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Case Report

Early-Onset Alzheimer's Disease, APOE4 And C9orf72 Expansion: A Case Report

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Abstract

Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and frontotemporal dementia (FTD) have become a focus of concern at the socio-sanitary and economic level due to their exponential growth as life expectancy for humans has increased through the years. Research into the genetic basis underlying the diseases should allow for earlier diagnosis, better prognosis and the possibility of discovering new therapeutic targets. In this article, we describe the case of a 45-year-old patient who presented with early-onset dementia with symptoms similar to AD, for which he was diagnosed. However, his genetic study revealed a mutation in C9orf72, a gene associated with FTD and amyotrophic lateral sclerosis. In addition, the patient is a heterozygous carrier of APOE c.338T>C (E4 allele), considered a risk factor for AD. This finding would suggest an involvement of C9orf72 in triggering AD.

Introduction

Dementia is a chronic and acquired loss of cognition in multiple cognitive domains which affects daily life, as it affects memory, language, visuospatial and other functions [1]. That condition affects approximately 47 million people worldwide and is expected to increase to 131 million by 2050. Among all types of dementia, Alzheimer's disease (AD) is the most common cause of dementia in clinical practice, accounting for 60-80% [2,3], followed by Lewy body dementia and, thirdly, frontotemporal dementia (FTD) [3].

AD is thought to begin 20 years before symptoms appear [4]. Memory loss is the key symptom. Forgetfulness is frequent, persistent, important and progressive. As the disease worsens, other symptoms appear [5].

AD is not genetically determined in most cases. Although certain genetic aspects can increase the risk of developing this disease, they are not determining factors for its appearance [2]. The genetic variant of the gene encoding apolipoprotein E (APOE), APOE4, is associated with a high risk of the disease, whereas the APOE2 allele is thought to lower the risk [6,7]. Only 1% of

the cases are caused by a mutation in autosomal dominant genes: Presenilin 1 (PSEN1), Presenilin 2 (PSEN2) and the amyloid precursor protein gene (APP).

FTD is caused by an atrophy of the brain's frontal and/or temporal lobes. It tends to appear at younger ages than other types of dementia. The symptoms of FTD vary depending on the brain region affected and the progression of the disease. There are 3 types of FTD: the behavioural variant (bvFTD) is the most frequent (60-70% of cases), which begins with behaviour changes and early alteration of executive functions. Another variant is primary progressive aphasia, with progressive language impairment. Finally, a variant of movement disorder, whose symptoms include tremors, stiffness, slowness or difficulty walking, and sometimes amyotrophic lateral sclerosis (ALS) [8].

The genetic influence of FTD is greater than in the case of AD. Between 15 and 30% of FTD cases are due to mutations in the C9orf72, GRN or MAPT genes, among others [9]. A pathogenic expansion of a hexanucleotide (G_4C_2) repeat in the regulatory region of C9orf72 was recently identified as a major cause of the disease amyotrophic lateral sclerosis and frontotemporal dementia10. In the human genome, the C9orf72 gene is located on chromosome 9p21. It is suggested that $G_4C_2 < 20$ repeats are normal, while $G_4C_2 \geq 30$ repeats are pathological repeat expansions. In ALS and FTD, G_4C_2 repeat copies of C9orf72 can reach up to 700-1600 units, which explains 4% to 8% of all the cases [11,12]. Substantial clinical heterogeneity between mutation carriers has been observed, and the age at onset is highly variable, from mid-20s to the ninth decade. Three possible hypotheses may explain the mechanism of how C9orf72 expanded repeats leads to these diseases: a loss-of-function effect causing C9orf72 haploinsufficiency, neurotoxic effect due to the generation of aggregates called dipeptide repeat proteins and a gain of function associated with the expression of abnormal bidirectionally transcribed RNAs carrying the repeat [13].

Clinical and pathological characteristics overlap among the common neurodegenerative diseases. However, the relationship between C9orf72 and AD remains unknown, and numerous studies are taking place to clarify it [2,6,10,11,14].

Here, we report the clinical, neuropsychological and genetics of a patient presenting early onset dementia with Alzheimer-like symptoms, carrier of the G_4C_2 expansion of C9orf72 and the APOE4 variant.

Case Report

A 45-year-old male with a family history positive for dementia. Our patient had 13 siblings, 4 of them deceased by dementia (unspecified), 1 diagnosed with AD (42 years old) and 1 diagnosed with FTD (50 years old). We do not have information about the parents.

The patient worked as a scrap merchant. He was referred to the neuropsychiatric department of the hospital due to memory loss and reduced cognitive functional capacity. He repeated the same story without realizing it and forgot some recent events. He had difficulty performing daily activities, such as remembering the shopping list or getting dressed. He also had orientation problems in which it was difficult to find way around and drive. Besides, he had aphasia-type language disorders of which the patient was aware.

In a subsequent evaluation (3 years after the onset of symptoms), a greater cognitive deterioration was observed, coupled with behavioural worsening, indicating that he required ongoing help and support. His caregivers reported irritability, explosive nature, and inconsistent eating.

Materials and Methods

Neuropsychological evaluation

Two neuropsychological evaluations were performed: one in January 2020 and the other in June 2023. The first evaluation included the following tests: Barther scale, Short Portable Mental State Questionnaire (SPSMQ), and Lawton Instrumental Activities of Daily Living Scale (IADL). The second evaluation included the three previous tests and added the Mini-Mental State Examination (MMSE) and the Clock Drawing Test. Additionally, a neuropsychiatric evaluation was performed in both evaluations.

FDG-PET

The patient underwent a PET brain scan with fluorodeoxyglucose (FDG-PET), injecting labelled glucose into the patient's peripheral vein and obtaining radiological images during the first 30 minutes.

Genetic analysis

Considering the doctor's recommendation, a panel was conducted to investigate the presence of mutation associated with AD, including the genes PSEN1, PSEN2, APP and APOE NGS sequencing. The DNA was extracted using MAXWELL 16 LEV (Promega Biotech Ibérica, SL, Spain.). An AmpliSeq Custom Panel (Life Technologies, CA, USA) was designed with Ion Ampliseq Designer for the exons of the genes related to AD PSEN1, PSEN2, APP and APOE. According to the manufacturer's instructions, library preparation was performed using Ion Ampliseq Library Kit 2.0 (Life Technologies) and Ion Xpress™ Barcode Adapter 1–96 Kit (Life Technologies). The library was finally sequenced with an Ion Torrent PGM. After sequencing, the exonic regions with a depth coverage below 20X were completed by Sanger sequencing.

Subsequently, exome NGS sequencing of the sample was performed. Coding regions of the DNA were captured using the Agilent Sure Select Human All Exon v6 capture kit (Agilent

Technologies, CA, USA) and sequenced in a NovaSeq 6000 platform (Illumina Inc., CA, USA). FastQ files were aligned using bwa [15], and Free Bayes [16] was used as a variant caller. Detected variants were annotated using an in-house pipeline. CNVs were detected by using Exome-Depth [17] and CONIFER [18]. The analysis of the variants was restricted to an in silico panel composed of genes related to early-onset dementia. The list of genes was obtained by combining HPO terms (dementia frontotemporal HPO:0002145), Panel App (Genomic England) panel for Early Onset Dementia and review of the bibliography. Final panel consisted in the following genes: APP, ATN1, CHCHD10, CHMP2B, CSF1R, DCTN1, DNAJC5, DNMT1, EPM2A, FUS, GRN, HNRNPA1, HTRA1, ITM2B, JPH3, MAPT, NHLRC1, NOTCH3, PRNP, PSEN1, PSEN2, SNCA, SNCB, TARDBP, TBK1, TREM2, TYROBP, UBQLN2, VCP, SERPINI1, SQSTM1 and TUBA4A.

Finally, the expansion of the C9orf72 gene was analyzed by primed PCR using the AmplideX PCRCE C9orf72 kit (Asuragen Inc., TX, USA). A PCR amplification of the hexanucleotide repeat element (G_4C_2) was performed in intron 1 of the C9orf72 gene. The size of the fragments was determined by capillary electrophoresis. The number of replicates was calculated based on the size of the PCR fragment generated. The kit range is set between 1 and 145 repeats; above 145 repeats, all alleles were reported as ">145 repeats".

Results

Neuropsychological and Neuropsychiatric evaluation

No focal deficits or gait disturbances were observed in either evaluation. The patient was negative for frontal release reflexes, and did not show Parkinsonism.

The results of the first evaluation were: Barther scale 95 over 100 (mild dependence), SPSMQ was 1 over 10 (normal intellectual functioning), IADL 3 over 5 (moderate dependence).

The results of the second evaluation were: Barther scale 90 over 100 (mild dependence), SPSMQ was 4 over 10 (deficient intellectual functioning), IADL 2 over 5 (moderate dependence). The MMSE was 24 over 30 (pathological suspicion), and the Clock Drawing Test was 2-2-0, indicating malfunction and cognitive impairment.

The neuropsychiatric evaluation did not detect delusions or hallucinations. In the second evaluation, the patient presented behavioural alterations such as childish behaviour, dependence on his wife, and possible confabulations; there was some social but not sexual disinhibition. Furthermore, the patient had irritability.

FDG-PET

The FDG-PET study did not reveal pathological findings from the metabolic point of view at the cortical and subcortical level that would suggest AD or FTD. The patient presented diffuse cortical atrophy.

The study does not show areas of significant hypometabolism at the cortical, subcortical or cerebellar level.

Genetic analysis

In the analysis of the AmpliSeq panel for the most common AD genes, the variant APOE(ENST00000252486.9):c.388T>C (p.Cys130Arg) was detected. This variant has conflicting interpretations in ClinVar: 2 pathogenic, 2 likely pathogenic and 2 VUS. However, this variant is considered a factor risk for late-onset AD [19]. No other significant variant was found in this panel.

The analysis of the in silico panel for early onset dementia did not show any pathogenic or likely pathogenic variant. The depth coverage obtained was 134X, covering nearly 99% of the coding region.

Finally, C9orf72 analysis disclosed the presence of an expansion of more than 145 repeats of hexanucleotides (G_4C_2) in intron 1 of the C9orf72 gene compatible with pathology.

Discussion

We present the case of a 45-year-old male suspected of having AD whose genetic study would further support the diagnosis of FTD. Initial symptoms showed more similarities to AD; however, as the disease progressed, they became more suggestive of FTD.

The genetic study for typical Alzheimer's genes was negative, and a pathological expansion was detected in C9orf72, a common gene for FTD [13]. Nevertheless, the patient was a heterozygous carrier of the E4 variant of APOE, which has been widely associated with an increased risk of AD, although not a sufficient cause for its development [20].

There are greys in this palette of neurodegenerative diseases. A variant of AD, the frontal variant (fvAD), has recently been described [21]. It shows a less frequent phenotype with symptoms that differ from classic AD. This variant is characterized by presenting executive alterations, progressive apathy, disinhibition and stereotyped behaviours. On the other hand, behavioural variant FTD (bvFTD) cases have been described, which present memory impairment similar to that of people with AD, impacting their linguistic and behavioural abilities [22,23].

Our patient presented symptoms that could be compatible with fvAD or atypical bvFTD. Furthermore, there were diagnosed cases of AD and FTD in his family. Given the clinical overlap of these two pathologies, the definitive diagnosis is currently only obtained through an autopsy. It has been seen that up to 40% of patients diagnosed with FTD had AD in the postmortem examination. Likewise, some patients diagnosed with FTD at

the pathological level had previously been diagnosed with AD [21]. Besides postmortem examination, genetics studies are also considered a definitive diagnostic method. Historically, mutations in C9orf72 had been associated with FTD. However, there are cases of AD in which a pathological expansion in C9orf72 has been found [2,12] complicating the genetic diagnostic method.

Over the past few years, significant efforts have been achieved in understanding the pathological mechanisms triggered by a high number of hexanucleotide repeats in C9orf72 [13]. It has been suggested that the severity of the disease seems to be more related to the type of repeated dipeptide than to the number of G_4C_2 repeats. The dipeptides produce the cytoplasmic accumulation of the TDP-43 protein, which is abnormally phosphorylated and aggregates, leading to the alteration of nuclear-cytoplasmic protein transport by altering the importin complex. This phenomenon generates an accumulation of proteins that aggregate in the cytoplasm [23].

It has been seen that 57% of AD cases have TDP-43 inclusions. AD patients harbouring TDP-43 pathology exhibit increased cognitive severity impairment in contrast to those lacking such pathology. Additionally, APOE4 correlates with an elevated incidence of TDP-43 pathology [24]. Furthermore, APOE4 could accelerate neurodegeneration in patients with FTD [25].

Considering all these data, we suggest that in our patient, APOE4 could act as a genetic modifier of C9orf72. The presence of the pathological expansion together with the APOE4 variant could cause the development of Alzheimer's disease instead of FTD.

Given the existence of the aforementioned atypical forms of AD and FTD and the described cases of AD that presented a mutation in C9orf72, we consider that the expansions of this gene should be analyzed in all patients with suspected familial AD, whose previous genetic study directed towards Alzheimer's genes have tested negative. Furthermore, a genetic study is key in this kind of patients due to the possibility of offering them genetic counselling to their relatives.

The relevance of genetic study also takes on importance in pharmacological treatment. To the date, approved drugs do not stop these neurodegenerative diseases. However, new disease-modifying therapies are currently being developed to delay its progression. The early and adequate diagnosis of these diseases is a challenge and to achieve this, it is essential to investigate the genetic bases of neurodegenerative pathologies in order to move towards personalized medicine [26,27].

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