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Case Report Open Access

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, intradiol ring-cleavage dioxygenase domain (2104 -2381 residues). *In silico* structural prediction of intradiol 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 and 24 causative genes have been identified. All types of inheritance are reported for HSP, including autosomal dominant, recessive and X-linked.

Autosomal recessive hereditary spastic paraplegia with thin corpus callosum (ARHSP-TCC) is one of the most prevalent forms of complex 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, intradiol 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 II3 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

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patient was noted by his younger brother. Seven years later, maximum walking distance with assistance was about 300-500 meter. By now, he is wheelchair-bound. Detailed neurological examination showed predominantly bilateral proximal pronounced paresis (3/4) of the lower limbs with hyperreflexia and positive bilateral Babinski sign. He also complains about urinary urgency. Mental retardation as illustrated by learning difficulties can be assumed but not confirmed due to the illiterate condition and the language problems of the patient. For the same reasons cognitive decline could not be followed up. The patient developed dysdiadochokinesis, but no cerebellar ocular signs were noted. Magnetic resonance imaging (MRI) showed periventricular white matter alterations and moderate diffuse cortical atrophy with pronounced thinning of the CC (Figure 1). Additional features included hyper-pigmented skin lesions on shoulder and genitals, conspicuous iris pigmentation and obesity with a body mass index above 33.

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.

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 (AAGTTGATAACTCTG) 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.K2296_L2300Dup5) Figure 2A. The c.6886_6900Dup15 mutation was found in both affected patients in homozygous state. The parents (I1 and 12) as healthy first-degree cousins, were typed to be heterozygous for this mutation (Figure 2B). Comparing the clinical features in affected individuals from this family with those reported in families harboring other *SPG11*-mutations [5-9], the phenotypic characteristics were remarkably similar concerning disease severity and progressive neurological impairment. Yet, the present mutation appears to cause comparably late age at onset of walking impairment.

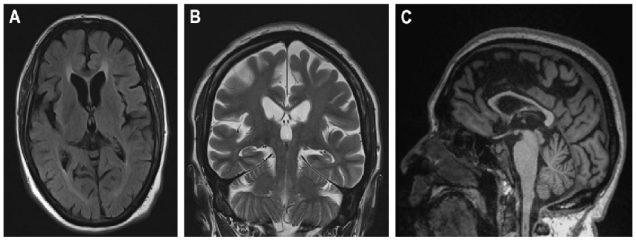

Figure 1: Brain magnetic resonance imaging of individual II3. (**A**) Axial FLAIR (**B**) T2-weighted coronary and T1-weighted sagittal images (**C**) show supratentorial brain atrophy with enlargement of the lateral ventricles and outer liquor spaces as well as pronounced thinning of the corpus callosum.

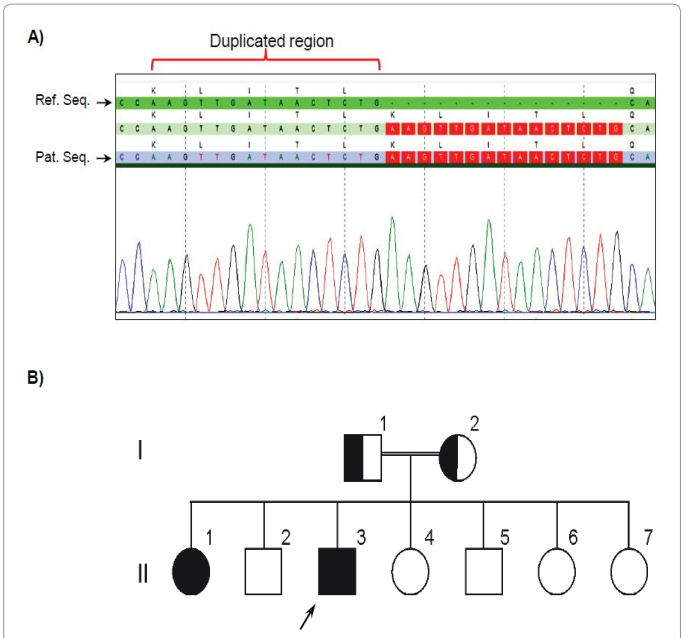

Figure 2: Identification of mutations in the *SPG11* gene. (**A**) The chromatogram represents the in-frame insertion mutation c.6886_6900Dup15 in exon 38 of the *SPG11* gene of the index patient (II3). The wild type (Ref. Seq.) nucleotide sequence is shown at the top (highlighted in green color) and index patient sequence (Pat. Seq.) is highlighted in light blue color. The insertion mutation is highlighted in red color. (**B**) Pedigree and segregation of the mutation in the HSP family. Square symbols represent men, the circles represent women. The index patient (II3) and his affected sister (II1) both carry the in-frame insertion mutation in the homozygous state. The parents are heterozygote carriers (I1 and I2).

The 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 altercation 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

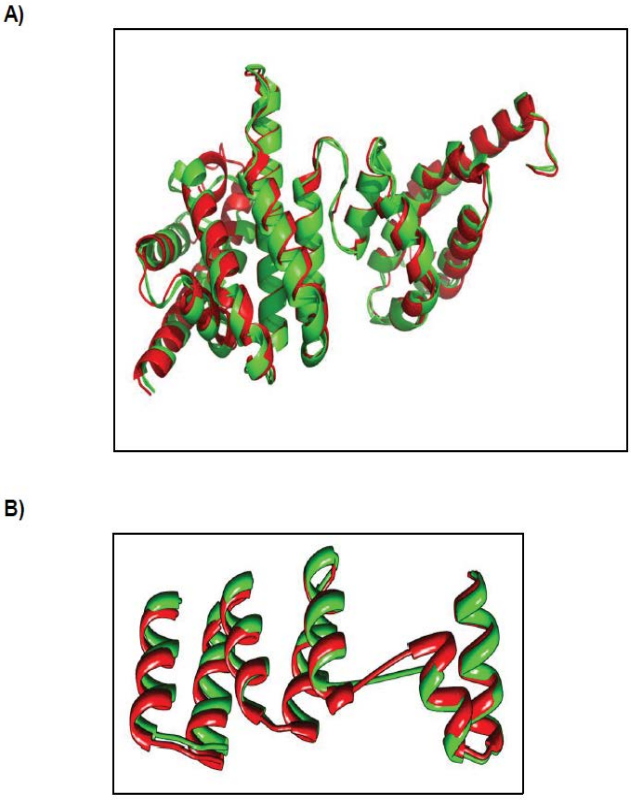

Figure 3: Structural models of Spatacsin's intradiol ring-cleavage dioxygenase domain (2104 -2381 residues). (**A**) Overlay of tertiary structure of intradiol domain of wild-type (green) and mutant Spatacsin (red) are shown as ribbon diagrams. No major conformational change was detectable in the mutated protein due to addition of five amino acids (lysine, leucine, isoleucine, threonine and leucine). (**B**) Structural prediction of core region of intradiol domain (2271 -2355 residues) revealed that insertion of five amino acids in the mutant Spatacsin (red) leads to an extra turn in α-helix and a slightly longer loop region.

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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References

1. Fink JK (1997) Advances in hereditary spastic paraplegia. Curr Opin Neurol 10: 313-318.
2. Fink JK (2003) The hereditary spastic paraplegias: nine genes and counting. Arch Neurol 60: 1045-1049.
3. Harding AE (1983) Classification of the hereditary ataxias and paraplegias. Lancet 1: 1151-1155.
4. Reid E (2003) Science in motion: common molecular pathological themes emerge in the hereditary spastic paraplegias. J Med Genet 40: 81-86.
5. Hehr U, Bauer P, Winner B, Schule R, Olmez A, et al. (2007) Long-term course and mutational spectrum of spatacsin-linked spastic paraplegia. Ann Neurol 62: 656-665.
6. Schüle R, Schlipf N, Synofzik M, Klebe S, Klimpe S, et al. (2009) Frequency and phenotype of SPG11 and SPG15 in complicated hereditary spastic paraplegia. J Neurol Neurosurg Psychiatry 80: 1402-1404.
7. Stevanin G, Paternotte C, Coutinho P, Klebe S, Elleuch N, et al. (2007) A new locus for autosomal recessive spastic paraplegia (SPG32) on chromosome 14q12-q21. Neurology 68: 1837-1840.
8. Stevanin G, Azzedine H, Denora P, Boukhris A, Tazir M, et al. (2008) Mutations in SPG11 are frequent in autosomal recessive spastic paraplegia with thin corpus callosum, cognitive decline and lower motor neuron degeneration. Brain 131: 772-784.
9. Stevanin G, Santorelli FM, Azzedine H, Coutinho P, Chomilier J, et al. (2007) Mutations in SPG1, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. Nat Genet 39: 366-372.
10. Alazami AM, Adly N, Al Dhalaan H, Alkuraya FS (2011) A nullimorphic ERLIN2 mutation defines a complicated hereditary spastic paraplegia locus (SPG18). Neurogenetics 12: 333-336.
11. Boukhris A, Feki I, Elleuch N, Miladi MI, Boland-Auge A, et al. (2010) A new locus (SPG46) maps to 9p21.2-q21.12 in a Tunisian family with a complicated autosomal recessive hereditary spastic paraplegia with mental impairment and thin corpus callosum. Neurogenetics 11: 441-448.
12. Hanein S, Martin E, Boukhris A, Byrne P, Goizet C, et al. (2008) Identification of the SPG15 gene, encoding spastizin, as a frequent cause of complicated autosomal-recessive spastic paraplegia, including Kjellin syndrome. Am J Hum Genet 82: 992-1002.
13. Simpson MA, Cross H, Proukakis C, Pryde A, Hershberger R, et al. (2003) Maspardin is mutated in mast syndrome, a complicated form of hereditary spastic paraplegia associated with dementia. Am J Hum Genet 73: 1147-1156.
14. Paisan-Ruiz C, Nath P, Wood NW, Singleton A, Houlden H (2008) Clinical heterogeneity and genotype-phenotype correlations in hereditary spastic paraplegia because of Spatacsin mutations (SPG11). Eur J Neurol 15: 1065-1070.
15. Murmu RP, Martin E, Rastetter A, Esteves T, Muriel MP, et al. (2011) Cellular distribution and subcellular localization of spatacsin and spastizin, two proteins involved in hereditary spastic paraplegia. Mol Cell Neurosci 47: 191-202.
16. Jaeckel S, Epplen JT, Kauth M, Miterski B, Tschentscher F, et al. (1998) Polymerase chain reaction-single strand conformation polymorphism or how to detect reliably and efficiently each sequence variation in many samples and many genes. Electrophoresis 19: 3055-3061.
17. Roy A, Kucukural A, Zhang Y (2010) I-TASSER: a unified platform for automated protein structure and function prediction. Nat Protoc 5: 725-738.
18. Zhang Y (2008) I-TASSER server for protein 3D structure prediction. BMC Bioinformatics 9: 40.
19. Kelley LA, Sternberg MJ (2009) Protein structure prediction on the Web: a case study using the Phyre server. Nat Protoc 4: 363-371.