- **Western blot analysis** using anti-human citrin antibody specific for the amino-terminal half detects little or no cross-reactive immune material in liver, cultured fibroblasts, or lymphocytes from individuals with *SLC25A13* mutations [Yasuda et al 2000, Takahashi et al 2006, Dimmock et al 2007, Tokuhara et al 2007, Fu et al 2011].

For laboratories offering biochemical testing for CTLN2, see Testing.

Molecular Genetic Testing

Gene. *SLC25A13* is the only gene in which mutations are known to cause citrin deficiency.

Table 4. Summary of Molecular Genetic Testing Used in Citrin Deficiency

Gene Symbol	Test Method	Mutations Detected	Mutation Detection Frequency by Test Method [1]	Test Availability
SLC25A13	Sequence analysis	Sequence variants [2]	>95% [3]	Clinical Testing
	Deletion / duplication analysis [4]	Exonic and whole-gene deletions	Unknown [5]	

Test Availability refers to availability in the GeneTests™ Laboratory Directory. *GeneReviews* designates a molecular genetic test as clinically available only if the test is listed in the GeneTests Laboratory Directory by either a US CLIA-licensed laboratory or a non-US clinical laboratory. GeneTests does not verify laboratory-submitted information or warrant any aspect of a laboratory's licensure or performance. Clinicians must communicate directly with the laboratories to verify information.

1. Because the criteria for clinical and biochemical diagnosis of citrin deficiency other than CTLN2 are not yet established, it is difficult to calculate the mutation detection frequency.

2. Examples of mutations detected by sequence analysis may include small intragenic deletions/insertions and missense, nonsense, and splice site mutations.

3. Kobayashi et al [1999], Yasuda et al [2000], Ben-Shalom et al [2002], Yamaguchi et al [2002], Saheki et al [2004], Lu et al [2005], Takaya et al [2005], Ko et al [2007a], Song et al [2008], Tabata et al [2008], Song et al [2009b], Xing et al [2010], Fu et al [2011], Song et al [2011], Wen et al [2011]

4. Testing that identifies deletions/duplications not readily detectable by sequence analysis of the coding and flanking intronic regions of genomic DNA; a variety of methods including quantitative PCR, long-range PCR, multiplex ligation-dependent probe amplification (MLPA), or targeted chromosomal microarray analysis (gene/segment-specific) may be used. A full chromosomal microarray analysis that detects deletions/duplications across the genome may also include this gene/segment. See array GH.

5. Takaya et al [2005], Wong et al [2008]

Interpretation of test results. For issues to consider in interpretation of sequence analysis results, click here.

Testing Strategy

Confirming/establishing the diagnosis of citrin deficiency in a proband (see Figure 2 and Figure 3)

The following testing strategy (see **Order of testing**) should be considered for:

- Infants who have had a positive newborn screening test for:
  - Citrullinemia and/or prolonged jaundice; or
  - Galactosemia, hypermethionemia or hyperphenylalanemia, who on follow-up diagnostic testing were found not to have one of these disorders.
- Children beyond age one year who present with failure to thrive and dyslipidemia;
- Older children and adults with hepatic encephalopathy with hyperammonemia, especially those with aversion to carbohydrate and fondness for protein- and lipid-rich foods;
- Children and adults with unexplained recurrent pancreatitis, hyperlipidemia, fatty liver or hepatoma.

Order of testing

- Perform quantitative plasma amino acid analysis (children age 1-4 months).
- Measure blood ammonia, plasma amino acids, PSTI, liver enzymes (when CTLN2 is suspected).
- Perform dietary assessment, including food preferences (particularly important if FFTDCD or CTLN2 is suspected).
- Perform molecular genetic testing:

Sequence analysis of *SLC25A13*, followed by deletion/duplication analysis if neither or only one disease-causing mutation is identified.

- Note: Western blotting for citrin protein is considered if no or only one disease-causing mutation is identified by molecular genetic testing.

Carrier testing for at-risk relatives requires prior identification of the disease-causing mutations in the family.

Note: Carriers are heterozygotes for this autosomal recessive disorder and are not at risk of developing the disorder.

Predictive testing for at-risk asymptomatic adult family members requires prior identification of the disease-causing mutations in the family.

Prenatal diagnosis and preimplantation genetic diagnosis (PGD) for at-risk pregnancies require prior identification of the disease-causing mutations in the family.

Note: It is the policy of *GeneReviews* to include in *GeneReviews*™ chapters any clinical uses of testing available from laboratories listed in the GeneTests™ Laboratory Directory; inclusion does not necessarily reflect the endorsement of such uses by the author(s), editor(s), or reviewer(s).

Genetically Related (Allelic) Disorders

CTLN2, NICCD, and FTTDCD are the only phenotypes currently known to be associated with mutations in *SLC25A13*.

Clinical Description

Natural History

Citrin deficiency can manifest in newborns as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), in older children as failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD), and in adults as recurrent hyperammonemia with neuropsychiatric symptoms in citrullinemia type II (CTLN2). Often FTTDCD and CTLN2 are characterized by fondness for protein-rich and/or lipid-rich foods and aversion to carbohydrate-rich foods. Individuals with CTLN2 may or may not have a prior history of NICCD or FTTDCD. The proportion of persons with NICCD or FTTDCD that evolves into CTLN2 is unknown.

Neonatal Intrahepatic Cholestasis Caused by Citrin Deficiency (NICCD)

Children under age one year with NICCD have transient intrahepatic cholestasis. Other findings include diffuse fatty liver with hepatomegaly and parenchymal cellular infiltration associated with hepatic fibrosis, low birth weight, growth retardation, hypoproteinemia, decreased coagulation factors, hemolytic anemia, variable (mainly mild) liver dysfunction, and/or hypoglycemia.

NICCD is generally not severe, although liver transplantation has been required in rare cases [Tamamori et al 2002, Kobayashi et al 2006]. Symptoms typically resolve by age one year with treatment, including fat-soluble vitamin supplementation and use of lactose-free formulas (for those with galactosemia) or formulas containing medium-chain triglycerides [Ohura et al 2003, Song et al 2010].

Starting around age one to two years, children show a strong preference for protein-rich and lipid-rich foods and an aversion to sugar-rich and carbohydrate-rich foods [Hachisu et al 2005, Saheki & Kobayashi 2005, Saheki et al 2008].

In the second or later decades, some individuals with citrin deficiency develop severe CTLN2 with neuropsychiatric symptoms [Saheki & Kobayashi 2002]. Typically the transition from the adaptation (and/or compensation) stage following NICCD to the onset of CTLN2 is gradual.

Failure to Thrive and Dyslipidemia Caused by Citrin Deficiency (FTTDCD)

FTTDCD has recently been proposed as a novel post-NICCD phenotype before the onset of CTLN2 [Song et al 2011]. The clinical and laboratory features of FTTDCD are still being elucidated. During this period (traditionally assumed to be an "apparently healthy" stage before CTLN2 onset) some children were found to have laboratory abnormalities (see Diagnosis) and/or clinical abnormalities including fatigue, growth retardation, hypoglycemia, and pancreatitis.

Citrullinemia Type II (CTLN2)

CTLN2 is characterized by recurring episodes of hyperammonemia and neurologic and psychotic symptoms that closely resemble those of hepatic encephalopathy or genetic urea cycle disorders, including nocturnal delirium, aberrant behaviors (aggression, irritability, and hyperactivity), delusions, disorientation, restlessness, drowsiness, loss of memory, flapping tremor, convulsive seizures, and coma. Brain CT is normal, and EEG shows diffuse slow waves.

Onset is sudden and usually between ages 20 and 50 years (range: 11-79 years; mean age: 34.4 ±12.8 years; n=103) [Yasuda et al 2000].

Many individuals with CTLN2 have a strong preference for protein-rich and/or lipid-rich foods (e.g., beans, peanuts, eggs, milk, cheese, fish, meat) and an aversion to carbohydrate-rich foods including rice, juice, and sweets. Symptoms are often provoked by alcohol and sugar intake, medication, and/or surgery.

Most individuals are thin. More than 90% have a body mass index lower than 20 and approximately 40% have a body mass index lower than 17 (range: 15.6-19.1; n=110) [Kobayashi et al 2006] (range in healthy Japanese individuals: 20-24 in males; 19-23 in females).

The following complications occur in more than 10% of individuals with CTLN2 [Kobayashi et al 2000]. Studies regarding these complications are ongoing.

- **Pancreatitis.** Juvenile-onset chronic pancreatitis and hepatocellular carcinoma without cirrhosis can precede the appearance of CTLN2 [Ikeda et al 2004].
- **Hyperlipidemia.** Hypertrigyceridemia is frequently observed if high carbohydrate meals are provided to individuals with citrin deficiency [Imamura et al 2003].
- **Fatty liver.** Most individuals with NICCD and CTLN2 have fatty liver, which is histologically identical to NASH (non-alcoholic steatohepatitis) [Takagi et al 2006, Fukumoto et al 2008, Komatsu et al 2008]. Mild fibrosis can also be seen [Kobayashi et al 2000].
- **Hepatoma** may be present, even before the diagnosis of CTLN2 is made [Tanaka et al 2002, Hagiwara et al 2003, Tsai et al 2006, Soeda et al 2008]

Intrahepatic cholestasis is rare; however, some individuals are noted in retrospect to have had signs of NICCD in early childhood [Kobayashi & Saheki 2004, Saheki & Kobayashi 2005]. For example, a 16-year-old with CTLN2 undergoing liver transplantation [Kasahara et al 2001] had had transient hypoproteinemia and jaundice in early infancy [Tomomasa et al 2001].

Pathologic findings include fatty infiltration and mild fibrosis of the liver despite little or no liver dysfunction.

Genotype-Phenotype Correlations

No significant correlation between *SLC25A13* mutation types and decreased level of hepatic enzyme ASS activity/protein or age of onset in individuals with CTLN2 is observed [Yasuda et al 2000].

Penetrance

The male-to-female ratio in NICCD is roughly equal (73:80) [Kobayashi & Saheki 2004].

The male-to-female ratio in CTLN2 is 2.4 to 1 (120:50) [Kobayashi & Saheki 2004].

The unequal male-to-female ratio in CTLN2 suggests that for unknown reasons, homozygous females are more resistant to the CTLN2 phenotype than males.

Nomenclature

NICCD. NICCD was known as "idiopathic neonatal hepatitis with fatty liver of unknown origin" [Ohura et al 1997] before molecular genetic testing confirmed the presence of *SLC25A13* mutations.

CTLN2. Miyakoshi et al [1968] reported that blood citrulline concentrations were increased in individuals with hyperammonemia and a unique chronic recurrent hepatocerebral degeneration. This hepatocerebral degeneration came to be known as "pseudo-ulegyric hepatocerebral disease" on the basis of pathologic brain changes, and "nutritional hepatocerebral disease" on the basis of metabolic disturbance resulting from a highly unbalanced diet or developmental disturbance caused by endocrine abnormalities.

Saheki et al [1981] reported this hepatocerebral disease as a type of citrullinemia with a qualitative and liver-specific decrease of the arginosuccinate synthetase activity/protein, and later Saheki et al [1985] named it "adult-onset type II citrullinemia."

Prevalence

In Japan, the frequency of homozygotes or compound heterozygotes for *SLC25A13* mutations is calculated to be 1:17,000 based on the carrier or heterozygote rate of 1:65 [Saheki & Kobayashi 2002, Tabata et al 2008]. This is similar to the observed prevalence of NICCD [Shigematsu et al 2002], but different from the observed prevalence of CTLN2 (1:100,000-1:230,000) [Kobayashi et al 2006]. Based on their observations, the authors believe that most homozygotes of Japanese heritage have NICCD.

Until recently, citrin deficiency was thought to be restricted to Japan; citrin deficiency is now recognized to be pan ethnic [Dimmock et al 2009]. Individuals with novel *SLC25A13* mutations have been identified in Israel, Pakistan, the US, the United Kingdom, China, and the Czech Republic [Ben-Shalom et al 2002, Hutchin et al 2006, Luder et al 2006, Dimmock et al 2007, Fiermonte et al 2008, Song et al 2008, Tabata et al 2008, Song et al 2009b, Song et al 2011].

The carrier frequency is also high in China (1/65), especially southern China including Taiwan (1/48), and in Korea (1/112) [Lu et al 2005, Lee et al 2011].

Differential Diagnosis

For current information on availability of genetic testing for disorders included in this section, see GeneTests Laboratory

Directory. —ED.

Plasma concentration of citrulline is increased in citrin deficiency as well as in the following disorders:

- **Citrullinemia type 1 (CTLN1; ASS deficiency).** CTLN1 presents as a wide spectrum of overlapping phenotypes: an acute neonatal form (the "classic" form), a milder late-onset form, a form without symptoms and/or hyperammonemia, and a form in which women have onset of severe symptoms during pregnancy or post partum [Gao et al 2003]. Shortly after birth, infants with the acute neonatal form develop hyperammonemia and its complications, from which they die without prompt intervention. Those who are treated promptly may survive for an indeterminate period of time, but usually with significant neurologic deficit. In the late-onset form, the episodes of hyperammonemia are similar to those seen in the acute neonatal form, but the initial neurologic findings may be more subtle.

  CTLN1 results from deficiency of the enzyme ASS, the third step in the urea cycle, in which citrulline is condensed with aspartate to form argininosuccinic acid. Untreated individuals with the severe form of CTLN1 have hyperammonemia, increased plasma concentration of citrulline, and decreased plasma concentration of arginine. Inheritance is autosomal recessive.

  In CTLN2, the liver-specific deficiency of the ASS protein is secondary by unknown mechanisms [Yasuda et al 2000] as no abnormalities are present in hepatic ASS mRNA or *ASS1*.
- **Argininosuccinic aciduria (argininosuccinate lyase [ASL] deficiency)** (see Urea Cycle Disorders Overview)
- **Lysinuric protein intolerance (LPI)**
- **Pyruvate carboxylase (PC) deficiency**
- **Renal insufficiency**
- **Galactosemia**. In one neonate, classic galactosemia presented as citrin deficiency [Feillet et al 2008].

Hyperammonemia occurs in citrin deficiency as well as in the urea cycle disorders, which result from defects in the metabolism of the nitrogen produced by the breakdown of protein and other nitrogen-containing molecules (see Urea Cycle Disorders Overview). Severe deficiency or total absence of activity of any of the first four enzymes (CPSI, OTC, ASS, ASL) in the urea cycle, the ornithine transporter, or the cofactor producer (NAGS) results in the accumulation of ammonia and other precursor metabolites during the first few days of life in most affected individuals.

Neonatal/infantile cholestasis occurs in citrin deficiency as well as the following disorders:

- **Idiopathic neonatal hepatitis (INH) and extrahepatic biliary atresia (EBA).** In comparison with INH and EBA, NICCD is associated with lower levels of serum direct bilirubin or ALT and higher levels of serum total bile acids and alkaline phosphatase. NICCD also has higher levels of serum γ-GTP and lower levels of serum AST activity than are seen in INH [Tazawa et al 2005].
- **Progressive familial intrahepatic cholestasis (PFIC, Byler disease).** The high-serum γ-GTP levels of NICCD may distinguish it from other intrahepatic cholestasis disorders with low-normal γ-GTP levels including PFIC and benign recurrent intrahepatic cholestasis (BRIC). PFIC is caused by mutations in *ATP8B1* (*FIC1*) or *ABCB11* (*BSEP*). Some cases of BRIC are caused by mutations in *ATP8B1.*

Hereditary jaundice and hyperbilirubinemia result from defects in the metabolism of bilirubin. These include disorders resulting in predominantly unconjugated (indirect) hyperbilirubinemia (UDP-glucuronosyltransferase 1-1 deficiency) and those resulting in predominantly conjugated (direct) hyperbilirubinemia (deficiency in canalicular ATP-dependent transporters: ABCC2 [MRP2], ABCB11, or ATP8B1).

Other

- Portal-systemic shunts can be excluded by angiography.
- More than 30% of individuals with CTLN2 have been misdiagnosed initially as having epileptic seizures and/or a psychological disorder (e.g., depression, schizophrenia); others may be diagnosed as having diseases such as hepatoma, pancreatitis, and hyperlipidemia.

Note to clinicians: For a patient-specific 'simultaneous consult' related to this disorder, go to SimulConsult®, an interactive diagnostic decision support software tool that provides differential diagnoses based on patient findings (registration or institutional access required).

- CTLN2
- NICCD

Management

Evaluations Following Initial Diagnosis

To establish the extent of disease and needs of an individual diagnosed with citrin deficiency the following are recommended by phenotype:

NICCD

- Assess the size of the liver and spleen.
- Seek evidence of fatty liver by abdominal US, CT, or MRI.
- Investigate feeding pattern.

FTTDCD

- Perform detailed anthropometric examination and evaluation using age- and gender-matched growth standards.
- Investigate feeding pattern.

CTLN2

- Investigate carbohydrate, protein, and lipid composition of the diet.

Treatment of Manifestations

NICCD. The symptoms in most children with NICCD resolve by age 12 months following supplementation with fat-soluble vitamins and use of lactose-free formula (in those with galactosemia) or formulas containing medium-chain triglycerides (MCT) [Ohura et al 2003]. Moreover, the efficacy of lactose-free and/or MCT-enriched therapeutic formulas has also been demonstrated in a Chinese NICCD cohort [Song et al 2010]. Two siblings improved after switching from breast milk to formula, which has higher proline content [Ben-Shalom et al 2002]. Some children with NICCD improve without treatment.

Four infants with NICCD and severe liver dysfunction were diagnosed as having tyrosinemia of unknown cause and underwent liver transplantation at age ten to 12 months [Tamamori et al 2002, Kobayashi et al 2006].

FTTDCD. Few treatment measures have been described for this novel citrin-deficient phenotype.

- A toddler with FTTDCD was fed in accordance with his own food preferences (including aversion to rice and fondness for fish); FTT improved gradually, with weight-for-age recovering beyond the thirrd percentile at age three years. The dyslipidemia also improved gradually [Song et al 2009a].
- In addition to dietary treatment, administration of sodium pyruvate may be effective in correcting growth retardation [Mutoh et al 2008, Saheki et al 2010].

CTLN2. The most successful therapy to date has been liver transplantation [Ikeda et al 2001, Kasahara et al 2001, Yazaki et al 2004, Hirai et al 2008], which prevents episodic hyperammonemic crises, corrects the metabolic disturbances, and eliminates preferences for protein-rich foods [Kobayashi & Saheki 2004]. Nearly all cases of CTLN2 need liver transplantation in the past, but this situation starts to change since introduction of arginine and sodium pyruvate.

- Administration of arginine was reported to be effective in decreasing blood ammonia concentration. Reducing calorie/carbohydrate intake and increasing protein intake ameliorates hypertriglyceridemia [Imamura et al 2003].
- Administration of sodium pyruvate was effective in several cases [Yazaki et al 2005; Mutoh et al 2008; Saheki et al 2010; Yazaki et al 2010; Ohura et al, personal communication; Okano et al, personal communication].

Prevention of Primary Manifestations

To prevent hyperammonemia and resolve failure to thrive, a diet rich in protein and lipids and low in carbohydrates is recommended [Saheki & Kobayashi 2005, Saheki et al 2006, Dimmock et al 2007, Saheki et al 2008, Dimmock et al 2009].

Avoid high-carbohydrate meals and alcohol.

Arginine administration may be effective in preventing hyperammonemic crisis.

Prevention of Secondary Complications

Vitamin D deficiency and zinc deficiency are common complications in NICCD [Song et al, in preparation]. Severe infection and liver cirrhosis have also been reported to be lethal complications in some individuals with NICCD. Therefore, vitamin D and zinc supplements and active infection control are recommended in NICCD.

Surveillance

To monitor for emergence of the FTTDCD phenotype in persons with citrin deficiency older than age one year: close surveillance of anthropometric indices, such as height, weight, and head circumference; serum lipid levels, including triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol.

It is recommended that the following be measured every several months:

- Plasma ammonia concentration (especially in the evening or 2 hours after feeding)
- Plasma citrulline concentration
- Serum PSTI concentration

Increases in plasma citrulline concentration and serum PSTI suggest onset of CTLN2 [Tsuboi et al 2001, Mutoh et al 2008] and should trigger initiation of treatment.

Agents/Circumstances to Avoid

Low-protein/high-caloric (high-carbohydrate) diet. Although a low-protein/high-caloric diet helps prevent hyperammonemia in urea cycle enzyme deficiencies, it is harmful for individuals with all forms of citrin deficiency (i.e., NICCD, FTTDCD, or CTLN2) [Saheki et al 2004, Saheki & Kobayashi 2005, Saheki et al 2006]. A high-carbohydrate diet may increase NADH production, disturb urea synthesis, and stimulate the citrate-malate shuttle, resulting in hyperammonemia, fatty liver, and hypertriglyceridemia [Saheki & Kobayashi 2002, Imamura et al 2003, Saheki et al 2006, Saheki et al 2007].

Infusion of sugars, such as glycerol, fructose, and glucose. Severe brain edema treated with glycerol-containing osmotic agents has resulted in continued deterioration and is contraindicated in those with CTLN2 [Yazaki et al 2005]. Degradation of large amounts of glycerol and fructose generates NADH in the liver, which may disturb liver function and produce toxic substances [Saheki et al 2004, Yazaki et al 2005, Takahashi et al 2006].

Infusion of high-concentration glucose may also exacerbate hyperammonemia [Tamakawa et al 1994, Takahashi et al 2006].

Note: Mannitol infusion appears to be safer [Yazaki et al 2005].

Alcohol. Drinking alcohol can trigger the onset of CTLN2 because alcohol dehydrogenase (ADH) generates NADH in the cytosol of the liver.

Medications. Acetaminophen and rabeprozole may trigger CTLN2 [Shiohama et al 1993, Imamura et al 2003].

Evaluation of Relatives at Risk

It is appropriate to test at-risk asymptomatic sibs of a proband for citrin deficiency so that appropriate dietary management of infants (discontinuation of breast feeding and introduction of lactose-free and/or MCT-enriched formulas) can be instituted before symptoms occur.

See Genetic Counseling for issues related to testing of at-risk relatives for genetic counseling purposes.

Therapies Under Investigation

Search ClinicalTrials.gov for access to information on clinical studies for a wide range of diseases and conditions. Note: There may not be clinical trials for this disorder.

Other

Glycerol or similar drugs containing glycerol and fructose for brain edema are not only ineffective but also dangerous for persons with citrin deficiency (see Agents/Circumstances to Avoid).

Genetics clinics, staffed by genetics professionals, provide information for individuals and families regarding the natural history, treatment, mode of inheritance, and genetic risks to other family members as well as information about available consumer-oriented resources. See the GeneTests Clinic Directory.

See Consumer Resources for disease-specific and/or umbrella support organizations for this disorder. These organizations have been established for individuals and families to provide information, support, and contact with other affected individuals.

Genetic Counseling

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members. This section is not meant to address all personal, cultural, or ethical issues that individuals may face or to substitute for consultation with a genetics professional. To find a genetics or prenatal diagnosis clinic, see the GeneTests Clinic Directory.

Mode of Inheritance

Citrin deficiency is inherited in an autosomal recessive manner.

Risk to Family Members

Parents of a proband

- The parents of an affected child are obligate heterozygotes and therefore carry one mutant allele.
- Occasionally a parent may have two mutated *SLC25A13* alleles without severe symptoms of CTLN2, a finding in two of 48 fathers and one of 54 mothers tested in 163 Japanese families with NICCD [Kobayashi et al 2006].
- Heterozygotes (carriers) are asymptomatic.

Sibs of a proband

- When both parents are carriers, each sib of an affected individual has, at conception, a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier.
- When one parent is a carrier and the other parent has two mutated *SLC25A13* alleles, each sib of an affected individual has, at conception, a 50% chance of inheriting two mutated *SLC25A13* alleles and being affected and a 50% chance of inheriting one mutated *SLC25A13* allele and being an asymptomatic carrier.
- Heterozygotes (carriers) are asymptomatic.

Offspring of a proband. The offspring of an individual with citrin deficiency are obligate heterozygotes (carriers) for a disease-causing mutation in *SLC25A13*.

Other family members of a proband. Each sib of the proband's parents is at a 50% risk of being a carrier.

Carrier Detection

Carrier testing for at-risk family members is possible once the mutations have been identified in the family.

Related Genetic Counseling Issues

See Management, Evaluation of Relatives at Risk for information on evaluating at-risk relatives for the purpose of early diagnosis and treatment.

Family planning

- The optimal time for determination of genetic risk, clarification of carrier status, and discussion of the availability of prenatal testing is before pregnancy.
- It is appropriate to offer genetic counseling (including discussion of potential risks to offspring and reproductive options) to young adults who are affected, are carriers, or are at risk of being carriers.

DNA banking is the storage of DNA (typically extracted from white blood cells) for possible future use. Because it is likely that testing methodology and our understanding of genes, mutations, and diseases will improve in the future, consideration should be given to banking DNA of affected individuals. See Testing for a list of laboratories offering DNA banking.

Prenatal Testing

Prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis [Zhao et al 2011] usually performed at approximately 15 to 18 weeks' gestation or chorionic villus sampling (CVS) at approximately ten to 12 weeks' gestation. The disease-causing mutations in the family must have been identified before prenatal testing can be performed.

Note: Gestational age is expressed as menstrual weeks calculated either from the first day of the last normal menstrual period or by ultrasound measurements.

Requests for prenatal testing for conditions which (like citrin deficiency) have treatment available are not common. Differences in perspective may exist among medical professionals and within families regarding the use of prenatal testing, particularly if the testing is being considered for the purpose of pregnancy termination rather than early diagnosis. Although most centers would consider decisions about prenatal testing to be the choice of the parents, discussion of these issues is appropriate.

Preimplantation genetic diagnosis (PGD) may be available for families in which the disease-causing mutations have been identified. For laboratories offering PGD, see Testing.

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Molecular Genetics

Information in the Molecular Genetics and OMIM tables may differ from that elsewhere in the GeneReview: tables may contain more recent information. —ED.

Table A. Citrin Deficiency: Genes and Databases

Gene Symbol	Chromosomal Locus	Protein Name	Locus Specific	HGMD
SLC25A13	7q21.3	Calcium-binding mitochondrial carrier protein Aralar2	SLC25A13 @ LOVD	SLC25A13

Data are compiled from the following standard references: gene symbol from HGNC; chromosomal locus, locus name, critical region, complementation group from OMIM; protein name from UniProt. For a description of databases (Locus Specific, HGMD) linked to, click here.

Table B. OMIM Entries for Citrin Deficiency (View All in OMIM)

603471	CITRULLINEMIA, TYPE II, ADULT-ONSET; CTLN2
603859	SOLUTE CARRIER FAMILY 25 (CITRIN), MEMBER 13; SLC25A13
605814	CITRULLINEMIA, TYPE II, NEONATAL-ONSET

Normal allelic variants. The normal *SLC25A13* gene comprises 18 exons [Kobayashi et al 1999, Sinasac et al 1999].

Pathologic allelic variants. To date, 59 pathologic allelic variants occurring in exons or introns resulting in missense mutations, predicted truncated forms of citrin, or abnormal mRNA splicing have been reported [Kobayashi et al 1999, Yasuda et al 2000, Ben-Shalom et al 2002, Yamaguchi et al 2002, Lu et al 2005, Takaya et al 2005, Hutchin et al 2006, Ko et al 2007a, Ko et al 2007b, Komatsu et al 2008, Song et al 2008, Tabata et al 2008, Wong et al 2008, Dimmock et al 2009, Hutchin et al 2009, Song et al 2009b, Xing et al 2010, Fu et al 2011, Lin et al 2011, Song et al 2011, Wen et al 2011]. Thirteen novel pathologic variations have been identified by the authors [Song et al, unpublished data].

- Two mutations (c.1177+1G>A and c.851-854del) account for the majority (~70%) of pathologic alleles in Japanese persons with citrin deficiency.
- In a cohort of 51 persons with citrin deficiency from 50 Chinese families, four mutations (c.851-854del, c.615+5G>A, c.1750+72_1751-4dup17insNM_138459.3: 2667, and c.1638_1660dup23) accounted for 87% of the mutated alleles [Song et al 2011].
- Only one mutation, p.Arg360X, has been found in both Japanese and Northern European populations [Tabata et al 2008].

Some of the 20 mutations identified in Japanese individuals have been found in Chinese, Vietnamese, and Korean individuals with citrin deficiency (NICCD or CTLN2) [Lu et al 2005, Lee et al 2006, Song et al 2006, Tsai et al 2006, Yeh et al 2006, Ko et al 2007a, Ko et al 2007b, Song et al 2008, Tabata et al 2008].

Different mutations were found in Israel, the United States, the United Kingdom, and China [Ben-Shalom et al 2002, Hutchin et al 2006, Luder et al 2006, Dimmock et al 2007, Song et al 2008, Tabata et al 2008, Song et al 2009b, Xing et al 2010, Fu et al 2011, Song et al 2011].

Table 10. Selected *SLC25A13* Pathologic Allelic Variants

DNA Nucleotide Change (Alias [1])	Protein Amino Acid Change	Reference Sequences	Reference
c.15G>A (Ex1-1G>A)	--	NM_014251.2 NP_055066.1	Tabata et al [2008]
c.550C>T	p.Arg184X		Saheki et al [2004]
c.615+5G>A (IVS6+5G>A)	--		
c.615+1G>C (IVS6+1G>C)	--		Lu et al [2005]
c.674C>A	p.Ser225X		Kobayashi et al [1999]
c.851_854del (851del4)	p.Met285ProfsX2		
c.1078C>T	p. Arg360X		Tabata et al [2008]
c.1177+1G>A (IVS11+1G>A)	--		Kobayashi et al [1999]
c.1311+1G>A (IVS13+1G>A)	--		
c.1592G>A	p.Gly531Asp		Tabata et al [2008]
c.1638_1660dup23 (1638ins23)	p.Ala554GlyfsX17		Kobayashi et al [1999]

c.1799dupA (1800_1801insA)	p.Tyr600X		Yasuda et al [2000]
c.1801G>T	p.Glu601X		Yamaguchi et al [2002]
c.1801G>A	p.Glu601Lys		
c.1813C>T	p.Arg605X		Yasuda et al [2000]
c.1750+72_1751-4dup17ins NM_138459.3: 2667 [2] (IVS16ins3kb)	--		Tabata et al [2008]
g.20984997_20985512del516 (Ex16+74_IVS17-32del516)	--	NT_007933.14	Takaya et al [2005]

See Quick Reference for an explanation of nomenclature. *GeneReviews* follows the standard naming conventions of the Human Genome Variation Society (www.hgvs.org).

1. Variant designation that does not conform to current naming conventions

2. A complex allele with an insertion of 2667 nucleotides of processed cDNA in antisense orientation of *NUS1* at 6q22.31 (reference sequence NM_138459.3); this insertion is flanked by the 17 nucleotide duplication of intron 16 sequences (NM_014251.2:c.1751-4_-22dup17) [Tabata et al 2008].

Normal gene product. Citrin and its homolog aralar [del Arco & Satrústegui 1998] are members of the SLC25 (solute carrier family 25) protein family. Both proteins are localized in the mitochondrial inner membrane and function as a Ca^{2+}-binding/-stimulated aspartate-glutamate carrier (AGC), a component of the malate-aspartate NADH shuttle [Palmieri et al 2001, Kobayashi & Saheki 2003]. Citrin is expressed in the liver; aralar in the brain and skeletal muscle; both are expressed in the kidney and heart [Kobayashi et al 1999]. Citrin as a liver-type AGC plays a role in various metabolic pathways, including aerobic glycolysis, gluconeogenesis, the urea cycle, and protein and nucleotide syntheses [Saheki & Kobayashi 2002, Saheki et al 2004, Saheki & Kobayashi 2005, Saheki et al 2006].

Abnormal gene product. Most *SLC25A13* mutations cause or predict truncation of the citrin protein or delete a loop between the mitochondrial transmembrane domains. The lack of significant citrin protein was confirmed by Western blot analysis using antibody against the N-terminal half of the human citrin protein, which detected little or no cross-reactive immune material in liver, cultured fibroblasts, and lymphocytes from individuals with *SLC25A13* mutations [Yasuda et al 2000, Takahashi et al 2006, Dimmock et al 2007, Tokuhara et al 2007, Fu et al 2011].

Resources

See Consumer Resources for disease-specific and/or umbrella support organizations for this disorder. These organizations have been established for individuals and families to provide information, support, and contact with other affected individuals. GeneTests provides information about selected organizations and resources for the benefit of the reader; GeneTests is not responsible for information provided by other organizations.—ED.

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Medical Genetic Searches: A specialized PubMed search designed for clinicians that is located on the PubMed Clinical Queries page PubMed

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Chapter Notes

Author Notes

The first author of this review, Keiko Kobayashi, PhD, died of colon cancer on December 21, 2010. The scientific community has lost a great scientist, teacher, and friend.

Keiko Kobayashi is recognized internationally as a pioneer in citrin deficiency research. An investigator with the research group of Professor Takeyori Saheki (Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University, Japan), in 1999 she cloned the gene in which mutation is causative (*SLC25A13*) and designated the term citrin. Kobayashi also played essential roles in the discovery and designation of NICCD and FTTDCD, two early onset forms of citrin deficiency. As an outstanding molecular geneticist, she identified over 50 mutations in *SLC25A13* and diagnosed over 500 citrin-deficient patients worldwide (Japan, Korea, China, Vietnam, Malaysia, Israel, Palestine, Australia, Czech, France, Britain, and the US). She also worked tirelessly to educate the medical community about citrin deficiency, thus improving the care and prognosis of affected patients worldwide. Less than a month before her death, Dr. Kobayashi delivered a lecture on citrin deficiency to the 9th Asia-Pacific Conference on Human Genetics.

Keiko Kobayashi, the “mother of citrin deficiency,” will be remembered and sorely missed by her friends, students, colleagues, and the citrin-deficient patients whom she diagnosed.

Acknowledgments

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Revision History

- 5 January 2012 (me) Comprehensive update posted live
- 1 July 2008 (me) Comprehensive update posted live
- 28 December 2006 (kk) Revision: sequence analysis for *SLC25A13* clinically available

16 September 2005 (me) Review posted to live Web site

- 5 November 2004 (kk) Original submission

Figures

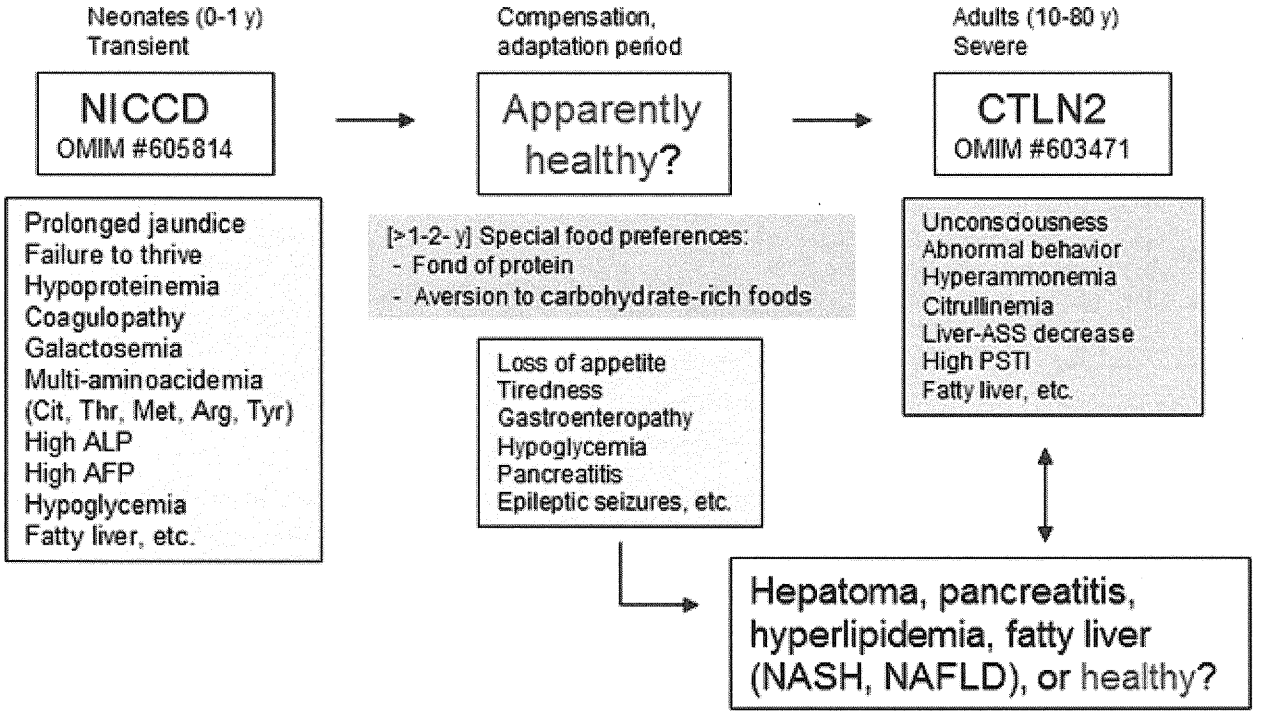

Figure 1. Citrin deficiency
NICCD = neonatal intrahepatic cholestasis caused by citrin deficiency
CTLN2 = adult-onset type II citrullinemia
Cit = citrulline
Thr = threonine
Met = methionine
Arg = arginine
Tyr = tyrosine
ALP = alkaline phosphatase
AFP = α-fetoprotein
ASS = argininosuccinate synthetase
PSTI = pancreatic secretory trypsin inhibitor
NASH = non-alcoholic steathepatitis
NAFLD = non-alcoholic fatty liver disease

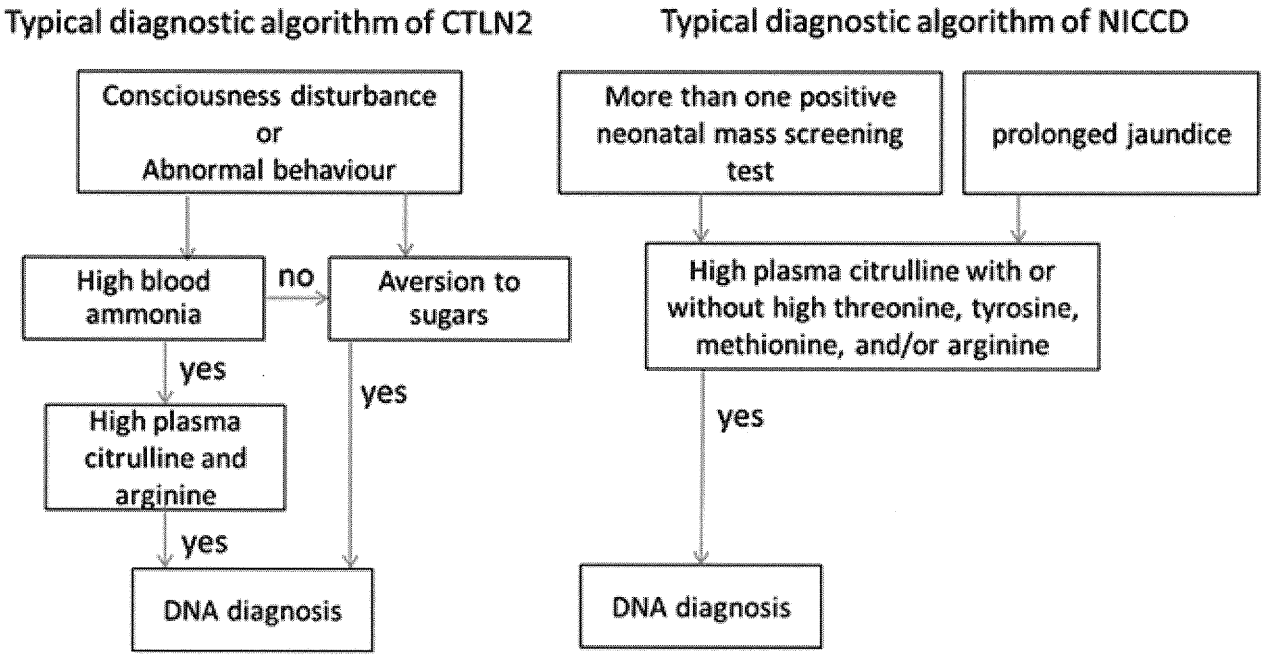

Fig. 2. Diagnostic algorithm of citrin deficiency. Note that food preferences (e.g., aversion to sugars) are important in the diagnosis of citrin deficiency, not only in typical CTLN2 but also in cases of growth retardation, hypoglycemia, pancreatitis, hypertriglyceridemia, etc. in both children and adults.

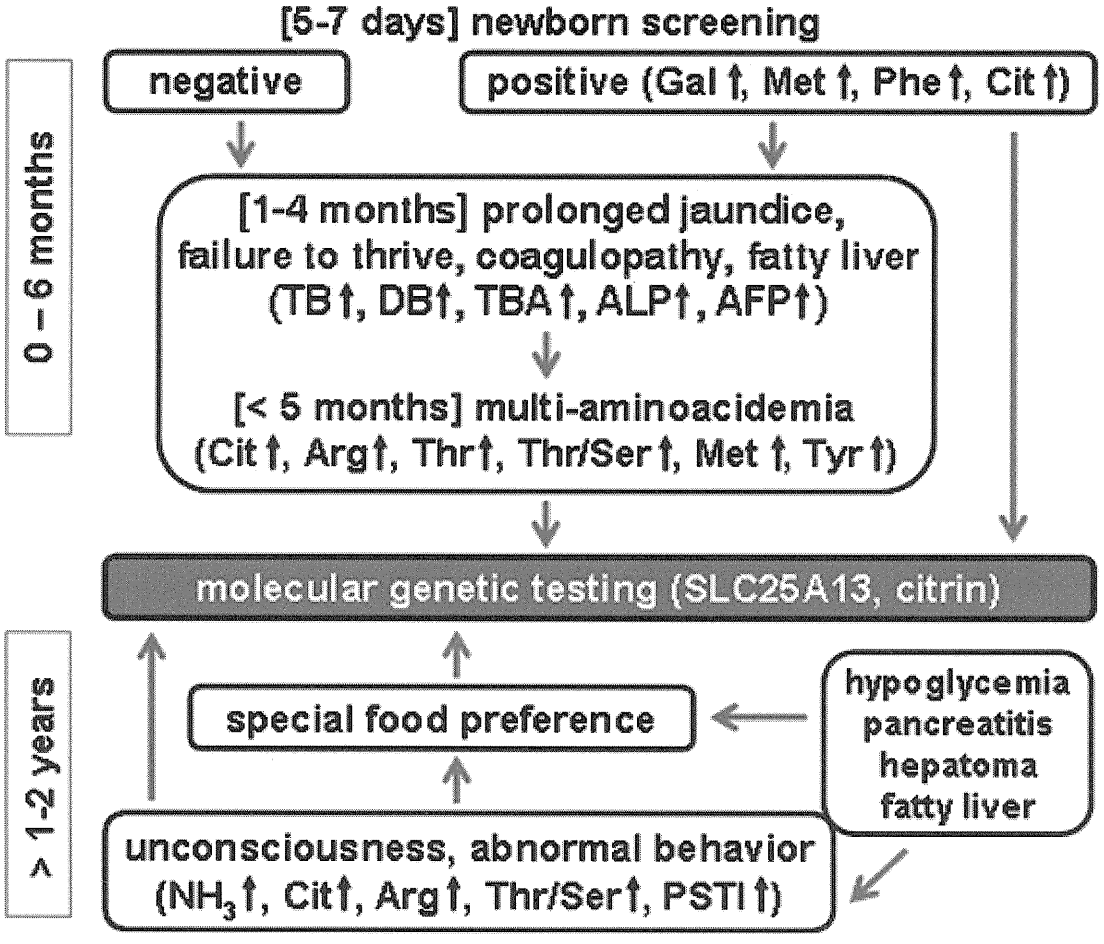

Figure 3. \Flow chart for diagnosis of citrin deficiency

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CASE REPORT

Neonatal intrahepatic cholestasis associated with citrin deficiency (NICCD): a case series of 11 Malaysian patients

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Abstract Citrin deficiency, aetiologically linked to mutations of SLC25A13 gene, has two clinical phenotypes, namely adult-onset type II citrullinaemia (CTLN2) and neonatal/infantile intrahepatic cholestasis, caused by citrin deficiency (NICCD). Malaysian patients with NICCD, especially of Malay and East Malaysian indigenous descent, have never been reported in the literature. We present the clinical features, biochemical findings and results of molecular analysis in 11 Malaysian children with NICCD. In this case series, all patients manifested prolonged cholestatic jaundice and elevated citrulline levels. The other more variable features included failure to thrive, bleeding diathesis, hypoproteinaemia, abnormal liver enzymes, prolonged coagulation profile, hyperammonaemia, hypergalactosaemia, multiple aminoacidaemia, elevated α-feto protein and urinary orotic acid as well as liver biopsies showing hepatitis and steatosis. DNA analysis of SLC25A13 revealed combinations of 851del4 (Ex9), IVS16ins3kb and 1638ins23. Most of our patients recovered completely by the age of 22 months. However, one patient had ongoing symptoms at the time of reporting and one had died of liver failure. Since a small percentage of children with NICCD will develop CTLN2 and the mechanisms leading to this is yet to be defined, ongoing health surveillance into adulthood is essential.

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Competing interests: None declared.

References to electronic databases: NICCD: OMIM #605814
Citrin: Solute Carrier Family 25, Member 13; SLC25A13 OMIM *603859

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Introduction

Citrin, a bipartite protein located in the mitochondrial inner membrane, functions as a calcium-binding/stimulated aspartate-glutamate carrier that is a component of the malate-aspartate NADH (nicotinamide adenine dinucleotide) shuttle. It is mainly expressed in the liver and plays an important role in the metabolic pathways of aerobic glycolysis, gluconeogenesis, urea cycle, and synthesis of proteins and nucleotides (Kobayashi et al. 1999; Saheki and Kobayashi 2002; Saheki et al. 2002, 2004; Tamamori et al. 2004). The deficiency of citrin results in two main clinical phenotypes: the adult-onset type II citrullinaemia (CTLN2) that mainly presents with neuropsychiatric symptoms of confusion, seizure, coma and death associated with hyperammonaemia, and the neonatal/infantile form of transient intrahepatic cholestatic hepatitis (NICCD: OMIM #605814) Table 1 and Table 2.

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Table 1 Summary of clinical characteristics of 11 infants with NICCD

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
Sex	Male	Female	Male	Female	Female	Female	Male	Female	Male	Male	Female
Ethnic group	Chinese	Malay	Chinese	Chinese	Malay	Malay	Iban	Kadazandusun	Malay	Chinese	Malay
Birth weight (kg)	2.5	2.3	2.46	2.95	2.44	2.1	2.8	2.5	2.5	2.6	2.5
Consanguinity	No	No	?	No	No	Yes	No	No	No	No	No
Type of feeding (duration)	BF (3 weeks)	Mix	IF	Mix	BF (5 weeks)	BF (6 months)	BF (3 months)	BF	Mix	Mix	BF
Age at Px[a] (weeks)	3	8	8	8	5	20	4	10	8	8	4
Px[a] complaint	J	J	J	J	J	J	J	J	J	J	J
FTT[b]	+	+	+	-	-	+	-	+	-	-	-
Hepatomegaly	+	+	-	-	-	+	+	+	-	+	+
Splenomegaly	+	+	-	-	-	+	-	+	-	-	-
Development	N	N	Mild delay	N	N	N	N	Mild delay	N	N	N
Resolution of symptoms and biochemistry (age in months)	6½	14	16	5	Ongoing	9	6	Died at 9 months	Ongoing	5	Ongoing

BF Breast fed, *IF* infant formula, *J* jaundice, *N* normal

[a] *Px* Presentation

[b] *FTT* Failure to thrive

Children with NICCD, a recessively inherited condition, are typically small for gestational age and present in infancy with prolonged cholestatic jaundice and failure to thrive. Biochemical findings include hypoproteinaemia, decreased coagulation factors, haemolytic anaemia, mild liver dysfunction, ketotic hypoglycaemia, hypergalactosaemia, multiple aminoacidaemia including high citrulline, arginine, threonine, methionine and tyrosine, and high levels of α-feto protein. Liver biopsy may show diffuse fatty infiltration and hepatic fibrosis. These symptoms generally disappear by 1 year of age and are followed by a peculiar aversion to carbohydrate-rich food and a preference for protein and lipid-rich food (Tazawa et al. 2004; Ohura et al. 2007). Although benign in most, some children with NICCD may develop liver failure or CTLN2 decades later requiring liver transplant (Tamamori et al. 2002; Tomomasa et al. 2001).

Citrin deficiency was initially reported solely in Japanese subjects. However, recent literature reports of non-Japanese patients have emerged, including those of Chinese origin (residing in China and Taiwan), Korean, Vietnamese (residing in Taiwan, China, Korea, Australia, France and Czech Republic), Palestinian, Israeli and Caucasian (residing in the United Kingdom and United States of America) (Ben-Shalom et al. 2002; Luder et al. 2006; Yeh et al. 2006; Dimmock et al. 2007; Ko et al. 2007). The SLC25A13 gene encodes citrin and, to date, 32 different mutations have been identified including the very specific IVS16ins3kb found relatively frequently in Japanese patients but not outside the East Asian population (Tabata et al. 2008).

Malaysia has a heterogenous population, with Malay being the predominant ethnic group followed by Chinese, Indian and indigenous subpopulations such as Iban and Kadazandusun. NICCD patients of Malay and East Malaysian indigenous descent have never been reported in the literature. We present 11 patients, including 5 Malay, 4 Chinese and 2 East Malaysian indigenous children, with clinical and biochemical features consistent with NICCD. DNA diagnosis for SLC25A13 mutations revealed combinations of the mutations 851del4(Ex9), IVS16ins3kb and 1638ins23 in our patients. The recently described mutation IVS16ins3kb was found solely in Japanese, Chinese and Korean patients. In this study, we report for the first time the IVS16ins3kb mutation in 4 patients of Malay descent.

Patients

Patient 1 had jaundice on day 3 of life which resolved with 4 days of phototherapy. He was the youngest of 4 children, all of whom had physiological jaundice in infancy. At 3 weeks old, he was re-admitted with increasing cholestatic jaundice, hypoproteinaemia and mildly elevated liver trans-

Table 2 Summary of laboratory data of 11 infants with NICCD at presentation

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
Citrulline (6 – 22 μmol/l)[a]	30	59	332	965	355	NA	128.63	61	176.12	265	440
threonine (89 – 232 μmol/l)	304	472	N	1054	352.1	NA	NA	126	NA	595	465
methionine (17 – 43 μmol/l)	74	149	311	360.1	123.4	NA	81.98	325	113.04	146	456
tyrosine (30 – 193 μmol/l)	145	130	N	365	N	NA	217.89	320	219.87	N	N
arginine (46 – 172 μmol/l)	237	164	220	364.3	124	NA	2.66	125	16.85	199	146
Urine reducing sugar	+	-	+	+	+	-	-	+	-	-	+
Total Galactose (DBS[b]) (NR <552 μmol/l)	99	NA	NA	NA	4,245	NA	NA	157	4,287	NA	7,060
T. bili[c] (3 – 17 μmol/l) [direct]	130.3 [68]	164 [53.3]	277 [173]	136 [75]	277 [111]	75 [31]	153 [76]	809 [368]	181 [127]	202 [158]	218 [168]
Albumin (35 – 50 g/l)	27	25	22	27	27	34	4.3	29	31	25	23
ALP[d] (117 – 390U/l)	1,058	611	1,379	1,144	958	780	1,015	661	1,374	1,475	1,815
AST[e] (0 – 50U/l)	104	153	95	112	NA	127	67	202	97	187	30
ALT[f] (6 – 39U/l)	53	49	29	48	20	41	27	67	35	48	31
Ammonia (14 – 55 μmol/l)	22.9	41	151	76.4	NA	124.5	46.9	110	NA	NA	NA
Hypoglycaemia	No	No	No	No	No	No	No	No	No	No	No
α-fetoprotein (ng/ml)[g]	30716.3	27480.5	NA	>35,000	NA	1307.6	NA	NA	NA	NA	NA
Hepatobiliary Ultrasound	Hepato-splenomegaly	Hepato-splenomegaly	N	N	N	Enlarge, fatty liver+ splenomegaly	NA	Hepatosplenomegaly +R hydronephrosis	N	NA	NA
Liver biopsy	NA	NA	NA	NA	NA	Hepatitis with steatosis	NA	Neonatal hepatitis	NA	NA	NA
SLC25A13 Mutation	Htz[i] 851del4 (Ex9)	Hmz[h] IVS16ins3kb	Hmz[h] 851del4 (Ex9)	Htz[i] 851del4 (Ex9)	None found	Hmz[h] IVS16ins3kb	None found	None found	Htz[i] 851del4 (Ex9) and Htz[h] IVS16in3kb	Htz[i] 851del4 (Ex9) and Htz[h] 1638ins23	Htz[i] IVS 16ins3kb

N Normal, *NA* Not applicable

[a] Normal range in parentheses

[b] Dried blood spot

[c] Total bilirubin

[d] Alkaline phosphatase

[e] Aspartate transaminase

[f] Alanine transaminase

[g] Normal range of α-fetoprotein in adult is <50 ng/ml. At birth, infants have 4 or more orders of magnitudes above this normal range, decreasing to it within the first 2 years of life

[h] Homozygote

[i] Heterozygote

aminases. A diagnosis of breast milk jaundice was made in light of normal G6PD, thyroid function, full blood count, negative Coomb's test and normal infective hepatitis screen. However, an increasing trend of conjugated hyperbilirubinaemia, unsatisfactory weight gain and hepatosplenomegaly prompted further investigations at 10 weeks old. Radio-imaging excluded biliary atresia. α-feto protein was markedly elevated and plasma amino acid profile was consistent with citrin deficiency. He was commenced on lactose free milk, vitamin K and multivitamins. Over the course of the next 4 months, he sustained good weight gain with normal developmental milestones and resolution of jaundice, hepatosplenomegaly and α-feto protein. Plasma amino acid normalised at 15 months old. At 17 months of age, he suffered 4 episodes of generalised tonic clonic convulsions, 2 of them associated with fever and 2 episodes unexplained. Electroencephalogram was normal. Growth, physical examination and biochemical data remained normal at 7 years old.

Patient 2 was first noticed to be jaundiced at 2 months old. Her older sister reportedly suffered physiological jaundice in infancy. Jaundice persisted at 4½ months old associated with 3-cm hepatomegaly and 2-cm splenomegaly below the costal margin. Abdominal ultrasonography excluded biliary atresia. Abnormal investigations were liver function tests, coagulation profile, lactate and α-feto protein. Urine orotic acid was mildly elevated at 5.2 Mmol/mol creat (NR 1.0–3.2). Extensive investigations ruled out infective hepatitis, storage diseases and neonatal haemachromatosis. She was empirically treated as having a mitochondrial respiratory chain disorder, and received multivitamins, folic acid, vitamin B complex and advice on avoidance of fasting. Serum amino acid subsequently revealed a profile consistent with NICCD and the diagnosis was supported by DNA analysis of SLC25A13. Mitochondrial cocktail was discontinued. Growth was slow in the first year of life with an average increase of 400 g per month; however, this eventually normalised. Citrullinaemia resolved at 8 months of age with normalisation of liver function and urine orotic acid at 14 months of age. In her second year of life, she was noticed to have a peculiar fondness for protein-rich food like peanuts and seafood and an aversion to carbohydrate-rich food. She is currently 6 years old and enjoys excellent health.

Patient 3 is a 4-year-old Chinese boy from the east of Malaysia. Antenatal and family history was unobtainable as he was an adopted child. He was admitted at 1 month old with cholestatic jaundice and elevated liver transaminases that were attributed to an overdose of Dothiepin and Diazepam, antidepressants taken by his biological mother. His urine reducing sugars were recorded as 4+. He was discharged well after this event but re-presented at 2 months old with increasing cholestatic liver dysfunction. NICCD was diagnosed based on serum amino acid and DNA analysis of SLC25A13. He was commenced on lactose free milk with supplementation of fat-soluble vitamins and MCT oil. Cholestasis resolved at 16 months old and Citrulline level normalised at 22 months of age. This patient also demonstrated a preference for protein-rich foods with an aversion to sugary foods. Currently, his growth is normal, but development is delayed especially in the area of speech.

Patient 4 is a 3½-year-old Chinese girl who had transient neonatal physiological jaundice on day 3 of life. This resolved with natural sunlight but subsequent intermittent jaundice led to further investigations at 2 months of age. NICCD was diagnosed based on clinical phenotype, conjugated hyperbilirubinaemia, plasma amino acid profile and markedly elevated α-feto protein. She received lactose free formula milk, supplementation of fat soluble vitamins and MCT oil. All investigations normalised at 5 months old. Her physical examination, growth and development are normal at the time of reporting.

Patient 5 had jaundice on day 4 of life which persisted at 5 weeks old. She was otherwise clinically well and thriving. Investigations including plasma amino acid profile and elevated total galactose level were consistent with NICCD. Breast feeding was continued supplemented by lactose free milk. At her current age of 11 months old, development and physical examination are normal. Plasma citrulline level remained high at 328.2 μmol/l.

Patient 6 is a 6-year-old Malay girl born via emergency caesarean section to parents who are second cousins. Of her 4 siblings, one older brother (current age 19 years) had a history of prolonged neonatal jaundice that resolved without sequelae. She had jaundice on day 3 of life which resolved with 2 days of phototherapy. She re-presented at 5 months old with cholestatic jaundice, poor weight gain, 3-cm hepatomegaly and 1-cm splenomegaly below the costal margins. Ultrasonography of the hepatobiliary system showed hepatosplenomegaly with features of fatty change. A liver biopsy showed generalised macrovesicular steatosis with bile stasis, portal tract expansion by lymphocytic infiltrate with associated interface hepatitis. Unfortunately, plasma amino acid was not performed at the acute stage, but elevated ammonia and α-feto protein accompanied by homozygocity for IVS16ins3kb mutation in SLC25A13 gene led to the diagnosis of NICCD. Urine orotic acid was elevated at 8 Mmol/mol creat. In addition, she had hypokalaemia and hypophosphataemia with normal anion gap metabolic acidosis that was attributed to renal tubulopathy. This resolved spontaneously without intervention. Jaundice resolved at the age of 9 months with normalisation of liver transaminases at the age of 1 year and 3 months. Plasma amino acid is now normal.

Patient 7, the 3rd child of Ibanese (Malaysian aborigines) parents, presented at 1 month old with jaundice which persisted to 6 months of age. Examination revealed 5-cm hepatomegaly below the costal margin. NICCD was diagnosed based on clinical phenotype and amino acid profile. He was commenced on lactose-free diet with supplementation of multivitamins. At his current age of 3 years, he remains clinically well.

Patient 8, the 4th child of Kadazandusun (Malaysian aborigines) parents, presented at 10 weeks old with prolonged conjugated hyperbilirubinaemia. Investigations ruled out biliary atresia and liver biopsy showed neonatal hepatitis. She was followed up in the district hospital as liver disease progressively worsened. At 7 months old, she was admitted in a decompensated state with liver failure, coagulopathy and gastrointestinal bleed precipitated by sepsis. Physical examination revealed growth parameters below the 3rd percentile, jaundice, ascites and hepatosplenomegaly recorded at 4.5 cm and 5 cm below the costal margins, respectively. Doppler ultrasound of the abdomen suggested cirrhotic changes in the liver, gross splenomegaly and mild right hydronephrosis. Liver enzymes and coagulation profile were abnormal with ongoing bleeding requiring rescue with fresh frozen plasma. Full blood count showed normocytic normochromic anaemia. Investigations for infective hepatitis, autoimmune hepatitis, hypothyroidism, Wilson's disease, α-1-Anti-Trypsin deficiency yielded negative results. Urine reducing sugars were positive but total galactose level was normal. Serum amino acid profile was consistent with citrin deficiency. She was treated with lactose free diet, multivitamins, folate and vitamin D. Unfortunately, she passed away from liver failure shortly after.

Patient 9 is a 6-month-old Malay boy, who had transient physiological jaundice at 2 days old, re-presented at 2 months old with conjugated hyperbilirubinaemia. Physical examination was unremarkable. Investigations ruled out biliary atresia, hypothyroidism and congenital infections. Plasma amino acid profile, elevated galactose level and DNA analysis of SLC25A13 led to the diagnosis of NICCD. He was commenced on lactose-free milk as a supplement with continuation of breast feeding.

Patient 10 is a 5-month-old Chinese boy who presented with prolonged cholestatic jaundice at 2 months old. Physical examination revealed a well and thriving child with jaundice and 3-cm hepatomegaly below the costal margin. Plasma amino acid profile was consistent with NICCD and this was supported by DNA analysis of SLC25A13.

Patient 11 is a 3-month-old Malay girl who presented with prolonged cholestatic jaundice at 1 month old. Clinically, she was jaundiced, has bilateral cataract and 3-cm hepatomegaly. Citrin deficiency was diagnosed based on clinical and biochemical data. She was commenced on lactose-free diet and examination at 3 months of age showed resolving jaundice and cataracts.

Molecular analysis

Fifteen known common mutations among Asians were screened according to the methods described previously by using GeneScan/SNaPshot and/or PCR/gel running (Tabata et al. 2008). These mutations are: (1) [851del4 (Ex9)]; (2) (g.IVS11+1 G>A); (3) [1638ins23 (Ex16)]; (4) [S225X (Ex7)], (5) (g.IVS13+1 G>A); (6) [1800ins1 (Ex17)]; (7) [R605X (Ex17)]; (8) [E601X (Ex17)]; (9) [E601K (Ex17)]; (10) (g.IVS6+5 G>A); (11) [R184X (Ex6)]; (14) (g.IVS6+1 G>C); (16) [G531D (Ex16)]; (19) (g.IVS16ins3kb); (20) (g.Ex16+74_IVS17-32del516).

Discussion

The diagnosis of NICCD can be reliably achieved by molecular studies as mutations of the SLC25A13 gene had been established as the cause of citrin deficiency. However, studies have estimated that mutations are not detected in about 15% of cases (Tokuhara et al. 2007). So far, 32 mutations have been described and novel mutations are still being discovered. The phenotype of NICCD patients have been delineated in studies over the past decade (Ohura et al. 2007; Saheki et al. 2002, 2004; Tazawa et al. 2004). The diagnosis of NICCD in our series is confirmed by molecular studies in 5 patients (patient 2, 3, 6, 9, 10); in the other 6 patients, published clinical phenotype and biochemical data form the basis of our diagnosis. Interestingly, all our patients who carry the mutation IVS16ins3kb are from the Malay ethnic group. This mutation was thought to have occurred historically very early on in East Asia and has never been reported outside the Japanese, Chinese and Korean populations. This may lend support to the theories regarding the origin of Malaysians of Malay descent. One theory postulates migration from Yunnan, China; another states that they are sea migrants originally from Taiwan. Genetic heterogeneity exists among different races in the mutations of SLC25A13. This may be the other reason that abnormal mutations were not found in patients 7 and 8, children of Iban and Kadazandusun descent. The Ibans are the original inhabitants of Borneo Island and Kadazandusun is an ethnic group indigenous to the state of Sabah in East Malaysia (North Borneo). With advancement in this field, these samples should be re-tested for novel mutations of SLC25A13.

The characteristic clinical feature of NICCD is neonatal hepatitis associated with cholestasis. Some patients are