厚生労働科学研究費補助金（難病・がん等の疾患分野の医療の実用化　研究事業）
分担研究報告書

次世代シークエンサーを用いた孤発性 ALS 遺伝子解析

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〔目的〕筋萎縮性側索硬化症(ALS) は上位および下位運動ニューロンを侵す神経変性疾患であり、その一部の原因として遺伝子異常が報告されている。これまで当科では家族性 ALS 患者の遺伝子解析を行っており、Superoxide dismutase 1 (SOD1), Fused in sarcoma/translated in liposarcoma (FUS/TLS)などの遺伝子変異を報告してきた。次世代シークエンサーを用いて家族性のみならず孤発性 ALS の原因および疾患関連遺伝子の解析を行う。
〔方法〕当科で収集した常染色体優性遺伝の遺伝形式が疑われる 95 家系について、引き続きサンガー法による既知の原因遺伝子解析を行う。孤発性 ALS に関しては臨床情報と一緒に検体の収集を行う。
〔結果および考察〕解析した家族性 95 家系では 23 家系に *SOD1* 異常、10 家系に *FUS/TLS* 遺伝子異常を認めたが、TAR DNA-binding protein 43 kDa (TDP43)、Valosin-containing protein (VCP)に関しては遺伝子異常を伴う家系は認められなかった。
〔結論〕今後は次々に明らかとなる新規原因遺伝子の解析を孤発性 ALS 患者を含めて進めていく必要がある。

A.研究目的

筋萎縮性側索硬化症(ALS) は上位および下位運動ニューロンを侵す神経変性疾患であり、その一部の原因として遺伝子異常が報告されている。これまで当科では ALS 全体の約 10%を占めると云われている家族性 ALS 患者に関する遺伝子解析を進めており、Superoxide dismutase 1 (SOD1), Fused in sarcoma/translated in liposarcoma (FUS/TLS)における遺伝子変異を報告してきた。次世代シークエンサーを用いて家族性のみならず孤発性 ALS の原因および疾患関連遺伝子の解析を行う。

B.研究方法

当科で収集した常染色体優性遺伝の遺伝形式が疑われる家族性 ALS 95 家系についてサンガー法にて遺伝子解析を行った。SOD1, FUS/TLS に加えて TAR DNA-binding protein 43 kDa (TDP43)、Valosin-containing protein (VCP)についての解析を加えて行った。

孤発性 ALS に関しては臨床情報と一緒に検体の収集を行った。

(倫理面への配慮)

すべての遺伝子操作は東北大学 DNA 組換え実験指針に従い、個人を同定できない形で発表し、個人情報は鍵のかかる戸棚に保管、DNA は連結可能匿名化で保存している。東北大学倫理委員会の承認を受けている。

C.研究結果

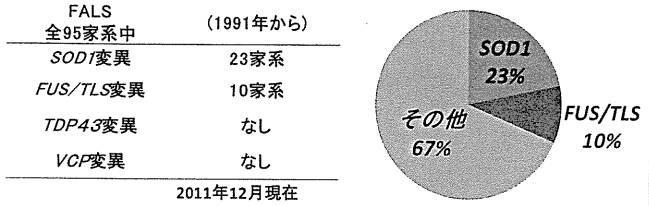
家族性 ALS 遺伝子解析

遺伝子解析を行ったところ 23 家系に *SOD1* 異常、10 家系に FUS/TLS 遺伝子異常を認めた。TDP43 に関しては遺伝子異常を伴う家系は認められなかった。今回新たに解析した VCP 遺伝子についても 95 家系内では遺伝子変異は認められなかった。(図 1)

次世代シークエンスについては解析プラットフォ

ームの検討を行った。

D.考察



FALS 全95家系中	(1991年から)
*SOD1*変異	23家系
*FUS/TLS*変異	10家系
*TDP43*変異	なし
*VCP*変異	なし
2011年12月現在	

図1：東北大学にて遺伝子検査を行った家系における遺伝子変異

今回の当科における 95 家系の解析では VCP 遺伝子変異を伴う家系は認められず、現在のところ、SOD1, FUS/TLS 遺伝子の変異が国内の家族性 ALS の原因遺伝子として約 10%程度をしめることが確認された。

FUS/TLS 変異を持つ ALS は若年発症でかつ病気の進行が非常に早いことが特徴である。変異部位は FUS/TLS の C 末端部分に集中しており、変異によっては発症、進行がやや緩やかな変異もあるようであった。R521C は全世界で共通してみられる重要な変異である。今後も新規報告されつつあり新規遺伝子の解析をサンガー法と次世代シークエンスの併用にて確認していく必要がある。さらには孤発性 ALS の検体数を増やす必要がある。

E.結論

東北大学にて遺伝子解析を行った家族性 ALS 95 家系について検討した。今後も孤発性を含めた患者検体を増やしつつ、新規遺伝子検索を進めていくことが重要である。

F.健康危険情報

特記事項なし

G.研究発表

1. 論文発表

なし

2. 学会発表

青木正志、鈴木直輝、割田　仁、加藤昌昭、水野秀紀、島倉奈緒子、今野秀彦、加藤信介、糸山泰人
FUS/TLS 遺伝子異常に伴う日本人家族性 ALS における遺伝子変異と臨床型、病理に関する検討
第 52 回日本神経学会総会　2011 年 5 月 18-20 日 名古屋

島倉奈緒子、鈴木直輝、加藤昌昭、割田　仁、水野秀紀、今野秀彦、加藤信介、糸山泰人、青木正志　日本人家族性 ALS における遺伝子変異と臨床型、病理に関する検討
第 56 回人類遺伝学会 2011 年 11 月 10-12 日　千葉県幕張

厚生労働科学研究費補助金（難病・がん等の疾患分野の医療の実用化　研究事業）
分担研究報告書

次世代シークエンサーを用いた孤発性の神経難病の発症機構の解明に関する研究

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研究要旨

タウオパチーとされる進行性核上性麻痺（PSP）や大脳皮質基底核変性症（CBD）を含むパーキンソン症候群（PS），前頭側頭葉変性症（FTLD）は，通常は孤発性であり，中年期以降に発症する緩徐進行性の変性疾患である．未だ有効な根治療法はなく，各疾患の関連や相違など疾患分類位置づけに関しても多くの議論がなされてきている．本研究では，各疾患の診断，病態解明，治療法の開発に寄与する臨床情報の整ったPS・FTLDの，遺伝子試料収集体制の整備を行うことを目的とし，鳥取県において地域での遺伝子試料収集体制の整備を進めると共に，全国多施設共同研究体制の整備を進めた．

A.研究目的

パーキンソン病（PD）は，振戦，筋強剛，無動，姿勢反射障害を四徴とした進行性の神経変性疾患である．パーキンソン症候群（PS）は，PDならびに，パーキンソンニズムとして四徴のいずれかを含めた神経徴候を呈する症候群であり，進行性核上性麻痺（PSP），大脳皮質基底核変性症（CBD）などの神経変性疾患が含まれる．一方，前頭側頭葉変性症（FTLD）は，前頭・側頭葉に限局して進行性の変性を呈し，行動障害や言語障害を主徴とする非アルツハイマー型変性性認知症の一群を指す臨床概念であり，臨床的には，前頭側頭型認知症，意味性認知症，進行性非流暢性失語の3つのサブタイプに分類される．また，FTLDにPSPやCBDも含める考え方もある．さらに，PSP・CBDやFTLDの一部はタウオパチーとしても捉えられている。これらの疾患では，中年以降に発症し，通常は孤発例である．治療法，予後も異なり，鑑別診断が重要であるが，臨床的に鑑別が困難な場合が少なくない．各疾患の関連や相違など疾患分類位置づけに関しても多くの議論がなされてきており，臨床的，遺伝学的，病理学的，生化学的な詳細な研究の推進が待たれている．

詳細な遺伝子解析を行うには，多数例の遺伝子試料が必要となるが，PS・FTLDは有病率が低く，遺伝子解析に活用できる多数例の収集は困難である．正確な臨床情報の整った遺伝子試料の収集とともに研究にすぐに活用できる遺伝子試料のバンク設立も求められている．

本研究では臨床調査個人票を活用して経時的変化も把握し，臨床情報を含めたPS・FTLD症例の遺伝子の収集を行う．本研究では，PSP・CBDなどのタウオパチーを中心に，タウオパチー以外のFTLD症例も含めて遺伝子試料収集を進める．収集した遺伝子試料を用いて，各疾患に影響を及ぼす遺伝的要因の候補を明らかにすることにより，病気の進行と共に変化する臨床症状と診断，病態解明，予後予測を可能とし，更に，治療法の開発に寄与し，臨床症状を改善することを目的としている．

B.研究方法

1) 鳥取県における患者登録体制の整備

鳥取県における特定疾患申請患者の把握，医療機

関調査を鳥取県難病相談・支援センターと連携して研究を進め，患者登録体制を整備した．

2) 全国 PS・FTLD 遺伝子試料収集共同研究体制

a) 厚生労働省科学研究費補助金事業難治性疾克服事業“神経変性疾患に関する調査研究”班（以下，“神経変性班”）との連携による研究体制の整備

“神経変性班”に協力を依頼し，全国的ネットワークによる多施設共同による遺伝子収集研究体制の整備を進めた．

b) 個別参加による医療機関との連携

上記研究班には属さないが，PS や FTLD 研究を積極的に進めている施設にも協力を依頼し，広く研究協力が得られる研究体制の整備を開始した．

3) 遺伝子試料の収集

同意が得られた患者から臨床情報と共にゲノム DNA を収集した．

（倫理面への配慮）

「ヒトゲノム・遺伝子解析研究に関する倫理指針」、「疫学研究に関する倫理指針」を遵守して研究を実施した．本研究について鳥取大学医学部倫理委員会の承認を得，本研究への参加者には，文書により研究内容や倫理的配慮について詳細に説明した後，書面にて同意を得て研究を実施した．

C.研究結果

1) 遺伝子試料の収集

PS・FTLD の患者登録を行なうとともに，2011 年 12 月までに，PSP 21 例，CBD 9 例，FTLD 5 例について臨床情報と共にゲノム DNA を収集した．

2) 全国共同研究体制の整備

“神経変性班”所属施設や，その他の PS・FTLD などの神経変性疾患の診療・研究に積極的に取り組んでいる施設に本研究協力を依頼した．

多施設の参加による収集体制の整備に向けて，本研究計画について，鳥取大学医学部倫理委員会に申請し，承認を受けた．この申請において、上述の“神経変性班”所属施設や，その他の参加予定施設についても共同研究機関として承認を得た．今後，参加各施設の倫理委員会で本研究の承認を得ていく予定である．

D.考察

鳥取県においては鳥取県難病相談・支援センターと連携し，地域における生体試料収集研究体制を整備した．PS・FTLD 患者の遺伝子試料収集が進めば国際的にも意義ある研究が可能となることが期待される．本研究を継続することにより一層の遺伝子試料収集が進み，国際的研究に発展するものと考える．

今後，“神経変性班”の構成班員施設などの PS・FTLD の診療・研究に積極的に関与してきている施設に本研究への協力を依頼し，各施設で倫理委員会の承諾を得て頂き，多施設での収集を進める．

E.結論

PS・FTLD の遺伝子試料収集研究体制の整備を行った．今後，本研究をさらに推進することにより，多数例での遺伝子試料収集が望まれる．

F.研究発表

1. 論文発表

（発表誌名巻号・頁・発行年等も記入）

1) Uemura Y, et al. Mild Parkinsonian signs in a community-swelling elderly population.) J Neurol Sci 304: 61-66, 2011.

2) Nomura T, et al. Utility of the REM sleep behavior disorder screening questionnaire (RBDSQ) in Parkinson's disease patients. Sleep Med 12: 711-713, 2011.

3) Nomura T, Inoue Y, Högl B, Uemura Y, Yasui K, Sasai T, Namba K, Nakashima K. Comparison of the clinical features of rapid eye movement sleep behavior disorder in patients with Parkinson's disease and multiple system atrophy. Psychiatry Clin Neurosci, 65: 264-271, 2011.

4) 中下聡子，ほか．多発性の大脳微小出血を認めたパーキンソニズム．神経内科 74: 324-326,

2011.
5) 和田健二, ほか. レビー小体型認知症. 臨床と研究 88: 9-12, 2011.
6) 古和久典, ほか. Parkinson 病患者における葉酸・ビタミンB12補充療法による高ホモシステイン血漿是正効果の臨床薬理学的検討一栄養障害関連因子の検討を含めて一. 臨床薬理の進歩 32: 46-52, 2011.
7) 和田健二, ほか. レビー小体型認知症 (DLB)・認知症を伴うパーキンソン病 (PDD) の治療. Geriatric Medicine 49: 787-794, 2011.
8) 野村哲志, ほか. レム睡眠行動異常症の原因とその対策. 水野美邦, 近藤智善, 監. よくわかるパーキンソン病のすべて. 改定 2 版, 大阪: 永井書店; 2011. p. 136-144.

2. 学会発表

1) Takigawa H, et al. Prevalence of Progressive Supranuclear Palsy in Yonago, Japan.
15th EFNS congress
2) Nakashita S, et al. Parkinsonism in a community-dwelling elderly population sample in Japan.
15th EFNS congress
3) Nomura T, et al. Comparison of polysomnographic findings and REM sleep behavior disorder related symptoms between patients with progressive supranuclear palsy and those with Parkinson's disease.
World sleep 2011
4) Nomura T, et al. Update on RBD Significance of REM sleep behavior disorders in synucleinopathies such as Parkinson's disease.
World sleep 2011
5) Nomura T, et al. Difference in RBD manifestation between tauopathy and alpha-synucleinopathy.
The 5th International REM Sleep Behavior Disorder Symposium
6) Takigawa H, et al. Plasma 25-(OH) vitamin D levels in the patients with Parkinson syndrome.
Advances and Controversies in B-Vitamins and Choline
7) Kowa H, et al. Hyperhomocysteinemia and cognitive dysfunction in Parkinson's disease
Advances and Controversies in B-Vitamins and Choline
8) 中下聡子, 他. 地域住民におけるパーキンソニズムの原因疾患についての検討
第 52 回日本神経学会総会
9) 瀧川洋史, 他. 鳥取県米子市における進行性核上性麻痺 (PSP) の疫学的検討 第 2 報
第 52 回日本神経学会総会
10) 野村哲志, 他. 睡眠医学と神経学 RLS と RBD
日本睡眠学会第 36 回定期学術集会
11) 野村哲志, 他. レム睡眠行動障害合併多系統萎縮症とパーキンソン病での MIBG 心筋シンチの相違
第 64 回日本自律神経学会
12) 野村哲志, 他. パーキンソン病, 多系統萎縮症における REM 睡眠行動障害の経過
第 41 回日本臨床神経生理学会
13) 瀧川洋史, 他. パーキンソン病における基礎代謝率と Ghrelin および Leptin に関する検討
第 27 回日本静脈経腸栄養学会
14) 瀧川洋史, 他. パーキンソン症候群における 25-(OH)ビタミン D に関する検討
第 27 回日本静脈経腸栄養学会

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

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
野村哲志，ほか	レム睡眠行動異常症の原因とその対策	水野美邦／近藤智善	改訂第2版 よくわかるパーキンソン病のすべて	永井書店	大阪	2011	136-144

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Taniguchi-Ikeda M, Kobayashi K, Kanagawa M, Yu CC, Mori K, Oda T, Kuga A, Kurahashi H, Akman HO, DiMauro S, Kaji R, Yokota T, Takeda S, Toda T.	Pathogenic exon-trapping by SVA retrotransposon and rescue in Fukuyama muscular dystrophy.	Nature	478	127-131	2011
Kuga A, Ohsawa Y, Okada T, Kanda F, Kanagawa M, Toda T, Sunada Y.	Endoplasmic reticulum stress response in P104L mutant caveolin-3 transgenic mice.	Hum Mol Genet	20	2975-2983	2011
Sun H, Satake W, Zhang C, Nagai Y, Tian Y, Fu S, Yu J, Qian Y, Qian Y, Chu J, Toda T.	Genetic and clinical analysis in a Chinese parkinsonism-predominant spinocerebellar ataxia type 2 family.	J Hum Genet	56	330-334	2011
Sharma M, Maraganore DM, Ioannidis JP, Riess O, Aasly JO, Annesi G, Abahuni N, Bentivoglio AR, Brice A, Van Broeckhoven C, Chartier-Harlin MC, Destée A, Djarmati A, Elbaz A, Farrer M, Ferrarese C, Gibson JM, Gispert S, Hattori N, Jasinska-Myga B, Klein C, Lesage S, Lynch T, Lichtner P, Lambert JC, Lang AE, Mellick GD, De Nigris F, Opala G, Quattrone A, Riva C, Rogaeva E, Ross OA, Satake W,	Role of sepiapterin reductase gene at the PARK3 locus in Parkinson's disease.	Neurobiol Aging	32	2108.e1-5	2011

Silburn PA, Theuns J, Toda T, Tomiyama H, Uitti RJ, Wirdefeldt K, Wszolek Z, Gasser T, Krüger R; Genetic Epidemiology of Parkinson's Disease Consortium.					
Popiel HA, Burke JR, Strittmatter WJ, Oishi S, Fujii N, Toda T, Wada K, and Nagai Y.	The Aggregation Inhibitor Peptide QBP1 as a Therapeutic Molecule for the Polyglutamine Neurodegenerative Diseases	J Amino Acids	doi:10.4061/2011/265084		2011
Ueda T, Kanda F, Aoyama N, Fujii M, Nishigori C, Toda T.	Neuroimaging features of xeroderma pigmentosum group A.	Brain Behav	doi: 10.1002/brb3.22		2011
Tachikawa M, Kanagawa M, Yu CC, Kobayashi K, Toda T.	Mislocalization of fukutin protein by disease-causing missense mutations can be rescued with treatments directed at folding amelioration.	J Biol Chem	287	8398-8406	2012
Kuga A, Kanagawa M, Sudo A, Chan YM, Tajiri M, Manya H, Kikkawa Y, Nomizu M, Kobayashi K, Endo T, Lu QL, Wada Y, Toda T.	Absence of post-phosphoryl modification in dystroglycanopathy mouse models and wild-type tissues expressing a non-laminin binding form of alpha-dystroglycan.	J Biol Chem	287	9560-9567	2012
Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BM, Schjeide LM, Meissner E, Zauft U, Allen NC, Liu T, Schilling M, Anderson KJ, Beecham G, Berg D, Biernacka JM, Brice A, Destefano AL, Do CB, Eriksson N, Factor SA, Farrer MJ, Foroud T, Gasser T, Hamza T, Hardy JA, Heutink P, Hill-Burns EM, Klein C, Latourelle JC, Maraganore DM, Martin ER, Martinez M, Myers RH, Nalls MA, Pankratz N, Payami H, Satake W,	Comprehensive Research Synopsis and Systematic Meta-Analyses in Parkinson's Disease Genetics: The PDGene Database.	PLoS Genet	8	e1002548	2012

Scott WK, Sharma M, Singleton AB, Stefansson K, Toda T, Tung JY, Vance J, Wood NW, Zabetian CP; 23andMe, The Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium; The International Parkinson's Disease Genomics Consortium (IPDGC); The Parkinson's Disease GWAS Consortium; The Wellcome Trust Case Control Consortium 2 (WTCCC2), Young P, Tanzi RE, Khoury MJ, Zipp F, Lehrach H, Ioannidis JP, Bertram L.					
Sharma M, Ioannidis JPA, Aasly JO, Brice A, Van Broeckhoven C, Annesi G, Bertram L, Bozi M, Crosiers D, Clarke C, Facheris MF, Farrer M, Gispert S, Auburger G, Vilarino-Guell , Garraux G, Hadjigeorgiou GM, Hicks AA, Hattori N, Jeon BS, Lesage S, Lill CM, Lin JJ, Lynch T, Lichtner P, Lang AE, Mok VCT, Jasinska-Myga B, Mellick GD, Morrison KE, Opala GM, Pramstaller PP, Pichler I, Park SS, Quattrone A, Rogaeva EA, Ross OA, Stefanis L, Stockton J, Satake W, Silburn P, Theuns J,	World-wide replication and heterogeneity in Parkinson disease genetic loci.	Neurology	in press		

Tan EK, Toda T, Tomiyama H, Uitti RJ, Wirdefeldt K, Wszolek ZK, Xiromerisiou G, Yueh KC, ZHAO YI, Gasser T, Maraganore DM, Krüger R.					
Iida A, Takahashi A, Kubo M, Saito S, Hosono N, Ohnishi Y, Kiyotani K, Mushiroda T, Nakajima M, Ozaki K, Tanaka T, Tsunoda T, Oshima S, Sano M, Kamei T, Tokuda T, Aoki M, Hasegawa K, Mizoguchi K, Morita M, Takahashi Y, Katsuno M, Atsuta N, Watanabe H, Tanaka F, Kaji R, Nakano I, Kamatani N, Tsuji S, Sobue G, Nakamura Y, Ikegawa S	A functional variant in ZNF512B is associated with susceptibility to amyotrophic lateral sclerosis in Japanese.	Hum Mol Genet	20	3684-3692	2011
Iida A, Takahashi A, Deng M, Zhang Y, Wang J, Atsuta N, Tanaka F, Kamei T, Sano M, Oshima S, Tokuda T, Morita M, Akimoto C, Nakajima M, Kubo M, Kamatani N, Nakano I, Sobue G, Nakamura Y, Fan D, Ikegawa S	Replication analysis of SNPs on 9p21.2 and 19p13.3 with amyotrophic lateral sclerosis in East Asians.	Neurobiol Aging	32	757.e13-14	2011
Iguchi Y, Katsuno M, Takagi S, Ishigaki S, Niwa JI, Hasegawa M, Tanaka F, Sobue G	Oxidative stress induced by glutathione depletion reproduces pathological modifications of TDP-43 linked to TDP-43 proteinopathies	Neurobiol Dis	45	862-870	2012
Amo T, Sato S, Saiki S, Wolf AM, Toyomizu M, Gautier CA, Shen J, Ohta S, Hattori N.	Mitochondrial membrane potential decrease caused by loss of PINK1 is not due to proton leak, but to respiratory chain defects.	Neurobiol Dis	41	111-118	2011
Hassin-Baer S, Hattori N, Cohen OS, Massarwa M, Israeli-Korn SD,	Phenotype of the 202 adenine deletion in the parkin gene: 40 years of follow-up.	Mov Disord	26	719-722	2011

Inzelberg R.					
Hayashi C, Funayama M, Li Y, Kamiya K, Kawano A, Suzuki M, Hattori N, Ikeda K.	Prevalence of GJB2 causing recessive profound non-syndromic deafness in Japanese children.	Int J Pediatr Otorhino-laryngol	75	211-214	2011
Kamagata K, Motoi Y, Hori M, Suzuki M, Nakanishi A, Shimoji K, Kyougoku S, Kuwatsuru R, Sasai K, Abe O, Mizuno Y, Aoki S, Hattori N.	Posterior hypoperfusion in Parkinson's disease with and without dementia measured with arterial spin labeling MRI.	J Magn Reson Imaging	33	803-807	2011
Kawajiri S, Saiki S, Sato S, Hattori N.	Genetic mutations and functions of PINK1.	Trends Pharmacol Sci	32	573-580	2011
Kawanabe T, Tanaka R, Sakaguchi Y, Akiyama O, Shimura H, Yasumoto Y, Ito M, Hattori N, Tanaka S.	Posterior reversible encephalopathy syndrome complicating intracranial hemorrhage after phenylpropanolamine exposure.	Neurol Med Chir (Tokyo)	51	582-585	2011
Morita A, Okuma Y, Kamei S, Yoshii F, Yamamoto T, Hashimoto S, Utsumi H, Hatano T, Hattori N, Matsumura M, Takahashi K, Nogawa S, Watanabe Y, Miyamoto T, Miyamoto M, Hirata K.	Pramipexole reduces the prevalence of fatigue in patients with Parkinson's disease.	Intern Med	50	2163-2168	2011
Noda K, Fukae J, Fujishima K, Mori K, Urabe T, Hattori N, Okuma Y.	Reversible cerebral vasoconstriction syndrome presenting as subarachnoid hemorrhage, reversible posterior leukoencephalopathy, and cerebral infarction.	Intern Med	50	1227-1233	2011
Ogaki K, Motoi Y, Li Y, Tomiyama H, Shimizu N, Takanashi M, Nakanishi A, Yokoyama K, Hattori N.	Visual grasping in frontotemporal dementia and parkinsonism linked to chromosome 17 (microtubule-associated with protein tau): a comparison of N-Isopropyl-p-[(123)I]-iodoamphetamine brain perfusion single photon emission computed tomography analysis with progressive supranuclear palsy.	Mov Disord	26	561-563	2011
Ross OA,	Association of LRRK2 exonic	Lancet Neurol	10	898-908	2011

Soto-Ortolaza AI, Heckman MG, Aasly JO, Abahuni N, Annesi G, Bacon JA, Bardien S, Bozi M, Brice A, Brighina L, Van Broeckhoven C, Carr J, Chartier-Harlin MC, Dardiotis E, Dickson DW, Diehl NN, Elbaz A, Ferrarese C, Ferraris A, Fiske B, Gibson JM, Gibson R, Hadjigeorgiou GM, Hattori N, Ioannidis JP, Jasinska-Myga B, Jeon BS, Kim YJ, Klein C, Kruger R, Kyratzi E, Lesage S, Lin CH, Lynch T, Maraganore DM, Mellick GD, Mutez E, Nilsson C, Opala G, Park SS, Puschmann A, Quattrone A, Sharma M, Silburn PA, Sohn YH, Stefanis L, Tadic V, Theuns J, Tomiyama H, Uitti RJ, Valente EM, van de Loo S, Vassilatis DK, Vilariño-Güell C, White LR, Wirdefeldt K, Wszolek ZK, Wu RM, Farrer MJ; Genetic Epidemiology Of Parkinson's Disease (GEO-PD) Consortium.	variants with susceptibility to Parkinson's disease: a case-control study.				
Saiki S, Sasazawa Y, Imamichi Y, Kawajiri S, Fujimaki T, Tanida I, Kobayashi H, Sato F, Sato S, Ishikawa K, Imoto M, Hattori N.	Caffeine induces apoptosis by enhancement of autophagy via PI3K/Akt/mTOR/p70S6K inhibition.	Autophagy	7	176-187	2011
Saiki S, Sato S, Hattori N.	Molecular pathogenesis of Parkinson's disease: update.	J Neurol Neurosurg Psychiatry	83	430-436	2011
Sato S, Hattori N.	Genetic mutations and	Parkinsons Dis	2011	979231	2011

	mitochondrial toxins shed new light on the pathogenesis of Parkinson's disease.				
Takamatsu Y, Shiotsuki H, Kasai S, Sato S, Iwamura T, Hattori N, Ikeda K.	Enhanced Hyperthermia Induced by MDMA in Parkin Knockout Mice.	Curr Neuropharma-col.	9	96-99	2011
Tanaka R, Sasaki-Ikesawa K, Shimura H, Nishioka K, Hattori N, Tanaka S.	Methotrexate leukoencephalopathy mimics acute progressive stroke.	J Neurol	258	2083-2085	2011
Teramoto S, Miyamoto N, Yatomi K, Tanaka Y, Oishi H, Arai H, Hattori N, Urabe T.	Exendin-4, a glucagon-like peptide-1 receptor agonist, provides neuroprotection in mice transient focal cerebral ischemia.	J Cereb Blood Flow Metab	31	1696-1705	2011
Usami Y, Hatano T, Imai S, Kubo S, Sato S, Saiki S, Fujioka Y, Ohba Y, Sato F, Funayama M, Eguchi H, Shiba K, Ariga H, Shen J, Hattori N.	DJ-1 associates with synaptic membranes.	Neurobiol Dis	43	651-662	2011
Usui C, Hatta K, Doi N, Kubo S, Kamigaichi R, Nakanishi A, Nakamura H, Hattori N, Arai H.	Improvements in both psychosis and motor signs in Parkinson's disease, and changes in regional cerebral blood flow after electroconvulsive therapy.	Prog Neuropsychoph armacol Biol Psychiatry	35	1704-1708	2011
Yamashiro K, Furuya T, Noda K, Urabe T, Hattori N, Okuma Y.	Convulsive movements in bilateral paramedian thalamic and midbrain infarction.	Case Rep Neurol	3	289-293	2011
Yasuda T, Hayakawa H, Nihira T, Ren YR, Nakata Y, Nagai M, Hattori N, Miyake K, Takada M, Shimada T, Mizuno Y, Mochizuki H.	Parkin-mediated protection of dopaminergic neurons in a chronic MPTP-minipump mouse model of Parkinson disease.	J Neuropathol Exp Neurol	70	686-697	2011
Shimura H, Tanaka R, Urabe T, Tanaka S, Hattori N.	Art and Parkinson's disease: a dramatic change in an artist's style as an initial symptom.	J Neurol	in Press		
Yoritaka A, Shimo Y, Shimo Y, Inoue Y, Yoshino H, Hattori N.	Nonmotor Symptoms in Patients with PARK2 Mutations.	Parkinsons Dis	in Press		
Uemura Y, Wada-Isoe K, Nakashita S, Nakashima K.	Mild Parkinsonian signs in a community-swelling elderly population	J Neurol Sci	304	61-66	2011

Nomura T, Inoue Y, Kagimura T, Uemura Y, Nakashima K.	Utility of the REM sleep behavior disorder screening questionnaire(RBDSQ)in parkinson's disease patients	Sleep Med	12	711-713	2011
Nomura T, Inoue Y, Högl B, Uemura Y, Yasui K, Sasai T, Namba K, Nakashima K.	Comparison of the clinical features of rapid eye movement sleep behavior disorder in patients with Parkinson's disease and multiple system atrophy.	Psychiatry Clin Neurosci	65	264-271	2011
久我敦, 金川基, 戸田達史	【筋疾患 update】 αジストログリカン異常症	BRAIN and NERVE	63巻11号	1189-1195	2011
金川基, 戸田達史	【筋ジストロフィーの分子病態から治療へ】福山型筋ジストロフィー症の成因	生体の科学	62巻2号	91-94	2011
戸田達史	国際共同研究における Genome-Wide Association Study(GWAS)	Medical Science Digest	37巻9号	346-347	2011
戸田達史, 佐竹渉	【パーキンソン病発症のメカニズム】パーキンソン病の分子遺伝学 ゲノム関連解析研究	BIO Clinica	26巻8号	701-705	2011
戸田達史	【変わりゆくパーキンソン病診療 早期診断から進行期患者の治療まで】孤発性パーキンソン病の分子病態機序はどこまで解明されたか	内科	107巻5号	759-766	2011
中下聡子, 和田健二, 足立芳樹, 渡辺保裕, 中島健二	多発性の大脳微小出血を認めたパーキンソニズム二次性レストレッグス症候群について	神経内科	74巻3号	324-326	2011
和田健二, 中島健二	レビー小体型認知症	臨床と研究	88巻6号	657-660	2011
古和久典, 瀧川洋史, 野村哲志, 安井建一, 中島健二	Parkinson病患者における葉酸・ビタミンB12補充療法による高ホモシステイン血漿是正効果の臨床薬理学的検討—栄養障害関連因子の検討を含めて—	臨床薬理の進歩	32号	46-52	2011
和田健二, 田中健一郎, 中島健二	レビー小体型認知症(DLB)・認知症を伴うパーキンソン病(PDD)の治療	Geriatric Medicine	49巻7号	787-794	2011

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

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Reprinted from Nature, Vol. 478, No. 7367, pp. 127–131, 6 October 2011

LETTER

doi:10.1038/nature10456

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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

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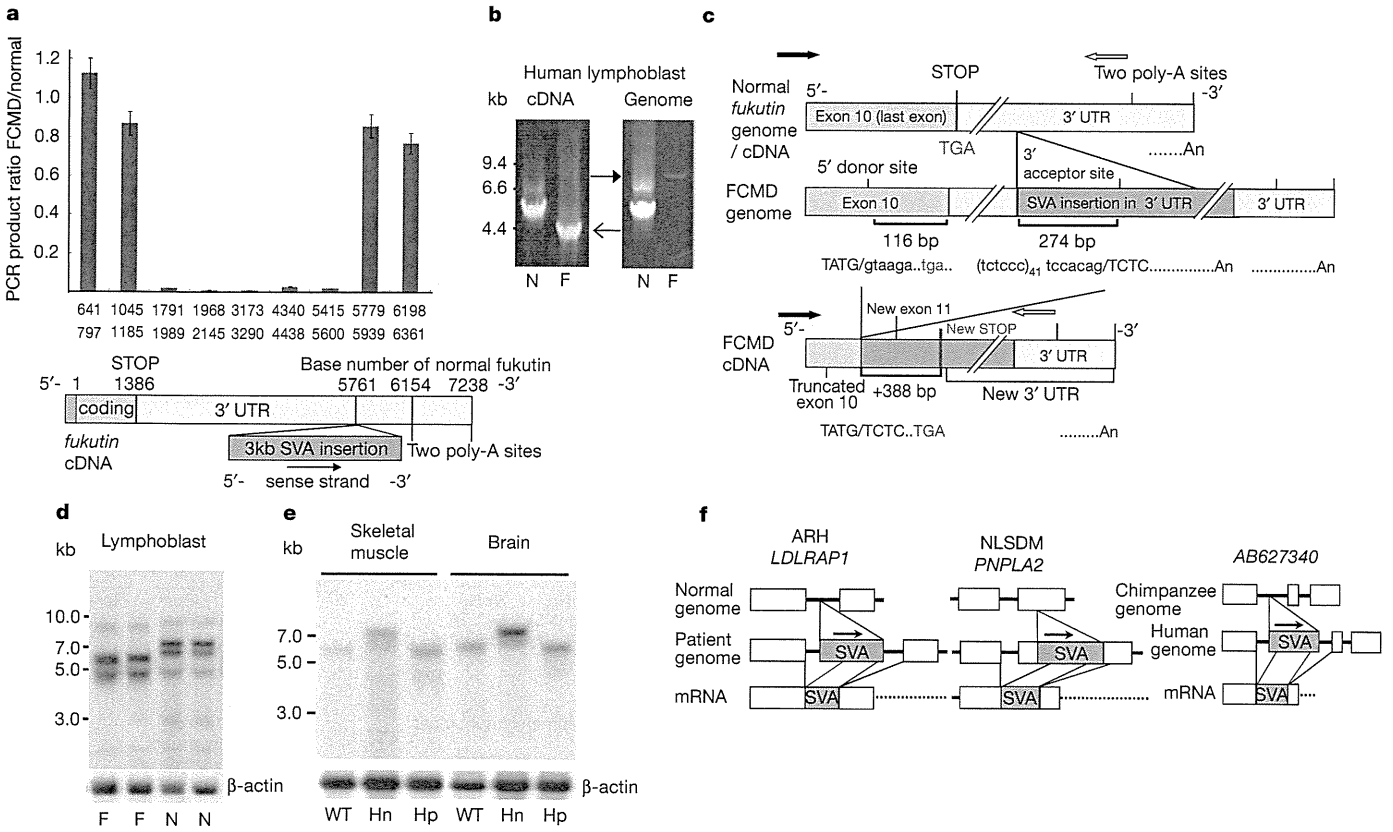

Figure 1 | An SVA retrotransposal insertion induces abnormal splicing in FCMD. **a**, Expression analysis of various regions of *fukutin* mRNA in lymphoblasts. Grey bar, the ratio of RT–PCR product in patients with FCMD relative to the normal control; numbers on the *x* axis, nucleotide positions of 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

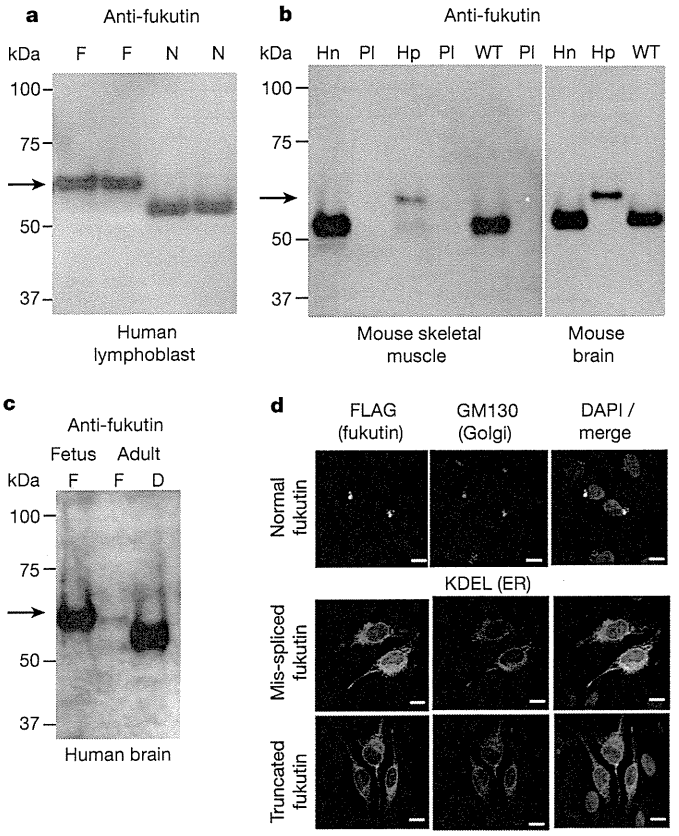

Figure 2 | Abnormal fukutin protein in FCMD. a–c, Immunoprecipitation analysis of fukutin protein in human lymphoblasts (**a**), both skeletal muscle and brain tissues from Hp/Hp mice (**b**) and brain tissue from patients with FCMD (**c**); filled arrow, abnormal fukutin; N, normal sample; F, sample 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.

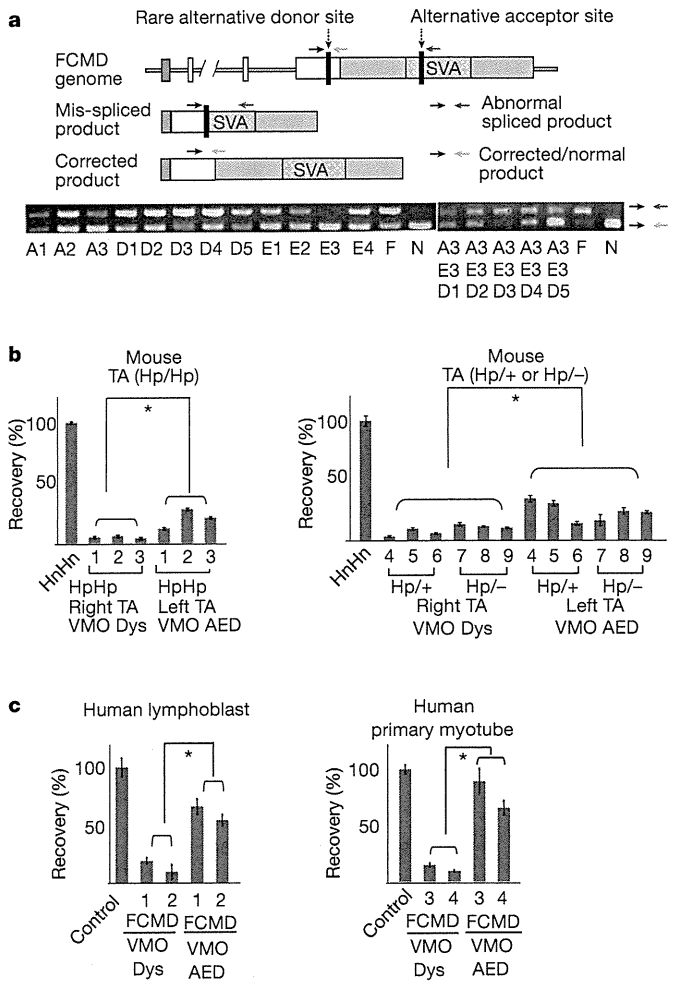

Figure 3 | AON cocktail rescues normal *fukutin* mRNA. a, RT–PCR diagram of three primers designed to assess normal *fukutin* mRNA recovery (upper). Black arrow, a common forward primer located on *fukutin* coding region; dark grey arrow, a reverse primer to detect the abnormal RT–PCR product (161 bp); 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 (vivo-morpholino, 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

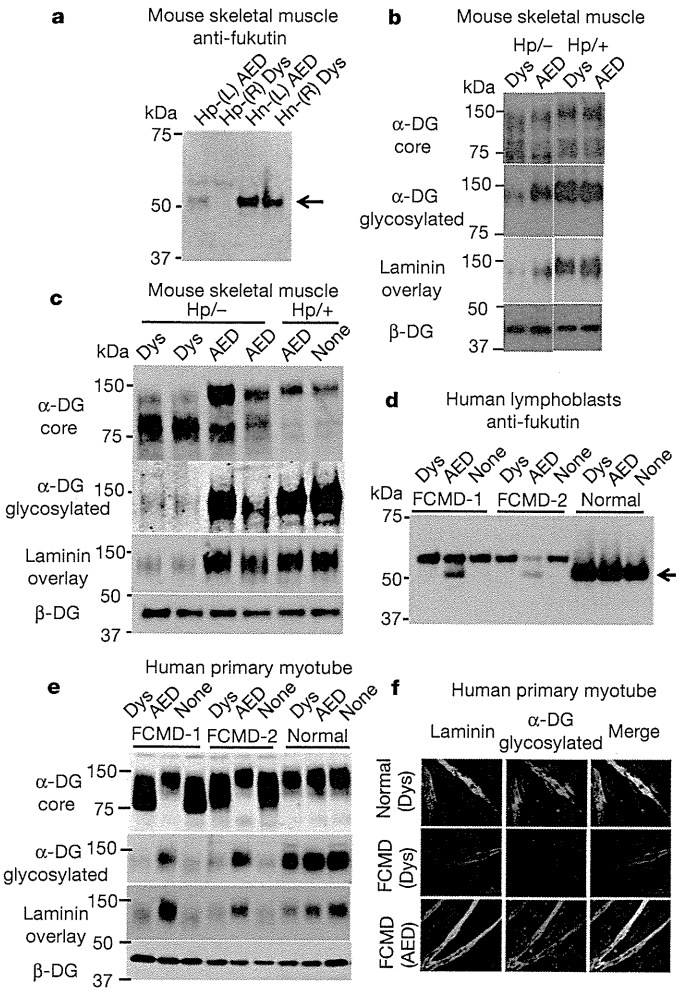

Figure 4 | AON cocktail treatment rescues normal fukutin protein and functional α-DG. a, d, Immunoprecipitation analysis of fukutin protein after local treatment with VMO (AED) in FCMD model mice (**a**) and human FCMD lymphoblasts (**d**). Arrow, normal fukutin protein. L, left tibialis anterior; R, 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.

Received 16 September 2010; accepted 12 August 2011.

1. Toda, T. *et al.* Localization of a gene for Fukuyama type congenital muscular dystrophy to chromosome 9q31–33. *Nature Genet.* **5,** 283–286 (1993).
2. Kobayashi, K. *et al.* An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. *Nature* **394,** 388–392 (1998).
3. Watanabe, M. *et al.* Founder SVA retrotransposal insertion in Fukuyama-type congenital muscular dystrophy and its origin in Japanese and Northeast Asian populations. *Am. J. Med. Genet. A.* **138,** 344–348 (2005).
4. Wilund, K. R. *et al.* Molecular mechanisms of autosomal recessive hypercholesterolemia. *Hum. Mol. Genet.* **11,** 3019–3030 (2002).
5. Akman, H. O. *et al.* Neutral lipid storage disease with subclinical myopathy due to a retrotransposal insertion in the *PNPLA2* gene. *Neuromuscul. Disord.* **20,** 397–402 (2010).
6. Hancks, D. C. *et al.* Exon-trapping mediated by the human retrotransposon SVA. *Genome Res.* **19,** 1983–1991 (2009).
7. Damert, A. *et al.* 5′-Transducing SVA retrotransposon groups spread efficiently throughout the human genome. *Genome Res.* **19,** 1992–2008 (2009).
8. Bantysh, O. B. & Buzdin, A. A. Novel family of human transposable elements formed due to fusion of the first exon of gene MAST2 with retrotransposon SVA. *Biochemistry (Mosc.)* **74,** 1393–1399 (2009).
9. Michele, D. E. *et al.* Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. *Nature* **418,** 417–422 (2002).
10. Barresi, R. & Campbell, K. P. Dystroglycan: from biosynthesis to pathogenesis of human disease. *J. Cell Sci.* **119,** 199–207 (2006).
11. Strichman-Almashanu, L. Z. *et al.* Retroposed copies of the HMG genes: a window to genome dynamics. *Genome Res.* **13,** 800–812 (2003).
12. Ostertag, E. M. *et al.* SVA elements are nonautonomous retrotransposons that cause disease in humans. *Am. J. Hum. Genet.* **73,** 1444–1451 (2003).
13. Bennett, E. A. *et al.* Natural genetic variation caused by transposable elements in humans. *Genetics* **168,** 933–951 (2004).
14. Wang, H. *et al.* SVA elements: a hominid-specific retroposon family. *J. Mol. Biol.* **354,** 994–1007 (2005).