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Genotype-phenotype analysis and long-term clinical outcome of MEN1-related pancreatic neuroendocrine tumor

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Genotype-phenotype analysis and long-term clinical outcome of MEN1-related pancreatic neuroendocrine tumor

Directed by Professor Chang Moo Kang

The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Juwan Kim

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December 2022

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ABSTRACT

Genotype-phenotype analysis and long-term clinical outcome of MEN1-related pancreatic neuroendocrine tumor

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(Directed by Professor Chang Moo Kang)

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a rare, autosomal dominant disease. Neuroendocrine tumor of the pancreas (PNET) is the leading cause of death in patients with MEN1. As pancreatic tumors are found in most patients with MEN1 during their lifetime, predicting the progression of PNET is important. Relatively few studies have been carried out on MEN1-related PNET in Asian countries; therefore, we summarized and reported the short- and long-term outcomes in patients with MEN1 after pancreatic resection. We also analyzed the clinical characteristics of patients with MEN1 according to the genotype and long-term oncologic outcomes of MEN1-related PNET.

Materials and Methods

A total of 71 patients diagnosed with MEN1 at Severance Hospital in Seoul, Korea, from January 2003 to September 2022 were retrospectively analyzed. Patients diagnosed herein were analyzed for mutations in *MEN1* using direct or next-generation sequencing (NGS); additionally, the mutation type and location were determined. PNET with malignant transformation was defined as lymph node or systemic metastasis of PNET. PNET progression in the observation group was detected through imaging and was defined as an increase in the number or size of the tumors. PNET recurrence after surgery was confirmed using imaging.

Results

Among the 71 patients with MEN1, 50 (70.4%) were diagnosed with PNET and twenty

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patients underwent pancreatic resection. During the follow-up period, the median long-term progression-free survival of the observation group was 4.5 years [95% confidence interval (CI): 2.5–6.5]. The median long-term recurrence-free survival of the surgery group was 6.0 years (95% CI: 0.6–11.4). Among the 43 families with MEN1, 10 families had mutations in exon 2, which is the most common mutation site in patients with MEN1. Six patients with MEN1 without mutations in the *MEN1* gene showed a significant difference in the penetrance of PNET compared to patients with confirmed mutations (16.7% vs. 70.4%, p=0.015). Patients with mutations in exon 2 and patients with truncating mutations in exon 2 showed significant differences in the age-related penetrance of PNET (p=0.028 and p=0.014, respectively). Families of MEN1 patients who had more than two family members with MEN1-related PNET were 9. When we investigated the clinical characteristics of PNET in MEN1 patients, the age at diagnosis as PNET was relatively similar among family.

Conclusions

Regarding PNET in patients with MEN1, as the tumor occurs in multiple locations, it is necessary to preserve the pancreatic parenchyma to improve the quality of life of the patient after surgery. Clinical manifestations may differ depending on the genetic mutation in MEN1 patients. A more individualized and detailed follow-up strategy may be required for young patients with MEN1 with mutations in exon 2 and truncating mutations. In addition, active surveillance would be beneficial for MEN1 patients who had kindreds with MEN1 related PNET.

Key words: multiple endocrine neoplasia type 1, genotype, phenotype, gastroenteropancreatic neuroendocrine tumor, pancreatectomy

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Genotype-phenotype analysis and long-term clinical outcome of MEN1-related pancreatic neuroendocrine tumor

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(Directed by Professor Chang Moo Kang)

I. INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) (OMIM*131100) is a rare autosomal dominant disease characterized by endocrine tumors of the parathyroid gland, pancreatic islets, and anterior pituitary gland.(1) Most patients with MEN1 have germline mutations in the tumor suppressor gene *MEN1*, with loss of heterozygosity at 11q13.(1) This results in the biallelic inactivation of *MEN1*. The *MEN1* gene contains 10 exons and encodes a 610-amino acid protein, menin, which interacts with the transcription factors and proteins involved in cell signaling regulation.(1) In patients with MEN1, various tumors occur, such as parathyroid gland tumors, which cause primary hyperparathyroidism (PHPT), pancreatic neuroendocrine tumor (PNET), pituitary adenoma, adrenal tumor, and neuroendocrine tumors of the thymus.(2) Among these tumors, PNET is the leading cause of death in patients with MEN1.(3) Active surveillance and early intervention should be performed to appropriately diagnose and treat PNET.(4) Discussion on the proper treatment of PNET is still in progress and varies according to the classification of PNET: insulinoma, gastrinoma, and non-functioning PNET (NF-PNET).(5, 6) In particular, as NF-PNET is mostly asymptomatic, it is necessary to determine the optimal treatment timing considering the risk of surgery and potential for malignancy. Likewise, treatment should be performed to minimize pancreatic insufficiency after resection, and the appropriate oncologic

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effect should be followed. Several studies have investigated the indications for surgery for PNET in patients with MEN1.(7, 8) The Dutch MEN1 Study Group and the French Endocrine Tumor Study Group reported that small NF-PNETs (<2 cm) were observed without resection in long-term observational studies.(7, 8) So far, the size of tumors diagnosed by imaging studies have been the only known indication for surgery.

The genotype of patients with MEN1 may be vital in predicting the prognosis of PNET; however, the correlation between the genotype and prognosis is still unclear.(9, 10) Recent studies have shown that the location and type of mutations in the *MEN1* gene, such as those in the JunD transcription factor-interacting domain, checkpoint suppressor 1 (Ches1)-interacting domain, and exon 2 in patients aged 20–40 years, are associated with tumor progression.(11-13) Further studies on gene mutations related to PNET progression will help make personalized clinical decisions. This study aimed to investigate whether genotype can provide a clue for identifying patients with MEN1 in Korea who need active surveillance at an earlier time and with aggressive intervention. Therefore, in this study, the differences in the prognosis and penetrance of PNET were analyzed according to the location of the exon with mutations, the domains where the menin protein interacts with other transcription factors, or the type of mutation, such as missense, nonsense, frameshift, and splicing site mutations.

PNET in patients with MEN1 is different from sporadic PNETs in terms of surgical procedures and postoperative outcomes.(14) There are several studies on the short- and long-term outcomes after pancreatic resection in patients with MEN1.(15-17) However, relatively few studies have been carried out on MEN1-related PNET in Asian countries; therefore, we summarized and reported the short- and long-term outcomes of patients after surgery.

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II. MATERIALS AND METHODS

1. Clinical data collection and patient selection

The clinical information of MEN1 patients at Severance Hospital, Seoul, Korea, from January 2003 to September 2022 was retrospectively reviewed. The study protocol was approved by the institutional ethical review board (approval number: 2019-3554-001). According to the MEN1 International Diagnostic Guideline,(6) patients were diagnosed with MEN1 according to the following three criteria. First, the clinical diagnostic criteria were as follows: the presence of two or more MEN1-associated endocrine tumors (i.e., parathyroid adenoma, gastroenteropancreatic neuroendocrine tumor (GEP-NET), and pituitary adenoma). Second, a familial diagnostic criterion is that the patient has one MEN1-associated tumor and a first-degree relative with MEN1. Third, the genetic diagnostic criteria included asymptomatic mutant gene carriers involved in genetic testing. A total of 79 patients were included in. To investigate the relationship between genotypes and clinical prognosis of MEN1 patients, we excluded MEN1 patients who did not show *MEN1* mutation in the genetic test or did not undergo genetic testing. Among the 79 MEN1 patients, we excluded 2 MEN1 patients who did not undergo genetic testing and 6 MEN1 patients who did not have mutations in the *MEN1* gene (5 patients on direct sequencing and 1 patient on NGS panel). Thus, 71 MEN1 patients were included in the analysis of genotype and clinical prognosis. By reviewing the electronic medical record system, data from outpatient records, surgical records, pathological results, and image study results of patients with MEN1 were collected. According to the surveillance protocol of this institution, PNET is diagnosed using computed tomography (CT), magnetic resonance imaging (MRI), and endoscopic ultrasound (EUS). In addition, 68 Gallium(68Ga)-DOTATOC positron emission tomography-computed tomography (PET-CT) and fluorodeoxyglucose (FDG) PET-CT were used to examine metastasis of neuroendocrine tumors. However, we defined that MEN1 patients were diagnosed with

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PNET or liver metastasis when the lesions were detected on CT, MRI, or EUS. Some patients in whom lesions were detected only on the DOTATOC scan, not on CT, MRI, or EUS, were excluded. The size and number of tumors were evaluated using CT, MRI, and EUS. Radioisotope scans did not reflect the exact tumor size. The patients included in this study were followed up through an outpatient clinic and regular imaging tests, including CT, MRI, and EUS.

In this study, we divided patients with MEN1 into two groups: those who underwent surgery and those who were observed without any intervention. In the observation group, the progression of PNET was defined as an increase in the size or number of PNET on CT, MTI, or EUS compared to the previous modality. PNET recurrence after surgery in the surgery group was also defined based on imaging modality. PNET with malignant transformation was defined as lymph node or systemic metastasis of PNET.

2. Genetic testing and analysis

In this institution, patients who were diagnosed with MEN1 based on clinical criteria or patients who were first-degree relatives of MEN1 patients underwent genetic testing through direct sequencing or NGS after providing their consent. Direct sequencing was performed as previously described.(18) Genomic DNA was isolated from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (QIAGEN, GmbH, Hilden, Germany). The exons and introns of *MEN1* were amplified using primers. PCR amplification was performed using a thermal cycler (model 9700; Applied Biosystems, Foster City, CA, USA). Amplicons were purified using Agencourt AMpure XP (Beckman Coulter Genomics, Danvers, MA, USA). Direct sequencing was performed using an ABI Prism 3730 and 3130 Genetic Analyzer (Applied Biosystems). A customized NGS panel that included 400 genes related to various endocrine disorders was

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used for targeted sequencing. DNA was extracted using the same method as that used for direct sequencing. Subsequent sequencing and data analyses were performed as previously described.(19, 20) The variants were interpreted using the 5-tier classification system recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines.(21) All variants identified in this study were confirmed using the NCBI single-nucleotide polymorphism and human gene mutation databases. Some patients underwent genetic testing at other institutions, and genetic data were collected from the records of the genetic tests. Owing to insufficient data, the genotype in three patients was described as a mutation of amino acid sequences rather than a codon.

Exons 2–10 and introns of *MEN1* were analyzed to determine the location of the mutation. The types of mutations examined were nonsense, frameshift, missense, splicing, and in-frame deletion (or insertion). Splicing mutations were excluded from truncated mutations. Owing to the lack of systematic cDNA-sequencing data, it was difficult to determine whether the mutations caused the formation of premature stop codons. The interaction domains of the functional partners of menin were mapped based on previous studies, as summarized in **Table 1**.(22)

Table 1. Domains of interacting transcriptional partners on the *MEN1* gene

Function of interacting partners	Protein (codon)
Transcriptional repressor	**JunD/AP-1** (codons 1–40, 139–242, and 323–428), **histone deacetylase1 (HDAC1**, codons 145–450),
	Nuclear factor-κB (NF-κB, codons 305–381), **mammalian switch independent 3A (mSin3A**, codons 371–387)
Transcription activator	**phosphorylated mothers against decapentaplegic3 (Smad3**, codons 40–278 and 477–606), **homeobox-containing protein (Pem**, codons 278–476)

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DNA repair	**Fanconi anemia complementation group D2 (FANCD2**, codons 219–395), **checkpoint kinase 1 (CHES1**, codons 428–610)
Proliferation	**NM23H1** (codons 1–486)
Cell cycle	**Replication protein A2 (RPA2**, codons 1–40 and 286–448)

3. Statistical analysis

Clinical characteristics are reported as mean ± standard deviation (SD) or median with interquartile range (IQR). Subgroups were compared using the chi-square test and Fisher's exact test. We conducted a subgroup analysis to determine the association between the age-related cumulative incidence of PNET and the location or type of mutation using the Kaplan–Meier method. Event-free or recurrence-free survival was also analyzed using the Kaplan–Meier method. We conducted Cox proportional hazard model analysis for the observation group, in which progression (the increase in size or number of tumors) was defined as "event." "Time" was defined as the time from diagnosis of PNET to progression. Similarly, we also used a Cox proportional hazard model for the pancreatectomy group. The recurrence was defined as "event," and "Time" was defined as the time from surgery to recurrence. In multivariate analysis of the Cox proportional hazard model, we included genotypes with statistical significance and general characteristics to adjust for other variables of general characteristics. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using the SPSS software version 23.0.0.0 for MAC (SPSS Inc., Chicago, IL, USA) and R 3.6.3.

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III. RESULTS

1. General characteristics of patients with MEN1

A total of 71 patients were diagnosed with MEN1 during the study period at Severance Hospital, Seoul, Korea. The general characteristics of MEN1 patients are summarized in **Table 2**. The median follow-up time was 6.5 years [IQR: 4.0; 9.2].

In total, 50 patients with MEN1 (50/71, 70.4%) were diagnosed with PNET on CT, MRI, or EUS. Furthermore, 6 patients (6/71, 8.5%) were suspected of having PNET on the DOTATOC scan, but no lesions were detected on CT, MRI, or EUS. Among the 50 patients with MEN1 and PNET, 4 (4/50, 8.0%) were diagnosed with insulinoma, 8 (8/50, 16.0%) with gastrinoma, and 38 (38/50, 76.0%) with non-functioning PNET (NF-PNET). A total of 20 MEN1 patients with PNET underwent pancreatic resection (20/50, 40.0%).

Table 2. General characteristics of MEN1 patients

Characteristics (N=71)	Number (%)
Age at diagnosis (years), mean	38.5 ± 15.9
Male/Female	26/45 (36.6% / 63.4%)
MEN1 manifestation	
-PHPT	50/71 (70.4%)
-Pituitary adenoma	40/71 (58.0%)
-PNET	50/71 (70.4%)
Type of PNET (N=50)	
-Insulinoma	4/50 (8.0%)
-Gastrinoma	8/50 (16.0%)

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-NF-PNET	38/50 (76.0%)
Pancreatic resection	20/50 (40.0%)
Largest size of the PNET, mm, median	12.5 [7.5; 20.0]
Number of PNETs, median	2.0 [1.0; 3.0]
Liver metastasis	4/71 (5.6%)

MEN1 multiple endocrine neoplasia type 1, *PHPT* primary hyperparathyroidism, *PNETs* pancreatic neuroendocrine tumors, *NF-PNET* non-functioning pancreatic neuroendocrine tumor

2. General characteristics of patients with MEN1 who underwent pancreatectomy for PNET

The general characteristics of the patients who underwent surgery are summarized in **Table 3**. A total of 20 MEN1 patients underwent surgery for PNET, and 6 patients underwent reoperation for recurrence. Of the 20 surgeries for first resection, 9 patients (45.0%) were laparotomies and 8 patients (40.0%) were laparoscopic surgery, and laparoscopic surgery in 3 patients (15.0%) was converted to open surgery. The short-term perioperative results and pathological characteristics of the first pancreatic resection in MEN1 patients are also summarized in **Table 3**. The median largest tumor diameter was 16.0 (13.5–25.0, IQR), which was significantly larger than that of the observation group (10.5; 7.0–14.0, IQR) p=0.007). According to the 2017 WHO classification, (23) 13 cases were grade 1, 5 cases were grade 2, and none of them resulted in carcinoma. Lymph node sampling was performed in 10 cases, and lymph node metastasis was confirmed in only 4 cases. Three patients were diagnosed with liver metastasis based on CT or MRI. A patient was suspected of having liver metastasis based on DOTATOC scan, but no liver metastasis was observed on CT or MRI. One patient with synchronous liver metastasis underwent pancreatic and hepatic wedge resections. The other two patients were diagnosed with liver metastasis after surgery and treated with TACE and chemotherapy.

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However, one of them died of spontaneous bacterial peritonitis 2 years after being diagnosed with liver metastasis.

Table 3. General characteristics of MEN1 patients who underwent pancreatic resection

Variables (N=20)	Number (%)
Age at diagnosis of PNET (years), mean	35.6 ± 12.2
Number of tumors at diagnosis, mean	2.1 ± 1.4
Size of the largest tumors at diagnosis (mm), median	16.0 [13.5; 25.0]
Type of PNET	
-Insulinoma	4 (20.0%)
-Gastrinoma	5 (25.0%)
-NF-PNET	11 (55.0%)
Types of first surgery **(N=20)**	
(Laparotomy: 9 / MIS: 8/ open conversion: 3)	
-Enucleation	1 (5.0%)
-Distal pancreatectomy	10 (50.0%)
-Pancreaticoduodenectomy	1 (5.0%)
-Total pancreatectomy*	8 (40.0%)
Types of re-operation **(N=6)**	
(Laparotomy: 4 / MIS: 2)	
-Subtotal pancreatectomy	2/6 (33.3%)
-Completion total pancreatectomy	3/6 (50.0%)

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-Duodenectomy**	1/6 (16.7%)
Recurrence after first surgery	13/20 (65.0%)
2017 WHO classification of PNET	
-Grade 1	13 (65.0%)
-Grade 2	5 (25.0%)
-Unclassified	2 (10.0%)
Lymph node metastasis	4 (20.0%)
Liver metastasis	3 (15.0%)

MEN1 multiple endocrine neoplasia type 1, *PHPT* primary hyperparathyroidism, *PNET* pancreatic neuroendocrine tumor, *NF-PNET* non-functioning pancreatic neuroendocrine tumor, *MIS* minimally invasive surgery, *WHO* World Health Organization
* One patient underwent duodenum-preserving total pancreatectomy.
** Duodenectomy was followed by duodenum-preserving total pancreatectomy because of recurrence at duodenal bulb.

The median hospital stay after surgery was 14 days (range 5–25). Postoperative complications occurred in 8 of the 26 surgeries (30.8%). According to the Clavien–Dindo classification, 3 cases were grade II and 5 cases were grade III. (24) Excluding total pancreatectomy, 2 of 14 pancreatic resection were resulted in postoperative pancreatic fistula. All cases of postoperative pancreatic fistula were biochemical leaks according to the International Study Group of Pancreatic Fistula Classification. (25) No 30-day or 90-day surgery-related mortality rates were observed. Among the 9 patients who underwent partial pancreatic resection, postoperative diabetes mellitus developed in 20% of patients, as shown in **Supplementary Table 1**. One patient required insulin treatment to control the blood sugar levels. On the other hand, all of patients who underwent total pancreatectomy need insulin treatment. The median HbA1c level of MEN1 patients who

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underwent partial resection was significantly lower than that of MEN1 patients who underwent total pancreatectomy (8.3 [8.0; 8.5] vs. 5.7 [5.5; 6.3], p=0.012).

Supplementary Table 1. Comparison of postoperative sugar control depending on the extent of pancreatic resection

Variables (N=20)	Total pancreatectomy (N=11)	Partial resection (N=9)	p value
Postoperative DM	11 (100.0%)	2 (22.2%)	<0.001
Insulin dependence	11 (100.0%)	1 (11.1%)	<0.001
HbA1c (%), median	8.3 [8.0; 8.5]	5.7 [5.5; 6.3]	0.012

DM diabetes mellitus, *HbA1c* hemoglobin A1c

3. Long-term outcomes of MEN1 patients with PNET

Regarding 50 patients with MEN1 and PNET, the mean follow-up duration for 20 patients who underwent surgery was 11.1 ± 5.5 years, and the mean postoperative follow-up time was 8.7 ± 6.1 years. In the surgery group, 13 of the 20 patients (65.7%) experienced recurrence after surgery, and 6 patients underwent re-operation. The median recurrence-free survival was 6.0 years (0.6–11.4, IQR). Among patients with recurrence after surgery, the longest follow-up time was approximately 12 years. Seven patients with recurrence who had not undergone reoperation were followed up for an average of 11.3 years, and three of them received chemotherapy without mortality.

On the other hand, the median follow-up duration for 30 patients without surgery was 6.0 years (3.5–7.2, IQR). In the observation group, the median size of the largest tumor at diagnosis was 10.5 mm (7.0–14.0, IQR); however, the median of the most recently measured size of the largest tumor was 11.5 mm (7.9–16.0, IQR). During the follow-up period, 13 of 30 patients without surgery (43.3%) showed disease progression (the number and size of tumors increased on follow-up CT, MRI, or EUS). The median progression-free survival of the observation group was 4.5 years (2.5–6.5, IQR).

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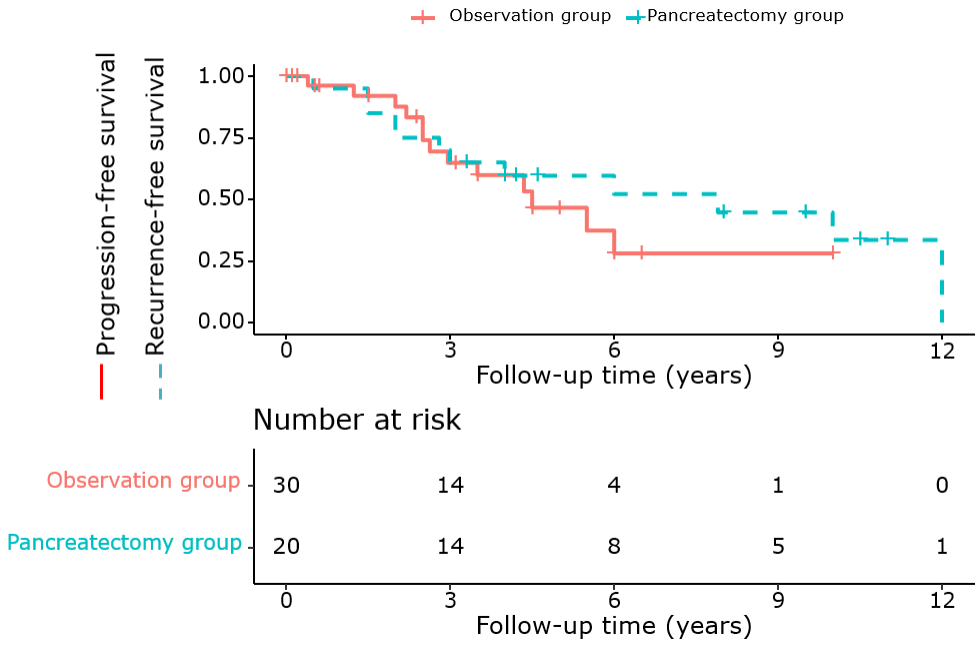

Figure 1. The long-term outcome of MEN1 patients in observation group and pancreatectomy group.

4. Distribution of germline mutation of *MEN1* and comparison with previously published mutation variants and variants in Japan

71 patients with MEN1 were confirmed to have mutations in the MEN1 gene. The distribution of germline mutation of MEN1 in patients with MEN1 are summarized in **Figure 2-(A)**. The mutations were scattered throughout all exons of the MEN1 gene. The most common mutation in MEN1 was in exon 2 (10 families), followed by exons 3, 9, and 7 (8, 5, and 4 families, respectively). **Figure 2-(B)** shows a comparison of the distribution of mutations in exons in the *MEN1* gene between this study and a multicenter study from Japan. The types of mutations revealed were frameshift mutations in 16 families (41.0%), missense mutations in 14 families (35.9%), and nonsense mutations in

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6 families (15.4%). Three families (7.7%) had mutations at splicing sites, as presented in **Figure 2-(C)**. Approximately 56% of the variants were truncating mutations.

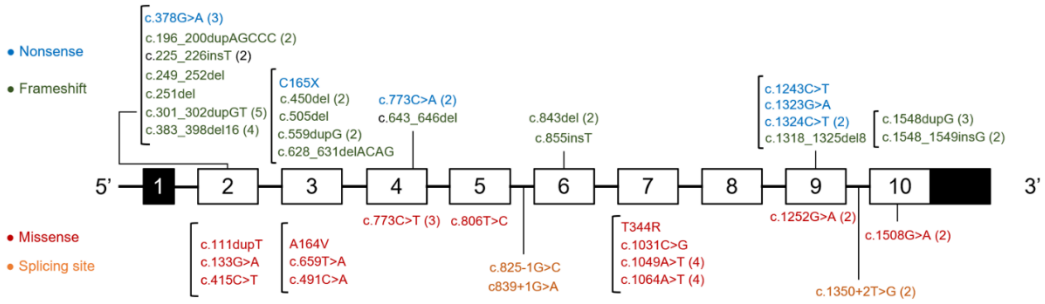

Figure 2-(A) Mutation patterns in the *MEN1* gene of patients with MEN1. The numbers in the parenctheses are the numbers of family's members in each variant.

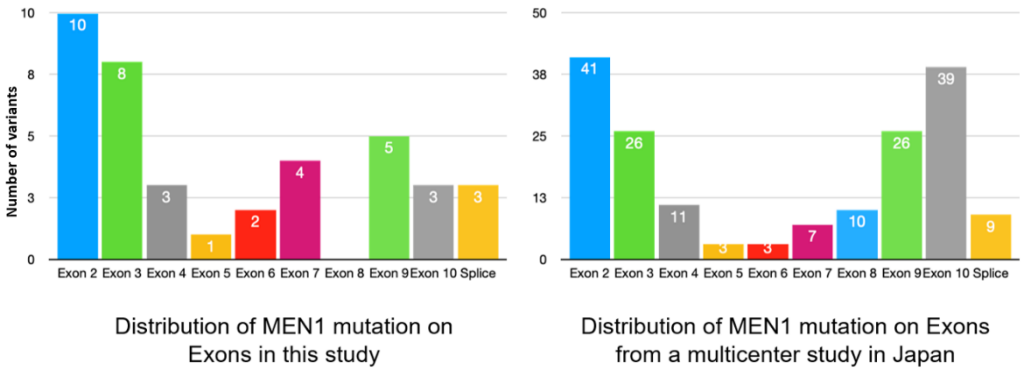

Figure 2-(B) Comparison of distribution of mutation in exons in the *MEN1* gene between this study and a multicenter study in Japan (23)

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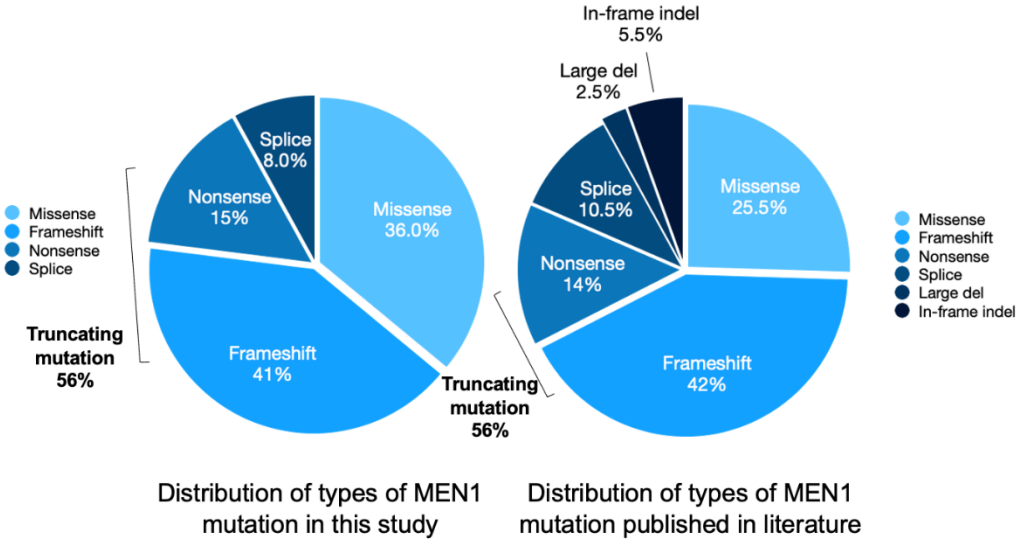

Figure 2-(C) Distribution of types of *MEN1* mutation in this study compared with published variants in literature (24, 25)

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5. Age-related penetrance of PNET in MEN1 patients according to genotypes

A total of 71 patients with confirmed germline mutations in *MEN1* were included to investigate the relationship between genotype and penetrance of PNET. Initially, six patients with MEN1 with no mutations in the *MEN1* gene had a significant difference in the penetrance of PNET compared with patients with confirmed mutations (16.7% vs. 70.4%, p=0.015). There was no significant difference in genotypes between patients with PNET and MEN1 patients without PNET. However, comparison of age-related penetrance of PNET in MEN1 patients by Kaplan–Meier method revealed that MEN1 patients with mutations in exon 2 were diagnosed with PNET at an earlier age, as shown in **Figure 3-(A)** (p=0.028). In addition, truncating mutations in exon 2 resulted in significantly higher age-related penetrance of PNET, as shown in **Figure 3-(B)** (p=0.014). MEN1 patients with truncating mutation showed no difference compared to patients with other types of mutation (p=0.150). Mutation in other exons or interacting domains of functional partners had no significant association with age-related penetrance of PNET.

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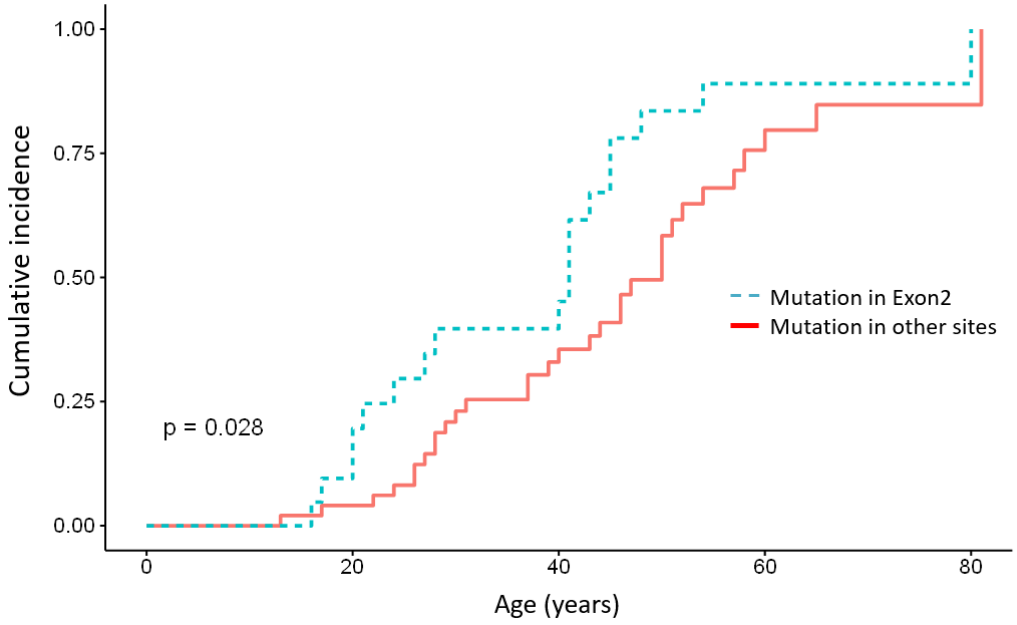

Figure 3-(A) Difference in age-related penetrance of PNET between patients with mutation in exon 2 and other patients with MEN1

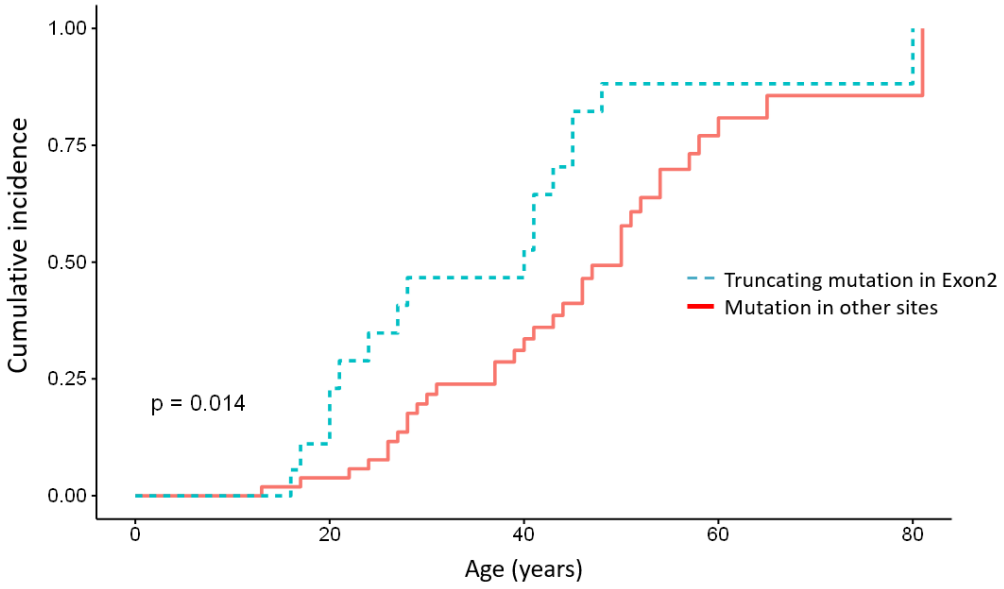

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Figure3-(B) Difference in age-related penetrance of PNET between patients with truncating mutation in exon 2 and other patients with MEN1

6. Comparison of general characteristics and genotypes between MEN1 patients with PNET-related malignancy and other patients with PNET

PNET patients with confirmed MEN1 mutations were 50 in this study. Eight patients were diagnosed with lymph node metastasis after surgery or with systemic metastasis on imaging. We compared these patients who were diagnosed as PNET with malignant transformation with 42 patients who weren't to investigate the risk factors for PNET with malignant transformation. The comparison of both groups was listed in **Table 4**. The mean age at diagnosis of PNET was significantly higher in patients with PNET with malignant transformation than other patients who had PNET. (47.5 vs. 28.0, p=0.017). Moreover, 50% of patients who had PNET with malignant transformation had gastrinoma, compared to only 7.3% of other patients who had MEN1-related PNET. Additionally, the proportion of patients who underwent pancreatectomy was higher in the PNET-related malignancy group (75.0% vs. 33.3%, p=0.047). There was no difference in the genotypes between the two groups.

Table 4. Comparison of general characteristics and genotypes between patients with MEN1-related PNET and other patients diagnosed with PNET with malignant formation

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Variables (N=50)	PNET with malignant transformation (N=8)	MEN1-related PNET (N=42)	p value
Age at diagnosis of PNET (years), median	47.5 [33.0;52.0]	28.0 [24.0;41.0]	0.017
Number of tumors at diagnosis, median	1.0 [1.0; 2.5]	2.0 [1.0; 3.0]	0.296
Size of the largest tumors at diagnosis (mm), median	13.5 [9.5; 26.5]	12.5 [7.0; 19.0]	0.458
Types of PNET			0.032
-NF-PNET	4 (50.0%)	34 (81.0%)	
-Insulinoma	0 (0.0%)	4 (9.5%)	
-Gastrinoma	4 (50.0%)	4 (9.5%)	
Pancreatectomy	6 (75.0%)	14 (33.3%)	0.047
Truncating mutation	5 (62.5%)	29 (69.0%)	>0.699
Exon 2	2 (25.0%)	16 (38.1%)	0.694
Exon 3	1 (12.5%)	6 (14.3%)	>0.999
Exon 7	1 (12.5%)	3 (7.1%)	0.514
Exon 9	2 (25.0%)	3 (7.1%)	0.176
Exon 10	0 (0.0%)	4 (9.5%)	>0.999
Ches1-interacting domain	2 (25.0%)	8 (19.0%)	0.653
JUND-interacting domain	3 (37.5%)	11 (26.2%)	0.670
RPA2-interacting domain	4 (50.0%)	7 (16.7%)	0.059

PNET pancreatic neuroendocrine tumor, *NF-PNET* non-functioning pancreatic neuroendocrine tumor, *CHES1* checkpoint kinase 1

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7. Investigation of clinical characteristics of PNET in families who were diagnosed as MEN1

Among 71 MEN1 patients, it was summarized whether there was any common clinical characteristics of PNET among family members. 9 families of MEN1 patients had more than two family members who were diagnosed as PNET. Clinical characteristics of PNET were summarized in **Table 5**.

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Table 5. Clinical characteristics of PNET 9 families of MEN1 patients had more than two family members who were diagnosed as PNET

Family	Mutation	Location of mutation	effect	Patient	Sex/Age	Age at diagnosis of PNET	Characteristics of PNET	Treatment	Progress
F1	c.196_200dupAGCCC	Exon 2	Frameshift	I-1	M/64	41	NFPNET, 1 tumor, 15mm-sized largest tumor	DP	Recur at head, a 12mm-sized tumor
				II-1	F/41	28	Insulinoma, 3 tumors, 17mm-sized largest tumor	TP	No recur for 9 yrs
F2	c.301_302dupGT	Exon 2	Frameshift	I-1	M/55	49	NFPNET, 2 tumors in pancreas, 22mm-sized largest tumor, 1 tumor in liver S3.	Lap TP with liver wedge rx.	No recur for 5 yrs
				I-2	F/51	45	NFPNET, 4 tumors, 7mm-sized largest tumor	Observation	Increase in size (7mm →9mm)
				II-1	M/26	20	NFPNET, 2 tumors, 5mm-sized largest tumor	Observation	Increase in size (5mm →8mm)
				II-2	F/25	19	NFPNET, 3 tumors, 65mm-sized largest tumor	Lap attempted DP	No recur for 3 yrs
				II-3	F/21	17	NFPNET, 4 tumors, 23mm-sized largest tumor	Observation	Stable for 4 yrs
F3	c.378G>A	Exon 2	Nonsense	I-1	F/57	47	NFPNET, 1 tumor, 25mm sized largest tumor	Lap DP, Lap Completion total PD	Recur in 2 yrs after 1st Sx, liver mets after 2nd Sx, died in 56 yr old d/t SBP
				I-2	F/55		PNET only on DOTATOC scan		
				II-1	M/29	26	NFPNET, 1 tumor, 6mm-sized largest tumor	Observation	Stable for 3 yrs

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F4	c.383_398del16	Exon 2	Frameshift	I-1	M/88	80	NFPNET, 1 tumor, 12mm-sized largest tumor	Observation	Increase in size (12mm →16mm)
				II-1	F/55	42	Insulinoma, 3 tumors, 25mm-sized largest tumor	Lap attempted PPPD with enucleation, Sandostatin	Recur at tail, a 12mm-sized tumor
				II-2	F/53	48	NFPNET, 2 tumors, 14mm-sized largest tumor	Observation	Stable for 5 years
				III-1	F/33	27	NFPNET, 2 tumors, 4mm sized largest tumor	Observation	Stable for 5 years
F5	c.773C>A	Exon 4	Nonsense	I-1	M/35	31	Gastrinoma, 3 tumors, 49mm-sized largest tumor	Lap TP	No recur for 3 years
				I-2	F/30	27	NFPNET, 5 tumors, 28mm-sized largest tumor	Lap DP	Recur at LN of great curvature
F6	c.1049A>T	Exon 7	Missense	I-1	66/F			No PNET	
				I-2	60/F	50	NFPNET, 1 tumor, 15mm-sized largest tumor	Lap TP, Somatostatin	Recur at liver
				II-1	35/M	30	NFPNET, 1 tumor, 10mm-sized largest tumor	Observation	Increase in size and number (10mm →11mm,1→2)
				II-2	31/M			No PNET	
F7	c.1064A>T	Exon 7	Missense	I-1	42/M			No PNET	
				I-2	39/F	36	NFPNET, 1 tumor, 14mm-sized largest tumor	Observation	Stable for 3 years
				I-3	33/F	31	NFPNET, 3 tumors, 22mm-sized largest tumor	Observation	Stable for 3 years
				I-4	30/F			No PNET	
F8	c.1350+2T>G	Intron 9	Splicing	I-1	50/F	39	NFPNET, 3 tumors, 12mm-sized largest tumor	Observation	Stable for 11 years

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				I-2	48/F	44	NFPNET, 1 tumor, 6.5mm-sized largest tumor	Observation	Stable for 4 years
F9	c.1548dupG	Exon 10	Frameshift	I-1	75/M	65	Gastrinoma, 3 tumors, 26mm-sized largest tumor	Sandostatin	Increase in size (26mm →36mm)
				I-2	69/F	60	NFPNET, 1 tumor, 13mm-sized largest tumor	Observation	Increase in size and number (13mm →17mm,1→2)

NFPNET; Nonfunctioning pancreatic nueoroendocrine tumor, DP; Distal pancreatectomy, TP; Total pancreatectomy, Liver wedge rx; Wedge resection of liver, PD; Pancreaticoduodenectomy, Sx; surgery, SBP; Spontaneous bacterial peritonitis, PPPD; pylorus-preserving pancreaticoduodenectomy

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IV. DISCUSSION

We analyzed the distribution of mutations in 71 patients with MEN1. We followed up 50 patients diagnosed with PNET to analyze the short- and long-term oncological outcomes in the pancreatectomy and observation groups. Patients with grade 1 and 2 PNET accounted for approximately 65% and 25% of the patients who underwent surgery, respectively. However, it was difficult to predict the progression of grade 1 PNET because of the high number of cases of lymph node metastasis or liver metastasis with grade 1 PNET. Among 13 patients with grade 1, 2 patients had liver metastasis (2/13, 15.4%), and 3 patients had lymph node metastasis (3/13, 23.1%). So, even in patients diagnosed with grade 1, surveillance is necessary that grade 1 PNET may develop lymph node metastasis or liver metastasis.

In addition, PNET or liver metastasis of PNET was detected subclinically on DOTATOC scan. In this study, PNET or liver metastasis was defined based on CT, MRI, and EUS; however, there were cases in which the lesion was not visible in these modalities but clearly showed uptake on the DOTATOC scan. As DOTATOC scan is performed on non-surgical patients with PNET as a surveillance protocol in our institution, it is expected that this procedure will be used to detect the lesions early. DOTATOC scans can evaluate not only lesions in the pancreas or liver but also other endocrine organs. (26) With these advantages, DOTATOC scan is expected to be more widely used in MEN1 patients.

this study thoroughly summarized the types of surgery, postoperative outcomes, and pathological results. We observed that surgery for MEN1-related PNET had different characteristics and prognoses from surgery for sporadic PNET. The multifocality of tumors is a distinctive characteristic of MEN1-related PNET. Patients with MEN1 tend to have multifocal tumors, in contrast to those with sporadic PNET, which has been described mainly as a solitary lesion in previous studies.(14, 27) Currently, there is still a discussion on the treatment for gastrinoma, but surgical resection is recommended for

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insulinoma or NF-PNET of 2-cm diameter or larger. When multiple tumors require surgical treatment,

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the range of surgical resection may be wider. Before surgery, EUS should be used to diagnose small lesions. In addition, enucleation accompanied by major resection should be considered instead of total pancreatectomy to completely remove the multifocal lesions. In this study, there was also a significant difference in new-onset diabetes between patients who underwent total pancreatectomy and those who underwent partial resection. A previous study conducted by this institution also showed differences in newly occurring diabetes according to the extent of resection, indirectly confirming differences in quality of life after surgery.(26) In addition, PNET related to hereditary syndrome showed a worse prognosis after surgery than sporadic PNET in previous studies.(14, 27) It will be important to establish a treatment plan for MEN1-related PNET considering these factors.

In this study, we summarized the genetic variants of *MEN1* families diagnosed in this institution. Some results were consistent with those of previous studies, whereas others were not. In this study, exon analysis was used to determine the location of mutations in the gene. Previously, Chung et al.(18) reported that the number of germline mutations in exons 7 and 8 were relatively high in Korean patients with MEN1 compared with those in patients from Western countries. In the present study, exon 7 mutations had a relatively high frequency. According to the data reported in Japan, the mutation frequency of exon 7 was higher than that of exons 5 and 6.(23) On the contrary, we found that frameshift mutation was the most common type of mutation, similar to the finding of Chung et al.(18) The overall distribution of mutation types of variants in this study was similar to that in literature.(24, 25) And there was no statistical difference between this study and variants in literature.($p=0.692$) All kinds of mutation types were distributed throughout exons, and no distinct hotspots were found.

Analysis of the differences in clinical progress according to genotype was performed in various subgroups in this study. First, we confirmed that there was a difference in the penetrance of PNET between the patient groups without mutation in *MEN1* and the patient group with *MEN1* mutations. De Laat et al.(28) reported that *MEN1* mutation-

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negative patients had a lower prevalence of duodenopancreatic neuroendocrine tumors (55.9% vs. 23.3%). These patients also showed apparent differences in the penetration of MEN1 manifestation and survival compared to *MEN1* mutation-positive patients.(28)

In this study, patients with mutations in exon 2 showed a tendency toward higher penetrance. The difference between the penetrance and aggressiveness of PNET according to the location of exons with mutations has been reported previously.(12, 14, 15) Bartsch et al. reported that nonsense and frameshift mutations in exons 2, 9, and 10 had a higher rate of malignant tumors (55% vs. 10%, $p < 0.05$).(15) Christakis I. et al. reported that PNET penetration was higher in young patients with MEN1 with exon 2 mutations.(12) Patients with mutations in exon 2 more frequently showed metastasis ($p=0.04$, OR=4.857).(14) In the comparison of age-related penetrance according to the location of mutations in this study, patients with mutations in exon 2 were also diagnosed earlier than other patients. The crystal structure of menin reveals that the N-terminal domain, domains of the central cavity in the thumb, and PALM are included in exon 2. Therefore, mutations in these domains may severely affect the function of menin.(29) Among 6 patients who were detected PNET on only DOTATOC scan, 5 patients had missense mutation in common. So, when we investigated the age-related penetrance of PNET of missense mutation in *MEN1* gene compared to the other types of mutation, there was not significantly different. ($p=0.538$)

In addition, other studies have reported mutations in CHES1- and JunD-interacting domains, which are associated with PNET aggressiveness and PNET-related mortality, respectively.(11, 13) However, in this study, there was no significant difference in the aggressiveness or penetrance of PNET according to mutations in the CHES1- or JunD-interacting domain.

In this study, approximately 50% of the patients with MEN1 had truncated mutations (nonsense and frameshift mutations), and the truncating mutation in exon 2 also showed a difference in age-related penetrance of PNET. Romanet et al.(30) summarized and compared 370 MEN1 variants and found truncating mutations in approximately 52.3% of

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the patients with MEN1. When comparing patients with large rearrangements, truncated variants, and non-truncated variants, there was a significant difference according to the genotype at the onset of the first clinical symptoms related to MEN1. However, when analyzing DP-NET alone, there were no significant differences in the cumulative incidence of DP-NET with age. Truncating mutations could cause deformity in menin and significantly affect its function, considering that menin is a scaffold protein.(31) Likewise previous study, truncating mutations showed no significant difference in PNET with malignant transformation, recurrence, or progression in the subgroup analysis.

When we investigated the clinical characteristics of PNET in MEN1 patients, the age at diagnosis as PNET was relatively similar among MEN1 family. Especially, a family who had c.773C>A mutation in *MEN1 gene*, the members of family were shown similar clinical course of PNETs: early onset and multiple tumors. Although the progress or aggressiveness differ from each member in a MEN1 family, the onset of PNET may be relative to the genotype and if patients had family members who diagnosed MEN1 with PNET in early age, they may be necessary to be in surveillance earlier.

In addition, it was confirmed that some genotypes were significantly associated with the clinical course of PNET. Cox regression analysis of the observation group revealed a significant difference in progression in patients with a mutation in exon 3 or 9 in **Supplementary Table 2**. On the contrary, Cox regression analysis of recurrence in patients who underwent surgery showed a significant difference in patients with a mutation in exon 10 in **Supplementary Table 3**. We confirmed that the difference in genotypes was relevant to the clinical course of patients with MEN1-related PNET, even when corrected with other variables related to the characteristics of the tumor. However, the genotype associated with the aggressive features of PNET was not consistent, and further discussion is needed to determine whether it has substantial significance.

In addition, it is interesting that recently, several studies have been conducted to analyze the functional changes in menin at the molecular level caused by mutations in *MEN1*.(32, 33) A recent study showed that the frequency of certain missense mutations in menin-

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binding pockets was reduced in mixed-lineage leukemia (MLL1/MLL2) interaction compared to JunD interaction in multi-omics analysis.(33) Biancaniello et al. showed that it was possible to predict the effect of amino acid variation on the structure of menin using a computational method.(32) These promising results could be the key to unveiling the association between each location of mutation in the *MEN1* gene and defects in the function of menin.

This retrospective study was conducted at a single institution and thus had several limitations. First, as this was a single-center study, the number of patients with MEN1 was insufficient to confirm the association between genotype and long-term outcomes. Functional PNET was also included in the study, and the treatment indications could be applied differently to each patient. In addition, some data that depended only on medical records from other institutions were insufficient.

Supplementary Table 2. Cox proportional hazard model for risk factors for tumor progression in the observation group

	Univariate			Multivariate		
Variables (N=30)	**HR**	**95% CI**	**p value**	**HR**	**95% CI**	**p value**
Age at diagnosis of PNET (continuous)	1.018	0.986–1.050	0.276	1.022	0.970–1.077	0.420
Types of PNET (ref: NF-PNET)						
- Gastrinoma	1.281	0.161–10.155	0.815	1.067	0.065–17.459	0.964
Number of tumors (continuous)	1.214	0.746–1.977	0.435	1.592	0.916–2.769	0.099
Tumor size ≥2 cm (ref: tumor size <2 cm)	1.121	0.245–5.127	0.883	1.746	0.316–9.650	0.523
Truncating mutation	0.719	0.240–2.149	0.557			
Mutation in exon 2	0.295	0.064–1.352	0.118			

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Mutation in exon 3	4.839	1.135–20.627	0.033			
Mutation in exon 7	1.298	0.282–5.981	0.754			
Mutation in exon 9	0.461	0.059–3.627	0.465			
Mutation in exon 10	5.283	1.349–20.692	0.017			
Mutation in exon 3 or 10	8.051	2.214–29.274	0.002	9.907	2.297–42.724	0.002
Mutation in Ches1-interacting domain	0.765	0.209–2.796	0.692			
Mutation in JUND-interacting domain	1.693	0.548–5.235	0.376			

HR hazard ratio, *CI* confidence interval, *PNET* pancreatic neuroendocrine tumor, *NF-PNET* non-functioning pancreatic neuroendocrine tumor, *CHES1* checkpoint kinase 1

Supplementary Table 3. Cox proportional hazard model analysis of risk factors for tumor recurrence in the pancreatectomy group

	Univariate			**Multivariate**		
Variables (N=20)	**HR**	**95% CI**	**p value**	**HR**	**95% CI**	**p value**
Age at diagnosis of PNET (continuous)	1.004	0.957–1.052	0.885	0.993	0.938–1.052	0.809
Types of PNET (ref: NF-PNET)						
- Insulinoma	1.443	0.349–5.958	0.612	4.173	0.465–37.452	0.202
- Gastrinoma	0.719	0.190–3.147	0.719	0.387	0.057–2.637	0.332
Number of tumors (continuous)	1.077	0.683–1.698	0.751	0.761	0.393–1.475	0.419
Tumor size ≥2 cm (ref: tumor size <2 cm)	0.858	0.228–3.233	0.821	0.978	0.144–6.644	0.982
WHO Grade (ref: Grade 1)						
- Grade 2	1.156	0.305–4.384	0.831	0.347	0.050–2.429	0.286

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Ki67, % (continuous)	1.083	0.919–1.277	0.342			
Mitotic counts, n/10 HPF (continuous)	0.973	0.635–1.492	0.902			
LN metastasis	0.248	0.031–1.966	0.187			
Truncating mutation	4.796	0.609–37.773	0.136			
Mutation in exon 2	0.511	0.157–1.661	0.265			
Mutation in exon 3	1.101	0.138–8.777	0.928			
Mutation in exon 7	1.700	0.210–13.756	0.619			
Mutation in exon 9	7.309	1.198–44.580	0.031	21.501	1.778–360.003	0.016
Mutation in exon 10	1.353	0.168–10.866	0.776			
Mutation in Ches1-interacting domain	3.232	0.823–12.692	0.093			
Mutation in JUND-interacting domain	1.045	0.280–3.899	0.948			

HR hazard ratio, *CI* confidence interval, *PNET* pancreatic neuroendocrine tumor, *NF-PNET* non-functioning pancreatic neuroendocrine tumor, *CHES1* checkpoint kinase 1, *WHO* World Health Organization

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V. CONCLUSION

Regarding PNET in patients with MEN1, as the tumor occurs in multiple locations, it is necessary to preserve the pancreatic parenchyma to improve the quality of life of the patient after surgery. The tumors, which are indications for surgery, should be actively treated, but this study confirmed that the progression of the tumor was relatively stable without surgery. Moreover, clinical manifestations may differ depending on the genetic mutation in MEN1 patients. In particular, a more individualized and detailed follow-up strategy is required for young patients with MEN1 with mutations in exon 2 and truncating mutations, as it showed a trend toward the penetrance of PNET. In addition, MEN1 patients had kindreds with MEN1 related PNET, active surveillance would be beneficial for these patients. Further studies are needed to determine the association between the prognosis of MEN1-related PNET and the genotype of germline mutations in *MEN1*.

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REFERENCES

1. Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, et al. Positional cloning of the gene for multiple endocrine neoplasia-type 1. Science. 1997;276(5311):404-7. Epub 1997/04/18. doi: 10.1126/science.276.5311.404. PubMed PMID: 9103196.
2. Marx SJ, Agarwal SK, Kester MB, Heppner C, Kim YS, Skarulis MC, et al. Multiple endocrine neoplasia type 1: clinical and genetic features of the hereditary endocrine neoplasias. Recent Prog Horm Res. 1999;54:397-438; discussion -9. Epub 1999/11/05. PubMed PMID: 10548885.
3. Goudet P, Murat A, Binquet C, Cardot-Bauters C, Costa A, Ruszniewski P, et al. Risk factors and causes of death in MEN1 disease. A GTE (Groupe d'Etude des Tumeurs Endocrines) cohort study among 758 patients. World J Surg. 2010;34(2):249-55. Epub 2009/12/02. doi: 10.1007/s00268-009-0290-1. PubMed PMID: 19949948.
4. Klein Haneveld MJ, van Treijen MJC, Pieterman CRC, Dekkers OM, van de Ven A, de Herder WW, et al. Initiating Pancreatic Neuroendocrine Tumor (pNET) Screening in Young MEN1 Patients: Results From the DutchMEN Study Group. J Clin Endocrinol Metab. 2021;106(12):3515-25. Epub 2021/08/02. doi: 10.1210/clinem/dgab569. PubMed PMID: 34333645.
5. Niederle B, Selberherr A, Bartsch DK, Brandi ML, Doherty GM, Falconi M, et al. Multiple Endocrine Neoplasia Type 1 and the Pancreas: Diagnosis and Treatment of Functioning and Non-Functioning Pancreatic and Duodenal Neuroendocrine Neoplasia within the MEN1 Syndrome - An International Consensus Statement. Neuroendocrinology. 2021;111(7):609-30.
6. Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). J Clin Endocrinol Metab. 2012;97(9):2990-3011. Epub 2012/06/23. doi: 10.1210/jc.2012-1230. PubMed PMID: 22723327.
7. Pieterman CRC, de Laat JM, Twisk JWR, van Leeuwaarde RS, de Herder WW, Dreijerink KMA, et al. Long-Term Natural Course of Small Nonfunctional Pancreatic Neuroendocrine Tumors in MEN1-Results From the Dutch MEN1 Study Group. J Clin Endocrinol Metab. 2017;102(10):3795-805. Epub 2017/09/25. doi: 10.1210/jc.2017-00372. PubMed PMID: 28938468.
8. Triponez F, Sadowski SM, Pattou F, Cardot-Bauters C, Mirallie E, Le Bras M, et al. Long-term Follow-up of MEN1 Patients Who Do Not Have Initial Surgery for Small </=2 cm Nonfunctioning Pancreatic Neuroendocrine Tumors, an AFCE and GTE Study: Association Francophone de Chirurgie Endocrinienne & Groupe d'Etude des Tumeurs Endocrines. Ann Surg. 2018;268(1):158-64. Epub 2017/03/07. doi: 10.1097/SLA.0000000000002191. PubMed PMID: 28263205; PubMed Central PMCID: PMC6012055.
9. Lemos MC, Thakker RV. Multiple endocrine neoplaslia type 1 (MEN 1): Analysis of 1336 mutations reported in the first decade following identification of the gene. Human Mutation. 2008;29(1):22-32. doi: 10.1002/humu.20605. PubMed PMID: WOS:000252143300005.

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10. Wautot V, Vercherat C, Lespinasse J, Chambe B, Lenoir GM, Zhang CX, et al. Germline mutation profile of MEN1 in multiple endocrine neoplasia type 1: search for correlation between phenotype and the functional domains of the MEN1 protein. Human Mutation. 2002;20(1):35. PubMed PMID: 197275886.
11. Bartsch DK, Slater EP, Albers M, Knoop R, Chaloupka B, Lopez CL, et al. Higher Risk of Aggressive Pancreatic Neuroendocrine Tumors in MEN1 Patients With MEN1 Mutations Affecting the CHES1 Interacting MENIN Domain. The Journal of Clinical Endocrinology & Metabolism. 2014;99(11):E2387-E91. doi: 10.1210/jc.2013-4432.
12. Christakis I, Qiu W, Hyde SM, Cote GJ, Grubbs EG, Perrier ND, et al. Genotype-phenotype pancreatic neuroendocrine tumor relationship in multiple endocrine neoplasia type 1 patients: A 23-year experience at a single institution. Surgery. 2018;163(1):212-7. doi: https://doi.org/10.1016/j.surg.2017.04.044.
13. Thevenon J, Bourredjem A, Faivre L, Cardot-Bauters C, Calender A, Murat A, et al. Higher risk of death among MEN1 patients with mutations in the JunD interacting domain: a Groupe d'étude des Tumeurs Endocrines (GTE) cohort study. Human Molecular Genetics. 2013;22(10):1940-8. doi: 10.1093/hmg/ddt039.
14. Soczomski P, Jurecka-Lubieniecka B, Krzywon A, Cortez AJ, Zgliczynski S, Rogozik N, et al. A Direct Comparison of Patients With Hereditary and Sporadic Pancreatic Neuroendocrine Tumors: Evaluation of Clinical Course, Prognostic Factors and Genotype-Phenotype Correlations. Front Endocrinol (Lausanne). 2021;12:681013. Epub 20210528. doi: 10.3389/fendo.2021.681013. PubMed PMID: 34122352; PubMed Central PMCID: PMC8194819.
15. Bartsch DK, Langer P, Wild A, Schilling T, Celik I, Rothmund M, et al. Pancreaticoduodenal endocrine tumors in multiple endocrine neoplasia type 1: surgery or surveillance? Surgery. 2000;128(6):958-66. Epub 2000/12/15. doi: 10.1067/msy.2000.109727. PubMed PMID: 11114630.
16. Nell S, Borel Rinkes IHM, Verkooijen HM, Bonsing BA, van Eijck CH, van Goor H, et al. Early and Late Complications After Surgery for MEN1-related Nonfunctioning Pancreatic Neuroendocrine Tumors. Ann Surg. 2018;267(2):352-6. Epub 2016/11/05. doi: 10.1097/SLA.0000000000002050. PubMed PMID: 27811505.
17. van Beek DJ, Nell S, Verkooijen HM, Borel Rinkes IHM, Valk GD, Vriens MR. Surgery for multiple endocrine neoplasia type 1-related insulinoma: long-term outcomes in a large international cohort. Br J Surg. 2020;107(11):1489-99. Epub 2020/05/01. doi: 10.1002/bjs.11632. PubMed PMID: 32352164; PubMed Central PMCID: PMC7540387.
18. Chung YJ, Hwang S, Jeong JJ, Song SY, Kim SH, Rhee Y. Genetic and epigenetic analysis in korean patients with multiple endocrine neoplasia type 1. Endocrinol Metab (Seoul). 2014;29(3):270-9. Epub 2014/09/25. doi: 10.3803/EnM.2014.29.3.270. PubMed PMID: 25309785.
19. Kim B, Won D, Jang M, Kim H, Choi JR, Kim TI, et al. Next-generation sequencing with comprehensive bioinformatics analysis facilitates somatic mosaic APC gene mutation detection in patients with familial adenomatous polyposis. BMC Med Genomics. 2019;12(1):103. Epub 2019/07/05. doi: 10.1186/s12920-019-0553-0. PubMed PMID: 31269945; PubMed Central PMCID: PMC6610853.
20. Kim B, Won D, Lee ST, Choi JR. Somatic mosaic truncating mutations of PPM1D in blood can result from expansion of a mutant clone under selective pressure of chemotherapy. PLoS One. 2019;14(6):e0217521. Epub 2019/06/27. doi: 10.1371/journal.pone.0217521. PubMed PMID: 31242196; PubMed Central PMCID: PMC6594580.
21. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the

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American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24. Epub 2015/03/06. doi: 10.1038/gim.2015.30. PubMed PMID: 25741868; PubMed Central PMCID: PMC4544753.
22. Lemos MC, Thakker RV. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. Human Mutation. 2008;29(1):22-32. doi: https://doi.org/10.1002/humu.20605.
23. Sakurai A, Suzuki S, Kosugi S, Okamoto T, Uchino S, Miya A, et al. Multiple endocrine neoplasia type 1 in Japan: establishment and analysis of a multicentre database. Clinical endocrinology. 2012;76(4):533-9.
24. Concolino P, Costella A, Capoluongo E. Multiple endocrine neoplasia type 1 (MEN1): An update of 208 new germline variants reported in the last nine years. Cancer Genet. 2016;209(1-2):36-41. Epub 20151214. doi: 10.1016/j.cancergen.2015.12.002. PubMed PMID: 26767918.
25. Romanet P, Mohamed A, Giraud S, Odou MF, North MO, Pertuit M, et al. UMD-MEN1 Database: An Overview of the 370 MEN1 Variants Present in 1676 Patients From the French Population. J Clin Endocrinol Metab. 2019;104(3):753-64. doi: 10.1210/jc.2018-01170. PubMed PMID: 30339208.
26. Jung D, Bari H, Hwang HK, Lee WJ, Kang CM. Short and long-term outcomes of minimally invasive central pancreatectomy: Comparison with minimally invasive spleen-preserving subtotal distal pancreatectomy. Asian Journal of Surgery. 2022. doi: https://doi.org/10.1016/j.asjsur.2022.08.084.
27. Gudmundsdottir H, Graham RP, Sonbol MB, Smoot RL, Truty MJ, Kendrick ML, et al. Multifocality is not associated with worse survival in sporadic pancreatic neuroendocrine tumors. J Surg Oncol. 2021;124(7):1077-84. Epub 20210726. doi: 10.1002/jso.26618. PubMed PMID: 34310723.
28. de Laat JM, van der Luijt RB, Pieterman CRC, Oostveen MP, Hermus AR, Dekkers OM, et al. MEN1 redefined, a clinical comparison of mutation-positive and mutation-negative patients. Bmc Medicine. 2016;14. doi: 10.1186/s12916-016-0708-1. PubMed PMID: WOS:000387729700001.
29. Nelakurti DD, Pappula AL, Rajasekaran S, Miles WO, Petreaca RC. Comprehensive Analysis of MEN1 Mutations and Their Role in Cancer. Cancers. 2020;12(9):2616. doi: 10.3390/cancers12092616. PubMed PMID: 32937789.
30. Romanet P, Mohamed A, Giraud S, Odou M-F, North M-O, Pertuit M, et al. UMD-MEN1 Database: An Overview of the 370 MEN1 Variants Present in 1676 Patients From the French Population. The Journal of Clinical Endocrinology & Metabolism. 2019;104(3):753-64. doi: 10.1210/jc.2018-01170.
31. Dreijerink KMA, Timmers HTM, Brown M. Twenty years of menin: emerging opportunities for restoration of transcriptional regulation in MEN1. Endocrine-Related Cancer. 2017;24(10):T135-T45. doi: 10.1530/ERC-17-0281.
32. Biancaniello C, D'Argenio A, Giordano D, Dotolo S, Scafuri B, Marabotti A, et al. Investigating the Effects of Amino Acid Variations in Human Menin. Molecules. 2022;27(5). Epub 2022/03/11. doi: 10.3390/molecules27051747. PubMed PMID: 35268848; PubMed Central PMCID: PMC8911756.
33. Dreijerink KMA, Ozyerli-Goknar E, Koidl S, van der Lelij EJ, van den Heuvel P, Kooijman JJ, et al. Multi-omics analyses of MEN1 missense mutations identify disruption of menin–MLL and menin–JunD interactions as critical requirements for molecular pathogenicity. Epigenetics & Chromatin. 2022;15(1):29. doi: 10.1186/s13072-022-00461-8.

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ABSTRACT (IN KOREAN)

다발성 내분비 종양 유형 1 환자에서 발생하는 췌장 신경내분비 종양의 장기적 예후와 유전자형에 따른 임상 양상에 대한 분석.

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서론

다발성 내분비 종양 유형 1 (MEN1) 은 상염색체 우성으로 발생하는 유전질환이며 매우 드물다. MEN1 환자들의 주된 사망원인은 췌장에서 발생하는 췌장 신경내분비 종양 (PNET) 이다. 대다수의 MEN1 환자들이 일생 동안에 PNET으로 진단되는 것을 고려할 때, MEN1 환자들에게서 PNET의 경과를 예측하는 것은 중요하다. MEN1은 유전자형과 표현형의 상관관계가 거의 없는 것으로 알려져 있지만, 우리는 PNET에 한정하여 MEN1 환자에게서 발생하는 돌연변이 유전자형을 통해 PNET의 발병양상과 임상적 경과를 미리 예측해 볼 수 있는지 분석해보았다. 또한 아시아 국가에서 MEN1 환자에게서 발생하는 PNET의 치료 경과 및 장기적 예후에 대한 보고가 거의 없기 때문에, 본 연구에서 수술 후 단기적, 장기적 예후에 대해서 정리하여 보고하였다.

연구 방법

2003년 1월부터 2022년 12월까지 한국의 연세의료원 단일 기관에서 MEN1으로

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진단받은 71명의 환자들을 대상으로 후향적 연구를 진행하였다. 유전자 검사는 타병원에서 시행받은 환자들은 결과지를 참고하였고, 본원에서 진단된 환자들은 Direct Sequencing 혹은 Next Generation Sequencing을 통해 MEN1 유전자의 돌연변이가 있는지 확인하였다. 돌연변이의 위치 및 종류에 대해서 분석하였다. 췌장내분비 종양의 악성화는 림파절 전이나 간전이를 기준으로 정의하였다. 또한 경과관찰중인 환자들에게서 종양의 '진행'은 연속된 영상학적 검사에서 종양의 크기나 수가 증가한 것으로 정의하였다. 수술 후 종양의 재발 또한 영상학적 검사를 기준으로 정의하였다.

결과

71명의 환자들 중에서 50명의 환자들 (67.8%)은 PNET으로 진단되었고, 그중 21명의 환자들이 췌장 절제술을 받았다. 수술받지 않고 경과관찰만 진행한 환자들은 경과관찰 기간 동안 암의 progression-free survival 중간값은 4.5 년이었다. (95% 신뢰구간: 2.5~6.5) 또한 수술받은 환자들은 연구 기간동안 recurrence-free survival 중간값은 6.0년이었다. (95% 신뢰구간: 2.5~6.5) exon 2에 돌연변이가 있던 가족은 10가구로 exon 들 중에 가장 많이 돌연변이가 발생한 위치였다. 돌연변이가 발견되지 않은 6명의 환자들은 71명의 돌연변이가 확인된 환자들에 비해 췌장 신경내분비 종양의 발병 빈도가 통계적으로 유의미하게 적었다. (16.7% vs 70.4%, p=0.015) 나이에 따른 발병 정도(age-related penetrance)를 비교해보았을 때도 exon2에 돌연변이가 있었던 환자들과 exon2에서 truncating mutation이 발생했던 환자들에게서 더 어린 나이에 PNET 이 발생하는 결과를 보였다.

결론

\MEN1 환자들은 대체로 병변이 다발적으로 발생하므로 췌장의 실질을 보존하는 것을 지향하며 치료를 하는 것이 수술 후 환자의 삶의 질을 향상시키는데 중요하다.

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PNET의 임상적 경과는 MEN1 환자들의 돌연변이의 유전자형에 따라 차이를 보일 수도 있다는 가능성을 확인하였다. 본 연구에 따르면 비록 적은 수의 환자들을 대상으로 연구를 진행하였지만, exon 2에 돌연변이가 있는 환자들이나 truncating mutation이 관찰된 환자들은 더욱 더 어린 나이부터 PNET에 대한 검사들을 시작하고 더 자주 검사를 하여 경과관찰 하는 것이 필요할 수도 있다. 또한 가족 구성원 중에 PNET이 진단된 MEN1 환자가 있는 경우는 PNET에 대한 검사들을 더 적극적으로 받는 것이 도움이 될 수 있다.

핵심되는 말 : 다발성 내분비 종양 유형 1, 췌장 신경내분비 종양, 유전자형, 표현형, 췌장절제술