These mutations in the *SPTBN2* spectrin repeats may affect the structure, binding properties and overall function of the protein, and also lead to SCA5. To date, no curative treatment for autosomal-dominant spinocerebellar ataxias has been found. Therefore, in addition to its value in genetic counseling, genetic knowledge of the relevant loci might help to identify the possible targets of pharmaceutical interventions.

Clinical presentations similar to those in our family have been reported in other SCA5 families.[3–6] The age at the onset is typically from the third to fifth decade, with highly variable symptoms that worsen over time. The major characteristics of SCA5 are slow progression with patients remaining ambulatory even after decades of suffering from the disease and having an approximately normal longevity.[15] The MRI findings in four patients were pronounced cerebellar atrophy, but the brainstem remained intact, in accordance with the major clinical presentation. Our study suggests that SCA5 should be considered in a pure form of autosomal-dominant cerebellar ataxia when SCA6 or SCA31 is excluded, particularly in Japan.

A single infantile-onset SCA5 patient with a heterozygous missense mutation (c.1438C>T, p.R480W) in the *SPTBN2* gene has been reported.[7] The parents of the patient were healthy and nonconsanguineous. This is a much more severe phenotype with ataxia and global development delay.[7] Meanwhile, a homozygous mutation in the *SPTBN2* gene has been reported in a consanguineous family with childhood developmental ataxia and cognitive impairment.[16,17] Thus, recessive mutations in the *SPTBN2* gene might cause a more severe disorder than SCA5, that is, resembling one infantile-onset SCA5.[7] This suggests that beta-III spectrin has an important role in both development and cognition in addition to its function in the cerebellum. Cognitive impairment is an integral part of a recessive ataxic syndrome, and the condition is called spectrin-associated autosomal-recessive cerebellar ataxia type 1 (SPARCA1).[13] The identification of SPARCA1 and normal heterozygous carriers of the stop codon in *SPTBN2* provides insights into the mechanism underlying the molecular dominance in SCA5 and demonstrates that the cell-specific repertoire of spectrin subunits underlies a novel group of disorders, namely, the neuronal spectrinopathies, which include SCA5, SPARCA1 and a form of West syndrome.[16]

In summary, there are increasing numbers of *SPTBN2* mutations being reported, and we added a novel *SPTBN2* mutation to the 'spectrinopathies', which will be useful for elucidating the molecular mechanism underlying the 'spectrinopathies'.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Accession codes: The nucleotide sequence data reported are available in the GenBank database under the accession number KJ567653.

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RESEARCH PAPER

Autosomal-recessive complicated spastic paraplegia with a novel *lysosomal trafficking regulator* gene mutation

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ABSTRACT

Background Autosomal-recessive hereditary spastic paraplegias (AR-HSP) consist of a genetically diverse group of neurodegenerative diseases characterised by pyramidal tracts dysfunction. The causative genes for many types of AR-HSP remain elusive. We tried to identify the gene mutation for AR-HSP with cerebellar ataxia and neuropathy.

Methods This study included two patients in a Japanese family with their parents who are first cousins. Neurological examination and gene analysis were conducted in the two patients and two normal family members. We undertook genome-wide linkage analysis employing single nucleotide polymorphism arrays using the two patients' DNAs and exome sequencing using one patient's sample.

Results We detected a homozygous missense mutation (c.4189T>G, p.F1397V) in the *lysosomal trafficking regulator* (*LYST*) gene, which is described as the causative gene for Chédiak–Higashi syndrome (CHS). CHS is a rare autosomal-recessive syndrome characterised by hypopigmentation, severe immune deficiency, a bleeding tendency and progressive neurological dysfunction. This mutation was co-segregated with the disease in the family and was located at well-conserved amino acid. This *LYST* mutation was not found in 200 Japanese control DNAs. Microscopic observation of peripheral blood in the two patients disclosed large peroxidase-positive granules in both patients' granulocytes, although they had no symptoms of immune deficiency or bleeding tendency.

Conclusions We diagnosed these patients as having adult CHS presenting spastic paraplegia with cerebellar ataxia and neuropathy. The clinical spectrum of CHS is broader than previously recognised. Adult CHS must be considered in the differential diagnosis of AR-HSP.

INTRODUCTION

Hereditary spastic paraplegias (HSP) comprise a genetically diverse group of inherited neurological disorders mainly exhibiting increased tone of the lower limb muscles with various associated symptoms. HSP are classified into two subtypes, that is, pure and complicated forms. The pure HSP presents with symptoms of progressive bilateral leg spasticity and weakness, exaggerated tendon reflexes and positive pathological reflexes. Meanwhile, the complicated HSP has additional neurological symptoms, as follows: mental impairment, extrapyramidal signs, cerebellar ataxia, peripheral neuropathy, muscle atrophy and optic atrophy, as well as symptoms other than neurological ones.[1]

HSP can be inherited in an autosomal-dominant (AD), autosomal-recessive (AR) or X-linked recessive (XR) pattern. Fifty-seven spastic paraplegia gene (SPG) loci have been assigned and about 40 causative genes have been identified to date. The pure HSP is mainly transmitted as an AD trait, whereas the complicated HSP exhibits AR or XR transmission. SPG4, the most common pure AD-HSP, is accounting for about 50% of such cases.[2] The most frequent AR-HSP is SPG11, which shows a complex phenotype, including cognitive impairment, a thin corpus callosum and peripheral neuropathy.[3] However, the majority of causative genes for AR-HSPs remain to be identified.

A number of pathogenic mechanisms underlying HSPs have been suggested by studies on several implicated genes for HSP. HSP is thought to be involved in intracellular trafficking, resulting from disruption of the axonal transport of molecules, organelles and other cargos, which predominantly affects the distal parts of motor neurons.[4]

Here, we present an AR-HSP family with cerebellar ataxia and neuropathy with a novel homozygous missense mutation in the *lysosomal trafficking regulator* (*LYST*) gene. *LYST* is known as the causative gene for Chédiak–Higashi syndrome (CHS, OMIM #214500),[5] which is a rare AR syndrome characterised by hypopigmentation, severe immune deficiency, a bleeding tendency and progressive neurological dysfunction.

METHODS

Patients

This study included two patients from a family with spastic paraplegia, cerebellar ataxia and peripheral neuropathy. The family tree is shown in figure 1C. The parents were first cousins. Two affected (II-1 and II-2) and two unaffected members (II-3, III-1) of the family underwent neurological examinations and blood analyses. All of the genomic DNA samples were obtained with written informed consent.

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Linkage analysis

We extracted genomic DNAs from blood samples in the two affected individuals (II-1 and II-2) (figure 1C). Then, we conducted multipoint parametric linkage analysis using a pipeline software, SNP high-throughput linkage analysis system (SNP HiTLink).[6] We can directly import SNP chip data for the Mapping 100k/500k array set and Genome-Wide Human SNP array V.6.0 (Affymetrix, Santa Clara, California, USA) and pass to a multipoint parametric linkage analysis program, Allegro, with this system.[7] We calculated Parametric logarithm of the odds (LOD) scores using Allegro V.2 with the parameter setting of an AR model with 100% penetrance.

Exome sequencing

We extracted Genomic DNA from leucocytes from one patient (II-1) and then sheared. We purified the sample using Agencourt AMPure XP beads. We prepared an adaptor-ligated library and clustered on the cBOT system (Illumina, San Diego, California, USA). We performed exon capture with a SureSelect Human All Exon 50 Mb Kit (Agilent). We carried out paired-end sequencing on an Illumina Hiseq 2000, which generated 101 bp reads. We aligned the sequences with the human genome reference sequence (hg19 build, http://hgdownload.cse.ucsc.edu/goldenPath/hg19/chromosomes/) using a Burrows-Wheeler Aligner for sequence alignment, variant calling and annotation. We carried out substitution calling with a Genome Analysis Toolkit (GATK, http://www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit). SNP calls were made with a GATK Unified Genotyper, and indel calls were made with a GATK IndelGenotyper V2. We performed SNP calling with reference to dbSNP135 (ftp://ftp.ncbi.nlm.nih.gov/snp/orgasms/human_9606/ASN1_flat/) and 1000 Genomes (ftp://1000genomes.ebi.ac.uk/vol1/ftp/release/20110521). All variants were annotated with reference to consensus coding sequences (CCDS) (NCBI release 20111122) and RefSeq (UCSC dumped 20111122).

Validation of mutations by Sanger sequencing

We amplified exon 12 of *LYST* and flanking intronic sequences using the genomic DNA of the two patients using a MJ Research PTC-100 Thermal Cycler (Marshall Scientific, Brentwood, New Hampshire, USA). The primer sequences were as follows: LYST ex12-F: agg aat gct gat atg tgt ggg; LYST ex12-R: cac att ttt acg gct caa gga. We performed Sanger sequencing according to an established standard protocol on an Applied Biosystems (ABI) 3730 capillary sequencer (Applied Biosystems, Carlsbad, California, USA). We also analysed genomic DNA samples from 200 Japanese subjects without apparent neurological disorders as controls.

The institutional review boards of the Jichi Medical University, the Shinshu University, the University of Tokyo and the University of Yamanashi approved this study.

RESULTS

Clinical features

The proband (II-2) was a 53-year-old man who was admitted to our hospital because of gait disturbance and slowly progressive weakness of the lower extremities. He was experiencing difficulties in walking up stairs and standing upright at age 48 years. Later he walked with a cane due to unsteadiness. His Mini-Mental State Examination (MMSE) score was 25/30. On neurological examination, he showed bilateral leg spasticity, and bilateral iliopsoas muscle weakness and atrophy. Tendon reflexes were exaggerated in all extremities except for diminished ankle jerks. He showed extensor bilateral plantar responses. We could observe subtle ataxia in the upper extremities. Brain MRI showed mild cerebellar atrophy, and spinal MRI revealed mild thoracic cord atrophy (figure 2A). Mild decreases of motor and sensory nerve conduction velocities in the lower extremities were revealed by a nerve conduction study, but SNAPs were not evoked in the sural nerves. Motor-evoked potential examination disclosed marked delay of the central motor conduction times in the corticospinal tracts. His eyes exhibited no areas of hyperpigmentation or hypopigmentation on ophthalmological examination. Pigmentary abnormalities of the skin were not observed. Peroxidase staining of peripheral blood revealed giant granules in granulocytes (figure 2B) and mild reduction of natural killer cell activity.

The second case, an older brother of the proband (II-1), showed almost the same clinical presentations except for the leg tonus. He suffered from gait difficulty at age 58 years and became unable to walk for a long time at age 62 years. A year later he walked with a cane and was admitted to our hospital for slowly progressive gait disturbance. His MMSE score was 16/30. Neurological examination at age 63 years showed bilateral iliopsoas muscle weakness and atrophy, but leg spasticity was not noted. Tendon reflexes were diminished in all extremities with extensor plantar responses bilaterally. Mild ataxia in the upper extremities was observed. Brain MRI showed mild cerebellar atrophy, whereas spinal MRI revealed no spinal cord atrophy. A nerve conduction study showed normal motor and sensory nerve conduction velocities in the upper and lower extremities, whereas the amplitudes of compound muscle action potentials and sensory nerve action potentials were decreased in all extremities, and F-wave conduction velocities were decreased in the lower extremities. Motor-evoked potential examination revealed prolongation of the central motor conduction times in the corticospinal tracts. Needle EMG showed chronic neurogenic patterns in his legs. A sural nerve biopsy disclosed a decreased number of nerve fibres of large diameter and residual axonal swelling, but no formation of onion bulb-like structure. Peripheral blood examinations showed peroxidase-positive giant granules in granulocytes and reduced natural killer cell activity.

Identification of candidate chromosome areas

We found linkages to a several parts of chromosomes 1 (rs3914503-rs2186205), 2 (rs12614444-rs4035021), 11 (rs11235880-rs120435) and 17 (rs7216464-rs4128515, rs7217461-rs11079098), with maximum cumulative LOD scores of 1.8 (figure 1A). These four chromosome parts did not contain previously reported HSP loci.

Exome sequencing allowed identification of the candidate gene substitutions

Exome sequencing covered 98.65% of the target region with an average sequence depth of 106.24X. All coding sequences in the four candidate chromosomal areas were covered at least eight times. The presence of consanguinity and two asymptomatic parents supported that the patients have homozygous disease-causing mutations. We selected all coding variants in the homozygous regions and then filtered them by discarding variants documented in the dbSNP135 and the 1000 Genomes Project, and heterozygous and synonymous substitutions. We identified three novel homozygous, non-synonymous single nucleotide variants: c.4189T>G (p.F1397V) in *LYST* in the chromosome 1 candidate area (figure 1B), c.5135G>A (p.G1712E) in *FAT3* in the chromosome 11 candidate area and

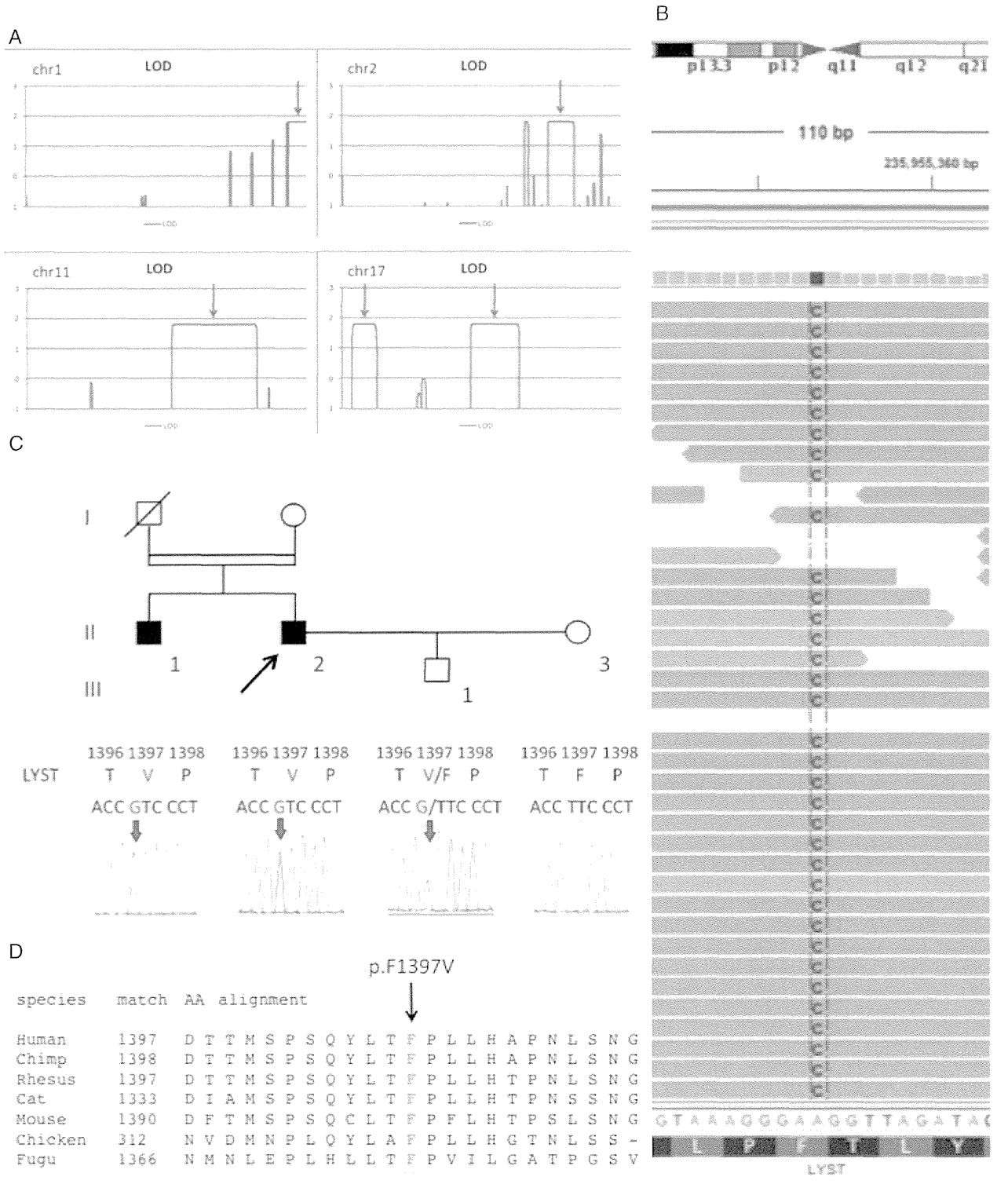

Figure 1 Linkage analysis and mutation of the *lysosomal trafficking regulator* (*LYST*) gene in the family. (A) Linkage analysis involving single nucleotide proteins revealed the highest logarithm of the odds (LOD) scores (about 1.8) in parts of chromosomes 1, 2, 11 and 17 (arrows). These four areas were thought to be candidate areas in which the causative gene was located. (B) Exome sequencing using one patient's DNA identified the mutation of the *LYST* gene on chromosome 1. This mutation was demonstrated with Integrative genomics viewer (IGV).[26] (C) Family tree and Sanger sequencing validation. Sanger sequencing confirmed the homozygous missense mutation (c.4189T>G, p.F1397V) of the *LYST* gene identified in the proband and the affected brother. This mutation was co-segregated with the disease in this family. (D) This amino acid substitution (p.F1397V) is located at a highly conserved residue within the concanavalin A (ConA)-like lectin domain (amino acid numbers 1390–1691) of the LYST protein.

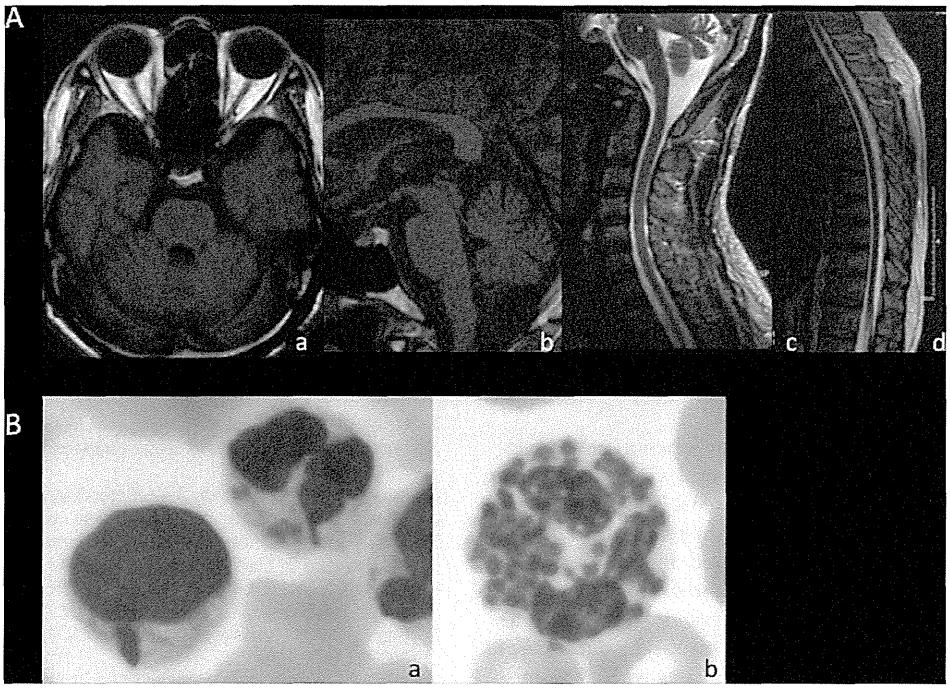

Figure 2 MRI and peripheral blood findings in the proband (II-2). (A) Brain and spinal MRI of the proband. Brain T1-weighted MRI (a, b) showed mild cerebellar atrophy, and spinal T2-weighted MRI (c, d) disclosed mild thoracic cord atrophy. (B) Peripheral blood leucocytes findings in the proband (May–Giemsa stain (a) and peroxidase stain (b)). We could observe large granules in the proband's leucocytes and large peroxidase-positive ones in granulocytes.

c.1045T>C (p.F349L) in *ANGPTL5* in the chromosome 11 candidate area with reference to dbSNP135. These three variations were validated by Sanger sequencing. No such variations were detected in the chromosome 2 and 17 areas. Subsequently, the two candidate gene variants in *FAT3* and *ANGPTL5* could be excluded as polymorphisms because these variants of the two genes were not co-segregated in the normal family members. The another candidate gene variant of *LYST* was predicted to be a functionally deleterious mutation with the prediction programs (PROVEAN, Polyphen-2 and Mutation Taster) and confirmed to be a homozygous missense mutation (c.4189T>G, p.F1397V) on Sanger sequencing in the two patients, II-1 and II-2 (figure 1C). This missense mutation was co-segregated within the family members (figure 1C) and not found in 200 Japanese control genomic DNA samples. This mutation is located at a highly conserved residue (figure 1D) within the concanavalin A (ConA)-like lectin domain (amino acids numbers 1390–1691).[8]

DISCUSSION

The present two patients exhibited spastic paraplegia, peripheral neuropathy and mild cerebellar ataxia with AR transmission. Autosomal recessive hereditary spastic paraplegia (AR-HSP) with cerebellar ataxia and neuropathy is considered to be SPG7 with the *Paraplegin* gene alteration linked to chromosome 16q24.3,[9] SPG21 with the *Maspardin* gene mutation linked to chromosome 15q22.31,[10] SPG27 linked to chromosome 10q22.1–q24.1[11] and SPG30 with the *KIF1A* mutation linked to chromosome 2q37.3.[12 13] However, linkage analysis did not show all reported HSP gene locus linkages, and whole exome sequence analysis did not disclose the *Paraplegin, Maspardin* and *KIF1A* gene mutations. According to these results, we could conclude that the causative gene in this family was not one of the previously reported HSP ones.

Through linkage analysis of the two patients' DNA and whole exome sequencing using one individual's DNA, we could identify a novel homozygous missense mutation in the *LYST* gene. This homozygous mutation was shared by the two patients. We considered the *LYST* gene mutation was causative of the neurological deficits in these two patients because it was co-segregated within their family members, located at a highly conserved amino acid, and not found in the normal controls. Moreover, large granules in leucocytes and reduced natural killer cell activity could support the diagnosis of CHS.

CHS is a rare, AR early-onset disorder characterised by severe immune deficiency, frequent bacterial infections, skin pigmentation or albinism, a bleeding tendency and progressive neurological dysfunction in most cases.[14] It is often complicated by a lymphoproliferative condition called the 'accelerated phase'. A classic diagnostic feature of CHS is enlarged granules in leucocytes, melanocytes, platelets and so forth. Most cases present in early childhood with haematological dysfunction, whereas a small number of cases with the adult form of CHS predominantly exhibit slowly progressive neurological dysfunction without apparent immunodeficiency or a bleeding tendency. Neurological involvement in CHS can include parkinsonism,[15] dementia,[16] cerebellar ataxia, peripheral neuropathy and spastic paraplegia.[17] Although the neurological phenotypes of our cases resembled those previously reported,[17] the main symptom in those patients was cerebellar ataxia that was more severe than that in our cases.

The gene responsible for CHS was identified in 1996, and was called *LYST*.[5 18] The *LYST* gene is a large gene that has 51 coding exons and an open reading frame of 11 403 kb.[6] The LYST protein, which is a large, putative cytosolic protein of 425 kDa (3801 amino acids), is ubiquitously expressed and involved in control of the exocytosis of secretory lysosomes.[5 19] The LYST protein has a BEACH (named after BEige And

Chédiak–Higashi) domain (amino acid numbers 3132–3422),[5] Trp-Asp (WD) 40 repeats (amino acid numbers 3477-3778) and a ConA-like lectin domain (amino acid numbers 1390–1691).[8] The LYST protein has been proposed to act as a scaffold protein in the mediation of fusion or a fission event of vesicles.[20] The mutation in this family (p.F1397V) is located within the ConA-like lectin domain. This domain could be involved in oligosaccharide binding associated with protein trafficking and sorting along the secretory pathway.[8]

Recently, *Drosophila* with a gene mutation of an *LYST* homologue was revealed to exhibit impaired autophagy.[21] The loss of function of some HSP-related proteins, TECPR2[22] and spastizin,[23] caused autophagic dysfunction and induced spastic paraplegia. Therefore, autophagic impairment might have resulted in spastic paraplegia in the CHS patients.

Karim *et al*[24] found apparent genotype–phenotype correlations in CHS, that is, that severe childhood CHS involved a functionally null mutation, whereas missense mutations were seen only in the two later-onset forms. They reported four missense mutations, two of which are located in the ConA-like lectin domain. Our cases correspond to late-onset, slowly progressive neurological CHS with a missense mutation of the *LYST* gene. According to the information on the Japanese cases in the literature,[24] the Japanese adult CHS cases with *LYST* missense mutations (R1563H or V1999D) showed spastic paraplegia, gaze nystagmus and diminished ATRs. Thus, their phenotypes were similar to those of our cases. Moreover, as far as we know, this family had one of the oldest adult CHS cases (onset of 58 years) with a *LYST* gene mutation in the literature. To date, a 57-year-old man has been reported who suffered from sensorimotor polyneuropathy and muscle wasting with a heterozygous *LYST* gene mutation (p.Y2026X).[25]

In this family, the proband showed spastic paraplegia dominantly as well as neuropathy and mild cerebellar ataxia, whereas the brother mainly showed peripheral neuropathy with a positive Babinski sign, cerebellar ataxia and dementia. These two patients did not exhibit parkinsonism. The phenotypic variety in this family might be explained by environmental factors or other modifier gene mutations.

In summary, we could diagnose these patients as having adult CHS presenting spastic paraplegia with neuropathy and cerebellar ataxia. As far as we know, this family includes one of the oldest adult CHS cases in the literature. The clinical spectrum of CHS is broader than previously recognised, and this family shows phenotypic variability. Adult CHS must be considered in the differential diagnosis of AR-HSPs. The linkage analysis and exome sequencing were useful for identifying the causative mutation in this family.

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Contributors HS was responsible for conception and design of the work, acquisition, analysis or interpretation of data, drafting the work and revising the work critically for important intellectual content. JH, TN and MN were responsible for acquisition and analysis of data. IN was responsible for analysis or interpretation of data. MY, KN, KY, S-il, HI, YF and YT were responsible for acquisition, analysis or interpretation of data. JG, ST and YT were responsible for conception and design of the work, interpretation of data and revising the work critically for important intellectual content.

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Data sharing statement Exome sequencing data are available (DDBJ Sequence Read Archive (DRA) accession number DRA000961).

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Autosomal-recessive complicated spastic paraplegia with a novel *lysosomal trafficking regulator* gene mutation

Haruo Shimazaki, Junko Honda, Tametou Naoi, et al.

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Notes

＜ Symposium　05-1 ＞　遺伝性痙性対麻痺の最新情報

遺伝性痙性対麻痺の最新情報

瀧山　嘉久[1)]

要旨：遺伝性痙性対麻痺（hereditary spastic paraplegia; HSP）は下肢の痙縮と筋力低下を呈する神経変性疾患群である．現時点でSPG1～72の遺伝子座と60を超す原因遺伝子が同定されているが，全国多施設共同研究体制であるJapan Spastic Paraplegia Research Consortium（JASPAC）により，本邦HSPの分子疫学が明らかになってきた．さらに，JASPACにより，はじめて*C12orf65*遺伝子変異や*LYST*遺伝子変異がHSPの表現型を呈することが判明した．今後，JASPACの活動がHSPの更なる新規原因遺伝子の同定，分子病態の解明，そして根本的治療法の開発へと繋がることが望まれる．

（臨床神経 2014;54:1009-1011）
Key words：遺伝性痙性対麻痺，JASPAC，遺伝子解析

はじめに

遺伝性痙性対麻痺（hereditary spastic paraplegia; HSP）は，臨床的には緩徐進行性の下肢痙縮と筋力低下を主徴とし，病理学的には脊髄の錐体路，後索，脊髄小脳路の系統変性を主病変とする神経変性症候群である．

随伴症状の有無により，純粋型と複合型に分けられ，前者は通常，痙性対麻痺のみを呈するが，時に膀胱直腸症状，振動覚低下，上肢の腱反射亢進をともなうことがある．後者はニューロパチー，小脳失調，脳梁の菲薄化，精神発達遅延，痙攣，難聴，網膜色素変性症，魚鱗癬などをともなう[1)]．

遺伝形式からは，常染色体優性（AD-HSP），常染色体劣性（AR-HSP），X連鎖性（XL-HSP）に分けられる．その頻度は，AD-HSPが多く，AR-HSPは少なく，XL-HSPはまれである．純粋型はAD-HSPにおいて一般的であり，複合型はAR-HSPやXL-HSPにみとめられやすい．

従来は，Hardingが提唱した臨床像と遺伝形式からみた分類法が受け入れられていたが[2)]，今日では分子遺伝学的分類がなされており，現時点でSPG1～SPG72が分類されている．本稿では，本邦の痙性対麻痺に関する全国多施設共同研究体制である，Japan Spastic Paraplegia Research Consortium（JASPAC）による本邦HSPの分子疫学と新規原因遺伝子の同定など，HSPの最新情報について概説する．

HSP診断基準（案）

本邦ではこれまで痙性対麻痺の診断基準が作成されていなかったので，筆者らは，いわゆる変性疾患としての痙性対麻痺を抽出しようとして診断基準（案）を作成した．今後，この診断基準（案）の感度と特異度の検証が必要であるが，参考までにTable 1に示す[3)]．

JASPAC

JASPACは，本邦HSPの分子疫学と病態の解明，および治療法の開発を目的として，2006年，厚生労働科学研究費補助金難治性疾患克服研究事業運動失調に関する調査研究班（西澤正豊班長）のプロジェクトの1つとして構築され，現在も活動を継続している（事務局は設立当時の自治医科大学神経内科から山梨大学神経内科に異動した）．2014年8月22日現在，全国47都道府県，211施設からHSP　587家系が登録され，index patient 465検体がJASPACに集められている．

本邦HSPの分子疫学

現在，東京大学神経内科で直接塩基配列決定法，CGHアレイによるrearrangement解析法，resequencing microarray解析法を組み合わせてHSPの網羅的遺伝子解析をおこなっている[4)]．

本邦AD-HSP 206家系中SPG4がもっとも多く，78家系（38%）を占めている．以下，SPG3A 11家系（5%），SPG31 10家系（5%），SPG10 3家系（2%），SPG8 1家系（1%）の順である（Fig. 1）．AD-HSPの半数では，既知の遺伝子変異をみとめず，遺伝子型が同定できていない．AR-HSPについては，網羅的遺伝子解析では12%しか遺伝子型が同定できなかった．そこで，当初AR-HSPがうたがわれた116例（JASPAC症例と東京大学神経内科の症例）についてエクソーム解析をおこなったところ，42%で遺伝子変異を同定できており，SPG11 14例（12%），SPG28 5例（4%），SPG46 4例（3%），SPG15 3例（3%）などであり，AR-HSPはきわめてheterogeneousな集団であることが判明した．さらに，家系図からAR-HSPがうたがわれたものの，遺伝子解析によりAD-HSPである症例があることも判明した．

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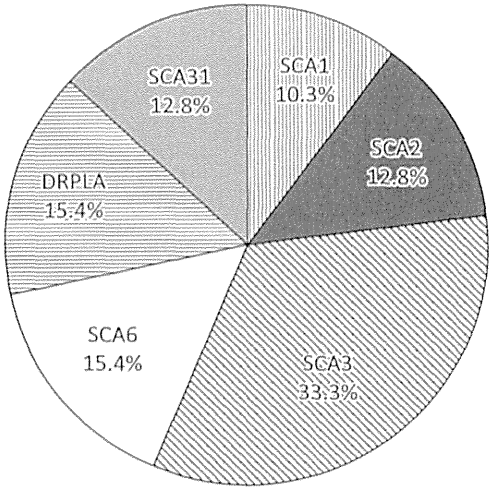

Fig. 1　Percentage of SCA types in our hospital (1998–2012). The graph shows that SCA3 is the most frequent (33.3%) subtype in our hospital. The second most frequent subtypes are DRPLA and SCA6 at the same percentage (15.4%), followed by SCA31 and SCA2 at the same percentage (12.8%) and SCA1 (10.3%). The percentage is calculated with the number of family.

「明らかにあり」,「おそらくあり」,「おそらくなし」,「明らかになし」の4段階の順序変数をもちい，順に +2点，+1点，−1点，−2点と点数化して両病型における平均点をMann-Whitney検定で比較した．遺伝子検査はSCA31の全例とSCA6の1例は東京医科歯科大学で，SCA6の5例は名古屋大学でおこなった．失調の評価にScale for the assessment and rating of ataxia（SARA）を，認知機能評価に改訂版長谷川式簡易知能スケール（HDS-R）をもちいた．

結　果

1．当院におけるSCAの病型の割合（Fig. 1）

最初に当院において，1998年～2012年に診療をおこない遺伝子診断で判明した遺伝性脊髄小脳変性症の内訳を示す．病型の判明した39家系のうち当院ではSCA3がもっとも多く13家系（33.3%）を占め，2番目にDRPLAとSCA6が同率で各6家系（15.4%），続いてSCA31とSCA2が同率で各5家系（12.8%），SCA1が4家系（10.3%）であった．

2．SCA31，SCA6の臨床症候の比較（Table 1）

a．発症年齢

SCA31の発症年齢は平均63.8歳（57～67歳），SCA6の発症年齢は平均40.7歳（28～60歳）で有意差をみとめた．

b．発症時の症候

SCA31の初発神経症候は歩行障害が4例，言語障害が1例，その両者を同時期に自覚したものが1例であった．SCA6の初発神経症候は歩行障害が2例，歩行障害および言語障害を同時期に自覚したものが2例，めまい感が1例，手の震えによる書字障害が1例であった．

c．神経症候

脳神経の所見は，水平方向の眼振はSCA31で全例にみとめたのに対し，SCA6では1例のみにみとめた．下向き眼振はSCA31ではみとめず，SCA6の2例でみとめた．難聴はSCA31では確認できた4例中3例あり，そのうち1例は小脳症候出現より20年以上前の40代から難聴を自覚し，73歳の検査時に両側性に高音域になるにしたがって強い難聴を示した．もう1例では片側の全音域の伝音性難聴と，両側の高音域の難聴がみとめられた．これらの症例の聴性脳幹反応（ABR）のI～V波の潜時はすべて正常であった．SCA6では1例検索したが正常であった．

運動系の所見は，SCA6で四肢筋力低下および筋萎縮をみとめたものが5例中1例あった．SCA31，SCA6ともに全例で経過中に体幹，四肢の小脳失調を呈し，小脳性言語障害をみとめた．筋トーヌスはSCA31の5例で低下していたが，SCA6では低下，正常，亢進がそれぞれ2例ずつ存在した．不随意運動はSCA31では1例のみ振戦をみとめ，SCA6では3例に振戦を，別の1例にミオクローヌスをみとめた．

感覚系では表在覚は全例で正常であった．深部感覚では，振動覚の低下をSCA31の3例，SCA6の5例中4例でみとめ，関節位置覚の障害はSCA6の5例中1例でみとめた．

精神神経症候として，認知機能はSCA31の全例で正常であり，SCA6の3例中1例で低下していた．精神症候として，SCA31の6例では精神症状を示すものはみられなかったが，SCA6では幼稚性，パニック発作，自殺，抑鬱および攻撃性がそれぞれ1例ずつみられ，頻度に有意差をみとめた．

その他の症候として，腱反射はSCA31では低下，正常，亢進がそれぞれ2例ずつ存在したのに対し，SCA6では5例中5例で亢進し有意差をみとめた．Babinski徴候はSCA6の4例中1例のみで陽性であった．自律神経症状はSCA6の1例で排尿障害をみとめた．SCA6ではめまい感を訴えるものが3例中2例存在したが，SCA31では皆無であった．

みとめられた小脳外症候すなわち小脳症状以外の神経症候の数の患者毎の平均値は，SCA31では平均1.5個（0～3個），SCA6では平均4.5個（2～9個）で有意差をみとめた．

d．罹病期間とADLの関係（Fig. 2）

SCA31では罹病期間が長いほどADLが低下していた．SCA6では罹病期間とADLは一定の傾向を示さず，罹病期間が短くても移動に車いすを必要とする患者もみられた．

3．画像所見（Fig. 3）

画像所見を2名の判定者で定性的に検討した．判定者間の一致度は重み付けκ係数＝0.94と高値であった．小脳はいずれの病型でも萎縮をみとめたが，萎縮の程度，部位に差異をみとめなかった．両者とも，とくに小脳虫部の上方で山頂や山腹に相当する部位に萎縮が強い傾向があった．一方，第4脳室の拡大，中小脳脚の萎縮については，SCA6で有意にみとめた．大脳にいずれの病型とも萎縮をみとめなかった．

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※本論文に関連し，開示すべきCOI状態にある企業，組織，団体はいずれも有りません．

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Abstract

Hereditary spastic paraplegia: up to date

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Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of neurodegenerative disorders that are clinically characterized by progressive spasticity and weakness of the lower limbs. HSP genetic loci are designated SPG1-72 in order of their discovery. In 206 Japanese families with autosomal dominant HSP, SPG4 was the most common form, accounting for 38%, followed by SPG3A (5%), SPG31 (5%), SPG10 (2%), and SPG8 (1%). We have identified novel mutations in the *C12orf65* gene and the *LYST* gene in several Japanese families with autosomal recessive HSP. JASPAC will facilitate gene discovery and mechanistic understanding of HSP. The future challenge will be the establishment of treatment of HSP.

(Clin Neurol 2014;54:1009-1011)

Key words: hereditary spastic paraplegia, JASPAC, gene analysis

Neurology® Clinical Practice

Characteristic MRI findings in beta-propeller protein-associated neurodegeneration (BPAN)

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Neurology® Clinical Practice

Cases

Characteristic MRI findings in beta-propeller protein-associated neurodegeneration (BPAN)

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Akiko Onohara, MD
Kimiko Obi, MD, PhD
Kazumasa Shindo, MD, PhD
Hirotomo Saitsu, MD, PhD
Naomichi Matsumoto, MD, PhD
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A 31-year-old woman presented with severe dystonia-parkinsonism. She had nonprogressive psychomotor retardation and cognitive dysfunction from childhood without evidence of dystonia or parkinsonism. At age 30, she then developed severe dystonia and gait disturbance. There was neither dystonia nor parkinsonism before age 30. MRI revealed cerebral atrophy and iron accumulation in the globus pallidus and substantia nigra (figure 1, A–D). The characteristic MRI findings were hyperintensity of the substantia nigra with a central band of hypointensity in T1-weighted axial slices (figure 1, B). Beta-propeller protein-associated neurodegeneration (BPAN) was diagnosed based on MRI findings and identification of a novel heterozygous mutation in the *WDR45* gene (NM_007075.3: c.519+1_519+3del) (figure 2). This is a neurodegeneration involving brain iron accumulation (NBIA) characterized by psychomotor retardation from childhood and dystonia-parkinsonism in midadulthood.[1,2] Although we could not analyze the father's gene since he had died, the mother had no mutation in the *WDR45* gene (figure 2). Thus, it might be a de novo mutation in the *WDR45* gene, as reported previously.[1,2]

L-Dopa/decarboxylase inhibitor treatment (200 mg/day orally) led to improvement of the rigidity and bradykinesia in our patient. She became able to move by herself by crawling on her hands and knees and no longer needed meal assistance. In addition, she became able to utter a few words like "thanks" and "bye," although the dystonia of the lower limbs remained unchanged. Since T1-weighted hyperintensity of the substantia nigra with a central band of hypointensity has not been found in other NBIA disorders, including neuroferritinopathy,

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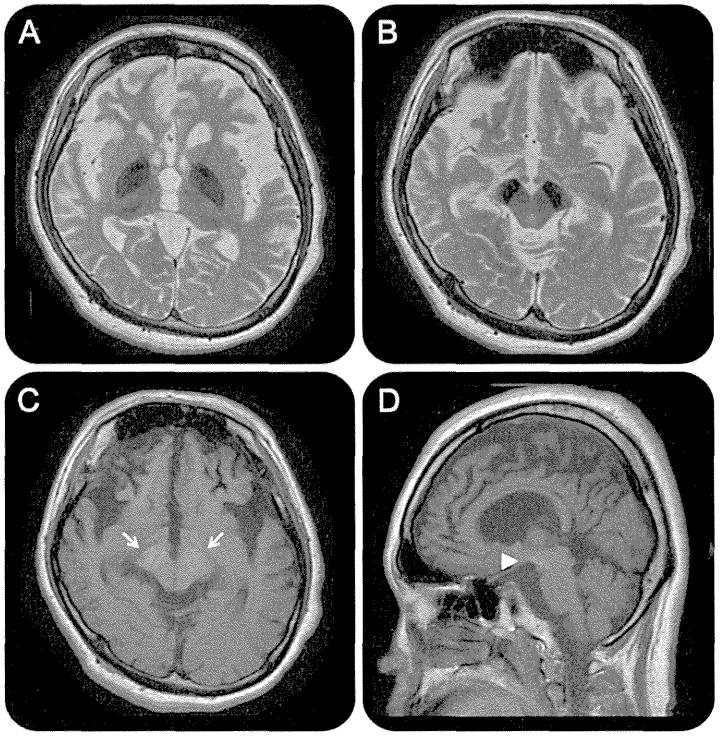
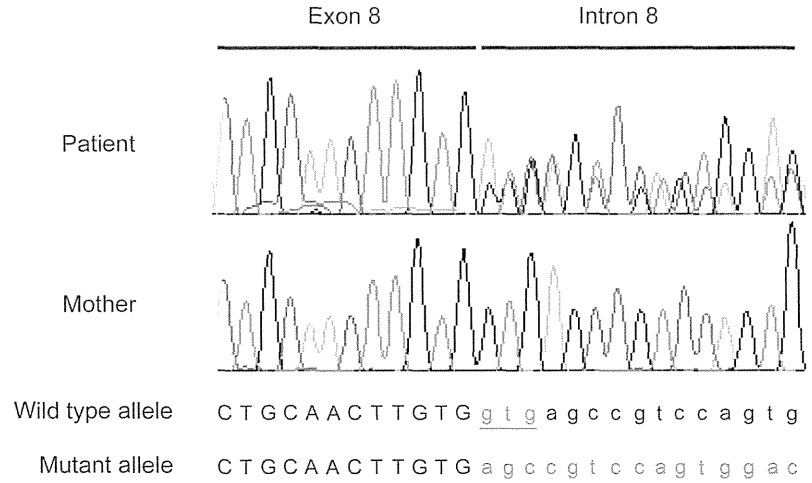
Figure 1 Brain MRI findings in the patient



(A, B) T2-weighted MRI shows marked hypointensity in the globus pallidus and substantia nigra in axial slices. (C) T1-weighted MRI shows hyperintensity of the substantia nigra with a central band of hypointensity in an axial slice (arrows). (D) T1-weighted MRI shows cerebral atrophy and hyperintensity of the substantia nigra (arrowhead) in a sagittal slice.

Figure 2 Identification of a novel heterozygous mutation in the *WDR45* gene



Electropherograms of the patient and her mother using a forward primer are shown. A novel heterozygous mutation (NM_007075.3: c.519+1_519+3del, underlined), which may result in abnormal splicing, was identified in intron 8 (splice donor site) of the *WDR45* gene in the patient, as shown by the double signals due to the 3 bp deletion. Meanwhile, the mother had no mutation in the *WDR45* gene. The colored peaks denote nucleotide bases as follows: black, guanine; red, thymine; blue, cytosine; and green, adenine.

aceruloplasminemia, and pantothenate kinase-associated neurodegeneration, this finding should facilitate the diagnosis of BPAN.

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Unique combination of hyperintense vessel sign on initial FLAIR and delayed vasoconstriction on MRA in reversible cerebral vasoconstriction syndrome: A case report

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Abstract

Background: Reversible cerebral vasoconstriction syndrome is characterized by thunderclap headache and reversible cerebral vasoconstriction on angiographic findings. It can be difficult to diagnose when initial angiography is normal.

Case results: A 30-year-old woman was admitted because of sudden-onset thunderclap headache and seizure on post-partum day 7. Brain MRI on fluid-attenuated inversion recovery (FLAIR) showed hyperintense vessel sign (HVS), which usually means slow flow due to severe proximal arterial stenosis. However, magnetic resonance angiography (MRA) indicated that proximal arteries was normal. After nicardipine treatment, her symptoms improved dramatically. Follow-up FLAIR on day 7 showed complete resolution of HVS, while a series of MRAs revealed reversible multifocal segmental vasoconstriction.

Conclusions: HVS on initial FLAIR is useful for an early diagnosis of reversible cerebral vasoconstriction syndrome. As the delayed vasoconstriction on MRA can be observed, reversible cerebral vasoconstriction syndrome may progress from distal small to proximal larger arteries.

Keywords

Thunderclap headache, hyperintense vessel sign, reversible cerebral vasoconstriction syndrome

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Introduction

Reversible cerebral vasoconstriction syndrome (RCVS) is a condition with clinical and radiological features characterized by sudden-onset severe thunderclap headache and reversible multifocal segmental constriction of cerebral arteries, with or without focal neurological deficits (1). Conventional cerebral angiography and magnetic resonance angiography (MRA) are used for the diagnosis and follow-up of vasoconstriction (1,2). Magnetic resonance imaging (MRI) of cerebral vasoconstriction shows various abnormalities such as non-aneurysmal cortical subarachnoid hemorrhage, intracerebral hemorrhages, cerebral intraparenchymal T2-hyperintense lesions compatible with posterior reversible encephalopathy syndrome, or border-zone cerebral infarctions (1,3). Additionally, hyperintense vessel sign (HVS) on fluid-attenuated inversion recovery (FLAIR) images has been reported in some cases of cerebral vasoconstriction (4–6). HVS is observed as hyperintense signals along the cerebral cortical surfaces, and mainly has been observed in acute stroke patients associated with large-vessel occlusion or severe stenosis (7). Chen et al. have reported in their large series that HVS has been observed in one-fifth of patients with RCVS and associated with severe vasoconstriction (8).

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However, they have not described the difference in timing between HVS and vasoconstriction on MRA. We report a patient with RCVS, showing HVS on initial FLAIR despite the absence of vasoconstriction on initial MRA.

Case report

A 30-year-old woman presented to an emergency room, reporting her worst-ever headache. The headache began as a throbbing pain at the bilateral temples and reached a peak within a few minutes. Her past medical history included migraine without aura. Seven days before onset, the patient had delivered a healthy infant. She had no signs of hypertension or proteinuria during the perinatal period. Clinical examination, cerebrospinal fluid examination and head computed tomography were normal. That night, she again developed a severe headache. Sumatriptan administration only slightly ameliorated the headache. The following morning, she was found by her mother in a confused state, and a few minutes later developed generalized tonic-clonic convulsions. She was immediately admitted to our hospital. She complained of a severe headache, nausea and blurred vision, and was disoriented to time and place. Her systolic blood pressure was 120–150 mmHg, her pulse rate was 100 beats per minute and temperature was 37.2°C. Her pupils were isocoric with 3 mm diameters and reactive to light. She presented with bilateral blindness. Her strength, sensation and tendon reflexes were all intact. Babinski sign and meningeal signs were absent. Routine hematological and biochemical test findings were normal except for a high level of plasma D-dimer (29.4 μg/ml). The anticardiolipin and antinuclear antibodies were negative. Brain magnetic resonance imaging (MRI) T2-weighted and FLAIR images showed multiple hyperintense areas in the bilateral cerebellum, occipital lobes and right frontal lobes. The lesions were isointense on diffusion-weighted images and hyperintense on apparent diffusion coefficient value maps, indicating vasogenic edema. FLAIR imaging also revealed HVS within the subarachnoid space along the cortical surfaces in the bilateral middle cerebral artery territory. Her brain MRA was normal at this time (Figure 1(a) and (b)).

Intravenous nicardipine, phenytoin and glycerol infusion rapidly and dramatically improved the patient's mental status and visual function with the severe headache almost disappearing. Follow-up MRI performed on day 7 of admission revealed complete resolution of HVS as well as the cerebrocerebellar intraparenchymal hyperintense areas, but MRA revealed multifocal segmental stenosis of the basilar, posterior and anterior cerebral arteries (Figure 1(c) and (d)). She was clinically diagnosed with RCVS because MRA on day 16 showed normalization of the arterial vasoconstriction.

Discussion

Our patient developed thunderclap headache in the postpartum period and subsequently generalized tonic-clonic seizure, which may have been triggered by the vasoactive drug sumatriptan. FLAIR sequence of the initial MRI showed posterior reversible encephalopathy syndrome (PRES) and HVS, while the initial MRA was apparently normal. Thus, we were not able to diagnose her condition at first. Because the follow-up MRA revealed reversible cerebral arterial vasoconstriction, we later diagnosed her condition as RCVS-PRES overlap syndrome according to the typical clinical and radiological features.

It is noteworthy that the first MRA did not show significant vasoconstriction. Ghia et al. reported a patient with RCVS who showed delayed vasoconstriction despite resolution of the headache (9). In a recent case series of 67 patients with RCVS, the first MRA did not detect arterial vasoconstriction in 14/67 (21%) patients (3). In this case series, cortical subarachnoid hemorrhage, intracerebral hemorrhages, seizures and posterior reversible encephalopathy syndrome associated with distal arteries, which cannot be detected by MRA (for example, distal cortical branches or pial arteries), were mainly observed during the first week after onset, and border-zone cerebral infarction, probably attributed to large arteries, mainly occurred during the second week (3). To explain these findings, the authors hypothesized that the initial arterial changes might start from distal small arteries and progress toward proximal large arteries. In contrast to MRA, HVS was observed in the FLAIR sequence of only the first MRI in our patient. Some previous reports have similarly described HVS appearing in the early stage of RCVS accompanied by multiple sites of vasoconstriction on MRA (4–6). HVS is indicated by dot-shaped or linear hyperintense signals within the subarachnoid space along the cortical surfaces, and was first described in acute stroke patients associated with proximal large-vessel occlusion or severe stenosis (8). HVS was also observed in patients with multiple cerebral arterial stenoses, including cerebral vasoconstriction in distal small arteries (3). The exact mechanisms of HVS remain unclear. One study suggested that the retrograde leptomeningeal collateral flows might be the origin of HVS (10), while another study indicated that HVS might reflect extremely dilated pial vasculature (11). In either hypothesis, HVS is likely to reflect slow blood flow within distal small arteries dilated to compensate for the hypoperfusion resulting from severe arterial stenosis. Thus, even when MRA indicates that

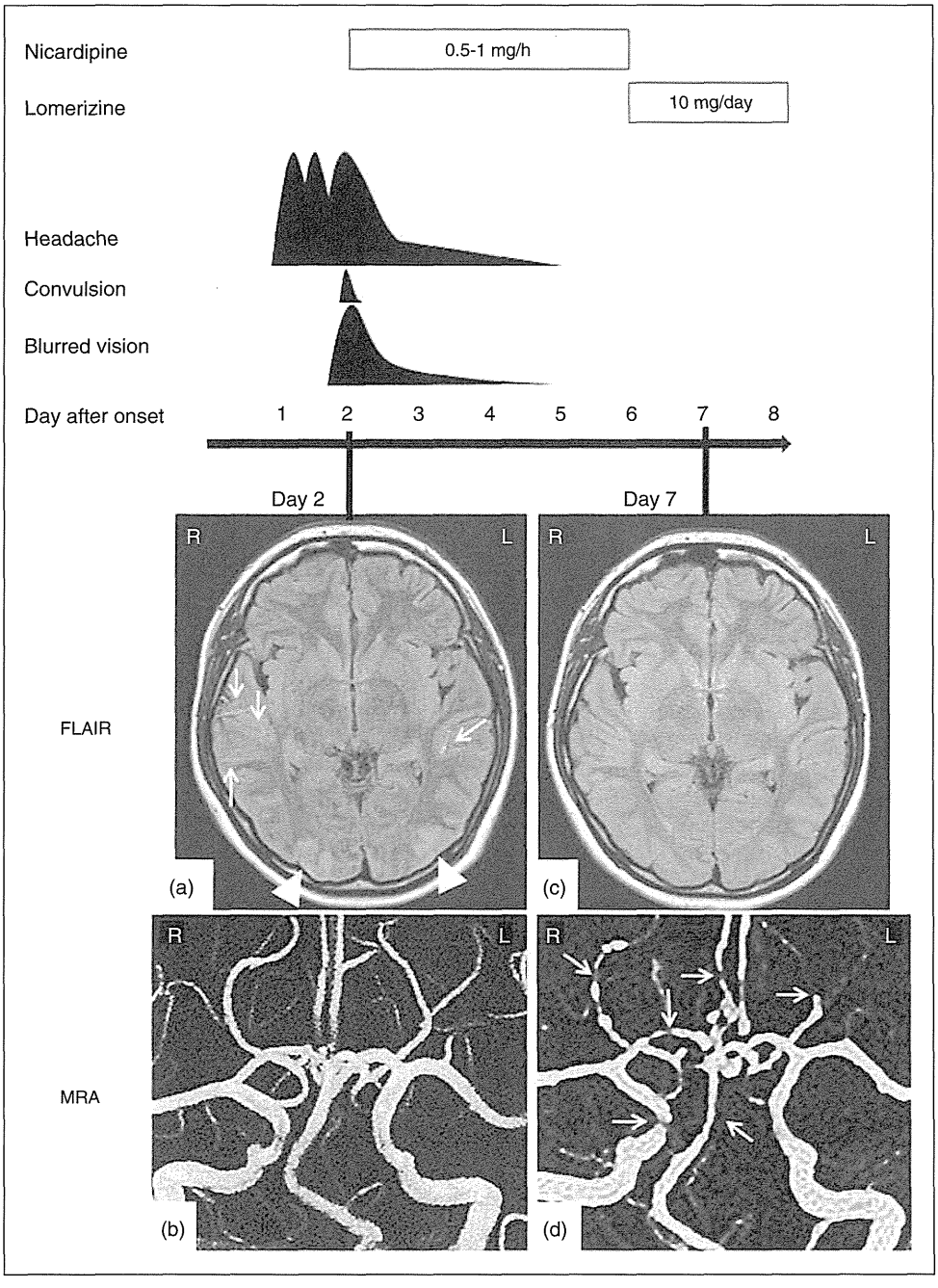

Figure 1. FLAIR image on admission (two days after the onset of thunderclap headache) showed hyperintense areas in the bilateral occipital lobes ((a) arrowheads), and multiple linear hyperintense signals along the cortical surfaces (hyperintense vessel sign) in the bilateral middle cerebral artery territories ((a) arrows). MRA on admission (b) was normal. FLAIR image on day 7 (c) showed complete resolution of the previous abnormalities. MRA on day 7 (d) showed multifocal segmental vasoconstriction (arrows) of the basilar, posterior and anterior cerebral arteries.
FLAIR: fluid-attenuated inversion recovery; MRA: magnetic resonance angiography.

proximal arteries are normal, HVS might suggest the result of more distal vasoconstriction.

In conclusion, the unique radiological findings in our case suggest that vasoconstriction in RCVS may progress from distal small to proximal large arteries. Importantly, HVS on initial FLAIR may be useful for an early diagnosis of RCVS. When HVS is detected in a patient with thunderclap headache, even if initial MRA is normal, RCVS should be considered among the probable differential diagnoses.

Clinical implications

- Hyperintense vessel sign on initial fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) is useful for an early diagnosis of reversible cerebral vasoconstriction syndrome (RCVS).
- The unique radiological findings (combination of hyperintense vessel sign on initial FLAIR and delayed vasoconstriction on magnetic resonance angiography (MRA)) suggest that vasoconstriction in RCVS may progress from distal small to proximal large arteries.

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Conflict of interest

None declared.

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