ELSEVIER

Journal of the Neurological Sciences 268 (2008) 131 – 135

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Screening of hereditary spastic paraplegia patients for alterations at *NIPA1* mutational hotspots

Christian Beetz [a,*], Rebecca Schüle [b], Stephan Klebe [c], Sven Klimpe [d], Thomas Klopstock [e], Arnaud Lacour [f], Susanne Otto [g], Anne-Dorte Sperfeld [h], Bart van de Warrenburg [i], Ludger Schöls [b], Thomas Deufel [a]

[a] *Institut für Klinische Chemie und Laboratoriumsdiagnostik, Universitätsklinikum, 07747 Jena, Germany*
[b] *Neurologische Klinik und Hertie-Institut für Klinische Hirnforschung, Universität Tübingen, Germany*
[c] *INSERM, UMR679, Federative Institute for Neuroscience Research, Pitié-Salpêtrière Hospital Paris, France*
[d] *Klinik und Poliklinik für Neurologie, Johannes-Gutenberg-Universität Mainz, Germany*
[e] *Neurologische Klinik am Klinikum Großhadern, Ludwig-Maximilians-Universität München, Germany*
[f] *Department of Neurology, Roger Salengro Hospital Lille, France*
[g] *Klinik für Neurologie, Ruhr-Universität Bochum, Germany*
[h] *Neurologische Klinik und Poliklinik, Universität Ulm, Germany*
[i] *Department of Neurology, Radboud University Medical Centre Nijmegen, The Netherlands*

Received 25 September 2007; received in revised form 23 November 2007; accepted 27 November 2007
Available online 14 January 2008

Abstract

Mutations in *NIPA1* cause hereditary spastic paraplegia type 6 (*SPG6* HSP). Sequencing of the whole gene has revealed alterations of either of two nucleotides in eight of nine *SPG6* HSP families reported to date. By analysing CpG methylation, we provide a mechanistic explanation for a mutational hotspot to underlie frequent alteration of one of these nucleotides. We also developed PCR RFLP assays to detect recurrent *NIPA1* changes and screened 101 independent HSP patients, including 45 index patients of autosomal dominant HSP families. Our negative finding in this cohort for which several other causes of HSP had been excluded suggests *NIPA1* alterations at mutational hotspots to be less frequent than previously thought. Nevertheless, the assays introduced represent a valid pre-screen easily implementable in the molecular diagnosis of HSP.
© 2007 Elsevier B.V. All rights reserved.

Keywords: CpG methylation; Hereditary spastic paraplegia; Mutational hotspot; *NIPA1*; *SPG6*

1. Introduction

The hereditary spastic paraplegias (HSPs) are a group of movement disorders caused by degeneration of motoneuronal axons in the spinal cord. The major and unifying clinical feature is progressive weakness and spasticity of the lower limbs. Presence of complicating symptoms, mode of inheritance, and age at onset vary widely. This heterogeneity is, in part, explained by the existence of >30 spastic paraplegia (SPG) loci [1]. The frequency of most of these genetic forms is assumed to be small but large scale screens are often lacking.

SPG6 HSP is caused by mutations in *NIPA1* [2,3]. A total of nine *SPG6* HSP families have been identified to date by sequencing of the whole gene; four of these had previously been linked to the locus [3–5] while the remaining five were amongst a total of 207 unlinked pedigrees or single patients screened [3,6–9]. All *NIPA1* mutations are associated with

* Corresponding author. Tel.: +49 3641 9325933; fax: +49 3641 9325937.
E-mail address: christian.beetz@med.uni-jena.de (C. Beetz).

0022-510X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2007.11.015

autosomal dominant (AD) inheritance of a pure phenotype; age at onset ranges from 5 to 40 years. The nine families carry four different changes affecting three *NIPA1* nucleotides: c.134C>G (2×), c.298G>A (1×), c.316G>A (4×), and c.316G>C (2×). The recurrent alterations of nucleotides 134 and 316, responsible for 88% (8 out of 9) of known cases of *SPG6* HSP, were found in ethnically differing populations; consequently, they have been suggested to represent alterations at mutational hotspots [3,5,6,8,9]. The most frequent of these mutations, c.316G>A, would be explained by spontaneous deamination of a methylated cytosine on the other strand. This mechanism is a significant cause of DNA sequence change, making C>T and G>A transitions the most frequent single-base-pair alterations [10].

In the present study, the methylation-based mutational hotspot hypothesis is tested. In addition, PCR restriction fragment length polymorphism (RFLP) assays to detect all recurrent *NIPA1* mutations are designed and subsequently applied for screening a large cohort of German HSP patients.

2. Patients and methods

2.1. Methylation analysis

Detection of methylated cytosines was based on the sodium bisulfite conversion method [11]. Using a commercial kit (EpiTect Bisulfite Kit, Qiagen), leukocyte-derived genomic DNA of six unrelated individuals was treated ("converted") as recommended by the manufacturer. The sequence of interest containing *NIPA1* nucleotide c.316 was amplified from treated DNA (oligonucleotides available upon request) and cloned into pCR®4-TOPO (Invitrogen). Three colonies per input DNA were sequenced using vector-specific primers.

2.2. Development of suitable PCR RFLP assays

Wild-type and mutant genomic sequence surrounding *NIPA1* nucleotides 134 and 316 were comparatively analysed *in silico* for creation and removal of restriction enzyme recognition sites (http://rebase.neb.com/rebase/rebase.html). Several such sites were identified for c.134C whereas no restriction enzyme recognition site was altered by replacement of c.316G. Mismatches 2–4 base pairs away from the nucleotide in question were therefore introduced and the analysis repeated.

Based on the results, PCR primers were designed and tested for ability to specifically amplify the desired fragments from leukocyte-derived genomic DNA. When successful, primers were extended to incorporate positive control restriction sites and binding sites for universal sequencing primers. End-labelling (IRD800; MWG Biotech) was implemented to allow detection of small size differences on a sequencing-type gel (LI-COR). A DNA with the c.316G>C mutation [9] was used as a positive control to assess sensitivity of the corresponding PCR RFLP assay.

2.3. Screening of HSP patients

A total of 101 HSP patients, recruited via the German Network for Hereditary Movement Disorders (GeNeMove), were included in the screen for recurrent alterations at *NIPA1* mutational hotspots; informed consent was obtained in all cases. Mutations in *SPG3A*, *SPAST* (*SPG4*), and *REEP1* (*SPG31)* had been excluded by direct sequencing and in *SPG3A*, *SPAST*, *SPG7*, and *REEP1* by multiplex ligation-dependent probe amplification [12, in preparation, unpublished data]. Family history was positive in 57 cases with evidence of autosomal dominant inheritance in 45 index patients. Pure and complicated phenotypes were evenly distributed in the sample (pure: $n=37$, complicated: $n=36$, information missing: $n=28$).

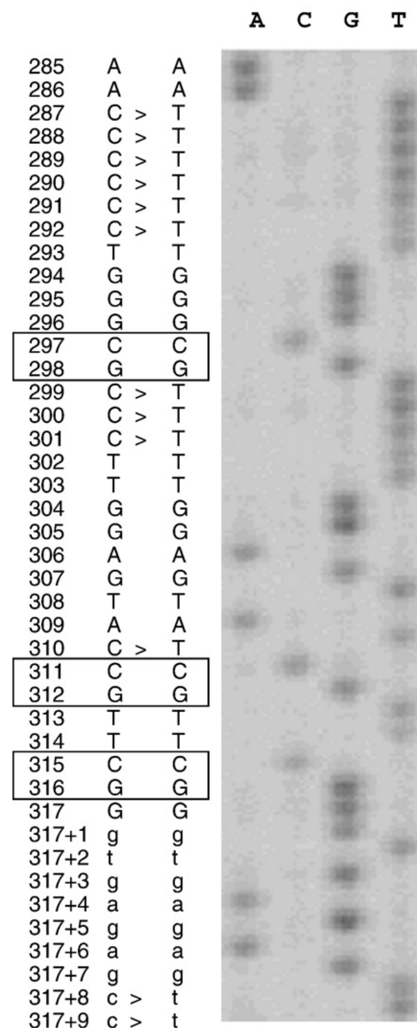

Fig. 1. Methylation analysis. Genomic DNA was treated with sodium bisulfite as outlined under Patients and methods. Treated DNA was then amplified, cloned, and sequenced. Most cytosines originally present (left sequence) are converted to thymines; three cytosines, however, remain (right sequence and image). This indicates methylation of the corresponding CpG dinucleotides (framed). Numbering indicates nucleotide position in reference to the *NIPA1* cDNA.

Download English Version:

https://daneshyari.com/en/article/1915754

Download Persian Version:

https://daneshyari.com/article/1915754

Daneshyari.com