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A Rare Case of Homozygous Mutation in TBK1 Gene in a Patient with ALS-FTD

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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that leads to death in 3-5 years. In up to 10% of cases there is a family history for ALS. Several genes have been associated with ALS and many of them are also involved in the development of Fronto-temporal dementia (FTD). Recently mutations in the TANK binding kinase 1 (TBK1) have been identified as a cause of ALS and FTD. Most TBK1 known mutations associated with ALS are nonsense and frameshift mutations, resulting in premature termination codons (TCs), causing the production of an incomplete and non-functional protein. Here we described a case of ALS associated with bv-FTD of a woman with a novel homozygous missense mutation in TBK1 gene. We used a silico computational software to evaluate the effect of this novel mutation and this analysis indicated, with high probability a detrimental effect of protein expression and activity of TBK1. The haploinsufficiency due to the homozygous mutations probably led to the development of ALS-FTD in this patient.

Keywords: ALS-FTD, novel mutation, TBK1, homozygous mutation, case report

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive loss of both upper and lower motor neurons that leads to a fatal paralysis and death, mainly due to respiratory failure[1] typically after 3-5 years post onset[2]. The average age of onset is between 50 and 65 years[3]. It is a multifactorial disorder with environmental and genetic components[4]. In up to 10%[5] of cases there is a family history positive for ALS. Mutations in several genes have been linked to the pathogenesis of ALS, such as SOD1, C9orf72, TARDPB, FUS, NEK1[5] and many of them are also associated with fronto-temporal dementia (FTD)[6], a heterogeneous neurocognitive syndrome characterized by the progressive impairment of language, executive functions and change in behavior. Two main FTD clinical variants are to date recognized: behavioral (bv-FTD) and primary progressive aphasia (PPA)[7]. The brain usually shows marked atrophy of frontal and temporal lobes[8]. ALS and FTD often overlap in their clinical presentation, genetic mutations, and physiopathology. For many researchers, they represent a spectrum of disease continuum[1, 9]. Recently, thanks to exome sequencing techniques, mutations in the TANK binding kinase 1 (TBK1) have been identified as a cause of ALS [10, 11] and FTD[12]. TBK1 is a multifunctional protein, member of the IKK kinase family, involved in the regulation of multiple processes as innate immunity, inflammation, autophagy, and cell proliferation[13].Here we present a case of ALS associated with bv-FTD of a woman with a novel homozygous mutation in TBK1 gene.

MATERIAL AND METHODS

This research was approved by the ethical committee of Policlinico Umberto I and the patient, and her family gave their written informed consent for this study.

The patientwas first seen at age of 58 years with a 6 months history of weakness of the right hand, without loss of sensibility. In the next few months, the patients noted a progressive gait impairment, with unsteadiness due to leg weakness. At the first evaluation, weakness of the right upper limb was noted, together with spastic hyper tone, brisk reflexes, fasciculations of upper and lower limbs, Babinski sign at both sides, Hoffmann sign at the right side, palmo-mental reflex, positive glabellar tap sign and absent jaw jerk. ALS Functional Rating Scale-Revised (ALSFRS-R) was 40. Electrophysiology

study was performed and widespread denervation in lower and upper limbs and cervical muscles was found. According to El Escorial criteria diagnosis of defined ALS was done, and the patient began therapy with Riluzole 50 mg twice a day.

Otherwise,she was healthy apart from celiac disease. She had not a family history consistent with ALS or other neurodegenerative diseases, but her parents were first-degree cousins as they were from a little village, in the center of Italy, with approximately 300 inhabitants.

Subsequently, the patient developed a depressive syndrome with anhedonia, lack of empathy, ideomotor slowdown with attention deficit.She performed a brain MRI that showed cerebral atrophy with marked dilation of the subarachnoid spaces in the temporal-insular para hippocampal region (figure 1).

The patient was administered Edinburgh Cognitive and Behavioral Amyotrophic Lateral Sclerosis (ALS) Screen (ECAS), as a tool for the detection of the cognitive and behavioral status of patients with ALS[14]. This test consists of 15 subtests across different domains: language, verbal fluency, executive functions, that generate a composite ALS-specific score, and memory and visuospatial functioning that form an ALS-nonspecific score.The total score for ECAS was 89/136 with specific ALS test score equal to 75/100 and a non-specific ALS test score of 14/36. Neuropsychological assessment revealed a mild degree of cognitive dysfunction in the context of executive functions. She and her family also noted some changes regarding her behavior, with an increased apathy and emotional lability, uncontrollable laughing or crying, and heightened irritability. Her speech was fluent, well-articulated, and grammatically correct. The cognitive and behavioral profile reflected a degree of predominantly frontal lobe dysfunction. After two years from ALS diagnosis, ALS FRS re-evaluation was performed, and the patient score was 30. The patient was unable to use the right hand (MRC for wrist and fingers flexion and extension equal to 0/5), she had marked spasticity of the limbs, greater in the right upper limb. She was unable to use stairs and completely dependent on others for her normal daily activities. She had no dysphagia. For the presence of initial signs of respiratory failure, with spirometry values of forced vital capacity (FVC, expressed as the percentage of predicted value) in standing posture equal to 74% and in sitting posture equal to 62%, it was recommended to start non-invasive ventilation for a few hours during the day. The patient performed again neuropsychological assessment and the total ECAS score was 66/136, with ALS specific sub-score of57/100 and non-ALS specific scoreof 9/36. There was a worsening of performances especially in the domain of executive and visuospatial functions. The patient became completely apathetic and anhedonic. She lost interest in any activity, withdrew into the home, minimizing interactions with other individuals. She performed another brain MRI and there was more atrophy, enlarged ventricles and the volumes of the hippocampi were reduced compared to the first scan.

After obtaining written informed consent, a peripheral blood sample was taken to perform the genetic test, including C9ORF72 repeat expansion evaluation, in view to determinate the possible role of genetic factors in the etiology of the patient clinical phenotype. The patient died after 4 years from the onset of symptoms due to respiratory failure.

Genetic Test

Whole blood (3 ml) was collected for exome analysis after obtaining informed consent. DNA was extracted using the Qiagen Bio Robot DNA extraction kit (Qiagen Benelux B.V., Venlo, the Netherlands) according to the manufacturer's instructions and quantified using Nanodrop spectral analysis (Thermo Fischer Scientific, Inc., Waltham, MA, USA). DNA fragmentation and degradation were evaluated by standard agarose gel electrophoresis (100 V, 30 min, 1.5% agarose gel in Tris-borate-EDTA buffer). DNA Library preparation and whole exon enrichment were performed employing Agilent All Exon V.6 kit (Agilent Technologies, Inc., Santa Clara CA, USA). Library sequences were obtained using the HiSeq2500 Illumina Sequencer (125-bp paired end sequence mode). Bioinformatics analysis included the following: Next-generation sequencing (NGS)

reads mapping to whole genomes using the Burrows-Wheeler Alignment tool with default parameters, polymerase chain reaction (PCR) duplicate removal using Picard (http://picard.sourceforge.net), single nucleotide polymorphisms and indel calling using the Genome Analysis Toolkit (GATK) Unified Genotyper, variant annotation using snpEff (http://snpeff.sourceforge.net) and false positive variant filtration using the GATK Variant Filtration module.

Exome sequencing data and reads alignment analysis were checked for coverage depth and alignment quality employing Bedtools software package. Phenotype driven analysis coupled with the employment of in silico multigene panels specific for motor neuron diseases was used to filter, select and interpret genetic variants.Variant analysis was performed employing bioinformatic prediction tools (Polyphen2, SIFT, Mutation Taster, PhyloP, CADD-Phred) and classification was conducted in accordance with the guidelines from the American College of Medical Genetics and Genomics. In brief, variants were classified as follows: I) pathogenic variants, i.e. sequence change known to be directly implicated to the development of disease; II) likely pathogenic variants, i.e. genetic changes not previously reported in literature, or with limited scientific evidence, likely implicated in sufficient informations to support a more definitive classification and III) benign or likely benign variants. NGS sequencing and variant interpretation highlighted the presence of the homozygous mutation p.Trp259Arg (c.775T>A) in the TBK1 gene. The presence of this change was confirmed by Sanger re-sequencing and the absence of a possible gene or exon deletion of the TBK1 gene was evaluated by Real-Time PCR (data not shown). To date, p.Trp259Arg (c.775T>A) mutation is not reported in literature, it is absent from Gnome AD, in silico computational analysis indicates with high probability a damaging effect on the structure or activity of the resulting protein (Polyphen2=0.848/1,00; SIFT=0.00/0.00, MutationTaster=1,00/1,00; CADD PHRED=22/20; Mutation assessor=2.81/5,00) and it is located in a conserved protein position (phyloP-Vertebrate= 4.44/6.42; phyloP-Primate= 0.448/0.65; PhastCons= 1.00/1.00)

DISCUSSION

We here report the case of a patient affected by progressive ALS with frontotemporal dementia, carrier of a novel missense homozygous mutation of the TBK1 gene.

TBK1 is a kinase protein involved in many cellular processes including inflammatory pathways, autophagy and replication[13] by phosphorylation of a wide range of substrates [15]. TBK1 protein has four domains: kinase domain (KD), a ubiquitin-like domain (ULD), an α -helical scaffold dimerization domain (SDD), and a C-terminal domain (CTD)[15]. Most TBK1 known mutations associated with ALS are [10, 16] nonsense and frame shift mutations, resulting in premature termination codons (TCs), causing the production of an incomplete and non-functional protein [17]. TBK1 haploinsufficiency is supposed to contribute to the development of ALS [16]., alone or combined with FTD[18]. The contribution to the ALS pathogenesis of missense mutations and single amino acid deletions is less known. It was proposed that missense mutations could influence the effect of kinase activity or substrate binding capacity [12] leading to a predisposition for the disease and not cause it directly [19]. Other possible mechanisms leading to the development of the disease are the reduction of protein expression or the loss of interaction with its adaptor s[20].The mutation p.Trp259Arg is located in a conserved position which falls in the serine/threonine kinase domain[21], essential for its pleiotropic functions and in silico computational software analysis indicated with high confidence a damaging effect on the expression and activity of TBK1 protein. We hypothesize that this missense mutation leads to a disruption of phosphorylation activity of TBK1, and the homozygous mutation contributes to a further reduction of protein functions and therefore to the development of the disease.A previous in vivo study demonstrated that the mutant mice with homozygous missense mutations in the kinase domain of TBK1 were non-viable[22]. Both parents of the patients should be carriers of this mutation (Real-Time PCR disclosed the presence of two mutated alleles in the Proband), but they had not any symptoms suggesting that a cumulative

disfunction of TBK1 kinase activity have an impact on disease development.A previous studyhas shown that mutations in the Kinase domain of TBK1 gene are associated with overlapped diseases ALS-FTD, and often with the bv FTD phenotype of frontotemporal dementia[18]. The clinical presentation of patients with TBK1 mutations is very broad, regarding age and site of onset, survival and cognitive impairment [18]. Our patient developed the first symptoms of disease in middle age, with weakness of the right arm. Impairment of executive functions and changes in her behavior, with apathy and social withdraw developed soon during the disease and the brain MRI revealed marked atrophy in temporal regions. The death occurred due to respiratory failure after 4 years.

CONCLUSION

Most of the TBK1 pathogenic mutations associated with ALS-FTD are known to be LoF mutations, but there are little data regarding the potential pathogenicity of missense mutations. TBK1 is a multifunctional kinase and even a minimal dysfunction due to a single variation could be relevant and affect the development of the disease[23]. This is the first report to demonstrate a mutation of p.Trp259Arg in homozygosity in the TBK1 gene in an Italian ALS-bvFTD patient. Clinical, neuropsychologic tests and electrophysiological evaluations confirmed the diagnosis of ALS-FTD. The silico computational software analysis indicated, with high probability a detrimental effect of protein expression and activity of *TBK1.* The haploinsufficiency due to the homozygous mutations probably led to the development of ALS-FTD in this patient.

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