An ITPR1 gene deletion causes SCA15 and 16; a genetic, clinical and radiological description

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2

ITPR1 gene deletion causes SCA15 and 16: a description of the phenotype

Abstract

Objective: To characterize a novel family with non-progressive pure spinocerebellar ataxia (SCA) caused by a deletion in the inositol 1, 4, 5-triphosphate receptor 1 (ITPR1) gene on chromosome 3. This is the first detailed clinical, genetic and radiological description of this genotype.

Background: Deletions in the ITPR1 gene have been shown to cause SCA15 and SCA16 in four families to date. A further Japanese family has been identified with an ITPR1 point mutation. The exact prevalence is unknown as only around 30 families have been screened worldwide.

Methods: The clinical phenotype of the family is described and videotaped clinical examinations presented. Serial brain MRIs were carried out on one affected individual and genetic analysis performed on all family members. Protein analysis confirmed the ITPR1 deletion.

Results: Affected subjects display a remarkably slow pure cerebellar syndrome. Serial MRIs show progressive midline cerebellar atrophy with mild inferior parietal and temporal cortical volume loss. Genetic analysis shows a deletion of 346.487Kb in the ITPR1 gene (the largest ITPR1 deletion reported to date), suggesting SCA15 is due to a loss of ITPR1 function. Western blotting of lymphoblastoid cell line protein confirms reduced ITPR1 protein levels.

Conclusions: SCA15 is a slowly or non-progressive pure cerebellar ataxia which appears to be caused by a loss of ITPR1 function and a reduction in the translated protein. This family highlights the disparity between progressive cerebellar atrophy on MRI and absent clinical progression, and suggests that patients with non-progressive or slowly progressive ataxia should be screened for ITPR1 defects.

Keywords: All clinical neurology [14], Genetic linkage [94], MRI [120], Spinocerebellar ataxia [298], Cerebellum [312]

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Introduction

According to Harding's classification (1), the autosomal dominant spinocerebellar ataxias (ADCAs) can be subdivided clinically into three groups: ADCA type I – ataxia associated with extracerebellar symptoms; type II – ataxia associated with pigmentary retinopathy and type III - pure cerebellar ataxia. ADCAIII, pure spinocerebellar ataxia, is by far the commonest type of ataxia seen (2, 3).

Spinocerebellar ataxia 15 (SCA15) was first described by Storey et al in 2001 (2). They reported an Australian kindred with slowly or non-progressive ataxia, slurred speech, dysphagia, disrupted eye movements and slightly brisk knee jerks. Some affected members of the family also had a tremor. Linkage analysis located the SCA15 locus in an 11.6-cM region on 3p24.2-3pter, a region which was noted to contain the ITPR1 gene. The genetic defects in two Japanese families with ADCAIII were found to overlap this linked region on 3p25.3-26.1 (4). A further Australian family was identified with a similar phenotype of non-progressive ataxia (5) linked to a region of chromosome 3 overlapping the original SCA15 locus; though in this family, affected members also had cognitive impairment.

Mouse models with ITPR1 deletions have a phenotype of ataxia and epilepsy in homozygous animals (6). In 2007, van de Leemput et al (7) identified an ataxic mouse with a small ITPR1 deletion and in light of this, revisited the genetic analysis of the original Australian SCA15 family and two additional UK families with a similar phenotype. A deletion involving both the 5' region

of the ITPR1 gene and the 3' region of the SUMF1 genes was identified in the affected members of all three families.

In addition, the two original and one further Japanese family with a similar phenotype (originally classed as having SCA16) were shown to have ITPR1 defects (8, 9). Of the two original Japanese families, an ITPR1 deletion was seen in affected members of one kindred and an ITPR1 point mutation in the other, confirming the pathogenicity of the ITPR1 gene and conclusively ruling out the role of SUMF1. In 2008, Gardner proposed that SCA15 and SCA16 are in fact the same condition: an ITPR1-associated ataxia (3).

We describe the clinical and genetic findings in a previously unreported UK family with a very slowly progressive pure cerebellar ataxia. All affected subjects have a deletion in the ITPR1 gene. Serial MRI brain scans were carried out to delineate the rate of cerebellar atrophy in comparison to clinical features. Immunoblotting of lymphocytes from one affected individual showed a reduction in the ITPR1 protein, suggesting the disease is due to a loss of ITPR1 function and haploinsufficiency.

Materials and Methods

Subjects. Ethical approval to investigate spinocerebellar ataxias was obtained from the local ION/NHNN ethical committee. Our index subject was identified through the Neurogenetics Clinic at the NHNN and her brother, uncle and two cousins were contacted through her. A family tree, consistent

with autosomal dominant inheritance, is shown (figure 1). All subjects gave a clinical history, underwent a clinical examination including SARA score and gave a blood sample for genetic analysis. The three affected subjects agreed to their clinical examination being videotaped. All subjects gave informed consent for this study.

Genetics. High density genome-wide genotyping was performed by using the Affymetrix SNP6.0 DNA array which has more than 906,600 single nucleotide polymorphisms (SNPs). 500ng of DNA from the proband was analysed according to the manufacturer's instructions by using the Whole Genome Sampling Assay (Kennedy GC 2003). Restriction digestion of 250ng fractions of each DNA sample was performed using NspI and StyI. The digested DNA was then ligated with adaptors, and the adaptor-ligated DNA amplified with a single primer complementary to the adaptor. The polymerase chain reaction (PCR) products were pooled, fragmented and end-labelled using biotinylated nucleotides. The labelled product was then hybridised to a SNP6.0 array, washed and stained in the fluidics station, and then detected with the GCS3000 scanner.

The large ITPR1 deletion was identified from the SNP6.0 array data. This deletion occurred over the SUMF1 gene and the ITPR1 gene, removing exons 1-3 of SUMF1 and exons 1-48 of ITPR1 (figure 2b). To identify the flanking regions of the deletion, we designed primers to PCR and amplify over the deletion in affected individuals. The PCR would not work in unaffected individuals or controls because of the large size of the deletion, so we added in an internal positive control to test for PCR amplification; this control consisted of primers to a known fragment of the BDNF gene (figure 2a). PCR was carried out using 10pmol of both forward and reverse genomic

primers for the SCA15 genomic deletion and the BDNF gene (both primers in the same reaction) and FastStart Taq DNA polymerase (www.roche-applied-science.com). Each purified product was then sequenced using forward or reverse SCA15 genomic primers with Applied Biosystems BigDye terminator v3.1 sequencing chemistry as per the manufacturer's instructions; the resulting reactions were resolved on an ABI3730XL genetic analyzer (Applied Biosystems, Foster city, CA) and analyzed with Sequencher 4.8 software (Gene Codes Corporation, Ann Arbor, MI). The primers that were used were: SCA15 genomic forward 5'-tcaagagatatgtcaccatac-3'; SCA15 reverse 5'-gtccatttaaaccacacac-3'; BDNF forward 5'-aaagaagcaaacatccgaggacaag- 3'; and BDNF reverse 5'-attcctccagcagaaagagaagagg-3'. The BDNF fragment was 276 bp, and the SCA15 fragment amplified over the deletion was 247 bp (figure 2).

Cell culture and preparation. Lymphoblastoid cell lines were created by isolating immature blood lymphoblasts and immortalizing them with Epstein-Barr virus. Lymphoblasts were cultured in a T75 flask in RPMI-1640 media (Sigma) containing 15% Fetal Bovine Serum (GIBCO) and 2mM L-Glutamine (GIBCO), and were fed every two days. After collection, the cells were pelleted by being centrifuged at 200g. The pellet was then resuspended in PBS (Sigma) containing protease inhibitor cocktail (Roche), then centrifuged again. The supernatant was discarded and the pelleted cells were stored at -80°C until further use.

Western blot. The cells were lysed in 50mM Tris (pH 8.0) containing 150mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate and protease inhibitors. Benzonase was added (50U/ml final concentration) and the cells were incubated on ice for fifteen minutes. Protein concentrations were

determined by BCA and adjusted to 1mg/ml. Gel samples were prepared by the addition of 5X sample buffer (0.625M Tris-HCl, pH 6.8, 50% glycerol, 10% (w/v) SDS, a trace of bromophenol blue and 10% β-mercaptoethanol) and heating at 95°C for five minutes. The proteins were then separated on 6% SDS gels and transferred to PVDF. Membranes were blocked with PBS/0.1% Tween 20 (PBS-T) containing 10% dried milk powder for 30 minutes, washed briefly in PBS-T and then incubated overnight at 4°C on a shaker with primary antibody diluted in PBS-T. This solution contained 1% BSA for anti-ITPR1, (Chemicon anti-IP3 receptor 1, diluted 1:2000) or 1% dried milk powder for anti-βactin (clone AC15, Sigma diluted 1:10,000). The membranes were washed five times in PBS-T then incubated at room temperature for 60 minutes with secondary antibody HRP conjugates diluted in PBS-T which contained 1% dried milk powder. They were then washed in PBS-T a further five times and developed with ECL reagents.

Imaging. A 3T MRI scan (Siemans TIM Trio) was performed on one affected individual (subject 2) and T1 weighted images were obtained (figure 3b). These images were compared with a 1.5T MRI scan (GE Sigma Horizon LX Echospeed Plus) obtained five years earlier (figure 3a).

Results

Clinical characteristics: case histories of affected subjects

Subject 1 (proband) is 40 years old. She attained normal motor milestones, but describes herself as having been clumsy and bad at sport at school. During her 20s, she developed mild unsteadiness when walking. This has slowly progressed since, but she remains independently ambulant. She has mild

slurring of her speech, which is more pronounced when she is tired. She intermittently notices that her eye movements are jerky, and, again, this is more marked when she is tired.

Subject 2 is 38 years old. He also attained normal motor milestones but was poor at sports at school, with particular problems balancing when trying to do gymnastics. He notes that he has been prone to falling over for as long as he can remember. His balance deteriorated from his teens, and during his late 20s, he noticed that his speech became slurred when he was tired. In his early 30s, his limb coordination and eye movements became jerky and he began to experience intermittent involuntary limb jerking (myoclonus). Around this time, he had an acute episode of hearing loss, vertigo, nausea and vomiting which was diagnosed as viral labyrinthitis. The acute symptoms resolved, but his balance deteriorated and never returned to its former level. He currently has mild gait ataxia.

Subject 3 is 56 years old. Since his teens, he was aware that he was unable to carry drinks from the bar without spilling them. He attributed this to difficulties with both walking and coordinating his hands. His brother was diagnosed with ataxia during subject 3's mid-30s, prompting him to try some clinical assessment tests. Rapid finger tapping and heel-shin coordination were impaired. He became more aware of his symptoms when his wife died when he was 38 years old, and his ataxic gait became more noticeable. He has never noticed his dysarthria himself, but other people have commented on it since his early 50s. He has a slightly slow, broad-based gait, but is able to walk for unlimited distances. He has minimal functional impairment: he works as a carer, though he avoids lifting clients as he is aware that his balance is impaired.

ITPR1 gene deletion causes SCA15 and 16: a description of the phenotype

Clinical characteristics: clinical examination. All three affected subjects show signs consistent with a cerebellar syndrome; namely ataxia, cerebellar dysarthria and abnormal eye movements. These findings are detailed in table 1. (SARA scores are given in supplement 2). In addition subject 3 had hyperreflexia. Subjects had full power in their limbs and normal sensation. There was no evidence of retinal disease and no cognitive dysfunction.

Subjects 4 (male; 27 years old) and 5 (male; 22 years old) were asymptomatic and had normal clinical examinations.

Imaging. The initial 1.5T MRI of subject 2 (figure 3a) showed midline superior and inferior cerebellar atrophy and mild posterior parietal and temporal volume loss. Brainstem structures were preserved. The follow-up 3T MRI (figure 3b) showed progression of the midline cerebellar atrophy, with the atrophy more marked superiorly than inferiorally. The mild cortical volume loss had not progressed.

Genetics. A large deletion (346.487Kb) including exons 1-3 of the SUMF1 and exons 1-48 of the ITPR1 gene in the 5' region of ITPR1 was identified in all affected individuals (figure 2b). This ITPR1 deletion is the largest reported to date, and segregates with pure ataxia in this ADCAIII family. It is not present in 100 UK controls. The location of the deletion, removing the first 48 exons, suggests that the function of the ITPR1 gene will be lost in the mutated allele. Analysis of lymphoblastoid derived protein on a western blot indicates that the ITPR1 translated protein is reduced compared with a control (figure 4), confirming the postulated genetic mechanism of loss of function and haploinsufficiency.

ITPR1 gene deletion causes SCA15 and 16: a description of the phenotype

Discussion

These results illustrate the clinical, radiological and genetic features of the SCA15 phenotype. Genetic analysis confirmed a deletion in the ITPR1 gene in all affected subjects; this segregated with the disease and was absent in all clinically unaffected individuals.

Affected members of this family display a slowly progressive, or non-progressive, relatively pure cerebellar syndrome, with the onset of symptoms in early adulthood. This pattern is in keeping with previous reports of SCA15 (2, 7). Hyperreflexia, as seen in subject 3 (our subject with the longest duration of symptoms), was also described in some members of the original Australian SCA15 family (2) and of the Japanese SCA15 (4) and SCA16 (10) families . Normal life spans have been achieved by most deceased symptomatic members of our family in the past, strengthening the current consensus that SCA15 does not shorten life expectancy. Serial MRI brain scans have not previously been reported in SCA15 patients, and it is notable that the progression in cerebellar atrophy seen on subject 2's MRI was visible over a five year period during which no clinical progression was detected by patient or clinician. This finding may prove relevent in the development of biomarkers for disease-modifying drug trials in the future. The mild volume loss seen in the parietal and temporal lobes is also interesting; cortical atrophy has not been observed in previous SCA15 imaging.

As expected, the almost complete deletion of the ITPR1 gene leads to a loss of mRNA transcription and reduced ITPR1 protein (seen on western blotting). The main function of the ITPR1 gene is to mediate the release of intracellular stores of calcium from the endoplasmic reticulum by playing a key role in the inositol messenger pathway. The ITPR1 gene is highly expressed in the cerebellum

in Purkinje cells and other neurons. The slow degeneration of these cells may therefore be due to a particular sensitivity to calcium homeostasis. The inositol triphosphate (IP3) pathway is also critical to calcium homeostasis, and this is reflected in the six spontaneously ataxic mouse models of genes involved in this cascade that have been identified to date. Mutant mice in this and associated pathways include Grm1 (11), Gnaq (12), Plcb4 (13), Itpr1 (6), Car8 (14), and Inpp4 (15).

Overall, our data extends the phenotype of ITPR1-associated ataxia, or SCA15, and confirms that ITPR1 deletion leads to loss of function in the translated protein. The phenotype and potential frequency of ITPR1 genetic defects suggests that this genetic test should be a routine DNA screen available in all neurogenetic clinics.

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Figure Legends

Figure 1 Family tree showing autosomal dominant pattern of inheritance.

Figure 2a PCR across the deleted region. The positive control primers (BDNF; 276bp, upper band) amplify in each reaction in all cases. The SCA15 primers (247bp, lower band) only amplify when the large SCA15 deletion is present. In this assay, therefore, affected subjects have two bands and unaffected subjects have just one.

Figure 2b Sequencing across the deleted region reveals the size of the deletion.

Figure 3a Initial MRI scan of subject 2. showed midline superior and inferior cerebellar atrophy and mild posterior parietal and temporal volume loss. Brainstem structures were preserved. The follow-up 3T MRI (figure 3b) showed progression of the midline cerebellar atrophy, with the atrophy more marked superiorly than inferiorally. The mild cortical volume loss had not progressed.

Figure 3b Follow-up MRI scan of subject 2 (carried out five years after the initial scan).

Figure 4 Western blot showing reduced levels of ITPR1-translated protein in SCA15 subjects in comparision to controls.

Table 1 Clinical examination of the three affected subjects

	Subject 1	Subject 2	Subject 3
Age (years)	40	38	56
Sex	Female	Male	Male
Gait and stance	Mild impairment of tandem walking; unable to stand in tandem position.	Mild impairment of tandem walking; unable to stand in tandem position.	Slowed walking with mild broad-based gait; marked impairment of tandem walking. Unsteady when standing with feet together.
Sitting	Normal	Normal	Normal
Speech disturbance	Minimal dysarthria	Minimal dysarthria	Minimal dysarthria
Finger-nose coordination	Low amplitude tremor only	Mild dysmetria with low amplitude tremor	Mild dysmetria with low amplitude tremor
Fast alternating hand movements	Normal	Normal	Slightly irregular
Heel-shin coordination	Slight incoordination	Slight incoordination	Slight incoordination
Eye movements - pursuit	Mildly jerky pursuit with one-two beats fine nystagmus in all directions.	Smooth pursuit with one-two beats nystagmus in all directions.	Jerky horizontal pursuit with one-two beats nystagmus to left
Eye movements - saccades	Hypermetria	Jerky horizontal saccades	Slightly slow initiation. Marked hypermetria
Reflexes	Normal	Normal	Hyperreflexia
Tone	Normal	Normal	Increased

1

2

ITPR1 gene deletion causes SCA15 and 16: a description of the phenotype