

## CASE REPORT

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

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### 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  $\text{Cl}^-/\text{HCO}_3^-$  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. (Gut 2001;48:724–727)

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.<sup>1–5</sup> 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.<sup>1</sup> 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.<sup>2</sup> 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<sup>3</sup> 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 proband 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

**Abbreviations used in this paper:** CLD, congenital chloride diarrhoea; SLC26A3, solute carrier family 26, member 3; PCR, polymerase chain reaction.

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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,  $\alpha_1$  antitrypsin, and  $\alpha_1$  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<sup>6</sup>) 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.<sup>2,7</sup> 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<sup>2</sup> from

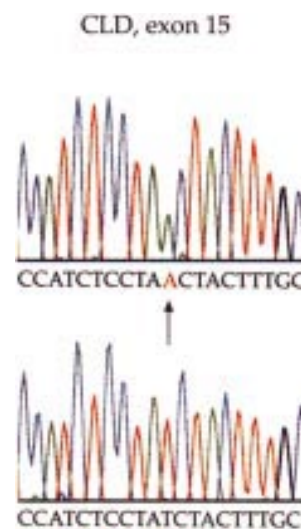


Figure 1 I544N mutation of the SLC26A3 exon 15 identified by sequencing in both siblings. (Top) Homozygous sequence change from patient No 1. (Bottom) Normal sequence from an unaffected control individual. The arrow shows the T to A mutation, changing an isoleucine residue at 544 to asparagine.

Table 1 Summary of all mutations reported in the SLC26A3 gene worldwide. The three founder mutations are highlighted

Mutation name	Exon/intro n	Nucleotide change	Effect on coding sequence, amino acid change	Codon	Predicted protein product	Patient origin	No	Ref
Missense								
G120S	Exon 4	G>A at 358	Glycine→serine at 120	120	Conserved residue	Poland, Sweden, Norway	3	3
H124L	Exon 4	A>T at 371	Histidine→leucine at 124	124	Conserved residue	Poland, Sweden (Polish origin)	3	1
P131R	Exon 5	C>G at 392	Proline→arginine at 131	131	Partially conserved	USA	2	3
S206P	Exon 6	T>C at 616	Serine→proline at 206	206	Conserved residue	Holland (Morocco origin)	1	17
D468V	Exon 12	A>T at 1403	Aspartic acid→valine at 468	468	Conserved residue	Poland	1	17
L496R	Exon 13	T>G at 1487	Leucine→arginine at 496	496	Partially conserved	USA, Hong Kong	3	2
I544N	Exon 15	T>A at 1631	Isoleucine→asparagine at 544	544	Conserved residue	Vietnam	2	17
Nonsense								
<b>G187X</b>	Exon 5	G>T at 559	Glycine→STOP at 187	187	Truncated	Saudi Arabia, Kuwait	9	2
Y305X	Exon 8	C>A at 915	Tyrosine→STOP at 305	305	Truncated	Poland	2	3
Q436X	Exon 11	C>T at 1306	Glutamine→STOP at 436	436	Truncated	Holland	1	17
Deletion								
344delT	Exon 4	Deletion of T at 344	Frameshift	115	Truncated	Poland	2	1
3.5 kb deletion	Intron 6–8	Deletion of 3.5 kb genomic DNA	Loss of exons 7 and 8, frameshift		Deleted	Japan	2	17
<b>V317del</b>	Exon 8	Deletion of GGT at 951–953	In frame loss of a valine at 317	317	Present, function abnormal	Finland, Sweden (Finnish origin)	41	1
1342–1343delTT	Exon 12	Deletion of TT at a342–1343	Frameshift	448	Truncated	Japan	1	16
1516delC	Exon 14	Deletion of C at 1516	Frameshift	505	Truncated	Poland	1	3
1548–1551delAACC	Exon 14	Deletion of AACC at 1548–1551	Frameshift	516	Truncated	Poland	1	3
Y527del	Exon 14	Deletion of TTA at 1578–1580	In frame loss of a tyrosine at 527	527	Deleted	Poland	1	3
1609delA	Exon 15	Deletion of A at 1609	Frameshift	537	Truncated	Canada	1	2
2116delA	Exon 19	Deletion of A at 2116	Frameshift	706	Truncated	Finland	1	3
Insertion								
177–178insC	Exon 3	Insertion of C at 177–178	Frameshift	60	Truncated	USA	1	3
268–269insAA	Exon 3	Insertion of AA at 268–269	Frameshift	90	Truncated	Hong Kong	3	3
<b>I675–676ins</b>	Exon 18	Insertion of ATC at 2025–2026	In frame addition of an isoleucine	676		Poland	12	3
Replacement								
2104–2105delGGins29bp	Exon 19	Replacement of GG with 29 bp insertion	Frameshift	703	Truncated	Norway	2	17
Splice defect								
IVS5–2A>G	Intron 5	A>G at 571–2	Destruction of the intron acceptor site AG			Canada	1	3
IVS5–1G>T	Intron 5	G>T at 571–1	Destruction of the intron acceptor site AG			USA	1	3
IVS11–1G>A	Intron 11	G>A at 1312–1	Destruction of the intron acceptor site AG			Poland	1	3
IVS12–1G>C	Intron 12	G>C at 1408–1	Destruction of the intron acceptor site AG			Germany (Palestine)	1	17
IVS13–2delA	Intron 13	Deletion of A at 1515–2	Destruction of the intron acceptor site AG			Kuwait	1	17

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<sup>8</sup> (table 1). Thus this amino acid substitution was considered to be responsible for the functional abnormality of the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> protein.<sup>5</sup>

### 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.<sup>8–11</sup> Early diagnosis is essential as hyponatraemic episodes in infancy may result in mental and psychomotor impairment.<sup>12</sup> 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.<sup>8</sup>

Patients who remain undiagnosed in early infancy and survive, like the proband, have a chronic course of the disease with persistent hypovolaemia and hypoelectrolytaemia that leads to growth retardation. Older patients with an undiagnosed and/or untreated disease tend to present more variation in their clinical

picture as dietary compensation, such as consumption of salt, varies between patients. Acute worsening of the clinical condition may follow common infections or vomiting. The proband was diagnosed as having CLD at the age of five years after a long period of chronic diarrhoea of unknown origin. Thereafter, compliance with electrolyte and fluid substitution was poor, inhibiting normal growth. In the long term, chronic contraction of the intravascular space predisposes these patients to complications such as renal impairment and gout.<sup>8 11 13 14</sup> To date, four patients diagnosed late or receiving suboptimal or no substitution for long periods of time have developed end stage renal failure leading to dialysis and renal transplantation (Holmberg, unpublished).

Therapeutic manoeuvres to reduce diarrhoea have met with little success.<sup>11</sup> Cholestyramine reduces stool volumes in the short term.<sup>15</sup> This may reflect the ability of cholestyramine to bind bile acids which reach the colon with increased ileal effluent and stimulate an additional chloride secretory response.<sup>11</sup> The only other situation in which diarrhoea is reduced is chronic intravascular volume contraction which, in the long term, leads to renal injury. Omeprazole, an inhibitor of the gastric proton pump, was suggested for CLD patients because of its ability to inhibit secretion of chloride by the gastric mucosa. In a patient with CLD and severe diarrhoea, treatment with omeprazole decreased the amount of stool chloride losses and stool volumes.<sup>6</sup> In that case, optimisation of oral salt substitution would probably have the same effect because over substitution exacerbates diarrhoea by an osmotic mechanism. Because chloride absorption in CLD patients occurs passively and is determined by the intraluminal concentration of chloride, it may be expected that diarrhoea could be reduced by decreasing the intraluminal Cl<sup>-</sup> concentration, either by inhibiting Cl<sup>-</sup> secretion or by limiting oral salt substitution. Consequently, these patients develop Cl<sup>-</sup> deficiency, chronic intravascular volume contraction, and are at high risk of developing end stage renal disease. Ideally, the concentration of electrolytes in the small intestine should be only slightly higher than in plasma, enabling net absorption of both salt and water. Omeprazole therapy had no beneficial effect in either of our patients.

There is no need to over substitute patients with CLD because diarrhoea and incontinence greatly reduce their quality of life. Monitoring hydration, electrolyte balance, pH, and excretion of chloride in urine provides valuable information on the delicate electrolyte balance of these patients, and should be carried out frequently during the first years of life and at regular intervals in adults. Furthermore, families should be provided with detailed instructions for additional substitution and/or contact their paediatrician during intercurrent infectious diseases or episodes of vomiting. In growing children, electrolyte doses need to be regularly increased to meet the requirements of

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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