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表1B 非古典21二次調査(詳細抜粋)　　2011.12.14.

表. 本邦の非古典型21水酸化酵素欠損症男性症例の臨床的特徴

症例番号		1	2	3	4	5	6
A 周産期	出生体重(g)	2704	3375	3785	2910	2404	−
	出生身長(cm)	50.0	50.0	−	−	−	−
	在胎週数	40週	39週	−	36週	38週	−
	新生児MS	要再検査	要再検査	要精密検査	要再検査	要再検査	−
B 家族歴	家族歴	なし	あり	なし	あり	あり	あり
C 初診時所見	乳幼児:年齢	2か月	36日	1か月	3歳3か月	27日	5日
	乳幼児:初診時の症状	MSで発見(無症状)	MSで発見(無症状)	MSで発見(無症状)	家族歴で精査(無症状)	MSで発見(無症状)	家族歴(症例5)で精査(無症状)
D 精査時検査所見	精査時の年齢	2か月	36日	1か月	3歳3か月	27日	5か月
	精査時の身長(cm)	58.8	−	−	92.7	−	64.0
	精査時の体重(kg)	6.0	5.3	5.0	13.4	−	8.1
	ACTH(pg/mL)	440.0	72-154	55.9	296.0	57.0	71.0
	17-OHP(ng/mL)	69.3	6.2-37.7	7.0	107.0	13.4	140.0
	17-OHP頂値(ACTH負荷)(ng/mL)	26.5	176.0	95.8	237.0	119.0	−
	コルチゾール頂値(ACTH負荷)(μg/dL)	16.3	−	−	18.4	25.5	−
	ARC(pg/mL)	197.0	−	−	−	−	−
	PRA(ng/mL/hr)	−	6.0-14.8	11.8	11.2	28.5	16.8
	尿プレグナントリオロン(mg/gCr)	11.0	10.0	−	−	−	−
	*CYP21A2*遺伝子:変異型	P30L/IVS2-13A>C	P30L/Gly110del8nt	P30L/IVS2-13A>G	P30L/I172M,R356W	R356W/del	R356W/del
D 治療	未治療/治療歴あり	治療歴あり	7歳まで未治療	6歳まで未治療	治療歴あり	治療歴あり	治療歴あり
	治療開始時:年齢	2歳4か月	−	−	8歳2か月	2歳10か月	6か月
	治療開始時:身長(cm)	89.3	−	−	126.1	89.2	68.2
	治療開始時:体重(kg)	12.9	−	−	24.5	12.7	8.9
	ヒドロコルチゾン(mg/日)	3	−	−	18	4	3
	フルドロコルチゾン(mg/日)	−	−	−	−	−	0.03
	治療を開始した理由	骨年齢促進	−	−	骨年齢促進,成長率増加	ACTH負荷後のコルチゾール頂値の低値	−

MS:マススクリーニング

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

本邦における非古典型21水酸化酵素欠損症の実態把握と診断・治療指針の作成

研究分担者　田島　敏広（北海道大学小児科　講師）

研究要旨

古典型の頻度は世界的にほぼ同一であるが、非古典型の頻度は本邦で不明である。そこで、非古典型の頻度を明らかにする目的で、札幌市での27年間の新生児マススクリーニングで発見された非古典型を検討した。その結果NC型の頻度は約48万に一人であり、欧米より稀である可能性が示唆された。

共同研究者

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石井智弘（慶応大学　小児科）
横田一郎（国立病院機構香川小児病院　内分泌科）

A. 研究目的

日本での21-OHDの非古典型(NC)の頻度は欧米に比較し、現在まで明らかではない。また新生児スクリーニングでNCの一部が発見されるとする報告がある。そこで今回、2010年末までの、札幌市と新潟県でのマススクリーニングを受けた新生児について追跡し、陽性者におけるNC型の存在について検討を行った。

B. 研究方法

2010年末まで27年間において札幌市マススクリーニング検査を受けた新生児の中で21-OHDと診断した患者、2010年末まで20年間において新潟県マススクリーニング検査新生児の中で、21-OHDと診断した患者について、その病型を、血清ナトリウム値、糖質コルチコイド/鉱質コルチコイド治療の有無によって検討した。

糖質コルチコイド治療に加え、血清ナトリウムの低下を認めた症例、鉱質コルチコイド治療、食塩投与が必要であった患者は塩喪失型(SW)、糖質コルチコイドのみの治療を行っている患者は単純男性型(SV)、NC型はスクリーニングで発見後、無治療で経過観察、その後男性化徴候出現のため、糖質コルチコイド投与を行った症例あるいは無治療で経過観察している症例とした。

（倫理面への配慮）

なし。

C. 研究結果

札幌市でのマススクリーニング27年間（1982.5.1～2010.3.31）の1次検査数は498,147人で精査対象者は146人（0.029%）だった。そのうち21-OHDは26人で、罹患率は1/19,159人であった。初回検査での精査対象者は24人、残り2人は再採血での精査対象者であった。重症度分類では、SW19人,SV6人,NC1人であった。新潟県マススクリーニング20年間（1989.4.1～2009.3.31）の1次検査数は478,337人で精査対象者は242人(0.05%)だった。そのうち21-OHDは26人で、罹患率は1/18,398人であった。初回検査での精査対象者は20人、残り6人は再採血での精査対象者であった。重症度分類では、SW18人,SV7人,NC1人であった。したがってこれらの結果からNSでみつかる本症の発症頻度は約48万に一人である。

札幌市の症例は17-OHP高値で見つかった女児であるが、外性器異常なく、体重増加良好、血清電解質は正常であった。生後1ケ月で行ったACTH負荷試験では17-OHPの基礎値(2.6 ng/ml)、負荷後の値(86.2 ng/ml)の上昇を認めた。Newらは21-水酸化酵素欠損症の血清17-OHPの反応について病型毎に、その基準を策定しているが、この患者の反応はＮＣ型のそれと一致した。その後も17-OHPは軽度高値(2~5 ng/ml)が続いている。現在10歳であるが、成長促進、男性化などの症状を認めていない。10歳時の尿中ステロイ

ド分析においても、スポット尿で尿中プレグナントリオール 4.434 mg/g creatinine (正常範囲　0.052~0.133 mg/g creatinine)と上昇を示している。

新潟県の症例は、17-OHP 高値でみつかった女児であるが、外性器異常などの他の症状は認めなかった。45 生日の血清 17-OHP 値は 110 ng/ml と異常高値であり、21-OHD NC 型と診断した。維持量の糖質コルチコイド補充を生後 2 ヶ月から 3 歳まで行った。現在 4 歳で、無治療で経過観察中であるが、成長促進、男性化などの症状を認めていないが、血清 17-OHP 軽度高値は続いている。

D. 考察

NC 型のスクリーニングでの発見例は過去に外国、日本で報告されている。日本では正確な NC 型の頻度は不明である。スクリーニングで見つかる NC 型の日本での頻度は百万に 1 人とする報告がある。今回の検討ではその頻度の約 2 倍であった。札幌市と新潟県という地域性、あるいは全例追跡調査が確実に行われた点の違いによる可能性もある。また過去の日本の報告では日本の NC 型には CYP21A2 遺伝子の P30L の変異が多いとされている。さらに本疾患では遺伝型と表現型が相関することが知られている。今後 NC 型の遺伝型の検討が必要である。

E. 結論

本邦において新生児スクリーニングで発見される NC 型の頻度は約 48 万に一人であった。

F. 健康危険情報

なし

G. 研究発表

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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

本邦における非古典型 21 水酸化酵素欠損症の実態把握と診断・治療指針の作成

研究分担者　横田　一郎（国立病院機構香川小児病院臨床研究部長）

研究要旨

非古典型 21 水酸化酵素欠損症女児の実態及び診断時臨床所見の特徴を明らかにする目的で、本邦及び海外における報告例の検討を行った。診断年齢が若い例では早発恥毛や思春期早発、思春期以降は多毛や生理不順、不妊が診断契機となっている例が多かった。少数であるが副腎不全を発症する例も見られた。本症の適切な診断・治療は特に女児患者の場合その QOL 向上のために重要と考えられた、

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田島敏広（北海道大学　小児科）

A. 研究目的

21 水酸化酵素欠損症は糖質コルチコイド、鉱質コルチコイドの欠乏だけでなく、副腎性アンドロゲンの過剰をきたすため、特に女児患者においては不妊や多毛などの原因となり、QOL を損ねることが報告されている。非古典型女児においては特にこのような点が主訴となり診断に至っていると考えられる。本症の早期診断・治療の方策を検討するために、非古典型女児の報告例より診断のきっかけや臨床症状の特徴を解析した。

B. 研究方法

非古典型 21 水酸化酵素欠損症の女性例の報告について、文献データベースを用いて検索し、臨床症状について記載のある文献を抽出した。症状の発現年齢や診断年齢による臨床症状の違い等について検討した。

（倫理面への配慮）

本研究は臨床症状についての過去の症例報告及び研究報告を検索し、まとめたものであり、個人情報等倫理面での問題は生じないと考える。

C. 研究結果

本邦における報告

① 新生児マス・スクリーニングで軽度 17(OH)P の上昇が認められ、遺伝子検査で非古典型と診断された女児３例（診断時１～３ヶ月）では、外性器異常や色素沈着などの異常は認められなかった。[1)]

② １３歳より多毛・無月経が出現した例では、２５歳時には陰核肥大も認めていた。[2)]

③ １４歳より多毛、陰核肥大を自覚しており、１５歳で初経を認めるも生理不順があった例では、２４歳時に不妊のため産婦人科受診し、テストステロン高値を指摘され、診断に至っている。[3)]

④ CT で副腎腫大を指摘されて診断に至った７２歳女性では、外性器異常を認めないが、身長の伸びは１２歳で停止していた。[4)]

⑤ 甲状腺機能亢進症の治療のため入院中に副腎不全症状を呈した例（75 歳女性）では、外性器異常などの所見は認めなかった。[5)]

海外における報告

① 238 人の早発恥毛を認めたフランス女児において、10 人で 17(OH)P の上昇等を認め、遺伝子解析の結果全例が非古典型 21 水酸化酵素欠損症と診断された。[6)]

② ギリシャにおいては、同じく４８人の早発 adrenarche の女児において、４例が非古典型 21 水酸化酵素欠損症と遺伝子解析で診断された。[7)]

③ 米国における多施設共同研究（２２０人）では、10 歳未満の患者２５例の内２３例に思春期早発を認

め陰核肥大、面皰を認めたのは５例だった。１０歳以上の患者では多毛を 59%、稀発月経を 54%、面皰を 33%、不妊を 13%、陰核肥大を 10%、禿頭を 8%、１次性無月経を 4%に認めた。多毛は年齢と共に増悪していた。[8)]

④　フランスからの１６１人（１３-５２歳）の非古典型 21 水酸化酵素欠損症女性の検討では、多毛を 78%、生理不順を 54.7%に、不妊を 12%に認めた。問診では、５人が小児期に陰核肥大を自覚しており、１人は外科的修復を行っていた。１７人は思春期早発を認めた。２人に急性副腎不全を認めていた。[9)]

⑤　米国からの報告では、本症女性患者の約半数に多嚢胞性卵胞を認めたとされている。[10)]

⑥　妊娠を望んだ非古典型の患者９５人のうち８５人（１８７妊娠）が妊娠し、内９９妊娠は診断前（無治療）の状態であった。しかしながら、流産率は無治療群、治療群で 28.3%と 6.5%であり、無治療の場合流産のリスクが高まることが考えられた。[11)]

D．考察

本邦報告例、海外報告例を総合すると、乳児期にマス・スクリーニングを契機に診断に至った例では最初目立った症状を認めていない。従って、マス・スクリーニングで陽性とならない場合乳幼児期には診断されない例が多いと考えられる。最も早期の診断のきっかけになる症状は早発恥毛や思春期早発と考えられ、陰核肥大などの外性器異常を伴っていない例が多いことから、本症の可能性も考慮して診断を行う重要性が示唆された。思春期年齢になると、多毛、生理不順、面皰などの症状が目立つようになるが、これらの症状の場合、必ずしも（小児）内分泌科医の診療を受けるとは考えにくく、診断に至っていない場合も多いことが考えられた。従って、非古典型 21 水酸化酵素欠損症という疾患概念を幅広く認識してもらい、適切に診断されるような体制を構築することは、本症患者が未診断による不利益を被らないためには重要と考えられた。また、陰核肥大などの症状がない例でも、急性副腎不全症状を呈した例が報告されている。本症男児においても同様の例は報告されており、非古典型においても、診断後は急性副腎不全の可能性を念頭においてフォローアップしていく必要があると考えられた。

E．結論

非古典型 21 水酸化酵素欠損症女児では、診断・治療されないことによる不利益は大きい。非古典型の存在を正しく認識し、適切に診断され、臨床症状の発現、増悪を起こさないようにすることは、本症女児の QOL の向上に大きく寄与することが期待できる。

F．健康危険情報

特になし。

G．研究発表

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H. 知的財産権の出願・登録状況

とくになし

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Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル	発表誌名 巻号 ページ	出版年
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Ⅳ. 研究成果の刊行物・別刷

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Original Article

Stress Doses of Glucocorticoids Cannot Prevent Progression of All Adrenal Crises

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Abstract. Adrenal crises (ACs) sometimes progress rapidly and can be fatal. The aims of the present study were to reveal whether stress doses of glucocorticoids (SDGs) can prevent progression of severe ACs and to suggest a method of prevention, through analysis of its clinical features. We studied 24 severe ACs (nine patients) that occurred after diagnosis of primary or secondary adrenal insufficiency, retrospectively. The following information was analyzed: 1) whether SDGs were given orally and/or sc; 2) duration from the time when some symptoms started to the time when the patient came to the hospital; and 3) presence of hypoglycemia and electrolyte disturbance (hyponatremia, hyperkalemia). Eleven crises occurred after taking SDGs. Ten crises progressed within 3 h. Six of these ten crises progressed to severe ACs despite the fact that the patients took SDGs. Six crises were observed in association with hypoglycemia, and five of these six crises occurred in patients under 5 yr of age. Three of the six crises in association with hypoglycemia progressed to ACs within 3 h. Two of the three crises progressed to severe status within 3 h despite the fact that the patients took SDGs. Electrolyte disturbance was observed in only one crisis. In conclusion, SDGs cannot prevent progression of all ACs. Progression can be associated with hypoglycemia, particularly in patients under 5 yr of age. Patients should be given guidance on an ongoing basis on how to prevent ACs and hypoglycemia.

Key words: adrenal crisis, stress doses of glucocorticoids, hypoglycemia, electrolyte disturbance, young children

Introduction

In patients with primary or secondary adrenal insufficiency, adrenal crises (ACs) may develop when they are subjected to stress, such as infection. Therefore, these patients require stress doses of glucocorticoids (SDGs).

As ACs can be fatal (1–3), it is pivotal that they be prevented and managed at home. Understanding the clinical features of severe ACs is helpful in their prevention. However, a search of Pubmed revealed no studies available in regard to clinical features in which a large number of cases of severe ACs were analyzed. In addition, there were also no studies that investigated the usefulness of SDGs, except for case reports asserting that SDGs failed to prevent

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hypoglycemia (4, 5).

Therefore, the aims of the present study were 1) to reveal whether SDGs can prevent progression of severe ACs and 2) to suggest a method of prevention, through analysis of their clinical features.

Subjects

This retrospective study was conducted using patients experiencing severe ACs after diagnosis of adrenal insufficiency (explained below).

The original cohort was eleven patients with primary or secondary adrenal insufficiency who were treated due to ACs at our hospital from January 1985 to June 2005. The total number of ACs was 49. Glucocorticoid replacement therapy using hydrocortisone (cortisol) was used for all patients. Mineralocorticoid replacement was also prescribed to patients with primary adrenal insufficiency. The daily doses of hydrocortisone were 13.1–18.8 and 1.6–15 mg/m^2/day in primary and secondary adrenal insufficiency, respectively.

The inclusion criteria of the present study were as follows; 1) patients and their parents were already instructed on how to take SDG (hydrocortisone orally at 80–100 mg/m^2/day divided into four doses per day, and a 20–25 mg/m^2/dose sc injection); 2) the crises occurred outside of the hospital; and 3) the symptoms satisfied the following definition of severe AC on arrival, so that patients who needed treatment for severe ACs could be selected. They must have experienced at least one of the following symptoms, unconsciousness, convulsion or hypotension (blood pressure was under 60 mmHg or pulse could not be palpated), or at least one symptoms indicating circulatory failure (pale facial color, weak pulsation or cold extremities) with definitive causes of adrenal crises (traffic accident, infection with CRP above 10 mg/dl or torsion of an ovarian cyst).

Consequently, 24 crises in nine patients were evaluated in the present study (Table 1). The median age at crisis was 4 yr and 0 mo of age (7 mo to 24 yr of age). As severe ACs in association with hypoglycemia were examined in the present study, patients with hypopituitarism who did not start GH therapy were excluded.

Methods

The following information were analyzed: 1) whether SDGs were given orally and/or sc before arrival to the hospital; 2) 'duration before arrival', which was the duration from the time some symptoms started that could be related to AC (for example, vomiting or fever) to the time when the patient came to hospital; and 3) presence of hypoglycemia and electrolyte disturbance (hyponatremia and hyperkalemia). In the present study, a serum glucose concentration of less than 40 mg/dl was defined as hypoglycemia, a serum Na concentration of less than 130 mEq/L was defined as hyponatremia and a serum K concentration of over 5.5 mEq/L was defined as hyperkalemia.

Results

SDGs at home

Eleven severe ACs (four patients; 7 mo to 21 yr of age) occurred even though SDGs were given at home (orally and/or sc; Table 1). Among these eleven crises, SDGs were given orally in seven crises and were given sc in six crises. Another eleven crises occurred in patients (seven patients; 1 to 24 yr of age) who came to the hospital without administration of glucocorticoid. In at least two of the latter eleven crises, the ACs progressed so rapidly that the patients had no time to take SDGs. No information was available for the remaining two crises.

Duration before arrival

Ten (five patients; 8 mo to 24 yr of age) of the 24 crises progressed rapidly within 3 h (Table 1). Six (four patients; 8 mo to 21 yr of age) of

Table 1 Details of the severe ACs

Patient	Diagnosis	Age at AC	SDGs	Duration before arrival	Glucose (mg/dl)	Na (mEq/L)	K (mEq/L)
Primary adrenal insufficiency							
1	CYP21A2 deficiency	11 mo	Orally	C	109	118	5.8
		4 yr	(–)	B	78	147	3.7
2	CYP21A2 deficiency	1 yr	Orally	C	45	134	4.8
3	CYP21A2 deficiency	1 yr	(–)	A	4	139	4.7
4	StAR mutation	16 yr	Orally	C	99	137	3.7
		18 yr	(–)	B	92	141	3.5
		19 yr	(–)	A	91	141	3.4
		21 yr	Orally and sc	A	67	144	3.6
		21 yr	Sc	A	76	140	3.8
Secondary adrenal insufficiency							
5	Septo-optic dysplasia	7 mo	Orally	B	80	146	4.9
		8 mo	Orally and sc	A	100	149	4.8
		1 yr	Sc	A	26	138	3.6
		2 yr	Sc	A	35	140	3.9
		2 yr	Sc	C	20	139	4.3
6	Septo-optic dysplasia	3 yr	(–)	A	93	137	3.5
		3 yr	Orally	A	96	141	4.1
7	Hypopituitarism*	3 yr	(–)	B	67	133	4.5
		4 yr	(–)	B	8	141	3.4
		8 yr	(–)	B	<5	136	5.1
8	Hypopituitarism*	13 yr	(–)	C	125	135	3.4
9	Hypopituitarism*	18 yr	N.A.	C	83	137	3.9
		18 yr	N.A.	C	75	130	3.8
		20 yr	(–)	B	105	131	3.4
		24 yr	(–)	A	105	142	2.9

Hypopituitarism*: Hypopituitarism with invisible pituitary stalk on MRI. N.A.: not available. Duration before arrival: A, <3 h; B, 3–24 h; C, 24–72 h. Severe ACs did not always occur repeatedly in the same patients. However, when there were specific causes of the crisis (for example, an ovarian cyst in patient 4 and usual appetite loss in patient 5), severe ACs could occur repeatedly.

these ten crises progressed to severe ACs within 3 h, even though SDGs were administered (Table 2). Duration before arrival was from 3 to 24 h in seven ACs (five patients) and was from 24 to 72 h in another seven crises (six patients; Table 1).

Hypoglycemia and electrolyte disturbances

Hypoglycemia: Six crises (three patients) in association with hypoglycemia (<5–35 mg/dl) were observed in the morning after overnight fast (Table 1). Unconsciousness was observed in one crisis, and convulsions were observed in the other five. Five of these six crises were observed in patients with secondary adrenal insufficiency, and the other one was observed in a patient with primary adrenal insufficiency.

In the above six crises with hypoglycemia, five crises (three patients) occurred in patients under 5 yr of age (1 to 4 yr of age; 13 of the 24 crises occurred in patients under 5 yr of age). The remaining crisis occurred in an 8-yr-old maltreated boy with hypopituitarism (Patient 7 in Table1). This patient died due to this crisis.

Table 2 Stress doses of glucocorticoids (SDGs)

	SDGs (+)	SDGs (–)	Total
Duration before arrival < 3 h[#1]	6	4	10
Hypoglycemia[#2]	3	3	6
Hypoglycemia + duration before arrival < 3 h[#3]	2	1	3

[#1] Ten crises progressed rapidly within 3 h. Six out of these ten crises progressed within 3 h despite SDGs. [#2] Six crises with hypoglycemia were observed. Hypoglycemia was observed in three patients despite SDGs. [#3] Duration before arrival was within 3 h in three of the six crises with hypoglycemia. Two of the three crises progressed to severe status within 3 h despite SDGs.

He had severe mental retardation due to frequent episodes of hypoglycemia.

Hypoglycemia was observed in three crises (one patient) even though the patient came to the hospital after sc injections of SDGs (Table 2). Duration before arrival was within 3 h in three of the six crises with hypoglycemia (Table 1). Two of the three crises progressed to severe status within 3 h despite SDGs (Table 2).

Electrolyte disturbance: In secondary adrenal insufficiency, no abnormalities in the serum Na or K concentrations were observed. Even in primary adrenal insufficiency, ACs progressed to severe states without electrolyte disturbance, with the exception of one crisis. This crisis occurred in an 11-mo-old girl with CYP21A2 deficiency (Na 118 mEq/L, K 5.8 mEq/L) despite SDGs (Patient 1 in Table 1).

Discussion

SDGs could not prevent progression of all severe ACs. Rapid progression and infantile hypoglycemia (under 5 yr of age) were observed despite SDGs. One possible reason why the SDGs could not prevent progression of all ACs is that the dose of glucocorticoids may be insufficient. This should be studied in the near future.

In regard to prevention of severe ACs, it is important 1) to take SDGs (the earlier, the better), 2) to come to the hospital if no improvement is seen after taking SDGs, and 3) to control hypoglycemia in young children. To make these possible, we first need to instruct patients and/or their patients on when to take SDGs (orally and sc) on an ongoing basis. To enable sc administration as early as possible, we should ensure that the patients most likely to have severe ACs, such as young children less than 5 yr of age, or their parents have a syringe of hydrocortisone. Furthermore, the patients should come to the hospital quickly, if they do not improve after taking SDGs. Finally, control of hypoglycemia is necessary to prevent severe ACs in young children less than 5 yr of age. There have been several reports of children with primary or secondary adrenal insufficiency presenting severe ACs with hypoglycemia (4–12), and some of these crises resulted in death (6, 10–12). We recommend measurement of the patient's glucose level at home, especially in the morning (12), in the same manner as is now widely performed in patients with type1 diabetes mellitus. In patients under stress, it is advisable to supply them with sugar-rich foods or drinks with SDGs, especially young children. This importance of prevention of hypoglycemia in AC has previously been indicated in case reports concerning congenital adrenal hyperplasia in the 1970s and 1980s (4, 5).

In conclusion, SDGs cannot prevent

progression of all ACs. Progression can be associated with hypoglycemia, particularly in patients under 5 yr of age. Patients should be given guidance on an ongoing basis on how to prevent ACs and hypoglycemia.

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

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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

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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.

(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

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TABLE 1. Summary of molecular analyses

Patients				*POR* mutations	
Case	Karyotype	Age (yr)	Inheritance	Nucleotide changes[a]	Aminoacid changes
Group A: homozygotes for R457H					
1	46,XY	5.0	Sporadic	1370G>A/1370G>A	R457H/R457H
2	46,XY	23.8	Familial-A	1370G>A/1370G>A	R457H/R457H
3	46,XY	22.6	Familial-A	1370G>A/1370G>A	R457H/R457H
4	46,XY	6.7	Sporadic	1370G>A/1370G>A	R457H/R457H
5	46,XY	0.4	Sporadic	1370G>A/1370G>A	R457H/R457H
6	46,XX	0.4	Sporadic	1370G>A/1370G>A	R457H/R457H
7	46,XX	0.4	Sporadic	1370G>A/1370G>A	R457H/R457H
8	46,XX	2.0	Sporadic	1370G>A/1370G>A	R457H/R457H
9	46,XX	14.1	Sporadic	1370G>A/1370G>A	R457H/R457H
10	46,XX	15.0	Familial-A (P)	1370G>A/1370G>A	R457H/R457H
11	46,XX	3.0	Sporadic	1370G>A/1370G>A	R457H/R457H
12	46,XX	0.2	Sporadic	1370G>A/1370G>A	R457H/R457H
13	46,XX	0.1	Sporadic	1370G>A/1370G>A	R457H/R457H
14	46,XX	18.0	Sporadic	1370G>A/1370G>A	R457H/R457H
Group B: compound heterozygotes for R457H and an apparently null mutation					
15	46,XY	16.8	Familial-B (P)	1370G>A/601C>T	R457H/Q201X
16	46,XY	15.7	Familial-B	1370G>A/601C>T	R457H/Q201X
17	46,XY	14.8	Sporadic	1370G>A/1329-1330insC	R457H/I444fsX449
18	46,XY	17.5	Sporadic	1370G>A/(15A>G)	R457H/Non-transcribed (G5G)[b]
19	46,XY	2.1	Sporadic	1370G>A/143delG	R457H/R48fsX63
20	46,XY	0.2	Sporadic	1370G>A/1665delG	R457H/Q555fsX612
21	46,XY	13.1	Sporadic	1370G>A/(−)[c]	R457H/DeltaExons 2–13[d]
22	46,XX	9.0	Sporadic	1370G>A/IVS7+1G>A	R457H/IVS7+1G>A
23	46,XX	14.8	Sporadic	1370G>A/1698-1699insC	R457H/Y567fsX574
24	46,XX	13.2	Sporadic	1370G>A/1329-1330insC	R457H/I444fsX449
25	46,XX	12.9	Familial-B	1370G>A/601C>T	R457H/Q201X
26	46,XX	6.6	Sporadic	1370G>A/(−)[c]	R457H/Non-transcribed[b]
27	46,XX	4.2	Sporadic	1370G>A/(−)[c]	R457H/Non-transcribed[b]
28	46,XX	17.0	Sporadic	1370G>A/1329-1330insC	R457H/I444fsX449
Group C: other compound heterozygotes					
29	46,XY	0.4	Sporadic	1370G>A/1386-1387insATCGCC	R457H/A462-S463insIA
30	46,XY	23.5	Familial-C (P)	1370G>A/1835-1858del[e]	R457H/L612-W620delinsR
31	46,XY	18.0	Familial-C	1370G>A/1835-1858del[e]	R457H/L612-W620delinsR
32	46,XY	17.9	Familial-D	1733A>G/1329-1330insC	Y578C/I444fsX449
33	46,XX	0.8	Sporadic	1370G>A/1738G>C	R457H/E580Q
34	46,XX	0.7	Sporadic	1370G>A/1042-1044delGTC	R457H/348delV
35	46,XX	0.5	Familial-D (P)	1733A>G/1329-1330insC	Y578C/I444fsX449

The genomic position corresponding to each mutation based on NC_000007.12 sequence at the National Center for Biotechnology Information database (Bethesda, MD) is as follows: R457H, 75452433G>A; Q201X, 75448386C>T; I444fsX449, 75452391-2insC; G5G, 75421261A>G; R48fsX63, 75421389delG; Q555fsX612, 75453099delG; IVS7 + 1G>A, 75448861G>A; Y567fsX574, 75453205-6insC; A462-S463insIA, 75452349-50insATCGCC; L612-W620delinsR, 75453432-55delTAAAGCAAGACCGAGAGCACCTGT; Y578C, 75453237A>G; E580Q, 75453245G>C; and 348delV, 75451086-88delGTC. Cases 1–3, 6–10, 15–18, 22–26, 29–33, and 35 have been reported previously (6, 8, 9), and the remaining 12 cases were first examined in this study. P, Proband.

[a] The A of the ATG encoding the initiator methionine residue of the predicted translation product is denoted position +1.

[b] The allele with G5G and the apparently normal alleles are not trasncribed into mRNA.

[c] The (−) symbol indicates the absence of a recognizable mutation on the exonic sequences.

[d] An intragenic microdeletion involving exons 2–13.

[e] 1835-1858delTAAAGCAAGACCGAGAGCACCTGT.

subjects of both sexes (854 males and 909 females), the M data of the patients were expressed as the SD score to allow for the comparison among patients of different sexes and ages.

Statistical analysis

Statistical significance of the frequency of clinical features was analyzed by the Fisher's exact probability test, and that of the median of nonpaired and paired variables was examined by the Mann-Whitney's *U* test and the Wilcoxon signed-rank test, respectively. $P < 0.05$ was considered significant.

Results

POR mutations

The results are summarized in Table 1. Direct sequencing revealed 12 types of mutations and one silent substitution (G5G) (Fig. 1A), with R457H being identified in 40 of the 58 alleles (~70%) in 25 sporadic cases and four probands of families A–D. Of the 12 mutations, R48fsX63, Q555fsX612, and 348delV were first identified in this study. These mutations were absent in 100 control subjects.

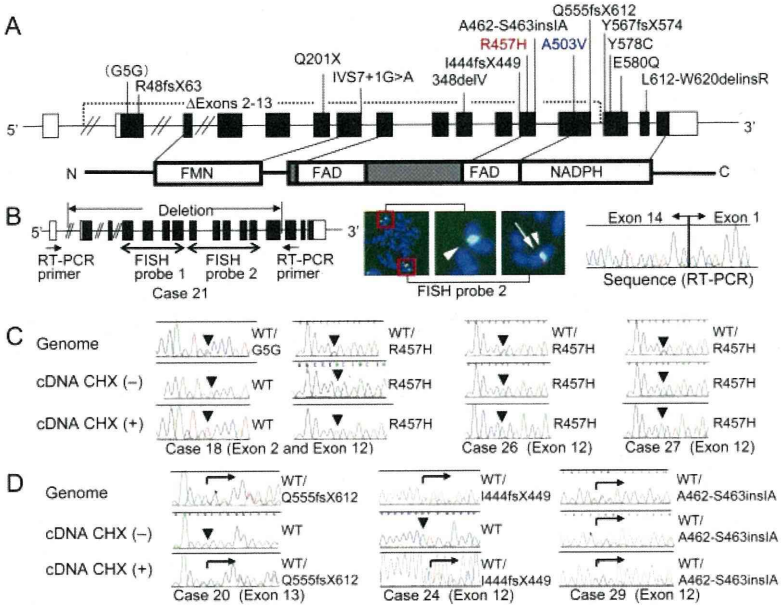

FIG. 1. Mutation analysis of *POR*. A, Schematic representation of the *POR* gene and the positions of identified mutations. The Japanese founder mutation R457H is shown in *red*, other disease-causing mutations in *black*, and the common A503V variant in *blue*. *Upper diagram*, The genomic structure comprising 16 exons. The *black* and *white boxes* denote the coding and the untranslated regions, respectively. *Lower diagram*, The protein structure consisting of the cofactor binding domains (FMN: flavin mononucleotide; FAD: flavin-adenine dinucleotide; and NADPH: nicotinamide-adenine dinucleotide phosphate, reduced) and the connecting domain (*stippled area*). B, FISH and RT-PCR sequencing analyses in case 21. *Left diagram*, The positions of the two FISH probes and those of the primers for RT-PCR. *Middle diagram*, FISH findings showing two signals for *D7Z1* (*arrowheads*) and a single signal for *POR* (*arrow*) delineated by the FISH probe 2. *Right diagram*, RT-PCR sequencing indicating the fusion between exons 1 and 14 (the deletion of exons 2–13). C, Transcription failure in cases 18, 26, and 27. Although heterozygosity for R457H is delineated for the genomic DNA, RT-PCR sequencing indicates absent expression of the wild-type (WT) alleles in the three cases. Similarly, although heterozygosity for G5G is shown for the genomic DNA of case 18, RT-PCR sequencing reveals no expression of the G5G allele. Such lack of transcripts is not recovered by CHX. D, Nonsense-mediated mRNA decay in cases 20 and 24 but not case 29. Although heterozygosity for the mutations is shown for the genomic DNA, RT-PCR sequencing delineates the WT alleles only before CHX treatment and the heterozygosity after CHX treatment in cases 20 and 24. The NMD is not observed in case 29.

Fifteen cases were apparently homozygous for R457H, and hemizygosity was excluded in 14 of the 15 cases by parental analysis indicating heterozygosity for R457H in both parents (cases 1–3, 6–11, and 13) and by FISH analysis with two FISH probes (cases 4, 5, 12, and 14). Notably, however, FISH analysis delineated a heterozygous microdeletion in case 21, and RT-PCR sequencing analysis revealed loss of exons 2–13 in this case (Fig. 1B). The mother was heterozygous for R457H, and the father was heterozygous for the intragenic microdeletion.

Three cases were apparently heterozygous for R457H (cases 18, 26, and 27), although case 18 also had G5G. However, RT-PCR sequencing analysis using lymphoblastoid cell lines showed nearly complete absence of mRNA derived from the apparently normal alleles in the three cases (Fig. 1C). The mRNA remained undetected after CHX treatment, indicating transcription failure.

Of the 11 other types of mutations, the nonsense and four frameshift mutations (Q201X, R48fsX63, I444fsX449, Q555fsX612, and Y567fsX574) leading to premature termination and the conserved splice donor site mutation (IVS7+1G>A) appeared to be null mutations, whereas the remaining five mutations (Y578C, E580Q, 348delV, A462-S463insIA, and L612-W620delinsR) were unknown for residual activities. Indeed, RT-PCR sequencing analysis performed before and after CHX treatment in three cases with available lymphoblastoid cell lines demonstrated that the alleles carrying Q555fsX612 and I444fsX449 underwent NMD, whereas the allele harboring A462-S463insIA escaped NMD (Fig. 1D).

The common A503V variant was absent from cases of group A and was identified in four cases of group B (cases 22, 23, 26, and 27) and four cases of group C (cases 29–31, and 34). The eight cases with A503V were all compound heterozygotes with R457H and another mutation, and direct sequencing for subcloned PCR products encompassing both 457th and 503rd codons revealed lack of coexistence of R457H and A503V. Thus, it was indicated that the A503V variant was absent from all of the 47 alleles carrying R457H and was present on alleles carrying IVS7+1G>A, Y567fsX574, A462-S463insIA, L612-W620delinsR, and 348delV and on the two nontranscribed alleles.

Classification of the patients

On the basis of the above results, the 35 cases were classified into three groups: group A, homozygotes for R457H (cases 1–14); group B, compound heterozygotes for R457H and one apparently null mutation (cases 15–28); and group C, other types of compound heterozygotes (cases 29–35) (Table 1). The residual POR activity was predicted to be higher in group A than group B, although it was unknown for group C. In addition, group B was subclassified into A503V-positive cases (cases 22, 23, 26, and 27) and negative cases (cases 15–21, 24, 25, and 28).

Clinical features

The prevalence of each clinical feature in groups A–C is summarized in Table 2, together with its comparison between groups A and B. The sex ratio was similar between groups A and B, as was the median age.

ABS-compatible skeletal features were definitely more prevalent in group B than group A (Table 2 and supplementary Fig. 1, published as supplemental data on The Endocrine Society's Journals Online Web site at http://jcem.endojournals.org). In particular, severe brachycephaly, elbow joint synostosis, and choanal stenosis were exclusively identified in group B.

Adrenal steroidogenic dysfunction was biochemically identified in all cases, with some difference between groups A and B. Blood ACTH was normal or elevated at the baseline, 17-OHP was normal or elevated at the baseline and above the normal range after ACTH stimulation, and cortisol was normal at the baseline but barely responded to ACTH stimulation (Fig. 2A). Significant difference between groups A and B was identified for basal 17-OHP value ($P = 0.044$) and basal and ACTH-stimulated cortisol values ($P = 0.018$ and $P = 0.022$). Urine Ms of progesterone and 17-OHP were elevated, whereas those of an-

TABLE 2. The prevalence of each clinical feature in groups A–C and its comparison between groups A and B

	Group A (n = 14)	Group B (n = 14)	Group C (n = 7)	Groups A *vs.* B (*P* value)
Sex (male:female)	5:9	7:7	4:3	0.35
Age (median, range, yr)	4.0 (0.1–23.8)	13.1 (0.2–17.5)	0.8 (0.4–23.5)	0.19
Skeletal features				
Any skeletal feature	7/14	14/14	7/7	0.0029
Brachycephaly (overt)	0/14	14/14	6/7[a]	0.000000025
Elbow joint synostosis[b]	0/14	7/14	4/7	0.0029
Arachnodactyly (overt)	5/14	14/14	7/7	0.048
Choanal stenosis	0/14	5/14	1/7	0.020
Joint contracture	7/14	14/14	7/7	0.0029
Adrenal dysfunction				
Adrenal crisis	0/14	4/14	1/7[c]	0.049
Detection by mass screening[d]	5/8	3/8	2/4	0.31
46,XY DSD				
Any genital feature at birth	1/5[e]	3/7[f]	3/4	0.42
Hypospadias	0/5	2/7	1/4	0.32
Cryptorchidism	0/5	3/7	2/4	0.16
Micropenis	1/5	2/7	3/4	0.64
46,XX DSD				
Any genital feature at birth	9/9[e]	7/7[f]	3/3	1.0
Clitoromegaly	8/9	5/7	3/3	0.40
Labial fusion	8/9	5/7	2/3	0.40
Common urogenital sinus	2/9	2/7	0/3	0.61
Maternal virilization	8/14	5/14	4/7	0.22
Pubertal failure, 46,XY				
Delayed (>2 SD) or no pubertal sign	0/2[g]	3/4[h]	2/3	0.20
Small testis (<2 SD)	0/2	2/4	1/3	0.40
Primary hypogonadism[i]	0/2	2/2	3/3	0.17
Pubertal failure, 46,XX				
Delayed (>2 SD) or no pubertal sign	3/3[g]	4/4[h]		1.0
Delayed (>2 SD) or no menses	0/2[j]	2/2		0.17
Primary hypogonadism[i]	3/3	3/3		1.0
Polycystic ovary	4/9	3/6	1/3	0.62

The denominators indicate the number of patients examined for the presence or absence of each feature, and the numerators represent the number of patients assessed to be positive for that feature; thus, the differences between the denominators and numerators denote the number of patients evaluated to be negative for that feature.

[a] Severe craniosynostosis is absent in case 33 with two missense mutations.

[b] Humeroradial, humeroulnar, or radioulnar synostosis.

[c] Adrenal crisis has been manifested by case 35 with Y578C and I444fsX449.

[d] The measurement of 17-OHP in the mass screening for 21-hydroxylase deficiency has been performed since 1988 in Japan.

[e,f] DSD is more frequent in 46,XX cases than 46,XY cases in groups A ($P = 0.0050$) and B ($P = 0.035$).

[g,h] The *P* values between 46,XY and 46,XX cases are 0.19 for group A and 0.50 for group B.

[i] Elevated gonadotropins (LH and/or FSH) and/or decreased T or E_2, as compared with age- and sex-matched reference data.

[j] Only a few vaginal spottings.

drostenedione, 11-deoxycortisol, cortisol, and aldosterone grossly remained within the normal range (Fig. 2B). The M ratio indicating 17α-hydroxylase activity remained almost normal, consistent with the elevation of both substrates and products, whereas the M ratios indicating 17,20 lyase and 21-hydroxylase activities were grossly decreased. Significant difference between groups A and B was identified for Ms of progesterone ($P = 0.044$), those of 17-OHP ($P = 0.022$), those of aldosterone ($P = 0.0084$), and M ratio indicating 17,20 lyase activity ($P = 0.011$). Adrenal crisis was observed only in group B with a significant difference between groups A and B, whereas the detection frequency of elevated 17-OHP in mass screening was similar between groups A and B (Table 2).

DSD was more prevalent in 46,XX cases than 46,XY cases in both groups A and B (Table 2, footnote, and supplementary Fig. 2). 46,XY DSD in group A was micropenis in one case, and that in group B included more severe phenotypes. By contrast, 46,XX DSD was invariably identified in both groups A and B. Maternal virilization during pregnancy was often found in groups A and B with a similar prevalence. Serum T of case 20, aged 0.2 yr in group B, was 6.5 and 7.6 nmol/liter (1.9 and 2.2 ng/ml) before and after hCG stimulation, respectively.

Pubertal development was apparently normal in two 46,XY cases of group A and one of four 46,XY cases in group B and was invariably affected in 46,XX cases in both groups A and B (Table 2). In family A of group A, cases 2 and 3 exhibited full pubertal development with testis volume of 20 ml, whereas case 10 had obvious pubertal failure with Tanner B2 stage. T value of case 18, aged 17.5 yr in group B, was low at the baseline (0.7 nmol/liter,

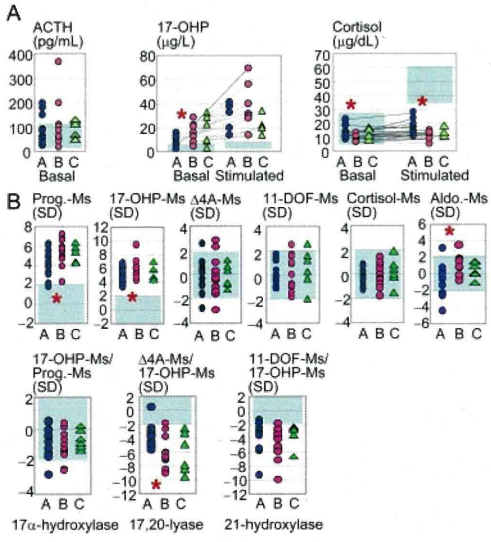

FIG. 2. Adrenal steroidogenic dysfunctions in groups A–C. *Light blue areas* represent the normal ranges. *Red asterisks* indicate the presence of significant differences between groups A and B. A, Basal and ACTH-stimulated blood hormone values. B, Basal urine steroid M values. Prog, Progesterone; Δ4A, androstenedione; 11DOF, 11-deoxycortisol; Aldo, aldosterone.

0.2 ng/ml) and poorly responded to hCG stimulation (1.0 nmol/liter, 0.3 ng/ml). PCO was observed in infantile or pubertal cases with a similar frequency between groups A and B, and cases 22 and 24 had ovarian torsion. Notably, bilateral ovarian cysts of case 10 markedly reduced in size after treatment with estradiol (E_2) (supplementary Fig. 3).

Long-term growth patterns were obtained in eight cases (Fig. 3). Whereas childhood heights tended to be high in both groups A and B, pubertal growth was different between the two groups. Cases in group A lacked obvious pubertal growth spurt but continued to grow for a long term, attaining tall adult heights, whereas those in group B showed rather compromised pubertal growth with worsening of scoliosis (supplementary Fig. 1).

There was no phenotypic difference between A503V-positive and -negative cases of group B (supplementary Table 2). In addition, the phenotypes in group C were grossly similar to those in group B (Table 2). In particular, craniosynostosis was identified in all cases except for case 33 with R457H and E580Q, and adrenal crisis was manifested by case 35 with Y578C and I444fsX449.

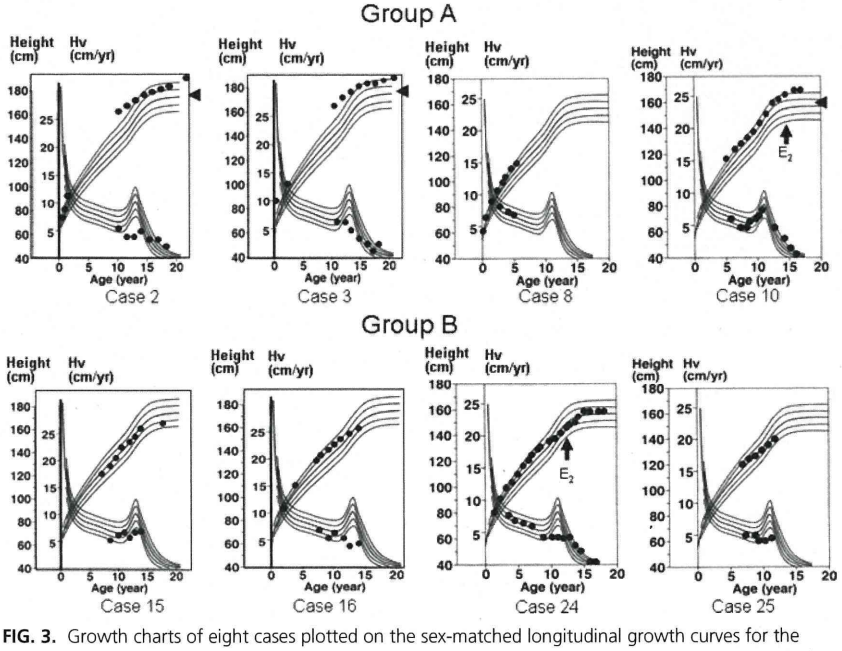

FIG. 3. Growth charts of eight cases plotted on the sex-matched longitudinal growth curves for the normal Japanese children (+2 SD, +1 SD, the mean, −1 SD, and −2 SD). The *triangles* in cases 2, 3, and 10 represent the target heights. Cases 10 and 24 are placed on E_2 replacement therapy. Hv, Height velocity.

Discussion

Molecular studies

Detailed molecular studies were performed in this study, providing two notable findings. First, all 35 cases were found to be homozygotes or compound heterozygotes for *POR* mutations including intragenic microdeletion and transcription failure. Because the microdeletion was found in case 21 with apparent R457H homozygosity, such a microdeletion might be hidden in the previously reported patients with apparent homozygosity (1, 5). Similarly, because transcription failure was invariably identified in cases 18, 26, and 27 with apparent heterozygosity, it may also underlie in the previously reported patients with apparent heterozygosity (4, 5, 10). In this regard, it is likely that the three cases carry a mutation in a hitherto unidentified *cis*-regulatory sequence(s) for the transcription of *POR*, as has been reported for several genes (24).

Second, RT-PCR sequence analysis indicated the occurrence of NMD in the two frameshift mutations (I444fsX449 and Q555fsX612). In this context, all the premature termination codons caused by the nonsense and the four frameshift mutations satisfy the positional conditions for the occurrence of NMD that functions as an mRNA surveillance mechanism to prevent the formation of aberrant proteins (13, 14). Thus, it is likely that the remaining three mutations (Q201X, R48fsX63, and Y567fsX574) are also null mutations subject to NMD *in vivo*.

Genotype-phenotype correlations

Genotype-phenotype correlations also provide several informative findings. Skeletal features were clearly different between groups A and B. Because cholesterol production in skeletal tissues is carried out in a simple one way manner (Fig. 4), this would explain why the skeletal phenotype is obviously dependent on the R457H dosage, reflecting the residual activity. It is likely that the threshold level for the development of severe skeletal phenotypes resides between a single copy and two copies of the R457H residual activity.

Adrenal steroidogenic dysfunction was grossly similar between groups A and B, although it was somewhat milder in group A than group B. Such a relatively minor role of R457H dosage in adrenal steroidogenesis