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Neonatal intrahepatic cholestasis caused by citrin deficiency: Clinical and laboratory investigation of 13 subjects in mainland of China☆

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ABSTRACT

Background: Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) is a novel inborn error of metabolism due to dysfunction of citrin protein, and much more information about this new disease is still needed for its clinical management.
Aims: To investigate in detail the clinical and laboratory features of NICCD.
Patients: 13 NICCD subjects in mainland of China diagnosed in our department since 2006.
Methods: The anthropometric parameters of the patients at birth were compared with controls, representative biochemical changes and metabolome findings were investigated cross-sectionally, and mutations in the causative gene *SLC25A13* were analyzed by protocols established previously.
Results: The patients showed reduced birth weight, length and ponderal index. Main clinical manifestations consisted of jaundice, hepato/hepatosplenomegaly and steatohepatosis on ultrasonography. Biochemical analysis revealed intrahepatic cholestasis, delayed switch of AFP to albumin, and elevated triglyceride, total cholesterol and LDL-cholesterol together with reduced HDL-cholesterol. Metabolome findings included co-existence of markers for galactosemia and tyrosinemia in urine, and elevated Cit, Met, Thr, Tyr, Lys, Arg and Orn in blood. Mutations of 851-854del, IVS6+5G>A, 1638-1660dup, A541D, IVS16ins3kb, R319X and G333D were detected in the gene *SLC25A13*.
Conclusions: The diagnosis of NICCD cannot be established based just on the numerous but non-specific clinical manifestations and biochemical changes. The relatively specific metabolome features provide valuable tools for its screening and diagnosis, while *SLC25A13* mutation analysis should be taken as one of the reliable tools for the definitive diagnosis. The body proportionality at birth, steatohepatosis on ultrasonography, delayed switch of AFP to albumin, dyslipidemia pattern, urinary metabolome features and the novel mutation G333D expanded the clinical spectrum of NICCD.

1. Introduction

Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD, OMIM 605814) is a novel entity of inherited metabolic disease due to dysfunction of citrin, a liver-type aspartate/glutamate carrier protein located within mitochondrial inner membrane [1,2]. The causative gene *SLC25A13* locating on chromosome 7q21.3 was identified by means of homozygosity mapping and positional cloning by Kobayashi's group in 1999 [3]. NICCD was first described in Japanese nearly at the same time in the year 2001 [4–6]. Since then more and more NICCD patients were diagnosed,

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however, most of them are Japanese [7–9] although some cases in such East Asians as Chinese, Vietnamese and Korean have also been reported in recent years [8–17]. Furthermore, NICCD is now recognized as a novel disease with the feature of world-wide distribution, since some Caucasian, Ashkenazi Jewish, Pakistani, and Israeli patients in USA, UK, Netherland, Czech Republic and Israel, although still quite rare in number, have also been identified by mutation analysis in our group [8,9,18–20]. In 2006, we reported a NICCD patient in mainland of China by international collaboration [11], and from then on, 13 such cases from 12 families have been definitely diagnosed by analysis of the gene *SLC25A13*. More recently, our selective screening investigation of inborn errors of metabolism revealed a second highest positive rate of NICCD just behind methylmalonic aciduria in high-risk Chinese population [21]. However, it should be recognized that much more clinical and laboratory information about NICCD still need further accumulation and exploration at current stage, which is absolutely necessary for pediatricians to make a correct diagnosis and therapeutic measures at an earlier stage. In this paper, we investigate the clinical manifestations of the 13 NICCD patients and report their *SLC25A13* mutations.

2. Subjects and methods

2.1. Subjects

Since 2006, 13 NICCD patients have been diagnosed in our department, all with origin of Chinese Han population. Eleven patients came from south China, including nine from Guangdong, one from Zhejiang and one from Shanghai, respectively. There are only two patients from north China, with one from Shandong and one from Hebei, respectively. None of the 13 subjects have parental consanguineous marriages or similar patients in the same family except P1194. After referral to our hospital, most patients were diagnosed clinically as NICCD according to the major evidences summarized in reference [11], however, three patients were, at first stage, misdiagnosed as tyrosinemia, galactosemia, and CMV hepatitis, respectively, with NICCD diagnosed after *SLC25A13* gene mutation analysis. After establishment of the diagnosis, therapeutic interventions were performed, and their prognosis followed up for at least 6 months by clinical visits, telephone and email.

2.2. Case-control study and statistics

We compared the anthropometry parameters at birth, including weight, length and ponderal index [PI, represented as weight (g) × 100/length(cm)^3] of 12 NICCD patients with that of 12 gestational age (GA), gender and deliver pattern-matched normal neonates. All parameters were presented as mean ± S.D., and Student test (*t*-test) was used to evaluate the significance of the differences between the patient and control groups.

2.3. Cross-sectional investigation

The major clinical manifestations, representative blood biochemical changes and metabolome findings were collected, analyzed, and described cross-sectionally. Some data were collected from the copies of clinic or hospitalization records in other hospitals, provided by parents of the patients at their referrals to the authors for diagnosis, treatment and genetic counseling.

2.4. Mutation analysis

Dried blood spots on filter paper of the subjects were collected with the informed consents of their parents. DNA was extracted and 13–17 known mutations in the gene *SLC25A13* were screened by means of PCR without or with restriction endonuclease digestion (PCR-RFLP), GeneScan and SNaPshot established and described previously by our group [3,8,10,22–24]. We used direct sequencing of DNA fragments amplified by genomic DNA-PCR to identify novel mutation in the gene *SLC25A13* [8]. Furthermore, we developed a protocol of PCR-RFLP for the molecular diagnosis of the mutation G333D. Sequences of the forward and backward primers for PCR amplification of the mutation are 5′-TGCCTGGCCTCAGTGATGTT-3′ and 5′-CCTGTCTTTGGAAGGCCTGA-3′, respectively. The restriction endonuclease is Mbo I. This study was approved by the Committee for Ethics of Kagoshima University Faculty of Medicine, and adheres to the principles of the Declaration of Helsinki.

3. Results

3.1. General clinical presentations

The NICCD subjects were comprised of seven females and six males, with onset ages as early as 15 (3–42) days (n = 13), but

Table 1
Anthropometric parameters at birth and *SLC25A13* mutations in the NICCD subjects.

Patients	Gender	Gestational age (weeks)	Birth weight (g)	Birth length (cm)	Ponderal Index	Mutations
P1071	Male	39.0	3050	49.0	2.59	851del4/1638-1660dup
P1194	Female	40.0	3250	50.0	2.60	851del4/A541D
P1194S	Female	40.0	2800	48.0	2.53	851del4/A541D
P1443	Male	40.0	3000	50.0	2.40	IVS6+5G>A/R319X
P1478	Female	40.0	2800	50.0	**2.24**	851del4/851del4
P1482	Male	unknown	unkown	unknown	unknown	851del4/851del4
P1495	Female	39.3	3350	50.0	2.68	851del4/G333D
P1513	Female	37.7	**2250**	46.0	2.31	851del4/IVS16ins3kb
P1628	Male	40.1	2550	49.0	**2.17**	851del4/IVS6+5G>A
P1638	Male	38.6	**2310**	45.0	2.53	851del4/1638-1660dup
P1643	Female	40.9	**2225**	49.0	**1.89**	851del4/?
P1644	Female	38.0	2750	48.0	2.49	851del4/IVS6+5G>A
P1648	Male	38.9	**2400**	46.0	2.47	851del4/851del4
Patients group (n = 12)		39.4 ± 1.0	2728 ± 387$^{\Delta}$	48.3 ± 1.8*	2.41 ± 0.22*	–
Control group (n = 12)		39.4 ± 1.0	3202 ± 254	49.8 ± 1.1	2.60 ± 0.14	–

The birth weight values in bold black indicate IUGR, while ponderal index (PI) values in bold black suggest asymmetric body proportionality. P1194S is the elder sister of P1194, and the mutations in the 24 *SLC25A13* alleles from the 12 families were listed.

* $p < 0.05$ compared with control.

$^{\Delta}$ $p < 0.01$ compared with control.