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Sequencing analysis of the *ITPR1* gene in a pure autosomal dominant spinocerebellar ataxia series

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Abstract

Spinocerebellar ataxia type 15 and 16 (SCA15/16) are autosomal dominant cerebellar ataxias that are slowly progressive with a predominantly pure ataxia phenotype (ADCA III). The locus for SCA15 was first mapped to 3p24.2–3pter and subsequently full or partial deletions in the *inositol 1,4,5-triphosphate receptor type 1* (*ITPR1*) gene were identified in several ADCA III families that segregated with the disease. A single missense coding variant has been described, but the pathogenicity of this change has not been proven. We sequenced the entire coding region and flanking regions of *ITPR1* in unrelated ADCA III families (*n*=38) that were negative for large deletions on whole genome arrays, and for which SCAs 1, 2, 3, 6, 7, 8, 11, 12, 14, 17 and the Friedreich's ataxia expansion were excluded in all probands. Mutation at SCA5, 10 and 27 was also excluded in some families. A number of coding and non-coding polymorphisms were identified but no *ITPR1* mutations were found. The results indicate that point mutations in *ITPR1* are at best a rare cause of ADCA III.

Keywords

ataxia; spinocerebellar; SCA15; genetics; inositol-1,4,5-triphosphate receptor type 1

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INTRODUCTION

Autosomal dominant spinocerebellar ataxias (ADCAs) are a group of clinically and genetically heterogeneous neurodegenerative diseases, characterized by progressive cerebellar ataxia of gait and limbs, variably associated with ophthalmoplegia, pyramidal and extrapyramidal signs, dementia, pigmentary and peripheral neuropathy. Disease onset is usually between 30 and 50 years of age.1 The prognosis is variable depending on the underlying cause of the ADCA subtype.2 ADCA type III includes relatively pure cerebellar ataxias where the degenerative process is limited to the cerebellum, although some affected individuals may also manifest mild pyramidal signs.

SCA15, first described in an Australian kindred, is a slow-progressive ADCA III.3 Age of onset ranges from mid-childhood to middle age. The phenotype is characterized by a mild degree of gait ataxia, a pattern of gaze-evoked nystagmus with and without rebound, brisk lower limb reflexes, and head tremor.4 We previously described the identification of deletion in the gene encoding inositol 1,4,5-triphosphate receptor type 1 (*ITPR1*) as the genetic cause of SCA15.5 To date hereditary ataxia in six families, five with SCA15 and one with SCA16, have been ascribed to mutations in *ITPR1* (thus SCA16 is really SCA15 6); five caused by full-length or partial deletions in *ITPR1* with or without partial deletion in *SUMF1*, and one possibly pathogenic missense mutation in *ITPR1*.5‚7‚8 The *ITPR1* gene has so far not been sequenced in a series of ADCA cases as the identification of deletions is recent and the gene is very large at 58 exons. We undertook a classical sequencing approach in a cohort of ADCA III cases to search for distinct *ITPR1* mutations causing this ataxic disorder.

SUBJECTS AND METHODS

Subjects

Probands from 38 unrelated families with an inherited autosomal dominant cerebellar ataxia, ascertained through neurology clinics in the UK, were analyzed in the present study. The UK population is diverse but families were mainly English origin, with three families originally from Vietnam, Spain and Brazil. Clinically all patients had an adult onset spinocerebellar ataxia (range 18 to 52 years), cerebellar atrophy was present on CT or MRI scanning. In two families there were additional cognitive signs, two families had additional myoclonus one family had dystonic posturing the upper limbs. SCAs 1, 2, 3, 6, 7, 8, 11, 12, 14, 17 and the Friedreich's ataxia expansion was excluded in all probands. SCA10 was excluded in the Brazilian family; exons known to harbor SCA5 and SCA27 causing mutations were excluded by sequencing. Ethical approval was obtained through the National Hospital for Neurology and Neurosurgery ethics committee. Patients were diagnosed at the National Hospital for Neurology and Neurosurgery based on clinical examination by a neurologist. Informed consent was given by all participants. Whole blood was collected in EDTA blood collection tubes. Genomic DNA was isolated using phenol/chloroform extraction.

ITPR1 sequence analysis

Large structural changes such as deletions, insertions and duplications in the *ITPR1* gene were excluded in all families by analysis on whole genome arrays (Infinium HumanHap550 beadchip, Illumina). Screening of the 58 coding exons and at least 30bp of the flanking introns of *ITPR1* was carried out in 38 families with autosomal dominant cerebellar ataxia. Polymerase chain reaction (PCR) amplification was performed in a final volume of 12μl containing 10ng genomic DNA, 10pmol transcript-specific forward and reverse primers (58 primer pairs were used; sequences available upon request) and 10μl FastStart PCR Master

mix (Roche). Primers for amplification were designed using the online tool ExonPrimer (http://ihg.gsf.de/ihg/ExonPrimer.html). Each purified product was sequenced with forward or reverse primers using BigDye-terminator version 3.1 (Applied Biosystems). Purified sequence reactions were run on an ABI3730XP automated sequencer as per the manufacturer's instructions (Applied Biosystems). Sequence data were analyzed using Sequencher software version 4.1.4 (Gene Codes Corporation). All changes that deviated from the reference sequence (*ITPR1* RefSeq NM_001099952.1; GenBank) were verified by PCR amplification using a fresh DNA aliquot to obtain sequence data in both forward and reverse directions.

RESULTS

A classical sequencing approach was undertaken in a cohort of 38 unrelated families with ADCA III in search for distinct *ITPR1* mutations. Sanger-based sequence analysis of the 58 coding exons and an additional 30bp of each flanking region to include splice sites identified several known polymorphisms, as well as an unknown synonymous variation in exon 26 and unknown variants in the region 5′ of exon 38 and 5′ of exon 44, and the region 3′ of exon 42 (table 1). The nucleotide changes found in exon 26 and 5′ of exon 38 were subsequently found in neurologically normal controls (NDPT020 and NDPT023 respectively; Coriell Cell Repositories, http://ccr.coriell.org). The variations identified in the regions flanking exon 42 and 44 of SCA patients were not found in over 400 neurologically normal controls (exon 42, n=415; exon 44, n=409; NDPT019, NDPT020, NDPT022, NDPT023, NDPT024; Coriell Cell Repositories, http://ccr.coriell.org). More controls are needed to be conclusive, however given the location of the variations, in the flanking regions 12bp and 18bp away from the exon, they are unlikely to be disease causing.

DISCUSSION

The initial reports of DNA array analysis on unrelated families with ADCA suggest that deletions in the *ITPR1* gene are a frequent cause of pure inherited spinocerebellar ataxia (unpublished data).5,7,8 In this study, sequence analysis of *ITPR1* in an ADCA III cohort did not lead to identification of distinct *ITPR1* mutations. To date, spinocerebellar ataxia in only one family has been ascribed to a missense mutation in *ITPR1*, a C8581→T variation resulting in substitution of leucine for proline (P1059L).8 However, pathogenicity of this change should be considered unproved until further characterization of the mutation or additional families have been identified. Taken together, these data suggest that small structural and point mutations in *ITPR1* are at best a rare cause of ADCA III. Further analysis, increased sample sizes and study of different ethnic groups as well as other types of ataxia would aid in elucidating the pathogenic role of *ITPR1* in spinocerebellar ataxia.

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Table 1

ITPR1 variants identified in ADCA III cohort using the ABI prism 3730 genetic analyser platform.

nucleotide	exon	rs.no.	variant	sample freq.	population freq.
c.2574G>A	20	rs41289636	p.A742A	GA_1, GG_{37}	n/a
c.3006A>C	22	rs58408221	p.L886L	AA_{27}, CA_9, CC_2	n/a
c.3528C>T	26	n/a	p.T1060T	CT_1, CC_{37}	n/a
c.4944C>T	35	rs34748547	p.S1532S	CT_1, CC_{37}	0.028 ± 0.115
c.5469C>T	39	rs61757111	p.N1707N	CT_1, CC_{37}	n/a
c.6315**C**>T	43	rs6442905	p.N1989N	CT_2, CC_{36}	0.031 ± 0.120
c.6900A>G	48	rs34491089	p.K2184K	AG_1, AA_{37}	0.025 ± 0.110
c.6921**A**>G	48	rs13079522	p.T2191T	AA_{22}, AG_{12}, GG_4	0.350 ± 0.229
c.7258C>**T**	50	rs57511443	p.L2304L	CC_{18}, CT_{16}, TT_4	n/a
c.7839C>T	54	rs60021678	p.T2497T	CC_{26}, CT_5, TT_7	n/a
c.7893A>**G**	54	rs901854	p.K2515K	AA_{18}, AG_{12}, GG_8	0.469 ± 0.120
	exon flanking				
g.4687413A>**G**	19[−1]	rs2306875	p.R669R	AA_{16}, AG_{19}, GG_3	0.496 ± 0.044
g.4700239G>A	26[+4]	rs2306878	n/a	GA_1, GG_{37}	n/a
n/a, C>T	38[−21]	n/a	n/a	CT_1, CC_{37}	n/a
n/a, G>A	42[+18]	n/a	n/a	GA_1, GG_{37}	n/a
n/a, G>T	44[−12]	n/a	n/a	GT_1, GG_{37}	n/a

Data based on *ITPR1* RefSeq NM_001099952.1, NP_001093422, obtained from GenBank. Detailed information on genetic variants from NCBI dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP), including ancestral allele, rs-number, variant found at protein level and population frequency. Nucleotide, in bold signifies ancestral allele (if in dbSNP); rs.no, reference number; sample frequency, genotype with in subscript the number of samples in which genotype found; population freq, average population frequency heterozygote ± standard error; c, cDNA; p, protein; g, genomic DNA.