Clinical and genetic studies in hereditary spastic paraplegia with thin corpus callosum

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Abstract—*Background:* A complicated form of recessive hereditary spastic paraplegias (HSPs) with thin corpus callosum (TCC) was first described in Japan, and most of the Japanese families showed linkage to chromosome 15q13–15. A recessive HSP locus (SPG11) has also been mapped to chromosome 15q13–15 in Italian and North American families with and without TCC, and it overlaps the region identified in the Japanese families. *Objective:* To study clinically and genetically 12 Italian families with HSP and TCC. *Methods:* The authors investigated 18 affected and 30 healthy individuals from 12 unrelated Italian families with recessive HSP-TCC. Clinical, neurophysiologic, and neuroradiologic studies were undertaken. All patients were negative for *SPG7* mutations. Genetic linkage analyses were carried out with polymorphic DNA markers on 15q13–15. *Results:* Five families were consistent with linkage, thus defining a 19.8-cM region between markers D15S1007 and D15S978, encompassing the SPG11 interval. In one consanguineous family, linkage could be firmly excluded, confirming genetic heterogeneity. Two families appeared not linked to the region, but this could not be firmly proved because of the small family size. The remaining four families were uninformative for linkage purposes. *Conclusion:* HSP-TCC is common in Italy. The phenotype is fairly homogeneous and is associated with impaired cognition. There are at least two loci for HSP-TCC, one of which is on chromosome 15q13–15.

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Autosomal recessive hereditary spastic paraplegia with thin corpus callosum (HSP-TCC) is characterized by normal motor development, slowly progressive spastic paraparesis, mental retardation, and extremely thin corpus callosum.[1,2] Additional symptoms include muscular atrophy, extrapyramidal symptoms, and cerebellar ataxia, and epileptic seizures may occur, often late in the disease progression.[3] HSP-TCC was described in Japan and is considered essentially a Japanese subtype of HSP.[4] In American and European countries, HSP-TCC appears to be rare: only six white families with HSP-TCC have been reported thus far, including one each from the United States, Italy, Spain, and Portugal and two from Brazil.[5-7] It is uncertain whether the frequency of HSP-TCC is underrated in Western countries.

In one North American and two Italian recessive HSP families, of which two had TCC, an SPG11 locus has been mapped to the long arm of chromosome 15.[5] Normal corpus callosum in one of these three families suggested phenotypic variability. The SPG11 region spans 10.2 cM and overlaps by 4.3 cM the locus responsible for Andermann's syndrome (AS, [MIM #218000]), a neurologic condition with peripheral neuropathy and agenesis of the corpus callosum.[8,9] More recently, linkage to chromosome 15q13–15 has been reported in 10 of 13 Japanese families with HSP-TCC.[10] The HSP-TCC interval identified spans 19.9 cM and overlaps by 4.6 cM the SPG11 region but not the AS locus (figure 1).[9]

We report clinical and genetic studies of 12 Italian families with HSP-TCC.

Methods. *Clinical study.* Twelve Italian families were studied. Clinical features of affected patients are summarized here. For identification codes for families and individuals, refer to figure 2.

Family OS. The proband is a 13-year-old boy (H27) born to healthy first-degree cousins. His elder sister (H30) is healthy. He

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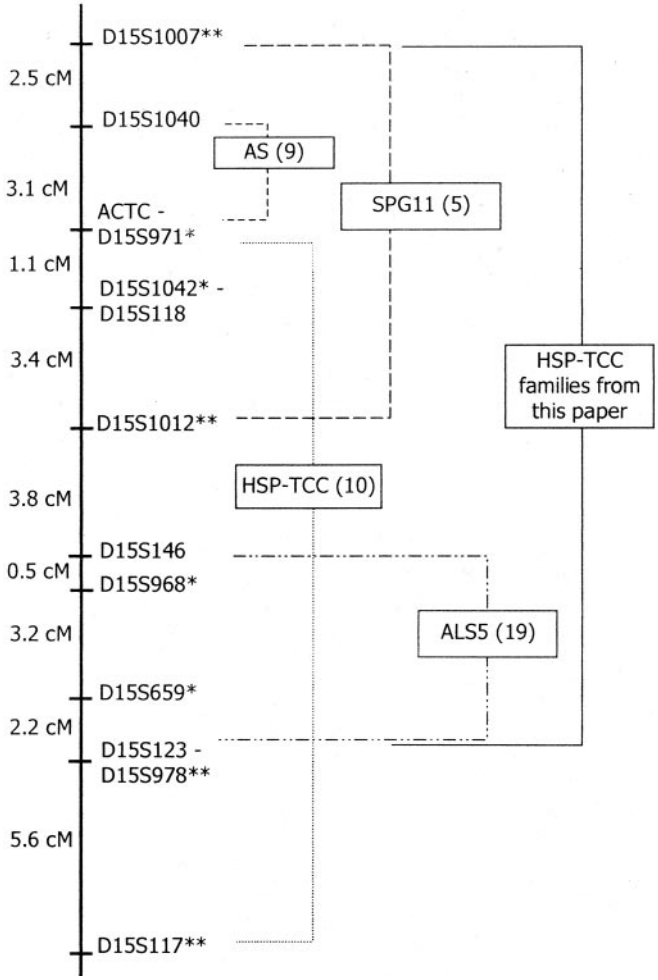

Figure 1. Schematic representation of the genetic map of part of human chromosome 15 showing the Andermann region as determined in reference 9 (flanking markers, D15S1040 and ACTC); the SPG11 region as determined in reference 5 (flanking markers, D15S1007 and D15S1012); the Japanese hereditary spastic paraplegia with thin corpus callosum (HSP-TCC) linked region as determined in reference 10 (flanking markers, D15S971 and D15S117); the ALS5 region as determined in reference 19 (flanking markers, D15S146 and D15S123); and the HSP-TCC region as determined in our families (flanking markers, D15S1007 and D15S978). Markers with one asterisk (*) are those genotyped in all 12 families, and markers with two asterisks (**) are those genotyped in only six families (see Results section and lane B, figure 2). Marker location and intermarker distances are taken from the Marshfield chromosome 15 genetic map and confirmed on the physical map of the University of California at Santa Cruz's Human Genome Working Draft.

achieved autonomous walking at age 16 months. Learning problems were reported during his first school years. At age 10 years, he started walking on tiptoe. Neurologic examination showed pyramidal tract signs in his lower limbs. Since then, his motor disability has slowly progressed, and he is now unable to walk unaided. Brain MRI showed TCC and diffuse periventricular white matter changes. A biopsy of deltoid muscle at age 12 years showed normal oxidative phosphorylation histochemically and biochemically. Nerve sensory conduction velocities, EEG, somatosensory evoked potentials (SSEPs), visual evoked potentials (VEPs), electroretinogram, and brainstem auditory evoked responses (BAERs) were normal. Nerve motor conduction velocity of the deep peroneal nerve was in the lower range (41 m/s) with reduced compound motor action potential (CMAP) amplitude (5.4 mV). Motor evoked potentials (MEPs) were markedly delayed for lower limbs and moderately delayed for upper limbs.

Family DKD. A 29-year-old woman (H82) born to healthy, nonconsanguineous parents was aged 13 years when she first had stiffness while walking. At that time she was attending school regularly and went on to obtain a high school diploma without difficulty. After age 17 years, her walking worsened, and she became unable to walk unassisted at approximately age 20 years. At that age, insidious mental deterioration ensued. She is now wheelchair-bound, severely dysarthric, and mentally impaired. Neurologic examination showed severe spasticity—more evident in her lower limbs—hand muscle amyotrophy, extremely brisk tendon reflexes, and bilateral Babinski sign. MRI showed striking TCC and white matter changes (figure 3). Her 24-year-old sister (H83) presented with hand tremor at age 17 years and received a diagnosis of essential tremor. At that age, a brain MRI was similar to that of her older sister. In the following years, walking difficulties, pyramidal tract involvement in the lower limbs, and progressive mental deterioration became evident, although to a lesser degree than in her sister's case.

Family NPE. In this family, two siblings are affected, whereas their unrelated parents are healthy. The first proband (H73), now aged 17 years, had normal motor and intellectual development until age 13 years, when motor disability first became apparent. At age 15 years, neurologic examination showed hyperreflexia in her lower limbs, extensor plantar reflexes, and increased muscle tone but normal strength. Cognitive status was normal. MRI showed TCC and periventricular white matter changes. She is still ambulant, but mild mental deterioration is now evident. Her 16-year-old brother (H70) began to experience walking difficulties at age 15 years after a normal motor and cognitive development. Neurologic examination showed normal intellectual abilities, increased muscle tone, hyperreflexia, extensor plantar reflexes, and normal strength. His MRI showed the same abnormalities as his sister. Nerve sensory and motor conduction velocities in upper and lower limbs were normal. Both patients had normal EEG, VEPs, electroretinogram, and BAERs. SSEPs with stimulation of upper limbs were normal, whereas stimulation of the lower limbs showed delayed responses. MEPs were delayed in the lower limbs only.

Family F1. Two patients, a 33-year-old man (H21) and a 28-year-old woman (H19), were born to healthy first-degree cousins. Motor development was normal. At age 12 years, difficulties in running and frequent falls were noted, and leg "stiffness" was reported. These symptoms slowly worsened until autonomous walking became impossible at age 19 years for the boy and at age 20 years for his sister. A recent neurologic examination showed severe bilateral lower limb spasticity, brisk tendon reflexes and Babinski sign, and dysarthric speech in both patients. Upper limbs are unaffected. Both patients attended secondary school with poor results. Neuropsychological examination showed poor performances on the Wechsler Adult Intelligence Scale (WAIS; IQ = 75). A brain MRI of H21 at age 32 years showed moderate cortical atrophy along with TCC in the brother, whereas the sister had only TCC at age 27 years. MRI was normal in the patients' mother. Two other sisters (H18 and H20) are normal.

Family PS. A 19-year-old man (H77) was born to healthy unrelated parents. He showed mild learning disabilities in primary school. After age 17 years, he presented progressive gait disturbances, urinary urgency, and cognitive impairment. The latter consists of emotional lability and childlike attitude along with difficulties in concentration. Neurologic examination revealed mild spastic paraparesis and hyper-reactive tendon reflexes and Babinski sign. Brisk reflexes were also noted in the upper limbs. MRI showed marked TCC and periventricular white matter changes. His 20-year-old brother (H80) is normal.

Family SM. A 27-year-old woman (H5) was born an only child of healthy unrelated parents. Learning disability was clearly evident at age 10 years and subsequently worsened to moderate dementia. Walking became difficult after age 12 years and impossible at age 17 years. MRI at that time showed TCC. She is now wheelchair-bound, severely dysarthric, and requires continuous assistance. Neurologic examination showed severe pyramidal tract involvement (more marked in her lower limbs), dysarthria, mild

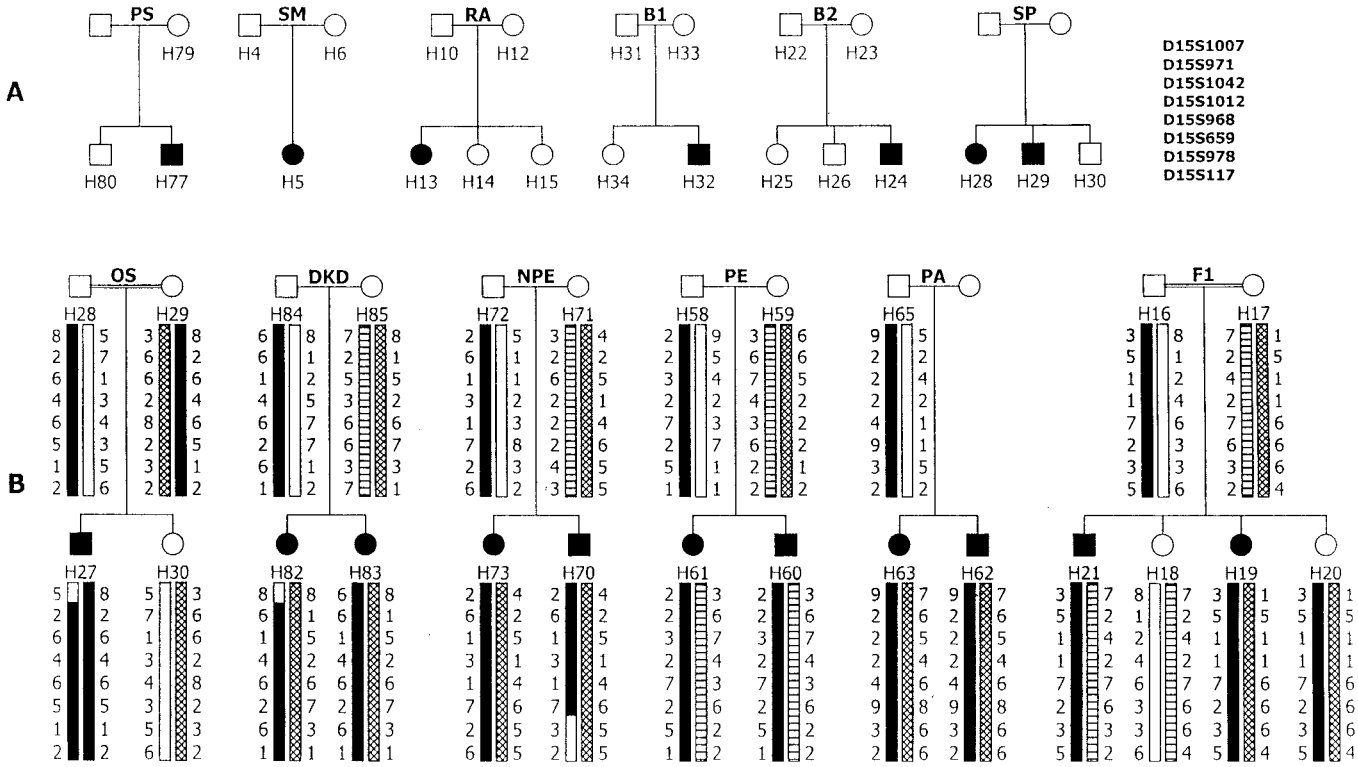

Figure 2. Pedigrees of the 12 Italian families with autosomal recessive spastic paraparesis and thin corpus callosum (HSP-TCC). (Lane A) Families uninformative or only partially informative for linkage purposes. (Lane B) Families OS, DKD, NPE, PE, and PA are consistent with linkage to the 15q13–15 chromosomal region, whereas in Family F1 linkage with the whole region has been definitely excluded. Black symbols denote individuals affected by HSP-TCC. Deceased members are marked with a diagonal bar, and individuals who were clinically examined and blood sampled are coded with the letter "H," followed by a number.

cerebellar signs, and dysphagia. At age 24 years, MRI showed unequivocal signs of disease progression: marked frontal and parietal cortical atrophy, white matter changes in periventricular areas, and further thinning of corpus callosum (see figure 3).

Family RA. A 26-year-old woman (H13) born to unrelated healthy parents began to have difficulties walking at approximately age 19 years. Gait disturbances have progressed insidiously along with mild mental changes, mainly represented by childlike behavior. Neurologic examination was consistent with marked spastic paraparesis. MRI showed TCC and frontal cortex atrophy. Two younger sisters (H14 and H15) aged 24 and 20 years are normal.

Family B1. The proband (H32) is now aged 17 years. His parents are healthy and unrelated. He achieved autonomous walking at age 16 months, and mental development was reportedly normal. He began to have learning disabilities during primary school. At age 11 years, he experienced walking difficulties. At age 14 years, a neurologic examination showed spasticity in his lower limbs. A brain MRI at that age showed TCC and periventricular frontal and occipital white matter changes. SSEPs with stimulation of the lower limbs were delayed. Nerve motor conduction velocity in his lower limbs was in the lower range (43 m/s) with reduced CMAP amplitude (3.2 mV). MEPs were markedly delayed in his lower limbs and moderately delayed in his upper limbs. At present, he requires support while walking and has moderate mental deficiency. An older sister (H34) is normal.

Family B2. A 6-year-old boy (H24) born to healthy unrelated parents was able to sit at age 7 months but had delayed psychomotor development. At age 4 years, he walked tiptoeing and could barely speak. Neurologic examination showed increased tendon reflexes and bilateral Babinski sign. MRI showed subtotal "agenesis" of corpus callosum and periventricular hyperintensities on T2-weighted images. MEPs were delayed in his lower limbs. Two older siblings (H25 and H26) are healthy.

Family PA. In this family, two siblings (H62 and H63) were born to healthy, unrelated parents. Mental and motor development was normal. The elder sibling, a 44-year-old man (H62), was examined for stiffness while walking and mental deterioration at age 16 years. Neuropsychological examination showed poor performances on the WAIS test (IQ = 60). Twenty years after disease onset, he was wheelchair-bound. His 31-year-old sister (H63) started walking on tiptoe at age 19 years. Subsequently, she developed progressive mental deterioration. Independent walking was impossible at age 35 years. Neurologic examination showed dysarthria, dysphagia, spastic paraplegia with weakness and distal wasting of the upper limbs, reduced vibration sense in the lower limbs, and urinary incontinence in both siblings, and claw foot in Patient H62. MRI showed TCC, cortical atrophy, and cerebral white matter changes in both siblings. Median nerve electrophysiologic studies performed in Patient H63 showed a motor conduction velocity in the lower range (52 m/s) and a reduced sensory velocity (38 m/s).

Family SP. Two siblings aged 20 and 13 years (H28 and H29) were born to unrelated healthy parents. A third brother (H30) is normal. Patient (H28) had normal motor milestones, but learning problems were evident during her first school years. After age 15 years, she had progressive gait disturbances. On the latest neurologic examination, she was still able to walk independently. Mild weakness, distal wasting, and increased tendon reflexes in lower limbs and extensor plantar response were found. WAIS IQ was 78. Her younger brother (H29) has shown progressive walking disability beginning at age 12 years. Neurologic examination showed spastic gait, mild weakness in his lower limbs, increased tendon reflexes and extensor plantar response, and claw foot. Mild cognitive impairment is also present (IQ = 89). Nerve motor conduction velocity of peroneal nerve is reduced (38 m/s). SSEPs are normal, whereas MEPs are delayed in both patients. MRI revealed

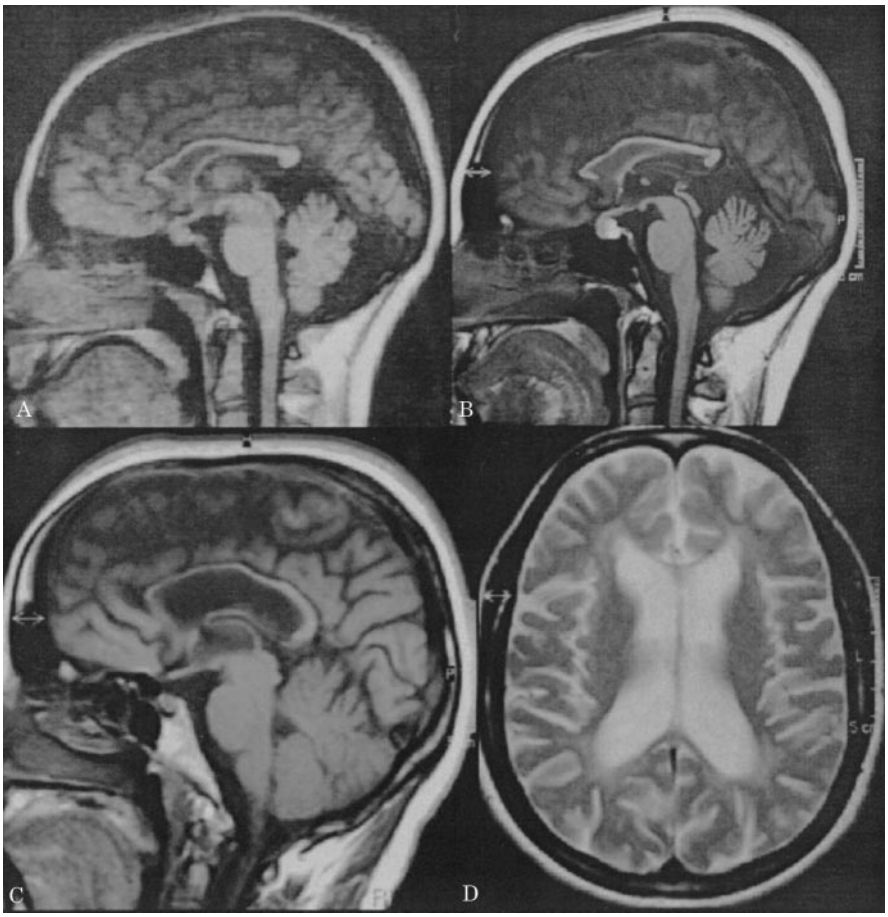

Figure 3. Brain MRI T1-weighted images on the sagittal plane in Patient H5 from Family SM show thinning of the corpus callosum (A) and progression of atrophy of the corpus callosum at follow-up scan obtained 10 years later (B). Brain MRI of Patient H82 from Family DKD shows severe thinning of body and genu of the corpus callosum on sagittal T1-weighted images (C) and hyperintensity of periventricular white matter on axial T2-weighted images (D).

changes of periventricular white matter in both siblings and of TCC in the elder.

Family PE. In this family, two affected siblings (H60 and H61) were born to healthy unrelated parents. The 18-year-old boy (H60) achieved autonomous walking at age 2 years and had delayed speech development. At age 13 years, he presented learning problems and difficulties in writing. One year later, he had progressive gait disturbance. Neurologic examination showed mild spastic gait with increased tendon reflexes in the upper and lower limbs and lower limb weakness, claw foot, and writing tremor. WAIS IQ is 66. MEPs were delayed. His younger sister has mild spastic gait and mild cognitive impairment. MRI showed TCC and white matter changes in both siblings.

All probands underwent biochemical screening tests based on the extensive list of differential diagnoses proposed by the HSP Working Group.[11]

Genetic analysis. DNA was extracted from peripheral blood leukocytes using standard protocols. In all affected patients, we had previously ruled out *SPG7* mutations by direct sequencing.[12] All available individuals from the 12 families were genotyped for four microsatellite markers spanning the region on chromosome 15q13–15 (D15S971, D15S1042, D15S968, and D15S659; see figure 1). Microsatellite markers were PCR amplified from genomic DNA using fluorescein-labeled primers and electrophoresed on a capillary 3100 DNA Sequencer (Applied Biosystems, Foster City, CA). DNA fragment size analysis was performed semiautomatically using the Genescan and Genotyper software (Applied Biosystems) to determine genotypes. Haplotypes were manually constructed, and phase was assigned based on the minimum number of recombinants. Pair-wise lod scores were obtained using the FASTLINK version of the MLINK program, under the assumption of equal male-female recombination rate, autosomal recessive inheritance, full penetrance, gene frequency of 0.001, and equal allele frequencies for each marker.[13] Multipoint lod scores were generated using the SIMWALK2 program.[14] Linkage homogeneity was tested in nine families with the HOMOG program using the multipoint lod scores at every 0.5 cM between markers D15S971 and D15S659.[15] Six families were genotyped for four additional microsatellite markers (D15S1007, D15S1012, D15S978, and D15S117; see figure 1 and the Results section). Marker location and genetic distances were based on the chromosome 15 genetic map of the Center for Medical Genetics, Marshfield Medical Research Foundation. Marker order was also confirmed on the physical map of the University of California at Santa Cruz's Human Genome Working Draft.

Results. *Clinical study.* This study includes 18 patients (9 men and 9 women), belonging to 12 families, with a fairly homogeneous clinical presentation that meets the clinical and radiologic criteria for the complicated form of HSP-TCC first reported in Japan.[2,10] The diagnosis was further supported by negative results of laboratory investigations, including serum creatine kinase, very long fatty acids, amino acids, urinary organic acids, lysosomal enzymes in leukocytes, and CSF isoelectrofocusing. Five cases were sporadic, whereas in seven families, there were two affected siblings, consistent with autosomal recessive transmission. Consanguinity was present in two families. The median age at first examination was 17 years (range, 4 to 21 years). The median age at onset for motor symptoms was 13 years (range, 4 to 20 years). Motor symptoms consisted mainly of walking difficulties and a tendency to walk on the toes. All patients had bilateral pyramidal tract involvement that started in the lower limbs, where it remained more evident after subsequent diffusion to the upper limbs over the years. Worsening of the motor signs, with relatively rapid course, was found in all patients on successive examinations. In at least eight patients, inde-

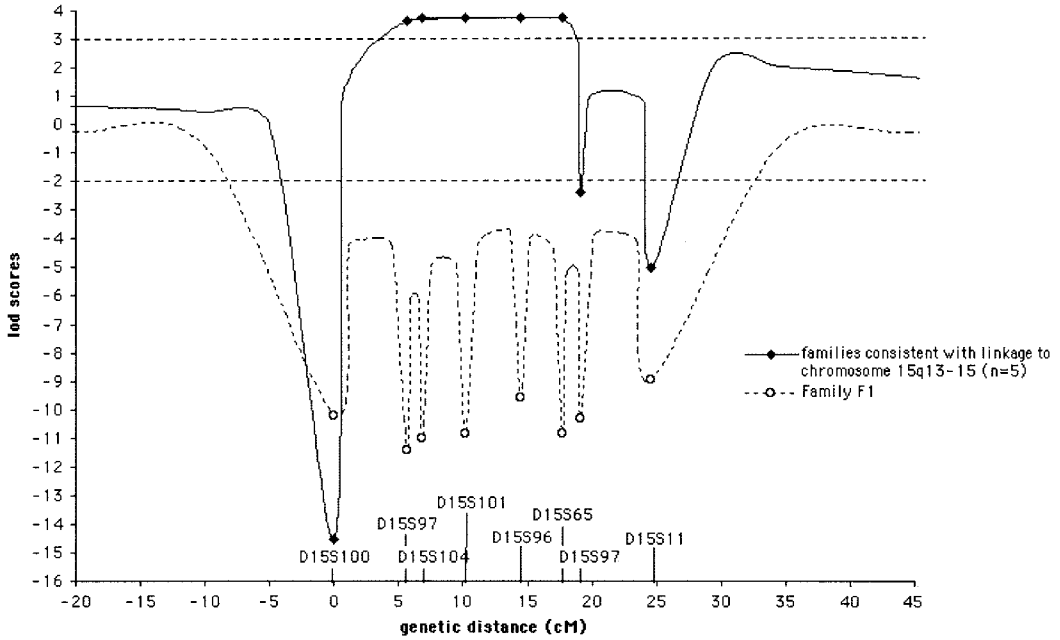

Figure 4. Multipoint lod scores between the disease and markers spanning the hereditary spastic paraplegia with thin corpus callosum (HSP-TCC) region. The black line indicates the cumulative multipoint analysis for families OS, DKD, NPE, PA, and PE, and the dashed line refers to Family F1 (see Results).

pendent walking was impossible after 8 or 9 years of disease progression. Late dysarthria was a constant feature in patients with severe advanced disease. The frequency of additional signs, including sphincter disturbances such as urinary urgency and frequency, seemed to increase with disease duration. Neurophysiologic studies showed delayed central MEPs and signs of motor peripheral neuropathy. All patients had mild to moderate cognitive impairment, often consisting of attention problems and "fatuous" behavior suggesting frontal lobe dysfunction. Cognitive impairment generally followed the onset of motor signs, but early learning disability preceding spastic paraparesis was also reported. Brain MRI in all patients showed TCC ranging from severe to extreme. In at least one patient, successive MRI examinations suggested progressive atrophy. Periventricular white matter changes were found almost constantly (15/18 patients, 83%). Frontal cortical atrophy seemed to be a late feature.

Linkage analysis. Linkage to the 15q13–15 chromosomal region was first tested with four microsatellite markers (D15S971, D15S1042, D15S968, and D15S659) in 48 individuals from the 12 families (18 affected members and 30 unaffected relatives). In three families (PS, SM, and B1), two-point lod scores were entirely uninformative at all recombination fractions for the four markers analyzed (range, 0.0 to 0.12). Moreover, haplotype construction did not allow either to exclude or to confirm linkage with the HSP-TCC region (data not shown). These families were excluded from further analysis. Linkage homogeneity was tested in the remaining nine families with the HOMOG program using the multipoint lod scores at every 0.5 cM between markers D15S971 and D15S659. The maximum likelihood was obtained for the condition of linkage and heterogeneity, with a maximum alpha value of 0.55.

Multipoint lod scores were positive across the entire region for families DKD, NPE, PE, PA, and OS (range, 0.65 to 1.32); the conditional probability in favor of linkage was 0.96 for Family OS and 0.87 for the other four families. Conversely, families F1, RA, and SP generated negative lod scores across the whole interval (range, −9.45 to −0.89), and the conditional probability in favor of linkage was equal or close to zero. Family B2 generated multipoint lod score values that ranged from not informative to mildly negative across the region. For this family, the conditional probability in favor of linkage was 0.68 but with broad support limits (0.00 to 0.94).

The five families with a high probability in favor of linkage (DKD, NPE, PE, PA, and OS) were genotyped for four additional microsatellite markers (D15S1007, D15S1012, D15S978, D15S117; see figure 1) to better define the genetic extension of the HSP-TCC interval. These markers were also genotyped in consanguineous Family F1, in which linkage could be firmly excluded (multipoint lod score values were <-2.00 across the entire interval tested), to ensure exclusion of linkage to the whole region and confirm genetic heterogeneity.

Haplotype construction. Five families (OS, DKD, NPE, PA, and PE) were consistent with linkage to the SPG11 region. Figure 2 shows the pedigrees of these families and haplotypes for the eight markers spanning the candidate interval on chromosome 15q13–15. In consanguineous Family OS, the affected individual (H27) was homozygous across the region, whereas his unaffected sister (H30) showed different haplotypes. In the other nonconsanguineous families, affected siblings shared identical haplotypes within the 15q13–15 region. The maximum cumulative two-point lod score for these five families was 3.35 for marker D15S659 at theta = 0.00. Cumulative multipoint linkage analysis is shown in figure 4. Affected individuals from each family showed different haplotypes, which excludes a common founder effect and suggests independent mutational events. In these five families, the upper extent

of the region is determined by recombinations detected in subjects H27 (Family OS) and H82 (Family DKD) between markers D15S1007 and D15S971, whereas the lower extent of the region is defined in individual H70 (Family NPE) between markers D15S659 and D15S978 (see figure 2). This identifies a 19.8-cM region encompassing the SPG11 interval reported by Martínez Murillo et al. and the AS region[5,9] and partially overlapping the minimal chromosomal region as defined in Japanese HSP-TCC families[10] (see figure 1). In consanguineous Family F1, linkage to the entire 15q13–15 region could be excluded. The two affected individuals (H21 and H19) showed different haplotypes across the whole interval, whereas one affected sibling (H19) shared identical haplotypes with one of his healthy sisters (H20). Negative two-point lod scores were obtained at all theta values for all eight microsatellite markers. This was confirmed by multipoint linkage analysis, which generated lod scores < -2.00 for the entire candidate interval (see figure 4). The clinical picture of Family F1 is not distinguishable from that of the other families consistent with linkage to the 15q13–15 region, which corroborates genetic heterogeneity.

Discussion. We report on the clinical and genetic investigations of 12 Italian families with HSP-TCC, for a total of 18 affected individuals—the largest group of patients investigated outside Japan. Clinical features closely matched those reported in Japanese patients, suggesting that HSP-TCC is more widespread than previously believed.[10] Such a high frequency has never been reported in other large series of autosomal recessive HSP, raising the possibility that HSP-TCC is underrated in Western countries.[6,16-18]

The phenotype of HSP-TCC consists of pyramidal tract involvement, initially confined to lower limbs with subsequent diffusion to upper limbs, and progressive cognitive impairment. This allows for a relatively easy clinical diagnosis. Motor dysfunction worsens progressively, and independent walking becomes impossible at approximately age 20 years, on average. Thinning—possibly progressive—of the corpus callosum is the neuroradiologically distinctive feature of this syndrome and further confirms the fairly homogeneous clinical presentation. Periventricular white matter changes and late cortical atrophy are additional, almost constant, features, which may be used in the differential diagnosis.

A maximum two-point lod score of 3.35 for marker D15S659 (at theta = 0.00) is consistent with linkage to the chromosome 15q13–15 region for five families. Affected individuals from different families did not share a common haplotype, which excludes a founder effect and suggests frequent independent mutational events in the Italian population (see figure 2) with a relative high frequency of carriers. Our results also show that HSP-TCC is genetically heterogeneous. In at least one case (Family F1), we excluded linkage to the whole region on chromosome 15. Our findings are in agreement with those reported by the Japanese study, in which only 10 of 13 HSP-TCC families were linked to chromosome 15q13–15.[10] It seems that HSP-TCC cannot be unequivocally identified with the SPG11 locus. Pending identification of the disease gene(s), patients without evidence of linkage must be considered as genetically different, although a distinct SPG locus number cannot yet be assigned.

Four families were uninformative for linkage purposes, and two families produced ambiguous results. Until the mutated gene is identified, these families cannot be considered either consistent with linkage or unlinked to SPG11. This is a frequent problem in autosomal recessive disorders because of the small size of families, which are often composed of only one affected individual with or without unaffected siblings.

The region on chromosome 15q13–15 identified in our families is broad and encompasses entirely the described SPG11 locus[5] and the AS locus.[8] Although a single gene—with variable expression—accounting for these neurologic conditions could be postulated, recent refinement of the AS locus does not support this hypothesis (see figure 1), suggesting that the causative genes are distinct.[9] Another neurologic condition affecting the motor pathways, an autosomal recessive form of ALS (ALS5), has been mapped to a 5.9-cM interval within the HSP-TCC region (see figure 1).[19] ALS5 families have onset in early adulthood and involvement of upper and lower motor neurons and mild mental retardation. Although the ALS5 region does not overlap the reported SPG11 region, the possibility that HSP-TCC and ALS5 may represent allelic disorders of the same gene cannot formally be excluded.[5] The identification of different mutations in the *ALS2* gene in juvenile primary lateral sclerosis (which often mimics HSP)[20] and early-onset recessive ALS[21] is a recent example of two clinically distinguished neurologic disorders of the motor pathways that later proved to be allelic.

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