図3 Unilateral thalamotomy and STN-DBS later on

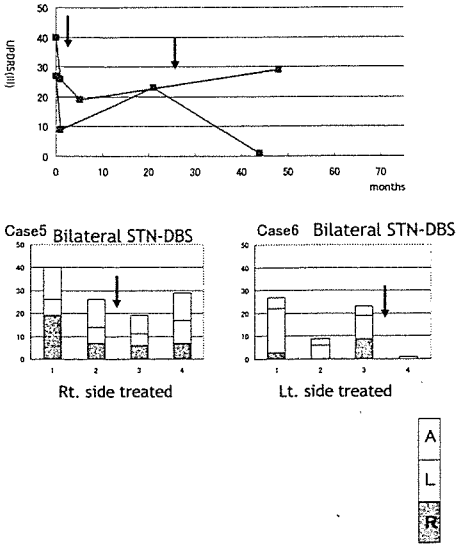


図5 Post-operative UPDRS Progresses in 4 cases of the thalamotomy and the STN-DBS simultaneously

- 66/M Rt. Thalamotomy + Rt. STN-DBS
  - Lt. STN-DBS (14mo later)
- 57/F Lt.Thalamotomy + Blt. STN-DBS
- 59/M Lt.Thalamotomy + Blt. STN-DBS
- 65/M Lt.Thalamotomy + Blt. STN-DBS

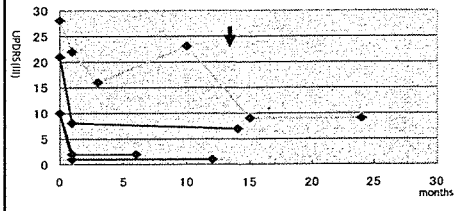


図4 Vim-DBS

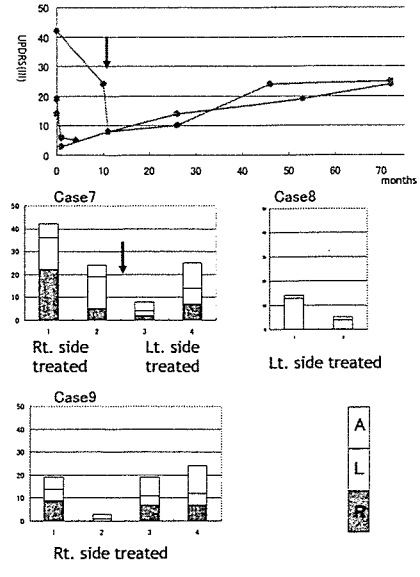
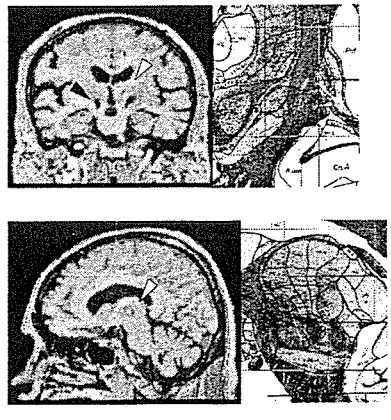


図6 Combination of the Thalamotomy and the Bilatral STN-DBS



厚生労働科学研究補助金研究報告書

パーキンソン病遺伝子治療でのPET計測に関する研究

研究者　佐藤俊彦　　宇都宮セントラルクリニック院長

研究要旨：パーキンソン病の主要原因である線条体ドーパミン合成能力の診断が可能なPET検査薬 6-[18F]fluoro-L-m-tyrosine(6-FMT)を合成し、臨床使用に必要な薬剤検定データを得た。6-FMTの合成収量は、①原料核種となる^{18}Fガスは、^{18}Oガスへの１０MeVの陽子線照射により約200mCi、②^{18}Fガスを原料とする合成プロセスによる6-FMTの収量は20-30mCi であった。本合成薬剤の検定データを３ロットでそれぞれ取得し、放射化学的純度:95%以上、エンドトキシン試験:陰性、無菌試験:陰性、比放射能:250-320 GBq/mmol 、不純物分析（Sn）：10ppb以下であり、臨床使用に問題ないことを確認した。

A、研究目的

パーキンソン病の遺伝子治療対象患者のスクリーニングが可能なPET用RI検査薬の６-FMT(6-[18F]fluoro-L-m-tyrosine)は、わが国では、合成及び使用実績が無いことから、この合成プロセスを確立すると同時に臨床使用が可能となる検定データを取得し、安全性を確認する。

B、研究方法

これまでの６-FMT合成事例を文献調査し、^{18}F ガスから合成するプロセスを選定した。本プロセスに必要な、^{18}Fガス製造及び回収装置とその後の標識反応に必要な合成装置を製作し、それらの合成性能を評価した。また、合成後の6-FMTの安全性を評価するために、放射化学的純度試験、エンドトキシン試験、無菌試験、比放射能評価試験、不純物分析（Sn）等を実施した。

① ^{18}F ガス製造試験

^{18}O ガスへの陽子線照射により^{18}Fガスを製造($^{18}O(p,n)\ ^{18}F$)するために、照射用ターゲット、ガス移送及び回収装置を製作する。本装置での^{18}Oガスの適正充填圧力と照射電流を試験的に決定する。また、^{18}Fガスの大部分は ^{18}O ガスを充填した金属ターゲット内壁に吸着されるため、この吸着 ^{18}Fの回収方法を検討する。

② 6-FMT標識反応試験

^{18}F ガスと反応前駆体との反応により ^{18}Fをフェニル基の６位置に置換する標識反応装置を製作する。反応前駆体として、N-Trifluoroacetyl-5-acetoxy-2-trimethylstannyl-L-phenylalanine ethyl esterを使用し、この前駆体のスタニル基と^{18}Fを置換する。その後、よう化水素酸による加水分解反応により6-FMTを合成し、さらに加水分解後の反応液から高速液体クロマトグラフィーにより６-FMTを分取する。

③ ６-FMTの検定試験

PET用標識薬剤の安全性の確認のために下記項目の検定データを３ロットについてそれぞれ取得する。

・ 放射化学的純度試験

ラジオ液体クロマトグラフフ法により測定

- エンドトキシン試験
  比濁法により測定
- 無菌試験
  血液培養システム法により測定
- 比放射能
  高速液体クロマトグラフ法により測定
- 不純物分析（Sn）
  ICP-MS 法により測定

（倫理面への配慮）

上記の３ロットによる検定試験データと日本核医学会「院内製造された FDG を用いて PET 検査を行うためのガイドライン」に従った製品標準書を作成し、これを病院内倫理委員会で審議した後、ヒトへの薬剤投与を実施する予定である。

C, 研究結果

今回製作した ^{18}F ガス製造装置及び 6-FMT 標識反応装置を用いて、^{18}F ガス製造試験、6-FMT 標識反応試験を実施し、以下の結果が得られた。

① ^{18}F ガス製造試験

第１プロセスとして、$^{18}O_2$ ガスの照射ターゲットへの移送及び回収、第２プロセスとしてターゲット内壁に吸着した ^{18}F ガスを回収可能な ^{18}F ガス製造装置を製作した。

（第１プロセスの試験結果）

アルミターゲット（容積 30mL）に、$^{18}O_2$ ガスを充填し、エネルギー１０MeV の陽子線をサイクロトロンにより６０分間照射。

$^{18}O_2$ ガス充填圧力：10Kg/cm2
照射電流　　：30 μA

（第２プロセスの試験結果）

第１プロセス終了後に、ターゲットから $^{18}O_2$ ガスを抜き出し、代わって、Ar＋F_2 ガス及び Ar ガスを充填し、陽子線を再度照射した後に ^{18}F ガスを回収した。照射時間、照射電流をパラメータとして試験を実施し、以下の最適条件を得た。

Ar＋F_2 ガス充填圧力：0.9Kg/cm2
Ar ガス充填圧力　　：10Kg/cm2
最適照射電流　　：20μA
最適照射時間　　：7.5分

照射終了後に上記ガスを回収し、^{18}F 約 200 mCi を得た。

② 6-FMT 標識反応試験

N-Trifluoroacetyl-5-acetoxy-2-trimethylstannyl-L-phenylalanine ethyl ester 20mg をフロン１１溶液に溶かし、$^{18}F_2$ ガスをマイナス５℃-マイナス１０℃に冷却したフロン溶液中に吹き込み、標識反応を実施した。反応液を常温に戻した後８分間静置し、その後よう化水素酸を加え、１３０℃で１０分間加水分解を行った。反応液を１８０℃で４分間維持し、よう化水素酸を留去し、さらに減圧下１５０℃で標識反応物を乾燥した。乾燥後、注射用水 1.5ml を加えて反応物をバブリングにより完全に溶解させ、本反応溶液から高速液体クロマトグラフィーHPLC により 6-FMT をフラスコに分取した。分取条件は以下である。

カラム：YMC-Pack ODS-AQ12S05
溶離液：　酢酸：ﾒﾀﾉｰﾙ：蒸留水
　　＝0.5:15:484.5
流速　：4ml/min
検出器：UV(280nm)
溶出時間：18 min

分取液を加熱乾燥することにより、溶離

液を除去し、生理食塩水 10ml を加えて注射用水とした。

合成収量は、３回平均で 25mCi、合成収率は、^{18}F ガスを基準に約 13%であった。

③ 6-FMT 検定試験

上記で合成した 6-FMT 注射用水３ロットをそれぞれ分析検定し以下の結果を得た。

- 放射化学的純度試験：95%以上
- エンドトキシン試験：陰性
- 無菌試験　　：陰性
- 比放射能　　：250-320 GBq/mmol
- 不純物分析（Sn）　：0.1 ppm 以下

その他の分析項目も含めて別表に検定試験結果を示した。既に臨床使用されている FDG 製剤の検定試験項目にスズ(Sn)の分析を加えたものであるが、検定試験結果は良好で、問題なく臨床使用可能となった。

D、考察

① ^{18}F ガスの製造試験

第２プロセスの ^{18}F ガスの回収では、回収ガス($Ar+F_2$)中の F_2 濃度を上げると回収量も増加するが、比放射能が低下した。今回実施条件は、比放射能と回収量のバランスを考慮して決定している。

② 6-FMT 標識反応試験

今回は、標識反応の溶媒としてフロン１１を使用した。フロン１１を使用することにより、^{18}F ガスによる直接標識反応が可能になると同時に溶媒の離脱プロセスが簡略化でき、結果として反応効率の向上が図れた。しかし、フロン１１は環境的な問題として大規模製造は中止になっている。合成時の使用量は、１回あたり 2-3 ml 程度で微小であり、ラボ用として購入可能であるが、購入不能になった場合の代替策も検討しておく必要がある。

③ 6-FMT 検定試験

３ロット検定試験では、使用に当たって問題になると思われるデータはなかった。今回の検定項目について、毎合成ごとに実施する項目と半年程度に１回実施する項目に分けて基準化し、倫理委員会で審議する予定である。

E、結論

^{18}F ガス製造試験、6-FMT 標識反応試験、6-FMT 検定試験により、ガス製造及び標識反応の最適条件を決定した。また、この条件により製造した 6-FMT の安全性を評価する検定試験データを取得し、臨床使用可能となった。

G、研究発表

１、論文発表

臨床データを取得した段階で発表予定。

２、学会発表

同上。

H、知的財産権の出願・登録状況

なし。

（別表）^{18}F－FMT　3ロット試験結果

		1月22日		1月23日		1月24日	
照射条件		30μA×60min		30μA×60min		30μA×60min	
放射能量		0.515GBq(13.92mCi)		1.041GBq(28.1mCi)		1.031GBq(27.86mCi)	
合成時間		80分		73分		75分	
試験項目	*規定値*	結果	判定	結果	判定	結果	判定
性状	*無色または微黄色の液体*	無色透明の液体	合格	無色透明の液体	合格	無色透明の液体	合格
pH	*4.0～8.0*	5.0	合格	6.0	合格	5.0	合格
メタノール濃度	*3000ppm以下*	検出限界以下	合格	検出限界以下	合格	検出限界以下	合格
ガンマ線スペクトル	*511keV及び1022keVにピーク*	511keVにピーク	合格	511keVにピーク	合格	511keVにピーク	合格
半減期	*105～115分*	112.55分	合格	106.0分	合格	114.55分	合格
放射能確認試験	*約7.8分にピークを認める*	7.98分にピーク	合格	7.87分にピーク	合格	7.81分にピーク	合格
放射化学的純度	*純度95%以上*	純度95%以上	合格	純度95%以上	合格	純度95%以上	合格
エンドトキシン試験	*0.25EU/mL以下*	0.25EU/mL以下	合格	0.25EU/mL以下	合格	0.25EU/mL以下	合格
無菌試験	*菌を認めない*	7日間培養、陰性	合格	7日間培養、陰性	合格	7日間培養、陰性	合格
残留スズ濃度	*20ppm以下*	<100ppb	合格	<100ppb	合格	<100ppb	合格

研究成果の刊行に関する一覧表

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
Ouyang Y, Takiyama Y, Sakoe K, Shimazaki H, Ogawa T, Nagano S, Yamamoto Y, Nakano I	Sacsin-related ataxia (ARSACS):Expanding the genotype upstream from the gigantic exon.	Neurology	66	1103-1104	2006
Ouyang Y, Sakoe K , Shimazaki H, Namekawa M, Ogawa T, Ando Y, Kawakami T, Kaneko J, Hasegawa Y, Yoshizawa K, Amino T, Ishikawa K, Mizusawa H, Nakano I, Takiyama Y	16q-linked autosomal dominant cerebellar ataxia: A clinical and genetic study.	Jounal of the Neurological Sciences	247	180-186	2006
Kamimura T, Shimazaki H, Morita M, Nakano I, Okazaki H, Minota S	Limited Wegenr's granulomatosis manifested by abducens nerve palsy resulting from pachymeningitis.	Journal of Clinical Rheumatology	12 (No.5)	259-260	2006
Ishihara K, Sugie M, Shiota J, Kawamura M, Kitamoto T, Nakano I	Severe cortical involvement in MV2 Creutzfeldt-Jakob disease: An autopsy case report.	Neuropathology	26	433-437	2006

Ishihara K, Araki S, Ihori N, Shiota J, Kawamura M, Nakano I	An autopsy case of frontotemporal dementia with severe dysarthria and motor neuron disease showing numerous basophilic inclusions.	Neuropathology	26	447-454	2006

研究成果の刊行に関する一覧表

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
Ideno, J., Mizukami, H., Kakehashi, A., Saito,Y., Okada,T., Urabe, M., Kume, A., Kuroki, M., Kawakami, M., Ishibashi, S., and Ozawa, K.	Prevention of diabetic retinopathy by intraocular soluble flt-1 gene transfer in a spontaneously diabetic rat model	Int J Mol Med	19	75-79	2007
Takei,Y., Mizukami, H., Saga,Y., Yoshimura, I., Hasumi,Y., Takayama, T., Kohno, T., Matsushita, T., Okada, T., Kume, A., Suzuki, M., Ozawa, K.	Suppression of ovarian cancer by muscle-mediated expression of soluble VEGFR-1/Flt-1 using adeno-associated virus serotype 1-derived vector	Int J Cancer	120	278-284	2006
Xin, KQ., Mizukami, H., Urabe, M., Toda, Y., Shinoda, K., Yoshida, A., Oomura, K., Kojima, Y., Ichino, M., Klinman, D., Ozawa K., Okuda, K.	Induction of robust immune responses against HIV is supported by the inherent tropism of AAV5 for DC	J Virol	80	11899-11910	2006
Mizukami, H., Mimuro, J., Ogura, T., Okada, T., Urabe, M., Kume, A., Sakata, Y., Ozawa, K.:	Adipose tissue as a novel target for *in vivo* gene transfer by using adeno-associated virus vectors.	Hum Gene Ther	17	921-928	2006

Ogura, T., Mizukami, H., Mimuro, J., Madoiwa, S., Okada, T., Matsushita, T., Urabe, M., Kume, A., Hamada, H., Yoshikawa, H., Sakata, Y., Ozawa, K.	Utility of intraperitoneal administration as a route of AAV serotype 5 vector-mediated neonatal gene transfer.	J Gene Med	8	990-997	2006

研究成果の刊行に関する一覧表

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
Li XG, Okada T, Kodera M, Nara Y, Takino N, Muramatu C, Ikeguchi K, Urano F, Ichinose H, Metzger D, Chambon P, Nakano I, Ozawa K, Muramatsu S	Viral-MediatedTemporally Controlled Dopamine Production in a Rat Model of Parkinson Disease	Mol Ther	13(1)	160-166	2006
Shibata H, Ageyama N, Tanaka Y, Kishi Y, Sasaki K, Nakamura S, Muramatsu S, Hayashi S, Kitano Y, Terao K, Hanazono Y	Improved Safety of Hematopoietic Transplantation with Monkey Embryonic Stem Cell in the Allogeneic Setting	Stem Cells	24(6)	1450-1457	2006
Wakamatu M, Ishii A, Iwata S, Sakagami J, Ukai Y, Ono M, Kanbe D, Muramatsu S, Kobayashi K, Iwatsubo T, Yoshimoto M	Selective loss of nigral dopamine neurons induced by overexpression of truncated human a-synuclein in mice.	Neurobiol Aging			in press

Sawada H, Hishida R, Hirata Y, Ono K, Suzuki H, Muramatsu S, Nakano I, Nagatsu T, Sawada M,	Activated microglia affect The nigro-striatal dopamine neurons differently in neonatal and aged mice treated with 1-methy1-4-phenyl-1,2,3,6-tetrahydropyridine has.	J Neurosci Res			in press

研究成果の刊行物・別刷

Sacsin-related ataxia (ARSACS): Expanding the genotype upstream from the gigantic exon

Abstract—The authors describe a Japanese autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) patient with a compound heterozygous mutation (32627-32636delACACTGTTAC and 31760delT) in a new exon of the *SACS* gene. The new exons upstream of the gigantic one should be analyzed when a case is clinically compatible with ARSACS, even without any mutation in the gigantic exon.

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Y. Ouyang, MD; Y. Takiyama, MD, PhD; K. Sakoe, PhD; H. Shimazaki, MD, PhD; T. Ogawa, MD; S. Nagano, MD, PhD; Y. Yamamoto, MD, PhD; and I. Nakano, MD, PhD

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS; MIM 270550) was first reported in the late 1970s.[1] ARSACS is characterized by early-onset spastic ataxia, dysarthria, nystagmus, distal muscle wasting, finger or foot deformities, and retinal hypermyelination.[1]

In 2000, the gene responsible for ARSACS (*SACS*) was identified in Quebec patients.[2] The *SACS* gene consists of a single gigantic exon spanning 12,794 bp with an 11,487-bp open reading frame and encodes the protein sacsin.[2] To date, over 20 mutations in the gigantic exon have been found in Quebec[2] and non-Quebec patients including ones in Japan,[3-5] Italy,[6] Tunisia,[7] Turkey,[8] and Spain,[9] and ARSACS thus shows a worldwide occurrence. We sometimes encounter patients with clinical features identical to those of ARSACS who have no mutation in the gigantic exon. Eight new exons located upstream of the gigantic one were recently found (GenBank, AL157766). We report a Japanese patient with ARSACS with a compound heterozygous mutation in a new exon of the *SACS* gene.

Methods. *Clinical study.* We encountered a 25-year-old woman with early-onset spastic ataxia. The patient was an only child born to nonconsanguineous parents. Detailed neurologic examinations were performed on the family members including the patient and her unaffected parents. In addition, we performed brain MRI and a nerve conduction study of the patient.

Molecular analysis. Blood samples were obtained with informed consent from the patient and her parents. Genomic DNA was extracted from peripheral blood leukocytes. Including the gigantic exon described previously,[2] nine exons were initially retrieved from the National Center for Biotechnology Information (NCBI). The accession number is AL 157766, and the protein product number is CAI 13923 (4579 aa) in NCBI. Exons 1 through 9 are shown according to their location in figure 1. Primer pairs were designed to amplify each exon including the gigantic one (exon 9) (the primer sequences are available on request). Each exon was amplified by PCR from 200 ng of genomic DNA and sequenced directly with an ABI PRISM 310 genetic analyzer. To confirm the mutations, the amplified fragments were subcloned into a TA-cloning plasmid vector (TOPO TA Cloning Kit; Invitrogen). Each mutation was screened in the chromosomes from 100 Japanese controls in order to exclude polymorphisms. This study was approved by the Medical Ethical Committee of Jichi Medical School.

Case report. This 25-year-old woman first walked at 18 months of age, but the speed of her gait and running was low in her first decade. In her school days, she could not run as fast as her classmates. Her gait disturbance progressed slowly, and from age 23, she needed some assistance when walking. Her speech also became slow and dysarthric at age 23.

Neurologic examination at age 25 revealed marked spasticity and moderate distal weakness in the lower extremities. Tendon reflexes were markedly increased with Babinski signs, but absent in the ankles. She showed limb and truncal ataxia, slurred speech, and a defect in conjugate pursuit ocular movements. Vibration sensation in the toes was reduced. She showed pes caves and pes varus. Her gait was markedly ataxic and spastic. Myelinated retinal nerve fibers were not observed. Brain MRI revealed cerebellar atrophy, especially in the upper vermis (data not shown). Motor nerve conduction velocity was mildly reduced in the median and ulnar nerves. A compound muscle action potential was not evoked in the posterior tibial nerve. A sensory nerve action potential was not evoked in the sural nerve (data not shown).

Results. *Molecular analysis.* No mutation in gigantic exon 9 was found in the patient, and a compound heterozygous deletion mutation (32627-32636delACACTGTTAC and 31760 delT) was identified in a new exon, 7, of the *SACS* gene, which results in a frameshift and a subsequent stop codon at amino acid residues 407 (W395-fsX407) and 713 (V687-fsX713) (figure 2A). This mutation leads to truncation of the predicted sacsin protein. This mutation was found in a heterozygous state in the unaffected father (32627-32636delACACTGTTAC) and mother (31760delT) (figure 2B and C). None of these mutations were found in the chromosomes from 100 Japanese controls.

Discussion. We consider the present compound heterozygous mutation (32627-32636delACACTGTTAC and 31760delT) responsible for our patient's condition. First, this mutation in the new coding exon results in premature termination of the predicted protein (W395-fsX407 and V687-fsX713). Second, 32627-32636delACACTGTTAC was found in the father and 31760delT in the mother, supporting autosomal recessive inheritance as in ARSACS.

All causative mutations previously reported were in the gigantic exon.[3-9] Our results, however, show

From the Department of Neurology (Y.O., Y.T., K.S., H.S. T.O., I.N.), Jichi Medical School, Tochigi, Japan; and the Department of Neurology (S.N., Y.Y.), Osaka University Graduate School of Medicine, Osaka, Japan.

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Address correspondence and reprint requests to Dr. Yoshihisa Takiyama, Department of Neurology, Jichi Medical School, Tochigi 329-0498, Japan; e-mail: ytakiya@jichi.ac.jp

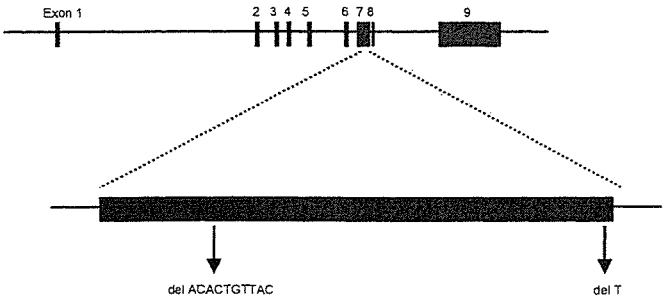

Figure 1. Schematic representation of the SACS gene. Nine exons of the SACS gene, including gigantic exon 9 originally described,[2] and their locations are shown. The arrows indicate the compound heterozygous mutation of 32627-32636delACACTGTTAC and 31760delT.

the need to analyze the new exons when a patient without any mutation in the gigantic one is clinically suspected to have ARSACS. A Turkish family with a mutation linked to the ARSACS region on chromosome 13q, the clinical symptoms being identical to those in the other ARSACS patients studied, showed

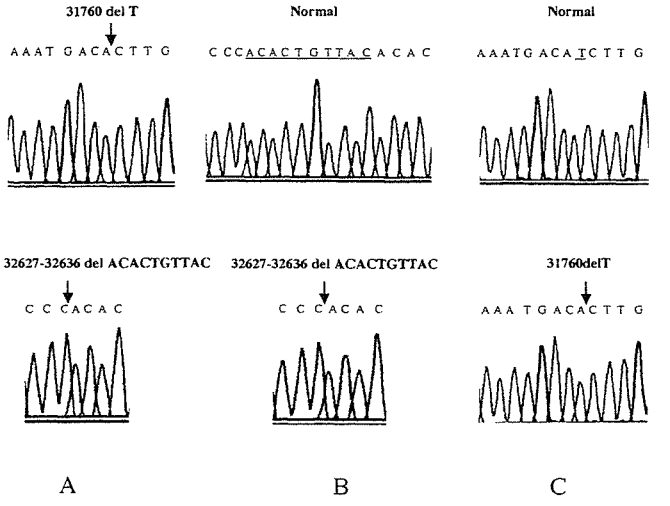

Figure 2. Identification of mutations of the SACS gene. The sequences in the patient (A), father (B), and mother (C) are shown. A compound heterozygous mutation (32627-32636delACACTGTTAC and 31760delT) was identified in exon 7 of the SACS gene in the patient, which results in a frameshift and a subsequent stop codon at amino acid residues 407 (W395-fsX407) and 713 (V687-fsX713). This mutation leads to truncation of the predicted sacsin protein. This mutation was found in a heterozygous state in the unaffected father (32627-32636delACACTGTTAC) and mother (31760delT).

no mutation in the gigantic exon of the *SACS* gene.[8] It is possible that this family also had a mutation in the new exons in the *SACS* gene.

In Quebec patients, two ancestral haplotypes (6594delT/6594delT and C5254T/6594delT in exon 9) have been identified,[2] and patients with these two haplotypes are known to show clinical homogeneity of ARSACS with the core clinical features of early-onset spastic ataxia and prominent myelinated retinal fibers.[1,2] Meanwhile, although early-onset spastic ataxia is the core clinical features in non-Quebec patients,[3-9] retinal hypermyelination and mental retardation are variable. Furthermore, we previously reported a phenotype without spasticity in a Japanese family with ARSACS,[4] reinforcing the notion that the clinical features are heterogeneous in non-Quebec patients. However, the genotype-phenotype correlation in ARSACS has remained unclear so far. In the present study, our patient showed the core clinical features of early-onset spastic ataxia without retinal hypermyelination, and we did not observe any difference in the clinical features between our patient with a truncated protein encoded by the new exon and patients with a truncated protein encoded by or a missense mutation in the original gigantic exon. However, as more *SACS* mutations are identified in the new exons, the clinical spectrum of sacsinopathies will expand, and a finer genotype-phenotype correlation study will be possible.

Acknowledgment

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References

1. Bouchard JP, Barbeau A, Bouchard R, Bouchard RW. Autosomal recessive spastic ataxia of Charlevoix-Saguenay. Can J Neurol Sci 1978;5:61–69.
2. Engert JC, Bérubé P, Mercier J, et al. ARSACS, a spastic ataxia common in northeastern Québec, is caused by mutations in a new gene encoding an 11.5-kb ORF. Nat Genet 2000;24:120–125.
3. Ogawa T, Takiyama Y, Sakoe K, et al. Identification of a SACS gene missense mutation in ARSACS. Neurology 2004;62:107–109.
4. Shimazaki H, Takiyama Y, Sakoe K, Ando Y, Nakano I. A phenotype without spasticity in sacsin-related ataxia. Neurology 2005;64:2129–2131.
5. Hara K, Onodera O, Endo M, et al. Sacsin-related autosomal recessive ataxia without prominent retinal myelinated fibers in Japan. Mov Disord 2005;20:380–382.
6. Criscuolo C, Banfi S, Orio M, et al. A novel mutation in SACS gene in a family from southern Italy. Neurology 2004;62:100–102.
7. El Euch-Fayache G, Lalani I, Amouri R, et al. Phenotypic features and genetic findings in sacsin-related autosomal recessive ataxia in Tunisia. Arch Neurol 2003;60:982–988.
8. Richter AM, Ozgul RK, Poisson VC, et al. Private SACS mutations in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) families from Turkey. Neurogenetics 2004;5:165–170.
9. Criscuolo C, Sacca F, De Michele G, et al. Novel mutation of SACS gene in a Spanish family with autosomal recessive spastic ataxia. Mov Disord 2005;20:1358–1361.

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16q-linked autosomal dominant cerebellar ataxia: A clinical and genetic study

Y. Ouyang [a], K. Sakoe [a], H. Shimazaki [a], M. Namekawa [a], T. Ogawa [a], Y. Ando [a], T. Kawakami [a], J. Kaneko [b], Y. Hasegawa [c], K. Yoshizawa [d], T. Amino [e], K. Ishikawa [e], H. Mizusawa [e], I. Nakano [a], Y. Takiyama [a,*]

[a] *Department of Neurology, Jichi Medical School, Tochigi 329-0498, Japan*
[b] *Kaneko Clinic, Gunma 373-0841, Japan*
[c] *Department of Neurology, Showa University School of Medicine, Tokyo 142-8555, Japan*
[d] *Department of Neurology, National Mito General Hospital, Ibaraki 311-3193, Japan*
[e] *Department of Neurology and Neurological Science, Graduate School, Tokyo Medical and Dental University, Tokyo 113-8519, Japan*

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Abstract

The autosomal dominant cerebellar ataxias (ADCAs) comprise a genetically and clinically heterogenous group of neurodegenerative disorders. Very recently, a C-to-T single nucleotide substitution in the *puratrophin-1* gene was found to be strongly associated with a form of ADCA linked to chromosome 16q22.1 (16q-linked ADCA; OMIM 600223). We found the C-to-T substitution in the *puratrophin-1* gene in 20 patients with ataxia (16 heterozygotes and four homozygotes) and four asymptomatic carriers in 9 of 24 families with an unknown type of ADCA. We also found two cases with 16q-linked ADCA among 43 sporadic patients with late-onset cortical cerebellar atrophy (LCCA). The mean age at onset in the 22 patients was 61.8 years, and that of homozygous patients was lower than that of heterozygous ones in one family. Neurological examination revealed that the majority of our patients showed exaggerated deep tendon reflexes in addition to the cardinal symptom of cerebellar ataxia (100%), and 37.5% of them had sensorineural hearing impairment, whereas sensory axonal neuropathy was absent. The frequency of 16q-linked ADCA was about 1/10 of our series of 110 ADCA families, making it the third most frequent ADCA in Japan.

Keywords: 16q-linked ADCA; *Puratrophin-1* gene; Heterozygote; Homozygote; Haplotype analysis

1. Introduction

Autosomal dominant cerebellar ataxias (ADCAs) comprise a genetically and clinically heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar ataxia that can be variably associated with other neurological features [1]. ADCAs are now classified on the basis of the causative genes or gene loci. To date, at least 26 subtypes of ADCA have been identified including spinocerebellar ataxia (SCA) type 1, 2, Machado-Joseph disease (MJD/SCA3), 4–8, 10–19/22, 21, 23, 25–28, and dentatorubral and pallidoluysian atrophy (DRPLA) [2,3].

Among these subtypes, SCA4 was mapped to chromosome 16q22.1 in a Scandinavian family residing in Utah and Wyoming in 1996 [4]. This family showed prominent sensory axonal neuropathy and pyramidal tract signs in addition to cerebellar ataxia. In 2003, a German family characterized by cerebellar ataxia and sensory axonal neuropathy was assigned to the same locus as SCA4 [5].

Meanwhile, the gene locus responsible for six Japanese families with ADCA was mapped to the same region as SCA4 in 2000 [6]. Although SCA4 and this form of ADCA might be allelic, the clinical features of the Japanese families

* Corresponding author. Tel.: +81 285 58 7352; fax: +81 285 44 5118.
E-mail address: ytakiya@jichi.ac.jp (Y. Takiyama).

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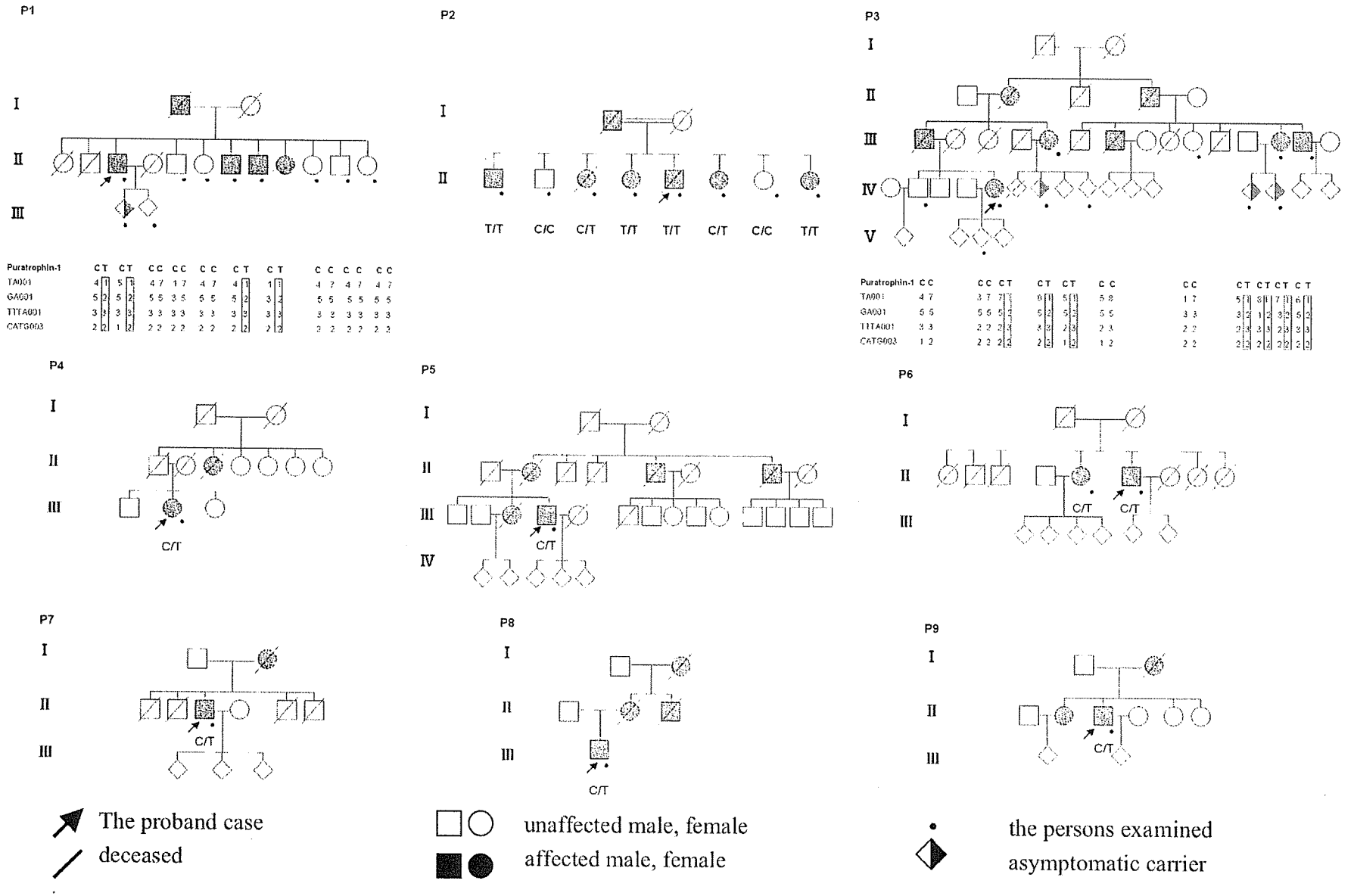

Fig. 1. The pedigrees of nine Japanese families with 16q-linked ADCA. In pedigrees 1 and 3, the gender is concealed in those individuals, including asymptomatic ones, denoted by diamonds to maintain the anonymity of the families.

were somewhat different from those in the case of SCA4, i.e., pure cerebellar ataxia without obvious evidence of extracerebellar neurological dysfunction. Therefore, the term "16q-linked ADCA" instead of "SCA4" was used to describe these Japanese families [7]. It is considered that 16q-linked ADCA shows prominent cerebellar ataxia with a later age at onset (>55 years) than that in SCA4 [8]. Very recently, a heterozygous C-to-T single nucleotide substitution in the 5′ untranslated region (UTR) of the *puratrophin-1* gene was found to be strongly associated with 16q-linked ADCA [9]. Thereafter, a substantial number of patients with this mutation showed progressive sensorineural hearing impairment in addition to cerebellar ataxia [10]. The clinical spectrum and the prevalence of 16q-ADCA, however, remain unclear.

We report here the clinical and molecular features of 20 patients including four homozygotes and four asymptomatic carriers in nine families, and two apparently sporadic patients with 16q-linked ADCA. Furthermore, we describe the frequency of 16q-linked ADCA in our series of 110 Japanese families with ADCA.

2. Subjects and methods

2.1. Clinical study

Clinical data were collected for 20 patients, four asymptomatic carriers in nine Japanese ADCA families, and two sporadic patients with a C-to-T substitution in the *puratrophin-1* gene (16q-linked ADCA). Fig. 1 shows the pedigrees of the nine families. Pedigrees 1 and 2 were partially described in the previous reports [6,8,9]. In pedigree 2, the parents (generation I) were first cousins, and thus consanguinity was present. In addition to neurological examination, brain MRI ($n=15$), peripheral nerve conduction studies ($n=8$), and audiograms ($n=8$) were performed in the patients as much as possible.

2.2. Molecular analysis

Blood samples were obtained with informed consent from 190 patients in 110 Japanese families with ADCA seen in the past 14 years (from 1992 to 2005). Genomic DNA was extracted from peripheral blood leukocytes. Screening for CAG repeat expansion for SCA1, SCA2, MJD/SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, and DRPLA was performed by PCR as described elsewhere [11–19]. In this study, the SCA10, SCA14, and FGF mutations were not analyzed.

The C-to-T substitution in the *puratrophin-1* gene were analyzed in 33 patients, 16 at risk individuals, and 5 normal spouses in 24 of 110 families after exclusion of SCA1, SCA2, MJD/SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, and DRPLA gene mutations (unknown ADCA families), and 43 sporadic patients with LCCA (late-onset cortical cerebellar ataxia without apparent extracerebellar signs or genetic inheritance). Using the primer pair of UK1-E1F1 (5′-CAGCGCGTTCACACTGAGA-3′) and UK1-E1R1 (5′-GGCCCTTTCTGACAGGACTGA-3′), exon 1 flanking the C-to-T change in the 5′ UTR of the *puratrophin-1* gene was amplified by PCR from 200 ng of genomic DNA [9], and then sequenced directly with an ABI PRISM 310 genetic analyzer; analysis was performed with Sequencing Analysis software, ver. 3.4.1 (Applied Biosystems). The PCR products of exon 1 with the primers were digested with *Eco*NI at 37 °C, subjected to electrophoresis on 2% agarose gels, and then stained with ethidium bromide. In addition, we performed haplotype analysis for the family members in pedigrees 1 and 3 using chromosome 16q markers TA001, GA001, TTTA001 and CATG003 [9].

This study was approved by the Medical Ethical Committee of Jichi Medical School.

3. Results

3.1. Clinical study

We found 20 patients (16 heterozygotes and four homozygotes) with 16q-linked ADCA and four asymptomatic carriers (two with and two without clinical signs) in 9 of 24 families with an unknown type of ADCA (Fig. 1). Furthermore, we found two sporadic patients with 16q-linked ADCA among 43 with LCCA.

Table 1
Clinical features in the patients with 16q-linked ADCA

Number of patients	22 (Male 13, Female 9)
Age at examination (years)	
Range	61–88
Mean	74.5
Age at onset (years)	
Range	50–83
Mean	61.8
Disease duration (years)	
Range	1–13
Mean	12.5
Initial symptoms (%)	
Unsteadiness of gait	77.3
Dysarthria	13.6
Tremor	9.1
Clinical features (%)	
Cerebellar	
Ataxic gait	100
Dysarthria	100
Nystagmus	77.3
Pyramidal	
Spasticity	13.6
Brisk DTRs	54.5
Babinski signs	0
Peripheral	
Depressed DTRs	13.6
Decreased vibration sense	13.6
Hearing impairment	37.5[a]
Tremor	13.6

[a] Audiograms revealed hearing impairment in three of the eight patients examined.

Table 1 summarizes the clinical features in the 22 patients with 16q-linked ADCA. The age at onset in the patients ranged from 50 to 83 years, the mean age at onset being 61.8 years. In pedigree 2, the mean age at onset in homozygous patients (n=4) was 55.6 years and that in heterozygous ones (n=2) was 68.5 years, showing an earlier age at onset in the former than in the latter. In pedigrees 1 and 3, anticipation was not noted.

The cardinal clinical feature was cerebellar ataxia including ataxic gait (100%), dysarthria (100%), and nystagmus (77.3%). Fifteen patients showed lateral gaze nystagmus, and two showed down-beat nystagmus. Oscillopsia was noted in one patient with down-beat nystagmus. Although external ophthalmoparesis was not evident, 13.6% of the patients complained of diplopia. Brisk deep tendon reflexes were found in the majority of the patients (54.5%), but Babinski signs were absent. In pedigree 3, three of the four patients examined showed moderate spasticity of the lower extremities in addition to brisk deep tendon reflexes. Meanwhile, 13.6% of the patients showed depressed deep tendon reflexes and depressed vibration sense in the toes. Audiograms revealed hearing impairment in three (37.5%) of the eight patients examined. Tremor was noted in 13.6% of the patients. Unfortunately, since we examined each homozygous or heterozygous patient in pedigree 2 only one time, we could not compare the disease course progression in them. However, there seemed to be no apparent differences in clinical phenotype between them. Among the four asymptomatic carriers, two individuals (mean, 46.0 years old) showed transient nystagmus and mild hyperreflexia.

Brain MRI (n=15) revealed cerebellar atrophy whereas the brainstem was of normal size and shape. Brain MRI of a homozygous (disease duration, 20 years) and a heterozygous patient (disease duration, 22 years) showed cerebellar atrophy of the same degree (Fig. 2). The results of a motor and sensory nerve conduction study (n=8) including two patients with depressed deep tendon reflexes or depressed vibration sense were normal, there being no sensory axonal neuropathy.

3.2. Molecular study

Fig. 1 shows the results of a heterozygous or homozygous C-to-T substitution of exon 1 in the *puratrophin-1* gene. Fig.

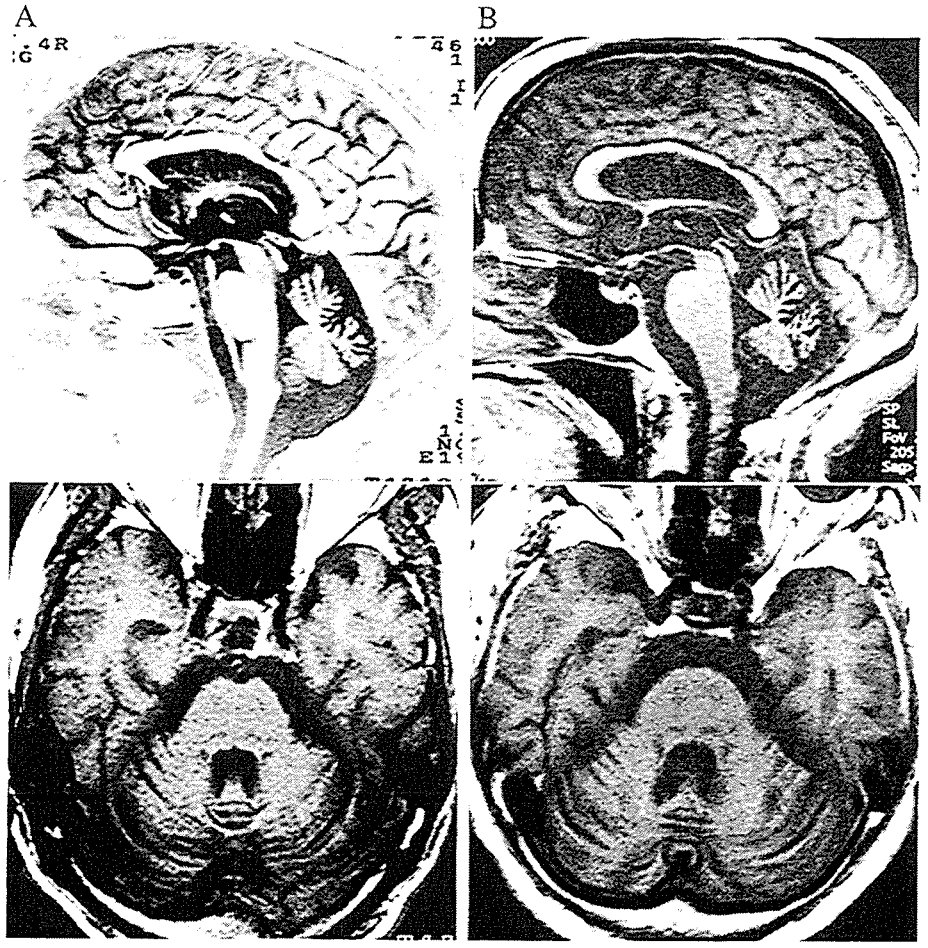

Fig. 2. (A) Brain MRI in a homozygous patient (disease duration, 20 years). Top: Reversed T2-weighted, sagittal slice. Bottom: T1-weighted, axial slice. (B) Brain MRI in a heterozygous patient (disease duration, 22 years). Top: T1-weighted, sagittal slice. Bottom: T1-weighted, axial slice. Both patients showed cerebellar atrophy of the same degree.

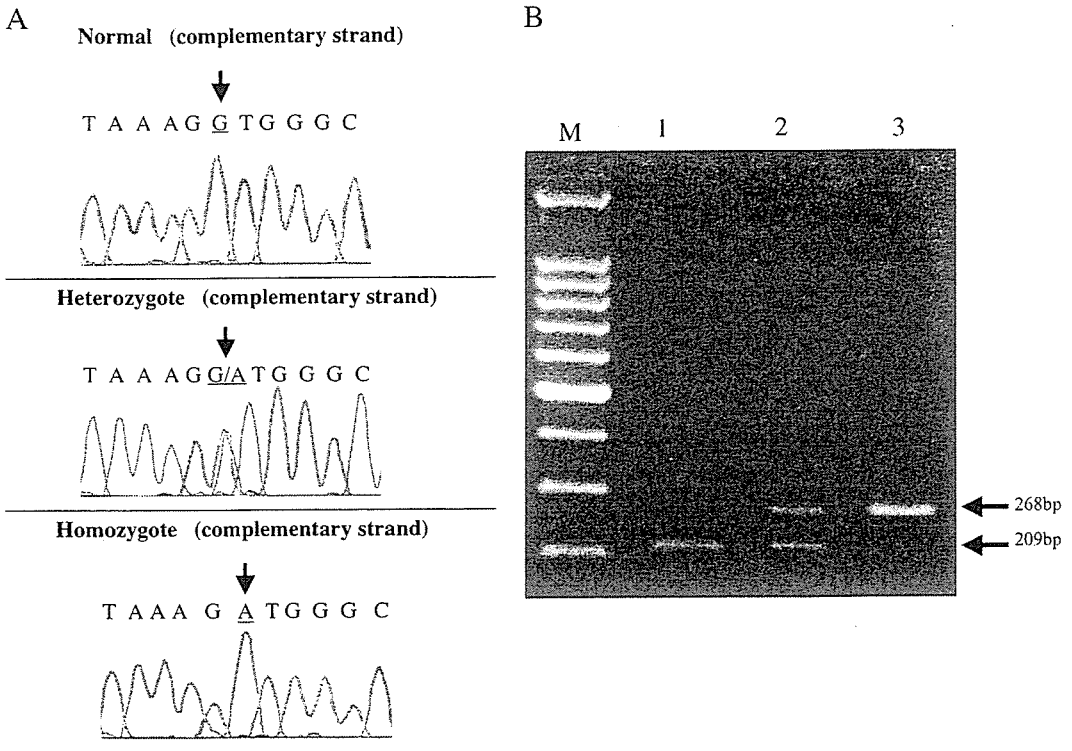

Fig. 3. (A) Nucleotide sequences of exon 1 in the *Puratrophin-1* gene. A G-to-A change (complementary strand) in a patient with a heterozygous or a homozygous state, and the normal sequence in a normal individual are shown. (B) The PCR products after *Eco*NI digestion. Lane M, 100 bp size markers; lane 1, a normal individual; lane 2, a patient with a heterozygous C-to-T change; lane 3, a patient with a homozygous C-to-T change. The wild-type *Eco*NI-digested PCR products gave three bands (209, 92, and 59 bp). Meanwhile, the *Eco*NI-digested PCR products with a heterozygous C-to-T change gave four bands (268, 209, 92, and 59 bp), and those with a homozygous C-to-T change gave two bands (268 and 92 bp). Two *Eco*NI-digested bands (92 and 59 bp) cannot be seen.

3A shows the results of GeneScan analysis of the nucleotide sequence flanking the C-to-T change (complementary strand) in the *puratrophin-1* gene in a heterozygous or homozygous patient and a normal individual in the same family. Fig. 3B shows the results as to the *Eco*NI-digested PCR products in the heterozygous or homozygous patient and the normal individual.

We constructed haplotypes for pedigrees 1 and 3, the two families large enough for haplotype analysis, and the results revealed that all patients in the two families were segregated with the haplotype 1-2-3-2 for the chromosome 16q markers TA001, GA001, TTTA001 and CATG003 (Fig. 1). The genotypes of the remaining patients and carriers were also identical with the haplotype 1-2-3-2 (data not shown). Furthermore, the specific allele, 2, of GA001 was only seen in all 22 patients and the four carriers with 16q-linked ADCA. The results indicate that the GA001 marker is very specific for the diagnosis of patients with16q-linked ADCA.

Table 2
Frequencies of various subtypes of ADCA in 110 Japanese families

	Number of families	%	Number of patients	%
MJD/SCA3	36	32.7	79	41.6
SCA6	27	24.5	44	23.1
16q-linked	9	8.2	20	10.5
DRPLA	9	8.2	11	5.8
SCA2	7	6.4	12	6.3
SCA1	6	5.5	6	3.2
SCA8	1	0.9	1	0.5
Unknown	15	13.6	17	9.0
Total	110	100	190	100

Approximately 80% of the 110 families were from the Kanto region, in a central region of the mainland of Japan. Five of the nine families with 16q-linked ADCA were from the Kanto region, whereas the remaining families were from the other regions of Japan.

3.3. Frequency of 16q-linked ADCA

The frequencies of various subtypes of ADCA in Japan are shown in Table 2. The results showed that the frequency of 16q-linked ADCA families is 8.2%, this being lower than those of MJD/SCA3 (32.7%) and SCA6 (24.5%) ones, and thus it is the third most frequent ADCA together with DRPLA in Japan. Similarly, concerning the number of patients, 16q-linked ADCA was the third-most frequent next to MJD/SCA3 and SCA6.

4. Discussion

In the present study, we found 22 Japanese patients with 16q-linked ADCA, and revealed some characteristic clinical features of this disease in comparison with those found in

earlier studies on 16q-linked ADCA in Japan, and SCA4 in Utah and Germany, most of which involved linkage analyses [4–10,20,21]. First, the mean age at onset in our patients was 61.8 years, this being later than those in two earlier reports on Japanese families with 16q-linked ADCA (mean, 55.9 and 56.7 years old) [6,20]. Moreover, the mean age at onset in our patients was much later than that in the SCA4 patients in Utah and Germany (mean, 39.3 and 38.3 years old) [4,5]. The age at onset in our patients with 16q-linked ADCA is much later than that in the patients with SCA6 (mean, 45.0 years old) [6], which indicates late-onset pure cerebellar ataxia. Therefore, 16q-linked ADCA appears to exhibit the oldest age at onset among the ADCA subtypes with assigned loci [9]. Second, we found that although cerebellar ataxia was the most common and predominant feature in 16q-linked ADCA, 54.5% of our patients showed exaggerated deep tendon reflexes. Furthermore, moderate spasticity in the lower limbs was noted in three of the four patients examined in pedigree 3. Thus, although we observed no Babinski signs in our patients, possible pyramidal tract signs can accompany cerebellar ataxia in 16q-linked ADCA, as described for SCA4 [4,5]. Since spasticity in the lower limbs was noted only in one pedigree, the presence of some modifying genetic factors for this phenotype is suggested. Meanwhile, the sensory axonal neuropathy described in SCA4 [4,5] was absent in our patients, as in the earlier reports of 16q-linked ADCA [6–10,20,21]. Sensorineural hearing impairment was considered to be another important clinical feature of the disease [9,10], and 6 (42.8%) of 14 families were reported to have this condition in addition to age-related hearing loss [9]. In our study, audiograms revealed that 37.5% of the patients examined had hearing impairment. However, since we examined only eight patients by means of audiograms, further examinations including audiograms and brainstem auditory evoked potential measurement will be necessary to clarify whether or not hearing impairment is associated with 16q-linked ADCA. Third, we found two asymptomatic carriers with transient nystagmus and mild hyperreflexia, suggesting they are early clinical signs of this disease.

It is noteworthy that we found two sporadic patients with 16q-linked ADCA who had been diagnosed as having LCCA. The parents of the two sporadic patients were all normal until death in their 40s and at 73, 74, and 94 years old, and there were no individuals with cerebellar ataxia in their families. Since the age at onset in our patients with 16q-linked ADCA is very late, the parents who could have harbored a C-to-T mutation in the *puratrophin-1* gene appeared to be neurologically free until their death. Otherwise, incomplete penetrance can be suspected in 16q-linked ADCA. Thus, there is a possibility that a patient with this disease can be misdiagnosed as having sporadic LCCA, and we should analyze the *puratrophin-1* gene even in an apparently sporadic case with cerebellar ataxia.

In pedigree 2, four of the six patients were homozygous for the C-to-T substitution in the *puratrophin-1* gene. Comparing the mean age at onset in homozygotes with that in heterozygotes in this pedigree, the former was earlier than the latter. Unfortunately, we could not accurately compare the phenotypic severity during the disease course in them. Since the number of observation is low, we should be prudent in interpretation for a gene dosage effect in 16q-linked ADCA. In SCA6, although a gene dosage effect is considered [22–24], the increase in the severity of symptoms with homozygosity is not as great as that observed in MJD/SCA3 [13]. Similarly, a gene dosage effect in 16q-linked ADCA, if one exists, might be mild and similar to that in SCA6. Further studies are required to clarify whether a gene dosage effect indeed exists in 16q-linked ADCA or not, because the brain MRI findings revealed similar atrophy of the cerebellum in a homozygous patient and a heterozygous one.

Our study revealed that 16q-linked ADCA was the third-most frequent subtype of ADCA next to MJD/SCA3 and SCA6 in 110 Japanese families with ADCA. Although SCA6, MJD/SCA3, and DRPLA are considered to be the most prevalent subtypes of ADCA in Japan despite considerable variation in the frequency of each subtype among districts [25], our study showed that 16q-linked ADCA is also frequently seen among Japanese patients with ADCA, and thus this disease may be widespread in Japan. Meanwhile, 13.6% of our ADCA families still remained to be caused by an unknown molecular basis. The clinical features of these families showed adult-onset cerebellar ataxia with or without extracerebellar neurological dysfunction. Although a linkage analysis could not be performed on these families because of a small number of the family members, we should elucidate the molecular etiology of these ADCA families in the near future.

We confirmed that a C-to-T single nucleotide substitution in the 5′ UTR of exon 1 in the *puratrophin-1* gene is strongly associated with a distinct form of ataxia. This substitution appears to be the mutation that causes 16q-linked ADCA for the following reasons. First, this change was completely segregated with the disease in 52 Japanese ADCA families, whereas such a change was not seen in 1000 control chromosomes [9]. Second, the C-to-T change resulted in reduced expression in the in vitro luciferase assay, which was consistent with the tendency for reduction in mRNA expression in the cerebellum in 16q-linked ADCA [9]. Third, *puratrophin-1* was aggregated in the major target neurons, i.e., Purkinje cells, in 16q-linked ADCA [9]. In the present study, we also confirmed that allele 2 of GA001 was only seen in all affected and asymptomatic carriers with the C-to-T substitution. Since allele 2 ("allele 4" in the previous report) has been seen in all affected individuals in all 52 families with 16q-linked ADCA, but in only 1 in 1000 control chromosomes, GA001 shows strong linkage disequilibrium [9]. Although we could perform haplotype analysis in only two families, the haplotype of "1-2-3-2" (TA001-GA001-TTTA001-CATG003) was common in the two families, suggesting a founder effect in 16q-linked ADCA. Similarly, a strong founder effect has been observed for 16q-linked ADCA in Japan [7, 9].