1	*FGFR2* c.1171_1172insATA p.(Cys391delinsTyrSer)	40.0	Frame shift		Tier III	COSV107405700, seen in endometrium	—					
	Domain 1	*PIK3CA* c.1634A>C p.(Glu545Ala)	41.0	Missense		Tier IID	COSV55873209, seen previously in multiple other cancer types	Probably damaging (0.995)	Probably damaging (0.935)	Deleterious (0)	27.6	Likely pathogenic (0.9665)	0.715

(continued on following page)

TABLE A2. Somatic Variants From Whole-Genome Sequencing With Classifications, COSMIC Details, Polyphen2 In-Silico Protein Change Prediction for Missense Variants (continued)

Patient	Domain	Variant	VAF (%)	Classification	ACMG Class	AMP Tier	COSMIC	Polyphen 2 (using HumDiv)	VEP PolyPhen2 (HumVar)	VEP SIFT	VEP CADD PHRED	VEP AlphaMissense	VEP REVEL
11	Domain 1	*TP53* c.673-2A>G	0.45;(LOH)	Splice variant	Class 5	Tier IID	COSV52682653, seen previously in multiple cancer types	—					
	Domain 2	*BCL9L* c.610_611delAG p.(Ser204GlnfsTer16)	14.0	Frame Shift	Class 5	Tier III	Not present in COSMIC	—					
	Domain 2	*MYB* c.1958C>T p.(Ser653Phe)	25.0	Missense	Class 3	Tier III	COSV57200595, seen previously in kidney cancer	—		Deleterious—low confidence (0)	25.4		0.404
	Domain 2	*NOTCH1* c.4620C>G p.(Phe1540Leu)	0.52;(LOH)	Missense	Class 3	Tier III	COSV99484021, seen previously in melanoma	Probably damaging (0.957)	Possibly damaging (0.696)	Deleterious—low confidence (0)	23.5	Likely pathogenic (0.9801)	—
	Domain 2	*PML* c.1909C>G p.(Arg637Gly)	22.0	Missense	Class 3	Tier III	Not present in COSMIC	Possibly damaging (0.910)	Benign (0.328)	Deleterious (0.02)	20.3	Likely benign (0.3285)	0.195
12	Domain 1	*AMER1* c.3367A>T p.(Ser1123Cys)	6.0	Missense		Tier III	Not present in COSMIC	—	Benign (0)	Deleterious (0)	18.2	Likely benign (0.1002)	—
	Domain 1	*POLE* c.2934A>C p.(Glu978Asp)	10.0	Missense		Tier III	Not present in COSMIC	Possibly damaging (0.949)	Probably damaging (0.979)	Deleterious (0)	21.6	Likely pathogenic (0.9935)	0.348
	Domain 2	*AFF4* c.745A>T p.(Met249Leu)	54.0	Missense			Not present in COSMIC	Possibly damaging (0.699)	Benign (0.031)	Deleterious (0.03)	24.3	Likely benign (0.1827)	0.256
	Domain 2	*ELF4* c.1187+1G>T	42.0	Splice variant			Not present in COSMIC	—			35		
	Domain 2	*IL2* c.203A>T p.(Lys68Met)	6.0	Missense			Not present in COSMIC	Probably damaging (1.0)	Unknown	Deleterious (0.02)	18.73	Likely benign (0.263)	0.147
	Domain 2	*KMT2D* c.11493G>T p.(Gln3831His)	20.0	Missense			Not present in COSMIC	Probably damaging (0.978)	Unknown	Deleterious—low confidence (0)	17.62	Likely benign (0.3009)	0.328
	Domain 2	*PREX2* c.2326C>T p.(Pro776Ser)	42.0	Missense			COSV55796805, previously seen in lung and basal cell carcinoma	Benign (0.0)	Benign (0)	Tolerated—low confidence (0.93)	1.53	Likely benign (0.0658)	0.071
13	Domain 1	*BAP1* c.757C>T p.(Gln253Ter)	48.0	Nonsense	Class 5	Tier IID	COSV56230964, seen previously in multiple cancer types	—			39		
	Domain 1	*FANCA* c.2602-9_2602-8delCT	35.0	Splice variant	Class 3	Tier III	COSV59798201, seen previously in prostate and hemangioblastoma	—			7.19		
	Domain 1	*KDM6A* c.907_909delGCC p.(Ala303del)	33.0	Inframe deletion	Class 3	Tier III	Not present in COSMIC	—					
	Domain 2	*PDGFRB* c.236G>A p.(Gly79Asp)	24.0	Missense			Not present in COSMIC	Probably damaging (0.999)	Probably damaging (0.955)	Tolerated (0.11)	18.2	Likely benign (0.103)	0.298
14	No small somatic variants detected												

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AdCC, adenoid cystic carcinoma; AMP, Association for Molecular Pathology; CADD, Combined Annotation Dependent Depletion; GIST, gastrointestinal stromal tumor; LOH, loss of heterozygosity; REVEL, Rare Exome Variant Ensemble Learner; SIFT, Sorting Intolerant From Tolerant; VAF, variant allele frequency; VEP, Variant Effect Predictor.