A novel form of juvenile recessive ALS maps to loci on 6p25 and 21q22

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Abstract

We describe a novel form of juvenile recessive ALS (JRALS) affecting four of six offspring from a consanguineous first-cousin marriage. The syndrome is characterized by early and prominent upper motor neuron signs, along with striking amyotrophy of the upper and lower limbs and bulbar involvement. After excluding linkage to loci with known association to ALS and other motor neuron diseases, we used a homozygosity mapping approach to identify loci on chromosomes 6p25 and 21q22, each with an equal probability of linkage to the trait (with a LOD score = 3.1, the maximum possible given the family structure). Mutation analysis of seven candidate genes that are expressed in the CNS or have roles in neuronal function did not reveal any pathogenic mutations. Identification of additional families will help to distinguish between which of the two autosomal loci contains the disease-causing gene, or whether this is a digenic trait.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is broadly characterized by upper and lower motor neuron disease with progressive severity. Symptoms include weakness, spasticity, atrophy, and hyper-reflexia that are progressive over time. Five to ten percent of ALS cases are considered familial [1–3]. Mutations in superoxide dismutase-1 (SOD1) gene on chromosome 21 (designated ALS1) account for 20% of familial ALS cases (OMIM #147450) [4,5]. To date, more than 125 different disease-causing mutations have been described in SOD1 [3]. A number of additional genetic loci have been identified in ALS families, including families with dominant, recessive and X-linked inheritance [2,3]. Loci on chromosomes 9q34 (ALS4), 2q33 (ALS2), and 15q (ALS5) have been associated with juvenile onset ALS [6–8]. In contrast to typical sporadic cases, with onset between 60 and 75 years and rapid progression to death in 3–5 years [9], juvenile ALS is typically inherited, with an age of onset in the first or second decade, and symptom progression over decades leading to death. Three different forms of juvenile ALS have been described based on clinical features [10]. Type 1 involves early, predominantly lower motor neuron signs with upper motor neuron involvement as disease progresses. Type 2 is a combination of lower and upper motor neuron involvement of primarily the lower limbs with bulbar sparing. Predominant upper motor neuron signs and a pseudo-bulbar affect are seen in type 3. Here we describe an apparently novel juvenile recessive motor neuron syndrome, affecting upper and lower motor neurons in both the limb and bulbar distribution in four of six offspring of a consanguineous first-cousin marriage. The proband was first examined by us when he was age 15 years with prominent upper motor neuron signs suggestive of type 3, but without a pseudobulbar affect and displaying striking and progressive distal amyotrophy of both upper and lower limbs, along with bulbar symptoms.

To further elucidate the underlying genetic factors responsible for disease in this family, we performed an initial genetic screen for known loci associated with ALS, as well as other neuromuscular disorders with overlapping phenotypes including, Charcot-Marie-Tooth (CMT) and hereditary spastic paraplegia (HSP). In the absence of linkage to known loci with motor neuron phenotypes, we performed a genome-wide screening using a homozygosity mapping strategy [11]. Using this approach we have identified two loci with equal probability, one on chromosome 6 and one on chromosome 21, associated with disease in this family.

2. Methods

2.1. Clinical

Clinical examination of all family members was performed by K.M.F. Each family member gave their informed consent (or, in

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the case of minor subjects, assent with parental consent) for this research under a University of Utah Institutional Review Board-approved protocol. Nerve conduction studies were performed on an Advantage EMG machine (Clarke-Davis).

2.2. Mapping strategy and genotyping

Initial genetic studies were performed to screen for homozygosity among affected individuals in our pedigree for known ALS loci and additional loci associated with overlapping neuromuscular disorders including CMT and HSP. (Markers and loci are listed in Supplemental Table 1.) Because of the absence of demyelinating features or myelin folding abnormalities on nerve biopsy, and the absence of hearing abnormalities, CMT4 loci were not evaluated in spite of the recessive pattern of inheritance associated with these loci.

A genome-wide screen for homozygosity [11] among affected individuals was performed using 192 microsatellite markers selected from the Utah Development Group index linkage mapping set [12]. Additional markers were selected from the ABI MD-10 mapping set (Applied Biosystems, Foster City, CA) with a goal for coverage of <20 cM between markers [13]. Candidate regions were identified at markers for which all 4 affected individuals shared identical alleles. Further evaluation at each candidate locus was performed by selecting nearby markers from the Genethon-CEPH integrated map, and Marshfield genetic map. Genotypes at these markers were obtained for all 11 individuals in the kindred including unaffected sibs, parents, and grandparents to identify regions of linkage to disease (Figs. 3 and 4).

The youngest sibling was clinically unaffected at the time of the initial genetic analysis. He was not included in the initial genomic screen due to his young age, and the possibility that he was evaluated prior to onset of symptoms. He remained unaffected by clinical exam age 12 and remains unaffected now at age 15 by report from his mother. As other affected individuals in the family had onset of symptoms at age 10 or less, we now consider this youngest sibling unaffected. He was subsequently genotyped at each candidate region, as discussed below.

2.3. Homozygosity mapping and linkage analysis

Homozygosity mapping was performed by the method proposed by Lander and Botstein [11], with goal for marker spacing of 20 cM or less [13]. Maximum-likelihood analysis was performed for candidate loci on chromosomes 6 and 21 by pairwise two-point linkage analysis using the MLINK subroutine within the FASTLINK linkage analysis program on the easyLINKAGE software package [14–16]. Allele frequency for the disease locus was set at .001 with 95% penetrance and a recessive model. Haplotype analysis was performed using the GENEHUNTER algorithm in the easyLINKAGE software [16], and plots made with HaploPainter software (http://sourceforge.net/projects/haplopainter/).

2.4. Evaluation of candidate genes

Mutational analysis of candidate genes was performed by direct sequencing of genomic DNA from the coding region and flanking intronic sequences to identify both coding and splice site variants. PCR primers were derived from published primers or designed from intronic sequence flanking each exon [17,18], and sequenced within the University of Utah DNA Sequencing and Genomics Core Facility. Sequence comparisons between the proband, one parent, and the reference sequence (NCBI build 36) were made using Sequencher software (GeneCodes Corp., Ann Arbor, MI).

3. Results

3.1. Clinical description

The proband (Fig. 1; patient IV-2, Figs. 3 and 4) was the product of a normal pregnancy and delivery. Developmental milestones were normal until the age of 2½ years, at which time he developed an aseptic brainstem encephalitis (with marked opsinclonus). He recovered well, with only mild residual ptosis and dysarthria. According to his mother his motor skills had nearly caught back up to his peers by the age of four, at which time he first developed difficulty with walking. Between the ages of four and 12 years he had progressive gait disturbance as well as progressive distal limb weakness and atrophy, and foot deformities. At the age of 12 years he began to require a wheelchair for daily use, as a result of gait instability, and underwent bilateral tendo-aichilles lengthening.

Brain MRI was performed at age 8, and repeated at age 12, at which time MRI of the cervical, thoracic, and lumbar spinal cord was also performed. The brain MRI showed a small (2 mm) area of increased signal intensity adjacent to the posterior portion of the right lateral ventricle, without interval change between ages 8 and 12, and mild cerebellar atrophy; spinal cord MRI scans were normal. Nerve conduction studies were performed at age 12, and are summarized in Table 1. Diminished sensory and compound motor action potentials were seen; conduction velocities and motor F-wave latencies were normal throughout. Concentric needle EMG of the anterior tibialis and abductor digiti quinti reportedly revealed abnormal spontaneous activity in both muscles; voluntary motor unit potentials of increased amplitude and decreased recruitment were seen in both muscles, whereas units of increased duration and polyphasia were seen only in the anterior tibialis. These changes were considered consistent with a motor and sensory neuropathy, and muscle and
nerve biopsies were performed. The muscle demonstrated moderate neurogenic changes (grouped atrophy and fiber type grouping). The sural nerve biopsy (obtained at the ankle) demonstrated minimal loss of axonal density, without evidence of ongoing degeneration or evidence of chronic demyelination or remyelination (e.g., thinly myelinated fibers or onion bulbs) (Fig. 2). His distal limb atrophy progressed between the ages of 12 and 16, during which time he developed progressive kyphoscoliosis and marked gynecomastia (Tanner stage 4 breast development, with the presence of both glandular tissue and fat by clinical exam). Endocrine evaluation revealed normal levels of testosterone, estradiol, and progesterone. A forced vital capacity was 44% of the predicted value.

At the age of 15, he was examined for the first time by K.M.F. This examination revealed striking kyphoscoliosis and gynecomastia (Fig. 1a). Mental status was normal by bedside exam. He had moderate asymmetric (right > left) ptosis; moderate facial weakness, and spastic dysphonia. Eye movements were normal, without nystagmus or saccadic abnormalities. Muscle bulk was decreased throughout, with marked atrophy in the distal arms and legs (Fig. 1b and c); no fasciculations were seen. Muscle strength was similarly weak throughout, in a symmetric fashion. Sensory examination revealed normal levels of light touch sensation but vibratory sensation was mildly decreased in the foot. Tendon reflexes were absent in the arms; testing of patellar reflexes resulted in several beats of clonus, and there was sustained clonus at the ankles. The Babinski sign was present bilaterally. Using a walker, he could stand and slowly walk.

Limited nerve conduction studies and EMG were again performed. No sural sensory response could be obtained. Concentric needle EMG was performed in three muscles. Abnormal spontaneous activity (low-amplitude positive waves and fibrillation potentials) was seen in the anterior tibialis and orbicularis oris muscles, but not in the tongue. Voluntary motor unit potentials demonstrated decreased recruitment and increased polyphasia (including in the tongue), and variably increased duration. Blink reflex studies demonstrated normal R1 latencies but prolonged ipsilateral R2 latencies bilaterally, and contralateral R2 responses were absent, suggesting pontine or medullary conduction block.

### 3.2. Additional family members

Although the parents initially thought that no other children were affected, additional family members (see pedigree, Figs. 3 and 4) were subsequently evaluated, and nerve conduction studies (limited studies, at parental request) were performed in patients IV-1 through IV-5 (see pedigree, Figs. 3 and 4). Findings on examination were normal for both parents (III-1 and III-2) and for two of the 5 siblings (IV-1 and IV-6). At the time of his initial evaluation, subject IV-6 was 5 years old and was considered to be too young for definitive exclusion of the trait; however, he remained unaffected at reexamination at age 15. Three other siblings (IV-2, IV-4, and IV-5) were determined to be affected, but in contrast to the proband, none had unusual secondary sex characteristics. Details of their clinical features are described below, and details of electrophysiologic testing are included in Table 1.

![Fig. 2. Epon-embedded, toluidine-blue stained section of sural nerve from the proband, showing only a minimal decrease in sensory nerve fiber density (100× oil immersion objective; bar = 10 μm).](image-url)
3.2.1. Subject IV-3 (at initial evaluation, age 10)

Mild weakness and wasting of the anterior tibialis and extensor digitorum brevis muscles bilaterally (4/5 on the Medical Research Council [MRC] scale); strength elsewhere was normal, although slight atrophy of the intrinsic hand muscles was present. Slightly decreased vibratory sensation in the toes (normal at the ankles), with normal temperature and pinprick sensation. Symmetrically brisk reflexes in the arms; pathologically brisk knee jerks with spread (crossed adduction); clonus (8–10 beats) at each ankle. Babinski sign was present bilaterally. Minimally spastic gait. An ophthalmologic consultant noted very mild posterior subcapsular lens opacities, and mild ptosis.

Fig. 3. Pedigree showing haplotypes at the chromosome 6 locus. Box marks homozygous haplotype. Arrows indicate observed recombination events. Microsatellite markers and their physical position in (Mb) are shown to the left (NCBI build 36.3).
3.2.2. Subject IV-4 (at initial evaluation, age 9)

Minimal asymmetric ptosis. Mild weakness of the anterior tibialis (MRC grade, 4/5 bilaterally) and moderate weakness of the extensor digitorum brevis (MRC grade, 3/5 bilaterally), each with mild associated atrophy. Intact sensation of pinprick, vibration, and temperature. Symmetrically brisk reflexes (without pathologic spread) in the arms and at the knees; clonus (3–4 beats) at each ankle. Babinski sign was present bilaterally. Mildly spastic gait.

3.2.3. Subject IV-5 (at initial evaluation, age 6)

Minimal bilateral ptosis. Normal limb strength except for a trace of anterior tibialis muscle weakness bilaterally. Slightly decreased vibratory sensation in the toes (normal at the ankles) with intact pinprick and temperature sensation. Symmetrically brisk reflexes in the arms and at the ankles (without clonus); pathologically brisk knee jerks (with crossed adductor response). Babinski sign absent bilaterally. Gait normal.

3.2.4. Summary

Over the 10 years following our initial evaluation, progression of disease was noted in all affected patients. The proband (patient IV-2) died of respiratory complications at age 21 after progression of scoliosis, dysphagia, and limb weakness, and autopsy was not performed. Patients IV-3 and 4 have developed progressive spasticity, weakness, and distal amyotrophy, as well as speech and swallowing difficulties. Patient IV-3 is unable to walk without...
assistance. Her sister (IV-4) can walk only short distances without assistance. Patient IV-5 is ambulatory, but has difficulty keeping up with peers. He has progression of distal amyotrophy and dysarthric speech.

3.3. Linkage studies

Affected individuals were initially screened for linkage to loci with known associations to juvenile ALS including ALS2, ALS4, and ALS5 and other overlapping neuromuscular phenotypes including CM2A2, CM2B2, and CM2D2, as well as SPG3, SPG4, SPG5, SPG6, SPG7, SPG8, SPG9, SPG10, and SPG11. Markers used to evaluate loci are listed in Supplemental Table 1. Among the four affected siblings, none of the loci examined was homozygous for all eight alleles, thus excluding these loci as causative of motor neuron disease in this consanguineous family.

A subsequent genome-wide scan identified 10 markers that each shared eight identical alleles among the four affected siblings. Microsatellite markers flanking each of these ten homozygous loci (within 0–2 cM) were identified, and genotyping of these additional markers was performed in the six siblings, their parents, and three grandparents. For two of these ten markers – UT1448 (D6S398) on chromosome 6 and UT7582 (D21S1413) on chromosome 21 – close adjacent markers revealed homozygous genotypes for the affected individuals, suggesting a region of homozygosity inherited from a common ancestor. Markers close to the remaining eight loci revealed no evidence for regions of homozygosity.

Screening with 13 additional informative markers in the region of UT1448 on chromosome 6 revealed a 7.6 cM region of homozygosity shared only by the affected individuals. The homozygous haplotype (Fig. 3) defines a locus of 63 Mb flanked by markers D6S1564 and D6S1577. At the UT7582 locus on chromosome 21, genotyping 10 additional informative markers revealed a 6.9 cM (3.9 Mb) region of homozygosity shared by all affected individuals bounded by markers D21S2049 and D21S1895 (Fig. 4). Two-point linkage analysis showed essentially equal probability for a disease-causing locus in either interval with maximum LOD scores of 3.11 (at \( \theta = 0 \)) for each locus (Table 2).

### Table 2

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<th>Marker</th>
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<td>D6S437</td>
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<td>D6S264</td>
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</tr>
<tr>
<td>D21S270</td>
<td>37.75</td>
<td>0.10</td>
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</table>

*Two-point LOD scores with assumption of: disease allele frequency 0.001, 95% penetrance, and a recessive model. Positions are relative physical position of markers per NCBI build 36.3. Markers in bold define the candidate interval.

3.4. Candidate gene screening

We evaluated each candidate region by searching for known or predicted genes using the published genome sequence data (NCBI build 36). We identified 46 known/predicted genes in the candidate interval on chromosome 6. Interesting candidate genes included ESRI, KATNA1, and ZBTB2. ESRI encodes the estrogen receptor and is a particularly interesting candidate because of the presence of gynecomasia in the proband (in the absence of detectable endocrine abnormalities) and by analogy to androgen receptor mutations in X-linked spinal and bulbar muscular atrophy (XSBMA). The mechanisms whereby androgen receptor abnormalities may cause motor neuron death remains obscure [19], but the possibility that estrogen receptor abnormalities may act along a similar pathway is intriguing. KATNA1 encodes the katanin p60 (ATPase containing) subunit A1. Katanin is a microtubule severing protein that has been shown to play a role in neurite outgrowth [20]. ZBTB2 encodes a zinc finger BTB binding protein. A similar gene, abrupt in Drosophila controls specificity of neuromuscular connections [21]. Direct sequencing of the coding exons and splice sites in these three genes revealed no mutations.

Thirty-four known/predicted genes were identified in the candidate interval on chromosome 21, including SOD1, SYN1, ITSNI, and ATP5O. SOD1 is of obvious interest due to its known association with ALS [4,5]. SOD1 encodes superoxide dismutase (Cu-Zn)-1, and maps within the critical interval. All five exons and flanking intronic sequences were evaluated by direct sequencing. No mutations were identified.

SYN1 encodes synaptojanin 1 and is important in clathrin-mediated vesicle fusion at nerve terminals [22,23]. Disruption of this process leads to aberrant synaptic development, and unstable nerve transmission [24,25]. Similarly ITSNI, encoding intersectin 1 modulates vesicle fusion at the synapse by recruiting dynamin to the synaptic endocytic zone [26]. Disruption of the interaction of dynamin/intersectin inhibits vesicle fusion. Furthermore, disruption of the ITSNI homolog Dap160 in drosophila has been shown to disrupt signaling at the neuromuscular junction [27,28]. Complete sequencing of the 32 exons of SYN1 and 40 exons of ITSNI revealed no disease-causing mutations. We identified a 6bp insertion site in exon 32 of the SYN1 gene in affected individuals (dsSNP ref# ss9307959). We screened for this insertion among 90 healthy control individuals. Our results indicated an allele frequency of 0.48 for the allele including the insertion, thus excluding it as a potential disease-causing allele.

ATP5O encodes ATP synthase mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein), and is part of a 16 protein complex important in oxidative phosphorylation. Given the known association of oxidative stress on neurodegeneration [29,30], and particularly the known association of SOD1, another gene important in oxidative metabolism with ALS, we sequenced the seven exons of ATP5O. No mutations were identified.
4. Discussion

4.1. Clinical classification

We describe a novel neurodegenerative syndrome inherited as an autosomal recessive trait. The nosology of this syndrome is arguable, but we have chosen the designation of juvenile recessive ALS for several reasons. Foremost among these are the clinical features with an overwhelming predominance of motor neuron signs. The four affected children each have motor neuron signs clearly referable to both upper and lower motor neurons—the sine qua non of ALS.

Prior to the genetic classification of juvenile recessive ALS, a clinical classification was proposed [10]. Recessive ALS Type 1, the more common form, is characterized by early and prominent involvement of the lower motor neurons, with atrophy and weakness of the hands and feet; the trait linked to the ALS5 locus on chromosome 15 (OMIM #602099) falls in this category [7]. In recessive ALS Type 2, symptoms are confined primarily to the lower limbs, resulting in a syndrome of spastic paraplegia with peroneal atrophy. Recessive ALS Type 3 is associated with early and predominant upper motor neuron signs, with spasticity of limbs, face, and speech, and only rare or mild limb atrophy; a pseudobulbar affect was a prominent feature. The trait linked to the ALS2 locus on chromosome 2 (OMIM #606352) falls in this group [31]. The syndrome we describe is associated with early and predominant upper motor neuron signs, but in contrast to other types of juvenile ALS lacks a pseudobulbar affect and is marked by striking atrophy of both the upper and lower limbs. Therefore we propose that this syndrome represents an additional clinical type of recessive ALS, Type 4. (The El Escorial criteria [32]—used for the diagnosis of sporadic ALS—are not met in the description of childhood recessive forms of ALS, and we do not propose that they are met in this family.)

Because of the predominance of upper motor neuron signs early in the course of disease, a categorization of this syndrome as a hereditary spastic paraplegia (HSP) might be considered. In contrast to HSP, however, each of the affected siblings has developed significant wasting and weakness over time. In this family, the degree of limb atrophy is much greater than that described in most cases of “complicated” HSP with only a few exceptions. A clinical syndrome similar to that which we describe was described in other motor neuron syndromes, including X-SBMA [37], sporadic ALS, and previously reported families with recessive ALS [27].

Some clinical features in the proband—particularly the gynecomastia and bulbar involvement—are reminiscent of those found in X-SBMA, but several features make this syndrome distinct. The first is the autosomal recessive pattern of inheritance, with two girls in the family affected. The second is the young age of onset; X-SBMA is an adult onset disorder, with weakness typically beginning in the third to fifth decade [37]. The third is the pattern of weakness; in X-SBMA, proximal weakness is prominent, and upper motor neuron signs are essentially absent. Finally—and most definitively—the proband did not carry an expanded trinucleotide repeat in the androgen receptor gene, as is seen in X-SBMA.

Although it is tempting to speculate that the proband’s childhood episode of brainstem encephalitis is in some way connected with his subsequent symptoms (and in particular is in some way responsible for his bulbar denervation), we conclude that his childhood illness had no causative relationship to his present condition based upon the similarity between the him and his affected siblings, none of whom had brainstem encephalitis.

In summary, based on the clinical presentation, examination, and nerve conduction studies in this family, we feel that this syndrome is best described as a hereditary motor system disease consistent with a fourth type of juvenile ALS combining a bilateral pyramidal syndrome with limb and bulbar amyotrophy [10].

4.2. Homozygosity screening and genetic mapping

Affected members of this family do not share regions of homozygosity around the previously described loci associated with juvenile recessive forms of ALS (ALS2, ALS5), arguing for a genetically distinct form of motor neuron disease. Currently accepted ALS loci are summarized in Table 3. Homozygous regions are also not found at loci associated with recessive hereditary spastic paraplegia (SPG5, SPG7, and SPG11), or dominantly-inherited axonal forms of CMT (CMT2A, 2B, or 2D).

As noted in Figs. 3 and 4, two distinct regions of homozygosity were defined by a full genome scan. Linkage analysis at each of these loci reveals LOD scores >3.0 for fully informative markers at $= 0.00$, suggestive of linkage—and representing the maximum possible LOD score given this family structure. Clarification of the gene or genes responsible for this novel form of motor neuron disease will be facilitated by the identification of other families affected by the same syndrome.

Our results demonstrate both the strengths and limitations of single-family homozygosity mapping analysis. As with another report which used homozygosity mapping in a single family to map achondroplasia [31], homozygosity screening resulted in the identification of more than one potential locus. We estimate the probability of a false positive linkage result given our family structure and marker density is 16% (see Supplemental data). In spite of the limitations of homozygosity mapping in single families, our single-
family study has identified only two restricted portions of the genome allowing a strategy of candidate gene analysis.

It is possible that this syndrome is not monogenic, and that each of two genes (one at chromosome 6 and one at chromosome 21) must have a mutation in order for manifestation of disease. A digenic mechanism has been described in a number of different disorders including retinitis pigmentosa, Bardet-Biedl syndrome, and Hirschspring’s disease among others [38]. A digenic mechanism might explain the absence of prior reports of the apparently novel syndrome we report, as mutations in two genes would be a very uncommon event in the general population. This possibility will be difficult to assess unless mutational analysis reveals pathogenic mutations in a gene at each locus, or another similarly affected family maps to either or both loci. If mutations in two different genes are required for manifestation of the syndrome we report, then it is possible that an intermediate phenotype, presumably consisting of less severe motor neuron degeneration, may be due to a mutation in a single gene at either locus. However, apart from ALS1, which we have ruled out by sequencing no clinically suggestive syndromes are known to map within or near these regions.

Definition of this novel syndrome may result in the identification of other affected families, leading to further refinement of the locus (or loci). Characterization of the genetic defect may shed light on mechanisms of motor neuron degeneration, particularly as those mechanisms occur in the more common forms of motor neuron disease.

Electronic database information

1. HUGO Gene Nomenclature Committee: www.genenames.org
2. Human Genome Database: www.gdb.org
5. Ensemble Genome Browser: www.ensembl.org
7. GenBank Accession Nos.: SOD1, NM_000454; KATNA1, NM_007044; ESR1, NM_000125; ATP5O, NM_000838; SYNJ1, NM_003895; ITSN1, NM_003024; ZBTB2, NM_020861.

Disclosure

The authors report no conflicts of interest.

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All experiments described comply with current laws of the USA.

Appendix A. Supplementary data


References


