1. 論文発表

なし

2. 学会発表

なし

H. 知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3.その他

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

性腺機能不全における治療効果の判定

研究分担者　堀川玲子　国立成育医療センター内分泌代謝科　医長

研究要旨

思春期後のPrader-Willi症候群(PWS)男性患者に対する性ホルモン補充効果について検討した．対象患者にテストステロンを1カ月ごとに投与し，BMI(body mass index)，%Fat，BMD (bone mineral density)，BMC(bone mineral content)，lean+BMC，lean を測定した．BMD，lean は有意に増加し，BMI・%FAT・BMC に変化は認めなかった．テストステロン投与に伴う有害事象は認めなかった．思春期後の PWS 男性患者に対する性ホルモン補充は，安全に施行できる有効な治療法である．

共同研究者

城戸　康宏　獨協医大越谷病院　小児科　助教

A. 研究目的

PWS 患者には性腺機能低下が必発であり，そのため筋量や骨量の減少が生じる．男性性腺機能低下に対する有効な治療としてテストステロン補充療法があるが，その副作用として攻撃性の増加や行動異常が生じる可能性があると寓話的に信じられている．本研究では，思春期後の PWS に対する性ホルモン補充の効果と有害事象を検討する．

B. 研究方法

当院で定期的に外来フォロー中の PWS 患者で思春期後の男性患者 19 名のうち検査データを取得できた 14 名を対象とした．対象 14 名にテストステロンとしてエナルモン デポー125mg または 250mg を 1 カ月ごとに筋注し，BMI (body mass index)，%Fat，BMD (bone mineral density)，BMC(bone mineral content)，除脂肪体重を測定した．テストステロン投与後の検査データは 6 カ月以降に取得した．統計解析には paired t-test を用い，$p<0.05$ を有意とした．また，解析データは中央値±標準誤差で記した．

(倫理面への配慮)

保護者および患者に対して本研究の目的および効果．有害事象の可能性について十分に説明し了解を取得した．また，対象患者の個人情報について特定できないよう十分に配慮した．

C. 研究結果

行動異常を示した症例は認めず，6 名は過敏性の改善を認めた．対象全例で皮膚の色素沈着とひげが増加し，自信を持つようになった．8 名では勃起を認め，そのうちの 1 名は月に 1 回の射精を認めた．BMDと除脂肪体重は有意に増加した．脂肪率と BMI には変化を認めなかった．

D. 考察

思春期後の PWS 男性患者に対してテストステロン補充療法を行っても，行動異常は増加しないばかりか，何人かでは行動の改善が認められた．また，投与されたものは全て自信を持つようになった．脂肪率と BMI は変化なかったが，BMD と除脂肪体重は増加した．

E. 結論

思春期後の PWS 男性患者に対する性ホルモン補充は，安全に施行できる有効な治療法である．

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

1. Fukami M, Nishimura G, Hommma K, Nagai T, Hanaki K, Uematsu A, Ishii T, Numakura C, Sawada

H, Nakacho M, Kowase T, Motomura K, Haruna H, Nakamura M, Ohishi A, Adachi M, Tajima T, Hasegawa Y, Hasegawa T, Horikawa R, Fujieda K, Ogata T. Cytochrome P450 Oxidoreductase Deficiency:Identification and Characterization of Biallelic Mutaions and Genotype-Phenotype Correlations in 35 Japanese Patients. J Clin Endocrinol Metab 94(5):1723-1731,2009

2. Isojima T, Yokoya S, Ito J, Horikawa R, Tanaka T. New reference growth charts for Japanese girls with Turner syndrome. Pediatr Int. 51(5):709-14,2009.
3. Isojima T, Yokoya S, Ito J, Horikawa R, Tanaka T. Inconsistent determination of overweight by two anthropometric indices in girls with Turner syndrome. Acta Paediatr. 98(3):513-8,2009.
4. 堀川玲子　早産児の二次性徴、生殖機能　周産期医学 39(5):609-614,2009.
5. 堀川玲子　思春期と内分泌　小児疾患診療のための病態生理 小児内科・小児外科　22-31: ,2009.
6. 堀川玲子　思春期における身体成熟　小児科 50(11): 1681-1689,2009.

学会発表

省略

F.知的財産権の出願・登録状況

なし。

1. 実用新案登録

なし

2. その他

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

Prader-Willi 症候群患者における向精神薬の使用状況

研究分担者　村上信行　獨協医科大学越谷病院小児科

研究要旨

Prader-Willi 症候群（PWS）患者の精神症状に有用な薬物療法を検討するために後方視的に向精神病薬の使用状況を調査した．セロトニン再吸収阻害剤とベンゾジアゼピン系薬剤の有用性が示唆された．

共同研究者

永井敏郎　獨協医科大学越谷病院小児科

A. 研究目的

Prader-Willi 症候群（PWS）においては精神神経症錠が思春期以降の中核症状と言われている．一方，PWS の診療経験が少ない医師が多いこと，また，向精神薬が必要な年齢が思春期であることなど，なかなか精神的な問題の治療を実践する機会は乏しい．当院ではおよそ 170 名の Prader-Willi 症候群（PWS）患者をフォローアップしておりその中から向精神薬治療を要した患者の経過をまとめ，今後の治療の指針を考察する．

PWS の現状

1．認知機能

PWS 患者の IQ は概ね 65－70 である．その他かで特徴的なことは短期記憶の脆弱性と視空間認知能が高いことがあげられる．遺伝子型，欠失・非欠失の違いで認知能に UPD で言語能力がやや高いが，視空間認知能でやや劣るといわれる．

2．不適応行動

PWS 患者の 85％に過度の行動障害が認められる．小児から成人の広い年齢で皮膚のひっかき，かんしゃく，衝動性，頑固さ，他者との言い争い，反抗的態度，盗食，食べ物ほしさの金品の盗み，強迫障害，引きこもり，不安障害など多岐にわたる．また問題行動，不適応行動は PWS 患者でも個人差が大きくは年齢によっても変化するので，保護者の対応，保護者の教育が必要になる．

2.1 強迫障害

PWS 患者は食事への過度のこだわりが協調されるが，そのほかにも強迫的な行動異常が見られる．たとえば収集癖（化粧品，ペン，紙切れ），色・形・大きさによって物を並べ替える，繰り返す問いただし，靴を繰り返しそろえ直したり，ひもを結び直したり，家事を繰り返したり，クーポン券を切り抜きなどしつこい動作を繰り返す．これらはとても時間を要する作業で完全な obsessive-compulsive disorder OCD といえる率は 40－80％に登る．PWS 以外の一般的な精神発達遅滞患者の OCD1－3％といわれ PWS で高頻度である．

2.2 過食

PWS 患者の過食は単なる食べ過ぎでなく，満腹感を得ない，常に空腹，吐かないといった特徴がある．原因は不明だが視床下部のオキシトシン分泌ニューロンの障害が候補に挙がっている．また食行動の異常として単なる過食ではなく，普通では食べないものを食べるという報告がある．たとえば，落とした食べ物，ゴミ箱から食べ物を探す，凍った肉を食べる，ペットフードを食べた，とてもまずいものを食べたなどの報告がある．

2.3

他の不適応行動，精神的な問題行動

PWS 患者は衝動性，精神病様症状，情緒的な問題を抱える頻度が高い．ほとんどの PWS 患者はかんしゃくや攻撃性を持つが，その程度は様々である．88％にか

んなしゃくがみられ，自傷は 42%，他者への危害も34%に登る．時に自傷，他障は年齢とともに頻度は減るがかんしゃくや頑固さは年齢と変わらないか，悪化する場合もある．

PWS 患児は非定型精神病の罹患率が高いことも知られている．これは突然発症し，抑鬱傾向が強いと言われる．それいがいにも精神病様症状や幻覚も見られる6.3%，12.1%．

また，抑鬱傾向が高く50%の PWS 患者は以下の項目に該当する，自分が不幸せだと感じていたり，悲しみを感じやすい，引きこもり，孤独感，自尊心の低さなどである．

3 治療

PWS 患者の治療は，食事，運動，ホルモン療法，生活指導であるが，向精神病薬も一定の効果が上げられる．

4.2 SSRI

選択的セロトニン再取り込み阻害剤（SSRI）は中枢神経系シナプスでのセロトニンバランスの正常はかる薬剤である．常同行動，強迫行動，衝動性，抑鬱，自傷，不安などの治療に効果的である．PWS 患者においても SSRI 投与により髄液中のセロトニン代謝産物である5－HIAAの上昇が報告されている．SSRIの一つであるfluoxetine はPWS 患者に投与された複数の報告があり，強迫症状，皮膚ひっかき行動，収集癖，かんしゃくに有効であった．また，SSRI では成人女性 PWS 患者に投与した場合，月経周期の確立が期待されるとの報告もある．

4.3 その他の薬剤

Litium, Carbamazepine, Topiramate も皮膚のひっかき行動を主体に有効であるとの報告が出つつある．

B. 研究方法

1998 年以降，獨協医大越谷病院小児科で診療した PWS 患児 170 名の診療録を調査した．向精神病薬を用いた患者について，後方視的に検討した．

C. 研究結果

PWS 患者 170 名のうち 9 名に向精神薬が投与されていた．セロトニン再吸収阻害剤（SSRI），ベンゾジアゼピン系薬剤（BNZ）の使用頻度が比較的高く各 4 名，6名であった．当院小児科外来で新規に投与を開始した患者は 2 名．他の 7 名は紹介元やその近隣の精神科で投与を開始されていた．性別は 9 名中男児 8 名，女児 1 名．核型は欠失型7名，非欠失型 2 名であった．投与開始年齢は 9 歳から 24 歳で平均 13.5 歳．1 名の女児も 13 歳から投与されていた．使用薬剤はベンゾジアゼピン系 6/9, SSRI4/9, パーキンソン病治療薬 3/9, フェノチアジン系 2/9,バルプロ酸 1/9, カルバマゼピン 1/9 であった．

SSRI 使用患者 4 人のうち 3 人は SSRI 単剤で加療，もう一名は SSRI とベンゾジアゼピン系薬剤を併用していた．

D. 考察

今回は心理検査，知能検査などは検討できていないが向精神病薬が PWS 患者の QOL に一定の効果があるとの印象が持てる．とくに我々が投与開始した 2 名，ともに SSRI，は投与開始により明らかに“焦燥感が改善”“パニックの頻度の減少”“盗食の頻度低下”が認められた．また，投与開始後から“作業所に通うことが楽しい”といった，抑鬱の改善と思われる様子も見られた．他院で投与開始された患者でも“セレネースを開始して人が変わった”といった家人の驚きもきかれた．

一方，向精神病薬の内服を開始したものの，なかなか社会生活に溶け込めない患者もいる．このような患者の共通点は，環境整備ができない点である．幼少期から家族の協力が得られない場合や，現状が整備されない場合は向精神病薬の効果がなかなか感じられないこともある．

E. 結論

PWS のしつこさ，強迫行動，鬱等に向精神病薬はある程度の効果があり，日常生活の質を上げられる期待は高い．今後は定量的な指標に基づいたより医学的な検討が必要となる．

また，今回調査したの PWS 患者の向精神病薬治療

の特徴がある.

1. 海外に比べ導入年齢が遅い.
2. おもに不適応行動を押さえるために投与されている.
3. 精神科の介入事例が多い.

1. 2. に関しては保護者が困って初めて向精神病薬を投与している可能性がある. もっと早い段階で投与できれば患者自体の症状の緩和に直接寄与できると考える. 海外ではスキンピッキングを診療にしている事例が目立ち, 若年者に投与が期待されている.

3. に関しては海外では小児精神科医にアクセスしやすい事もあげられるが, 今回の調査では, 成人の精神科医師が向精神病薬を処方している事例が多い. 日本では小児精神科医が少なく, 専門医に受診が困難であることから, 小児科医, 小児内分泌科医, 小児神経科医が積極的に PWS 患児の向精神病薬投与を開始する啓蒙活動がこれからの課題になる.

F. 健康危険情報

G. 研究発表

1. 論文発表

2. 学会発表

(発表誌名巻号・頁・発行年等も記入)

H. 知的財産権の出願・登録状況

(予定を含む。)

1. 特許取得

2. 実用新案登録

3. その他

研究成果の刊行一覧表

別紙4

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Nakamura Y, Nagai T, Iida T, Ozeki S, Nohara Y.	Epidemiological aspects of scoliosis in a cohort of Japanese patients with Prader-Willi syndrome.	Spine J	9	809-816	2009
Fukami M, Nishimura G, Homma K, Nagai T, Hanaki K, Uematsu A, Ishii T, Numakura C, Sawada H, Nakacho M, Kowase T, Motomura K, Haruna H, Nakamura M, Ohishi A, Adachi M, Tajima T, Hasegawa Y, Hasegawa T, Horikawa R, Fujieda K, Ogata T	Cytochrome P450 oxidoreductase deficiency: identification and characterization of biallelic mutations and genotype-phenotype correlations in 35 Japanese patients.	J Clin Endocrinol Metab	94 (3)	1723-1731	2009
Yuka Wada, Gen Nishimura, Toshiro Nagai, Hideaki Sawai, Magumi Yoshitaka, Shinichiro Miyagawa, Takushi Hanita, Seiji Sato, Tomonobu Hasegawa, Shumpei Ishikawa, Tsutomu Ogata	Mutation analysis of SOX9 and single copy number variant analysis of the upstream region in eight acampomelic campomelic dysplasia	Am J Med Genet Part A	149A	2882-2885	2009
Kagami M, O'Sullivan MJ, Green AJ, Watabe Y, Arisaka O, Masawa N, Matsuoka K, Fukami M, Matsubara K, Kato F, Ferguson-Smith AC, Ogata T	The IG-DMR and the MEG3-DMR at Human Chromosome 14q32.2: Hierarchical Interaction and Distinct Functional Properties as Imprinting Control Centers	*PLoS Genetics*	in press		
Hosoki K, Kagami M, Tanaka T, Kubota M, Kurosawa K, Kato M, Uetake K, Tohyama J, Ogata T, Saitoh S	Maternal uniparental disomy 14 syndrome demonstrates Prader-Willi syndrome-like phenotype	Journal of Pediatrics	155 (6)	900-903	2009
Kobayashi H, Yamada K, Morita S, Hiura H, Fukuda A, Kagami M, Ogata T, Hata K, Sotomaru Y, Kono T	Identification of the mouse paternally expressed imprinted gene Zdbf2 on chromosome 1 and its imprinted human homolog ZDBF2 on chromosome 2	*Genomics*	93 (5)	461-472	2009
Nakamura Y, Nagai T, Iida T, Ozeki S, Nohara Y.	Epidemiological aspects of scoliosis in a cohort of Japanese patients with Prader-Willi syndrome.	Spine J	9	809-816	2009
Fukami M, Nishimura G, Homma K, Nagai T, Hanaki K, Uematsu A, Ishii T, Numakura C, Sawada H, Nakacho M, Kowase T, Motomura K, Haruna H, Nakamura M, Ohishi A, Adachi M, Tajima T, Hasegawa Y, Hasegawa T, Horikawa R, Fujieda K, Ogata T	Cytochrome P450 oxidoreductase deficiency: identification and characterization of biallelic mutations and genotype-phenotype correlations in 35 Japanese patients.	J Clin Endocrinol Metab	94 (3)	1723-1731	2009

別紙4

Yuka Wada, Gen Nishimura, Toshiro Nagai, Hideaki Sawai, Magumi Yoshitaka, Shinichiro Miyagawa, Takushi Hanita, Seiji Sato, Tomonobu Hasegawa, Shumpei Ishikawa, Tsutomu Ogata	Mutation analysis of SOX9 and single copy number variant analysis of the upstream region in eight acampomelic campomelic dysplasia	Am J Med Genet Part A	149A	2882-2885	2009
2. Isojima T, Yokoya S, Ito J, Horikawa R, Tanaka T.	New reference growth charts for Japanese girls with Turner syndrome.	Pediatr Int.	51(5)	709-714	2009
3. Isojima T, Yokoya S, Ito J, Horikawa R, Tanaka T.	Inconsistent determination of overweight by two anthropometric indices in girls with Turner syndrome.	Acta Paediatr	98(3)	513-518	2009
4. 堀川玲子	早産児の二次性徴、生殖機能	周産期医学.	39(5)	609-614	2009

研究成果の刊行物・別刷り

ELSEVIER

The Spine Journal 9 (2009) 809–816

THE SPINE JOURNAL

Clinical Study

Epidemiological aspects of scoliosis in a cohort of Japanese patients with Prader-Willi syndrome

Yutaka Nakamura, MD[a,*], Toshiro Nagai, MD, PhD[b], Takahiro Iida, MD, PhD[a], Satoru Ozeki, MD, PhD[a], Yutaka Nohara, MD, PhD[c]

[a]*Department of Orthopaedic Surgery, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama 343-8555, Japan*
[b]*Department of Pediatrics, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama 343-8555, Japan*
[c]*Department of Orthopedic Surgery, Dokkyo Medical University Hospital, Mibu, Tochigi, 321-0293, Japan*

Abstract

BACKGROUND CONTEXT: The prevalence of scoliosis in Prader-Willi syndrome (PWS) is high; however, the prevalence of PWS is rare, with one person in 10,000 to 20,000 affected. The etiology and characteristics of scoliosis associated with PWS remain unidentified. In addition, it is believed that the speedup of growth associated with growth hormone (GH) supplement treatment may influence the deterioration of scoliosis in PWS.

PURPOSE: To investigate scoliosis in Japanese patients with PWS.

STUDY DESIGN: Retrospective observational study.

PATIENT SAMPLE: We investigated 101 patients (67 men, 34 women) who were followed up from November 2002 to January 2008. All patients were diagnosed using fluorescence in situ hybridization or the methylation test. Of the 101 patients, 80 had an inherited deletion of chromosome 15q11–13 (deletion) and 21 patients had no deletion, including those with uniparental disomy.

OUTCOME MEASURES: 1) Prevalence of scoliosis; 2) association of scoliosis with GH treatments; 3) association of scoliosis with genotype; 4) clarification of PWS scoliosis characteristics; and 5) analysis of severe PWS scoliosis patients (Cobb angle greater than 40°). Scoliosis for our study was defined as scoliosis with a Cobb angle greater than 10°.

METHODS: To investigate PWS-associated scoliosis, we used spinal X-ray examinations. The pattern of scoliosis was classified into three types: primary single lumbar or thoracolumbar curve (Type 1), double curve (Type 2), and primary single thoracic curve (Type 3). For statistical analysis, chi-square tests for the distribution of patients were used ($p<.05$).

RESULTS: 1) Scoliosis was found in 38.6% (39/101) of patients with PWS. 2) There was no statistical difference in the prevalence of scoliosis between the GH treatment group (32.8%) and the GH nontreatment group (group with no GH treatments) (46.5%) ($p=.16$, chi-square test). 3) There was no statistical difference in the prevalence of scoliosis between the deletion group (38.8%) and the nondeletion group (38.1%) ($p=.84$, chi-square test). 4) Scoliosis was classified into three types, according to single or double curve scoliosis and position of scoliosis. The prevalence of these groups was 61.5% for Type 1 (primary single lumbar and thoracolumbar curve), 48.7% for lumbar curve convex on the left side, 28.2% for Type 2 (double curve), and 10.3% for Type 3 (primary single thoracic curve). 5) Severe scoliosis was found in nine patients (8.9%, 9/101). Type 2 was found in 66.7% (6/9) of patients with severe scoliosis. During the follow-up period, two patients changed from Type 1 to Type 2.

CONCLUSIONS: Scoliosis in PWS can be classified into three types. A lumbar curve convex on the left side was found in most patients. In addition, severe deterioration of scoliosis was found in Type 2 patients. Therefore we recommend careful, ongoing observations for patients showing double curve tendencies.

Keywords: Prader-Willi syndrome; Scoliosis; Growth Hormone; Genotype; Severe spinal deformity; Surgery

FDA device/drug status: not applicable.
Author disclosures: none.

* Corresponding author. Department of Orthopedic Surgery, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama 343-8555, Japan. Tel.: (81) 489-65-1111 ext 2626; fax: (81) 489-65-4700.
E-mail address: nakayuta@dokkyomed.ac.jp (Y. Nakamura)

1529-9430/09/$ – see front matter
doi:10.1016/j.spinee.2009.06.017

EVIDENCE & METHODS

Context
Prader-Willi syndrome is rare, and the associated spinal deformity is not fully characterized.

Contribution
This study provides a radiographic characterization of scoliosis in 101 affected patients. They found that while scoliosis is common, severe scoliosis occurred in only 9% of patients, and less than 6% went on to operative intervention.

Implications
Monitoring scoliosis in these patients is justified, but parents may be reassured that surgical intervention is often not warranted.

—*The Editors*

Introduction

Prader-Willi syndrome (PWS) is caused by abnormalities of chromosome 15 and is characterized by weakness of muscle tension, imperfect function of the hypothalamus and the pituitary gland, hypogonadism, overeating, and obesity. Orthopedic characteristics include scoliosis, hip dysplasia, and lower limb alignment abnormality. In addition, bone fracture because of osteoporosis is problematic.

The prevalence of scoliosis in PWS is 15% to 86% [1–5]; however, the prevalence of PWS is rare, with 1 person in 10,000 to 20,000 affected. The etiology of scoliosis in PWS is not well defined. Recently, growth hormone (GH) supplement treatment has been used worldwide. GH treatment has contributed to improvements in height, body composition, bone density, and breathing function [6,7]; however, the possibility that increased rates of growth because of GH treatment can adversely affect scoliosis is a concern.

In this report, scoliosis in PWS was examined. We investigated 1) the prevalence of scoliosis; 2) the association of scoliosis with GH treatments; 3) the association of scoliosis with genotype; 4) the clarification of PWS scoliosis characteristics; and 5) the characteristics of severe PWS scoliosis patients.

Methods

We investigated 101 patients (67 men, 34 women), who had been followed up by the pediatric department in our hospital from November 2002 to January 2008. All patients were diagnosed for PWS using fluorescence in situ hybridization or the methylation test. The median age was 13 years (range 1–51 years). Of the 101 patients, 80 patients had an inherited deletion of chromosome 15q11–13 (henceforth referred to as "deletion"), and 21 patients had no deletion, including those with uniparental disomy. Patients were investigated using spinal X-ray examinations.

Standing posteroanterior radiographs were taken of children who were able to stand. Patients with scoliosis were followed up by spinal X-ray examination in our orthopedic surgery department every 6 months. Patients with early progression, defined as progress of greater than 10°, were examined every 3 months. The same senior spinal surgeon evaluated the Cobb angle of the scoliosis, with angles greater than 10°, and rotation of the vertebrae defined as scoliosis. Cobb angles greater than 20° required brace treatment. Patients with Cobb angles greater than 40° were defined as having severe scoliosis. The age of onset of scoliosis was determined as the age at first diagnosis.

We focused on 1) the prevalence of scoliosis in Japanese patients with PWS, with respect to age distribution; 2) the association of GH treatments with the prevalence of PWS scoliosis and the association of GH treatments with the progression of PWS scoliosis; 3) the association of PWS with genotype and the prevalence of scoliosis with respect to genotype; 4) analysis of PWS scoliosis characteristics, as classified into three types; and 5) the analysis of severe PWS scoliosis patients (Cobb angle greater than 40°). "1" and "2" were classified by the age criteria: infants (younger than 3 years), juveniles (ages from 3 to 10 years), and adolescents/adults (aged 10 years and older). For "4," scoliosis was divided into single and double curve scoliosis, using the following classification, based on standing whole spinal X-rays:

Type 1: Primary single lumbar or thoracolumbar curve
Type 2: Double curve (thoracic curve and lumbar or thoracolumbar curve)
Type 3: Primary single thoracic curve

Patients were investigated according to age, gender, genotype, position of the apex of the scoliosis, Lenke classification system [8], average Cobb angle, thoracic kyphotic angle (T1–T12), lumbar lordosis angle (L1–S1), progression of scoliosis, and the severity of scoliosis.

For "5," scoliosis type, presence of surgery, surgical method, fusion level, age at surgery, change in Cobb angle (during follow-up in our hospital), and any change between types were assessed.

For statistical analysis, chi-square tests for the distribution of patients were used. p Values less than .05 were considered as statistically significant.

Results

Prevalence of scoliosis

Scoliosis was found in 38.6% (39/101) of patients with PWS: 27 men and 12 women. The average follow-up period was 4 years 5 months (from 6 months to 12 years 9 months).

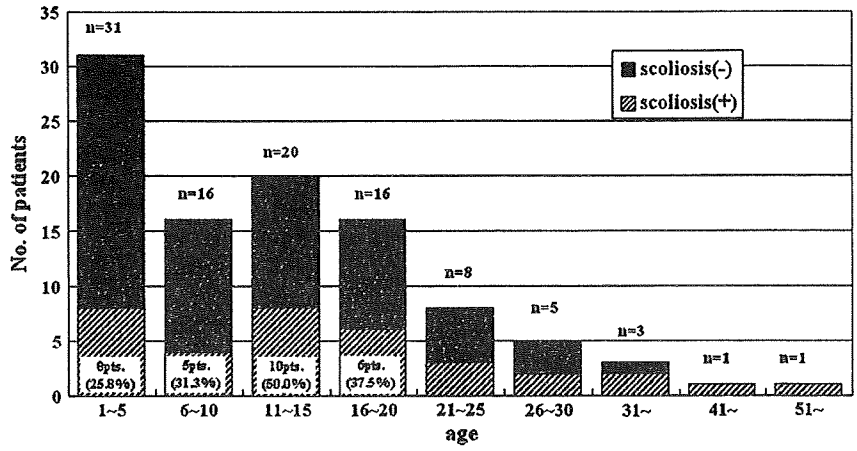

Fig. 1. The prevalence of scoliosis with respect to age distribution. pts, patients.

There were no patients who had congenital spinal anomalies. The prevalence of PWS patients with scoliosis with respect to age was 25.8% (8/31) for 1- to 5-year olds, 31.3% (5/16) for 6- to 10-year olds, 50.0% (10/20) for 11- to 15-year olds, and 37.5% (6/16) for 16- to 20-year olds. The prevalence of scoliosis was highest in 11- to 15-year olds (Fig. 1). In addition, the prevalence of infants with scoliosis was 44.4% (4/9), juveniles was 27.3% (9/33), and adolescents/adults was 44.8% (26/58). Scoliosis with a Cobb angle of greater than 20° was found in 23.8% (24/101) of patients: 17 men and 7 women. The brace treatment was used in nine patients, which excluded patients who had mental retardation.

Association of GH treatments with the prevalence of scoliosis

The prevalence of scoliosis in the GH treatment group was 32.8% (19/58) and in the GH nontreatment group was 46.5% (20/43). There were no statistically significant differences between these groups (p=.16, chi-square test) (Table 1). Analyzing the GH treatment and nontreatment groups with respect to age, the frequency was 44.4% (4/9) in the infant GH treatment group, 30.4% (7/23) in the juvenile GH treatment group, and 30.8% (8/26) in the adolescent/adult GH treatment group. In GH nontreatment groups, scoliosis was absent in infants but was 18.2% (2/11) in the juvenile group and 56.3% (18/32) in the adolescent/adult group. There were no statistically significant differences in prevalence between treatment and nontreatment groups at juvenile and adolescent/adult ages (Table 2).

Table 1
The association between GH treatment and the prevalence of scoliosis*

Scoliosis	+	−
GH use	19 (32.8%)	39 (67.2%)
GH nonuse	20 (46.5%)	23 (43.4%)
Total	39 (38.6%)	62 (63.2%)

GH, growth hormone.

* There was no significant difference between the two groups (p=.16, chi-square test).

Association of GH treatments with the progression of scoliosis

In the GH treatment group, scoliosis deteriorated in 61.5% (8/13) of patients and in the GH nontreatment group in 38.5% (5/13) of patients. In the GH treatment group, no change was seen in 44.0% (11/25) of patients and in the GH nontreatment group in 56.0% (14/25) of patients. The condition of one patient in the GH nontreatment group improved (Table 3). There were no statistically significant differences in the progression of scoliosis deterioration between GH treatment and nontreatment groups (p=.47, chi-square test).

Association of scoliosis with genotype

With respect to genotype, the prevalence of scoliosis was 38.8% (31/80) for the deletion group and 38.1% (8/21) for the nondeletion group. There were no statistically significant differences between groups (p=.84, chi-square test; Table 4).

Table 2
The association of GH treatment with the prevalence of scoliosis, with respect to age

GH therapy	(+)	(−)	Test*
Infants	4/9 (44.4%)	0/0 (0%)	
Juveniles	7/23 (30.4%)	2/11 (18.2%)	p=.12
Adolescent/adults	8/26 (30.8%)	18/32 (56.3%)	p=.38

GH, growth hormone.

* There was no significant difference between the two groups (chi-square test).

Table 3
Patients with scoliosis

Age (y)	Gender	Genotype	Follow-up period (mo)	GH	Lenke classification	Change of Cobb angle (°)	Progression
Type 1: Primary single lumbar and thoracolumbar scoliosis							
1	M	Deletion	41	Use	5A	T8–L2:11→12	No change
1	F	Deletion	32	Use	5A	L1–L5:11→11	No change
2	F	UPD	42	Use	5A	T11–L4:11→12	No change
2	F	Deletion	75	Use	5C	T4–T11:30→ope→34	Deterioration*
3	F	UPD	41	Use	—	T9–L3:14→21	Deterioration
4	M	UPD	28	Use	5A	T9–L3:15→18	No change
4	F	Deletion	58	Use	5B	T11—L4:14→10	No change
6	M	Deletion	44	Use	5B	T11–L4:12→14	No change
13	F	Deletion	44	Use	—	T9–L5:25→37	Deterioration
14	M	Deletion	127	Use	5A	T11–L4:24→22	No change
14	M	Deletion	37	Use	5A	T11–L5:20→21	No change
15	M	UPD	120	Use	5C	T9–L3:31→38	No change
17	M	Deletion	105	Use	5C	T8–L2:46→55	Deterioration
38	F	Deletion	45	Use	5C	L1–L5:16→18	No change
8	F	Deletion	153	Nonuse	5C	T8–L3:70→ope→30	Deterioration*
11	F	Deletion	25	Nonuse	5B	T12–L5:13→6	improvement
13	M	Deletion	45	Nonuse	5C	T10–L5:50→ope→38	Deterioration
16	M	Deletion	24	Nonuse	5A	T12–L5:10→13	No change
19	M	Deletion	44	Nonuse	5A	T12–L5:11→13	No change
22	M	Deletion	47	Nonuse	—	T7–L4:18→20	No change
22	M	Deletion	79	Nonuse	5B	T12–L5:11→10	No change
23	M	Deletion	25	Nonuse	5C	T12–L5:23→26	No change
24	M	Deletion	54	Nonuse	5B	T12–L4:16→13	No change
50	M	Deletion	54	Nonuse	5A	T12–L5:10→13	No change
Type 2: double curve							
5	M	Deletion	40	Use	2A	T11–L5:23→26	No change
9	M	UPD	32	Use	3C	T6–T12:49→ope→45	Deterioration
9	F	Deletion	21	Use	3C	T4–T11:49→ope→55	Deterioration
12	M	Deletion	24	Use	3B	T4–L1:39→59	Deterioration
16	F	UPD	71	Use	2C	T9–L2:13→ope→27	Deterioration
9	M	UPD	45	Nonuse	3A	T11–T5:18→22	No change
11	M	Deletion	72	Nonuse	2A	T11–L5:14→18	No change
15	M	Deletion	46	Nonuse	3A	T5–L2:35→39	Deterioration
20	M	Deletion	53	Nonuse	3A	T7–T11:28→31	No change
37	M	Deletion	50	Nonuse	2C	T4–T11:36→38	No change
42	M	Deletion	89	Nonuse	2B	T6–L1:35→32	No change
Type 3: primary single thoracic curve							
12	F	Deletion	26	Nonuse	—	T2–T11:23→22	No change
16	M	Deletion	24	Nonuse	—	T8–L2:46→55	Deterioration
27	M	UPD	6	Nonuse	—	T3–T11:52→57	Deterioration
28	M	Deletion	85	Nonuse	—	T3–T12:12→11	No change

GH, growth hormone; M, Male; F, Female; UPD, uniparental disomy.
Change of Cobb angle indicates the level of scoliosis and the difference between the first X-ray and the final X-ray during follow-up.
* Case changed from Type 1 to Type 2 during the follow-up period.

Clarification of the characteristics of PWS scoliosis

Type 1 (primary single lumbar or thoracolumbar curve) (Fig. 2) was found in 61.5% (24/39) of patients, 15 men and 9 women (Table 3). The median age of the patients was 13 years (range, 1–50 years), with the inherited deletion form found in 20 patients and the nondeletion form found in 4 patients. In 23 patients, scoliosis was convex on the left side (T12, 3 patients; L1, 2 patients; L2, 12 patients; L3, 6 patients), and in only one patient, scoliosis was convex on the right side (L3). Lenke classification system indicated 5A in nine patients, 5B in five patients, 5C in seven patients, and three other patients were not distinguishable because of a long C curve. Lumbar curve convex on the left side was seen in 48.7% (19/39) of patients, which was the most common PWS presentation seen. The average Cobb angle was 21.3°, the average thoracic kyphosis (T1–T12) angle was 34.6°, and the average lumbar lordosis angle (L1-S1) was 34.7°. Scoliosis deteriorated in 5 patients, 18 patients showed no change, and 1 patient showed improvement. Severe scoliosis was found in one patient and in two additional patients, who changed from Type 1 to Type 2 during the follow-up period.

Type 2 (double curve [thoracic curve and lumbar or thoracolumbar curve]) (Fig. 3) was found in 28.2% (11/39) of

Table 4
Association between genotypes and the prevalence of scoliosis*

Scoliosis	+	−
Deletion	31 (38.8%)	49 (61.2%)
Nondeletion	8 (38.1%)	13 (61.9%)
Total	39 (38.6%)	62 (61.4%)

* There was no significant difference between the two groups (p=.84, chi-square test).

patients, 9 men and 2 women. The median age of the patients was 12 years (range, 5–42 years). The inherited deletion form was found in eight patients and the nondeletion form was found in three patients. The apex of scoliosis in all patients was convex on the left side of the lumbar or thoracolumbar curve (T11, two patients; L1, two patients; L2, five patients; L3, two patients) and convex on the right side of the thoracic curve (T6, one patient; T7, one patient; T8, one patient; T9, eight patients). Lenke classification system indicated 2A in two patients, 2B in one patient, 2C in two patients, 3A in three patients, 3B in one patient, and 3C in two patients. The average Cobb angle was 38.6° for the lumbar or thoracolumbar curve and 33.6° for the thoracic curve. The average thoracic kyphosis angle (T1–T12) was 30.3°, and the average lumbar lordosis angle (L1–S1) was 33.3°. Scoliosis deteriorated in five patients, and six patients showed no change. Severe scoliosis of Type 2 was found in six patients, including two patients who changed from Type 1 to Type 2 during the follow-up period.

Type 3 (primary single thoracic curve) (Fig. 4) was found in 10.3% (4/39) of patients, three men and one woman. The median age of the patient was 16 years (range, 12–28 years). The inherited deletion form was found in three patients and the nondeletion form in one patient. The apex of scoliosis in all patients was convex on the right side (T8, two patients; T9, one patient; T11, one patient). There were no cases found that fit the Lenke classification system. The average Cobb angle was 33.3°, the average thoracic kyphosis angle (T1–T12) was 45.0°, and the average lumbar lordosis angle (L1–S1) was 48.0°. Scoliosis

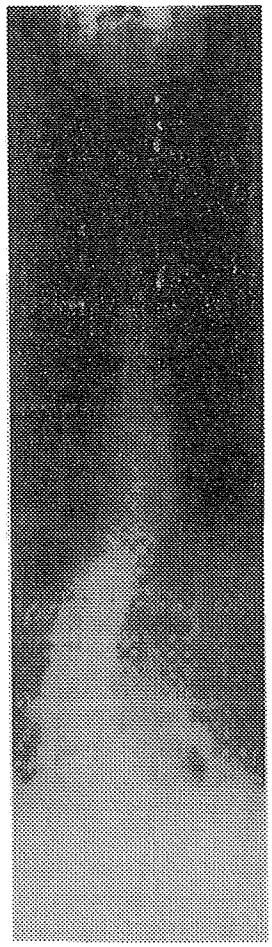

Fig. 2. A case of Type 1. The patient is an 18-year-old man. There was an apex of scoliosis in L2 and a lumbar curve convex on the left side. The Cobb angle was 47° (T11–L4).

Fig. 3. A case of Type 2. The patient is a 17-year-old male. There was a lumbar curve convex on the left side with the apex at L2 (the Cobb angle was 58° between T12 and L4), and a thoracic curve convex on the right side with the apex at T7 (the Cobb angle was 65° between T1 and T12).

Fig. 4. A case of Type 3. This patient is a 27-year-old man. There was an apex of scoliosis at T8 and a thoracic curve convex on the right side. The Cobb angle was 57° (T2–T11).

deteriorated in two patients and two patients showed no change. Severe scoliosis was found in two patients.

Analysis of severe PWS scoliosis patients

Severe scoliosis was found in nine patients (8.9%, 9/101). Of these, 66.7% (6/9) were Type 2, 11.1% (1/9) were Type 1, and 22.2% (2/9) were Type 3. Spinal correction and fusion was performed in six patients (anterior method in two patients, posterior method in two patients, and a combined method in two patients). Three patients did not undergo surgery. Two patients were juvenile and four patients were older than adolescent at surgery. Change from Type 1 to Type 2 was found in two patients after evaluation of scoliosis progression (Table 5).

Discussion

Scoliosis is an important component of PWS that requires monitoring for possible progression. In this study, the prevalence of scoliosis in PWS was 38.6% (39/101). Prevalence of scoliosis in PWS has been reported at 15% to 86% [1–5]. de Lind van Wijngaarden et al. [5] studied 96 patients, reporting a 37.5% prevalence and a median age of 6.9 years. Kroonen et al. [3] studied 31 patients, reporting a 58% prevalence and an average age of 22 years. We studied 101 patients and found a 38.6% prevalence and a median age of 13 years. One reason for the different frequencies observed in these studies is the difference in patient age distribution.

In this study, the prevalence of scoliosis was 31.3% (5/16) at ages 1–5 years, 50.0% (10/20) at ages 11–15 years, and 37.5% (6/16) at ages 16–20 years. Holm and Laurnen [2] reported in a study of 32 PWS patients with scoliosis, "the scoliosis is present from an early age and remains stable during childhood, but progresses in 15 to 20 per cent of the cases." As patients age, general long-term muscle weakness, paravertebral muscular weakness, obesity, and congenital soft-tissue abnormality worsen. We consider PWS characteristics, such as low height, osteoporosis, hip dysplasia, and bone maturity imperfections, including imperfections of the vertebral end plate, as factors in the etiology of scoliosis in PWS.

GH therapy corrects osteopenia and improves lean body mass, physical strength, and agility. There has been a concern that the speedup of growth with GH treatment can exacerbate scoliosis. Whether GH treatment does indeed contribute to the progression of scoliosis remains arguable. Docquier et al. [9] reported that GH may increase the risk of scoliosis progression and, furthermore, that this progress is frequently rapid. However, there are reports indicating that scoliosis in PWS was not affected by GH treatment [10] and that Turner syndrome can be treated with GH, indicating the safety of GH treatment [11]. Nagai et al. [4] reported in 2006 that GH therapy increases the height velocity of patients with PWS and also that the scoliosis in these patients does not necessarily progress. Moreover, our current data showed no statistical differences between the frequency of scoliosis and GH treatment or nontreatment. In addition, no meaningful statistical difference was found, in juvenile and adolescent/adult groups, between the prevalence of scoliosis and the presence or absence of GH treatment; the prevalence of scoliosis increased in juvenile and adolescent/adults regardless of GH treatment.

There were also no statistically significant differences in the progression of scoliosis deterioration between the GH treatment and nontreatment groups. In the present study, the number of cases increased with age, but no correlation was found between GH treatment and the prevalence and progression of scoliosis. However, because GH treatment has only been used for 7 years, the effects of long-term GH treatment are not yet known. Until such effects are better understood, caution is necessary.

There have been few reports documenting inherited PWS in Japanese patients, so this study of 101 Japanese patients with PWS is significant. Lin et al. [12] reported that PWS with deletion, characterized by hypogonadism, small hands and feet,

Table 5
Patients with severe scoliosis

Age, y (at onset)	Gender	Follow-up period	Scoliosis type	Surgical methods (fusion level)	Age, y (at surgery)	Change in Cobb angle (per 6 mo) level: degree of Cobb angle
13	M	3 y 9 mo	1	First: ACF (L1–3)	15	T4–T10: 50*→30†→40→43→38→38
						T10–L5:40*→27†→38→40→40→35→39
2	F	6 y 3 mo	1→2	First: ACF (T11–L3)	6	T3–T9: 0→0→50→→46*→31†→40→→35
						T4–T11:30→29→37→T9–L3: 80→70→89→→88*→26†→41→→34
9	F	1 y 11 mo	2	First: Growing rod (T3–L3)	12	T4–T11: 49 →53→72*→55†
						T11–L5:55→48→51*→51†
9	M	2 y 8 mo	2	First: ACF (T10–L2)	15	T6–12: 49 →66→69*→42†→45
				Second: PCF (T2–L2)	16	T12–L4:35→52→53*→38†→38
16	F	5 y 11 mo	2	First: PCF (T3–L3)	15	T4–T9: 18→→→→→34→30→32*→24†→22→26
						T9–L2: 13→→→→→32→47→44*→27†→27→27
8	F	12 y 9 mo	1→2	First: ACF (T10–L2)		T1–T8: 44*→40†→41→38→42→45→37→→45→51→→
						T8–L3: 70→81→87→86*→43†→46→48→53→51→53→→64→53→→
				Second: PCF (T3–L3)	9	T1–T8: 55→→60→→60→66→64→48→→59→54*→28†
						T8–L3: 60→→ 72→→68→59→69→58→→58→46*→30†
12	M	20 mo	2	No surgery		T4–L1: 39→46→45→59
						L1–L5: 37→36→33→33
16	M	21 mo	3	No surgery		T8–L2:46→52→55→55
27	M	6 mo	3	No surgery		T3–T11:52→57

ACF, anterior correction fusion; PCF, posterior correction fusion; M, male; F, female.
* Before surgery.
† After surgery.

and hypopigmentation, was more prevalent than PWS with uniparental disomy. In this study, the prevalence of PWS scoliosis with genotype was examined, but we found no significant difference between deletion and nondeletion groups.

It has been frequently reported that thoracic convex curve to the right side is a characteristic of idiopathic scoliosis [13]. In our study, however, there was a prevalence of lumbar convex curve to the left side, 48.7% (19/39); we recognize this type of scoliosis as a characteristic of scoliosis in PWS. The cause of these clear differences is unknown, but the etiology of PWS is general muscle weakness, suggesting that paravertebral muscle weakness more easily influences the lumbar vertebrae compared with the thoracic vertebrae, which are surrounded by the rib cage.

It is important to monitor scoliosis in PWS to determine whether the scoliosis will progress over time. First, a classification scheme is necessary to evaluate the characteristics of progressive scoliosis in PWS. Holm and Laurnen [2] recommended spinal X-ray examination of patients with PWS during follow-up, but only one X-ray of the whole spinal anterior-posterior orientation is acceptable because of the effects of X-rays on younger patients. The standard evaluation is thus necessarily based on one X-ray, but to judge correctly a compensation curve and a construction curve using only one X-ray is difficult.

de Lind van Wijngaarden et al. [5] distinguished two types of scoliosis in children with PWS: long C-curve type scoliosis and scoliosis resembling idiopathic scoliosis. In this study, we classified scoliosis into three types, depending on the location and the presence of a single or double curve. Holm and Laurnen [2] reported two surgical patients with severe Type 3 scoliosis, Gurd and Thompson [14] reported one patient with severe Type 3, and Rees et al. [15] also reported one surgical patient with Type 3. There have been many reports of thoracic curve (Type 3) in severe scoliosis. However, in this study, there was severe scoliosis in nine patients, with 11.1% (1/9) in Type 1, 66.7% (6/9) in Type 2, and 22.2% (2/9) in Type 3. Type 2 had the highest rate of severe scoliosis. In addition, the average Cobb angle in patients with Type 2 scoliosis was 33.6° for the thoracic curve and 30.3° for the lumbar or thoracolumbar curve. These curve angles were higher than the 21.3° of Type 1 and similar to the 33.3° of Type 3. During the follow-up, two patients were deteriorated from Type 1 to Type 2. Previously reported Type 2 patients include one surgical patient reported by Accadbled et al. [16], one patient reported by Yamada et al. [17], and one surgical patient reported by Rees et al. [15]. In this study, there were few cases of Type 3. However, we considered that careful observation is needed for patients showing a double curve tendency and that this is an important factor in observing the progress of scoliosis in patients with PWS.

Severe scoliosis was found in nine patients (8.9%), and two additional patients were confirmed to change from Type 1 to Type 2 after a comparatively long follow-up (Table 5). It was observed that in PWS, scoliosis in the lumbar spine progressed to the thoracic spine.

References

[1] Butler JV, Whittington JE, Holland AJ, et al. Prevalence of and risk factors for, physical ill-health in people with Prader-Willi syndrome: a population based study. Dev Med Child Neurol 2002;44:248–55.

[2] Holm VA, Laurnen EL. Prader-Willi syndrome and scoliosis. Dev Med Child Neurol 1981;23:192–201.

[3] Kroonen LT, Herman M, Pizzutillo PD, Macewen GD. Prader-Willi syndrome: clinical concerns for the orthopedic surgeon. J Pediatr Orthop 2006;26:673–9.

[4] Nagai T, Obata K, Ogata T, et al. Growth hormone therapy and scoliosis in patients with Prader-Willi syndrome. Am J Med Genet A 2006;140:1623–7.

[5] de Lind van Wijngaarden R, de Klerk LW, Festen DA, Hokken-Koelega AC. Scoliosis in Prader-Willi syndrome: prevalence, effects of age, gender, body mass index, lean body mass and genotype. Arch Dis Child 2008;93:1012–6.

[6] Myers SE, Whitman BY, Carrel AL, et al. Two years of growth hormone therapy in young children with Prader-Willi syndrome: physical and neurodevelopment benefits. Am J Med Genet A 2007;143:443–8.

[7] Mogul HR, Lee PD, Whitman BY, et al. Growth hormone treatment of adults with Prader-Willi Syndrome and growth hormone deficiency improves lean body mass, fractional body fat, and serum triiodothyronine without glucose impairment: results from the United Sates multicenter trial. J Clin Endocrinol Metab 2008;93:1238–45.

[8] Lenke L, Edwards C II, Birdwell KH. The Lenke classification of adolescent idiopathic scoliosis: how it organizes curve patterns as a template to perform selective fusions of the spine. Spine 2003;28: 199–207.

[9] Docquier PL, Mousny M, Jouret M, et al. Orthopaedic concerns in children with growth hormone therapy. Acta Orthop Belg 2004;70:299–305.

[10] Odent T, Accadbled F, Koureas G, et al. Scoliosis in patients with Prader-Willi syndrome. Pediatrics 2008;122:e499–503.

[11] Quigley CA, Gill AM, Crowe BJ, et al. Safety of growth hormone treatment in pediatric patients with idiopathic short stature. J Clin Endocrinol Metab 2005;90:5188–96.

[12] Lin HY, Lin SP, Chuang CK, et al. Genotype and phenotype in patients with Prader-Willi syndrome in Taiwan. Acta Paediatr. 2007;96: 902–5.

[13] King HA, Moe JH, Bradford DS, Winter RB. The selection of fusion levels in thoracic idiopathic scoliosis. J Bone Joint Surg Am 1983;65: 1302–13.

[14] Gurd AR, Thompson TR. Scoliosis in Prader-Willi syndrome. J Pediatr Orthop 1981;1:317–20.

[15] Rees D, Jones MW, Owen R, Dorgan JC. Scoliosis surgery in the Prader-Willi syndrome. J Bone Joint Surg Br 1989;71: 685–8.

[16] Accadbled F, Odent T, Moine A, et al. Complications of scoliosis surgery in Prader-Willi syndrome. Spine 2008;33:394–401.

[17] Yamada K, Miyamoto K, Hosoe H, et al. Scoliosis associated with Prader-Willi syndrome. Spine J 2007;7:345–8.

balt1/zeg-jcem/zeg-jcem/zeg00509/zeg6456-09a | angnes | S=6 | 4/7/09 | 21:45 | 4/Color Figure(s): F1,F2,F4 | Art: 08-2816 | Input-ss

Cytochrome P450 Oxidoreductase Deficiency: Identification and Characterization of Biallelic Mutations and Genotype-Phenotype Correlations in 35 Japanese Patients

Maki Fukami, Gen Nishimura, Keiko Homma, Toshiro Nagai, Keiichi Hanaki, Ayumi Uematsu, Tomohiro Ishii, Chikahiko Numakura, Hirotake Sawada, Mariko Nakacho, Takanori Kowase, Katsuaki Motomura, Hidenori Haruna, Mihoko Nakamura, Akira Ohishi, Masanori Adachi, Toshihiro Tajima, Yukihiro Hasegawa, Tomonobu Hasegawa, Reiko Horikawa, Kenji Fujieda, and Tsutomu Ogata*

Context: Cytochrome P450 oxidoreductase (POR) deficiency is a rare autosomal recessive disorder characterized by skeletal dysplasia, adrenal dysfunction, disorders of sex development (DSD), and maternal virilization during pregnancy. Although multiple studies have been performed for this condition, several matters remain to be clarified, including the presence of manifesting heterozygosity and the underlying factors for clinical variability.

Objective: The objective of the study was to examine such unresolved matters by detailed molecular studies and genotype-phenotype correlations.

Patients: Thirty-five Japanese patients with POR deficiency participated in the study.

Results: Mutation analysis revealed homozygosity for R457H in cases 1–14 (group A), compound heterozygosity for R457H and one apparently null mutation in cases 15–28 (group B), and other combinations of mutations in cases 29–35 (group C). In particular, FISH and RT-PCR sequencing analyses revealed an intragenic microdeletion in one apparent R457H homozygote, transcription failure of apparently normal alleles in three R457H heterozygotes, and nonsense mediated mRNA decay in two frameshift mutation-positive cases examined. Genotype-phenotype correlations indicated that skeletal features were definitely more severe, and adrenal dysfunction, 46,XY DSD, and pubertal failure were somewhat more severe in group B than group A, whereas 46,XX DSD and maternal virilization during pregnancy were similar between two groups. Notable findings also included the contrast between infrequent occurrence of 46,XY DSD and invariable occurrence of 46,XX DSD and pubertal growth pattern in group A mimicking that of aromatase deficiency.

Conclusions: The results argue against the heterozygote manifestation and suggest that the residual POR activity reflected by the R457H dosage constitutes the underlying factor for clinical variability in some features but not other features, probably due to the simplicity and complexity of POR-dependent metabolic pathways relevant to each phenotype. (***J Clin Endocrinol Metab* 94: 1723–1731, 2009**)

Cytochrome P450 oxidoreductase (POR) deficiency (PORD) is a rare autosomal recessive disorder caused by mutations in the gene encoding an electron donor for all microsomal P450 enzymes and several non-P450 enzymes (1–4). Salient clinical features of PORD include skeletal dysplasia referred to as Antley-Bixler syndrome (ABS), adrenal dysfunction, 46,XY and 46,XX disorders of sex development (DSD), and maternal virilization during pregnancy (3, 4). Such features are primarily ascribed to impaired activities of POR-dependent CYP51A1 (lanosterol 14α-demethylase) and SQLE

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2008-2816 Received December 29, 2008. Accepted February 24, 2009.
First Published Online March 3, 2009
*Author Affiliations are shown at the bottom of the page 2

Abbreviations: ABS, Antley-Bixler syndrome; CHX, cycloheximide; DSD, disorders of sex development; E_2, estradiol; FISH, fluorescent *in situ* hybridization; hCG, human chorionic gonadotropin; M, metabolite; NMD, nonsense-mediated mRNA decay; PCO, polycystic ovary; POR, cytochrome P450 oxidoreductase; PORD, POR deficiency; 17-OHP, 17α-hydroxyprogesterone; T, testosterone.

<zjs;> • <zjss;1,11>
<zshorttitle>Cytochrome P450 Oxidoreductase Deficiency: Genotype-Phenotype Correlations in Japanese

(squalene monooxigenase) involved in cholesterologenesis and CYP17A1 (17α-hydroxylase and 17,20 lyase), CYP21A2 (21-hydroxylase), and CYP19A1 (aromatase) involved in steroidogenesis (3, 4).

PORD has been identified in multiple patients (4). Mutations are diverse, including missense, nonsense, frameshift, and splice site mutations (4). Notably, however, A287P is the most common mutation in Caucasian patients, and R457H is the most prevalent founder mutation in Japanese patients (1–8). In addition, there is no patient with two apparently null mutations, suggesting that absence of a residual POR activity is incompatible with life (4–6). Clinical features are also variable, with a wide range of expressivity and penetrance. Indeed, ABS-compatible skeletal features and DSD are severely manifested by some patients and apparently absent in other patients (4–6). In addition, adrenal crisis remains relatively rare (4, 6), and maternal virilization is not a consistent feature (5, 6, 9).

To date, however, several critical matters remain to be clarified. First, although about 12% of patients have one apparently normal *POR* allele (4), it is uncertain whether such patients represent manifesting heterozygotes or have hidden aberrations in nonexamined region(s) (4, 10). Second, the underlying factors for the clinical diversity remain to be determined, although variable supporting activities of different POR mutants for target enzymes would have a certain role (5, 11, 12). Third, pubertal development and longitudinal growth have poorly been investigated.

To examine these matters, we analyzed the *POR* gene in affected patients and performed genotype-phenotype correlations in terms of the dosage effect of the R457H mutant.

Patients and Methods

Patients

This study consisted of 35 Japanese patients aged 0.1–23.8 yr (16 patients with 46,XY and 19 patients with 46,XX), including previously reported 23 cases (6, 8, 9) (Table 1). Of the 35 patients, 25 were sporadic cases and the remaining 10 were familial cases from families A–D. Twenty-three sporadic cases and four probands (cases 10, 15, 30, and 35) were ascertained by skeletal features and/or DSD, two sporadic cases (cases 1 and 5) by newborn mass screening for 21-hydroxylase deficiency, and the remaining six cases by familial studies.

Molecular analysis

This study was approved by the Institutional Review Board Committee at National Center for Child Health and Development. The primers used in this study are shown in supplementary Table 1, published as supplemental data on The Endocrine Society's Journals Online Web site at http://jcem.endojournals.org. After taking written informed consent, peripheral blood samples were obtained from all the patients and the parents of 19 sporadic cases and two familial cases (families A and C). Subsequently, genomic DNA samples were subjected to direct sequencing for the *POR* exons 1–16, together with their flanking splice sites. To confirm a heterozygous mutation, the corresponding PCR products were subcloned with a TOPO TA cloning kit (Invitrogen, Carlsbad, CA), and the two alleles were sequenced separately.

When lymphoblastoid cell lines were available, fluorescent *in situ* hybridization (FISH) analysis was performed with two long PCR products spanning exons 4–7 (probe 1) and exons 8–12 (probe 2). The two probes were labeled with digoxigenin and detected by rhodamine anti-digoxigenin. A spectrum green-labeled probe for *D7Z1* (CEP7) (Abbott, Abbott Park, IL) was used as an internal control. For a case with a probable microdeletion, RT-PCR was performed with a variety of primers, to determine the deletion size. Furthermore, to examine the occurrence of transcription failure in cases with apparent heterozygosity and that of the nonsense-mediated mRNA decay (NMD) in cases with premature truncation mutations, the lymphoblastoid cell lines available were incubated for 8 h with and without an NMD inhibitor cycloheximide (CHX; 100 μg/ml; Sigma, St. Louis, MO), and direct sequencing was performed for RT-PCR products (13, 14).

In addition to disease-causing mutations, we also examined the presence or absence of a common A503V variant that has been shown to have a mildly decreased supporting activity at least for CYP17A1 (~60%) (15), to investigate whether the A503V variant can function as a modifier of the clinical phenotype. To examine whether the A503V variant resides on the same allele carrying R457H, PCR products encompassing both the 457th and 503rd codons were subcloned and subjected to direct sequencing.

Clinical assessment

Skeletal features were assessed by bone survey. Adrenal function was evaluated by basal and ACTH-stimulated blood hormone values [250 μg/m^2 (maximum 250 μg) bolus iv; blood sampling at 0 and 60 min] and by urine steroid profiles determined by the gas chromatography/mass spectrometry using first morning urine samples in cases aged older than 6 months (16) (several urine steroid metabolites cannot be measured precisely during the first 6 months of age due to interference of unknown steroids derived from the fetal adrenocortex). DSD was clinically evaluated, as was pubertal development in boys aged older than 14.3 yr (mean +2 SD age for pubic stage 2) and in girls aged older than 12.8 yr (mean +2 SD age for breast stage 2) (17). When possible, basal blood pituitary-gonadal hormone values were also obtained as well as human chorionic gonadotropin (hCG)-stimulated testosterone (T) values (3000 IU/m^2 per dose im for 3 consecutive days; blood sampling on d 1 and 4). In addition, clinical records were surveyed for the data of 17-hydroxyprogesterone (17-OHP) values at the newborn mass screening, adrenal crisis, maternal virilization during pregnancy, polycystic ovary (PCO) in female cases, and body measurement.

Penile length, clitoral size, Tanner stage, testis size, age of menarche, and statural growth were assessed by age- and sex-matched Japanese reference data (17–20), as were hormone values (21–23). Because urine steroid metabolites (Ms) expressed in a logarithm scale grossly followed the normal distribution and showed marked change with age in control

Research Institute (M.F., T.O.) and Hospital (R.H.), National Center for Child Health and Development, Tokyo 157-8535, Japan; Division of Radiology (G.N.) and Endocrinology and Metabolism Unit (Y.H.), Tokyo Metropolitan Kiyose Children's Hospital, Kiyose 204-8567, Japan; Departments of Laboratory Medicine (K.Ho.) and Pediatrics (T.I., T.H.), Keio University Hospital, Tokyo 160-8582, Japan; Department of Pediatrics (T.N.), Dokkyo Medical University Koshigaya Hospital, Koshigaya 343-8555, Japan; Department of Pediatrics and Perinatology (K.Ha.), Tottori University Hospital, Yonago 683-8503, Japan; Division of Endocrinology and Metabolism (A.U.), Shizuoka Children's Hospital, Shizuoka 420-8660, Japan; Department of Pediatrics (C.N.), Yamagata University Hospital, Yamagata 990-9585, Japan; Department of Pediatrics (H.S.), University of Miyazaki Hospital, Miyazaki 889-1692, Japan; Department of Pediatrics (M.Nakac.), Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka 594-1101, Japan; Department of Pediatrics (T.K.), Gunma University Hospital, Maebashi 371-8511, Japan; Division of Pediatrics (K.M.), Nagasaki University Hospital, Nagasaki 852-8102, Japan; Department of Pediatrics and Adolescent Medicine (H.H.), Juntendo University Hospital, Tokyo 113-8421, Japan; Department of Pediatrics, Kagoshima University Hospital (M.Nakam.), Kagoshima 890-8520, Japan; Department of Pediatrics (A.O.), Hamamatsu University Hospital, Hamamatsu 431-3192, Japan; Division of Endocrinology and Metabolism (M.A.), Kanagawa Children's Medical Center, Yokohama 232-8555, Japan; Department of Pediatrics (T.T.), Hokkaido University, Sapporo 060-8638, Japan; and Department of Pediatrics (K.F.), Asahikawa Medical Collage Hospital, Asahikawa 078-8510, Japan