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Multiple endocrine neoplasia type 1 caused by mosaic mutation: what about clinical follow up and genetic counseling?

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Abstract (136/250):

MEN1 is an autosomal dominant hereditary syndrome characterized by several endocrine tumors, in most cases affecting the parathyroid glands, pancreas, and anterior pituitary. It is the result of inactivating mutations in the tumor suppressor gene *MEN1*. More than 1300 different mutations have been identified in this gene. Mosaic *MEN1* mutations have been previously described in only a few patients in the literature. In this paper we provide a review of six cases of *MEN1* mosaicism reported in the literature supplemented with six additional cases described by the French TENgen network of laboratories. This review highlights that (i) *MEN1* mosaicism is not associated with a mild phenotype, (ii) results in the same natural history as heterozygous *MEN1* mutation, and (iii) that more systematic detection of *MEN1* mosaic mutation enables improvements in both patient monitoring and genetic counseling.

MAIN TEXT

BACKGROUND

Multiple endocrine neoplasia type 1 (MEN1) is a rare disease caused by inactivating mutations in the *MEN1* gene (1). MEN1 is characterized by a broad spectrum of clinical manifestations from which the three cardinal lesions are primary hyperparathyroidism (PHPT), pituitary neuroendocrine tumors (PitNETs), and neuroendocrine duodeno-pancreatic tumors (2). Other neuro-endocrine tumors (NETs) such as adrenal cortical tumors, bronchopulmonary NETs, or thymic NETs may also be associated. Twenty-eight to 70% of

MEN1 patients die due to the disease, particularly due to the pancreatic and carcinoid lesions (3). In 2012 a group of experts provided guidelines for the evaluation, treatment, and genetic testing of MEN1 disease (4). MEN1 is diagnosed based on clinical criteria in all patients with two or more MEN1-related tumors, on family criteria in patients having one MEN1-related tumor and a first-degree relative with MEN1, and on genetic criteria in all patients, symptomatic or not, who carry a *MEN1* pathogenic variant. The MEN1 diagnosis allows patients to benefit from a multidisciplinary follow-up program which enables early detection and treatment of lesions (4, 5).

MEN1 is a tumor suppressor gene (1, 6) and *MEN1* pathogenic or likely pathogenic variants are detected in 90% to 95% of family cases, and in 30-45% of sporadic cases bearing the classical triad (7, 8, 9). A false-negative genetic test can occur in cases where a *de novo* mutation occurs during postzygotic development, that is, after fertilization. In these cases, only a proportion of cells in the organism harbor the variation; a feature termed mosaicism or somatic mosaicism. In mosaicism, germline genetic testing may be negative if the fraction of mutated alleles in the blood is below the detection threshold. Only a few cases of *MEN1* mosaicism have been reported in the literature (10, 11, 12, 13, 14). The impact of *MEN1* mosaicism on patient phenotype and on the natural history of the disease is unknown. Here we review all published cases of mosaicism and additionally consider six unpublished cases from the TEngen group (French network of laboratories working on NETs), in order to clarify the clinical presentation and natural history of *MEN1* mosaicism and then discuss appropriate genetic counseling.

REPORT

To date, only 6 cases of *MEN1* mosaicism have been reported in the literature (see Table). The first description was published by Klein *et al* in 2005 (10) who used molecular subcloning to identify a *MEN1* truncating mutation with a 10 % allele frequency in a 27 year-old woman who presented all typical *MEN1* lesions (PHPT, prolactinoma, and a pancreatic lesion, patient #1). The second case (patient #2), a woman diagnosed with PHPT at age 52, was reported by Farook *et al* in 2011(11). She presented several recurrences of PHPT and her daughter also developed PHPT, leading to genetic testing. The index case later developed a pituitary microadenoma and two pancreatic lesions. A mosaic mutation in *MEN1* was identified at a level of 10% and the same mutation found in her daughter at the heterozygous level. Mauchlen *et al* also reported a case of *MEN1* mosaicism with 15 % of mutated alleles in a 43-year-old patient with a parathyroid adenoma and pancreatic gastrinoma (patient #3) (12).

The three other cases were reported in relatively older patients. Two were identified by Coppin *et al* (13) using NGS. The first was a patient who developed PHPT at age 57 and a pancreatic NET at age 66 (patient #4). The mutation (6 % mutated alleles) had previously been identified at the heterozygous level in her daughter. The second case was a 68-year-old patient with hyperparathyroidism and a PitNET (patient #5) with 15 % mutated alleles. Later Beijers *et al* described an entire *MEN1* gene mosaic deletion, using MLPA, in a 63-year-old patient with PHPT and metastatic pancreatic NETs (patient #6, 14). Again, the deletion had previously been identified at a heterozygous level in his 2 children.

In addition to cases from the literature review, we present here 6 new cases of *MEN1* mosaicism identified by next-generation sequencing (NGS) during recent years in the French TENgen network. Three were identified in young patients. The first showed a severe clinical presentation: the patient developed PHPT at age 28, an endocrine thymic tumor at age 35 and

a pituitary adenoma at age 37 (patient #7). The proportion of mutated alleles was 6%. The second case was a 24-year-old man harboring a *MEN1* mosaic mutation at a level of 10 % (patient #8). He presented with PHPT and a duodenopancreatic NET and developed a PitNET at age 27. In the third case, a *MEN1* mosaic mutation was found in a 24-year-old man at a level of 11 % with a milder clinical presentation, only a PitNET being diagnosed (patient #9).

Another *MEN1* mosaic mutation was identified in an older woman first reported in 2013 by Cuny et al (15) who developed PHPT and a PitNET before age 45. Her daughter was diagnosed with a heterozygous frameshift insertion of 26 nucleotides in *MEN1*, discovered after she developed a macroprolactinoma at age 13 and PHPT at age 20. Using NGS we retrospectively identified the same truncating mutation as in her child, at a level of 6.3 % mutated alleles (patient #10).

We also detected *MEN1* mosaicism in two older patients with severe MEN1. The first patient presented with PHPT, a PitNET and duodenopancreatic NETs at age 46 and an endocrine thymic tumor was diagnosed a year later (patient #11). The proportion of mutated alleles identified by NGS was 2.5%. Additionally, *MEN1* mosaicism was identified at 9.4% in a patient who presented at age 43 with PHPT and a metastatic thymic NET. He later developed duodenopancreatic NETs at age 53 (patient #12).

We compared the age of occurrence of the first, second and third lesions of the classic MEN1 triad (PHPT , PitNET, and duodeno-pancreatic NET) between these 12 patients with *MEN1* mosaicism and the 497 index cases with pathogenic and likely pathogenic variants from the UMD-MEN1 database as previously described (16). No difference in the cumulative incidence of lesions was found (Figure 1).

DISCUSSION

Only six cases of *MEN1* mosaicism have been reported in the literature to date (10, 11, 12, 13, 14). We report here six new patients, thus constituting the largest series ever reported. Some studies have suggested that the *MEN1* phenotype may be attenuated in patients with *MEN1* mosaicism (11). This review clearly demonstrates that patients with *MEN1* mosaicism can develop the classical triad (patients #1, #3, #4), even early in life (patients #1, #4). Patients with *MEN1* mosaicism appear to develop the classic *MEN1* lesions at the same age as others (Figure 1). Moreover *MEN1* can become a very aggressive disease with multiple lesions (6 patients reported here showed at least 3 *MEN1* lesions) or show metastatic lesions (patients #6, #12) even in patients harboring *MEN1* mosaicism. For this reason careful monitoring in these patients must not be relaxed.

In terms of the nature of the variant, we observed that all types of alterations were represented in patients with mosaicism (5/12 frameshift variants, 3/12 non-sense variants, 2 variants affecting splice junctions, 1 entire gene deletion, 1 missense variant). A relationship between mutated allele frequency and the severity of the disease was not established. In patient #11, a very low mutated allele frequency of 2.5% was responsible for disease affecting multiple tissues, whereas mosaicism at 10% can cause complete *MEN1* disease at a young age. For the moment, there is no argument in favor of modifying the follow-up program of *MEN1* patients with mosaicism in relation to either age of onset or frequency.

MEN1 mosaicism can be discovered in index cases with a symptomatic child harboring a heterozygous mutation (patient #2, #4, #6, and #10). First-line negative genetic screening by Sanger sequencing could lead to the conclusion of a phenocopy in the symptomatic parent and the exclusion of other siblings from presymptomatic genetic screening. We suggest that

physicians should consider that a symptomatic parent with a child presenting with an apparently *de novo* mutation may harbor a mosaic mutation until proven otherwise. We note that in the literature there is only one symptomatic parent of a child carrying a *MEN1* mutation, in whom genetic explorations were negative, even by deep NGS (17). This man presented with hypercalcemia and elevated PTH levels (10.9 mg/mL, 80 pg/mL (normal assay range 14–72)) at age 63, but imaging data (ultrasound and Sestamibi parathyroid scan) provided no information. The analysis of abnormal tissue in this case could have made it possible to definitively exclude mosaicism.

MEN1 mosaicism can also be identified in sporadic index cases bearing one or several *MEN1* lesions. Success in identifying *MEN1* mosaicism in these patients is really fortunate for the patient and his/her family. Firstly, it allows confirmation of the *MEN1* diagnosis in the patient, even in a patient with an isolated PitNET (patient #9), or late multiple lesions (patient #5). Additionally it clarifies genetic counseling. Since the disease is a mosaic, it confirms the appearance of the disease in the patient. Consequently, the patient's parents and siblings can be reassured and excluded from a *MEN1* follow-up program. Presymptomatic genetic screening can also be started for the patient's children. In fact, the component of genetic counseling that addresses the risk of recurrence remains challenging in mosaic genetic diseases, since it depends on the proportion of germ cell progenitors that harbor the mutation. One way in which to determine this would be to genotype the sperm of male patients (18, 19). However, no study has thus far been conducted to determine the reliability of a negative result. Nevertheless, as NGS can detect mosaic variants at low AF (13, 20, 21, 22, 23); we suggest geneticists and physicians keep in mind that mosaicism is likely underestimated in unresolved *MEN1* patients.

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Ethics declaration: Informed consent was obtained from the subjects involved in the study. The study was conducted according to the guidelines of the Declaration of Helsinki and the UE General Data Protection Regulation.

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312 Figure 1: Cumulative incidence of the first, second and third MEN1 cardinal lesions in patients
313 with MEN1 mosaicism and in index cases from the UMD-MEN1 database.

314 A total of 497 index cases with heterozygous MEN1 (likely) pathogenic variants, and for which
315 the age of occurrence of the lesions of the classic MEN1 triad was known were included.

316 Patients from the UMD MEN1 database with incomplete clinical data were excluded. A) Age
317 at occurrence of the first MEN1 cardinal lesion. B) Age at occurrence of the second MEN1
318 cardinal lesion. C) Age at occurrence of the third MEN1 cardinal lesion.

319 Log rank test performed with Prism V9 (Graphpad) showed no difference between the 2
320 groups for the 3 conditions.

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