 IMPORTANCE Sodium iodide symporter (NIS) expression in adenoid cystic carcinoma (ACC) has not been fully elucidated in the literature, and it is unclear whether radioactive iodine may be a potential therapeutic modality. To our knowledge, the present study includes the largest ACC tumor sample size to evaluate for NIS expression.

 OBJECTIVE To assess whether ACC of the head and neck expresses NIS by using immunohistochemical staining techniques, as well as assess whether the presence or intensity of staining correlates with tumor or patient variables.

 DESIGN, SETTING, AND PARTICIPANTS Immunohistochemical analysis of NIS expression was performed on 20 ACC specimens from various head and neck subsites obtained from January 1, 1988, to May 6, 2013, at a single academic tertiary care medical center. Staining intensity was graded on a scale of 0 to 3+ (higher numbers indicate greater staining intensity) and was analyzed according to multiple patient and tumor variables. Tumors were eliminated from the study if the patient had undergone prior surgical resection, chemotherapy, or radiotherapy, or was receiving thyroid hormone supplementation at the time of the operation.

 MAIN OUTCOMES AND MEASURES Presence or absence of staining was the primary outcome measured; secondary outcomes were the intensity and localization of staining.

 RESULTS Sodium iodide symporter staining was positive in 15 of the 20 tumor specimens (75%). Staining was largely localized to the cytoplasm and was of low intensity. There was no significant association between the presence or intensity of staining and the tumor subtype, tumor location, or any of the patient variables assessed (P > .05). No association between staining intensity and tumor growth pattern was shown on χ² analysis: 1+ (P = .53), 2+ (P = .14), or a combination of 1+ and 2+ staining (P = .64). Parotid control tissue demonstrated intense membranous staining of the striated parotid gland ducts.

 CONCLUSIONS AND RELEVANCE Sodium iodide symporter was expressed in the cytoplasm with low intensity in most of the tumor specimens examined in this study. These staining characteristics are also commonly found in thyroid cancer cells. Further investigation is required to determine the significance of this finding. We are optimistic that future studies using endogenous NIS stimulation and identification of genes associated with NIS plasma membrane localization could be applied to the treatment of ACC with radioactive iodine techniques.
The sodium iodide symporter (NIS) plays a fundamental role in the transportation of iodide into the thyroid follicular cell. The application of radioactive iodide in the treatment of well-differentiated thyroid cancer depends on this symporter for successful therapy. It is well known that NIS expression is present in extrathyroidal tissues, including the salivary glands.1,2 This is evidenced by physiologic uptake of iodide by the major and minor salivary glands on routine scintigraphic studies, as well as by the posttreatment sialoadenitis that patients may experience following radioactive iodine (RAI) therapy.3 The NIS RNA transcripts expressed in the parotid gland are identical to those of the thyroid, as confirmed by complementary DNA sequencing.4

Expression of NIS in salivary gland malignant neoplasms has not been fully elucidated in the literature. To date, there have been 2 studies5,6 investigating NIS expression in salivary gland neoplasms. Given the limited research thus far, it is unclear whether RAI may be a potential therapeutic modality in salivary gland malignant neoplasms.

We chose to assess NIS expression in adenoid cystic carcinoma (ACC) for several reasons. First, ACC is a slow-growing malignant neoplasm with a fairly mild histopathologic appearance. Tumors usually demonstrate a uniform cellular appearance within the tubular and cribriform components, with rare mitotic activity. Given these findings, it is possible that these cells may retain many of their normal cellular processes, including the potential for NIS expression. The relatively indolent behavior histologically makes ACC refractory to strategies intended to target rapidly dividing cells typically seen in more aggressive tumors. Nonetheless, clinical presentation of the disease is far from benign. Treatment resistance has resulted in distant metastatic disease in almost half of patients with ACC and undesirable disease-free survival rates despite locally aggressive therapy.7 Currently, the response rates to chemotherapy are low and the response is short-lived.8

In some ways, ACC resembles well-differentiated thyroid cancer. Both tumors tend to exhibit slow growth rates and derive from glands with the potential for iodide uptake. We hypothesized that, given the unique histologic features of ACC and the need for improved systemic control, RAI may be a potential therapeutic modality. In this study, we used immunohistochemical methods to assess the expression of the NIS in a variety of ACC tumor specimens. To our knowledge, this study includes the largest ACC tumor sample size to evaluate for NIS expression.

Methods

Subjects

The study protocol was approved by the Cleveland Clinic Foundation Institutional Review Board, and consent was obtained for the use of residual human malignant tissue from the Protocol Monitoring Review Committee for Case Comprehensive Cancer Center. Patient data were deidentified. The Cleveland Clinic surgical pathology database was searched over a 25-year period (January 1, 1988, to May 6, 2013) for all formalin-fixed, paraffin-embedded head and neck ACC specimens with retrievable material. Tumor specimens were eliminated from the study if the patient had undergone prior surgical resection, chemotherapy, or radiotherapy, or was receiving thyroid hormone supplementation at the time of surgery. Paraffin-embedded control specimens, including normal parotid, normal thyroid, and Grave thyroid tissue, were also obtained. Tumor slides were reviewed by a staff pathologist (D.J.C.) and the diagnosis of ACC was verified prior to study inclusion. A total of 20 ACC tumor specimens were included from various subsites including the oral cavity (n = 6), parotid gland (n = 8), submandibular gland (n = 2), larynx (n = 2), sinonasal (n = 1), and oropharynx (n = 1). Slides were created using conventional whole-tissue sectioning. Pertinent patient information, including sex, age at diagnosis, tumor location, nodal status, extranodal extension, metastatic disease, perineural invasion, lymphovascular invasion, margin status, and living status, was extracted from the electronic medical record. Cancer stages were labeled N0 if the neck was clinically negative and M1 if there was evidence of progressive metastatic lesions. The Mx stage was assigned in 3 patients owing to the presence of pulmonary nodules on chest imaging that were of unclear significance because of the lack of further imaging or loss of follow-up.

Immunochemistry

Staining of the NIS was performed with an automated immunostainer (Ventana Benchmark XT; Ventana Medical Systems Inc) using a detection kit (OptiView DAB IHC Detection Kit with CC2; Ventana Medical Systems Inc) for 24 minutes. Primary antibody, monoclonal mouse antisodium/iodide symporter (MAB3564; EMD Millipore Corp) was diluted 1:200 and applied for 1 hour without heat. Components of the detection kit (H2 Universal Linker and HRP Multimer; Ventana Medical Systems Inc) were applied sequentially for 12 minutes each, and slides were counterstained with hematoxylin II (Ventana Medical Systems Inc) for 4 minutes; bluing reagent (Ventana Medical Systems) was then applied for 4 minutes. The stained slides were dehydrated and permanently mounted for viewing. Individual tumor samples were also sectioned and stained with hematoxylin-eosin for comparison. Appropriate positive (normal parotid tissue, normal thyroid tissue, and Grave thyroid tissue) and negative (with omission of the primary antibody) control samples were included with each run.

Evaluation of Immunostaining

The slides were individually reviewed by an expert pathologist (D.J.C.) under light microscopy. Immunohistochemical staining for NIS was qualitatively graded (using the positive controls for comparison and reproducibility) as follows: 0, no staining; 1+, pale cytoplasmic staining with fine granules; 2+, dark, dotlike cytoplasmic staining with coarse granules; and 3+, intense membranous staining (Figure 1). The tumor growth patterns were classified as follows: tubular (n = 1), cribriform (n = 6), solid (n = 0), mixed tubular and cribriform (n = 12), mixed tubular and solid (n = 1), or mixed cribriform and solid (n = 0). In tumors that demonstrated mixed histopathology, both components were given individual gradings.
Statistical Analysis
Statistical analysis was performed using the Fisher exact test to compare cribriform or tubular NIS staining and the following patient variables: sex, age at diagnosis, tumor location, node status, extranodal extension, metastatic disease, perineural invasion, lymphovascular invasion, margin status, and living status. The analysis was limited to cribriform and tubular staining owing to only 1 instance of a nonzero solid staining measure. Analysis with the χ² test was used to compare cribriform staining intensity with tubular staining intensity. P < .05 was considered significant.

Results
The NIS immunostaining of parotid control tissue demonstrated strong 3+ staining of the basolateral membrane of striated duct cells; weaker 2+ dark dotlike cytoplasmic staining with coarse granules was identified in the intercalated ductal cells. The excretory ducts demonstrated the weakest staining, with 1+ fine, granular cytoplasmic staining. The acinar cells were negative for staining (Figure 1). These results confirm the findings of others. Normal thyroid control tissue demonstrated a heterogeneous patchy distribution of membranous staining with occasional nuclear and cytoplasmic staining as shown in Figure 1. The Grave thyroid control tissue demonstrated similar findings to normal thyroid tissue including both membranous and cytoplasmic staining. The Table summarizes the patients’ characteristics and NIS staining results. Staining was positive in 15 of 20 tumors (75%) and was largely localized to the cytoplasm in all cases. Five tumors (25%) were negative for staining, as demonstrated in Figure 2. A total of 8 tumors (40%) exhibited 1+ staining (Figure 3), 3 tumors (15%) had 2+ staining (Figure 4), and 4 tumors (20%) had a mixture of 1+ and 2+ staining. Using the Fisher exact test, the following variables were analyzed with respect to the presence of staining in cribriform and tubular tumors, respectively: sex (P = .37 and P = .65), age at diagnosis (P = .39 and P = .89), tumor location (P = .56 and P = .35), nodal status (P > .99 and P = .30), extranodal extension (P > .99 and P = .49), metastatic disease (P > .99 and P = .11), perineural invasion (P = .33 and P = .01), lymphovascular invasion (P > .99 and P = .17), margin status (P = .02 and P = .59), and living status (P = .16 and P = .22). Perineural invasion and margin status did not remain significant after correction for multiple comparisons. There were no significant associations between the presence or intensity of staining and the tumor subtype, tumor location, or the following patient variables: sex, age at diagnosis, nodal status, presence of extranodal extension, metastatic disease, perineural invasion, lymphovascular invasion, surgical margin status, or living status (all P > .05). The assessment of whether staining intensity was associated with either cribriform or tubular growth patterns was conducted with χ² analysis. There was no significant growth pattern association with 1+ (P = .53), 2+ (P = .14), or a combination of 1+ and 2+ staining patterns (P = .64). During the slide review, we noted focal staining to be more common in luminal cells rather than myoepithelial-derived components in tumors with positive stain-
Discussion

The cloning and molecular characterization of human NIS gene (GenBank U66088) in 1996 has allowed for extensive research on NIS. Despite the importance of NIS in thyroid cancer treatment, it has become increasingly evident that other bodily tissues share the capacity to accumulate iodide. Research is underway to explore the potential for RAI therapy in extrathyroidal cancer. Promising results have been found in other exocrine gland neoplasms including breast carcinoma and prostate adenocarcinoma.

Our staining results were in accordance with those of La Perle et al, with the highest salivary gland NIS expression in the basolateral membranes of striated ducts and weaker staining expressed in intercalated and excretory duct cells. Their study also noted a higher percentage of NIS-positive cells in the submandibular gland compared with the parotid gland. We did not find that ACC tumors of submandibular gland origin differed from those of parotid gland origin in the presence or intensity of staining. La Perle et al also observed low-intensity staining in 1 of 5 ACC tumors. Disparities in the detection of NIS among investigators may be explained by the use of antibodies directed toward different epitopes as well as differences in slide preparation. Their analysis used microarrays with 1.5-mm cores; our analysis used conventional slide staining, hence avoiding the problems inherent to microarrays. They also used a polyclonal antibody, but our study was performed with a monoclonal antibody.

There are several limitations to our study. First, our sample size was small, including only 20 tumors. However, ACC is a very rare neoplasm and the present investigation was intended to be an exploratory pilot study. With this sample size, there is a less than 5% chance that NIS staining would not be detected if staining is present in only 15% of ACC tumors. We believed that if less than 15% of tumors expressed NIS, the clinical usefulness for RAI therapy would be limited. Second, the intracytoplasmic NIS expression found in most of our tumor specimens is yet to be elucidated. We do not believe that the staining identified in the tumor specimens is due to nonspecific background staining because this was not found in our

### Table. Summary of Patient Characteristics and NIS Staining

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Primary Site</th>
<th>Histologic Finding</th>
<th>TNM Cancer Stage</th>
<th>PNI/LVI/ECE</th>
<th>NIS Staining*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/73</td>
<td>Submandibular gland</td>
<td>C + Tu</td>
<td>T1N0M0</td>
<td>+/NA/−</td>
<td>2+</td>
</tr>
<tr>
<td>2/M/36</td>
<td>Oral cavity</td>
<td>C</td>
<td>T1N0M0</td>
<td>NA/NA/NA</td>
<td>0</td>
</tr>
<tr>
<td>3/F/66</td>
<td>Sinonasal</td>
<td>C</td>
<td>T4N0M0</td>
<td>NA/NA/NA</td>
<td>0</td>
</tr>
<tr>
<td>4/F/82</td>
<td>Parotid gland</td>
<td>C</td>
<td>T1N0M0</td>
<td>NA/NA/NA</td>
<td>0</td>
</tr>
<tr>
<td>5/M/48</td>
<td>Parotid gland</td>
<td>C</td>
<td>T2N1M0</td>
<td>NA/NA/−</td>
<td>1+</td>
</tr>
<tr>
<td>6/F/39</td>
<td>Parotid gland</td>
<td>C</td>
<td>T2N0M0</td>
<td>−/−/NA</td>
<td>1+</td>
</tr>
<tr>
<td>7/F/56</td>
<td>Parotid gland</td>
<td>Tu + S</td>
<td>T4N0M0</td>
<td>+/−/−</td>
<td>1+/2+</td>
</tr>
<tr>
<td>8/F/72</td>
<td>Oral cavity</td>
<td>T</td>
<td>T4N0M0</td>
<td>+/−/−</td>
<td>0</td>
</tr>
<tr>
<td>9/M/53</td>
<td>Oral cavity</td>
<td>C + Tu</td>
<td>T2N0M0</td>
<td>+/−/NA</td>
<td>1+</td>
</tr>
<tr>
<td>10/M/62</td>
<td>Parotid gland</td>
<td>C</td>
<td>T4N0M1</td>
<td>+/NA/NA</td>
<td>1+/2+</td>
</tr>
<tr>
<td>11/M/65</td>
<td>Oral cavity</td>
<td>C + Tu</td>
<td>T2N0M0</td>
<td>−/NA/NA</td>
<td>1+</td>
</tr>
<tr>
<td>12/M/76</td>
<td>Parotid gland</td>
<td>C</td>
<td>T3N0M0</td>
<td>+/NA/NA</td>
<td>1+</td>
</tr>
<tr>
<td>13/F/59</td>
<td>Parotid gland</td>
<td>C + Tu</td>
<td>T4N0M1</td>
<td>+/−/−</td>
<td>1+/2+</td>
</tr>
<tr>
<td>14/M/81</td>
<td>Larynx</td>
<td>C + Tu</td>
<td>T4N0M1</td>
<td>+/−/NA</td>
<td>0/2+</td>
</tr>
<tr>
<td>15/F/46</td>
<td>Parotid gland</td>
<td>C + Tu</td>
<td>T2N0M0</td>
<td>−/−/−</td>
<td>0</td>
</tr>
<tr>
<td>16/M/48</td>
<td>Oral cavity</td>
<td>C + Tu</td>
<td>T1N2Mx</td>
<td>+/+/+</td>
<td>0/1+</td>
</tr>
<tr>
<td>17/M/41</td>
<td>Oropharynx</td>
<td>C</td>
<td>T2N2M0</td>
<td>+/+/+</td>
<td>1+</td>
</tr>
<tr>
<td>18/F/47</td>
<td>Larynx</td>
<td>C + Tu</td>
<td>T1N0Mx</td>
<td>−/−/−</td>
<td>1+/2+</td>
</tr>
<tr>
<td>19/M/43</td>
<td>Submandibular gland</td>
<td>C + Tu</td>
<td>T1N0Mx</td>
<td>−/−/−</td>
<td>1+</td>
</tr>
<tr>
<td>20/F/59</td>
<td>Oral cavity</td>
<td>C + Tu</td>
<td>T4N0M1</td>
<td>+/NA/NA</td>
<td>1+/2+</td>
</tr>
</tbody>
</table>

Abbreviations: C, cribriform; ECE, extracapsular extension; LVI, lymphovascular invasion; M, metastatic; N, nodal; NA, information not available; NIS, sodium iodide symporter; PNI, perineural invasion; S, Solid; T, tumor; Tu, tubular; +, present; −, absent.

* Higher numbers indicate greater staining intensity.
control tissues or in the normal salivary tissue located adjacent to the tumor tissue on multiple slides. Weak intracytoplasmic staining is a frequent finding in follicular and papillary thyroid cancer cells as well as in benign thyroid adenomas.\textsuperscript{10-12} Whether the cytoplasmic localization is a result of faulty targeting and/or insufficient retention of NIS in the plasma membrane is unclear, and determining the cause for these findings was not the goal of the present study. Given the subcellular localization of the gene product, it would appear that NIS is not biologically active in ACC. However, as with any immunohistochemical study, the results reflect a visual distribution and localization of the NIS protein, but do not assess the functionality of the protein of study. To assess functional tumor protein expression, other techniques may be used, such as radionuclide imaging with sodium iodide I\textsubscript{123} and technetium 99m pertechnetate scintigraphy. A recent study by Akai et al\textsuperscript{6} examined the functional uptake of technetium in parotid gland tumors and compared the results with those of NIS immunohistochemical staining. The investigators found that all technetium-positive specimens stained positive with NIS and all technetium-negative specimens were NIS-negative. They concluded that poor tumor excretory function compared with normal parotid tissue, in addition to uptake of technetium via NIS, may be involved in technetium accumulation in Warthin tumors and oncocytomas. The analysis did not include ACC tumors.

When considering RAI therapy in nonthyroid neoplasms, several important limiting factors need to be considered. First, when using RAI as a strategy to treat extrathyroidal neoplasms, it is necessary to inhibit iodide uptake by the thyroid gland to allow for effective concentrations to be reached in extrathyroidal tumor tissue. Successful techniques have been applied by using a combination of triiodothyronine and methimazole to selectively downregulate NIS expression, as well as inhibit organification in thyrocytes.\textsuperscript{13} This technique could potentially be used in the treatment of salivary gland malignant neoplasms since normal salivary tissue NIS expression is independent of thyroid-stimulating hormone levels.\textsuperscript{14} Second, the tissue must maintain the ability to express NIS at the plasma membrane to transport RAI effectively into the cell. Moreover, the cell must retain the radioisotope for a reasonable time period to allow for the adequate delivery of its radiation dose. Unfortunately, most research efforts have found that few patients would benefit from RAI therapy in the native tumor state. This is largely because of NIS expression being limited to low levels, as well as cytoplasmic localization of expression, similar to the findings in our study. For this reason, an extensive amount of investigation has been undertaken to identify compounds to stimulate endogenous tumor NIS expression in an effort to improve goal therapeutic concentrations.

Research is underway to find ways to improve NIS expression to allow for more effective RAI therapy in both thyroid and nonthyroid neoplasms.\textsuperscript{15} A study by Liu and Xing\textsuperscript{16} revealed that simultaneously suppressing mitogen-activated protein kinase as well as phosphatidylinositol-3 kinase and protein kinase B pathways and histone deacetylase could induce robust expression and increased radioiodine uptake in certain nonthyroid human cancer cell lines including melanoma, hepatic, gastric, colon, and breast carcinoma cell lines. Given the recent concerns regarding cross-contamination of ACC cell lines, further research to explore the effect of these inhibitors on ACC cell expression at this time is difficult.\textsuperscript{17} Production of newer and more reliable ACC cell lines is in process.

**Conclusions**

The NIS was expressed with low intensity in 15 of 20 tumor specimens. Cytoplasmic localization of the gene product is
similar to that seen in other well-differentiated thyroid cancers. Whether these findings represent a possible biologically interesting change in subcellular localization of NIS with potential implications for therapy remains in question; further study is required to determine the explanation for these findings. Whether the cytoplasmic localization is the result of faulty targeting and/or insufficient retention of NIS in the plasma membrane, we are optimistic that future studies using endogenous NIS stimulation and identification of genes associated with NIS plasma membrane localization could be applied to the treatment of ACC with RAI techniques. Elucidation of the underlying regulatory pathways involved in the intense expression of NIS in salivary gland striated ducts may also enhance our knowledge regarding the functional NIS expression in extrathyroidal neoplasms.

REFERENCES