Development of a Questionnaire Method of Screening for Citrin Deficiency in Schoolchildren

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Abstract

Citrin deficiency is a congenital metabolic disorder of autosomal recessive inheritance that is caused by mutations in the SLC25A13 gene. The prevalence of homozygotes of these mutations is 1/17,000, with a corresponding prevalence of 0.015 for heterozygotes 5 in Japan. It is difficult to detect citrin deficiency before the onset of adult onset type II citrullinemia (CTLN2) during the asymptomatic period.

To detect citrin deficiency during the asymptomatic period, we distributed a parent-administered questionnaire, which focused on specific food preferences of disliking sweets and preferring high protein and high fat foods, to 62,895 children in elementary and junior high school. We obtained 16,468 responses, and 84 children had these specific food preferences. After excluding children whose parents did not want a telephone contact and those without available contact details, we asked the parents of 32 children if they would allow their child to undergo a genetic test of the SLC25A13 gene. DNA extracted from the collected saliva of these 13 children was examined for 6 prevalent mutations in the SLC25A13 gene. Although two of these 13 children were heterozygous carriers, one child with c.851_854delGTAT and one with c.1177+1G>A, no homozygous carrier was detected. We plan to expand the number of study subjects to improve the questionnaire screening for citrin deficiency in an ongoing genome cohort study.

Keywords: Citrin deficiency; Screening test; NICCD; CTLN2; SLC25A13

Introduction

Citrin deficiency is one of the most frequent diseases among congenital metabolic disorders in Japan. Based on the time of its onset, citrin deficiency is classified into neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD; OMIM # 603471) and adult onset type II citrullinemia (CTLN2; OMIM # 605814). Citrin deficiency has been found to be caused mainly by mutations in the SLC25A13 gene, and the prevalence of homozygotes for SLC25A13 polymorphism is 1/17,000 in Japan [1]. The prevalence of heterozygous carriers is 0.015 in

Japan, which is higher than that in the western countries [1]. Nevertheless, since the prevalence of CTLN2 is 1/100,000 to 1/230,000 [2,3], it is estimated that there are a considerable number of patients who have not received a definitive diagnosis or who have spent all their lives without symptoms of citrin deficiency. One of the reasons why the actual prevalence of citrin deficiency differs from the theoretical prevalence is that no simple screening methods for definitive diagnosis have been established.Only about 40% of NICCD is detected by newborn mass screening [4]. NICCD is easily overlooked in some cases because its symptoms are normalized by 6 months to around 1 year of age or because it remains asymptomatic [5,6].

In particular, the period between onset of NICCD and onset of CTLN2 is called the phase of adaptation/compensation because patients have minor or no symptoms during this period. This lack of symptoms makes it difficult to predict the age of onset of CTLN2 [7]. The age of onset of CTLN2 ranges widely from 10 years of age to the 70s [6-16]. Symptoms of CTLN2 include disturbance of consciousness [8,12,13], behavior abnormality [6], hepatic dysfunction [12,13,17], hyperammonemia [8], depression [18], and epilepsy [17]. There is a possibility that it is often diagnosed as another disease such as a mental disease or a hepatic disease.

Fortunately, it is known that patients with citrin deficiency have specific food preferences. They prefer high-protein foods such as beans and high-fat foods such as fried food, and they dislike sweets such as candy [19] even in the phase of adaptation/compensation [19,20]. If potential patients with citrin deficiency could be detected before the onset of CTLN2 by a simple questionnaire method focusing on specific food preferences, it may be possible to prevent many patients from suffering disease aggravation or severe symptoms by using appropriate diet therapy or it may be possible to refrain from concentrated glycerin/fructose injection or high calorie infusion. In this study, we examined the possibility of using a questionnaire that focused on food preferences and childhood clinical symptoms as a screening test for citrin deficiency.

Methods

Design

This study is part of the Tohoku Medical Megabank Organization (ToMMo) Child Health Study, which was a cross-sectional study of a parent-administered questionnaire survey of schoolchildren in 28 out of 35 municipalities of Miyagi Prefecture in Japan from 2012 to 2015 [21,22]. This study was approved by the institutional review board of Tohoku University Graduate School of Medicine (No. 2014-1-440).

Study Population

We examined children in public elementary schools and public junior high schools located in southern areas of Miyagi Prefecture in Japan, in 2012, 2013 and 2014, and in northern areas of Miyagi Prefecture in 2013, 2014 and 2015 [21]. In the first year, we examined children in the 2nd, 4th, 6th, and 8th grades, and in the second year, we examined those in the 1st, 3rd, 5th, and 7th grades. Since students progressed to the next grade the following year, the 2nd, 4th, 6th, and 8th grade students were examined at that 69time. As a result, among all children, there was no duplicate participant [21]. The questionnaire was distributed to the children in all the public school located in the study area. The total number of distributed questionnaires was 62,895, which consisted of 3,505, 12,742, 28,159, and 18,489 questionnaires in 2012, 2013, 2014 and 2015, respectively. The children's parents or guardians then completed the questionnaire and sent them to our laboratory by mail. A total of 17,020 questionnaires were returned to our laboratory (response rate 27.1%), which consisted of 1,369, 4,080, 7,197 and 4,374 questionnaires in 2012, 2013, 2014 and 2015, respectively. We excluded 552 questionnaire responses due to missing values of age or sex, or for being outside the targeted grades (n=551),

and due to missing values for questionnaire items about citrin deficiency (n=1). Ultimately, 16,468 questionnaires were included in the present analysis (Figure 1).

Questionnaire

The questionnaire included items regarding birth year, birth month, past medical history, and history of hospitalization. The questionnaire item of past medical history was free description type questionnaire. History of hospitalization was defined as hospitalization for any reason. The questions regarding specific food preferences were designed by pediatricians based on their clinical experiences, and questionnaire was revised annually, resulting in the use of three versions of the questionnaire.

In the 2012 survey version, we asked parents with a true or false question whether their child does not eat Japanese sweets such as 'yokan' (sweet bean jelly with sugar), does not drink sweet soda or any other soft drink at all, or becomes sick after consuming such items. When the response was "true", we defined the specific food preferences as positive.

In the 2013 survey version, the questionnaire included five food items in relation to food preference (Figure 2) and an item regarding birth weight. If the criterion described in the following number 1 was met, we defined the specific food preferences as positive. We also defined the specific food preferences as positive if the criteria stated in both the following numbers 2 and 3 were simultaneously met.
1. Response to "Japanese sweets with anko (sweet bean paste with sugar)" or to "Apple juice" was "gets sick after taking".
2. Among the following five criteria, four or more responses met the criteria.
2.1. Response to "Japanese sweets with anko (sweet bean paste with sugar)" was "does not take at all".
2.2. Response to "Apple juice" was "does not take at all".
2.3. Response to "Karaage (fried chicken)" was "takes a lot".
2.4. Response to "Edamame (green soybeans)" was "takes a lot".
2.5. Response to "Peanuts" was "takes a lot".
3. The child's birth weight was less than 2,900 grams according to the questionnaire.

In the 2014 and 2015 survey version, the questionnaire included 11 items of food in relation to food preference (Figure 3). The respondents were asked to check the most appropriate box among the following five answer choices of "Takes very often", "Takes a lot", "Takes moderately", "Takes a little", and "Does not take at all". In addition, the respondent was also asked to check in parentheses the item of "Gets sick after taking". For each box, we assigned a score of -1, 0 or +3 (Figure 3). Similarly, for each parenthesis, we assigned a score of 0 or +5 (Figure 3). The total score was defined as the sum of the scores of the individual boxes and those of the parentheses for all 11 food items. Thus, the total score can range from -11 to 58 points. We defined the specific food preferences as positive when the total score was more than 28 points. The cut-off point of 28 was derived from the results of the 2014 survey; this cut-off point corresponded to our predefined criterion of the top 0.3 percentile of the distribution of the total score in the 2014 survey.
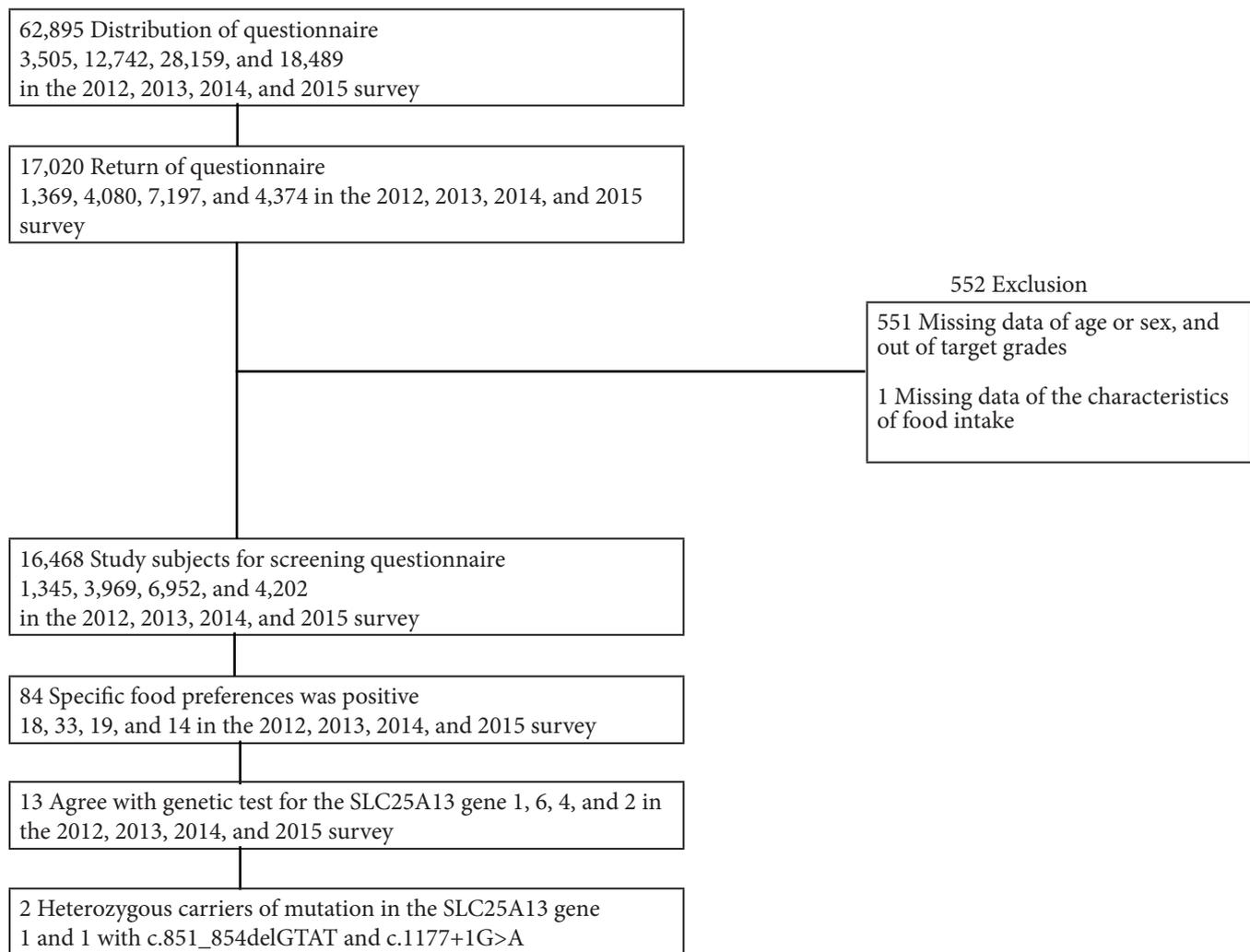
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62,895 Distribution of questionnaire
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552 Exclusion

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551 Missing data of age or sex, and
out of target grades

1 Missing data of the characteristics
of food intake
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16,468 Study subjects for screening questionnaire
1,345, 3,969, 6,952, and 4,202
in the 2012, 2013, 2014, and 2015 survey
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84 Specific food preferences was positive
18, 33, 19, and 14 in the 2012, 2013, 2014, and 2015 survey
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13 Agree with genetic test for the SLC25A13 gene 1, 6, 4, and 2 in
the 2012, 2013, 2014, and 2015 survey
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2 Heterozygous carriers of mutation in the SLC25A13 gene
1 and 1 with c.851_854delGTAT and c.1177+1G>A
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Figure 1: Outline of the study
The genetic test of SLC25A13 examined the presence or absence of six kinds of mutations in the SLC25A13 gene: c.851_854del-GTAT, c.1177+1G>A, c.1638_1660dup, c.674C>A, c.1230+1G>A, g.IVS16ins3kb.

Please check the most appropriate box for your child about the following food / drink (multiple answers allowed).

	Takes very often	Takes a lot	Takes a little	Does not take at all	Gets sick after taking
1. Japanese sweets with anko (sweet bean paste with sugar)	☐	☐	☐	☐	☐
2. Apple juice	☐	☐	☐	☐	☐
3. Karaage (fried chicken)	☐	☐	☐	☐	☐
4. Edamame (green soy-beans)	☐	☐	☐	☐	☐
5. Peanuts	☐	☐	☐	☐	☐

Figure 2: Questionnaire for specific food preferences of the 2013 survey version
We defined the specific food preferences of these five food items and birth weight according to the questionnaire.
Please check the most appropriate box for your child about the following each food / drink.
In addition, please put cross in parentheses of the items, if your child gets sick after taking the following 1 to 11 food / drink.

Please check the most appropriate box for your child about the following each food / drink.
In addition, please put cross in parentheses of the items, if your child gets sick after taking the following 1 to 11 food / drink.

	Takes very often	Takes a lot	Takes moderately	Takes a little	Does not take at all	Gets sick after taking
1. Yokan (sweet bean jelly with sugar)	☐ -1	☐ 0	☐ 0	☐ +3	☐ +3	( ) +5
2. Edamame (green soybeans)	☐ +3	☐ +3	☐ 0	☐ 0	☐ -1	( ) 0
3. Apple juice	☐ -1	☐ 0	☐ 0	☐ +3	☐ +3	( ) +5
4. Karaage (fried chicken)	☐ +3	☐ +3	☐ 0	☐ 0	☐ -1	( ) 0
5. Lollipop	☐ -1	☐ 0	☐ 0	☐ +3	☐ +3	( ) +5
6. Potato crisp	☐ +3	☐ +3	☐ 0	☐ 0	☐ -1	( ) 0
7. Peanuts	☐ +3	☐ +3	☐ 0	☐ 0	☐ -1	( ) 0
8. Sweets with anko (sweet bean paste with sugar)	☐ -1	☐ 0	☐ 0	☐ +3	☐ +3	( ) +5
9. Milk	☐ +3	☐ +3	☐ 0	☐ 0	☐ -1	( )0
10. Cheese	☐ +3	☐ +3	☐ 0	☐ 0	☐ -1	( )0
11. Caramel candy	☐ -1	☐ 0	☐ 0	☐ +3	☐ +3	( )+5

Figure 3: Questionnaire for specific food preferences of the 2014 and 2015 survey version.
The numbers beside the boxes or parentheses are the scores for each answer. The scores were not shown in the real questionnaire. The total score was defined as the sum of the scores of the boxes and those of the parentheses for all 11 food items.

Recruitment of Participants for the Genetic Test

After excluding children whose parents or guardians did not want contact from a researcher and those without available contact details, we sent a letter about citrin deficiency to the parents or guardians whose child had been screened positive in the questionnaire regarding specific food preferences for citrin deficiency information. Subsequently, over the phone, we explained the purpose of the genetic test to them.

After a face-to-face detailed explanation in our laboratory, an informed consent procedure for genetic testing of citrin deficiency was explained to the parent or guardian in our laboratory. We also explained that this study project was conducted in close cooperation with department of pediatrics, Tohoku University hospital. If the homozygous of mutations were founded among their children, the children will be referred to the Tohoku University hospital according to the parents' or guardians' request. After written informed consent was obtained from the parent or guardian, we asked them additional questions regarding their child's health condition from the neonatal period to the present day, inquiring into fatigue in daily life, a prolonged or severe symptom of neonatal jaundice, phototherapy for neonatal jaundice, and poor hepatobiliary function in the neonatal period.

Genetic Test

We collected 0.75 ml saliva from the children using ®Oragene - DNA (DNA Genotek, Inc) after confirming with the children that they had not taken anything orally for 30 minutes before saliva collection.

Genomic DNA was extracted from the saliva specimens in the laboratory of Tohoku University, and a melting-peak analysis was performed using real-time PCR. The presences or absences of 6 different mutations in the SLC25A13 gene (NM_014251) were tested: c.851_854delGTAT, c.1177+1G>A, c.1638_1660dup, c.674C>A, c.1230+1G>A, g.IVS16ins3kb [1].

These 6 mutations in the SLC25A13 gene were chosen because they are known to explain 91% of Japanese mutations of citrin deficiency [1]. However, when a child is a heterozygous carrier of a high frequency mutation in the SLC25A13, he/she has a possibility being a homozygous patient with low frequency mutations in it [1]. For this reason, we also examined other mutations in the SLC25A13 gene by Sanger sequencing when any of these 6 mutations were detected. Although there might be overlooking of new significant deletion and insertion mutations in the SLC25A13 gene [23], those are very rare cases among Japanese patients, thus we did not take into account them.

Results

Of the total 16,468 children provided with the screening questionnaire regarding specific food preferences, the number of children who participated in the study was 1,345, 3,969, 6,952, and 4,202 in the 2012, 2013, 2014 and 2015 survey, respectively. The mean ± standard deviation of age in these 16,468 children was 10.5 ± 2.2 years old. The number of boys, girls and children with a history of hospitalization was 8,136 (49.4%), 8,332 (50.6%) and 4,845 (29.4%), respectively. Birth weight was an average of 3,026 ± 450 grams in 14,849 children (Table 1).

Out of the 16,468 children, there were 84 children whose specific food preferences were considered positive, consisting of 18 (1.3%), 33 (0.81%), 19 (0.26%), and 14 (0.32%) children in the 2012, 2013, 2014 and 2015 survey, respectively. The mean age of the 84 children was 10.8 ± 2.3 years old. The number of boys was 35 (41.7%). Birth weight was an average of 2,897 ± 506 grams (N=66). The number of children with a history of hospitalization was 26 (31.0%). In the 2014 and 2015 surveys, the total score of specific food preferences averaged 12.4 ± 5.8 points (range, - 2 to 42) and 12.8 ± 5.9 (range, -1 to 33), respectively.

	Total	2012 survey	2013 survey	2014 survey	2015 survey
N	16,468 (100.0)	1,345 (100.0)	3,969 (100.0)	6,952 (100.0)	4,202 (100.0)
Grade 2nd	4,451 (27.0)	317 (23.6)	1,098 (27.7)	1,867 (26.9)	1,169 (27.8)
4th	4,334 (26.3)	358 (26.6)	1,123 (28.3)	1,815 (26.1)	1,038 (24.7)
6th	4,336 (26.3)	375 (27.9)	1,014 (25.6)	1,784 (25.7)	1,163 (27.7)
8th	3,347 (20.3)	295 (21.9)	734 (18.5)	1,486 (21.4)	832 (19.8)
Boys	8,136 (49.4)	642 (47.7)	1,948 (49.1)	3,422 (49.2)	2,124 (50.6)
History of hospitalization	4,845 (29.4)	352 (26.2)	1,432 (36.1)	1,965 (28.3)	1,096 (26.1)
Birth weight, gram (N=14,849)	3,026 ± 450	No data	3,027 ± 432	3,023 ± 457	3,030 ± 457

Data are expressed as number (%) in categorical variable and mean ± standard deviation in continuous variable. History of hospitalization was defined hospitalization from any reason.
Birth weight was not considered by gestational age.

Table 1: Characteristics of the survey participants

One girl among the 16,468 children had a past history of citrin deficiency according to a questionnaire answer in the 2014 survey but was not screened as positive because her total score for specific food preferences was 26 points, which was under the cut-off point of 28. Of the 84 screened children, after excluding children whose parents did not want a telephone contact and those without available contact details, we were able to contact 32 of their parents by phone; 5, 19, 4, and 4 in the 2012, 2013, 2014 and 2015 survey, respectively. We confirmed with them the criteria of screening for citrin deficiency and their intention to cooperate with this study. We had sent the information regarding citrin deficiency to them by mail in advance. The children whose parents we could not contact or whose parents did not wish them to participate in this study were excluded from genetic testing.

Ultimately, a total of 13 children: 1, 6, 4, and 2 in the 2012, 2013, 2014 and 2015 survey, respectively, came to Tohoku University-related facilities with the intention of participating in this study. We obtained written informed consent from all 13 sets of parents for participation of their children in this study and then collected saliva from the 13 children. Genomic DNA was extracted from all collected saliva specimens and was analyzed using real-time PCR. The mean age of these 13 children was 11.3 ± 2.9 years old, the mean birth weight was 2,786 ± 557 grams and there were 6 boys. Of these 13 children, 5 had experienced a prolongation of neonatal jaundice or received phototherapy, 1 child had been indicated as having poor hepatobiliary function in the neonatal period and poor weight gain at infancy, and 2 children were easily fatigued on a daily basis.

The analysis of 6 high-frequency mutations in the SLC25A13 gene found that two children were each a carrier of a different type of mutation and 11 children had none of the 6 types of mutations (Table 2). Of these two children with a mutation, one was a heterozygous carrier of c.851_854delGTAT, and the other was a heterozygous carrier of c.1177+1G>A. Exons 1-18 in the SLC25A13 gene (NM_014251) of these two children were then directly sequenced but no other significant mutation was found in it. We did not detect any homozygous carrier.

Discussion

We conducted this study to develop a questionnaire for screening of elementary and junior high school students for citrin deficiency by specific food preferences. Out of the 16,468 responses, 84 children had these specific food preferences. Of these 84 children, we examined 13 children for mutations in the SLC25A13 gene. We did not detect any children who were homozygous for high-frequency mutations, but we did detect two children who were heterozygous for such a mutation. Given that the prevalence of homozygotes of mutations in the SLC25A13 gene was reported as 1/17,000 in Japan [1], it was not surprising that no homozygotes of mutations in the SLC25A13 gene were detected among our present study subjects of 16,468 children. Furthermore, only 13 of the 84 children (15.5%) with specific food preferences underwent genetic testing.

If the discovery of citrin deficiency is delayed and a concentrated glycerin/fructose injection or a high calorie infusion is administered to a patient, the symptoms might worsen rapidly [12,15]. Therefore, to prevent the onset of CTLN2 and to offer proper treatment upon its onset, it is particularly important to detect potential patients who had been asymptomatic during infancy or those who had not received a definitive diagnosis as NICCD, during an apparently healthy period before the onset of CTLN2 [11]. Moreover, for prevention of its onset, it is necessary to detect patients at school child age because CTLN2 develops in the teenage years at the earliest [6,10]. For these reasons, development of a simple screening method for the detection of citrin deficiency among schoolchildren is very important.

The questionnaire we used was revised annually to quantitatively evaluate distinctive food preferences of citrin deficiency. In the latest version of the questionnaire, we adopted a scoring system of food preference, and increased the questionnaire items of food to 11 foods that consisted exclusively of high-protein foods, high-fat foods and sweets. In the planned next revision of the questionnaire of the specific food preferences of citrin deficiency, we will consider adding high-arginine food as one of the food items, because patients of citrin

No.	Sex	Age, y	Birth weight, gram[*1]	BMI, kg/m^2	Easily fatigued	Neonatal jaundice[*2]	Poor hepatobiliary function[*3]	Survey year	Total score of questionnaire about food preferences[*4]	Gene mutation[*5]
1	Girl	15	3,100	18.8	Yes	No	No	2012	NA	Hetero [I]
2	Girl	13	2,600	16.8	No	No	No	2013	NA	No
3	Girl	10	2,900	18.1	No	No	No	2013	NA	No
4	Boy	14	2,200	14.5	No	No	No	2013	NA	No
5	Boy	8	2,900	14.5	No	Yes	No	2013	NA	No
6	Girl	9	2,500	15.3	No	Yes	No	2013	NA	No
7	Boy	10	2,200	15.9	No	Yes	Yes	2013	NA	No
8	Girl	15	3,300	24.7	No	No	No	2014	31	No
9	Girl	9	3,600	26.9	No	No	No	2014	42	No
10	Boy	15	3,100	28.2	Yes	Yes	No	2014	33	Hetero [II]
11	Boy	13	3,500	18.9	No	No	No	2014	28	No
12	Girl	8	1,700	13.4	No	Yes	No	2015	30	No
13	Boy	8	2,600	16.5	No	No	No	2015	30	No

*1 Birth weight was rounded to the nearest 100 and was not considered by gestational age. *2 Prolonged or sever symptom of neonatal jaundice or phototherapy for neonatal jaundice. *3 Poor hepatobiliary function in the neonatal period.
*4 Total score was defined as sum of the point of the boxes and those of the parentheses for all the 11 food items in Fig. 3.
*5 Mutation in the SLC25A13 gene. Hetero [I] and hetero [II] indicates heterozygous mutation of c.851_854delGTAT, and that of c.1177 + 1G>A, respectively.

Table 2: PCR results and characteristics of the genetic test participants

deficiency have been reported to actively take foods such as soybean products that contain a lot of arginine in protein [7,8,20]. The selection of food was of critical importance in designing the questionnaire. In addition to the characteristics of the food as described above, the food items must be familiar to the children in daily life. In the following cases, the results of the questionnaire are considered unreliable. For children from different ethnic groups, the items of food in the questionnaire may not be familiar to them, and could be inappropriate for them. It was also considered that the answers of respondents to the questionnaire about food preference could be affected by dietary habits in the child's home such as the parents' own food preferences, dietary restrictions on some foods due to food allergies, or confectionery restriction as a childcare policy of the parents. In public elementary and junior high schools in Japan, there is a school lunch program which ensures that children get basic nutrition and menus decided at school are provided to children. Therefore, in future study, we may remove the influence of home dietary habits to some extent by adding questions to children or parents about school lunch leftovers as well as food preferences.

Citrin deficiency is a congenital metabolic disorder of autosomal recessive inheritance. We therefore expected that a series of symptoms of citrin deficiency including specific food preferences would appear only in homozygous patients.

However, contrary to our expectations, heterozygous children were detected frequently in the questionnaire of specific food preferences of citrin deficiency. We found two out of 13 children with a mutation in the SLC25A13 gene, which corresponded to a prevalence of 0.15 that was much higher than the reported prevalence of 0.015 for heterozygotes in Japan (P=0.0001) [1, 24], or than the reported prevalence of 0.024 for heterozygotes in Miyagi prefecture in Japan (P=0.005) [25]. Although there was a report that heterozygous carriers with cholestasis in infancy were negative in the blood test of citrin protein [26], new genetic mutations were discovered from allelic inheritance later [1]. Furthermore, we did not confirm the citrin protein by the blood test. Further research is needed to explore whether a heterozygous carrier displays partial symptoms or characteristics of citrin deficiency and whether such symptoms are clinically relevant or not. It is expected that these questions will be clarified by a cohort study of the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study (The TMM Bir Three Cohort Study) [22]. The TMM Bir Three Cohort Study is an ongoing genome cohort study in which 70,000 subjects including 30,000 children have participated. The 11-item specific food preference questionnaire i.e. the 2014 and 2015 survey versions of the present study, was adopted by the TMM Bir Three Cohort Study.

One girl who had a past history of citrin deficiency according to the answer to the patient-reported past history in the present questionnaire was not detected as positive in the 11-item specific food preference questionnaire.

However, her total score of specific food preference was 26 points, which was in the top 1.0 percentile of the distribution of the score. This result may suggest that our predefined criterion of positive in the questionnaire screening as being in the top 0.3 percentile may be too strict. Generally, there is a trade-off relationship between sensitivity and specificity. To validate this criterion, further studies with Receiver Operating Characteristic (ROC) curve analysis are needed.

There are five limitations to this study.
1) Selection bias may affect our results because response rate of questionnaire was low (27.1%). Parents or guardians with strong anxiety about their child's health might have more replied the questionnaire.
2) This study was based on parent-administered questionnaire. Parent of guardian may not completely know their children's food preferences.
3) The questionnaire of specific food preferences may not be applicable to other ethnic groups.
4) We examined not all mutations in the SLC25A13 gene. However, 6 high-frequency mutations which we examined would explain about 91% of Japanese citrin deficiency patients.
5) We used three different versions of questionnaires. However these questionnaires consistently asked a specific food preference. Thus, we consider that the results of this study could be reliable for qualitative analyses.

In conclusion, we tried to develop a questionnaire of specific food preferences to detect citrin deficiency patients. Using this questionnaire, we detected two heterozygous carriers of mutations in the SLC25A13 gene. However, no homozygous carrier was detected. We plan to expand the number of study subjects to improve the questionnaire screening method for citrin deficiency in an ongoing genome cohort study, the TMM Bir Three Cohort Study.

Acknowledgments

Conflicts of Interest

The authors have no conflicts of interest to declare.

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