Objective: We sought to explore the genetic and molecular causes of Troyer syndrome, one of several complicated hereditary spastic paraplegias (HSPs). Troyer syndrome had been thought to be restricted to the Amish; however, we identified 2 Omani families with HSP, short stature, dysarthria and developmental delay—core features of Troyer syndrome—and a novel mutation in the \textit{SPG20} gene, which is also mutated in the Amish. In addition, we analyzed \textit{SPG20} expression throughout development to infer how disruption of this gene might generate the constellation of developmental and degenerative Troyer syndrome phenotypes.

Methods: Clinical characterization of 2 non-Amish families with Troyer syndrome was followed by linkage and sequencing analysis. Quantitative polymerase chain reaction and in situ hybridization analysis of \textit{SPG20} expression were carried out in embryonic and adult human and mouse tissue.

Results: Two Omani families carrying a novel \textit{SPG20} mutation displayed clinical features remarkably similar to the Amish patients with Troyer syndrome. \textit{SPG20} mRNA is expressed broadly but at low relative levels in the adult brain; however, it is robustly and specifically expressed in the limbs, face, and brain during early morphogenesis.

Interpretation: Null mutations in \textit{SPG20} cause Troyer syndrome, a specific clinical entity with developmental and degenerative features. Maximal expression of \textit{SPG20} in the limb buds and forebrain during embryogenesis may explain the developmental origin of the skeletal and cognitive defects observed in this disorder.

Hereditary spastic paraplegias (HSPs) comprise several disorders commonly divided into 2 subgroups: “pure” HSPs characterized by progressive spasticity in the lower limbs due to pyramidal tract degeneration and “complicated” HSPs, where lower limb spasticity is associated with a variety of other neurological signs and clinical features. Complicated HSPs are clinically heterogeneous, mainly autosomal recessive syndromes, frequently described and mapped in sporadic families within inbred populations. Because of this heterogeneity, diagnosis...
and recommendations for genetic testing in these disorders have been daunting tasks.

Troyer syndrome (Online Mendelian Inheritance in Man #275900) is a complicated HSP associated with short stature, skeletal abnormalities, dysarthria, and developmental delay, first described in the Old Order Amish.\textsuperscript{3,5} Since the original description in 1967, several Troyer-like syndromes have been reported,\textsuperscript{6–9} but they often differed from classical Troyer syndrome in their neurological or skeletal features. The Amish founder mutation is a single nucleotide deletion in the \textit{SPG20} gene,\textsuperscript{10} leading to the loss of the spartin protein\textsuperscript{11}; however, no additional \textit{SPG20} mutations were subsequently identified,\textsuperscript{5} and it was suspected that Troyer syndrome may be restricted to the Amish.

We identified an Omani kindred presenting with clinical features resembling Troyer syndrome. All affected Omani individuals had a novel homozygous null mutation in \textit{SPG20}, and their clinical descriptions matched closely to those of Amish Troyer syndrome individuals of comparable ages. Because Troyer syndrome is associated with developmental features, such as short stature, skeletal abnormalities, and global developmental delay, we investigated the sites of action of \textit{SPG20} during development in humans and mice. \textit{SPG20}/\textit{Spg20} expression in the adult is relatively modest and widespread in the nervous system; however, maximal and focal expression is observed in the embryonic limb buds, face, and forebrain during early morphogenesis, which might explain the developmental phenotypic changes involving the extremities, face, and brain.

**Subjects and Methods**

**Enrollment and Clinical Studies**

The subjects belonged to 2 families residing in a remote region of Oman and were originally ascertained by a local clinical geneticist. Detailed family and medical histories were obtained by a genetic counselor, who is a native Arabic speaker, and a developmental pediatrician, also a native Arabic speaker, conducted a developmental assessment. A pediatric neurologist performed a standard neurological examination, and a second clinical geneticist obtained a second clinical geneticist obtained anthropometric measurements. The height of all subjects and head circumference of subjects under the age of 36 months were plotted on the Centers for Disease Control and Prevention 2000 growth charts,\textsuperscript{12} and head circumference of subjects above 36 months and all other anthropometric measurements were plotted on standard charts.\textsuperscript{13} Written, informed consent was obtained from the subjects or their legal guardians. The Ministry of Health in Oman and the institutional review board of Children’s Hospital Boston approved this study.

**Genomic DNA was purified from lymphocytes separated from peripheral blood using commercial kits (Qiagen, Valencia, CA). Six hundred nanograms of genomic DNA was used to hybridize**

Affymetrix Human SNP Array 6.0 at the Genomic Analysis Core of the University of North Carolina (UNC) Neuroscience Center of the UNC School of Medicine, Chapel Hill, North Carolina. After removal of low-quality calls and of Mendelian and non-Mendelian errors using Merlin software,\textsuperscript{14} the single nucleotide polymorphism (SNP) data were analyzed using Allegro software\textsuperscript{15} under a fully penetrant autosomal recessive model. For microsatellite analysis, highly polymorphic microsatellite markers were chosen from the Marshfield database in the University of California at Santa Cruz Genome Browser. Fluorescently labeled polymerase chain reaction (PCR) primers (Applied Biosystems, Foster City, CA) were used to amplify DNA samples using standard conditions, and PCR products were resolved on an Applied Biosystems 3130xl Genetic analyzer.

**SPG20 Sequencing Analysis**

Sequencing of \textit{SPG20} (NM_015087; NM_001142294-6) coding region was performed by SeqWright (Houston, TX) on PCR products after amplification of genomic DNA. PCR primers were designed for each exon including at least 50bp of flanking intronic sequences. Primer sequences are available on request. Once a putative change was identified, at least 96 control DNAs (192 alleles) were tested to exclude the possibility of a benign polymorphic change.

**Patient Cell Lines and Mutated Protein/mRNA Detection**

Transformed lymphoblastoid cell lines were established from peripheral blood of affected and unaffected individuals at the Partners Center for Personalized Genetic Medicine (Cambridge, MA). Cell lines were maintained in RPMI medium supplemented with 10% fetal bovine serum, glutamine (2mM) and penicillin/streptomycin (100U/100\(\mu\)g) (all from Gibco, Grand Island, NY). Protein lysates were obtained by boiling a cell pellet in Laemmlı sample buffer (Bio-Rad Laboratories, Hercules, CA). Western blotting was performed using standard protocols on Bio-Rad equipment. Presence of spartin was assessed using a rabbit anti-SPG20 antibody (Proteintech Group, Chicago, IL) and a goat anti-rabbit IRDye-800CW secondary antibody (LI-COR Biosciences, Lincoln, NE) detected on an Odyssey Imager (LI-COR Biosciences).

Total RNA was purified from patient cell lines using the mirVana RNA Isolation Kit (Ambion Applied Biosystems, Foster City, CA). Random primed cDNA was generated using reverse transcriptase (Promega, Madison, WI) and analyzed by quantitative PCR (qPCR) using SYBR Green reagents (Applied Biosystems) on an ABI 7500 qPCR platform as previously described.\textsuperscript{16} Primer sequences are available in Supplementary Table.

**SPG20/Spg20 Expression Analysis**

cDNA samples from human fetal and adult postmortem brain tissue were obtained commercially (BioChain Institute, Hayward, CA and Clontech, Mountain View, CA). cDNA from embryonic and postnatal mouse brains was generated as described above. qPCR was performed as described above (see Supplementary Table for primer sequences). DNA templates for in situ hybridization probes were cloned using a 550bp fragment of the Spg20
coding sequence using the following primers: Spg20-BamHI 5' -
ctacatggatccgatatcaaccggaggagcagccaaagtcagc-3' and Spg20-SalI
5' -gtccttgtcgactgcccctggcttctcttcacctg-3'. Digoxigenin-labeled
riboprobes were synthesized and in situ hybridization was per-
formed as previously described.17 Video images of the hybridized
sections were obtained on a Wild Leitz (Heerbrugg, Switzerland)
photomicroscope or Leica (Nussloch, Germany) DMR micro-
scope under consistent illumination conditions.

Results
Identification of a Novel SPG20 Mutation in
an Omani Kindred
We examined 2 related Omani families presenting with
short stature, spasticity, dysarthria, and developmental delay. Affected individuals are in black and
unaffected in white. The status of individual 2-8 (in gray) could not be determined, because she is too young to properly
assess neurological symptoms. All numbered individuals were examined, but genomic DNA was only collected from individ-
uals for whom microsatellite analysis is shown. Microsatellite analysis revealed common maternal (in yellow) and paternal
(in blue) haplotypes in all affected. Microsatellite markers in the linkage region identified by the single nucleotide poly-
morphism analysis are in bold. One homozygous marker (D13S219) is common to all affected individuals (highlighted in orange). (B) The homozygous region contains 6 genes, including SPG20 (in orange). (C) The affected individuals carried a homozygous 2bp deletion in SPG20, which was present in heterozygosity in the carriers. (D) Western blot analysis of patient cell lines showed that full-length SPG20 protein is missing in the affected individuals (A) compared with a nonaffected noncarrier sibling (NA). (E) Quantitative polymerase chain reaction analysis of cDNA from the patient cell lines indicated that
SPG20 mRNA is not present in the affected individual. deltaRN = signal magnitude expressed by the difference in the
normalized reporter (RN) values.

FIGURE 1: Affected individuals carry a homozygous null mutation in the SPG20 gene. (A) Pedigree of 2 related Omani
families affected with short stature, spasticity, dysarthria, and developmental delay. Affected individuals are in black and
unaffected in white. The status of individual 2-8 (in gray) could not be determined, because she is too young to properly
assess neurological symptoms. All numbered individuals were examined, but genomic DNA was only collected from individ-
uals for whom microsatellite analysis is shown. Microsatellite analysis revealed common maternal (in yellow) and paternal
(in blue) haplotypes in all affected. Microsatellite markers in the linkage region identified by the single nucleotide poly-
morphism analysis are in bold. One homozygous marker (D13S219) is common to all affected individuals (highlighted in orange). (B) The homozygous region contains 6 genes, including SPG20 (in orange). (C) The affected individuals carried a homozygous 2bp deletion in SPG20, which was present in heterozygosity in the carriers. (D) Western blot analysis of patient cell lines showed that full-length SPG20 protein is missing in the affected individuals (A) compared with a nonaffected noncarrier sibling (NA). (E) Quantitative polymerase chain reaction analysis of cDNA from the patient cell lines indicated that
SPG20 mRNA is not present in the affected individual. deltaRN = signal magnitude expressed by the difference in the
normalized reporter (RN) values.

in Fig 1A). The fathers (individuals 1-2 and 2-1) were
brothers, and the mothers (1-1 and 2-2) were aunt and
niece. Family 1 had 9 children: 4 were reportedly unaf-
fected females, who were married and lived elsewhere, 1
was a male (1-4), who was unaffected by clinical exami-
nation, 3 were affected males (1-3, 1-6, and 1-7), and 1
was an affected female (1-5). Family 2 had 8 children: 4
unaffected girls and 1 unaffected boy, 3 of whom were
present at the time of our evaluation (2-4, 2-5 and 2-6),
1 affected male (2-3), 1 affected female (2-7). The young-
est child (2-8) was small for her age and could neither
talk nor walk, but did not display overt neurological
symptoms; we were not able to obtain DNA from her for
genotyping or sequencing.
Clinical Characterization of Patients Carrying a Novel SPG20 Mutation

To ascertain phenotypic similarities or differences among individuals carrying SPG20 mutations, we compared the clinical presentations of the Omani individuals with the clinical description of 21 Amish patients by Proukakis et al. All affected individuals in the Omani cohort presented with short stature and dysarthria, and were delayed in reaching motor and cognitive developmental milestones. They all had some difficulties walking, with clumsy, mildly spastic gait, which their parents reported to be worsening over time. The most common physical features were relative hypertelorism and overgrowth of the maxilla leading to overbite, as well as hand and feet anomalies such as brachydactyly (5/6 patients), hammer toes, and pes cavus (Fig 2). Additional nonspecific skeletal malformations observed in the hands were clinodactyly, camptodactyly, and hypoplastic 5th middle phalanges (see Fig 2E, F). Most affected individuals had persistent cognitive deficits and poor performance in school, but no emotional lability was reported. Detailed neuropsychological testing was not available.

The neurological examination revealed distal amyotrophy (4/6 patients), dysmetria in the upper extremities (3/6 patients), hyperreflexia, which was more severe in the lower limbs (4/6 patients), and ankle clonus (3/6 patients). Heel cords were tight in the 4 older affected individuals. Brain magnetic resonance imaging (MRI) was performed in 2 individuals (1-7 at 14 years and 2-3 at 19 years). Both individuals had mild atrophy of the cerebellar vermis, mild white matter volume loss, and revealed periventricular white matter hyperintensity in T2-weighted images, consistent with gliosis (see Fig 2I, J).

The mean age of our cohort (16.6 years) was younger than the Amish cohort, which might explain the milder and more variable phenotype observed in the Omani individuals; therefore, we subdivided the Amish cohort into a younger subset (Amish I) comparable in age to our cohort (<27 years; mean age, 15 years) and an older subset (Amish II) (>28 years; mean age, 43.5 years). Our cohort closely matched the description of the Amish I group, whereas the Amish II group was more severely affected, consistent with the progressive, degenerative aspects of Troyer syndrome (see Table).

Expression of Spg20 in the Developing and Adult Nervous System

There appear to be 2 major components in Troyer syndrome: an early developmental aspect and a neurodegenerative process. The combination of phenotypes, including limb, craniofacial, and behavioral anomalies, is common in complex genetic disorders affecting early morphogenetic events, and the relevant genes are often specifically expressed in the embryonic rudiments of the affected adult structures. We first analyzed SPG20 expression in the developing and adult human brain using qPCR. SPG20 was expressed at modest levels in the fetal
and adult human brain compared to a highly expressed neurally specific gene, synaptophysin (SYN; Figs 3A, B). Levels of SPG20 were highest in the amygdala, cortex, and thalamus, and lowest in the hippocampus and cerebellum. Although clearly detectable, SPG20 was expressed at substantially lower levels than SYN. Expression levels and distribution of the mouse orthologue of SPG20, Spg20, in the adult mouse brain parallel those of the human, with some divergence; particularly, relative expression of Spg20 in the hippocampus was slightly elevated compared with humans. In addition, we analyzed Spg20 expression in mouse spinal cord and brainstem and found that it was highest in the spinal cord of all brain regions measured. As in the human, Spg20 was expressed at comparatively lower levels than Syn. Spg20 is not a brain-specific gene, as it is also expressed in several other organs.10,19 It is, however, developmentally regulated, as expression was maximal at midgestation and embryonic day (E)10, and declined precipitously thereafter (see Fig 3C–F).

We then used in situ hybridization to localize Spg20 in adult, fetal, and embryonic mouse brain (Fig 4). Spg20 was expressed at relatively low levels in neurons and glia throughout the adult brain, including glia in fiber tracts. Modestly elevated expression was seen throughout hippocampal stratum pyramidale of the CA fields and dentate gyrus and the transitional parahippocampal/entorhinal cortex. Low expression was seen in the cerebral cortex with no apparent laminar or cell class specificity. The only additional site of elevated expression in the forebrain was the habenular complex, including the habenular recess appendent to the corpus callosum. Spg20 is expressed throughout the cerebellum in Purkinje cells, granule cells, and scattered cells in the molecular layer. In the brain-

| TABLE: Clinical Features of the Omani Troyer Syndrome Individuals and Comparison with the Amish Cohort |
|-----------------------------------------------|---|---|---|---|---|---|
| Characteristics | 1–3 | 1–5 | 2–3 | 1–6 | 1–7 | 2–7 |
| Age (yr) | 27 | 19 | 19 | 17 | 14 | 4 |
| Sex | M | F | M | M | M | F |
| Height (cm) | 149 | 133 | 145 | 146 | NA | 83 |
| % | <5th | <5th | <5th | <5th | <5th | <5th |
| OFC (cm) | 52.4 | 51.6 | 52.8 | 54.8 | 53.0 | 48.4 |
| % | 3rd–10th | <5th | 10th | 25th–50th | 10th–25th | 3rd–10th |
| Developmental milestones and cognition | | | | | | |
| Talked at | 2 years | 2 years | 3 years | Late | Late | Late |
| Walked at | 2 years | 3 years | 1.5 years | 2.5 years | 3 years | 1.5 years |
| Poor school performance | + | + | + | − | + | NA |
| Emotional lability | − | − | − | − | − | − |
| Dysarthria | + | + | + | + | + | NA |
| Tongue dyspraxia | + | − | + | + | + | NA |
| Distal amyotrophy | + | − | + | + | + | − |
| Hyperreflexia | Upper | − | − | + | + | − |
| Lower | − | − | + | + | + | − |
| Ankle clonus | − | − | + | + | + | − |
| Clumsy, spastic gait | + | + | + | + | + | + |
| Dysmetria | − | − | + | + | + | − |
| Skeletal abnormalities | Overbite | + | + | + | + | + |
| Hypertelorism | + | + | + | − | + | + |
| Hand/foot abnormalities | + | + | + | + | + | + |

OFC = occipitofrontal circumference; NA = not available.
stem, there is robust expression in large neurons, probably motor neurons, and in the facial nucleus. Spg20 is also expressed in cells distributed throughout the spinal cord with no noticeable discontinuities. As predicted from the qPCR data, expression in the fetal brain is relatively low, with no apparent regional distinctions. Aside from elevated expression in the lens placode and pigment epithelium, cochlear epithelium, and condensing mesenchyme at sites of myogenic or cartilage formation, there is little focal expression at fetal stages (see Fig 4L–N; E12.5 and E14.5 are shown).

In contrast to the later stages, and, as predicted from the qPCR analysis, Spg20 expression in the midgestation embryo is elevated and highly patterned. In the E10.5 embryos, shortly after neural tube closure, Spg20 is specifically expressed in the initial frontonasal mass/forebrain, craniofacial structures, aortic arch/heart primordium, and limb buds during morphogenesis, with lowest expression in the heart (Fig 4O, P). qPCR analysis confirms this distribution; expression levels in limb buds, branchial arches, and frontonasal mass/forebrain are substantially elevated compared with the heart as well as with whole E10.5 embryo (Fig 4R). Our observations suggest that Spg20 has its most specific and maximal activity in the limbs, face and forebrain during early morphogenesis. This is remarkable, because the sites of phenotypic manifestations in the affected members of the Omani Troyer syndrome kindred described here include anomalies of the limb extremities, face, and brain.

**Discussion**

Our identification of a novel, disease-associated SPG20 mutation in an Omani kindred indicates for the first time that Troyer syndrome is not restricted to the Amish, as previously proposed.\(^2\) Complicated autosomal recessive HSPs are heterogeneous disorders often lacking clear clinical and molecular diagnostic guidelines. Of the 17 distinct loci for complicated HSP identified to date, more
than half have been described in single families or isolated populations.\textsuperscript{1,2} In the past 4 decades, several HSP syndromes with Troyer-like features have been reported, but none matched the clinical presentation in the Amish cases.\textsuperscript{5} Some of these disorders were later mapped to different loci such as SPG26,\textsuperscript{6,20} SPG39,\textsuperscript{21} and ARSACS.\textsuperscript{22,23} Even within the isolated region where these Omani families reside, we had described a similar extended pedigree with dysarthria, mental retardation, cerebral palsy, and microcephaly.\textsuperscript{24} Linkage analysis ruled out the SPG20 locus in the second kindred, confirming that the 2 disorders are separate clinical and genetic entities (A.R., R.S.H., C.A.W., unpublished data). Four individuals in this extended family (N = 45 individuals) also were heterozygous carriers for the SPG20 mutation, highlighting its presence in this isolated population. Thus, this novel mutation in SPG20 is geographically and genetically distinct from that in the Amish population; nevertheless, both mutations result in the phenotypic spectrum associated with Troyer syndrome.

![Figure 3: Quantitative polymerase chain reaction analysis of SPG20/Spg20 expression in human and mouse tissues.](image_url)

(A) There is modest variation in SPG20 local expression in the adult human brain when distinct regions are compared to whole brain samples. (B) Human SPG20 expression is substantially lower than that of the brain-specific gene synaptophysin (SYP) across all brain regions with the exception of the amygdala. (C) A similar profile of regionally variable Spg20 expression, with some modest differences, is seen in the mouse brain. (D) Lower Spg20 expression is observed when compared with Syp. (E) Spg20 is expressed in several murine tissues beside the brain. (F) Spg20 is developmentally regulated in the mouse embryo with highest expression levels in the whole embryo at E10.5. ΔCT = ; Bas Gang = basal ganglia; DeltaCT = signal magnitude expressed by the difference in CT values. Hippoc = hippocampus; Mesen = mesenchyme; Cerebel = cerebellum; Sp Cord = spinal cord; Sk Muscle = skeletal muscle; Rel. = relative.
Although a clear Troyer syndrome diagnosis is difficult to reach in young subjects due to the variability and mildness of their symptoms, direct comparison of clinical features showed that the Omani cohort closely resembled the age-matched Amish Troyer syndrome group (Amish I), suggesting that SPG20 null mutations cause a well-defined phenotype. As in the Amish, we observed short stature, skeletal abnormalities in the extremities, developmental delay, and dysarthria of possible cerebellar origin from an early age. Therefore, a diagnosis of Troyer syndrome must be considered when this constellation of phenotypes is present in young children. Facial dysmorphism...
and skeletal features are subtle, and in single cases in non-consanguineous populations this disorder could be initially diagnosed simply as cerebral palsy. Spasticity and distal amyotrophy appeared in the teenage years and worsened slowly over time. In the Amish, brain MRI revealed white matter abnormalities, which were less severe in the youngest patient examined (age 15 years), suggesting a progressive worsening of the condition. MRI analysis indicates that the Omani Troyer syndrome individuals of comparable ages also have mild white matter abnormalities, supporting the hypothesis that white matter degeneration accompanies disease progression, although direct neuronal degeneration cannot be ruled out. In addition, we identified mild cerebellar atrophy, which is consistent with the cerebellar signs. Serial MRI scans of the Omani individuals might resolve whether progressive neurological symptoms are correlated with increased white or gray matter degeneration. Despite many similarities to the Amish cohort, a few specific differences were observed in the affected Omani individuals, including the presence of hypertelorism and a pronounced overbite, and the absence of inappropriate emotional responses. As new cases are identified it will be interesting to assess whether these traits have variable penetrance or are population specific.

Functional studies on spartin, the protein encoded by SPG20, have identified multiple roles in protein ubiquitination and lipid droplet formation, and a possible link to endothelial growth factor receptor signaling. Previous expression analyses by Northern blot or qPCR identified widespread SPG20/spartin localization in the brain and in other tissues, however, it was unclear whether regional and cellular dynamism in SPG20 expression could explain the phenotypes observed in the affected individuals. Our study shows that whereas SPG20/Spg20 expression is modest and virtually ubiquitous in the adult and developing brain, early stages of embryonic development show maximal levels of Spg20 in the limbs, face, and forebrain primordia. This localized expression suggests a parallel role for SPG20/Spg20 in morphogenesis and differentiation at these phenotypic sites; accordingly, a loss of function mutation may contribute to the phenotypic spectrum of Troyer syndrome.

Acknowledgment
This work was supported by the NINDS (grant RO1 NS 35129, C.A.W.), NICHD (grant RO1 HD02917S, A.-S.L.), the Dubai Harvard Foundation for Medical Research (C.A.W.), the Manton Center for Orphan Disease Research (C.A.W.), the Muscular Dystrophy Association (Development Grant, M.C.M.), and the University of North Carolina (Reynolds Faculty Fellowship, A.-S.L.). C.A.W. is an Investigator of the Howard Hughes Medical Institute. We thank the families for their cooperation and their hospitality; the local nurses and doctors for their assistance; Megumi Aita at the University of North Carolina In Situ Core for help with the in situ analysis; Daniel Rakic at Children’s Hospital, Boston for help with the sample preparation and analysis; and the following colleagues at Children’s Hospital Boston for helpful discussion on the clinical features of the patients: Hope E. Dickinson, a speech pathologist from the Department of Otolaryngology and Communication Enhancement, and Janice Ware, a child psychologist and associate director of the Developmental Medicine Center.

Authorship
M. Chiara Manzini and Anna Rajab contributed equally and are co-first authors.

Potential Conflicts of Interest
Nothing to report.

References
1. Harding AE. Classification of the hereditary ataxias and paraplegias. Lancet 1983;1:1151–1155.
2. Depienne C, Stevanin G, Brice A, Durr A. Hereditary spastic paraplegias: an update. Curr Opin Neurol 2007;20:674–680.
3. Salinas S, Proukakis C, Crosby A, Warner TT. Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms. Lancet Neurol 2008;7:1127–1138.
4. Cross HE, McKusick VA. The Troyer syndrome. A recessive form of spastic paraplegia with distal muscle wasting. Arch Neurol 1967;16:473–485.
5. Proukakis C, Cross H, Patel H, et al. Troyer syndrome revisited. A clinical and radiological study of a complicated hereditary spastic paraplegia. J Neurol 2004;251:1105–1110.
6. Farag TI, el-Badramany MH, al-Sharkawy S. Troyer syndrome: report of the first “non-Amish” sibship and review. Am J Med Genet 1994;53:383–385.
7. Farah S, Sabry MA, al-Shubaili AF, et al. Hereditary spastic paraparesis with distal muscle wasting, microcephaly, mental retardation, arachnodactyly and tremors: new entity? Clin Neurol Neurosurg 1997;99:66–70.
8. Auer-Grumbach M, Fazekas F, Radner H, et al. Troyer syndrome: a combination of central brain abnormality and motor neuron disease? J Neurol 1999;246:556–561.
9. Bertini E, Sabatelli M, Di Capua M, et al. Familial spastic paraplegia, axonal sensory-motor polyneuropathy and bulbary amyotrophy with facial dysmorphism: new cases of Troyer-like syndrome. Eur J Paediatr Neurol 1999;2:245–254.
10. Patel H, Cross H, Proukakis C, et al. SPG20 is mutated in Troyer syndrome, an hereditary spastic paraplegia. J Neurol 2004;251:1105–1110.
11. Bakowska JC, Wang H, Xin B, et al. Lack of spartin protein in Troyer syndrome: a loss-of-function disease mechanism? Arch Neurol 2008;65:520–524.
12. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. Adv Data 2000;(314):1–27.
13. Hall J, Allanson J, Gripp K, Slavotinek A. Handbook of physical measurements. New York, NY: Oxford University Press, 2007:520.
14. Leykin I, Hao K, Cheng J, et al. Comparative linkage analysis and visualization of high-density oligonucleotide SNP array data. BMC Genet 2005;6:7.
15. Gudbjartsson DF, Jonasson K, Frigge ML, Kong A. Allegro, a new computer program for multipoint linkage analysis. Nat Genet 2000;25:12–13.
16. Maynard TM, Meechan DW, Dudevoir ML, et al. Mitochondrial localization and function of a subset of 22q11 deletion syndrome candidate genes. Mol Cell Neurosci 2008;39:439–451.
17. Meechan DW, Maynard TM, Gopalakrishna D, et al. When half is not enough: gene expression and dosage in the 22q11 deletion syndrome. Gene Expr 2007;13:299–310.
18. LaMantia AS. Forebrain induction, retinoic acid, and vulnerability to schizophrenia: insights from molecular and genetic analysis in developing mice. Biol Psychiatry 1999;46:19–30.
19. Robay D, Patel H, Simpson MA, et al. Endogenous spartin, mutated in hereditary spastic paraplegia, has a complex subcellular localization suggesting diverse roles in neurons. Exp Cell Res 2006;312:2764–2777.
20. Wilkinson PA, Simpson MA, Bastaki L, et al. A new locus for autosomal recessive complicated hereditary spastic paraplegia (SPG26) maps to chromosome 12p11.1-12q14. J Med Genet 2005;42:80–82.
21. Rainier S, Bui M, Mark E, et al. Neuropathy target esterase gene mutations cause motor neuron disease. Am J Hum Genet 2008;82:780–785.
22. Bouchard JP, Barbeau A, Bouchard R, Bouchard RW. Autosomal recessive spastic ataxia of Charlevoix-Saguenay. Can J Neurol Sci 1978;5:61–69.
23. Engert JC, Berube P, Mercier J, et al. ARSACS, a spastic ataxia common in northeastern Quebec, is caused by mutations in a new gene encoding an 11.5-kb ORF. Nat Genet 2000;24:120–125.
24. Rajab A, Yoo SY, Abdulgalil A, et al. An autosomal recessive form of spastic cerebral palsy (CP) with microcephaly and mental retardation. Am J Med Genet A 2006;140:1504–1510.
25. Eastman SW, Yassaee M, Bieniasz PD. A role for ubiquitin ligases and Spartin/SPG20 in lipid droplet turnover. J Cell Biol 2009;184:881–894.
26. Edwards TL, Clowes VE, Tsang HT, et al. Endogenous spartin (SPG20) is recruited to the endosomes and lipid droplets and interacts with the ubiquitin E3 ligases AIP4 and AIPS. Biochem J 2009;423:31–39.
27. Milewska M, McRedmond J, Byrne PC. Identification of novel spartin-interactors shows spartin is a multifunctional protein. J Neurochem 2009;111:1022–1030.