Development of a Multiplex Ligation-Dependent Probe Amplification Assay for Diagnosis and Estimation of the Frequency of Spinocerebellar Ataxia Type 15

Devika Ganesamoorthy,1 Damien L. Bruno,1 Jacqueline Schoumans,2 Elsdon Storey,3 Martin B. Delatycki,4 Danqing Zhu,5 Morgan K. Wei,5 Garth A. Nicholson,5 R.J. McKinlay Gardner,4 and Howard R. Slater1,4,*

1 Victorian Clinical Genetic Services and Murdoch Children’s Research Institute, University of Melbourne, Department of Paediatrics, Royal Children’s Hospital, Parkville, Victoria, Australia; 2 Department of Molecular Medicine and Surgery, Karolinska Institute, Karolinska University Hospital Solna, Stockholm, Sweden; 3 Department of Medicine, Alfred Hospital, Monash University, Melbourne, Australia; 4 Genetic Health Services Victoria, Melbourne, Australia; 5 ANZAC Research Institute, University of Sydney, Department of Medicine, Concord Hospital, Sydney, Australia; * address correspondence to this author at: VCGS Cytogenetics Laboratory, Royal Children’s Hospital, Parkville VIC 3052, Australia. Fax +61-3-83416366; e-mail howard.slater@ghsv.org.au.

BACKGROUND: Spinocerebellar ataxia type 15 (SCA15) is a slowly progressive neurodegenerative disorder characterized by cerebellar ataxia. Mutation of the ITPR1 gene (inositol 1,4,5-triphosphate receptor, type 1) has been identified recently as the underlying cause, and in most cases the molecular defect is a multiexon deletion. To date, 5 different SCA15 families have been identified with ITPR1 gene deletion.

METHODS: We have designed a synthetic, dual-color multiplex ligation-dependent probe amplification (MLPA) assay that measures copy number with high precision in selected exons across the entire length of ITPR1 and the proximal region of the neighboring gene, SUMF1 (sulfatase modifying factor 1). We screened 189 idiopathic ataxic patients with this MLPA assay.

RESULTS: We identified ITPR1 deletion of exons 1–10 in the previously reported AUS1 family (4 members) and deletion of exons 1–38 in a new family (2 members). In addition to the multiexon deletions, apparent single-exon deletions identified in 2 other patients were subsequently shown to be due to single-nucleotide changes at the ligation sites.

CONCLUSIONS: The frequency of ITPR1 deletions is 2.7% in known familial cases. This finding suggests that SCA15 is one of the “less common” SCAs. Although the deletions in the 5 families identified worldwide thus far have been of differing sizes, all share deletion of exons 1–10. This region may be important, both in terms of the underlying pathogenetic mechanism and as a pragmatic target for an accurate, robust, and cost-effective diagnostic analysis.

The spinocerebellar ataxias (SCAs) are a heterogeneous group of clinically and genetically distinct disorders characterized by progressive cerebellar ataxia. Currently, there are 28 different SCA loci, listed as SCA1 through to SCA30 (SCA9 and SCA24 being unassigned). The SCAs may be classified according to their frequency, i.e., the more common SCAs (e.g., SCAs 1–3), the less common SCAs (e.g., SCAs 5, 7, 10), and the “private” SCAs, seen only in a single kindred (e.g., SCAs 20, 30) (1).

Spinocerebellar ataxia type 15 (SCA15) is a slowly progressive neurodegenerative disorder characterized by pure cerebellar ataxia. The condition was first described in an Australian family (AUS1) (2), and after mapping of the disease locus to 3p26.2 (3), the gene itself was identified as ITPR1 (inositol 1,4,5-triphosphate receptor, type 1) (4). In the original AUS1 family, the causative mutation consisted of a deletion of exons 1–10 plus deletion of sequence extending into the 5’ region of the adjacent gene, SUMF1 (sulfatase modifying factor 1). ITPR1 deletions of different sizes were found in 4 other SCA15 kindreds, 2 from England (4) and 2 from Japan (5, 6). Another Japanese family with a SCA15 phenotype presented a heterozygous nonconservative missense mutation in ITPR1, and the investigators proposed that this mutation was likely causative of the disease (6). Mutation in ITPR1 is a biologically plausible cause of ataxia in that its protein product, a receptor for the intracellular messenger inositol 1,4,5-triphosphate, is highly produced by Purkinje cells (4, 6). In addition, homozygous partial deletion of ITPR1 causes ataxia in mice (4). The family described by Iwaki et al. (5) was instructive in that the deletion involved only the ITPR1 gene, the SUMF1 gene being intact, thus suggesting that ITPR1 was in fact the SCA15 gene.

The identification of 5 new SCA15 kindreds over a short period of time and from 2 different ethnic groups indicates at the least that SCA15 is not a “private”

6 Nonstandard abbreviations: SCA, spinocerebellar ataxia; SCA15, SCA type 15; MLPA, multiplex ligation-dependent probe amplification; RPH, relative peak height.

2 Human genes: ITPR1, inositol 1,4,5-triphosphate receptor, type 1; SUMF1, sulfatase modifying factor 1.
ataxia. Therefore, it is of interest to learn how common the condition might be. Furthermore, it is apparent that adding SCA15 testing to the armamentarium of routinely testable SCA genes could be useful once the recognized common SCAs have been excluded. Facilitating such testing requires an accurate, robust, and cost-effective assay. We chose to develop a test based on multiplex ligation-dependent probe amplification (MLPA) (7). Because commercial probes are manufactured by cloning with M13 vectors, a time-consuming process, we took the approach of designing synthetic MLPA probes of chemically synthesized oligonucleotides (8). To accommodate the desired number of targets, we increased the multiplexing scale within the assay via the use of 2-color MLPA (9).

Materials and Methods

Patient Selection

Blood samples were obtained from cerebellar ataxia patients at the neurogenetic clinics in Melbourne, Sydney, and Canberra, Australia. The samples were collected between 1990 and 2007 for testing for the SCA genes available at the time. Storey et al. (10) describes the patient population to 1999 with respect to testing for SCAs 1, 2, 3, 6, and 7. DNA preparations were made, and DNA aliquots from 189 samples were stored in the laboratories of the VCGS Pathology, Melbourne, and the Department of Molecular Medicine at the Concord Hospital, Sydney. Clinical and pedigree information was comprehensive for some patients, and 93 patients from 73 kindreds were known to have a familial ataxia; others were described simply as “ataxic,” with no reference to family history.

Assay

Synthetic MLPA probes were designed to unique targets (9) for selected exons and introns in ITPR1 and SUMF1. ITPR1 exons were selected after taking into account the previous reports of deletion breakpoints. The primary screening probe set included the first 10 ITPR1 exons and then approximately every fifth exon or intron of the ITPR1 gene. Additional MLPA probes, including probes targeting the first 4 exons of SUMF1, were used to determine the breakpoints of the detected deletions more accurately. Details of the probes, reaction conditions, and data analysis, are given in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol55/issue7.

Results

Deletions in ITPR1 or SUMF1 were found in 6 of the 189 individual patient samples. Four of the samples, part of our original collection of samples from idiopathic ataxia patients, were from the previously described AUS1 family (4). ITPR1 exons 1–10 and SUMF1 exons 1–3 were deleted in all members of the AUS1 family tested. A different deletion was found in 2 members of a second family (AUS2). The primary exon screen showed that at least exons 1–35 of ITPR1 were deleted (Fig. 1A). Because the targeted adjacent probe for ITPR1 intron 38 was not deleted, we designed an auxiliary probe set to locate the breakpoint more accurately and found exons 36–38 to be deleted. This set also targets SUMF1; therefore, we mapped the breakpoints to SUMF1 intron 1 and ITPR1 exon 38 (Fig. 1B). No polymorphisms occurred within the probe binding sites at these breakpoints (sequencing data not shown).

The mean relative peak height (RPH) for measurements of all 24 exons in 279 tests of nonpathologic samples was 1.01 (SD, 0.09). The mean (SD) RPH was 0.52 (0.08) for the 10 deleted exons assayed in 18 tests of AUS1 family samples and 0.51 (0.07) for the 16 deleted exons in 7 tests of samples from the AUS2 family (Fig. 1A). The RPHs for assays of deleted exons were highly significantly different from the RPHs of assays of undelated exons, within or between patients (P < 0.001, 1-tailed t-test).

There appeared to be deletions of a single exon (exon 15 and exon 35) in 2 of the other tested patient samples. Sequencing revealed a synonymous change (c.4545C>T; G1515G) in exon 35 in one case and a nonsynonymous change (c.1480G>A; V494I, rs41289628) in exon 15 in the other; both were within the probe-ligation sites (data not shown).

Discussion

Deletions in ITPR1 have previously been reported in 5 different families: 1 Australian family (AUS1) with deletion of exons 1–10, 2 British families with deletion of exons 1–40 and of exons 1–44 (4), and 2 Japanese families with deletion of exons 1–48 (5) and of exons 1–58 (6). Among the 189 samples tested, we identified a new deletion encompassing ITPR1 exons 1–38 and SUMF1 intron 1 in 2 related individuals (the AUS2 family). The proximal and distal breakpoints in this new deletion are distinctive and add to the known variation documented for the previously reported cases (Fig. 1C). Notably, all of the reported deletions include exons 1–10, which may represent a minimal critical region of overlap for SCA15.

The AUS2 family has had affected individuals in at least 3 generations. The mutation has caused very slowly progressive, relatively pure cerebellar ataxia. The affected individuals were all poor at sports in childhood and noticed definite symptoms from their 30s or 40s. The eldest was in her 80s and still ambulatory,
albeit with a walking frame. The 2 affected family members, who have been examined by one of the authors (E.S.), both had down-beating nystagmus and hypermetric saccades.

The 2 apparent single-exon deletions found within the test sample are almost certainly artifacts caused by single-nucleotide polymorphism interference with probe binding, a conclusion supported by DNA sequencing of the relevant exons, i.e., exon 15 in one case and exon 35 in the other. The heterozygous $ITPR1$ missense mutation reported in one Japanese SCA family (6) was the first case of a point mutation to be recognized; it is located within a modulatory and transducing domain of the protein and has been proposed to be pathogenic. The missense mutation (c.1480G>A, V494I) detected in the present study is located within the highly conserved binding domain of this receptor protein and therefore may also be pathogenic. Although nothing more is currently known about pathogenic point mutations in $ITPR1$, such mutations need to be considered as an alternative etiology.

Given the relatively small size of the test sample ($n = 189$) and the lack of precise patient-selection criteria, only an approximate estimate of the incidence of SCA15 caused by $ITPR1$ deletion can be given. Of the 189 samples tested, 93 originated from 73 kindreds with documented familial ataxia other than SCAs 1, 2, 3, 6, and 7. Two deletions were found within this group.

Fig. 1. Analysis of dosage of $ITPR1$ exons with MLPA.
(A), Top panel, dosage measurement of $ITPR1$ exons with MLPA (mean ± 2 SD) in 183 nonpathologic samples ($n = 279$); middle panel, mutation in the AUS1 family [van de Leemput et al. (4)] with a known deletion of $ITPR1$ exons 1–10 ($n = 18$); bottom panel, the novel deletion in the AUS2 family involves at least $ITPR1$ exons 1–35 ($n = 7$). The data for each are pooled to show the variation (mean ± 2 SD) in dosage measurement. (B), Assay of additional probes in $ITPR1$ and SUMF1 introns and exons. The deletion breakpoint in the AUS2 family (cases 5 and 6) was mapped to SUMF1 intron 1 to $ITPR1$ exon 38. The proximal breakpoint of the AUS1 family (case 2) was mapped to SUMF1 exon 3. Ctrl, control. (C), Comparison of reported deletions in SUMF1/$ITPR1$ showing that the minimal region of overlap consists of $ITPR1$ exons 1–10.
(families AUS1 and AUS2) to yield a frequency per kindred of 2.7%. No deletions were found among the other 96 “apparently” sporadic cases of ataxia, some of which, however, may be unrecognized familial cases. This supposition allows a preliminary conclusion that SCA15 is probably one of the “less common” SCAs.

Given how little we know currently of the incidence of SCA15 among patients with idiopathic ataxia and the efficiency of the test, testing could be targeted not only to patients presenting a “pure” ataxia and manifesting a slow rate of progression and a relatively mild disease pattern, but also more broadly to any patient with slowly progressive ataxia for whom “standard” SCA testing has returned normal results.

The dual-color synthetic MLPA method we have reported is accurate and robust. This assay displays the same high level of precision in discriminating copy number as previously reported for other tests (11, 12). Deletion-specific PCR (5) and microarrays (4, 6) have been used to detect the other known ITPR1 deletions. Both techniques have the required multiplexing capacity to measure the variation in deletion size, but MLPA has the advantage of a higher sample throughput. We have spanned the region of deletion by targeting more than half of the exons in the ITPR1 gene and the first 4 exons in the SUMF1 gene. This coverage can be easily increased by designing additional probes within the region. If the minimal region of overlap from ITPR1 exons 1–10 is confirmed when additional positive cases are found, testing might be directed to this specific region alone. This change would further enhance the potential of the assay as a simpler and cost-effective test for SCA15.

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References


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