2004；5：273-281
9) 中田洋二郎，上林靖子，藤井和子，ほか．障害の告知に親が求めるもの―発達障害，児の母親のアンケート調査から―．小児の精神と神経 1997；37：187-196
10) 長谷川知子．先天異常医療におけるインフォームド・コンセント．小児内科 1994；26：549-554
11) 玉井真理子．「障害」の告知の実態―母親に対する質問紙調査の結果および事例的考察―．発達障害研究 1993；15：223-229
12) 秋山泰子．発達障害児（者）のライフサイクル計画，有馬正高．熊谷公明（編），発達障害医学の進歩 No.7，診断と治療社，1995，pp1-7
13) T.Matsumoto, T.Kondoh, N.Niikawa, et al. A genetic counseling system in Nagasaki prefecture：The course and current status of the Genetic Counseling Unit in Nagasaki University Hospital. Acta Med Nagasaki 2001；46：7-10
14) T.Kondoh, T.Matsumoto, S.Izumi, et al. Genetic Counseling System in Nagasaki University Hospital and Genetic Services in Nagasaki Prefecture - Now and Future. Clin Pediatr Endocrinol 2002；11：1-7
15) 近藤達郎，和泉志津子，松本正，ほか．我が国における遺伝医療に関する状況及び長崎大学医学部附属病院遺伝カウンセリング室の現状．長崎県小児科医会会報 2003；97-105
16) 近藤達郎，石丸忠之．患者・家族への心理的・社会的サポート―遺伝カウンセリング室における患者・家族へのサポート―．病院 2003；62：300-303
17) 土井知己，近藤達郎，船越康智，ほか．ダウン症候群児・者を取り巻く環境の変移．日本遺伝カウンセリング学会誌 2004；25：81-87
18) 近藤達郎．長崎県における遺伝カウンセリングの現状と問題点．純心人文研究 2005；11：201-208
19) 近藤達郎．遺伝カウンセリングの現状と問題点（長崎での試みを中心として）．日本遺伝看護学会誌 2006；4：43-48
20) 玉井真理子，加部一彦．本邦におけるダウン症の告知をめぐる現状と課題 第1報 患児の親を対象にした全国調査より明らかになった告知の早期化傾向．日本周産期・新生児医学会雑誌 1995；31-2：310-317
21) 玉井真理子，加部一彦．本邦におけるダウン症の告知をめぐる現状と課題 第2報 告知の際の「父母同席」に関する検討．日本周産期・新生児医学会雑誌 1995；31-2：318-321

How should we explain to parents the need for chromosomal tests and notify their results? －Implications for the desirable way of notification and counseling from questionnaire investigations

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To understand current situation about explanation of the need for chromosomal tests and notification of their results to parents who have children with chromosomal aberrations, questionnaire investigation was performed to the parents, pediatricians and obstetricians. More than 50% of the parents were not satisfied with explanation and notification that they received. However, parents with younger patients tend to have greater satisfaction with them than those with elder ones. There is a great gap between the current status and an idealized image of notification and counseling, so is between pediatricians' and obstetricians' views. While many obstetricians considered that definite diagnosis be done before 22 weeks of gestational age, many pediatricians did not regard the timing of diagnosis as important. It is thought that the doctors involved should have sufficient knowledge about the natural history and social circumstances of the respective patients with chromosomal aberration in order to provide proper explanation, notification and counseling to patients' families. Furthermore, the organization of a team consisting of specialists in various fields is essential for establishment of an desirable support system.

日本小児科学会雑誌　116巻8号　1239～1243（2012年）

原　著

塩酸ドネペジル療法により日常生活能力と成長率の改善がみられたDown症候群の1例

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要　旨

ダウン症候群（DS）患者は，加齢と伴にアルツハイマー型認知症（AD）発症頻度が増える一方で，20歳前後をピークとして日常生活能力が比較的短期間に衰退（急激退行）することがある．さらに10歳未満のDS患児でコリン作動性の低下によると思われる排尿障害も合併する．DS患者にみられるこれらの病態に対して，アセチルコリンエステラーゼ阻害剤である塩酸ドネペジルの有効性が示されているが，これまで幼児への使用報告例はなかった．今回，我々は強い拒食で食事摂取が困難になり，種々の治療で改善を認めなかった5歳のDS患児に塩酸ドネペジル療法を行い，良好な結果を得た．本児においては日常生活能力の改善のみならず，血中インスリン様成長因子1（IGF-1）誘導による成長率の改善も示唆された．本例は，DS患者はAD発症には早すぎる幼児期にも急激退行を認めること，またそのような状況で塩酸ドネペジルが有効であることから，コリン作動性の一過性・可逆的障害が病態に関与することを臨床的に強く示唆する貴重な症例であると思われる．

キーワード：ダウン症候群，退行，日常生活能力，塩酸ドネペジル，アルツハイマー型認知症

はじめに

ダウン症候群（DS）患者は，加齢と伴にアルツハイマー型認知症（AD）に見られる神経病理学的変化が増加する[1]一方で，20歳前後をピークとして日常生活能力が比較的短期間に衰退（急激退行）することがある．さらに10歳未満のDS患児でコリン作動性の低下によると思われる排尿障害も合併するとされる[2]．DS患者の退行症状に対してアセチルコリンエステラーゼ阻害剤である塩酸ドネペジルの有効性が示されているが[3][4]，これまで幼児への使用報告例はみられない．今回，我々は強い拒食で食事摂取が困難になり，種々の治療で改善を認めなかった5歳のDS患児に塩酸ドネペジル療法を行い良好な結果を得たので報告する．

症　例

症例は5歳男児．妊娠37週5日，2,340gにて出生した．Apgarスコアは，1分9点，5分10点で仮死なく出生した．生下時，心雑音があり，筋緊張低下，特異顔貌などよりDSを疑い，染色体検査にて21トリソミーを確認した．心エコーにて，房室中隔欠損を認め，生後5か月時に根治術施行．中耳炎に頻回に罹患し，鼓膜チューブ留置歴あり．肺炎で数回の入院歴あり．

乳児期より体重はDS患児発育曲線の－2SD前後で推移していたが，平成21年9月中旬より原因不明の食欲低下による体重減少，活動性低下，強固な便秘で1週間に1回程度の排便状況であった．5歳6か月時に当科外来受診し，緩下剤や浣腸で排便コントロールを行ったが食欲の改善なく，4日後には水分摂取も不可能となり，その翌日に拒食，体重減少，活動性低下に関しての精査目的にて入院となった．

入院時の身体所見は，身長96.2cm（－2.9SD，DS身長曲線－1.3SD），体重11.7kg（－2.4SD，DS体重曲線－2.2SD），体温35.9℃，心拍数74回/分，血圧84/46 mmHg．意識清明であったが，倦怠様表情で活気なく発語もなかった．心音呼吸音は異常なく，腹部は平坦，軟で肝脾腫なし．ツルゴールは軽度低下していたが，末梢循環不全は認めなかった．血液・尿検査では，表1に示すように軽度のBUN上昇と代謝性アシドーシスを認めた．またfT4，fT3の低下を認めたが，TSHの上昇は認めなかった．頭部MRIや心臓超音波等の画像検索を行ったが特記所見は認めなかった．

入院後の臨床経過を図1に示す．入院時より甲状腺ホルモン補充を開始したが，甲状腺値が正常化しても

（平成23年4月14日受付）（平成24年1月25日受理）
別刷請求先：（〒857-8511）佐世保市平瀬町9—3
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表1　血液検査所見

＜血球検査＞		＜生化学＞		＜検尿＞	
WBC	3.4×10^3 /ul	AST	45 IU/l	比重	1.022
RBC	4.34×10^6 /ul	ALT	33 IU/l	蛋白	(−)
Hb	13.6 g/dl	LDH	193 IU/l	糖	(−)
Plt	204×10^3 /ul	BUN	22.9 mg/dl	ケトン体	(2+)
		Cr	0.51 mg/dl	潜血	(−)
＜血液ガス＞		Na	135 mEq/l	赤血球	1～2 /F
pH	7.331	K	5.3 mEq/l	白血球	1～2 /F
pCO2	31.2 mmHg	Cl	95 mEq/l		
pO2	32.5 mmHg	CRP	0.3 mg/dl		
HCO3	16.0 mmol/l				
BE	−8.4	＜内分泌＞			
		TSH	1.20 uIU/ml		
		fT4	0.62 ng/dl		
		fT3	1.10 pg/ml		

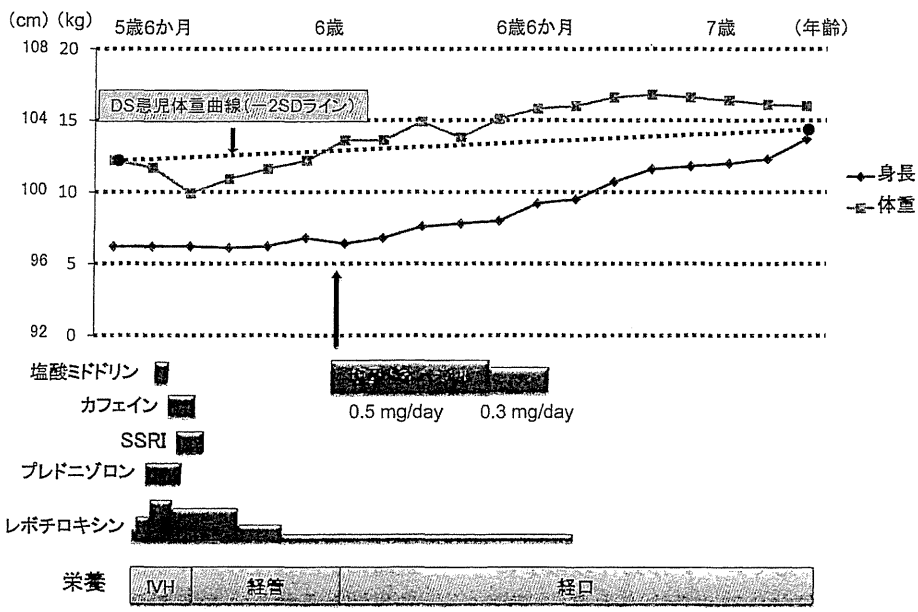

図1　経過表

入院時より甲状腺ホルモン，選択的セロトニン再取り込み阻害薬（SSRI），塩酸ミドドリン，カフェインを投与したが，効果は得られなかった．栄養維持のために高カロリー輸液，経管栄養を併用し，体重はやや増加傾向となったが経口摂取困難は持続した．入院4か月後に塩酸ドネペジル0.5mg/日（1日1回）を開始し，10日目頃から経口摂取可能となった．体重は増加傾向となり，周囲への興味の改善や表情・感情の表出を認め，投与開始後2週間で退院できた．その後徐々に活動性が増し，多動とも思える程の状況が見られたため内服開始5か月目に減量中止とした．内服中止後も体重は維持でき，身長は増加傾向である．

症状の改善は認めず，さらに当初内服できていた薬剤も受け付けなくなった．経鼻栄養の維持に非常な困難を伴ったため，高カロリー輸液を開始し，その後経管栄養も併用した．うつ的症状の可能性も否定できず選択的セロトニン再取り込み阻害薬(SSRI)，また塩酸ミドドリンやカフェインも使用したが，効果は得られなかった．短期間のステロイド使用中のみ，一過性に食欲増進を認めたが持続せず，その際に経口摂取もできたことより嚥下や消化機能に問題がないことを確認できた．

入院後1か月間で体重は減少傾向で，活気はなく無気力・倦怠様表情を示し，日中のほとんどがベッド上臥床の状態となった．また，入院経過中に感染症や肝機能障害などを認めた．経管栄養により体重はやや増加傾向となったものの，活気低下，経口摂取困難は持続した．通常の治療で効果がほとんど乏しいため，家

表2　DS患者へのアリセプト療法

	報告例（文献2より抜粋）	自験例
年齢	13～58歳	5歳
投与量	3～5mg/day※	0.5mg/day
血中濃度	13～33ng/ml	4.3ng/ml
効果発現までの期間	1～3ヶ月	1～2週間
効果	自立的行動出現，改善 言語機能向上 精神的安定 排尿機能障害改善	拒食の改善 対人関係の改善 感情表出の改善 排尿機能障害改善
副作用	肝機能障害，下痢，興奮	下痢

※現在，成人DS患者には3mg/dayが適正量と考えられている．

族への充分なインフォームドコンセントのもと長崎大学ダウン症候群研究プロジェクトによる「DS患者へのアリセプト投与におけるガイドライン」に準じ，入院4か月後，肝機能など改善したことを確認してから，塩酸ドネペジル療法を導入した．0.5mg/日（1日1回）で開始し，内服14日目の血中濃度（トラフ値）は4.3ng/mlであった．開始10日目頃から経口摂取可能となり，経管栄養中止後も体重は増加傾向であった．家族は周囲への興味や表情・感情の表出に関して良い印象を持ったようだが，近藤らも指摘するように客観的評価法には的確に反映されず[2]，治療前後の評価法は今後の検討が必要である．体調を崩した時期以前の状態に回復し，塩酸ドネペジル投与開始後2週間で退院できた．その後徐々に活動性が増し，多動とも思える程の状況が見られたため，内服開始5か月目に減量中止とした．その後半年以上経過した現在も生活能力は維持されている．また，本例の塩酸ドネペジル開始時の血中IGF-1濃度は15.9ng/mlであり，投与半年後には111.9ng/mlと急激な上昇を認め，投与開始前1年間の成長率2.9cmに比し，投与開始後1年間は6.2cmと著明な改善を認めた．

考　察

DS患者における急激退行とは，日常生活能力が比較的短期間で急激に低下する現象で20歳前後に好発するとされる．①動作・行動面，②対人面，③情緒・性格面，④身体面の4つの側面に表れる．具体例としては，a. 緩慢・表情の乏しさ・会話減少，b. 対人関係の場での過度の緊張・人を意識しない，c. 興味消失・頑固・固執傾向・興奮，d. 睡眠障害・食欲減退・体重減少が挙げられる．頑固，無気力，拒食などが初期症状として表れやすい[5]．精神神経科領域では，「退行」または「急激退行」は「赤ちゃんがえり」を意味する言葉として定着しており，そのため混乱が生じうる．また，上記症状をうつ的状態（depressive illness）と見なす医学的立場もあり，実際抗うつ薬の投与や環境整備により改善した例は存在する．しかし，様々な治療法で改善が見られない例も存在する[6]．

長崎大学ダウン症候群研究プロジェクトチーム（近藤ら）は，平成14年より脳内コリン作動性の改善がDS患者の日常生活能力を向上させることを期待して，DS患者50名以上（投与開始年齢13～58歳）に本薬剤を投与し日常生活能力の改善を認めるとともに，急激退行を認めた症例においてもその効果を確認し報告している（表2）[2)~4)]．しかし，塩酸ドネペジルは本来AD治療薬で高齢者が対象となっており，低年齢者への投与報告が全くみられない．我々のこれまでの使用経験例の最年少は12歳DS女児で，体重は40kgと成人とほとんど変わらなかった．国外の報告では8歳児への使用例があり，日常生活能力の改善が報告されている[7]．そのため，今回の使用例（5歳，体重9kg）は年齢，体重ともに最小例だったと考えられる．DS者では塩酸ドネペジルの血中濃度が非DS者より高い事が知られており，成人では1日量3mgを基準に投与している[2]．今回のDS患児の体重が非DS児の1歳相当であることから，Von Harnackの換算表より算定し，一般量の1/4（0.75mg）より少し低い量である0.5mgを設定した．成人DS患者に3mg投与した場合の血中濃度（20ng/ml前後）と比べてかなり低い血中濃度（4.3ng/ml）であったにもかかわらず，比較的典型的なDSの退行症状（頑固な拒食，無気力など）を示していた本患児において臨床的には良好な結果を得ることができた．

塩酸ドネペジルは中枢選択性の高いアセチルコリンエステラーゼ阻害薬であり，ADの進行抑制目的で使用される．ADと臨床的（記憶力の低下，記銘力障害，認知障害）および神経病理学的（老人斑［βアミロイド蛋白の沈着］や神経原線維変化等）特徴を共有するDS患者においても，コリン作動性障害が指摘されている．脳内アセチルコリンに関する酵素活性の異常が30歳代後半から起こるとされているが[8]，胎児期からの異常を示唆する報告もある[9]．DS動物モデルでも加齢とと

もにコリン作動性ニューロンの変性を認めるとされている[10]．膀胱収縮にもアセチルコリンが深く関わっており，DS患者の70%程度に排尿障害を認めるが，10歳未満のDS患児にも認められている[2]．このような事実から，DS患者にはもともとアセチルコリン作動性の障害がベースにあり，これに30歳代以降のAD様の症状が付加されることが推測される．

本例において塩酸ドネペジル療法が活動性や経口摂取を劇的に改善したということは，DSにおいてADとの関係が薄いと思われる幼児期にも退行現象が起こりうること，そしてその機序として脳内コリン作動性の低下が関与することを示唆するものと思われた．しかし，塩酸ドネペジルのADに対する有効血中濃度よりはるかに低い数値で効果を示した理由は不明で，効果発現も報告例に比し非常に早かった．この年齢にみられるコリン作動性の障害が軽微で可逆的であって，ADとは質的にも異なる部分があり，そのために治療反応性が高かった可能性も考えられるため，今後症例を重ねて検討していく必要がある．

また今回塩酸ドネペジル療法開始後に血中IGF-1濃度の上昇を認め，それとともに成長率は著明に改善した．DS患者のIGF-1濃度は思春期ステージや性腺ホルモンにより変動するが，年齢別基準値は同年齢健常者よりやや低値であるものの，その85%程度の値をとるとされる[11]．本例のIGF-1低値の原因として，GH分泌能の評価は行っていないが，発育不全を認めGH分泌不全の可能性があること，また甲状腺ホルモン低値にかかわらずTSHの上昇を認めないことから下垂体機能低下の影響が示唆される．塩酸ドネペジルによるIGF-1への影響に関して，2005年に塩酸ドネペジル内服が高齢の健常人男性の血中IGF-1濃度を上昇させることが報告されている[12]．またNarimatsuらはマウスを用いた検討で，塩酸ドネペジルが消化管の知覚神経刺激作用を介して海馬のIGF-1濃度を上昇させ，その結果，海馬での血管再生，神経再生が促進され空間認知機能が改善することを報告している[13]．本例のIGF-1濃度上昇については，食事摂取や栄養状態の改善によることも否定できないが，上記の機序により上昇した可能性も示唆される．

本例で見られたように，DS患者における日常生活能力の低下や退行は，本人だけでなくその家族に多大な問題を引き起こす．塩酸ドネペジル療法は，認知症の有無やその程度，年齢，IQレベルに関係なく，DS治療薬としての可能性が示されている．今後，適切なガイドライン構築や保険診療のもとでのDS治療法として確立されることを期待する．さらに，幼少期に塩酸ドネペジルを投与することは，発達促進に付加的な効果やIGF-1誘導による発育向上にも意義深い可能性もあり，今後の更なる検討が待たれる．

まとめ

退行症状を示したDS幼児に塩酸ドネペジル療法を行い，良好な結果を得た．本例は，DS患者はAD発症には早すぎる若年期にも急激退行を認めること，またそのような状況で塩酸ドネペジルが有効であることから，コリン作動性の一過性・可逆的障害が病態に関与することを臨床的に示唆する貴重な症例であると思われた．

本論文の要旨は第53回日本小児神経学会総会（2011年5月　横浜）にて発表した．

日本小児科学会の定める利益相反に関する開示事項はありません．

文　献

1) Heinrich G. Amyloid A4 protein and its precursor in Down's syndrome and Alzheimer's disease. N Engl J Med 1989；321：1197.
2) 近藤達郎，森内浩幸．ダウン症候群患者への塩酸ドネペジル療法．日児誌　2010；114：15—22.
3) Kishnani PS, Sullivan JA, Walter BK, et al. Cholinergic therapy for Down's syndrome. Lancet 1999；353：1064—1065.
4) Lott IT, Osann K, Doran E, et al. Down syndrome and Alzheimer disease：response to donepezil. Nelson L Arch Neurol 2002；59：1133—1136.
5) 菅野　敦．退行を示した青年期・成人期知的障害者に対する地域生活支援と社会参加の促進に関する研究—退行の類型と予防—．発達障害支援システム学研究　2005；4：35—46.
6) Capone G, Goyal P, Ares W, et al. Neurobehavioral disorders in children, adolescents, and young adults with Down syndrome. Am J Med Genet 2006；142C：158—172.
7) Spiridigliozzi GA, Heller JH, Crissman BG, et al. Preliminary study of the safety and efficacy of donepezil hydrochloride in children with Down syndrome：a clinical report series. Am J Med Genet Part A 2007；143A：1408—1413.
8) Yates CM, Simpson J, Gordon A, et al. Catecholamines and cholinergic enzymes in pre-senile and senile Alzheimer-type dementia and Down's syndrome. Brain Res 1983；280：119—126.
9) Flórez J, del Arco C, González A, et al. Autoradiographic studies of neurotransmitter receptors in the brain of newborn infants with Down syndrome. Am J Med Genet Suppl 1990；7：301—305.
10) Holtzman DM, Santucci D, Kilbridge J, et al. Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. Proc Natl Acad Sci USA 1996；93：13333—13338.
11) Ragusa L, Valetto MR, Proto C, et al. IGF-I levels

in prepubertal and pubertal children with Down syndrome. Minerva Endocrinol 1998 ; 23 : 31—36.
12) Obermayr RP, Mayerhofer L, Knechtelsdorfer M, et al. The age-related down-regulation of the growth hormone / insulin-like growth factor-1 axis in the elderly male is reversed considerably by donepezil, a drug for Alzheimer's disease. Exp Gerontol 2005 ; 40 : 157—163.
13) Narimatsu N, Harada N, Kurihara H, et al. Donepezil improves cognitive function in mice by increasing the production of insulin-like growth factor-I in the hippocampus. HJ Pharmacol Exp Ther 2009 ; 330 : 2—12.

The Efficacy of Donepezil Treatment on the Activity of Daily Life and Growth Rate in a 5-Year-Old Boy with Down Syndrome

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Down syndrome (DS) patients share certain neuropathological features with Alzheimer disease (AD) patients. Aside from AD-like dementia, DS patients occasionally develop neurobehavioral disorders of unknown causes that lead to rapidly progressive deterioration of their activities of daily lives in adolescence or early adulthood. Some DS children aged 10 or younger develop voiding dysfunction, possibly associated with cholinergic abnormalities. The anti-AD drug donepezil, a cholin esterase inhibitor, has been shown to effectively improve the activities of daily life in DS patients ; however, its efficacy and safety in children with DS remain unknown. We herein report the successful treatment with donepezil of a 5-year-old boy with DS who had been suffering from intractable anorexia and an autistic state. To the best of our knowledge, he is the youngest patient treated with donepezil, and his clinical state and prompt response to donepezil treatment suggest that even young children with DS have a risk of progressive deterioration of behavioral and mental state that apparently results from transient or reversible cholinergic abnormalities.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

MBTPS2 Mutation Causes BRESEK/BRESHECK Syndrome

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Received 25 July 2011; Accepted 17 October 2011

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

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

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

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

Patients

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

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

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

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

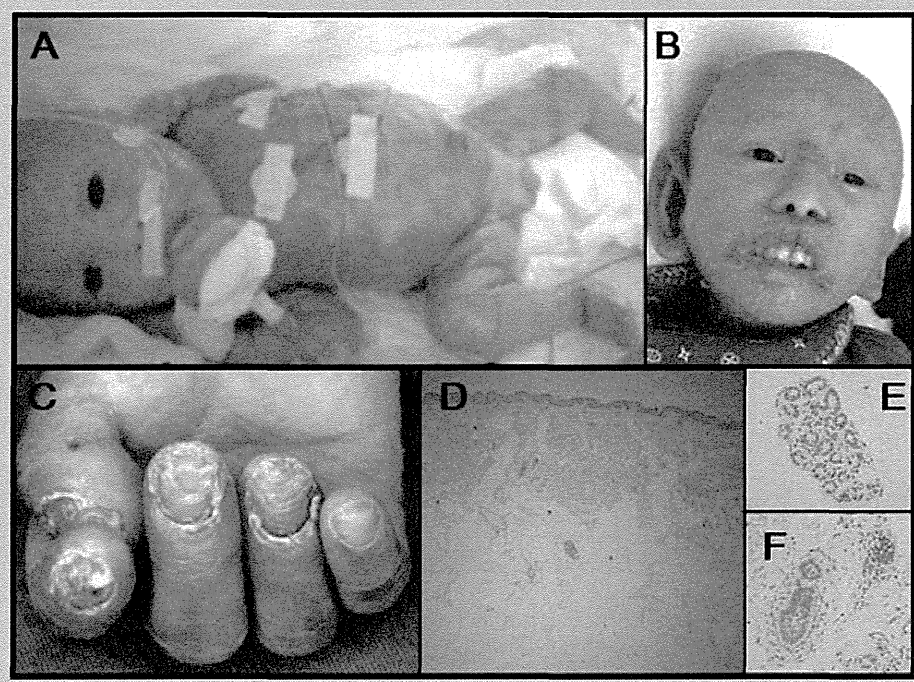

FIG. 1. Clinical appearance and dermatological findings of the patient. A: Lateral view of the patient at birth. Note the generalized alopecia with an absence of scalp hair, eyebrows, and eyelashes. The skin was dry and scaly, and an itchy erythema was observed over the entire body. B: Frontal view of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

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

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

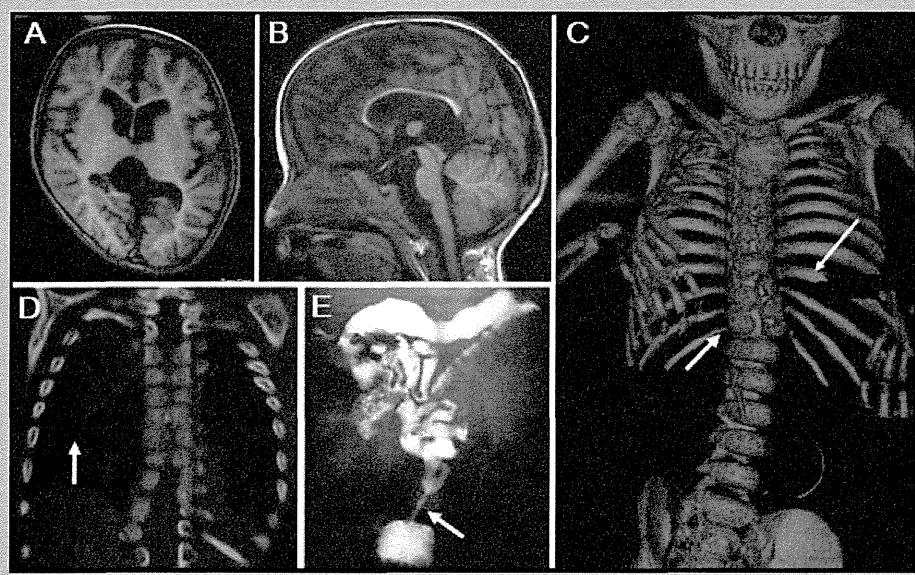

FIG. 2. CT and MRI findings of the patient. A,B: Brain MRI (T1-weighted image) at 3 years of age showed decreased volume of the cortex in the frontal and parietal lobes, the presence of a subdural cyst in the corpora quadrigemina, and dilatation of the lateral and fourth ventricle. C: A bone survey performed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

Chromosomal and Molecular Genetic Studies

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

RESULTS

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

DISCUSSION

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

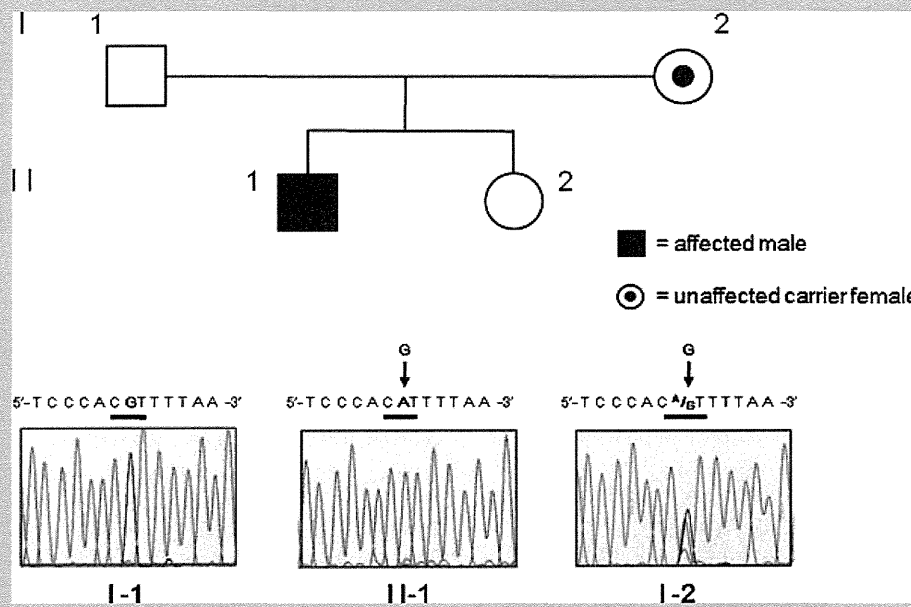

FIG. 3. Identification of a disease mutation. The sequence analyses of the patient (II-1) showed a c.1286G>A variant in exon 10 of *MBTPS2*, which predicts p.Arg429His, as indicated by the arrow (middle panel). The mother (I-2) was heterozygous for the mutation ($C^A/_GT$) (right panel).

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

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

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

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

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

ACKNOWLEDGMENTS

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

REFERENCES

Aten E, Brasz LC, Bornholdt D, Hooijkaas IB, Porteous ME, Sybert VP, Vermeer MH, Vossen RH, van der Wielen MJ, Bakker E, Breuning MH, Grzeschik KH, Oosterwijk JC, den Dunnen JT. 2010. Keratosis follicularis spinulosa decalvans is caused by mutations in MBTPS2. Hum Mutat 31:1125–1133.

Asada R, Kanemoto S, Kondo S, Saito A, Imaizumi K. 2011. The signalling from endoplasmic reticulum-resident bZIP transcription factors involved in diverse cellular physiology. J Biochem 149:507–518.

Cursiefen C, Schlötzer-Schrehardt U, Holbach LM, Pfeiffer RA, Naumann GOH. 1999. Ocular findings in ichthyosis follicularis, atrichia, and photophobia syndrome. Arch Ophthalmol 117:681–684.

Elias PM, Williams ML, Holleran WM, Jiang YJ, Schmuth M. 2008. Pathogenesis of permeability barrier abnormalities in the ichthyoses: Inherited disorders of lipid metabolism. J Lipid Res 49:697–714.

Kondo S, Murakami T, Tatsumi K, Ogata M, Kanemoto S, Otori K, Iseki K, Wanaka A, Imaizumi K. 2005. OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. Nat Cell Biol 7:186–194.

MacLeod JMH. 1909. Three cases of 'ichthyosis follicularis' associated with baldness. Br J Dermatol 21:165–189.

Martino F, D'Eufemia P, Pergola MS, Finocchiaro R, Celli M, Giampà G, Frontali M, Giardini O. 1992. Child with manifestations of dermotrichic syndrome and ichthyosis follicularis alopecia photophobia (IFAP) syndrome. Am J Med Genet 44:233–236.

Oeffner F, Fischer G, Happle R, König A, Betz RC, Bornholdt D, Neidel U, Boente Mdel C, Redler S, Romero-Gomez J, Salhi A, Vera-Casaño A, Weirich C, Grzeschik KH. 2009. IFAP syndrome is caused by deficiency in MBTPS2, an intramembrane zinc metalloprotease essential for cholesterol homeostasis and ER stress response. Am J Hum Genet 84:459–467.

Reish O, Gorlin RJ, Hordinsky M, Rest EB, Burke B, Berry SA. 1997. Brain anomalies, retardation of mentality and growth, ectodermal dysplasia, skeletal malformations, Hirschsprung disease, ear deformity and deafness, eye hypoplasia, cleft palate, cryptorchidism, and kidney dysplasia/hypoplasia (BRESEK/BRESHECK): New X-linked syndrome? Am J Med Genet 68:386–390.

Sakai J, Duncan EA, Rawson RB, Hua X, Brown MS, Goldstein JL. 1996. Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavages, one within a transmembrane segment. Cell 85: 1037–1046.

Sato-Matsumura KC, Matsumura T, Kumakiri M, Hosokawa K, Nakamura H, Kobayashi H, Ohkawara A. 2000. Ichthyosis follicularis with alopecia and photophobia in a mother and daughter. Br J Dermatol 142:157–162.

Traboulsi E, Waked N, Mégarbané H, Mégarbané A. 2004. Ocular findings in ichthyosis follicularis–alopecia–photophobia (IFAP) syndrome. Ophthal Genet 25:153–156.

Tumialán LM, Mapstone TB. 2006. A rare cause of benign ventriculomegaly with associated syringomyelia: BRESEK/BRESHECK syndrome. Case illustration. J Neurosurg 105:155.

Ye J, Rawson RB, Komuro R, Chen X, Davé UP, Prywes R, Brown MS, Goldstein JL. 2000. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell 6:1355–1364.

Zelenski NG, Rawson RB, Brown MS, Goldstein JL. 1999. Membrane topology of S2P, a protein required for intramembranous cleavage of sterol regulatory element-binding proteins. J Biol Chem 274:21973–21980.

European Journal of Endocrinology (2012) **166** 829–838 ISSN 0804-4643

CLINICAL STUDY

Mass screening of newborns for congenital hypothyroidism of central origin by free thyroxine measurement of blood samples on filter paper

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Abstract

Objective: To evaluate the effectiveness of mass screening of newborns for congenital hypothyroidism of central origin (CH-C) by measurement of free thyroxine (FT_4) and thyroid-stimulating hormone (TSH).
Design: Questionnaire-based survey of CH-C patients born between 1999 and 2008 in Kanagawa prefecture, Japan.
Methods: TSH and FT_4 levels in dried blood spots on filter paper were measured using ELISA kits, and CH-C was diagnosed at FT_4 levels below a cutoff of 0.7 ng/dl (9.0 pmol/l). Survey results were collated with the database created by the screening organizer.
Results: Twenty-four CH-C patients (18 males) were identified, 14 of whom had multiple pituitary hormone deficiencies (group M), eight had isolated CH-C (group I), and two had undetermined pituitary involvement (group U). In groups M, I, and U, the number of patients with FT_4 levels below the cutoff value at screening was five (36%), seven (88%), and one (50%) respectively; other patients had been diagnosed clinically. Thus, 13 patients were true positives, while nine were false negatives, yielding screening sensitivity of 59.1% and positive predictive value of 11.5%. The calculated sensitivity was 81.8% at a higher cutoff value of 0.9 ng/dl (11.6 pmol/l). The overall incidence of CH-C was estimated at 1 in 30 833 live births, while that of CH of thyroidal origin (CH-T) is 1 in 3472 live births in Kanagawa prefecture (CH-T/CH-C, 8.9).
Conclusions: Newborn screening with combined FT_4 and TSH measurements can identify a significant number of CH-C patients before manifestation of clinical symptoms, but a more appropriate FT_4 cutoff value should be considered.

European Journal of Endocrinology **166** 829–838

Introduction

Screening of newborns for congenital hypothyroidism (CH) is now routinely used in most of the developed world and in an increasing number of developing countries, which has prevented serious intellectual sequelae in a considerable number of patients with CH (1, 2). While most CH cases are due to CH of thyroidal origin (CH-T) manifesting as thyroid dysgenesis or thyroid hormone synthesis defects, a significant number of CH cases are due to inadequate thyroid-stimulating hormone (TSH) secretion from the anterior pituitary (3, 4, 5, 6, 7, 8, 9). The latter category of CH cases is termed as CH of central origin (CH-C). The incidence of CH-C is estimated to be ~1 in 20 000–30 000 live births (3, 5, 6, 7, 10), which is much higher than previously thought. Nevertheless, CH screening in Japan is mainly based on the detection of elevated TSH levels in dried blood samples on filter paper (primary TSH strategy). This assay has demonstrated high sensitivity in detecting CH-T (11, 12) but failed to identify newborns with CH-C. On the other hand, screening based on the detection of low T_4 levels (primary T_4 strategy) can identify CH-C newborns only inefficiently, as false-positive cases are inevitable due to both thyroxine-binding globulin (TBG) deficiency and transient low T_4 levels in critically ill newborns.

To overcome this situation, The Netherlands has implemented a system of assaying TSH, T_4, and TBG, which can eliminate false-positive results caused by TBG deficiency (5, 6). Assaying free T_4 (FT_4) may be an alternative solution because FT_4 is less influenced by TBG than T_4. Moreover, determination of FT_4 seems to be superior to that of T_4 because this reduces false-positive cases in premature newborns, according to the report of a smaller difference between full-term and

DOI: 10.1530/EJE-11-0653
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preterm newborns in FT_4 levels than in T_4 levels measured in dried blood samples on filter paper (13). Therefore, in Kanagawa prefecture, we have adopted a strategy of simultaneously measuring TSH and FT_4 in all newborns using a filter paper assay (9). Sapporo city has also adopted the same screening system. The report of a 5-year audit in Sapporo city was released in 2004, in which six CH-C cases were identified through this screening (7). However, the study in Sapporo included only patients showing positive screening results, which preclude evaluation of the sensitivity of screening in detecting CH-C. In addition, the annual birth rate in Sapporo is approximately one-fourth of Kanagawa prefecture.

To evaluate the effectiveness of our CH-C screening system, we have conducted a detailed, comprehensive survey of CH-C patients from Kanagawa region, Japan. In this study, all CH-C cases detected via screening and diagnosed clinically were included and used to estimate the sensitivity and positive predictive value (PPV) of the screening method.

Subjects and methods

Outline of newborn screening system

Kanagawa prefecture, in which Yokohama is the main city, is located in the central region of the Japanese islands, neighboring the Tokyo metropolitan area. The annual number of births in Kanagawa prefecture has been ~70 000 in recent years. The incidence of CH-T in Kanagawa prefecture is estimated to be 1 in 3472 births. Neonatal screening is exclusively conducted by the Neonatal Mass-screening Committee (NMC) of the Kanagawa Prefecture Medical Association (KPMA), which comprises executive officers, technical experts, gynecologists, general pediatricians, and pediatric endocrinologists. The screening procedure adopted by the NMC-KPMA is based on the determination of TSH and FT_4 in dried blood spots on filter paper obtained 4 to 7 days after birth (median sampling day was the fifth day). According to the standard practice followed, newborns with high TSH levels ($\geq$30 μIU/ml serum) are immediately sent to one of the several pediatric endocrine units within the prefecture. A second filter paper sampling is requested for those with borderline TSH levels (15–30 μIU/ml serum) or low FT_4 levels ($<$0.7 ng/dl of serum (9.0 pmol/l)). If the results again indicate borderline TSH or low FT_4, the baby is sent for a thorough evaluation. Thus, CH-C is suspected if FT_4 levels are low in two consecutive samples. To eliminate cases with transient low FT_4 due to prematurity, samples taken from the newborns with birth weight $<$2000 g are considered to be preliminary, and the results are sent to each attending physician as an unofficial report. Once the baby attains a weight of 2500 g or reaches 30 days of age, the first sample is requested.

TSH levels in filter paper samples were determined by ELISA using mouse monoclonal antihuman TSH antibodies (Eiken Chemical Co. Ltd., Tochigi, Japan). To determine FT_4 levels in filter paper samples, ENZAPLATE N-FT_4 was used (Siemens Healthcare Diagnostics K.K., Tokyo, Japan), which is an ELISA kit based on a competitive reaction between sample FT_4 and peroxidase-tagged human T_4 to bind to rabbit polyclonal antihuman T_4 antibody (first antibody). A 3 mm disc is punched out from the filter paper and is incubated with peroxidase-tagged T_4 and the first antibody in a reaction mixture of 150 μl for 4 h at 18–25 °C in a micro-well plate with immobilized caprine antirabbit IgG antibodies (second antibody). After removal of the filter paper disc and washing five times, *O*-phenylenediamine is added, and the absorbance is then measured at 492 nm. A calibration curve is established using standard filter paper samples of known FT_4 concentrations, which are provided by the manufacturer. FT_4 level in the sample is then determined by comparison with the calibration curve.

The performance of this kit, of which there is only one study, reported in a Japanese journal (14), is as follows. The FT_4 determination range is 0.5–5.0 ng/dl, which is based on a precision level lower than 15% of the coefficient of variation (CV). Intra-assay CV is 7.6–15.0%, whereas inter-assay CV is 9.4–18.5%. The correlation between the FT_4 levels measured by this kit and the electrochemiluminescence immunoassay (ECLIA) kit (Elecsys FT_4; Roche Diagnostics) is shown in Fig. 1.

Preliminary survey

A preliminary survey was conducted in December 2008. Questionnaires were sent to all 139 hospitals with a pediatric section in Kanagawa prefecture. The questionnaire included questions about the number of CH-C patients born in Kanagawa prefecture between January 1999 and December 2008 and treated continuously with levothyroxine (L-T_4). CH-C was defined as CH considered to be of hypothalamic or pituitary origin, excluding acquired sequelae of head trauma, brain tumor, etc., and irrespective of involvement of other pituitary functions. Cases of hypothyroxinemia due to prematurity were excluded.

Secondary survey

In April 2009, we requested the corresponding doctors caring for the probable CH-C patients identified in the preliminary survey to provide detailed information, including patient profile, medical complications, data on newborn screening, and results of thyroid function, thyroid imaging studies, and pituitary function tests with imaging information.

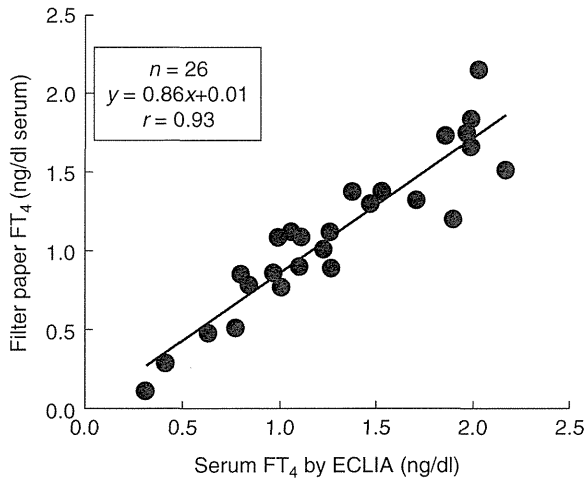

Figure 1 Correlation between FT_4 levels in dried blood samples on filter paper measured by ELISA and FT_4 serum levels measured by ECLIA for newborns and young infants. Filter paper blood specimens and serum samples were collected simultaneously from 26 infants younger than 2 months. FT_4 levels of blood samples on the filter paper were measured by an ELISA kit, whereas serum FT_4 was measured by ECLIA.

Collation study

After completion of the secondary survey, we collated the list of CH-C patients identified through the above surveys with the NMC-KPMA database, in which information from the first-line investigation at the pediatric endocrine unit and the screening results for all patients with positive screening results had been compiled.

Patient categorization

CH-C patients identified through the secondary survey and collation study were categorized into three groups according to the involvement of other pituitary hormones. Group M comprised CH-C patients with at least one pituitary hormone deficiency other than insufficient TSH secretion. These patients were considered to have congenital hypopituitarism with multiple pituitary hormone deficiencies. The diagnosis of each pituitary hormone deficiency was based on the attending physician's evaluation, except for GH deficiency, which was verified by at least one pharmacological stimulation test. Group I comprised isolated CH-C patients without pituitary involvement other than TSH insufficiency. Group U consisted of CH-C patients for whom pituitary involvement was undetermined.

Statistical analysis

Statistical analysis was carried out using Microsoft Office Excel 2007 (Microsoft Corporation). Correlation between the assay results of FT_4 (ELISA) in filter paper samples and serum FT_4 (ECLIA) was evaluated by linear regression analysis. Mann–Whitney *U*-test was used to compare FT_4 values between groups M and I. Fisher's exact probability test was used to compare the incidence of screening positive patients according to the etiological categories (groups M and I). *P* values of <0.05 were considered to be significant.

The Ethics Committee of Kanagawa Children's Medical Center reviewed and approved the study procedures.

Results

Out of the 139 hospitals from Kanagawa prefecture to which the preliminary survey questionnaire was sent, responses were obtained from 94 hospitals, including 14 hospitals stating that they currently had no pediatric section. Accordingly, the actual response rate was calculated to be 64.0% (80/125 hospitals with pediatric sections). Through this primary survey, 42 patients with probable CH-C (2–11 years old) were identified at 14 out of the 80 hospitals.

Figure 2 shows the number of CH-C patients, both probable and confirmed, identified through the surveys. The preliminary survey identified 42 probable patients, of which 20 patients were considered to represent true CH-C cases. After collation with the NMC-KPMA database, 24 CH-C patients (of which 18 were male) were finally identified. Details of each patient are summarized in Tables 1 and 2. As the total number of newborns screened during the study period was 740 003, we calculated the minimal incidence of

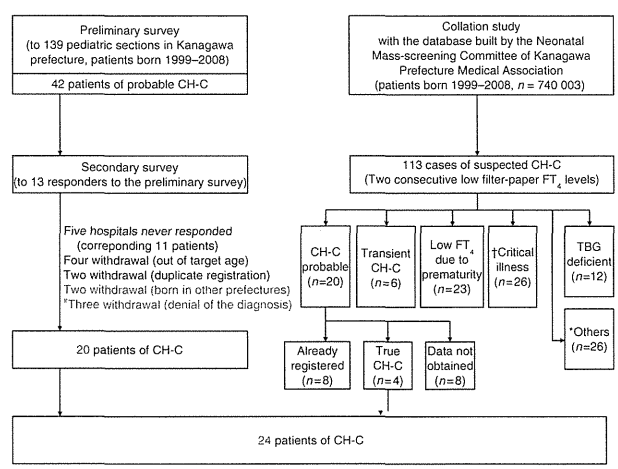

Figure 2 Overview of the study. #Three patients were excluded because they were judged not to have CH-C. Of these, two patients were diagnosed with CH-T with delayed TSH elevation, while the third patient had transient low FT_4, possibly because of an emotional deprivation syndrome. †Including one newborn with hydranencephaly. *Patients from whom we could not obtain detailed information.

Table 1 Characteristics of 14 patients with CH-C with multiple pituitary hormone deficiencies (group M). Patient 13 was already on L-thyroxine treatment at the time of screening.

	At birth			FT_4 values at Sc (ng/dl)		At first presentation			
Pt. no., sex	Year	Wt (g)	**Diagnostic symptom** (age)	1st sample (day)	2nd sample (day)	Serum TSH (μIU/ml)	Serum FT_4 (ng/dl)	**Deficient pituitary hormones**	**MR imaging of the CN**
1, F	1999	2872	Low vision (4M)	0.90 (5)		1.59	0.86	TSH, GH, AVP	APS, EPP, ONH, ASP
2, F	2000	3352	SS (1Y)	1.42 (4)		4.34	0.75	TSH, GH, LH/FSH	APS, EPP, PH
3, F	2000	2994	SS (4Y)	1.80 (5)		0.79	0.84	TSH, GH, AVP	Normal
4, M	2000	4420	Icterus (2M)	0.81 (26)		2.90	1.00	TSH, GH, ACTH	APS, EPP, ONH, ASP
5, M	2001	3240	Shock (1D)	0.83 (7)		7.40	0.70	TSH, GH, ACTH, LH/FSH	APS, EPP, ONH
6, F	2002	3150	Shock (1D)	0.58 (5)		10.28	0.65	TSH, GH, ACTH, LH/FSH	APS, EPP, ONH
7, M	2002	2342	Seizure (1Y)	0.81 (7)		1.71	0.42	TSH, GH, ACTH	APS, EPP, PH
8, M	2004	2275	Sc (23D)	0.48 (5)	0.50 (23)	3.77	0.76	TSH, ACTH	Normal
9, M	2005	3135	Sc (22D)	0.55 (5)	0.38 (22)	6.58	0.66	TSH, GH, ACTH, LH/FSH, AVP	APS, APP, ONH, ASP
10, M	2005	2972	SS, micropenis (1Y)	2.02 (5)		3.85	0.99	TSH, LH/FSH, PRL	Normal
11, M	2005	3168	Sc (31D)	0.37 (14)	0.62 (31)	3.29	0.53	TSH, ACTH	PH
12, M	2007	1786	Follow-up of HP (4M)	1.10[a] (6)	0.83 (28)	0.19	0.96	TSH, GH, ACTH, AVP	APS, APP, HP
13, M	2007	3122	Hypoglycemia (2D)	Not tested		3.34	0.88	TSH, GH, ACTH, LH/FSH	APS, EPP, PH
14, M	2007	3445	Sc (31D)	0.43 (6)	0.50 (31)	2.35	0.60	TSH, GH, ACTH, LH/FSH	EPP, PH

Pt. no., patient number; Wt, weight; Sc, screening; D, days old; M, months old; Y, years old; AVP, arginine vasopressin; PRL, prolactin; TSH, thyroid-stimulating hormone. APS, absent pituitary stalk; EPP, ectopic posterior pituitary; APP, absent posterior pituitary; ONH, optic nerve hypoplasia; ASP, absent septum pellucidum; PH, pituitary hypoplasia; HP, holoprosencephaly; SS, short stature; MR, magnetic resonance.
[a]This patient was born with low birth weight and hence this value was treated as unofficial.

Table 2 Characteristics of ten patients with CH-C categorized into isolated CH-C (group I; patients 15–22) and those with undetermined pituitary involvement (group U; patients 23 and 24).

	At birth			FT_4 values at Sc (ng/dl)		At first presentation			
Pt. no., sex	Year	Weight (g)	**Diagnostic symptom** (age)	1st sample (day)	2nd sample (day)	Serum TSH (μIU/ml)	Serum FT_4 (ng/dl)	**Basis for diagnosis of hypothyroidism**	**MR imaging of the CN**
15, M	2003	3370	Sc (14D)	0.14 (5)	0.48 (14)	2.86	0.45	Delayed TSH-R to TRH (5Y) Low FT_4 of 0.10 ng/dl (5Y)	Normal
16, M	2004	2770	SS (2Y)	1.79 (5)		2.20	0.55	Low FT_4 of 0.55 ng/dl (2Y)	Normal
17, M	2006	3450	Sc (15D)	0.60 (4)	0.47 (15)	2.79	1.01	Low FT_4 of 0.99 ng/dl on L-T_4 therapy (5Y) Requirement of high dose of L-T_4 (55 μg) to achieve NFR (5Y)	ND
18, M	2008	3060	Sc (24D)	0.68 (13)	0.68 (24)	1.86	0.94	Low TSH-R (6.90 μIU/ml) to TRH (1M)	Normal
19, M	2008	3868	Sc (12D)	0.43 (5)	0.66 (12)	3.02	0.72	Requirement of high dose of L-T_4 (55μg) to achieve NFR (11M)	ND
20, F	2007	3262	Sc (13D)	0.50 (5)	0.60 (13)	2.28	0.73	Low TSH-R (0.59 μIU/ml) to TRH (3M) Requirement of high dose of L-T_4 (55 μg) to achieve NFR (2Y)	ND
21, M	2008	3440	Sc (13D)	0.69 (4)	0.53 (13)	2.13	0.70	Low TSH-R (0.01 μIU/ml) to TRH (6M) Low FT_4 of 0.70 ng/dl (20D) and 1.10 ng/dl (6M)	PH
22, M	2008	3145	Sc (20D)	0.21 (5)	0.50 (20)	2.34	0.43	Low TSH-R to TRH (26D) Low FT_4 of 0.43 ng/dl (26D)	Normal
23, F	2007	668	Follow-up of low birth weight (1M)	0.27[a] (4)		Unknown	0.80	Low FT_4 of 0.70 ng/dl on L-T_4 therapy (2M)	Normal
24, M	2008	2542	Sc (15D)	0.57 (4)	0.57 (15)	1.24	0.98	Low FT_4 of 0.98 ng/dl (5M)	ND

Pt. no., patient number; D, days old; M, months old; Y, years old; L-T_4, levothyroxine; PH, pituitary hypoplasia; SS, short stature; Sc, Screening; TSH-R, TSH response; NFR, normal FT_4 range; MR, magnetic resonance; ND, not done.

[a]This patient had low birth weight, and the data obtained at 4 days of age were treated as unofficial. L-Thyroxine therapy was initiated before her first official sample was obtained.

CH-C in Kanagawa prefecture as 1 in 30 833 births (24/740 003).

Among the 24 patients, 14 patients (58%, ten males) were categorized into group M (Fig. 3). Group M ($n=14$) consisted of five patients with septo-optic dysplasia, five patients with pituitary hypoplasia, one with holoprosencephaly, and three with normal pituitary morphology. Eight other patients out of the 24 (33%) were considered to have isolated CH-C, without pituitary involvement, and they were hence categorized as group I (Fig. 3). Pituitary function in the remaining two patients could not be fully evaluated because of their younger age, and they were therefore categorized as group U (Fig. 3).

Twelve patients (50%) were identified as having CH-C solely via the newborn screening system in Kanagawa prefecture (Fig. 3). Of these, four patients belonged to group M, seven patients to group I, and one patient to group U. In addition, patient 6 in group M was clinically diagnosed with CH-C because this patient exhibited shock; however, the screening result was actually positive (low FT_4 levels), and hence, this was considered as a true-positive case of CH-C. Therefore, the total number of true-positive CH-C cases was 13. In contrast, nine other patients out of 24 (38%, eight patients in group M and one in group I) had normal screening results and were revealed to have CH-C through the evaluation of clinical symptoms such as shock and/or hypoglycemia during the neonatal period ($n=2$), short stature ($n=4$), and other features ($n=3$). These nine patients were considered to be false negatives. The remaining two patients (one in group M and one in group U, depicted as '?' in Fig. 3) were already on L-T_4 treatment before screening, and hence, they were excluded from the judgment as to whether the screening results were positive or negative as they had already been diagnosed with CH-C. Patients in group I were significantly identified more frequently through the screening program than those in group M: 88% (7/8) vs 29% (4/14), $P<0.01$.

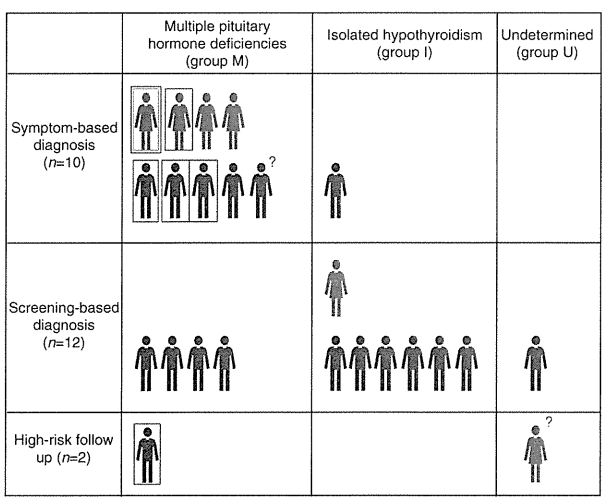

Figure 3 Summary of the 24 patients with CH-C, categorized by presence/absence of other pituitary hormone deficiencies and diagnostic symptoms. The red and blue figures indicate female and male patients respectively. Figures within a single-line box indicate CH-C patients who could have been identified as having CH-C by screening if the FT_4 cutoff values were 0.9 ng/dl. The figure within a double-line box indicates the patient diagnosed with septo-optic dysplasia presenting with shock, who had FT_4 levels <0.7 ng/dl according to the results of the filter paper assay. $^{?}FT_4$ data with the filter paper assay were not available for two patients; L-thyroxine treatment was initiated in one male patient at 2 days of age. One female patient had low birth weight, and the data obtained at 4 days of age were treated as unofficial. L-Thyroxine therapy was initiated before the first official sample was obtained from this patient.

Out of the 24 CH-C patients, for 22 patients the filter paper assay for FT_4 showed clear positive or negative results during screening (Fig. 4). The remaining two patients had been started on L-T_4 therapy before screening. Because no blood samples were collected from any patient between 8 and 11 days of age, FT_4 measurements were arbitrarily divided into those obtained on or before 10 days of age (FT_4 before 10D, $n=18$, collected from 18 patients) and those obtained on or after 11 days of age (FT_4 after 11D, $n=16$, collected from 14 patients). Overall, the FT_4 level before 10D was 0.82 ± 0.56 ng/dl (median, 5 days of age; range, 4–7 days), whereas the FT_4 level after 11D was 0.57 ± 0.13 ng/dl (median, 17.5 days; range, 12–31 days; Fig. 4). In addition, when we analyzed the data exclusively obtained from patients whose FT_4 levels had been determined twice ($n=10$), no significant difference was observed between FT_4 before 10D (0.46 ± 0.05 ng/dl) and FT_4 after 11D (0.52 ± 0.02 ng/dl). Thus, FT_4 values in CH-C patients appeared to be stable during the neonatal period. A comparison of FT_4 levels in group M ($n=17$) with those in group I ($n=15$) also did not show a statistically significant difference (group M, 0.81 ± 0.49 ng/dl; group I, 0.60 ± 0.37 ng/dl), indicating that the severity of hypothyroidism did not differ significantly between these two groups, differentiated by pituitary involvement.

Evaluation of the performance of the screening system is depicted in Table 3. Our screening system yielded 13 true positives and nine false negatives, so that the sensitivity of detection of a true positive was calculated to be 59.1%. Specificity and PPVs were calculated to be 99.99 and 11.5% respectively. A total of 740 003 newborns were screened during the study period and 113 newborns were sent for thorough evaluation based on two consecutive FT_4 measurements. The cutoff level used was 0.7 ng/dl serum (9.0 pmol/l). In the next step, we simulated the performance of the screening system with higher cutoff values. As depicted in Fig. 4, FT_4 levels for nine patients who were not identified in the screening ranged from 0.81 to 2.02 ng/dl (median, 0.9 ng/dl), which was substantially lower than the reference range of

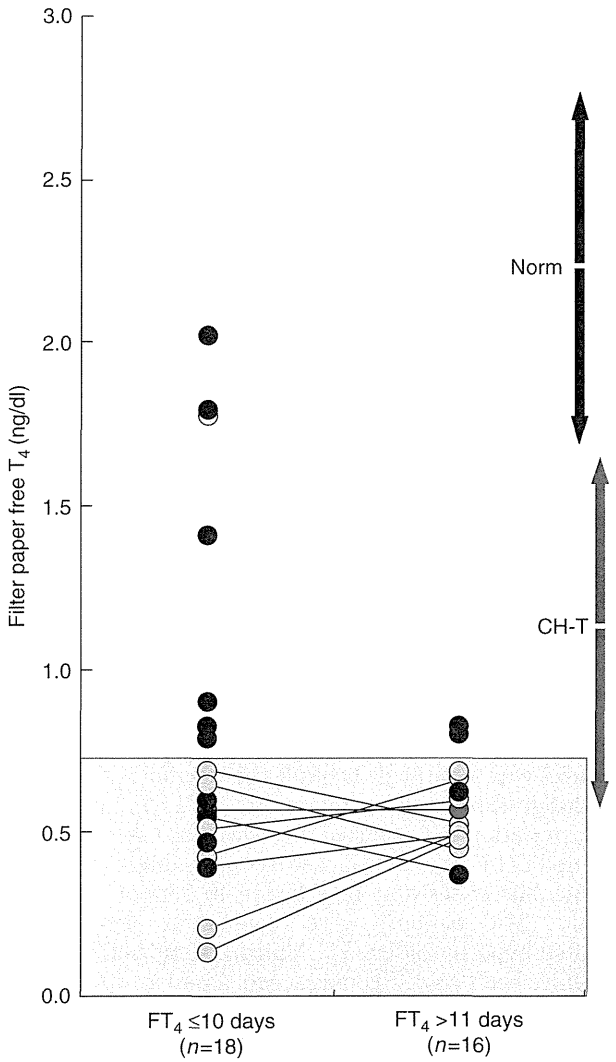

Figure 4 Distribution of FT_4 values from the filter paper assay for 22 patients with CH-C. FT_4 measurements obtained before 10 days of age (FT_4 before 10D) and those obtained after 11 days (FT_4 after 11D) did not differ significantly. The black circles indicate FT_4 obtained from patients in group M (with multiple pituitary hormone deficiencies), the yellow circles indicate FT_4 obtained from patients in group I (isolated hypothyroidism), and the red circles indicate FT_4 obtained from patient 24. The shaded area indicates FT_4 values below the cutoff of 0.7 ng/dl. Determinants from the same individuals are connected by solid lines. The black arrow (norm) indicates the mean ± 1 S.D. (2.22 ± 0.58 ng/dl) of FT_4 values from the filter paper assay conducted on 67 933 normal newborns. The blue arrow (CH-T) indicates the mean ± 1 S.D. (1.08 ± 0.54 ng/dl) of FT_4 values from filter paper assay on 61 patients diagnosed with CH-T.

1.64–2.80 ng/dl (21.1–36.0 pmol/l; data obtained from the 67 933 normal newborns). If the cutoff value is raised to 0.9 ng/dl serum (11.6 pmol/l), then an additional five patients would have been found to be positive by the screening, and the estimated sensitivity would be increased by 81.8%.

Discussion

In Japan, two types of ELISA-based kits are available for measuring FT_4 levels in dried blood samples on filter paper; one developed by Siemens Healthcare Diagnostics K.K and another by Eiken Chemical Co. Ltd. Because TSH and FT_4 can be measured with a common detection module, additional costs for FT_4 measurements are only those incurred for reagents: 465 yen for TSH alone vs 705 yen for TSH and FT_4 determination per newborn examined. Most of the screening centers adopt a primary TSH and backup FT_4 system: the filter paper method is used for measuring TSH in all newborns, while it is used for measuring FT_4 only in those with high TSH values for confirmation of possible hypothyroidism (11, 12). To detect CH-C, certain areas, including Kanagawa prefecture and Sapporo city, have adopted a combined primary TSH–FT_4 screening system (7, 9). After the report from Sapporo city (7), this report is the second audit of this CH-C newborn screening system, conducted on a larger population and for a longer study period. We also tried to trace CH-C patients not identified by neonatal screening (false-negative cases).

The ELISA-based filter paper FT_4 kits are almost exclusively used in Japan. One may argue against its accuracy in determining FT_4 levels, considering that some TBG-deficient patients were falsely detected to have low FT_4 levels and that the equilibrium dialysis method is the gold standard (15, 16). However, it has been difficult to introduce the equilibrium dialysis method in newborn screening because it requires a larger volume serum sample and longer measurement times. On the other hand, to use the FT_4 index instead of FT_4, tri-iodothyronine (T_3) uptake must also be measured, which increases cost. FT_4 determined by ELISA on filter paper blood samples seems to correctly reflect FT_4 status in newborns because most (88%) FT_4 values in CH-C patients were more than 2 S.D. below the mean of normal newborns and because FT_4 levels in CH-T were distributed in a substantially low range (0.04–2.32 ng/dl; Fig. 4). Moreover, Fig. 4 shows that the FT_4 levels measured using the filter paper method may be consistent even at lower concentrations of FT_4. Thus, we believe that although FT_4 levels determined using the filter paper samples may not be identical to those measured by the equilibrium dialysis method, the assay is a promising, practical alternative for use in CH-C screening. Because combined TSH-T_4 is recommended as the ideal strategy for detecting both CH-C and CH-T by the American Thyroid Association and Pediatric Endocrine Societies in the US and Europe (2), we think it is justified to continue implementation of our combined TSH–FT_4 system as a new version of the TSH–T_4 system.

From our survey, the incidence of CH-C was calculated as 1 in 30 833 live births, while that of CH-T was 1 in 3472 live births. Thus, the CH-T/CH-C ratio in this study was 8.9, which is close to the ratio 8.4 reported from

Table 3 Simulation of sensitivity and PPV of the screening system for CH-C based on varying FT_4 cutoff values.

FT_4 cutoff[a] (ng/dl (pmol/l) serum)	Newborns screened	Newborns asked for second sample (% of the total)	Newborns sent for evaluation (% of the total)	CH-C patients identified by screening (true positives)	CH-C patients missed by screening (false negatives)	Sensitivity (%)	PPV (%)
Kanagawa prefecture results (1999–2008)							
0.7 (9.0)	740 003	1220 (0.16)	113 (0.015)	13[b]	9	59.1	11.5
Simulation of data from Kanagawa prefecture							
0.9 (11.6)	740 003	3735 (0.50)	Unknown	18[c]	4	81.8	Unknown
1.0 (12.9)	740 003	6656 (0.90)	192[d]	18[c]	4	81.8	9.4
Sapporo city results (2004–2008, reference (7))							
1.0 (12.9)	83 232	629 (0.76)	22 (0.026)	6	Unknown	Unknown	27.3

[a]FT_4 cutoff of 0.7 ng/dl (9.0 pmol/l) was used during the survey.
[b]Twelve patients identified entirely via screening (screening-based diagnosis in Fig. 3) and one who developed shock and had FT_4 below 0.7 ng/dl at screening (patient 6 in Table 1, who is indicated by the figure surrounded by double lines in the symptom-based diagnosis in Fig. 3).
[c]Twelve patients identified entirely via screening (screening-based diagnosis in Fig. 3) and six who were diagnosed clinically but had FT_4 below 0.9 ng/dl (figures surrounded by a single line in the symptom-based diagnosis in Fig. 3).
[d]This figure was deduced from the incidence of 0.026% in Sapporo city (reference (7)).

The Netherlands (6). Although we previously reported a much lower CH-C incidence (1 in 160 516 births) (9), that survey was based on only the cases detected through screening. The incidence rate of 1 in 30 833 reported here is likely to be underestimated because this study was based on a questionnaire survey and false-negative cases may not have been recorded. Indeed, we could not obtain follow up data on 11 cases identified in the preliminary survey, as well as on eight patients with positive screening results. In addition, because correct diagnosis of CH-C is difficult (17, 18), especially in those with isolated hypothyroidism, some cases may have been overlooked. Moreover, as shown in Fig. 4, the mean values and range of FT_4 in CH-C patients were lower than those in CH-T patients, suggesting that milder forms of CH-C may escape detection.

A remarkable finding in this study is that isolated hypothyroidism (group I) was detected in one-third of the total CH-C population. Previous studies have found that 78% (5) to 98% (8) of CH-C patients had multiple pituitary hormone defects such as septo-optic dysplasia. There are some explanations for this discrepancy. First, isolated CH-C patients present less prominent symptoms than those with multiple pituitary hormone deficiencies (19, 20, 21, 22) and hence may be missed in the absence of screening. Indeed, all but one patient in group I was identified through the newborn screening. A Dutch screening system with TSH, T_4, and TBG determination (5) reported a prevalence rate of 22% of isolated CH-C, which is closer to our findings. Secondly, ethnic differences may be a factor: in Sapporo city, two of six CH-C patients were reported to demonstrate isolated hypothyroidism (7). Thirdly, some patients may not have been correctly diagnosed: a patient (patient 21 in Table 2) with pituitary hypoplasia is likely to have other hormone deficiencies. Finally, transient hypothyroidism may not be definitively ruled out, especially in younger patients. However, the authors are aware of a patient in group I (patient 15) who demonstrated severe hypothyroidism when L-T_4 therapy was tentatively interrupted. Reevaluation of all other patients in group I will determine the true incidence of isolated hypothyroidism.

Our current system yielded a sensitivity of 59.1% and PPV of 11.0% in detecting CH-C. In fact, 12 patients were diagnosed with CH-C entirely on the basis of low FT_4 levels at newborn screening. Above all, the presence of four patients in group M, who were overlooked clinically but in whom low FT_4 levels were detected at screening, underscores the usefulness of our combined primary TSH–FT_4 system. The sensitivity of 59.1% seems superior to the reported sensitivity of 19.0% in the state of Indiana, USA, where T_4 measurement was used (8). On the other hand, a study from The Netherlands reported the sensitivity to be 71.4% (6). Because our study relied on responses to a questionnaire, the actual sensitivity of our screening system may be lower: physicians who did not respond may have