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Novel pathogenic *VPS13A* gene mutations in Japanese patients with chorea-acanthocytosis

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Abstract

Objective

To identify mutations in *vacuolar protein sorting 13A* (*VPS13A*) for Japanese patients with suspected chorea-acanthocytosis (ChAc).

Methods

We performed a comprehensive mutation screen, including sequencing and copy number variation (CNV) analysis of the *VPS13A* gene, and chorein Western blotting of erythrocyte ghosts. As the results of the analysis, 17 patients were molecularly diagnosed with ChAc. In addition, we investigated the distribution of *VPS13A* gene mutations and clinical symptoms in a total of 39 molecularly diagnosed Japanese patients with ChAc, including 22 previously reported cases.

Results

We identified 11 novel pathogenic mutations, including 1 novel CNV. Excluding 5 patients with the unknown symptoms, 97.1% of patients displayed various neuropsychiatric symptoms or forms of cognitive dysfunction during the course of disease. The patients carrying the 2 major mutations representing over half of the mutations, exon 60–61 deletion and exon 37 c.4411C>T (R1471X), were localized in western Japan.

Conclusions

We identified 13 different mutations in *VPS13A*, including 11 novel mutations, and verified the clinical manifestations in 39 Japanese patients with ChAc.

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Glossary

cDNA = complementary DNA; **ChAc** = chorea-acanthocytosis; **CNV** = copy number variation; **gDNA** = genomic DNA; **qPCR** = quantitative PCR; ***VPS13A*** = *vacuolar protein sorting 13A*.

Chorea-acanthocytosis (ChAc) is a rare, autosomal recessive neurodegenerative disease characterized by adult-onset chorea, involuntary orofacial movement, peripheral acanthocytes, and various neuropsychiatric symptoms with loss-of-function mutations in *vacuolar protein sorting 13A* (*VPS13A*), which consists of 73 exons spanning approximately 250 kb of chromosome 9q21. *VPS13A* encodes a protein with a molecular weight of approximately 360 kDa, named chorein.[1,2] It is estimated that there are likely around 1000 ChAc cases in the world.[3] Although more than 100 patients with ChAc have so far been reported in Japan, the distribution of *VPS13A* mutations in Japan has not been conclusively determined. In this study, we report novel mutations in Japanese patients with ChAc. In addition, we investigate their clinical symptoms.

Methods

Mutation analysis

Coding and flanking regions of *VPS13A* (NC_000009.11) were analyzed by Sanger sequencing on an ABI PRISM 3130 Avant Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA).[4,5] For patients 16 and 17, we performed copy number variation (CNV) analysis that has been previously described in detail.[4,6]

Chorein analysis

We performed chorein Western blotting analysis that has been previously described in detail[4,7] with minor modifications. We used polyvinylidene difluoride membranes from GE Healthcare (Little Chalfont, United Kingdom) or Merck Millipore

Table 1 Profiles of the patients with ChAc in this study

Pt no.	AO	S	C	Ac	Ch	O	Ep	NPS	FS	CK	AS	Res or Ori
1	35	M	ND	?	?	+	−	DI, Pica	?	+	+	Kochi
2	26	F	−	+	?	?	+	OCS, AOP, CDc	Sei	+	+	Tokyo
3	25	F	−	+	+	+	−	Ins	IMTL	+	−	Fukushima
4	18	F	−	+	+	+	+	Del, OCS, DI, CDc	Sei	+	+	Hokkaido
5	18	F	−	+	+	+	+	EI, Hal, FLD, CDc	Sei	+	+	Hokkaido
6	35	M	−	+	+	+	−	DI, EFD	Cho	+	+	Tokyo
7	34	M	−	+	+	+	−	?	GD	+	+	Hyogo
8	39	M	−	+	+	+	−	CDc	OIM	+	+	Nagano
9	33	F	−	+	?	+	+	LOM, Vio, CDc	Sei	−	+	Nagano
10	42	F	−	+	?	−	+	Dem	Sei	+	+	Akita
11	36	F	+	?	+	−	−	OCS, CDc	OCS	+	+	Saitama
12	25	F	−	+	+	+	−	Cop	OIM	+	+	Kagawa
13	25	F	−	+	+	+	+	Mon, CDc	Sei	+	+	Ibaraki
14	20s	F	−	+	+	+	−	CDc	Cho	+	+	Shizuoka
15	37	M	−	+	+	+	?	Mon	OIM	+	+	Tokyo
16	23	M	−	+	+	+	+	Irr, CDc	OIM	+	+	Nara
17	26	F	−	+	+	+	+	CDc	Sei	+	+	Nara

Abbreviations: ? = unknown; Ac = acanthocyte; AO = age at onset of first signs or symptoms (y), S = sex; AOP = alteration of personality; AS = atrophy of the corpus striatum on MRI or CT; C = chorein; CDc = cognitive decline; Ch = chorea; CK = elevated creatine kinase; Cop = coprolalia; Del = delusion; Dem = dementia; DI = disinhibition; EFD = executive function disorder; EI = emotional instability; Ep = epileptic episode; F = female; FLD = frontal lobe dysfunction; FS = first signs or symptoms; GD = gait disturbance; Hal = hallucination; IMTL = involuntary movement of the tongue and limbs; Ins = insomnia; Irr = irritability; LOM = lack of motivation; M = male; Mon = monologue; ND = not determined; NPS = neuropsychiatric symptom; OCS = obsessive-compulsive syndrome; OIM = orofacial involuntary movement; Pt = patient; Res or Ori = place of residence or origin (Japanese prefecture); Sei = seizure; Vio = violence.

Table 2 *VPS13A* mutations identified in this study

Mutation no.	Position	DNA change[b]	Protein change[b]	Type of mutation	Patient ID (genotype)
1[a]	Exon 3	c.145-2A>T	Unknown	Splice	Pt 2 (ht)
2[a]	Exon 11	c.799C>T	p.R267X	Nonsense	Pt 10 (hm)
3[a]	Exon 25	c.2532dupT	p.D845X	Nonsense	Pt 12 (ht)
4[a]	Exon 25	c.2593C>T	p.R825X	Nonsense	Pt 6 (ht)
5[a]	Exon 26	c.2824+1 G>T	Unknown	Splice	Pt 4 (ht), Pt 5 (ht)
6[a]	Exon 33	c.3562C>T	p.Q1182X	Nonsense	Pt 16 (ht), Pt 17 (ht)
7[a]	Exon 36-45	c.4115-459_5991+6444dup[c]	Unknown	Large duplication	Pt 16 (ht), Pt 17 (ht)
8	Exon 37	c.4411C>T	p.R1471X	Nonsense	Pt 3 (hm), Pt 6 (hm), Pt 7 (hm), Pt 8 (hm), Pt 9 (hm), Pt 13 (hm)
9[a]	Exon 45	c.5881C>T	p.R1961X	Nonsense	Pt 14 (hm)
10[a]	Exon 60	c.8325 G>A	p.K2775K	Splice	Pt 4 (ht), Pt 5 (ht)
11	Exon 60-61	c.8211+1232_8472-245delinsTC	p.V2738AfsX5	Large deletion	Pt 1 (hm), Pt 2 (ht), Pt 6 (ht), Pt 11 (ht), Pt 15 (hm)
12[a]	Exon 63	c.8653dupT	p.Y2885LfsX2	Small insertion	Pt 12 (ht)
13[a]	Exon 63	c.8667+3A>T	Unknown	Splice	Pt 11 (ht)

Abbreviations: hm = homozygous; ht = heterozygous; Pt = patient.
[a] Novel mutation.
[b] Mutations are described according to the nomenclature recommended by the Human Genome Variation Society (hgvs.org).
[c] The DNA change was predicted by the sequencing of the duplication breakpoints.

(Carrigtwohill, County Cork, Ireland). We used 2 primary antibodies, a commercially available rabbit polyclonal antibody against chorein (NBP1-85641; Novus Biologicals, Littleton, CO) and a generated rabbit polyclonal antibody against a synthetic oligopeptide antigen corresponding to amino acid residues 1816–1830 (ESDPEEENYKVPEYK) encoded by exon 43 of the *VPS13A* gene (Asahi Techno Glass, Chiba, Japan). Images were recorded by digital analyzers (Fujifilm LAS-1000; Fujifilm, Tokyo, Japan, or Fusion-Solo.7S; Vilber Lourmat, Collégien, France).

Patients

As the results of mutation analysis and chorein analysis, 17 Japanese patients were molecularly diagnosed with ChAc (table 1). We extracted the patient's symptoms based on the clinical records.

Standard protocol approvals, registrations, and patient consents

Total DNA, RNA, and erythrocyte membrane protein from peripheral blood samples were taken from participants who had given written informed consent. Total DNA and RNA from postmortem brains were collected after written informed consent was obtained from a family member. The research protocol and consent form were approved by the Institutional Review Board of Kagoshima University.

Data availability statement

The data sets pertaining to the current study are available from the corresponding author upon reasonable request.

Results

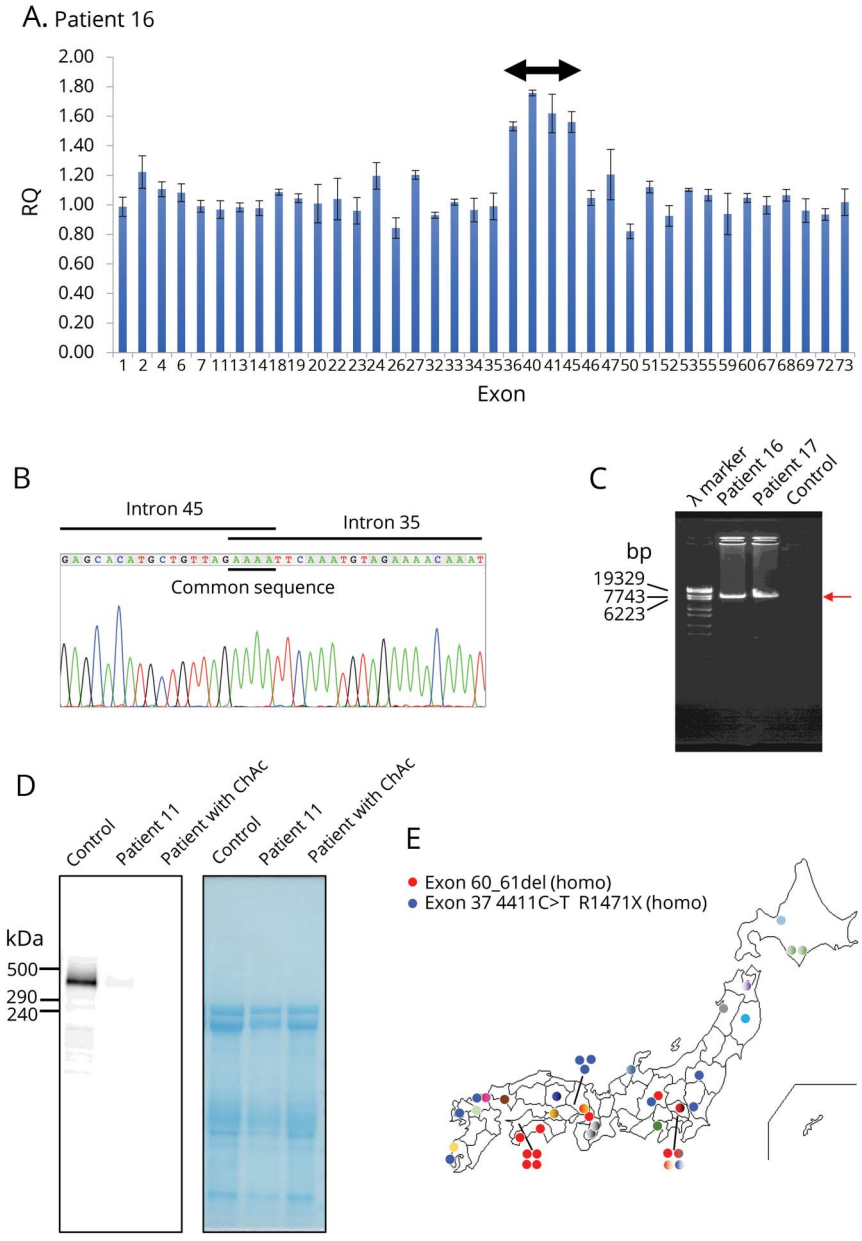
Mutations identified by Sanger sequencing analysis

Using Sanger sequencing, we identified 10 novel mutations and 2 previously reported mutations in 15 patients (table 2). These comprised homozygous or compound heterozygous mutations. Five novel nonsense mutations (799C>T, 2532dupT, 2593C>T, 3562C>T, and 5881C>T) were found in 6 patients. In addition, 4 splice site mutations were found among 4 patients. These splice site mutations (145-2A>T, 2824+1 G>T, 8325G>A, and 8667+3A>T) were predicted to lead to exon skipping because of the loss of a functional splice acceptor or donor site. Exon skipping events in exons 3, 26, and 60 caused by 145-2A>T, 2824+1 G>T, and 8325G>A, respectively, were predicted to cause a frameshift resulting in a premature stop codon. On the other hand, exon 63 skipping caused by 8667+3A>T does not result in a frameshift because exon 63 consists of 114 bp multiples of codon length. Nonsense mutation of 4411C>T in exon 37 and gross deletion of exons 60–61, which have been previously reported,[1,4,8] were found in 6 and 5 patients, respectively. A single nucleotide insertion mutation, which would cause a frameshift and premature stop codon, was found in patient 12.

Mutations identified by CNV analysis

CNV analysis was performed in samples from patients 16 and 17, for whom only a single heterozygous mutation was found by Sanger sequencing analysis. Quantitative PCR (qPCR)

Figure Results of duplication analysis and patient 11's chorein analysis and geographical distribution of *VPS13A* mutations



Results of qPCR for each exon of the *VPS13A* gene (A), results of genomic PCR performed with a forward primer located in intron 45 and reverse primer located in intron 35 (B), breakpoint in genomic sequence (C), Western blotting for patient 11 (D), and geographical distribution of *VPS13A* mutations (E). (A) The RQ value of normal controls is approximately 1.0. The extent of a predicted duplication is indicated by arrows. The figure shows the results of qPCR for each exon of the *VPS13A* gene in patient 16. Comparable results were observed in patient 17. These results suggest heterozygous duplication of exon 36–45. (B) A direct connection between introns 45 and 35 was observed in the genomic DNA of patients. In this connection, a repeated AAAA sequence, which was common between the 5′ end of intron 35 and the 3′ end of intron 45, was observed. (C) Forward primer was located in intron 45. Reverse primer was located in intron 35. Genomic PCR using this combination of primers led to an approximately 7,300 bp PCR product (theoretically 7,319 bp long; arrow) including the predicted junction in 2 patients, but no PCR product in the control. (D) Equal loading was shown by staining with Memcode Reversible Protein Stain (Thermo Fisher Scientific, Waltham, MA), shown in the right panel. Chorein immunoreactivity at 360 kDa was observed in the normal control, but not in patients with ChAc other than patient 11. A considerable reduction in chorein levels was observed for patient 11. (E) Solid-colored circles represent patients who have homozygous mutations. Gradient-colored circles represent patients who have heterozygous mutations. Single red circles indicate exon 60–61 deletion, and single blue circles indicate exon 37 4411C>T mutations. The patients carrying these 2 mutations are localized in western Japan. Some patients who could not identify their ancestral origin provided their address. The map was obtained from aoki2.si.gunma-u.ac.jp/map/map.cgi. ChAc = chorea-acanthocytosis; qPCR = quantitative PCR; *VPS13A* = *vacuolar protein sorting 13A*.

and long-range PCR suggested a single gross duplication of exons 36–45 because the relative quantification values for these exons were approximately 1.5 fold (figure, A). Consequently, we performed individually designed PCR assays for both patients to enable sequencing of the duplication breakpoints. Sanger sequencing analysis, in which the PCR template included the junction of the duplication, revealed an abnormal sequence connecting exons 45 and 36 (figure, B). Long-range PCR of gDNA covering the junction between exons 45 and 36 in both patients revealed bands corresponding to approximately 7,300 bp (figure, C). Exons 36–45 were tandemly duplicated, according to cDNA sequencing. The cDNA length of the duplication was 3754 bp, which would cause a frameshift and premature stop codon.

Chorein analysis

We performed chorein Western blotting of erythrocyte membranes of 16 patients. Western blotting revealed the complete absence of chorein in 15 patients. However, in patient 11, chorein immunoreactivity was markedly reduced, although the chorein band remained faintly present (figure, D).

Summary of 39 patients with ChAc

A summary of the distribution of *VPS13A* gene mutations and clinical symptoms in a total of 39 Japanese patients with ChAc, including 22 previously reported cases,[4] can be given as follows: (1) average onset age was 29.9 ± 7.0 years; (2) the main symptoms at onset were involuntary movements,

epilepsy, neuropsychiatric symptoms, and/or cognitive dysfunction; (3) excluding 4 patients with the unknown data, all patients showed peripheral acanthocytosis; (4) excluding 2 patients with the unknown imaging results, 97.3% of patients showed atrophy of bilateral caudate heads in brain MRI or CT; (5) excluding 5 patients with the unknown symptoms, 97.1% of patients showed various psychiatric symptoms or forms of cognitive dysfunction; (6) excluding 5 patients with the unknown symptoms, 94.3% of patients showed involuntary orofacial movement; (7) excluding 2 patients with the unknown data, 91.9% of patients showed elevated creatine kinase; (8) excluding 6 patients with the unknown symptoms, 90.9% of patients showed chorea affecting all 4 limbs and trunk; (9) 55.1% of the mutations in Japanese patients with ChAc carried the 2 major mutations, exon 37 4411C>T (R1471X) and deletion of exons 60–61; and (10) there were individually different mutations in the remaining 44.9% of Japanese patients with ChAc.

Discussion

In the present study, we identified 11 novel pathogenic mutations and 2 previously reported mutations[1,4,8] in 17 patients with ChAc and verified the clinical manifestations in 39 Japanese patients with ChAc. These mutations were distributed throughout the *VPS13A* gene, as were those in previous reports.[4,8] Although we could not identify genotype-phenotype correlations, over a half of the Japanese patients with ChAc carried exon 37 4411C>T (R1471X) or deletion of exons 60–61. The patients carrying these mutations were mainly localized in Tokyo and western Japan, suggesting partial founder effects (figure, E).

In the CNV analysis, we found c.4115-459_5991+6444dup. At the break point junction, a repeated AAAA sequence, which was common between the 5′ end of intron 35 and the 3′ end of intron 45, was observed. This is presumed to be a microhomology-mediated break-induced replication.[9]

Patient 11 carried an exon-intron junction mutation resulting in the removal of exon 63 during splicing. Although exon 63 consists of 114 bp with multiple codon lengths, chorein Western blotting revealed a considerable reduction of chorein in patient 11 (figure, D). Because the region of chorein corresponding to exon 63 contains a tetratricopeptide repeat motif, which has been reported to be involved in protein-protein interaction domains, we suggest that exon 63 is essential in the critically important protein interaction function of chorein.

In addition to the motor symptoms, patients with ChAc displayed high frequency of psychiatric symptoms, which may explain the previous report that *VPS13A* mutations predispose individuals to psychiatric disorders.[6]

In the present study, we summarized the distribution of *VPS13A* mutations and manifestations in Japanese patients with molecularly diagnosed ChAc. To understand the natural disease history and for accurate prediction of ChAc prognosis, much longer monitoring periods of the disease course are required.

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Disclosure

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Continued

Appendix *(continued)*

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