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IS THAT THE R171Q AMINO ACID VARIANT IN EXON 3 OF THE MEN1 GENE IS RELATED TO AGE, TO PLAY A MUTATING ROLE?

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Keywords: *MEN1, polymorphism, mutation, heterozygote, young patients.*

Abstract

Background/aim: Several studies demonstrated that the R171Q amino acid variant in exon 3 of MEN1 gene is a polymorphism, and in some new studies it is probably a mutation. We found in our study of twelve cases, two young cases have this variant and developed multiple endocrine neoplasia type1.

Materials and methods: twelve MEN1 young patients (7 female, 5 male) aged between 20 and 40 years old, were included in our study.

After investigating each patient, biochemical and molecular researches is done. We sequenced exon 3 of the MEN1 gene of patients and some members of their families.

Results: Ten patients have MEN1 syndrome and they have no mutation in MEN1 gene. Two patients from separated families have a c.512G>A heterozygote variant. Phenotypically the two cases have hyperparathyroidism in young age. One of them developed others tumors later.

Conclusion: The R171Q variant is a mutation in some cases; causes hyperparathyroidism and will develop further MEN1 lesions later, and it is just a polymorphism in other cases. We believe that when this polymorphism combines with young age in severe depression, it will lead to MEN1 syndrome, which will make this polymorphism considered a genetic mutation

Introduction

Multiple Endocrine Neoplasia type1 (MEN1) is an autosomal dominant inherited syndrome with high penetrance. It is estimated that more than 90% of subjects carrying a mutation in the MEN1 gene have or will develop one or more of the clinical and / or laboratory signs of the disease before the age of 60 years [1–2].

This genetic disease predisposes to the development of hyperplastic and tumor lesions of the endocrine glands, in particular parathyroid, pancreatic endocrine, antehypophyseal, adrenal cortex and in the diffuse endocrine cellular components of the thymus and bronchi [3]. Some patients can also develop carcinoid tumors, angiofibromas of the

MEN1 is a syndrome generally due to mutations of the MEN1 gene, located on chromosome 11 at 11q13 encoding Menin, a protein known as a tumor suppressor. Some cases in MEN1 occur without any mutation in the MEN1 gene [6].

The change of the amino acid arginine to glutamine at position 171 (R171Q) in exon 3 of the MEN1 gene has been seen in the general population with a frequency ranging from 1.4% to 5% [7].

New MEN1 gene mutations (L301R, C354X and G28A) in two familial cases of MEN1 and one sporadic case, and was found to be the only alteration of the MEN1 gene in three other subjects with sporadic MEN1 [8].

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Materials and methods

1. Patient selection

Twelve young patients (7 female, 5 male) aged between 24 and 40 years old, who were diagnosed with MEN1 at the Constantine's University Hospital Center between 2013 and 2019. A MEN1 diagnosis was made if there were at least two clinical cardinal lesions out of the five main ones (parathyroids, pancreas, pituitary gland, adrenals, bronchi / or thymus) [9]. (Table 1). We also divided the patients according to the type of first organ with lesion (Graph 1).

Table 1. Distribution of MEN1 patients according to the type of lesion

Atteintes	Fréquences
Hyperparathyroidism	75%
Tumor of Pancreas	16.7%
Tumor of Ptuitary gland	16.7%
Tumor of Adrenals	33.3%

2. Clinical, biochemical, and molecular research

We have collected all the basic information about patients and their families. We also recorded everything related to the lifestyle, the environment, and the type of patients' lives, whether difficult, tense or quiet.

Sample :The whole blood samples for the genetic study were taken from the elbow crease after placing a tourniquet. Peripheral blood DNA was not studied in these patients.We collected for each patient :two sterile vacutainer tubes of 5ml each containing EDTA as anticoagulant, which is also a nuclease inhibitor.Informed consent is requested from the patient.

DNA extraction: It was performed on fresh blood. In certain cases of technical impossibility, the extraction was postponed and then carried out on blood stored at + 4°C. Genomic DNA was extracted using an inorganic solvent NaCl.

Polymerase Chain Reaction (PCR): was performed using 2 Primers for the exon 3 of MEN1 gene and we used several PCR protocols (Table 2, Table 3). Sequencing: It is done by use of the 3500 sequencer from APLLIED BIOSYSTEM.

Table 2. Sequences of primers used

Primer sequence (5'-3')	Product size (bp)
F:GCACAGAGGACCCTCTTTCATTAC R:CTTGCCGTGCCAGGTGAC	197 bp
F:CTCGCCCTGTCTGAGGATCATG R:TGGGTGGCTTGGGCTACTACAG	190 bp

Table 3. PCR protocols for exon 3

	P 1	P 2	P3	P 4
H2o	11.3 µl	11.3 µl	11.3 µl	34.3 µl
Tampon	5 µl	5 µl	5 µl	5 µl
Mgcl2 (25mM)	2.5 µl	2.5 µl	2.5 µl	2.5 µl
dNTP (5mM)	3 µl	2 µl	2 µl	6 µl

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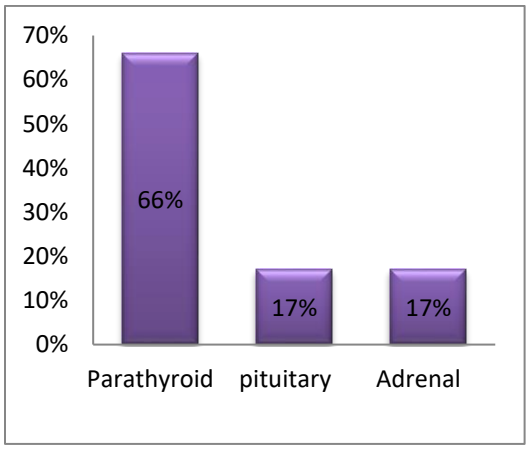
Primer F (30 pmol)	1 µl	1 µl	1 µl	2 µl
Primer R (30 pmol)	1 µl	1 µl	1 µl	2 µl
Taq (1Units/5ul)	0.2 µl	0.1 µl	0.1 µl	0.2 µl
MIX	24 µl	24 µl	24 µl	50 µl
DNA	1 µl	1 µl	1 µl	1 µl
Temperatures	94°c/ 5min 94°c/ 1min 64.5°c/1min 72°c/ 5min	94°c/ 5min 94°c/ 1min 64.5°c/1min 72°c/ 5min	98.5°c/5min 92°c/ 30sec 64.5°c/30sec 72°c/ 5min	94°c/ 5min 94°c/ 1min 64.5°c/1min 72°c/ 5min
P: protocol				

Results

In result of our study about exon 3 of the MEN1 gene, we found that all the patients have no variant except two of them that have the R171Q variant (one patient is man and the second is woman).

The two patients share many characteristics: young age (24 -27 years old), stress, severe depression, having primary HPT, Constant pain in the limbs, stomach ulcer, and no familial antecedents. The female patient developed other lesions later (pancreatic tumor, liver tumor and breast tumor); While the male patient developed a tumor in the thyroid gland.



Graph 1.Distribution of patients according to the first organ affected

Conclusion

We suppose that the R171Q variant, when it occurs in young cases in sporadic form, it will be considered a mutation, which later leads to MEN1 syndrome.

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PhD student at the University of Biology in Algeria, specializing in Molecular Genetics. I am currently doing my Ph.D. graduation thesis. This article was the result of my research in the laboratory of Constantine Hospital, which is a research parallel to the research of my graduation thesis.