De novo mutation in *MEN1* is not associated with parental somatic mosaicism

Dear Editor,

Multiple endocrine neoplasia type 1 (MEN1-OMIM #131100) is characterized by the co-occurrence of tumors in at least two of the following three endocrine tissues: parathyroid, endocrine pancreas and anterior pituitary (Thakker 2014). Familial MEN1 follows an autosomal dominant mode of inheritance, and germline mutations in the *MEN1* gene at 11q13 can be detected in affected family members (Chandrasekharappa *et al.* 1997). Mutations in the *CDKN1B* gene (*CDKN1B*, *KIP1* and OMIM #600778) have been also reported in MEN1 families but are far less frequent (Pellegata *et al.* 2006). Germline mutations in the *MEN1* gene have also been detected in seemingly sporadic MEN1 cases and have been referred to as *de novo* mutations. Indeed, an often quoted number in MEN1-focused reports is 10% of the cases showing a *de novo* mutation (Bassett *et al.* 1998). The occurrence of a mutation in an affected individual with none of the parents exhibiting MEN1-suggestive features may be attributed to non-paternity, non-penetrant mutant allele, gonadal mosaicism or parental germline/somatic mosaicism (Koper & Lamberts 2000). The assignment of a *de novo MEN1* gene mutation in previous studies was based on lack of a parental phenotype (Peppa *et al.* 2009), inferred haplotype (Bassett *et al.* 1998) or lack of the proband's mutation in either parent using Sanger sequencing (Teh *et al.* 1998, Rix *et al.* 2004). No published study used deep sequencing to assess the possibility of parental, low-level mosaicism.

The diagnostic criteria of MEN1 include having at least two of the three predominant neoplasms in at least one case (Thakker 2014). Clinical associations other than benign and malignant tumors are scarce in MEN1. Notably, there is a single report of an MEN1-affected individual who also exhibited a distinct phenotype that includes autism and dysmorphic features and harbors a 570 kb deletion at 11q13 encompassing the *MEN1* gene (Mohrmann *et al.* 2011). Here we evaluated the possibility of low-level germline parental mosaicism in a Jewish Ashkenazi family where the proband also exhibits autism.

Patients and clinical phenotype

This study was approved by the local Ethics Committee, and each participant gave an informed consent. Participants were clinically evaluated by history and physical examination and diagnosis was further supported by biochemical testing and imaging analyses. The proband, a Jewish Ashkenazi male born in 1989, was diagnosed with mild autistic disorder at age 5 years and is treated since with Clotiapine 2.5 mg/day. At age 24, he was diagnosed with pituitary micro-adenoma based on elevated prolactin (PRL) levels (2200 IU/L (45–375)), and MRI showing 7 mm pituitary micro-adenoma. He was treated with Cabergoline 0.5 mg/week resulting in a return of the PRL levels to near normal. At age 26 years, he was diagnosed with primary hyperparathyroidism with elevated levels of calcium and PTH (11.3 mg/mL, 40 pg/mL (normal range for kit 4.6–38), respectively) with imaging (ultrasound and Sestamibi parathyroid scan) showing a single adenoma of the lower left parathyroid. Based on persistent hypercalcemia at a young age, osteoporosis (bone density at age 26 years (hologic) *Z* scores at femoral neck –3.0 and lumbar spine –2.9) and ongoing anorexia, surgical removal of the parathyroid gland was performed in November of 2015. At surgery, the lower left parathyroid adenoma and the thymus were surgically resected with intra-operative PTH reduction from 355 pg/mL to 63 pg/mL (normal range for kit 14–72), with normocalcemia and normo-PTH achieved and maintained until the time of reporting.

The proband's father was diagnosed with primary hyperparathyroidism at age 63 years based on hypercalcemia and elevated PTH levels (10.9 mg/mL, 80 pg/mL (normal range for kit 14–72)). Imaging studies

(ultrasound and Sestamibi parathyroid scan) could not document any abnormal parathyroid tissue.

Both parents were unrelated to each other, and there is no family history of endocrine tumors on either parental side. The proband has a healthy brother born 1994.

Germline mutation analysis of the *MEN1* gene

Genomic DNA was isolated from peripheral blood leukocytes of the proband and both parents using a commercially available kit (wizard genomic DNA purification kit, Promega) and using the manufacturer's suggested protocol. The proband's DNA was sent by the treating physician for analysis of a multi-gene panel (InVitae, San Francisco, CA, USA). Subsequent to defining the proband's mutation, his DNA and that of his parents underwent genotyping for the specific *MEN1* mutation by using both Sanger sequencing and deep sequencing (using Miseq platform). The primers used for PCR of the genotyped mutation-containing region are Forward 5-CTGATCTGTGCCCTCCCTTC-3; Reverse 5-TCAAAACGGCCTAGGGACTG-3.

Paternity testing

Paternity was ascertained by applying a commercially available kit (Aneufast, Wollerau, Switzerland) and following the manufacturer's protocol to the proband and both his parents.

MEN1 genetic analysis

Invitae testing showed that the proband harbored a germline heterozygous nonsense mutation c.1213C->T in exon 9 of the *MEN1* gene, which leads to a premature stop codon, p.Gln405X. In addition, a heterozygous variant was detected in the *KIT* gene, rs138585275 p.Thr274Met, which is classified as likely benign.

The existence of the *MEN1* gene mutation in the proband was subsequently confirmed using Sanger sequencing and repeat deep sequencing using a different DNA extraction. Neither parent harbored the pathogenic *MEN1* mutation using either Sanger sequencing or deep sequencing (X1000 coverage of the mutation). The father harbored the rare p.Thr274Met sequence variant in the *KIT* gene, and paternity testing revealed that both parents are indeed the proband's parents (data not shown).

In the present study, the *MEN1* truncating mutation undoubtedly underlies the MEN1-phenotype in the proband. Although the *MEN*1 germline mutation could be detected both by using Sanger sequencing as well as deep sequencing in the proband, neither parent was shown to carry the mutation by either sequencing technique. This finding conclusively excludes low-level parental germline mosaicism as the cause of this *de novo MEN1* mutation. As non-paternity was excluded, the other molecular mechanisms that may account for this phenomenon are gonadal mosaicism or a *bona fide de novo* mutation restricted to the proband. Interestingly, Rix and coworkers described a *de novo MEN1* mutation (p.Leu444Pro) in a phenotypically sporadic MEN1 case, and the mutation could not be detected in either parent by Sanger Sequencing (Rix *et al.* 2004). Yet, an identical *MEN1* mutation was previously reported in a large Italian family (Cetani *et al.* 1999), raising the possibility of a low-level parental mosaicism in the case by Rix and coworkers (Rix *et al.* 2004). In the *MEN1* mutation database (http://www.umd.be/MEN1/), a truncating mutation in the same codon (p.Leu444X) in a single family is also noted. Previous studies have not effectively excluded the possibility of a low-level parental mosaicism, as they did not employ deep sequencing analysis. It remains to be seen what the relative contribution of each of these possible molecular mechanisms to the emergence of *de novo MEN1* mutations.

Based on the fact that the father was diagnosed with biochemical primary hyperparathyroidism (despite the lack of any demonstrable parathyroid tumor), one would expect the father to harbor the familial *MEN1* mutation. The fact that he does not have any evidence of harboring the familial mutation may imply that the father represents a phenocopy of primary hyperparathyroidism in the context of MEN1, a phenomenon not previously described, to the best of our knowledge.

Another issue raised by this case is the clinical association between MEN1 phenotype and autism. This is only the second case (Mohrmann *et al.* 2011) in which such an association was reported, and the first where no major deletions involving the *MEN1* gene have been noted. In all likelihood, this is a co-occurrence of two distinct disease entities, as the rate of autism is reportedly 1:68 individuals and even higher (1:42) among boys (http://www.cdc.gov/ncbddd/autism/data.html).

In conclusion, a *de novo MEN1* gene mutation in this family is not associated with low-level parental mosaicism, the father may represent the first described case of MEN1 phenocopy.

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