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The importance of MLPA technique in the diagnosis of multiple endocrine neoplasia type 1



La importancia de la técnica MLPA en el diagnóstico de neoplasia endocrina múltiple tipo 1

Multiple endocrine neoplasia type 1 (MEN1), or Wermer syndrome, is a rare entity with an autosomal dominant inheritance pattern with high penetrance due to pathogenic variants in the germ line of the MEN1 tumor suppressor gene, which encodes a protein called menin1. Its estimated prevalence is 2 cases per 100,000 inhabitants and is characterized by a predisposition to primarily endocrine neoplasms (most commonly parathyroid, enteropancreatic, and pituitary). The diagnosis of MEN1 in a patient has relevant implications for family members, as first-degree relatives have a 50% chance of inheriting the pathogenic variant and can be identified through MEN1 sequential analysis.^{1,2}

We present the case of a patient with a personal history of several neoplasms and initial negative genetic study by MEN1 gene sequencing, but with subsequent detection of a heterozygous deletion using multiplex ligation-dependent probe amplification (MLPA), which eventually confirmed the diagnosis.

A 65-year-old woman with a personal history of essential hypertension, type 2 diabetes mellitus with good metabolic control, and several previous neoplasms. At the age of

45, she underwent surgery for primary hyperparathyroidism through right hemithyroidectomy and multiple parathyroidectomy (3 and a half glands), with pathological anatomy consistent with adenoma in the right inferior gland and hyperplasia in the right superior gland. Two years later, she underwent cephalic duodenopancreatectomy due to the finding of one mass in the head of the pancreas after significant unintentional weight loss, which was both analytically and histologically consistent with pancreatic glucagonoma (2 tumors) and subsequently left adrenal adenoma treated with left adrenalectomy. At age 59, she underwent right hemicolectomy for moderately differentiated adenocarcinoma of the colon pT2N0. She has currently been referred to for hereditary cancer screening and for a left breast nodule incidentally detected on follow-up computed tomography one month earlier. Further study was completed with mammography (BI-RADS 3–4A) and biopsy, which were consistent with the presence of intraductal carcinoma with estrogen receptor expression. A multidisciplinary team decided to perform seed-guided tumorectomy along with radiotherapy and hormonal treatment with anastrozole. Given the clinical phenotype of MEN1, genetic testing was performed 4 years earlier using polymerase chain reaction (PCR) amplification of the coding exons and splicing regions of the MEN1 gene and subsequent analytical sequencing, with no pathological findings. Given the personal history of multiple neoplasms, genetic sequencing of genes related to breast, ovarian, and colorectal cancer (BRCA1, BRCA2, CDH1, STK11, TP53, ATM, BRIP1, CHEK2, PALB2, MSH6, RAD51D, MSH2, MLH1, APC, MUTYH, EPCAM, and PMS2) was performed, with no

pathological findings either. Subsequently, genetic study was expanded by examining copy number variation using next-generation sequencing (NGS) technique and confirmation by MLPA of the AIP, CDC73, CDKN1B, MEN1, RET genes. This study reported a heterozygous deletion of the entire MEN1 gene, confirming the diagnosis and providing genetic counseling to family members. The same deletion was also confirmed in her brother.

The MEN1 gene study is indicated in 4 situations: (1) cases with 2 or more endocrine tumors associated with MEN1 (parathyroid adenoma, gastroenteropancreatic tumors, and pituitary adenoma, primarily); (2) patients with suspected or atypical presentation of MEN1, such as parathyroid adenoma in patients younger than 30 years or multigland disease; presence of gastrinoma or multiple pancreatic neuroendocrine tumors at any age, or cases with 2 or more tumors associated with MEN1 different from the classic triad; (3) asymptomatic first-degree relatives of carriers of a pathogenic variant in MEN1 and (4) first-degree relatives of carriers of a pathogenic variant in MEN1 with compatible expression (for example, confirmation of a tumor associated with MEN1 or clinical, radiological, or biochemical changes suggestive of tumor associated with MEN1).³ Genetic testing in the aforementioned indications is important to confirm the clinical diagnosis and identify the two carriers in the family who may benefit from a follow-up plan and genetic counseling, and non-carriers. The diagnostic sequence will depend on the presence or absence of a family history with genetically confirmed MEN1. In the former case, gene-targeted sequencing will be performed to confirm or rule out the presence of the known pathogenic variant. In the latter case, PCR amplification of coding exons and splicing regions will be performed as a first step, followed by sequencing.⁴ If no abnormalities are found, the next step is to perform MLPA to detect large deletions of the MEN1 gene. If no changes are found either, the next step is to perform sequencing of gene panels associated with conditions similar to MEN1 (MEN1-like), such as CDKN1A, CDKN1B, CDKN2A, CDKN2C, CDC73, CASR, GNA11, AP2S1, GCM2, and AIP. Finally, if clinical suspicion is high and the genetic cause has not been identified, sequencing of the clinical exome (WES) or whole genome (WGS)⁴ could be performed. In 2015, a review of published pathogenic variants of the germ line identified 576 unique pathogenic variants, and in 2019 the Universal Mutation Database for the MEN1 gene (UMD-MEN1) reported an additional 181 unique pathogenic variants of the germ line.^{5,6} The obviously pathogenic category of germ line variants (69%) predicts premature truncation of menin from nonsense pathogenic variants (14%), frameshift pathogenic variants (42%), splice-site pathogenic variants (10.5%), and large deletions (2.5%).⁴ We should mention that gene sequencing techniques have high sensitivity rates for detecting small intragenic deletions/insertions, nonsense variants, missense variants, and splice-site variants, but as a technical limitation, they may not detect deletions or duplications of specific genomic regions (single exon, multi-exon, or large deletions/duplications), so it is essential to perform MLPA in cases of negative sequencing to identify them. In fact, the combined use of sequencing and deletion/duplication

(del/dup) analysis can identify heterozygous pathogenic germ line variants of MEN1 in 70% up to 90% of families with typical MEN1 characteristics. Advances made in genetic diagnosis have offered the opportunity to recognize conditions related to MEN1 in patients with MEN1 pathogenic germ line variants. Currently, additional use of *in silico* validation offers multiple advantages in simulating specific gene variants, such as medium-sized deletions that may be difficult to detect by NGS, which must be subsequently confirmed by MLPA. The importance of accurate molecular diagnosis is crucial in this entity, as it has a high degree of penetrance (up to 100% in carriers older than 55 years), and it is essential to provide genetic counseling to patients and their relatives, informing them that the risk of transmitting the pathogenic variant to their offspring is 50%.

In this case, the presence of a large deletion in the MEN1 gene was not detected in the PCR amplification previously performed, hence the importance of repeating and expanding the genetic study using MLPA in cases with a clinical phenotype and no alterations in genetic sequencing, where, although infrequent (2.5%), such deletions may be present, thus allowing both their diagnostic confirmation and proper follow-up and genetic counseling.

Funding

None declared.

Conflicts of interest

None declared.

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