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なし

G.知的財産権の出願・登録状況
(予定を含む。)

1. 特許取得
なし

2. 実用新案登録
なし

3.その他

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

難治性疾患克服研究事業「孤発性パーキンソン病遺伝子同定と創薬・テーラーメード研究」班

研究分担者　香川県立中央病院神経内科　主任部長　山本光利

**研究要旨**

対象となったパーキンソン病患者数は224例であった。生活上支障となる程度の姿勢異常を示した患者は約15%であり、重症ほど割合は増加した。また脊椎の圧迫骨折が危険因子としてあげられた。薬剤による誘発、悪化例が3例認められた。薬剤との関連について今後さらに啓示的調査を行い、関連を調査する計画である。

**A.研究目的**

テーラーメイド医療の一環として副作用の事前予防は重要である。近年ドパミンアゴニストによる副作用が注目されているが、今回は脊柱異常とドパミン受容体刺激薬との関連が注目されている。副作用の有無とD3受容体SNPとの関連を調査する。

**B.研究方法**

当施設における同意の得られたパーキンソン病患者全例において臨床背景、パーキンソン病重症度の評価、姿勢異常の評価、AOLの状態、姿勢異常例ではCT等の画像検査、骨代謝関連メーカーの検索も併せて実施した。

(倫理面への配慮)

IRB 承認の下に書面における同意を得た実施した。

**C.研究結果**

対象となったパーキンソン病患者数は224例であった。生活上支障となる程度の姿勢異常を示した患者は約15%であり、重症ほど割合は増加した。また脊椎の圧迫骨折が危険因子としてあげられた。薬剤による誘発、悪化例が3例認められた。3例とも薬剤中止により回復した。

**D.考察**

パーキンソン病での姿勢異常は生活に支障を来す程度の患者は15%程度であった。しかし、患者のQOLにどの程度影響を及ぼしているかは今後の解析に待たねばならない。

**E.結論**

パーキンソン病における姿勢異常と薬剤の関連は明確でないが、明らかに薬剤誘発例が存在しているので、薬理作用の検討や遺伝的背景等を含めての調査が必要である。

**F.研究発表**

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G.知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得

なし。

2. 実用新案登録

なし。

3.その他

なし。

# III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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# IV. 研究成果の刊行物・別刷

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

nature

# Pathogenic exon-trapping by SVA retrotransposon and rescue in Fukuyama muscular dystrophy

Mariko Taniguchi-Ikeda[1,2]*, Kazuhiro Kobayashi[1]*, Motoi Kanagawa[1], Chih-chieh Yu[1], Kouhei Mori[1], Tetsuya Oda[1], Atsushi Kuga[1], Hiroki Kurahashi[3], Hasan O. Akman[4], Salvatore DiMauro[4], Ryuji Kaji[5], Toshifumi Yokota[6], Shin'ichi Takeda[7] & Tatsushi Toda[1]

[1]*Division of Neurology/Molecular Brain Science, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan.* [2]*Division of General Pediatrics, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan.* [3]*Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Aichi 470-1192, Japan.* [4]*Department of Neurology, Columbia University Medical Center,NewYork, NY10032, USA.* [5]*Department of Clinical Neuroscience, The University of Tokushima Graduate School, Tokushima 770-8503, Japan.* [6]*Department of Medical Genetics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2H7, Canada.* [7]*Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo 187-8502, Japan.*

**These authors contributed equally to this work.*

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# LETTER

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# Pathogenic exon-trapping by SVA retrotransposon and rescue in Fukuyama muscular dystrophy

Mariko Taniguchi-Ikeda[1,2]*, Kazuhiro Kobayashi[1]*, Motoi Kanagawa[1], Chih-chieh Yu[1], Kouhei Mori[1], Tetsuya Oda[1], Atsushi Kuga[1], Hiroki Kurahashi[3], Hasan O. Akman[4], Salvatore DiMauro[4], Ryuji Kaji[5], Toshifumi Yokota[6], Shin'ichi Takeda[7] & Tatsushi Toda[1]

**Fukuyama muscular dystrophy (FCMD; MIM253800), one of the most common autosomal recessive disorders in Japan, was the first human disease found to result from ancestral insertion of a SINE-VNTR-*Alu* (SVA) retrotransposon into a causative gene[1–3]. In FCMD, the SVA insertion occurs in the 3′ untranslated region (UTR) of the *fukutin* gene. The pathogenic mechanism for FCMD is unknown, and no effective clinical treatments exist. Here we show that aberrant messenger RNA (mRNA) splicing, induced by SVA exon-trapping, underlies the molecular pathogenesis of FCMD. Quantitative mRNA analysis pinpointed a region that was missing from transcripts in patients with FCMD. This region spans part of the 3′ end of the fukutin coding region, a proximal part of the 3′ UTR and the SVA insertion. Correspondingly, *fukutin* mRNA transcripts in patients with FCMD and SVA knock-in model mice were shorter than the expected length. Sequence analysis revealed an abnormal splicing event, provoked by a strong acceptor site in SVA and a rare alternative donor site in *fukutin* exon 10. The resulting product truncates the fukutin carboxy (C) terminus and adds 129 amino acids encoded by the SVA. Introduction of antisense oligonucleotides (AONs) targeting the splice acceptor, the predicted exonic splicing enhancer and the intronic splicing enhancer prevented pathogenic exon-trapping by SVA in cells of patients with FCMD and model mice, rescuing normal *fukutin* mRNA expression and protein production. AON treatment also restored fukutin functions, including *O*-glycosylation of α-dystroglycan (α-DG) and laminin binding by α-DG. Moreover, we observe exon-trapping in other SVA insertions associated with disease (hypercholesterolemia[4], neutral lipid storage disease[5]) and human-specific SVA insertion in a novel gene. Thus, although splicing into SVA is known[6–8], we have discovered in human disease a role for SVA-mediated exon-trapping and demonstrated the promise of splicing modulation therapy as the first radical clinical treatment for FCMD and other SVA-mediated diseases.**

FCMD (incidence 1/34,000 births) shares phenotypic similarities with other severe muscular dystrophies, including muscle–eye–brain disease and Walker–Warburg syndrome. All show deficiencies in *O*-glycosylation of α-DG, an extracellular protein anchored on the plasma membrane. Insufficient *O*-glycosylation interferes with the ability of α-DG to interact with extracellular matrix proteins such as laminin[9,10]. For this reason, FCMD, muscle–eye–brain disease and Walker–Warburg syndrome are categorized as 'α-dystroglycanopathies (α-DGpathy)'[10]; so far, no effective treatments exist for these conditions. SVA is a hominid-specific, composite non-coding retrotransposon that contains SINE (short interspersed sequence), VNTR (variable number of tandem repeat), and *Alu* sequences. It is still active in humans, polymorphic and mobilized by the human LINE-1 *in trans*[6,11–15].

In previous work, we showed that *fukutin* mRNA (10 exons, 7.4- and 6.4-kilobase (kb) cDNAs in size with two poly-A sites, 461-amino-acid protein with calculated molecular mass of 53.7 kDa) was not detectable by northern blot analysis in patients with FCMD carrying the SVA insertion[2]. To investigate the aetiology of this decreased expression, we have now analysed whole *fukutin* mRNA in lymphoblasts from patients with FCMD using quantitative PCR with reverse transcription (qRT–PCR). PCR products corresponding to the protein-coding region of *fukutin*, as well as those including sequences in the distal part of the 3′ UTR (and thus downstream of the SVA insertion), were similar in abundance to those from an unaffected control (Fig. 1a). However, products located at sequence positions within the 3′ UTR were markedly decreased relative to the control. From these results and along with previous reports of many 3′ and 5′ splice sites within SVA elements[6–8], we hypothesized that abnormal splicing occurs somewhere between the end of the fukutin protein-coding region and the SVA insertion.

We then performed long-range RT–PCR using primers that flank the region corresponding to decreased expression. In patients with FCMD, we detected a single 3-kb PCR product, which is shorter than the 5-kb product seen in the normal control (Fig. 1b). This observation was consistent in several tissue types from patients with FCMD (Supplementary Fig. 1). PCR from genomic DNA produced an 8-kb product in patients with FCMD, compared with a 5-kb product in the control (Fig. 1b). Sequence analysis of the 3-kb product from FCMD cDNA revealed a splicing event (Supplementary Fig. 2). This event generates a new donor-side breakpoint within the final coding exon (exon 10), located 116 base pairs (bp) upstream from the authentic stop codon. A rare alternative donor site at that position is activated and trapped by an alternative acceptor site located within the inserted SVA, creating an additional and aberrant exonic sequence (exon 11) (Fig. 1c). The acceptor-side breakpoint is located 274 bp downstream from the start of the SVA insertion, between ag and TC (Fig. 1c). The acceptor site has not been described in the previous reports of SVA splicing[6,7]. This location is preceded by a pyrimidine-rich stretch, the SVA $(TCTCCC)_{41}$ hexamer at the 5′ end of the SVA element, with a possible favourable branch point. Predicted exonic splicing enhancer sites occur around 70 bp downstream from the new acceptor site. We confirmed that the aberrant splicing event can be abolished by replacing AG with GG at the acceptor junction in cultured cells transfected with a *fukutin* construct carrying SVA insertion (Supplementary Fig. 3). *Fukutin* expression was not altered by cycloheximide treatment, indicating that the transcript was not subject to nonsense-mediated mRNA decay, possibly because this exon-trapping occurred within the last

[1]Division of Neurology/Molecular Brain Science, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan. [2]Division of General Pediatrics, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan. [3]Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Aichi 470-1192, Japan. [4]Department of Neurology, Columbia University Medical Center, New York, NY 10032, USA. [5]Department of Clinical Neuroscience, The University of Tokushima Graduate School, Tokushima 770-8503, Japan. [6]Department of Medical Genetics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2H7, Canada. [7]Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo 187-8502, Japan.
*These authors contributed equally to this work.

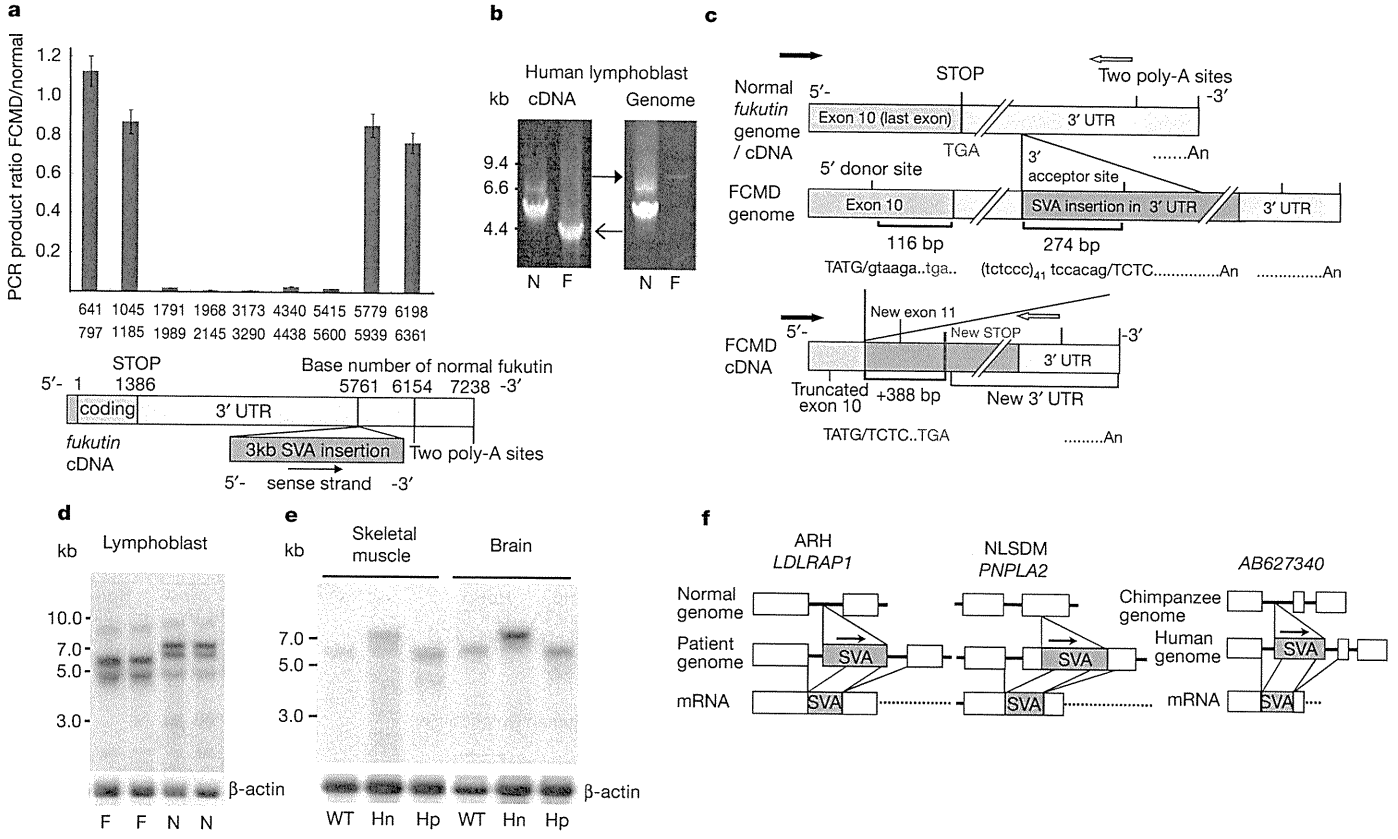


**Figure 1 | An SVA retrotransposal insertion induces abnormal splicing in FCMD.** **a**, Expression analysis of various regions of *fukutin* mRNA in lymphoblasts. Grey bar, the ratio of RT–PCR product in patients with FCMD relative to the normal control; numbers on the *x* axis, nucleotide positions of both forward and reverse primers in *fukutin*. Error bars, s.e.m. **b**, Long-range PCR using primers flanking the expression-decreasing area (nucleotide position 1,061–5,941) detected a 3-kb PCR product in FCMD lymphoblast cDNA (open arrow) and an 8-kb product in FCMD genomic DNA (filled arrow). In the normal control, cDNA and genomic DNA both showed 5-kb PCR products. The 8-kb band was weak, probably because VNTR region of SVA is GC-rich (82%). **c**, Representation of genomic DNA and cDNA in FCMD. Black and white arrows, forward and reverse sequencing primers. The intronic sequence in FCMD is indicated in lower case. The authentic stop codon is coloured red, and the new stop codon is coloured blue. **d, e**, Northern blot analysis of *fukutin* in human lymphoblasts (**d**) and model mice (**e**); F, FCMD; N, normal control. The wild-type mouse *fukutin* mRNA was detected at a size of 6.1 kb. Both skeletal muscle (left) and brain (right) showed smaller, abnormal bands in Hp/Hp mice. WT, wild type; Hn, Hn/Hn mice; Hp, Hp/Hp mice. **f**, Representation of genomic DNA and cDNA in ARH (*LDLRAP1*, left), NLSDM (*PNPLA2*, middle) and human (*AB627340*, right).

exon, and the new stop codon exists downstream of the new last exon–exon junction (Supplementary Fig. 4).

We have recently generated knock-in mice that carry a humanized fukutin exon 10, which either includes (Hp allele) or excludes (Hn allele) the SVA insertion, and bred these strains with heterozygous fukutin knockout mice to obtain compound heterozygotes (Hp/−)[16]. Knock-in mice that are homozygous (Hp/Hp) and compound heterozygous (Hp/−) are representative of the human FCMD alleles. These mice exhibit hypoglycosylation of α-DG in skeletal muscle, which is the most significant characteristic in α-DGpathy[16]. Quantitative RT–PCR in various tissues from Hp/Hp mice revealed an aberrant splicing pattern identical to that seen in human patients (Supplementary Fig. 5). Northern blot analysis detected abnormally spliced *fukutin* mRNA species at the expected sizes of 5.6 and 4.6 kb in patients with FCMD, whereas the normal *fukutin* mRNAs appeared at 7.4 and 6.4 kb (Fig. 1d and Methods). We replicated these results in the knock-in model mice (Fig. 1e and Supplementary Fig. 6a). The consistent observations between patients with FCMD and knock-in model mice lead us to conclude that a splicing abnormality underlies the pathogenesis of FCMD.

Abnormal splicing excises the authentic stop codon and produces another stop codon located 388 bp downstream from the 5′ side of the new exon 11 (Fig. 1c). The predicted protein lacks the C-terminal 38 amino acids of fukutin, instead containing 129 amino acids derived from the SVA sequence (Supplementary Fig. 7). Endogenous fukutin is scarce and difficult to detect; however, we were able to identify both normal and aberrant forms of the protein in human and mouse using immunoprecipitation followed by western blot analysis. The abnormal fukutin protein in FCMD displayed the predicted mobility shift (Fig. 2a–c and Supplementary Fig. 6b).

We introduced normal and aberrantly spliced *fukutin* cDNA constructs into mammalian cell lines. Whereas normal fukutin localized to the Golgi apparatus, the aberrantly spliced fukutin protein is displaced completely from the Golgi to the endoplasmic reticulum (Fig. 2d and Supplementary Fig. 8). Further examination showed that a *fukutin* construct lacking the C-terminal 38 amino acids also mislocalized to the endoplasmic reticulum (Fig. 2d and Supplementary Fig. 8), suggesting that the C-terminal domain of fukutin is important for localization to the Golgi. Thus, impairment of this domain may lead to fukutin dysfunction in FCMD. The mislocalization is unlikely to be toxic because FCMD is an autosomal recessive disease and heterozygous carriers of the SVA insertion have no symptoms.

We next tested if exon-trapping occurs in other diseases with SVA insertion[6]. In a patient with autosomal recessive hypercholesterolemia (ARH), a 2.6-kb SVA was inserted within intron 1 of the *LDLRAP1* gene[4]. A patient with lipid storage disease with subclinical myopathy (NLSDM) also had a 1.9-kb SVA insertion in exon 3 of the *PNPLA2* gene[5]. We found abnormally spliced products induced by SVA exon-trapping in these patients' fibroblast (Fig. 1f left and middle panels, Supplementary Figs 9 and 10, and Supplementary Table 1). Cycloheximide treatment to fibroblasts from these patients increased expression of the genes (Supplementary Figs 9a and 10a), suggesting

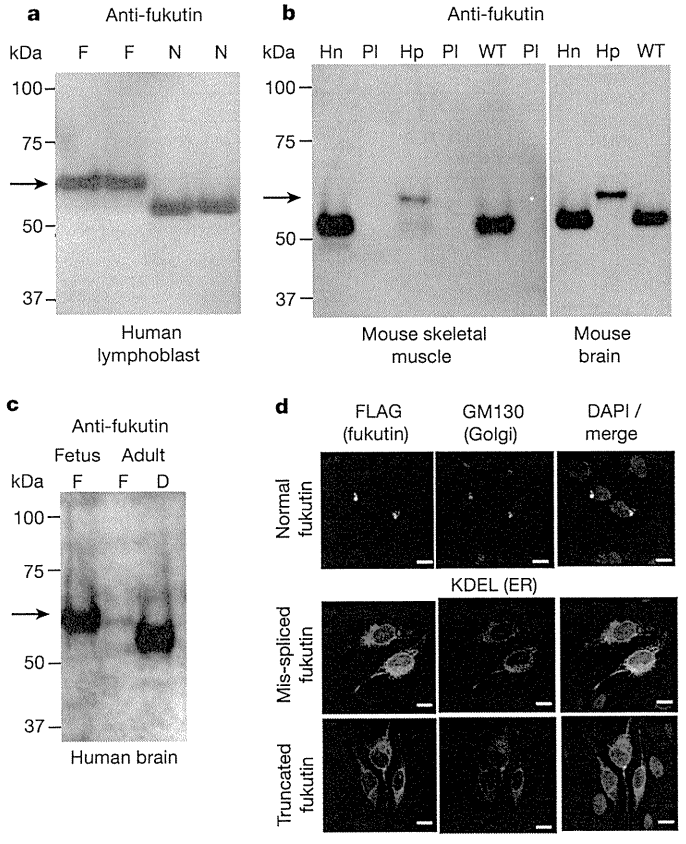


**Figure 2 | Abnormal fukutin protein in FCMD. a–c**, Immunoprecipitation analysis of fukutin protein in human lymphoblasts (**a**), both skeletal muscle and brain tissues from Hp/Hp mice (**b**) and brain tissue from patients with FCMD (**c**); filled arrow, abnormal fukutin; N, normal sample; F, sample from patient with FCMD; Hn, Hn/Hn mice; Hp, Hp/Hp mice; PI, pre-immune serum; D, patient with Duchenne muscular dystrophy. **d**, The subcellular localization of fukutin. Top, normal fukutin; middle, mis-spliced fukutin; bottom, truncated fukutin. Stained with anti-FLAG (left, to detect fukutin), anti-GM130 (middle, Golgi marker, top) and anti-KDEL (endoplasmic reticulum marker, middle and bottom), and merge (right, with DAPI stain). Scale bar, 10 μm.

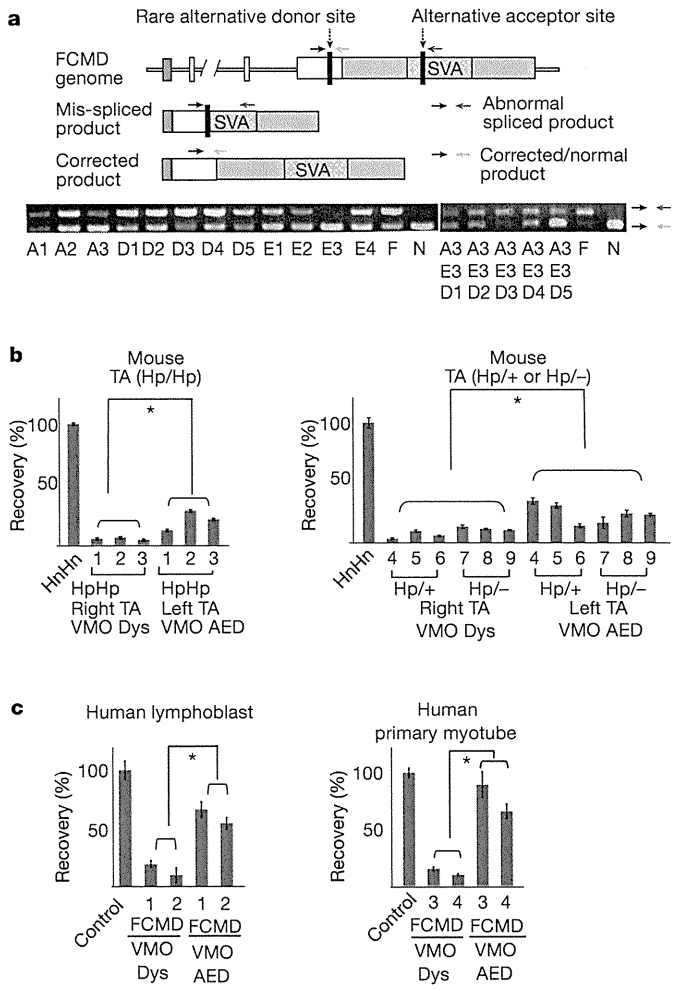


**Figure 3 | AON cocktail rescues normal *fukutin* mRNA. a**, RT–PCR diagram of three primers designed to assess normal *fukutin* mRNA recovery (upper). Black arrow, a common forward primer located on *fukutin* coding region; dark grey arrow, a reverse primer to detect the abnormal RT–PCR product (161 bp); light grey arrow, the other reverse primer to detect the restored normal RT–PCR product (129 bp). The effect on Hp/Hp ES cells treated with each single or a cocktail of AONs (lower). F, FCMD; N, normal sample. **b**, Rescue from abnormal splicing in VMO-treated Hp/Hp and Hp/− mice. Local injection of AED cocktail into tibialis anterior ($n = 3$). Dys, a negative control. **c**, Rescue from abnormal splicing in VMO-treated human FCMD lymphoblasts (left, $n = 2$) and myotubes (right, $n = 2$). The $y$ axis shows the percentage recovery of normal mRNA ($^*P < 0.01$ by Student's $t$-test). TA, tibialis anterior. Error bars, s.e.m.

that the SVA-trapped transcripts are likely to be subjected to nonsense-mediated mRNA decay[6,17]. In a search for the same events using the same acceptor site as FCMD in the human genome, we located two expressed sequence tags on human chromosome 4 (DA436529 and DA060755) that represent a spliced transcript induced by an SVA element. We found exonization in a human-specific insertion of SVA (AB627340) into a small gene (Fig. 1f right panel and Supplementary Fig. 11). The human-specific exon-trapping of SVA in the small gene might influence human evolution and development.

FCMD alleles of the *fukutin* gene contain a fully intact protein coding sequence, raising the possibility that FCMD could be treated by restoring translation of the full-length protein through splicing modulation with AONs. To identify promising target sequences in various cell lines, we produced 25-mer 2′-O-methyl phosphoramidite (2′OMePS) AONs targeted to the acceptor (A1–A3), donor (D1–D5) and exonic splicing enhancer sites (E1–E4) in *fukutin* pre-mRNA (Supplementary Fig. 12). We introduced the AONs into various cell types and assessed the recovery of normal processing and restoration of the authentic stop codon (Fig. 3a). Cells with A3 and E3 showed strong suppression of SVA-derived splicing. The greatest recovery of *fukutin* mRNA, to levels of more than 40% of the normal control, was achieved with a combination of A3, E3 and D5 (AED) (Fig. 3a). The D5 sequence overlaps with a predicted intronic splicing enhancer site within the aberrant intronic sequence; in normal *fukutin*, this sequence resides in exon 10 (Supplementary Fig. 12).

We injected octa-guanidine morpholino oligonucleotide (vivomorpholino, VMO)[18] AED cocktail locally into skeletal muscle of knock-in mice and evaluated the therapeutic effect by calculating the percentage recovery of normally processed mRNA. In the AED-treated tibialis anterior and gastrocnemius of Hp/Hp and Hp/− mice, the amount of corrected *fukutin* mRNA increased significantly relative to mice treated with control VMO (Fig. 3b and Supplementary Fig. 13). We assessed fukutin protein recovery in injected skeletal muscle tissue from Hp/Hp mice. Consistent with the significant increase of restored normal mRNA, normal fukutin protein was rescued (Fig. 4a). We examined α-DG glycosylation in AED-treated Hp/− mice. Deficiently glycosylated α-DG, at the predicted smaller size, was reduced in abundance, whereas normal-sized α-DG increased after AED treatment (Fig. 4b). The signal intensity for glycosylated α-DG was clearly increased, and a shift in the α-DG core was observed, indicating that the rescued fukutin is functional. Laminin overlay assays revealed a marked increase in α-DG laminin-binding ability, indicating that α-DG

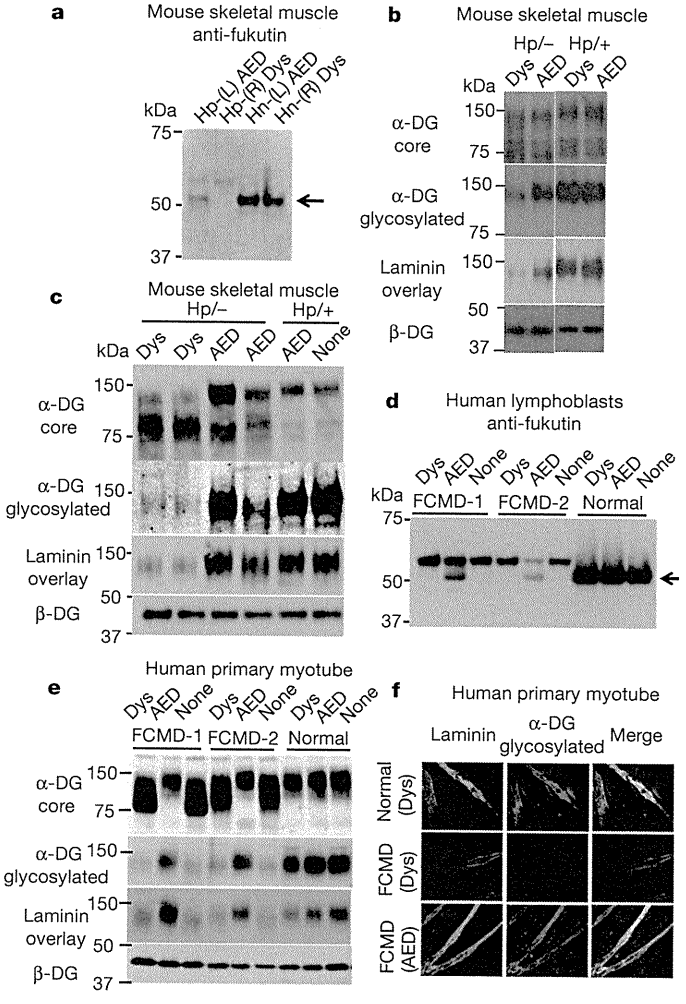


**Figure 4 | AON cocktail treatment rescues normal fukutin protein and functional α-DG. a, d,** Immunoprecipitation analysis of fukutin protein after local treatment with VMO (AED) in FCMD model mice (**a**) and human FCMD lymphoblasts (**d**). Arrow, normal fukutin protein. L, left tibialis anterior; R, right tibialis anterior; Dys, negative control. **b, c, e,** Tibialis anterior muscle after local (**b**) or systemic (**c**) treatment with AED and human FCMD lymphoblasts treated with the AED (**e**) were analysed by western blot using antibodies against α-DG core protein (top panel) and glycosylated α-DG (second), and by a laminin overlay assay (third). Bottom, β-DG (internal control). **f,** Laminin clustering assay. Left, anti-laminin; middle, anti-glycosylated α-DG; right, merged images. Upper, normal myotubes treated with control VMO; middle, FCMD patient myotubes treated with control VMO; bottom, FCMD patient myotubes treated with AED.

function also is recovered (Fig. 4b). We next tested systemic AED treatment by intravenous injection of Hp/− mice. This treatment also showed the recovery of normally glycosylated α-DG in AED-treated mice (Fig. 4c).

We administered the VMO AED cocktail to human lymphoblasts and myotubes. As in knock-in mice, we observed successful correction of the splicing abnormality. The corrected *fukutin* mRNA was restored to 50% or more of the levels seen in normal controls (Fig. 3c). We believe this to be sufficient recovery, considering that unaffected FCMD carriers have only 50% of normal *fukutin* mRNA. Finally, we tested recovery of the fukutin protein and the glycosylation of α-DG in the cells of patients with FCMD. Not only was normal fukutin protein expression significantly rescued in AED-treated lymphoblasts (Fig. 4d), but also we observed recovery of normally glycosylated α-DG in AED-treated myotubes (Fig. 4e). Immunofluorescence staining also showed immensely increased glycosylated α-DG (Fig. 4f). A laminin clustering assay showed increased laminin clustering ability, which is characteristically absent in α-DGpathy[19] (Fig. 4f). These data show that AED treatment effectively rescues normal fukutin, confirming our observation of abnormal *fukutin* splicing and raising the possibility of splicing modulation therapy as the first treatment for FCMD. To treat neuronal migration disorder of FCMD, prenatal treatment may be necessary, but it is currently difficult for ethical and technical reasons. Nevertheless, improving even only the muscular symptoms would greatly ameliorate quality of life of the patients as well as their families.

Retrotransposons account for nearly half of the human genome[20]. Increased numbers of reports have highlighted positive and negative contributions of retrotransposons to human health and disease[21,22]. In addition to being the causative factor for FCMD, ARH and NLSDM, SVA insertions have also been implicated in hereditary elliptocytosis, X-linked agammaglobulinemia, neurofibromatosis type 2 and X-linked dystonia-Parkinsonism[12,23–26]. It has been suggested that SVA insertions cause such diseases through genomic deletion, reduced mRNA expression or skipping of neighbouring exons[17,22]. Recently, SVA splicing has been suggested to generate variation within and across species by activating functional 3′ splice sites within SVAs across the human genome, controlling gene transcription, creating alternative splicing by exon-trapping, or inducing premature stop codons, and was experimentally demonstrated[6]. Our findings emphasize the importance of SVA functions in human disease and support the possibility of radical treatment against SVA-induced disease by splicing modulation therapy. AONs have become one of the most promising and practical candidate chemicals for splicing modulation therapy in cancer[27], infectious diseases[28] and Duchenne muscular dystrophy[29,30]. In demonstrating the ability of AONs to rescue fukutin function in FCMD, we introduce a novel clinical role for them in treating FCMD and other SVA-mediated diseases, while providing new insights about the influence of SVAs on human evolution, development and disease.

## METHODS SUMMARY

For AON treatment, 25-mer 2′OMePS (GeneDesign and Invitrogen) and octaguanidine morpholino (VMO; Gene-Tools) were used. The knock-in mouse was produced as described previously[16].

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Contributions** M.T.-I., K.K., M.K. and T.T. designed the study. M.T.-I. performed most of the experiments. K.K. developed a system to detect endogenous fukutin protein. M.K. performed biochemical analysis of VMO-injected mice. C.Y. produced the *fukutin* cDNA constructs for transfection experiments. K.M., T.O., and A.K. performed analyses of AON treatment in mice and various cell types. H.K., T.Y. and S.T. provided intellectual input. H.O.A., S.D. and R.K., provided patients' samples. M.T.-I., K.K. and T.T. wrote the paper.

**Author Information** The patient *fukutin* and a chimpanzee mRNA sequences are deposited in GenBank/European Molecular Biology Laboratory/DNA Data Bank of Japan under accession numbers AB609007 and AB627340, respectively. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to T.T. (toda@med.kobe-u.ac.jp).

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# Endoplasmic reticulum stress response in P104L mutant caveolin-3 transgenic mice

**Atsushi Kuga[1,2], Yutaka Ohsawa[2], Tadashi Okada[2], Fumio Kanda[1], Motoi Kanagawa[1], Tatsushi Toda[1] and Yoshihide Sunada[2,*]**

[1]Division of Neurology/Molecular Brain Science, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Kobe, Hyogo 650-0017, Japan and [2]Department of Neurology, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama 701-0192, Japan

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**Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy 1C (LGMD1C). However, the precise molecular pathogenesis of caveolin-3-related muscular dystrophy remains uncertain. Here, we demonstrate the effect of gene dosage on the severity of the myopathic phenotype in P104L mutant caveolin-3 (mCav3$^{P104L}$) transgenic mice, a model of LGMD1C. We analyzed the endoplasmic reticulum (ER) stress response in the transgenic mice and found upregulated transcription of the molecular chaperone, glucose-regulated protein (GRP78). Moreover, signaling downstream of GRP78 in the myofibers was activated toward apoptosis. However, terminal transferase dUTP nick end labeling assays detected a few apoptotic nuclei in transgenic mouse skeletal muscle, probably due to the transcriptional activation of Dad1, an anti-apoptotic factor in the ER. These findings suggest that the ER stress response caused by mCav3$^{P104L}$ plays a role in the pathogenesis of LGMD1C as a toxic gain of function effect.**

## INTRODUCTION

Caveolae are characterized as flask-shaped invaginations of the plasma membrane (1). Caveolin is a major structural component of caveolae (2) and it also functions as a scaffolding protein to concentrate and regulate many classes of signaling molecules. Distinct genes encode the isoforms, caveolin-1, -2 and -3. Caveolin-3 is specifically expressed in muscle cells (3). Co-expressed caveolins-1 and -2 form hetero-oligomers in non-muscle cells, whereas caveolin-3 forms homo-oligomers in muscle cells. Different mutations in the human caveolin-3 gene have been associated with several muscle diseases that are collectively called caveolinopathies and include limb-girdle muscular dystrophy, distal myopathy and rippling muscle disease (4,5). A mutant caveolin-3 with a single amino acid substitution from proline to leucine at amino acid residue 104 (mCav3$^{P104L}$) was originally identified from a genetic analysis of autosomal dominant limb-girdle muscular dystrophy 1C (LGMD1C) (6).

Since LGMD1C is inherited as an autosomal dominant trait, mCav3$^{P104L}$ presumably has a dominant-negative effect on the molecular pathogenesis of caveolinopathy. Studies *in vitro* have shown that homo-oligomers of wild-type caveolin-3 translocate to the cell membrane via the endoplasmic reticulum (ER)–Golgi network, whereas mCav3$^{P104L}$ does not target the cell membrane (7). The hetero-oligomers formed between wild-type caveolin-3 and mCav3$^{P104L}$ in the ER–Golgi system are degraded by the ubiquitin–proteasome proteolytic pathway, which might lead to the loss of caveolin-3 in LGMD1C (7,8). Targeted downregulation of caveolin-3 gene in differentiating C2C12 myoblasts can inhibit myotube formation (9). Expression of mCav3$^{P104L}$ can trigger a loss of caveolin-3 during C2C12 cell differentiation (10). These results suggest that the secondary loss of caveolin-3 due to mCav3$^{P104L}$ is associated with the molecular pathology of LGMD1C. However, whether mCav3$^{P104L}$ has a gain of function effect that contributes to the pathogenesis of LGMD1C remains uncertain.

The ER stress response to the accumulation of a mutant protein has recently garnered interest among those investigating the pathogenesis of neurodegenerative disorders. For instance, the cytoplasmic aggregation of alpha-synuclein that is a pathological hallmark of Parkinson's disease is accelerated in a form of familial Parkinson's disease caused by a dominant mutation of the alpha-synuclein gene (11). In addition, several genes responsible for autosomal recessive Parkinson's disease

*To whom correspondence should be addressed. Tel: +81 864621111; Fax: +81 864621199; Email: ysunada@med.kawasaki-m.ac.jp