以上のことを理解した上で、自由意思に基づき、本研究に協力することに同意します。

なお、遺伝子解析の結果については、以下のように希望します。（以下のどちらかに○をつけてください。）

（　）　遺伝子解析の結果を知りたい。

（　）　遺伝子解析の結果を今は知りたくないが、私がのちに(5年以内)希望したときには教えてほしい。

上記の内容をご確認の上、以下にご署名ならびにご連絡先などをご記入ください。

ご協力くださる方の名前：＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

患者さんとの関係＿＿＿＿＿＿

（本人署名・代筆）　※ あなたが16歳以上の場合にはご自身で署名してください。

保護者の方の署名：　＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

※ ご協力くださる方が未成年の場合には、保護者の方の署名をお願いいたします。

連絡先住所：　〒＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

電 話 番 号：＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

記　入　日：　平成＿＿＿＿年＿＿＿＿月＿＿＿＿日

以下、医師が記入のこと

説明者の氏名および職名　＿＿＿＿＿＿＿＿　＿＿＿＿＿＿＿＿

説明者の署名または記名・捺印＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

【説明医師への注意】

※ 別紙の説明文書を用いて、上記説明者より説明を受けたことを、検体提供者となる患者さんご本人もしくはその保護者によって確認していただき、□内にチェックを入れてもらってください。また、結果の開示についても、ご希望を確認の上、○をつけてもらってください。

※ ご協力くださる方の名前の欄は、提供者が16歳以上もしくはご本人が十分に理解されている場合には、ご本人の署名を求め、その際に「本人署名」に○をつけておいてください。16歳未満で本人署名が難しい対象者の場合には、保護者もしくは医師が提供者の名前を記載してください。（この際は「代筆」に○をお願いします。）

※本同意書のコピーを必ず一部もらってください。

厚生労働科学研究費補助金（子ども家庭総合研究事業）
分担研究報告書

遺伝子診断の拠点化に伴う倫理的基盤の確立に関する研究(2)
－診断コンサルテーション・システムの検討－

分担研究者　掛江　直子　国立成育医療センター研究所成育政策科学研究部
成育保健政策科学研究室長

研究要旨

本分担研究では、小児先天性疾患および難治性疾患における遺伝子診断の拠点化に伴う様々な倫理的問題を検討し、その基盤を確立することを目指した。具体的には、稀少な小児先天性疾患および難治性疾患の診断の際に、拠点となる遺伝子診断可能施設に対し、他の医療施設から診断コンサルテーションを行う際の倫理的問題の検討を行った。

研究協力者
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A．研究目的

本分担研究では、小児先天性疾患および難治性疾患における遺伝子診断の拠点化に伴う様々な倫理的問題を検討し、その基盤を確立することを目指した。

具体的には、稀少な小児先天性疾患および難治性疾患の診断の際に、拠点となる遺伝子診断可能施設に対し、他の医療施設から診断コンサルテーションを行う際の倫理的問題の検討として、顔貌情報を含むセンシティブ・データを用いた遠隔相談のあり方について検討した。

B．研究方法

稀少疾患の診断に際しては、診断経験の少ない医療機関から、対象稀少疾患の診断経験があると推察される拠点医療施設もしくは研究者に対して、診断コンサルテーションがなされてきた。これらは、患者の診断・治療を目的とした医療専門家間の意見交換として、慣例的に許容されてきた行為である。しかしながら、近年のインターネットや電子メールの普及に伴い、これらの診断相談がネットを介して行われる状況がでてきている。当然のことながら、診断に必要な情報というのは、患者のプライバシー情報であり、かつ極めてセンシティブな情報であることから、こういった情報の取扱いについて、倫理的な側面から検討することが必要となった。

本分担研究では、まずこれらのセンシティブ情報が診断コンサルテーションに不可欠であるかについて検討し、次にこれらの情報が不可欠である場合に、個人情報保護の観点から適切かつ安全に診断コンサルテーションを行うためのシステムの検討を行った。

（倫理面への配慮）

本分担研究は、人ならびに人由来資料を用いることのない研究であることから、倫理的問題はないと考える。

C．研究結果

先天性疾患の診断においては、顔貌情報、奇形部位の写真など、センシティブな情報が不可欠である。また、遺伝子診断による確定診断が必要となる場合も少なくないことから、家系情報や遺伝子解析結果なども用いなければならない場合もある。このようなセンシティブな情報を用いなければならない診断について、施設外に診断コンサルテーションを行う場合には、その情報の取り扱いについて、慎重な対応が求められる。

従来は、電話や郵便という手段による施設外への

コンサルテーションが主であったが、現在はインターネットの普及により、電子メール等を用いた相談も増加しているようである。これは、迅速に専門家に相談できるという点で、患者にとっても利益は大きいのであるが、反面セキュリティの状況などによっては非常に大きな個人情報漏洩のリスクを孕んでいる。

そこで、本分担研究では、1）患者のセンシティブ情報を取り扱う、2）相談者（医師）は不特定である、3）複数の専門家による議論をもとに診断助言をする、4）患者情報の保護は不可欠であるという前提で、安全な診断コンサルテーション･システムの構築に取り組むこととした。結果は末尾資料に示した通りである。

1. コンサルテーション・システムの概要

成育医療センターに当該研究班による先天性疾患の診断コンサルテーション･システムを設置することを前提とした。全国の小児科医から、診断の難しい稀少疾患等の診断コンサルテーションを、インターネットを介して受けることを想定している。受けた相談は、診断精度を高めるため、専門家グループにより議論し、結果を相談者に対して助言として送付し、完結する。

2. 依頼時のセキュリティ

依頼者を特定医療機関等に限定すると認証などの手続きが簡便になるが、反面、対象医療機関以外を受診した患者についてのコンサルテーションが受けられなくなる。したがって、今回は、相談者を全国の不特定の医師という前提でシステムを検討した。その結果、診断を依頼する際には、1）暗号化された通信（https）を用いて、2）最小限の相談内容の送付に限定すること（指定の相談シートを作成）により、個人情報保護に努めることとした。相談時にIDとパスワードを発行し、その後の認証を行う。

3. 相談・診断の際のセキュリティ

相談内容については、相談者からの情報を主に掲載する画面（相談記録登録画面）に掲載され、コンサルテーション･チームのメンバーである専門家らUSBトークンによるユーザー認証を行った上で画面にアクセスし、さらに議論をするために設置された画面（共有情報登録画面）において議論を行う。これらの情報は、アプリケーションにより使用権限を制御する。その結果を、相談者に対して助言の形で返信し、それらの記録を診断情報データベースに蓄積する。これらに対する不正アクセスについては、ファイアウォールを設けて防止する。

D．考察

本分担研究において、患者のセンシティブ情報を用いた遠隔診断コンサルテーション・システムの構築について検討を重ねた。既に、類似のシステムはいくつか存在するが、それらの利用も含めて検討した結果、独自にプログラムすることにより、詳細なかつ正確な情報を直接的に取り扱い、より正確な診断助言を行うことができると判断した。

E．結論

本年度は診断コンサルテーション・システムの枠組みの検討を行ったが、次年度以降、実際に当該研究班で対象としている先天性疾患等を対象として、実際に運用し、当該システムの評価・検討ならびに改善を行う予定である。

F．研究発表

1.　論文発表

なし。

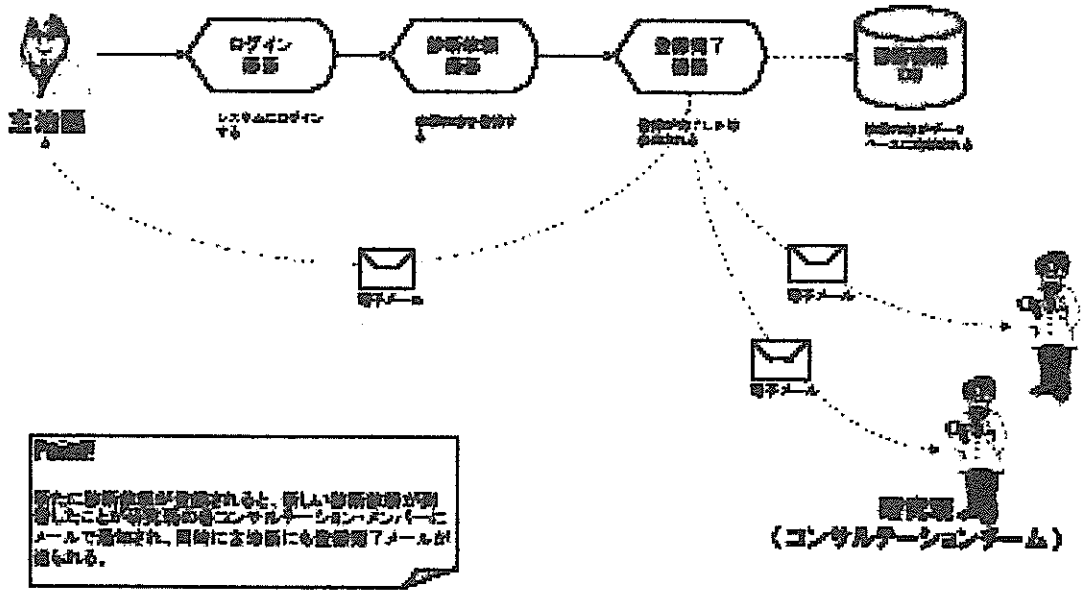
2.　学会発表

なし。

G．知的財産権の出願・登録状況

なし。

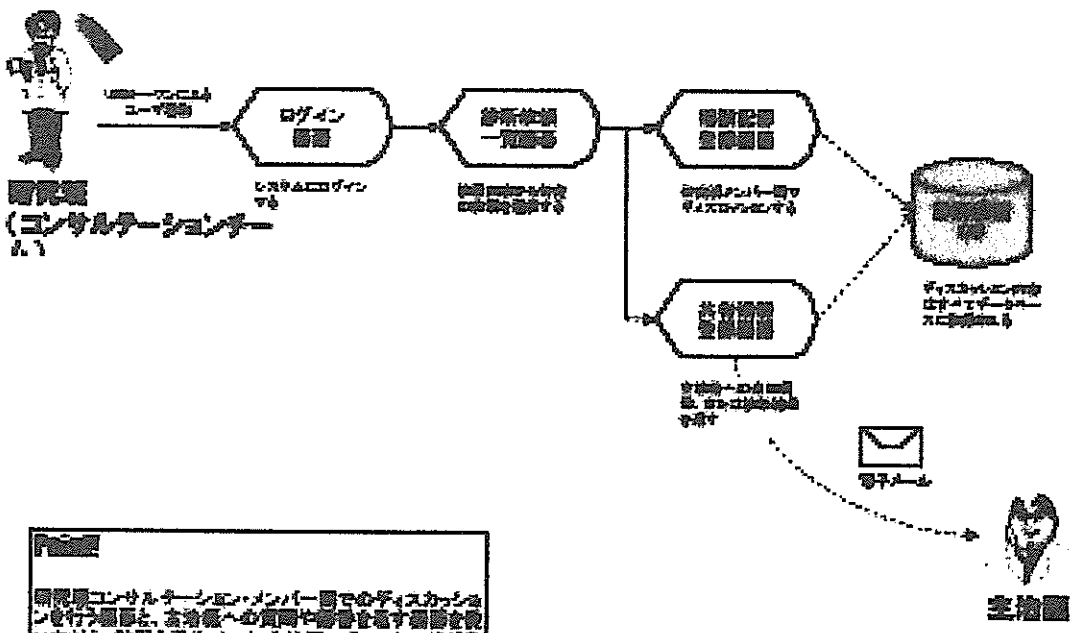
利用1．コンサルテーションチームに診断を依頼する場合フロー



Point!

新たに診断依頼が登録されると、新しい診断依頼が到着したことが専門医の全コンサルテーション・メンバーにメールで通知され、同時に主治医にも登録完了メールが送られる。

利用2．コンサルテーションメンバーによるディスカッションの場合フロー



Point!

利用者コンサルテーション・メンバー間でのディスカッションを行う場合と、主治医へ必要時や都合を尋ねる場合などがあるが、診断を進めていく。主治医へのメッセージが書き込まれると自動的に主治医にメールが届く。

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

雑誌

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研究成果の刊行物・別刷り

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CXorf6 is a causative gene for hypospadias

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46,XY disorders of sex development (DSD) refer to a wide range of abnormal genitalia, including hypospadias, which affects ~0.5% of male newborns. We identified three different nonsense mutations of *CXorf6* in individuals with hypospadias and found that its mouse homolog was specifically expressed in fetal Sertoli and Leydig cells around the critical period for sex development. These data imply that *CXorf6* is a causative gene for hypospadias.

A gene responsible for 46,XY DSD has been postulated near *MTM1*, the gene on Xq28 underlying myotubular myopathy, on the basis of the finding that genital development is normal in individuals with intragenic *MTM1* mutations[1] and invariably abnormal in six individuals with microdeletions involving *MTM1* (refs. 2–4). Subsequent studies have suggested that *CXorf6* (ref. 5; GenBank accession number NM_005491) is an excellent candidate gene for 46,XY DSD because it is deleted in the six individuals with 46,XY DSD, and no other candidate gene has been identified within the commonly deleted region (refs. 2–4 and J.L., unpublished observations). However, there has been no direct evidence for the relevance of *CXorf6* to 46,XY DSD.

We performed direct sequencing for coding exons 3–6 of *CXorf6* and their flanking splice sites in 166 individuals with various types of 46,XY DSD (152 sporadic cases and 14 probands of familial cases; **Supplementary Table 1** online). When we identified a substitution, we attempted to examine other family members. Consequently, we identified three nonsense mutations in Japanese individuals with hypospadias: 370G→T (E124X) in maternally related half-brothers from family A (individuals 1 and 2), 589C→T (Q197X) in an individual from family B (individual 3) and 1957C→T (R653X) in an individual from family C (individual 4) (**Fig. 1a**; for all the primers used in this study, see **Supplementary Table 2** online). The mothers of families A and C were heterozygous for the mutations; the mother of family B was not studied. These mutations were absent in 150 Japanese control males.

We found P286S in a Swedish individual with hypospadias that was absent in his brother and nephew with the same phenotype (family D). Similarly, we detected Q507R in Italian brothers with feminized genitalia, but it was absent in a nephew with a similar phenotype (family E). Thus, these two missense substitutions seem to be nonpathological variants. In addition, we found P286S in 8 of 110 Swedish control subjects, although Q507R was absent in 200 European control subjects. We also found the previously reported polymorphism N589S (rs2073043) in two Japanese affected individuals, one European affected individual and four Japanese control males.

Individuals 1–4 showed penoscrotal hypospadias as the conspicuous phenotype (**Fig. 1b**) and had seemingly normal pituitary-gonadal serum hormone values. For example, the human chorionic gonadotropin–stimulated testosterone level was normal in individual 1 at age 2 years 5 months. Similarly, basal testosterone levels were normal in individual 2 at 1 month of age and in individual 4 at 3 months of age, when serum testosterone is physiologically elevated[6] (**Supplementary Table 3** online). We sequenced the androgen receptor gene (*AR*) and the 5α-reductase gene (*SRD5A2*) in individuals 1–4 and did not find any mutation. There were no other individuals with 46,XY DSD in families A–C.

The three nonsense mutations are predicted to cause nonsense-mediated mRNA decay because of their positions[7] (**Fig. 1c**). Consistent with this, RT-PCR for leukocytes indicated markedly reduced transcripts in individuals 1–4.

We performed PCR-based screening of human cDNA samples (either purchased from Invitrogen and Clontech, or prepared using Invitrogen Superscript III reverse transcriptase) and found ubiquitous expression of *CXorf6*, including in fetal testis (**Supplementary Fig. 1** online). In addition, we identified two in-frame splice variants, a major form with exon 4 and a minor form without exon 4, as reported previously[5].

Next, we performed *in situ* hybridization (ISH) analysis for the homologous mouse gene (*G630014P10Rik*). Expression patterns in the fetal gonads are shown in **Figure 2a**. In the testis, the mouse homolog was weakly expressed in the internal region at embryonic day (E) 11.5 and clearly expressed in Sertoli cells and in a small number of Leydig cells at E12.5. At E14.5, it was still clearly expressed in Sertoli cells and in the majority of Leydig cells. In the ovary, the mouse homolog was expressed in a small number of somatic cells, primarily at the boundary to the mesonephros at E11.5 and E12.5, and weakly expressed in a small number of somatic cells in the internal region at E14.5. In extragonadal tissues at E12.5, the mouse homolog was not expressed in the adrenals and weakly and diffusely expressed in the external genital region, including the genital tubercle, at a level similar

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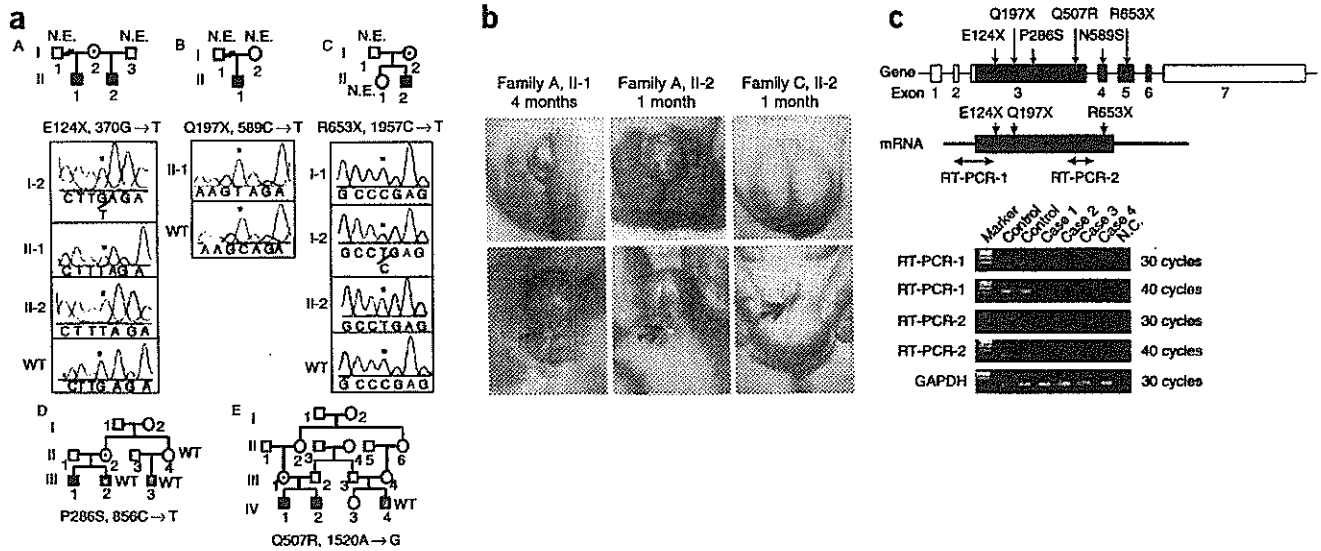

Figure 1 Molecular and clinical findings. (**a**) Pedigrees and electrochromatograms. The black and gray squares indicate individuals with 46,XY DSD with mutant or variant *CXorf6* and wild-type (WT) *CXorf6*, respectively. The asterisks in the chromatograms indicate mutant and corresponding wild-type nucleotides. N.E.: not examined. (**b**) Appearance of external genitalia in three affected individuals from families A and C. The arrows indicate the urethral meatus. (**c**) RT-PCR analysis. The black and gray filled boxes represent the coding regions, and the open boxes denote the untranslated regions. The positions of the mutations and variations are shown. R653X (1957C→T) resides at a position >100 nucleotides apart from the junction between exons 5 and 6. RT-PCR for the two regions (RT-PCR-1 and -2) failed to produce any bands after 30 cycles and yielded very faint bands after 40 cycles in individuals 1–4. *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase gene; N.C.: negative control.

to that detected in the neighboring extragenital tissues. It was also clearly expressed in the Müllerian ducts, forebrain, somites, neural tube and pancreas.

Expression patterns in the postnatal gonads are shown in **Figure 2b**. In the testis, we found weak expression of the mouse homolog within the cords until 1 week of age, and expression was faint thereafter. In the ovary, its expression was barely detected until 2 weeks of age and was clearly identified in granulosa cells at the perifollicular regions of most of Graafian follicles at 3 and 8 weeks of age.

The present study implies that *CXorf6* is a causative gene for 46,XY DSD. One may argue that hypospadias in individual 4, with R653X on exon 5, is inconsistent with the previous report of apparently normal genital development in a boy with a microdeletion involving *MTM1* that resulted in the generation of a fusion gene between exons 1–4 of *CXorf6* and exons 3–16 of *MTMR1* (locus order: *CXorf6-MTM1-MTMR1*)[8]. However, in contrast to the positive expression of the fusion gene[8], R653X (as well as E124X and Q197X) is predicted to cause nonsense-mediated mRNA decay and disrupt the function of *CXorf6*, a prediction supported by RT-PCR analysis. In addition, the apparently normal genitalia of the boy could be due to incomplete penetrance.

The salient phenotype in *CXorf6* abnormalities is hypospadias. Individuals 1–4 have penoscrotal hypospadias, and five of the six reported affected individuals with microdeletions involving *CXorf6* manifest hypospadias of various types, although the one remaining

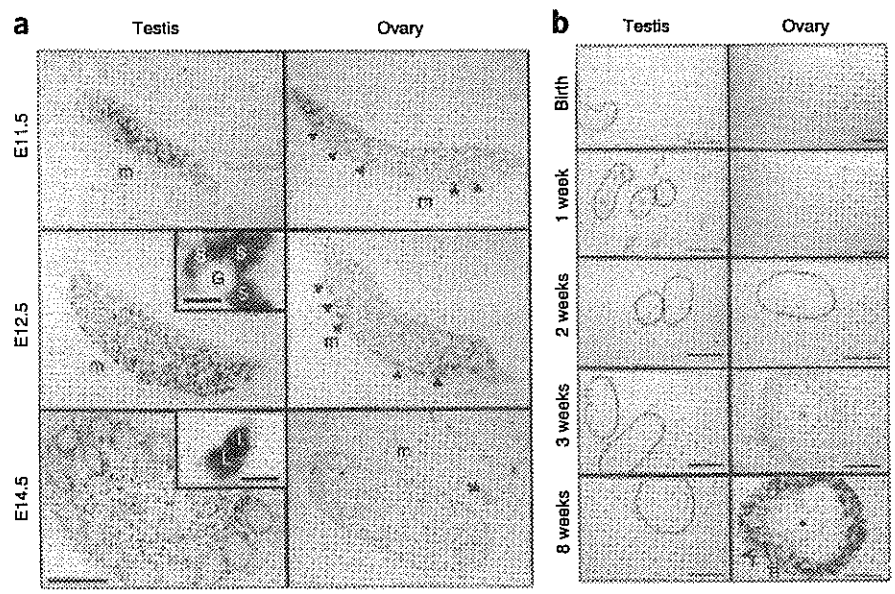

Figure 2 *In situ* hybridization analysis of the mouse *CXorf6* homolog. (**a**) Expression patterns in the fetal gonads. The blue signals are derived from *in situ* hybridization using a digoxigenin-labeled probe for the mouse homolog, and the brown signals from immunohistochemical analysis for Sf1, which serves as a marker for Sertoli and Leydig cells[10]. Arrowheads indicate the border between the ovary and the mesonephros, and an arrow denotes the Müllerian duct. m: mesonephros; G: germ cell; S: Sertoli cell; and L: Leydig cell. The scale bars in the low- and high-power fields represent 200 μm and 20 μm, respectively. (**b**) Expression patterns in the postnatal gonads. O: oocyte; T: thecal layer; B: basal membrane. The dotted circles indicate some of the testicular cords and a developing follicle. Scale bars: 100 μm.

individual has ambiguous genitalia[2–4]. In this regard, several matters are noteworthy: (i) structural abnormalities of the external genitalia, including hypospadias, are usually caused by either defective testicular androgen production or impaired responsiveness of external genitalia to androgens around the critical period for sex development[9], (ii) the mouse homolog is specifically expressed in Leydig and Sertoli cells around the critical period (E12.5–E14.5), with no significant expression in the external genital region, and (iii) individuals 1–4 have apparently normal serum testosterone values and are free from *AR* and *SRD5A2* mutations. These findings suggest that the genital phenotype is primarily ascribed to transient testicular (Leydig cell) dysfunction and the resulting compromised testosterone production around the critical period.

Molecular analyses of human subjects were approved by the Institutional Review Board Committees of National Research Institute for Child Health and Development (Tokyo) and Institut de Génétique et de Biologie Moléculaire et Cellulaire (Strasbourg, France). Informed consent was obtained from each subject or the parent(s). Mouse experiments were approved by the Ethical Committees for Animal Experiments of the National Institute for Basic Biology (Okazaki, Japan) and the Center for Animal Resources and Development (Kumamoto, Japan).

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

Mutation analysis was performed by M.F., Y.W., G.C., C.K. and A.B.-B.; human cDNA screening and RT-PCR by M.F.; mouse expression analysis by K.M., G.Y. and K.M. and phenotype assessment by I.N., T.H., J.L. and T.O. The study was designed and coordinated by J.L. and T.O., and the paper was written by T.O.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Urine Steroid Hormone Profile Analysis in Cytochrome P450 Oxidoreductase Deficiency: Implication for the Backdoor Pathway to Dihydrotestosterone

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Context: Although the "backdoor" pathway to dihydrotestosterone has been postulated in the fetal-to-early-infantile period of patients with cytochrome P450 oxidoreductase deficiency (PORD), clinical data in support of this pathway remain limited.

Objective: The objective of this study was to obtain clinical evidence for the presence of the backdoor pathway in PORD.

Setting: This was a collaboration study between laboratories and hospitals.

Subjects: Twenty-two Japanese patients with molecularly confirmed PORD and 1763 control subjects participated in this study.

Intervention: Urine steroid profile analysis was performed by gas chromatography/mass spectrometry. In five patients and 776 control subjects, urine samples were obtained before 12 months of age.

Main Outcome Measure: The main outcome measure was identification of a urine steroid(s) indicating the backdoor pathway.

Results: In the PORD patients, pregnanediol, pregnanetriolone, and pregnanetriol were obviously elevated, and the urine steroid ratios reflecting CYP17A1 and CYP21A2 activities were decreased throughout the examined ages. Furthermore, etiocholanolone and 11-hydroxyandrosterone, which should originate almost exclusively from androstenedione in the conventional "frontdoor" pathway, were grossly normal or somewhat decreased since early infancy, whereas androsterone, which can be derived not only from androstenedione and dihydrotestosterone in the conventional frontdoor pathway but also from 5α-pregnane-3α,17α-diol-20-one in the backdoor pathway, was increased during early infancy and remained grossly normal thereafter. Thus, the androsterone to etiocholanolone ratio was increased during early infancy and remained grossly normal thereafter. 5α-Pregnane-3α,17α-diol-20-one was elevated throughout the examined ages.

Conclusions: The increased androsterone excretion during early infancy, as compared with the etiocholanolone and 11-hydroxyandrosterone excretions in the same period, suggests the presence of the backdoor pathway in PORD. (***J Clin Endocrinol Metab* 91: 2643–2649, 2006**)

CYTOCHROME P450 OXIDOREDUCTASE (POR) deficiency (PORD) is an autosomal recessive disorder caused by mutations in the gene encoding a flavoprotein that serves as an electron donor to all microsomal P450 enzymes such as CYP51A1 (lanosterol 14α-demethylase) involved in cholesterol biosynthesis and CYP17A1 (17α-hydroxylase and 17,20 lyase), CYP21A2 (21-hydroxylase), and CYP19A1 (aromatase) involved in steroidogenesis (1–3). Clinical phenotypes of this condition are highly variable, and include abnormal skeletal development referred to as Antley-Bixler syndrome and insufficient glucocorticoid production with increased 17α-hydroxyprogesterone (17-OHP) in patients of both sexes, undermasculinization during the fetal and pubertal periods in male patients, and virilization during the fetal life and poor pubertal development without worsening of virilization in female patients, together with maternal virilization during pregnancy (1–7). Because the complete absence of POR activity is assumed to be lethal (1), some residual POR activity should be present in the patients. Indeed, all the reported patients have at least one missense mutation that is likely to preserve some residual activity (2–7).

Therefore, PORD is a unique disorder that can lead to impaired fetal sex development in both sexes. In particular,

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Abbreviations: Δ^4A, Androstenedione; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; M, metabolite; 11-OHAn, 11-hydroxyandrosterone; 17-OHP, 17α-hydroxyprogesterone; PD, pregnanediol; POR, cytochrome P450 oxidoreductase; PORD, POR deficiency; PT, pregnanetriol; PT5, pregnenetriol; Ptl, pregnanetriolone; T, testosterone; THA, tetrahydro-11-dehydrocorticosterone; THB, tetrahydrocorticosterone; THE, tetrahydrocortisone; THF, tetrahydrocortisol.

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whereas masculinization can be preserved normally in a relatively large fraction of male patients, virilization is exhibited by nearly all female patients (2–7) except for one adult woman who presented with primary amenorrhea (2). The defective sex development is primarily consistent with impaired gonadal CYP17A1 activity resulting in compromised testosterone (T) production in genetic males and defective placental CYP19A1 activity leading to accumulation of placental T as well as androstenedione (Δ^4A) that is transferred into the fetal and maternal circulations in both genetic male and female patients (2–4). In this situation, genetic male patients have nearly normal to underdeveloped external genitalia because of the combined effects of reduced gonadal androgens and accumulated placental androgens, whereas genetic female patients have virilized genitalia because of the effects of placenta-derived androgens. However, the virilization in genetic female patients appears to be inexplicable by defective placental CYP19A1 function alone: 1) in CYP19A1 deficiency, only female infants with nearly complete loss of enzyme activity (<1%) have virilization (8); and 2) in PORD, the supply of dehydroepiandrosterone (DHEA), the precursor of Δ^4A and T, from the fetus to the placenta is reduced (4, 9).

Thus, the backdoor pathway to dihydrotestosterone (DHT) has been postulated in PORD (3, 10). The backdoor pathway, which has been demonstrated in the tammar wallaby pouch young testis (11) and in the immature mouse testis (12), is driven by the accumulation of 17-OHP, leading to the sequential conversion of 17-OHP into DHT via a T-independent route. Although the backdoor pathway is mediated by CYP17A1 as well as 5α-reductase (presumably type 1) and other enzymes, the substrate for CYP17A1 in the backdoor pathway, 5α-pregnane-3α,17α-diol-20-one, is known to have a much higher affinity for CYP17A1 than 17-OHP (13). Thus, the backdoor pathway would function better than the conventional frontdoor pathway in PORD. In this study, we report urine steroid profile data obtained in PORD patients that suggest the presence of the backdoor pathway in this condition.

Patients and Methods

Patients

Twenty-two Japanese patients with PORD (11 genetic male patients with 46,XY and 11 genetic female patients with 46,XX) were studied (Table 1). Fifteen of the 22 patients have been reported previously (2, 4, 6, 7). In all the patients, molecular analysis indicated the diagnosis of PORD, and the patients were divided into two groups in terms of the mutation type: 1) those with two missense mutations, mostly homozygotes for R457H with some residual activity (2, 3) (cases 1–3 and cases 12–17) (group 1); and 2) those with one missense mutation, mostly R457H, and one non-missense mutation that probably has no or very little residual activity (cases 4–11 and cases 18–22) (group 2). Skeletal phenotype was much milder in group 1 than in group 2, masculinization in genetic male patients was somewhat better preserved in group 1 than in group 2, and virilization in genetic female patients and maternal virilization during pregnancy appeared to be similar between groups 1 and 2; case 21 only exhibited normal female genitalia. This study was approved by the institutional review board committee of each investigator, and the mutation analysis was performed at the National Research Institute for Child Health and Development after obtaining written informed consent from each patient or the parents.

Urine steroid hormone profile analysis

Urine steroid hormone profile was determined by gas chromatography/mass spectrometry (14), using 24-h urine samples or random spot urine samples. The age at the time of urine sampling in each case is shown in Table 1. Analyzed urine steroids included pregnanediol (PD), pregnenetriol (PT5), pregnanetriolone (Ptl), pregnanetriol (PT), tetrahydrocorticosterone (THB), tetrahydro-11-dehydrocorticosterone (THA), tetrahydrocortisol (THF), tetrahydrocortisone (THE), DHEA metabolites (Ms) (the sum of DHEA, androstenediol, 16α-hydroxy-DHEA, 16β-hydroxy-DHEA, 16-oxoandrostenediol, and androstenetriol), 11-hydroxyandrosterone (11-OHAn), etiocholanolone, androsterone, 5α-pregnane-3α,17α-diol-20-one, and 5β-pregnane-3α,17α-diol-20-one (Fig. 1) (the formal steroid names are described in Ref. 14). This analysis was performed after obtaining written permission from each patient or the parents.

For comparison, cross-sectional data obtained from 854 males and 909 females were used (part of the control data has been reported previously) (4, 7, 14, 15). Only a single urine sample was obtained from each subject. The subject age at the time of urine sampling and the urine collection methods are summarized in Table 2. The control urine samples were collected from healthy neonates, children with microhematuria or mild upper respiratory infection at the recovered phase, and healthy volunteers of various ages. The urine sampling was approved by the institutional review board committee at Keio University Hospital; each subject or the parents agreed to the urine sampling on the condition that the sample is only used to make the control data for the diagnosis of adrenal disorders after discarding personal information except for age and sex.

Results

PD, PT5, Ptl, PT, THE, DHEA-Ms, 11-OHAn, etiocholanolone, androsterone, 5α-pregnane-3α,17α-diol-20-one, and 5β-pregnane-3α,17α-diol-20-one were measurable since birth, as was aldosterone-M. However, THB, THA, and THF, as well as the Ms of pregnenolone, deoxycorticosterone, and 11-deoxycortisol, could not be measured precisely during the first 6 months of age because of interference from unknown steroids that are probably derived from the fetal adrenocortex. Overall, the results were similar between genetic male and female patients and between groups 1 and 2 patients, including the particular case 21 with apparently normal female genitalia.

Representative results for the assessment of the conventional frontdoor pathway are shown in Fig. 2A (the data are expressed using a logarithm scale). Throughout the examined ages, PD, Ptl, and PT were obviously elevated, and PT5 tended to be increased in the PORD patients, whereas THF and THE, as well as aldosterone-M, remained grossly normal, and THB and THA tended to be elevated (data not shown). Furthermore, the (THF + THE) to (THB + THA) ratio reflecting CYP17A1 (17α-hydroxylase) activity was reduced, whereas the PT to PD ratio remained within the normal range (data not shown). Similarly, the THE to PT ratio reflecting CYP21A2 activity, the DHEA-Ms to PT5 ratio reflecting CYP17A1 (17,20 lyase) activity for the Δ^5-steroid, and the 11-OHAn to PT ratio reflecting CYP17A1 (17,20 lyase) activity for the Δ^4-steroid were all decreased in the PORD patients.

Representative data for the evaluation of the alternative backdoor pathway are shown in Fig. 2B (the data are expressed using a logarithm scale, except for the androsterone to etiocholanolone ratio, which is expressed using a linear scale). Throughout the examined ages, DHEA-Ms tended to be low, 11-OHAn was normal or low, and etiocholanolone