SHORT COMMUNICATION

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Novel *MEN1* gene mutations in familial multiple endocrine neoplasia type 1

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Abstract The recent isolation of the gene responsible for multiple endocrine neoplasia type 1 (MEN 1) has enabled direct genetic diagnosis for people with endocrine tumors and family members of affected patients. Although MEN 1 is rarely recognized in the Japanese population compared to its prevalence in Caucasians, we have previously reported a high prevalence of this disease in a limited area (Nagano Prefecture; population, 2.15 million). In this communication, we report mutations of the *MEN1* gene in kindreds living in Nagano Prefecture. The absence of a common mutation among these kindreds indicates that the high prevalence of MEN 1 in this area is not due to a regional accumulation of patients descended from a common ancestor. This result implies that the prevalence of MEN 1 in other areas of Japan could also be higher than had been thought.

Key words Multiple endocrine neoplasia type 1 · Menin · Endocrine tumor · Mutation · Founder effect

Introduction

Multiple endocrine neoplasia type 1 (MEN 1) is a syndrome characterized by a hyperplastic or neoplastic disorder of endocrine organs. The gene responsible for MEN 1, designated *MEN1*, has recently been identified by two independent groups (Chandrasekharappa et al. 1997; The European Consortium on MEN1 1997). The *MEN1* gene consists of one non-coding exon and nine coding exons that encode a 610-amino-acid protein, “menin”. Germline mutations of the *MEN1* gene have been identified in most people with familial and sporadic MEN 1 (Chandrasekharappa et al. 1997; The European Consortium on MEN1 1997; Agarwal et al. 1997; Bassett et al. 1998). No apparent hot spots have been observed, suggesting that the entire coding region is important for the as yet unknown functions of the menin protein.

Although several *MEN1* gene mutations have been reported in Japanese patients (Shimizu et al. 1997; Tanaka et al. 1998), MEN 1 has rarely been recognized in Japan (Yoshimoto and Saito 1991). We have previously reported that the prevalence of this syndrome in a limited area of Japan (Nagano Prefecture; population, 2.15 million) is not significantly different from that in Caucasians, and the apparently low prevalence of MEN 1 in other areas of Japan may be due to an incomplete recognition of this disease (Katai et al. 1997). To exclude the possibility that Nagano prefecture is a geographic exception, as are Tasmania and Newfoundland (Shepherd, 1991; Petty et al. 1994) where patients originating from common ancestors reside in islands, we analyzed the *MEN1* gene mutations in patients living in Nagano Prefecture.

Subjects and methods

The pedigrees of affected kindreds and clinical features of the subjects have been described previously (Sakurai et al. 1996; Katai et al. 1997). Except for one family, all families

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whose analysis is reported in this communication have lived in Nagano Prefecture for more than three generations. Blood samples were drawn from patients and family members, with their written informed consents, and high-molecular weight DNA was isolated, as described previously (Sakurai et al. 1996). Oligonucleotide primers for amplification and sequencing of the *MEN1* gene were prepared according to the information available at the web site, <http://www.nhgri.nih.gov/DIR/LGT/MEN1/table1.html> (Chandrasekharappa et al. 1997). DNA fragments amplified by polymerase chain reaction (PCR) were purified and directly sequenced using the ABI PRISM310 Genetic Analyzer (Perkin-Elmer, Foster City, CA, USA) with appropriate sequencing primers. Mutations were confirmed either by sequencing both strands or by restriction digestion when suitable enzymes were available.

Results

Among 14 families studied for mutations in the *MEN1* gene, we identified eight different germline mutations in 11 families. These are summarized in Table 1. There were two in-frame deletions in three families, four frame-shift deletions in four families, and two frame-shift insertions in four families. In three families, no mutations were found in exons 2 to 10 and exon/intron boundaries. Three mutations, 359del4, K119del, and 1657insC were identical to those previously reported (Table 1). When appropriate restriction sites were available, family members were screened for mutations by digestion of amplified DNA fragments. Cosegregation of the *MEN1* gene mutations and disease phenotype was observed in all families examined. As has been reported (Bassett et al. 1998), no apparent genotype-phenotype correlation was observed among the patients.

Discussion

The absence of a common ancestral mutation among patients living in Nagano Prefecture indicates that the high

prevalence of MEN 1 in this area is not due to a founder effect, and this may allow the prediction of a similar prevalence in other areas of Japan. As we have previously reasoned (Katai et al. 1997), many patients may still be left unrecognized in Japan. The diagnosis of MEN 1 early in its clinical course is crucial for a better prognosis (Wilkinson et al. 1993), and collection of clinical information on patients will enable better management of this disease.

Although there are no apparent hot spots in the *MEN1* gene, there is a relative accumulation of mutations in some regions, such as codons 83–85, 209–211, and 516. Several different mutations in these regions have been reported in Caucasian families, and two of them were, despite the ethnic difference, identical to the mutation we have identified (359del4 and 1657insC). Bassett et al. (1988) reported that mutations in codons 83/84, 210/211, and 516 accounted for 6%, 11%, and 6%, respectively, of the mutations they identified. Five of the 14 families (36%) in our study also harbored mutations in one of these regions (Table 1). The accumulation of mutations in such regions may reflect a structural susceptibility to mutation, rather than being of functional importance (Bassett et al. 1998).

Identification of the responsible gene has enabled the genetic diagnosis of MEN 1. Before the isolation of the *MEN1* gene, family members of patients needed to undergo an annual biochemical and radiological screening for early management of clinical manifestations (Vasen et al. 1989). With genetic analysis, early diagnosis and better clinical management can be achieved for gene carriers. And for non-carriers, the annual screening of endocrine organs will become unnecessary. Also, for patients with single endocrine manifestations such as hyperparathyroidism, pituitary tumor, or pancreatic endocrine tumor, evaluation of the *MEN1* gene in germline DNA will help to determine whether these lesions are initial manifestations of MEN 1 and whether future follow-up is necessary for other endocrine organs. However, it is to be noted that no mutations were found in some families with MEN 1 (Shimizu et al. 1997; Bassett et al. 1998). Some of these families may have a large deletion in the *MEN1* gene (Kishi et al. 1998), but a family with the clinical features of MEN 1 but not linked to

Table 1 Germline mutations of the *MEN1* gene

Exon	Codon	Type of mutation	Amino acids (aa)	Abbreviation	Restriction site ^a	No. of families	Previous report
2	83–84	4 Base deletion	Frame shift	359del4	(–)	1	^b
2	119	3 Base deletion	1 aa (119) deleted	K119del	– <i>Mbo</i> II	1	^c
3	171–173	9 Base deletion	3 aa (171–173) deleted	621del9	– <i>Fok</i> I	2	New mutation
3	210–211	4 Base deletion	Frame shift	738del4	+ <i>Bsr</i> I	1	New mutation
7	345	1 Base deletion	Frame shift	1143delG	– <i>Eae</i> I	1	New mutation
9	438	1 Base insertion	Frame shift	1422insA	(–)	1	New mutation
10	455–456	5 Base deletion	Frame shift	1473del5	+ <i>Fsp</i> I	1	New mutation
10	516	1 Base insertion	Frame shift	1657insC	(–)	3	^d
Mutations not found						3	

^a + (plus) or – (minus) signs placed to the left of restriction enzyme names indicate either the generation or the loss of restriction sites by mutation

^b The European Consortium on MEN 1 1997; Bassett et al. 1998

^c Agarwal et al. 1997; Shimizu et al. 1997

^d Bassett et al. 1998

the *MEN1* locus has also been reported (Stock et al. 1997). Thus, absence of the *MEN1* gene mutation, as determined by PCR and the following sequencing, cannot completely rule out the possibility that MEN 1 is present.

In summary, we have identified a number of *MEN1* gene mutations in families with MEN 1. The collection of many mutations, as well as functional analysis of wild-type and mutant menin protein, will facilitate our understanding of the molecular mechanism responsible for the tumorigenesis in MEN 1, and this, in turn, will enable better clinical management of patients with this syndrome.

References

- Agarwal SK, Kester MB, Debelenko LV, Heppner C, Emmert-Buck MR, Skarulis MC, Doppman JL, Kim YS, Lubensky IA, Zhuang Z, Green JS, Guru SC, Manickam P, Olufemi SE, Liotta LA, Chandrasekharappa SC, Collins FS, Spiegel AM, Burns AL, Marx SJ (1997) Germline mutations of the *MEN1* gene in familial multiple endocrine neoplasia type 1 and related states. *Hum Mol Genet* 6: 1169–1175
- Bassett JH, Forbes SA, Pannett AA, Lloyd SE, Christie PT, Wooding C, Harding B, Besser GM, Edwards CR, Monson JR, Sampson J, Wass JA, Wheeler MH, Thakker RV (1998) Characterization of mutations in patients with multiple endocrine neoplasia type 1. *Am J Hum Genet* 62: 232–244
- Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J, Boguski MS, Agarwal SK, Kester MB, Kim YS, Heppner C, Dong Q, Spiegel AM, Burns AL, Marx SJ (1997) Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* 276: 404–407
- Katai M, Sakurai A, Itakura Y, Ikeo Y, Nakajima K, Hara M, Iijima S, Kaneko T, Kobayashi M, Ichikawa K, Aizawa T, Hashizume K (1997) Multiple endocrine neoplasia type 1 is not rare in Japan. *Endocr J* 44: 841–845
- Kishi M, Tsukada T, Shimizu S, Futami H, Ito M, Kanbe M, Obara T, Yamaguchi K (1998) A large germline deletion of the *MEN1* gene in a family with multiple endocrine neoplasia type 1. *Jpn J Cancer Res* 89: 1–5
- Petty EM, Green JS, Marx SJ, Taggart RT, Farid N, Bale AE (1994) Mapping the gene for hereditary hyperparathyroidism and prolactinoma (*MEN1_{Burin}*) to chromosome 11q: Evidence for a founder effect in patients from Newfoundland. *Am J Hum Genet* 54: 1060–1066
- Sakurai A, Katai M, Itakura Y, Nakajima K, Baba K, Hashizume K (1996) Genetic screening in hereditary multiple endocrine neoplasia type 1: Absence of a founder effect among Japanese families. *Jpn J Cancer Res* 87: 985–994
- Shepherd JJ (1991) The natural history of multiple endocrine neoplasia type 1. Highly uncommon or highly unrecognized? *Arch Surg* 126: 935–952
- Shimizu S, Tsukada T, Futami H, Ui K, Kameya T, Kawanaka M, Uchiyama S, Aoki A, Yasuda H, Kawano S, Ito Y, Kanbe M, Obara T, Yamaguchi K (1997) Germline mutations of the *MEN1* gene in Japanese kindred with multiple endocrine neoplasia type 1. *Jpn J Cancer Res* 88: 1029–1032
- Stock JL, Warth MR, Teh BT, Coderre JA, Overdorf JH, Baumann G, Hintz RL, Hartman ML, Seizinger BR, Larsson C, Aronin N (1997) A kindred with a variant of multiple endocrine neoplasia type 1 demonstrating frequent expression of pituitary tumors but not linked to the multiple endocrine neoplasia type 1 locus at chromosome region 11q13. *J Clin Endocrinol Metab* 82: 486–492
- Tanaka C, Yoshimoto K, Yamada S, Nishioka H, Ii S, Moritani M, Yamaoka T, Itakura M (1998) Absence of germ-line mutations of the multiple endocrine neoplasia type 1 (*MEN1*) gene in familial pituitary adenoma in contrast to *MEN1* in Japanese. *J Clin Endocrinol Metab* 83: 960–965
- The European Consortium on MEN1 (1997) Identification of the multiple endocrine neoplasia type 1 (*MEN1*) gene. *Hum Mol Genet* 6: 1177–1183
- Vasen HFA, Lamers CBHW, Lips CJW (1989) Screening for the multiple endocrine neoplasia syndrome type 1. A study of 11 kindreds in the Netherlands. *Arch Int Med* 149: 2717–2722
- Wilkinson S, The BT, Davey KR, McArdle JP, Young M, Shepherd JJ (1993) Cause of death in multiple endocrine neoplasia type 1. *Arch Surg* 128: 683–690
- Yoshimoto K, Saito S (1991) Clinical characteristics in multiple endocrine neoplasia type 1 in Japan (in Japanese). *Folia Endocrinol Japon* 67: 764–774