fifth metacarpal bone with fusion of the fourth and the fifth proximal phalanges on the left and complete absence of the metacarpal to phalangeal bones on the right (Fig. 2a,b). X-rays of both her feet showed four metatarsal bones, a lack of the fifth metatarsal bone, and cutaneous syndactyly of toes III to V (Fig. 2c). Furthermore, she had bilateral congenital conductive hearing impairment. She had bilateral narrow auditory canals. Auditory brainstem response (ABR) showed mild hearing loss bilaterally.

Brain computed tomography (CT) and echocardiography revealed no abnormal findings. Abdominal ultrasonography showed hypoplasia of the right kidney without abnormal renal function. We tested development using the Japanese ordinary developmental quotient test, known as the Enjoji Infant Developmental Scale. Her developmental quotient at the age of 2 years and 5 months was 110 in motor, 120 in social, and 72 in the verbal field. Her mild verbal disturbance was more serious in recognition than in speech.

Her chromosomal analysis showed normal karyotype, 46XX. Genomic DNA was extracted from the peripheral blood of the patient and her parents. To investigate copy number change (CNC) of the patient, we performed microarray-based copy number analysis using Cytogenetics Whole-Genome 2.7 M Array and Chromosome Analysis Suite software (Affymetrix, Santa Clara, CA, USA). Copy number analysis revealed that 33 CNC, including 18 copy number gains and 10 losses were negligible as benign copy number variants (CNV), because they were registered in our original Japanese CNV database (unpublished) and/or Database of Genomic Variants (http://projects.tcag.ca/variation/). Real-time polymerase chain reaction (PCR) analysis of the three samples using Universal Probe Library (Roche, Basel, Switzerland) for reconfirmation of the results of the microarray studies revealed that all of remaining CNC, including four copy number gains and one loss, were inherited from one of the parents. The patient's *DHODH* gene was studied by PCR amplification and direct sequencing. Primers of all exons of the *DHODH* gene were designed according to Ng *et al.*[2] PCR-direct sequence method was performed according to ordinary procedure. The patient was found to be compound heterozygote for missense mutations in her *DHODH* gene, L28P (T→C) in exon 2 and A347T (G→A) in exon 8. These mutations were novel. Her mother and father were heterozygous for L28P in exon 2 and A347T exon 8, respectively.

Discussion

Miller syndrome is a rare autosomal recessive acrofacial disorder including peculiar facies such as severe micrognathia, cleft lip and/or palate, coloboma of the eyelids, supernumerary nipples, and hypoplasia or aplasia of the postaxial elements of the limbs.[3] Facial features are similar to Treacher Collins syndrome, Goldenhar syndrome and Nager syndrome, thus differential diagnosis is necessary.

Coloboma is present in the lower eyelid in Treacher Collins syndrome, Nager syndrome, and Miller syndrome. On the other hand, Goldenhar syndrome has coloboma in the upper eyelid.[4] The above syndromes are distinguished from Miller Syndrome by the limb anomalies. Postaxial limb deficiency is a cardinal feature in Miller syndrome. Treacher Collins syndrome and Goldenhar syndrome usually have no limb anomalies. Nager syndrome shows preaxial limb anomalies. Our patient had postaxial limb deficiencies, but her facial features were not typical. These were mild for Miller syndrome. She did not have respiratory problems, the cleft palate seen in 90% of patients with Miller syndrome,[3] in-curving arms, or abdominal findings needing surgical intervention. The most distinctive facial features of our patient were lower eyelid clefts, short palpebral fissures, and small and low set ears. These could be cardinal key points for diagnosis of first and second branchial arch-related disorders. We could clinically diagnose her with Miller syndrome with limb anomalies and mild facial features.

The cause of her verbal developmental delay is unclear, because most of patients with Miller syndrome have normal intelligence/development. Re-examinations showed mild hearing impairment, however, this created few obstacles in daily life. We will follow her developmental course carefully.

Treacher Collins syndrome and Nager syndrome are generally considered to be autosomal dominant disorders.[5,6] Some patients of Nager syndrome an reveal autosomal recessive trait.[7] Treacher Collins syndrome is caused by mutations in the *TCOF1* gene located on 5q32-q33.1. Haploinsufficiency of the *TCOF1* gene in Treacher Collins syndrome patients results in the inhibition of production of properly modified mature rRNA in addition to inhibition of rDNA gene transcription, which consequently affects proliferation and proper differentiation of specific embryonic cells during development.[8] On the other hand, disruption of DHODH activity in the fetuses of mice causes a wide range of limb and craniofacial defects. The *DHODH* dysfunction inhibits NF-κB activity directly, and the interruption of NF-κB signaling during development can result in disrupted cell migration, diminished cellular proliferation, and increased apoptosis. These observations suggest that the malformations observed in individuals with Miller syndrome could be caused by perturbed NF-κB signaling due to loss of the *DHODH* function.[2] *TCOF1* and *DHODH* genes are quite different; however, mutations in either gene can cause similar dysfunctions of cell proliferation, migration, and differentiation. So, these mutations would lead to similar phenotypes.

Miller syndrome had been hypothesized to be an autosomal recessive disorder. The genetic cause of Miller syndrome, the *DHODH* gene was discovered using exome sequencing.[2] The *DHODH* gene is located on 16q22 and composed of 9 exons. DHODH is a monofunctional protein which, in most eukaryotic organisms, is located on the outer surface of the inner mitochondrial membrane, and catalyzes the fourth enzymatic step in de novo pyrimidine biosynthesis. The human *DHODH* gene, which is reported as the causable gene of Miller syndrome, was cloned in 1992.[9] This gene exists in various species. Our patient has compound hetero mutations in 28 L and 347A in the transmembrane domain and in the β7~α11 region, respectively. Her parents had one of these mutations each. The 28 L region was conserved from zebrafish and 347A from drosophila (Fig. 3).[10] These regions are essential for the preservation of various species. Thus,

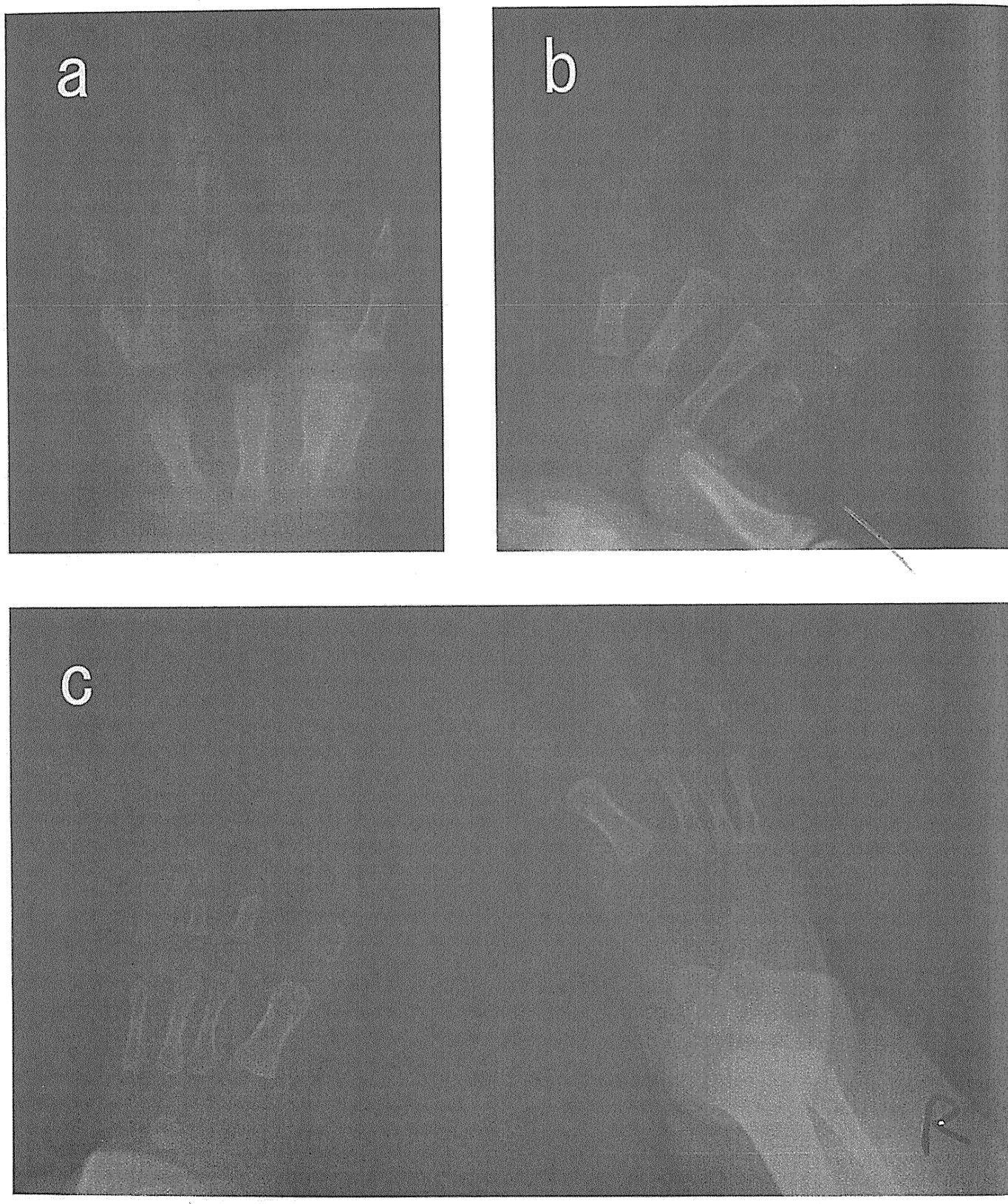

Fig. 2 (a) X-ray findings of left hand. The fifth metacarpal bone is absent, and the fourth and fifth proximal phalanges are fused. (b) Aplasia of the fifth digit in the right hand. (c) Both feet showing absence of the fifth metatarsal bone.

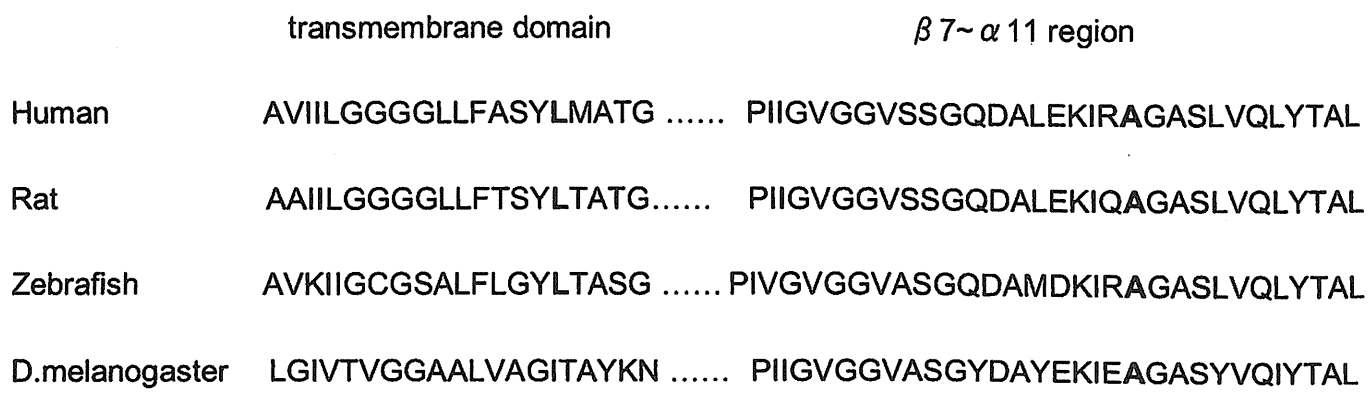

Fig. 3 Homology of the amino acid sequence in the *DHODH* gene. Transmembrane domain and β7~α11 region are shown. 28 L and 347A in the human genome are in bold.

missense mutations of these regions may be a significant etiological mechanism.

A total of 13 mutations of the *DHODH* gene are reported in Miller syndrome including our case, with a spread from exon 2 to exon 9. Human *DHODH* mutations have not yet been registered on the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/) except those reported by Ng *et al.*[2] The mutations of our patient were not found in the Miller patients described by Ng *et al.* Therefore, we think the mutation is novel. Further study is needed to elucidate the genotype/phenotype correlation.

In summary, we report a girl with Miller syndrome who was compound heterozygote of novel missense mutations in the *DHODH* gene. Facial features may not always be typical in this syndrome. Some patients with Miller syndrome have developmental delay, so a close follow-up system is needed for development as well as limb anomalies.

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Congenital anterior neck cysts classified as 'thyroglossal anomalies'

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Key words demoid cyst, Sistrunk operation, thyroglossal duct cyst.

The most frequent congenital anterior neck cyst is the thyroglossal duct remnant cyst and the second most frequent is the dermoid cyst.[1,2] A dermoid cyst in the anterior neck is considered to have arisen from abnormal invagination of the surface ectoderm that forms the face and neck.[3] A thyroglossal duct remnant cyst is caused by failure of obliteration of the thyroglossal duct when it descends from the foramen cecum to the infrahyoid region in early embryologic life. In this context, both congenital lesions are etiologically distinct but are considered by some to have a close relationship, and such lesions are sometimes collectively called 'thyroglossal anomalies'.[4–7]

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Received 21 May 2010; revised 28 August 2010; accepted 28 September 2010.
doi: 10.1111/j.1442-200X.2010.03273.x

《シンポジウム》

リハビリテーション促進的薬物治療の新たな展開

座長／田中　信行・江藤　文夫

ダウン症候群患者のQOL向上のための塩酸ドネペジル療法

重症心身障害児・者施設 みさかえの園むつみの家
近藤　達郎

Japanese Journal of Rehabilitation Medicine
VOL. 48 NO. 5　2011年5月

序については，上肢の回復が有意で，下肢の回復は認められなかったが，10 m歩行では速度の改善が有意にみられており，PETの所見と合わせると，上肢の回復については脳血流の改善に伴う効果，脳卒中で減少しているnorepinephrineの補充によるものなどが推測され，下肢についてはcentral spinal motor theoryが関与を指示する結果かと思われた．

ダウン症候群患者のQOL向上のための塩酸ドネペジル療法*

重症心身障害児・者施設 みさかえの園むつみの家
近藤 達郎

はじめに

ダウン症候群（DS）は，常染色体異常の中で最も多い疾患の1つであり，主に21番染色体過剰の21トリソミーによって生じる[1,2]．我が国のDSの出生頻度は1975年の1.07/1,000出生（1/935出生）から2006年の1.77/1000出生（1/565出生）に増加している[3]．西オーストラリア在住DS患者のコホート研究では，2000年時点での平均寿命が58.6歳，25%が62.9歳以上まで生存し，最高齢者は73歳であった[4]．我が国は長寿国であるから日本のDS患者の平均寿命はそれ以上と期待され，DS患者がはつらつとした人生を歩むことは非常に重要である．

しかしDS患者において，ある時期に，これまでできていたことが比較的短期間にできなくなることが時に経験される．筆者らの経験からは20歳前後および20歳代後半に多いような印象がある．菅野は上記の退行について，(1) 自然な衰え・低下による老化・退行タイプ，(2) 身体疾患退行タイプ，精神疾患退行タイプや青年期・成人期のDSに起こる「急激退行」タイプが含まれる稀に生じる低下・退行，および (3) その他の3つに大別できるとしている[5,6]．その中で「急激退行」は20歳前後のDS患者において日常生活能力が急激に低下するもので，具体的には，急に元気がなくなり，引きこもりが始まり，日常生活への適応に様々な困難や支障を生じるものと定義している．この「急激退行」という言葉は医学的に

* 本稿は第47回日本リハビリテーション医学会学術集会シンポジウム「リハビリテーション促進的薬物治療の新たな展開」（2010年5月21日，鹿児島）の講演をまとめたものである．

はさほど浸透されていない．Capone らは，上記の症状をうつ的状態（Depressive Illness）と見なし，「うつ病や軽微な気分障害（気分変調）の主な症状としては，うつ的状況，泣く，興味消失，行動の緩慢さ，疲れやすさ，食欲や体重の変化，および睡眠障害があげられる」と紹介している[7]．しかし，その中には抗うつ薬による治療や環境整備，ストレスの除去などに努めても効果が非常に乏しい DS 患者もいる[7]．これらを整理してみると，「急激退行」とはコリン作動性の障害をベースに甲状腺機能異常をはじめとする様々な身体疾患やうつ病などの精神疾患が併発して生じる複合的な病態なのか，または単にアルツハイマー型認知症（AD）の初期症状なのか，それともそれら両者が重複することで発症する複雑な状態であるのか，十分に検討する必要がある．さらにうつ的状況として表出されていても，それがうつ病そのものなのか AD の初期症状なのかを判別することもしばしば困難である．さらには，DS 患者は必ずしも諸検査に協力的でないため，精神医学的評価の困難さはさらに深まってしまう．

35 歳以降の DS 患者では，記憶力の低下，視覚性記銘力の障害，言語運動障害および認知の障害などが高率に出現し，さらに人格変化を示す例が多い[8,9]．60 歳以上の DS 患者の 75％が AD の症状を示すとされている[1]．DS で認知症を合併した患者の脳は AD に類似した病理学的変化を示す．老人斑は AD の場合と同様に主として β-アミロイドで構成されており，早いもので DS 者の 10 歳代にみられ，30 歳代には全例出現する．神経原線維変化は老人斑より遅れて現れ，DS 者では 30 歳以降にみられることが多い[10]．DS 患者は 21 番染色体上（21 q 21）にあるアミロイド前駆体蛋白（APP）遺伝子が 3 コピーあるために，APP が過剰産生されるためと考えられている[11]．APP は前脳基底核のコリン作動性ニューロン内での神経成長因子を抑制することが知られている[12]．

DS とコリン作動性障害の問題については，数多くの報告がある．DS 小児の脳では，アセチルコリンエステラーゼ（AChE）活性が高いという報告もある[13]．高齢になり，特に AD を合併した DS 患者の脳ではコリンアセチルトランスフェラーゼ（ChAT）の活性は明らかに減少する[14]．DS においてコリン作動性ニューロンの異常を来す時期は不明である．AD の発症の前におそらくコリン作動性ニューロンの数の減少を認める可能性がある[15]．DS 胎児のニューロンを定量した研究では，コリン作動性，モノアミン作動性，セロトニン作動性のいずれも異常を認めないという報告もあれば[16]，胎児期から異常があるという報告もある[17]．このコリン作動性の障害は，中枢にも末梢にも起こることが報告されている[17,18]．我々は，DS 患者で高頻度に排尿機能障害，特に膀胱収縮能の低下が起こることを経験しているが，膀胱収縮にはアセチルコリンが重要な役割を果たす．

塩酸ドネペジル（DH）は，アセチルコリンエステラーゼ阻害作用を有する薬剤で，AD の進行を抑制する薬剤として使用されている．上述の AD と DS の類似点から，脳内コリン作動性の改善が DS 患者の日常生活能力を向上させることを期待して DS の成人患者に DH の投与が試みられているが，効果の程度には大きな幅がある[19)~21)]．副作用についても，中断せざるを得ない程重度とする報告[20]と大きな問題はなかったという報告[19]が混在している．

今回，わが国の成人 DS 者がどのような生活をされているのかのアンケート調査，排尿障害の検討と QOL 能力改善に向けての DH 療法の試みについて報告する．

DS の自然歴アンケート調査

成人 DS 者の現状を把握するために中学校を卒業した方へアンケート調査を行った．家族を対象とした時間的経過を含む調査と施設職員などに回答を依頼する現状を中心とした調査の 2 つを用意した．家族を対象にしたものを第一にして，それが難しい場合に施設職員へのアンケートを行い，同一人物に複数アンケートが行かないように徹底した．回収数は 551 であった．

551 名の内訳は，男性 296 名，女性 254 名で 60 歳以上は 18 名（3.3％）で，最年長は女性 65 歳，

男性63歳であった．アンケートに回答した中の約75％が長崎県在住で，もっと規模を増しての調査が必要と思われるが長崎県の人口分布と比較することでの平均寿命は57.8歳であった．

生活の場は，30～34歳で在宅と施設入所が同じ数になり，それ以降は施設入所者の数が多くなってくる．移動能力，言語機能と日常生活能力の年代別の変化をみると，移動運動は高齢になっても比較的保たれるに際し，言語機能と日常生活能力は30歳代から徐々に低下することが分かった．全般的に在宅者より施設入所者の方が重症であった．

身体的にも知的にもピークであった年齢は，15～19歳であった．ピーク時と比べての現状を問うと，時に介護が必要以上の方が26.3％と少なくなかった．この中で，介護が関わっても困難困難なことがある6.4％を検討すると，20歳前後を中心に起こっていることが多く，良い状態から厳しい状況になる期間が1～2年と短い「急激退行」が約半数を占めた．この群はその後も改善せず，そのまま厳しい状況が持続していることが多かった．

DSの排尿機能

DSの排尿機能障害についての報告はほとんどないものの，排尿回数の減少やいきんで排尿をしているとのDS者家族から意見があった．そのため，長崎大学泌尿器科との共同研究でDS児・者の排尿機能を調べた．

残尿の出現は，15歳以上と未満のDS者で有意差があり，年齢とともに残尿出現頻度の増加を認めた．DS児・者88名と健常児21名でウロフロメトリー検討を行い，そのパターンによって，ベル型（正常），プラトーパターン（尿速が弱く時間が長くかかるパターン），腹圧排尿パターン（腹圧をかけることで排尿ができるパターン）に分類した．

その結果ベル型を示したのは，健常児86％に対し，DS児・者は22％であった．DS患者では低年齢であっても排尿障害が非常に高頻度で起こっていることも分かった．膀胱収縮には，アセチルコリンがムスカリン受容体を介して重要な役割をしていることから，末梢においてもコリン作動性が悪いのではないかと推察される．

DS者への塩酸ドネペジル療法

我々は長崎大学倫理審査委員会の承認を得て，2002年6月から現在約60名のDS者にDHを服用している[22]．

副作用としては，副交感神経亢進と思われる消化器症状が最も頻度が高いものであった．予測される副作用の出現頻度より遥かに高い印象があるため，本薬剤の血中濃度（トラフ値）を測定した．DS患者におけるDHの血中濃度は，同じ用量を服薬した健康成人と比較して高い傾向があり，t検定で$p<0.001$の危険度で有意差を認めた[22,23]．通常DHの使用量は3 mg/日の低容量で開始した後，5 mg/日に増量して維持されているが，血中濃度でみる限り3 mg/日の低用量のままで維持するのが良いと思われる．これまで8年以上治療を継続しているDS患者も少なくないが，以上の諸点に注意する限りこのような長期使用でも問題はないようである．

DHの効果としてはこれまでの結果から，起床や食事などの生活パターンが確立できた，自分から絵や文字を初めて書いた，新聞の社会面に対するコメントを発するようになった，一人で交通機関の利用することができるようになった，電話での対応能力が向上したなど，日常生活上の自立的な行動の出現または改善が認められた．また患者自らの積極的な意志による行動，作業性の向上，他者への興味なども認められ，同時に精神的に安定な状態が維持された[22]．さらに，言語表現力，語彙数，構文構成力を含む表出言語機能の改善や有意味語の理解力などを含む受容的言語機能の改善など言語機能全般の向上が認められた．

患者家族は上述のようにDH治療の効果について良い印象を保持していたにもかかわらず，それは客観的評価法には必ずしも的確に反映されなかった．行動適応尺度（ABS），田中ビネー知能検査，絵画語い発達検査，SM社会生活能力検査や田中・ビネー知能検査を使用してみたが，ABS

で部分的に効果を認める症例が存在するのみで[24]，全例に対応できる評価法はなかった．その後，国際生活機能分類（ICF）をもとに東京学芸大学で作成された心身機能チェックリスト(2007）を使用したところ，知的能力が低いDS患者群で適応しやすいことが判明した．東京学芸大学の菅野らは，このチェックリストを用いてDS患者の日常生活能力の推移を追い，本検査の妥当性を確認している[25]．

これを用いて，重症心身障害児(者)施設のDS者21名のダブルブラインド検討を行った[26]．21名を10名と11名に分け，DHグループとフラセボグループとして24週ダブルブラインド検討を行った．その結果をSASシステムを用いてp値の検討を行ったところ，全体でのp値が0.0001とDH群とプラセボ群において日常生活改善度に有意差を認めた（図）．同様の検定で，全体的精神機能，個別的精神機能，音声と発話の機能において有意差を持って改善したが，他の機能では有意差を認めなかった（図）．

さらにDH投与前後で排尿機能に改善が見られるのかを21名女性DS者で検討した．対象は平均45.1歳で，その1/3に残尿を認めていた．DH服用後5カ月には有意差を持って残尿量が減少した．ウロフロメトリーの結果からは，21名中9名（47.4%）で改善を認めた．これは，長期フォローの意味からも重要と思われる．非常に興味深いことに，DS者の排尿機能障害について末梢性アセチルコリンエステラーゼ阻害剤での改善報告はほとんど認められない．以上よりDS者の排尿障害は末梢だけでなく中枢性の関与も考えられる．

考察とまとめ

DS者のDH療法を行う動機としては，精神的な諸問題か排尿障害を持つ場合が想定される．精神的な問題としては，内向的な問題と外向的な問題があるが，DS者では内向的な問題の方が多い．

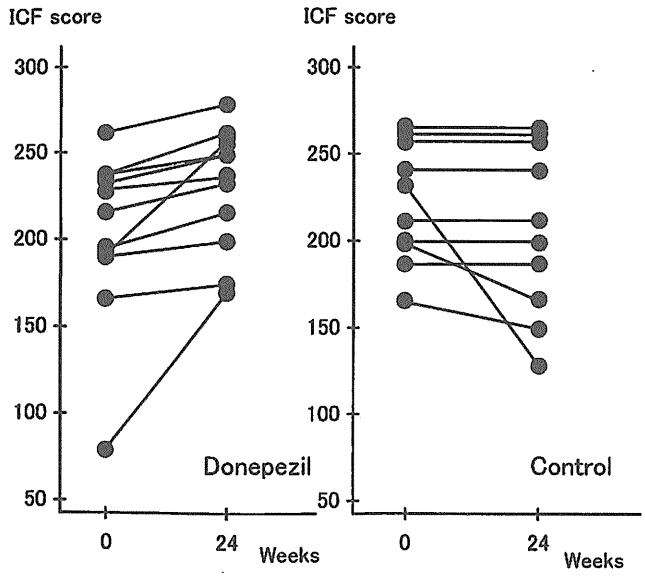
DHは使用した多く，特に内向的な精神諸問題のあるDS者に効果を示している．ただ，途中でイライラが高まったり，時にパニック様症状など問題を生じる場合もある．これはいろいろなことに対して理解力が深まることにより，新たに感じるストレスに対応することができないことに起因するのかもしれない．その際には環境整備やDH減量などが必要なこともある．排尿障害にはある程度の投与量が必要である．

DS者の一般的な状況としては15～20歳をピークとして30～40歳代まで良い状態を維持し，徐々に後退現象が認められることが多い．30～40歳頃より日常生活能力が減退していくことをこれまでは「老化」と称することもあるが，再考する必要があるかもしれない．

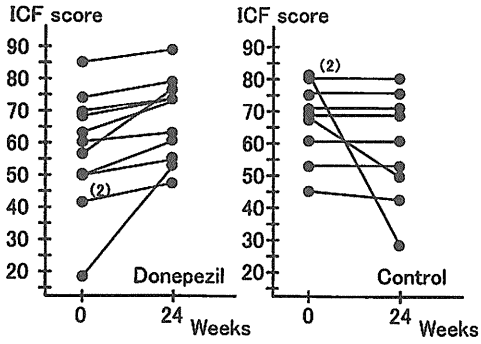
DHはADの進行を遅らせることを目的とした治療薬であるが，DSの脳内コリン作動性システムを考慮すると，ADと同様に有効であると推測された．実際に治療に用いた結果からは，効き幅に個人差を認めるものの，おそらくAD患者における改善度を凌駕する有効性があると思われる．

本薬剤は，認知症の有無やその程度，年齢，IQレベルに関係なくDS治療薬として使用できそうである．DS患者とその家族にとって，日常生活におけるQOLの向上を実現できると大きな福音となる．その反面，自己表現や自己主張ができるようになることが，家族に別のストレスを与えることもあり得る．従って，短絡的に薬物療法のみで問題が解決すると考えるのではなく，環境整備を含めた総合的な取組みをないがしろにしてはならない．さらに，排尿機能障害に対しても効果を示すDS患者が少なくないことも分かった．いずれにしても，本薬剤による治療に対するDS家族からの要望は高く，本薬剤の適応基準や用法用量を厳格に定めた治療ガイドラインを構築していく必要がある．そして何よりも，本薬剤がDS治療薬としての保険適応を得て，保険診療の元で安心して治療することができるようになることが強く求められる．

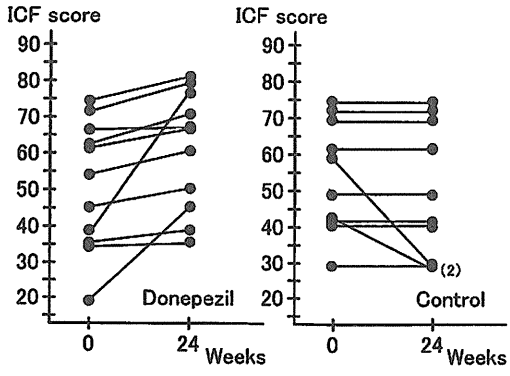
DHは，染色体異常症であるDSに対する薬物療法という新しい領域を開く可能性のある薬剤であり，今後の研究でその地位を確固たるものにすることが期待される．



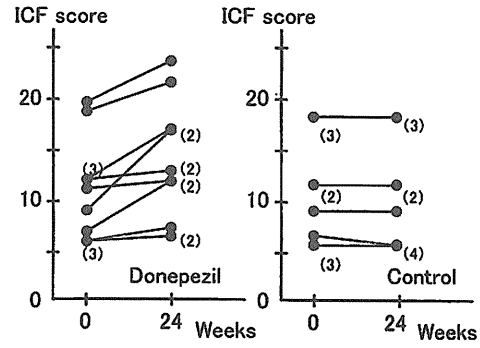
全体　p=0.0001



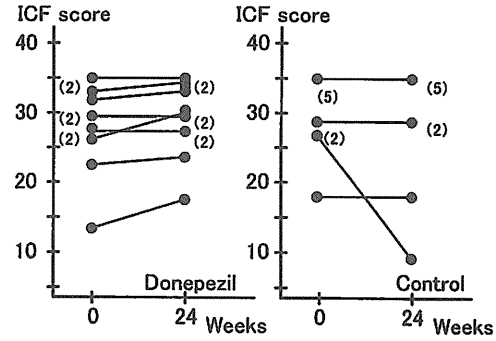
A. 全般的精神機能 p=0.0001



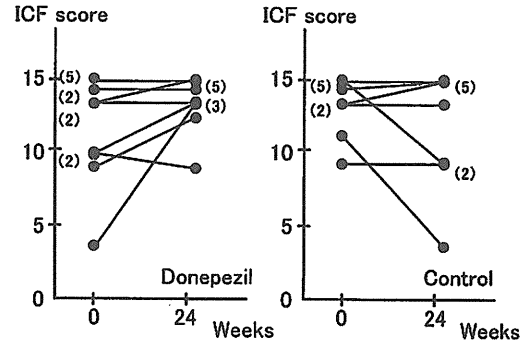
B. 個別的精神機能 p=0.0002



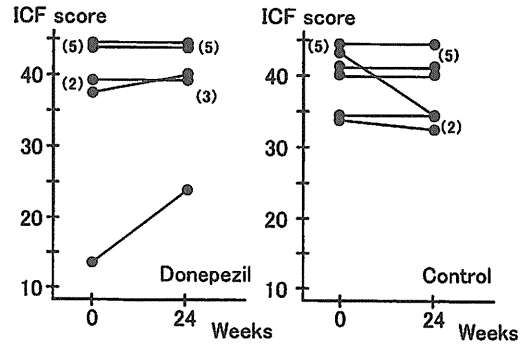
C. 音声と発話の機能 p=0.0005



D. 消化器系の機能



E. 尿路機能



F. 運動に関連する機能

図　SASシステムを用いてのダブルブラインド検討結果（文献26より改変）

本研究は2002年から開始し，これまでに以下の多くの方々の協力を得て推進することができた．(長崎大学医学部小児科学教室) 森内浩幸教授，天本なぎさ先生，土井知己先生，濱田仁美先生，北村 (田中) 温子先生，本田涼子先生；(同泌尿器科) 野口満先生；(同精神神経科) 中根秀之先生；(同創薬科学) 池田正行教授；(同遺伝情報) 柴田義貞先生；(同附属病院薬剤部) 中嶋幹郎教授，佐々木均教授；(長崎大学教育学部) 小島道生先生，相川勝代教授，原田純治教授；(長崎大学教育学部附属養護学校) 青木瑞惠先生，田中龍彦先生；(東京学芸大学) 菅野敦教授，伊藤浩先生；(独協医科大学越谷病院小児科) 永井敏郎教授；(横浜十愛病院精神神経科) 野崎秀次先生；(みさかえの園むつみの家) 福田雅文先生；(国立成育医療センター) 奥山虎之先生，小崎里華先生，掛江直子先生に深謝いたします．

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蛋白同化ホルモンのリハビリテーションへの応用*

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岡本さやか

はじめに

リハビリテーション（以下，リハ）において，筋力増強，日常生活動作（ADL）向上をめざすことは重要な課題となっている．当院でも，回復期リハ病棟においてFIT program（Fulltime Integrated Treatment）を導入し，ADL向上に努めている．しかし，実際は，麻痺が重度であったり，意欲低下や，廃用，併存症などから，十分な筋力増強訓練を行えない患者が多くみられる．そこで我々は，このような患者でも，より一層の筋力増強，さらにはADL向上が得られないか，と考え，蛋白同化ホルモン（Anabolic Steroid：AS）に着目した．

臨床における蛋白同化ホルモン

ASは，testosteroneの蛋白同化作用を強化した合成ホルモンペプチドである．医学的には，一般に骨粗鬆症や再生不良性貧血の治療に用いられているが，スポーツ界においては，筋力増強効果や筋肥大作用が注目され，ドーピングとして使用されている．その機序は，サテライト細胞の活性化を促すことにより，筋細胞が増加するとされている[1,2]．また，GH-IGF-1系を刺激し，間接的な筋力増強にも関与しているといわれている[3]．しかし，そのトリガー因子などは不明な点が多い．臨床においては，欧米では古くから，高齢健常者にASを投与した結果，握力やハムストリングスの筋力が増加した，という報告がいくつかみられて

* 本稿は第47回日本リハビリテーション医学会学術集会シンポジウム「リハビリテーション促進的薬物治療の新たな展開」（2010年5月21日，鹿児島）の講演をまとめたものである．

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

MBTPS2 Mutation Causes BRESEK/BRESHECK Syndrome

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Received 25 July 2011; Accepted 17 October 2011

BRESEK/BRESHECK syndrome is a multiple congenital malformation characterized by brain anomalies, intellectual disability, ectodermal dysplasia, skeletal deformities, ear or eye anomalies, and renal anomalies or small kidneys, with or without Hirschsprung disease and cleft palate or cryptorchidism. This syndrome has only been reported in three male patients. Here, we report on the fourth male patient presenting with brain anomaly, intellectual disability, growth retardation, ectodermal dysplasia, vertebral (skeletal) anomaly, Hirschsprung disease, low-set and large ears, cryptorchidism, and small kidneys. These manifestations fulfill the clinical diagnostic criteria of BRESHECK syndrome. Since all patients with BRESEK/BRESHECK syndrome are male, and X-linked syndrome of ichthyosis follicularis with atrichia and photophobia is sometimes associated with several features of BRESEK/BRESHECK syndrome such as intellectual disability, vertebral and renal anomalies, and Hirschsprung disease, we analyzed the causal gene of ichthyosis follicularis with atrichia and photophobia syndrome, *MBTPS2*, in the present patient and identified an p.Arg429His mutation. This mutation has been reported to cause the most severe type of ichthyosis follicularis with atrichia and photophobia syndrome, including neonatal and infantile death. These results demonstrate that the p.Arg429His mutation in *MBTPS2* causes BRESEK/BRESHECK syndrome.

Key words: BRESEK/BRESHECK syndrome; IFAP syndrome; *MBTPS2*; mutation; S2P

How to Cite this Article:
Naiki M, Mizuno S, Yamada K, Yamada Y, Kimura R, Oshiro M, Okamoto N, Makita Y, Seishima M, Wakamatsu N. 2011. *MBTPS2* mutation causes BRESEK/BRESHECK syndrome.
Am J Med Genet Part A .

INTRODUCTION

BRESEK/BRESHECK syndrome (OMIM# 300404), a multiple congenital malformation disorder characterized by brain anomalies, intellectual disability, ectodermal dysplasia, skeletal deformities, Hirschsprung disease, ear or eye anomalies, cleft palate or cryptorchidism, and kidney dysplasia/hypoplasia [Reish et al., 1997]. The acronym BRESEK refers to the common findings, whereas BRESHECK refers to all manifestations. Because the first two patients were maternally related half brothers, an X-linked disorder was proposed. Although each symptom of these patients is often observed in other congenital diseases, the combination of all symptoms is rare, and only one additional patient with BRESEK has been reported to date [Tumialán and Mapstone, 2006]. Here, we present the fourth male patient with multiple anomalies. The patient presented with a variety of clinical features that were consistent with those of the previously reported BRESHECK syndrome.

The syndrome of ichthyosis follicularis with atrichia and photophobia (IFAP, OMIM# 308205), an X-linked recessive oculocutaneous disorder, is characterized by a peculiar triad of ichthyosis follicularis, total or subtotal atrichia, and varying degrees

Grant sponsor: Takeda Science Foundation; Grant sponsor: Health Labour Sciences Research Grant.
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Published online 00 Month 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34373

of photophobia [MacLeod, 1909]. Martino et al. [1992] reported a male patient with IFAP syndrome presented with short stature, intellectual disability, seizures, hypohidrosis, enamel dysplasia, congenital aganglionic megacolon, inguinal hernia, vertebral and renal anomalies, and the classic symptom triad of IFAP syndrome. This report broadened the clinical features of IFAP syndrome. It should be noted that the clinical symptoms of this patient are quite similar to those of BRESHECK syndrome, with the exception of cleft palate, cryptorchidism, and photophobia (Patient 5; Table I). The gene mutated in patients with IFAP syndrome, *MBTPS2* (GenBank reference sequence NM_015884), was identified from a variety of clinical features of IFAP syndrome, including the triad and neonatal death [Oeffner et al., 2009]. Thus, the mode of inheritance and several clinical features are common to both BRESEK/BRESHECK and IFAP syndromes. These findings prompted us to perform mutation analysis of *MBTPS2* in the present patient, resulting in the identification of a missense mutation.

MATERIALS AND METHODS

Patients

Written informed consent was obtained from the parents of the patient. Experiments were conducted after approval of the institutional review board of the Institute for Developmental Research, Aichi Human Service Center. The patient (II-1; Fig. 3) was born to a 31-year-old mother (I-2) and a 31-year-old father (I-1), both healthy Japanese individuals without consanguinity. His mother miscarried her first child at 5 weeks. The pregnancy of the patient reported here was complicated with mild oligohydramnios, and he was delivered by caesarean because of a breech position at 38 weeks of gestation. His birth weight was 1,996 g (−2.6 SD), and he measured 44 cm (−2.6 SD) in length with an occipitofrontal circumference of 32.5 cm (−0.5 SD). Apgar scores at 1 and 5 min were four and eight, respectively. The patient exhibited generalized alopecia and lacked eyelashes, scalp hair, and eyebrows (Fig. 1A). The skin on the entire body was erythematous with

TABLE I. Clinical Features of BRESEK/BRESHECK and IFAP Syndromes and *MBTPS2* Mutation

	BRESEK/BRESHECK syndrome				IFAP syndrome		
Patient	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Clinical features							
Gender	M	M	M	M	M	M	M
Gestational age (weeks)	32	40	ND	38	30	ND	ND
Birth weight (g)	990	2,230	ND	1,996	2,040	ND	ND
Intrauterine growth retardation	+	+	ND	+	−	ND	ND
Major features							
Follicular ichthyosis	−	−	ND	−	+	+	+
Atrichia	+	+	+	+	+	+	+
Photophobia	−	−	−	+	+	+	+
Brain malformation	+	+	+	+	+	−	+
Mental and growth retardation	+	+	+	+	+	+	+
Skeletal (Vertebrate) anomalies	+	+	+	+	+	+	+
Hirschsprung disease	−	+	+	+	+	+	+
Eye malformation or	+	+	+	−	+	−	−
Large ears	+	+	+	+	+	−	−
Cleft lip/palate or	−	+	−	−	−	+	−
Cryptorchidism	+	+	−	+	−	−	−
Kidney malformation	+	+	−	+	+	+	+
Other features							
Microcephaly	+	+	+	+	+	−	+
Seizures	−	+	+	+	+	−	+
Deafness	−	+	−	+	−	−	−
Hand anomalies	+	+	+	−	+	+	+
Cardiac anomalies	−	−	+	−	−	−	+
Inguinal hernia	−	−	−	−	+	+	+
Trachea anomalies	−	−	−	+	−	−	−
Regression	−	−	−	+	−	−	−
Age	6 h d	7 y	1.5 y	8 y	3 y	9 m d	14 m d
MBTPS2 mutation	NP	NP	NP	R429H	NP	R429H	R429H

+, present; −, not present; M, male; ND, not described; NP, not performed; h, hour; d, dead; m, month; y, year; R429H, Arg429His; BRESEK/BRESHECK syndrome, (Patients 1-4); IFAP syndrome, (Patients 5–7); Patients: 1, Reish et al. [1997] patient 1; 2, Reish et al. [1997] patient 2; 3, Tumialán and Mapstone [2006]; 4, present case; 5, Martino et al. [1992]; 6, Oeffner et al. [2009] 3-III:3; 7, Oeffner et al. [2009] 3-III:4.

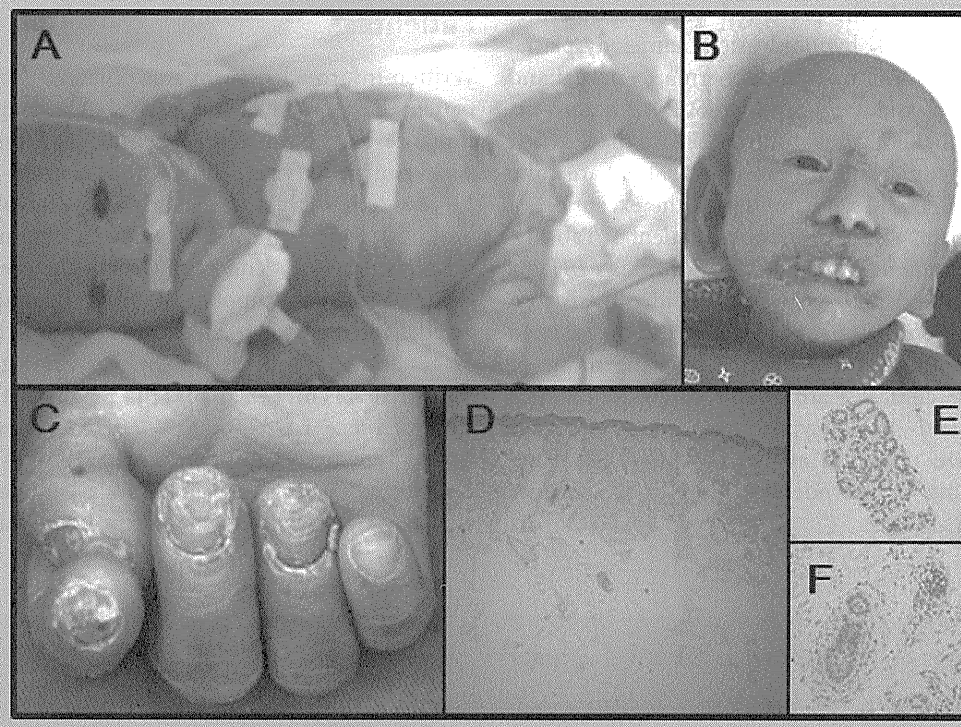

FIG. 1. Clinical appearance and dermatological findings of the patient. A: Lateral view of the patient at birth. Note the generalized alopecia with an absence of scalp hair, eyebrows, and eyelashes. The skin was dry and scaly, and an itchy erythema was observed over the entire body. B: Frontal view of the patient at 4 years of age. Note the characteristic facial appearance with long, malformed ears, a relatively high nasal bridge, and a wide nasal base. C: The patient had normal-sized but deformed and thickened nails. D–F: Histologic examination of the abdominal skin at the age of 15 months showed a reduced number of hair follicles (D), normal eccrine glands (E), and hypoplastic hair follicles (F).

continuous desquamation (Fig. 1A). He had malformed large ears, an inferiorly curved penis, and a bifid scrotum. The testicles were not palpable. He experienced persistent constipation, and total colonic Hirschsprung disease was confirmed through barium enema (Fig. 2E) and rectal biopsy at 2 months. A bone survey performed using three-dimensional (3D) computed tomography (CT) showed abnormal imbalanced hemivertebrae in the two lowest thoracic vertebral bodies (Fig. 2C). The patient's right kidney was smaller than normal. Brain magnetic resonance imaging (MRI) at 3 years of age demonstrated decreased volumes of the frontal and parietal lobes and thinning of the corpus callosum with dilatation of the ventricles (Fig. 2A,B). There were no abnormalities of the eyes or optic nerves. We concluded that the patient had BRESHECK syndrome. The patient had seizures at 5 months of age with an apneic episode and cyanosis. Electroencephalographic (EEG) analysis showed abnormal patterns of sharp waves in the posterior lobe. The seizures were almost completely controlled with phenobarbital. The patient was allergic to milk. At 7 months, tracheal endoscopy revealed subglottic tracheal stenosis and abnormal segmentation of the left lung. A chest CT performed at 3 years of age showed a congenital cystic adenomatoid malformation (CCAM) in the right upper lobe (Fig. 2D). Auditory brain stem responses showed bilateral 80 dB hearing loss at 8 months of age.

The patient exhibited delayed psychomotor development during his infancy. He could drink from a bottle at the age of 3 months and could sit up unsupported at 15 months. Abdominal skin biopsy at 15 months revealed reduced number of hair follicles (Fig. 1D). The eccrine glands were normal (Fig. 1E), and most of his hair follicles appeared to be hypoplastic (Fig. 1F). These findings were similar to ichthyosiform erythroderma. Photophobia was noted when the patient left the hospital and first went outside at 18 months of age. At 2 years and 6 months of age, he had a series of epileptic episodes. He experienced a maximum of 100 seizures per day, and EEG analysis showed continual abnormal spikes in the posterior lobe. The seizures were controlled with clonazepam therapy. At 2 years and 9 months of age, he could stand with support and displayed social smiles when interacting with other people. However, the patient developed psychomotor regression at the age of 3 years. He exhibited a progressive loss of emotional response to others, developed hypotonia, and could not stand or sit alone. At 4 years of age, he became bedridden and showed almost no response to people. He had highly desquamated skin, similar to that seen in ichthyosis (Fig. 1B), and easily developed erythema on the skin of the entire body. The patient had deformed and thickened nails (Fig. 1C). He had persistent corneal erosions, but ophthalmoscopy could not be performed at the age of 4 years because of corneal opacification.

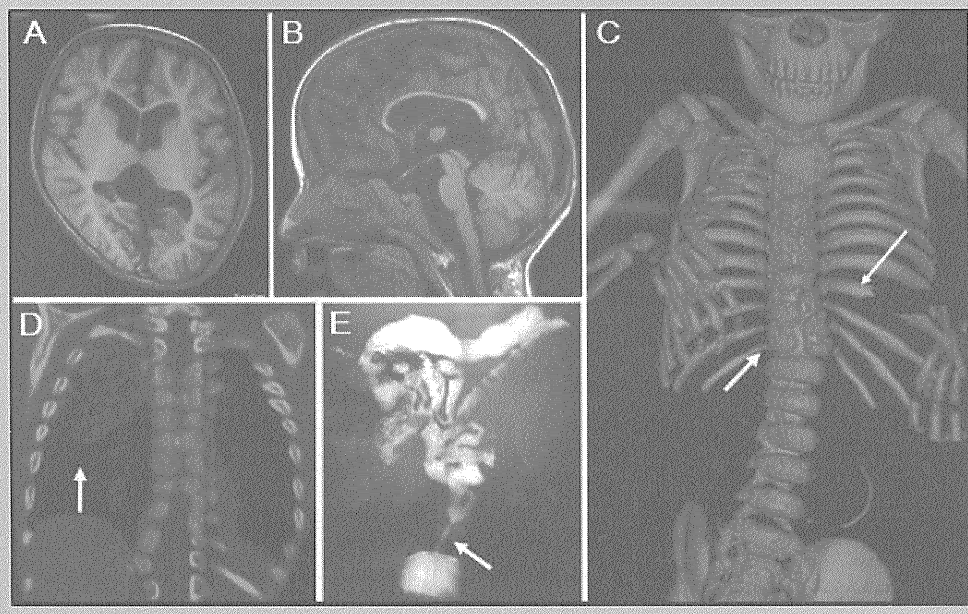

FIG. 2. CT and MRI findings of the patient. A,B: Brain MRI (T1-weighted image) at 3 years of age showed decreased volume of the cortex in the frontal and parietal lobes, the presence of a subdural cyst in the corpora quadrigemina, and dilatation of the lateral and fourth ventricle. C: A bone survey performed using 3D CT showed abnormal segmentation of the ninth rib and an imbalanced hemivertebrae in the two lowest thoracic vertebral bodies (shown with arrows). D: CT of the chest showed CCAM (indicated by the arrow) in the right upper lobe. E: Barium enema showed a reduced caliber rectum (indicated by the arrow), suggesting that the patient had Hirschsprung disease.

Chromosomal and Molecular Genetic Studies

Genomic DNA isolated from the patient's peripheral white cells by phenol/chloroform extraction was used for *MBTPS2* mutation analysis. PCR-amplified DNA fragments were isolated using the QIAEX II Gel Extraction Kit (Qiagen, Valencia, CA) and purified using polyethylene glycol 6000 precipitation. PCR products were sequenced with the Big Dye Terminator Cycle Sequencing Kit V1.1 and analyzed with the ABI PRISM 310 Genetic Analyzer (Life Technologies, Carlsbad, CA). We also performed G-banded chromosome analysis at a resolution of 400–550 bands, genome-wide subtelomere fluorescence in situ hybridization (FISH) analysis, and array comparative genomic hybridization (array CGH) using Whole Human Genome Oligo Microarray Kits 244K (Agilent Technologies Inc., Palo Alto, CA) to identify genomic abnormalities.

RESULTS

G-banded chromosome analysis and genome-wide subtelomere FISH analyses did not show chromosomal rearrangements in the patient. Array CGH analysis did not show copy number changes in the patient's genome with the exception of known copy-number variations (CNVs). Since some patients with IFAP syndrome have been reported to present with several clinical features of BRESEK/BRESHECK syndrome, including severe intellectual disability, vertebral and renal anomalies, and Hirschsprung disease, we conducted a comprehensive sequencing analysis of all exons and intron–exon boundaries of *MBTPS2*. This analysis identified a missense mutation (c.1286G>A, [p.Arg429His]) in exon 10, which was previously reported for IFAP syndrome (Fig. 3). The mutation was also found in one allele of the mother (I-2), indicating that the mutation was of maternal origin and that the mother was a heterozygous carrier (Fig. 3).

DISCUSSION

In this report, we describe the fourth male patient with BRESHECK syndrome in whom we identified a missense mutation (c.1286G>A, [p.Arg429His]) in *MBTPS2*, which is the causal gene for IFAP syndrome. *MBTPS2* encodes a membrane-embedded zinc metalloprotease, termed site-2 protease (S2P). S2P cleaves and activates cytosolic fragments of sterol regulatory element binding proteins (SREBP1 and SREBP2) and a family of bZIP membrane-bound transcription factors of endoplasmic reticulum (ER) stress sensors (ATF6, OASIS), after a first luminal proteolytic cut by site-1 protease (S1P) within Golgi membranes [Sakai et al., 1996; Ye et al., 2000; Kondo et al., 2005; Asada et al., 2011]. The SREBPs control the expression of many genes involved in the biosynthesis and uptake of cholesterol, whereas ATF6 and OASIS induce many genes that clean up accumulated unfolded proteins in the ER. Dysregulated SREBP activation, impaired lipid metabolism, and accumulation of unfolded proteins in the ER caused by *MBTPS2* mutations could lead to disturbed differentiation of epidermal structures, resulting in the symptom triad of IFAP syndrome [Cursiefen et al., 1999; Traboulsi et al., 2004; Elias et al., 2008]. Oeffner et al. [2009] first identified five missense mutations in *MBTPS2* in patients with IFAP

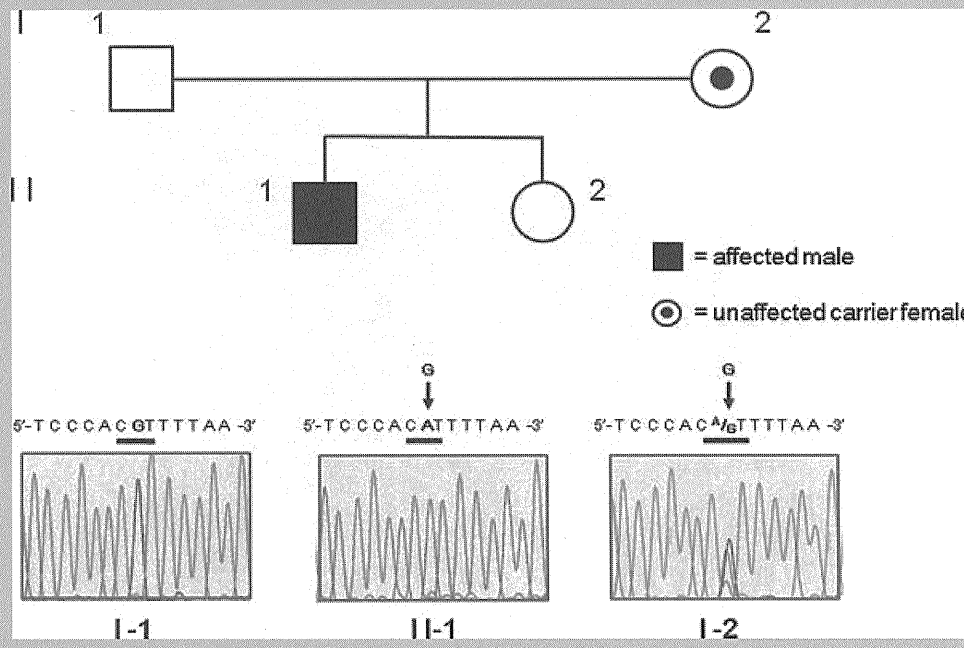

FIG. 3. Identification of a disease mutation. The sequence analyses of the patient (II-1) showed a c.1286G>A variant in exon 10 of *MBTPS2*, which predicts p.Arg429His, as indicated by the arrow (middle panel). The mother (I-2) was heterozygous for the mutation ($C^A/_GT$) (right panel).

syndrome. Transfection studies using wild type and mutant *MBTPS2* expression constructs demonstrated that the five *MBTPS2* mutations did not affect S2P protein amount and localization in the ER. However, enzyme activities, as measured by sterol responsiveness, were decreased in S2P-deficient M19 cells when the mutant *MBTPS2* was transiently expressed. Interfamilial phenotypic differences between male IFAP patients and the properties of mutants in functional assays predict a genotype–phenotype correlation, ranging from mild forms of the triad with relatively high enzyme activity (~80%) to severe manifestations of intellectual disability, various developmental defects, and early death with low enzyme activity (~15%). The identified p.Arg429His mutation in the patient reported here is one of the five missense mutations with the lowest enzyme activity. It was previously reported that all four patients harboring the p.Arg429His mutation died within 14 months of birth. The five mutations were not located in the HEIGH motif (amino acids [aa] 171–175) or in the $LD_{467}G$ sequence, both of which are regions important for coordinating the zinc atom at the enzymatic active site for protease activity in the Golgi membrane [Zelenski et al., 1999]. However, among the five mutations, the p.Arg429His mutation is located closest to the intramembranous domain, and it strongly reduced the enzymatic activity and caused a severe phenotype. This finding suggests that mutations in the HEIGH motif or in the $LD_{467}G$ sequence are fatal because they lead to a null function of the S2P. Although the detailed skin findings of the four patients with the p.Arg429His mutation have not been reported, it should be noted that one of the four patients (3-III:4) with the p.Arg429His mutation had brain anomaly, seizures, psychomotor retardation, vertebrae anomaly, Hirschsprung disease, absence of a kidney, atrial septum defect, and inguinal hernia, in addition to the symptom triad of IFAP syndrome [Oeffner et al., 2009]. These symptoms overlap with the majority of symptoms observed in BRESHECK syndrome (BRESHK; six of eight symptoms observed in BRESHECK) (Table I), and the present patient has BRESHECK syndrome. Collectively, these observations suggest that the most severe form of the syndrome caused by the p.Arg429His mutation in *MBTPS2* shows features quite similar or identical to those of BRESEK/BRESHECK syndrome.

There are two major differences in the definitions of IFAP syndrome and BRESEK/BRESHECK syndrome. Ichthyosis follicularis, one of the triad symptoms of IFAP syndrome, is a clinical condition of the skin. However, several studies on IFAP syndrome have reported various skin eruptions such as psoriasis-like and ichthyosis-like eruptions [Martino et al., 1992; Sato-Matsumura et al., 2000]. In contrast, patients with BRESEK/BRESHECK syndrome showed severe lamellar desquamation with diffuse scaling [Reish et al., 1997], similar to that observed in the present patient. This could be because of the difference in features of the skin, namely, ichthyosiform erythroderma-like appearance versus ichthyosis follicularis, in patients with the most severe forms of *MBTPS2* mutation and patients with IFAP syndrome who were described earlier, respectively.

The second difference is that photophobia was not described in the reported three male patients with BRESEK/BRESHECK syndrome [Reish et al., 1997; Tumialán and Mapstone, 2006]. In the present patient, photophobia became evident after he was diagnosed with BRESHECK syndrome. Photophobia is a symptom of epithelial disturbances of the cornea, such as ulceration and vascularization, which result in corneal scarring [Traboulsi et al., 2004]. In the most severe cases of *MBTPS2* mutation, such as

patients with severe intellectual disability who are bedridden and die early, it is likely that the patients were treated in the hospital without being exposed to sunlight. Therefore, it would be difficult to observe photophobia as a main symptom in those cases. Moreover, two previously described patients with BRESEK/BRESHECK syndrome had initial maldevelopment of one eye or small optic nerves. In these patients, photophobia may not have been obvious because of malformations of the eyes and optic nerves [Reish et al., 1997]. In our study, the patient showed clinical features of BRESHECK syndrome and photophobia with *MBTPS2* mutation, indicating that the clinical features of the present patient are extremely broad compared to the features of IFAP syndrome caused by *MBTPS2* mutation that have been previously reported [MacLeod, 1909].

Recently, a missense mutation (c.1523A>G, [p.Asn508Ser]) in *MBTPS2* was identified from 26 cases of three independent families with keratosis follicularis spinulosa decalvans (KFSD; OMIM# 308800), which is characterized by the development of hyperkeratotic follicular papules on the scalp followed by progressive alopecia of the scalp, eyelashes, and eyebrows in addition to childhood photophobia and corneal dystrophy [Aten et al., 2010]. A significant association was found between KFSD and the p.Asn508Ser mutation. The specific localization of alopecia to the scalp, eyelashes, and eyebrows and the limited childhood photophobia of KFSD indicate that KFSD has a relatively mild phenotype. The authors postulate that IFAP syndrome and KFSD are within the spectrum of one genetic disorder with a partially overlapping phenotype and propose that a new name should be chosen for KFSD/IFAP syndrome with an *MBTPS2* mutation. In contrast, the BRESHECK syndrome observed in the present patient has a severe phenotype caused by the p.Arg429His mutation. The present patient and the two patients (3-III:3 and 3-III:4) with the p.Arg429His mutation displayed broader clinical features, including eight features (BRESHECK) and six features (RESHCK and BRESHK) of BRESEK/BRESHECK syndrome, respectively (patients 4, 6, and 7; Table I) [Oeffner et al., 2009]. There is a debate regarding whether the two patients harboring six features were correctly diagnosed with BRESEK/BRESHECK syndrome since the patients did not have "BRESEK" but rather a combination of six other clinical features. To better understand and clearly distinguish the clinical features of the present patient from those of the reported patients with *MBTPS2* mutations, we propose the nomenclature of "BRESHECK/IFAP syndrome" for the present patient because he has clinical features of BRESHECK syndrome. We also suggest that the BRESHECK/IFAP syndrome be used for a broader definition that would include patients harboring most features of BRESHECK syndrome, including the previously reported two patients (3-III:3 and 3-III:4) with p.Arg429His mutation in *MBTPS2* [Oeffner et al., 2009]. Data from further genetic and clinical studies on more patients are required to determine which genes or *MBTPS2* mutations are associated with BRESEK/BRESHECK or BRESHECK/IFAP syndrome, respectively.

ACKNOWLEDGMENTS

We thank the patient and his family for participating in the study. This study was supported by the Takeda Science Foundation (to N.W.) and by the Health Labour Sciences Research Grant (to S.M. and N.W.).

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Clinical and Genomic Characterization of Siblings With a Distal Duplication of Chromosome 9q (9q34.1-qter)

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Received 21 July 2010; Accepted 23 May 2011

We report herein on two female siblings exhibiting mild intellectual disability, hypotonia in infancy, postnatal growth retardation, characteristic appearance of the face, fingers, and toes. Their healthy mother had a translocation between 9q34.1 and the 13pter. FISH and array CGH analysis demonstrated that the two children had an additional 8.5 Mb segment of the 9q34.1-qter at 13pter. The clinical features of the present cases were similar to those of previously reported 9q34 duplication cases; however, the present cases did not exhibit other abnormal behaviors, such as autistic features or attention deficit disorders, those are reportedly associated with 9q34 duplications. A 3.0 Mb region (9q34.1-q34.3) within 9q34 duplication in our patients are overlapped with duplication region of previously reported cases and is proposed to be critical for the presentation of several phenotypes associated with 9q34 duplications.

Key words: 9q34 duplication; intellectual disability; array CGH; dysmorphism

How to Cite this Article:
Mizuno S, Fukushi D, Kimura R, Yamada K, Yamada Y, Kumagai T, Wakamatsu N. 2011. Clinical and genomic characterization of siblings with a distal duplication of chromosome 9q (9q34.1-qter).
Am J Med Genet Part A 9999:1–7.

INTRODUCTION

Duplications of a distal region of the long arm of chromosome 9 (9q34) are rare and few cases have been reported. The first association between 9q34 duplications and phenotypic abnormalities were demonstrated in seven cases in a large pedigree [Allderdice et al., 1983]. The patients had low birth weight, initial poor feeding and thriving, slight psychomotor retardation, characteristic appearance of the face, fingers, and toes. Hyperactive behavior, heart murmur, and ptosis and strabismus were also noted. In another case, a girl of 3 years and 2 months carried a 9q34 duplication and a deletion of 3p26-pter due to a balanced translocation in her mother [Hodou et al., 1987]. This patient presented with dolichocephaly, characteristic facial appearance, and long thin fingers and toes, all of which are phenotypes noted in previous cases of 9q34 duplication; she also exhibited features associated with 3p terminal monosomy. In addition, duplication of 9q34-qter and monosomy of a small region on 12p13.3 in a male infant was described by Spinner et al. [1993]. The same patient was followed up at 18 years of age, and the duplicated and deleted regions were determined in detail by array-based comparative genomic hybridization (array CGH) analysis [Youngs et al., 2010]. The patient exhibited autistic features, hyperactivity, and attention deficit disorder in addition to the features associated with 9q34 duplications reported previously. Gawlik-Kuklinska et al. [2007] reported the case of a 17-year-old girl with an interstitial 7.4 Mb duplication of 9q34.1-q34.3 determined by array CGH analysis and compared the clinical features of the patient with those of previous cases. This patient exhibited the features common to patients with 9q34 duplications and three additional phenotypes of food-seeking behavior, obesity, and secondary amenorrhea.

In this report, we present two female siblings with 9q34.1-qter duplications and compare the clinical features and 9q34 duplication region of these patients with those of two previously reported cases using array CGH analysis. We also discuss the loci potentially responsible for the several phenotypes associated with a specific segment of 9q34.

Additional supporting information may be found in the online version of this article.

Grant sponsor: Takeda Science Foundation; Grant sponsor: Health Labour Sciences Research Grant.

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Published online 8 August 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34160

CLINICAL REPORTS

Patient 1. The patient was a 4-year-old girl and the first child of healthy, non-consanguineous Japanese parents. The family history was unremarkable. She was born at 40 weeks of gestation weighing 2,564 g and measuring 47.3 cm in length with an occipitofrontal circumference (OFC) of 33 cm, all within the standard range (10th–90th centile) for female Japanese neonates. The child was first evaluated at a cardiology clinic to investigate a heart murmur in the neonatal period. She was diagnosed with Ebstein anomaly, which was surgically repaired when she was 2-month old. At the age of 4 months, she was referred to our hospital due to generalized hypotonia and developmental delay. She rolled over at 12 months and sat up at 18 months. She stood with support at 24 months and started to walk unaided at 2.5 years. At 3 years of age, her height was 84 cm (−2.2 SD), body weight was 12.4 kg (−0.7 SD), and OFC was 49 cm (−0.2 SD). She could speak several meaningful words and understand simple sentences. Her developmental quotient (DQ) was 67, indicating mild intellectual disability. She was a sociable and friendly girl.

Clinical examination revealed that she had a characteristic facial appearance, including a round face, hypertelorism, almond-shaped palpebral fissures, telecanthus, depressed nasal bridge, short nose, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip (Fig. 1A). Her fingers were slender but not tapered (Fig. 1C). Neurological examination revealed that the cranial nerves were intact except for strabismus. Ocular fundi were normal. She walked slowly, but no ataxia was evident. Muscle tonus of the extremities was normal. Tendon reflexes of extremities were normal, and pathological reflex was absent. There was no evidence of epilepsy. Routine laboratory investigations were normal.

Patient 2. The patient was a 3-year-old girl and was the second child of the parents of Patient 1. She was born at 40 weeks of gestation weighing 2,874 g, measuring 49 cm in length with an OFC of 34.3 cm (all normal values for female Japanese neonates). She exhibited generalized hypotonia, but no feeding problems were observed during the neonatal period. She was referred to our hospital at the age of 19 months due to developmental delay. She exhibited head control at the age of 4 months. She rolled over at 9 months, sat at 10 months, and cruised between 11 and 12 months. She started to walk unaided at 18 months. Her height at 3 years was 88 cm (−2.4 SD), body weight was 10.1 kg (−2.7 SD), and OFC was 47 cm (−0.7 SD). DQ at the age of 3 was 72, indicating mild intellectual disability. She routinely exhibited affectionate and sociable behavior. She also had a round face with full cheeks, hypertelorism, almond-shaped palpebral fissures, telecanthus, depressed nasal bridge, short nose, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip (Fig. 1B). Ultrasonography of the abdomen showed no urogenital defects. No ophthalmic anomalies other than strabismus were found on routine evaluation. Neurological examination was not remarkable except strabismus. No epileptic seizures were observed. Routine laboratory investigations were normal. The clinical features of both patients and two previously reported cases of 9q34 duplication are summarized in Table I.

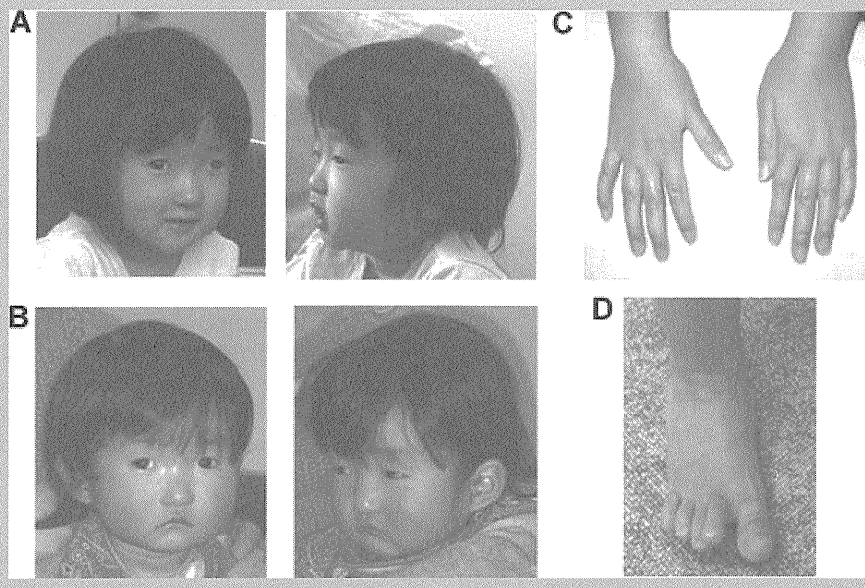

FIG. 1. A: Frontal and lateral views of Patient 1 at 3 years of age. Phenotypes include round face, hypertelorism, telecanthus, short nose, depressed nasal bridge, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip. B: Frontal and oblique view of Patient 2 at 2 years of age. Phenotypes include round face, hypertelorism, almond-shaped palpebral fissures with telecanthus, short nose, depressed nasal bridge, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip. C: Hands of Patient 1 with long and thin fingers. D: The right foot of Patient 1. She has long toes with increased space between the first and second toes.

TABLE I. Clinical Features of Patients With a 9q34.1-qter Duplication

Phenotypic features	Gawlik-Kuklinska et al. [2007]	Youngs et al. [2010]	Patient 1	Patient 2
General				
Hypotonia	+	+	+	+
Failure to thrive	+	−	−	−
Intellectual disability	Mild	Mild	Mild	Mild
Cardiac anomalies	−	+	+	−
Overweight/obesity	+	+	−	−
Scoliosis	+	−	−	−
Facial characteristics				
Dolichcephaly	+	+	−	−
Facial asymmetry	+	+	−	−
Narrow horizontal palpebral fissures	+	+	−	−
Deep-set eyes	+	+	−	−
Long nose	+	+	−	−
Prominent chin	+	+	−	−
Microstomia	+	+	+	+
Microretrognathia	+	+	+	+
Short philtrum	+	−	+	+
Round face	−	−	+	+
Hypertelorism	−	−	+	+
Depressed nasal bridge	−	−	+	+
Almond-shape palpebral fissures	−	−	+	+
Telecanthus	−	−	+	+
Short nose	−	−	+	+
Extremities				
Long and thin fingers	+	+	+	+
Increased space between first and second toes	+	+	+	+

+, present; −, absent.

MATERIALS AND METHODS

Cytogenetic Analysis

Cultured lymphoblastoid cells isolated from each patient were treated with colchicine (Sigma–Aldrich, St. Louis, MO) for 1 hr at a concentration of 20 ng/ml in culture medium, and then incubated in a hypotonic solution of 75 mM KCl at 37°C for 30 min. After incubation, cells were fixed with Carnoy's fixative (3:1 mixture of methanol and acetic acid), spread on glass slides in a humid atmosphere and air-dried. Chromosomal analysis was carried out on GTG banded chromosomes at a resolution of 400–550 bands. Fluorescence in situ hybridization (FISH) was performed on metaphase chromosome spreads from each patient. Commercial probes covering subtelomeric regions were used according to the manufacturer's protocols (ToTelVysion, Abbott Laboratories. Abbott Park, IL) [Flint et al., 1995]. In order to confirm the chromosomal rearrangement in detail, additional FISH analysis was carried out from the patients and their parents using a series of bacterial artificial chromosome (BAC) clones (Clontech Laboratories, Inc., Mountain View, CA) that map to chromosome regions 9q34 and 13q31.

Array CGH Analysis

Genomic DNA was isolated from peripheral blood lymphocytes of the two patients, their parents, and three normal controls by phenol/chloroform extraction. Array CGH analysis was performed using the Agilent Human Genome CGH 244K microarray platform (Agilent Technologies, Santa Clara, CA) according to standard protocols provided by the manufacturer. This array spans the entire human genome at a median resolution of approximately 8.9 kb. Genomic copy numbers were analyzed with Genomic Workbench (Standard Edition 5.0.14; Agilent Technologies).

Southern Blot Analysis

Genomic DNA samples (10 μg) from the patients, their parents, and the normal controls were digested with *Hin*dIII, separated on a 0.9% agarose gel, and transferred by the alkaline method to a nylon membrane (Hybond-N+; GE Healthcare, Tokyo, Japan). The membrane was sequentially hybridized with [α-^{32}P]dCTP-labeled *ABCA6* (exons 17–19) and *SP2* (exons 4–7) cDNA. A 301 bp *ABCA6* or a 798 bp *SP2* cDNA probe was prepared by amplifying the cDNA library of human lymphoblastoid cells with AmpliTaq-