In-Frame Insertion Mutation in the SPG11 Gene Causes Autosomal Recessive Spastic Paraplegia with Thin Corpus Callosum “In A” Turkish Family with Late Age of Onset of the Phenotype

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Abstract

Autosomal recessive hereditary spastic paraplegia with thin corpus callosum (ARHSP-TCC) is one of the most prevalent forms of complex ARHSP. Mutations in the SPG11 gene are the most common cause for ARHSP-TCC and accounts for up to 70% of all cases. The mutational spectrum of SPG11 gene is broad as all types of DNA alterations are detected in the gene and most mutations lead to a premature truncation of the protein, suggesting “loss of function” as the likely pathogenic mechanism. In the current study, we report a consanguineous Turkish family with ARHSP inheritance manifesting white matter abnormalities including TCC with relatively late age of onset. Sequencing of SPG11 gene revealed a homozygous insertion of 15 nucleotides at position 6886 in exon 38 (c.6886_6900Dup15) leading to an in-frame insertion of five amino acids at codon 2296 (p.K2296_L2300Dup5), which resides within a predicted, highly conserved, intradial ring-cleavage dioxygenase domain (2104 -2381 residues). In silico structural prediction of intradial domain of the mutated Spatacsin protein revealed that the duplication of five amino acids leads to an extra turn in α-helix and a slightly longer loop region. Our structural analysis suggests that it is unlikely that insertion mutation (c.6886_6900Dup15) causes dysfunctional protein rather the minor conformation changes may elicit a “gain of function”, which may be detrimental to endogenous function of Spatacsin thus cause HSP.

Keywords: Spastic paraplegia; SPG11; Thin corpus callosum; In-frame insertion

Introduction

Hereditary spastic paraplegias (HSP) are a heterogeneous group of neurodegenerative disorders, in which the prominent clinical features are progressive spasticity and weakness of the lower limbs [1-4]. On the basis of clinical phenotypes, HSP can be classified into two forms: the pure and the complex HSP. In pure HSP, spasticity occurs in relative isolation. However, when spasticity is associated with additional neurological and/or non-neurological symptoms then it is termed as complex HSP [2,4] Up till now, over 48 HSP loci were mapped as complex HSP [2,4] Up till now, over 48 HSP loci were mapped [5,6]. Among ARHSP loci, the TCC spastic paraplegia, mild mental retardation with learning difficulties ARHSP. Besides TCC, the disease is characterised by progressive spastic paraparesis, mild mental retardation with learning difficulties and severe peripheral neuropathy [5-8]. Among ARHSP loci, the TCC phenotype is manifested at least in six sub-types namely, SPG11, SPG15, SPG18, SPG21, SPG32 and SPG46, of which four causative genes have been identified to date, Spatacsin (SPG11), Spastizin (SPG15), ERLIN2 (SPG18) and ACP33 (SPG21) [9-13]. However, the most frequently mutated gene in ARHSP is SPG11 [5,7,8] and accounts for 41-77% of all ARHSP-TCC cases, depending upon the ethnic origin of the selected cohort of patients.

SPG11 gene spans 40 exons and encodes 2443 amino acids Spatacsin protein. The molecular function of Spatacsin is unknown. Spatacsin show broad expression in central nervous system with high expression in cerebellum, cerebral cortex, hippocampus and pineal gland. In silico sequence analysis revealed that Spatacsin consists of a Leucine zipper, a short-coiled coil domain, a Myb domain, a glycosyl hydroxylase F1 signature, intradial ring-cleavage dioxygenase domain and four transmembrane domains [14]. The presence of such domain/ motif suggests that Spatacsin is likely involved in vesicular transport. A recent study reported that Spatacsin partially colocalizes with microtubules and vesicles [15].

Screening for mutations in the SPG11 gene by various groups has identified over 120 different mutations in most exons of the gene. The SPG11 mutations are summarized in the Human Gene Mutation Database Professional release 7.1 (http://www.biobase.de/hgmd/pro/ start.php). All types of DNA alterations are detected in the SPG11 gene, including missense, nonsense, splice site mutations and insertions/ deletions. With exception of few missense mutations all mutations cause a premature truncation of the protein suggesting “loss of function” as the likely pathogenic mechanism.

Case Report

Patient I13 is the index patient who was first examined at the age of 30 years and followed up for more than 14 years. He manifests a short stature and mild macrocephalus. Initially, gait disturbance in the
Results and Discussion

Due to the observation of HSP symptoms and the autosomal recessive mode of inheritance, genetic analysis was requested. After informed consent was provided, blood samples were obtained from the probands and genomic DNA was extracted from leukocytes. After excluding mutations in the SPG7 gene (Refseq: NM_003119.2), the coding sequence and splice site boundaries of the 40 exons of the SPG11 gene (Refseq: NM_025137.3) were amplified by polymerase chain reaction (PCR). Single-strand conformation polymorphism (SSCP) analysis according to standard procedure was used to identify band shifts which were confirmed by direct sequencing [16]. The analyses revealed a homozygous insertion of 15 nucleotides (AAGTTGATAAUCTGT) at position 6886 in exon 38 (c.6886_6900dup15). This duplication of 15 base pairs leads to an in-frame insertion of five amino acids [lysine, leucine, isoleucine, threonine and leucine (KLITL)] at codon 2296 (p.K2296L2300dup5) Figure 2A. The c.6886_6900Dup15 mutation was found in both affected patients in homozygous state. The parents (I1 and I2) as healthy first-degree cousins, were typed to be heterozygote carriers (I1 and I2).

Patient II1 is a sister of the index patient. She first noticed gait disturbance and spasticity in the lower extremities together with nocturnal leg cramps and pain attacks in the upper limbs at age of 28 years. Neurological examination also showed the classic spastic paraplegia symptoms such as proximal weakness of the lower limbs and profound proximal spasticity, Hyperreflexia of the lower as well as the upper limbs was also observed. Brain MRI findings were similar to that of patient II3.

However, our structural analysis suggest that it is unlikely that insertion of five amino acids (KLITL) at codon 2296 due to c.6886_6900Dup15 mutation occurs within a predicted, highly conserved, intradiol ring-cleavage dioxygenase domain (2104 - 2381 residues) (Table S1) [5]. To evaluate the effect of KLITL insertion on the protein conformation, we performed structural prediction of intradiol domain of wild-type and mutant Spatacsin protein by using I-TASSER protein structure prediction software [17,18] and images of the structures were produced using the PyMOL Molecular Graphics System (Version 1.3, Schrödinger, LLC). Overlaying of the tertiary model of mutant protein (red) over wild-type intradiol domain (green) revealed no major conformational changes in the mutated protein due to addition of five amino acids (Figure 3A). However, when structural prediction of the core region (Table S1) was performed using PHYRE2 prediction server [19], it revealed that the presence of additional five amino acids in the mutant Spatacsin (red) leads to an extra turn in α-helix and a slightly longer loop region (Figure 3B).

In conclusion, we identified a novel SPG11 gene mutation in a consanguineous Turkish family causing a form of ARHSP with relatively late age of onset and white matter abnormalities including TCC. In silico structural prediction of intradiol domain of the mutated Spatacsin protein revealed only minor alteration in the conformation of the domain. Majority of SPG11 gene mutations lead to loss of function. However, our structural analysis suggest that it is unlikely that insertion mutation (c.6886_6900Dup15) causes dysfunctional protein rather the neurological impairment. Yet, the present mutation appears to cause comparably late age at onset of walking impairment.
minor conformation changes may elicit a gain of function, which may be detrimental to endogenous function of Spatacsin thus causes HSP.

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