

Adrenocortical Carcinoma Is a Lynch Syndrome–Associated Cancer

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ABSTRACT

Purpose

Adrenocortical carcinoma (ACC) is an endocrine malignancy with a poor prognosis. The association of adult-onset ACC with inherited cancer predisposition syndromes is poorly understood. Our study sought to define the prevalence of Lynch syndrome (LS) among patients with ACC.

Patients and Methods

One hundred fourteen patients with ACC were evaluated in a specialized endocrine oncology clinic and were prospectively offered genetic counseling and clinical genetics risk assessment (group 1). In addition, families with known mismatch repair (MMR) gene mutations that were recorded in the University of Michigan Cancer Genetics Registry were retrospectively reviewed for the presence of ACC (group 2). ACC tumors from patients with LS were tested for microsatellite instability and immunohistochemistry (IHC) to evaluate for MMR deficiency.

Results

Ninety-four (82.5%) of 114 patients with ACC underwent genetic counseling (group 1). Three individuals (3.2%) had family histories suggestive of LS. All three families were found to have MMR gene mutations. Retrospective review of an additional 135 MMR gene–positive probands identified two with ACC (group 2). Four ACC tumors were available (group 1, 3; group 2, 1). All four tumors were microsatellite stable; three had IHC staining patterns consistent with germline mutation status.

Conclusion

The prevalence of LS among patients with ACC is 3.2%, which is comparable to the prevalence of LS in colorectal and endometrial cancer. Patients with ACC and a personal or family history of LS tumors should be strongly considered for genetic risk assessment. IHC screening of all ACC tumors may be an effective strategy for identifying patients with LS.

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INTRODUCTION

Lynch syndrome (LS) is an inherited cancer predisposition syndrome associated with elevated cancer risks. The lifetime colorectal cancer risk is estimated to be as high as 35% to 80% and the endometrial cancer risk in women is estimated to be elevated to 34% to 71%.¹⁻⁴ Identifying individuals with LS and subsequently enrolling them in screening and surveillance protocols significantly decreases cancer-related morbidity and mortality.⁵ Focus has thus shifted to consider other potentially related cancers that have previously gone unrecognized owing to a lower prevalence than the classic LS-associated cancers. Elevated risks of other cancers, including gastric, ovarian, urinary tract, pancreas, brain tumors, and sebaceous tumors have been established in patients with LS.⁶ However, when considering tumors that are rare in the general population, recognizing

an association with an inherited cancer predisposition syndrome can be difficult. The ability to recognize an association of a rare tumor with an inherited cancer predisposition syndrome increases the ability to diagnose the syndrome and to provide appropriate interventions for patients and their families.

Adrenocortical carcinoma (ACC) is a rare cancer with an incidence of 0.72 cases per million individuals per year (300 new diagnoses are made in the United States per year).⁷ While the association of childhood ACC with hereditary cancer syndromes is well described, the association of ACC in adult patients and inherited predisposition syndromes is far less well understood. ACC is known to be a core tumor in Li Fraumeni syndrome (LFS) because of germline *TP53* mutations, and a diagnosis of ACC in childhood is strongly associated with this condition. Fifty percent to 80% of all children diagnosed with ACC have an underlying diagnosis of LFS.⁸⁻¹¹ This

association is not as strong in adult-onset ACC. LFS has been identified as the underlying cause in 3.9% to 5.8% of patients with adult-onset ACC.^{12,13} Adult-onset ACC has also been described in multiple endocrine neoplasia type 1^{14,15} and has been reported in patients with familial adenomatous polyposis and LS.¹⁶ Our aim was to uncover the prevalence of inherited cancer syndromes in patients with ACC.

PATIENTS AND METHODS

Patients

Prospective analysis (group 1). Our study population included patients who presented with a diagnosis of ACC to the University of Michigan Multidisciplinary Endocrine Oncology program between December 1, 2009 and October 31, 2011 (probands).¹³ This clinic is a national and international referral center, with expertise in the diagnosis and interdisciplinary management of patients with ACC. Patients met with a genetic counselor who obtained a four-generation cancer-focused pedigree and performed a clinical genetics risk assessment. Family cancer histories were confirmed where records were available. All patients were offered *TP53* germline genetic testing for LFS through a Clinical Laboratory Improvement Amendments–certified clinical lab based on the Chompret criteria as previously described.^{13,17} Other genetic testing was offered based on the clinical cancer genetics risk assessment.

Retrospective chart review (group 2). The University of Michigan Cancer Genetics Registry was queried for all probands with mismatch repair (MMR) gene mutations and a personal diagnosis of ACC. In this retrospective group, probands are defined as the first person presenting to the University of Michigan Cancer Genetics program. This registry was initiated in 2002, and participation in the registry is offered to all probands and their family members who are evaluated in the University of Michigan Cancer Genetics program. Probands enrolled in the Cancer Genetics Registry through the Multidisciplinary Endocrine Oncology program were excluded from the retrospective chart review.

Formalin-fixed paraffin-embedded (FFPE) tumor samples of ACC were obtained for those patients identified as having LS.

Permission for human research was approved through the University of Michigan institutional review board.

DNA Extraction

Hematoxylin and eosin–stained slides cut from FFPE tissues were evaluated by a pathologist (L.V.F.), and the areas of the slide representing tumor and normal (no malignant tissue) were identified and manually dissected from 10- μ m FFPE tissue sections using Pinpoint Slide DNA Isolation System (Zymo Research Corporation, Irvine, CA). Areas selected for dissection ranged from 2 mm to 1 cm in diameter. The proportion of tumor cells in the material used for the extraction of DNA exceeded 50% in all of the patients. Corresponding normal tissue was available in two of the four selected patients (patients 3 and 4; Appendix Fig A2, online only). Tissue samples were digested with 5 μ L of Proteinase K (Zymo Research Corporation) in 50- μ L extraction buffer (Zymo Research Corporation) for 4 hours at 55°C. Samples were then heated to 95°C for 10 minutes and vortexed. No additional purification of the DNA was performed.

Genomic DNA from peripheral blood lymphocytes was obtained and used as control for the two patients in whom only tumor tissue was identified by histologic examination (patients 1 and 2; Appendix Fig A1, online only). DNA from peripheral blood lymphocytes was extracted using standard procedures according to the manufacturer's instructions (Gentra Puregene Cell kit, Qiagen, Valencia, CA).

Microsatellite Instability Assay

Microsatellite instability (MSI) testing was performed with the MSI Analysis System, version 1.2, according to the manufacturer's protocol, (Promega Corporation, Madison, WI). In short, five mononucleotide repeat markers are used for MSI determination (BAT-26, NR-21, BAT-25, MONO-27, and NR-24) and two pentanucleotide markers for establishing sample identity (Penta C and Penta D).¹⁸ Amplicons were separated by capillary electrophore-

sis using an automated ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Carlsbad, CA). A positive amplification control and a no-template control were set up with each run. Data were analyzed using GeneMapper 4.0 software (Applied Biosystems, Carlsbad, CA). For analysis, allelic profiles of normal versus tumor tissues were compared, and MSI was scored as the presence of alterations in the length of short tandem repeats in tumor DNA compared with normal DNA.¹⁹⁻²¹ Alleles present in the tumor sample that were not present in the corresponding normal tissue indicate MSI. MSI was defined as instability (\geq one of five mononucleotide microsatellite markers) in tumor DNA compared with normal DNA. Tumors with instability in \geq two of five mononucleotide microsatellite markers was defined as MSI-high. MSI-low was defined as instability in one of five mononucleotide markers in tumor DNA compared with normal DNA. Tumors with no instability (none of five mononucleotide markers altered) were defined as microsatellite stable (MSS).^{22,23}

Immunohistochemical Staining

Immunoperoxidase staining was performed on FFPE tissues as previously described.²⁴ The primary antibodies were MLH1 predilute mouse monoclonal M1 from Ventana Medical Systems, MSH2 predilute mouse monoclonal G219-1129 from Ventana Medical Systems, MSH6 predilute mouse monoclonal 44 from Ventana Medical Systems, and PMS2 predilute rabbit monoclonal ERP3974 from Ventana Medical Systems. Positive and negative controls were stained appropriately, and any convincing nuclear staining was considered positive.

RESULTS

One hundred fourteen patients with ACC were evaluated in the University of Michigan Endocrine Oncology Program during the study period. Ninety-four patients (82.5%) met with a genetic counselor. Thirty-three patients (35.1%) were men and 61 patients (64.9%) were women. The majority of patients were white (87.2%). Average age at ACC diagnosis was 44.7 years, with a range of three to 82 years old at diagnosis (Table 1). Fifty-three patients underwent germline *TP53* mutation analysis, and four patients (7.4%) were positive for a germline *TP53* mutation as previously described.¹³

In the prospective analysis (group 1), after completion of a four-generation cancer genetics pedigree, three probands were identified as having family histories suggestive of LS (three of 94; 3.2%); two probands had family histories that met clinical diagnostic criteria for LS (Amsterdam I criteria; Table 2), and a third proband had a family

Table 1. Characteristics of Patients in the Endocrine Oncology Program (n = 94)

Characteristic	No. of Patients	%
Sex		
Female	61	64.9
Male	33	35.1
Race		
White	82	87.2
African American	8	8.5
Asian	2	2.1
Other	2	2.1
Age at diagnosis, years		
Mean	44.7	
Range	3-82	
Median	46	

Table 2. Clinical Diagnostic Criteria for LS**Amsterdam I Criteria:**

Three or more individuals diagnosed with colorectal cancer *plus* all of the following

- One individual must be a first-degree relative of the other two
- At least two successive generations are affected
- At least one individual is diagnosed with cancer before age 50 years
- Familial polyposis has been excluded

Amsterdam II Criteria:

Three or more individuals diagnosed with an LS-associated tumor, including colorectal, endometrial, small bowel, ureter, or renal pelvis cancer *plus* all of the following

- One individual must be a first-degree relative of the other two
- At least two successive generations are affected
- At least one individual is diagnosed with cancer before age 50 years
- Familial polyposis has been excluded

Abbreviation: LS, Lynch syndrome.

history suggestive of LS (Table 3). All three probands underwent germline *TP53* analysis and were negative for any germline mutations by sequencing and deletion/duplication analysis. Two of three probands underwent germline MMR genetic testing and were positive for MMR gene mutations (proband 1, *MSH2*; proband 3, *MSH6*). LS work-up in the third family (proband 2) was completed after the proband died and the brother tested positive for an *MLH1* mutation.

In the retrospective chart review (group 2), an additional 135 probands from independently ascertained families with MMR gene mutations enrolled onto the University of Michigan Cancer Genetics Registry (*MLH1*, n = 44; *MSH2*, n = 66; *MSH6*, n = 18; *PMS2*, n = 7) and revealed two probands with personal histories of ACC (*MSH2*, n = 2; probands 4 and 5; Table 3). At initial consultation, proband 4 tested negative for germline *TP53* mutations by sequencing and deletion/duplication analysis in accordance with Chompret guidelines to exclude the possibility of second inherited predisposition syndrome.

ACC tumor tissue was available for four of five patients (Table 3). All of the tested samples were MSS (Appendix Figs A1 and A2). The ACC tumor from the two probands with *MSH2* mutations had immunohistochemical (IHC) staining demonstrating the absence of *MSH2* and *MSH6* with retention of *MLH1* and *PMS2*. The ACC tumor from the proband whose brother tested positive for an *MLH1* mutation had IHC staining demonstrating the absence of *MSH2* and *MSH6* with retention of *MLH1* and *PMS2*. The ACC tumor from the proband whose brother tested positive for an *MLH1* mutation had IHC staining, demonstrating absence of *MLH1* and *PMS2* with retention of *MSH2* and *MSH6*. The ACC tumor from the proband with an *MSH6* mutation had a normal IHC staining pattern with retention of all four proteins (Fig 1; Table 3).

DISCUSSION

Childhood ACC is strongly associated with LFS, an inherited cancer predisposition syndrome, and ACC is a core malignancy of LFS.⁹ Though the majority of adult-onset ACC has been thought to be sporadic in nature, there is increasing evidence of an association between adult ACC and inherited conditions, including LFS and multiple endocrine neoplasia type 1.^{12-14,16} Our current study defines LS as

another hereditary cancer syndrome to be considered in the evaluation of patients with ACC.

Identification of individuals with LS is important in routine medical care and oncologic care.^{1,25} The National Comprehensive Cancer Network²⁶ advocates for tumor screening of colorectal cancers and endometrial cancers to analyze for high levels of MSI and absent IHC staining in an effort to identify those individuals and subsequently their families who are at elevated risk for cancer, which has been shown to decrease morbidity and mortality⁵ and has been demonstrated as cost-effective.¹

In this prospective series of patients with ACC, three (3.2%) of 94 patients were subsequently diagnosed with LS. This prevalence is significantly higher than observed in the general population (one of 440 patients; $P = .0185$; Fisher's exact test).²⁷ The prevalence of LS among patients with ACC is similar to the prevalence of LS among all patients with colorectal cancer (2% to 4%) and endometrial cancer (1% to 5%).²⁸⁻³² Furthermore, the identification of two patients with ACC among 135 probands in the Cancer Genetics Registry suggests a significant relative risk increased over the general population (approximately 0.72 diagnoses per million persons per year). Case reports of ACC in patients with LS date back to the initial reports defining the syndrome. One of the first families described by Lynch et al,³³ Family N, was ascertained through a proband who died at age 44 years with a history of ACC with a striking family history of young-onset endometrial cancer and young-onset, multiple primary colorectal cancer. In addition, there have been four single case reports of ACC in conjunction with LS and germline *MSH2* mutations.³⁴⁻³⁷

The description of the five patients presented in our study brings the total number of reports of ACC and LS to 10 and expands the spectrum of germline mutations to include *MLH1* and *MSH6*. These findings give weight to the consideration of including ACC as an LS-associated tumor. Including ACC as an LS-associated tumor in the Amsterdam II criteria (Table 2) for a clinical diagnosis of LS would increase the diagnostic yield. It also holds the potential to increase the true-positive rate, while reducing the false-negative rate. In our prospective series, the clinical diagnostic yield would increase from two of five patients to three of five patients (40% v 60%; Table 3) with proband 5 meeting these enhanced Amsterdam II criteria.

In an attempt to understand a relationship with LS, we analyzed MSI and loss of MMR gene protein expression by IHC. MSI analysis revealed all four ACC tumors to be MSS. This is in contrast with findings in the classic LS cancer, colorectal cancer, in which the majority of these cancers in patients with LS demonstrate high levels of MSI.²⁹ Although intriguing, our findings concur with those of previous studies that report a lower incidence of MSI on polymerase chain reaction testing in rare cancers shown to be associated with LS.³⁴⁻³⁷ These observations suggest that DNA MMR gene deficiency in these tumors may be a secondary event that occurs in a later stage in carcinogenesis, therefore preventing the accumulation of detectable MSI.³⁵ In our series, IHC analysis identified loss of expression for *MSH2* and *MSH6* in the ACC tumor from both *MSH2* mutation carriers and loss of *MLH1* and *PMS2* expression in the ACC tumor from the *MLH1* family, consistent with the molecular phenotype of LS. However, staining for *MSH6* was retained in the ACC tumor from the proband with the *MSH6* mutation. This normal staining pattern in the *MSH6* mutation carrier is not an unexpected finding and is consistent with the IHC findings in colorectal and endometrial cancers, in which not

Table 3. Characteristics of Patients With ACC and LS and Results of MSI and IHC Screenings for LS in ACC Tumors

Group and Proband	Sex	Age at Diagnosis (years)	Right or Left Side	ACC Stage	MSI	IHC	Germline Mutation	Other LS Cancer History* and Age (years)	Family History of LS Cancer* in First- and Second-Degree Relatives and Their Ages (years)†
Group 1									
Proband 1	M	52	Right	III	MSS	Absence of MSH2/MSH6; retention of MLH1/PMS2	MSH2 c.2131C>T p.R711X	SebCa; 53	Amsterdam I
Proband 2	M	47	Right	III	MSS	Absence of MLH1/PMS2; retention of MSH2/MSH6	MLH1# c.2246T>C p.L749P	—	Amsterdam I
Proband 3§	M	39	Right	II	MSS	Normal	MSH6 c.2141C>G p.S714C	—	F: CRC; 45
Group 2									
Proband 4	F	42	Right	I	MSS	Absence of MSH2/MSH6; retention of MLH1/PMS2	MSH2 c.792+1G>A	SebAd; 47	PGF: CRC; late 40s
Proband 5	F	23	Left	III	—	—	MSH2 c.942+3A>T	SebCa; 49	M: OvCa, 54; MA: CRC, 83

NOTE. Group 1 refers to patients recruited prospectively through the endocrine oncology program. Group 2 refers to patients identified through retrospective review of the Cancer Genetics Registry. ACC tumor was not available for Proband 5. Full clinical description of patients, including details of endocrine evaluation, are available in Appendix Table A1 (online only).

Abbreviations: ACC, adrenocortical carcinoma; CRC, colorectal cancer; F, father; FDG-PET, fluorodeoxyglucose–positron emission tomography; IHC, immunohistochemistry; LS, Lynch syndrome; M, mother; MA, maternal aunt; MSI, microsatellite instability; MSS, microsatellite stable; OvCa, ovarian cancer; PGF, paternal grandfather; SebAd, sebaceous adenoma; SebCa, sebaceous carcinoma.

*LS cancers include the following cancers: colorectal, endometrial, ovary, stomach, small intestine, urinary tract, renal pelvis, bile duct, pancreas, glioblastoma multiforme, and sebaceous gland tumors.

†First- and second-degree relatives include children, parents, siblings, aunts, uncles, and grandparents.

#Mutation identified in proband's brother; germline testing not available for proband.

§Pathology review revealed oncocytic adrenocortical tumor of uncertain malignant potential. Tumor grew significantly over a 2-year course and was FDG-PET–positive; thus, patient is receiving follow-up with a diagnosis of ACC.

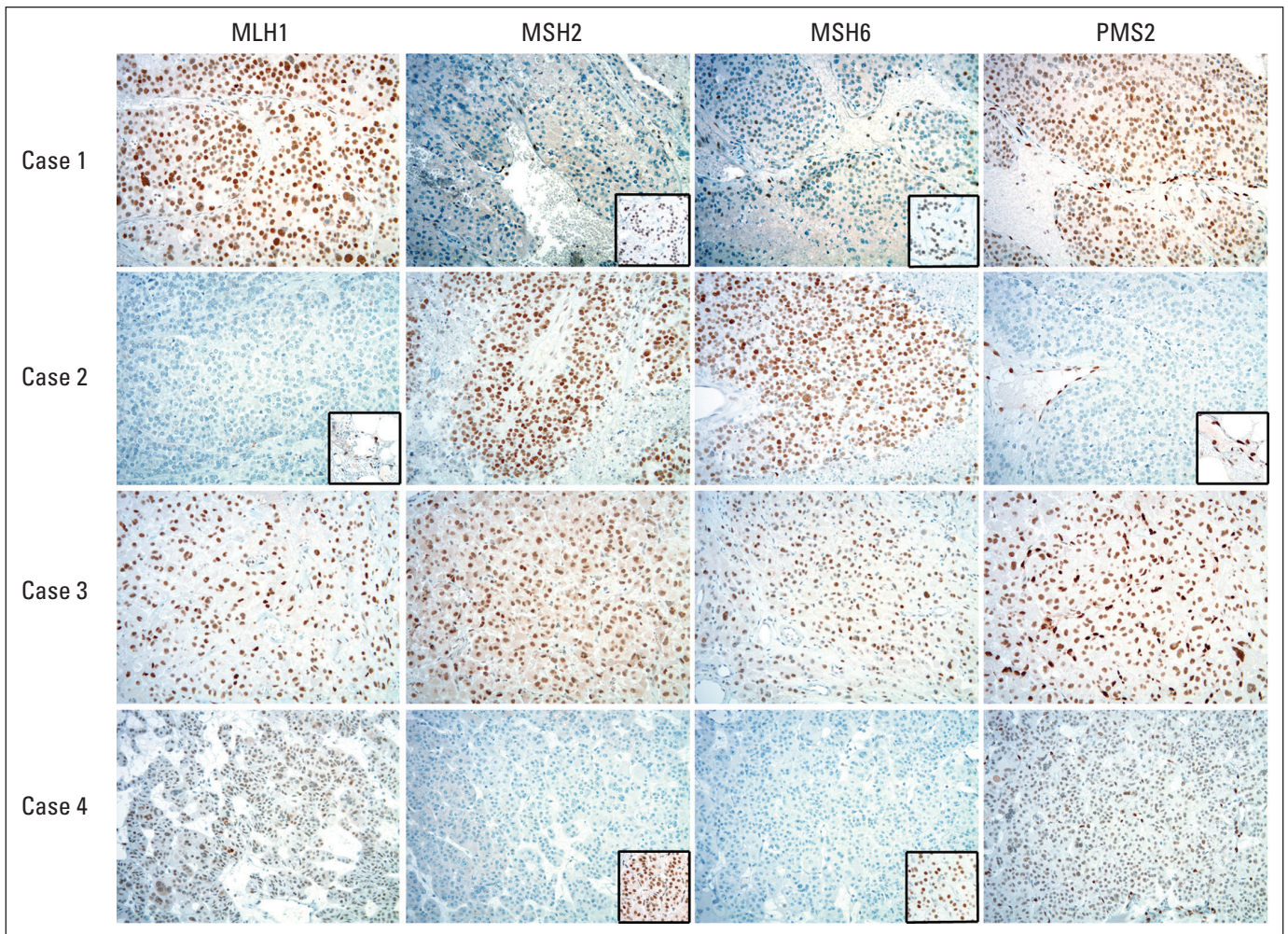


Fig 1. Immunohistochemistry stains of adrenocortical carcinoma (ACC) tumors. Patient 1 demonstrates absence of nuclear staining for MSH2 and MSH6 in the ACC tumor with positive nuclear staining in the adjacent normal adrenal tissue as shown in the inset photo. Retention of nuclear staining of MLH1 and PMS2 in the ACC tumor is shown. Patient 2 demonstrates absence of nuclear staining in the ACC tumor for MLH1 and PMS2 and positive nuclear staining in the adjacent normal adrenal tissue as shown in the inset photo. Retention of nuclear staining of MSH2 and MSH6 in the ACC tumor is shown. Patient 3 demonstrates retention of the nuclear staining in the ACC tumor tissue for all four proteins (MLH1, MSH2, MSH6, and PMS2). Patient 4 demonstrates absence of nuclear staining in the adjacent normal adrenal tissue as shown in the inset photo. Retention of nuclear staining of MLH1 and PMS2 in the ACC tumor is shown.

all patients with LS have informative tumor screening.^{28,29} Furthermore, IHC analysis has been shown to be inconsistent in other LS tumors in *MSH6* mutation carriers.^{38,39} In the combined data set of eight patients with ACC with tumor analysis, four patients presented here and four single case reports,³⁴⁻³⁷ IHC was informative and indicated a lack of expression corresponding with the gene harboring the mutation in six (75%) of eight. MSI analysis was not informative in any of the eight patients.

ACC is a cancer diagnosis with a very low incidence in the general population and a low incidence within our retrospective chart review. Screening for ACC in MMR mutation carriers may not necessarily be recommended, however, in patients with LS, incidentally found adrenal tumors and symptoms and signs of adrenal hormone excess should be clinically evaluated and treated with an increased suspicion for malignancy.

Our study is a prospective, unselected series of patients with ACC. Patients who underwent a genetic evaluation for LS had

personal and/or family histories that were suggestive of LS. The prevalence of 3.2% may indeed be an underestimate. Future work to understand the overall prevalence of LS in ACC is needed. This can be accomplished via routine IHC screening of ACC tumors as has been carried out in colorectal cancer tumors and endometrial cancer tumors.^{28,29}

Genetic evaluation for LS should be considered in all patients with ACC and personal or family histories of LS-associated tumors including, but not limited to, young onset, multiple primary colorectal, endometrial, ovarian, gastric, small intestine, urinary tract, renal pelvis, pancreas, and bile duct cancers; glioblastoma multiforme; and sebaceous gland tumors. On the basis of the tumor IHC results, we suggest IHC for the MMR proteins as the primary molecular screening tool for ACC tumors.

Given the relatively few cases of ACC diagnosed annually, approximately 300 cases in the United States, it would be reasonable to consider IHC screening for all patients with ACC to help identify

patients with LS, similar to the recommendations for colorectal cancers, endometrial cancers, and sebaceous tumors.^{31,40,41} In addition, given the prevalence of ACC in this prospective series, ACC should be considered an LS-associated tumor and should be included in the spectrum of cancers in the Amsterdam II clinical diagnostic criteria for LS. The identification of MMR mutations and LS as the underlying cause of the development of ACC would have significant implications on cancer screening as well as aiding in the identification of other relatives at risk for this autosomal dominant cancer predisposition condition.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy,

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Appendix

Table A1. Full Clinical Data of the Five Probands With Adrenocortical Carcinoma and Lynch Syndrome

Proband	Size (cm)	Grade	Stage	Clinical Features			Biochemical Evaluations		
				Hypercortisolism	Androgen Secretion	Other	Hypercortisolism	Androgen Secretion	Other
1	14.5	High	III	No	No	HTN	NA	NA	Normokalemia
2	11	High	III	Possible	No	HTN	Elevated AM cortisol	No	Hypokalemia; normal aldosterone
3	7	Low	II	No	No	NA	No	NA	No
4	5	Low	I	No	No	NA	No	No	No
5	14	NA	III	Yes	Yes	NA	NA	NA	NA

Abbreviation: AM, morning; HTN, hypertension; NA, not assessed.

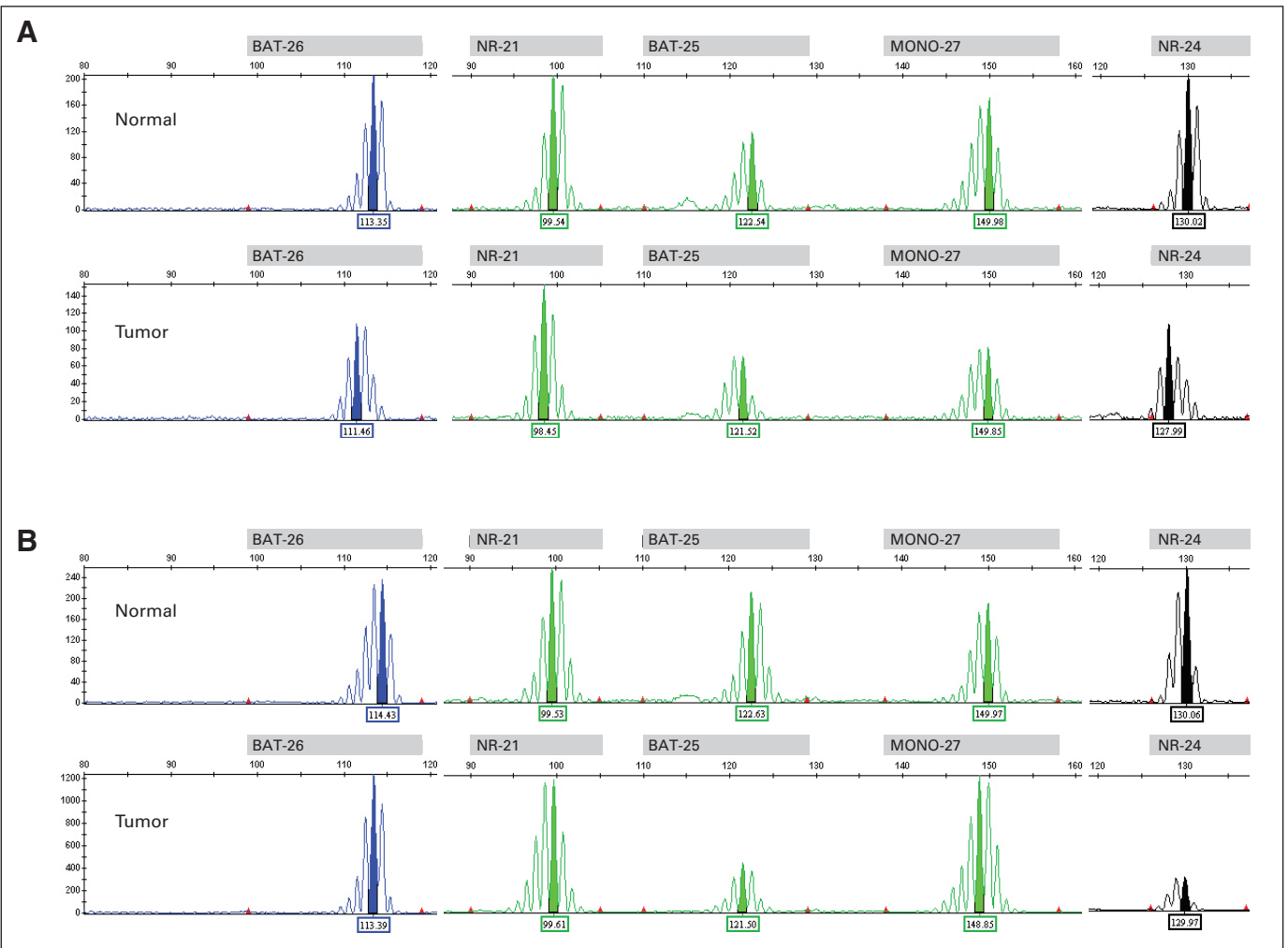


Fig A1. Microsatellite instability assay profiles of adrenocortical carcinomas of (A) Proband 1 and (B) Proband 2 demonstrating a microsatellite stable pattern in normal versus tumor.

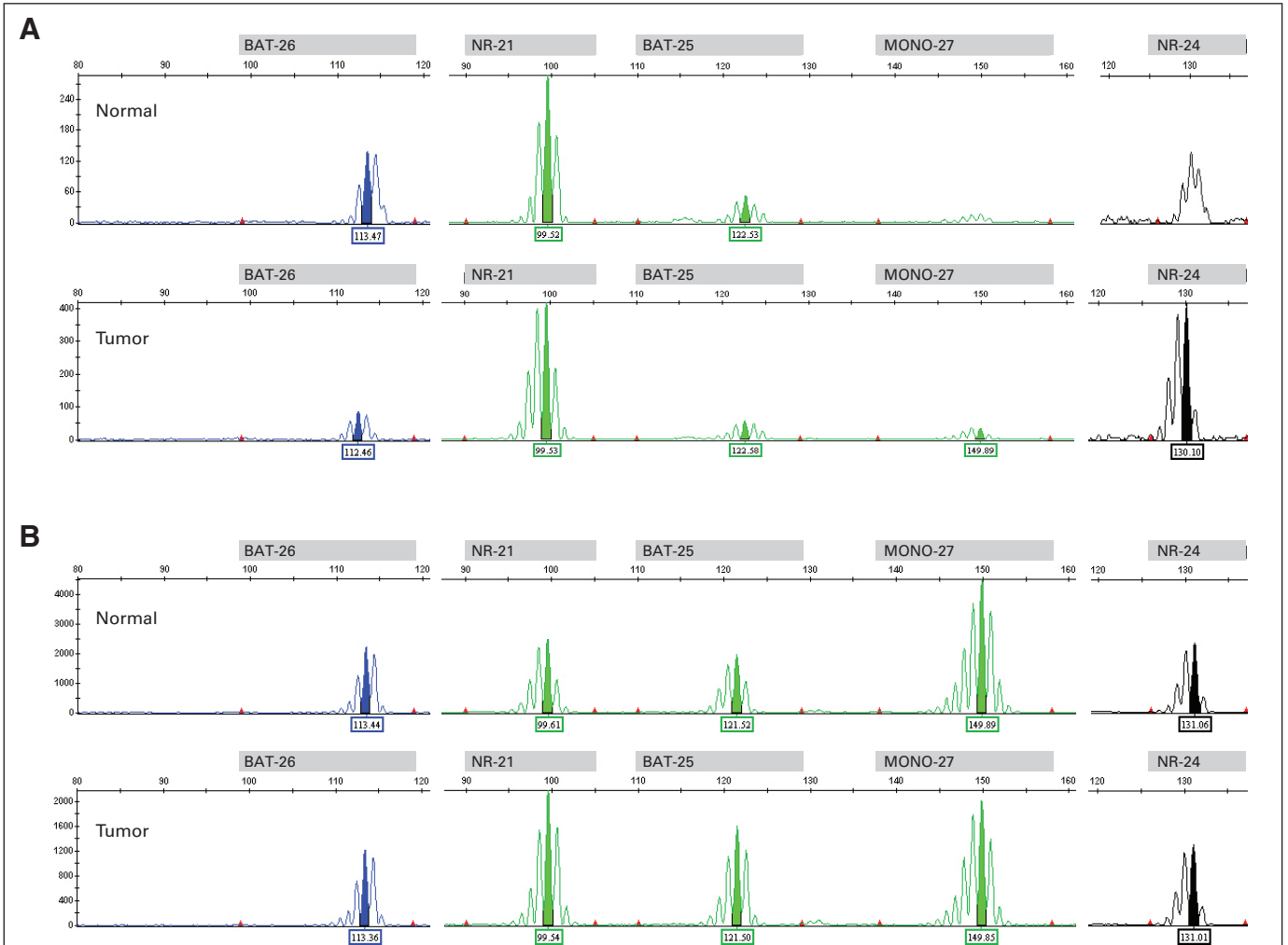


Fig A2. Microsatellite instability assay profiles of adrenocortical carcinomas of (A) Proband 4 and (B) Proband 3 demonstrating a microsatellite stable pattern in normal and tumor.