Herediter spastik paraparezi:Fenotipik heterojenite ve SPG11 lokusunun doğrulanması

Hereditary Spastic Paraparesis: Phenotypic Heterogeneity and Confirmation of the SPG11 Locus

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Abstract

Hereditary spastic paraplegias (HSPs) are a genetically and clinically heterogeneous group of upper motor neuron disorders. Although the primary feature of HSP is lower extremity weakness ("uncomplicated" form), sequelae and clinical features of this disorder may include other neurological deficits such as dementia, neuropathy, retinopathy, mental retardation, and seizures ("complicated" form). To date, more than 56 different genetic loci and 41 HSP-related genes have been described as causative for autosomal dominant, recessive or X-linked HSP. One such locus on chromosome 15q, also known as locus SPG11 (OMIM 604360), has been shown to link to a complicated autosomal recessive form of disease known as HSP with thin corpus callosum (HSP-TCC).

Herein we describe the identification and clinical presentation of a new family from Eastern Turkey with autosomal recessive HSP associated with mental retardation, epilepsy and a thinned corpus callosum on MRI. Using array-based SNP genotyping, we demonstrate linkage to the SPG11 locus on chromosome 15q13-15. Array-based copy number variation (CNV) analysis was also performed. Our results not only expand the phenotypic heterogeneity associated with the SPG11 locus to include an earlier age of onset with epilepsy, but also confirm the linkage to a 13 Mbp interval on chromosome 15q. This data, when added to those previously reported, support the notion that SPG11 is a phenotypically and genetically heterogeneous disorder.

Key Words: SPG11, Chromosome 15, Linkage analysis, hereditary spastic paraparesis, Microarray

Öz

Herediter spastik paraplejiler (HSP) üst motor nöron hastalıklarının genetik ve klinik olarak heterojen bir grubudur. HSP' nin temel özelliği alt eksremite güçsüzlüğü olmasına rağmen (komplike olamayan tip), bu hastalığın sekelleri ve klinik özellikleri demans, nöropati, retinopati, mental retardasyon ve nöbetler gibi diğer nörolojik bozuklukları kapsayabilir (komplike tip). Günümüze kadar, HSP'nin sorumlu nedeni olarak otozomal resesif, otozomal dominant veya X' e bağlı 56' dan fazla farklı genetik bölge ve 41 HSP ilişkili gen tanımlanmıştır. Kromozon 15q daki aynı zamanda SPG11 (OMIM 604360) olarak bilinen bir çeşit bölgenin, hastalığın ince korpus kallozumlu HSP (HSP-TCC) olarak bilinen komplike otozomal resesif formuyla bağlantılı olduğu gösterilmiştir.

Bu araştırmada, mental gerilik, epilepsi ve MRG' de ince korpus kallozumun eşlik ettiği otozomal resesif kalıtım gösteren HSP li Türkiyenin doğusundan yeni bir aile tanımlanmış ve klinik özellikleri tarif edilmiştir. Chip temelli SNP genotiplemesi kullanarak, kromozon 15q13-15' da SPG11 bölgesine bağlantı saptanmıştır. Aynı zamanda chip temelli kopya sayısı değişkenliği (CNV) analizi uygulanmıştır. Sonuçlarımız sadece SPG11 bölgesi ile ilişkili erken yaşta epilepsi ile başlayan fenotipik heterojeniteyi genişletmemiş, aynı zamanda 15q kromozomunun 13 Mbp aralığına bağlantıyıda doğrulamıştır. Bu veri, daha önceki çalışmalara eklendiğinde, SGP11'in fenotipik ve genotipik olarak heterojen hastalık olduğu gerçeğini desteklemektedir.

Anahtar Kelimeler: Herediter spastik paraparezi-Mikro-chip-SPG11-kromozom 15-Bağlantı analizi

Introduction

The hereditary spastic paraplegias (HSP), also known as familial spastic paraparesis (FSP), are a genetically and clinically heterogeneous group of neurological disorders characterized by progressive lower extremity spasticity. HSPs can be associated with other neurological sequelae including neuropathy, retinopathy, dementia, icthyosis, mental retardation, deafness and seizures ("complicated" form), or by upper motor neuron findings including lower-extremity spasticity and neurogenic bladder alone ("uncomplicated" form). The diagnosis is confirmed through neurological testing, muscle biopsy, EMG, MRI, and detailed genetic history. The majority of HSP familial forms reported to date, up to 80%, demonstrate autosomal dominant patterns of expression, while the remainder demonstrate autosomal recessive and X-linked recessive inheritance patterns (1-3).

The first association of HSP with mental retardation and epilepsy, designated "SPERM" (OMIM 182610), was reported as a novel genetic disorder with an autosomal dominant pattern of inheritance (4). The family described was excluded from 8 previously described autosomal dominant HSP loci by linkage (5). Other families with complicated forms of HSP have been reported including an autosomal recessive form with a relatively constant clinical presentation of pyramidal tract signs in the lower extremities which progress to the upper extremities, gradual cognitive impairment, an onset before age 20, and radiographic findings of thinning of the corpus callosum (CC) and cortical atrophy known as HSP-TCC, or SPG11 (OMIM 604360)(6-12). Herein we describe a new family from Eastern Turkey with autosomal recessive HSP associated with early-onset mental retardation, epilepsy and a variably thinned corpus callosum on MRI demonstrating linkage to the SPG11 locus at 15q13-15. This data, when added to those previously reported, support the notion that SPG11 is a phenotypically and genetically heterogeneous disorder.

Material and Methods

Family Identification and Phenotype Assignment

The family was identified in Southeastern Turkey after the index case, a product of a consanguineous marriage, presented to medical attention with spastic paraparesis, mental retardation, and epilepsy. Clinical testing included magnetic resonance imaging (MRI), electromyography (EMG), electroencephalogram (EEG), cerebrospinal fluid analysis, and other specific laboratory examinations such as routine blood work, blood thyroid hormone, ammonium, lactate levels, urine analysis.

Collection of Blood Samples and Isolation of Genomic DNA

This study was approved by the Yale HIC/IRP (protocol number: 7680) and The Istanbul University Ethics Committee. Blood samples were collected from all available family members after the attainment of informed consent. Total genomic DNA was isolated as previously described (13,14).

Single Nucleotide Polymorphism Genotyping

Genotyping was performed using the GeneChip Mapping 50K XbaI Array (Affymetrix Inc., Santa Clara, CA) containing 56,860 single nucleotide polymorphism (SNP) markers for genome-wide linkage analysis, according to the company's protocols. Affymetrix Micro-Array Suite 5.0 software was utilized to obtain raw microarray feature intensities, the results of which were processed to derive SNP genotypes using the Affymetrix Genotyping Tools software package.

Genechip Data Analysis

The Genome Analysis programs provided by Affymetrix were used for basic analysis of the Genechip data. Multipoint linkage analysis was performed using our previously described, UNIX-based program (Chunky) (15) followed by the Allegro software (DeCode Genetics, Inc) (16). We assumed an autosomal recessive inheritance pattern and assigned a 70% penetrance and a phenocopy rate 0.001. Allele frequencies for the GeneChips SNPs were obtained from Affymetrix.

Confirmation of Linkage Using Microsatellite Short Tandem Repeat Markers

Genomic regions with LOD scores approaching the theoretical maximum were further characterized and verified using microsatellite short tandem repeat (STR) markers within said regions according to the physical map data from the University of California at Santa Cruz (UCSC) Genome Browser (http://genome. ucsc.edu/ index. html?org=Human, May 2004). All available members of the family, both affected and unaffected, were genotyped. This strategy is often referred to as a 2-stage design in linkage analysis (17). All genotyping for microsatellite analysis was performed using PCR, with detection of fluorescent products on an ABI 3700 sequencer equipped with the Genescan and Genotyper software (ABI, Norwalk, CT). STS markers used include: D15S994, D15S641, D15S780, D15S783, D15S659, D15S1032 and D15S1016.

Canidate Gene Mutational Analysis

Exon-intron boundaries of the candidate genes SPATA5L1 and SEMA6D within the linked interval were determined based on the University of California at Santa Cruz (UCSC) Genome Browser (NCBI Build 36.1). PCR primers were designed using PRIMER3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Exon amplicons were amplified and sequenced using standard techniques.

Array CGH for Copy Number Analysis

Isolated DNA from the patients V-1 and V-2 were submitted for whole genome and chromosome 15-specific array-based comparative genomic hybridization (aCGH) analysis by high-resolution, tiled microarray (NimbleGen Systems, Madison, WI) to determine copy number variations (CNVs). These arrays employ 385,000 probes spanning all non-repetitive regions of the human genome on a single chip, tiling the full genome at a median probe spacing of 6,000 bp. The chromosome 15-specific aCGH contains 385,000 oligomeric probes of lengths between 45–85mers with a median probe spacing of 10 bp.

Array Data Analysis

All arrays were scanned with a GenePix 4000B

Scanner (Molecular Devices Corporation, Sunnyvale, California) and normalized using QSPLINE (18) within the NimbleScan software package (Nimblegen Inc., Madison, WI).

The normalized intensities were subsequently analyzed with the Circular Binary Segmentation (CBS) algorithm (19) to determine the significant breakpoints in log 2 intensities along the chromosomes. Using average window sizes of 1X, 5X, 10X, and 20X (X=the median inter-probe distance), we determined the possible segments of the genome that were different between our patients and pooled, population-matched control samples. A segment (y) was considered to be significant if $y>0.3$ or $y<-0.3$.

The normalized intensities were also analyzed with our Seed algorithm (Mason et al., in prep.), which creates windowed 5-probe averages along the genome after removing outlier data points (Dixon test) (20) to detect additional small CNVs occasionally missed by CBS. Two patients with large-scale, known duplications in their genome were used to empirically determine a threshold for the Seed algorithm that kept sensitivity above 90% and specificity above 99.99%. We considered segment (y) to be significant in Seed if $y>0.22$ or $y<-0.22$.

Results

Phenotype Assessment

Parents of the affected children were normal and consanguineous, providing evidence for an autosomal recessive inheritance pattern. Affected status was assigned after clinical documentation of prominent lower followed by upper extremity paraparesis with long (pyramidal) tract signs (spasticity, hyperreflexia, and bilateral Babinski sign), and epileptic discharges on electroencephalogram (EEG) (Figure 1.).

Case 1 (V-1)

The index case is a four year-old female, a product of a consanguineous marriage, who presented with neurological decline. The patient was neurologically normal until the age of 6 months when parents became concerned with the child's lack of interactivity. By the age of 9 months, the child developed seizures which were controlled medically. On neurologic examination, the patient demonstrated motor and mental retardation, spasticity in the lower limbs, hyperreflexia, and an inability to sit without support. An MRI revealed a thinned corpus callosum on coronal T2 and sagittal T1 imaging (Figure 2a-c).

Case 2 (V-2)

The second affected is the brother of the index case, a twelve year-old male. The patient developed normally but, as with Case 1, began having seizures at 10 months of age. He also suffered from mental retardation, spasticity in lower and upper limbs, aphasia, hyperreflexia, and an inability to sit without support (imaging unavailable).

Case 3(V-3)

The third affected individual is a first cousin of the index case, also a product of a consanguineous marriage. This ten-year-old boy also presented with seizures and mental retardation at age 6 months. He has difficulty swallowing solid food. Neurological examination revealed mental retardation, spasticity in the lower limbs, hyperreflexia, and inability to sit without support. MRI revealed mild, diffuse atrophy of grey and white matter with a thinned corpus callosum (Figure 2d-f).

In all 3 patients, EEG studies showed myoclonic generalized epileptic discharges. Laboratory values provided no evidence for a lysosomal, mitochondrial, or peroxisomal disorder or a disturbance of amino acid or organic acid metabolism. CSF analysis was unremarkable.

Single Nucleotide Polymorphism Genotyping

We performed array-based genotyping on all available affected individuals and their parents (n=7). The 50K SNP arrays provide estimated information with a mean marker distance of 26 kb with an average of 53970 genotypes scored per subject (SNP call-rates ranged between 92%–97%). Multipoint linkage analysis demonstrated a mean LOD score of >3 (maximum: 3.6287) within a 9.8 cM region between markers rs10518676 and rs2129773 on chromosome 15q15.1-q21.3 (Figures 3 and 4).

Linkage Using Microsatellite Short Tandem Repeat Markers

The linkage interval was verified and confirmed using seven highly polymorphic di- and tetranucleotide microsatellite repeats across our linkage interval. As expected, the parents were heterozygous for the affected haplotype while the children with the HSP-TCC phenotype were homozygous for the affected haplotype (Figure 1).

Mutation screen

Screening of the candidate genes within the linkage interval, SPATA5L1 and SEMA6D failed to reveal any polymorphisms that segregated with the phenotype (data not shown).

Array CGH for Copy Number Analysis

Whole genome aCGH and chromosome 15-specific aCGH identified several copy number variations throughout the genome. None of these variations, however, were within the linkage interval and none segregated with the disease. (Figure 5)

Discussion

In this manuscript, we report a new family from Eastern Turkey with autosomal recessive HSP, epilepsy, mental retardation, and a thinned corpus callosum on MRI demonstrating linkage to the SPG11 locus at 15q15.1-q21.3, a region between 38.1-51 Mb.

The clinical criteria for HSP-TCC are defined as normal motor development followed by a slowly progressive spastic paraparesis, mental retardation, and a very thin corpus callosum on imaging (10-12). Other clinical signs that have been reported include extrapyramidal signs, hyperreflexia, dysphagia, dysarthria, amyotrophy, urinary incontinence, muscle atrophy, and peripheral neuropathy with cortical atrophy and white matter changes on imaging (6-9). The average age of onset has been reported to be in the second decade (6).

Significant phenotypic variability exists within affected members of families with respect to clinical signs and radiographic signs. For example, some affected patients demonstrate various degrees of thinning of the CC on MRI, thought to represent a progressive finding of neuronal loss (6, 8-10). The family reported here differs from those previously reported with respect to the uniformly early onset of disease (in the first year of life) including epileptic seizures and a mildly-thinned CC on MRI, further expanding the phenotype. Interestingly, no cases of epilepsy were reported in any of the families demonstrating linkage to SPG11. The only prior reported case of HSP with epilepsy was from the family with the so-called autosomal dominant "SPERM" syndrome, (4) though others have reported affected patients with seizures late in disease progression (21).

Linkage analysis of the SPG11 locus was first reported by Martinez Murillo et al. (12) in 7 families from Italy and North America, between markers D15S1007 and D15S1012 on chromosome 15q13-15, a region of approximately 6.9 cM corresponding to 5 Mb on chromosome 15 (Figure 6). This linkage region was subsequently expanded (11) to between

markers D15S971 and D15S117, a region of 26.7 cM corresponding to 23 Mb in 10 Japanese families with HSP-TCC. Originally described in the Japanese population, (21-23) there have been several reports in the literature of families with HSP-TCC from different ethnicities, with the majority of families appearing to originate from the Mediterranean region (6-9,12). Genetic linkage results in many of these families have confirmed or narrowed the SPG11 locus (6, 8-10). A recently published paper by Olmez et al. reported linkage in 4 Turkish families in a region between markers D15S968 and D15S132. The authors argue that their results narrow the centromeric end of the region and, in effect, exclude the region defined by Martinez Murillo et al. (7,12) Our linkage data confirm the results of Olmez et al., also excluding the first reported interval (Figure 6).

Chromosomal copy number alterations can lead to overactivation or inactivation of genes in humans leading to cancer or disease phenotypes. Comparative Genomic Hybridization (CGH) is a method to detect chromosomal copy number by comparing hybridization intensity of a patient's DNA to a control DNA sample (24) and has become an important tool in rapid identification of functional mutations within linkage intervals (25-27). We hypothesized that CNVs within the linkage interval might be causative of the disease phenotype. We performed array-based whole-genome and chromosome 15-specific CGH in the family reported here. Analysis of the array results did not show any CNV within the linkage interval in affected patients. Furthermore, mutational analysis of the potential candidate genes SPATA5L1 and SEMA6D in this region did not reveal any causative mutations (6,9).

Another congenital neurological disorder demonstrates linkage to the same interval as SPG11. Amyotrophic lateral sclerosis type 5 (ALS5) is characterized by gait disturbance, spasticity, mental retardation, and severe bulbar and pseudobulbar findings and demonstrates linkage between markers D15S146 and D15S123 (28). It is possible that this phenotype is a part of the same spectrum of neurological disorders as HSP-TCC, caused by different mutations within the same gene (that ALS5 is an allelic disorder to HSP-TCC) supporting the idea that HSP-TCC represents a syndrome with broad phenotypic and genetic heterogeneity. This hypothesis isn't entirely impossible as it was previously demonstrated that ALS2 is allelic to infantile ascending hereditary spastic paralysis (IAHSP) (29). More compelling is the fact that many families with autosomal recessive HSP-TCC (families with affected members fitting the clinical criteria of the syndrome) exclude by linkage the reported SPG11 locus (6,8,10). This clearly argues in favor of genetic heterogeneity of the disorder.

Our results confirm previously published linkage analysis in families demonstrating linkage to the SPG11 locus on 15q and demonstrate the absence of CNV within the linkage interval in this family. The phenotype reported here adds to the broad spectrum of clinical findings within the HSP-TCC syndrome. These results, when dovetailed with those previously published, demonstrate phenotypic heterogeneity of a common clinical entity with a common genetic cause. The explanation of phenotypic variation within patients linking to the SPG11 locus is likely multifactorial and a combination of non-genetic, compound genetic, and mutation-specific causes.

Figure 1

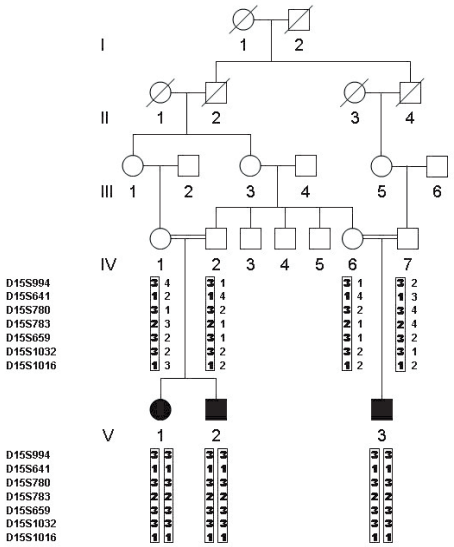

Figure 1: Family Pedigree with STS marker haplotypes. Filled symbols show affected individuals, males are represented with square symbols, females with circles. The pattern, given the consanguinity, suggests autosomal recessive inheritance. Below affected family members V1-3 and their respective parents note the haplotype as determined by STS marker genotyping between markers D15S994 and D15S1016.

Figure 2

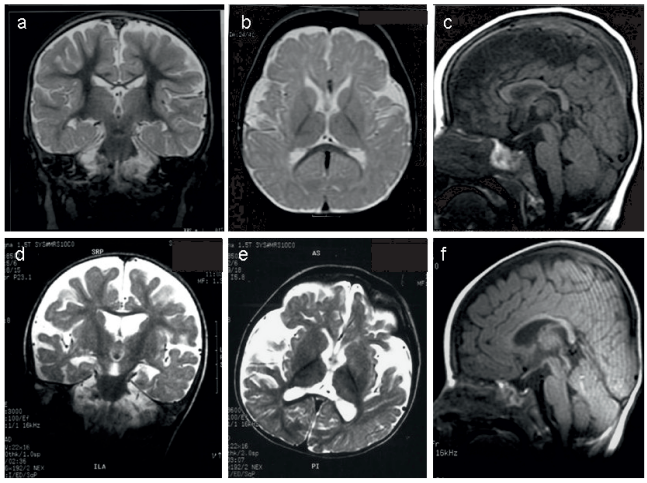

Figure 2: Representative MRI images from V1 and V3.

Patient V1(a-c). a Coronal and b. axial T2-weighted MRI images through the thalamus and 3rd ventricle demonstrate diffuse enlargement of the subarachnoid spaces along the convexities likely due to gray and white matter loss. Note the thinned corpus callosum. c. Midline sagittal T1 weighted MRI demonstrates the thinned corpus callosum. The cerebellum, pons, and medulla appear normal. Patient V3(d-f). d. coronal and e. axial T2- weighted MRI images through the thalamus and 3rd ventricle again demonstrate diffuse enlargement of the subarachnoid spaces along the convexities likely due to gray and white matter loss at similar sections to a. and b. f. Midline sagittal T1 weighted MRI similarly demonstrates the thinned corpus callosum.

Figure 3

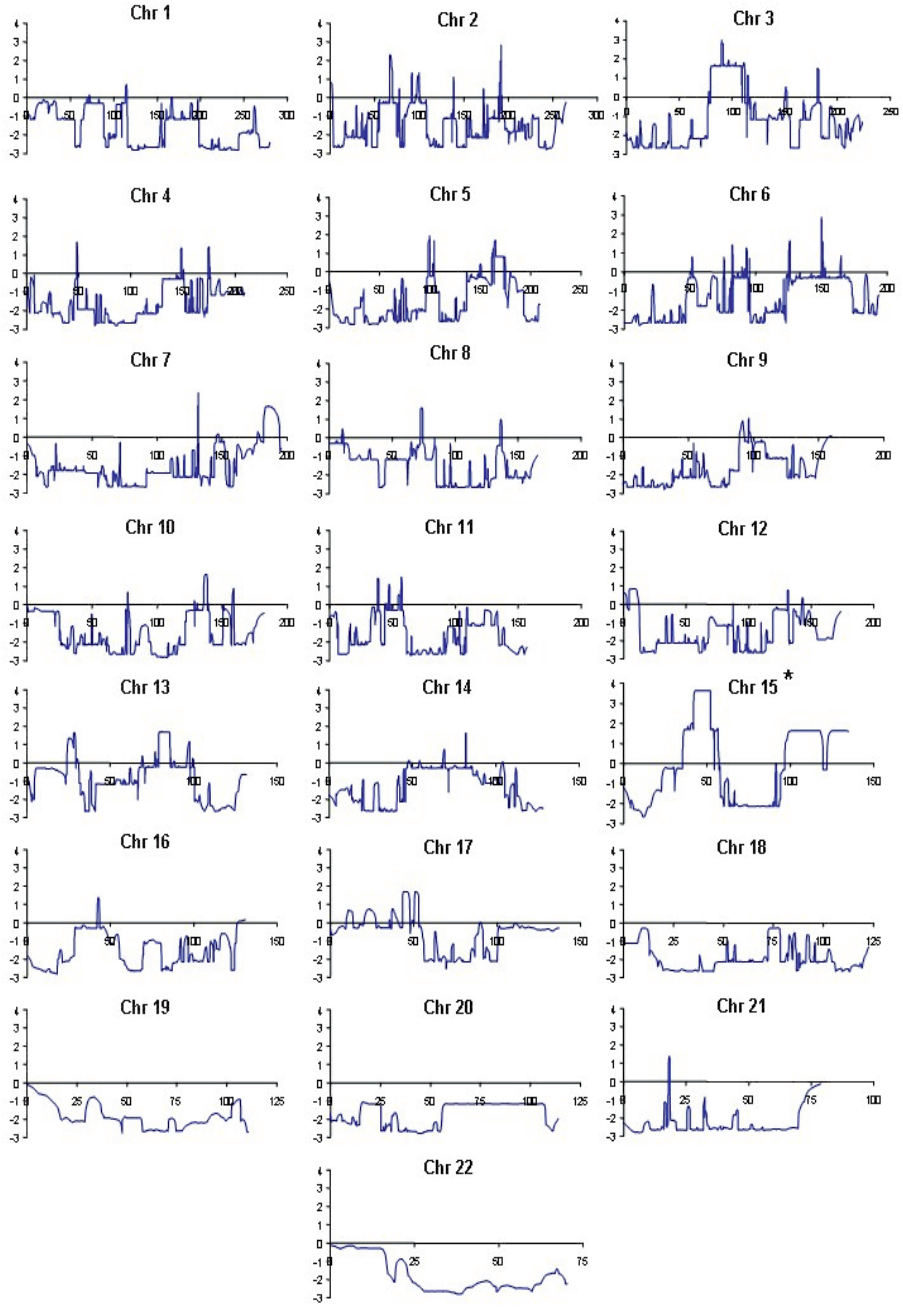

Figure 3: Results of Array-Based SNP genotyping

Genotyping results using the GeneChip Mapping 50K XbaI Array. Figure demonstrates multipoint linkage analysis plots for each chromosome. X axis = cM distance along the chromosome. Y axis = LOD score. Results are plotted after analysis using Chunky followed by the Allegro software. * denotes chromosome 15 results.

Figure 4

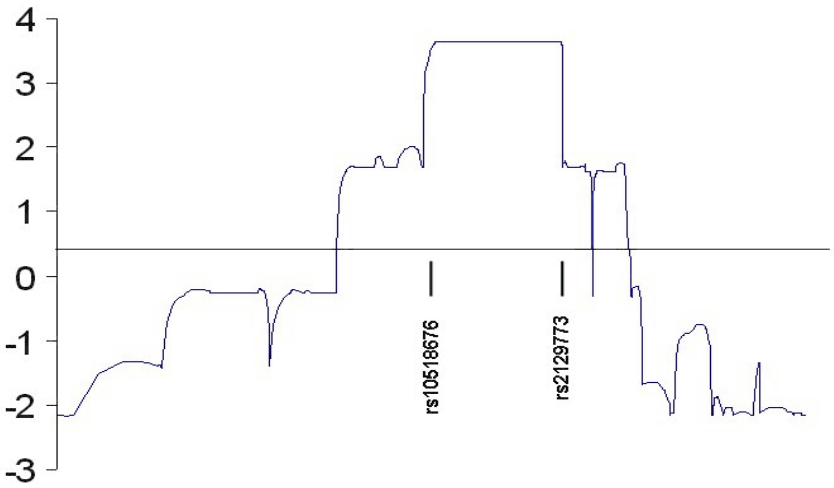

Figure 4: Validated Linkage Interval of 15q15.1-q21.3

Graphical demonstration of multipoint linkage analysis demonstrating a LOD score of >3 (maximum: 3.6287) within a 9.8 cM region between markers rs10518676 and rs2129773 on chromosome 15q.

Figure 5

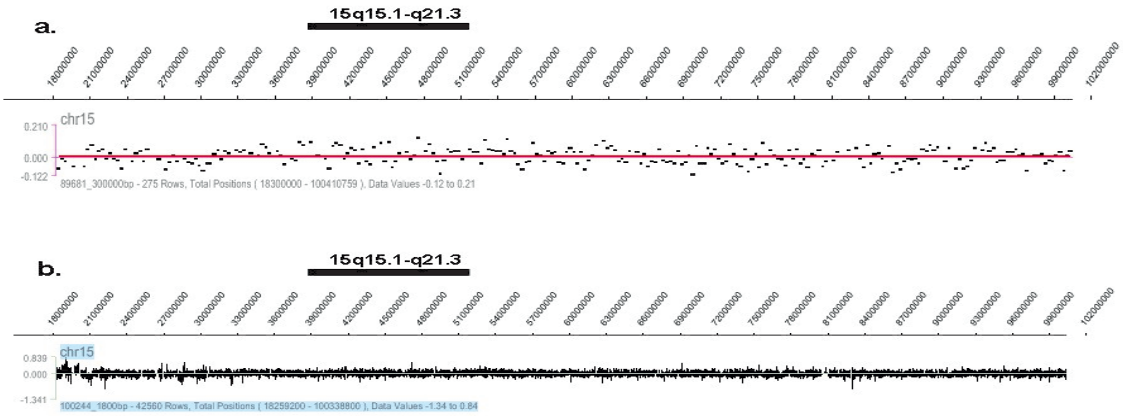

Figure 5: Whole-Genome and Chromosome 15-specific aCGH

Graphical representation of a Whole-Genome and b. Chromosome 15-specific copy number variation (CNV) analysis using array-based comparative genomic hybridization (aCGH). Detailed analysis did not demonstrate any CNVs within the sensitivity of the assay.

Figure 6.

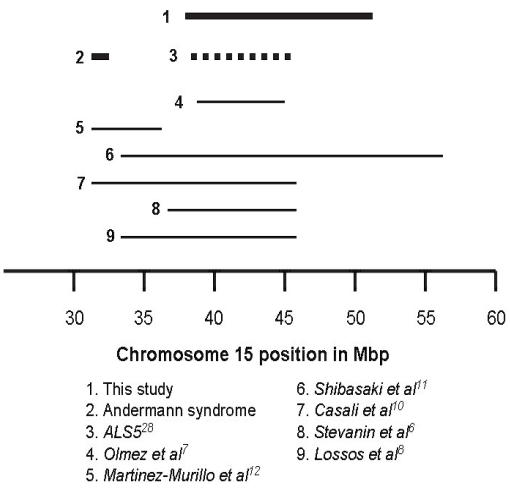

Figure 6: Schematic of Chromosome 15 Linkage Results for SPG11

X-axis designates the distance and position along chromosome 15q in million base pairs. The top bar represents the linkage interval reported in this study (3). The remainder of the linkage intervals reported for SPG11 are shown (4-9) as are the two known neurological syndromes within the region (Andermann syndrome and ALS5; 1,2).

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