Whole-genome sequencing of a multiple endocrine neoplasia type 1 proband identifies gross deletion in MEN1

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Abstract

Background

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant tumor syndrome with neuroendocrine tumorigenesis of the parathyroid and pituitary glands and pancreatic islet cells. This hereditary cancer is caused by germline mutations in the *MEN1*, located on chromosome 11q13. Among the approximately 3,000 cancer patients, in which multi-omics analysis was performed, we had a patient with multiple tumors including pancreatic neuroendocrine tumor, thymic carcinoid and adenoma parathyroid, who showed no family history of MEN1.

Methods

We performed whole genome sequencing (WGS) of peripheral blood samples and three isolated tumor tissues (pancreas, thymus, and parathyroid). Sanger sequencing was used to validate *MEN1* mutations; meanwhile, *MEN1* mRNA levels on tumor samples were obtained by microarrays. *MEN1* copy numbers and protein expression levels were determined by real-time PCR, and immunohistochemistry, respectively.

Results

MEN1 mRNA levels of pancreas and parathyroid tissues from patient-derived tumor samples were remarkably reduced, compared to those of 3,095 tumor and normal tissue samples. WGS data indicated the thymus sample with *MEN1* nonsense mutation (W203*), while no *MEN1* mutations were observed in the other two samples. WGS also identified *MEN1* gross deletion of 18.5 kbp (chr11:64569322 – chr11:64587796; GRCh37/hg19 assembly) in peripheral blood samples and all the three tumor tissues. In addition, no *MEN1* copy numbers on pancreas and parathyroid tumors were detected, whereas peripheral blood and the thymic carcinoid had one copy. Immunostaining of all the tissues detected very low levels of the MEN1 protein, Menin.

Conclusion

We detected a hemizygous *MEN1* germline deletion in blood sample of the MEN1 proband. All the three tumor samples had second hit with a deleterious mutation or normal alleles lacked to generate loss of heterozygosity at the MEN1 locus, suggesting loss of tumor suppressive function for MEN1.

Background

Multiple endocrine neoplasia type 1 (MEN1, OMIM 131100) is an autosomal-dominant inherited syndrome. MEN1 is caused by germline mutations in MEN1, located on chromosome 11q13 [1]. Patients with MEN1 typically present primary hyperparathyroidism, pituitary adenoma, and pancreatic neuroendocrine tumors, which are less frequently accompanied by carcinoid tumors at different sites and non-endocrine tumors of the adrenal cortex and thymus. Multicentric tumors develop in multiple organs

[2]; however, extensive evaluations of the genetic changes in these multicentric tumors have thus far not been performed.

MEN1 gene encodes the histone modifier Menin, and its inactivation drives a variety of phenotypes, including widespread transcriptional dysregulation via histone modification [3], activation of mTOR through AKT expression [4], suppression of homologous recombination of DNA damage response genes [5], and dysregulation of TERT [6]. Structural analysis and biological studies have identified Menin as a scaffold protein [7], involved in the normal maintenance of the cell cycle [8]. Chromosome abnormalities were observed in various endocrine tumors carrying MEN1 mutations, and loss of the wild-type MEN1 in some sporadic endocrine tumors was also reported [9].

In this study, we conducted multi-omics analysis of samples from patients who underwent cancer surgery from 2014 at Shizuoka Cancer Center and analyzed more than 3,000 cases since then [10]. Previously, we have shown a case of familial MEN1 with a novel germline mutation detected by whole-exome sequencing [11].

Hereditary cancer syndromes are rare; consequently, there are relatively few opportunities to analyze somatic mutations in multiple tumors obtained from single patients. During the extensive clinical data analysis of approximately 3,000 cases, we identified a patient with multiple tumors, including pancreatic neuroendocrine tumor and thymic carcinoid. Therefore, MEN1 expression levels and mutations, as well as analysis of all types of genomic mutations were investigated on blood and tumor tissue samples from this patient by using whole genome sequencing (WGS).

Methods

Clinical samples

Shizuoka Cancer Center launched the multi-omics cancer study, Project HOPE in 2014 [10]. Tumor tissue samples dissected from surgical specimens, along with whole blood samples were obtained at the institution according to protocols approved by the institutional review board at the Shizuoka Cancer Center (Authorization Number: 25–33). Written informed consents were obtained from all patients enrolled in the study. Furthermore, all experiments using clinical samples were carried out in accordance with the approved guidelines.

Tumors from pancreas, thymus, and parathyroid, along with their respective surrounding normal tissues (≥ 100 mg) were dissected from surgical specimens immediately after resection of the lesion containing more than 50% tumor content, as visually assessed by a clinical pathologist in our hospital.

DNA and RNA isolation

All samples were fresh-frozen in liquid nitrogen. Genomic DNA was extracted from surgically resected tissues and buffy coat cells of peripheral blood from each patient using the QIAamp DNA Blood Midi Kit (Qiagen, Germany). DNA concentrations and purities were assessed using Qubit and Nanodrop (Thermo

Fisher Scientific, USA). For RNA extraction, fresh tissues were submerged in QIAzol Lysis Reagent (Qiagen) and disrupted using a TissueLyser (Qiagen). Total RNA was isolated using the RNeasy Mini Kit (Qiagen) and quantified on an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) [12].

Gene expression analysis

RNA samples with an RNA integrity number ≥ 6.0 were used for microarray analysis. Total RNA (100 ng) was amplified and fluorescently labeled using the One-Color Low Input Quick Amp Labeling Kit (Agilent Technologies), according to the manufacturer's instructions. The gene expression data were obtained from our cancer genome project [10, 17].

Wgs

We performed WGS on extracted DNA from peripheral blood and pancreatic, thymic carcinoid, and parathyroid tumors (Table 2). Mutations were annotated using an in-house pipeline similar to that of the WES analysis [14]. These mutations were classified according to Ensembl Variation with SnpEff annotations [20, 21] (Table 3). Exonic non-synonymous mutations were confirmed by visual check using Integrative Genomics Viewer. Not overlap and very few mutations in each tissue were observed (Table 4). The thymic carcinoid tumor presented the MEN1 nonsense mutation (W203*).

Identification of MEN1 gross deletion

WGS revealed the gross deletion of the MEN1 locus in blood and three tumor tissue samples (Fig. 3a). In addition, MAP4K2, located 3'side of MEN1, presented a partial deletion, including intron 5 and its upstream. Sanger sequencing confirmed the deletion position as 18.474 kb, (chr11:64569322 – chr11:64587796; GRCh37/hg19 assembly) (Fig. 3b).

Loss of heterozygosity (LOH) at the MEN1 locus

MEN1 LOH was predicted on the thymic carcinoid tumor sample, due to the combination of the pathogenic MEN1 mutation (W203*) and loss of an allele at the MEN1 locus. To clarify the LOH status in other two tumor tissues, we performed MEN1 CNV (copy number variation) analysis. No MEN1 copy numbers were detected in tumor samples from pancreas and parathyroid; whereas, peripheral blood and the thymic carcinoid tumor had one copy (Fig. 4a). Therefore, these results indicated loss of both alleles at the MEN1 locus in the pancreas and parathyroid tumor samples.

Taken together, all the three tumor samples presented a deleterious mutation or normal allele loss to generate LOH at the MEN1 locus, suggesting the loss of MEN1 tumor suppressive function.

Menin protein expression

Next, we performed immunostaining to confirm the expression of Menin protein. More than 90% nuclei of the non-neoplastic tissues were stained with anti-Menin antibody. While, pancreatic tumor was stained 30% nuclei, thymic carcinoid and parathyroid tumors were stained 40% nuclei. (Fig. 4b-d). The thymic carcinoid tumor (Fig. 4c), which contained the mutant allele (W203*), showed similar staining results as those of MEN1 null tumor tissues from pancreatic (Fig. 4b) and parathyroid (Fig. 4d) samples. It was considered that these results supported the result of WGS and CNV.

Results

Patient and tumor sample characteristics

This patient was a 58-year-old male who underwent endoscopic curative resection for early gastric cancer at the Shizuoka Cancer Center hospital. Upon a follow-up computed tomography scan, he was accidentally diagnosed with multiple enhanced tumors in the pancreas, solitary tumor in the thymus, and swollen parathyroid glands (Fig. 1a-c(i)). The hormone levels were mostly within the normal range (Table 1). Distal pancreatectomy, thoracoscopic thymus gland resection, and subtotal parathyroidectomy were subsequently performed (Fig. 1a-c (ii)). Pathological diagnosis revealed multiple neuroendocrine tumors of the pancreas (maximum 25 mm in diameter), carcinoid of the thymus (15 mm in diameter), and adenoma of the parathyroid gland. After the surgery, he still presented tiny residual tumors in the pancreatic head because total pancreatectomy was not performed; however, no tumor progression or symptoms were detected for three years. As shown in Fig. 1d, the patient had no family history of MEN1.

MEN1 mRNA expression

Since the pancreatic tumor sample showed characteristics of neuroendocrine tumor, we investigated MEN1 mRNA expression levels using our microarray-based expression data [17]. When compared to 3,095 tumors and normal tissues samples, the patient-derived tumor samples from pancreas and parathyroid exhibited remarkable reduction of MEN1 mRNA levels (Fig. 2)

Discussion

We had a studied the case of a patient with multiple tumors, including pancreatic, thymic carcinoid, and parathyroid tumors, with no family history of MEN1. The levels of hormone and calcium in this patient were mostly within the normal ranges. However, MEN1 mRNA expression levels were remarkably reduced, compared to those of other 3,095 tumor samples derived from various origins. This urged us to investigate genomic alterations, which revealed a MEN1 proband carrying MEN1 gross deletion of an allele at germline level and a deletions mutation or another normal allele loss at somatic level in three different tissues.

Using WGS, we were able to precisely identify the location of the 18.474 kbp deleted region. WGS data of the pancreatic and parathyroid tumor samples indicated that both MEN1 alleles were likely deleted

(Fig. 3a), which was confirmed by CNV (Fig. 4a) and MEN1 immunostaining (Fig. 4b-d) analyses. We observed no structural variants including fusion-genes and chromothripsis. Recently, long-read sequencing was used as a structural change in the genome was speculated [22]. However, in our study, we did not consider this option because the tumors had large deletions that could be analyzed with short-reads sequencing.

MEN1 contributes to chromosomal instability [23, 24]. WGS detected chromosomal abnormality based on B allele frequency and sequencing read ratio. As shown in Fig. 5a, the pancreatic tumor had a large chromosomal abnormality, which was confirmed by array comparative genomic hybridization (data not shown). Farnebo et al. reported a familial MEN1 case with few genomic alterations [23]. In fact, WGS data of pancreatic and duodenal tumor samples from the familial MEN1 case [11] indicated that the unstable regions were restricted to chromosome 11 (Additional file 1: Fig. S1a). Thus, we speculated that the MEN1 case in this study was sporadic.

Different mutational processes generate unique combinations of mutational types, termed “mutational signatures” [25]. Scarpa et al. showed a novel mutational signature with G:C > T:A transversion, specific to samples from pancreatic neuroendocrine tumors bearing pathogenic germline MUTYH mutations [26]. This prompted us to investigate if any mutational signatures specific to MEN1 inactivation were present. According to the WGS data, the three MEN1 tissue samples displayed age-related signature 1 at the highest contribution rate (Fig. 5b and Additional file 2: Fig. S2) In addition, two tissue samples from the familial MEN1 also exhibited signature 1 (Additional file 1: Fig. S1b and additional file 2: Fig. S2). The difference between the two MEN1 cases was the C:G > A:T mutational signature, in which the rate of TCA:TGA > TAA:TTA transversion was higher in the familial MEN1. Interestingly, all the tumor samples from both MEN1 cases exhibited T:A > C:G transition signature characterized by ATA:TAT > ACA:TGA and TTT:AAA > TCT:AGA. Although more MEN1 samples need to be investigated, it is likely that these mutational signatures are characteristic of MEN1 inactivation.

Conclusion

In MEN1 patients, the possession of a germline mutation in the MEN1 was the only commonality. Menin encoded by MEN1 has been reported to interact with many proteins, but it is not clear how the interaction is changed by those mutations. Therefore, further study and elucidation of the MEN1 multicentric tumorigenesis mechanism should be performed, WGS analysis are useful those study.

Abbreviations

MEN1
Multiple endocrine neoplasia type 1
WGS
whole genome sequencing
mTOR

mechanistic target of rapamycin kinase
AKT
AKT kinase
TERT
telomerase reverse transcriptase
CNV
Copy number variation
LOH
Loss of heterozygosity
IGV
Integrated Genome Viewer

Declarations

Ethics approval and consent to participate

The studies were approved by the Institutional Review Board of the Shizuoka Cancer Center (Authorization Number: 25-33).

Consent for publication

The patients provided written informed consent for detailed genetic examination.

Availability of data and materials

The datasets used or analyzed during the study are available from the corresponding authors upon reasonable request

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was received for this study.

Authors' contributions

AN and SO led analysis and writing of the manuscript. TN performed WGS data analysis. KH and KO performed gene expression analysis. KH and TN performed mutational signature analysis. YH, YK, HM performed genetic counseling for MEN1 patient. MA, TO, TK, and TS performed immunostaining and histological analyses. TO, MI, YO, TS, TI, and KU were a major contributor in writing the manuscript. MS, SO, KU YA, MK, KY supervised and edited the manuscript. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, Tables 1 - 4 are only available for download from the Supplementary Files section.

Supplementary Files Legend

Additional file 1: Figure S1. Chromosomal condition and mutational signature for familial MEN1 patient.

Additional file 2: Figure S2. Mutation signature rates.

Figures

Figure 1

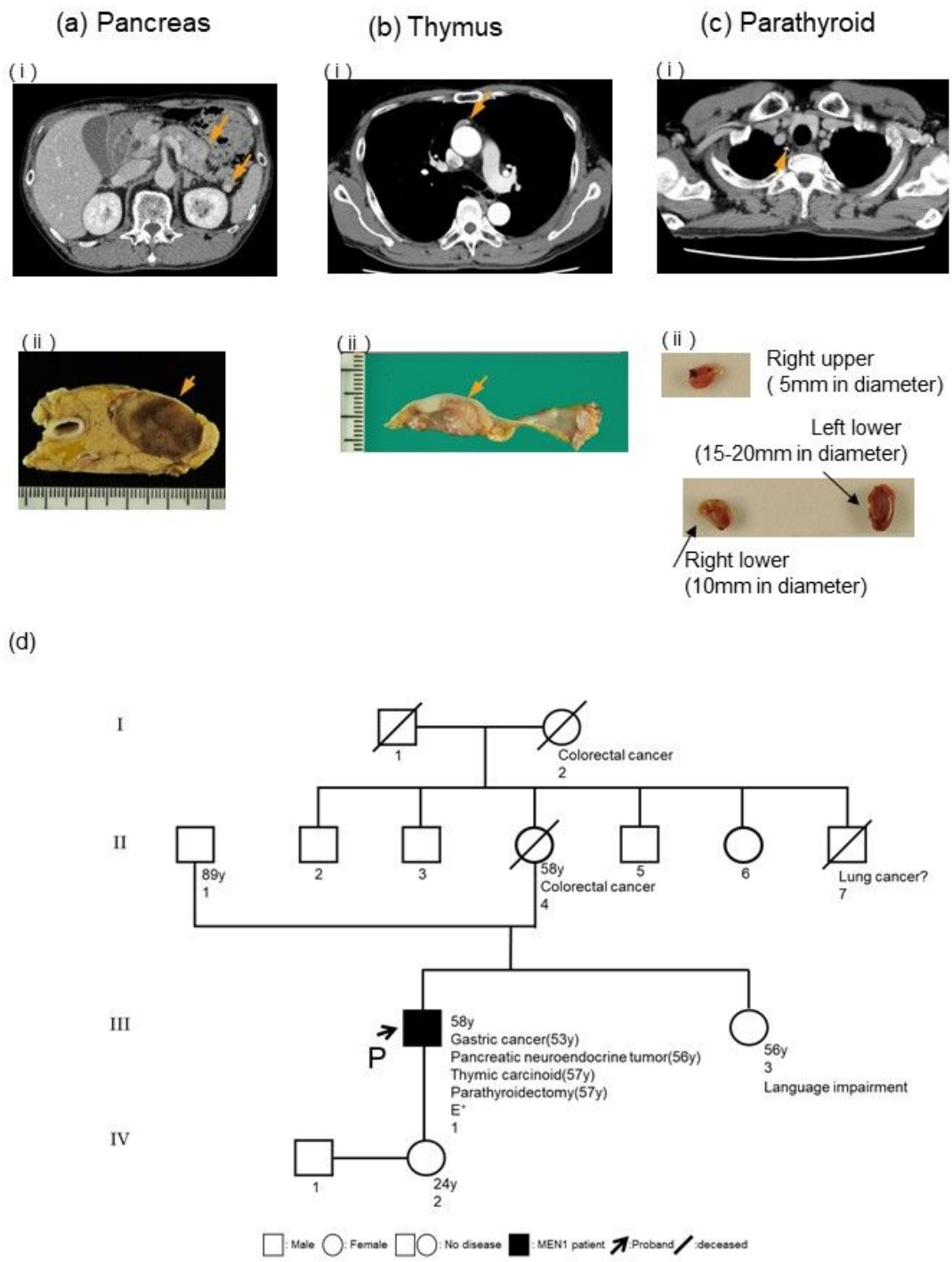

Figure 1

Clinical and pathological manifestations of MEN1. (i) Computed tomography images of the pancreas (a), thymus (b), and parathyroid (c). (ii) Photograph of excised tissue. Orange arrows indicate the tumor. Four nodules up to 2.5 mm in diameter were identified in the pancreas. During the surgery, the pancreas body and tail were resected; however, a 1 cm tumor in the head was not removed. Histologically, the tumors were identified as G2 [29] neuroendocrine tumors, and immunohistochemical analysis revealed that some

of them contained insulin and somatostatin. (Fig. 1a) In the anterior thymus, a single tumor measuring 20×7 mm in a flat configuration was detected. In the atrophic thymus, the carcinoid gland was identified as G2 [29]. (Fig. 1b) Subtotal parathyroidectomy was performed. (Fig. 1c) Adenoma was confirmed in the three glands within the normal rim of the parathyroid gland. (d) Pedigree analysis. P indicates the proband and E+ indicates germline mutation of MEN1.

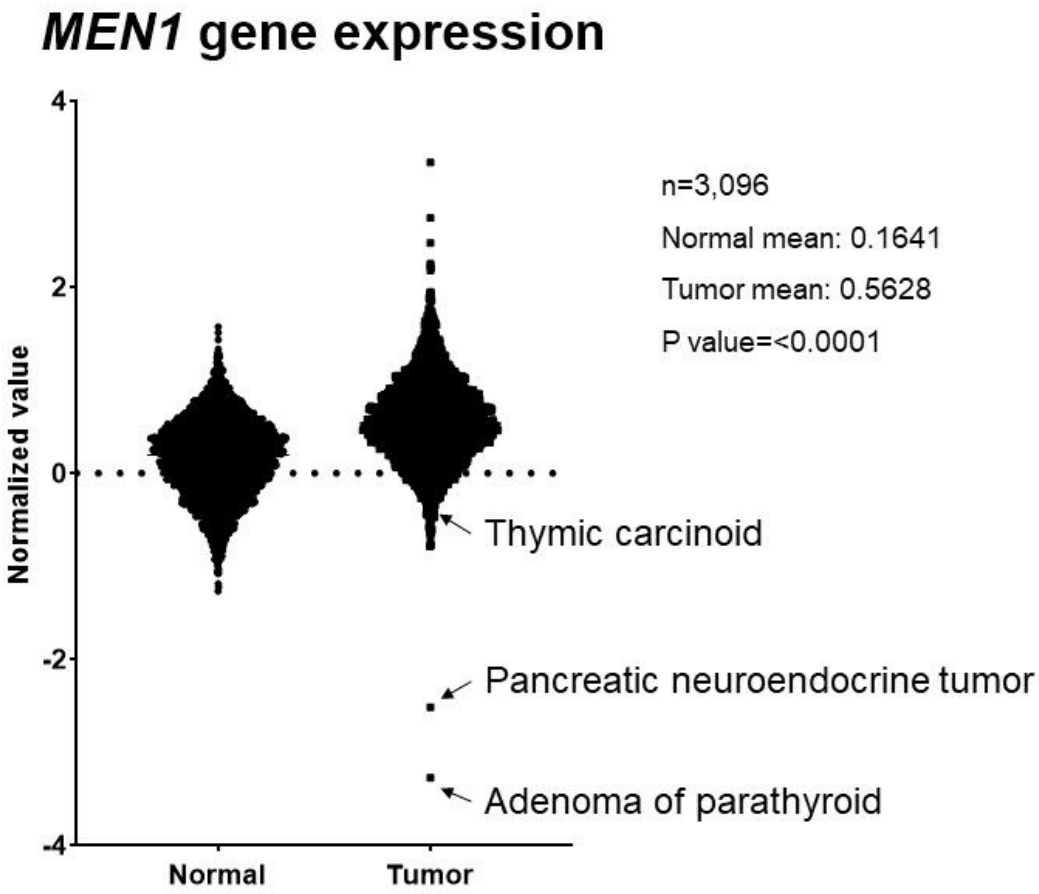

Figure 2

Comparison of MEN1 gene expression. Comparison of 3,095 tumors and normal tissue samples for MEN1 expression levels by using microarray. The pancreas and parathyroid MEN1 patient-derived samples showed remarkable reduction in MEN1 mRNA levels. The thymic carcinoid tumor had normal MEN1 expression levels mostly.

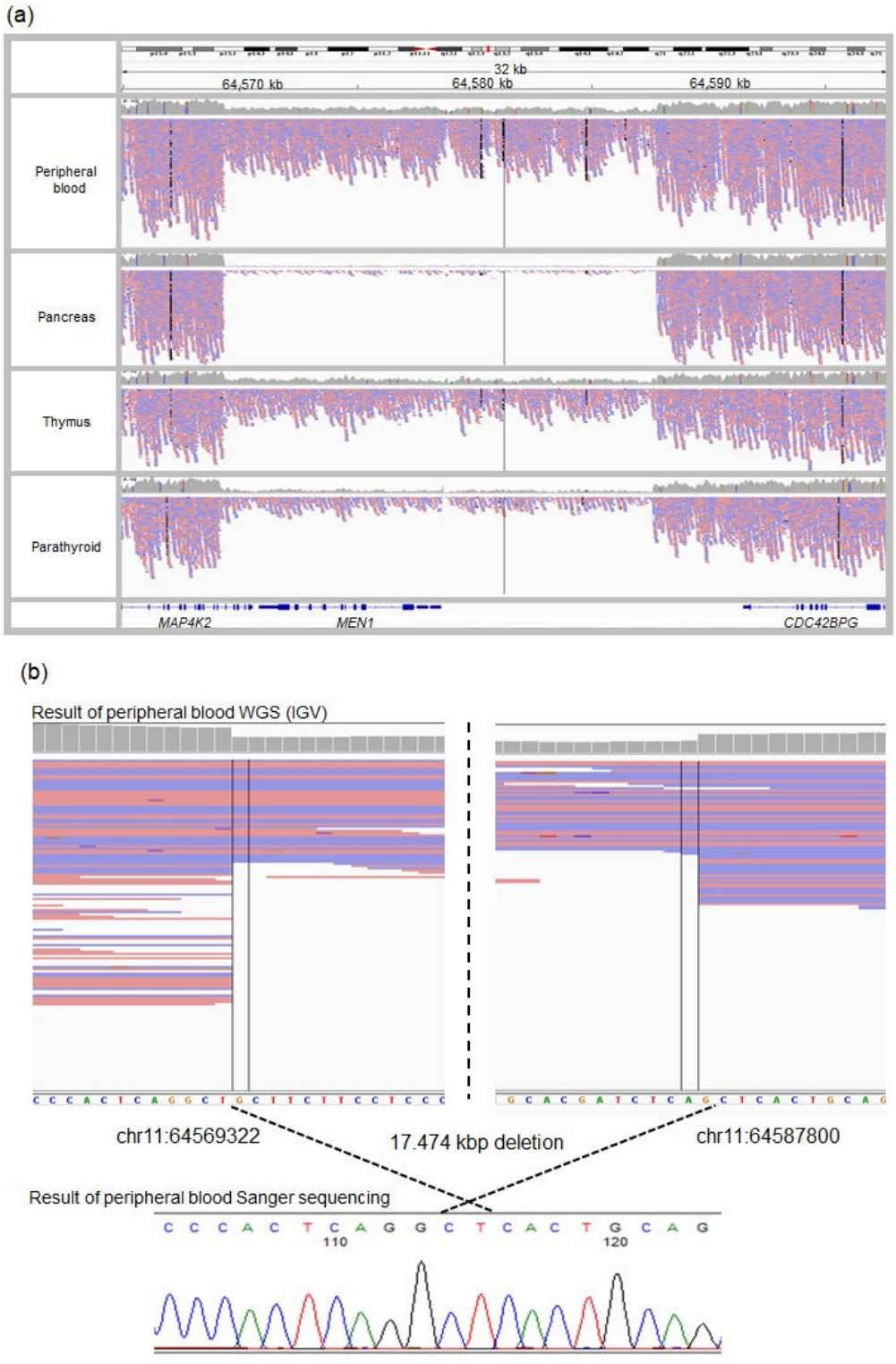

Figure 3

Result of WGS analysis in MEN1. We performed WGS and Sanger sequencing to confirm the deletion position at 18.474 kb (chr11:64569322 – chr11:64587796; GRCh37/hg19 assembly). (a) Integrative Genomics Viewer (IGV) analysis and MEN1 gross deletion. (b) Result of Sanger sequencing.

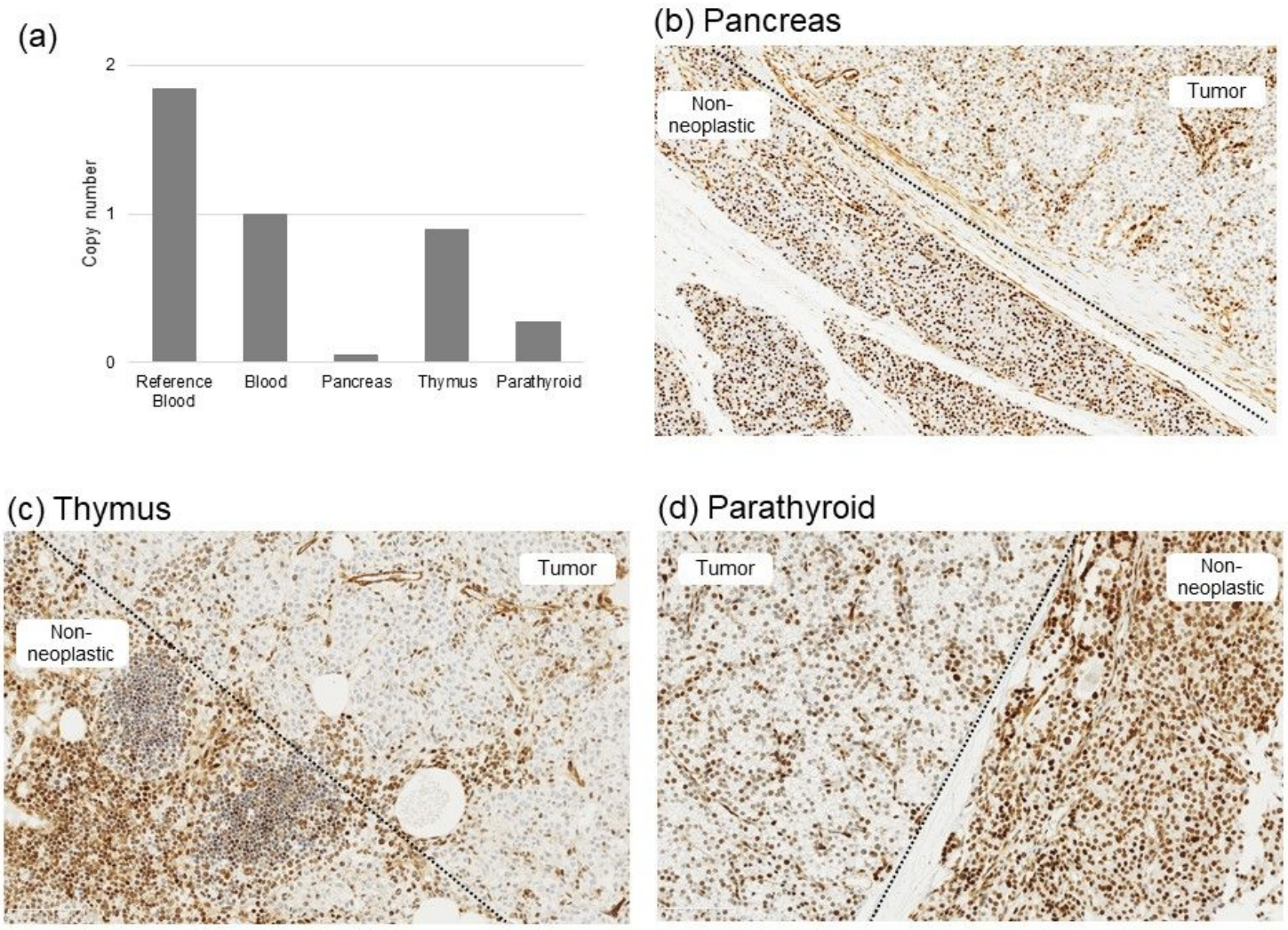

Figure 4

MEN1 phenotype and zygosity. (a) Copy numbers of MEN1 were determined by real-time PCR analysis. The blood sample of the MEN1 patient contained one MEN1 copy, as calculated in reference to the blood sample of a previously reported familial MEN1 case. (b-d) Menin staining of the sample isolated from MEN1 patient was performed using the N-terminal Menin antibody. (b) Pancreatic neuroendocrine tumor, (c) carcinoid of the thymus, and (d) adenoma of the parathyroid.

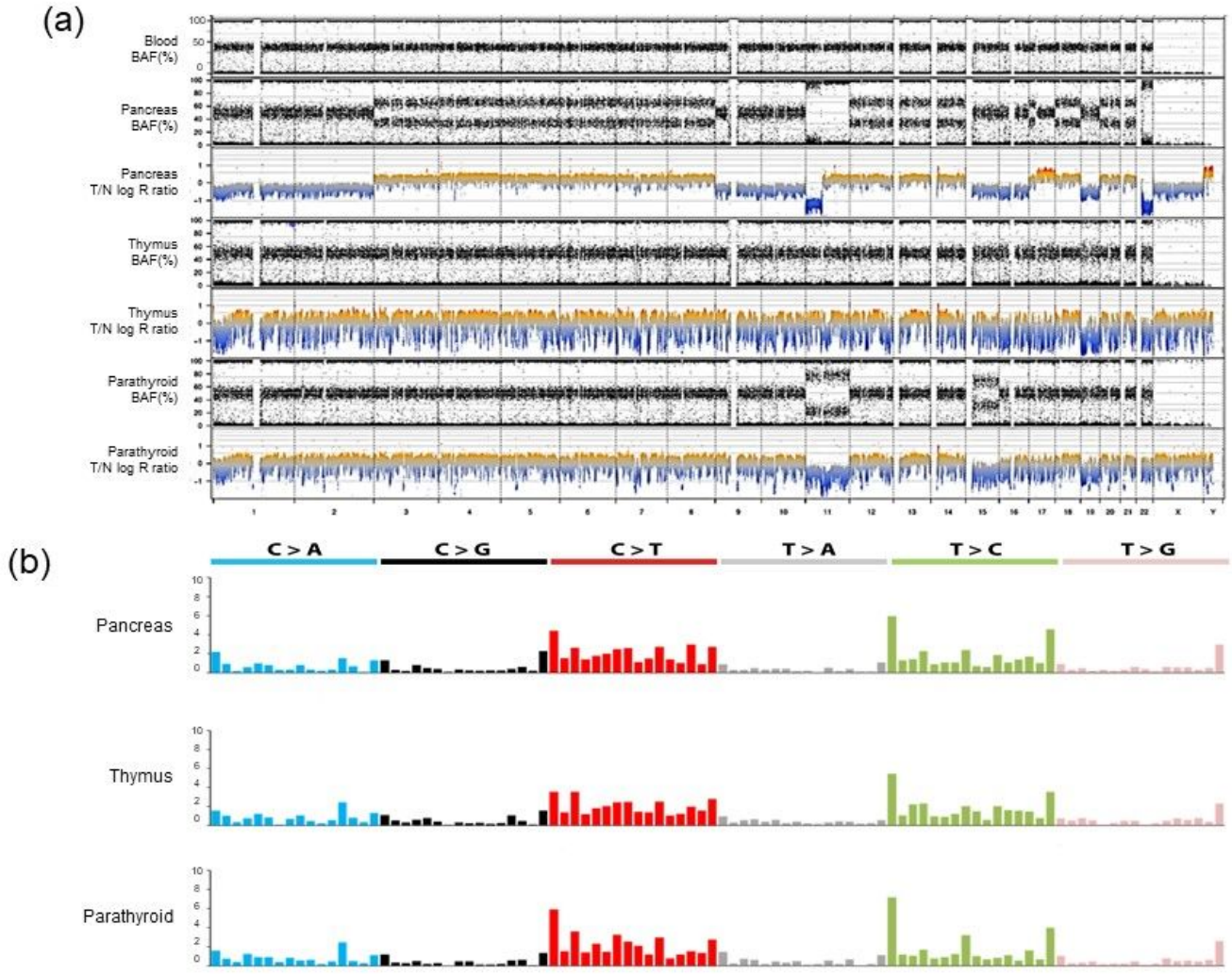

Figure 5

The chromosomal condition and mutational signature. (a) The upper lane shows the B allele frequency, and the lower lane indicates the tumor/normal read of the log R ratio calculated from the WGS result. The horizontal axe represents the chromosomal number. (b) The probability bars for the six types of substitutions are displayed in different colors. The mutation types are on the horizontal axes, whereas vertical axes depict the percentage of mutation pattern.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS2.jpg
- Table3Summaryofsomaticmutations.docx
- Table4Somaticmutationontheexonicregion.docx
- Table2Summaryofwholegenomesequencing.docx

- Table1Patientinfomation.docx
- FigureS1.jpg