employed methods of detection in the field of metabolome [25]. However, the metabolomic technologies including GC-MS and MS-MS analysis in this study sometimes yields nonspecific and nondiagnostic abnormalities. An example is the metabolome feature of NICCD. This entity was prone to be misdiagnosed as tyrosinemia or galactosemia in case just based on the GC-MS and/or MS-MS findings [26, 27].

Citrin deficiency and a rare chromosome karyotype were found in this study as novel causes for GDD and MR, respectively, further expanding the etiology profile of NDDs. Many "new" IEMs have been discovered in the recent years, and some of them have been found to have features of NDDs [28]. Citrin deficiency, first described in Japan and East Asia, is a newly-established IEM now considered as a panethnic disease distributed worldwide [27, 29]. We have no direct evidence to explain why the 2 citrin-deficient patients in this study presented with syndromic GDDs. However, their GDD manifestations could not be explained with brain injury directly due to deficient citrin, since this aspartate/glutamate carrier (AGC) is predominantly found in liver, kidney, heart, and intestine, but not in brain [29]. Interplay of multiple factors may represent the possible underlying mechanism. For example, secondary galactosemia and tyrosinemia in citrin deficiency, including the 2 cases in this paper, are quite common [26, 27], and brain injuries caused by disturbances in the metabolism of galactose and tyrosine have been reported [12, 30]. The detailed mechanism(s) for the child with novel karyotype to develop MR remains unclear at the current stage. However, the chromosome aberration in this patient produced 4 chromosome breakpoints, changing the genome architecture inevitably, and thus the syndromic MR may result from one or more mechanisms including gene interruption, gene fusion, position effect, unmasking of a recessive allele, presence of a functional polymorphism, and gene transvection effect [11, 31], as proved in other disorders of genome architecture such as acute lymphoblastic leukemia [32] and retinitis pigmentosa [33].

5. Conclusions

Although the etiologies in nearly half of the patients still remain an unresolved issue in this NDD cohort, genetic diseases including IEMs and congenital dysmorphic diseases constituted the commonest identified etiology category. GC-MS and MS-MS, the two key experimental technologies in the field of pediatric metabolomics, proved to be substantially helpful for the exploration of the NDD etiologies in our clinical practice. The findings in this paper provided latest epidemiologic information on the etiology distribution of NDDs in Chinese, and syndromic NDDs caused by citrin deficiency and the novel chromosomal karyotype, respectively, further expanded the etiology spectrum of NDDs.

Acknowledgments

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

[1] M. Shevell, "Global developmental delay and mental retardation or intellectual disability: conceptualization, evaluation, and etiology," *Pediatric Clinics of North America*, vol. 55, no. 5, pp. 1071–1084, 2008.

[2] M. Shevell, S. Ashwal, D. Donley et al., "Practice parameter: evaluation of the child with global developmental delay: report of the quality standards subcommittee of the American Academy of Neurology and The Practice Committee of the Child Neurology Society," *Neurology*, vol. 60, no. 3, pp. 367–380, 2003.

[3] J. Chelly, M. Khelfaoui, F. Francis, B. Chérif, and T. Bienvenu, "Genetics and pathophysiology of mental retardation," *European Journal of Human Genetics*, vol. 14, no. 6, pp. 701–713, 2006.

[4] M. Yeargin-Allsopp, C. C. Murphy, J. F. Cordero, P. Decouflé, and J. G. Hollowell, "Reported biomedical causes and associated medical conditions for mental retardation among 10 year old children, metropolitan Atlanta, 1985 to 1987," *Developmental Medicine and Child Neurology*, vol. 39, no. 3, pp. 142–149, 1997.

[5] Y. W. Jiang, "Enhance neurogenetic etiologic studies actively and standardize diagnosis of neurodevelopmental disorders," *Zhonghua Er Ke Za Zhi*, vol. 47, pp. 561–564, 2009 (Chinese).

[6] Y. Z. Song, B. X. Li, H. Hao et al., "Selective screening for inborn errors of metabolism and secondary methylmalonic aciduria in pregnancy at high risk district of neural tube defects: a human metabolome study by GC-MS in China," *Clinical Biochemistry*, vol. 41, no. 7-8, pp. 616–620, 2008.

[7] L. S. Han, J. Ye, W. J. Qiu, X. L. Gao, Y. Wang, and X. F. Gu, "Selective screening for inborn errors of metabolism on clinical patients using tandem mass spectrometry in China: a four-year report," *Journal of Inherited Metabolic Disease*, vol. 30, no. 4, pp. 507–514, 2007.

[8] D. H. Cheng, Y. Q. Tan, Y. F. Di, L. Y. Li, and G. X. Lu, "Crypt Y chromosome fragment resulting from an X;Y translocation in a patient with premature ovarian failure," *Fertility and Sterility*, vol. 92, no. 2, pp. 828–e3, 2009.

[9] Y. Z. Song, M. Ushikai, K. Kobayashi, and T. Saheki, "Citrin deficiency is an important etiology for cholestatic liver disease in children," *Zhonghua Er Ke Za Zhi*, vol. 47, no. 8, pp. 624–627, 2009 (Chinese).

[10] M. G. Mohaupt, R. H. Karas, E. B. Babiychuk et al., "Association between statin-associated myopathy and skeletal muscle damage," *Canadian Medical Association Journal*, vol. 181, no. 1-2, pp. E11–E18, 2009.

[11] D. Kumar, "Disorders of the genome architecture," *Genomic Medicine*, vol. 2, no. 3-4, pp. 69–76, 2008.

[12] A. M. Bosch, H. D. Bakker, A. H. van Gennip, J. V. van Kempen, R. J. A. Wanders, and F. A. Wijburg, "Clinical features of galactokinase deficiency: a review of the literature," *Journal of Inherited Metabolic Disease*, vol. 25, no. 8, pp. 629–634, 2002.

[13] J. B. Moeschler, "Genetic evaluation of intellectual disabilities," *Seminars in Pediatric Neurology*, vol. 15, no. 1, pp. 2–9, 2008.

[14] J. T. Glessner, K. Wang, G. Cai et al., "Autism genome-wide copy number variation reveals ubiquitin and neuronal genes," *Nature*, vol. 459, no. 7246, pp. 569–572, 2009.

[15] P. Szatmari, A. D. Paterson, L. Zwaigenbaum et al., "Mapping autism risk loci using genetic linkage and chromosomal rearrangements," *Nature Genetics*, vol. 39, no. 3, pp. 319–328, 2007.

[16] L. A. Weiss, Y. Shen, J. M. Korn et al., "Association between microdeletion and microduplication at 16p11.2 and autism," *The New England Journal of Medicine*, vol. 358, no. 7, pp. 667–675, 2008.

[17] X. Zhao, A. Leotta, V. Kustanovich et al., "A unified genetic theory for sporadic and inherited autism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 31, pp. 12831–12836, 2007.

[18] A. P. Aldenkamp, G. Baker, O. G. Mulder et al., "A multicenter, randomized clinical study to evaluate the effect on cognitive function of topiramate compared with valproate as add-on therapy to carbamazepine in patients with partial-onset seizures," *Epilepsia*, vol. 41, no. 9, pp. 1167–1178, 2000.

[19] D. W. Loring and K. J. Meador, "Cognitive side effects of antiepileptic drugs in children," *Neurology*, vol. 62, no. 6, pp. 872–877, 2004.

[20] J. H. Cross, "Neurodevelopmental effects of anti-epileptic drugs," *Epilepsy Research*, vol. 88, no. 1, pp. 1–10, 2010.

[21] L. McDonald, A. Rennie, J. Tolmie, P. Galloway, and R. McWilliam, "Investigation of global developmental delay," *Archives of Disease in Childhood*, vol. 91, no. 8, pp. 701–705, 2006.

[22] L. S. Han, J. Ye, W. J. Qiu et al., "Diagnosis of inborn errors of metabolism using tandem mass spectrometry and gas chromatography mass spectrometry," *Zhonghua Yi Xue Za Zhi*, vol. 88, no. 30, pp. 2122–2126, 2008 (Chinese).

[23] X. P. Luo, M. T. Wang, H. Wei et al., "Application of gas chromatography-mass spectrometry analysis on urine filter paper in the high-risk screening and diagnosis of inherited metabolic diseases," *Zhonghua Er Ke Za Zhi*, vol. 41, no. 4, pp. 245–248, 2003 (Chinese).

[24] A. R. Spitzer and D. Chace, "Proteomics- and metabolomics-based neonatal diagnostics in assessing and managing the critically ill neonate," *Clinics in Perinatology*, vol. 35, no. 4, pp. 695–716, 2008.

[25] S. G. Villas-Bôas, S. Mas, M. Åkesson, J. Smedsgaard, and J. Nielsen, "Mass spectrometry in metabolome analysis," *Mass Spectrometry Reviews*, vol. 24, no. 5, pp. 613–646, 2005.

[26] T. Ohura, K. Kobayashi, Y. Tazawa et al., "Clinical pictures of 75 patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD)," *Journal of Inherited Metabolic Disease*, vol. 30, no. 2, pp. 139–144, 2007.

[27] Y. Z. Song, B. X. Li, F. P. Chen et al., "Neonatal intrahepatic cholestasis caused by citrin deficiency: clinical and laboratory investigation of 13 subjects in mainland of China," *Digestive and Liver Disease*, vol. 41, no. 9, pp. 683–689, 2009.

[28] M. A. Kayser, "Inherited metabolic diseases in neurodevelopmental and neurobehavioral disorders," *Seminars in Pediatric Neurology*, vol. 15, no. 3, pp. 127–131, 2008.

[29] T. Saheki, K. Inoue, A. Tushima, K. Mutoh, and K. Kobayashi, "Citrin deficiency and current treatment concepts," *Molecular Genetics and Metabolism*, vol. 100, pp. S59–S64, 2010.

[30] A. M. Sgaravatti, B. A. Vargas, B. R. Zandoná et al., "Tyrosine promotes oxidative stress in cerebral cortex of young rats," *International Journal of Developmental Neuroscience*, vol. 26, no. 6, pp. 551–559, 2008.

[31] J. R. Lupski and P. Stankiewicz, "Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes," *PLoS Genetics*, vol. 1, no. 6, article e49, 2005.

[32] C. M. Rubin, J. J. Carrino, M. N. Dickler, D. Leibowitz, S. D. Smith, and C. A. Westbrook, "Heterogeneity of genomic fusion of BCR and ABL in Philadelphia chromosome-positive acute lymphoblastic leukemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 8, pp. 2795–2799, 1988.

[33] L. Köhn, S. J. Bowne, L. S. Sullivan et al., "Breakpoint characterization of a novel ~59 kb genomic deletion on 19q13.42 in autosomal-dominant retinitis pigmentosa with incomplete penetrance," *European Journal of Human Genetics*, vol. 17, no. 5, pp. 651–655, 2009.

Neonatal intrahepatic cholestasis caused by citrin deficiency in two Malaysian siblings: outcome at one year of life

Thong M K, Boey C C M, Sheng J S, Ushikai M, Kobayashi K

ABSTRACT

We report two Malaysian siblings with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). The younger sibling, a six-month-old Chinese girl, presented with prolonged neonatal jaundice, and was investigated for biliary atresia. Urine metabolic screen showed the presence of urinary-reducing sugars, and she was treated with a lactose-free formula. NICCD was suspected based on the clinical history, examination and the presence of urinary citrulline. Mutation study of the SLC25A13 gene showed the compound heterozygotes, 851del4 and IVS16ins3kb, which confirmed the diagnosis of NICCD in the patient and her three-year-old female sibling, who also had unexplained neonatal cholestasis. Long-term dietary advice, medical surveillance and genetic counselling were provided to the family. The diagnosis of NICCD should be considered in infants with unexplained prolonged jaundice. DNA-based genetic testing of the SLC25A13 gene may be performed to confirm the diagnosis retrospectively. An awareness of this condition may help in early diagnosis using appropriate metabolic and biochemical investigations, thus avoiding invasive investigations in infants with neonatal cholestasis caused by NICCD.

Keywords: citrin deficiency, citrullinaemia type 2, metabolic liver disease, molecular genetics, neonatal intrahepatic cholestasis, neonatal jaundice, prolonged jaundice

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INTRODUCTION

Citrin is a mitochondrial inner membrane aspartate-glutamate carrier that functions as part of the malate-aspartate shuttle, transferring cytosolic-reduced nicotinamide adenine dinucleotide into the mitochondria. It plays a role in the synthesis of urea, protein and nucleotide by supplying mitochondrial aspartate to the cytosol in the liver.[1] There are two disease entities attributed to the deficiency of citrin: neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD; OMIM#605814) and adult-onset Type II citrullinaemia (CTLN2; OMIM#603471).[2] Infants with NICCD often present with transient intrahepatic cholestasis with mild liver dysfunction, hepatomegaly, transient multiple aminoacidaemia (citrulline, methionine, tyrosine) and hypoglycaemia. To the best of our knowledge, these two conditions and their outcomes have not been published in the English medical literature from Southeast Asia.

CASE REPORT

A six-month-old Malaysian Chinese baby girl of non-consanguineous parents was referred to the University of Malaya Specialist Centre at six weeks of life for investigation of prolonged neonatal jaundice. The antenatal period was uneventful, and she was delivered at term with a birth weight of 2.75 kg. She was initially breastfed but later received additional formula feeds. Her stools were pale yellow in colour. Although she was thriving well, she was noted to be jaundiced from the first month of life and was investigated for prolonged jaundice. On review of the family history, it was found that the patient had an elder sister, aged three years old, who also had jaundice from one to three months of age. Her urinary-reducing sugar was positive. The sister was initially treated in another hospital. Her jaundice had disappeared after lactose-free formula was started so no other investigation was performed.

The physical examination confirmed the presence of jaundice in the patient. She was thriving well and had no dysmorphic features. The abdominal examination revealed a soft palpable liver 2 cm below the right subcostal margin. The rest of the findings were non-contributory. The investigations showed hyperbilirubinaemia 210 (control range 3–17) umol/L with conjugated fraction 126 (control range 0–3) umol/L, serum alkaline phosphatase 9,813 (control range 50–136) IU/L, serum alanine aminotransferase 47 (control range 30–65) IU/L, serum aspartate aminotransferase 168 (control range 15–37) IU/L and gamma glutamyl transferase 285 (control range 5–55) IU/L. The full blood

Genetics and Metabolism Unit, Department of Paediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

Thong MK, MBBS, MPaeds, MD Clinical Geneticist and Professor of Paediatrics

Gasteroenterology, Hepatology and Nutrition Unit

Boey CCM, FRCP, MD, PhD Professor of Paediatrics

Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8544, Japan

Sheng JS, MSc Scientist

Ushikai M, MSc Scientist

Kobayashi K, PhD Associate Professor and Molecular Geneticist

Correspondence to: Dr Meow-Keong Thong Tel: (60) 3 7949 2065 Fax: (60) 3 7955 6114 Email: thongmk@ um.edu.my

count, hypothyroid screen and glucose-6-phosphate dehydrogenase screen were normal. The alpha-1antitrypsin and serum TORCH screens were normal. Ultrasonography of the liver and abdomen was normal, but the isotope study with hepatobiliary iminodiacetic acid scan did not show any excretion. An on-table cholangiogram, however, showed good excretion into the small intestine, excluding the possibility of biliary atresia. The liver biopsy showed marked cholestasis with ballooning degeneration and fibrosis of the portal tracts. No inflammatory cell was noted.

The urine metabolic screen showed the presence of urinary-reducing sugars (positive) and tyrosine (positive) on two occasions. In view of the suspicion of galactosaemia, she was empirically started on a lactose-free formula, and breastfeeding was stopped. The urinary-reducing sugars subsequently became negative. The galactose-1-phosphate uridyl transferase assay and the urinary succinylacetone level were normal, while the urine organic acid showed a marked increase in 4-hydroxyphenylacetic acid, 4-hydroxyphenyllactic acid, 4-hydroxyphenylpyruvic acid and n-acetyl tyrosine, which was consistent with liver impairment. The urine amino acid showed a marked elevation of urine citrulline and a slight increase in tyrosine, histidine and alanine, with an absence of succinylacetone and argininosuccinic acid, suggesting NICCD. Plasma amino acid tests were not done at that time. Mutation analysis of the SLC25A13 gene was performed at the Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan. The results showed that the patient was a compound heterozygote for two different mutations: a known 851del4[3] and a novel IVS16ins3kb that was reported recently.[4]

On review at five months of age, the patient had recovered well. The liver function test, serum ammonium, urine and plasma amino acid assays were normal. The caregivers were counselled regarding the condition. Appropriate dietary advice was provided to the parents, who were told to ensure that the patient maintained a high-protein and low-carbohydrate diet, and avoided paracetamol and other antiinflammatory medications. The patient was well at one year of life. After the diagnosis of NICCD was made in the patient, the older sibling was tested and found to have the identical compound heterozygous mutations, 851del4 and IVS16ins3kb. Following this molecular diagnosis, the parents were counselled that both their daughters have NICCD. The older sister has remained well and has no specific food preference.

DISCUSSION

While NICCD during infancy is not considered a life-threatening disease, it is vital to note that three affected individuals had undergone liver transplantation.[5] The clinical features may include intrahepatic cholestasis, diffuse fatty liver with parenchymal cellular infiltration associated with hepatic fibrosis and variable liver dysfunctions, such as coagulopathy and hypoalbuminaemia. This may lead to extensive investigations in the affected infant to exclude biliary atresia. Other clinical features include ketotic hypoglycaemia, hepatomegaly and haemolytic anaemia in childhood. As awareness of NICCD is generally low, these patients may be empirically treated for galactosaemia with lactose-free formula. After weaning, the patient with NICCD may show an aversion to carbohydrate-rich foods and a fondness for protein-rich foods.

Unfortunately, some individuals with NICCD may develop CTLN2 with neuropsychiatric features during early adulthood.[2] CTLN2 is a severe condition characterised by recurrent episodes of hyperammonaemia and neuropsychiatric symptoms, such as nocturnal delirium, irritability, delusions, disorientation, changes in sensorium, convulsions and coma. Death may result from cerebral oedema. The onset is sudden, and the precipitating factors may include alcohol ingestion, medications or surgery. The liver may show fatty infiltration and mild fibrosis, with minimal liver dysfunction. Although the prognosis of CTLN2 is guarded, it is amenable to liver transplantation.[6]

The differential diagnoses may include citrullinaemia Type 1 (CTLN1), argininosuccinic aciduria and pyruvate carboxylase deficiency in patients presenting with increased urinary or plasma citrulline. Hyperammonaemia may lead one to suspect urea cycle defect and organic acidemias. The neonate with cholestasis may be investigated for galactosaemia, biliary atresia, Byler disease and other forms of hereditary hyperbilirubinaemia. The diagnosis of NICCD is made based on the history and the above mentioned physical examination findings, and supported by transient aminoacidaemia (citrulline, methionine, tyrosine), transient galactosuria, an increased alpha foetoprotein concentration and the presence of mild liver dysfunction. The symptoms and abnormal biochemical findings disappear by one year of age. Due to the transient nature of the biochemical abnormalities in NICCD, genetic testing is useful when the patient is seen after the first year of life, or when the parents are considering prenatal diagnosis. In addition, the parents of

patients with NICCD confirmed by genetic testing may receive early counselling on the risk of CTLN2 for their children, which will change their long-term management and health surveillance program. For example, as the outcome of NICCD patients is not always benign, the confirmation of their status as NICCD by genetic testing may mean a life-time of dietary modifications and early preparation for liver transplantation, if they develop recurring neuropsychiatric symptoms.[4] A patient with "idiopathic" neonatal cholestasis may not be subjected to similar restrictions or concerns. Therefore, continuing research and follow-up of these families are important for future reference.

The frequency carriers for the SLC25A13 mutation is one in 65 and one in 48 in the Japanese and Southern Chinese populations, respectively.[4,7] Recently, NICCD was reported in patients from Israel,[4,8] the United States of America,[9] the United Kingdom[4,10] and the Czech Republic.[4] Increasingly, this condition is being detected through expanded newborn screening programmes.[11,12] There is a need for greater awareness of this disease entity in this part of Southeast Asia. This is to avoid unnecessary and potentially hazardous investigations in infants. As such, it would be reasonable to consider this diagnosis in the workup for patients with neonatal cholestasis. This condition is also amenable to dietary management, which consists of a high-protein and low-carbohydrate diet, and avoidance of risk factors, such as alcohol and certain drugs. As this an autosomal recessive condition, genetic counselling should be provided to the affected family. Mutation study of the SLC25A13 gene may be used when the diagnosis is uncertain, or when prenatal diagnosis is being considered. In our case, the molecular diagnostic approach had confirmed the diagnosis of NICCD in both patients. Long-term health and medical surveillance has been provided for the family. Therefore, the diagnosis of NICCD in infants with unexplained prolonged jaundice should be considered, with a DNA-based genetic testing of the SLC25A13 gene performed to confirm the diagnosis.

The detailed methods for the identification and diagnosis of the novel mutation, IVS16ins3kb, which was first found and characterised in a Japanese CTLN2 patient, have been described in the literature.[4] However, the clinical and biochemical data have previously been reported mainly in Japanese,[13,14] Korean[15,16] and Chinese[4] NICCD patients with the IVS16ins3kb mutation, and it is well known that many patients

and carriers of the mutation 851del4 are found widely in East Asia.[4,7] Therefore, the presence of both these mutations in Malaysian Chinese patients, as reported in this case, suggests that the IVS16ins3kb mutation, like the 851del4 mutation, may also be widely distributed in East Asia.[3]

REFERENCES

1. Saheki T, Kobayashi K. Physiological role of citrin, a liver-type mitochondrial aspartate-glutamate carrier, and pathophysiology of citrin deficiency. Recent Res Devel Life Sci 2005; 3:1-15.
2. Saheki T, Kobayashi K. Mitochondrial aspartate glutamate carrier (citrin) deficiency as the cause of adult-onset type II citrullinemia (CTLN2) and idiopathic neonatal hepatitis (NICCD). J Hum Genet 2002; 47:333-41.
3. Kobayashi K, Sinasac DS, Iijima M, et al. The gene mutated in adult-onset type II citrullinaemia encodes a putative mitochondrial carrier protein. Nat Genet 1999; 22:159-63.
4. Tabata A, Sheng JS, Ushikai M, et al. Identification of 13 novel mutations including a retrotransposal insertion in SLC25A13 gene and frequency of 30 mutations found in patients with citrin deficiency. J Hum Genet 2008; 53:534-45.
5. Tamamori A, Okano Y, Ozaki H, et al. Neonatal intrahepatic cholestasis caused by citrin deficiency: severe hepatic dysfunction in an infant requiring liver transplantation. Eur J Pediatr 2002; 161:609-13.
6. Saheki T, Kobayashi K, Iijima M, et al. Adult-onset type II citrullinemia and idiopathic neonatal hepatitis caused by citrin deficiency: involvement of the aspartate-glutamate carrier for urea synthesis and maintenance of the urea cycle. Mol Genet Metab 2004; 81 Suppl 1:S20-6.
7. Lu YB, Kobayashi K, Ushikai M, et al. Frequency and distribution in East Asia of 12 mutations identified in the SLC25A13 gene of Japanese patients with citrin deficiency. J Hum Genet 2005; 50:338-46.
8. Ben-Shalom E, Kobayashi K, Shaag A, et al. Infantile citrullinemia caused by citrin deficiency with increased dibasic amino acids. Mol Genet Metab 2002; 77:202-8.
9. Dimmock D, Kobayashi K, Iijima M, et al. Citrin deficiency: a novel cause of failure to thrive that responds to a high protein, low carbohydrate diet. Pediatrics 2007; 119:e773-7.
10. Hutchin T, Preece MA, Hendriksz C, et al. Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) as a cause of liver disease in infants in the UK. J Inherit Metab Dis 2009, June 11[E-pub ahead of print].
11. Lee NC, Chien YH, Kobayashi K, et al. Time course of acylcarnitine elevation in neonatal intrahepatic cholestasis caused by citrin deficiency. J Inherit Metab Dis 2006; 29:551-5.
12. Ohura T, Kobayashi K, Tazawa Y, et al. Clinical pictures of 75 patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). J Inherit Metab Dis 2007; 30:139-44.
13. Tazawa Y, Kobayashi K, Abukawa D, et al. Clinical heterogeneity of neonatal intrahepatic cholestasis caused by citrin deficiency: case reports from 16 patients. Mol Genet Metab 2004; 83:213-9.
14. Tokuhara D, Iijima M, Tamamori A, et al. Novel diagnostic approach to citrin deficiency: analysis of citrin protein in lymphocytes. Mol Genet Metab 2007; 90:30-6.
15. Kim SZ, Jeon YM, Song WJ, et al. Two cases of citrin deficiency detected by newborn screening in Korea. J Inherit Metab Dis 2006; 29 Suppl 1:84.
16. Ko JM, Kim GH, Kim JH, et al. Six cases of citrin deficiency in Korea. Int J Mol Med 2007; 20:809-15.

[Improving the diagnostic method for the SLC25A13 gene 851del4 mutation and analysis of the common mutation frequencies in Quanzhou area.]

[Article in Chinese]

Gao H, Li Q, Zhuang X, Keiko K, Miharu U, Takeyori S, Hu W, Zhou C, Lin L.

The Second Clinical College, Fujian Medical University, Quanzhou, Fujian, 362000 P.R.China.

fmufuer@public.qz.fj.cn.

Abstract

OBJECTIVE: To ascertain whether the carrier rate is high in Quanzhou which is next to Taiwan in South of the Yangtze River.

METHODS: Population analysis of three SLC25A13 mutations, i.e. 851del4, 1638-1660 dup, and IVS6+ 5G to A was carried out in 450 healthy individuals. DNA diagnostic method of 851del4 was improved by using PCR-restriction fragment length polymorphism(PCR-RFLP) with restriction enzyme HpyCH4 IV, and the results were confirmed by GeneScan method.

RESULTS: Six carriers with 851del4, 3 with 1638-1660 dup and 3 with IVS6+ 5G to A was found.

CONCLUSION: The high carrier rate (0.027, 12/450) obtained from testing of only three mutations indicated that there must be a certain number of patients with citrin deficiency in Quanzhou, even in Fujian. Therefore, it is important for physicians in Quanzhou, Fujian province to learn about citrin deficiency, and to diagnose and treat the patients correctly.

Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) in three Malay children.

Ngu HL, Zabedah MY, Kobayashi K.

Genetics Department, Kuala Lumpur Hospital, Jalan Pahang, 50586 Kuala Lumpur, Malaysia. ngulh@hotmail.com

Abstract

Citrin deficiency is an autosomal recessive disorder caused by mutation in the SLC25AJ3 gene. It has two major phenotypes: adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestatic caused by citrin deficiency (NICCD). NICCD is characterized by neonatal/infantile-onset cholestatic hepatitis syndrome associated with multiple amino acidemia and hypergalactosemia. NICCD is self-limiting in most patients. However, some patients may develop CTLN2 years later, which manifests as fatal hyperammonemia coma. We report three unrelated Malay children with genetically confirmed NICCD characterised by an insertion mutation IVS16ins3kb in SLC25A13 gene. All 3 patients presented with prolonged neonatal jaundice which resolved without specific treatment between 5 to 10 months. Of note was the manifestation of a peculiar dislike of sweet foods and drinks. Elevated plasma citrulline was an important biochemical marker. NICCD should be considered in the differential diagnosis of cholestatic jaundice in Malaysian infants regardless of ethnic origin.

Reliability and validity of the PedsQL™ Multidimensional Fatigue Scale in Japan

Kyoko Kobayashi · Yoshiyuki Okano · Naohiro Hohashi

Abstract

Purpose To examine the reliability and validity of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale and to investigate the agreement between child self-reported fatigue and parent proxy-reported fatigue.

Methods The Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale was administered to 652 preschoolers and schoolchildren aged 5–12 and their parents, and to 91 parents of preschool children aged 1–4.

Results Internal consistency reliability was 0.62–0.87 for children and 0.81–0.93 for parents. Known-group validity was examined between a group of healthy samples ($n = 530$) and chronic condition sample ($n = 102$); the chronically ill group reported a significantly higher perceived fatigue problem. Correlations between child self- and parent proxy reports ranged from poor to fair. In subgroups identified by cluster analysis based on child self-reported scores, the greatest agreement between child and parent reports was seen in the good HRQOL group, while the least occurred in the poor HRQOL group. The parents overestimated their child's fatigue more when the child's HRQOL was low.

Conclusion The Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale demonstrated good reliability and validity and could be useful in evaluating Japanese children in school and health care settings.

Keywords Health-related quality of life · Fatigue · Children · Parents · Agreement · PedsQL™

K. Kobayashi (✉)
Department of Family Nursing, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
e-mail: kyoko-ko@umin.ac.jp

Y. Okano
Department of Pediatrics, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
e-mail: okano@med.osaka-cu.ac.jp

N. Hohashi
Division of Family Health Care Nursing, Kobe University Graduate School of Health Sciences, 7-10-2, Tomogaoka, Suma-ku, Kobe, Hyogo 654-0142, Japan
e-mail: naohiro@hohashi.org

Background

In the last decade, the utility of pediatric health-related quality of life (HRQOL) measurement was closely examined, and HRQOL is now recognized as an essential point of reference in clinical trials, health care settings, and school health care. Numerous HRQOL measurements have been developed for children and adolescents [1–4], and the Pediatric Quality of Life Inventory (PedsQL™), developed in the United States, has demonstrated satisfactory psychometric properties in both diseased and healthy children [5–10]. The PedsQL™ includes well-established methods of child self-reporting and parent proxy reporting, which have been utilized in clinical trials in Japan and other countries worldwide [11–16].

Fatigue is a health problem that significantly impacts HRQOL and it is a frequent and ubiquitous complaint among patients with chronic disease [17]. Fatigue is also a frequent complaint in the general school population, and an association between fatigue and school absenteeism has been reported [18, 19]. A previous Japanese study reported that preschool children and elementary school children

suffer from fatigue more frequently than adolescents do [20]. Fatigue is a subjective and multidimensional phenomenon that is defined by how the affected individual describes it [21, 22]. Therefore, a child's fatigue may be more completely evaluated when self-reporting is included. Japanese measurements that have been developed, including a subjective fatigue scale for young adults [23], a child fatigue symptoms scale [24], and a questionnaire of subjective symptoms of fatigue for schoolchildren [25], make it possible for children to express their subjective fatigue. However, those measurements are limited by the child's age and are difficult to adapt to longitudinal evaluation. The PedsQL™ Multidimensional Fatigue Scale was designed as a generic symptom-specific instrument to measure fatigue in pediatric patients aged 2–18 years from the perspective of the children, adolescents, and their parents [7, 8, 26]. As the PedsQL™ Multidimensional Fatigue Scale would be a useful tool for measuring the fatigue of Japanese children, the primary aim of this study was to develop and test a Japanese-language version of this scale.

Although the primary source of information regarding HRQOL or fatigue is children themselves, obtaining child self-reporting poses a challenge because of cognitive developmental limitations. In addition, when children have insufficient cognitive or communication abilities, are too young, experience severe symptom distress, or find an interview to be too physically or emotionally burdensome, the use of parent proxy reports may be an effective means of obtaining information about the children. However, several studies on HRQOL and fatigue have reported imperfect agreement between child self-reports and parent proxy reports [27, 28]. Lower agreement has been found for internalized problems (e.g., pain, emotional problems) than for external problems (e.g., physical movement) [28]. In previous studies, correlations (r, ICC) between the scores of self-reports and proxy reports were measured by using total scores or domains of the measurement. These studies shed light on the degree of agreement on specific domains of HRQOL or fatigue (e.g., a high degree of agreement in external functioning was seen). Thereafter, questions were raised about how the child's age, health status, and HRQOL level affected the extent of agreement between child self-reports and parent proxy reports. Although previous studies have compared the self-reports and proxy reports of adult patients and their significant others by the levels of the patients' health status [29, 30], few have compared those of children and parents.

Consequently, the objective of this study was to examine the reliability and validity of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale for children aged 2–12 years as well as to investigate the agreement between child self-reported fatigue and parent proxy-reported fatigue.

Method

Participants

The participants were children aged 5–12 years ($n = 652$) and their parents ($n = 652$). Parents with children aged 1–4 years also participated in the survey ($n = 91$). Thus, 652 child self-reports and 743 parent proxy reports were obtained. Of the participants, 63 (9.7%) of the children and 154 (20.7%) of the parents were recruited from a preschool, and 589 (90.3%) of the children and 589 (79.3%) of the parents were recruited from an elementary school. The preschool and elementary school were located in Osaka, the second largest city of Japan, which had a population of 8,840,372 in 2009 and a gross prefecture product of 38,808,582 yen in 2006. All the children aged 5–12 years ($n = 652$) filled out the questionnaire sheet, and 20 parents did not complete the questionnaire. The average age of the 743 children (652 children with self-reporting and 91 children with only proxy reporting) studied was 8.74 years (SD = 2.41); there were 373 boys (50.2%) and 370 girls (49.8%). Of those who completed proxy reports, 704 were mothers (97.4%), 18 were fathers (2.5%), 1 was a grandmother (0.1%), and 20 were missing data. Of the 743 children, 125 (19.7%) had a chronic condition (e.g., asthma, atopic dermatology). The children with chronic conditions were those whose parents reported the presence of a chronic health condition that required control by regular medication and/or visiting an outpatient clinic.

Measures

The PedsQL™ Multidimensional Fatigue Scale (Japanese-language version)

The PedsQL™ Multidimensional Fatigue Scale was designed on the basis of the concept that disease-specific symptoms are causal indicators of generic HRQOL [31]. The measurements were developed in the United States by Dr. J. W. Varni [5] on the basis of a modular approach model measuring HRQOL in healthy children and adolescents aged 2–18 years and in those with acute and chronic health conditions [7, 8]. The PedsQL™ Multidimensional Fatigue Scale consists of 18 items that are divided into three subscales: (1) general fatigue (six items, e.g., "I feel tired"), (2) sleep/rest fatigue (six items, e.g., "I spend a lot of time in bed"), and (3) cognitive fatigue (six items, e.g., "It is hard for me to keep my attention on things"). The PedsQL™ Multidimensional Fatigue Scale comprises parallel child self-report and parent proxy report formats. Child self-reports exist for children aged 5–7, 8–12, and 13–18 years. Parent proxy reports exist for children aged 2–4 (toddler), 5–7 (young children), 8–12 (children), and

13–18 (adolescent) years, and they are used to assess the parent's perception of their child's fatigue. In this study, formats for children aged 2–4, 5–7, and 8–12 years were used. The instructions for the format for children aged 8–12 asked how much of a problem each item had during the past month. For the self-report format for children aged 5–7, the recall time was shortened to a few weeks. For both of these older age groups, a 5-point Likert scale (0: never a problem; 1: almost never a problem; 2: sometimes a problem; 3: often a problem; 4: almost always a problem) was used. For the self-report format for the younger children (aged 5–7), the response choice was simplified to a 3-point scale (0: not at all a problem; 2: sometimes a problem; 4: a major problem). The scores are calculated by using a linearly transformed reversed score range from 0 to 100 (0: 100, 1: 75, 2: 50, 3: 25, 4: 0). Higher scores indicate less fatigue. Scale scores are calculated as the sum of items divided by the number of items answered; however, when more than 50% of the items on the scale are missing, the scale score is not calculated.

The Japanese-language version was developed in three phases according to the suggested guidelines [6]: (1) forward translation (English–Japanese translation), (2) back translation (Japanese–English translation), and (3) pretesting with 21 children (5–18 years old) and 27 parents. Dr. J. W. Varni reviewed the reports of the results of each phase, and his approval was obtained before proceeding to the next phase or to the final Japanese-language version, which was used in this validation study.

The PedsQL™ 4.0 Generic Core Scales (Japanese-language version)

The Japanese-language version of the PedsQL™ 4.0 Generic Core Scales was used to assess the child's general HRQOL. The original PedsQL™ 4.0 Generic Core Scales were developed according to the definition of health given by the World Health Organization [32], and they consist of 23 items belonging to four subscales: (1) physical functioning (eight items, e. g., "It is hard for me to walk more than one block"), (2) emotional functioning (five items, e.g., "I feel afraid or scared"), (3) social functioning (five items, e.g., "I have trouble getting along with other kids"), and (4) school functioning (five items, e.g., "It is hard to pay attention in class").

The Japanese-language version of the PedsQL™ Generic Core Scales was developed by Kobayashi and Kamibeppu [16]. The Cronbach's alpha coefficients for healthy children were 0.64 for the self-reports of young children, 0.85 for the self-reports of older children and adolescents, 0.90 for the parent proxy reports for toddlers and young children, and 0.92 for the parent proxy reports for older children and adolescents [16]. A 5-point Likert scale was

used with the children aged 8 and older, and a 3-point Likert scale was used with those aged between 5 and 7 years. In this study, the formats used for children aged 2–4, 5–7, and 8–12 were the same as those used for the PedsQL™ Multidimensional Fatigue Scale. Four scores can be calculated: the total score, subscale score (referring to each subscale), physical health summary score (referring to the score of the physical functioning subscale), and psychosocial health summary score (referring to the emotional functioning, social functioning, and school functioning subscales). Higher scores represent a better HRQOL.

Procedure

Children aged 8–12 years and all parents completed the measures without assistance, whereas children aged 5–7 years received assistance to complete the measures in preschool or elementary school. For children aged 2–4 years, only a proxy report was completed by the parents. All the participants received a written document that explained our procedure for the protection of their privacy and the voluntary nature of their participation. After the questionnaire and explanatory document were distributed to the parents and children, written parental informed consent and child assent were obtained. This study was approved by the Institutional Review Boards of Kobe University Graduate School of Health Sciences.

Statistical analysis

Reliability and validity

The feasibility of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale was determined by the percentage of missing values for each item [33]. Internal consistency reliability was determined by Cronbach's coefficient alpha [34].

Construct validity was examined by using the known-group method [35]. This method compares scale scores across groups known to differ in the health construct being investigated. In the given survey, the samples were divided into two groups that differed in known health condition—a healthy group ($n = 530$) and a chronic condition group of participants who needed medicine or hospital visits for treatment of their condition ($n = 102$). Independent sample t tests were used to compare the two groups. To determine the magnitude of the anticipated differences between the healthy and chronic condition groups, the effect sizes were computed by taking the difference between the healthy sample mean and the chronic sample mean, divided by the healthy sample standard deviation. The effect sizes were

designated as small (0.20), medium (0.50), and large (0.80) [36].

Construct validity was further examined by computing the intercorrelations between the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale and the Japanese-language version of the PedsQL™ Generic Core Scales. Computing the intercorrelations provides additional information on the construct validity of an instrument [37]. We hypothesized that more severe fatigue, as indicated by scores on the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale, would be associated with lower scores on the Japanese-language version of the PedsQL™ Generic Core Scales, on the basis of the concept of disease-specific symptoms as causal factors of generic HRQOL [31]. According to Cohen [36], Pearson's correlations effect sizes were considered as small (0.10–0.29), moderate (0.30–0.49), and large (≥0.50).

The factor structure of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale was extracted using confirmatory factor analysis (CFA) to assess the original three-subscale model: general fatigue, sleep/rest fatigue, and cognitive fatigue. As indices of goodness of fit, the chi-squared test, root mean squared error of approximation (RMSEA), comparative fit index (CFI), goodness of fit index (GFI), and adjusted goodness of fit index (AGFI) were used. Fit indices and reasonable values of these indices were considered as RMSEA less than 0.1 [38] and CFI, GFI, and AGFI less than 0.9 [39, 40]. Factor structure was further examined by using exploratory factor analysis (EFA). Principal components analysis with varimax rotation of the 18 items of the Japanese-language version of PedsQL™ Multidimensional Fatigue Scale was performed.

Agreement

Agreement between a child's self-report score and a parent proxy report score was examined by calculating the Pearson's correlation coefficient and intraclass correlation coefficients (ICCs). It was hypothesized that the correlations between child self-report scores and parent proxy report scores would show poor to fair correlation because fatigue is thought to be an internalized problem. In addition to correlation, the mean of the absolute differences between self-reports and proxy reports for total score and for all subscales of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale was calculated as a further indicator of the level of agreement between child and parent responses. Further, to evaluate the presence of a systematic tendency of parents to rate their children as having a better or poorer level of fatigue status than did the children themselves, the means of directional difference were calculated (proxy-reported score minus

self-reported score). The mean of directional difference indicates the direction of the differences between parent proxy-reported scores and child self-reported scores [29, 41]. The mean values of directional differences were standardized (d) by relating a given score to its standard deviation to determine the statistical magnitude of any observed systematic bias. The size of the bias of a standardized difference (d) was labeled as small when it was 0.2, medium when it was 0.5, and large when it was 0.8.

To investigate how dependent the extent of agreement between child self-report and parent proxy report was on child health status, age, and HRQOL level, we calculated the ICC, the mean of the absolute difference, and the mean of the directional difference for three kinds of child–parent dyad groups. The first was determined by the child's age; the second by the parent-reported presence of the child's chronic health condition (healthy sample and chronic condition sample); and the third by the child's reported HRQOL level. With regard to the analysis of agreement levels by the child's HRQOL level, the child–parent dyads were divided into subgroups determined by cluster analysis by using the child's self-reported scores of all three subscales of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale (general fatigue, sleep/rest fatigue, and cognitive fatigue) and all four subscales of the Japanese-language version of the PedsQL™ Generic Core Scales (physical functioning, emotional functioning, social functioning, and school functioning). To determine the content of the subgroups, Ward's method was used. The ICC, the mean of the absolute difference between child self-reports and parent proxy reports, and the mean of directional differences of total score and subscales were calculated by identified subgroup. Paired t tests between the children's self-reports and the parents' proxy reports of total scores and subscales were also conducted. An analysis of variance (ANOVA) was performed to clarify the statistically significant differences of the mean of absolute difference, directional differences, and mean scores of parent proxy report across subgroups.

All the statistical analyses were conducted by using SPSS 14.0 and Amos 5.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Feasibility

For the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale, the percentage of data missing from child self-reports and parent proxy reports was calculated to assess the feasibility. The percentages were 0.008 and 0.169%, respectively.

Table 1 Scale descriptives and internal consistency reliability (Cronbach's alpha) for the PedsQL™ Multidimensional Fatigue Scale child self-reports and parent proxy reports

Scale	Total sample Mean (SD)	Healthy sample Mean (SD)	Chronic sample Mean (SD)	Difference	Effect size (ES)	Internal consistency reliability α			
						Total sample	Age 2–4	Age 5–7	Age 8–12
Child self-report	$n = 652$	$n = 530$	$n = 102$			$n = 652$		$n = 95$	$n = 557$
Total fatigue	76.7 (16.3)	77.6 (16.0)	72.3 (17.7)	5.3[b]	0.33	0.87	–	0.77	0.89
General fatigue	80.6 (19.0)	81.7 (18.3)	74.9 (22.1)	6.8[b]	0.37	0.81	–	0.61	0.84
Sleep/rest fatigue	71.9 (18.7)	72.8 (18.6)	67.6 (19.4)	5.2[a]	0.28	0.62	–	0.47	0.65
Cognitive fatigue	77.9 (20.2)	78.5 (20.4)	74.5 (19.4)	4.0	0.20	0.82	–	0.68	0.84
Parent proxy report	$n = 743$	$n = 594$	$n = 125$			$n = 743$	$n = 91$	$n = 95$	$n = 557$
Total fatigue	84.7 (13.9)	85.6 (13.6)	80.2 (14.4)	5.4[b]	0.40	0.93	0.93	0.91	0.94
General fatigue	84.0 (15.8)	84.9 (15.6)	79.4 (16.3)	5.5[b]	0.35	0.88	0.89	0.83	0.88
Sleep/rest fatigue	85.2 (15.4)	86.2 (14.8)	79.9 (17.2)	6.3[b]	0.43	0.81	0.73	0.80	0.82
Cognitive fatigue	85.1 (16.4)	85.8 (16.1)	81.3 (17.4)	4.5[b]	0.28	0.92	0.94	0.91	0.92

Twenty child self-reports and 24 parent proxy reports of chronic condition group were missing data

α Cronbach's coefficient alpha

ES effect size. Effect sizes are designated as small (0.20), medium (0.50), and large (0.80)

[a] The scores of children with a chronic condition were significantly lower than the scores of healthy children at $P < 0.05$ based on an independent sample student t test

[b] The scores of children with a chronic condition were significantly lower than the scores of healthy children at $P < 0.01$ based on an independent sample student t test

Internal consistency reliability

Internal consistency reliability alpha coefficients for the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale are shown in Table 1. The alpha coefficients of the sleep/rest fatigue scale of the total sample fell below the reliability standard of 0.70, which is required to compare groups [42]; however, the rest of the scales exceeded the satisfaction criterion. The alpha coefficients of younger children (aged 5–7 years) were lower than those of older children (aged 8–12 years).

Construct validity

Table 1 shows the mean scores and standard deviations of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale scores for child self-reports and parent proxy reports for the total sample, as well as for the healthy and chronic condition samples that were extracted out of the total sample. The scores of child self-reports and parent proxy reports of children with chronic conditions indicated that these children had statistically significant worse fatigue than the healthy children; the effect sizes were in the medium range (0.20–0.37 for child self-report and 0.28–0.43 for parent proxy report). Table 2 shows the intercorrelations between the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale and the Japanese-language version of the PedsQL™ Generic Core

Scales. Scores of the PedsQL™ Multidimensional Fatigue scale and the PedsQL™ Generic Core Scales were significantly correlated, which indicated that worse fatigue correlated with worse generic HRQOL. The effect sizes (r) of Pearson's correlations were in the medium to large effect size range (for child self-report, r was 0.39–0.75; for parent proxy report, r was 0.35–0.73).

The results of the CFA for the 3-subscale model for both child self-report and parent proxy report are shown in Table 3. The GFI for child self-report was greater than 0.90; however, the CFI and AGFI for both child self-report and parent proxy report were slightly less than 0.9. Goodness of fits for the child self-report were better than the parent proxy report, which did not meet the pre-established criterion for RMSEA. Factor analysis with varimax rotation (EFA) extracted three factors for both child self-report and parent proxy report: Factor 1 (cognitive fatigue), Factor 2 (general fatigue), and Factor 3 (sleep/rest fatigue). The eigenvalues cutoff of 1.0, total variances explained were 39.6 and 58.1% variance for self-report and proxy report, respectively (Table 4). Four items of child self-report were loaded highest on a factor other than the a priori hypothesized factor structure. At the more detailed level, Items 8, 9, and 11 ("It is hard for me to sleep through the night," "I feel tired when I wake up in the morning," and "I take a lot of naps") split into general fatigue (Factor 2). Similarly, Item 5 ("I have trouble finishing things") loaded highest on cognitive fatigue (Factor

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Table 2 Intercorrelations among the PedsQL™ Multidimensional Fatigue Scale and the PedsQL™ Generic Core Scales for child self-reports and parent proxy reports

Scale	Child self-report				Parent proxy report			
	Total fatigue	General fatigue	Sleep/rest fatigue	Cognitive fatigue	Total fatigue	General fatigue	Sleep/rest fatigue	Cognitive fatigue
Total score	0.75	0.69	0.59	0.62	0.72	0.66	0.61	0.61
Physical	0.62	0.59	0.49	0.50	0.45	0.42	0.43	0.35
Psychosocial	0.71	0.66	0.56	0.59	0.73	0.68	0.60	0.65
Emotional	0.62	0.54	0.52	0.49	0.60	0.56	0.52	0.49
Social	0.56	0.54	0.39	0.48	0.63	0.58	0.49	0.59
School	0.55	0.50	0.43	0.46	0.65	0.66	0.61	0.61

Pearson's correlations, effect sizes are designated as small (0.10), moderate (0.30), and large (0.50)

* $P < 0.01$ for all figures

Physical physical functioning, *psychosocial* psychosocial health summary score, *emotional* emotional functioning, *social* social functioning, *school* school functioning

Table 3 Results of confirmatory factor analysis for the 3-factor model

Model	χ^2	df	χ^2/df	RMSEA	CFI	GFI	AGFI
Child self-report	575.8*	132	4.36	0.072	0.88	0.90	0.87
Parent proxy report	1,148.4*	132	8.12	0.102	0.88	0.83	0.78

* $P < 0.001$

1). Regarding parent proxy reports, three items were split into a factor other than the a priori scale; Items 5 and 6 ("I have trouble finishing things" and "I have trouble starting things") were loaded on cognitive fatigue (Factor 1); and Item 9 ("I feel tired when I wake up in the morning") was loaded on general fatigue (Factor 2).

Agreement

There was no significant difference between the scores of male children and those of female children. Figure 1 shows how the age of the child affected directional differences between child self-report scores and parent proxy report scores on the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale. Children who were 10 years old demonstrated the smallest biases for total scores and all subscales. For most child age groups, parents overestimated their children's fatigue. The mean of the directional differences was significant across age groups.

Table 5 shows the agreement level by child health status. The ICCs of the chronic condition sample were slightly higher than those of the healthy sample; however, the mean differences of the means of the absolute difference and directional difference did not differ significantly between the healthy sample and chronic condition sample.

To identify subgroups by the children's HRQOL levels, the scores of 650 child self-reports for all subscales of the PedsQL™ Multidimensional Fatigue Scale and the Peds-QL™ Generic Core Scales were entered into the cluster analysis. A three-cluster solution was obtained, where one subgroup ($n = 317$, 48.8%) included those with high scores on all subscales (good HRQOL group); a second subgroup ($n = 229$, 35.2%) included samples with intermediate level scores on all subscales (intermediate HRQOL group); and a third subgroup ($n = 104$, 16.0%) had low scores on all subscales (poor HRQOL group). Significant differences were seen in the average age of children found in the three subgroups: children in the intermediate HRQOL group were younger than children in the good HRQOL group (Tukey post hoc test, $P = 0.02$). The frequency among the subgroups of the number of children who had a chronic condition was significantly different (chi-square test, $P = 0.04$). Table 6 shows the means of the scores of the PedsQL™ Generic Core Scales by the subgroups identified by cluster analysis. Table 7 shows the means, standard deviations, correlation r and ICC, absolute difference, and directional difference (standardized difference d) of the child self-reported and parent proxy-reported total scores on the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale. The total scores of child self-reports and parent proxy reports and all the subscale scores of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale were significantly different among subgroups. The good HRQOL group had the highest total scores, subscale scores, and correlation r's and ICCs, and the poor HRQOL group had the lowest. The absolute and directional differences among the three subgroups were significantly different, with the largest differences seen in the poor HRQOL group, and the smallest differences, in the good

Table 4 Factor loadings for the Japanese version of PedsQL™ Multidimensional Fatigue Scale

Scale/item	Factor 1		Factor 2		Factor 3	
General fatigue						
1. I feel tired	.230	.196	**.549**	**.682**	.437	.338
2. I feel physically weak (not strong)	.313	.320	**.497**	**.634**	.148	.265
3. I feel too tired to do things that I like to do	.177	.215	**.717**	**.732**	.140	.314
4. I feel too tired to spend time with my friends	.074	.283	**.706**	**.642**	.136	.266
5. I have trouble finishing things	**.594**	**.553**	.490	.484	−.066	.213
6. I have trouble starting things	.465	**.540**	**.478**	.495	−.045	.235
Sleep/rest fatigue						
7. I sleep a lot	.066	.119	.030	.405	**.286**	**.514**
8. It is hard for me to sleep through the night	.109	.223	**.292**	.219	.263	**.337**
9. I feel tired when I wake up in the morning	.211	.172	**.497**	**.554**	.435	.470
10. I rest a lot	.370	.301	.280	.464	**.372**	**.584**
11. I take a lot of naps	.111	.169	**.307**	.207	.097	**.570**
12. I spend a lot of time in bed	.242	.260	.255	.240	**.398**	**.630**
Cognitive fatigue						
13. It is hard for me to keep my attention on things	**.541**	**.649**	.295	.317	.085	.192
14. It is hard to for me to remember what people tell me	**.622**	**.837**	.259	.233	.181	.141
15. It is hard for me to remember what I just heard	**.549**	**.775**	.123	.167	.255	.261
16. It is hard for me to think quickly	**.673**	**.752**	.129	.268	.159	.170
17. I have trouble remembering what I was just thinking	**.540**	**.773**	.119	.148	.294	.292
18. I have trouble remembering more than one thing at a time	**.588**	**.815**	.086	.135	.303	.176

Total variance explained was 39.6% for child self-report and 58.1% for parent proxy report
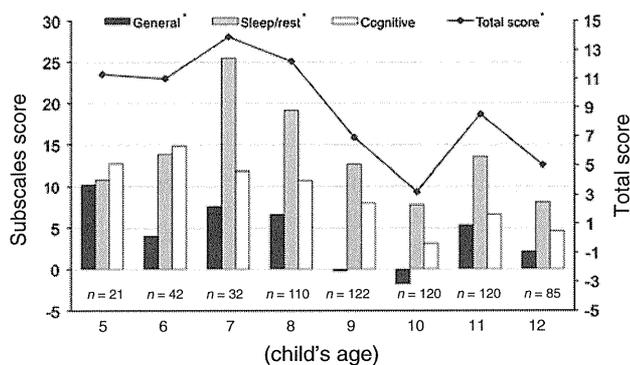
Highest factor loadings for each item are shown in bold

* Statistically significant across age groups, ANOVA ($p < 0.05$).

Fig. 1 Directional differences between child self-reports and parent proxy reports of scores on the PedsQL™ Multidimensional Fatigue Scale, by age of child. *Statistically significant across age groups, ANOVA ($P < 0.05$)

HRQOL group. Similarly, accounting for the direction, the good HRQOL group showed the smallest bias, and parents of this subgroup gave slightly lower ratings on the Peds-QL™ Multidimensional Fatigue Scale than the children did, which means that parents estimated their children's fatigue worse than the children themselves. In contrast, the poor HRQOL group showed the largest bias, and the

ratings of parents in that subgroup were higher than those of their children, by approximately 1 point of the Peds-QL™ Multidimensional Fatigue Scale (where 1 point scores 25). The parents of children in the poor HRQOL group estimated their children's fatigue as less severe when compared to the severity of fatigue reported by their children.

Discussion

Reliability and validity

The results of this study supported the reliability and validity of the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale with specified report formats for toddlers (2–4 years old), young children (5–7 years old), and older children (8–12 years old). The lack of missing responses for items on the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale indicated the simplicity of each item on the scale, and children of all ages and their parents were willing and able to provide good quality data regarding the child's fatigue.

The internal consistency reliability of the total sample for both child self-reported and parent proxy-reported

Table 5 Agreement between child self-reports and parent proxy reports by children's health status

	n	PedsQL™ Fatigue Score		Difference t score[b]	Correlation r[c]	ICC[d]	Absolute child–parent difference[e] Mean (SD)	Directional difference[f] (systematic bias) Mean (SD)	d[g]
		Child	Parent						
Healthy sample									
PedsQL™ fatigue	530	77.6[a]	85.6[a]	−9.5**	0.17	0.29	16.1 (13.3)	8.0 (19.3)	0.41
General fatigue	525	81.7[a]	84.9[a]	−3.1**	0.20	0.32	16.0 (15.0)	3.0 (21.7)	0.14
Sleep/rest fatigue	526	72.8[a]	86.2[a]	−14.1**	0.19	0.31	19.9 (15.9)	13.4 (21.7)	0.62
Cognitive fatigue	526	78.5	85.8[a]	−7.3**	0.12	0.20	19.1 (17.0)	7.7 (24.4)	0.32
Chronic condition sample									
PedsQL™ fatigue	102	72.3[a]	80.2[a]	−3.6**	0.37	0.53	14.6 (13.3)	7.2 (18.4)	0.39
General fatigue	101	74.9[a]	79.4[a]	−1.2	0.24	0.38	18.2 (16.3)	3.0 (24.3)	0.12
Sleep/rest fatigue	101	67.6[a]	79.9[a]	−5.3**	0.32	0.49	18.6 (16.0)	11.5 (21.8)	0.53
Cognitive fatigue	101	74.5	81.3[a]	−3.1**	0.37	0.53	15.9 (14.9)	7.1 (20.7)	0.34

[a] Statistically significant between healthy sample and chronic condition sample, independent student t test at ($P < 0.05$)

[b] Paired student t test between child and parent scores, statistically significant difference between child and parent mean scores (** $P < 0.01$)

[c] Pearson's correlation

[d] Intraclass correlation coefficient

[e] Absolute difference between child and parent scores (indicator of agreement)

[f] Difference between child and parent scores (indicator of bias)

[g] Standardized difference d = mean direction of difference/standard deviation of direction of difference ($d = 0.2$ small, 0.5 medium, and 0.8 large bias)

Table 6 The characteristics of subgroups identified by the cluster analysis

PedsQL™ Generic Core	n	Score [mean(SD)]		Chronic condition[a]
		Child[b]	Parent[c]	Chronic/healthy (%)
All samples	650	80.3 (13.0)	87.5 (12.0)	102/530 (19.2)
Physical health summary score	650	86.1 (13.7)	88.7 (19.1)	
Psychosocial health summary score	650	78.4 (14.3)	87.2 (11.7)	
Good HRQOL group	317	89.9 (5.5)	89.4 (10.9)	41/270 (15.2)
Physical health summary score	317	93.8 (6.5)	89.8 (18.9)	
Psychosocial health summary score	317	88.7 (6.6)	89.3 (10.2)	
Intermediate HRQOL group	229	76.2 (7.2)	87.5 (12.7)	37/183 (20.2)
Physical health summary score	229	83.3 (12.9)	88.9 (19.3)	
Psychosocial health summary score	229	73.9 (8.4)	87.0 (12.5)	
Poor HRQOL group	104	59.7 (10.1)	82.0 (12.1)	24/77 (29.9)
Physical health summary score	104	68.8 (13.3)	84.7 (19.1)	
Psychosocial health summary score	104	56.7 (12.2)	81.1 (12.4)	

[a] Statistically significant across 3 subgroups, chi-square test ($P = 0.04$)

[b] Statistically significant across 3 subgroups, ANOVA ($P < 0.01$)

[c] Statistically significant across 3 subgroups for total score and psychosocial health summary score, ANOVA ($P < 0.01$)

scores achieved the recommended minimal Cronbach's alpha coefficient standard (0.70) for total score and for all the subscales except the self-reported score of the sleep/rest fatigue subscale, where the alpha coefficient was 0.62 and therefore fell short of the standard. It should be noted that

in previous studies of the PedsQL™ Multidimensional Fatigue Scale, the sleep/rest fatigue subscale exhibited the lowest alpha value of the three subscales [43, 44]. Furthermore, the participants in this study were younger than those previously studied, and the low alpha value may be

Table 7 Agreement between child self-reports and parent proxy reports by subgroups based on cluster analysis

	n	PedsQL™		Difference	Child–parent correlation		Absolute difference[d]	Directional difference[e] (systematic bias)	
		Fatigue score		t score[a]	r[b]	ICC[c]	Mean (SD)[g]	Mean (SD)[g]	d[f]
		Child	Parent						
All samples	650	76.7	84.7	−10.4**	0.22	0.32	15.9 (13.3)	7.9 (19.1)	0.41
General fatigue	630	80.6	83.6	−3.4**	0.22	0.36	16.4 (15.2)	3.0 (22.2)	0.14
Sleep/rest fatigue	631	71.8	84.9	−15.2**	0.23	0.36	19.7 (15.9)	13.1 (21.7)	0.60
Cognitive fatigue	631	77.9	85.6	−8.1**	0.16	0.28	18.6 (16.7)	7.6 (23.8)	0.31
Good HRQOL group	317	89.3	86.7	3.4**	0.18	0.25	10.4 (9.4)	−2.7 (13.7)	−0.20
General fatigue	310	93.1	85.8	7.8**	0.12	0.18	12.6 (12.6)	−7.2 (16.3)	−0.44
Sleep/rest fatigue	311	83.6	87.0	−3.4**	0.16	0.27	13.7 (11.5)	3.4 (17.6)	0.19
Cognitive fatigue	311	91.5	87.4	4.3**	0.18	0.27	11.9 (12.4)	−4.1 (16.7)	−0.25
Intermediate HRQOL group	229	71.4	84.7	−12.7**	0.07	0.11	17.4 (11.1)	13.4 (15.7)	0.85
General fatigue	219	76.5	83.4	−5.3**	0.12	0.22	16.0 (12.5)	6.9 (19.1)	0.36
Sleep/rest fatigue	219	65.7	85.4	−14.8**	0.15	0.26	23.0 (15.6)	19.7 (19.6)	1.01
Cognitive fatigue	219	71.6	85.2	−9.2**	0.06	0.10	21.4 (14.5)	13.6 (22.0)	0.62
Poor HRQOL group	104	49.8	78.2	−15.7**	0.01	0.01	29.5 (17.0)	28.7 (18.3)	1.57
General fatigue	100	50.7	76.7	−10.8**	0.09	0.17	29.2 (20.1)	26.0 (24.2)	1.07
Sleep/rest fatigue	100	48.4	77.4	−12.7**	0.05	0.09	31.1 (19.9)	29.0 (22.9)	1.27
Cognitive fatigue	100	49.4	80.5	−12.7**	0.01	0.02	33.4 (21.1)	31.0 (24.5)	1.27

[a] Paired student t test between child and parent scores, statistically significant difference between child and parent mean scores (** $P < 0.01$)

[b] Pearson's correlation

[c] Intraclass correlation coefficient

[d] Absolute difference between child and parent scores (indicator of agreement)

[e] Difference between child and parent scores (indicator of bias)

[f] Standardized difference d = mean direction of difference/standard deviation of direction of difference (d = 0.2 small, 0.5 medium, and 0.8 large bias)

[g] Statistically significant difference across 3 subgroups for total score and all subscales, poor > intermediate > good based on ANOVA with Tukey's post hoc test

related to the child's age. Similarly, lower alphas for the formats of the self-reports and proxy reports for younger children may be influenced by the smaller sample size of younger children [45] as well as the child's age.

The Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale generally performed as hypothesized when the known-group method was used to compare fatigue in healthy children with that in children with chronic conditions. As a group, the children with chronic conditions manifested significantly more fatigue than the healthy children. The Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale demonstrated good construct validity and usefulness in a school population. It was also sensitive enough to detect differences in children even if the children's health levels are good enough to attend school or preschool. At the more detailed level, the cognitive fatigue subscale demonstrated the smallest effect sizes of the three fatigue subscales for both child and parent reports, which corresponded with the results of previous studies that used the PedsQL™

Multidimensional Fatigue Scale in cases involving rheumatology, type 1 diabetes, fibromyalgia, and bowel disease [7, 26, 43, 46]. However, in cases involving brain tumors, the cognitive fatigue effect sizes for both child self-reports and parent proxy reports were larger than the effect sizes for sleep/rest fatigue [47], and the effect sizes were larger than those in this study or in other previous studies. Perhaps cognitive fatigue is not easily influenced by disease, but brain tumors do influence children's cognitive fatigue.

The intercorrelations between the Japanese-language version of the PedsQL™ Multidimensional Fatigue Scale and the Japanese-language version of the PedsQL™ Generic Core Scales were consistent with the concept of disease-specific symptoms as causal indicators of HRQOL [31], and this result emphasizes the importance of recognizing the effect of fatigue on children's HRQOL.

The results of the factor structure analyses were generally consistent with the a priori hypothesized factor structure of the original PedsQL™ Multidimensional Fatigue Scale. First, we conducted CFA to examine the goodness of fit of

the original a priori factor structure, and the results indicated some exceptions. Then, EFA was conducted to examine the initial construct validity of the PedsQL^{TM} Multidimensional Fatigue Scale for children aged 2–12 years in Japan. The result of EFA confirmed the exceptions with regard to four items for child self-report and three items for parent proxy report, respectively; otherwise, the factors that emerged were consistent with the a priori hypothesized factor structure. Regarding the child self-report, Item 8 ("It is hard for me to sleep through the night") and Item 9 ("I feel tired when I wake up in the morning") of sleep/rest fatigue were loaded on general fatigue. The same results were reported with a university student population [44]. Previous surveys of preschool children and schoolchildren reported that approximately 50% of preschool children and 80% of schoolchildren answered that sleepiness was a symptom of fatigue in Japan [48, 49]; therefore, it is possible that children of these ages think of sleepiness as general fatigue. Furthermore, Item 5 ("I have trouble finishing things") and Item 6 ("I have trouble starting things") were loaded on cognitive fatigue. Perhaps doing things is more related to cognitive fatigue than general fatigue in Japan.

Agreement

The findings of this study demonstrated poor to fair correlations between reports by children and their parents. The results supported our hypothesis, and they were similar to those reported by Varni et al. [26, 50] in previous studies that focused on children with type 1 diabetes and their parents as well as obese children and their parents.

We examined agreement across age groups, and the results showed that the older the child, the smaller the bias. Cremeens et al. [28] reported that age affects the correlation between child and parent ratings, with a higher agreement for older children with respect to psychosocial aspects of health compared to a higher agreement for younger children concerning physical health. Our study supported this finding and added further implications for the child–parent agreement by examining the bias between child and parent ratings.

Differences in the mean of absolute difference and directional difference between the healthy sample and chronic condition sample were not significant. This finding indicates the possibility that the child's health status does not affect the child–parent agreement level. However, Yeh et al. [51], who investigated the agreement between children with cancer and their parents, reported greater agreement of ICC for off-treatment children than for on-treatment children and they stated that the severity of cancer was a significant predictor of the level of disagreement. On the other hand, the majority of the chronic

condition children in this study had asthma or atopic dermatitis that were adequately controlled by regular hospital visits and/or medication, and these children were not expected to manifest severe fatigue symptom such as those presented by children with cancer, rheumatology, heart disease, or other severe chronic illnesses. It is possible that the difference in illness severity among the participants caused these contrary findings (children with cancer versus children with a chronic condition that can be treated at home). Further investigation with participants varying in severity of illnesses will be of significance in this regard. However, the Japanese-language version of PedsQL^{TM} Multidimensional Fatigue Scale showed significant differences between the scores of healthy children and those with a chronic condition. These results supported that the Japanese-language version of the PedsQL^{TM} Multidimensional Fatigue Scale is adequately sensitive to small differences in child's fatigue.

Accounting for agreement between children and parents by the subgroups that were identified by cluster analysis based on the children's self-reported scores (in other words, the children's perceived HRQOL), the number of children who had chronic conditions was significantly different among the three identified subgroups, which supports the reliability of child self-reports. Integrating the results of the indicators we used in this study (r, ICC, absolute difference, directional difference) indicated that a maximum level of agreement was seen in the good HRQOL group, and a minimum level was seen in the poor HRQOL group. The parents estimated their child's fatigue as less severe when compared to the severity reported by their children, when the child's HRQOL was worse. On the other hand, the parents of children with high HRQOL rated them as having more fatigue. The average absolute difference between the child and parent scores in the poor HRQOL group was approximately 1 point. This suggests that in cases of high and low HRQOL, parents sometimes provide different information on fatigue than their children do themselves. Taken together, the findings suggest that the information on fatigue provided by the parent proxy report is not equivalent to that of the child self-report. However, this should not be interpreted as evidence of the inaccuracy of parent proxy-reported fatigue because child self-reports and parent proxy reports demonstrated acceptable reliability in this study. Thus, it will be helpful to understand the existence of differences and to identify the patterns of differences on the basis of the child's health status, child's age, parent's health status, and the child/parent relationship. This study showed that when a child is aged 5–12 years and is healthy enough to attend school, agreement between child and parent reports is high for children perceived to be in the good HRQOL group and low for those in the poor HRQOL group. Further, parents perceived

their children's fatigue as less severe when compared to the severity reported by their children, when the children had poor HRQOL scores. Our findings suggest that the child's age and HRQOL level could affect child–parent disagreement and that the child's health status would not affect disagreement as much as the age and HRQOL level.

The present study has several potential limitations. First, we did not examine the validity and agreement of the format for children aged 13–18 years. The PedsQL™ original version is formatted for children aged 13–18 years; therefore, validating the format for children aged 13–18 years will increase the value and usefulness of the Japanese version of the PedsQL™ Multidimensional Fatigue Scale. Based on the foregoing, additional documentation in this age range is needed, and we will address this issue in an ongoing study of fatigue of in adolescents. Second, we compared scores between healthy children and chronic condition children. However, these two groups were determined using parent reports of the presence of a chronic health condition that needed to be controlled by regular medication and/or visiting an outpatient clinic. In addition, the influence of illness severity was not considered, which could limit the generalizability of the findings. Illness severity may influence the HRQOL rating provided by children and parents and it could possibly explain the differences between the scores of children with chronic condition who belonged to the good HRQOL group or poor HRQOL group.

References

1. Langgraf, J. M., & Abetz, L. N. (1997). Functional status and well-being of children representing three cultural groups: Initial self-reports using the CHQ-CF87. *Psychology & Health, 12,* 839–865.
2. Feeny, D., Furlong, W., & Barr, R. D. (1998). Multiattribute approach to the assessment of health-related quality of life: Health Utilities Index. *Medical and Pediatric Oncology, 1*(Supplement), 54–59.
3. Vogels, T., Verrips, G. H., Verloove-Vanhorick, S. P., Fekkes, M., Kamphuis, R. P., Koopman, H. M., et al. (1998). Measuring health-related quality of life in children: The development of the TACQOL parent form. *Quality of Life Research, 7,* 457–465.
4. Ravens-Sieberer, U., & Bullinger, M. (1998). Assessing health-related quality of life in chronically ill children with the German KINDL: First psychometric and content analytic results. *Quality of Life Research, 7,* 399–407.

5. Varni, J. W., Seid, M., & Rode, C. A. (1999). The PedsQL™: Measurement model for the pediatric quality of life inventory. *Medical Care, 37,* 126–139.
6. Varni, J. W., Seid, M., & Kurtin, P. S. (2001). PedsQL™ 4.0: Reliability and validity of the Pediatric Quality of Life Inventory™ version 4.0 generic core scales in healthy and patient populations. *Medical Care, 39,* 800–812.
7. Varni, J. W., Burwinkle, T. M., & Szer, I. S. (2004). The PedsQL™ Multidimensional Fatigue Scale in pediatric rheumatology: Reliability and validity. *The Journal of Rheumatology, 31,* 2494–2500.
8. Varni, J. W., Burwinkle, T. M., Katz, E. R., Meeske, K., & Dickinson, P. (2002). The PedsQL™ in pediatric cancer: Reliability and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. *Cancer, 94,* 2090–2106.
9. Varni, J. W., Burwinkle, T. M., Jacobs, J. R., Gottschalk, M., Kaufman, F., & Jones, K. L. (2003). The PedsQL™ in type 1 and type 2 diabetes: Reliability and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales and Type 1 Diabetes Module. *Diabetes Care, 26,* 631–637.
10. Varni, J. W., Burwinkle, T. M., & Seid, M. (2006). The PedsQL™ 4.0 as a school population health measure: Feasibility, reliability, and validity. *Quality of Life Research, 15,* 203–215.
11. Seid, M., Varni, J. W., Gidwani, P., Gelhard, L. R., & Slymen, D. J. (2010). Problem-solving skills training for vulnerable families of children with persistent asthma: Report of a randomized trial on health-related quality of life outcomes. *Journal of Pediatric Psychology.* http://jpepsy.oxfordjournals.org/cgi/reprint/jsp133v1?maxtoshow=&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&resourcetype=HWCIT. Accessed 10 May 2010.
12. Clarke, S. A., Skinner, R., Guest, J., Darbyshire, P., Cooper, J., Shah, F., et al. (2010). Health-related quality of life and financial impact of caring for a child with Thalassaemia Major in the UK. *Child: Care, Health and Development, 36,* 118–122.
13. Tahirović, E., Begić, H., Nurkić, M., Tahirović, H., & Varni, J. W. (2010). Does the severity of congenital heart defects affect disease-specific health-related quality of life in children in Bosnia and Herzegovina? *European Journal of Pediatrics, 169,* 349–353.
14. Varni, J. W., Limbers, C. A., Newman, D. A., & Seid, M. (2008). Longitudinal factorial invariance of the PedsQL™ 4.0 Generic Core Scales child self-report version: One year prospective evidence from the California State Children's Health Insurance Program (SCHIP). *Quality of Life Research, 17,* 1153–1162.
15. Eiser, C., Vance, Y. H., Horne, B., Glaser, A., & Galvin, H. (2003). The value of the PedsQL™ in assessing quality of life in survivors of childhood cancer. *Child: Care, Health and Development, 29,* 95–102.
16. Kobayashi, K., & Kamibeppu, K. (2010). Measuring quality of life in Japanese children: Development of the Japanese version of PedsQL™. *Pediatrics International, 52,* 80–88.
17. Swain, M. G. (2000). Fatigue in chronic disease. *Clinical Science, 99,* 1–8.
18. Bakker, R. J., van de Putte, E. M., Kuis, W., & Sinnema, G. (2009). Risk factors for persistent fatigue with significant school absence in children and adolescents. *Pediatrics, 124,* 89–95.
19. Sankey, A., Hill, C. M., Brown, J., Quinn, L., & Fletcher, A. (2006). A follow-up study of chronic fatigue syndrome in children and adolescents: Symptom persistence and school absenteeism. *Clinical Child Psychology and Psychiatry, 11,* 126–138.
20. Saito, K. (2000). Fatigue and stress in children. *The Journal of Child Health, 59,* 131–138. (in Japanese).
21. Gibson, F., Mulhall, A. B., Richardson, A., Edwards, J. L., Ream, E., & Sepion, B. J. (2005). A phenomenologic study of fatigue in adolescents receiving treatment for cancer. *Oncology Nursing Forum, 32,* 651–660.

22. Edwards, J. L., Gibson, F., Richardson, A., Sepion, B., & Ream, E. (2003). Fatigue in adolescents with and following a cancer diagnosis: Developing an evidence base for practice. *European Journal of Cancer, 39*, 2671–2680.

23. Kobayashi, H., Demura, S., Goshi, F., Sato, S., & Noda, M. (2000). Construction of a subjective fatigue scale for adolescent students. *Japanese Journal of Public Health, 47*, 638–646. (in Japanese).

24. Mitsuoka, S., Horii, S., Ohmura, N., Kasagara, M., Suzuki, M., & Oyama, M. (2003). The relationships between child fatigue and symptoms and children's lifestyles using a Child Fatigue Symptoms Scale. *The Journal of Child Health, 62*, 81–87.

25. Maehashi, A., & Ogata, M. (1993). Constructing a questionnaire to clarify the subjective symptoms of fatigue for elementary schoolchildren. Part 1: An assessment of the questions. *Kawasaki Medical Welfare Journal, 3*, 75–86. (in Japanese).

26. Varni, J. W., Limbers, C. A., Bryant, W. P., & Wilson, D. P. (2008). The PedsQL™ Multidimensional Fatigue Scale in type 1 diabetes: Feasibility, reliability, and validity. *Pediatric Diabetes, 10*, 321–328.

27. Drotar, D. (Ed.). (1998). *Measuring health-related quality of life in children and adolescents: Implications for research and practice*. Hillsdale, NJ: Lawrence Erlbaum Associates.

28. Cremeens, J., Eiser, C., & Blades, M. (2006). Factors influencing agreement between child self-report and parent proxy-reports on the Pediatric Quality of Life Inventory™ 4.0 (PedsQL™) generic core scales. *Health and Quality of Life Outcomes, 4*, 58–65.

29. Sneeuw, K. C. A., Aaronson, N. K., Sprangers, A. G., Detmar, S. B., Wever, L. D. V., & Schornagel, J. H. (1998). Comparison of patient and proxy EORTC QLQ-C30 ratings in assessing the quality of life of cancer patients. *Journal of Clinical Epidemiology, 51*, 617–631.

30. Williams, L. S., Bakas, T., Brizendine, E., Plue, L., Tu, W., Hendrie, H., et al. (2006). How valid are family proxy assessment of stroke patients' health-related quality of life? *Stroke, 37*, 2081–2085.

31. Fayers, P. M., & Machin, D. (2000). *Quality of life: Assessment, analysis, and interpretation*. New York, NY: Wiley.

32. World Health Organization. (1948). *Constitution of the World Health Organization basic documents*. Geneva: World Health Organization.

33. McHorney, C. A., Ware, J. E., Jr., Lu, J. F., & Sherbourne, C. D. (1994). The MOS 36-item Short-form Health Survey (SF-36): III. Tests of data quality, scaling assumptions, and reliability across diverse patient groups. *Medical Care, 32*, 40–66.

34. Cronbach, L. J. (1951). Coefficient alpha and the internal structure of tests. *Psychometrika, 16*, 297–334.

35. McHorney, C. A., Ware, J. E., & Raczek, A. E. (1993). The MOS 36-item short-form health survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. *Medical Care, 31*, 247–263.

36. Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum Associates.

37. Pedhazur, E. J., & Schemelkin, L. P. (1991). *Measurement, design, and analysis: An integrated approach*. Hillsdale, NJ: Lawrence Erlbaum Associates.

38. Browne, M. W., & Cudeck, R. (1993). Alternative ways of assessing model fit. In K. Bollen & J. S. Long (Eds.), *Testing structural equation models* (pp. 147–162). Newbury Park, CA: Sage Publications.

39. Bentler, P. M. (1995). Comparative fit indexed in structural models. *Psychological Bulletin, 107*, 238–246.

40. Bentler, P. M., & Bonett, D. G. (1980). Significance tests and goodness of fit in the analysis of covariance structures. *Psychological Bulletin, 88*, 588–606.

41. Sneeuw, K. C. A., Aaronson, N. K., Osoba, D., Muller, M., Hsu, M., Yung, W. K. A., et al. (1997). The use of significant others as proxy rates of the quality of life of patients with brain cancer. *Medical Care, 35*, 490–506.

42. Nunnally, J. C., & Bernstein, I. H. (1994). *Psychometric theory* (3rd ed.). New York, NY: McGraw-Hill.

43. Varni, J. W., Burwinkle, T. M., Limbers, C. A., & Szer, I. S. (2007). The PedsQL™ as a patient-reported outcome in children and adolescents with fibromyalgia: An analysis of OMERACT domains. *Health and Quality of Life Outcomes, 5*, 9–20.

44. Varni, J. W., & Limbers, C. A. (2008). The PedsQL™ Multidimensional Fatigue Scale in young adults: Feasibility, reliability and validity in a university student population. *Quality of Life Research, 17*, 105–114.

45. DeVellis, R. E. (2003). *Scale development: Theory and applications* (2nd ed.). Thousand Oaks, CA: Sage Publications.

46. Marcus, S. B., Strople, J. A., Neighbors, K., Weissberg-Benchell, J., Nelson, S. P., Limbers, C., et al. (2009). Fatigue and health-related quality of life in pediatric inflammatory bowel disease. *Clinical Gastroenterology and Hepatology, 7*, 554–561.

47. Palmer, S. N., Meeske, K. A., Katz, E. R., Burwinkle, T. M., & Varni, J. W. (2007). The PedsQL™ Brain Tumor Module: Initial reliability and validity. *Pediatric Blood & Cancer, 49*, 287–293.

48. Shibaki, M., Taniyama, N., Fujii, A., Nasaki, M., & Sasajima, Y. (2010). A study on symptoms of fatigue in preschool children (first report)—the relationship between fundamental lifestyle and eating habits. *Journal of Hokkaido University of Education, 60*, 83–96. (in Japanese).

49. Nakamura, K., Ueya, K., Miyamaru, G., Maebashi, A., Kawazoe, K., Yamada, N., et al. (1997). A study on lifestyle, physical activities, and subjective symptoms in schoolchildren. *Proceedings of the Congress of the Japanese society of Physical Education, 48*, 391. (in Japanese).

50. Varni, J. W., Limbers, C. A., Bryant, W. P., & Wilson, D. P. (2010). The PedsQL™ Multidimensional Fatigue Scale in pediatric obesity: Feasibility, reliability and validity. *International Journal of Pediatric Obesity, 5*, 34–42.

51. Yeh, C., Chang, C., & Chang, P. (2005). Evaluating quality of life in children with cancer using children's self-reports and parent-proxy reports. *Nursing Research, 54*, 354–362.
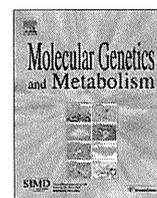
Citrin deficiency and current treatment concepts

Takeyori Saheki [a,*], Kanako Inoue [a], Anmi Tushima [a], Kozo Mutoh [b], Keiko Kobayashi [c]

[a] Institute for Health Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan
[b] Department of Pediatrics, Shimada Municipal Hospital, Shimada 427-8502, Japan
[c] Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8544, Japan

ABSTRACT

In this paper, we describe the historical aspects of citrin and citrin deficiency, characteristic food preference and food aversion of citrin-deficient subjects, and carbohydrate toxicity in relation to ureogenesis and issues of the conventional treatment procedures for hyperammonemia in citrin deficiency, leading to current treatment concepts for citrin deficiency. We also emphasize the importance of a citrin deficiency mouse model in elucidating the pathophysiology and developing novel therapeutics based on the pathophysiology, such as sodium pyruvate.

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Historical aspect citrin and citrin deficiency

Human citrin deficiency is a newly-established disease entity [1,2] that encompasses both adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestasis (NICCD), and results from mutations in the SLC25A13 gene that encodes citrin [3]. It was first described in Japan and East Asia, but is now a panethnic disease [4]. Citrin and the closely-related protein aralar (encoded by SLC25A12) [5] are isoforms of the mitochondrial aspartate (Asp)–glutamate (Glu) carrier (AGC) in the inner mitochondrial membrane and are responsible for the exchange of matrix Asp for cytosolic Glu and a H^+ ion, which is an electrogenic process [6]. Citrin is predominantly found in liver, kidney, heart and small intestine, while aralar is found in brain, skeletal muscle, kidney and heart [3,7,8]: citrin can be thought of as the liver-type, while aralar as the brain- and muscle-type, AGC. The function of the AGC is to participate in gluconeogenesis from lactate and transporting cytosolic NADH-reducing equivalents into mitochondria as part of the malate Asp shuttle, in addition to providing Asp from mitochondria to the cytosol for the synthesis of proteins, nucleotides and urea (Fig. 1).

Citrullinemia is caused by a deficiency of the urea cycle enzyme, argininosuccinate synthetase (ASS), which catalyzes the ligation of citrulline (Cit) and Asp to form argininosuccinate at the expense of ATP utilization. Saheki et al. [9] classified citrullinemia into classical or type one citrullinemia (CTLN1) caused by mutations in the ASS gene [10,11] and CTLN2 caused by mutations in SLC25A13 [3]. CTLN2 is characterized by a liver-specific decrease in ASS protein [12] without any detectable abnormalities in the ASS gene or hepatic ASS mRNA levels [13,14]. The hepatic loss of ASS protein in CTLN2 patients is secondary to citrin deficiency [15], although its cause still remains to be clarified. Patients with CTLN2 suffer from recurring neuropsychiatric symptoms associated with hyperammonemia, including disorientation, delirium, seizures, and coma that can lead to death from brain edema [1,2,12].

Identification of mutations in SLC25A13 of CTLN2 patients led to the discovery that patients with a type of neonatal hepatitis were also caused by the same mutations [16–18]. Since the neonatal symptoms were markedly different from those of adult CTLN2 patients, we named the neonatal presentation NICCD (neonatal intrahepatic cholestasis caused by citrin deficiency) [1,19]. Patients with NICCD show multiple metabolic abnormalities, including aminoacidemias (Cit, threonine, methionine, tyrosine, and arginine) accompanied by an increased threonine/serine ratio, galactosemia, hypoproteinemia, cholestasis, and fatty liver [2]. Human citrin deficiency therefore results in NICCD during the first few months of life, with symptoms usually self-resolving by the first year in most cases [20]. Following an apparently healthy period that can last from one to several decades, some patients with human citrin deficiency go on to develop severe CTLN2 [1,17,21].

* Corresponding author. Address: Institute for Health Sciences, Tokushima Bunri University, 180 Yamashiro-cho, Tokushima 770-8514, Japan. Fax: +81 88 622 2503.
E-mail address: takesah@tokushima.bunri-u.ac.jp (T. Saheki).