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Sporadic Adrenocorticotropic Hormone-Secreting Lung Carcinoids: Outcomes of Long-Term Clinical Follow-Up at a Single Center

ABSTRACT

Objective: Lung carcinoids represent a small portion of all lung tumors and about 1%-5% are associated with ectopic adrenocorticotropic hormone secretion, representing 1%-10% of Cushing's syndrome. They occur both sporadically and rarely in association with multiple endocrine neoplasia type 1. *MEN1* variations were detected in approximately 16% of sporadic cases with seldom reports about disease prognosis. This study aimed to identify *MEN1* variants in our cohort of sporadic lung carcinoids to associate with disease outcome following surgery.

Methods: Pathologically confirmed 5 lung carcinoid cases were retrospectively analyzed in terms of age, gender, imaging studies, clinicopathologic features, and long-term disease outcome. Genetic analysis was performed to detect copy number variations and point mutations in *MEN1*.

Results: Totally 1 female and 4 males with cushingoid features underwent thoracoscopic lobectomy for ectopic Cushing's syndrome. Histopathological examinations revealed 2 atypical (males) and 3 typical (1 female and 2 males) carcinoids. One underwent Wedge resection, the others underwent lobectomy; none of them had any post-operative complications. Median follow-up period was 11 years (range, 5-19); all patients were alive with no recurrence or metastases, up to date. Genetic analysis revealed a novel *MEN1* variant [c.1623G>T, p.(Gln541His)] in only 1 atypical carcinoid. Despite this variant, this case did not develop other components of MEN1 syndrome during long-term follow-up.

Conclusion: We detected the frequency of *MEN1* variation as 20%. Further studies are required to clarify the role of this variant.

Keywords: Ectopic ACTH secretion, lung carcinoids, MEN1 gene, prognosis

Introduction

Lung carcinoids occur both sporadically and rarely (5%) in association with multiple endocrine neoplasia type 1 (MEN1).[1] MEN1 is an inherited tumor syndrome associated with tumors of the endocrine glands, resulting from mutations of the tumor suppressor gene *MEN1* that encodes the protein menin. The most common tumor types observed are tumors ofparathyroid, pancreas, and pituitary origin. Other endocrine tumors associated with MEN1 are comprised of tumors of the adrenal cortex, neuroendocrine tumors (formerly known as carcinoid tumors), and rarely pheochromocytoma and tumors of other parts of the digestive tract. MEN1 syndrome is characterized by neoplasms of both the endocrine and the nervous systems with skin lesions. MEN1 phenotype shows variation among affected cases, even among the members of a single family genotype–phenotype correlations are hardly made.[2-4] According to the guidelines on MEN1 diagnosis, *MEN1* mutations lead to the onset of MEN1 syndrome with a nearly complete penetrance before the age of 50.[1] Furthermore, to date, all of the mutation-bearing individuals have been recorded to develop at least one of the MEN1-associated tumors during their lifetime. Moreover, should one carry a *MEN1* mutation, lifelong specific clinical surveillance is suggested, as more than 90% can develop one or more MEN1 components.[5]

Neuroendocrine tumors of the bronchopulmonary tracts occur most frequently with incidences of 1 per 100 000 individuals per year. Typical carcinoids (TC) and atypical carcinoids (AC) are the 2 major histological types of lung carcinoids, representing a small portion of all lung tumors (2% and 0.2%, respectively).[6] 1%-5% of these tumors secrete ectopic ACTH, causing Cushing's syndrome (CS) that account for 1%-10% of all CS cases.[7] The majority of these tumors are located in the perihilar region and patients often present with recurrent

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pneumonia, cough, hemoptysis, or chest pain. Ectopic ACTH syndrome (EAS) has rapid onset and accounts for the highest morbidity of this severe form of hypercortisolism.[8] Determining whether ACTH secretion is of pituitary or non-pituitary causes remain one of the most difficult challenges in clinical endocrinology, requiring all the skill of endocrinologists, radiologists, nuclear medicine physicians, and pathologists. These ectopic tumors are highly vascularized and they occur as polypoid masses of less than 3-4 cm in size.[9] Among them, around 1 cm carcinoids can occur, which are characterized by their slow growth and high vasculature that makes them prone to confusion with pulmonary vessels. For this reason, in 50% of cases, computed tomography (CT) and magnetic resonance imaging can fail to detect these tumors, which may appear several years following ECS diagnosis.[10] Radiological studies may sometimes disclose abnormalities with no functional significance or inflammatory conditions. Therefore, for the detection of pituitary and non-pituitary sources of ACTH, biochemical analysis should be performed prior to imaging modalities.[11,12] For lung carcinoids, surgery is the primary therapy for local disease, which is the most significant prognostic factor.[13,14] In this respect, the majority of the cases are localized, and for this, resection is the first-line treatment for better outcomes.[15] On the other hand, complete surgical resection is an option to treat bronchial carcinoids, which usually confers good prognosis, particularly in typical carcinoids.[11] Moreover, a variety of factors comprising intrabronchial obstruction, ability to metastasize, and overall production of vasoactive amines are taken into account in the prognosis of bronchial carcinoids.[16]

Germline *MEN1* mutations have also been reported in approximately 16% of sporadic lung carcinoids, while loss of heterozygosity of the region encompassing *MEN1* was estimated to occur in ~36% of the cases.[17,18] Due to the increasing incidence of bronchial carcinoids and their occurrence regardless of smoking history of the patients, genetic delineation of these tumors has been of increased interest.[19] Moreover, their occurrence as a component of the MEN1 syndrome made *MEN1* a candidate gene in the onset of sporadic bronchial carcinoids.[20,21] In this respect, a limited number of studies reported *MEN1* gene alterations in bronchial carcinoid cases along with the clinical implications.[22,23]

Therefore, our study aimed both to evaluate clinicopathologic data, surgery outcome, and prognosis for the long-term follow-up of sporadic bronchial carcinoids with ectopic CS referred to the endocrinology department of our university hospital and to perform mutational analysis of the candidate *MEN1* gene for its impact on the disease prognosis.

MAIN POINTS

- 1%-5% of lung carcinoids secrete ectopic ACTH causing Cushing's syndrome (CS) accounting for 1%-10% of all CS cases.
- Approximately 16% of lung carcinoid cases were found to exhibit *MEN1* gene alterations.
- Limited number of studies involving *MEN1* gene alterations and clinical implications on lung carcinoids are available.
- Our study aimed to evaluate long-term clinical outcomes of sporadic lung carcinoid cases and to determine the effect of *MEN1* gene variations on these cases.

Materials and Methods

Patients

For this study, the records of the patients, who were diagnosed and treated for CS caused by ectopic ACTH secretion at the endocrinology clinic of İstanbul University Medical Faculty between 2007 and 2011 and who were followed up regularly to date, were retrospectively examined. Patients, who were not given any other treatment at post-operative long-term follow-up, were accepted as in remission. In this respect, 5 cases (4 males and 1 female) with lung carcinoids (3 TC and 2 AC) that were post-operatively survived and regularly followed up to date were included in this study. The ectopic ACTH source was confirmed by a combination of dynamic non-invasive tests, imaging studies, and histopathological evaluations. The median age was 36 years (range: 27-48). At each follow-up visit, detailed clinical examination and biochemical data were obtained to assess disease control (such as plasma ACTH and cortisol at 08.00 AM), while imaging modalities were performed when necessary (using thorax High-resolution computed tomography (HRCT) and Positron Emission Tomography/Computed Tomography [PET-CT]). Hematoxylin and eosin-stained, and immunohistochemically stained sections of formaldehyde-fixed, paraffin-embedded tissue samples of each tumor were re-evaluated according to the latest World Health Organization (WHO) 2015 classification. The differential diagnosis between TC and AC was made with mitotic rate and necrosis status. Diagnosis of AC was made by a well-differentiated morphology between 2 and 10 mitoses per mm^2 and/or presence of focal necrosis.[7]

Genetic Analyses

Genetic material was obtained from the peripheral blood of the patients after obtaining their written informed consents in accordance with Istanbul University, Istanbul Faculty of Medicine, Clinical Ethics Committee (2021/1959). The work was conducted in accordance with Helsinki Declaration Principles. DNA was isolated using either the Qiagen Maxi kit (Qiagen, Inc., Hilden, Germany) or the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, Calif, USA). Large deletions and insertions in the *MEN1* gene were assessed utilizing multiplex ligation-dependent probe amplification (MLPA), for which commercially obtained reagents and probe-mixes were used according to the manufacturer's instructions (P244-AIP-MEN1-CDKN1B, MRC-Holland, the Netherlands). If initial screening with MLPA came negative, then the patients' *MEN1* gene was screened for mutations via Sanger sequencing. For sequencing, whole gene was polymerase chain reaction (PCR)-amplified, which comprised of the coding regions (exons), exon–intron boundaries, and the untranslated regions. The primers used in PCR and in Sanger sequencing of the amplicons are shown in Table 1. Sanger sequencing was performed by the service of Macrogen Korea, Incorporation.

Evaluation of the Genetic Findings

Coffalyser Net platform was used in MLPA analyses. Sequence analyses of the PCR amplicons were performed using the CLC Main Workbench 6.5 against the reference NM_000244.3 sequence, at our genetics laboratory. All available databases including Leiden Open Variation Database, gnomAD, Exome Variant Server, Mine Data Browser, UMD-MEN1, and The Human Gene Mutation Database (HGMD) database were screened to evaluate the frequency of the *MEN1* variants depicted in our cohort. In addition, literature search for the variants detected was performed with special attention to the

Table 1. Primer Sequences Used in *MEN1* Gene Amplification and Sanger Sequencing

Amplicon	Forward Primer Sequence (5′-3′)	Reverse Primer Sequence (5′-3′)	Amplicon Size (bp*)
1[a]	ACGCACAGCTCCCCTGCTT	CATTTTCCAGAAGGCACTGC	577
2	CCGAACCTCACAAGGCTTAC	TGGAACCTTAGCGGACCCT	647
3[b]	CCCACAGCAAGTCAAGTCTG	CTTTCCCCATGTTAAAGCACAG	660
4[c]	CCCAACACACAAAGTTCTCTTC	TGGTCCCTGTTGGTTCTGAC	405
5	ACGAGGGTGGTTGGAAACTG	TCAGCCAGCAGTCCTGTAGAC	402
6	CCCTAATCCCGTACATGCAG	CCTCCTCATTCTTGCTTTCTTC	399
7	GGCCAGAAAAGTCTGACAAGC	CCTCTGCTAAGGGGTGAGTAAG	298
8[d]	CTAGGGTTTGGGTAGAGGTGAG	CAGAGCAGGGTCCTGGAGTT	675
9[e]	AGCCAGAAGACAGAGGGAAG	TCACCTAGAGCCAGACCAAC	586
10[f]	GGACCTGTGCTCCTTGGGTT	TTCCAGAGTGAGAAGATGAAGG	580

[a]5′UTR; [b]encompasses exons 2, 3, and the intronic region in between; [c]encompasses exons 4, 5, and the intronic region in between; [d]contains 3′UTR along with exon 9; [e]3′UTR; [f]3′UTR.
bp, base pairs.

review on *MEN1* mutations detected in 2007-2015.[24] Moreover, the functional impact of the detected variants was evaluated utilizing in silico prediction tools including Sorting Tolerant From Intolerant release 63 (SIFT, http://sift.jcvi.org), Polyphen (http://genetics.bwh.harvard.edu/pph2), Combined Annotation Dependent Depletion (http://cadd.gs.washi ngton.edu), and Mutation Taster (http://www.mutationta ster.org). Due to the possibility of a population-specific variant, an exome variant database that consist of 1182 individuals from Turkey, who were diagnosed with different conditions, were used as a population control against the variants detected in our cohort.

Results

All the cases presented with signs and symptoms of hypercortisolemia with Cushingoid phenotype. The median follow-up of the patients was 11 years (range: 5-19). Clinical data and general features of the cases are summarized in Table 2. Basal hormonal evaluations, radiologic and radioisotope imaging, treatment modalities, and prognosis up to date are shown in Table 3. One patient (case 2), who was not detected to have ectopic ACTH focus, underwent bilateral adrenalectomy, because of severe comorbidities of hypercortisolemia. Another case (case 3) underwent maxillary sinus surgery due to false octreotide-scintigraphy positivity, but the material was diagnosed as chronic sinusitis. In these cases, lung carcinoids could be visualized a year after bilateral adrenalectomy and 4 months after sinusitis surgery.

All lobectomy materials showed positive staining for ACTH, chromogranin and synaptophysin but negative for thyroid transcription factor 1. Except for case 2 (a TC case), Ki-67 index ranged between 1% and 2%. Mitotic count changed between 0 and 3/2 mm^2 and necrosis was not detected in any of the samples. Therefore, 3 cases were diagnosed as typical and 2 cases were diagnosed as atypical lung carcinoid (Table 4).

Genetic evaluations of the *MEN1* gene did not reveal any large deletions or insertions by MLPA analyses, while 1 novel *MEN1* variant [c.1623G>T, p.(Gln541His)] was detected only in case 5 by sequence analysis (Figure 1). Currently, this patient neither develop any components specific to MEN1 syndrome nor he developed metastasis for lung carcinoid during 8 years of follow-up. Thus, the frequency of *MEN1* gene variation was detected as 20% in our cohort. Cushingoid phenotypes resolved in all cases and neither recurrence nor metastasis was seen in the long-term follow-up period in these carcinoids (Table 4).

Discussion

The role of *MEN1* gene mutations in the pathogenesis of lung carcinoids was first described by Debelenko et al.[20] but the precise genetic abnormality in lung carcinoids prior to the identification of the *MEN1* gene was not possible. Thereafter, the lung neuroendocrine tumor genetics has been rarely studied in regards to the variations in the *MEN1* gene, while gene expression was found to be heterogeneous in this group of patients.[20,21,25,26]

Table 2. General Features of the Cases with Sporadic Lung Carcinoids

Case Number	1	2	3	4	5
Age (year)/sex	30/M	27/M	45/F	30/M	48/M
Duration of complaints (months)	24	12	4	12	12
Abdominal weight gain	+	+	+	+	+
Stria/bruising	+/+	+/+	+/+	+/+	+/+
Proximal myopathy	+	+	+	+	+
Hypertension (>140/90 mm Hg)	150/90	150/100	155/80	180/100	160/90
Hyperglycemia (>126 mg/dL)	–	–	150	142	–
Hypokalemia (<3.5 mEq/L)	3.8	2.8	2.3	3.2	2.5
Hyperlipidemia	+	+	+	–	+
Osteoporosis/fracture	+/Ribs	+/Spine and ribs	–/–	+/Spine	+/Spine and ribs

+, yes; –, no; F, female; M, male.

Table 3. Evaluation of Patients in Terms of Diagnosis and Treatment of Hypercortisolemia

Case Number	1	2	3	4	5
Basal cortisol (range: 12-24 µg/dL)	32.1	37.3	32.4	34.0	33.8
ACTH (range: 0-46 pg/mL)	216	46.0	56.0	129	36.6
LDDST (2 mg) basal cortisol (normal <1.8 µg/dL)	30.0	36.8	19.2	25.0	23.2
Thorax HRCT (mm)	Nodule on right lobe inferior segment	No pathological finding	Non-specific lesion in the left lobe lingula segment	No pathological finding	Non-specific lesion in the upper posterior segment of the right lobe
Abdomen MRI	–	Negative	Negative	Negative	Negative
CRH+IPSS	Ectopic source	Non diagnostic	Non diagnostic	Ectopic source	Ectopic source
Octreotide-scintigraphy (OCT-SCT)	Positive uptake at nodule	Negative uptake	A solitary uptake in the right maxillary sinus	–	–
PET-CT	–	Negative	–	Lesion in the right lung hilar region	–
Surgical treatment	Right lobectomy	Bilateral adrenalectomy	Resection of maxillary sinus material*	Right lobectomy	Right wedge resection
Course following surgery	–	Lung tumor detected in thorax HRCT a year later.	Lung tumor detected in second OCT-SCT 4 months later.	–	–

–, could not be applied; * The non-specific lung lesion in the left lobe became apparent in the second OCT-SCT after the resection of the inflammatory tissue in the maxillary sinus. Subsequently, the patient underwent lobectomy.
LDDST, low-dose dexamethasone suppression test; CRH+IPSS, inferior petrosal sinus sampling with corticotropin releasing hormone; ACTH, adrenocorticotropic hormone; MRI, magnetic resonance imaging.

Recently, 148 lung neuroendocrine tumors (LNETs), composed of 53 TCs and 35 ACs (defined according to WHO classification categories), were subjected to next-generation sequencing (NGS). The results depicted *MEN1* as prone to having the most frequent variations in carcinoids (11.4%); particularly in ACs (20%). Moreover, mutations in this gene were associated with poor prognosis in AC, though they were exclusive to carcinoids.[23] In another study, the frequencies of allelic deletions and mutations of the *MEN1* gene in lung carcinoids were reported as 20% and 18%, respectively.[21] Among their patients, 1 AC was found to be a carrier of *MEN1* germline splice donor site mutation (5178–9 G→A), despite having none of the MEN1 components and any family history of the disease.[21] Similarly, our study determined the *MEN1* variation frequency as 20% by detecting a novel germline *MEN1* variant in a case of sporadic AC, which seems to be compatible with the above study. In addition to the above studies, Swarts et al[26] reported a significant relationship between *MEN1* gene deregulation and clinical aggressiveness in lung carcinoids. Moreover, ACs have been described as aggressive malignancies with the need to perform radical surgical resection and they have a guarded prognosis having high metastasis and recurrence

Table 4. Histopathological Characteristics and Latest Outcomes

Case Number	1	2	3	4	5
Type of lung surgery	Lobectomy	Lobectomy	Lobectomy	Lobectomy	Wedge resection
Pathological size of tumor (mm)	16	10	8	10 and 0.5	8
Mitotic count (per 2 mm^2)	1	0	1	3	3
Necrosis	Not detected	Not detected	Not detected	Not detected	Not detected
Number of lymph node metastasis	0/5	1/5	0/8	1/10	Lymph node analysis was not possible**
Ki67 index (%)	2	Could not re-count*	<1	<1	1
Histotype of carcinoids	TC	TC	TC	AC	AC
Total follow-up time (years)	12	16	11	5	8
Recurrence and/or metastasis	None	None	None	None	None

*Because the preparation is too old; **Due to Wedge resection.
TC, typical carcinoid; AC, atypical carcinoid.

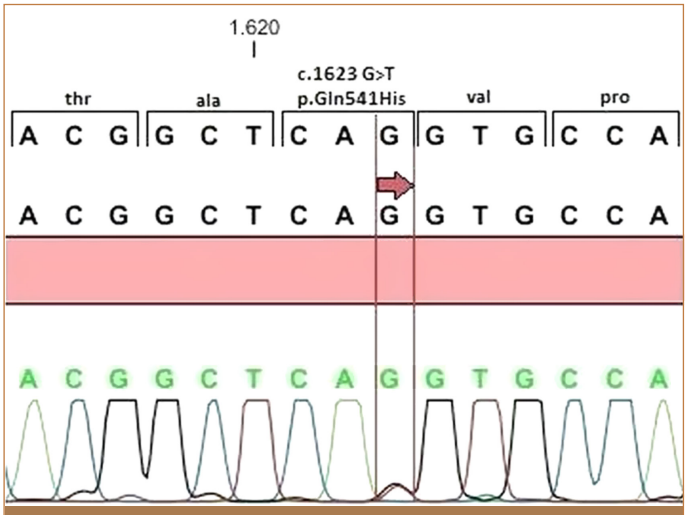

Figure 1. Chromatogram belonging to the heterozygous novel *MEN1* c.1623G>T, p.(Gln541His) variation is depicted.

tendencies.[21] It has been reported that the recurrence rate of ACs in median 3 years is significantly more likely than that of TCs.[27]

In our study, *MEN1* gene analysis revealed a novel germline variant [p.(Gln541His)] in only 1 AC. In silico evaluations predicted this variant as benign. None of our cases, including case 5 with *MEN1* variant, had local recurrence and/or distant metastases. Since this variant was absent from all available databases and the exome variant database of 1182 individuals from Turkey, it is attributed as novel that needs to be screened in larger cohorts. In our case, we are still following up this patient for any future prognostic difference possibility.

Previously, it has been reported that the best 5-year survival rate after surgery is 76.6% for bronchopulmonary carcinoid, and in these, invasive growth or metastatic spread is reported to be 27.2%.[25] Whereas in recent years, surgery was chosen to treat both TC and AC that showed 5- and 10-year survival rate greater than 90% for TC and a 5-year survival rate ranging from 56% to 87% in AC.[8,28] In our study, post-operative survival rate was 100% in 5 years and over in 2 AC and they did not develop any metastasis. The other patients (3 TCs) are still in remission proceeding lobectomy (in 16 years of follow-up) and the survival rate was 100% in over 10 years in these cases. On the basis of our result, the AC displaying *MEN1* gene variant is not characterized by more aggressive prognosis compared with the other carcinoids (3 TCs and 1 AC) without *MEN1* gene alterations. We also observed that clinical phenotype was independent of the presence of *MEN1* variant in case 5. Moreover, no other *MEN1* variations were detected in other carcinoids, which suggests the presence of other candidate genes yet to be associated with lung carcinoid onset.

Since our study comprised of long-term clinical follow-up of rare sporadic ACTH-secreting lung carcinoids at a single center, our sample size formed the limitation of our study. However, we presented detailed long-term clinical findings of these pathologically confirmed cases and detected a novel genetic variant in the candidate gene *MEN1*, which can be replicated in larger cohorts to delineate its role in disease prognosis.

Conclusion

This study highlights the importance of *MEN1* screening and long-term follow-up of the patients in order to make new phenotype–genotype correlations. The novel *MEN1* p.(Gln541His) variant could be related to well-differentiated lung carcinoids. Since the long-term follow-up of this patient did not yield another MEN1 component, this variant might provide information on good prognosis. For a definitive conclusion, functional impacts of these variants need to be assessed. Moreover to complement histology in better diagnostic definition and prognostic stratification of lung carcinoids, molecular profiling may be of use. In order to delineate the role of *MEN1* variations in the tumorigenesis of lung carcinoids, larger cohorts need to be screened.

Ethics Committee Approval: The study was approved by the Istanbul University, Istanbul Faculty of Medicine, Clinical Ethics Committee (2021/1959).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.Y.; Design – S.Y., F.N.T.; Supervision – S.Y.; Funding – S.Y., F.N.T.; Materials – S.Y., F.N.T., Ö.S.S., E.S.; Data Collection and/or Processing – S.Y., F.N.T., Ö.S.S., E.S.; Analysis and/or Interpretation – S.Y., F.N.T.; Literature Search – S.Y., F.N.T., Ö.S.S.; Writing Manuscript – S.Y., F.N.T., Ö.S.S.; Critical Review – S.Y., F.N.T.

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Declaration of Interests: The authors declare that they have no competing interest.

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