**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.
spastic paraparesis with mild to moderate cognitive impairment and may include dysarthria, dysphagia, distal amyotrophy, optic atrophy, and cerebellar ataxia. 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. The SPG11 or KIAA 1840 maps to chromosome 15q13-15, and encodes the protein spatacsin, which is expressed ubiquitously in the nervous system and most prominently in the cerebral cortex, hippocampus, and pineal gland. 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. To date SPG11 mutations are overwhelming nonsense alterations, splice site mutations, or insertions or deletions (indels) within the coding region. Denora et al recently identified a missense substitution as did Crimelia et al. 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. 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 Two. 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 genu and body of corpus callosum (white arrow heads).
Table 1 - Variants found across spatacsin (KIAA1840) genes studied.

<table>
<thead>
<tr>
<th>Family</th>
<th>Exon</th>
<th>Mutation</th>
<th>Predicted protein change</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>22</td>
<td>c.3804-06, ins23</td>
<td>p.L1268LfsX</td>
</tr>
<tr>
<td>F2</td>
<td>10</td>
<td>c.1953 C/T</td>
<td>p.R651X</td>
</tr>
<tr>
<td>F3</td>
<td>30</td>
<td>c.5769delT</td>
<td>p.S1923RfsX</td>
</tr>
<tr>
<td>F4</td>
<td>6</td>
<td>c.1021 C/T</td>
<td>p.Q3.341X</td>
</tr>
</tbody>
</table>

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 wheelchair 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 DYEEnatic 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 a31267 (Figure 1F1). The indel (del3835-37, inser23) leads to a frameshift and premature stop codon at a31268 (L1268L fX). 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
Spastic paraplegia in Saudi Arabia … Wākil et al

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


