Title: Mutational spectrum of the SPG4 (SPAST) and SPG3A (ATL1) genes in Spanish patients with hereditary spastic paraplegia

Authors:

Victoria Alvarez (victoria.alvarez@sespa.princast.es)
Elena Sánchez-Ferrero (tohelli@hotmail.com)
Christian Beetz (Christian.Beetz@med.uni-jena.de)
Marta Díaz (martaediaz@yahoo.es)
Belén Alonso (belen.montanel@hotmail.com)
Ana I Corao (anacora@hotmail.com)
Josep Gámez (pepgamez@gmail.com)
Jesús Esteban (jesteban.hdco@salud.madrid.org)
Juan F Gonzalo (juanchogonzalo@yahoo.es)
Samuel I Pascual-Pascual (ipascualp.hulp@salud.madrid.org)
Adolfo López de Munain (ADOLFO.LOPEZDEMUNAINARREGUI@osakidetza.net)
Germán Moris (gmorist@gmail.com)
Renne Ribacoba (rribacoba@gmail.com)
Celedonio Márquez (celedoniomarquez@gmail.com)
Jordi Rosell (jordi.rosell@ssib.es)
Maria J García-Barcina (MAJESUS.GARCIABARCINA@osakidetza.net)
Rosario Marín (genetica.hpm.sspa@juntadeandalucia.es)
Emilia del Castillo (genemalaga.hch.sspa@juntadeandalucia.es)
Carmen Benito (victoriaalvaremartinez@yahoo.es)
Eliecer Coto (eliecer.coto@sespa.princast.es)

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Author’s response to reviews: see over
Reviewer's report
Title: Mutational spectrum of the SPG4 (SPAST) and SPG3A (ATL1) genes in Spanish patients with hereditary spastic paraplegia
Version: 1 Date: 5 July 2010
Reviewer: Filippo M. Santorelli
Reviewer's report:
In my opinion the manuscript is of merit but can be improved, as follow:

Abstract:
- It should be stated how many index cases were examined in a total of 370, and how many were presenting with a pure phenotype:
The 370 were unrelated index cases, and 83% with a pure phenotype.
- It should be stated how many familial cases harbored mutations in one of the two genes and how many sporadic/unclear transmission patients proved to be positive upon full gene testing:
A total of 141 (31%) were familial cases, and we found a higher frequency of mutation carriers among these compared to apparently sporadic cases (38% vs. 5%).

Introduction
- Gene names should be italicized.
We have italicized the Gene names.
- For the sake of clarity, indicate at first mention the spastin and atlastin genes as SPAST/SPG4 and ATL1/SPG3A, respectively.
This has been corrected, including the abstract.
- Add latest date of consultation of the gene specific database.
Done.
- References should be presented consistently following the journal guidelines (no superscript references, please)
References were corrected and presented according to the Journal Guidelines.

Patients & Methods
- Considering the number of patients, state unequivocally index cases only.
The 370 were non-related patients (index cases). This was indicated in the ms.

- Divide them in familial (and n of families) (that is, those with a positive family history) and “apparent” sporadic those with parents who were alive and did not present subjective neurological complains or could not be examined or unsure past medical history.
A total of 141 patients (38%) were classified as familial ADHSP based in the family history, while 229 patients (62%) were classified as “apparently” sporadic cases. In 177 of these the two parents were alive and did not have symptoms consistent with HSP, while in 52 the inheritance pattern could not be established because no clinical data were available from relatives. We indicated these facts in the ms.

- MLPA analysis section should precede Analysis of SPAST transcripts
This section was moved.
- Avoid the use of not canonical jargon (for example SP).
We avoided non canonical terms (such as SP, using HSP)
- It would be important to have comparisons between disease duration, severity
and quality of life at least in ADHSP with mutations in SPAST, ATL1 and patients with no mutations.

We compared the mean onset age and disease duration between patients with mutations in SPAST and ATL1 and patients with no mutations. However, we have no data from all the patients about the disease severity and quality of life, and a phenotype-genotype correlation could not be established for these parameters.

Results
- See above for index patients and relative frequency of mutations. We indicated this in the results.
- State clearly criteria adopted to assess pathogenicity for variants identified in this study. For missense changes, the main criteria were the absence of the change among the 400 controls, the amino acid conservation between species, and a predicted effect on protein structure/function. We indicated the criteria in the methods, and also in the results.
- Since the disease-related status seems uncertain, the p.I328L mutation should be removed from the list of pathogenic mutations. Although it could be related to a clinical phenotype with reduced penetrance, the variant has a low predicted deleterious value and it seems not sufficient per se to cause spastic paraplegia. In my opinion, it is safer to list p.I328L among polymorphisms. In supplementary table 2 it could be just added a note to indicate uncertainty. We considered this as a change of uncertain effect, and moved it from the mutations to the polymorphisms.
- Genotype-phenotype correlations could be improved if one considers severity scores and Barthel index. The severity scores and quality-of-life index were not available for all the patients. For this reason, we did not include these in the genotype-phenotype comparisons. The neurologists who participate in this study are currently recruiting this information (that is difficult to obtain in some cases), and we could try to describe a more complete genotype-phenotype in the future.

Discussion
- Re-writing the Results section might help to have a more focused discussion. As an example, it is sufficient to discuss the relative frequency of SPAST and ATL1 mutation in the Authors’ cohort in comparison with published data, indicate the type of mutations identified (including sense mutations), a few words missense variants affecting splicing when they reside in exon-enhancement sequencing for splicing, and a relatively low frequency of multi-exon deletions/duplications.
We agree, and we have shortened the discussion because approximately 50% of this section was redundant with the results.

- Based on the fortunate large number of molecularly-tested patients in this study, readers would appreciate a sort of flowchart for molecular strategies in case of pure or complex phenotypes, familial or apparently sporadic patients, and early or late-onset disease onset.
We agree and tried to summarize this idea in the ms: the genetic screening should be more relevant in patients with a family history of the disease (because mutations are more likely found in these cases). However, the fact that a significant number of apparently sporadic cases showed a mutation suggested that these cases should not be excluded from the study. However, the mutational report could be of limited value to predict the phenotype associated to these mutations, as demonstrated by the heterogeneous behavior of most of the mutations (even within the same family).

Figures and Tables.
Quality of Supplem. Figure can be improved. Please, use a different ID code for patients in Supplementary fig. Commonly, SPGXX is used to indicate HSP loci or genes and it is better not to use in patients to avoid confusion in readers. Families are identified as HSP, instead of SPG.
Reviewer: Kirsten Svenstrup

Reviewer's report:
Major Compulsory Revisions

1) The manuscript should be significantly reduced in length (to at least 50%). The language could generally be more precise.

We have reduced the ms. length, especially in the introduction and the discussion. To reduce the length, we tried to limit the redundancies between the results and the discussion.

2) The references should be more precise. It is not clear which criterias references are chosen from when many references are cited. Example: To date >150 SPAST mutations have been reported (http://www.hgmd.cf.ac.uk/hgmd0.html), mainly in patients with pure HSP although other associated neurological symptoms/findings (cognitive impairment, mental retardation, cerebellar ataxia, neuropathy) have been found in some families[7-12]. Several referenced are not cited correctly. Examples: The disease is classified as "pure" when spasticity is the only clinical finding, and as “complicated” when other clinical features such as seizures, dementia, cerebellar ataxia, epilepsy, or peripheral neuropathy, are also present [1]. And: This suggested that both, haploinsufficiency and “toxic” gain of function could explain the pathogenic mechanism of SPAST mutations [17-19]. Ref 24 and 25 do not support “SPG3A is usually found in early onset cases (childhood or adolescence)” since only early onset cases are included in these studies.

-A number of reports have described the mutational spectrum of SPAST, ranging from small to large groups of patients. Because there are many works that could be referenced, we have chosen the references based on the next criteria:

Reference 7- This work from 2000 reported the first large cohort for SPAST mutations.
Reference 8- This paper from 2002 studied 150 European patients (one of the first for an European cohort).
Reference 9- This work analysed patients from Southern Europe, and also used DHPLC (a technique used in our study for the screening of the mutations in the controls).
Reference 10- Reported the mutational spectrum in Southern European population (that could be compared with our results from Spain).
Reference 11- This study described a large cohort of sporadic patients.
Reference 12- In this work, a large cohort of patients from Southern Europe was studied, and we think the results could be compared with our data.

-Reference 1 was wrong, and we changed this to: Harding AE. Lancet 1983: 1: 151-1155
3) The analysis of SIFT scores should be excluded. The power of the reported study is that conclusions of pathogenity of mutations can be drawn from the presented data and not from an algorithm. Pedigrees of all new mutations should be presented in a figure.

We deleted the paragraph about the bioinformatic analysis in the methods, and indicated the reference for each program (web page) in the results.

In suppl fig.1 we showed some of the pedigrees with new mutations. We did not illustrated all the pedigrees from new mutations in a single figure, we think the number of mutations makes this impractical. We could add more figures with these pedigrees, but this would increase the ms. length.

Minor Essential Revisions:
1) Human gene names should be in italics
   This was corrected..
2) The DNA variants are stated to follow guidelines from HGVC but they use one letter amino acid code
   We used the three letter code for amino acids
3) Figures should include both family identification and mutation on cDNA and protein level
   The figures were corrected.
4) On which criterias was AD suspected and how were siblings with healthy parents categorized (Patients).

   A total of 141 patients (38%) were classified as familial ADHSP based in the family history, while 229 patients (62%) were classified as “apparently” sporadic cases. In 177 of these the two parents were alive and did not have symptoms consistent with HSP, while in 52 the inheritance pattern could not be established because no clinical data were available from relatives. This was indicated in the revised ms.

5) Existence of pyramidal signs and examination by a qualified neurologist do not fullfill the criterias for HSP (patients).
In the revised ms. we have included the sentence: Spastic paraplegia (HSP) was diagnosed by qualified neurologists on the basis of Harding criteria.

6) What is the difference between neuropathy and periferal neuropathy? (Patient characteristics)
   There is no difference, we referred to peripheral neuropathy in the revised ms.

7) I do not understand the sentence: Our work showed a heterogeneous behaviour for these mutations and this would make difficult to establish a genotype-phenotype correlation (Conclusion)
We mean that Most of the SPAST and ATL1 mutations were associated to heterogeneous phenotypes, even between mutation carriers from the same family. We included this sentence at the end of the ms (conclusions):

Thus, the genetic screening should be more relevant in patients (pure and complicated phenotype) with a family history of the disease. However, the fact that a significant number of apparently sporadic cases showed a mutation suggested that these cases should not be excluded from the study. The mutational report could be of limited value to predict the phenotype associated to these mutations, as demonstrated by the heterogeneous behavior of most of the mutations (even within the same family).

8) In fig 2: What does P M Cand G mean?

In figure 2 we indicated the meaning of P, M, and G.
Reviewer's report
Reviewer: Stephan Zuchner
Reviewer's report:
The manuscript details the results of a large mutation screen of the two major HSP genes SPAST and ATL1 in a large sample of Spanish HSP patients. The further establishment of genotype/phenotype correlations in HSP is important. The report is very well written, carefully structured and the available literature is sufficiently researched. I have no major concerns or critique.