Germline and somatic mosaicism in a family with multiple endocrine neoplasia type 1 (MEN1) syndrome

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Abstract

Context: Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disease caused by mutations in the tumor suppressor gene *MEN1* and can be diagnosed based on clinical, familial and/or genetic criteria. We present a family in which we found both germline and somatic mosaicism for MEN1.

Family description: In our proband, we diagnosed MEN1. The mutation was not detected in her parents (DNA extracted from leucocytes). When her brother was found to harbor the same MEN1 mutation as our proband and, around the same time, their father was diagnosed with a neuroendocrine carcinoma, this tumor was investigated for the MEN1 mutation as well. In the histologic biopsy of this tumor, the same MEN1 mutation was detected as previously found in his children. Re-analysis of his blood using multiplex ligation-dependent probe amplification (MLPA) showed a minimal, but consistently decreased signal for the *MEN1*-specific MLPA probes. The deletion was confirmed in his son by high-resolution array analysis. Based on the array data, we concluded that the deletion was limited to the MEN1 gene and that the father had both germline and somatic mosaicism for *MEN1*.

Conclusions: To our knowledge, this is the first reported family with combined germline and somatic mosaicism for MEN1. This study illustrates that germline mosaicism is important to consider in apparently sporadic *de novo* MEN1 mutations, because of its particular importance for genetic counseling, specifically when evaluating the risk for family members and when considering the possibility of somatic mosaicism in the parent with germline mosaicism.

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Background

Multiple endocrine neoplasia type I (MEN1) is an autosomal dominant disease caused by mutations in the tumor suppressor gene *MEN1*. It is characterized by the combined occurrence of parathyroid adenomas, pituitary adenomas and duodenopancreatic neuroendocrine tumors (1). According to the present clinical practice guidelines, MEN1 is diagnosed based on clinical, familial and genetic criteria (2). Therefore, MEN1 can be diagnosed if (1) at least two out of three primary MEN1

manifestations occur or if (2) one MEN1-associated tumor occurs in a first-degree relative of a patient with a clinical diagnosis of MEN1 or if (3) a pathogenic germline *MEN1* mutation is found in an asymptomatic individual.

Since the discovery of the *MEN1* gene in 1997 (3), more than 1300 somatic and germline mutations have been identified (1, 2). Somatic mutations can spontaneously occur in any cell of the body and are limited to the descendants of the original cell that developed the

mutation and will not be passed on to future generations. A germline mutation is present in all cells of the body, including the germ cells and can therefore be passed on to future generations. The probability of finding a germline mutation is dependent on what features are present. In patients with a single MEN1-associated tumor and a negative family history, there is approximately a 15% chance of finding a *MEN1* mutation. This chance increases to up to 90% in case of multiple MEN1-related tumors and a positive family history (4). In more than 10% of the patients, the mutation has occurred 'de novo'; parents of the index patient are asymptomatic and the mutation cannot be detected in their lymphocytes.

Mutations occurring at later stages of embryonic development in a differentiated cell give rise to a phenotype confined to the affected tissue, referred to as somatic mosaicism. Somatic mutations are frequently found in single sporadic tumors of the MEN1 spectrum (5, 6). For example, 44% of the pancreatic neuroendocrine tumors have inactivating somatic mutations in *MEN1* (7). In addition 5–50% of sporadic endocrine tumors have been found to have loss of heterozygosity (LOH) at the 11q13 locus, harboring *MEN1* (8).

In case a mutation occurs before the separation to germinal cells (and if this mutation is present in both somatic and germ cells), the carrier of a somatic and germline mosaicism may be symptomatic with various symptoms of the disease and will have a risk of transferring the mutation to his offspring.

In this manuscript, we present a family with combined germline and somatic mosaicism for MEN1, which was detected when two siblings were found to harbor the same *MEN1* mutation, which was not detected in the lymphocytes of their parents.

Family presentation

The index patient (III:4, Fig. 1) of this family presented at the age of 16 years with primary amenorrhea. She was found to have a microprolactinoma for which she was treated with dopamine agonists. At the age of 26 years she developed primary hyperparathyroidism and at this point she was screened for *MEN1*. DNA analysis (UMC Utrecht) showed a deletion of *MEN1* c.(?-1)_(?1?)del. She underwent parathyroid surgery and prophylactic thymectomy. Further screening showed two small (approximately 1 cm) hypoechogenic lesions in the pancreas which were regarded as neuroendocrine pancreatic tumors.

Figure 1

Pedigree. Boxes indicate male subjects; circles indicate female subjects. Black boxes/circles indicate the presence of the *MEN1* mutation. Oblique line indicates that the respective subject has deceased. The small black box in patient II:3 indicates the presence of germline and somatic mosaicism. Arrow points at index patient.

DNA analysis in her two children showed the *MEN1* mutation only in her youngest daughter (IV:4, age 5.5 years at diagnosis). She was referred to the pediatric endocrinologist. Up till now (current age 13 years) there are no signs of MEN1-associated pathology. In the parents of the index patient, the mutation was not found. Her father (II:3), and her father's father (I:1) were at the time of screening both known with kidney stones. Because both parents did not have the mutation, at that point we concluded that, most likely, the DNA mutation in the index patient was a sporadic *de novo* mutation.

However, to rule out germline mosaicism, we advised her otherwise healthy brother (III:1) to have a DNA analysis as well. Surprisingly, he was found to have the same *MEN1* mutation as his sister, which suggested germline mosaicism in one of their parents. He was referred to an endocrinologist and diagnosed with primary hyperparathyroidism (age 38 years) and underwent parathyroid surgery and prophylactic thymectomy. Later he developed an atypical thymus carcinoid which was radically removed. Up till now further screening revealed no abnormalities. DNA analysis of his two children showed the *MEN1* mutation only in his son (IV:1, age 9 years at diagnosis). He

was referred to the pediatric endocrinologist. At last follow-up (age 15 years) there were, besides a mild primary hyperparathyroidism, no signs of MEN1-associated pathology.

Around the same time, the DNA mutation was found in the brother (III:1) of the index patient, their father (II:3) was diagnosed with a pancreatic tumor with liver metastases (age 63 years), which was initially diagnosed as an adenocarcinoma by a pathologist in another hospital. However, as his clinical condition remained stable for a long time, revision of the histologic biopsy of the liver lesion showed a well-differentiated neuroendocrine carcinoma instead. He was previously known with kidney stones and when he was screened for MEN1-associated diseases, he was additionally diagnosed with primary hyperparathyroidism. There were no other MEN1-related diseases found.

DNA isolated from this liver biopsy showed the same *MEN1* mutation as previously found in his son and daughter. Parallel re-analysis of his peripheral blood did not show the *MEN1* mutation. We then performed a skin biopsy, which revealed the same *MEN1* mutation. A third analysis of the blood using multiplex ligation-dependent probe amplification (MLPA) showed a minimal, but consistently decreased signal for the *MEN1*-specific MLPA probes (Fig. 2A). Array analysis on blood did not show a *MEN1* mutation. To get insight in the size of the deletion analysis on blood of his son (III:1) was performed. With high-resolution array analysis (CytoscanHD (Thermo Fisher)) the deletion in the *MEN1* gene was confirmed. Based on the array data we concluded that the deletion was limited to the *MEN1* gene (Fig. 2B) (please see online Supplementary data for the methods used for DNA, MPLA and array analysis, see section on supplementary data given at the end of this article). Unfortunately, LOH analysis could not be performed. To summarize, we concluded that the father had both germline and somatic mosaicism for *MEN1*. The pancreatic tumor could not be resected and was eventually treated with lutetium octreotate. Unfortunately, the patient died 2 years later due to progression of his hepatic metastases.

Discussion

In this report, we describe a family in which we found both germline and somatic mosaicism for *MEN1*. In mosaicism two or more cell lines are present, occurring as a postzygotic event. The mosaicism is often reported as germline, somatic (involving only somatic cells and not the

Figure 2

MLPA result. (A) MLPA results of the *MEN1* deletion in DNA extracted from peripheral blood of the index (red circles) and his father (green squares and blue triangles correspond to two different blood drawings, purple diamonds to DNA from an oral mucosa swap). Each data point is an average of two analyses. Gene dosage was calculated by dividing the peak area of the probes by the combined area of the control probes. This was compared to the ratio of five control samples using MLPA kit P017-C1. (B) High-resolution array analysis (CytoscanHD (Thermo Fisher)) showing the deletion in the *MEN1* gene. The deletion is limited to the *MEN1* gene with the proximal breakpoint within *MEN1* (in exon 8/intron 8) and the distal breakpoint in (5' of intron 7) or upstream of *MEN1* (based on NM_130799.2). The location of the deletion is indicated by the arrow.

germline) or gonosomal (involving both the somatic cells and the germline). The clinical manifestations depend on both the timing during embryonic development and the cell type in which the somatic change had occurred (9). Germline mosaicism for autosomal dominant disorders is well known in genetic counseling and a recurrence risk for apparent *de novo* mutations is often referred to as 1–6% (10).

Given that in our family the mutation was transmitted to more than one sib, it was concluded that there must have been germline mosaicism in one of the parents. Because the clinical features present in the father of our

proband were suggestive of somatic mosaicism, additional tissues were investigated and somatic mosaicism could be proven.

To our knowledge, somatic mosaicism for *MEN1* in peripheral blood has only once been described (11). In that report, a 27-year-old woman with hyperparathyroidism, prolactinoma and pancreatic mass, a low level of mosaicism for the p.Gln405* mutation in exon 9 was found in peripheral blood DNA. It is not known whether this patient had germline mosaicism as well. Although this case report is up till now the first one to describe combined somatic and germline mosaicism for *MEN1*, this phenomenon is known for other diseases (10, 12, 13, 14, 15, 16).

Sporadic tumors in the *MEN1* spectrum frequently harbor somatic pathogenic variants in *MEN1*. The recognition of a tumor being *MEN1*-related has important consequences for the patient and his family. First, neuroendocrine tumors deserve a tailored treatment. Second, in case of somatic mosaicism for *MEN1*, the disease might be more widespread than the occurrence of a single tumor and routine surveillance by biochemical analysis and imaging procedures is recommended. Third, in case the gonads are included in the somatic mosaicism, and there is in fact germline mosaicism as well, first-degree relatives have up to 50% risk of having *MEN1*.

The fact that this is a case of combined germline and somatic mosaicism suggests that the *MEN1* mutation has occurred very early in the embryo, before the separation to germinal layers. This is supported by our findings that the mutation that was found in the tumor was not found in the leucocytes (of patient II:3). Indeed leucocytes, used for conventional DNA analysis, are derived from the mesoderm, whereas the pancreas is of endodermal origin. It has also been shown that if germline mosaicism is discovered through the birth of more than one affected child, the mutation was also present in somatic cells of the mutation-carrying parent in 50% of the cases (10).

In summary, this is the first reported family with combined germline and somatic *MEN1* mosaicism. In addition, this case report illustrates that germline mosaicism is important to consider in apparently sporadic *de novo* *MEN1* mutations, because it has major implications for the surveillance, treatment and familial screening.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-18-0778>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this case report.

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Patient consent

Written informed consent was obtained from the index patient and her family for publication of this case report and any accompanying images.

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