CASE REPORT

Distinct outcomes of chloride diarrhoea in two siblings with identical genetic background of the disease: implications for early diagnosis and treatment

P Höglund, C Holmberg, P Sherman, J Kere

Abstract

Background—Congenital chloride diarrhoea (CLD, OMIM 214700) is a serious inherited defect of intestinal electrolyte absorption transmitted in an autosomal recessive fashion. The major clinical manifestation is diarrhoea with high chloride content which can be balanced by substitution. The molecular pathology involves an epithelial Cl/HCO₃− exchanger protein, encoded by the solute carrier family 26, member 3 gene (SLC26A3), previously known as CLD or DRA (downregulated in adenomas). To date, almost 30 different mutations in the SLC26A3 gene have been identified throughout the world. No clear genotype-phenotype correlation has been established.

Patients/methods—Two siblings presenting with CLD were studied for disease history, supplementation, or other treatments, and for mutations in the SLC26A3 gene.

Results—Mutation analysis revealed a homozygous I544N mutation in both patients. However, despite the uniform genetic background of CLD in this family, the clinical picture and outcome of the disease were remarkably different between siblings. The older sibling had a late diagnosis and chronic course of the disease whereas the younger one, who was diagnosed soon after birth and immediately received supplementation therapy, grows and develops normally.

Conclusion—Time of diagnosis, substitution therapy, compliance, and compensatory mechanisms are more important modulators of the clinical picture of CLD than the type of mutation in the SLC26A3 gene.

Keywords: chloride diarrhoea; SLC26A3 gene;

Patients with congenital chloride diarrhoea (CLD, OMIM 214700; http://www.ncbi.nlm.nih.gov/omim) have been reported worldwide in Caucasian, Oriental, and Black populations, and mutations in the solute carrier family 26, member 3 (SLC26A3) gene at chromosomal location 7q31 have been found to be responsible for the phenotype in all patients studied. In general, CLD is thought to be a rare disease but there are three populations with a higher disease incidence. In east central Finland, genetic founder effects (bottleneck phenomenon) reduced the pool of alleles among a limited number of settlers in the 16th century. Subsequent expansion of the isolated founder population during the 18th century led to enrichment of a single V317del mutation, with an estimated incidence of 1 in 20 000. In Poland, genetic studies have revealed at least three similar but more recent and more local founder effects, which together with a predominant I675–676ins founder mutation revealed an incidence of 1 per 200 000 live births in Poland. Less is known about low incidence and total absence of the Polish major mutation in other European countries, especially in those neighbouring Poland. Solitary case reports exist, supporting CLD as a known entity. Highest frequencies (up to 1 in 3200) have been reported among Arabic people where parental consanguinity is common; the G187X mutation is responsible for more than 90% of these cases. Sporadic patients from other populations usually have two previously uncharacterised unique mutations in their SLC26A3 alleles with no previous suspicion of CLD in the family. Evidently, mutations in the SLC26A3 gene appear to occur relatively frequently, and the actual number of patients may be underestimated. The variety of mutations has made it possible to evaluate the phenotype-genotype correlation, if present.

Case reports

PATIENT NO 1

The propositus was born as the first child of unrelated parents of North Vietnamese origin. After eight months’ gestation the baby was delivered by caesarean section because of maternal polyhydramnios in a refugee camp in Hong Kong. His birth weight was 2500 g. From birth he was noted to have watery stools. Shigellosis was treated but diarrhoea persisted leading to severe malnourishment and a
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Prolonged period of time receiving total parenteral nutrition. His motor developmental milestones were slightly delayed. At the age of 4.8 years, oral substitution with 300 mg/day KCl was started with a beneficial effect on weight gain.

By age 5, the family had moved to Canada and the patient was re-evaluated because of chronic diarrhoea. *Giardia lamblia* infection was treated with metronidazole. However, his stools remained watery and increased in frequency to up to seven times per day. His height and weight were below the third percentile for age. Laboratory tests showed normal erythrocyte sedimentation rate, immunoglobulin levels, serum urea, creatinine, alkaline phosphatase, and albumin. Faecal quantitative fat, lactose hydrogen breath test, α, antitrypsin, and α, antitrypsin clearance were normal. There was no metabolic alkalosis and aldosterone (120 pmol/l) and renin (1.10 ng/l/s) levels were within the normal range. Plasma angiotensin converting enzyme levels were slightly elevated (80 U/l, normal adult range 0–75 U/l). Serum Na, Cl, Ca, Mg, and phosphate concentrations were within the normal range but he was slightly hypokalaemic (serum potassium 2.7–3.4 mmol/l). Urine analysis was normal. Faecal Cl concentration was 152 mmol/l, Na 88 mmol/l, and K 38 mmol/l, fulfilling the two diagnostic criteria of CLD (faecal chloride content >90 mmol/l and faecal cationic gap F-Na+ +K+ <Cl−). Supplementation therapy was initiated with a dose of 6 mmol/kg chloride per day, divided equally between NaCl and KCl.

He continued to have watery stools 6–8 times per day but serum potassium concentration normalised. A trial of omeprazole (20 mg/day) had no effect on stool frequency or consistency. Episodes of marked hypokalaemia and slight metabolic alkalosis resulted from discontinuation of substitution therapy. The patient refused to take electrolyte substitution and no catch up growth spurt has been observed. At the age of 14 years his height and weight still remain below the third percentile. He attends a normal school on a regular basis and participates fully in extracurricular activities.

**PATIENT NO 2**

In the next pregnancy, maternal polyhydramnios was noted and the baby was delivered after 36 weeks' gestation by caesarean section. Apgar scores were 9/9 and birth weight was 2750 g. At birth, the boy was noted to have abdominal distention, and no meconium was observed. After a barium enema, he started to pass stools. He was discharged home at the age of six days. Three days later he was readmitted because of jaundice, abdominal distention, and apnoea. Bowel obstruction was suspected. His serum electrolytes were: K 2.4 mmol/l, Na 99 mmol/l, and Cl 60 mmol/l, and he was markedly dehydrated with a metabolic alkalosis. With intravenous and subsequent oral rehydration his condition stabilised. Re-evaluation of the family history prompted a study of stool electrolyte concentrations. A diagnosis of CLD was confirmed on the basis of high faecal chloride concentration (120 mmol/l). Electrolyte supplementation was started and his total electrolyte intake was 10.6 mmol/kg Na, 5.4 mmol/kg K, and 11.3 mmol/kg Cl per day. He recovered and returned home at 26 days of age. He has been passing watery stools 4–8 times per day. He had a brief trial of omeprazole (10 mg/day) but was discontinued without any effects on his diarrhoea. On follow up, growth and development are normal, height being at the 75–90th percentile and weight at the 50th percentile for age. His serum electrolyte concentrations and kidney function are normal, and he excretes chloride in urine.

**SEARCH FOR MUTATIONS IN THE SLC26A3 GENE**

Genomic DNA from blood samples from both brothers and their parents was prepared according to standard procedures. Exon specific primers and conditions used in the polymerase chain reaction (PCR) amplification of genomic DNA have been described previously.17 PCR fragments were recovered and sequenced using an automated sequencer (ABI373A). Sequencing of the whole coding region and exon-intron boundaries of the SLC26A3 gene resulted in identification of a single novel missense mutation (fig 1). Both siblings were found to be homozygous for a T to A change at the nucleotide position 1631 in exon 15, and their parents were heterozygous carriers. The change leads to an isoleucine to asparagine change at codon 544 (I544N) in the predicted SLC26A3 transmembrane protein sequence. The codon 544 resides at a site which is highly conserved among the human, animal, and plant members of the SLC26 family (previously known as “sulphate transporter family”). These proteins share high sequence homology to each other and most act as anion transporters, but structurally they are clearly distinct from the “classical” anion exchanger family. Analysis of healthy individuals from

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**Figure 1** 
**I544N mutation of the SLC26A3 exon 15** identified by sequencing in both siblings. (Top) Homozygous sequence change from patient No 1. (Bottom) Normal sequence from an unaffected control individual. The arrow shows the T to A mutation, changing an isoleucine residue at 544 to asparagine.
Coriell Institute’s DNA Polymorphism Discovery Resource (n=44) and anonymous Finnish blood donors from the Finnish Red Cross (n=30) revealed no carriers of this nucleotide change. No other sequence changes in the coding region or in the exon-intron boundaries of the SLC26A3 gene were identified in these patients, and no locus heterogeneity is known for CLD (Table 1). Thus this amino acid substitution was considered to be responsible for the functional abnormality of the Cl−/HCO3− exchanger.

**Discussion**

Despite the uniform genetic background of CLD in this family, the clinical picture and outcome of the disease were remarkably different in the siblings. A neonate that rapidly develops a severe potentially fatal state of dehydration, hypoelectrolytaemia, and hyperbilirubinaemia is the classical presentation of CLD. They do not pass meconium and Hirschsprung’s disease may be suspected. Watery diarrhoea may go unnoticed for some time because it is easily confused with urine. An infant may lose over 10% of its weight in the first day of life. Early diagnosis is essential as hyponatraemic episodes in infancy may result in mental and psychomotor impairment. A family history may provide valuable information. Here, the well documented history of the younger sibling of the proband fits these features of CLD. He had a fulminant hypoelectrolytaemia and dehydration postnatally which, together with the family history, alerted the paediatricians to suspect CLD. Supplementation therapy was started during the neonatal period providing the opportunity for normal growth and development. The optimal dose of chloride varies between 6 and 8 mmol/kg/day in neonates (given as 2:1 NaCl:KCl), and smaller doses (4 mmol/kg/day) are sufficient in older patients, maintaining serum chloride levels within the normal range with chloride excretion in urine.

Patients who remain undiagnosed in early infancy and survive, like the proband, have a chronic course of the disease with persistent hypovolaemia and hypelectrolytaemia that leads to growth retardation. Older patients with an undiagnosed and/or untreated disease tend to present more variation in their clinical outcome of the disease were remarkably different in the siblings. A neonate that rapidly develops a severe potentially fatal state of dehydration, hypoelectrolytaemia, and hyperbilirubinaemia is the classical presentation of CLD. They do not pass meconium and Hirschsprung’s disease may be suspected. Watery diarrhoea may go unnoticed for some time because it is easily confused with urine. An infant may lose over 10% of its weight in the first day of life. Early diagnosis is essential as hyponatraemic episodes in infancy may result in mental and psychomotor impairment. A family history may provide valuable information. Here, the well documented history of the younger sibling of the proband fits these features of CLD. He had a fulminant hypoelectrolytaemia and dehydration postnatally which, together with the family history, alerted the paediatricians to suspect CLD. Supplementation therapy was started during the neonatal period providing the opportunity for normal growth and development. The optimal dose of chloride varies between 6 and 8 mmol/kg/day in neonates (given as 2:1 NaCl:KCl), and smaller doses (4 mmol/kg/day) are sufficient in older patients, maintaining serum chloride levels within the normal range with chloride excretion in urine.

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Increased weight, but should not exceed 4 mmol chloride/kg/day in the older child and adult. Non-compliant and under substituted patients should have glomerular filtration rate measured at regular intervals.

We conclude that the time of diagnosis, optimal substitution therapy, compliance, and compensatory mechanisms, such as dietary habits and activation of the renin-aldosterone system, are more important modulators of the clinical manifestations of the disease than the specific type of mutation in the SLC26A3 gene. Screening of faecal chloride concentration should be performed in all patients, especially neonates with a family history of congenital chloride diarrhoea or an undefined intestinal absorption defect with a clinical picture including polyhydramnios, prematurity, and chronic watery diarrhoea. Early diagnosis and maintenance of optimal treatment will increase the likelihood of these children growing and developing normally, and avoiding long term complications in adulthood.

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