Staphylococcus aureus、1例が *Pantoea agglomerans*)であり、1例が肺炎(*Moraxella catarrhalis, Streptococcus oralis*)であり、グラム陽性菌による重症細菌感染発生率が高い傾向が認められた。

D. 考察

先天代謝異常症における周術期のストレスや好中球減少などは術後感染症のリスクファクターになることが想定されたものの、菌血症の発生率は他の基礎疾患と同等であった。糖原病 Ib 型の患者においては、周術期にグラム陽性菌による感染症を複数例認め、エンピリック治療を行う上で考慮すべき点と考えられた。一方で好中球減少をきたすこれら患者に対して GCSF の術前投与を行ったことが、効奏した可能性が指摘される。

E. 結論

先天代謝異常症患者に対する生体肝移植におけるリスクは他の疾患と同等であり、適切な周術期管理で適応を考える場合の障害にはならない。

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H. 知的財産権の出願・登録状況

なし

分担研究報告書

有機酸代謝異常症（メチルマロン酸血症・プロピオン酸血症）、尿素サイクル異常症（CPS1欠損症・OTC欠損症）、肝型糖原病の新規治療法の確立と標準化研究班

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研究要旨
重症プロピオン酸血症に対して、生体肝移植を施行後長期経過観察できた症例3例について検討した。いずれも生着し9－12年間の経過観察が行えた。うち1例で大脳基底核壊死を伴う重症アシドーシス発作、他の1例が重症乳酸アシドーシス発作を経験し、3例目はアシドーシス発作なく基底核壊死をきたした。肝移植後アシドーシス発作は減少するが、代謝の是正は部分的であり、発作を経験する可能性があること、発作がなくても中枢神経症状をきたすことがあることが明らかになった。このうちの女児1例に対して、脳内の代謝状態を検討する目的で3H-Magnetic Resonance Spectrometry（MRS）施行したところ、脳内における乳酸の蓄積が示唆された。再度タンパク摂取制限の厳格化、カルニチンに加えてビタミンB群、ビオチンの追加投与を行ったところ、MRS上の変化所見の改善傾向が示唆された。

研究分担者
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A 研究目的
プロピオン酸血症を含む、重症有機酸血症に対して、生体肝移植が有効であることは既に報告されている。移植により、重症アシドーシス発作が減少することは確実で、食事療法や薬物療法の緩和も可能である。しかしながら、プロピオニルCoAカルボキシラーゼは肝のみに局在せず、全身に広く存在することが知られており、移植後も尿有機酸分析や血清アシルカルニチン分析ではプロピオン酸代謝産物の減少はほとんど見られない。また、重症アシドーシス発作が移植により回避されたにもかかわらず、長期経過後に基底核壊死などの中枢神経後遺症を発症する症例があることも報告されている。本研究では、本邦において生体肝移植を受けた重症プロピオン酸血症患者の長期予後を調査するとともに、移植後経過のフォローアップの指標としての脳プロトンMRSの有用性について検討した。

B　研究方法
（1）京都大学移植外科で1999年—2002年にかけて生体肝移植をうけた3例の重症プロピオン酸血症患者の長期予後を検討した。
（2）うち1例について、移植後12年の時点で脳3H-MRSを検討した。撮像はTR 6800 s, TE 84.7 sで行った。患児は重症発作がないことからL-カルニチン 100 mg/kg/日のみでタンパク制限についてはほぼフリーの状態となっていたが、初回検査で脳内局所での乳酸の蓄積が疑われたため、再度の食事制限、ビタミンB1 100mg/日、ビオチン 10 mg/日の投与を追加して再度MRSを行った。

C　研究結果
（1）3例の重症プロピオン酸血症患者が生体肝移植をうけた（移植時年齢 症例1 2歳、症例2　1歳、症例3　5歳）
いずれも生着し9－12年間の経過観察が行えた。うち1例で大脳基底核壊死を伴う重症アシドーシス発作、他の1例が重症乳酸アシドーシス発作を経験し、3例目は明らかなアシドーシス発作なく基底核壊死をきたした。すなわち、長期観察例の全員に重度アシドーシス発作ないし基底核壊死が発症した。移植後の血清アシルカルニチンのプロフィルは移植前に比べて必ずしも改善しておらず、食事療法が緩和された症例ではむしろ悪化しているときもあった。尿有機酸分析によるプロピオン酸代謝産物についても同様であった。
（2）うち1例についての移植後12年での3H-MRSでは、ベースライン検査で脳内の

一部に乳酸を示唆するピークが観察された。食事の厳格化、内服薬の追加による管理の厳格化によりこのピークは減少傾向となった。撮像部位が若干移動していたため、再度撮像を繰り返す予定である。

D　考察

（1）プロピオン酸血症の生体肝移植後では、検査上プロピオン酸代謝物が残るだけではなく、慢性合併症とくに中枢神経合併症が必ずしも予防できないことが明らかになった。移植後患者の脳 MRS による乳酸のピークの存在は、局所でのミトコンドリア機能異常が続いている可能性を示唆している。プロピオニルカルニチンはミトコンドリア呼吸鎖複合体を阻害することが知られており、移植後晩期後遺症としての基底核壊死の発症に関与している可能性があると考えられた。

（2）プロピオン酸血症肝移植後の代謝状態のフォローアップに MRS が有用である可能性が示唆された。

E　結論

（1）プロピオン酸血症に対する生体肝移植では慢性神経合併症が予防できない可能性がある。

（2）脳 MRS は、プロピオン酸血症肝移植後患者のフォローアップに有用な可能性が示唆された。

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G．知的所有権の取得状況

特になし

研究成果の刊行に関する一覧

別紙4

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研究成果の刊行物・別刷

Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 881 – 887
Metabolism
Clinical and Experimental
www.metabolismjournal.com

CD36 deficiency predisposing young children to fasting hypoglycemia

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Received 23 January 2010; accepted 21 August 2010

Abstract

Fatty acid (FA) β-oxidation defects cause hypoglycemia. Our aim was to determine if CD36—a membrane transporter for long-chain FAs—deficiency predisposes children to hypoglycemia. After overnight fasting, we measured parameters for carbohydrate and FA metabolisms at 12-, 14-, and 16-hour fasting points in 51 preschool children with histories of episodic hypoglycemia and 49 age-matched healthy controls. Simultaneously, the expressions of CD36 on platelets and monocytes were examined to determine the phenotypes. Six of the 51 hypoglycemic children and none of the 49 control children were diagnosed as having type I CD36 deficiency. Four and 3 children were diagnosed as having type II CD36 deficiency, respectively. Hypoglycemia was often recurrent in the type I CD36 group. At any fasting point, the type I CD36 group showed significantly lower blood glucose and insulin concentrations than the other groups: glucose, $P < .001$ vs control group and $P < .01$ or $P < .001$ vs type II/wild-type CD36 hypoglycemic groups; insulin, $P < .001$ vs control group and $P < .01$ vs type II/wild-type CD36 hypoglycemic groups. Free FA concentration in the type I group was always 1.5- to 2.0-fold higher than that in the other groups, whereas the total ketone body concentration was consistently about two thirds of that in the other groups. Among the type II, wild-type, and control groups, there were no significant differences in the parameters except that the wild-type group showed significantly lower FFA concentration ($P < .05$). These results suggested that type I CD36 deficiency but not type II CD36 deficiency predisposes preschool children to hypoglycemia.

1. Introduction

CD36 is a multifunctional membrane-associated glycoprotein with a molecular weight of 88 kd [1-5]. CD36 is a receptor for collagen and thrombospondin on platelets and oxidized low-density lipoproteins on macrophages [1-4]. CD36 also plays an important role in the uptake of long-chain fatty acids (LCFAs) in the heart, skeletal muscle, adipose tissue, and small intestine [5].

Based on its expression patterns on platelets and monocytes, CD36 deficiency is classified into 2 subgroups: type I and II [6]. Type I CD36 deficiency lacks CD36 expression on

Authors' contributions: This study was conducted through the leadership of Dr Takashi Miida. Hironori Nagasaka, Takashi Miida, Ken-ichi Hirano, and Hitoshi Chiba made a design for this study. Hironori Nagasaka, Tohru Yorifuji, Tomozumi Takatani, Yoshiyuki Okano, Hirokazu Tsukahara, and Tetsuya Ito collected blood samples from the enrolled children after the informed consents from the children's parents. Hidekatsu Yanai, Satoshi Hirayama, and Ken-ichi Hirano performed statistical analyses together with the determinations of CD36 phenotypes. Tomozumi Takatani and Tohru Yorifuji performed gene analyses. Takashi Miida, Hidekatsu Yanai, Shu-Ping Hui, and Satoshi Hirayama interpreted the data and described the figures. Satoshi Hirayama, Hironori Nagasaka, and Takashi Miida described this manuscript.

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0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.08.008

both cell types, whereas type II CD36 deficiency lacks expression only on platelets. Most type I cases are either homozygous or compound heterozygous for CD36 gene mutations, whereas type II cases are often free of CD36 gene mutations [7-9]. CD36 deficiency is one of the common genetic disorders in Japan [9-10]. As the metabolic manifestations of CD36-deficient adult subjects, decreased insulin sensitivity and postprandial hypertriglyceridemia have been reported [10-13]. However, for children, its clinical manifestations and the prevalence have been scarcely studied [14,15].

Hypoglycemia is highly prevalent in children, but its underlying disease or condition cannot be identified in most cases [16-18]. Children with fatty acid (FA) β-oxidation defects often show profound hypoglycemia [19].

The present study aimed to elucidate whether CD36 deficiency is attributable to the development of hypoglycemia in young children as other FA β-oxidation defects. We examined the prevalence of CD36 deficiency among preschool children with histories of hypoglycemia and examined the glucose and FA metabolism in them with special reference to CD36 phenotype.

2. Subjects and methods

2.1. Subjects

From 2004 to 2008, we prospectively screened 198 consecutive preschool children brought to our local affiliated hospitals for unconsciousness and/or seizures in the morning (Fig. 1).

Fifty-three children (23 girls and 30 boys, aged 1.8-5.2 years) were found to have hypoglycemia. Their blood glucose (BG) concentrations were only 25 to 42 mg/dL. Blood gas analyses revealed that their base excess ranged from −2.7 to −6.1 mEq/L. All these children had bradycardia or tachycardia with excessive sweating, suggesting hypoglycemia-induced autonomic responses. Their symptoms disappeared immediately after intravenous glucose infusions.

The 53 children were referred to our institutions for further examinations at 1 to 3 months after their episodes of hypoglycemia. At the time of admission for an extended fasting test, their ages were 2.1 to 5.5 years; and they were free of symptoms suggestive of any disorders. As age-matched healthy controls, we enrolled 49 children (22 girls and 27 boys) aged 2.1 to 4.6 years.

Both hypoglycemic and control children had no medical problems during their newborn and infancy periods and had grown completely healthy. There were no significant differences in birth weight and gestational age between both children: hypoglycemic children—2673 to 3462 g and 37 to 41 weeks; control children—2790 to 3369 g and 38 to 40 weeks.

Informed consent was obtained from the parents before enrolling these children in this study. The protocol was approved by the medical ethics committees of the participating institutions.

2.2. Study design

Firstly, we excluded children with metabolic or hormonal diseases that cause hypoglycemia. To diagnose hyperinsulinemia; hyperthyroidism; growth hormone deficiency; FA β-oxidation disorders; organic acidemia; fructose-1,6-diphosphatase deficiency; and glycogen storage disease, we specifically examined the profiles of blood amino acids and acylcarnitine, and blood concentrations of ammonia, lactate, insulin, growth hormone, insulin-like growth factor–1, free thyroxine, free thyronine, thyroid-stimulating hormone, and cortisol. We also examined profiles of urinary organic acids.

Secondly, the children with histories of hypoglycemia were divided into 3 subgroups according to the CD36 expression patterns on the platelets and monocytes by flow cytometry: Type I CD36, type II CD36, and wild-type hypoglycemic groups. The CD36 expression patterns in the control children were also examined.

For the 3 hypoglycemic groups and the control group, extended fasting tests were performed. At 7:00 to 7:30 PM on the day before blood sampling, we provided the children with suppers containing one third of the daily required calories for children of these ages. Fasting blood samples were collected from cubital veins for biochemical assays 3 times in the morning (12, 14, and 16 hours after supper). Body weight and height SD scores were also recorded for all enrolled children.

2.3. Biochemical assays

Fasting BG and insulin concentrations were determined by an enzymatic method and an enzyme immunoassay using a commercial kit (TOSOH-II; Tosoh, Tokyo, Japan), respectively. Serum total cholesterol and triglycerides were measured enzymatically using an automated analyzer. Low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were determined by homogenous assays. Serum concentrations of free FA (FFA) and total ketone bodies (TKB) were measured by enzymatic methods using commercial kits (NEFA-SS kit EIKEN; Eiken Chemicals, Tokyo, Japan, and Total-ketone body kit; Kainos Laboratories, Tokyo, Japan, respectively). Acylcarnitine profiles were examined by tandem mass spectrometry as described previously [20].

2.4. CD36 phenotyping

Phenotypes of CD36 were determined by flow cytometry using platelets and monocytes as described previously [6]. Fasting venous blood was drawn into a tube containing EDTA-K_2 to prepare platelet-rich plasma (PRP). In monocyte assays, PRP was processed in a Multi-Q-Prep (Coulter, Miami, FL) for hemolysis and fixation. The prepared PRP was then mixed with a fluorescein isothiocyanate (FITC)–conjugated monoclonal antibody (Mab) (FA6-152; Immunotech, Miami, FL) [6]. To detect CD36 expressions on platelets or monocytes, the CD36 signal was gated with either a phycoerythrin-conjugated anti-CD42b Mab (AN51; Dako, Copenhagen, Denmark) using an EPICS Profile II flow cytometer (Coulter, Miami, FL) or an

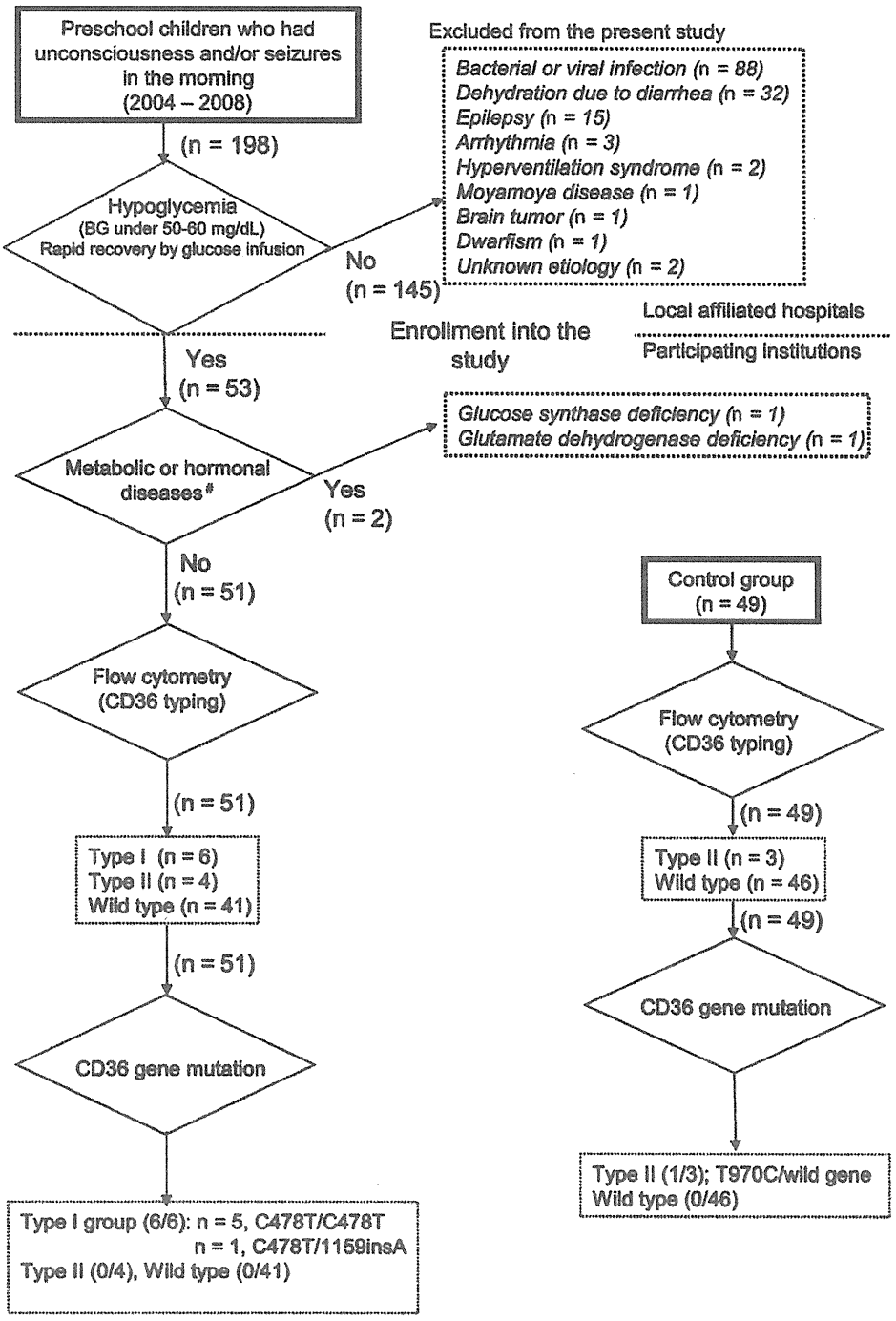

Fig. 1. Flowchart for classifying hypoglycemic children. #Profiles of blood amino acids and acylcarnitine, plasma ammonia, and lactate levels were measured. Insulin, growth hormone, insulin-like growth factor–1, free thyroxine, free thyronine, thyroid-stimulating hormone, and cortisol were also examined to detect endocrinologic disorders leading to hypoglycemia.

FITC-conjugated anti-CD14 Mab (MY4-FITC, Coulter) using an XL-MCL flow cytometer (Coulter).

2.5. CD36 gene analysis

In children with types I or II CD36 deficiencies, 3 common mutations of the CD36 gene, irrespective of histories with hypoglycemic episodes, were determined: (*a*) a substitution of T for C at nt 478 in exon 4 (C478T), (*b*) an AC deletion at nt 539 in exon 5 (539delAC), and (*c*) an A insertion at nt 1159 in exon 10 (1159insA) [7-9,14]. A previous study showed that 478T mutation impairs the maturation of the CD36 precursor, leading to CD36 defects on both platelets and macrophages [7]. Both