Atypical pituitary adenoma with MEN1 somatic mutation associated with abnormalities of DNA mismatch repair genes; MLH1 germline mutation and MSH6 somatic mutation

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Abstract. The mechanism of pituitary tumorigenesis remains largely unknown. Lynch syndrome is an autosomal, dominantly inherited syndrome caused by a defective mismatch repair (MMR) mechanism involved in the development of various tumors at an early age. In this case study, we showed the occurrence of pituitary tumors associated with Lynch syndrome for the first time and performed genetic and immunohistochemical analysis to evaluate the genetic aberrations that might be related to the tumorigenesis and proliferation. A 68-year-old female patient with Lynch syndrome due to mutL homolog 1 (MLH1) gene mutation suffered from hypersecretion of adrenocorticotrophic hormone (ACTH), hypercortisolism and a rapidly progressive pituitary tumor. We performed genetic analysis by whole genome sequencing with genomic DNA of the pituitary tumor and peripheral blood leukocytes, as well as immunohistochemical analysis of MMR proteins. Genetic analysis revealed that the tumor had homozygous gene mutation of MEN1 associated with pituitary tumorigenesis and mutS homolog 6 (MSH6) gene. Furthermore, immunohistochemical analysis showed that MLH1 and MSH6 immunoexpression were negative. We reveal for the first time that MMR abnormality could cause somatic mutation of MEN1 and pituitary tumor occurrence is associated with Lynch syndrome. We suggest that the identified gene mutations, especially those of MSH6 and MLH1 genes, may be involved in the pathogenesis and proliferation of pituitary tumor. The knowledge obtained from our case study is important to elucidate the pathogenesis and proliferation mechanisms of pituitary tumors.

Key words: Atypical pituitary adenoma, MEN1, MLH1, MSH6, Lynch syndrome
least one of the four MMR genes, namely the MLH1, PMS1 homolog 2 (PMS2), mutS homolog 2 (MSH2) and MSH6 genes. Lynch syndrome is involved in the development of various tumors at an early age [11], and the accumulation of genetic mutations due to the abnormality in the MMR mechanism results in the pathogenesis of colorectal, endometrial, ovarian, stomach, pancreatic, ureter, renal pelvis, hepatobiliary tract and central nervous system cancers (glioblastoma), among others [12]. In addition, MMR gene expression has been studied in pancreas neuroendocrine tumors (pNETs) and is suggested to play a role in pNET pathogenesis and proliferation [13].

Here, we describe a case of pituitary tumor associated with Lynch syndrome. Although there are no previous reports of pituitary tumors associated with Lynch syndrome, we hypothesized that abnormalities of DNA MMR genes might be related to the pathogenesis of pituitary tumors and thus carried out genetic and immunohistochemical analysis of these genes in this tumor.

Case

A 68-year-old female patient was admitted to our hospital for investigation of diplopia and a right drooping eyelid that had been rapidly worsening for two months. Although magnetic resonance imaging (MRI) showed the absence of a tumor five years previously (Fig. 1A(a)), enhanced MRI in the recent admittance revealed a 24 × 18 mm tumor invading the right cavernous sinus with a weak contrast (Fig. 1A(b)). The patient’s medical history prior to this tumor included ascending colorectal cancer, ovarian cancer and descending colorectal cancer at ages 44, 45 and 49 respectively. At age 49, she was also diagnosed with Lynch syndrome due to large deletion in the MLH1 gene based on the Amsterdam II criteria and revised Bethesda criteria including genetic analysis (Fig. 1B(a)) [14]. Additionally, she suffered from breast cancer, pancreatic cancer and urinary tract papilloma at ages 62, 63 and 67 respectively. Her second daughter, who was 40 years old, had also been diagnosed with colorectal cancer and Lynch syndrome due to same mutation at 20 years of age (Fig. 1B(b)).

We suspected Cushing’s disease due to the presence of hypercortisolism caused by autonomous hypersecretion of ACTH (Supplemental Table 1). Because her eye symptoms developed rapidly, we immediately performed endoscopic endonasal transspHENoidal surgery (eTSS). Her eye symptoms improved transiently after tumor removal but worsened again one and a half months later. MRI showed rapid enlargement of the tumor (Fig. 1A(c) (d)) with about two-fold enlargement in two months. Notably, previous studies reported that the tumor volume doubling time (TVDT) of pituitary adenoma was about three to five years [15-17]. Therefore, we performed eTSS again and were able to remove most of the tumor (Fig. 1A(e)). Based on our hypothesis that abnormalities of DNA MMR genes might contribute to the pathogenesis and proliferation of pituitary tumors, we carried out genetic and immunohistochemical analysis of DNA MMR genes in the patient’s pituitary tumor. In addition, we analyzed tissues removed from the patient’s breast cancer, urinary tract papilloma and colorectal cancer and compared the results.

Materials and Method

Informed consent was obtained from the patient for the collection, analysis, and publication of personal, familial and clinical data. It was also obtained for performing genetic tests on leukocytes, pituitary tumor and other tumor-derived DNA and for expression and immunohistochemical studies on the specimen of the pituitary tumor and other tumors. This study was performed with approval of the ethics committee of Wakayama Medical University.

Whole genome sequencing and Sanger sequencing analysis

Genomic DNA was extracted from the pituitary tumor, peripheral blood leukocytes and the formalin-fixed paraffin-embedded tissues (FFPE) of the patient’s breast cancer, urinary tract papilloma and colorectal cancer using Maxwell® 16 Tissue DNA Purification Kit (AS1030; Promega), QIAamp® DNA Blood Midi Kit (51185; QIAGEN) or Maxwell® 16 FFPE Plus LEV DNA Purification Kit (AS1135; Promega). We performed whole genome sequencing (Illumina, TruSeq Nano DNA Sample Prep Kit) of genomic DNA of the pituitary tumor and peripheral blood leukocytes. Sequencing was performed on an Illumina HiSeq X platform in a paired-end 150-bp configuration. In addition, patient genomic DNA samples were screened for MLH1, MSH6, and menin 1 (MEN1) gene mutations detected by whole genome sequencing and previous study using polymerase chain reaction
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were purchased from MRC (Amsterdam, Holland). All thermal reactions were carried out on a standard thermal cycler. A sample of 100 ng of DNA was diluted in 5 μL deionised water and denatured at 98 °C for 5 min before the addition of 3 μL MLPA probe mix and buffer. The reaction mixture was denatured for 1 min and incubated for 16 h at 60 °C to ensure specific hybridization of the oligonucleotide probes with their target sequences. After the hybridization step, a buffer/ligase mixture (32 μL) was added according to the manufacturer’s instructions and incubated at 54 °C for another 15 min to stabilize the ligation reaction. Ligation was terminated by heating to 98 °C for 5 min. A fluorescent multiplex PCR amplification using a single universal primer pair suitable for all 40 probes in each kit was carried out using standard conditions (annealing temperature

**Multiplex ligation dependent probe amplification (MLPA) analysis**

Screening for all exons of the **MEN1** and **MSH6** genes required two independent MLPA kits (SALSA P244-C1-0815 and P072-C1-0613). MLPA reagents were purchased from MRC (Amsterdam, Holland). All thermal reactions were carried out on a standard thermal cycler. A sample of 100 ng of DNA was diluted in 5 μL deionised water and denatured at 98 °C for 5 min before the addition of 3 μL MLPA probe mix and buffer. The reaction mixture was denatured for 1 min and incubated for 16 h at 60 °C to ensure specific hybridization of the oligonucleotide probes with their target sequences. After the hybridization step, a buffer/ligase mixture (32 μL) was added according to the manufacturer’s instructions and incubated at 54 °C for another 15 min to stabilize the ligation reaction. Ligation was terminated by heating to 98 °C for 5 min. A fluorescent multiplex PCR amplification using a single universal primer pair suitable for all 40 probes in each kit was carried out using standard conditions (annealing temperature
Fig. 2  A: Histopathological and immunohistochemical analysis of the pituitary adenoma. (a)-(c) The pituitary tumor showed pituitary adenoma with positive immunostaining of ACTH and CAM5.2, and had a Ki-67 proliferation index >20% and positive immunostaining of p53 protein. (f)-(i) MLH1, MSH6 and PMS2 immunoexpression was negative, while that of MSH2 was positive. B: Genetic analysis of the MEN1 gene. c.1546_1547insC in the MEN1 gene as detected by whole genome sequencing was also a homozygous mutation only in the pituitary adenoma. C: Genetic analysis of MLH1 and MSH6 genes. (a) PCR detection of large deletion of the MLH1 gene. MLH1 large deletion was heterozygous in leukocytes as previously reported, but showed loss of heterozygosity in pituitary adenoma. (b) Genomic DNA sequence of the MSH6 gene. c.3253_3254insC in the MSH6 gene as detected by whole genome sequencing was a homozygous mutation in the pituitary adenoma but not in leukocytes. D: Multiplex ligation dependent probe amplification analysis for MEN1 and MSH6 gene in the pituitary adenoma. The copy number ratio of MEN1 gene (a) and MSH6 gene (b) was value 0.7-1.3 defined as normal. E: Histopathological and immunohistochemical analysis of other tumors. (a)-(c) Breast cancer, (d)-(f) Urinary tract papilloma, (g)-(i) Colorectal cancer. As in the pituitary adenoma, MLH1 immunoexpression was negative in all three other cancers. However, in contrast with the pituitary adenoma, MSH6 immunoexpression was positive.
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Histopathological and immunohistochemical analysis

FFPE sections of the pituitary tumor were examined histopathologically and immunohistochemically using the following antibodies: ACTH (02A3; DAKO), cytokeratin (CAM5.2; Becton Dickinson), Ki-67 (MIB-1,790-4286; Ventana), p53 (M7001; DAKO), MLH1 (M3640; DAKO), PMS2 (M3647; DAKO), MSH2 (#2017; Cell Signaling), MSH6 (M3646; DAKO) and menin (ab2605; Abcam). Immunohistochemistry was performed using the BenchMark Ultra automated system (Ventana). FFPE sections of breast cancer, urinary tract papilloma and colorectal cancer were examined likewise using the above antibodies with the exception of ACTH and CAM5.2.

Results

Histopathological and immunohistochemical analysis of the pituitary tumor showed pituitary adenoma with positive immunostaining of ACTH and CAM5.2, which had a Ki-67 proliferation index of >20% and positive immunostaining of p53 protein. Based on the 2004 World Health Organization classification [18], we diagnosed the patient’s pituitary tumor as an ACTH-producing pituitary atypical adenoma without metastasis because of its invasiveness, with a Ki-67 proliferation index ≥3% and extensive nuclear staining for p53 protein (Fig. 2A).

A previous study suggested that the patient had Lynch syndrome based on a large deletion in the MLH1 gene in the germ line [14]. This led us to hypothesize that a DNA MMR abnormality due to the gene mutation may have resulted in somatic mutations in genes involved in pituitary tumor development and these mutations may have led to the development of atypical pituitary adenoma. Therefore, using the genomic DNA extracted from her pituitary adenoma and leukocytes, we performed whole genome sequencing and compared the respective results. Among all mutations detected by whole genome sequencing, we focused on approximately 15,000 gene mutations within exons and splicing sites. In addition, among 15,000 gene mutations, 41 homozygous gene mutations (Table 1) and 433 heterozygous gene mutations were identified only in the pituitary adenoma and not in the leukocyte.

Eighteen of the homozygous gene mutations, including MEN1, MSH6, EZH2 and CLU, may lead to functional changes in the encoded protein (Table 1). Whole genome sequencing revealed that mutations in ubiquitin specific peptidase 8 (USP 8) gene and aryl hydrocarbon receptor interacting protein (AIP) gene, which cause Cushing’s disease, were not detected among gene mutations identified in pituitary tumor. We specifically analyzed the tumor suppressor gene MEN1 and the MMR genes, including MLH1 and MSH6.

Analysis of genomic DNA sequences showed that c.1546_1547insC in the MEN1 gene, detected by whole genome sequencing, was homozygous only in the pituitary adenoma. Furthermore, the mutation was not detected in peripheral blood leukocytes or other tumors (Fig. 2B, Supplemental Fig. 1). This mutation resulted in p.Arg516ProfsX15. In addition, this mutation site occurred in the coding short repeat microsatellite sequence, where cytosine is repeated.

PCR to detect gene mutations in MLH1 showed a heterozygous large deletion in the germ line as previously reported, but loss of heterozygosity in the pituitary adenoma (Fig. 2C(a)). In addition, c.3253_3254insC in the MSH6 gene detected by whole genome sequencing was homozygous only in the pituitary adenoma. Furthermore, the mutation was not detected in peripheral blood leukocytes and other tumors (Fig. 2C(b), Supplemental Fig. 1). This mutation resulted in p.Phe1088LeufsX5. Like the MEN1 mutation, this mutation site also occurred in the microsatellite sequence.

A MLPA analysis for all exons of the MEN1 and MSH6 genes showed that the copy numbers ratio values were 0.7-1.3 defined as normal (Fig. 2D). Therefore, the MEN1 and MSH6 genes were not lost in the pituitary adenoma and the MEN1 and MSH6 gene mutations as above were homozygous, not hemizygous.

An immunohistochemical analysis of MLH1,
| No. | Gene       | RefSeq genes | Type    | Function  | Amino acid mutation | SIFT prediction |
|-----|------------|--------------|---------|-----------|---------------------|-----------------|
| 1   | MEN1       | NM_130799.2  | INS     | Frameshift| p.Arg516ProfsX15    | DAMAGING        |
| 2   | MSH6       | NM_000179.2  | INS     | Frameshift| p.Phe1088LeufsX5    | DAMAGING        |
| 3   | EZH2       | NM_00456.4   | DEL     | Frameshift| p.Gln512AlafsX8     | DAMAGING        |
| 4   | ATRX       | NM_000489.4  | DEL     | Frameshift| p.Gln883ArgfsX21    | DAMAGING        |
| 5   | NBPF11     | NM_183372.5  | INS     | Frameshift| p.Arg521ProfsX16    | DAMAGING        |
| 6   | SMAP1      | NM_001044305.2 | DEL   | Frameshift| p.Lys169LysfsX48    | DAMAGING        |
| 7   | CFAP44(WDR52) | NM_018338.3 | DEL     | Frameshift| p.Lys667ArgfsX15    | DAMAGING        |
| 8   | ZFR        | NM_016107.3  | DEL     | Frameshift| p.Glu359GlufsX4     | DAMAGING        |
| 9   | ARHGEF2    | NM_00162383.1 | SNV    | Nonsense  | p.Arg529X           | Damaging due to stop |
| 10  | DGKD       | NM_152879.2  | SNV     | Nonsense  | p.Arg80X            | Damaging due to stop |
| 11  | PDE3B      | NM_000922.3  | DEL     | Deletion  | p.Phe129del         | DAMAGING        |
| 12  | TREG       | NM_007180.2  | DEL     | Deletion  | p.Lys409del         | DAMAGING        |
| 13  | ACE        | NM_000789.3  | SNV     | Missense  | p.Ile502Thr         | DAMAGING        |
| 14  | ACOT11     | NM_015547.3  | SNV     | Missense  | p.His230Arg         | DAMAGING        |
| 15  | ARP2P21    | NM_001267619.1 | SNV | Missense  | p.Thr290Ile         | DAMAGING        |
| 16  | CLU        | NM_001831.3  | SNV     | Missense  | p.Thr416Ala         | DAMAGING        |
| 17  | INSRR      | NM_014215.2  | SNV     | Missense  | p.Leu562Val         | DAMAGING        |
| 18  | RND3       | NM_001254738.1 | SNV  | Missense  | p.Thr231Met         | DAMAGING        |
| 19  | ADAMTS7    | NM_014272.3  | SNV     | Missense  | p.Leu1293Pro        | TOLERATED       |
| 20  | AIM1L       | NM_001039775.3 | SNV | Missense  | p.Phe542Ser         | TOLERATED       |
| 21  | APBB2      | NM_004307.1  | SNV     | Missense  | p.Arg423Leu         | TOLERATED       |
| 22  | C2CD3      | NM_001286777.1 | SNV  | Missense  | Intron              | TOLERATED       |
| 23  | DNAH1      | NM_015512.4  | SNV     | Missense  | p.Arg1354His        | TOLERATED       |
| 24  | DRD4       | NM_000797.3  | SNV     | Missense  | p.Gly272Asp         | TOLERATED       |
| 25  | FGFR1       | NM_001174067.1 | SNV  | Missense  | Intron              | TOLERATED       |
| 26  | GPR179      | NM_001004334.3 | SNV  | Missense  | p.Gly1712Glu        | TOLERATED       |
| 27  | GYP1        | NM_002099.6  | SNV     | Missense  | p.Glu24Asp          | TOLERATED       |
| 28  | JARID2      | NM_004973.3  | SNV     | Missense  | p.Val559Ile         | TOLERATED       |
| 29  | NWD2       | NM_00144990.1 | SNV  | Missense  | p.Leu546Met         | TOLERATED       |
| 30  | KIF13A     | NM_022113.5  | SNV     | Missense  | p.Ala364Thr         | TOLERATED       |
| 31  | LGR4        | NM_018490.2  | SNV     | Missense  | p.Arg374His         | TOLERATED       |
| 32  | NPIPA5      | NM_001277325.1 | SNV  | Missense  | p.Cys93Arg          | TOLERATED       |
| 33  | NPIPB6      | NM_001282524.1 | SNV  | Missense  | p.Thr285Pro         | TOLERATED       |
| 34  | PRAMEF5     | NM_00103407.2 | SNV  | Missense  | p.Gln144Arg         | TOLERATED       |
| 35  | PTCHD2      | NM_020780.1  | SNV     | Missense  | p.Thr1276Met        | TOLERATED       |
| 36  | SAMD8       | NM_001174156.1 | SNV  | Missense  | 5' UTR              | TOLERATED       |
| 37  | NBPF8, NBPF14 | SNV      | Missense | Intron | Not scored          |                 |
| 38  | MUC12       | NM_001164462.1 | SNV  | Missense  | p.Asp868Gly         | Not scored       |
| 39  | CCLAL2      | NM_001291475.1 | SNV  | Missense  | p.Gly78Arg          | Not scored       |
| 40  | AP3M1       | NM_207012.2  | DEL     | Splice acceptor site | Splice acceptor site | Not scored |
| 41  | CACNA2D2    | NM_001174051.2 | SNV  | Splice donor site | Splice donor site | Not scored |

Eighteen homozygous gene mutations may lead to functional changes in the encoded proteins.
MSH6, MSH2 and PMS2 in the pituitary adenoma showed that MLH1 and MSH6 immunoexpression was negative, reflecting the results of the genetic analysis. PMS2 immunoexpression was also negative (Fig. 2A). Because MLH1 and PMS form a heterodimer and function, it was considered that a decrease in MLH1 expression may have caused a decrease in the PMS2 expression. Also, immunohistochemical analysis of menin in the pituitary adenoma showed that menin immunoexpression was negative, reflecting the results of the genetic analysis (Supplemental Fig. 2).

A genetic analysis of MSH6 and MEN1 gene mutations of the patient’s breast cancer, urinary tract papilloma and colorectal cancer did not show the same mutations as above (Supplemental Fig. 1). Also, immunohistochemical analysis of these other tumors showed that, unlike the pituitary adenoma, MLH1 immunoexpression was negative but MSH6 and menin immunoexpression were positive (Fig. 2E, Supplemental Figs. 2–5).

Discussion

There are no previous reports of pituitary tumors associated with Lynch syndrome. In this case study, whole-genome sequencing of the leukocytes and pituitary tumor in a patient with Lynch syndrome revealed a germline mutation in the MLH1 gene and homozygous somatic mutations in the MEN1 and MSH6 gene in the pituitary adenoma. MEN1 gene mutation (p.Arg516fsProfsX15) and MSH6 gene mutation (p.Phe1088LeufsX5) in this case could cause a decrease in the MSH6 and menin protein by nonsense-mediated mRNA decay, resulting in the pathogenesis of the pituitary tumor and abnormalities of MMR mechanism.

DNA MMR is a system for recognizing and repairing erroneous insertion, deletion, and mis-incorporation of bases that can arise during DNA replication and recombination, as well as repairing some forms of DNA damage. DNA MMR proteins, MLH1 and MSH6, function in sequential steps to initiate repair of DNA mismatches and the abnormalities of these proteins results in the tumorigenesis in colorectal cancer. The mechanism of DNA MMR in humans can be divided into four main steps: (a) mismatched DNA base pairs are recognized by the heterodimers MSH2/MSH6 (MutSα) and MSH2/MSH3 (MutSβ), which depend on ATP; (b) among the heterodimers MLH1/PMS2 (MutLα), MLH1/PMS1 (MutLβ) and MLH1/MLH3 (MutLγ), primarily MutLα is activated in an ATP-dependent manner and binds to MutSα or MutSβ; (c) MutLα with endonuclease activity causes a nick in the mismatch DNA and the DNA strand containing the incorrect nucleotide is removed by exonuclease EXO1; and (d) the excision gap is resynthesized by the replicative DNA polymerase δ using the remaining DNA strand without the incorrect nucleotide as a template [9, 10]. In colorectal cancer with Lynch syndrome, tumor progression is accelerated by the rapid accumulation of mutations in coding repetitive sequences (microsatellite sequence) of target genes with growth-related functions, such as growth factor receptors (TGFB2 and IGF2R) and genes involved in apoptosis (BAX) and DNA repair (MSH3 and MSH6) [7, 19]. We considered that in addition to the abnormal excision of mismatched DNA due to the MLH1 large gene deletion, the abnormal recognition of mismatched DNA due to a MSH6 gene frameshift mutation resulted in an increased mutation frequency and the accumulation of genetic abnormalities might have resulted in the pathogenesis of the pituitary tumor in the present case.

Among homozygous mutations that occur only in the pituitary adenoma, loss-of-function mutation of MEN1 gene results in the pathogenesis of multiple endocrine neoplasia type 1 (MEN1) tumors in various endocrine organs such as the parathyroid, endocrine pancreas, anterior pituitary and adrenal cortex [20, 21]. CLU is reported to be involved in the pathogenesis and proliferation of nonfunctioning pituitary adenoma (NFA) [22].

Over 1,300 MEN1 gene mutations have been reported to date. Among them, c.1546_1547insC (p.Arg516fsProfsX15) mutation is a potential mutational hot spot which accounts for 2.7% of MEN1 gene mutation. In addition, c.1546delC has also been reported to occur frequently at 1.8% of them [23]. As this mutation site is a microsatellite sequence where errors tend to occur during DNA replication, mutations due to MMR abnormality are likely to occur. Recently, it has been shown that MMR gene abnormality is associated with pNET pathogenesis and proliferation [13]. Therefore, since MEN1 gene may be a target gene of MMR abnormality, it is necessary to further investigate the association between MEN1 gene and MMR gene abnormality. Also, menin encoded by MEN1 has been shown to interact with a number of proteins that are involved in transcriptional regulation, genome stability, cell division and proliferation [24, 25]. We hypothesized
that loss-of-function of menin by p.Arg516fsProfsX15 might affect the binding between menin and SMAD family member 3 (SMAD3), transcriptional activators and cell signaling proteins [26], forkhead box N3 (FOXN3, CHES1), transcriptional repressors [27] and activator of S-phase kinase (ASK) [28], a protein involved in cell cycle (Fig. 2B) [23]. Although the detailed mechanism of the pituitary tumor caused by MEN1 mutation has not been fully elucidated, this homozygous mutation could be involved in the pathogenesis of pituitary tumors [23, 29].

CLU is an intra- and extra-cellular glycoprotein that is also variously known as apolipoprotein J, transient receptor potential cation, subfamily M, member 2; or sulfated glycoprotein-2 [30, 31]. CLU is constitutively induced in many benign tumors or early stage cancers [32], whereas in more advanced cancer stages it is often down-regulated [33]. In nonfunctioning pituitary adenoma (NFA), gonadotroph-derived tumors show high expression of CLU and intracellular pituitary CLU restrains gonadotroph tumor cell proliferation by inducing the cyclin-dependent kinase (Cdk) inhibitors p15, p16 and p27 [34]. In NFA of gonadotroph origin, CLU also suppresses pituitary tumor transforming gene (PTTG) [22] and thus protects tumor cells from excessive proliferation and oncogenic transformation [35]. Therefore, although the pituitary tumor in our case was ACTHoma, CLU mutation (p.Thr416Ala) might be associated with the pathogenesis and proliferation of pituitary tumors.

Enhancer of zeste homolog 2 (EZH2) is a core epigenetic regulator, playing a crucial role in cell cycle regulation. The protein is known to be associated with proliferation and worse outcomes in several tumor entities. A previous study revealed a distinct correlation between immunohistochemically detectable EZH2 and Ki-67 expression in pituitary adenomas [36]. In vitro experiments using the pituitary cell line AtT-20 revealed a significant decrease of tumor cell proliferation using the EZH2 inhibitor GSK126 [36]. Therefore, frameshift mutation of EZH2 gene (p.Gln512AlafsX8) leads to a decrease in the function of EZH2, which might suppress tumor cell proliferation.

Thus, we speculate that the accumulation of genetic abnormalities, including MEN1 and CLU mutation, which might result from the dysfunction of both MLH1 and MSH6, could be associated with the pituitary tumorigenesis and proliferation in this case. We consider that MSH6 mutation associated with abnormalities of MLH1 resulted in the collapse of MMR system and increased mutation frequencies.

On the other hand, the pituitary tumor in the present case showed rapid growth. Generally, the TVDT in pituitary adenoma is approximately three to five years [15-17], whereas in our case it was about two months. According to the immunohistochemical analysis of Ki-67 proliferation index and p53, the pituitary tumor was categorized as an atypical pituitary adenoma. It was reported that MSH6 was involved in cell apoptosis as well as DNA MMR [37]. Matsuno et al. showed that MSH6 immunoeexpression was decreased in four of 13 cases of pituitary carcinomas and atypical pituitary adenomas [38]. In addition, a case was reported in which loss of MSH6 occurred during the progression from an atypical pituitary adenoma to a pituitary carcinoma [39]. Therefore, although there is no evidence for the association of MSH6 gene with aggressive pituitary tumor growth so far, we suspect that loss of function due to the MSH6 gene mutation (p.Phe1088LeufsX5) might have been involved not only in DNA MMR but also directly in regulation of cell proliferation in our case.

In conclusion, the occurrence of a pituitary tumor associated with Lynch syndrome was demonstrated for the first time by genetic and immunohistochemical analysis. The MEN1 gene mutation identified in this case study was considered to be involved in the pathogenesis of the pituitary tumor in this case. Additionally, we have shown that MEN1 can be a target gene of MMR abnormality. We suggest that the loss-of-function of MSH6 could be involved in promotion of tumor proliferation in atypical pituitary adenoma. Further studies are required to reveal the relationship between pituitary tumor growth and the MSH6 gene. The knowledge obtained from this case study is important for elucidating the pathogenesis and proliferation of pituitary tumors.

Acknowledgments

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Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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Supplemental Table 1  Laboratory investigations at presentation

| Parameter            | Reference range |
|----------------------|-----------------|
| WBC (10^2/μL)        | 79.0            |
| Neutrophil (%)       | 82.5            |
| Eosinophil (%)       | 0.0             |
| Lymphocyte (%)       | 14.4            |
| Alb (g/dL)           | 3.9             |
| CK (IU/L)            | 100.0           |
| AST (IU/L)           | 25.0            |
| ALT (IU/L)           | 23.0            |
| LDH (IU/L)           | 351.0           |
| ALP (IU/L)           | 126.0           |
| Alb (g/dL)           | 3.9             |
| Cre (mg/dL)          | 0.6             |
| BUN (mg/dL)          | 21.0            |
| cGFR (mL/min/1.73m^2)| 79.0            |
| Na (mEq/L)           | 139.0           |
| K (mEq/L)            | 4.7             |
| TG (mg/dL)           | 235.0           |
| T-Chol (mg/dL)       | 314.0           |
| PG (mg/dL)           | 101.0           |
| HbA1c (%)            | 6.2             |
| ACTH (pg/mL)         | 263.8           |
| Cortisol (µg/dL)     | 20.4            |
| LH (mIU/mL)          | 0.2             |
| FSH (mIU/mL)         | <0.1            |
| E2 (pg/mL)           | <10.0           |
| GH (ng/mL)           | 0.3             |
| IGF-1 (ng/mL)        | 86              |
| PRL (ng/mL)          | 19.3            |
| TSH (µIU/mL)         | 0.015           |
| FT3 (pg/mL)          | 1.34            |
| FT4 (ng/dL)          | 0.83            |
| WBC (10^2/μL)        | 79.0            |
| Neutrophil (%)       | 82.5            |
| Eosinophil (%)       | 0.0             |
| Lymphocyte (%)       | 14.4            |
| Alb (g/dL)           | 3.9             |
| CK (IU/L)            | 100.0           |
| AST (IU/L)           | 25.0            |
| ALT (IU/L)           | 23.0            |
| LDH (IU/L)           | 351.0           |
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| GH (ng/mL)           | 0.3             |
| IGF-1 (ng/mL)        | 86              |
| PRL (ng/mL)          | 19.3            |
| TSH (µIU/mL)         | 0.015           |
| FT3 (pg/mL)          | 1.34            |
| FT4 (ng/dL)          | 0.83            |

Supplemental Table 2  Primers for PCR amplification of MLH1, MSH6, and MEN1 genes

| Gene     | Primer name | Sequence                              |
|----------|-------------|---------------------------------------|
| MLH1     | F1          | 5’-TTCCATTTGGCCCTGTACTTC-3’            |
|          | F2          | 5’-GTTGGCAGTGGGTGCTACT-3’              |
|          | R1          | 5’-CCCTCTGAATGTTAGTGG-3’               |
|          | R2          | 5’-GAGTAGCCACCTGTTGGTC-3’              |
|          | R3          | 5’-GCTTCCATGGGACTAAGACG-3’             |
| MSH6     | F1          | 5’-GGGGAGATCGTTGGACTGTA-3’             |
|          | R1          | 5’-CAGGGAGTAAATTTCCCTTG-3’             |
| MEN1     | F1          | 5’-CCCTTGCTCTACCTTGCTCT-3’             |
|          | R1          | 5’-CCGCTTTGAGAAAGACAG-3’               |

Supplemental Fig. 1  Genetic analysis of MEN1 and MSH6 genes in other tumors
c.1546_1547insC in the MEN1 gene and c.3253_3254insC in the MSH6 gene were not identified in breast cancer, urinary tract papilloma or colorectal cancer.

Supplemental Fig. 2  Immunohistochemical analysis for menin of pituitary adenoma, breast cancer, urinary tract papilloma, and colorectal cancer

In the pituitary adenoma, menin immunoreexpression was negative. Unlike the pituitary adenoma, menin immunoreexpression was positive in all three other cancers.
Supplemental Fig. 3  Histopathological and immunohistochemical analysis of breast cancer
As in the pituitary adenoma, MLH1 immunoexpression was negative. However, in contrast with the pituitary adenoma, MSH6 and PMS2 immunoexpression was positive.

Supplemental Fig. 4  Histopathological and immunohistochemical analysis of urinary tract papilloma
As in the pituitary adenoma, MLH1 and PMS2 immunoexpression was negative. However, in contrast with the pituitary adenoma, MSH6 immunoexpression was positive.

Supplemental Fig. 5  Histopathological and immunohistochemical analysis of colorectal cancer
As in the pituitary adenoma, MLH1 immunoexpression was negative. However, in contrast with the pituitary adenoma, MSH6 and PMS2 immunoexpression was positive.
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