Ⅳ. 研究成果の刊行物・別冊

3 βケトチオラーゼ欠損症

β-ketothiolase deficiency

概　念

本症はミトコンドリア・アセトアセチル-CoAチオラーゼ(以下，T2と略す)の欠損症で，常染色体劣性遺伝病である．1971年にDaum，Scriverらによって，イソロイシンの中間代謝の異常による有機酸代謝異常症としてLancet誌に初めて報告された．1988年山口らにより，β酸化系の短鎖チオラーゼで，ケトン体代謝に重要なT2が原因酵素であることが明らかにされた．このことから本症は，イソロイシン中間代謝異常としての有機酸代謝異常症であるとともに，ケトン体代謝異常症でもある．本遺伝子は11q22.1-23.1に存在し，酵素蛋白はホモテトラマーである．

臨床所見

1. 臨床症状

感染(発熱，嘔吐)などが引き金となって，重篤なケトアシドーシスをきたす．2歳までに90%が初発発作をおこす．それまでは通常，まったく無症状であり，発作間歇期も無症状である．

2. 一般検査所見

急性期は著しいケトアシドーシスを呈し，血液ガスにてpHは7.2を下回る．発作時に低血糖や高アンモニア血症を認めることもある．

治療と予後

1. 発作時治療

十分なブドウ糖補給が重要である．通常のケトアシドーシスの治療に準じてアシドーシス補正を行うが，炭酸水素ナトリウム過剰投与による高ナトリウム血症に注意が必要である．

2. 非発作時の治療

感染時，絶食時などの早期に経静脈ブドウ糖輸液を行い，発作を未然に防ぐことが重要である．日常の管理として，摂取蛋白質制限(1.5～2.0 g/kg/日)，およびレボカルニチン投与が有効である．

3. 予　後

発作中に死亡もしくは発作後に精神発達遅滞を生じることがある．本症は，適切な診断がつけば重篤な発作を防ぐことができ，成長とともに発作をきたさなくなる．重篤な発作が予防されれば，予後はよい．

化学診断のポイント

1. 異常代謝産物

典型例では2-メチルアセト酢酸，2-メチル-3-ヒドロキシ酪酸，チグリルグリシンが尿中に増加し，尿中有機酸分析で化学診断可能である(**図1**)．典型例では間歇期でも診断可能であるが，残存活性をもつ軽症変異例では，ごくわずかな2-メチル-3-ヒドロキシ酪酸の排泄があるのみのため，正常と判定されてしまうこともある(**図2**)．

2. 鑑別診断

著しいケトアシドーシスをきたすすべての有機酸代謝異常症が鑑別診断となる．除外診断として尿中有機酸分析が重要である．臨床像からはサクシニル-CoA：3-ケト酸-CoAトランスフェラーゼ(SCOT)欠損症が鑑別にあげられる(別稿「ケトン体代謝異常」*p*.112参照)．最終診断は酵素診断，遺伝子診断で行う．その他，有機酸プロフィールからは重篤な精神運動発達遅滞をきたす2-メチル-3-ヒドロキシブチリル-CoA脱水素酵素欠損症が鑑別診断としてあげられるが，日本では報告がない．

タンデムマス所見

アシルカルニチン分析ではC5:1，C5-OHの増加がみられる．また，軽症型では診断が困難なこともある．

参考文献

・深尾敏幸：ケトン体代謝異常症：特にアセトン血性嘔吐症と鑑別すべきサクシニル-CoA：3-ケト酸CoAトランスフェラーゼ欠損症を中心に．日児誌111: 723-739, 2007

· Mitchell GA, Fukao T : Inborn errors of ketone body metabolism. Metabolic and Molecular Bases of Inherited Disease, 8th ed., McGraw-Hill, New York, 2327-2356, 2001

· Fukao T : Beta-ketothiolase deficiency. Orphanet (internet resource), 2004 (www.orpha.net/data/patho/GB/uk-t2.pdf)

（深尾敏幸）

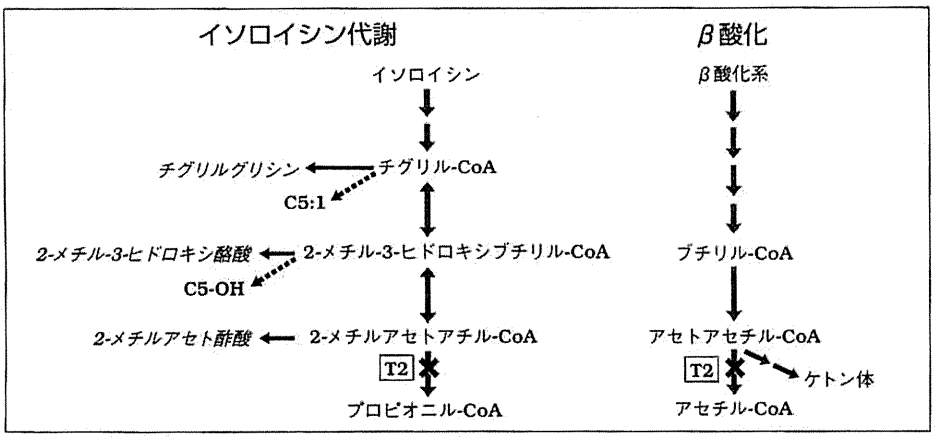


図1　βケトチオラーゼ欠損症の代謝経路

T2 の肝外組織でのケトン体代謝については別稿「ケトン体代謝異常」の図 1 を参照（*p.113*）．T2：ミトコンドリア・アセトアセチル-CoA チオラーゼ，➡：生成される異常代謝産物，⇢：アシルカルニチン抱合，*斜体*：GC/MS で検出される本症に特徴的な異常有機酸，C5:1：チグリルカルニチン，C5-OH：2-メチル-3-ヒドロキシブチリルカルニチン（タンデムマスで検出される特徴的アシルカルニチン）

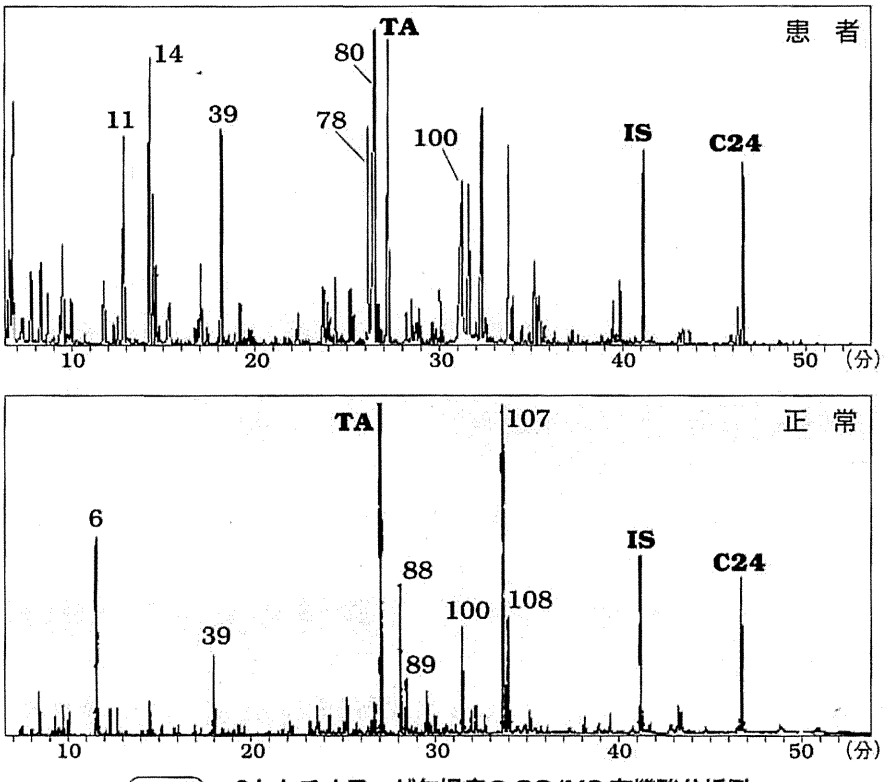


図2　βケトチオラーゼ欠損症の GC/MS 有機酸分析例

6：シュウ酸，11：3-ヒドロキシ酪酸，14：2-メチル-3-ヒドロキシ酪酸，39：コハク酸，78・80：チグリルグリシン，88：2-ケトグルタル酸，89：4-ヒドロキシフェニル酢酸，100：アコニット酸，107：クエン酸，108：馬尿酸，TA：トロパ酸（内部標準 1），IS：ヘプタデカン酸（内部標準 2），C24：テトラコサン（内部標準 3）

11 3-ヒドロキシイソ酪酸血症

3-Hydroxyisobutyric acidemia

概　念

3-ヒドロキシイソ酪酸は，代表的なケトン体である3-ヒドロキシ酪酸の異性体であり，バリンの中間代謝産物である(**図 1**)．3-ヒドロキシイソ酪酸血症は3-ヒドロキシイソ酪酸が大量に排泄される疾患であり，反復性のケトアシドーシスをきたす．

世界で10例程度，日本では2家系が知られている[1)~3)]．最初1991年にKoらが報告した症例は，三角形の顔，耳介低位，長い人中など特異的顔貌を示し，反復性のケトアシドーシス，成長障害，慢性高乳酸血症をきたしている．

病因として，バリン中間代謝における3-ヒドロキシイソ酪酸脱水素酵素(3HiBDH)欠損または下流のメチルマロン酸セミアルデヒド脱水素酵素(MMSADH)欠損，その両者でもない症例が存在し，いまだ病因には不明な点が多い．

臨床所見

1. 臨床症状

症例が限られており病因遺伝子も確定しておらず，単一疾患といってよいのか不明な点も多い．Koらの報告例および日本の1例(3HiBDH活性低下例)[1)]のどちらも，小さな三角形の顔，耳介低位，長い人中など特異的顔貌を示していた．一方，日本のもう1家系の兄弟例(3HiBDH活性正常)では特異的顔貌は認めなかった[2)]．共通するのは反復性のケトアシドーシスである．

2. 一般検査所見

ケトアシドーシス，高乳酸血症を呈する．

治療と予後

1. 発作時治療

通常のケトアシドーシス治療に準じる．

2. 非発作時の治療

バリン制限食や蛋白制限食(2 g/kg)が発作予防に有効と報告されている．レボカルニチン投与が併用されている．

3. 予　後

日本人兄弟例の一方は，ケトアシドーシス発作からてんかん重積状態で死亡．もう一方は右側脳室後角周囲の白質にT2高信号などのMRI変化をきたした[3)]．日本人の特異的顔貌を示した児は重度精神運動発達遅滞をきたしている．単一疾患でない可能性が高く，海外の報告例でも予後はさまざまである．

化学診断のポイント

1. 異常代謝産物

尿中有機酸分析で3-ヒドロキシイソ酪酸が増加し，バリン投与で3-ヒドロキシイソ酪酸排泄増加とケトアシドーシスを再現できたと報告されている．代表的ケトン体である3-ヒドロキシ酪酸と3-ヒドロキシイソ酪酸は分子量も同じで，通常の有機酸分析では同じ保持時間に出てくる．マススペクトルでは，3-ヒドロキシ酪酸には出ないフラグメントが3-ヒドロキシイソ酪酸では認められるので鑑別可能である．

ケトーシス時は大量の3-ヒドロキシ酪酸排泄のため3-ヒドロキシイソ酪酸の増加がわかりにくい．回復期，非発作時の解析ではむしろ，相対的に3-ヒドロキシイソ酪酸の排泄が多く，これを同定することが診断につながる(**図 2**)．

2. 鑑別診断

3-アミノイソ酪酸排泄増多を伴う場合は，メチルマロン酸セミアルデヒドロゲナーゼ欠損の可能性が高い[4)]．

タンデムマス所見

特異的所見はみられない．

文　献

1) Sasaki M et al.: A severely brain-damaged case of 3-hydroxyisobutyric aciduria. *Brain Dev* **23**: 243-245, 2001
2) Sasaki M et al.: 3-Hydroxyisobutyric aciduria in two brothers. *Pediatr Neurol* **18**: 253-255, 1998
3) Sasaki M et al.: Basal ganglia lesions in a patient with 3-hydroxyisobutyric aciduria. *Brain Dev* **28**: 600-603, 2006
4) Loupatty FJ et al.: Clinical, biochemical, and molecular findings in three patients with 3-hydroxyisobutyric aciduria. *Mol Genet Metab* **87**: 243-248, 2006

（深尾敏幸）

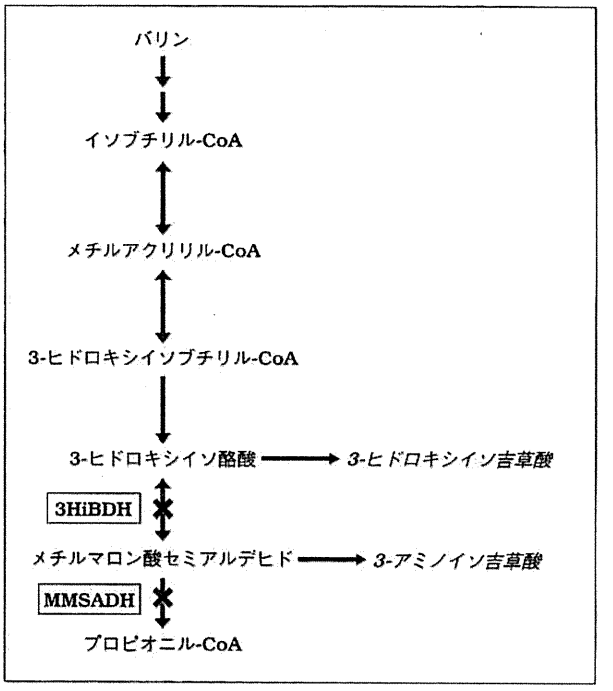


図1　バリン代謝経路

3HiBDH：3-ヒドロキシイソ酪酸脱水素酵素，**MMSADH**：メチルマロン酸セミアルデヒド脱水素酵素，*斜体*：GC/MS で検出される本症に特徴的な異常有機酸，→：生成される異常代謝産物

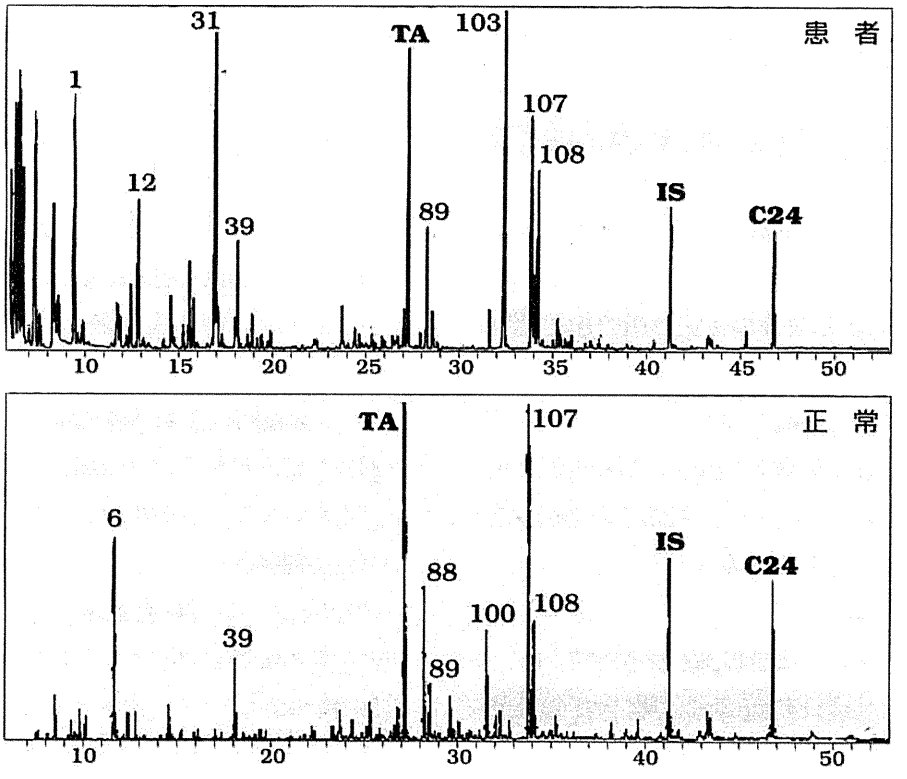


図2　3-ヒドロキシイソ酪酸血症の GC/MS 有機酸分析例

1：乳酸，**6**：シュウ酸，**12**：3-ヒドロキシイソ酪酸，**31**：グリセロール，**39**：コハク酸，**88**：2-ケトグルタル酸，**89**：4-ヒドロキシフェニル酢酸，**100**：アコニット酸，**103**：ホモバニリン酸，**107**：クエン酸，**108**：馬尿酸，**TA**：トロパ酸(内部標準 1)，**IS**：ヘプタデカン酸(内部標準 2)，**C24**：テトラコサン(内部標準 3)

29 ケトン体代謝異常

Disorders in ketone body metabolim

概　念

ケトン体は，肝臓でβ酸化の結果生じるアセチル-CoAとアセトアセチル-CoAから，ミトコンドリア3-ヒドロキシ-3-メチルグルタリル-CoA(HMG-CoA)合成酵素(HMGS)とHMG-CoAリアーゼ(HMGL)の働きによってHMG-CoAを介して生成される(**図1**)．

ケトン体はアセト酢酸(AAA)と3-ヒドロキシ酪酸(3HBA)であり，この両者はミトコンドリア内の酸化還元状態〔還元型ニコチンアミドアデニンジヌクレオチド/ニコチンアミドアデニンジヌクレオチド(NADH/NAD)比〕によってバランスが保たれている．すなわち，ミトコンドリア内のNADH/NAD比が高いとき3HBA/AAA比は高くなる．

炭水化物からのエネルギー供給が低下したとき(長時間空腹時など)や強いストレスのかかったとき(カテコラミンの分泌が亢進したとき)などにケトン体生成が亢進する．肝臓で生成されたケトン体は，サクシニル-CoA:3-ケト酸-CoAトランスフェラーゼ(SCOT)とミトコンドリア・アセトアセチル-CoAチオラーゼ(T2)の働きによって肝外組織で利用される．

ケトアシドーシス発作を主徴とする先天性ケトン体代謝異常として，T2欠損症，SCOT欠損症が有名である．これら疾患では，肝外組織でのケトン体の利用障害によって重篤なケトアシドーシスに傾きやすい．

ケトン体産生に働くHMGSやHMGLが欠損すると，ケトン体産生障害がおこる．これらの欠損症も広義のケトン体代謝異常といえるが，これらは非(低)ケトン性低血糖発作をおこす．

臨床所見(表)

1. βケトチオラーゼ(T2)欠損症

T2欠損症では重篤なケトアシドーシス発作を主徴とする．T2はイソロイシン代謝過程の2-メチルアセトアセチル-CoAの解裂にも働くため，イソロイシンの中間代謝過程に特徴的な有機酸が上昇するので，尿中有機酸分析で化学診断が可能である(*p*.56参照)．

2. サクシニル-CoA:3-ケト酸-CoAトランスフェラーゼ(SCOT)欠損症

SCOT欠損症も重篤なケトアシドーシス発作を主徴とするが，GC/MSではケトーシスを示すのみで特異的所見はみられない．新生児期に約半数がケトアシドーシス発作をきたす．乳児期以降，感染などを契機に頻回のケトアシドーシス発作をおこす例や，無症状でもつねに尿ケトン体陽性であったりケトン体血中濃度の高いような例では，本症を疑うべきである．

3. 3-ヒドロキシ-3-メチルグルタリル-CoAリアーゼ(HMGL)欠損症

間歇的に自家中毒様の発作がおこる．半数は新生児期に重篤な低血糖発作をおこす．低血糖にもかかわらず，ケトン体が低値であることが特徴である．ロイシンなどの中間代謝過程の特徴的な有機酸が上昇するため，尿中有機酸分析で化学診断が可能である(*p*.66参照)．

4. 3-ヒドロキシ-3-メチルグルタリル-CoA合成酵素(HMGS)欠損症

胃腸炎などに伴い，非ケトン性の重篤な低血糖をきたす．遊離脂肪酸(FFA)は高いのにケトン体が低値で，FFA/ケトン体比は脂肪酸β酸化異常症と同様，高くなる．

化学診断のポイント(表)

1. βケトチオラーゼ(T2)欠損症(図2)

2-メチル-3-ヒドロキシ酪酸，2-メチルアセト酢酸，チグリルグリシンなどが増加する(*p*.56参照)．

2. 3-ヒドロキシ-3-メチルグルタリル-CoA リアーゼ(HMGL)欠損症

3-ヒドロキシ-3-メチルグルタル酸，3-メチルグルタル酸，3-メチルグルタコン酸が増加する．

3. サクシニル-CoA：3-ケト酸-CoA トランスフェラーゼ(SCOT)欠損症

ケトーシスの所見のみであり，特異的有機酸の上昇はみられない．

4. 3-ヒドロキシ-3-メチルグルタリル-CoA 合成酵素(HMGS)欠損症

臨床的に非ケトン性低血糖症を呈するが，特異的有機酸の上昇はない．

5. 血中ケトン体と遊離脂肪酸(FFA)の関係

血中 FFA/ケトン体のモル比が 0.3 以下ならばケトン体利用異常，2.0 以上ならばケトン体産生もしくは脂肪酸 β 酸化異常症を疑う．

タンデムマス所見

T2 欠損症では C5:1，C5-OH の上昇，HMGL 欠損症では C5-OH の上昇がみられる．SCOT 欠損症，HMGS 欠損症では特異的所見はみられない．

●参考文献

- 深尾敏幸：ケトン体代謝異常症：特にアセトン血性嘔吐症と鑑別すべきサクシニル-CoA：3-ケト酸 CoA トランスフェラーゼ(SCOT)欠損症を中心に．日児誌 111：723-739, 2007
- Mitchell GA, Fukao T：Inborn errors of ketone body metabolism. Metabolic and Molecular Bases of Inherited Disease, 8th ed., McGraw-Hill, New York, 2327-2356, 2001
- Fukao T：Succinyl-CoA：3-ketoacid CoA transferase(SCOT)deficiency. Orphanet(internet resource), 2004(www.orpha.net/data/patho/GB/uk-scot.pdf)

(深尾敏幸)

表　広義のケトン体代謝異常症

	ケトン体	低血糖	特異的有機酸
T2 欠損症	+++	−/+	2M3HBA, MAA, TG
SCOT 欠損症	+++	−/+	なし
HMGS 欠損症	−	+++	なし
HMGL 欠損症	−	+++	HMGA, MGCA,MGA

T2：ミトコンドリア・アセトアセチル-CoA チオラーゼ(β ケトチオラーゼの欠損酵素)，SCOT：サクシニル-CoA：3-ケト酸-CoA トランスフェラーゼ，HMGS：3-ヒドロキシ-3-メチルグルタリル-CoA 合成酵素，HMGL：3-ヒドロキシ-3-メチルグルタリル-CoA リアーゼ，2M3HBA：2-メチル-3-ヒドロキシ酪酸，MAA：2-メチルアセト酢酸，TG：チグリルグリシン，HMGA：3-ヒドロキシ-3-メチルグルタル酸，MGCA：3-メチルグルタコン酸，MGA：3-メチルグルタル酸

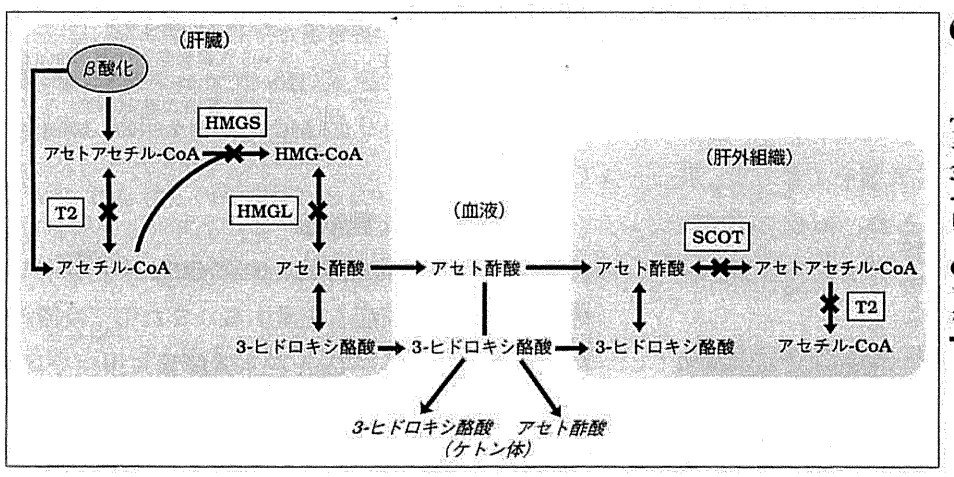


図1　ケトーシスを伴ったケトン体代謝異常の代謝経路

T2：ミトコンドリア・アセトアセチル-CoA チオラーゼ，HMGS：3-ヒドロキシ-3-メチルグルタリル-CoA 合成酵素，HMGL：3-ヒドロキシ-3-メチルグルタリル-CoA リアーゼ，SCOT：サクシニル-CoA：3-ケト酸-CoA トランスフェラーゼ，*斜体*：GC/MS で検出される本症に特徴的な異常有機酸，→：生成される異常代謝産物

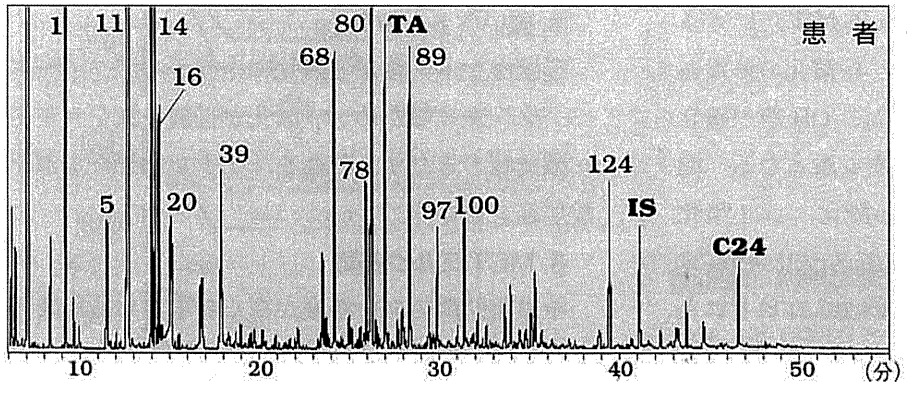


図2　βケトチオラーゼ(T2)欠損症の GC/MS 有機酸分析例

1：乳酸，5：グリオキシル酸，11：3-ヒドロキシ酪酸，14：2-メチル-3-ヒドロキシ酪酸，16：3-ヒドロキシイソ吉草酸，20：尿酸，39：コハク酸，68：アジピン酸，78・80：チグリルグリシン，89：4-ヒドロキシフェニル酢酸，97：スベリン酸，100：アコニット酸，124：3-ヒドロキシセバシン酸，TA：トロパ酸(内部標準 1)，IS：ヘプタデカン酸(内部標準 2)，C24：テトラコサン(内部標準 3)

31 ケトン性ジカルボン酸尿症

Ketotic dicarboxylic aciduria

概　念

ケトン体(3-ヒドロキシ酪酸，アセト酢酸)増加とともにジカルボン酸の増加した状態を，ケトン性ジカルボン酸尿症という．

炭水化物からのエネルギー供給が低下したとき，グリコーゲン分解や糖新生系によって血糖が維持される．さらに脂肪酸β酸化系が作動し，代替エネルギーとしてケトン体が産生される．小児では表に示すように，長時間の飢餓状態や感染・発熱・下痢などのエネルギー消耗状態，あるいは強い精神ストレス時(遠足，運動会の前日など)にケトーシスをおこす．ストレスを受けた際に分泌されるカテコラミンやグルカゴンはケトン体産生を促進し，一方，インスリン分泌はケトン体産生抑制にはたらく．ケトーシスのときに食事をとったりブドウ糖輸液をすると，インスリン分泌がおこりケトーシスは消失する．

小児期にケトーシスをきたすことが多い理由として，ホルモン調節の未熟性のほか，臓器が小さいためアラニンなど糖新生基質の予備能が少ないことなどが考えられる．β酸化の産物としてのケトン体が過剰産生されるとβ酸化にネガティブフィードバックがかかり，β酸化経路の中間体が蓄積する．蓄積した中間体は，図1に示すようにω酸化を受けてジカルボン酸，あるいは3-ヒドロキシジカルボン酸，不飽和ジカルボン酸になる．中間体がω-1酸化を受けると(ω-1)ヒドロキシ脂肪酸(9-ヒドロキシデカン酸，7-ヒドロキシオクタン酸など)も増加する．

表　おもなケトン性ジカルボン酸尿症をきたす疾患(明らかな有機酸代謝異常症を除く)

1. 生理的ケトーシス(発熱，胃腸炎などに伴う)
2. 周期性嘔吐症
3. ケトン性低血糖症
4. 糖尿病性ケトアシドーシス
5. 糖原病
6. 成長ホルモン単独欠損症
7. SCOT欠損症
8. βケトチオラーゼ欠損症(T2欠損症)
9. サリチル酸中毒

SCOT：サクシニル-CoA：3-ケト酸-CoAトランスフェラーゼ，
T2：ミトコンドリア・アセトアセチル-CoAチオラーゼ

臨床所見

嘔吐，腹痛，頭痛，蒼白など，いわゆる「自家中毒」の症状を呈する．

一般検査所見としては，遊離脂肪酸(FFA)の増加，血中ケトン体の増加がみられる．糖新生の障害を伴うと，低血糖(ケトン性低血糖症)もみられることがある．

治療と予後

1. 治　療

糖分補給，ブドウ糖輸液を行う．この他，必要に応じて電解質補正，基礎疾患に対する治療を考慮する．

2. 予　後

いわゆる「周期性嘔吐症」ならば，ブドウ糖輸液などによって短時間のうちに回復する．年齢が上がるにつれて(8歳以降には)みられなくなる．しかし，意識低下を伴うほどの強いケトーシスでは，治療が遅れると後遺症を残すこともある．とくに，頻回の発作，強いケトーシスを繰り返すような症例では，ケトン体代謝異常との鑑別が必要である．

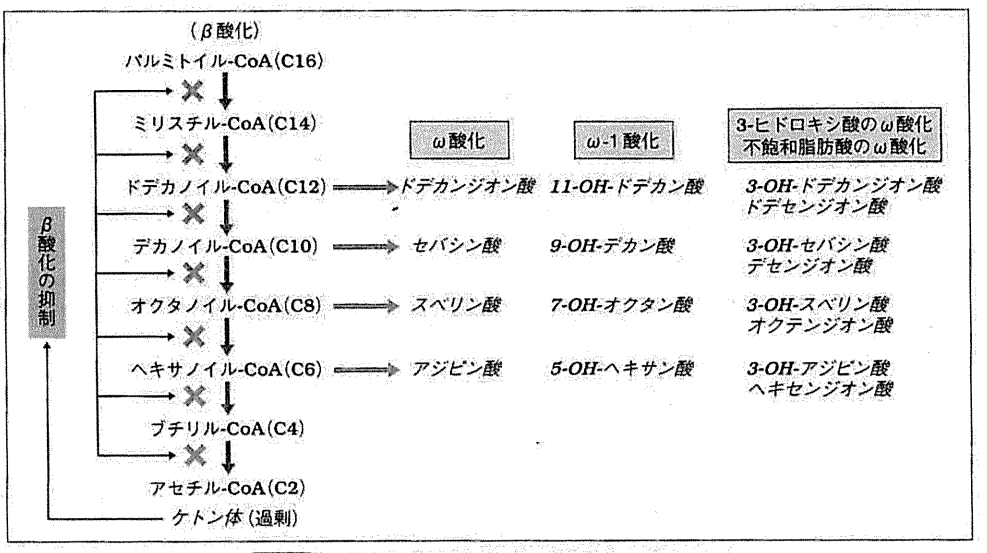
化学診断のポイント

ケトン体の大量排泄と同時に，ジカルボン酸(アジピン酸，スベリン酸，セバシン酸，ドデカンジオン酸など)，3-ヒドロキシジカルボン酸(3-ヒドロキシセバシン酸)，あるいは(ω-1)ヒドロキシ脂肪酸(7-ヒドロキシオクタン酸など)の増加がみられる(図2)．

タンデムマス所見

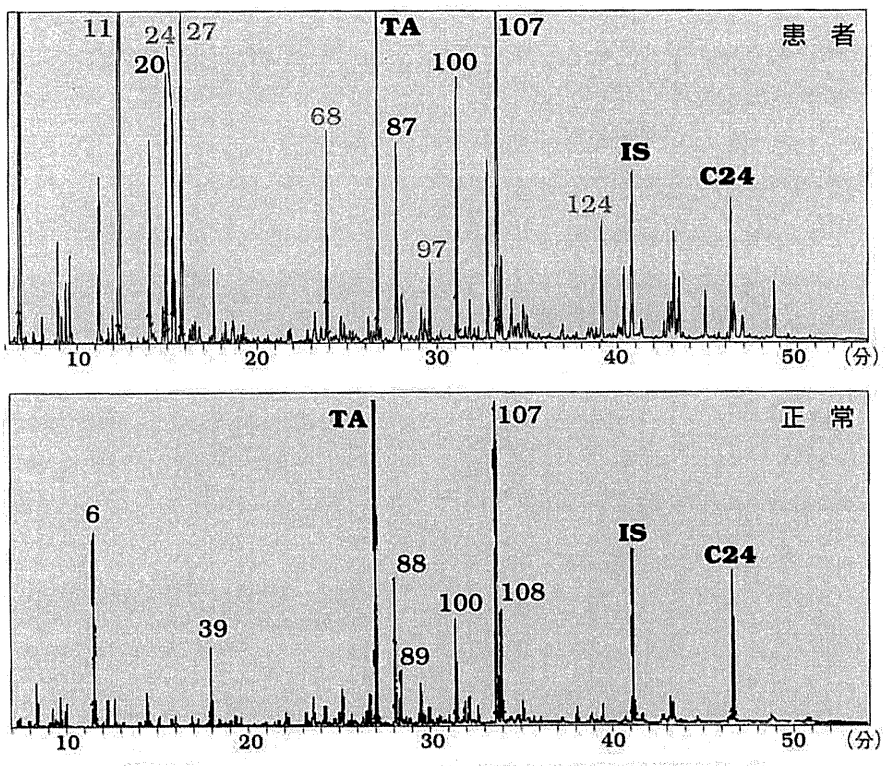
通常，特徴的所見がみられない．時に，C4-OH(3-ヒドロキシブチリルカルニチン)の上昇がみられることもある．

（渡邊宏雄，深尾敏幸）



図Ⅰ　**ケトン性ジカルボン酸尿症の代謝経路**

C16, C14：脂肪酸の炭素鎖長．ケトン体とは3-ヒドロキシ酪酸とアセト酢酸をさす．
斜体：GC/MSで検出される本症に特徴的な異常有機酸，➡：生成される異常代謝産物



図Ⅱ　**ケトン性ジカルボン酸尿症のGC/MS有機酸分析例**

6：シュウ酸，**11**：3-ヒドロキシ酪酸，**20**：尿素，**24**・**27**：アセト酢酸，**39**：コハク酸，**68**：アジピン酸，**87**：4-ヒドロキシ安息香酸，**88**：2-ケトグルタル酸，**89**：4-ヒドロキシフェニル酢酸，**97**：スベリン酸，**100**：アコニット酸，**107**：クエン酸，**108**：馬尿酸，**124**：3-ヒドロキシセバシン酸，**TA**：トロパ酸(内部標準1)，**IS**：ヘプタデカン酸(内部標準2)，**C24**：テトラコサン(内部標準3)

32 非ケトン性ジカルボン酸尿症

Non-ketotic dicarboxylic aciduria

概　念

ジカルボン酸尿症のうち，ケトン体の排泄がジカルボン酸の排泄に比べ著しく少ない場合を非ケトン性(または低ケトン性)ジカルボン酸尿症という．一般に，脂肪酸β酸化障害を反映する．非ケトン性ジカルボン酸尿症とは疾患名でなく所見名であり，表に示すような疾患が含まれる．

臨床所見

乳幼児期には長期間の飢餓状態や感染，下痢などに伴う急性脳症[1]，Reye 症候群，乳幼児突然死症候群(SIDS)様症状，低血糖によるけいれんなどがみられる．また，非ケトン性ジカルボン酸尿症を呈するβ酸化障害の急性期には，肝機能障害，高アンモニア血症，横紋筋融解症がみられる．学童～思春期以降に発症する例では，筋痛，脱力，易疲労感などの筋症状が主症状であることが多い．

一般検査所見

β酸化異常症では，CK，乳酸脱水素酵素(LDH)，アンモニアなどの上昇がみられ，運動後にミオグロビン尿をきたすこともある．低血糖症になりやすい．

治療と予後

1. 急性期

高張ブドウ糖液の点滴を行い，ブドウ糖からのエネルギー供給を行う．横紋筋融解症，Reye 症候群に至れば集中治療が必要である．

2. 慢性期

年齢に応じた食事間隔を指導し，食事がとれないときや感染症罹患時などでは早めのブドウ糖輸液が必要である[2]．夜間のコーンスターチ療法も有効なことがある．二次性のカルニチン欠乏に注意する．

3. 予　後

重症型では乳幼児期早期に死亡し，年長児，成人では筋症状，全身けいれんの発作があるが，知能は原則として正常である．

病態と化学診断

一般に，無症状のときには異常を示さないことが多いため，脱水，感染などの症状がみられるときに分析を行うことが重要である．

脂肪酸β酸化系が障害されるため，各炭素鎖長の脂肪酸の中間体(アシル-CoA)が蓄積される．それらはミクロソームにてω酸化もしくはω-1 酸化される．ω酸化されたアジピン酸，スベリン酸，セバシン酸，ドデカンジオン酸の排泄増加がみられる(図 1)．また，3-ヒドロキシジカルボン酸，不飽和ジカルボン酸，アシルカルニチンも排泄される(図 2)．

タンデムマス所見

各疾患特有のアシルカルニチンパターンから診断される(表)．

表　非ケトン性ジカルボン酸尿症をきたす疾患とアシルカルニチン所見

	疾患・状態	上昇するおもなアシルカルニチン
先天代謝異常	1．VLCAD 欠損症	C14:1, C16
	2．MCAD 欠損症	C8, C10, C6
	3．TFP 欠損症	C16-OH, C16
	4．CPT-1 欠損症	C16, C18, C0(高値)
	5．CPT-2 欠損症	C16, C18
	6．グルタル酸血症Ⅱ型	C6, C8, C10, C12, C14, C16
	7．CACT 欠損症	C16, C18
	8．全身性カルニチン欠乏症	C0(低下)，(全体に低下)
	9．ペルオキシソーム病	特異的所見なし
	10．HMGS 欠損症	特異的所見なし
	11．Reye 症候群	特異的所見なし
後天的要因	12．MCT ミルク(オイル)摂取時	特異的所見なし
	13．バルプロ酸服用中	特異的所見なし
	14．β酸化系の障害された病態(薬剤，細菌毒素など)	不明

VLCAD：極長鎖アシル-CoA 脱水素酵素，**MCAD**：中鎖アシル-CoA 脱水素酵素，**TFP**：ミトコンドリア三頭酵素，**CPT-1** および **CPT-2**：カルニチンパルミトイルトランスフェラーゼⅠ型とⅡ型，**CACT**：カルニチンアシルカルニチントランスロカーゼ，**HMGS**：3-ヒドロキシ-3-メチルグルタリル-CoA 合成酵素，**MCT**：中鎖トリグリセリド

文　献

1）山口清次：ミトコンドリアβ酸化異常症(概論)．骨格筋症候群，日本臨牀(別冊)領域別症候群シリーズ **36**：60-64, 2001

2）山口清次，小林弘典：先天性脂肪酸代謝異常症．小児科臨床 **59**：643-651, 2006

(渡邊宏雄，深尾敏幸)

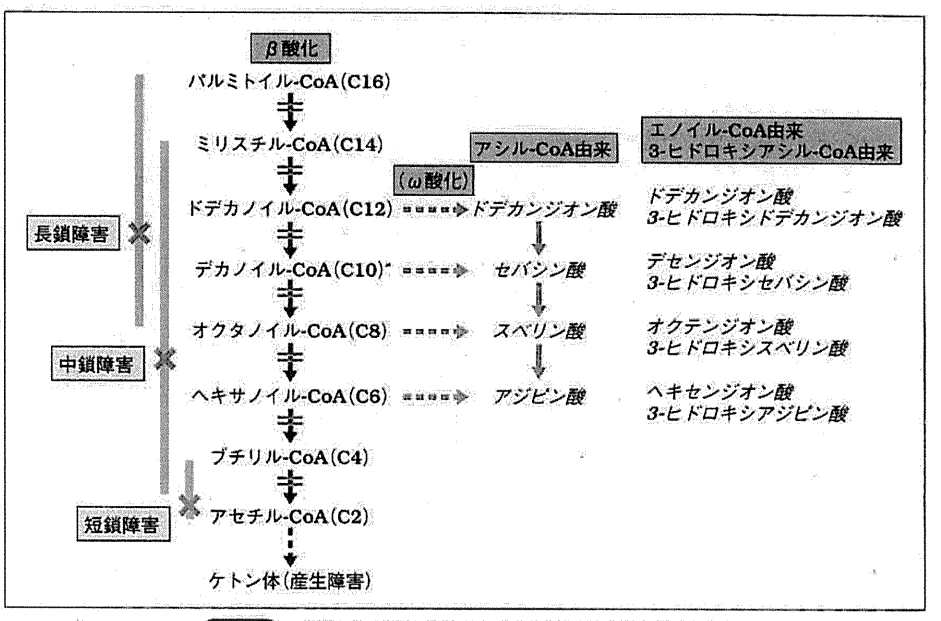


図1　非ケトン性ジカルボン酸尿症の代謝経路

C16, C14, C12 …は脂肪酸の炭素鎖長を表す．ω酸化を受けて生成されたジカルボン酸自体のβ酸化はペルオキシソームも関与する，*斜体*：GC/MS で検出される本症に特徴的な異常有機酸，→：生成される異常代謝産物，⇢：アシルカルニチン抱合

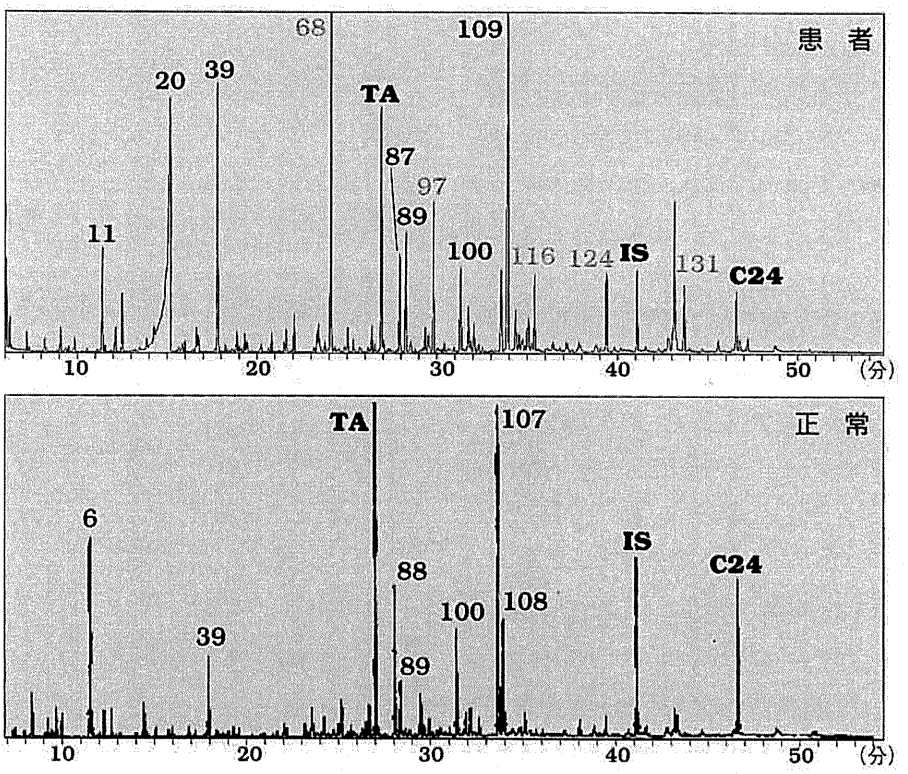


図2　非ケトン性ジカルボン酸尿症の GC/MS 有機酸分析例

6：シュウ酸，**11**：3-ヒドロキシ酪酸，**20**：尿素，**39**：コハク酸，**68**：アジピン酸，**87**：4-ヒドロキシ安息香酸，**88**：2-ケトグルタル酸，**89**：4-ヒドロキシフェニル酢酸，**97**：スベリン酸，**100**：アコニット酸，**107**：クエン酸，**108**：馬尿酸，**109**：ホモゲンチジン酸，**116**：セバシン酸，**124**：3-ヒドロキシセバシン酸，**131**：3-ヒドロキシドデカンジオン酸，**TA**：トロパ酸(内部標準 1)，**IS**：ヘプタデカン酸(内部標準 2)，**C24**：テトラコサン(内部標準 3)

55 3-ヒドロキシジカルボン酸尿症

3-Hydroxydicarboxylic aciduria

概　念

脂肪酸β酸化系において，第三段階の3-ヒドロキシアシル-CoA脱水素酵素(HAD)の部位でブロックされると3-ヒドロキシアシル-CoAが蓄積し，3-ヒドロキシジカルボン酸の排泄増加がみられる(図1)．非ケトン性ジカルボン酸尿症の所見に加えて，3-ヒドロキシジカルボン酸の排泄が目立つような有機酸所見を3-ヒドロキシジカルボン酸尿症という．これは，診断名ではなく有機酸所見である．

おもな疾患

1. ミトコンドリア三頭酵素(TFP)欠損症

ミトコンドリア三頭酵素(TFP)はα鎖，β鎖の二つのサブユニットからなり，α鎖はエノイル-CoAヒドラターゼ(EH)と長鎖3-ヒドロキシアシル-CoA脱水素酵素(LCHAD)を，β鎖は3-ケトアシル-CoAチオラーゼ(3KAT)の活性をもっている．1989年に，三つの活性のうちLCHADの活性のみが低下したLCHAD欠損症が報告され[1]，現在までに約60例以上が知られている．三つの酵素活性がすべて低下した場合をTFP欠損症といい，世界で50例，日本でも5例[2]が知られている．

2. ミトコンドリア・電子伝達系異常症

急性期に3-ヒドロキシジカルボン酸尿症の所見がみられることがある．その原因として，還元型ニコチンアミドアデニンジヌクレオチド(NADH)の酸化障害によってNADHが蓄積し，間接的にHAD反応を阻害すると考えられる．しかし，この所見は非特異的で，つねにみられるわけではない．

臨床所見

1. ミトコンドリア三頭酵素(TFP)欠損症

他の脂肪酸β酸化系異常症の所見と同様である．乳幼児期には感染，下痢などのときに乳幼児突然死症候群(SIDS)様症状，Reye症候群などで発症し，心筋障害，突然死をきたすこともある．年齢が長じると，筋痛，脱力，易疲労感など筋症状で気づかれることが多い．検査では，尿ケトン体は一般に低く，低血糖やCK，乳酸脱水素酵素(LDH)，アンモニアなどの上昇がみられる．また，運動後にミオグロビン尿をきたすこともある．

2. ミトコンドリア異常症

検査では血中の乳酸，ピルビン酸の上昇を伴う．髄液中の乳酸のみが上昇していることもあるので注意を要する．

治療と予後

他の脂肪酸β酸化系異常症と同様に，急性期には高張ブドウ糖液の点滴を行う．

L-カルニチンの二次欠乏にも注意する．中鎖トリグリセリド(MCT)食やコーンスターチが有用なこともある．末梢神経障害や網膜症の合併が報告されている．

病態と化学診断

無症状のときには異常を示さないことも多い．

脂肪酸β酸化系障害では，しばしば非ケトン性ジカルボン酸尿を示す．すなわち，アジピン酸，スベリン酸，セバシン酸，ドデカンジオン酸などのジカルボン酸が排泄される．また，3-ヒドロキシアジピン酸，3-ヒドロキシスベリン酸，3-ヒドロキシセバシン酸，3-ヒドロキシドデカンジオン酸などの3-ヒドロキシジカルボン酸尿の排泄増多もしばしばみられる(図2)．そして，TFP欠損症(LCHAD欠損症を含む)では，3-ヒドロキシジカルボン酸尿がより顕徴である．

ミトコンドリア異常症や電子伝達系障害では3-ヒドロキシジカルボン酸尿症の所見に加え，乳酸，ピルビン酸，3-ヒドロキシ酪酸，アセト酢酸の排泄が増加しており，とくに乳酸/ピルビン酸(L/P)比，3-ヒドロキシ酪酸/アセト酢酸(3HBA/

AAA)比の上昇がみられる.

タンデムマス所見

TFP 欠損症では，C16：0，C16：1，C18：0，C18：1，C16-OH，C18-OH の上昇が特徴的である.

文　献

1) Wanders RJ et al.: Sudden infant death and long-chain 3-hydroxyacyl-CoA dehydrogenase. *Lancet* **2**: 52-53, 1989
2) Purevsuren J et al.: Clinical and molecular aspects of Japanese patients with mitochondrial trifunctional protein deficiency. *Mol Genet Metab* **98**: 372-377, 2009

（渡邊宏雄，深尾敏幸）

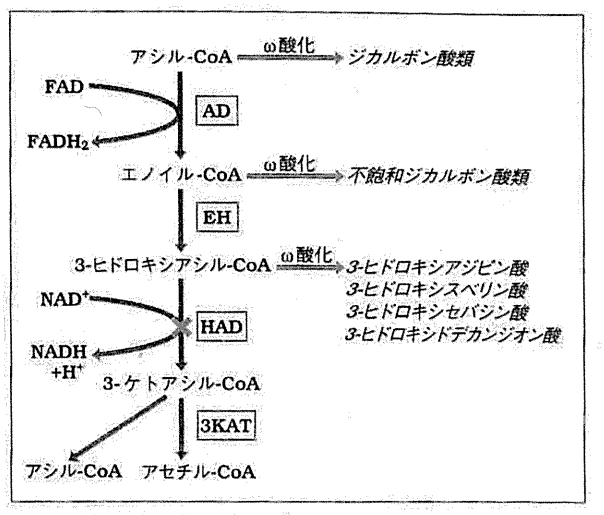


図1　3-ヒドロキシジカルボン酸尿症の代謝経路（β酸化経路）

AD：アシル-CoA 脱水素酵素，**EH**：エノイル-CoA ヒドラターゼ，**HAD**：3-ヒドロキシアシル-CoA 脱水素酵素，**3KAT**：3-ケトアシル-CoA チオラーゼ，**FAD**：フラビンアデニンジヌクレオチド，**$FADH_2$**：還元型フラビンアデニンジヌクレオチド，**NAD**：ニコチンアミドアデニンジヌクレオチド，**NADH**：還元型ニコチンアミドアデニンジヌクレオチド，*斜体*：GC/MS で検出される本症に特徴的な異常有機酸，→：生成される異常代謝産物
3-ヒドロキシジカルボン酸尿症はβ酸化系の第三段階(HAD)の障害

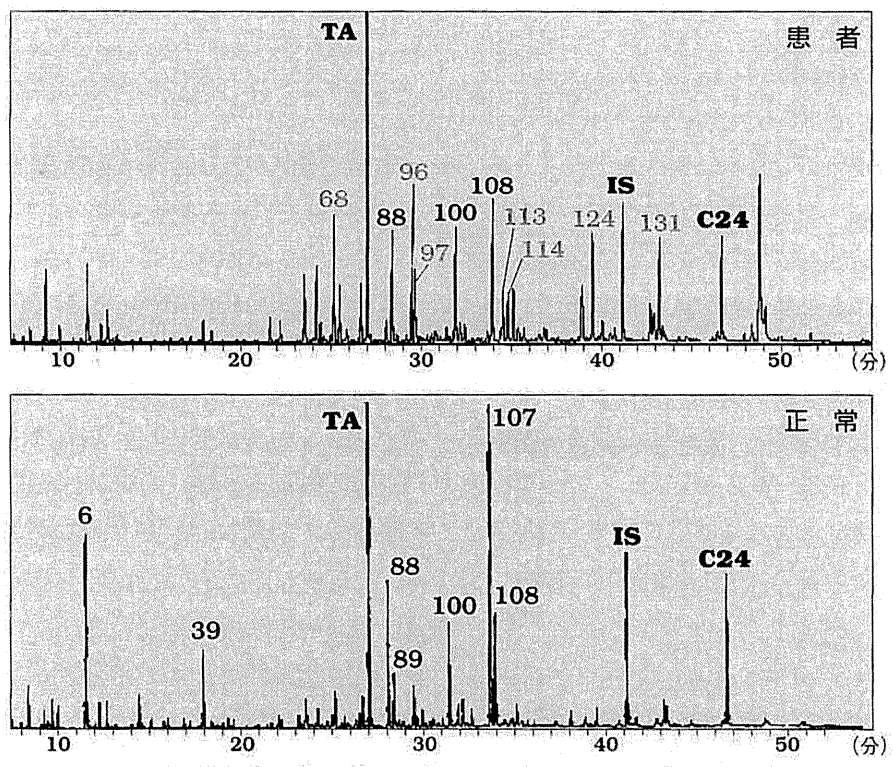


図2　3-ヒドロキシジカルボン酸尿症の GC/MS 有機酸分析例

6：シュウ酸，**39**：コハク酸，**68**：アジピン酸，**88**：2-ケトグルタル酸，**89**：4-ヒドロキシフェニル酢酸，**96**：3-ヒドロキシアジピン酸，**97**：スベリン酸，**100**：アコニット酸，**107**：クエン酸，**108**：馬尿酸，**113**：3-ヒドロキシオクテンジオン酸，**114**：3-ヒドロキシスベリン酸，**124**：3-ヒドロキシセバシン酸，**131**：3-ヒドロキシドデカンジオン酸，**TA**：トロパ酸(内部標準 1)，**IS**：ヘプタデカン酸(内部標準 2)，**C24**：テトラコサン(内部標準 3)

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Clinical and molecular characterization of five patients with succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency

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ABSTRACT

Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency is an inborn error of ketone body metabolism and causes episodic ketoacidosis. We report clinical and molecular analyses of 5 patients with SCOT deficiency. Patients GS07, GS13, and GS14 are homozygotes of S405P, L327P, and R468C, respectively. GS17 and GS18 are compound heterozygotes for S226N and A215V, and V404F and E273X, respectively. These mutations have not been reported previously. Missense mutations were further characterized by transient expression analysis of mutant cDNAs. Among 6 missense mutations, mutants L327P, R468C, and A215V retained some residual activities and their mutant proteins were detected in immunoblot analysis following expression at 37 °C. They were more stable at 30 °C than 37 °C, indicating their temperature sensitive character. The R468C mutant is a distinct temperature sensitive mutant which retained 12% and 51% of wild-type residual activities at 37 and 30 °C, respectively. The S226N mutant protein was detected but retained no residual activity. Effects of missense mutations were predicted from the tertiary structure of the SCOT molecule. Main effects of these mutations were destabilization of SCOT molecules, and some of them also affected catalytic activity. Among 5 patients, GS07 and GS18 had null mutations in both alleles and the other three patients retained some residual SCOT activities. All 5 developed a first severe ketoacidotic crisis with blood gas pH <7.1, and experienced multiple ketoacidotic decompensations (two of them had seven such episodes). In general, the outcome was good even following multiple ketoacidotic events. Permanent ketosis or ketonuria is considered a pathognomonic feature of SCOT deficiency. However, this condition depends not only on residual activity but also on environmental factors.

1. Introduction

Ketone bodies, produced mainly in the liver, are an important source of energy for extrahepatic tissues [1]. Succinyl-CoA: 3-ketoacid CoA transferase (SCOT; EC 2.8.3.5, gene symbol OXCT1) is a mitochondrial homodimer essential for ketone body utilization. SCOT catalyzes acetoacetate activation to acetoacetyl-CoA in mitochondria. The human OXCT1 gene is mapped to 5p13 and consists of 17 exons [2,3]. Human SCOT cDNA encodes a precursor subunit of 520 amino acids.

Patients with SCOT deficiency (OMIM 245050) experience episodic ketoacidosis and are usually asymptomatic between episodes. Fewer than 30 affected individuals are known [2–22]. Urinary organic acid analysis and acylcarnitine analysis show non-specific profiles in this disorder. Hence, in vitro methods of diagnosis, such as enzyme assay and mutation analysis, are essential for the definite diagnosis. Permanent ketosis or ketonuria is a pathognomonic feature of this disorder but is not always present [17,20,22]. We previously identified 11 mutations of the OXCT1 gene [6,8–14] in 12 SCOT-deficient families.

Recently, the human SCOT tertiary structure has become available (PDB entry 3DLX). This has enabled us to evaluate effects of missense

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mutations more precisely than homology modeling using porcine SCOT structure [20].

We herein describe 5 SCOT-deficient patients and characterize their mutations by transient expression analysis of mutant cDNAs and discuss the mutation sites on the tertiary structure of human SCOT.

2. Patients, materials and methods

2.1. Patients

GS07: Clinical findings of GS07 have been reported previously [11]. Briefly, he presented with two episodes of ketoacidosis during infections at 17 and 25 months of age. He was followed until age 6 years without any further episodes.

GS13: Some aspects of GS13 have already been published [14]. Although parents are not known to be related, the family originates from a small socially isolated area, making consanguinity not unlikely. After an initial severe crisis at age 6 months, the girl had three additional, but milder crises during infancy. After 6 h of fasting during a test performed after the first crisis, her blood pH was 7.42, HCO_3 12.5 mmol/L and BE −9.1 mmol/L, with high serum ketones 3.55 mmol/L and low FFA of 0.29 mmol/L. At the age of 12.5 years she is now doing well, attending a regular school. She usually takes her last meal at about 10 p.m. and takes first morning meal in school at about 9 to 10 a.m. without any signs or symptoms. Acid-base balance was checked three times at about 8 a.m. and was always normal.

GS14: This female patient of 21 years of age was born to consanguineous parents in October 1988. She had many hospitalizations in the pediatric ward for episodes of hypoglycemia. Her first hospitalization was at 19 months for seizures and coma. On physical exam she had deep respiration. Her blood glucose was 1.3 mmol/L, She had severe metabolic acidosis with pH 6.93, HCO_3 6 mmol/L and ketonuria 3+. She had six similar episodes of hypoglycemia with ketoacidosis without seizures, usually after episodes of infections. Her neuro-developmental status was normal. She had persistent ketonuria between episodes of decompensation.

GS17: She is a Caucasian girl born to non-consanguineous parents at full-term with a birth weight of 2230 g. She had low blood glucose levels of 1.6 mmol/L on the first day of life and received intravenous glucose for 1–2 days. She was discharged on day 5 of life. She was well until 3 years of age when she developed tachypnea and lethargy following gastroenteritis. Her blood pH was 6.99 with BE −25 mmol/L and her blood glucose was 7.5 mmol/L. Urine analysis showed massive ketones. She was intubated and admitted to the intensive care unit. She responded to intravenous glucose and bicarbonate and was discharged after 7 days. Six months later she was readmitted to a local hospital with mild lethargy, tachypnea and ketoacidosis which developed during a febrile upper respiratory infection. The patient's mother reported that the patient has had trace to moderate ketonuria, even when she was in good health. SCOT deficiency was confirmed by an enzyme assay using fibroblasts. The patient has not had any more episodes of ketoacidosis for 6 years. She is doing well and receives a mildly protein-restricted diet (2.0 g/kg/day), avoids prolonged fasting and adheres to "sick day" precautions such as increasing calories from carbohydrates in her diet and intravenous glucose as needed.

GS18: The male patient, first child of healthy non-consanguineous parents from Vietnam, was born at 39 weeks with a birth weight of 3390 g. After a normal clinical presentation during the first days of life he was readmitted to hospital at the age of 3 days with polypnea. Biochemically he presented with severe metabolic acidosis (pH 7.08, pCO_2 25 mmHg, BE −22.6 mmol/l) and pronounced ketonuria. Beside ketones, metabolic screening revealed unremarkable urinary organic acids. With intravenous fluid with sodium bicarbonate the patient recovered within hours. During the first year of life the patient was hospitalized three times because of episodes of severe ketoacidosis (minimal pH 6.98, pCO_2 15 mmHg, BE −28 mmol/l). At the age of 1 year, SCOT deficiency was confirmed by an enzyme assay using his lymphocytes and platelets. He was on treatment that consisted of avoidance of prolonged fasting and moderate protein restriction (1.5 g/kg/d). Subsequently the patient has had three more severe episodes of ketoacidosis in the course of intercurrent diseases. Otherwise, he has permanent mild ketonuria. At his present age of 10 years psychomotor and physical development are normal.

Table 1 summarizes the clinical presentations and laboratory data of these 5 SCOT-deficient patients. This study has been approved by The Ethical Committee of Graduate School of Medicine, Gifu University.

2.2. Enzyme assay and immunoblot analysis

Assays for acetoacetyl-CoA thiolase and for SCOT were performed as described [7,23], using acetoacetyl-CoA as a substrate and measuring its disappearance spectrophotometrically.

2.3. Mutation analysis

Total RNA was purified from peripheral blood mononuclear cells with an ISOGEN kit (Nippon Gene, Tokyo, Japan). RT-PCR was as described [2]. Mutations were detected by amplifying cDNA spanning the full-length coding sequence, and sequencing more than 5 clones.

Genomic DNA was purified with a Sepa Gene kit (Sanko Junyaku, Tokyo, Japan). Mutation analysis at the genomic level was done by PCR for each exon and its intron boundaries (at least 20 bases from the exon/intron boundaries for both directions) followed by direct sequencing [3].

2.4. Construction of eukaryote transient expression vectors

Wild-type full-length SCOT cDNAs [3] were subcloned into the pTZ18U and pCAGGS eukaryote expression vectors [24] and designated the pTscotWild-type and pCAGGSscotWild-type, respectively. Mutations were introduced into the pTscotWild-type using a QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla CA), confirmed by sequencing, and then transferred into pCAGGS.

2.5. Transient expression analysis

Wild-type and mutant SCOT expression vectors (4 μg) were first transfected using Lipofectamine 2000 (GIBCO BRL Invitrogen Inc., Carlsbad, CA) in ~10^5 SV40-transformed SCOT-deficient fibroblasts of GS01[2]. One microgram of the cytosolic acetoacetyl-CoA thiolase (CT)-expressing vector, pCAGGSct [25], was cotransfected to monitor transfection efficiency. Transfection was done at 37 °C for 24 h was followed by a further 48-h incubation at 37 or 30 °C. The cells were harvested and stored at −80 °C until SCOT and CT activities were assayed. Immunoblot was done using a mixture of anti-[human SCOT] antibody and anti-[human CT] antibody as the first antibody [26]. The quantity of mutant protein was estimated densitometrically, and was compared to the signal intensities of serially diluted samples of the wild-type SCOT protein.

2.6. Tertiary structural model of human SCOT

To analyze the putative structural implications of the SCOT mutations, the recently determined crystal structure from the Structural Genomics Consortium (PDB entry 3DLX) of human SCOT was taken as a starting point. Prior to the analysis, the structure was subjected to further refinement in PHENIX [27] and COOT [28], including the addition of missing side chains and rebuilding of the solvent network. The figures describing the structural details were prepared with PyMOL.

Table 1
Clinical presentation and mutation of SCOT-deficient cases.

GS number	Nationality	Sex	Consanguinity	Affected siblings	First ketoacidotic crisis						Persistent ketonuria	Frequency of crisis	Present age	Prognosis	Gene mutation		SNP		
					Onset	Symptom	Blood gas pH	HCO3	BE	Glucose					Paternal	Maternal	rs75134564 c.173C>T(T58M)	rs1136453 c.315 G>C(S105S)	c.1497 A>G(Q499Q)
GS07	France	M	+	–	1y5m	Polypnea, lethargy	6.92	4		na	+	2	(6y)	NP	c.1213T>C (S405P)	c.1213T>C (S405P)	C/C	C/C	G/G
GS13	Croatia	F	*	–	6 m	Appetite loss, coma	6.90	3.2	−27	7.8	–	2	12y	NP	c.980T>C (L327P)	c.980T>C (L327P)	C/C	C/C	G/G
GS14	Tunisia	F	+	–	1y7m	Seizure, coma	6.93	6		1.3	+	7	21y	NP	c.1402C>T (R468C)	c.1402C>T (R468C)	C/C	C/C	G/G
GS17	USA	F	–	–	3y	Polypnea	6.99	5	−25	7.5	+	2	9y	NP	c. 644C>T (A215V)	c.677G>A (S226N)	C/C	C/C	G/G
GS18	Germany**	F	–	+	3d	Polypnea, lethargy	7.08		−22.6	9.1	+	5	10y	NP	c.817G>T (E273X)	c.1210G>T (V404F)	C/T	G/G	A/A

NP: nothing particular.
* Possible consanguinity.
** GS18 is Vietnamese.

3. Results and discussion

3.1. Enzyme assay

Enzyme assay data for 4 patients are shown in Table 2. All four patients' fibroblasts presented with decreased SCOT activity, whereas they had a potassium-ion activated acetoacetyl-CoA thiolase activity which was a specific character of mitochondrial acetoacetyl-CoA thiolase (T2). In immunoblot analysis, SCOT protein was scarcely detected in these patients' cells, whereas T2 protein was clearly detected (data not shown). Lymphocytes and platelets from GS18 had no apparent SCOT activity (data not shown). These results confirmed the diagnosis of SCOT deficiency in the 5 patients.

3.2. Mutation analysis

Both genomic mutation analysis and cDNA analysis were done in all the cases except for GS18 of whom RNA was not available. The results of mutation analyses are shown in Table 1. Three patients with definite or possible consanguinity had homozygous mutations (c.1213T>C (S406P) in GS07; c.980T>C (L327P) in GS13; c.1402C>T (R468C) in GS14). GS17 is a compound heterozygote of c.644C>T (A215V) from the father and c.677G>A (S266N) from the mother. GS18 is also a compound heterozygote of c.817G>T (E273X) from the father and c.1210G>T (V404F) from the mother. We also detected three single nucleotide polymorphisms. Among them, c.173C>T (T58M) (rs75134564) was previously identified in a Japanese patient (GS02) and demonstrated not to reduce enzyme activity [13].

3.3. Transient expression analysis of mutant cDNAs

We performed transient expression analysis of wild-type and mutant cDNAs in SCOT-deficient SV40-transformed fibroblasts. Following expression of SCOT cDNAs for 48 h at either 37 or 30 °C, an enzyme assay and immunoblots were performed (Fig. 1). The transfection of wild-type SCOT cDNA produced high SCOT activity, whereas that of mock cDNA produced no demonstrable enzyme activity at any temperature. Among 6 missense mutations, S226N, V404F, and S405P did not retain residual SCOT activity. The A215V, L327P and R468C mutants retained detectable residual activities, 3.5, 4.7 and 12% of the wild-type value, respectively, in expression at 37 °C. Their relative residual SCOT activities to wild-type in expression at 30 °C were 3- to 4-fold higher than those in expression at 37 °C. In particular, R468C mutants retained a 51% activity of wild-type value in the expression at 30 °C. In immunoblot analysis, V404F and S405P protein was not detected in expression at 30 and 37 °C. S226N protein was clearly detected in the expression at 30 °C without any detectable residual activity, indicating that S226N protein was an inactive protein. The relative amount of the A215V, L327P, and R468C mutant proteins, as compared to the wild-type, was estimated to be 30%, 30%, and 50%, respectively, in expression at 30 °C. These proteins were more stable at 30 °C than at 37 °C. Specific activities (activity/protein) of A215V, L327P, and R468C mutants could be calculated to about 50%, 50%, and 100% that of wild-type, respectively.

Table 2
Enzyme assay using fibroblasts.

	Acetoacetyl-CoA thiolase			SCOT	SCOT/+K^+
	−K^+	+K^+	+K^+/−K^+		
GS07	5.9	12.9	2.2	1.9	0.2
GS13	4.3	9.1	2.1	0.8	0.1
GS14	3.6	7.3	2.0	1.2	0.2
GS17	7.0	14.9	2.1	1.2	0.1
Controls ($n=5$)	5.0 ± 0.7	10.8 ± 0.9	2.2 ± 0.3	6.7 ± 2.1	0.6 ± 0.2

Enzyme activity is expressed as nmol/min/mg protein.
Fibroblasts from GS18 were not available.

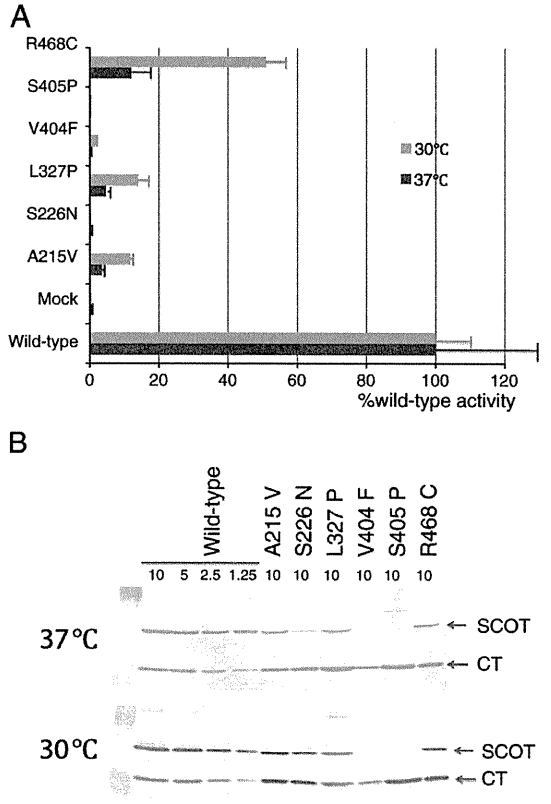

Fig. 1. Transient expression results for wild-type and mutant SCOT cDNAs. Wild-type and mutant SCOT expression vectors (4 μg) were transfected together with 1 μg of the cytosolic acetoacetyl-CoA thiolase (CT)-expressing vector, pCAGGSct, to SV40-transformed fibroblasts of GS01 of which the mutation is S283X/S283X. Transient expression was done 37 and 30 °C. Mock, transfection of 1 μg of pCAGGSct and 4 μg of pCAGGS vectors without insert. (A) SCOT activities relative to those in wild-type transfection are shown. The mean values are displayed together with the SD of three independent experiments. (B) Immunoblots for SCOT and CT are shown. The protein amounts applied are shown above the lanes. The first antibody was a mixture of an anti-CT (cytosolic thiolase) antibody and anti-SCOT antibody. The positions of the bands for CT and SCOT are indicated by arrows.

3.4. Tertiary structural model of human SCOT and mutations

A number of mutations have been characterized for SCOT deficiency, and several of them have been structurally analyzed before [3,20,22], based on homology models of human SCOT, made with the help of the pig SCOT crystal structure [20]. Fig. 2A shows a dimer of human SCOT, with presently and previously identified mutations highlighted on the SCOT monomer in Fig. 2B. It is noteworthy that most of the mutations are located around two 'hot-spots' in 3D space; these areas correspond to a small beta sandwich domain in the N-terminal lobe, and a larger beta sandwich structure close to the C terminus. Sporadic mutations are also seen closer to the active site cavity.

The mutation A215V involves the residue A215, which in the wild-type protein is in the middle of a beta sheet, pointing inwards into the protein. The terminal carbon atom of A215 is only 3.6 Å away from the terminal methyl group carbons of L269 in an opposing beta sheet. Thus, even a small valine residue cannot be incorporated into this position without structural strain and changes. The position is located at a small beta sandwich domain involved in SCOT dimer formation. A215 is, furthermore, in the very close vicinity of the previously characterized SCOT mutations G219E and V221M [3]. These observations on the tertiary structure are in accord with the results that the main mutant effect of A215V is the instability of the mutant protein since the mutant protein amount was 12.5% and 30% relative to wild-type at 37 and 30 °C, respectively.

S226 is located close to the dimerization interface, although not being directly involved in it. The side chain is hydrogen-bonded to the backbone carbonyl of D362 and *via* a buried water molecule to N345; N345 is vicinal to the crucial catalytic residue E344 (Fig. 2D). Thus, the S226N mutation is likely to disturb the structure at least locally, and could also affect the properties of the catalytic site. As expected from the view of tertiary structure, the S226N mutant protein was revealed to be unstable and non-functional protein was detected in transient expression analysis.

L327 locates to an alpha helix on the SCOT surface, close to the active site entrance (Fig. 2D). The side chain of L327 is solvent-exposed and disordered in the crystal. This helix could form part of the CoA substrate-binding site, and a proline mutation in the central part of this helix may both perturb the helical structure and affect the functional mobility of this region, especially since the neighboring residue (326) is also a proline. A main mutant effect of L327P is the instability of the mutant protein since the mutant protein amount was 12.5% and 30% relative to wild-type at 37 and 30 °C, respectively.

V404 locates to the C-terminal beta sandwich domain of SCOT (Fig. 2E). The side chain points inwards, into a tightly packed hydrophobic core between two beta sheets. There clearly is no room for a large Phe residue, as there are several short distances from the side chain of V404 to residues from the opposing beta sheet. S405 is neighboring V404, at the end of a beta strand, preceding a short tight beta turn (Fig. 2E). In light of this, the side chain hydroxyl group is within hydrogen bonding distance from three main-chain NH groups from residues 407–409. Such an arrangement would be completely destroyed upon mutation of residue 405 to proline. As expected from the tertiary structure, these mutant proteins were too unstable to detect in either the 37 or 30 °C expression.

R468 is an exposed residue, present in the beta sheet opposite to that harboring V404 and S405. Its side chain is a central residue in a salt bridge network with E488, R308, E312, K436, and D477 (Fig. 2F). Mutation of the R468 residue will be detrimental to this large hydrophilic region on the surface of the C-terminal domain of SCOT. Since its specific activity is similar to wild-type, R468C does not affect catalytic activity; the active site residues are far from this mutation. The main mutational effect of R468C is also instability of the molecule.

3.5. Clinical phenotypes and genotypes

We reported herein 5 SCOT-deficient patients and their clinical and molecular aspects are summarized in Table 1. They developed the first ketoacidotic episodes from 3 days of age to 3 years of age. The episodes were associated with very severe metabolic acidosis with blood pH ranging from 6.90 to 7.08. They all recovered from the first ketoacidotic crises and were well managed after the diagnosis of SCOT deficiency was made. We previously reported clinical and molecular characters for 12 SCOT-deficient families and now have added to those 5 more.

Permanent ketosis or persistent ketonuria is pathognomonic feature of SCOT deficiency. We, however, previously showed that SCOT-deficient patients with "mild" mutations may have no permanent ketosis. V221M, R268H, or T435N homozygotes and T435N/null mutation compound heterozygotes did not show permanent ketosis or permanent ketonuria [3,17,20,22]. V221M, R268H and T435N mutations retained 10%, 34% and 25%, respectively, relative activity to wild-type in 37 °C expression using the same expression system. In the present study, L327P and R468H mutations retained 4.7% and 12%, respectively, relative activity in 37 °C expression. A L327P homozygote, GS13, did not have permanent ketonuria but a R468H homozygote; GS14 did. To our knowledge, patients with null mutations all showed permanent ketonemia or ketonuria. Hence "mild" mutation with

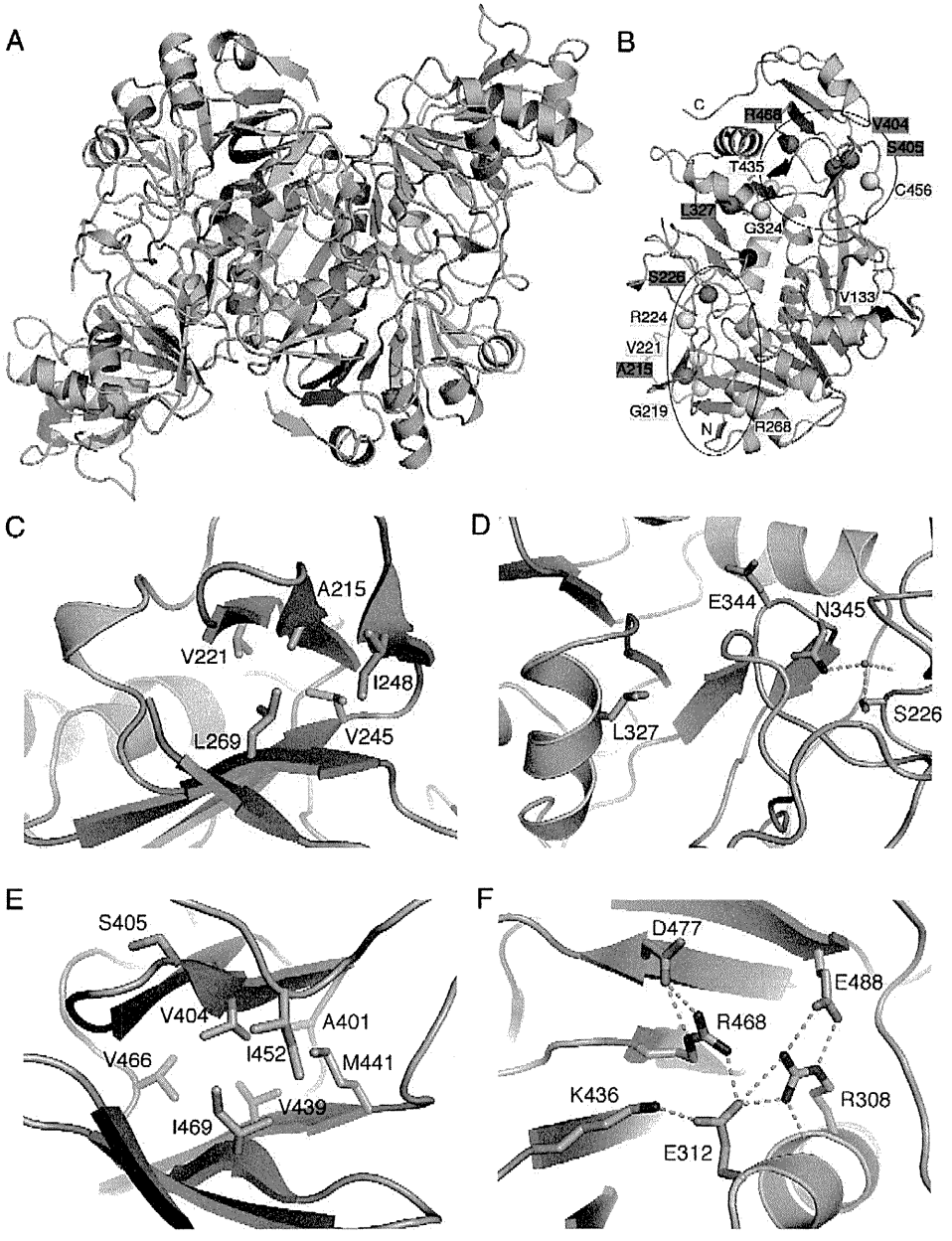

Fig. 2. Mutation sites on the tertiary structure of SCOT monomer. (A) An overall structure of human SCOT dimmer (PDB entry 3DLX). (B) A monomer of human SCOT. The N and C termini are labelled. The two clusters of mutations are indicated by ellipsoids. The positions of the mutations identified in this study are in red, and the ones previously identified are shown in yellow. The position of the catalytically active glutamate residue 344 is marked with a blue sphere. (C) The environment of the A215V mutation in the tightly packed hydrophobic core of the small beta sandwich. The N terminus of the crystal structure is at the bottom front. (D) S226 lies close to the active site, and interacts with N345 via a water-mediated hydrogen bond (green) and by van der Waals interactions. The catalytic residue is E344. L327 also lies close to the entrance of the catalytic cavity and could be involved in substrate binding. (E) V404 and S405 are next to each other, V404 being buried at the hydrophobic core of a beta sandwich unit. S405 interacts with the backbone amides of a tight turn (blue). (F) R468 plays a central role in a salt bridge network linking a beta sheet and a helix.

residual activity may be a necessary condition but not a sufficient condition for the absence of permanent ketonemia or ketonuria. Environmental factors may also affect a clinical phenotype of persistent ketonuria.

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References

[1] G.A. Mitchell, T. Fukao, Chapter 102. Inborn errors of ketone body catabolism, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), Metabolic and molecular bases of inherited disease, 8th edition, McGraw-Hill, Inc, NewYork, 2001, pp. 2327–2356.

[2] S. Kassovska-Bratinova, T. Fukao, X.Q. Song, A.M. Duncan, H.S. Chen, M.F. Robert, C. Perez-Cerda, M. Ugarte, C. Chartrand, S. Vobecky, N. Kondo, G.A. Mitchell, Succinyl CoA: 3-oxoacid CoA transferase (SCOT): human cDNA cloning, human chromosomal mapping to 5p13, and mutation detection in a SCOT-deficient patient, Am. J. Hum. Genet. 59 (1996) 519–528.

[3] T. Fukao, G.A. Mitchell, X.Q. Song, H. Nakamura, S. Kassovska-Bratinova, K.E. Orii, J. E. Wraith, G. Besley, R.J. Wanders, K.E. Niezen-Koning, G.T. Berry, M. Palmieri, N. Kondo, Succinyl-CoA:3-ketoacid CoA transferase (SCOT): cloning of the human SCOT gene, tertiary structural modeling of the human SCOT monomer, and characterization of three pathogenic mutations, Genomics 68 (2000) 144–151.

[4] M. Cornblath, R.L. Gingell, G.A. Fleming, J.T. Tildon, A.T. Leffler, R.A. Wapnir, A new syndrome of ketoacidosis in infancy, J. Pediatr. 79 (1971) 413–418.

[5] J.T. Tildon, M. Cornblath, Succinyl-CoA: 3-ketoacid CoA-transferase deficiency. A cause for ketoacidosis in infancy, J. Clin. Invest. 51 (1972) 493–498.

[6] C. Perez-Cerda, B. Merinero, P. Sanz, A. Jimenez, C. Hernandez, M.J. Garcia, M. Ugarte, A new case of succinyl-CoA: acetoacetate transferase deficiency, J. Inherit. Metab. Dis. 15 (1992) 371–373.

[7] H. Sakazaki, K. Hirayama, S. Murakami, S. Yonezawa, H. Shintaku, Y. Sawada, T. Fukao, H. Watanabe, T. Orii, G. Isshiki, A new Japanese case of succinyl-CoA: 3-ketoacid CoA-transferase deficiency, J. Inherit. Metab. Dis. 18 (1995) 323–325.

[8] C.J. Pretorius, G.G. Loy Son, F. Bonnici, E.H. Harley, Two siblings with episodic ketoacidosis and decreased activity of succinyl-CoA:3-ketoacid CoA-transferase in cultured fibroblasts, J. Inherit. Metab. Dis. 19 (1996) 296–300.

[9] T. Fukao, X.Q. Song, H. Watanabe, K. Hirayama, H. Sakazaki, H. Shintaku, M. Imanaka, T. Orii, N. Kondo, Prenatal diagnosis of succinyl-coenzyme A:3-ketoacid coenzyme A transferase deficiency, Prenat. Diagn. 16 (1996) 471–474.

[10] K.E. Niezen-Koning, R.J. Wanders, J.P. Ruiter, L. Ijlst, G. Visser, W.C. Reitsma-Bierens, H.S. Heymans, D.J. Reijngoud, G.P. Smit, Succinyl-CoA:acetoacetate transferase deficiency: identification of a new patient with a neonatal onset and review of the literature, Eur. J. Pediatr. 156 (1997) 870–873.

[11] M.O. Rolland, N. Guffon, G. Mandon, P. Divry, Succinyl-CoA:acetoacetate transferase deficiency. Identification of a new case; prenatal exclusion in three further pregnancies, J. Inherit. Metab. Dis. 21 (1998) 687–688.

[12] S.E. Snyderman, C. Sansaricq, B. Middleton, Succinyl-CoA:3-ketoacid CoA-transferase deficiency, Pediatrics 101 (1998) 709–711.

[13] X.Q. Song, T. Fukao, H. Watanabe, H. Shintaku, K. Hirayama, S. Kassovska-Bratinova, N. Kondo, G.A. Mitchell, Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency: two pathogenic mutations, V133E and C456F, in Japanese siblings, Hum. Mutat. 12 (1998) 83–88.

[14] I. Baric, V. Sarnavka, K. Fumic, M. Maradin, D. Begovic, J.P. Ruiter, R.J. Wanders, A new case of succinyl-CoA:acetoacetate transferase deficiency: favourable course despite very low residual activity, J. Inherit. Metab. Dis. 24 (2001) 81–82.

[15] G.T. Berry, T. Fukao, G.A. Mitchell, A. Mazur, M. Ciafre, J. Gibson, N. Kondo, M.J. Palmieri, Neonatal hypoglycaemia in severe succinyl-CoA: 3-oxoacid CoA-transferase deficiency, J. Inherit. Metab. Dis. 24 (2001) 587–595.

[16] N. Longo, T. Fukao, R. Singh, M. Pasquali, R.G. Barrios, N. Kondo, K.M. Gibson, Succinyl-CoA:3-ketoacid transferase (SCOT) deficiency in a new patient homozygous for an R217X mutation, J. Inherit. Metab. Dis. 27 (2004) 691–692.

[17] T. Fukao, H. Shintaku, R. Kusubae, G.X. Zhang, K. Nakamura, M. Kondo, N. Kondo, Patients homozygous for the T435N mutation of succinyl-CoA:3-ketoacid CoA transferase (SCOT) do not show permanent ketosis, Pediatr. Res. 56 (2004) 858–863.

[18] T. Fukao, S. Sakurai, M.O. Rolland, M.T. Zabot, A. Schulze, K. Yamada, N. Kondo, A 6-bp deletion at the splice donor site of the first intron resulted in aberrant splicing using a cryptic splice site within exon 1 in a patient with succinyl-CoA: 3-ketoacid CoA transferase (SCOT) deficiency, Mol. Genet. Metab. 89 (2006) 280–282.

[19] K. Yamada, T. Fukao, G. Zhang, S. Sakurai, J.P. Ruiter, R.J. Wanders, N. Kondo, Single-base substitution at the last nucleotide of exon 6 (c.671G>A), resulting in the skipping of exon 6, and exons 6 and 7 in human succinyl-CoA:3-ketoacid CoA transferase (SCOT) gene, Mol. Genet. Metab. 90 (2007) 291–297.

[20] T. Fukao, P. Kursula, E.P. Owen, N. Kondo, Identification and characterization of a temperature-sensitive R268H mutation in the human succinyl-CoA:3-ketoacid CoA transferase (SCOT) gene, Mol. Genet. Metab. 92 (2007) 216–221.

[21] S. Merron, R. Akhtar, Management and communication problems in a patient with succinyl-CoA transferase deficiency in pregnancy and labour, Int. J. Obstet. Anesth. 18 (2009) 280–283.

[22] T. Fukao, T. Ishii, N. Amano, P. Kursula, M. Takayanagi, K. Murase, N. Sakaguchi, N. Kondo, T. Hasegawa, A neonatal-onset succinyl-CoA:3-ketoacid CoA transferase (SCOT)-deficient patient with T435N and c.658–666dupAACGTGATT p. N220_I222dup mutations in the OXCT1 gene, J. Inherit. Metab. Dis. (2010).

[23] T. Fukao, X.Q. Song, G.A. Mitchell, S. Yamaguchi, K. Sukegawa, T. Orii, N. Kondo, Enzymes of ketone body utilization in human tissues: protein and messenger RNA levels of succinyl-coenzyme A (CoA):3-ketoacid CoA transferase and mitochondrial and cytosolic acetoacetyl-CoA thiolases, Pediatr. Res. 42 (1997) 498–502.

[24] H. Niwa, K. Yamamura, J. Miyazaki, Efficient selection for high-expression transfectants with a novel eukaryotic vector, Gene 108 (1991) 193–199.

[25] X.Q. Song, T. Fukao, S. Yamaguchi, S. Miyazawa, T. Hashimoto, T. Orii, Molecular cloning and nucleotide sequence of complementary DNA for human hepatic cytosolic acetoacetyl-coenzyme A thiolase, Biochem. Biophys. Res. Commun. 201 (1994) 478–485.

[26] X.Q. Song, T. Fukao, G.A. Mitchell, S. Kassovska-Bratinova, M. Ugarte, R.J. Wanders, K. Hirayama, H. Shintaku, P. Churchill, H. Watanabe, T. Orii, N. Kondo, Succinyl-CoA:3-ketoacid coenzyme A transferase (SCOT): development of an antibody to human SCOT and diagnostic use in hereditary SCOT deficiency, Biochim. Biophys. Acta 1360 (1997) 151–156.

[27] P.D. Adams, P.V. Afonine, G. Bunkoczi, V.B. Chen, I.W. Davis, N. Echols, J.J. Headd, L. W. Hung, G.J. Kapral, R.W. Grosse-Kunstleve, A.J. McCoy, N.W. Moriarty, R. Oeffner, R.J. Read, D.C. Richardson, J.S. Richardson, T.C. Terwilliger, P.H. Zwart, PHENIX: a comprehensive Python-based system for macromolecular structure solution, Acta Crystallogr. D Biol. Crystallogr. 66 (2010) 213–221.

[28] P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics, Acta Crystallogr. D Biol. Crystallogr. 60 (2004) 2126–2132.

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Differences between Human and Rodent Pancreatic Islets

LOW PYRUVATE CARBOXYLASE, ATP CITRATE LYASE, AND PYRUVATE CARBOXYLATION AND HIGH GLUCOSE-STIMULATED ACETOACETATE IN HUMAN PANCREATIC ISLETS[*][S]

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Anaplerosis, the net synthesis in mitochondria of citric acid cycle intermediates, and cataplerosis, their export to the cytosol, have been shown to be important for insulin secretion in rodent beta cells. However, human islets may be different. We observed that the enzyme activity, protein level, and relative mRNA level of the key anaplerotic enzyme pyruvate carboxylase (PC) were 80–90% lower in human pancreatic islets compared with islets of rats and mice and the rat insulinoma cell line INS-1 832/13. Activity and protein of ATP citrate lyase, which uses anaplerotic products in the cytosol, were 60–75% lower in human islets than in rodent islets or the cell line. In line with the lower PC, the percentage of glucose-derived pyruvate that entered mitochondrial metabolism via carboxylation in human islets was only 20–30% that in rat islets. This suggests human islets depend less on pyruvate carboxylation than rodent models that were used to establish the role of PC in insulin secretion. Human islets possessed high levels of succinyl-CoA:3-ketoacid-CoA transferase, an enzyme that forms acetoacetate in the mitochondria, and acetoacetyl-CoA synthetase, which uses acetoacetate to form acyl-CoAs in the cytosol. Glucose-stimulated human islets released insulin similarly to rat islets but formed much more acetoacetate. β-Hydroxybutyrate augmented insulin secretion in human islets. This information supports previous data that indicate beta cells can use a pathway involving succinyl-CoA:3-ketoacid-CoA transferase and acetoacetyl-CoA synthetase to synthesize and use acetoacetate and suggests human islets may use this pathway more than PC and citrate to form cytosolic acyl-CoAs.

Understanding the enzymatic makeup of human pancreatic islets is fundamental to developing strategies for designing artificial beta cells and beta cells differentiated from stem cells as treatments for type 1 diabetes, as well as modulating beta cell metabolism for the treatment of type 2 diabetes. Until recently, most of the information about normal insulin secretion came from studies of rodent islets or clonal cell lines. Although a recent study showed human pancreatic islets respond to insulin secretagogues similarly to rodent islets (1), what is still unknown is whether the use of intracellular pathways of secretagogue metabolism is the same in human islets as in rodent islets and cell lines. During the last few years, human islet preparations from human donors have become more readily available to researchers. By studying the levels of enzymes, the functional units of metabolism, the recent abundant supply of human islets has enabled our laboratory to discover clues suggesting differences in metabolic pathways between pancreatic islets of humans and rodents that have implications for better understanding normal human beta cell physiology.

Anaplerosis, the biosynthesis of citric acid cycle intermediates (2), is widely believed to be important for insulin secretion (3). Pyruvate carboxylase (PC)[2] is the key anaplerotic enzyme in this process and plays a central role in insulin secretion in the pancreatic beta cell of rodents and clonal insulin cell lines (3–8). In rat pancreatic islets, the level of PC is as high as in liver and kidney (4, 9–11), two organs in which PC plays a role in gluconeogenesis. In islets, which do not seem to require gluconeogenesis (12, 13), PC is concentrated in the beta cell (11). Our laboratory (4, 14–16) and, subsequently, Kahn *et al.* (17) previously showed that the rate of carboxylation of glucose-derived pyruvate catalyzed by PC is very high in rat pancreatic islets and equal to the rate of decarboxylation of glucose-derived pyruvate catalyzed by the pyruvate dehydrogenase complex. Lu *et al.* (18) showed that the rate of pyruvate cycling through PC is proportional to the capacity for glucose-stimulated insulin release from various INS-1 cell lines, and other studies have also found evidence for cycling of pyruvate through PC in rodent clonal beta cell lines (19, 20) and mouse pancreatic islets (21). More recently, we used RNAi knockdown technology to produce a series of cell lines derived from the rat insulinoma cell line INS-1 832/13 that expressed a range of PC levels. In these cell lines insulin release stimulated by glucose, as well as other metabolizable insulin secretagogues, was inhibited in proportion to the severity of PC knockdown (22). Severe knockdown

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[2] The abbreviations used are: PC, pyruvate carboxylase; BCH, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid; mGPD, mitochondrial glycerol phosphate dehydrogenase; PCC, propionyl-CoA carboxylase; PDC, pyruvate dehydrogenase complex; SCOT, succinyl-CoA:3-ketoacid-CoA transferase; BMI, body mass index.