CASE REPORT

Acromegaly in a multiple endocrine neoplasia type 1 (MEN1) family with low penetrance of the disease

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Abstract

Multiple endocrine neoplasia type 1 (MEN1) is an inherited syndrome that is characterised by the occurrence of tumours in the parathyroid glands, the endocrine pancreas, the pituitary gland and the adrenal glands and by neuroendocrine carcinoid tumours, often at a young age. The penetrance of MEN1 among gene carriers is reported to be high; 82–99% at age 50. We present a patient with a history of parathyroid adenomas also showing signs of acromegaly. He turned out to be a carrier of a MEN1 germ-line mutation in intron 3 (IVS3-6C > G). This germ-line mutation was also found in nine of his family members. However, none of these relatives have developed any MEN1-related lesion yet, although several are older than 60 years. To our knowledge, a MEN1 family with as few clinical features as this family has not been reported to date. Because MEN1 patients have an increased risk of developing acromegaly, insulin-like growth factor (IGF-I) levels are monitored periodically. We investigated whether IGF-I levels might serve as a presymptomatic marker for acromegaly; 9% (3/33) of MEN1 patients showed temporary IGF-I elevations. One patient (1/3) later developed clinical signs of acromegaly. Possibly, acromegaly in MEN1 is preceded by a transient preacromegalic state.

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Introduction

Multiple endocrine neoplasia type 1 (MEN1) (see Online Mendelian Inheritance in Man (OMIM), no. 131100; URL: www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) is an autosomal dominantly inherited disorder, characterised by the occurrence of tumours of the parathyroid glands, the pituitary gland, the pancreatic islets and the adrenal cortex and neuroendocrine carcinoid tumours, often at a young age. Non-endocrine manifestations include several other tumour types, such as collagenomas, angiofibromas, lipomas and leiomyomas (1). MEN1 is caused by inactivation of germ-line mutations of the MEN1 tumour suppressor gene, which is located on chromosome 11q13 (2, 3). The MEN1 gene product, termed 'menin', is a 610-amino-acid protein that is expressed in all tissues, but predominantly in the nucleus (4). Menin is a regulator of gene transcription; it has been found to interact with several transcription factors, including JunD, Smad3 and NF- κ B (5–7).

The identification of the MEN1 gene has enabled DNA diagnostics by mutation analysis. Identifying germ-line mutation carriers at an early age is important, since periodical clinical monitoring of mutation

carriers enables presymptomatic diagnosis and treatment of lesions associated with MEN1, most likely improving both life expectancy and quality of life (8).

More than 400 different germ-line mutations have been identified, scattered throughout the entire MEN1 gene. An overview of MEN1 germ-line mutations can be found at the website of the Human Gene Mutation Database (www.hgmd.org). According to the literature, disease penetrance among MEN1 germ-line mutation carriers is 82–99% at age 50 (9–11). Overall, a clear genotype–phenotype correlation has not been established (12, 13). However, in a limited number of families, MEN1 gene germ-line mutations seem to cause isolated primary hyperparathyroidism (14). Furthermore, the MEN1-Burin mutation is associated with a high prevalence of prolactinoma (15).

In this report, we present a patient with hyperparathyroidism and acromegaly. MEN1 mutation analysis showed a previously unknown MEN1 germ-line mutation. Nine family members proved to be carriers of the same mutation. Remarkably, none of these relatives showed any clinical manifestation of MEN1. Since the pathophysiological background of acromegaly is still largely unknown, and MEN1 carriers

may develop acromegaly, we analysed 33 MEN1 patient records of our outpatient clinic for presymptomatic signs of acromegaly, particularly insulin-like growth factor I (IGF-I) levels.

Index patient

At age 38, the index patient underwent resection of two parathyroid adenomas. One year later, hypercalcaemia recurred, and he was referred to our hospital. By venous sampling, an additional parathyroid adenoma was found to be located retrosternally, embedded in thymus tissue. This tumour was removed. During physical examination, enlarged hands, coarse facial features and several collagenomas were noticed. The family history of the patient revealed that his mother died at age 62 from a medullary thyroid carcinoma with lung, bone, liver and skin metastases. His father died at age 55 from lung cancer. The postoperative workup for acromegaly revealed unsuppressible growth hormone (GH) levels during an oral glucose tolerance test (OGTT) (19 mU/l) (Fig. 1). From radiographic images of the skull and computed tomography scan, the presence of a tumour in the pituitary gland was suspected. A trans-sphenoidal resection was performed. Histopathological examination confirmed that a GH-producing adenoma had been removed (Fig. 2). The patient declined additional radiotherapy. Postoperatively, GH levels during an OGTT were normal (2 mU/l) (Fig. 1). None of the other pituitary functions had been affected.

After several years, periodical monitoring showed increasing IGF-I levels of 380–520 ng/ml (age- and gender-adjusted reference ± 2 s.d.: 70–200 ng/ml), with normal prolactin levels. Recurrence of the pituitary adenoma was suspected. However, magnetic resonance imaging (MRI) did not show recurrence of the pituitary adenoma, nor were there any abnormalities in the pancreas. Although the features of acromegaly had not increased, at age 55, the patient was

readmitted to our hospital. Diurnal GH levels and OGTT were normal (nadir 1.8 mU/l), and colonoscopy revealed no polyps or other signs of malignancy. Combined treatment with long-acting octreotide and cabergoline reduced IGF-I levels to normal (169 ng/ml).

Patients and methods

A patient is considered a MEN1 patient when three of five major MEN1-associated lesions are present: tumours in the parathyroid glands, the pituitary gland, the pancreatic islets and the adrenal glands and neuroendocrine carcinoid tumours. A suspected MEN1 patient is defined as having two MEN1-associated lesions and/or multiple lesions within one organ and/or a MEN1-associated lesion at an early age (< 35 years) (16).

DNA mutation analysis

In the Netherlands, MEN1 mutation analysis is available to MEN1 patients, relatives of MEN1 patients or MEN1 gene mutation carriers, and suspected MEN1 patients. DNA analysis is performed by sequencing exons 1–10 of the MEN1 gene, as well as intron/exon boundaries with intron primers, from two independent blood samples, as described previously (3).

Periodical clinical monitoring of MEN1 patients

Periodical clinical monitoring of MEN1 patients, mutation carriers and suspected MEN1 patients consists of a biannual physical examination, and measurement of serum levels of ionised calcium, chloride, phosphate, parathyroid hormone, glucose, prolactin, IGF-I, gastrin, insulin, C-peptide, glucagon, pancreas polypeptide, platelet serotonin and chromogranin A, starting at the age of 5. In addition, starting at the age of 15, MRI of the upper abdomen and the mediastinum (in males; to detect thymus carcinoids, which

Figure 1 Results of an oral glucose tolerance test (OGTT) of the index patient 10 months before and 2 months after trans-sphenoidal surgery. GH levels were determined before and 15, 30, 60, 90, 120 and 150 min after intake of 75 g glucose.

Figure 2 Histological and immunohistochemical analysis of the removed pituitary tissue. HE staining of (A) normal pituitary tissue and (B) the adenomatous tissue. GH staining of (C) normal pituitary tissue, containing only a few GH-positive cells, and (D) the GH-producing adenoma. Magnification, 200 X.

occur only in males), and MRI of the pituitary gland are performed every 2 years. OGTT is performed when clinical features of acromegaly are present, and/or when IGF-I levels are elevated. During OGTT, GH levels are determined before and 15, 30, 60, 90, 120 and 150 min after intake of 75 g glucose. Levels below 5 mU/l (corresponding to 2 ng/ml) are considered normal.

IGF-I measurements

IGF-I levels were determined by chemiluminescence (Nichols Diagnostics, San Clemente, CA, USA, Advantage System). In this assay, interference of IGF-binding proteins is prevented by adding excess IGF-II to the reaction. IGF-I measurements in patient records of 33 MEN1 patients (20 females and 13 males) from 16 MEN1 families that are being monitored at the outpatient department of endocrinology over a period of 4–6 years were retrospectively analysed, and compared with gender- and age-adjusted references for IGF-I.

Results

Because the index patient had developed two tumour types associated with MEN1, and multiple parathyroid adenomas, he met the criteria for MEN1-suspected patients, and DNA analysis was performed. A C to G

substitution was found in intron 3 of the MEN1 gene (intervening sequence (IVS)3-6C > G). A C to T substitution was also identified at position 7264. The latter single-nucleotide change represents a common neutral polymorphism of the MEN1 gene, designated D418D. DNA analysis of family members led to the identification of nine MEN1 gene mutation carriers, including the patient's 64- and 66-year-old brothers and his 68-year-old sister (Fig. 3).

The (IVS)3-6C>G mutation affects an acceptor splice site within exon 3 of the MEN1 gene. Calculated prediction of the change in affinity of the acceptor splice site due to this mutation indicates that the affinity of the splice site is slightly reduced compared with the wild-type, possibly leading to skipping of exon 4 or the use of an alternative cryptic splice site (17, 18). In peripheral leucocytes from the index patient, however, with primers directed at exons 2 and 5 of the MEN1 cDNA, no aberrantly spliced mRNA could be detected by RT-PCR.

IGF-I measurements of 33 MEN1 patients were retrospectively analysed over a period of 4–6 years. In three patients (two female and one male; 9%), elevations (age- and gender-adjusted reference ± 2 S.D.) were found. In two patients, these elevations were mild. OGTT performed in these patients did not show aberrant responses. However, one patient showed gradually increasing IGF-I elevations (344–390 ng/ml) (age- and gender-adjusted reference ± 2 S.D.: 70–200 ng/ml) even during treatment with octreotide for bronchial

Figure 3 Pedigree of the family. Generations are indicated by Roman numerals I–III (squares represent males, circles females and diamonds males or females; black indicates clinical MEN1 (the index case, II:5), and grey indicates asymptomatic MEN1 mutation carriers). In family III:1–7, three family members were tested, and one turned out to be a carrier of the mutation.

carcinoid tumours. Four years after the first IGF-I elevation, this patient had developed signs of active acromegaly, including enlarged feet, coarsened facial features and hyperhidrosis. Since this patient had diabetes, OGTT was not performed. MRI did show slight asymmetry, but no tumour of the pituitary gland.

Discussion

Low penetrance of MEN1

In the literature, the disease penetrance of MEN1 is 82–99% at age 50 (10, 11). However, an age of onset of 86 years has been reported (10), and three of the family members of our index patient are older than 60. To our knowledge, a MEN1 family showing as few clinical features as the family described here (10%) has not been reported to date.

An intriguing question is how to explain the low penetrance in this family. One explanation could be the nature of the mutation itself. The MEN1 germ-line mutation that was found in this family, IVS3-6C>G, has not been reported before as a MEN1 mutation, nor as a polymorphism. In a previous study, a C to T substitution was found at the same position (IVS36C>T), leading to an acceptor splice site with increased affinity upstream of exon.

This increased affinity was shown to cause skipping of exon 3, resulting in aberrant messenger RNA (mRNA) (16). This patient had both pancreatic and adrenal tumours. The same IVS3-6C>T germ-line mutation has also been found in a patient with a sporadic pituitary tumour (19). Two other mutations that affect the same acceptor splice site are disease-related: IVS3-1G>T and IVS3-3A>G (19, 20).

Our inability to identify aberrantly spliced MEN1 mRNA may indicate that the change of the affinity of the acceptor site might lead to only partially or tissue-specific aberrant mRNA splicing. Thus, normally spliced mRNA might still be present even if the wild-type MEN1 allele is lost. So-called leaky mutations

have been reported in other inherited tumour syndromes, such as familial paraganglioma (21). Possibly, an additional predisposition that is present in the index patient, but not in his relatives, is required for this MEN1 gene mutation to be pathogenic.

Alternatively, the use of less stringent criteria for DNA analysis and the identification of more asymptomatic gene mutation carriers may eventually reveal that disease penetrance in MEN1 has been overestimated and that the true penetrance of the disease may in fact be lower, at least for certain types of mutations. This hypothesis could also explain the 'milder' MEN1 phenotypes such as familial isolated hyperparathyroidism, since a unique genotype for this phenotype has not been established (14).

Aetiology and pathophysiology of acromegaly in MEN1

Acromegaly is usually caused by a GH-secreting pituitary adenoma. The annual incidence of sporadic acromegaly is approximately 3 per million and the prevalence about 40 per million (22). GH-producing tumours causing acromegaly occur in 3–6% of MEN1 patients (9, 23). Two large studies showed that 13–23% of pituitary tumours in MEN1 produce GH (19, 24). Rarely, GH-releasing hormone (GHRH)-producing carcinoid tumours may cause pituitary hyperplasia and consecutive acromegaly (25–28). Since the index patient had clearly developed a primary GH-producing pituitary adenoma, GHRH levels were not determined.

The mean age at diagnosis is about 40–45 years in sporadic acromegaly (29), which is, in general, comparable to the age at diagnosis in MEN1 (9, 19). However, the earliest presentation of a GH/prolactin (PRL)-producing tumour in MEN1 was in a 5-year-old boy (30). The size and invasiveness of MEN1-associated, GH-producing tumours do not seem to differ significantly from sporadic tumours (31).

Periodical clinical monitoring of MEN1 mutation carriers (who are susceptible to development of GH-producing tumours) offers an opportunity to study early signs of acromegaly. Analysis of IGF-I measurements in MEN1 patients that are monitored periodically in our hospital showed temporary elevations in 9% of MEN1 patients. Indeed, one patient with persistent IGF-I elevations later developed clinical signs of acromegaly. Interestingly, the potential existence of a 'preacromegalic' state has been suggested in two other syndromes that are characterised by GH excess: Carney complex (CNC) and the McCune–Albright syndrome. CNC is an inherited complex of spotty skin pigmentation, myxoma, increased endocrine activity and schwannoma. About 80% of CNC patients show paradoxical GH responses or raised IGF-I levels, whereas 10% develop active acromegaly (32, 33). In a recent study, 21% of McCune–Albright syndrome patients had elevated GH levels during OGTT, and in 17%, IGF-I levels were elevated. In the group with IGF-I elevations, 4/10 patients (40%) had a GH-producing tumour (34).

Conclusion

We identified a MEN1 family with a IVS3(-6)C>G germ-line mutation with an unusually low penetrance of the disease. Furthermore, 9% of MEN1 patients showed elevations in IGF-I levels, which, in a single case, preceded active acromegaly. This proves the value of using IGF-I as a presymptomatic marker for detecting acromegaly in MEN1 patients.

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