られれば周囲も毎日のことなので気にならなくなるようです。具体的な説明をクラスメートにしても理解できる発達段階ではありませんので、例えば“熱が出るとすぐに体調が悪くなるから、それを防ぐ薬”“体調を壊しやすいのを防ぐ薬”などでもよいでしょう。その子どもにとって“説明しやすい”“説明に使ってもよいフレーズ”を親子でともに考えていくことも、子どもが自分の病気への理解を深めるために有効でしょう。

学童期になり内服管理を子どもに任せおくと、薬の飲み忘れがおこってきます。病気の理解が十分でないため薬の必要性が理解できておらず、発達段階的にも自己管理が完成するにはまだ早いことなどがその理由です。子ども自身による内服管理に移行することはとても大事ですが、全てを子ども一人で行うことはまだ無理なため、親は飲んでいるかの確認や見守りを続けてください。また、この自己管理への移行は子どもに病気の説明をしていく良いきっかけともなります。子どもへの病気の説明は、タイミングが重要です。

相談にのりますので気軽に医療者へお声掛けください。

X. 思春期の課題

思春期には心の変化、からだの変化があらわれ、ご両親もとまどうことが多いと思います。このような変化にともなって、内服薬の量や種類の微調整がたびたび必要になる子どももいます。こどもの心身が揺れると、ご両親も気持ちが揺れることがあると思います。

思春期の課題をお話しましょう。

1. こころの変化

①安心・安定を求めて友達(親友)を捜し求める

思春期になると、自分のことを深く理解してくれる友達を求めるようになります。自分の悩みや将来の夢など、語り合うことによって、絆が深くなってきます。時には、親しくなってから、離れていくこともあります。傷ついたり、さびしくなったりしますが、出会いと別れを繰り返す中で、人への思いやりが深くなっていきます。

②劣等感(コンプレックス)が強くなる

もっと成長したい、あの人のようになりたい・・・、という理想を持つと、自分とのギャップを自覚して、劣等感が大きくなります。そこから、理想や夢に向かって努力するという行動が生まれてくるので、劣等感は悪いものではありません。努力する自分自身を大切に思うこと、自分への思いやりも育てていきたいです。

③性に関する興味が大きくなる

同性の友達だけでなく、異性とのおつきあいについて興味が出てくるのは、自然なことです。インターネットや雑誌、テレビなどで過激な情報があふれていますが、まどわされることなく、無理することなく、自分らしいおつきあいをしたいですね。

特に、セックスの関係になると、「デートバイオレンス」の危険性が高くなります。相手を支配する、独占するという気持ちを乗り越えて、相手の成長を応援する関係になるためには、よほど心が成熟していないとむずかしいです。セックスから得られる安らぎ以上に、「望まない妊娠」や「性感染症」などのリスクにびくびくする可能性も大きくなります。高校生には、NO SEX を強くすすめます。安心してセックスをするためには、心とからだの準備が必要です。深いお付き合いに進みそうなら、気軽に看護師に相談してください。

2. からだの変化

①第2次性徴

- 自分らしいからだつき　保健体育の教科書には、男女のからだについて、ひとつのパターンだけが描かれていますが、からだは顔と同じくらい個性的であっていいのです。
- 発毛　発毛の範囲や程度もそれぞれにちがいます。毛深いのが気になる人は、カミソリで剃っている人もあります。
- 乳房　テレビや雑誌など、さまざまなメディアで「巨乳」がもてはやされていますが、将来の母乳の量と乳房の大きさは関係ありません。男性全部が巨乳好みでもありません。あせって豊胸

手術をすると、その後に後悔する人も多いです。ホルモン剤が含まれるバストアップサプリメントを飲んだりすると、月経異常が起こることもあります。極端なダイエットをすると、乳房を形作っている脂肪が減少するので、標準体重を目安に健康的なからだ作りが大切です。

②月経：16歳になっても、月経が始まらないときは、医師に相談して、性ホルモンの検査をします。しばらく月経を起こすためのホルモン治療をすることで、自然に順調な月経が起こる人が多いです。月経時には、ほとんどの女性が下腹痛や腰痛を感じていますが、痛みが気になるようなら、鎮痛剤（痛み止め）を飲みましょう。生理痛が強くなってから痛み止めを飲むのではなく、「そろそろ痛くなりそう」といった早めのタイミングで飲むほうが、効果的です。

CAHの女性には、膣が狭い人がいて、月経血の排出がスムーズにいかない場合があります。月経血が子宮の中に溜まると、強い下腹痛が起こります。そのような場合は、月経血がスムーズに流れるように手術をします。そのため、初潮（はじめての月経）が近づいて来たら、医師から内視鏡の検査をすすめられることがあります。

③毛深さ：毛深さが気になる場合は、カミソリで剃ったり、脱色して目立たなくすることができます。友達と一緒にプールや温泉に行くときだけ、剃っている人もいます。

3. 内服薬の調整

①コントロールの目安

思春期になると、からだの成長スピードの変化があるために、薬の量をたびたび調節することが必要になります。自分勝手に薬の量を調節しないで、必ず医師に相談してください。

②体重

思春期になると、女性ホルモンの作用で、からだ全体が丸みをおびて、ぽっちゃりする人が多いです。CAHの薬の作用で、食欲が増して、体重が増え続けるときは、医師に相談してください。

③皮膚の色素沈着

薬の量が足りないときに、乳首や手指の節などの色が濃くなることがあります。気になるときは、医師に相談してください。

4. こころとからだの相談

思春期のデリケートな悩みの相談ができる様に、大阪府立母子保健総合医療センターの小児外来（ストーマ外来）には、セクシュアリティ外来があります。性の悩み、からだの悩み、おつきあいの悩み、避妊や性感染症の予防など、気軽にご相談ください。親子での相談や、親だけ、子どもだけの相談にも応じています。『からだとこころ BOOK』というイラスト教材を見ながら、性の問題に対して、明るく相談に乗っています。

次ページからは、思春期の段階的なチェックリスト例を記しています。
病院や家庭で、話し合う際の参考にしてください。

例）思春期初期のチェックリスト（10 歳から 12 歳）

1 自分の障がいをきちんと理解し、周囲にもわかりやすく説明をして、生活上必要なサポートを合理的に要求することができる
   ① 自分の病気について、自分で説明させる
   ② 両親と一緒に、自分の経過を振り返る
   ③ 検診ごとに、本人に問診する

2 ヘルスケア行動の自立
   ① 薬や治療の必要性を話し合う
   ② 課題や困難な問題を話し合う
   ③ 医療用 ID タグの目的や、他者からの援助が必要な場合について話し合う

3 性的健康
   ① 第 2 次性徴について話し合う
   ② 友達との違いや健康状態への影響などについて話し合う
   ③ セクシュアリティに関する情報を得る方法・場所について話し合う

4 心理社会的支援
   ① 両親が将来への不安や子どもの自立にともなう喪失感について、話し合える機会を提供する
   ② 子どもと社会活動や友人関係や協力関係について話す

5 教育や職業についての計画
   ① 本人と家事分担や家での責任について話す
   ② 教育上の制限や余暇活動の制限（現実に起きているものと、推測されること）について話し合う

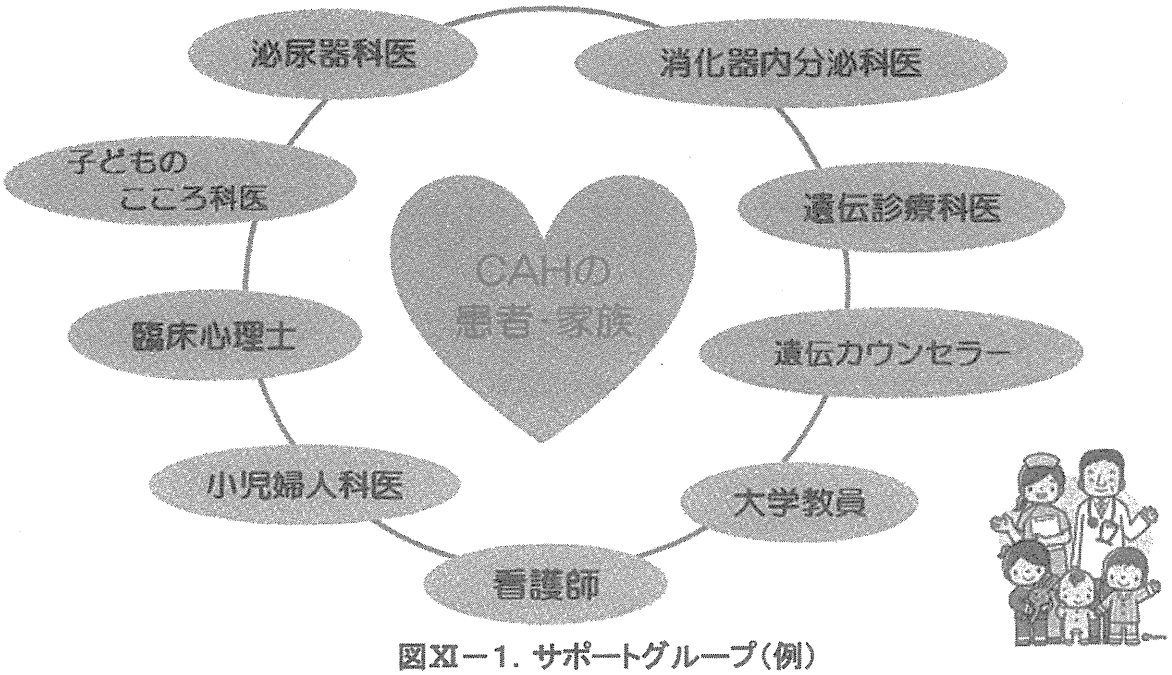
6 健康と生活習慣
   ① たばこ、アルコール、ドラッグについて質問する
   ② 上記の行動が、健康や幸福に及ぼす影響について話し合う

Sandy Whitehouse, MD, FRCPC, Clinical Associate Professor of Pediatrics at British Columbia's Children's Hospital. Mary C. Paone MSN , Nurse Clinician with Youth transition services at British Columbia's Children's Hospital.より許可を得て、翻訳・引用

XI. サポートグループについて

最近はインターネットで多くの情報を得ることができようになりました。しかし、その情報は正しいものばかりではなく、偏った情報や誤った情報も含まれており誤解や不安を抱く原因にもなっています。また、病気の特性から相談できる場所や対象が限られるといった声をよく患者さんやご家族の方々から耳にしています。

そのため、患者さんやご家族がさまざまな医療スタッフと話し合える場、例えば「CAHの患者・家族会(仮称)」が作られることが望まれます。



図XI－1. サポートグループ(例)

会の目的:

①患者さんとご家族に正しい情報を伝える

②患者さんやご家族たちが交流する機会をつくる

③ピアサポート*1の形成

＊1)ピアサポートとは、同じ課題や環境を体験する人同士がたがいに支えあうこと

患者さんやご家族同士が共に学び語り合うことで、子どもの健やかな成長発達を支援でき、充実した時間を過ごす機会となるでしょう。

まずは気軽な気持ちで参加していただき、共に過ごす時間を楽しみましょう。

XII. よくあるQ&A

Q:乳幼児へ薬の上手な飲ませ方はありませんか。
A:薬の味を嫌がるお子さんもいますので、ミルクおよび食事の前に飲ませるほうが良いでしょう。少量のお水で溶かしてスポイドかスプーンで飲ませます。乳児の場合は薬を少量のお水で練り上顎に塗る方法も効果的です。
そのうち子どもは薬を飲むことを食事の一部として習慣化していき、嫌がらずに飲めるようになります。ただし、ミルクに混ぜるとミルク嫌いになったり、ミルクを残して薬が飲めないこともあるので注意してください。

Q:なぜ、薬を飲まないといけないのですか。
A:副腎で糖質コルチコイド(コートリル)、鉱質コルチコイド(フロリネフ)を自分で作ることが出来ないため外から補う必要があります。(詳しくは、p3,p11 をごらんください。)

Q:熱がでたり吐いたりしたときは、どのように対応したらよいですか。
A:薬が飲めなかったり嘔吐するとき、ぐったりしたときは医療機関を受診し、点滴を受けてください。(詳しくは、p. 12 をごらんください。)

Q:今飲んでいる薬は、風邪薬と一緒に飲んでも大丈夫ですか。また、一緒に飲んではいけない薬はありますか。
A:今飲んでいる薬は補充療法で、生理的な機能を補っています。従って、三度の食事のような感覚で薬を考えてください。予防接種は通常通りおこない、風邪薬など一緒に飲んで問題ありません。カゼでも38℃以上の発熱を伴う場合は、倍量飲んでください。

Q:この病気による身長への影響はありますか。
A:薬が多いと身長が伸びにくくなります。一方で薬の量が足りないと骨年齢が進み将来的に低身長になる可能性があります。

Q:女の子の場合、妊娠や出産はできますか。
A:妊娠や出産に問題ありません。コントロールが悪いと男性ホルモンが高値になり卵巣機能に悪影響を及ぼし、月経不順や無月経、無排卵の原因になります。多のう胞性卵巣を引き起こすこともあります。月経不順があれば婦人科を受診したほうが良いでしょう。
出産方法は経腟分娩ができるかどうかを、担当の泌尿器科医に問い合わせてください。

Q:私の娘は子どもの遊びや好みが男の子っぽいのですが、どのように対応したら良いでしょうか。
A:子どもはお母さんのお腹の中にいる時に、すでに「男型の脳」「女型の脳」に分かれると考えられており、それを決めているのが男性ホルモンと女性ホルモンと言われています。どちらのホルモ

ンがどの程度脳に影響を及ぼすかは良く分かっていませんが、人によって「男だけども女の子らしいことが好き」「女だけども男らしいことが好き」といったことが、程度の差がありつつも出てくるのです。

一般に男型脳では空間認識や図形処理を司る部位が先に発達しやすいと言われ、この点がいわゆる「男の子っぽい」遊びを生み出すと考えられています。一方で女型脳は言語能力を司る部位が先に発達しやすく、脳全体の連絡網がよく発達すると言われています。そのため、女の子ではいろいろなお話をしたり、まわりをよく見ていることが多いと考えられます。

また、「男の子だから男の子らしい」「女の子だから女の子らしい」行為を社会的に求めている点もあり、そこから外れることで集団から阻害される感じを持たれる現状もあると思われます。親が自然と男の子らしさ、女の子らしさを求めているのかもしれません。

ご心配のように「男の子っぽい」ことは子どもが生まれ持ってきた性格ですから、どちらかの性に偏った行動を求めるのでなく、すなおに良い面を伸ばすように勧めてあげてください。

Q:子どもへの病気の説明について

A:特にCAHの子どもたちは小さな頃から頻繁に病院に通って診察を受け、薬を飲むことが日常となっています。幼稚園や保育園といった集団に属する年代になると、病院に通院し、お薬を飲むことが「どうも特別なことだ」とわかってきます。3歳すぎの「ねえねえ、なんでなんで？」といった疑問を親にぶつけてくる年代になると、特に自分に起こっていることが疑問の主題ともなり、親御さんは答えに窮することになるかもしれません。

子供の頃に正しい知識を教えられ、望ましい体験を積んでいることが、安定した大人になるためには必要だと言われます。誤魔化したり間違ったことで言い逃れるのではなく、そのときの子どもの年齢に応じ、理解できる言葉を使って本当のことを説明するよう心がけましょう。

幼年時期には病気になって治すためにしているということを中心に、学童期になれば病気の内容とその治療内容を中心に、青年期以降には病態も含めて伝えておくことが大事でしょう。

子どもに病気のことを教えるのに病名が大事なのではありません。今、子どもの体に何が起こっていて、どうしなければいけないのか、しなければどうなるのかといった事柄を正しく伝えて行きましょう。

小さな子どもは、親が手本であり、親が常に正しいことを言って行なっていると思っています。それは子どもから親への信頼の証であると言えるでしょう。ですから、親も子どもにしっかり向きあって伝えていくことがとても大事なことなのです。

Q:この病気で使える公費負担制度はありますか。

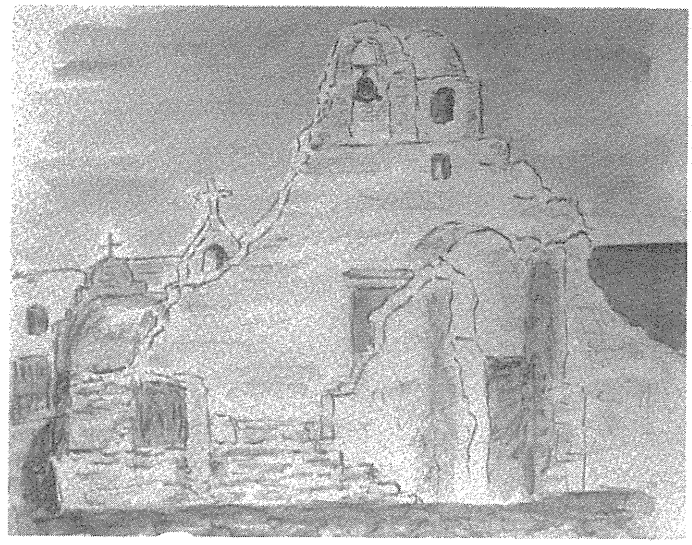
A:小児慢性特定疾患治療研究事業医療給付が、18歳以後20歳まで延長が可能です。成人以降の公的な給付制度はまだ確立されていません。

編集後記

これまでもCAHのご家族から、同じ病気をもつ子どものお父さん、お母さんと話ができないでしょうか、という問い合わせが幾度もありました。私たち医療グループはどのような形で患者さんとご家族のご希望に沿った活動ができるかと幾度も話し合いを重ねました。

その結果、まずは始めることだ、そして続けることだ、と2012年1月から「大阪母子センターCAHの会」を立ち上げたのです。これまで3回、このCAHの会を開き、いろいろの角度から話題を提供し、ご家族の意見もお聞きしました。そのなかでスタッフから、これまで話した内容を判りやすい読みものにしてはどうか、との提案があり作り上げたのがこの小冊子です。内容を読んで頂ければお分かりのように、CAHの子どもさんをもつご家族が抱いておられる心配や疑問にできるだけ答えようと、私たちグループがそれぞれの専門知識を持ち寄り書き上げ、編集しました。

これからも皆様のご意見やご質問、要望をどしどし寄せていただき、さらに版を重ねてより良い冊子が作られることを願っております。



27

執筆者（五十音順）

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この小冊子は、
平成 25 年度　厚生労働科学研究費補助金　難治性疾患克服研究事業「性分化疾患の実態把握と病態解明ならびに標準的診断・治療指針の作成」（分担研究者：島田憲次）の助成を受けて、企画・製作されました。

CAH の子どもをもつ家族のガイドブック

編集：　島田憲次
発行年月日：　平成 25 年　 3 月　 3 日
印刷：キクイ印刷工芸社　TEL072-956-6881

第46回 日本小児内分泌学会学術集会・特別企画

DSDセミナー in Osaka

～DSDをめぐる諸問題の解決をめざして～

開催日時：平成24年9月29日（土） 15：30-17：00
場　　所：大阪国際会議場 10階 1003会場

セミナー事務局長：大阪府立母子保健総合医療センター
消化器内分泌科 位田忍

司 会

島田 憲次　大阪府立母子保健総合医療センター 泌尿器科
三善 陽子　大阪大学大学院医学系研究科 小児科学

プログラム

1、性分化疾患（DSD）の考え方、とらえ方の移り変わり
　　島田 憲次
2、性分化疾患のマネージメント
　　堀川 玲子　国立成育医療研究センター 内分泌代謝科
3、性分化疾患の子どもと親への看護支援
　　石見 和世　大阪府立母子保健総合医療センター看護部
4、性分化疾患の子どもと親への思春期からの
　　セクシュアリティー支援
　　佐保 美奈子　大阪府立大学 地域保健学域 看護学類
5、フリーディスカッション

第2回 DSDセミナー in 大阪

DSD:Disorder of sex development(性分化疾患)

多職種の関わりを基盤にした支援を目指して

医師・看護師・助産師・保健師・遺伝カウンセラー・臨床心理士など
多職種の皆様のご参加をお待ちしています。

日時：平成25年(2013年)12月14日(土) 11:00－16:30(受付開始10:30)
場所：千里ライフサイエンスセンター 8階 801・802号室(地図裏面)
参加費：1000円(資料・昼食代を含む)

第1部 講演 11:00 - 13:00

座長 大阪府立母子保健総合医療センター 泌尿器科 島田憲次
大阪大学大学院医学系研究科小児科学 大薗恵一

1. 性分化疾患初期対応の手引き解説 — 大阪府立母子保健総合医療センター 消化器・内分泌科 位田 忍
2. 性の健康と権利 — 大阪府立大学 人間社会学域 東 優子
3. 性自認 — こども心身研究所(野瀬クリニック) 仲野 由季子
4. DSDの診断と治療の実際 — 自治医科大学とちぎ医療センター 泌尿器科 中井 秀郎

第2部 ケースカンファレンス 13:20 - 14:50

座長 大阪大学大学院医学系研究科小児科学 三善陽子
大阪府立母子保健総合医療センター 看護部 石見和世

1. 滋賀医科大附属病院
2. 佐賀大学医学部附属病院・長崎大学病院
3. 大阪府立母子保健総合医療センター

第3部 ワールド・カフェ 15:00 - 16:00

進行 大阪府立大学 地域保健学域 佐保美奈子

参加申し込み：11月30日までに下記までメールでお申し込みください。(先着90名)
dsd@mch.pref.osaka.jp

主 催：DSDセミナー in 大阪 実行委員会(代表 島田憲次、位田忍、大薗恵一)
事務局：大阪府立母子保健総合医療センター 看護部 石見和世 0725-56-1220(代)

Clinical Endocrinology (2013) doi: 10.1111/cen.12379

ORIGINAL ARTICLE

IMAGe syndrome: clinical and genetic implications based on investigations in three Japanese patients

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Summary

Objective Arboleda *et al.* have recently shown that IMAGe (intra-uterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita and genital abnormalities) syndrome is caused by gain-of-function mutations of maternally expressed gene *CDKN1C* on chromosome 11p15.5. However, there is no other report describing clinical findings in patients with molecularly studied IMAGe syndrome. Here, we report clinical and molecular findings in Japanese patients.

Patients We studied a 46,XX patient aged 8·5 years (case 1) and two 46,XY patients aged 16·5 and 15·0 years (cases 2 and 3).

Results Clinical studies revealed not only IMAGe syndrome-compatible phenotypes in cases 1–3, but also hitherto undescribed findings including relative macrocephaly and apparently normal pituitary-gonadal endocrine function in cases 1–3, familial glucocorticoid deficiency (FGD)-like adrenal phenotype and the history of oligohydramnios in case 2, and arachnodactyly in case 3. Sequence analysis of *CDKN1C*, pyrosequencing-based methylation analysis of KvDMR1 and high-density oligonucleotide array comparative genome hybridization analysis for chromosome 11p15.5 were performed, showing an identical *de novo* and maternally inherited *CDKN1C* gain-of-function mutation (p.Asp274Asn) in cases 1 and 2, respectively, and no demonstrable abnormality in case 3.

Conclusions The results of cases 1 and 2 with *CDKN1C* mutation would argue the following: [1] relative macrocephaly is consistent with maternal expression of *CDKN1C* in most tissues and biparental expression of *CDKN1C* in the foetal brain; [2] FGD-like phenotype can result from *CDKN1C* mutation; and [3] genital abnormalities may primarily be ascribed to placental dysfunction. Furthermore, lack of *CDKN1C* mutation in case 3 implies genetic heterogeneity in IMAGe syndrome.

(Received 1 October 2013; returned for revision 24 November 2013; finally revised 26 November 2013; accepted 29 November 2013)

Introduction

IMAGe syndrome is a multisystem developmental disorder named by the acronym of intra-uterine growth restriction (IUGR), metaphyseal dysplasia and adrenal hypoplasia congenita common to both 46,XY and 46,XX patients, and genital abnormalities specific to 46,XY patients.[1] In addition to these salient clinical features, hypercalciuria has been reported frequently in IMAGe syndrome.[1,2] This condition occurs not only as a sporadic form but also as a familial form.[1–3] Furthermore, transmission analysis in a large pedigree has revealed an absolute maternal inheritance of this condition, indicating the relevance of a maternally expressed gene to the development of IMAGe syndrome.[3]

Subsequently, Arboleda *et al.*[4] have mapped the causative gene to a ~17·2-Mb region on chromosome 11 by an identity-by-descent analysis in this large pedigree and performed targeted exon array capture and high-throughput genomic sequencing for this region in the affected family members and in other sporadic patients. Consequently, they have identified five different missense mutations in the maternally expressed gene *CDKN1C* (cyclin-dependent kinase inhibitor 1C) that resides on the imprinting control region 2 (ICR2) domain at chromosome 11p15.5 and encodes a negative regulator for cell proliferation.[4–6] Notably, all the missense mutations are clustered within a specific segment of PCNA-binding domain, and functional studies have implicated that these mutations have gain-of-function effects.[4] Thus, IMAGe syndrome appears to constitute a mirror image of Beckwith–Wiedemann syndrome (BWS) in terms of the

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CDKN1C function, because multiple *CDKN1C* loss-of-function mutations have been identified in BWS with no mutation shared in common by IMAGe syndrome and BWS.[4,5]

However, several matters remain to be clarified in IMAGe syndrome, including phenotypic spectrum and underlying mechanism(s) for the development of each phenotype in *CDKN1C*-mutation-positive patients, and the presence or absence of genetic heterogeneity. Here, we report clinical and molecular findings in three patients with IMAGe syndrome and discuss these unresolved matters.

Patients and methods

Patients

We studied one previously described 46,XX patient (case 1)[7] and two hitherto unreported 46,XY patients (cases 2 and 3). In cases 1–3, no pathologic mutations were identified in the coding exons and their splice cites of *NR5A1* (*SF1*) and *NR0B1* (*DAX1*) relevant to adrenal hypoplasia,[8] and *MC2R*, *MRAP*, *STAR* and *NNT* involved in familial glucocorticoid deficiency (FGD).[9]

Ethical approval and samples

This study was approved by the Institutional Review Board Committee at Hamamatsu University School of Medicine. Molecular studies were performed using leucocyte genomic DNA samples of cases 1–3 and the parents of cases 1 and 2, after obtaining written informed consent.

Sequence analysis of CDKN1C

The coding exons 1 and 2 and their flanking splice sites were amplified by polymerase chain reaction (PCR) (Fig. 1a), using primers shown in Table S1. Subsequently, the PCR products were subjected to direct sequencing from both directions on ABI 3130 autosequencer (Life Technologies, Carlsbad, CA, USA). In this regard, if a nucleotide variation were present within the primer-binding site(s), this may cause a false-negative finding because of amplification failure of a mutation-positive allele. Thus, PNCA-binding domain was examined with different primer sets. To confirm a heterozygous mutation, the corresponding PCR products were subcloned with TOPO TA Cloning Kit (Life Technologies), and normal and mutant alleles were sequenced separately.

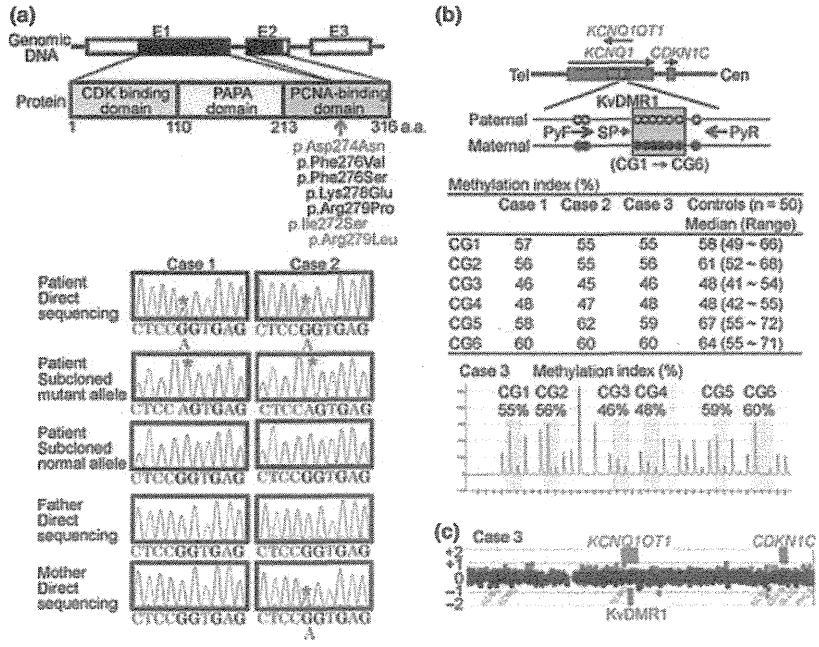

Fig. 1 Summary of molecular studies. (a) Sequence analysis of *CDKN1C*. *CDKN1C* consists of three exons (E1–E3), and the black and white boxes denote the coding regions and the untranslated regions, respectively. CDKN1C protein is composed of 316 amino acids and contains CDK binding domain, PAPA domain and PCNA-binding domain. The p.Asp274Asn mutation found in this study and the previous study[4] is shown in red. The four mutations written in black have also been identified in IMAGe syndrome.[4] The pIle272Ser mutation written in green has been detected in atypical IMAGe syndrome lacking skeletal lesion,[22] and the pArg279Leu mutation written in blue has been found in SRS.[24] Electrochromatograms denote a *de novo* p.Asp274Asn mutation in case 1 and a maternally inherited p.Asp274Asn mutation in case 2. (b) Methylation analysis of KvDMR1 at the ICR2 domain. The cytosine residues at the CpG dinucleotides are unmethylated after paternal transmission (open circles) and methylated after maternal transmission (filled circles). *KCNQ1OT1* is a paternally expressed gene, and *KCNQ1* and *CDKN1C* are maternally expressed genes. The six CpG dinucleotides (CG1→CG6) examined by pyrosequencing are highlighted with a yellow rectangle, and the positions of PyF & PyR primers and SP are shown by thick arrows and a thin arrow, respectively. A pyrogram of case 3 is shown. (c) Array CGH analysis for chromosome 11p15.5 encompassing the ICR2 domain in case 3. A region encompassing KvDMR1 and *CDKN1C* is shown. Black, red and green dots denote signals indicative of the normal, the increased (>+0·5) and the decreased (<−1·0) copy numbers, respectively. Although several red and green signals are seen, there is no portion associated with ≥3 consecutive red or green signals.

Methylation analysis of KvDMR1 and array CGH analysis for chromosome 11p15.5

Increased expression of *CDKN1C*, as well as gain-of-function mutations of *CDKN1C*, may lead to IMAGe syndrome. Such increased *CDKN1C* expression would occur in association with hypermethylated KvDMR1 (differentially methylated region 1) at the ICR2 domain, because *CDKN1C* is expressed when the *cis*-situated KvDMR1 is methylated as observed after maternal transmission and is repressed when the *cis*-situated KvDMR1 is unmethylated as observed after paternal transmission.[5] Thus, we performed pyrosequencing analysis for six CpG dinucleotides (CG1–CG6) within KvDMR1, using bisulphite-treated leucocyte genomic DNA samples (Fig. 1b). In brief, a 155-bp region was PCR-amplified with a primer set (PyF and PyR) for both methylated and unmethylated clones, and a sequence primer (SP) was hybridized to single-stranded PCR products (for PyF, PyR and SP sequences, see Table S1). Subsequently, methylation index (MI, the ratio of methylated clones) was obtained for each CpG dinucleotide, using PyroMark Q24 (Qiagen, Hilden, Germany). To define the reference ranges of MIs, 50 control subjects were similarly studied with permission.

Increased *CDKN1C* expression may also result from a copy number gain of the maternally inherited ICR2 domain. Thus, we performed high-density array CGH (comparative genomic hybridization) using a custom-build 33 088 oligonucleotide probes for chromosome 11p15.5 encompassing the ICR2 domain, together with ~10 000 reference probes for other chromosomal regions (Agilent Technologies, Santa Clara, CA, USA). The procedure was carried out as described in the manufacturer's instructions.

Results

Clinical findings

Detailed clinical findings are shown in Table 1. Cases 1–3 exhibited characteristic faces with frontal bossing, flat nasal root, low set ears and mild micrognathia, as well as short limbs. They had IUGR and postnatal growth failure. Notably, while birth and present length/height and weight were severely compromised, birth and present occipitofrontal circumference (OFC) were relatively well preserved. Radiological examinations revealed generalized osteopenia, delayed bone maturation and metaphyseal dysplasia with vertical sclerotic striations of the knee in cases 1–3, slender bones in cases 1 and 2, scoliosis in cases 2 and 3, arachnodactyly in case 3 and broad distal phalanx of the thumbs and great toes in case 2 (Fig. 2). Cases 1 and 3 experienced adrenal crisis in early infancy and received glucocorticoid and mineralocorticoid supplementation therapy since infancy. Case 2 had transient neonatal hyponatremia and several episodes of hypoglycaemia without electrolyte abnormality in childhood and was found to have hypoglycaemia and hyponatremia without hyperkalemia when he had severe viral gastroenteritis at 15·5 years of age. Thus, an adrenocorticotropic hormone stimulation test was performed after recovery from gastroenteritis, revealing poor cortisol response. Thereafter, he was placed on glucocorticoid supplementation therapy. As serum electrolytes were normal, mineralocorticoid supplementation therapy was not initiated. Genital abnormalities included cryptorchidism and small testes in cases 2 and 3, and hypospadias in case 3. However, pituitary-gonadal endocrine function was apparently normal in cases 1–3. Urine calcium secretion was borderline high or increased in cases 1–3, although serum calcium and calcium homeostasis-related factors were normal. In addition, feeding difficulties during infancy were observed in cases 1 and 2, but not in case 3, and oligohydramnios was noticed during the pregnancy of case 2. There was no body asymmetry in cases 1–3. Thus, clinical studies in cases 1–3 revealed not only IMAGe syndrome-compatible phenotypes, but also hitherto undescribed clinical finding (Table 2).

Sequence analysis of CDKN1C

A heterozygous identical missense mutation (c.820G>A, p.Asp274Asn) was identified in cases 1 and 2 (Fig. 1a). This mutation occurred as a *de novo* event in case 1 and was inherited from the phenotypically normal mother in case 2. No demonstrable mutation was identified in case 3.

Methylation analysis of KvDMR1 and array CGH analysis for chromosome 11p15.5

The MIs for CG1–CG6 were invariably within the normal range in cases 1–3 (Fig. 1b), and no discernible copy number alteration was identified in cases 1–3 (Fig. 1c). The results excluded maternal uniparental disomy involving KvDMR1, hypermethylation (epimutation) of the paternally inherited KvDMR1 and submicroscopic duplication involving the maternally derived ICR2 domain, as well as submicroscopic deletion affecting the paternally derived ICR2 domain.

Discussion

CDKN1C mutations in IMAGe syndrome

We identified a heterozygous *CDKN1C* missense mutation (Asp274Asn) in cases 1 and 2. This mutation has previously been detected in a patient with IMAGe syndrome.[4] Furthermore, *de novo* occurrence of the mutation in case 1 argues for the mutation being pathologic, and maternal transmission of the mutation in case 2 is consistent with *CDKN1C* being a maternally expressed gene. Thus, our results provide further evidence for specific missense mutations of *CDKN1C* being responsible for the development of IMAGe syndrome.

Clinical features in CDKN1C*-mutation-positive cases 1 and 2*

Several matters are noteworthy with regard to clinical findings in *CDKN1C*-mutation-positive cases 1 and 2. First, although

Table 1. Clinical findings of cases 1–3

	Case 1*	Case 2	Case 3
Karyotype	46,XX	46,XY	46,XY
Present age (year)	8·5	16·5	15·0
Characteristic face	Yes	Yes	Yes
Pre- and postnatal growth			
Gestational age (week)	35	37	38
Birth length (cm) (SDS)	37·0 (−3·5)	40·0 (−4·0)	41·0 (−4·3)
Birth weight (kg) (SDS)	1·34 (−2·9)	2·03 (−3·5)	1·71 (−3·4)
Birth OFC (cm) (SDS)	30·7 (−0·3)	32·0 (−0·9)	33·0 (−0·1)
Birth BMI (kg/m^2) (percentile)	9·8 (<3)	12·7 (50)	10·1 (<3)
BMI (kg/m^2) at 2 years of age (SDS)	14·2 (−1·8)	13·0 (−3·4)	Unknown
Present height (cm) (SDS)	92·8 (−6·2)	124·7 (−7·8)	135·2 (−5·1)
Present weight (kg) (SDS)	16·0 (−1·9)	25·4 (−3·5)	30·4 (−2·6)
Present OFC (cm) (SDS)	52·0 (−0·2)	53·0 (−2·5)	Unknown
Present BMI (kg/m^2) (SDS)	18·6 (+1·6)	16·3 (−2·6)	16·6 (−1·7)
Skeletal abnormality			
Examined age (year)	5·5	16·5	15·0
Generalized osteopenia	Yes	Yes	Yes
Delayed maturation	Yes	Yes	Yes
Metaphyseal dysplasia	Yes	Yes	Yes
Slender bones	Yes	Yes	No
Scoliosis	No	Yes	Yes
Arachnodactyly	No	No	Yes
Broad thumbs & big toes	No	Yes	No
Adrenal dysfunction			
Examined age (year) before therapy	0·1 (39 days)	15·5	0·5 (6 months)
MRI/CT	Undetectable	Undetectable	Undetectable
ACTH (pg/ml)	9010 [19·9 ± 8·8]	427 [22·9 ± 6·2]	>1000 [22·9 ± 6·2]
Cortisol (μg/dl)	8·4 [8·3 ± 3·4]	6·9 [9·5 ± 2·9]	<1·0 [9·5 ± 2·9]
After ACTH stimulation†	N.E.	9·4 [> 20]	<1·0 [> 20]
Plasma renin activity (ng/ml/h)	N.E.	6·0 [1·0 ± 0·1]	>25 [1·01 ± 0·14]
Active renin concentration (pg/ml)	21 400 [2·5–21·4]	N.E.	N.E.
Aldosterone (ng/dl)	6·9 [9·7 ± 4·5]	5·2 [8·5 ± 1·4]	4·1 [7·4 ± 2·2]
Na (mEq/l)	122 [135–145]	141 (127‡) [135–145]	126 [135–145]
K (mEq/l)	8·0 [3·7–4·8]	4·2 (4·0‡) [3·7–4·8]	6·5 [3·7–4·8]
Cl (mEq/l)	86 [98–108]	103 (98‡) [98–108]	89 [98–108]
Glucocorticoid therapy	Yes (since 2 months)	Yes (since 15·5 years)	Yes (since 6 months)
Mineralocorticoid therapy	Yes (since 2 months)	No	Yes (since 6 months)
Genital abnormality			
Examined age (year)	8·5	16·5	15·0
Hypospadias	–	No	Yes (operated at 2 years)
Cryptorchidism	–	Yes (B) (operated at 2 years)	Yes (operated at 2 years)
Micropenis	–	No	No
Testis size (R & L) (ml)	–	5 & 8 [13–20]	4 & 10 [11–20]
Pubic hair (Tanner stage)	1 [10·0 ± 1·4 years]§	4 [14·9 ± 0·9 years]¶	4 [14·9 ± 0·9 years]¶
LH (mIU/ml)	<0·1 [<0·1–1·3]	3·9 [0·2–7·8]	4·8 [0·2–7·8]
After GnRH-stimulation**	3·5 [1·6–4·8]	N.E.	N.E.
FSH (mIU/ml)	0·7 [<0·1–5·4]	4·2 [0·3–18·4]	17·6 [0·3–18·4]
After GnRH-stimulation**	12·0 [10·7–38·1]	N.E.	N.E.
Testosterone (ng/ml)	–	4·3 [1·7–8·7]	3·7 [1·7–8·7]
Calcium metabolism			
Examined age (year)	8·5	16·5	15·0
Calcium (mg/dl)	9·7 [8·8–10·5]	9·2 [8·9–10·6]	9·8 [8·9–10·6]
Inorganic phosphate (mg/dl)	3·9 [3·7–5·6]	4·6 [3·1–5·0]	3·8 [3·2–5·1]
Alkaline phosphatase (IU/l)	458 [343–917]	623 [225–680]	309 [225–680]
Intact PTH (pg/ml)	23 [10–65]	43 [10–65]	28 [10–65]
PTHrP (pmol/l)	N.E.	<1·1 [<1·1]	N.E.

(continued)

Clinical Endocrinology (2013), **0**, 1–8

Table 1. (continued)

	Case 1*	Case 2	Case 3
$1,25(OH)_2$ vitamin D (pg/ml)	50 [13–79]	67 [13–79]	50 [13–79]
Urine calcium/creatinine ratio (mg/mg)	0·82 [<0·25]	0·24 [<0·25]	0·44 [<0·25]
%TRP	92 [80–96]	95 [80–96]	94 [80–96]
Others	Feeding difficulties	Feeding difficulties Oligohydramnios	

SDS, standard deviation score; OFC, occipitofrontal circumference; BMI, body mass index; MRI, magnetic resonance imaging; CT, computed tomography; ACTH, adrenocorticotropic hormone; R, right; L, left; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; PTH, parathyroid hormone; PTHrP, PTH-related protein; TRP, tubular reabsorption of phosphate; N.E., not examined; and B, bilateral.
Biochemical values indicate basal blood values, except for those specifically defined.
Birth and present length/height, weight, OFC and BMI have been assessed by sex- and gestational- or age-matched Japanese reference data reported in the literature[26,27] and in the Ministry of Health, Labor, and Welfare Database (http://www.e-stat.go.jp/SG1/estat/GL02020101.do).
The values in brackets represent age- and sex-matched reference values in Japanese children.[28]
The conversion factor to the SI unit: 0·220 for ACTH (pmol/l), 27·6 for cortisol (nmol/l), 0·028 for aldosterone (nmol/l), 3·46 for testosterone (nmol/l), 0·25 for calcium (nmol/l), 0·323 for inorganic phosphate (nmol/l), 0·106 for intact PTH (pmol/l), 2·40 for $1,25(OH)_2$ vitamin D (pmol/l) and 1·0 for plasma renin activity (μg/l/h), active renin concentration (ng/l), Na (nmol/l), K (nmol/l), Cl (nmol/l), LH (IU/l), FSH (IU/l), alkaline phosphatase (IU/l) and PTHrP (pmol/l).
*Clinical findings before 3 years of age have been reported previously.[7]
†ACTH 0·25 mg bolus i.v.; blood sampling at 60 min.
‡Electrolyte values at the time of severe gastroenteritis; other biochemical data in reference to adrenal dysfunction were obtained after recovery from gastroenteritis and before glucocorticoid supplementation therapy.
§Reference age for Tanner stage 2 breast development in Japanese girls.[29]
¶Reference age for Tanner stage 4 pubic hair development in Japanese boys.[29]
**GnRH 100 μg/m^2 bolus i.v.; blood sampling at 0, 30, 60, 90, and 120 min.

Table 2. Summary of clinical features of cases 1–3

	Case 1	Case 2	Case 3
CDKN1C mutation	Yes	Yes	No
Previously reported IMAGe syndrome-compatible phenotype			
IUGR	Yes	Yes	Yes
Metaphyseal dysplasia	Yes	Yes	Yes
Adrenal hypoplasia	Yes*	Yes*	Yes*
Genital abnormality	(Female)	Yes	Yes
Hypercalciura†	Yes	No	Yes
Hitherto undescribed findings			
Body habitus	Relative macrocephaly	Relative macrocephaly	Relative macrocephaly
Skeletal			Arachnodactyly Lack of slender bones
Adrenal		FGD-like phenotype with no obvious mineralocorticoid deficiency	
Genital	Apparently normal pituitary-gonadal endocrine function	Apparently normal pituitary-gonadal endocrine function	Apparently normal pituitary-gonadal endocrine function
Others	Feeding difficulties	Feeding difficulties Oligohydramnios	

IUGR, intrauterine growth retardation; and FGD, familial glucocorticoid deficiency.
*Undetectable on magnetic resonance imaging and/or computed tomography.
†Frequent but not invariable feature.

pre- and postnatal body growth was severely impaired, pre- and postnatal OFC was relatively well preserved. In this regard, while *CDKN1C* is preferentially expressed from the maternal allele in most tissues, it is biparentally expressed at least in the foetal brain.[10] This expression pattern would be relevant to the relative macrocephaly in IMAGe syndrome. Notably, the combination of severely compromised body growth and well-preserved OFC is also characteristic of Silver–Russell syndrome (SRS) resulting

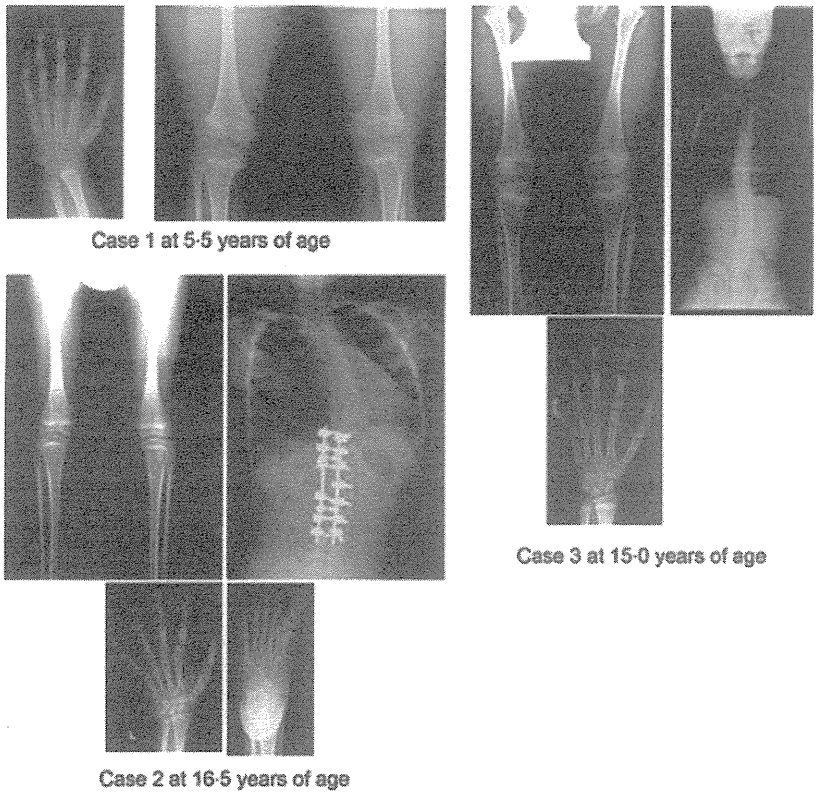

Fig. 2 Representative skeletal roentgenograms in cases 1–3.

from *H19*-DMR hypomethylation (epimutation),[11] and this is primarily consistent with paternal expression of the growth-promoting gene *IGF2* in the body and biparental expression of *IGF2* in the brain.[12] Thus, loss-of-imprinting in the brain tissue appears to underlie relative macrocephaly in both IMAGe syndrome and SRS.

Second, skeletal abnormalities including metaphyseal dysplasia were identified in cases 1 and 2. In this regard, skeletal phenotype of mice lacking *Cdkn1c* is grossly opposite of parathyroid hormone-related protein (PTHrP)-null phenotype,[13,14] and PTHrP permits skeletal development at least in part by suppressing *Cdkn1c* expression.[15] Thus, while serum calcium and calcium homeostasis-related factors were normal in cases 1 and 2, dysregulated PTHrP and/or PTH/PTHrP receptor signalling might be relevant to skeletal abnormalities in patients with gain-of-function mutations of *CDKN1C*. In addition, such a possible signalling defect might also be relevant to the frequent occurrence of hypercalciuria in IMAGe syndrome.

Third, adrenal dysfunction was mild in case 2, while case 1 experienced adrenal crisis in infancy as previously reported in patients with *CDKN1C* mutations.[1,3,4] Indeed, adrenal phenotype of case 2 is similar to that of patients with FGD rather than adrenal hypoplasia.[8,9] Our results therefore would expand the clinical spectrum of adrenal dysfunction in patients with *CDKN1C* mutations. For adrenal dysfunction, cortisol and aldosterone values remained within the normal range at the time of adrenal crisis in case 1 (Table 1). However, as adrenocorticotropic hormone and active renin concentrations were markedly increased, the overall results would be consistent with primary hypoadrenalism, as has been described previously.[16] This notion would also apply to the adrenal dysfunction in case 3 who had apparently normal aldosterone value and markedly increased plasma renin activity at the time of adrenal crisis.

Lastly, although male case 2 had bilateral cryptorchidism and small testes, pituitary-gonadal endocrine function was apparently normal as was secondary sexual development. Previously reported patients with *CDKN1C* mutations, as well as those who have not been examined for *CDKN1C* mutations, also have undermasculinized external genitalia in the presence of apparently normal endocrine function and pubertal development.[1–4,17,18] Notably, an episode of oligohydramnios was found in case 2 and has also been described in a 46,XY IMAGe syndrome patient with cryptorchidism.[19] This may imply the presence of placental hypoplasia and resultant chorionic gonadotropin deficiency as an underlying factor for genital anomalies.[11] In support of this notion, imprinted genes are known to play a pivotal role in body and placental growth,[20] and SRS is often associated with oligohydramnios, placental hypoplasia and undermasculinization.[11,21]

Genetic heterogeneity in IMAGe syndrome

Molecular data in case 3 imply the presence of genetic heterogeneity in IMAGe syndrome. Indeed, there was neither demonstrable *CDKN1C* mutation nor evidence for increased *CDKN1C* expression, while a pathologic mutation leading to gain-of-function or

increased expression of *CDKN1C* might reside on an unexamined region(s) such as promoter or enhancer sequences. In this regard, while case 3 showed IMAGe syndrome-compatible clinical features such as IUGR, metaphyseal dysplasia, adrenal hypoplasia and genital abnormalities, case 3 lacked slender bones and had arachnodactyly, in contrast to *CDKN1C*-mutation-positive cases 1 and 2. Such mild but discernible phenotypic variation might reflect the genetic heterogeneity. This matter might be clarified in the future by extensive studies such as exome or whole-genome sequencing. In particular, when such *CDKN1C*-mutation-negative patients with IMAGe syndrome-compatible phenotype have been accumulated, a novel gene(s) mutated in such patients may be identified. In this regard, if such a gene(s) exist, it is predicted to reside in the signal transduction pathway involving *CDKN1C*.

Relevance of CDKN1C mutations to atypical IMAGe syndrome and SRS

CDKN1C mutations have also been identified in atypical IMAGe syndrome and SRS (Fig. 1a). Hamajima *et al.* revealed a maternally inherited p.Ile272Ser mutation in three siblings (two males and one female) who manifested IUGR and adrenal insufficiency, and male genital abnormalities, but had no skeletal lesion.[22] Similarly, Brioude *et al.* found a maternally transmitted p.Arg279Leu mutation in six relatives (all females) from a four-generation family who satisfied the SRS diagnostic criteria,[23,24] after studying 97 SRS patients without known causes of SRS, that is, hypomethylation (epimutation) of the *H19*-DMR, duplication of the ICR2 and maternal uniparental disomy for chromosome 7 (upd(7)mat).[24] Notably, although both mutations had no significant effect on a cell cycle, they were associated with increased protein stability that appears to be consistent with the gain-of-function effects.[22,24] Such increased stability was also found for IMAGe-associated missense mutant proteins,[22] and an altered cell cycle with a significantly higher proportion of cells in the G1 phase was shown for an IMAGe-associated p.Arg279Pro mutation.[24] It is possible therefore that relatively severe *CDKN1C* gain-of-function effects lead to IMAGe syndrome and relatively mild *CDKN1C* gain-of-function effects result in SRS, with intermediate *CDKN1C* gain-of-function effects being associated with atypical IMAGe syndrome.[24]

Thus, it would not be surprising that cases 1–3 also met the SRS diagnostic criteria (Table 3).[23,24] Indeed, cases 1–3, as well as *CDKN1C*-mutation-positive SRS patients,[24] exhibited pre- and post-natal growth failure with relative macrocephaly and frequently manifested feeding difficulties and/or low body mass index (BMI) at two years of age. However, while relative macrocephaly is usually obvious at birth in SRS patients with *H19*-DMR epimutations and upd(7)mat,[21,23,25] it is more obvious at 2 years of age than at birth in *CDKN1C*-mutation-positive SRS patients.[24] Furthermore, *CDKN1C*-mutation-positive SRS patients are free from body asymmetry,[24] as are typical and atypical IMAGe syndrome patients described in this study and in the previous studies.[1–4,7,22] Thus, SRS caused by *CDKN1C* mutations may be characterized by clinically discernible macrocephaly at two years of age and lack of body asymmetry.

Table 3. Silver–Russell syndrome phenotypes in cases 1–3 and in affected relatives reported by Brioude *et al.*

	Case 1	Case 2	Case 3	Brioude *et al.**
Mandatory criteria				
IUGR†	Yes	Yes	Yes	4/4
Scoring system criteria				
Postnatal short stature (≤−2 SDS)	Yes	Yes	Yes	4/4
Relative macrocephaly‡	Yes	Yes	Yes	4/4§
Prominent forehead during early childhood	Yes	Yes	Yes	4/4
Body asymmetry	No	No	No	0/4
Feeding difficulties during early childhood and/or low BMI (<−2·0 SDS) around 2 years of age	Yes	Yes	Unknown	3/4 (1/4 & 2/4)¶

IUGR, Intrauterine growth retardation; SDS, standard deviation score; and BMI, body mass index.
The SRS diagnostic criteria proposed by Netchine *et al.*[23] and Brioude *et al.*[24] (low BMI around 2 years of age is included in Brioude *et al.*, but not in Netchine *et al.*): The diagnosis of SRS is made, when mandatory criteria plus at least three of the five scoring system criteria are observed. For detailed clinical features in cases 1–3, see Table 1.
*While six relatives were found to have *CDKN1C* mutation, detailed clinical features have been obtained in four mutation-positive relatives.[24]
†Birth length and/or birth weight ≤−2 SDS for gestational age.
‡SDS for birth length or birth weight minus SDS for birth occipitofrontal circumference ≤−1·5.
§Relative macrocephaly is more obvious at 2 years of age (4/4) than at birth (2/4).
¶One patient is positive for feeding difficulties, and other two patients are positive for low BMI.

Conclusion

In summary, we studied three patients with IMAGe syndrome. The results provide implications for phenotypic spectrum, underlying factor(s) in the development of each phenotype and genetic heterogeneity in IMAGe syndrome, as well as a phenotypic overlap between IMAGe syndrome and SRS. Further studies will permit to elucidate such matters.

Funding

This study was supported in part by Grants-in-Aid for Scientific Research (A) (25253023) and for Scientific Research on Innovative Areas (22132004-A01) from the Ministry of Education, Culture, Sports, Science and Technology, by Grant for Research on Intractable Diseases from the Ministry of Health, Labor and Welfare (H24-048), and by Grants from National Center for Child Health and Development (23A-1, 24-7 and 25-10).

Declaration of interest

The authors have nothing to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primers utilized in this study.