Autosomal recessive hereditary spastic paraplegia with thin corpus callosum among Saudis

Salma M. Wakil, *PhD,* ***Hatem N. Murad,*** *MD,* ***Batoul M. Baz,*** *MSc,* ***Samiya T. Hagos,*** *BSc,* ***Rana A. Al-Amr,*** *MSc,* ***Suad A. Al-Yamani,*** *MD,* ***Salem M. Al-Wadaee,*** *MSc,* ***Brian F. Meyer,*** *PhD,* ***Saeed A. Bohlega,*** *MD.*

ABSTRACT

الأهداف: تقييم الطفرات الجينية والصفات السريرية المرتبطة بالشلل التشنجي السفلي مع دقة في الحجم الثفني (ARHSP-TCC).

الطريقة: أُجريت هذه الدراسة في مستشفى الملك فيصل التخصصي ومركز الأبحاث، الرياض، المملكة العربية السعودية وذلك خلال الفترة من فبراير 2008م إلى مارس 2011م. أُدرج في هذه الدراسة 4 أسر من مناطق مختلفة في المملكة العربية السعودية دون أي صلة قرابة، ومصابة بالشلل السفلي التشنجي (ARHSP-TCC)، حيث شملت 13 فرداً مصاباً. وتتميز الصفات العرضية بخلل في طريقة المشي لوحظ مابين سن 2-18 عاماً، كما وتشمل تطور سريع في الشلل السفلي التشنجي، يرافق ذلك فقدان بسيط إلى متوسط في الإدراك، ولوحظ اعتلال عصبي طرفي علوي في أسرتين. وقد أظهر الرنين المغناطيسي دقة في حجم الجسم الثفني مُصاحب لتغير في المادة البيضاء في نسيج الدماغ ما قبل تجويف البطين، وضمور في قشرة الدماغ.

النتائج: أثبت تحليل الصبغة الجينية الصلة بين المنطقة الجينية (SPG11). وأظهر التحليل الجيني 4 طفرات جينية. الأولى تكون من إزاحة (3bp) و(23bp)، وإدخال (L1268L fsX)، والثانية كانت إزاحة من (S1923R fsX) (1bp)، أما الثالثة والرابعة فكانت من النوع الاستبدالي (Q342X and R651X). وأدت جميع هذه الطفرات إلى تقطع مبكر في بروتين الأسباستاسين.

خاتمة: تصف هذه الدراسة طفرات جديدة في جين الأسباستاسين، ويعد أحد أكبر الإضافات الجينية التي تم نشرها فيما يخص هذا الجين. وعززت هذه الدراسة أيضاً العلاقة بين الشلل السفلي التشنجي (ARHSP-TCC) و (SPG11).

Objective: To assess the mutational and clinical spectrum of spatacsin associated with autosomal recessive hereditary spastic paraplegia (ARHSP) with thin corpus callosum (TCC).

Methods: A retrospective study was carried out at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia from February 2008 until March 2011. Four unrelated Saudi Arabian families with ARHSP-TCC were studied, totaling 13 affected individuals. Clinical presentations included gait disturbance at variable ages (2-18 years), spastic paraplegia with mild to moderate cognitive impairment and evidence of peripheral neuropathy in 2 families. Brain MRI showed TCC accompanied by periventricular white matter changes and cortical atrophy.

Results: A genome wide scan demonstrated linkage to the SPG11 locus. Sequencing revealed 4 mutations. The first is an insertion/deletion (indel) consisting of a 3 base pair (bp) deletion and 23 bp insertion (L1268L fsX), the second is a one bp deletion (S1923R fsX), and the third and the fourth are nonsense mutations (Q341X and R651X). All mutations predict premature truncation of the spatacsin protein.

Conclusion: We report 2 novel mutations in this gene, including an indel considerably larger than any other identified to date. The identification of these mutations further confirms the causative link between SPG11 and ARHSP-TCC in these families.

Neurosciences 2012; Vol. 17 (1): 48-52

From the Departments of Genetics (Wakil, Baz, Hagos, Al-Amr, Al-Wadaee, Meyer, Bohlega), and Neurosciences (Murad, Al-Yamani, Bohlega), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Received 8th June 2011. Accepted 6th December 2011.

Address correspondence and reprint request to: Prof. Saeed A. Bohlega, Professor of Neurology, Department of Neurosciences, King Faisal Specialist Hospital & Research Centre, PO Box 3354, Riyadh 11211, Kingdom of Saudi Arabia. Tel. +966 (1) 4647272 Ext. 32827. Fax. +966 (1) 4424763. E-mail: boholega@kfshrc.edu.sa

Autosomal recessive hereditary spastic paraplegia with thin corpus callosum (ARHSP-TCC) is a frequent phenotype of complicated hereditary spastic paraplegia (HSP) characterized by slowly progressive

spastic paraparesis with mild to moderate cognitive impairment and may include dysarthria, dysphagia, distal amyotrophy, optic atrophy, and cerebellar ataxia.[1,2] Among the 5 distinct loci identified in this condition: the major locus is SPG11 (OMIM 604360), which accounts for around half of ARHSP-TCC cases.[3,4] The SPG11 or KIAA 1840 maps to chromosome 15q13-15,[5] and encodes the protein spatacsin, which is expressed ubiquitously in the nervous system and most prominently in the cerebral cortex, hippocampus, and pineal gland.[3] The function of spatacsin is unknown, but putative co-localization to both mitochondria and the cytoskeleton has been reported to cause an abnormality of active transport that leads to axonal degeneration.[3,4] To date SPG11 mutations are overwhelming nonsense alterations, splice site mutations, or insertions or deletions (indels) within the coding region.[3-9] Denora et al[10] recently identified a missense substitution as did Crimelia et al.[11] In our study, we identified 4 different SPG11 mutations in 4 Arab families with ARHSP-TCC. All mutations were homoallelic coding region alterations, and predicted premature truncation of the protein. Our study objective is to assess the mutational and clinical spectrum of spatacsin associated with ARHSP with TCC.

Methods. The King Faisal Specialist Hospital & Research Centre is a major tertiary care center in Saudi Arabia. The medical records database was reviewed, and all cases of familial spastic paraplegia were included. Out of 20 families identified, 2 had autosomal dominant inheritance and the rest had autosomal recessive HSP. Out of the 18 with autosomal recessive HSP, 7 had a pure familial form (no other neurological or systemic involvement). The remaining 11 families had a complicated form of HSP, and from this group 4 families (13 affected individuals), fulfilled the criteria of ARHSP-TCC.[1,2] Below is the clinical description of these families. The study was carried out according to the principles of the Helsinki Declaration, and informed consent was obtained from all the individuals in accordance with the ethics approved proposal obtained for this study by King Faisal Specialist Hospital and Research Centre Research Advisory Council.

Family One. Parents were normal and first-degree cousins. Five (3 males and 2 females) of their 11 children were affected (**Figure 1F1**). The eldest affected (IV-1) is a male seen at age 37 years with a history of difficulty walking and falls started at age 14. He became wheelchair-bound at age 18 with a progressive and severe dysarthria, noted over 12 years. At age 30 he became totally dependent on others; he managed to finish grade 6 in school with great difficulty. He was anarthric with pseudobulbar affect with no movement in the legs and only a flicker of movement in the hands. There was moderate amyotrophic wasting in the hands, arms, and legs. The second affected (IV-2) is a 30-year-old man who was well until the age of 12 when walking became difficult. Dysarthria and learning difficulties were noted simultaneously and progressed. He walked with assistance on his tip-toes. The third affected was a female (IV-3) seen at age 27 and who had difficulty walking since the age of 13. She became wheelchair-bound at age 18. She had moderate wasting of arms, hands, and legs, dysarthric speech and pyramidal weakness. The fourth (IV-4) was a female seen at the age of 15 with a one-year history of weakness and falls with average school performance at eighth grade. She was spastic and walked independently on her tip-toes. The fifth (IV-5) is a 17-year-old female who was first seen at the age of 7 years. Difficulty walking started at the age of 2, with tip-toe walking and falls. School performance was poor. The head circumference was above the 50th percentile with cognitive impairment and decreased verbal memory. Over 10 years of follow-up she remained ambulatory independently. An MRI was carried out in 3 affected individuals, and showed, with variable severity, thinning of the corpus callosum and multiple hyperintensities in the white matter of the centrum semiovale.

Family 2. The index case was a man of 28 years (II-1) born to healthy first-degree cousins (**Figure 1F2**). Three of his 12 siblings were affected. At the age of 17 years, he complained of leg weakness. He could not stand or walk unassisted and became wheelchair-bound around 22 years of age. His speech was dysarthric. Psychological testing revealed limited reading and writing skills with basic arithmetic and vocabulary abilities. Abstract ability was trace, and his IQ was 78 on the Wechsler adult intelligence scale. An MRI showed diffuse thinning of the CC with bilateral periventricular non-enhancing patchy T2 hyperintensity lesions. An MRI of the spine showed thoracolumbar spinal cord atrophy. The MR spectroscopy showed elevated choline. The N-acetyl aspartate (NAA) peak was slightly decreased and lactate peak noted at parieto occipital and fronto white matter. Motor evoked potential showed severe conduction delay in legs more than arms, needle electromyography (EMG) and nerve conduction study showed neurogenic denervation in all the muscles. His sister (II-2) noted leg heaviness at the age of 18. Poor school performance was

Disclosure. All the authors have no conflicting interest and this project is not supported or funded by any drug company. This study was supported by the King Faisal Specialist Hospital Research Advisory Council with Project No. 2090011.

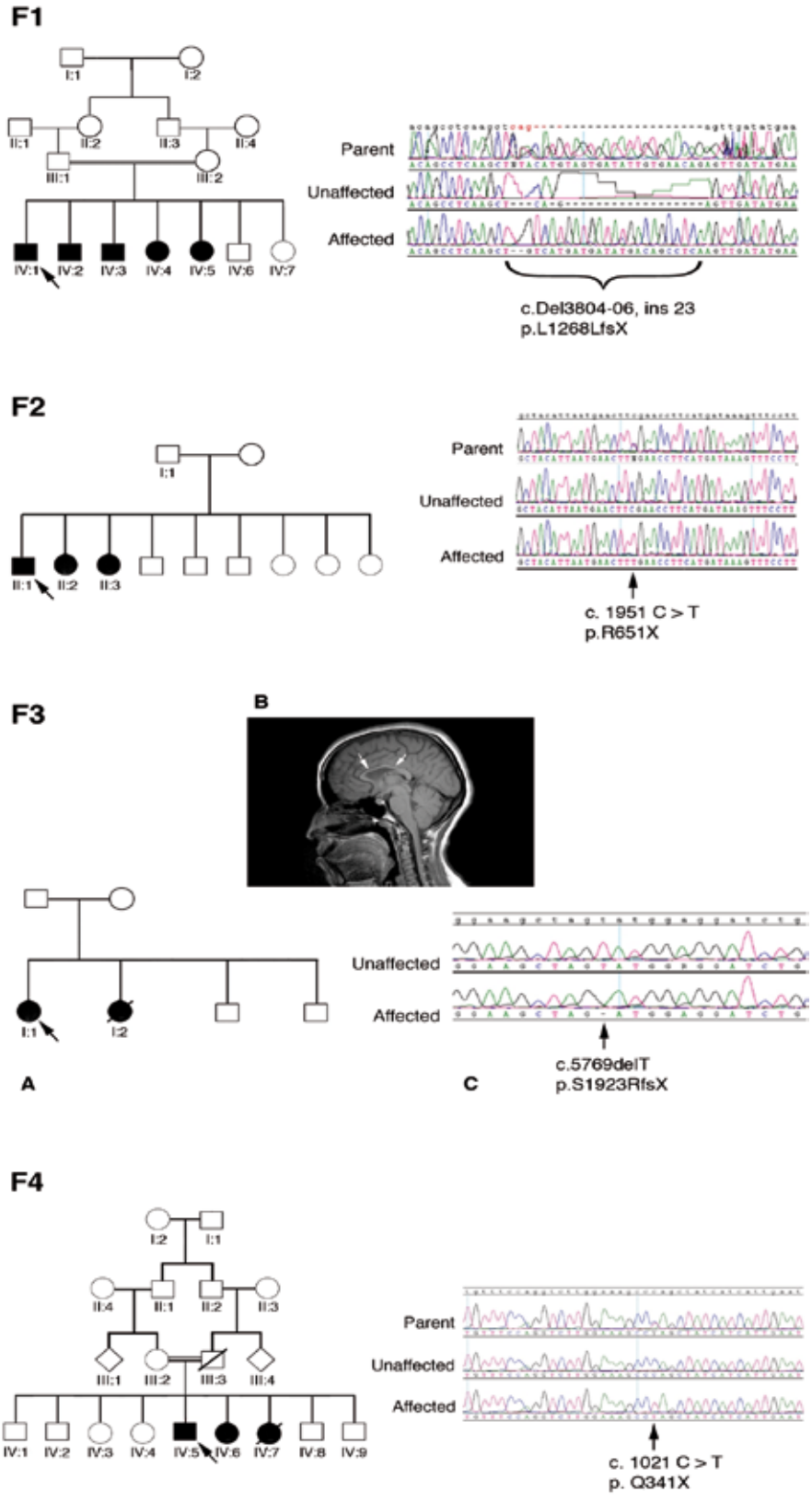

Figure 1 - Pedigrees of Family 1: **F1**, Family 2: **F2**, Family 3: **F3**, and Family 4: **F4** showing affected status individual identification as per text, also showing representative section of sequence chromatograms of genomic deoxyribonucleic acid (DNA) fragments encompassing the different exons of SPG-11 gene. **F3:B** Brain MRI T1-weighted image on the sagittal plane in the index case of Family 3, showing thinning of the corpus callosum affecting the geno and body of corpus callosum (white arrow heads).

Table 1 - Variants found across spatacsin (KIAA1840) genes studied.

Family	Exon	Mutation	Predicted protein change
F1	22	c.Del3804-06, ins23	p.L1268LfsX
F2	10	c.1953 C>T	p.R651X
F3	30	c.5769delT	p.S1923RfsX
F4	6	c.1021 C>T	p.Q.341X

noted at age 8. She was able to finish grade 6 of school at age 14. She was oriented, poor fund of knowledge, and no dysarthria. Spastic and weak legs with Babinski sign were able to walk with assistance at the age of 21. The second affected sister (II-3) noted slowness of gait, toe walking at the age of 17. She finished grade 9 of school with difficulty. She had marked dysarthria, spastic arms and legs and required assistance for walking. Her brain MRI showed similar abnormalities to her brother.

Family 3. The parents were second-degree cousins (**Figure 1F3**). She was the sixth offspring of a sibship of 8. She was seen at age 22 complaining of difficulty walking since age 13. She required a walking aid at age 20. She had spastic parietic legs. A brain MRI showed thinned CC, particularly its median part (**Figure 1F3,B**) and hyperintensities in the periventricular white matter. Motor more than sensory evoked potentials were prolonged. One sister died at the age of 40. She presented at the age of 16 with gait problems, spasticity, lost autonomous walking at the age of 20, and relentless disease progression affected her arms, speech, and bulbar muscles. Sphincteric impairment was noted at age 25. She also had marked cognitive deterioration.

Family 4. The index case for family 4 is a 26-year-old man. The parents are second cousins (**Figure 1F4**). Three out of 9 siblings were affected. Progressive spasticity and paraparesis were noted at age 16. He had to quit school at grade 8 because of cognitive problems. His global IQ is 82. His sister had a similar disease and died because of pulmonary embolism at age 27, which was 4 years after being wheel-chaired. The other sister of 29-years-old had an earlier disease onset, and she has been wheel-chaired for 6 years. The brain MRI showed scattered white matter abnormalities and thin CC, and spinal cord atrophy mainly at the dorsal region.

Genetic linkage. Blood samples were obtained from all the affected patients, their parents, and unaffected siblings after they had given informed consent in accordance with the local ethics committee. Multipoint parametric linkage analysis was performed using the GeneHunter module of Easy Linkage analysis Software (version 4.0). A recessive model of inheritance was used with a population disease allele frequency of 0.0001 and Asian SNP allele frequencies.

Mutation screening. Direct sequencing of the SPG11 gene (Gene Bank accession number NM-025137) was performed via automated sequencing (Primer sequences are available on request). The PCR products were generated using the DNA Engine PTC-200 (MJ Research Watertown, MA, USA), then sequenced using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway, NJ, USA) on a Megabase 1000 DNA Analysis System (Molecular Dynamics, Sunnyvale, CA, USA). Sequence data were processed and analyzed with Seqman II (DNAstar, Madison, WI, USA) and then compared to reference Gene Bank Sequence NM-025137.

Results. Multipoint parametric linkage analysis assuming a recessive model of inheritance and complete penetrance revealed linkage on chromosome 15 (SPG11) for Family one. A maximum logarithm of the odds (LOD) score of 3.73 was achieved at 15q14-q21. Since the spatacsin gene lies in this region, we sequenced all exons and discovered a homozygous indel consisting of a 3 base pair (bp) deletion accompanied by a 23 bp insertion at aa1267 (**Figure 1F1**). The indel (del3835-37, inser23) leads to a frameshift and premature stop codon at aa1268 (L1268L fsX). This variant was heterozygous in both parents and was absent in 200 Saudi normal control chromosomes (**Table 1**). In Family 2, a homozygous base substitution in exon 10 was found in all 3 affected subjects that resulted in nonsense mutation R651X. Five members from this family and their parents were found heterozygous for this change (**Table 1**). In Family 3, a homozygous T deletion was found in one affected subject in exon 30, which creates a frame shift change and a premature stop codon (S1923R) (**Table 1**). In Family 4, a homozygous base substitution in exon 6 of spatacsin was identified in all the 4 affected patients resulting in a nonsense mutation (Q341X). The heterozygous individuals in these families did not show any clinically apparent neurological abnormalities.

Discussion. This study included 13 patients from 4 unrelated native Saudi Arabian families with clinical and radiological findings that meet the published criteria for the complicated form of ARHSP-TCC.[1,2] The diagnosis was further supported by excluding other metabolic, infectious, and immune-related disorders. Consanguinity was noted in all families and inheritance was consistent with autosomal recessive transmission. The onset of motor symptoms was 2-18 years (median 13 years) with symptoms related to spasticity and progressive leg weakness like falls and toe-walking. Spasticity progressed to affect arms in 11 individuals and bulbar muscles findings in 2. Spastic dysarthria was noted in most patients and was mild to severe. Cognitive impairment was also seen in all except one

patient and preceded the motor involvement in these patients. Loss of ambulation was variable and ranged from 4-14 years after onset, which is similar to previous reports.[4] Additional signs in our patients include: tremor, neurogenic atrophy, and sphincteric disturbance seen in some with disease progression. Neurophysiological studies showed prolonged motor central latencies and neurogenic motor evoked potentials; thinning of CC and white matter abnormalities ranged from mild to extreme;[1] in one patient lactate peak was seen on MRI spectroscopy. Inter- and intra-familial phenotypic variability was noticed in our families. The clinical heterogeneity in cases with SPG 11 has been noted frequently.[6-10] Of interest, there were rare cases where the imaging of the CC was noted to be normal.[11]

The ARHSP-TCC cases account for approximately 20% of families with autosomal recessive hereditary spastic paraplegia. This is reflected in our own cohort, as SPG11 mutations were causative in 4 out of 19 families (21%) with autosomal recessive HSP. The disease is genetically heterogeneous and seen in patients from different ethnicities but mostly from the Mediterranean basin. Although also associated with mutations in SPG4 and SPG21, mutations in SPG11 account for 41-77% of published cases thus far.[2] The SPG11 encodes a predicted 2,443 amino acid with full-length 8-kb transcript, the function of which is unknown. There are several putative functional domains and 2 regions, I and II corresponding to structurally similar domains based on their hydrophobicity.[3] The presence of a leucine zipper motif suggests involvement in the regulation of gene expression.[3,4] The pathobiology may involve a dying back effect of the terminal end of the cortico-spinal tract axon due to defective axonal trafficking with loss of function in the truncated protein.[3,12]

In summary, we describe clinical manifestations associated with 4 SPG11 mutations, of which 2 are novel, and 2 previously reported.[10,11] Since all 4 of our ARHSP TCC families contained causative mutations in SPG11, this suggests that SPG11 is the major locus for ARHSP TCC involvement in the Arab population. Further work is necessary to genetically categorize the remaining families in our HSP cohort, and to ascertain whether the percentages for involvement of known HSP genes in Arab patients is reflective of what is seen worldwide.

Acknowledgments. *We thank Dr. Fahad Al-Mohaileb for reviewing Figure 1 for its clarity and correctness. Special thanks to Ms. Alma Rose Tabogoc for preparation of the manuscript.*

References

1. Winner B, Uyanik G, Gross C, Lange M, Schulte-Mattler W, Schuierer G, et al. Clinical progression and genetic analysis in hereditary spastic paraplegia with thin corpus callosum in spastic gait gene 11 (SPG11). ***Arch Neurol*** 2004; 61: 117-121.
2. Depienne C, Stevanin G, Brice A, Durr A. Hereditary spastic paraplegias: an update. ***Curr Opin Neurol*** 2007; 20: 674-680. Review.
3. Stevanin G, Santorelli FM, Azzedine H, Coutinho P, Chomilier J, Denora PS, et al. Mutations in SPG11, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. ***Nat Genet*** 2007; 39: 366-372.
4. Stevanin G, Azzedine H, Denora P, Boukhris A, Tazir M, Lossos A, et al. Mutations in SPG11 are frequent in autosomal recessive spastic paraplegia with thin corpus callosum, cognitive decline and lower motor neuron degeneration. ***Brain*** 2008; 131: 772-784.
5. Del Bo R, Di Fonzo A, Ghezzi S, Locatelli F, Stevanin G, Costa A, et al. SPG11: a consistent clinical phenotype in a family with homozygous Spatacsin truncating mutation. ***Neurogenetics*** 2007; 8: 301-305.
6. Hehr U, Bauer P, Winner B Schule R, Olmez A, Koehler W, et al. Long-term course and mutational spectrum of spatacsin-linked spastic paraplegia. ***Ann Neurol*** 2007; 62: 656-665.
7. Paisan-Ruiz C, Dogu O, Yilmaz A, Houlden H, Singleton A. SPG11 mutations are common in familial cases of complicated hereditary spastic paraplegia. ***Neurology*** 2008; 70: 1384-1389.
8. Liao SS, Shen L, Du J, Zhqo GH, Wang XY, Yang Y, et al. Novel mutations of the SPG11 gene in hereditary spastic paraplegia with thin corpus callosum. ***J Neurol Sci*** 2008; 275: 92-99.
9. Samaranch L, Riverol M, Masdeu JC, Lorenzo E, Vidal-Taboada JM, Irigoyen J, et al. SPG11 compound mutations in spastic paraparesis with thin corpus callosum. ***Neurology*** 2008; 71: 332-336.
10. Denora PS, Schlesinger D, Casali C, Kok F, Tessa A, Boukhris A, et al. Screening of ARHSP-TCC patients expands the spectrum of SPG11 mutations and includes a large scale gene deletion. ***Hum Mutat*** 2009; 30: 500-519.
11. Crimella C, Arnoldi A, Crippa F, Mostacciuolo ML, Boaretto F, Sironi M, et al. Point mutations and a large intragenic deletion in SPG11 in complicated spastic paraplegia without thin corpus callosum. ***J Med Genet*** 2009; 46: 345-351.
12. Bauer P, Winner B, Schüle R, Bauer C, Häfele V, Hehr U, et al. Identification of a heterozygous genomic deletion in the spatacsin gene in SPG11 patients using high-resolution comparative genomic hybridization. ***Neurogen*** 2009; 10: 43-48.