Molecular Genetics and Metabolism Reports 17 (2018) 3–8

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Molecular and clinical characterization of citrin deficiency in a cohort of Chinese patients in Hong Kong

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ARTICLE INFO

Keywords:
Citrin deficiency
NICCD
FTTDCD
CTLN2
Hemangioendothelioma

ABSTRACT

Background and objectives: This retrospective study analysed a case series of subjects with citrin deficiency, and aims to present the molecular and clinical characterization of this disease in the Hong Kong Chinese population for the first time. Patients and Methods: Data from medical records of eighteen patients with citrin deficiency (years 2006–2015) were retrieved. Demographic data, biochemical parameters, radiological results, genetic testing results, management, and clinical outcome were collected and analysed. Results: Eighteen patients with diagnosis of citrin deficiency were recruited. All 18 patients carried at least one common pathogenic variant c.852_855delTATG in *SLC25A13*. Prolonged jaundice (neonatal intrahepatic cholestasis caused by citrin deficiency, NICCD) was the most common presenting symptom, in conjunction with elevated plasma citrulline, threonine, alkaline phosphatase, and alpha-fetoprotein levels. The abnormal biochemical parameters including liver derangement returned to normal range in most of the cases by 6 months of age after the introduction of a lactose-free formula. There were a few cases with atypical presentations. Two subjects did not present with NICCD, and were subsequently diagnosed later in life after their siblings presented with symptoms of citrin deficiency at one month of age and subsequently received a molecular diagnosis. One patient with citrin deficiency also exhibited multiple liver hemangioendotheliomas, which subsided gradually after introduction of a lactose-free formula. Only one patient from this cohort was offered expanded metabolic screening at birth. She was not ascertained by conducted newborn screening and was diagnosed upon presentation with cholestatic jaundice by 1 month of age. Conclusion: This is the first report of the clinical and molecular characterization of a large cohort of patients with citrin deficiency in Hong Kong. The presentation of this cohort of patients expands the clinical phenotypic spectrum of NICCD. Benign liver tumors such as hemangioendotheliomas may be associated with citrin deficiency in addition to the well-known association with hepatocellular carcinoma. Citrin deficiency may manifest in later infancy period with an NICCD-like phenotype. Furthermore, this condition is not always ascertained by expanded newborn metabolic screening testing.

1. Introduction

Citrin deficiency is an autosomal recessive condition that encompasses a broad phenotypic spectrum of clinical phenotypes including neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD: OMIM#605814) [1], the intermediate type failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) [2], and the adult-onset citrullinemia type II (CTLN2: OMIM#603471) [1,3,4]. The condition is caused by biallelic pathogenic variants in *SLC25A13* on chromosome 7q21.3 [5,6]. Citrin deficiency can manifest in the

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https://doi.org/10.1016/j.ymgmr.2018.08.002
Received 26 May 2018; Received in revised form 18 August 2018; Accepted 18 August 2018

neonatal period or infancy with an NICCD phenotype. NICCD is characterized by neonatal or infantile onset intrahepatic cholestatic jaundice with raised plasma citrulline, arginine, ammonia, alpha-fetoprotein, and liver parenchymal cellular infiltration [7]. Most of the NICCD patients respond well with a dietary management of lactose-free formula. Very rarely, liver transplant was needed in few affected subjects [8,9]. Some of the subjects with citrin deficiency will develop an intermediate phenotype, FTTDCD beyond neonatal period [10], while some remain asymptomatic until adulthood. This intermediate phenotype is mostly associated with failure to thrive and dyslipidemia. The factors that would confer a higher risk for subjects with NICCD or FTTDCD to develop CTLN2 remain unknown for the most part. Subjects with this latter condition may present with recurrent late childhood or adult onset hyperammonemic episodes with liver dysfunction and neuropsychiatric symptoms [1,3,4]. The initial case series were reported in Japanese and other Asian populations. However, subjects have now been reported from different ethnicities [1,2,4,10,11]. Further research studies are essential to elucidate the pathophysiology underlying this diverse spectrum. This study aims to present the clinical and molecular spectrum of citrin deficiency in a cohort of Chinese patients in Hong Kong and to discuss a rare incidence of liver hemangioendothelioma in one of the subjects.

2. Materials and methods

2.1. Subjects

Medical records of paediatric patients aged 0–18 with the diagnosis of citrin deficiency from 2007 to 2017 were retrieved from the medical record system of a Joint Metabolic Clinic located at Prince of Wales Hospital, New Territories, Hong Kong Special Administrative Region. Eighteen patients were identified with a diagnosis of citrin deficiency. Demographic data including age, gender, birth weight, gestation, ethnicity, age of presentation, clinical symptoms and signs, and biochemical findings were collected. The treatment and outcome of the patients were also retrieved. Since subjects 1 and 2 in the cohort did not present with NICCD and were diagnosed late in life, their biochemical data were not used to compare with the data from the other 16 patients who presented with NICCD.

2.2. Newborn screening

Only one patient out of the 18 patients in this cohort received newborn metabolic screening testing. All the other patients did not receive newborn metabolic screening.

2.3. Molecular testing

Tiered molecular testing was conducted. It included targeted mutation analysis by Sanger sequencing for coding exons 1, 6, 7, 9, 11, 14, 16, 17 of the *SLC25A13* gene. Flanking introns (20 nucleotides) were directly sequenced in both directions. Long range PCR analysis was performed to screen for the 3 kb insertion mutation in intron 16 (IVS16ins3kb) currently known as c.1750+72_1751-4dup17insNM_138459.3:2667. If tiered testing was not informative, Sanger sequencing of the remaining exons of *SLC25A13* was performed.

3. Results

3.1. Patient demographics

Twelve female and six male patients with diagnosis of citrin deficiency were recruited in this study. Out of the 18 patients with citrin deficiency, two subjects did not exhibit NICCD and were ascertained after their siblings were molecularly diagnosed with citrin deficiency. Sixteen patients presented with an NICCD phenotype with onset from 1 month to 4 months of life. The median age of presentation was 48 days of life (range: 32 days to 4 months). The median birth weight was 2.65 kg (range: 2.07–3.48 kg). (Fig. 1, Table 1).

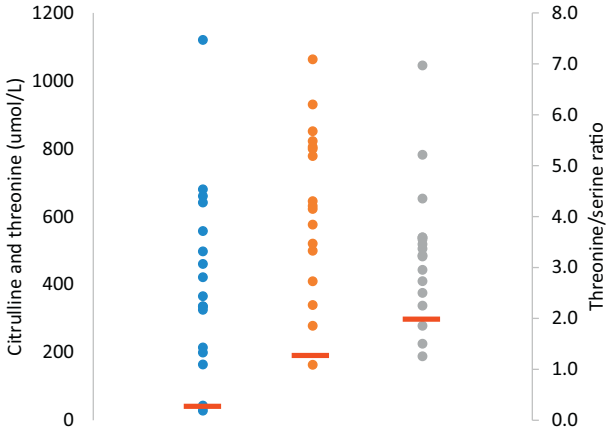

Fig. 1. Plasma amino acid levels for 18 subjects at presentation (citrulline, threonine, threonine to serine ratio).
"—" represents the normal cut off levels of amino acids citrulline, threonine, and threonine/serine ratio in plasma samples.
"●" represents the distribution of levels of plasma amino acid of each subjects. Blue dots represent plasma citrulline values, red dots represent plasma threonine values, and green dots represent plasma threonine/serine ratios.

Table 1
Biochemical characteristics of 16 citrin deficiency patients with NICCD phenotype.

Biochemical findings	Number of positive case (%)
Anaemia (Hb < 10 g/dL) at presentation	4 (25)
Prothrombin time (> 12.4 s)	12 (75)
Hypoglycemia (Glucose < 3.9 mmol/L)	2 (12.5)
Raised total bilirubin (> 17 umol/L)	16 (100)
Raised direct bilirubin (> 5 umol/L)	16 (100)
Elevated ALT (> 67 IU/L)	1 (6)
Elevated GGT (> 95 U/L)	13 (81)
Hypoalbuminaemia (< 35 g/L)	12 (75)
Raised AFP at presentation (at least twice the upper normal range for age)	16 (100)
Raised ALP at presentation (> 380 IU/L)	13 (81)
Raised Triglyceride (> 1.7 mmol/L)	1 (6)

3.2. Dietary preference

All 18 patients have a strong preference toward protein-rich foods especially fish and egg, and dislike high carbohydrate foods such as rice, sweets, and noodles. Four patients reported nausea after intake of high carbohydrate foods.

3.3. Biochemical findings

All 16 (100%) NICCD patients presented with cholestatic jaundice, and 81% of them had significantly raised alkaline phosphatase at presentation. Sixteen subjects (100%) had markedly elevated alpha-fetoprotein (AFP) levels at presentation (Fig. 1, Table 1). The biochemical abnormalities began to improve within a month and returned to a normal range within six months after switching the formula to a soy based or lactose free formula milk in 15 out of 16 patients. The liver function of one patient (subject 12) did not improve soon after initiation of dietary modifications consisting of a lactose-free formula in conjunction with fat-soluble vitamin supplements. It took four months for the cholestatic jaundice to resolve and ten months for his liver panel