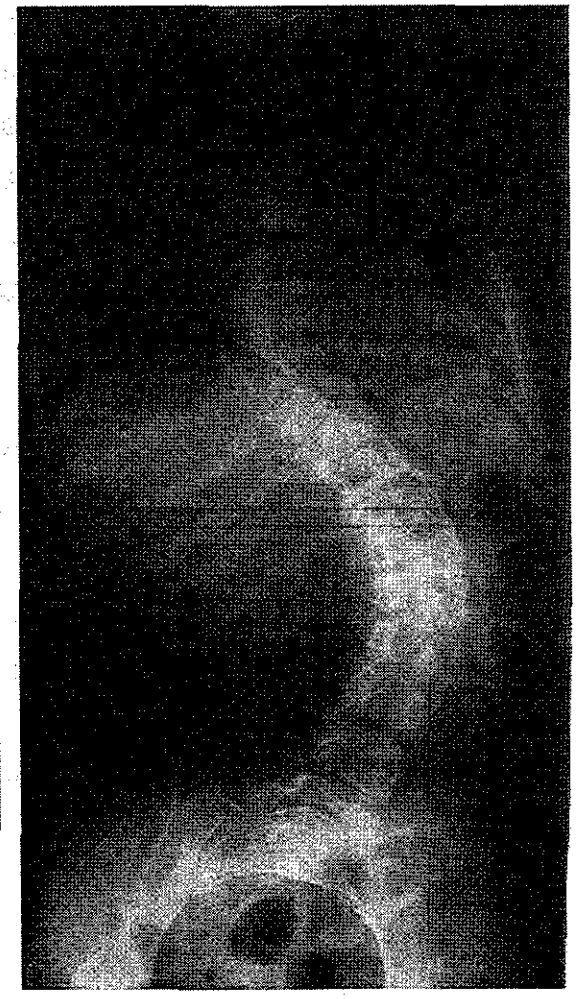
2）リラクセーション－筋肉の緊張が低下する楽な姿勢を探し、その姿勢を長時間とれるようにする、坐位保持装置や特殊な構造の車イスも含まれます
3）自発運動を誘発する－音や触覚による快刺激で本人からの動きを引き出す
などがあります。個人により好みがありますが、プールが好きな子はプールに入ると、上記の1）2）3）が全部できたりします。

側弯はレット症候群のリハビリテーションで最大の問題です。次の写真と、レントゲンフィルムを見て下さい。



背中を見せてくれているお子さんは高校生です。長期間リハビリテーションをしていますが、背骨が本人の左側を外にして大きく曲がり、車イスに乗って良い姿勢を維持するのも困難です。右のレントゲン写真もほぼ同じ年齢の別のお子さんですが、幼児期からS字状の側弯が進行し、リハビリテーションとコルセットの使用をしましたが、結果的には進行を止められませんでした。このように側弯が進行すると、呼吸や循環機能に障害をきたし、股関節の形にも悪影響がきます。着がえや入浴などの日常の介護も困難になります。高度の側弯に対しては、背骨をまっすぐに伸ばす手術が試みられていますが、大がかりな手術なので、実施するかどうかはなかなか迷うところです。

痙性が進行した場合は、筋肉の緊張を落とす薬剤を併用するとリハビリテーションの効果が上がります。股関節の内転（股関節をかたく閉めてしまう動き）に対しては神経ブロックが一時的ですが有効です。足関節の伸展（尖足）に対しては、アキレス腱延長術が効果があります。

２．手の機能

手の機能、特に把握保持機能が早期に障害される、同時に手の常同運動が出現するのがレット症候群の特徴です。これまで常同運動を減らし、把握機能を回復させようと、いろいろ試みられてきました。ひじや手首を固定して、常同運動を止める方法は効果がありませんでした。むしろ本人をイライラさせてしまい、逆効果だったようです。ミトンのような手袋も同様に手もみ防止の効果はないようでした。ただしミトンは、唾液による汚染や手のあれを防止する効果はあります。

絵カードやおもちゃを使って刺激を加え、手の有目的運動を誘発する方法は、多少は効果があるようです。先の音楽療法もこれを取り入れています。

３．情緒やコミュニケーション

レット症候群のお子さんは言語機能を失ってしまいますが、視覚や聴覚による刺激入力は可能で、内面的な感情はかなり保たれていると考えます。このため先の音楽療法や、視覚と聴覚とを組み合わせた入力は、本人の反応を引き出す効果が期待できます。また触刺激を加えるため、ボールプールや泡風呂も効果があります。

その他

バランス機能の低下が、一部のレット症候群のお子さんで観察されます。このような場合は、バランスボードや天井からつるした器具に乗るリハビリテーションを行い、平衡感覚に刺激を加えます。

第８章　レット症候群の歯科の問題

この章は、拙著　レット症候群介護マニュアル　に原稿を寄せて頂いた、東京都立東大和療育センター歯科医長中村全宏先生から御許可を頂き、同書第５章歯科の問題のダイジェスト版を御紹介します。

１．レット症候群の子では、生まれつきの歯の異常はない　口腔保健は一般と同じ

歯の２大疾病は、むし歯と歯周病です。これらを予防することが口腔保健の目的です。それには歯みがきをきちんと行い、歯垢を取り除くことによって、この２大疾病を予防することです。

２．レット症候群の子に出やすい歯科の病気がふたつある

レット症候群の症状のひとつに歯ぎしりがあります。起きている間ずっと長時間、強い歯ぎしりをしている例もあります。人のあごの関節の力（かみ合わせる力）は大変強いので、歯ぎしりをすると歯に大きな力がかかります。このため咬耗（こうもう）と咬合性外傷という、ふたつの問題が生じます。

１）咬耗

歯のかみ合わせ面（咬合面）には凹凸がついています。これは食べ物をかみ切る、あるいは食べ物をすりつぶすという目的のためです。歯ぎしりがくり返されると、上下の咬合面がお互いに強くこすり合わされるので、だんだんとすり減ってきます。そうすると表面の丈夫なエナメル質がなくなり、その下から象牙質が出てきます。さらに進行すると歯髄が露出し、歯髄壊死をおこし、また感染を起こして、適切な治療をしないと、抜歯しなくてはならなくなります。もちろんあまり咬耗すると、摂食機能にも障害をきたします。

２）咬合性外傷

歯は歯槽骨によって支えられています。歯ぎしりが強いと、歯と歯槽骨との接着部位に横向きの強い力が働き、その繰り返しの結果、接着部位に炎症を起こします。そうすると歯槽骨が歯を支える力が低下し、歯の脱落の原因になります。

レット症候群における咬耗と咬合性外傷とは、一般の方のむし歯と歯槽膿漏とに相当します。予防策としては、歯ぎしりの強いときにはプラスチック製のプロテクターを作製して使用します。これによって咬耗と咬合性外傷とを予防し、また歯ぎしり時の音も予防されます。ただし長時間プロテクターを装着することは精神的ストレスの増加にもつながるので、時間を決めて使用するという方法をとるのが現実的です。

第９章　お父さんの手記

若林勇司（東京都）

「さすが麻友さん、パパの子名人！」

麻友の将来を考えると不安で仕方ない。

「これから先、毎日麻友は普通に食事を摂ることができるんだろうか？」「麻友の背骨の側弯はこのまま進行し続けてしまうのだろうか？」「麻友は毎日ニコニコでやっていけるのだろうか？」「私たち両親が年老いた時、大きくなった麻友の世話は十分にできるのだろうか？」「私たち両親がいなくなったら、誰が麻友の面倒を見るのだろうか？」「30 年後、40 年後、麻友の生活環境はどうなっているんだろう？」「レット症候群の治療法が見つかって、麻友は会話をしたり、お散歩したりできるのだろうか？」「レット症候群の治療法は間に合うんだろうか？」　――――将来の不安は書き切れないほどいっぱいある。

まわりでは、子どもの中学受験の話が飛び交っている。テレビや新聞では、小学生・中学生の犯罪の被害者・加害者になるニュースが連日報道されている。モラルに欠けた子どもたちの実態を報じる映像もよく目にする。そんな子どもを持つ親のしつけや教育の大変さも日に日に深刻化している。街へ出ると、ギャーギャー喚き、親の言うことを聞かない子どもが多く、ムカムカさせられることも多い。そんな時、何か残念感みたいなものを感じながら、一方で、ふと「麻友さんでよかった」と、そんな幾多の余計な不安も不必要な麻友に感謝させられたりもする。

普段の麻友は私にとって最高の“癒し系アイドル”である。

平日は決まって深夜帰りのため、もちろん麻友と遊んだりはできない。それでも深夜の駅からの帰り道、遣り残した仕事のことで頭が一杯の時に限って、麻友の顔を必ず思い浮かべる。するとそこから先、家に着くまでずっと麻友の表情を想像しながら歩を早めることになる。傍から見ればニヤニヤデレデレしながら早足で歩く危ないオヤヂに見られても仕方ないのだが、深夜だから誰とすれ違うこともない。毎晩こんな調子である。家に帰れば、決まって麻友の寝顔を覗き込んではほっぺたに触りながら、「まゆちん」「まゆきち」「まゆたろう」などと声を掛け、起こしてはいけないとわかっていながらも、そのギリギリのところを毎晩楽しんでいる。たまに迷惑そうな声を出したり、眉間に皺を寄せ「何時だと思ってんだ！」と文句を言わんばかりの険しい顔をすることもある。そんな麻友を見ては、ニコニコしている麻友を見るのと同じくらい楽しくて毎晩止められないでいる。要は、麻友が反応さえしてくれれば、それで楽しいのである。はたまた、その日一日の麻友の“お利口ぶり”を妻からつぶさに聞き出しては、さらにほっぺたを弄り回すことになる。結局は、半々の割合で麻友を起こしてしまっている。翌朝は前夜に聞いた麻友の“お利口ぶり”を褒めちぎりながら、ほっぺたを弄り回しながら、「さすが麻友さん、〇〇名人！」と連呼する。すると、麻友も

ニターッとしてくる。その顔を見てはすかさず「さすが麻友さん、パパの子名人！」と顔を撫でくり回す。きりがない。そんな毎日である。

麻友を取り巻く今の環境は非常に恵まれている。特に麻友は、お世話をして下さるたくさんの“人”に恵まれている。

都立府中養護学校の先生方や職員さん、都立東大和療育センターの看護士さんやヘルパーさんたち、自宅でお風呂に入れてくれるボランティアの方などなど、皆さんの見事なまでの献身的なお世話ぶりには、本当に頭の下がる思いである。もちろん、いつも一番麻友の近くにいる妻にも頭が上がらない。自分を犠牲にして、周りの目に見えない敵と闘って麻友を守り続ける姿は尊敬ものである。

麻友の世話も十分にできない私も、そんな方々のお役に立つサポートをしたい、あるいは麻友と同じような状況の子どもや家族の役に立ちたいと思い、資格を取って転職を、とまで考えたことも何度かあった。しかし、思い切りも悪く惰性に流され何も出来ず、今に至ってしまっている。ましてや麻友の障害や将来のことを正面で捉えることが出来ず、面倒なことからいつも逃げてばかりで妻に任せっきり。周囲にも麻友のことを言えないでいる。情けない次第。

今年の**4**月、いつも**5**歳くらいの感覚で接している麻友もついに中学生になる。「うそだろー！」という気持ちが正直のところ。麻友のお世話をして下さるたくさんの方々に支えられ、毎日をニコニコで過ごす麻友に癒される日々がしばらくは続くだろう。でも、そろそろ真剣に将来に向けて気持ちの切替えをしていかなくてはと思う。

拙著＜レット症候群介護マニュアル＞では、５人のお母さんの手記を掲載し、大変好評でした。今回は若林麻友ちゃんのお父さんが手記を寄せて下さいました。本当に有難うございました。感謝します。

おわりに

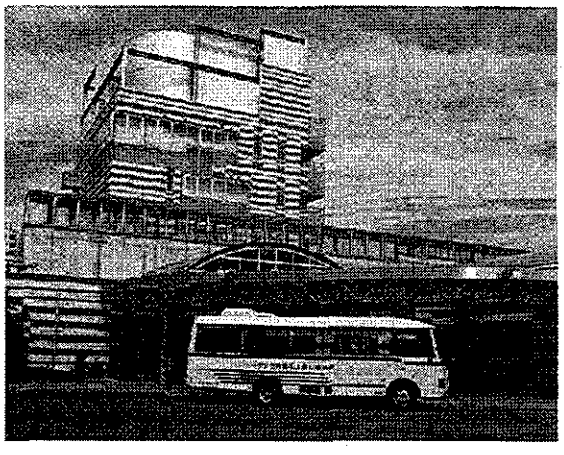
関係者の皆さんから御協力をいただき、この本もなんとか完成にこぎつけました。あらためて皆さんに感謝を申し上げます。

レット症候群に関してはまだまだ不明な点が多いのですが、目の前のお子さんに対して＜まだ分からない点が多いので、研究成果が上がるまで待っててね、ごめんね＞と言うわけにもいきません。手探り状態でも前進するのが医師のつとめでしょう。この本はその＜手探りの手＞の一部分のつもりです。

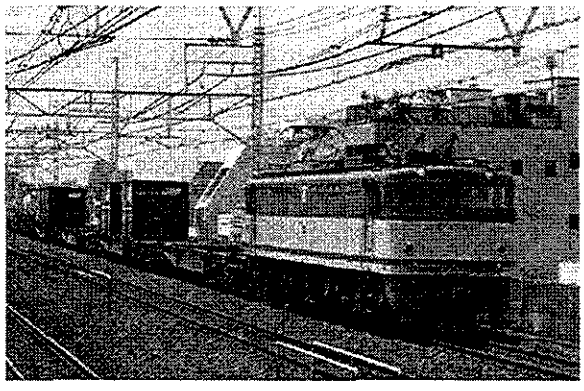
くり返しますがこの本はあくまで入門書です。一般の方には日本レット症候群協会から出版されている各種図書が、専門家の方には多数の学術論文が参考になります。また拙著＜レット症候群介護マニュアル＞（１９９８年）は品切れですが、御希望の方は下記まで御連絡下さい。御相談いたします。

東京都立東大和療育センター　鈴木文晴
〒　２０７－００２２　東大和市桜が丘３－４４－１０
電　話　０４２－５６７－０２２２
ＦＡＸ　０４２－５６７－０２２４

最後におまけを少々・・・



東京都立東大和療育センターの外観と通所バス
この建物は　日本建築学会賞　受賞です。
でも雨もりするんですよね。



私の好きな電気機関車ＥＦ６５型
（ＪＲ貨物）

印刷：グラパックジャパン株式会社
〒102-0082
東京都千代田区一番町17-2-1F

厚生労働省科学研究費補助金（障害保険福祉総合研究事業）
「知的障害のある人への適正な医療の提供に関する研究」報告書

— Angelman 症候群と睡眠障害 —

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研究要旨

Angelman 症候群（以下 AS）は、重度精神遅滞，てんかん，失調性歩行，睡眠障害，笑い発作，特徴的な顔貌を特徴とする染色体異常症である。原因遺伝子は 15 番染色体長腕 q11- q13 領域に存在する UBE3A 遺伝子であることが報告されている。AS の 20％～80％に認められるとされる睡眠障害は、特に幼少期（2～6 歳）に認められ、睡眠覚醒のリズム異常や夜間睡眠の減少、中途覚醒増加など ＡＳ児を育てる上で多くの家族が直面する問題といえる。

今回、我々は本大学病院に受診中の AS 患児 3 名を対象に AS の臨床像について検討し、睡眠障害に対してメラトニンが有効であった。またパソコンを用いた睡眠覚醒リズム解析プログラムが治療効果の判定に有効であることが明らかになった。

A, 研究目的

AS における臨床症状は多彩であり、表 1 に示すように重度精神遅滞は全例にまた、睡眠障害は 2～6 歳では 80％と高率であり、親も極度の睡眠不足におちいり、緊急の対応が必要である。今回、メラトニンの有効性を睡眠覚醒リズム解析を用いて検討する。

B, 対象

症例：ＦＩＳＨにて 15 番染色体長腕の欠失を認めたＡＳ3 例（Ａ：男児 5 歳、Ｂ：男児 15 歳、Ｃ：女児 8 歳）で睡眠障害を含む臨床像について検討した。

C，方法

臨床症状については以下のチェック項目（Williams CA.,et al）について主治医が評価した。

Ｉ群：必ずみられる症状（100％）として、「発達の遅れ（機能的には重度）」、「言語障害」、「動作や平衡の異常（失調性歩行や四肢の振戦様運動）」、「特異な行動（すぐに笑ったり

微笑んだりする)」、「見るからに嬉しそう」、「すぐに興奮する性格」、「動いてばかりいる」、「集中力に欠ける」、の 8 項目。Ⅱ群：多く見られる症状（80％以上）として、「頭囲の増加の遅れまたは不均衡（小頭症)、「痙攣（3 歳までに)」、「脳波異常（高振幅徐波)」の 3 項目。Ⅲ群：ときにみられる症状（20～80％）として、「斜視」、「皮膚や眼球の低色素」、「舌を突き出す（吸綴や嚥下の異常)」、「腱反射の亢進」、「乳児期の哺乳・摂食障害」、「歩行中に腕を持ち上げ曲げる」、「下顎の突出」、「熱に対する感覚過敏」、「大きな口」、「間隙の空いた歯」、「睡眠障害」、「頻回の流涎」、「舌の呈出」、「水が大好きで引き付けられる」、「噛んだりもぐもぐする動作の過剰」、「後頭部扁平」の 14 項目、それぞれについて現在の症状および状態と、これまでの発達過程において認められた時期について評価した。(表 1)

さらに睡眠障害については睡眠記録用紙をＡＳ児の保護者に配付し、ＡＳ児の睡眠時間（入眠と起床）について記入してもらい、メラトニン投与前と投与後について睡眠障害の改善を検討した。

D，結果

チェック項目を用いて臨床像の検討をしたところ、現在幼少期にあるＡ男児とＣ女児は、Ⅰ、Ⅱ群の項目全ての臨床症状が認められた。Ⅲ群においては「噛んだりもぐもぐする動作の過剰」以外の臨床症状が認められた。C男児は現在 15 歳であり、すでに軽快している症状もありⅢ群においては、「舌を突き出す（吸綴や嚥下の異常)」、「下顎の突出」、「大きな口」、「間隙の空いた歯」、「睡眠障害」、「頻回の流涎」、「舌の呈出」、「後頭部扁平」の 8 項目の臨床症状が認められた。

睡眠障害について睡眠記録表に記入してもらい、メラトニン投与前、投与後で入眠時間、夜間の覚醒の有無、起床時間、夜間の睡眠時間、昼間の睡眠時間などを比較した。その結果、メラトニン投与前と比べ投与後では、入眠時間が約 1 時間半早くなり、起床時間がほぼ一定した。さらに夜間の中途覚醒の回数が減少した。1 日の総睡眠時間に対する夜間の睡眠時間の割合が増え、昼間の睡眠時間が減少した。このことは、夜間の十分な睡眠により、昼間の活動が増えたことも示唆している。（図 1,A,B）ＡＳ児の家族にとっても夜間の中途覚醒が減少したことにより、家族の睡眠時間が維持でき、昼夜のメリハリのある生活が送れるようになってきている。現在では、昼間の活動量が多いとメラトニンを服用しなくても、

入眠でき、夜間の中途覚醒もない日を過ごすことができている。

E，結論

ＡＳ児の臨床像と睡眠障害の程度とメラトニンによるその改善を検討した。ＡＳ児の睡眠覚醒リズム障害は、生後数ヶ月より認められ、長期に持続する。[1)~3)]。これは、共に暮らす家族にとっても大きな問題である。今回、睡眠記録表に記入してもらうことにより、ＡＳ児の睡眠が断片化しており、家族にとっては夜間の中途覚醒が最も問題であることがわかった。メラトニン服用により、それらの睡眠の断片化が減少し、昼夜の睡眠覚醒リズムがとれるようになった。さらにそのリズムが出来上がると、メラトニンを服用しなくても昼間の活動量を維持できれば夜間の入眠がスムーズで中途覚醒も認められない。したがって、早期の段階に一定期間メラトニンを服用することで、睡眠覚醒のリズムを確立することができると思われる。

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MUTATION IN BRIEF

Novel *TSC2* Mutations and Decreased Expression of Tuberin in Cultured Tumor Cells with an Insertion Mutation

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by hamartomas in many organs. Two genes responsible for TSC, *TSC1* and *TSC2*, were recently identified. *TSC1* and *TSC2* encode the proteins hamartin and tuberin, respectively, and 337 different mutations have been reported in these genes thus far. Here, we report six novel *TSC2* mutations including one missense mutation, two nonsense point mutations, two frameshifts, and an insertion mutation. The insertion mutation is unique because of its location at an exon/intron boundary that results in triplication of a 34-bp sequence. Cultured tumor cells from the patient with this insertion mutation exhibited a decreased level of tuberin as revealed by Western blotting, suggesting that the mRNA of *TSC2* is not translated as efficiently or the translated protein exhibits reduced stability. Five novel polymorphisms of *TSC2* were also identified. As previously reported, the missense mutations were located in the GTPase activating protein-related domain of *TSC2* encoded in exons 34-38. No *TSC1* mutations were identified in the present subjects. © 2004 Wiley-Liss, Inc.

KEY WORDS: giant cell astrocytoma; Japanese; mutation; *TSC2*; tuberin; tuberous sclerosis complex

INTRODUCTION

Tuberous sclerosis complex (TSC) (MIM# 191100) is an autosomal dominant disorder characterized by the development of hamartomatous growth in many different organs, most commonly in the brain, heart, kidney and skin (Gomez et al., 1999). Involvement of the brain is associated with the most problematic clinical manifestations

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of TSC, including intellectual handicap, epilepsy and abnormal behavior (Cheadle et al., 2000). Approximately two-thirds of the cases are sporadic, without family history, reflecting a high spontaneous mutation rate in the underlying genes (Osborne et al., 1991, Sampson et al., 1994).

Two TSC-related genes were previously identified by positional cloning. *TSC2* (MIM# 191092) is located on chromosome 16p13.3 and consists of 41 exons, whereas *TSC1* (MIM# 191100) is located on 9q34 and consists of 23 exons (The European Chromosome 16 Tuberous Sclerosis Consortium 1993; van Slegtenhorst et al., 1997). *TSC2* encodes the 200-kDa protein tuberin that contains a GTPase activating protein (GAP)-related domain (The European Chromosome 16 Tuberous Sclerosis Consortium 1993). Hamartin, the 130-kDa predicted product of *TSC1*, is a novel protein that is predicted to form a complex with tuberin (van Slegtenhorst et al., 1997). Loss of heterozygosity (LOH) of either *TSC1* or *TSC2* in affected tissues indicates that each acts as a tumor suppressor (Green et al., 1994; Henske et al., 1995; Sepp et al., 1996).

At least, 131 and 343 different disease causing mutations have been reported in *TSC1* and *TSC2*, respectively (Cheadle et al., 2000; The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html). Here, we used single-strand conformational polymorphism (SSCP) analysis of genomic DNA to identify six novel mutations in Japanese TSC patients. Tuberin expression was decreased in cultured tumor cells from a patient with a unique insertion mutation in *TSC2*.

MATERIALS AND METHODS

Patients

Clinical information for all patients is summarized in Table 1. Patient 1 was a 9-year-old female. Her early developmental milestones were normal. She suffered from febrile convulsions at age 2, and at age 5 was diagnosed with TSC due to sebacea and brain calcifications revealed by computed tomography (CT). At 6 years of age, she presented with afebrile convulsions, and electroencephalography (EEG) revealed right parietal focal spikes and waves. Her mental milestones indicated mild retardation.

Table 1. Summary of the Clinical Information of the Patients

Patient	Age	Gender	Skin	Neurological Findings	Brain Radiological Findings	Others
Patient 1 (s)	9	F	+	seizures, mild MR	calcification	
Patient 2 (nd)	20	M	+	seizures, severe MR	?	bilateral renal tumor
Patient 3 (f)	15	F	+	seizures, MR	+ (no detailed information)	bilateral renal tumor, lung lymphangima
Patient 4 (nd)	16	F	?	(normal intelligence)	calcification	left renal tumor
Patient 5 (s)	1	M	+	West, mild developmental delay	PN, CD, heterotopia	cardiac tumor
Patient 6 (s)	2	F	+	West	PN, CD, heterotopia	cardiac tumor
Patient 7 (f)	15	F	+	West to Lennox, severe MR	PN, tubers	cardiac tumor
Patient 8 (f)	24	F	+	West syndrome, moderate MR	PN, calcification	brain tumor
Patient 9 (s)	2	F	+	seizures	calcification	
Patient 10 (s)	7	F	+	West syndrome	PN, tubers	

skin, skin involvement; s, sporadic case; f, familial case; nd, not detected; F, female; M, male; ?, unknown

MR, mental retardation; PN, periventricular nodules; CD, cortical dysplasia

Patient 2 was a 20-year-old male. At 10 months of age, he suffered febrile convulsions and was diagnosed with a developmental delay. At 17 months of age, a left renal tumor was surgically removed, the pathological diagnosis of which was renal cell carcinoma. At age 17 years, right renal angiolipoma was identified. His mental state was one of severe retardation, and he displayed white skin patches.

Patient 3 was a 15-year-old girl. In infancy, she suffered from seizure attacks and subsequently exhibited delayed psychomotor development. Periventricular nodules were noted by radiological examination. White skin

patches were also noted. At 13 years of age, bilateral renal angiolipomas were identified and surgically removed. At age 14, she had a first incidence of spontaneous pneumothorax with recurrent episodes in subsequent years. A chest X-ray revealed bilateral lung cystic lesions that were suspected to be lymphangiomas.

Patient 4 was a 16-year-old girl. At age 3 months, a right renal tumor was surgically removed and found to be cystic dysplasia. Her first epileptic episode occurred at age 1 year. At present, she has normal intelligence in spite of brain calcification. She has no cystic lesions in her left kidney but has an angiomyolipoma in the liver.

Patient 5 was the second child of healthy parents, and was immediately diagnosed with multiple cardiac tumors just after delivery. At 1 month of age, he presented with white skin patches and developed tonic spasms. Brain CT showed periventricular nodules and MRI showed left fronto-parietal cortical dysplasia and heterotopia. Now, at 1 year of age, he exhibits mild developmental delay.

Patient 6 was a 2-year-old girl. At 4 months of age, she was afflicted with infantile spasms, and an EEG indicated hypsarhythmia. She had white skin patches and cardiac rhabdomyoma that was identified by echocardiography. Brain MRI showed small nodules in periventricular regions.

Patient 7 was a 15-year-old girl. Although her parents were healthy, her younger brother had retinal hamartomas. She had white patches, facial angiofibromas and ungual fibroma. Radiological findings suggested subependymal nodules and tubers in the brain and cardiac rhabdomyoma. In infancy, she had West syndrome that later developed into Lennox syndrome. Her present intelligence quotient (I.Q.) is below 20.

Patient 8 was a 24-year-old female. At age 11 months she suffered infantile spasms. Many white spots were present on her skin. Brain radiological examinations revealed a right anterior ventricular tumor and periventricular calcifications. When she was 8 months old, she had chronic left facial palsy that may have been related to a tumor, and her symptoms disappeared after resection of a tumor that was diagnosed as a giant cell astrocytoma (cells were cultured from the resected tumor tissues, and were used for Western blotting). Presently, she exhibits moderate mental retardation. Although her parents and an elder sister are healthy, her father has white macules.

Patient 9 was a 2-year-old girl. Following her first epileptic attack, a detailed investigation suggested TSC based on white macules and brain calcifications.

Patient 10 was a 7-year-old boy that displayed white macules. He developed West syndrome at 5 months of age. Radiological examination showed periventricular nodules and some tubers. Presently, he shows moderate mental retardation. His parents are healthy.

Molecular analysis

DNAs were extracted from peripheral lymphocytes using a standard method. Sixty-four normal control DNAs were also obtained from blood samples of healthy Japanese volunteers and used for a population study. Informed consent for genomic examinations was obtained from all patients and volunteers. The Caucasian Population Panel 100 was provided by the Coriell Institute for Medical Research (NJ, USA) and fifty DNAs samples were used for population study. Polymerase chain reaction (PCR) was used to amplify all exons of *TSC1* (GenBank accession number AF013168.1) and *TSC2* (GenBank accession number X75621.1) from genomic DNAs using standard methods with primers described elsewhere (Zhang et al., 1999; Pipo et al., 2000; Yamamoto et al., 2002). The PCR products were subjected to SSCP analysis using a minigel (10 cm X 10 cm). The samples were analyzed under four different electrophoresis conditions from a combination of two sets of gel mixtures (12% polyacrylamide gel with or without 5% (w/v) glycerol) and two temperatures (4°C or 22°C) (Zhang et al., 1999). DNA bands were visualized by silver staining. The PCR products that gave aberrant bands during the SSCP analysis were sequenced directly using the BigDye terminator cycle sequencing kit (Applied Biosystems, CA, USA) and the ABI PRISM 3100 genetic analyzer (Applied Biosystems). Each PCR product was sequenced in both directions using PCR primers.

Western blotting

Tumor cells of patient 8 were cultured from the resected tumor tissues, and harvested and homogenized by sonication in buffer (10 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA) supplemented with 1% (w/v) Triton X-100 and protease inhibitor cocktail (Boehringer Ingelheim, Ingelheim, Germany). The suspensions were centrifuged at 12,000 x g for 30 min at 4°C and the protein concentration of the supernatant was determined by the BCA protein assay (Bio-Rad, CA, USA). Protein (10 μg) was subjected to SDS-polyacrylamide gel

electrophoresis on a 10% gel, and the proteins were electrophoretically transferred to an Immobilon membrane (Millipore, Bedford, MA). Following a 1-h preincubation in 5% (w/v) skim milk, the membrane was incubated overnight at 4°C with the tuberin antibody, tuberin (C-20) (cat. # sc-893; Santa Cruz Biotechnology, Inc., USA; diluted 1:1000). Tuberin bands were detected using avidin-biotin-alkaline phosphatase (Vector ABC-AP kit).

RESULTS AND DISCUSSION

Table 2. Summary of the Disease-Causing *TSC2* Mutations

Patient	Location	Nucleotide Change*	Amino Acid Change	Type	Novel or Reported by
Patient 1 (s)	ex4	c.469G>T	p.E157X	nonsense	novel
Patient 2 (nd)	ex19	c.2163del G		frameshift	novel
Patient 3 (f)	ex24	c.2767_2768insC		frameshift	novel
Patient 4 (nd)	ex28	c.3355C>T	p.Q1119X	nonsense	novel
Patient 5 (s)	ex37	c.4952A>G	p.N1651S	missense	Maheshwar et al., 1997
Patient 6 (s)	ex37	c.4958C>T	p.S1653F	missense	novel
Patient 7 (f)	ex38	c.5024C>T	p.P1675L	missense	Maheshwar et al., 1997
Patient 8 (f)	IVS38	c.5068+20_5068+21ins34		splicing?	novel
Patient 9 (s)	ex40	c.5238_5255del	p.H1746_R1751del	deletion	Beauchamp et al., 1998
Patient 10 (s)	ex40	c.5238_5255del	p.H1746_R1751del	deletion	Beauchamp et al., 1998

s, sporadic case; c., complementary DNA No.; f, familial case; del, deletion; ins, insertion

*GenBank X75621.1. Nucleotide numbering, with A of the initiator ATG as +1

The mutation nomenclature according to the website (http://www.HGVS.org/mutnomen/).

Six novel and three previously known mutations of *TSC2* (Table 2) were identified. All of the patients exhibited mutations in *TSC2* only, and no *TSC1* mutations were identified despite the comprehensive screening of both genes. Two new nonsense mutations and two new frameshift mutations resulted from respective deletion and insertion of 1 bp would be definitely disease causing.

A novel missense mutation within exon 37, complementary DNA No. c.4958C>T (p.S1653F) in patient 6 was not found in healthy control subjects (64 Japanese and 50 Caucasians) suggesting that they are pathogenic for TSC. The known missense mutation, c.5024C>T (p.P1675L) within exon 38 in patient 7, was recurrent and thus represents a relatively common mutation (Beauchamp et al., 1998; Zhang et al., 1999). Interestingly, all three patients (patients 5, 6 and 7) with missense mutations presented with cardiorhabdomyoma. As reported elsewhere, these missense mutations were located in the GAP-related domain of *TSC2* encoded in exons 34-38 (Cheadle et al., 2000). None of the mutations were located in the sequence CpG, a dinucleotide sequence in which nucleotide alterations are prevalent.

Interestingly, both patient 2 and 3 having a novel frameshift mutation had renal cancers, and patient 3 also suffered from lung lesions. TSC with lung lesions is relatively rare and constitutes a distinct subset of the disease termed pulmonary TSC (Kalassian et al., 1997; Sullivan 1998). A common pulmonary lesion is a parenchymal cyst that is often associated with dyspnea or pneumothorax. Typically, patients with pulmonary TSC are women of childbearing age whose pulmonary lesions may be influenced by hormonal changes (Kalassian et al., 1997; Sullivan 1998).

An 18-bp deletion (c.5238_5255del) was identified within exon 40 in both sporadic patients 9 and 10. As this in-frame deletion has been frequently identified in TSC patients (Dabora et al., 2001), this region may constitute a hot spot. Another 18-bp in-frame deletion, c.5256_5273del (adjacent to 18-bp deletion at c. 5238_5255) was also identified frequently in four unrelated sporadic cases (Jones et al., 1999). These deletions occur in the sequence context of a direct repeat of eleven nucleotides with seven intervening nucleotides, and are likely the product of

slipped mispairing during replication (Cooper and Krawczak 1991). The recurrent 18-bp deletion in exon 40 of *TSC2* lies within the putative rabaptin-binding domain (Xiao et al., 1997).

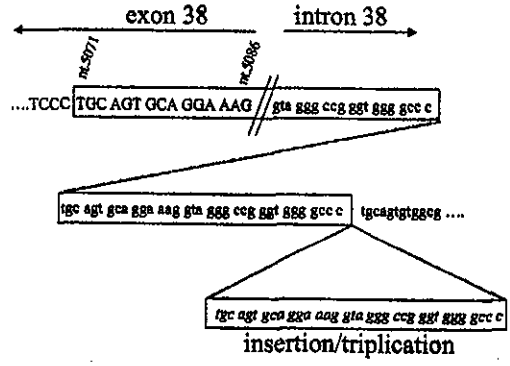

Figure 1. Schematic representation of the sequence around the site of the insertion detected in patient 8. Open boxes indicate the set of 34-bp sequences that is duplicated in the normal sequence and triplicated in patient 8. Uppercase and lowercase nucleotides indicate exonic and intronic sequences, respectively.

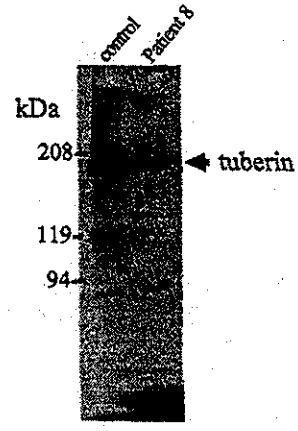

Figure 2. Western blot analysis. It was confirmed that the same amount of protein had been loaded in each lane and transferred by staining gel and unused portion of membrane with Coomassie brilliant blue (data not shown). The arrowhead indicates tuberin, the expression of which is decreased in the sample from patient 8 compared with that of control. The migration of molecular weight markers is shown to the left. C, control sample derived from an autopsied brain.

A 34-bp insertion (c.5068+20_5068+21ins34) was identified in patient 8. In the normal genomic sequence, this 34-bp sequence (TGC AGT GCA GGA AAG GTA GGG CCG GGT GGG GCC C) is duplicated across the boundary of exon 38 and intron 38 (Fig. 1). Patient 8 had three repeats of this 34-bp sequence and there was no other mutation in any of the exons of *TSC1* and *TSC2*. This patient was a familial case, but attempts to obtain samples from family members were unsuccessful. Thus, to exclude the possibility that the triplication represented benign polymorphisms, we analyzed this region in normal Japanese controls as well as in the Caucasian Population Panel 100. As expected, this duplication was not observed in any of the control samples. To test whether this insertion influences pre-mRNA splicing, cDNA was analyzed around this exon 38 using reverse-transcription PCR. However, no aberrant splicing was observed (data not shown). To estimate the impact of this genomic mutation, tuberin expression was assessed in primary-cultured cells from the patient's giant cell astrocytoma.

Tuberin expression was decreased compared with normal brain tissue (Fig. 3). This result is compatible with mutational loss of *TSC2* (Wienecke et al., 1997). However, we cannot exclude the possibility that secondary effects such as hamartin or rap1 expression may negatively influence tuberin expression.

Unlike many other symptoms that show age-dependant penetration, intellectual disability in TSC is almost invariably present from early childhood and rarely escapes detection. However, patient 4 (carrying a nonsense mutation) exhibited normal intellectual development. Patients 9 and 10 carried the same mutation, but their clinical features were different from one other. Thus, the extent of the protein truncation expected from mutations in *TSC2* does not necessarily correlate with the severity of the clinical symptoms. Therefore, the severity is likely dependent on other somatic mutations within the pathogenic lesions. Future determination of the pathogenesis of these genomic mutations will require the development of a functional assay for tuberin activity.

Five novel variations of *TSC2* were also identified (Table 3). The each variation was coincidentally identified in only one control sample which was used for population study. Thus, these variation would be very rare and not disease causing.

Table 3. Summary of the *TSC2* Polymorphisms

Location	Nucleotide change*	Amino Acid Change	Type	Novel or Reported by	Frequency#
ex14	c.1593C>T	p.I531	silent	Yamashita et al., 2000	
ex16	c.1819G>A	p.A607T	missense	novel	1/114
ex22	c.2585C>T	p.A862V	missense	novel	1/114
ex33	c.4285G>T	p.A1429S	missense	novel	1/114
ex33	c.4349C>G	p.P1450R	missense	novel	1/114
IVS33	c.4493+17C>T			novel	1/114
IVS39	c.5161-9C>A			Jones et al., 1999	
ex40	c.5202T>C	p.D1734	silent	Au et al., 1997	
ex41 (3' non-coding region)	c.5424+55_5424+58delTAAA			Kumar et al., 1995	

c., complementary DNA No.; *GenBank X75621.1. Nucleotide numbering, with A of the initiator ATG as +1

#Frequency of each variation was described as 1/114, because the each variation was detected in only one sample among 64 healthy Japanese volunteers and 50 samples of Caucasian DNA panel.

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Axonal dystrophy of dorsal root ganglion sensory neurons in a mouse model of Niemann–Pick disease type C

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Abstract

Niemann–Pick disease type C (NP-C) is a progressive and fatal neurological disorder characterized by intracellular accumulation of cholesterol and glycolipid. A Balb/c-npc1 mutant strain is a genetically authentic murine model of NP-C, and homozygous mice show progressive weight loss and tremor or ataxia until death at 12–14 weeks of age. Neuropathologically, this model is known to faithfully reproduce the cardinal histologic features of NP-C including neuronal storage, appearance of swollen axons (spheroids), and neuronal loss, although the cellular mechanisms of neural degeneration are largely unknown. To investigate the mode of neural degeneration of sensory neurons in NP-C, we studied the central processes of dorsal root ganglion (DRG) neurons at the level of the medullary dorsal column nuclei and the spinal dorsal horn with special attention to the ultrastructural changes of presynaptic axon terminals. The appearance of axonal spheroids in the dorsal column nuclei and the loss of axons in the spinal nerve roots were assessed quantitatively. We show that the gracile nuclei develop numerous axonal spheroids after only 3 weeks. At 6 and 9 weeks, dystrophic axons, which were separated from simple axonal spheroids by the ultrastructural presence of distinctive tubulo-vesicular elements, progressively increased in size and number. These neuropathological findings are identical to those of gracile axonal dystrophy (GAD) of the normal aging mouse. Presynaptic elements were exclusively involved in spheroid formation. The cuneate nuclei and the spinal dorsal horn revealed fewer axonal spheroids and only rare dystrophic changes. This was associated with a significant drop in the number of L4–5 dorsal root axons in NP-C mouse at 9 weeks of age compared with controls. These results support the existence of a length-dependent axonopathy in the central processes of DRG neurons and are consistent with the view that altered axonal transport, which is implicated in the pathogenesis of GAD in physiological aging, may be an underlying mechanism in neuronal degeneration in NP-C. Clinically, the premature development of GAD may be responsible for ataxia, one of the early manifestations of this disease.

Keywords: Niemann–Pick disease type C; Axonal dystrophy; Dorsal root ganglion neurons

Introduction

Niemann–Pick disease type C (NP-C) is an autosomal recessive disorder of lipid storage metabolism presenting with a variety of neurological symptoms including cerebellar ataxia, dementia, and supranuclear ophthalmoplegia (Pentchev et al., 1995). The defective gene responsible for this disorder is NP-C-1, which causes a disturbance in the trafficking of free cholesterol from lysosome or endosome to other membrane sites (Cruz et al., 2000; Davies et al., 2000; Neufield et al., 1999). In the monkey central nervous system (CNS), the NP-C-1 protein has been shown to reside in astrocytic processes adjacent to neural processes at synapses, suggesting its role in regional homeostasis (Patel et al., 1999). However, the cellular mechanisms of neurodegeneration in NP-C are largely unknown.

Neuropathologically, the widespread appearance of swollen neurites (spheroids) and the accumulation of intraneuronal cytoplasmic inclusions with neuronal loss (Higashi et al., 1991; Vanier and Suzuki, 1998) are the most characteristic histologic features of the NP-C brain. The distribution of

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