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unusual pattern and increases susceptibility to schizophrenia. Proc. Nat. Acad. Sci. vol. 99, 3717-3722, 2002.

Ⅳ．第１回三渕班会議プログラム

三　渕　班　班　会　議

厚生労働科学研究費補助金（難治性疾患克服研究事業）主任研究者　三渕　浩

研究課題名：　高プロリン血症の臨床的多様性の解明と新しい診断治療基準および
長期フォローアップ体制の確立

会　　場：　ザ・ナハテラス　「ガーデンルーム」　沖縄県那覇市おもろまち2-14-1
日　　時：　平成２１年７月２４日（金）　16：00～18：00
参 加 者：　三渕　浩、遠藤文夫、安東敏彦、知念安紹、久原とみ子、粟田久多佳、
宮野　博、井原健二、小須賀基道、城戸　淳（敬称略）

― 式　次　第 ―

I.　班長挨拶

熊本大学医学部附属病院　三渕 浩

II　研究発表

（1）プロリン代謝の先天代謝異常症について

熊本大学医学部　遠藤文夫教授

（2）尿の有機酸分析で診断された高プロリン血症について

金沢医科大学　久原とみ子教授

コーヒーブレーク

（3）アミノ酸分析の新しい方法

味の素研究所　宮野 博部長

（4）成人における血中プロリンが高い集団について

味の素研究所　安東敏彦部長

III.　総合討論　　司会　三渕 浩

情報提供：地方におけるアミノ酸代謝異常症のスクリーニングについて

琉球大学小児科　知念安紹先生

― 午後６時終了 ―

情報交換会：　同会場にて６時３０分開始

プロリンおよびヒドロキシプロリン関連の先天性代謝異常症の解説

熊本大学医学部付属病院新生児学寄付講座　　三渕　浩

1）遺伝性高プロリン血症にはⅠ型（[MIM239500]hyperprolinemia, type1)とⅡ型([MIM239510]hyperprolinemia, type2)の2つのタイプが記載されている。いずれもプロリン代謝経路に異常があり、血中プロリンが上昇する。Ⅰ型ではproline oxidaseが欠損している。Ⅱ型ではΔ1-pyrroline-5-carboxylate(P5C) dehydrogenaseが欠損している。
いずれも常染色体劣性遺伝である。
Ⅰ型高プロリン血症の臨床症状は報告によって一定していない。臨床症状を全く示さない例から、難治性けいれんや精神発達遅滞を示す例が報告されている。最近の研究では22q11deletion syndromeにおいて血中プロリンレベルとIQの間に逆の相関が見出された。さらに興味あることに、proline oxidase遺伝子と成人および早期発症統合失調症と関連していることが明らかとなった。Ⅱ型高プロリン血症は通常、難治性けいれんと精神発達遅滞を示すことで知られているが不明の点も多い。ラットでは血中のプロリン高値は脳内では酸化ストレスを誘導することが報告されている。このことは高プロリン血症が人においても何らかの神経学的機能異常を引き起こす可能性が高いことを示唆するものであろう。
2）遺伝性の低プロリン血症が最近報告された。P5C synthaseの欠損によって引き起こされる。精神発達遅滞、白内障、関節過伸展を示す。
3）Hyperhydroxyprolinemia[MIM 237000]はhydroxyproline oxidaseの欠損で引き起こされる常染色体劣性遺伝性疾患である。これまでの報告からは特に症状を示さない良性の代謝異常と考えられる。
4）Prolidase deficiency[MIM 170100]は常染色体劣性の遺伝性疾患である。prolidaseはカルボキシル末にプロリンを有するジペプチド、オリゴペプチドを加水分解する。患者では尿中にプロリンを含むイミドペプチドの排泄がみられる。多彩な皮膚症状、知能低下、顔貌の異常をを伴う。

Disorders of proline and hydroxyproline metabolism

In hereditary hyperprolinemia、hyperprolinemia type I (HPI) (MIM239500) and hyperprolinemia, typ II (HPII) (MIM239510) are mentioned, and both diseases are caused by the defect in proline metabolic pathway, so blood level of proline elevates. HPI is caused by deficiency of proline oxidase, and HPII is due to deficiency of Δ 1-pyrroline-5-carboxylate(P5C) dehydrogenase. Both are apparently inherited as autosomal recessive traits.

Clinical phenotypes of HPI are different from each reports. For example, some cases show mental retardation and refractory convulsion, other cases does not quite show clinical manifestation. By recent studies, reverse correlation was found between blood proline level and IQ in the patients of 22q11 deletion(including the region of praline oxidase gene) syndrome. In addision, relationship between the proline oxidase gene and adult onset or early onset schizophrenia became clear. HPII usually shows refractory convulsion and mental retardation, but its mechanisms is not clear. In rats, high level of blood proline induces oxidative stress in the brain of the rat. These result suggests that hyperprolinemia likely to induces oxidative stress and causes dysfunction of the neuron in human too.

The hereditary cases with low level of blood proline were presented recently. It is caused by absence of P5C synthase and shows mental retardation, cataract and arthrosis hyperextension.

Hyperhydroxyprolinemia[MIM 237000] is an autosomal recessive inheritance disorder caused by the defficiency of hydroxyproline oxidase. It does not show a symptom and is thought with the benign metabolic disorder.

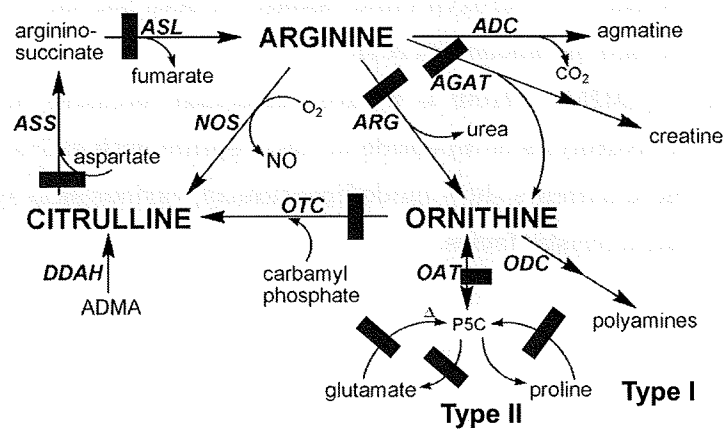
Prolidase deficiency[MIM 170100] is a rare autosomal recessive hereditary disease. Prolidase catalyzes hydrolysis of dipeptide or oligopeptide with a C-terminal proline or hydroxyproline. These patients show imidodipeptiduria, various skin symptoms, mental retardation and characteristic facies.

Inborn errors of proline metabolism

Fumio Endo, M.D. PhD.
Hiroshi Mitsubuchi , M.D. PhD.

Department of Pediatrics
Kumamoto Univerisity Scool of Medicine

Inherited enzyme deficiencies related to urea cycle and proline metaboslim



ADC, arginine decarboxylase; AGAT, arginine:glycine amidinotransferase; ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; OTC, ornithine transcarbamylase; P5C, Δ^1-pyrroline-5-carboxylate.

L-Proline metabolism and human disease

- Monogenic inborn errors of metabolism

  hyperprolinamia type I
  hyperprolinemia type II
  P5C synthetase deficiecy
  ornithine aminotransferase deficiency
  hydroxyprolinemia
  iminoglycinuria

- Neuropsychiatric disorders
- Prolidase deficiency

J Inherit Metab Dis. 2007 Aug;30(4):515-21.

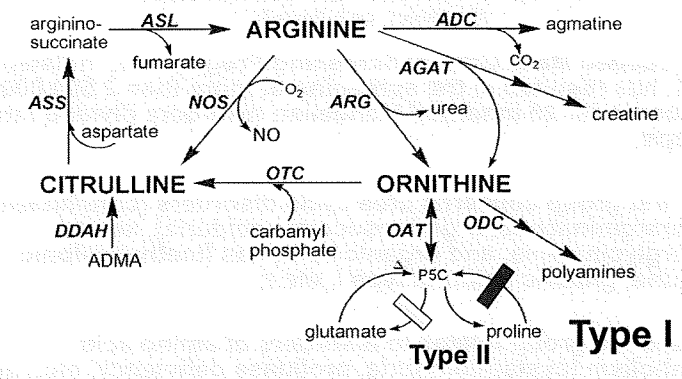
Quebec neonatal mass urinary screening programme: from micromolecules to macromolecules.

Auray-Blais C, Cyr D, Drouin R.

- The Quebec Mass Urinary Screening Programme, initiated in 1971, has resulted in the screening of more than 2 500 000 newborns for 25 inherited Mendelian disorders divided into two groups.
- The first group concerns urea cycle disorders (citrullinaemia, hyperargininaemia, argininosuccinic aciduria), ketotic hyperglycinaemia, and organic acidurias (methylmalonic aciduria, glutaric aciduria type I, etc.);
- the second group relates to disorders of amino acid metabolism (cystathioninuria, prolidase deficiency, etc.) and transport (Fanconi syndrome, cystinurias, Hartnup syndrome, etc.).
- There are two cases of prolidase deficiency, no case of hyperprolinemias.

Type I hyperprolinemia

Type I hyperprolinemia



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Cloning of human proline oxidase(dehydrogenase)

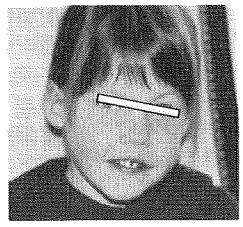
- Campbell et al. (1997) cloned the complete coding region for a human homolog of the Drosophila melanogaster 'sluggish-A' (slgA) and yeast 'PUT1' genes, previously shown to encode proline oxidase activity in these organisms.
- The predicted 516-residue human protein shows 51% amino acid sequence identity to the Drosophila protein, indicating that this human gene may encode proline oxidase.
- Northern analysis showed that the gene is expressed in human lung, skeletal muscle, and brain, to a lesser extent in heart and kidney, and weakly in liver, placenta, and pancreas.
- Campbell, H. D.; Webb, G. C.; Young, I. G. :
  A human homologue of the Drosophila melanogaster sluggish-A (proline oxidase) gene maps to 22q11.2, and is a candidate gene for type-I hyperprolinaemia. *Hum. Genet.* 101: 69-74, 1997.

Type I hyperprolinemia

- **Type I hyperprolinaemia (MIM 239500) is a rare metabolic disorder which is biochemically characterised by a defect of the proline dehydrogenase (oxidase) enzyme involved in the conversion from proline to glutamate.**
- **Jacquet et al (2002) identified, in a child with a severe form of type I hyperprolinaemia with severe psychomotor delay and status epilepticus associated with a very high level of plasma proline level (2246 µmol/l), a complete homozygous deletion of the *PRODH* gene located on chromosome 22q11.**
- **These studies shows unambiguously that the severe form of type I hyperprolinaemia, characterised by neurological manifestations, results from homozygous inactivating alterations of the *PRODH* gene.**

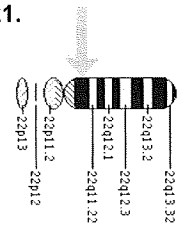
Velo-Cardio-Facial Syndrome

Velo-Cardio-Facial syndrome (VCFS) is a genetic, autosomal dominant condition defined by Shprintzen in 1978. Its frequency is estimated at 1 per 4000 live births. In most patients, a deletion on chromosome 22q11.2 is responsible for the syndrome. The increased prevalence of schizophrenia among patients was noticed. Most of these deletions occur spontaneously (are not inherited from parent to child).

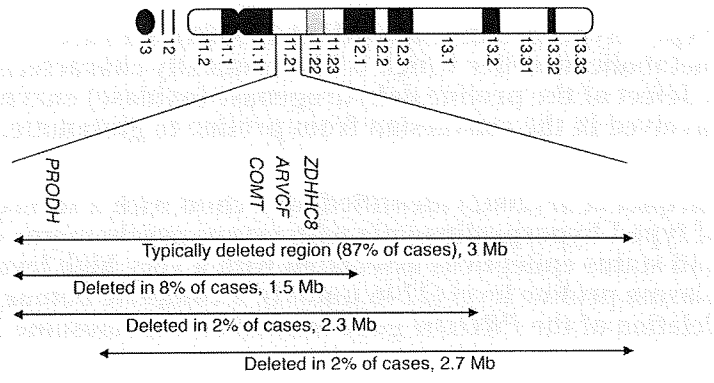


A 4-year-old girl with Velo-Cardio-Facial syndrome

The PRODH gene is located on the long (q) arm of chromosome 22 at position 11.21.



Chromosome 22 and the location of the VCFS deletions



Kirov, G. et al. J. Clin. Invest. 2005;115:1440-1448

Figure 3
Chromosome 22 and the location of the VCFS deletions. The positions of candidate genes within the typically deleted region that are discussed in this paper are also indicated. The frequencies of the deleted regions are taken from Shaikh et al.

Human Molecular Genetics, 2002, Vol. 11, No. 19 2243-2249

PRODH mutations and hyperprolinemia in a subset of schizophrenic patients

- The increased prevalence of schizophrenia among patients with the 22q11 interstitial deletion associated with DiGeorge syndrome has suggested the existence of a susceptibility gene for schizophrenia within the DiGeorge syndrome chromosomal region (DGCR) on 22q11.
- This heterozygous deletion was associated with hyperprolinemia in the schizophrenic patients.
- In addition, two heterozygous *PRODH* missense mutations (L441P and L289M), detected in 3 of 63 schizophrenic patients but in none among 68 controls, were also associated with increased plasma proline levels.

Table 3. Correlation between *PRODH* genotypes and plasma proline levels

Genotype	Proline level (μmol/l) *n*<290
L441P/L441P	1255
L441P/L441P+R453C	800 (range 413–1745)[a]
L441P/R431H[b]	694
Del/R453C[c]	538
L289M/wt; E521R/wt[d]	377
L441P/wt[b]	360
L289M/wt; R431H/wt[d]	345
Del/wt[c]	338
R453C/wt; A455S/wt[d]	312
R453C/wt[e]	221
R453C/wt[e]	179
Del/wt[c]	172

aProline levels indicated in (13).
bDetected in the F2 family.
cDetected in the F1 family.
dThe phase of the two substitutions was not determined.
eDetected in a control subject.

Human Molecular Genetics, 2002, Vol. 11, No. 19 2243-2249

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Human Molecular Genetics, 2002, Vol. 11, No. 19 2243-2249

The American Journal of Human Genetics, volume 76 (2005), 409–420

Functional Consequences of PRODH Missense Mutations

Hans-Ulrich Bender, Shlomo Almashanu, Gary Steel, Chien-An Hu, Wei-Wen Lin, Alecia Willis, Ann Pulver, and David Valle

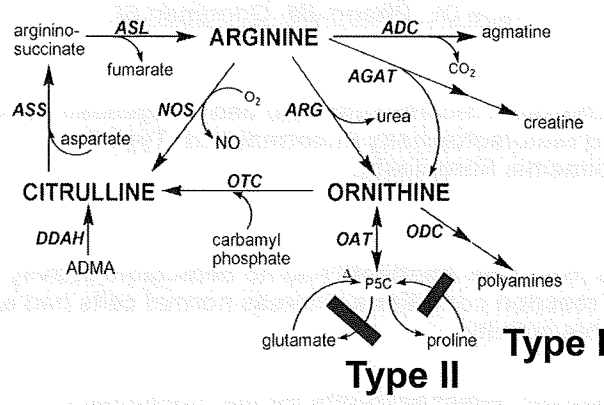
At least 16 *PRODH* missense mutations have been identified in studies of type I hyperprolinemia (HPI) and schizophrenia, 10 of which are present at polymorphic frequencies.

- **Although there is limited information on plasma proline levels in individuals of known *PRODH* genotype, extant data suggest that severe hyperprolinemia (>800 M) occurs in individuals with large deletions and/or *PRODH* missense mutations with the most-severe effect on function (*L441P* and *R453C*), whereas modest hyperprolinemia (300~ 500 M) is associated with *PRODH* alleles with a moderate reduction in activity.**

- **Interestingly, three of the four alleles associated with or found in schizophrenia (*V427M, L441P,* and *R453C*) resulted in severe reduction of POX activity and hyperprolinemia.**

type II Hyperprolinemia

Hyperprolinemia type II



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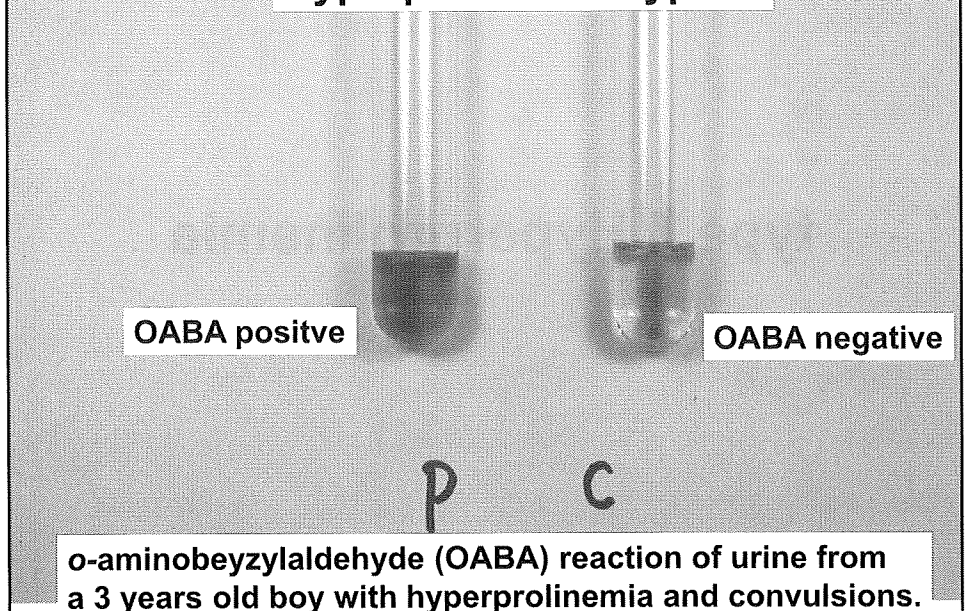
Hyperprolinemia type II



o-aminobeyzylaldehyde (OABA) reaction of urine from a 3 years old boy with hyperprolinemia and convulsions.

Science. 1974 Sep 20;185(156):1053-4.

Type 2 hyperprolinemia: absence of delta1-pyrroline-5-carboxylic acid dehydrogenase activity.

Valle DL, Phang JM, Goodman SI.

- **Delta(1)-Pyrroline-5-carboxylic acid dehydrogenase activity was measured radioisotopically in normal and Type 2 hyperprolinemia fibroblasts.**
- **The cells from type 2 patients had no detectable activity over a range of reaction conditions whereas normal cells had easily measurable activity.**
- **This enzymatic defect accounts for the biochemical abnormalities in type 2 hyperprolinemia.**

Hum Mol Genet. 1998 Sep;7(9):1411-5.

Mutations in the Delta1-pyrroline 5-carboxylate dehydrogenase gene cause type II hyperprolinemia.

Geraghty MT, Vaughn D, Nicholson AJ, Lin WW, Jimenez-Sanchez G, Obie C, Flynn MP, Valle D, Hu CA.

- Authors surveyed Delta1-pyrroline 5-carboxylate dehydrogenase genes from four patients with hyperprolinemia type II.
- They found four mutant alleles, two with frameshift mutations [A7fs(-1) and G521fs(+1)] and two with missense mutations (S352L and P16L).
- To test the functional consequences of three of these, they expressed them in a P5CDh-deficient strain of Saccharomyces cerevisiae . In contrast to wild-type human P5CDh, yeast expressing S352L and G521fs(+1) failed to grow on proline and had no detectable P5CDh activity.
- Interestingly, the G521fs(+1) allele segregates in the large Irish Traveller pedigree used to define the HPII phenotype. This is the first description of the molecular basis for this inborn error.

Arch Dis Child. 1989 Dec;64(12):1699-707

Type II hyperprolinaemia in a pedigree of Irish travellers (nomads)

MP Flynn, MC Martin, PT Moore, JA Stafford, GA Fleming and JM Phang

- This paper describes a study of 312 subjects in 71 families near related to a proband with type II hyperprolinaemia.
- The subjects were Irish travellers (nomads) among whom consanguineous marriage and high fertility are common.
- Thirteen additional cases of type II hyperprolinaemia were discovered. A further 50 subjects were found to have mild hyperprolinaemia.
- There is a strong association between type II hyperprolinaemia and seizures during childhood but no significant association with mental handicap.
- Most adults with type II hyperprolinaemia enjoyed normal health.

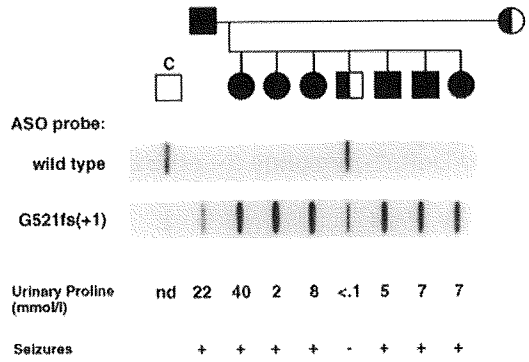
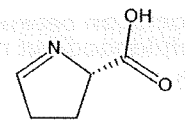


Figure 4. Segregation of G521fs(+1) in a small segment of the Irish Traveller HPII pedigree by ASO analysis. The father and six children are all homozygous for the mutant allele; the unaffected child is heterozygous. The biochemical and clinical phenotypes are indicated below. nd, not done.
Hum Mol Genet. 1998 Sep;7(9):1411-5.

J. Biol. Chem., April 27, 2001; 276(18): 15107 - 15116.

Pyridoxal Phosphate De-activation by Pyrroline-5-carboxylic Acid.
INCREASED RISK OF VITAMIN B6 DEFICIENCY AND SEIZURES IN HYPERPROLINEMIA TYPE II
R. D. Farrant, V. Walker, G. A. Mills, J. M. Mellor, and G. J. Langley

- **Pyrroline-5-carboxylic acid is found to be a unique endogenous vitamin antagonist.**
- **Vitamin B6 de-activation may contribute to seizures in hyperprolinemia type II, which are so far unexplained, but they may be preventable with long term vitamin B6 supplementation.**



L-Δ^1-Pyrroline-5-carboxylic acid (P5C)

厚生労働科学研究費補助金(難治性疾患克服研究事業)
主任研究者　三渕　浩　先生

研究課題名：高プロリン血症の臨床的多様性の解明と新しい診断
治療基準および長期フォローアップ体制の確立

尿メタボローム解析で診断された高プロリン血症について

金沢医科大学総合医学研究所　人類遺伝学研究部門　久原とみ子

平成21年7月24日(金)　16:00～18:00　ザ・ナハテラス「ガーデンルーム」

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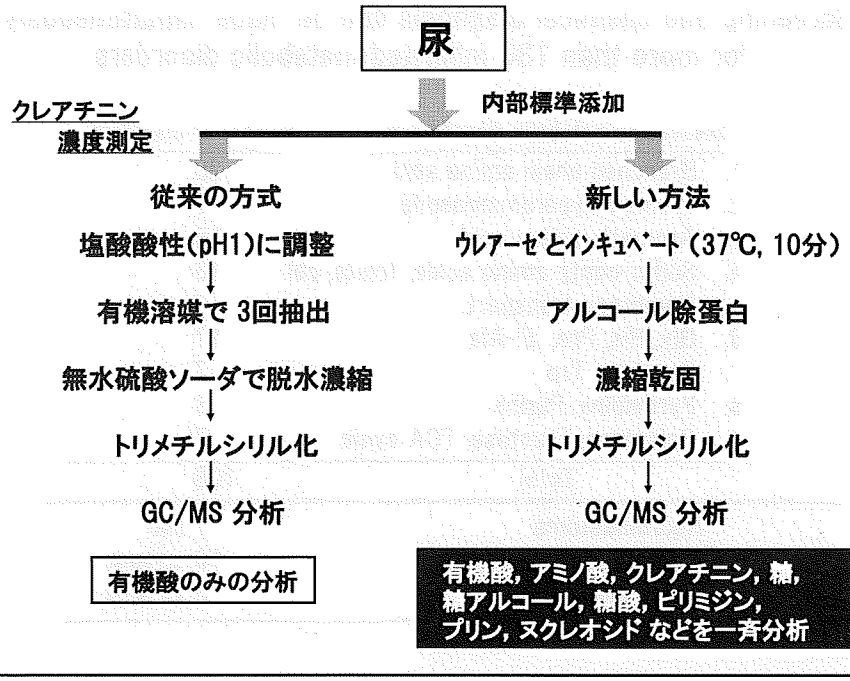

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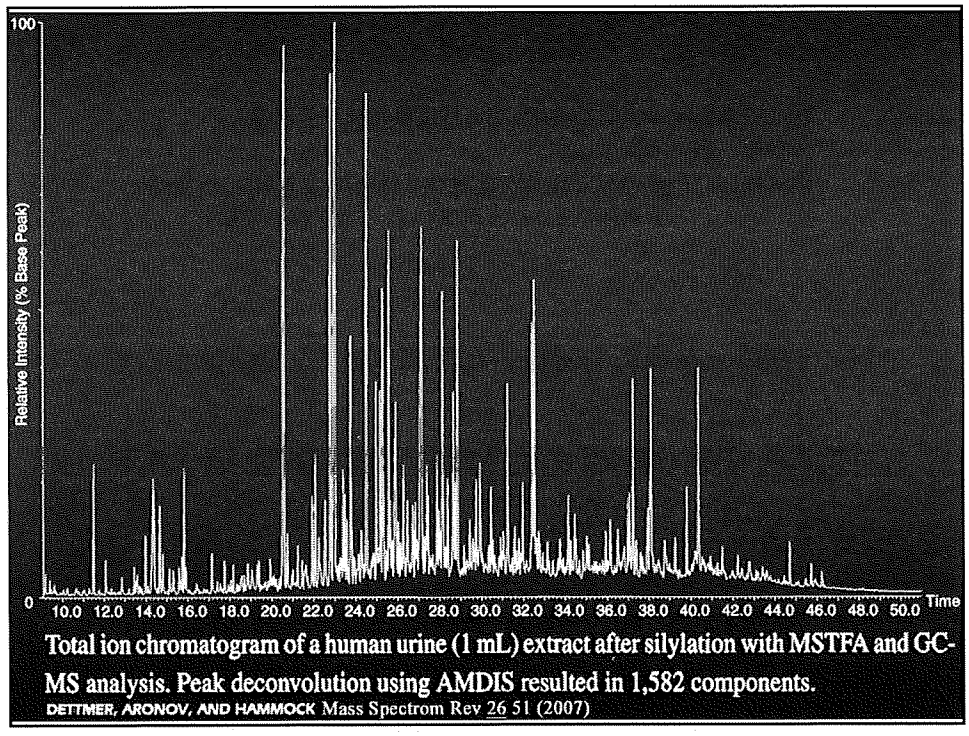

Total ion chromatogram of a human urine (1 mL) extract after silylation with MSTFA and GC-MS analysis. Peak deconvolution using AMDIS resulted in 1,582 components.

DETTMER, ARONOV, AND HAMMOCK Mass Spectrom Rev 26 51 (2007)

Screening and chemical diagnosis can be made simultaneously for more than 130 inherited metabolic disorders

Diseases or metabolic disorders in	Number of disorders
1. Branched chain amino acid	20
2. Primary hyperammonemia	9
3. Aromatic amino acid	15
4. Sulfur-contg amino acids, folate, cbl	16
5. Membrane transport	9
6. Gly, His, Pro, β-Ala	17
7. Orn, Lys, Trp	7
8. Pyrimidine, Purine	8
9. Galactose, Fructose, TCA cycle	7
Total	108
Neuroblastoma	1 DMD
Primary lactic acidemia	16 screened
Fatty acid oxidation	5 screened

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Hyperprolinemia and Other Disorders

Disorders	AA	OA	Others
	pro	Δ5PC	
Hyperprolinemia type I	++		+
Hyperprolinemia type II	+++	++	+
Iminoglycinuria	++		+
Generalized Amino Aciduria	+		++
Urea Cycle Disease	+		+++
Lactic acidemia	+		++
Some DM	+		+++

Δ[1]- pyrroline-5-carboxylate (Δ5PC)

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高プロリン血症の化学診断

症例	年齢	性別	症状	尿中異常増加化合物	判定
SM	1歳	女児	精神運動発達遅滞，痙攣，脳波異常	Proline, 4-Hydroxyproline, Glycine	高プロリン血症
IY	1歳	男児	無症状	(血中 Proline)	高プロリン血症
KE	3生月	女児	痙攣，退行性変性脳症	Proline, 4-Hydroxyproline, Glycine, (血中，髄液中 Proline)	高プロリン血症
TK	4歳	女児	精神発達遅滞，性分化異常，甲状腺機能低下，尿路結石	Proline, 4-Hydroxyproline, Glycine, Δ[1]- pyrroline-5-carboxylate (-)	高プロリン血症I型
MK	31歳	男性	糖尿病，心疾患	Glucose, Proline, 4-Hydroxyproline	高プロリン血症 二次性？ 独立事象？
TA	4歳	男児	意識混濁，四肢強直性痙攣	Proline, 4-Hydroxyproline, Glycine, Δ[1]- pyrroline-5-carboxylate	高プロリン血症II型
T (baby)	1生日	不明	全身性点状出血，高ビリルビン血症	Proline, 4-Hydroxyproline, Glycine, Δ[1]- pyrroline-5-carboxylate	高プロリン血症II型

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