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Clinical and Genetic Features of Patients with Multiple Endocrine Tumors Who Have neither Family History nor *MEN1* Germline Mutations

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Multiple endocrine neoplasia type 1 (MEN1) is an hereditary tumor syndrome that involves specific endocrine organs such as parathyroids, anterior pituitary gland, and endocrine pancreas. The responsible gene for this syndrome, *MEN1*, has been isolated and that enabled genetic diagnosis for patients with endocrine tumors and early detection of asymptomatic gene carriers in affected families. Nevertheless, there are a considerable number of patients with MEN1 who have neither family history nor germline *MEN1* mutations. In this article, clinical features of such patients are described. Among 53 MEN1 patients we have seen during the last 20 yr, five patients who did not have either *MEN1* germline mutation or family history were categorized as MEN1 phenocopy. During the same period, we have also experienced three patients who had primary hyperparathyroidism and adrenocortical tumor but had no apparent family history of endocrine tumors. These patients were considered as MEN1 phenocopy variants and included in the study. The mean age of MEN1 phenocopy patients (including variants) at diagnosis was 48 yr, which was not significantly different from that of probands of familial MEN1 (46 yr) who carry heterozygous *MEN1* gene mutations. In the majority of MEN1 phenocopy patients, primary hyperparathyroidism was due to a single parathyroid adenoma. In contrast to a previous report, we found that MEN1 phenocopy patients are not necessarily older than probands of familial MEN1. Phenotypic expression of such patients is variable, thus differentiation of familial MEN1 and MEN1 phenocopy cannot be made based on age and clinical phenotype alone.

Key Words: Multiple endocrine neoplasia type 1; phenocopy; incidentaloma.

Introduction

Multiple endocrine neoplasia type 1 (MEN1, OMIM #131100) is an hereditary syndrome characterized by predisposition to hyperplastic and neoplastic disorders arising predominantly from endocrine organs such as parathyroids, anterior pituitary gland, and endocrine pancreas *(1)*. The locus of the responsible gene for this syndrome was assigned to the long arm of chromosome 11 in 1988 *(2)*, and the gene itself was isolated in 1997 *(3)*. The reported *MEN1* gene contained 10 exons, and encoded a 610-amino-acid protein, designated menin. Heterozygous germline mutations of the *MEN1* gene have been identified in over 90% of patients with MEN1 *(4)*, and more than 300 germline *MEN1* gene mutations have been reported to date *(1)*. Mutations distribute widely in the coding region and exon–intron boundaries of the gene, and no apparent genotype–phenotype correlation has been observed *(5)*, although some investigators suggested weak correlation *(6)*. Identification of the *MEN1* gene rapidly changed the standard procedure of screening for family members from a combination of biochemical measurement and radioimaging to the more reliable genetic test.

Subjects with MEN1 without either family history or *MEN1* germline mutations have occasionally been observed. Hai et al. categorized such patients as MEN1 phenocopy and reported that MEN1 phenocopy patients tend to be older than patients with familial MEN1 and the most common clinical feature seen in MEN1 phenocopy patients is a combination of parathyroid adenoma and growth hormone (GH) secreting pituitary adenoma *(7)*. Although the precise molecular mechanisms of tumorigenesis in those patients are not known, coincidental occurrence of relatively common endocrine tumors is likely the reason *(7,8)*. Differential diagnosis of "true" MEN1 and such patients is important for appropriate follow-up of patients and also for consideration of family screening.

During the last 20 yr, we have encountered five patients with MEN1 who had neither *MEN1* germline mutation nor family history of MEN1. We have also experienced three patients with concurrent endocrine tumors who did not meet the criteria of MEN1. Here we report characterization of clinical and genetic features of such patients.

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Table 1
Clinical Features of Patients with Multiple Endocrine Tumors

Patient	Sex	Age at diagnosis	Age at present	PHPT[a]	Pituitary tumor[b] (size, mm)	Adrenal tumor[c] (size, mm)	Other neoplastic diseases
#1	F	43	46	+	PRL (8 × 7 × 7)	COR* (45 × 30 × 25)	chondrosarcoma,* leukemia
#2	F	31	48	+	NF* (40 × 32 × 27)		
#3	F	47	63	+	GH* (14 × 13)		
#4	F	37	44	+	GH? (5 × 4 × 4)		paraganglioma*
#5	F	58	77	+	NF* (28 × 23 × 17)		islet cell tumor,*[c] ovarian tumor
#6	F	62	75	+		COR* (50 × 40 × 20)	colon polyps*
#7	F	56	63	+		NF* (35 × 25 × 20)	
#8	F	55	62	+		ALD (8 × 7 × 7)	breast cancer*

[a]PHPT, primary hyperparathyroidism.
[b]Oversecreted hormone from each tumor is indicated. COR, cortisol; ALD, aldosterone; NF, non-functioning.
[c]Produces ACTH and immunologically positive for ACTH and β-endorphin.
*Operated.

Table 2
Endocrine Function of Patients with Multiple Endocrine Tumors[a]

Patient	Calcium (mg/dL)	Intact PTH (pg/mL)	Pathology of parathyroids[b]	GH (ng/mL)	IGF-1 (ng/mL)	PRL (ng/mL)	ACTH (pg/mL)	Cortisol (μg/dL)	PRA (ng/mL/h)	Aldosterone (pg/mL)
#1	11.7	170	H (4)	1.4	206	151	6.8/2.4/7.7	17.1/16.8/17.4	2.1	235[c]
#2	12.7	163	A (1)	NA	NA	30.2	6.0/–/–	1.9/–/–	NA	NA
#3	11.0	63	H (1)	13.7	NA	14.3	12.3/19.6/12.3	15.4/14.7/1.5	NA	NA
#4	10.1	133	A (1)	12.1	217	6.9	20.8/15.2/3.2	18.6/11.7/3.8	1.9	129.9
#5	12.6	1.63(PTH-C)	A (1)	1.4	NA	24.3	93.1/99.0/75.5	39.4/45.3/33.5	1.1	74.1
#6	10.2	357	A (2)	0.4	NA	3.8	<5/<5/<5	15.1/14.4/14.5	1.3	74.8
#7	10.8	160	A (1)	4.2	246	4.1	10/7/<5	14.2/4.7/3.5	0.9	47.3
#8	12.1	460	A (1)+H (1)	0.66	136	14.0	20/18/<5	8.5/4.6/2.0	4.2	223

[a]Normal range of each measurement is (albumin-adjusted) calcium, 8.7–9.9 mg/dL; intact PTH, 14–66 pg/mL; PTH-C, <0.5 ng/mL; GH, <5 ng/mL; IGF-1, 121–436 ng/mL (adult female); PRL, 1.4–14.6 ng/mL (adult female); ACTH, 9–60 pg/mL; cortisol, 5–15 μg/dL; plasma renin activity (PRA), 0.2–3.1 ng/mL/h; aldosterone, 50–180 pg/mL. Three values of ACTH and cortisol are those obtained at 0700 h/1400 h/2300 h on the same day. NA, data not available.
[b]H and A indicate hyperplasia and adenoma, respectively. Number of affected glands is indicated in parenthesis.
[c]Normalized after adrenalectomy.

Results

During the last 20 yr, we have had 53 patients with MEN1—44 patients with family history, and 9 patients without it. Among the latter nine patients, genetic analysis of the *MEN1* gene was performed in six patients and a germline mutation was found in one patient. Five patients who had no detectable *MEN1* germline mutations were categorized as MEN1 phenocopy. We could not obtain sufficient information on family history of the remaining three patients because those patients were dead or dropped out of follow-up program. During the same period, we also have had three patients with concomitance of primary hyperparathyroidism and an adrenocortical tumor. None of the three had family history of endocrine tumors and they were included in the present study.

Phenotype and laboratory data of eight patients are summarized in Tables 1 and 2. All were female and the age at diagnosis ranged from 31 to 62 yr. All eight patients had primary hyperparathyroidism. Pathology of surgically removed parathyroid glands was variable from a single adenoma to the four-gland hyperplasia. In patient #8, one gland was pathologically diagnosed as adenoma and the other gland as hyperplasia.

Patients #1–5 had primary hyperparathyroidism and pituitary adenoma, thus met the practical definition of MEN1 *(9)*. In patient #4, the serum level of growth hormone was high but the level of IGF-1 remained within the normal range (Table 2). She had no acromegalic features. The levels of GH and IGF-1, and the size of the pituitary tumor, have been evaluated periodically for over 7 yr without progression.

Patient #5 had an islet cell tumor, which was immunologically positive for ACTH and β-endorphin, while a surgically resected pituitary tumor was negative for ACTH. Thus, Cushing's disease in this patient was considered owing to ectopic production of ACTH by the islet cell tumor, which metastasized to the liver. Surgical enucleation of metastatic tumors had been performed several times, and she has recently been treated with octreotide and periodic transcatheter arterial embolization. Plasma level of ACTH is, however, gradually increasing and recent values exceed 1000 pg/mL.

Patients #6–8, who did not meet the criteria of MEN1, had primary hyperparathyroidism and an adrenal tumor; patient #6 had a cortisol-producing adenoma and patient #7 had a non-functioning adenoma. In patient #8, the initial adrenal function test revealed elevated levels of aldosterone without suppression of plasma renin activity (Table 2), but later measurements showed suppression of plasma renin activity (0–0.2 ng/mL/h) accompanied by elevated levels of serum aldosterone (218.4–235.8 pg/mL). Although the size of the adrenal tumor had not changed for 7 yr, development of primary aldosteronism was anticipated. The patient has been observed under medication with spironolactone and blood pressure and serum potassium levels have been kept within the normal range throughout the follow-up period. Detail of patient #1, who manifested typical features of MEN1 as well as bone tumor and leukemia, will be reported elsewhere *(10)*.

Primary hyperparathyroidism was the initial disorder in patients #7 and #8. In patients #1–6, primary hyperparathyroidism was found during biochemical screening after diagnosis of the pituitary or adrenal tumors. Adrenal tumors in patients #7 and #8 were found during work up for MEN1. Symptoms related to growth hormone or cortisol excess were the initial manifestation in patients #3, #5, and #6, and secondary amenorrhea was the initial symptom in patient #2. Except for patients #1 and #8, only enlarged parathyroid glands were surgically removed, and no patients experienced recurrence of hyperparathyroidism 3–18 yr after operation.

Possible germline mutations in the *MEN1* gene were analyzed in all eight patients after a written informed consent had been obtained. This analysis was reviewed and approved by the Ethics Review Committee for Human Gene Analysis Research of Shinshu University. High-molecular-weight DNA was isolated from peripheral mononuclear cells and entire coding regions and exon–intron boundaries of the *MEN1* gene were amplified and directly sequenced as previously described *(3,11)*. No patients harbored germline *MEN1* gene mutations in coding regions or exon–intron boundaries. Heterozygosity of two intragenic polymorphisms in the *MEN1* gene in each patient is summarized in Table 3 *(4,7)*. At least one of two polymorphisms was heterozygous except in patients #2 and #6, indicating the possibility of hemizygosity of the *MEN1* gene, which have been reported in two families *(12,13)*, was unlikely.

Table 3
Benign Polymorphisms in the *MEN1* Gene

Patient	D418D	A541T
#1	GAC/GAT	GCA/ACA
#2	GAC/GAC	ACA/ACA
#3	GAC/GAT	GCA/ACA
#4	GAC/GAT	ACA/ACA
#5	GAC/GAT	GCA/ACA
#6	GAC/GAC	ACA/ACA
#7	GAC/GAC	GCA/ACA
#8	GAC/GAC	GCA/ACA

Discussion

In this article, we described eight patients with two or more endocrine tumors but no apparent family history of endocrine tumors or *MEN1* germline mutation. The mean age of those patients at diagnosis was 48 yr and that was not significantly different from that of probands of familial MEN1 seen in our department (46 yr) *(14)*. In a previous report, the patients who met the criteria of MEN1 but lack both family history and *MEN1* germline mutation tended to be older than familial MEN1 patients *(7)*. It was also mentioned that those patients were predominantly female, and a combination of primary hyperparathyroidism and GH-producing pituitary tumor was most frequently observed. In accordance with such observations, all cases reported herein were female, which presumably reflects higher incidence of sporadic primary hyperparathyroidism in females. Indeed, pathological finding of the parathyroid glands in five of eight patients was a single adenoma, that is uncommon in familial MEN1. The MEN1 phenocopy patients reported herein were younger than those of the previous report and clinical features were more variable. Among our eight patients, a combination of hyperparathyroidism and acromegaly was seen only in one case (patient #3), and she was 47 yr old when diagnosis of both diseases were confirmed. The reason for the difference the between the previous report and our experience is not clear. Our institution has continued systematic regional survey of MEN1 for 20 yr *(14)*, whereas in the report by Hai et al., patients have been seen in several hospitals *(7)*. Clinically silent pituitary tumors such as prolactinoma and non-functioning adenoma could be discovered only through a systematic survey *(14)*. On the other hand, under the regular clinical work, GH-producing adenoma will be more frequently recognized due to clinical symptoms.

We have experienced three cases with hyperparathyroidism and the adrenocortical tumor. One patient had a cortisol-producing tumor and others had an incidentally identified non-functioning tumor. Interestingly, one patient (#8) developed primary aldosteronism during follow-up of a non-functioning tumor. Recently, adrenal incidentaloma is more

Table 4
Patients with MEN1 Who Manifest Only Hyperparathyroidism and Adrenal Tumor

Patient	Sex	Age at diagnosis	Cues to diagnosis	Family history	*MENI* mutation	PHPT[a]	Adrenal tumor[b]	Other lesions (follow-up period)
#11	M	39	hypertension	+	Q166X	+[c]	ALD[c]	none (5 yr)
#12	M	54	family screening	+	621del9	+[c]	NF	none (11 yr)
#13	F	26	obesity	+	1473del5	+[c]	COR[c]	none (6 yr)
#14	M	31	family screening	+	1657insC	+	NF	none (6 yr)

[a]PHPT, primary hyperparathyroidism.
[b]Oversecreted hormone from each tumor is indicated. ALD, aldosterone; COR, cortisol; NF, non-functioning.
[c]Operated.

frequently detected largely due to improvement in the radioimaging technique, so that the most recent figure for its prevalence is 1–4% *(15)*. This frequency is much higher than that of acromegaly, of which estimated prevalence is less than 1 per 10^5 *(16)*. Although the adrenal tumor is not considered as a classical triad of MEN1, we have experienced four cases with genetically confirmed MEN1 who have manifested only hyperparathyroidism and adrenal adenoma (Table 4). Two patients (#11 and #13) had no family history at the time of diagnosis, but subsequent screening of their families identified other affected members. Interestingly, other MEN1-related tumors (namely, pituitary tumor and enteropancreas tumor) have not occurred in these patients during the follow-up for 5–11 yr. Patient #12, whose hyperparathyroidism and non-functioning adrenal tumor were found during family screening, has not manifested any other diseases until now at the age of 65. These findings suggest that patients with hyperparathyroidism and adrenal incidentaloma should be considered as having a high possibility of MEN1, and further examination including genetic tests for those patients are warranted *(9)*.

The rate of sporadic cases within all MEN1 patients considerably differs among reports; that is probably attributable to thoroughness of family screening. In a series at the National Institute of Health (NIH), 23 sporadic cases with MEN1 have been reported through a surveillance of 58 families *(17)*. We experienced 6 sporadic cases in 19 families, which is similar to that the prevalence in NIH: a previous Japanese report showed a higher fraction of sporadic cases *(18)*. It is possible that some of our patients reported herein have family members with unrecognized endocrine tumors, but the high fraction of familial cases among all MEN1 patients in our experience indicate that our survey procedure is appropriate and sufficient.

In conclusion, we reported cases with multiple endocrine tumors without apparent family history and *MEN1* germline mutation. Our experience indicates that MEN1 phenocopy patients are not necessarily older than familial MEN1 patients. Phenotypic expression of such patients is variable, so that differentiation of familial MEN1 and MEN1 phenocopy cannot be made based on age and clinical phenotype alone. Considering that the pathology of parathyroid tumors in most of our patients was a single adenoma and that none of them experienced recurrence of hyperparathyroidism, occurrence of multiple tumors in those patients was likely incidental. However, as the molecular background of occurrence of multiple tumors is currently unknown, it is not certain if disorders seen in each patient are inheritable to the next generation. Indeed, clinical features of some patients, namely, patients #1 and #5, were indistinguishable from that of genetically confirmed MEN1 patients. Patient #1 manifested four-gland parathyroid hyperplasia, prolactinoma, and adrenal tumor. Patient #5 developed hyperparathyroidism, pituitary tumor, and pancreatic islet cell tumor metastasized to the liver.

Because *MEN1* germline mutation cannot be detected in about 10% of MEN1 patients *(4)*, it is possible that these patients acquired *de novo* mutation in the *MEN1* gene that is undetectable by current method. Alternatively, mutations in another gene or genes in these patients may have produced a phenotype identical to that seen in patients with mutations in *MEN1* gene. In any case, periodic screening of endocrine and other disorders for those patients and family members seems rational.

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References

1. Gagel, R. F. and Marx, S. J. (2002). In: *Textbook of endocrinology: multiple endocrine neoplasia.* Larsen, P. R., Kronenberg, H. M., Melmed, S., and Polonsky, K. (eds.). W. B. Saunders: Philadelphia, PA.
2 Larsson, C., Skogseid, B., Oberg, K., Nakamura, Y., and Nordenskjold, M. (1988). *Nature* **332,** 86–87.
3 Chandrasekharappa, S. C., Guru, S. C., Manickam, P., et al. (1997). *Science* **276,** 404–407.
4 Agarwal, S. K., Kester, M. B., Debelenko, L. V., et al. (1997). *Hum. Molec. Genet.* **6,** 1169–1175.
5 Wautot, V., Vercherat, C., Lespinasse, J., et al. (2002). *Hum. Mut.* **20,** 35–47.
6 Kouvaraki, M. A., Lee, J. E., Shapiro, S. E., et al. (2002). *Arch. Surg.* **137,** 641–647.

7 Hai, N., Aoki, N., Shimatsu, A., Mori, T., and Kosugi, S. (2000). *Clin. Endocrinol.* **52,** 509–518.
8 Burgess, J. R., Nord, B., David, R., et al. (2000). *Clin. Endocrinol.* **53,** 205–211.
9 Brandi, M. L., Gagel, R. F., Angeli, A., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 5658–5671.
10 Nishimura, Y., Yamashita, K., Yumita, W., et al. (2004). *Endocr. J.,* in press.
11 Sakurai, A., Shirahama, S., Fujimori, M., et al. (1998). *J. Hum. Genet.* **43,** 199–201.
12 Kishi, M., Tsukada, T., Shimizu, S., et al. (1998). *Jpn. J. Cancer Res.* **89,** 1–5.
13 Cavaco, B. M., Domingues, R., Bacelar, M. C., et al. (2002). *Clin. Endocrinol.* **56,** 465–473.
14 Katai, M., Sakurai, A., Itakura, Y., et al. (1997). *Endocr. J.* **44,** 841–845.
15 Bertherat, J., Mosnier-Pudar, H., and Bertagna, X. (2002). *Curr. Opin. Oncol.* **14,** 58–63.
16 Bengtsson, B. A., Eden, S., Ernest, I., Oden, A., and Sjogren, A. (1988). *Acta Med. Scand.* **223,** 327–335.
17 Marx, S., Spiegel, A. M., Skarulis, M. C., Doppman, J. L., Collins, F. S., and Liotta, L. A. (1998). *Ann. Intern. Med.* **129,** 484–494.
18. Yoshimoto, K. and Saito, S. (1991). *Folia Endocrinol. Japon.* **67,** 764–774.