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【 CASE REPORT 】

# A Japanese SPG4 Patient with a Confirmed *De Novo* Mutation of the *SPAST* Gene

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**Abstract:**

Spastic paraplegia type 4 (SPG4) is caused by mutations of the *SPAST* gene and is the most common form of autosomal-dominantly inherited pure hereditary spastic paraplegia (HSP). We herein report a Japanese patient with SPG4 with a confirmed *de novo* mutation of *SPAST*. On exome sequencing and Sanger sequencing, we identified the heterozygous missense mutation p.R460L in the *SPAST* gene. This mutation was absent in the parents, and the paternity and maternity of the parents were both confirmed. The patient showed a pure SPG4 phenotype with an infantile onset. This study may expand the clinical and genetic findings for SPG4.

**Key words:** hereditary spastic paraplegia, SPG4, *SPAST*, *de novo* mutation, Japanese

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## Introduction

Hereditary spastic paraplegias (HSPs) are clinically and genetically heterogeneous neurodegenerative disorders characterized by progressive weakness and spasticity in the lower limbs due to pyramidal tract dysfunction (1). Spastic paraplegia type 4 (SPG4) is due to heterozygous mutations of the *SPAST* gene and is the most frequent cause of both familial and sporadic HSP (2). However, sporadic SPG4 patients are generally attributed to common mechanisms like incomplete penetrance, somatic mosaicism, non-paternity, and inadequate clinical assessment of the parents (3). True *de novo* occurrence of a *SPAST* mutation, where both parents of the patient are proven not to have the mutation in lymphocytes, appears to be rare. Thus far, true *de novo* *SPAST* mutations have been reported in American, Brazilian, Canadian, Czech, Dutch, French, German, Greek, Italian, and Polish SPG4 families (3-13). However, the paternity and maternity of the parents have rarely been assessed to confirm the *de novo* occurrence.

We herein report a Japanese patient with a clinically pure phenotype of SPG4 with a *de novo* mutation of *SPAST*.

## Case Report

A 23-year-old woman (Figure A, II-2) was the second of two siblings born to healthy, unrelated parents. Her 26-year-old brother was unaffected. She was born by vaginal delivery after an uneventful pregnancy. Her parents initially became concerned when she had not begun to walk by 12 months of age. She began to walk independently at two years old, and her gait became increasingly slow and spastic over time. However, the symptoms progressed slowly during the first two decades of her life, and she was able to run until graduation from high school. At age 20, however, she developed gait unsteadiness with frequent falling and difficulty climbing stairs.

On a neurological examination, she presented with increased muscle reflexes of the lower limbs, a positive Babinski's sign, contractures of the joints, and slight paresis of the extensors in the lower limbs. She was intellectually normal, and no cerebellar, sensory, or autonomic dysfunction was detected. Metabolic and routine blood investigations were unremarkable. Magnetic resonance imaging (MRI) of the brain and spine were normal.

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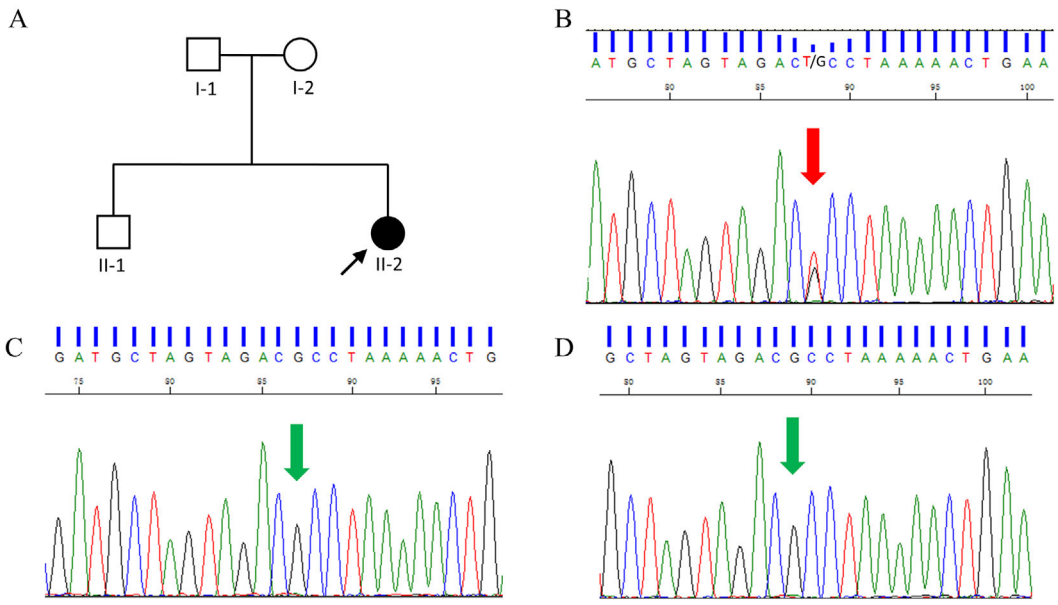


**Figure.** **A: Pedigree of the HSP family. The patient is indicated (arrow). Squares indicate men; circles, women; shaded (black) symbol, individual with HSP, unshaded symbols, individuals without HSP. B: A sequence analysis revealed the c.1379 G>T mutation in exon 11 of *SPAST* in the patient. The red arrow indicates the position of the c.1379 nucleotide. C: A sequence analysis revealed no mutation in exon 11 of *SPAST* in the patient's father. The green arrow indicates the position of the c.1379 nucleotide. D: A sequence analysis revealed no mutation in exon 11 of *SPAST* in the patient's mother. The green arrow indicates the position of the c.1379 nucleotide.**

**Table 1.** **Genes Known to Be Responsible for HSP.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ATL1 | SLC16A2 | WDR48 | GJA12 | CYP7B1 | KIF1A | IBA57 | c12orf65 |
| SPAST | Xp25 | ARL6IP1 | NT5C2 | SPG7 | FAM134B | MAG | CYP2U1 |
| NIPA1 | HACE1 | ERLIN1 | GBA2 | ALDH18A1 | ALS2 | MTCO3 | TFG |
| KIAA0196 | LYST | AMPD2 | AP4B1 | SPG11 | EXOSC3 | MTTI | KIF1C |
| ALDH18A1 | ALS2 | ENTPD1 | KIAA0415 | ZFYVE26 | SPOAN | MTND4 | USP8 |
| KIF5A | SACS | ARSI | TECPR2 | ERLIN2 | GAD1 | MTATP6 | FA2H |
| RTN2 | SPPRS | PGAP1 | AP4M1 | SPG20 | ARSACS | L1CAM | PNPLA6 |
| HSPD1 | BICD2 | FLRT1 | AP4E1 | ACP33 | MAG | PLP | c9orf12 |
| BSCL2 | CHS | RAB3GAP2 | AP4S1 | B4GALNT1 | ARSPG75 | Xq11 | ZFYVE27 |
| ATSV | IFIH1 | MARS | VPS37A | DDHD1 | REEP2 | CPT1C | SLC33A1 |
| REEP1 | CCT5 | ZFR | DDHD2 | ATSV | UBAP1 | | |

We carried out whole-exome sequencing of genomic DNA from the patient. Genomic DNA was extracted from peripheral blood. Exome capture was performed with a SureSelect Human All Exon V6+UTR (89Mb) Kit (Agilent Technologies, Santa Clara, USA). Paired-end sequencing was carried out on a HiSeq2500 (Illumina, San Diego, USA) using a HiSeq SBS Kit V4 (Illumina), which generated 100-bp reads. The reference databases utilized included hg38 (GRCh38) (http://genome.ucsc.edu), The Human Gene Mutation Database (HGMD) (https://portal.biobase-international.com), Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org), the Genome Aggregation Database (GnomAD) (http://gnomad.broadinstitute.org), and the Single Nucleotide Polymorphism Database (dbSNP) (https://www.ncbi.nlm.nih.gov/SNP). We examined variants of 86 genes known to be responsible for HSP (Table 1). Through this analysis, we identified a heterozygous missense mutation (c.1379G>T, p.Arg460Leu) in exon 11 of the *SPAST* gene in the patient and ruled out the possibility of other causative genes. We then examined exon 11 of the *SPAST* gene in the patient as well as the patient's father (Figure A, I-1) and mother (Figure A, I-2) via polymerase chain reaction (PCR). The genomic DNA of the patient's parents was also extracted from peripheral blood. On Sanger sequencing, we reconfirmed the p.R460L mutation in exon 11 of the *SPAST* gene, which was in a heterozygous state in the patient (Figure B). Arginine was replaced by leucine in an area evolutionarily conserved among the human, rhesus monkey, mouse, dog, elephant, chicken, western clawed frog, and zebrafish species. Bioinformatic analyses using the

**Table 2. Paternity and Maternity Testing by Analysis of Forensic Short Tandem Repeat (STR) Markers in the Family Members.**

| | Father | | Daughter | | Mother | | Probability of Maternity | Likelihood Ratio (LR) | Probability of Paternity | Likelihood Ratio (LR) |
|---|---|---|---|---|---|---|---|---|---|---|
| D3S1358 | 15 | 18 | 17 | 18 | 12 | 17 | 0.552486187845304 | 1.234568 | 0.8855827134254 | 7.73993808 |
| vWA | 16 | 18 | 18 | 18 | 16 | 18 | 0.734176657587349 | 2.237136 | 0.9454008329360 | 2.237136465 |
| D16S539 | 10 | 11 | 11 | 12 | 9 | 12 | 0.800388145990311 | 1.451800 | 0.9788351386246 | 2.670940171 |
| CSF1PO | 9 | 11 | 11 | 12 | 12 | 12 | 0.826078806001666 | 1.184553 | 0.9911489487954 | 2.421307506 |
| TPOX | 8 | 9 | 8 | 8 | 8 | 9 | 0.840105573791601 | 1.106195 | 0.9919918451746 | 1.10619469 |
| D8S1179 | 12 | 14 | 12 | 14 | 12 | 13 | 0.914186190238607 | 2.027575 | 0.9966497467238 | 2.401536984 |
| D21S11 | 31 | 31 | 29 | 31 | 29 | 30 | 0.915285808510090 | 1.014199 | 0.9996609381179 | 9.910802775 |
| D18S51 | 16 | 19 | 16 | 19 | 16 | 17 | 0.953105141863687 | 1.881114 | 0.9999751728685 | 13.66120219 |
| D2S441 | 11 | 14 | 11 | 14 | 11 | 14 | 0.985057307313079 | 3.243523 | 0.9999889840496 | 2.253775073 |
| D19S433 | 14 | 15 | 14 | null | 16.2 | null | 0.999981797094757* | 833.333333* | 0.9999923845519 | 1.446531792 |
| TH01 | 6 | 9 | 6 | 9 | 6 | 9 | 0.999989671501180 | 1.762410 | 0.9999952989702 | 1.619957881 |
| FGA | 21 | 22 | 21 | 24 | 24 | 24 | 0.999996723777069 | 3.152585 | 0.9999987786682 | 3.849114704 |
| D22S1045 | 15 | 16 | 16 | 17 | 17 | 17 | 0.999998601050182 | 2.341920 | 0.9999993922649 | 2.009646302 |
| D5S818 | 9 | 12 | 11 | 12 | 11 | 11 | 0.999999193924637 | 1.735509 | 0.9999997198340 | 2.169197397 |
| D13S317 | 11 | 12 | 11 | 12 | 11 | 12 | 0.999999656429549 | 2.346173 | 0.9999998802570 | 2.339728591 |
| D7S820 | 10 | 10 | 10 | 12 | 11 | 12 | 0.999999685976599 | 1.094092 | 0.9999999729141 | 4.42086649 |
| SE33 | 18 | 31.2 | 18 | 25.2 | 16 | 25.2 | 0.999999923001444 | 4.078303 | 0.9999999965005 | 7.73993808 |
| D10S1248 | 13 | 13 | 13 | 15 | 13 | 15 | 0.999999959106875 | 1.882922 | 0.9999999980798 | 1.822489521 |
| D1S1656 | 13 | 18.3 | 14 | 18.3 | 14 | 15 | 0.999999986161766 | 2.955083 | 0.9999999998018 | 9.689922481 |
| D12S391 | 18 | 18 | 18 | 21 | 21 | 21 | 0.999999996936215 | 4.516712 | 0.9999999999496 | 3.93236335 |
| D2S1338 | 19 | 20 | 19 | 20 | 19 | 20 | 0.999999999119441 | 3.479363 | 0.9999999999838 | 3.107520199 |
| Amelo. | X | Y | X | X | X | X | - | Total LR: 1135641941.9485 | - | Total LR: 61666289424.4637 |

*The frequency of allele "null" was set as the lowest allele frequency, "0.0003", in the database we used (14).
Since realistically, the allele "null" has not been found in the database, its frequency is expected to be less than 0.0003. Therefore, both the probability of maternity and the likelihood ratio are expected to be greater than those calculated at the lowest frequency.

Mutation Taster (http://www.mutationtaster.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), Protein Variation Effect Analyzer (PROVEAN), and SIFT (http://provean.jcvi.org/genome_submit_2.php) software programs predicted that this variant was disease-causing, probably damaging, deleterious and damaging, respectively. On the other hand, the patient's parents did not exhibit the mutation on Sanger sequencing (Figure C, D). In this family, the patient harbored a mutation that was absent in her parents, and her sibling was healthy. This suggests that the mutation occurred *de novo* in the patient.

Finally, to genetically confirm the paternity and maternity of the parents, we performed a paternity test. Twenty-one of the most polymorphic autosomal short tandem repeat (STR) markers commonly used for paternity testing in Japanese populations and a sex-identification marker (Amelogenin locus) were genotyped (14). The STR loci, D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, and Amelogenin, were amplified using a Globalfiler™ Amplification Kit (Thermo Fisher Scientific, Waltham, USA). The amplified products were detected with an ABI PRISM® 310 Genetic Analyzer (Thermo Fisher Scientific). Fragment sizes were determined using the GeneScan Analysis v3.7 software program (Thermo Fisher Scientific), and the alleles were typed using the GenoTyper v3.7 software program (Thermo Fisher Scientific). The numbers of repeats in each STR marker in the family members and the bio-statistical computations are shown in Table 2. The bio-statistical calculation was performed using a spreadsheet as described previously (15). The genotypes of all 21 loci showed that the child inherited one allele from her father, and the genotypes of all 21 loci except D19S433 showed that the child inherited one allele from her mother. The DNA samples from the mother and the daughter might have shown some microsatellite instability in the D19S433 STR locus; however, the bio-statistical computations strongly supported the maternity relationship. Therefore, the paternity and maternity of the parents were both confirmed in this case.

## Discussion

The p.R460L mutation of the *SPAST* gene was first reported as a disease-causing mutation in a European family with autosomal dominant pure HSP. This mutation is located in the AAA ATPase cassette of spastin (from amino acid 342 to 616), which is crucial for microtubule-severing activity (16). This mutation was not present in the patients who were reported to have true *de novo SPAST* mutations in the literature (3-13). Since the causative mutation of the *SPG4*

gene in Japanese was first confirmed in 2001 (17), true *de novo SPAST* mutations in cases of Japanese or Asian ethnicity have rarely been reported. After we obtained DNA samples from the patient's father (54 years old) and mother (51 years old), who are both currently unaffected, we were able to establish that the p.R460L mutation was a *de novo* event, as both parents exhibited normal sequencing.

True *de novo* occurrence of *SPAST* mutations was the topic of focus for the first time in the report by Schieving et al. in 2019 (3). They reported that most of the *SPAST* mutations that occur *de novo* are also present in families with multiple generations with pure HSP. Furthermore, they suggested that the majority of patients (81%) with *de novo* mutations have an extremely early onset of the disease. This finding fits our patient. However, it is possible that this is because patients with early-onset disease simply tend to undergo a trio analysis. The relationship between the age of onset and the *de novo* occurrence of the mutation in *SPAST* may need further study.

It has been reported that 5.7% of SPG4 cases occur sporadically (16). However, it is very difficult to identify true *de novo* occurrence from incomplete penetrance or non-paternity because both parents need to be examined and genetically tested. Therefore, the frequency of *de novo* variants causing SPG4 is unknown. We reported a proven case of a *de novo* mutation in the *SPAST* gene in a Japanese patient. We were unable to rule out the possibility of gonadal mosaicism in either of the unaffected parents, even though it would still represent a *de novo* event. We suggest also including genes exhibiting an autosomal dominant mode of inheritance in patients with apparently sporadic HSP if a genetic analysis is performed.

Of the previously reported 27 patients with a *de novo SPAST* mutation identified, 9 (33%) harbored the common c.1496G>A mutation (3-13). Although the low number of cases did not allow for any conclusions to be drawn, more clinical cases should be evaluated in order to determine if there are any mutational hot spots for the *de novo* occurrence of *SPAST.*

There are many kinds of mutations in *SPAST*, and all of them arose *de novo* at some point in the past. It has been suggested that some mutations in *SPAST* identified in certain populations had a founder effect (18), while some pathogenic variants of genetic disorders arose only once in human history (19). Our study indicates that a *de novo* mutation of *SPAST* can arise in an Asian population independently, thus contradicting the possibility of sharing a common ancestral origin with European populations.

In conclusion, we encountered a case of a pure SPG4 phenotype with an infantile onset caused by a *de novo SPAST* mutation in a Japanese patient. The paternity and maternity of the parents were both confirmed in this case. This study may expand the clinical and genetic findings for SPG4.

The present clinical and genetic study was approved by the institutional review board of Yamanashi University, and written informed consent was obtained from all participating individuals.

**The authors state that they have no Conflict of Interest (COI).**

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## *References*

1. Fink JK. Hereditary spastic paraplegia. Curr Neurol Neurosci Rep **6**: 65-76, 2006.
2. Lo Giudice T, Lombardi F, Santorelli FM, Kawarai T, Orlacchio A. Hereditary spastic paraplegia: clinical-genetic characteristics and evolving molecular mechanisms. Exp Neurol **261**: 518-539, 2014.
3. Schieving JH, de Bot ST, van de Pol LA, et al. De novo SPAST mutations may cause a complex SPG4 phenotype. Brain **142**: e31, 2019.
4. Blair MA, Riddle ME, Wells JF, Breviu BA, Hedera P. Infantile onset of hereditary spastic paraplegia poorly predicts the genotype. Pediatr Neurol **36**: 382-386, 2007.
5. Burguez D, Polese-Bonatto M, Scudeiro LAJ, et al. Clinical and molecular characterization of hereditary spastic paraplegias: a next-generation sequencing panel approach. J Neurol Sci **383**: 18-25, 2017.
6. Gillespie MK, Humphreys P, McMillan HJ, Boycott KM. Association of early-onset spasticity and risk for cognitive impairment with Mutations at amino acid 499 in SPAST. J Child Neurol **33**: 329-332, 2018.
7. Meszarosova AU, Putzova M, Cermakova M, et al. SPAST mutation spectrum and familial occurrence among Czech patients with pure hereditary spastic paraplegia. J Hum Genet **61**: 845-850, 2016.
8. Depienne C, Fedirko E, Faucheux JM, et al. A de novo SPAST mutation leading to somatic mosaicism is associated with a later age at onset in HSP. Neurogenetics **8**: 231-233, 2007.
9. Aulitzky A, Friedrich K, Glaser D, et al. A complex form of hereditary spastic paraplegia in three siblings due to somatic mosaicism for a novel SPAST mutation in the mother. J Neurol Sci **347**: 352-355, 2014.
10. Polymeris AA, Tessa A, Anagnostopoulou K, et al. A series of Greek children with pure hereditary spastic paraplegia: clinical features and genetic findings. J Neurol **263**: 1604-1611, 2016.
11. Battini R, Fogli A, Borghetti D, et al. Clinical and genetic findings in a series of Italian children with pure hereditary spastic paraplegia. Eur J Neurol **18**: 150-157, 2011.
12. Crippa F, Panzeri C, Martinuzzi A, et al. Eight novel mutations in SPG4 in a large sample of patients with hereditary spastic paraplegia. Arch Neurol **63**: 750-755, 2006.
13. Elert-Dobkowska E, Stepniak I, Krysa W, et al. Molecular spectrum of the SPAST, ATL1 and REEP1 gene mutations associated with the most common hereditary spastic paraplegias in a group of Polish patients. J Neurol Sci **359**: 35-39, 2015.
14. Koji F, Haruhiko W, Yusuke M, et al. Allele frequencies for 21 autosomal short tandem repeat loci obtained using GlobalFiler in a sample of 1501 individuals from the Japanese population. Legal

Medicine **17**: 306-308, 2015.

**15.** Aoki Y, Hashiyada M, Morioka A, et al. Spreadsheets of a conventional application software for calculation of plausibility of paternity: application to parentage testing with highly polymorphic markers in deceased party. Nihon Hoigaku Zasshi **51**: 196-204, 1997(in Japanese, Abstract in English) .

**16.** Parodi L, Fenu S, Barbier M, et al. Spastic paraplegia due to SPAST mutations is modified by the underlying mutation and sex. Brain **141**: 3331-3342, 2018.

**17.** Namekawa M, Takiyama Y, Sakoe K, et al. A large Japanese SPG 4 family with a novel insertion mutation of the SPG4 gene: a clinical and genetic study. J Neurol Sci **185**: 63-68, 2001.

**18.** Meijer IA, Dupré N, Brais B, et al. SPG4 founder effect in French Canadians with hereditary spastic paraplegia. Can J Neurol Sci **34**: 211-214, 2007.

**19.** Rafehi H, Szmulewicz DJ, Bennett MF, et al. Bioinformatics-based identification of expanded repeats: a non-reference intronic pentamer expansion in RFC1 causes CANVAS. Am J Hum Genet **105**: 151-165, 2019.