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Genetic Background of Congenital Chloride Diarrhea in High-Incidence Populations: Finland, Poland, and Saudi Arabia and Kuwait

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Summary

Congenital chloride diarrhea (CLD) is an inherited intestinal disorder caused by mutations in the down-regulated in adenoma gene. In Finland, the disease is prevalent because of a founder effect, and all but one of the CLD-associated chromosomes carry the same mutation, V317del. In Poland, another area with a high incidence of CLD, as many as seven different mutations have been detected so far. A third known cluster of CLD, around the Persian Gulf, has not been genetically studied. We studied the allelic diversity of CLD in Poland, in Saudi Arabia and Kuwait, and in three isolated families from North America and Hong Kong. Altogether, eight novel mutations were identified, making a total of 19 known CLD gene mutations. The Polish major mutation I675–676ins was found in 47% of the Polish CLD-associated chromosomes. Haplotype analysis and clustering of the I675–676ins mutation supported a founder effect and common ancestral origin. As in Finland, a major founder effect was observed in Arab patients: 94% of the CLD-associated chromosomes carried a nonsense mutation, G187X, which occurred in either a conserved ancestral haplotype or its derivative. Our data confirm that the same locus is mutated in all cases of CLD studied so far. In Poland, a relatively common founder mutation is likely to highlight a set of rare mutations that would very rarely produce homozygosity alone. This suggests that mutations in the CLD locus are not rare events. Although the disease is thought to be rare, undiagnosed patients may not be uncommon.

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Introduction

Mutations in the down-regulated in adenoma gene (DRA [MIM 126650]) recently have been shown to cause congenital chloride diarrhea (CLD [MIM 214700]; McKusick 1994), an intestinal disorder of chloride and bicarbonate transport (Höglund et al. 1996). The DRA gene initially was cloned on the basis of its absent expression in colonic neoplasia (Schweinfest et al. 1993). Since then, its involvement in intestinal sulfate, oxalate, (Silberg et al. 1995) and chloride transport has been demonstrated (R. H. Moseley, P. Höglund, D. G. Silberg, S. Haila, C. Holmberg, A. de la Chapelle, G. D. Wu, and J. Kere, unpublished data). The predicted CLD protein is a transmembrane protein that, by amino acid homology, belongs to the sulfate permease gene family. Despite the clinical observation of both chloride and bicarbonate trafficking defects in CLD, this set of transporters is distinct from the genes encoding known human anion exchangers (AE1–AE3).

The clinical picture of CLD includes a life-long watery diarrhea with a high concentration of chloride, electrolyte disturbances, and metabolic alkalosis (Darrow 1945; Gamble et al. 1945; Perheentupa et al. 1965; Norio et al. 1971; Holmberg et al. 1977). Patients receiving adequate salt and fluid supplementation grow and develop normally and may have a normal life span (Holmberg 1986). Complications such as renal impairment and gout result from chronic dehydration and vascular contraction (Pasternack and Perheentupa 1966; Holmberg et al. 1977; Holmberg 1986; Nuki et al. 1991) and are prevented most effectively by supplementation therapy. A few reported patients with a later-age diagnosis of CLD probably have coped with their disease by the consumption of salty foods (Pearson et al. 1973; Holmberg et al. 1977; Leskinen 1996); their long-term renal prognosis is unknown.

The disease is known worldwide but, in most populations, has been reported only occasionally. Three regions with elevated incidence include Finland, Poland,

and countries around the Persian Gulf. In Poland, there are ~40 known patients with CLD (Tomaszewski et al. 1987; J. Socha, unpublished data), and one or two new patients emerge annually. No specific ethnic groups have been recognized as having increased incidence. The diagnostics of childhood chronic diarrhea has been focused in one hospital (Center of Child Health, Warsaw), whose clinicians are familiar with this disorder. Thus the number of undiagnosed patients with CLD is probably low in this country, and the disease incidence is ~1: 200,000. Because of the dispersed health-care services in Saudi Arabia and Kuwait, the incidence of CLD is more difficult to estimate. So far, 46 patients have been reported (Lubani et al. 1989; Shaltout et al. 1989; Abdullah et al. 1990; Khan and Yaish 1992; Kagalwalla 1994; Al-Abbad et al. 1995). Since a significant number of patients are likely to remain undiagnosed, incidence figures as high as 1:5,500 have been estimated (Kagalwalla 1994). Approximately one-fourth of all patients worldwide occur in the Finnish population, where at least 52 patients are currently known. In Finland, the high incidence (1:10,000 in the high-risk area; Höglund et al. 1995) is caused by genetic drift in combination with two successive founder effects, the latest of which occurred during the 16th century (Norio et al. 1971; Norio 1981; de la Chapelle 1993). Not surprisingly, a single ancestral mutation, V317del, has been found in all but one of the Finnish CLD chromosomes studied (Höglund et al. 1996, 1998).

In the present investigation, we studied the genetic background of CLD in two other high-incidence areas: (1) Poland and (2) Saudi Arabia and Kuwait. We constructed CLD-associated haplotypes spanning 800 kb of genomic DNA around the CLD gene, searched for additional mutations by SSCP and genomic sequencing, and compared the spectrum of mutations versus the haplotype data and genealogical information.

Subjects, Material, and Methods

Subjects and Samples

Altogether, 32 patients diagnosed as having CLD were studied. The Polish patients were 11 males and 9 females born during 1969–95. DNA samples were available from these 20 patients and from 32 parents. The four Saudi Arabian families consisted of five patients, three unaffected siblings, and eight parents. The Kuwaiti families consisted of five patients and six parents. In addition, two isolated cases of CLD, one from Canada and one from the United States, were studied, as was a family with three affected members, from Hong Kong. The clinical phenotype of all patients was characterized by a profuse diarrhea with a high chloride content (>90 mmol/liter) in watery stools, justifying the diagnosis of CLD. There were no obvious phenotypic differences between these patients. The severity of the intestinal chloride transport defect cannot be predicted on the basis of the absolute concentration of chloride in stools, since it fluctuates, depending on diet, hydration status, salt intake, and electrolyte balance. To test the population frequency of each sequence change observed, DNA samples from Finnish blood donors were used as controls. DNA was prepared from blood, as described elsewhere (Lahiri and Nurnberg 1991). Appropriate informed consent was obtained from each individual participating in the study.

Mutation Analyses

Mutations in the coding region of the CLD gene were detected and identified as described elsewhere (Höglund et al. 1998). In brief, each exon of the CLD gene was amplified by use of specific intronic primers (Haila et al., in press). The amplified exons were screened, by SSCP analysis, for a mobility change, and suggested sequence changes were identified by sequencing using dye-terminator chemistry and an automated sequencer (ABI 373A; Applied Biosystems). The observed mutations were screened in control individuals by either direct visualization of the altered PCR fragment size in polyacrylamide gel (for mutations 268–269insAA, I675–676ins, and 1609delA), SSCP (for mutations G187X and IVS11-G→A), or detection of either novel restriction sites (for mutations IVS5-2A→G and L496R) or loss of restriction sites present in wild-type alleles (for mutation IVS5-1G→T).

Haplotype Analysis

DNA was amplified by use of four polymorphic markers—D7S2420, D7S496, D7S2459, and D7S2456 (Dib et al. 1996)—as described elsewhere (Höglund et al. 1995). These markers define a haplotype that, altogether, spans 0.8 cM (0.8 Mb). The intermarker distances are as follows: D7S2420–0 cM, 300 kb–D7S496–0 cM, 150 kb–D7S2459–0.8 cM, 350 kb–D7S2456. The PCR products were separated by electrophoresis in 6% polyacrylamide gels, and the fragments were visualized by silver staining. The CLD-associated haplotypes were constructed manually. When parental samples were available from one parent only (in the case of two Polish families and four Kuwaiti families), haplotype identities were inferred on the basis of the associated most conserved haplotype.

Results

Analysis of the coding region and exon/intron boundaries of the CLD gene led to the identification of 8 novel mutations, making the total number of all known mutations 19 (fig. 1*E* and tables 1 and 2).

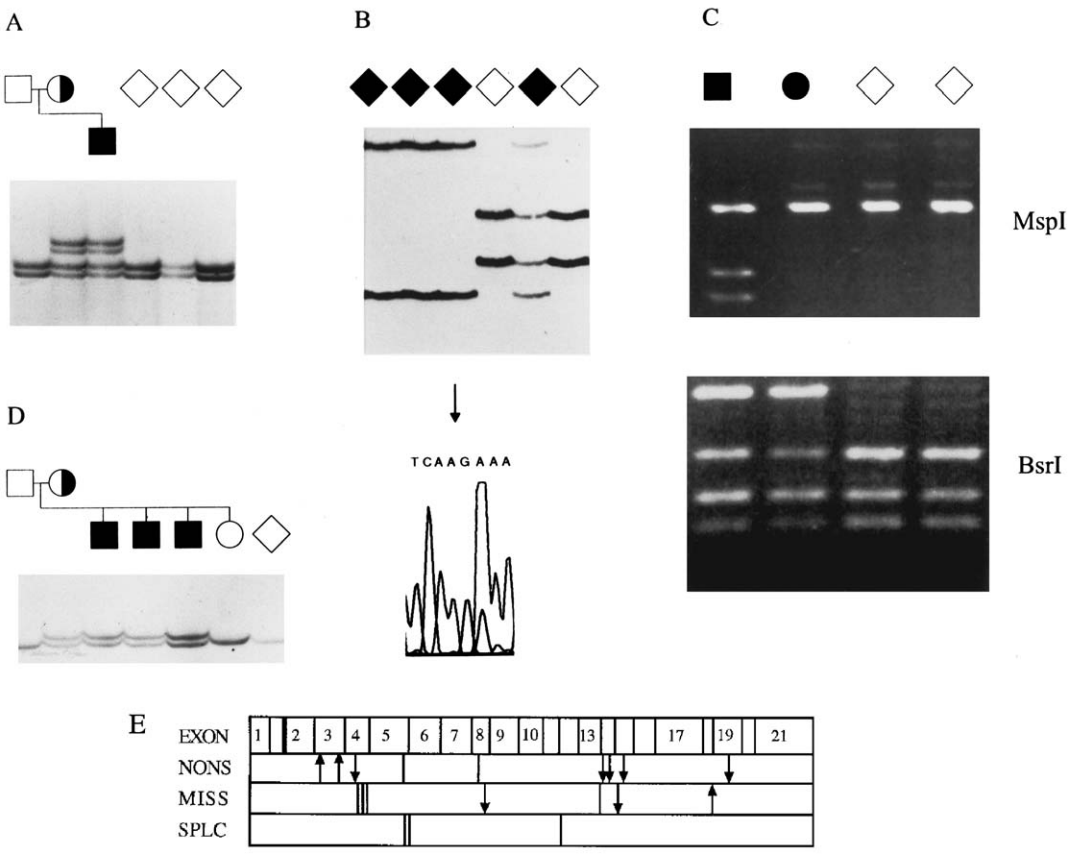

Figure 1 Identification of five novel mutations of the CLD gene. The patients with CLD are denoted by blackened symbols (squares denote males, circles denote females, and diamonds denote individuals whose gender is not known), and healthy control individuals and family members are denoted by unblackened symbols (for those with no depicted mutation) or half-blackened symbols (for carriers). The 3-bp insertion responsible for the Polish major mutation, I675–676ins, was identified, by PAGE, as an altered size of a genomic PCR fragment (*A*). The Saudi Arabian and Kuwaiti major mutation, G187X, was screened for by SSCP analysis. The arrow indicates the reverse-strand homozygous C→A sequence change leading to the mutation (*B*). The identification of two different nucleotide changes of the intron 5 acceptor site AG (IVS5-1G→T and IVS5-2A→G) in two unrelated North American patients was accomplished by two separate restriction analyses. Both of the mutations led to a loss of a restriction site for enzyme *Bsr*I, and the A→G mutation generated an additional restriction site for *Msp*I (*C*). An insertion mutation (268–269insAA) in a Chinese family with three affected children is shown in a silver-stained polyacrylamide gel (*D*). The distribution of all known CLD gene mutations is shown (*E*). Exons are denoted by numbered boxes (numbers have been omitted from short exons). The thicker vertical bar in exon 2 marks the translation-initiation codon. Mutations are indicated are belonging to three classes: nonsense mutations (NONS), caused by point mutations resulting in a stop codon (*vertical bar*), out-of-frame insertions (*upward-pointing arrows*), or deletions (*downward-pointing arrows*); missense mutations (MISS), including amino acid substitutions (*vertical bars*), single-amino-acid insertions (*upward-pointing arrrow*), or deletions (*downward-pointing arrows*); and splice-site mutations (SPLC), all in acceptor sites of the corresponding introns.

Poland

The most prevalent Polish mutation was an insertion of three nucleotides (ATC) between nucleotides 2025 and 2026, which resulted in an in-frame addition of an isoleucine in the predicted intracellular C-terminus of the CLD protein (I675–676ins; see fig. 1). This insertion mutation was found in 47% of all Polish disease-associated chromosomes. Another Polish mutation characterized here was a G→A substitution in the splice-acceptor site AG at intron 11, leading to the loss of the splice site (IVS11-1G→A).

In studying a set of 17 Polish families by using four polymorphic markers spanning 400 kb on both sides of the CLD gene, we found 16 different CLD-associated haplotypes (fig. 2*A*). An identical core haplotype could be assumed in four sets of haplotypes with a unique mutation each, supporting the hypothesis that these mutations have the same ancestral origin. Genealogical studies showed no close consanguinity between the families. The distribution of the birthplaces of all known Polish CLD grandparents (fig. 3*A*) shows clustering in the middle and southern parts of the country. The pattern of CLD parents (fig. 3*B*) is less informative, a result that is understandable in light of recent migrations.

Table 1

CLD Patients Ascertained in the Present Study

Origin and No. of Patients	Mutations
Hong Kong:	
3	268–269insAA, L496R
Canada:	
1	IVS5-2A→G, 1609delA
United States:	
1	IVS5-1G→T, P131R
Kuwait:	
4	G187X, G187X
1	G187X, unknown
Saudi Arabia:	
4	G187X, G187X
Poland:	
1	IVS11-1G→A, H124L
2	I675–676ins, H124L
1	I675–676ins, 344delT
1	I675–676ins, Y527del
1	I675–676ins, 1548–1551delAACC
1	I675–676ins, G120S
2	I675–676ins, unknown
4	I675–676ins, I675–676ins
2	Unknown, unknown

However, not a single case was observed in the northern cities. If we take a closer look at the spectrum of mutations in separate geographical regions, the four mutations each found in more than one CLD chromosome showed clustering. The major insertion mutation was most prevalent in the southern part of the country, whereas Y305X, H124L, and 344delT occurred in the eastern, southeastern, and middle parts of Poland, respectively (fig. 3). Of the 34 Polish CLD chromosomes, 5 carried a unique mutation and 4 an unidentified mutation.

Saudi Arabia and Kuwait

The most frequent Saudi Arabian and Kuwaiti mutation was a G→T transversion at nucleotide position 559. The predicted amino acid change was a substitution of glycine by the termination codon at 187 (G187X; see fig. 1). The haplotypes segregating with G187X were well conserved and suggested a common origin for this mutation (fig. 2*B*). The geographical distribution of the Saudi Arabian patients was throughout the country, whereas the Kuwaiti patients reside within the Al-Jahra region, which has $\leqslant$300,000 inhabitants (of whom 240,000 are Bedouins; data not shown).

North America and Hong Kong

So far, the most proximal mutation detected in this gene is a 2-bp insertion (AA) between nucleotides 268 and 269. The insertion causes a frameshift leading to a nonsense change at codon 91, followed by a termination at codon 93 (fig. 1). The insertion mutation was present in three affected sisters and their mother, from Hong Kong, in heterozygous form. The second mutation segregating in this family was a T→G transversion at nucleotide position 1487, causing a leucine→arginine amino acid substitution at codon 496. The three novel North American mutations all were found in single disease-associated chromosomes only. The first one was a deletion of nucleotide A at position 1609, leading both to a frameshift and nonsense change at codon 537 and to a termination codon at 575 in the predicted amino acid sequence. The other mutation in this patient was an A→G change at the AG acceptor site of intron 5, leading to the loss of a splice site (IVS5-2A→G; see fig. 1). At the same splice-acceptor site, in another North American patient, who elsewhere had been reported to have a P131R mutation in her other CLD-associated chromosome (Höglund et al. 1998), there was a G→T change (IVS5-1G→T; see fig. 1). The haplotypes of these isolated patients were not studied.

Discussion

The detection of a set of 19 mutations in the CLD gene in patients with CLD originating in several different populations confirms that there is no locus heterogeneity in CLD. Since the disease is believed to be rare worldwide, the presence of clusters of CLD families residing in certain geographic areas can, a priori, be considered to be due to founder effects in these areas. In the case of Finland, this hypothesis holds up well, since all but one of the Finnish CLD patients are homozygous for the same mutation, V317del (Höglund et al. 1996, 1998), that abolishes chloride-transport activity in vitro (R. H. Moseley, P. Höglund, D. G. Silberg, S. Haila, P. Holmberg, A. de la Chapelle, G. D. Wu, and J. Kere, unpublished data).

In Saudi Arabia and Kuwait, a founder effect combined with a high frequency of consanguineous marriages, which increase locus homozygosity, are likely to be responsible for the higher-than-average incidence of CLD. In the Al-Jahra area, whence all our Kuwaiti patients originated, the overall rate of consanguineous marriages is as high as 85%, and, of these, 95% are paternal-first-cousin marriages. Parental consanguinity was known in all Saudi Arabian and Kuwaiti CLD families included in this study.

The G187X mutation was found in all Saudi Arabian and all but one of the Kuwaiti CLD-associated chromosomes, and, in all cases, the 800-kb haplotype surrounding the disease locus supported a common origin of the mutation (fig. 2*B*). The Kuwaiti patients with G187X in both chromosomes all carried a homozygous 800-kb haplotype (2-1-2-6 or 2-1-2-3), reflecting not only close parental consanguinity (first-cousin mar-

Table 2

Summary of All Mutations Observed in the CLD Gene in CLD Patients

Population(s) (No. of Patients)	cDNA Change	Exon	Codon	Predicted Coding Change	Screening Method	Proportion of Control Chromosomes
North American (1)	177–178insC[a]	3	60	Frameshift leading to nonsense change at codon 60, followed by stop at codon 70	Altered PCR-fragment size (PAGE)	0/80
Hong Kong (3)	268–269insAA[b]	3	90	Frameshift leading to nonsense change at codon 91, followed by stop at codon 93	Altered PCR-fragment size (PAGE)	0/80
Polish (3)	344delT[b,c]	4	115	Frameshift leading to nonsense change at codon 115, followed by stop at codon 133	Altered PCR-fragment size (PAGE)	0/336
Polish, Swedish (2)	358G→A[a,b]	4	120	Glycine→serine, G120S	Gain of novel site for restriction enzyme *Eco*57I	0/100
Polish (3)	371A→T[b,c]	4	124	Histidine→leucine, H124L	Gain of novel site for restriction enzyme *Hin*fI	0/316
North American (2)	392C→G[a]	5	131	Proline→arginine, P131R	Altered PCR-fragment mobility (SSCP)	0/104
Kuwaiti, Saudi Arabian (9)	559G→T[b]	5	187	Glycine→stop, G187X	Altered PCR-fragment mobility (SSCP)	0/130
North American (1)	IVS5-1G→T[b]	…	…	Change in the intron acceptor site AG at nt −1 of intron 5, leading to loss of a splice site	Loss of a site for restriction enzyme *Bsr*I	0/112
North American (1)	IVS5-2A→G[b]	…	…	Change in the intron acceptor site AG at nt −2 of intron 5 leading to the loss of a splice site	Gain of novel site for restriction enzyme *Bsr*I, loss for enzyme *Msp*I	0/108
Polish (2)	915C→A[a]	8	305	Tyrosine→stop, Y305X	Gain of novel site for restriction enzyme *Bsu*36I	0/82
Finnish (32)	951–953delGGT[c]	8	317	In-frame loss of a valine, V317del	Altered PCR-fragment size (PAGE)	3/872
Polish (1)	IVS11-1G→A[b]	…	…	Change in the intron acceptor site AG at nt −1 of intron 11 leading to the loss of a splice site	Altered PCR-fragment mobility (SSCP) after digestion with *Rsa*I	0/84
Hong Kong (3)	1487T→G[b]	13	496	Leucine→arginine, L496R	Gain of novel site for restriction enzyme *Cvi*JI	0/156
Polish (1)	1516delC[a]	14	505	Frameshift leading to nonsense change at codon 505, followed by stop at codon 534	Altered PCR-fragment size (PAGE)	0/124
Polish (1)	1548–1551delAACC[a]	14	516	Frameshift leading to nonsense change at codon 518, followed by stop at codon 534	Altered PCR-fragment size (PAGE)	0/124
Polish (2)	1578–1580delTTA[a]	14	527	In-frame loss of a Tyrosine, Y527del	Altered PCR-fragment size (PAGE)	0/124
North American (1)	1609delA[b]	15	537	Frameshift leading to nonsense change at codon 537, stop at codon 575	Loss of site for restriction enzyme *Xmn*I	0/142
Polish (12)	2025–2026insATC[b]	18	676	In-frame addition of an isoleucine, I675–676ins	Altered PCR-fragment size (PAGE)	0/168
Finnish (1)	2116delA[a]	19	706	Frameshift leading to nonsense change at codon 706, stop at codon 711	Altered PCR-fragment size (PAGE)	0/170

[a] Source: Höglund et al. (1998).
[b] Source: present study.
[c] Source: Höglund et al. (1996).

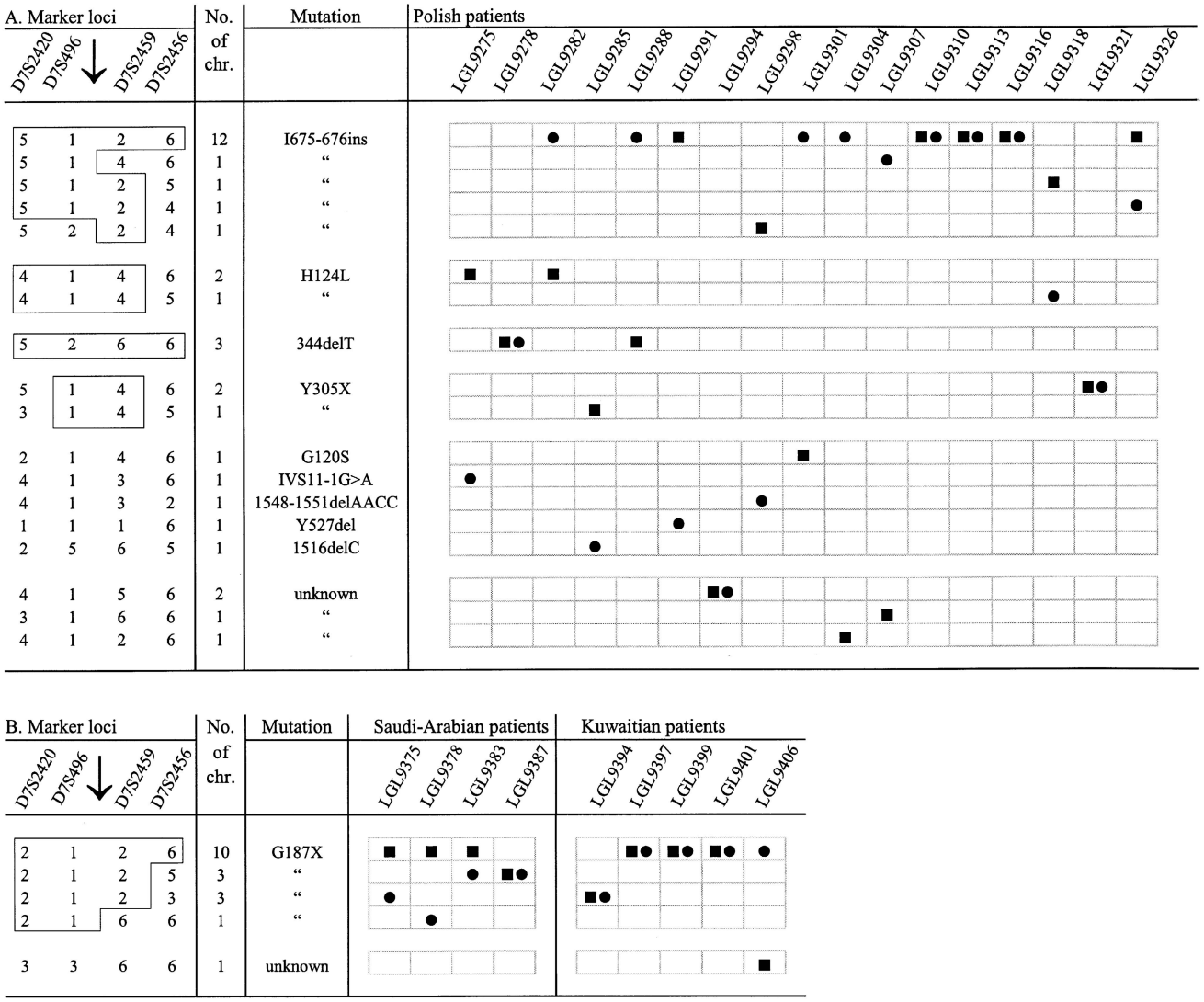

Figure 2 CLD-associated 800-kb haplotypes across the CLD gene region, in (*A*) a set of 17 unrelated Polish CLD families and (*B*) in four Saudi Arabian and five Kuwaiti families. The associated mutations and their parental origin are denoted by blackened circles (maternal) and blackened squares (paternal). The most likely localization of the CLD gene is indicated by the downward-pointing arrows.

riages) but, also, close interfamilial connections. In the Saudi Arabian families, in which the degree of parental consanguinity could not be specified, only one of the patients carried a fully homozygous 800-kb haplotype, and, as well, the overall spectrum of the haplotypes was wider than that in the Kuwaitis.

Further evidence for the common origin of the major G187X mutation in Kuwait and Saudi Arabia was provided by the population-historical data. Both the Saudi Arabian population and the majority of the Kuwaiti population descend from common ancestors, the Arabic Bedouins who reside in the Persian Gulf desert areas. Not only geographically but, also, historically and culturally, Kuwait belongs to the Arabian Peninsula, with a high rate of recent and present marriages between the Kuwaiti and Saudi Arabian families. The accurate estimation of the age or origin of the Arabic major mutation is complicated both by migration and by the high rate of consanguinity. It can be speculated that the mutation might be younger than some of the most prevalent European CLD mutations (e.g., I675–676ins), since the common ancestral haplotype is, in general, relatively well conserved, especially among the Kuwaitis.

In Poland, the situation is more complicated, in that the finding of many different mutations in a disease that is considered very rare was unexpected. To understand this phenomenon, we have haplotyped CLD chromosomes and have investigated the geographical distribution of the mutations in Poland, to trace their origins. The most common mutation, I675–676ins, was found

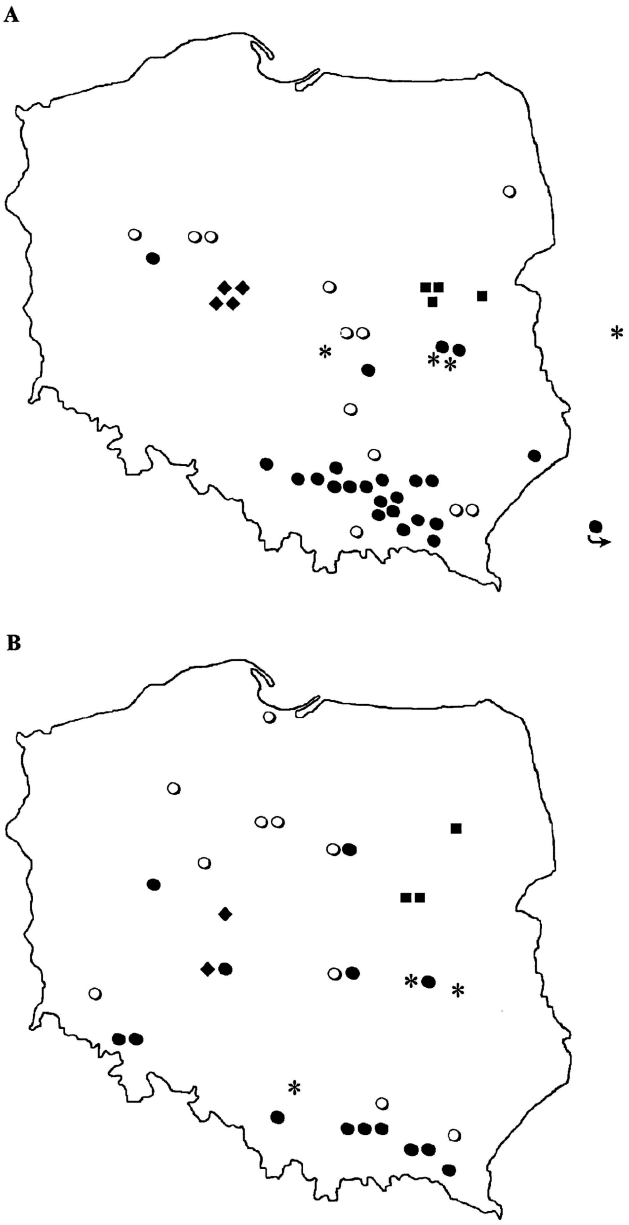

Figure 3 Distribution of the birthplaces for all known grandparents (*A*) and parents (*B*) of the Polish CLD patients. The parents carrying I675–676ins, the major insertion mutation, are denoted by blackened circles, the H124L mutation by asterisks, the 344delT mutation by diamonds, and the Y305X mutation by blackened squares. The mutations present either in a single chromosomes only or in chromosomes with unidentified an mutation are denoted by unblackened circles. Since the carrier status of the grandparental chromosomes was not studied, half of the symbols in *A* are not associated with CLD.

to be present in 16/34 (47%) of all CLD chromosomes of Polish origin. The study of flanking markers delineated a common ancestral haplotype, which supported a single origin of this mutation. Of chromosomes carrying the I675–676ins mutation, 75% (12/16) shared the same 800-kb haplotype, 5-1-2-6, whereas the remaining four chromosomes with this mutation had haplotypes 5-1-4-6, 5-1-2-5, 5-1-2-4, and 5-2-2-4, which are likely to be derivatives of the single ancestral mutated chromosome. The mutation is likely to be at least several hundred years old, since recombinations and microsatellite mutations have had time to introduce changes within the 800-kb haplotype in four chromosomes. For comparison, of 48 Finnish CLD chromosomes carrying the major mutation, V317del, with an "age" estimated to be 450 years, or 19 generations (Höglund et al. 1996), as many as 42/47 (89%) carry the identical 800-kb haplotype segregating with the disease (data not shown).

As in Finland, the geographic distribution of the I675–676ins mutation in Poland shows clustering, although it is more diffuse than that in Finland (fig. 3*A*). The tendency of the clustering to become weaker for parental versus grandparental birthplaces (fig. 3*B*) suggests that migration in Poland has been much more active during the past few decades than, for example, that in Finland, where distinct geographical clustering of CLD cases is still evident (Norio et al. 1971; Höglund et al. 1995). Furthermore, contrary to the situation in Finland, the presence of a set of other mutations increases the number of patients who are compound heterozygous for the major mutation.

In Poland, one of the mutations (344delT; see fig. 2*A*) segregated with a fully conserved 800-kb haplotype in three chromosomes. In fact, the conservation of these haplotypes was found to extend as far as 5.8 cM (data not shown). This finding, together with the putative geographical clustering of this mutation (fig. 3), suggests that this mutation is relatively recent. Two of the mutations (Y305X and H124L; see fig. 2*A*), with identical 200-kb and 550-kb core haplotypes in three disease-associated chromosomes, may be older mutations, because there has been enough time for at least one (in the case of H124L) or two (in the case of Y305X) ancient recombinations to occur in the ancestral mutation-carrying chromosomes. Thus, altogether, there are one major and three more-local founder effects in the Polish population that have made CLD a relatively common disease. The rest of the mutations were found only in a single chromosome each (fig. 2*A*).

In the absence of a predominant founder mutation, private mutations occurring alone in the population would result in a CLD incidence much lower than we have observed. Since, altogether, 12/17 patients (71%) were either homozygotes or compound heterozygotes for the major, I675–676ins mutation, only five CLD cases would have occurred if this founder mutation did not occur in Poland. Approximately half (16/34) of the CLD chromosomes studied carry the I675–676ins mutation. If random mating is assumed, results from the application of the Hardy-Weinberg equation suggest that the

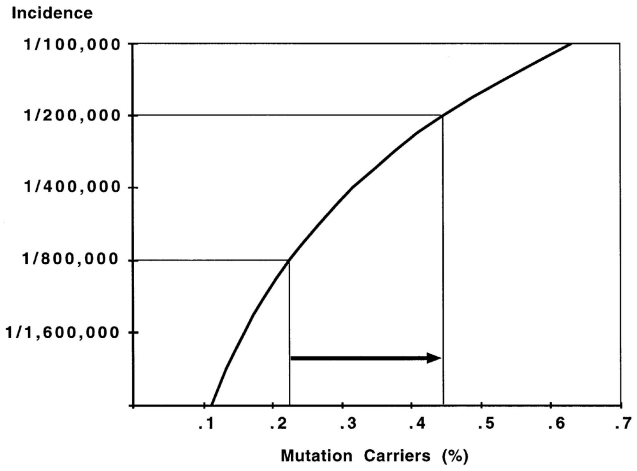

Figure 4 Effect of the major insertion mutation (I675–676ins) in Poland. Mutation-carrier status (*horizontal axis*) has been correlated with CLD incidence (*vertical axis*), to Hardy-Weinberg equilibrium. A doubling of carrier frequency, due to a founder mutation (*right-pointing arrow*), leads to a fourfold increase in CLD incidence.

effect of the I675–676ins mutation increases approximately fourfold the incidence of CLD in Poland (fig. 4), increasing it from ~1/800,000 births to 1/200,000 births.

Multiple mutations in a specific gene restricted to a certain geographical area have been reported in homogeneous isolates, such as that in Réunion Island (Allamand et al. 1995). The finding, in the calpain gene, of multiple distinct mutations (at least seven) that are responsible for recessively inherited limb-girdle muscular dystrophy type 2A has been proposed as being due to either digenic inheritance (Allamand et al. 1995; Beckmann 1996) or a high mutation rate (Zlotogora et al. 1996). In the digenic model, the disease manifestations are controlled by two unlinked genes whose mutations are required if the disease phenotype is to arise. Inactivation of one of these genes would have little or no effect on the biological phenotype, but mutations in both would result in the disease. The second gene should be more frequently mutated in the Réunion Island isolate than elsewhere (Allamand et al. 1995; Beckmann 1996). The functional evidence that the Finnish V317del mutation alone abolishes the chloride-transport activity of the CLD protein (R. H. Moseley, P. Höglund, D. G. Silberg, S. Haila, P. Holmberg, A. de la Chapelle, G. D. Wu and J. Kere, unpublished data) makes the digenic model a relatively unattractive explanation of the situation in Poland. However, mutations in the CLD gene may be more frequent than previously has been assumed. The well-conserved disease-associated haplotypes segregating with some of the Polish mutations, together with the observation of multiple different mutations in single disease-associated chromosomes, are compatible with a relatively recent occurrence of several mutations in the CLD gene. There is, of course, no evidence that mutation rates in the Polish population are generally higher than those in other populations. Moreover, there is no known selective advantage conferred to the carriers of the CLD mutations, which is in contrast to the situation with either cystic fibrosis (Chao et al. 1994) or thalassemia. It follows that multiple different mutations should occur in all populations.

The diagnosis of CLD in a newborn may be difficult if the physician is not aware of this "rare" possibility. Even in countries, such Saudi Arabia, that have a high incidence of CLD, decentralized health-care services result in the failure to diagnose a significant number of patients. This is mainly because gastrointestinal problems and, in particular, diarrheal illnesses are among the most common causes of morbidity and mortality among infants. Even though the diagnosis of CLD is seemingly simple (by fecal-chloride measurement), watery diarrhea can be confused with urine, and an infant may succumb undiagnosed. Provided that an undiagnosed infant survives its first 6 mo, it is likely to adopt a compensatory diet with salty food, which improves its prognosis. In such cases, complications are likely to shorten the life span. The oldest known living patients are ages 32 years, in Finland; 28 years, in Poland; and 14 years, in Saudi Arabia. Our study suggests that the mutation frequency in the CLD locus is not low, so CLD patients may be born in all populations, and some of the Polish CLD mutations may occur also in neighboring countries, where CLD may be a more significant clinical problem than previously has been thought. The importance of an early diagnosis of CLD is stressed by the fact that accurate therapy allows these children to grow and develop normally and, probably, also to avoid complications in the long term.

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Accession numbers and URLs for data in this article are as follows:

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References

Abdullah AMA, Shahed MM, Katugampola S, Patel PJ (1990) Congenital chloride diarrhoea: case report and review of the literature. Ann Trop Paediatr 10:71–74

Al-Abbad A, Nazer H, Sanjad S, Al-Sabban E (1995) Congenital chloride diarrhea: a single center experience with ten patients. Ann Saudi Med 15:466–469

Allamand V, Broux O, Richard I, Fougerousse F, Chiannilkulchai N, Bourg N, Brenguier L, et al (1995) Preferential localization of the limb-girdle muscular dystrophy type 2A gene in the proximal part of a 1-cM 15q15.1-15q3 interval. Am J Hum Genet 56:1417–1430

Beckmann JS (1996) The reunion paradox and the digenic model. Am J Hum Genet 59:1400–1402

Chao AC, de Sauvage FJ, Dong YJ, Wagner JA, Goeddel DV, Gardner P (1994) Activation of intestinal CFTR Cl-channel by heat-stable enterotoxin and guanylin via cAMP-dependent protein kinase. EMBO J 13:1065–1072

Darrow DC (1945) Congenital alkalosis with diarrhea. J Pediatr 26:519–532

de la Chapelle A (1993) Disease gene mapping in isolated human populations: the example of Finland. J Med Genet 30:857–865

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Gamble JL, Fahey KR, Appleton J, McLachlan E (1945) Congenital alkalosis with diarrhea. J Pediatr 26:509–518

Haila S, Höglund P, Scherer SW, Lee JR, Kristo P, Coyle B, Trembath R, et al. Genomic structure of the human chloride diarrhea (CLD) gene. Gene (in press)

Holmberg C (1986) Congenital chloride diarrhea. Clin Gastroenterol 15:583–602

Holmberg C, Perheentupa J, Launiala K, Hallman N (1977) Congenital chloride diarrhea: clinical analysis of 21 Finnish patients. Arch Dis Child 52:255–267

Höglund P, Haila S, Gustavson K-H, Taipale M, Hannula K, Popinska K, Holmberg C, et al (1998) Clustering of private mutations in the congenital chloride diarrhea/down-regulated in adenoma gene. Hum Mutat 11:321–327

Höglund P, Haila S, Socha J, Tomaszewski L, Saarialho-Kere U, Karjalainen-Lindsberg M-L, Airola K, et al (1996) Mutations in the down-regulated in adenoma (DRA) gene cause congenital chloride diarrhea. Nat Genet 14:316–319

Höglund P, Sistonen P, Norio R, Holmberg C, Dimberg A, Gustavson K-H, de la Chapelle A, et al (1995) Fine mapping of the congenital chloride diarrhea gene by linkage disequilibrium. Am J Hum Genet 57:95–102

Kagalwalla AF (1994) Congenital chloride diarrhea: a study in Arab children. J Clin Gastroenterol 19:36–40

Khan SN, Yaish HM (1992) Misdiagnosis of congenital chloride-loosing diarrhea. J Perinatol 12:112–114

Lahiri DK, Nurnberg JI (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 19:5444

Leskinen M (1996) Metabolic alkalosis in a five-year old patient with diarrhea (in Finnish) . Duodecim 112: 608–612

Lubani MM, Doudin KI, Sharda DC, Shaltout AA, Al-Shab T, Abdul Al YK, Said MA, et al (1989) Congenital chloride diarrhea in Kuwaiti children. Eur J Pediatr 148:333–336

McKusick VA (1994) Mendelian inheritance in man, 11th ed. Johns Hopkins University Press, Baltimore and London

Norio R (1981) Diseases of Finland and Scandinavia. In: Rothschild H (ed) Biocultural aspects of disease. Academic Press, New York, pp 359–415

Norio R, Perheentupa J, Launiala K, Hallman N (1971) Congenital chloride diarrhea, an autosomal recessive disease. Clin Genet 2:182–192

Nuki G, Watson ML, Williams BC, Simmonds HA, Wallace RC (1991) Congenital chloride losing enteropathy associated with tophaceous gouty arthritis. Adv Exp Med Biol 309A:203–208

Pasternack A, Perheentupa J (1966) Hypertensive angiopathy in familial chloride diarrhoea. Lancet 1047–1049

Pearson AJG, Sladen GE, Edmonds CJ, Tavill AS, Wills MR, McIntyre N (1973) The pathophysiology of congenital chloridorrhoea. Q J Med 167:453–466

Perheentupa J, Eklund J, Kojo N (1965) Familial chloride diarrhoea ("congenital alkalosis with diarrhoea"). Acta Paediatr Scand Suppl 159:119–120

Schweinfest CW, Henderson KW, Suster S, Kondoh N, Papas TS (1993) Identification of a colon mucosa gene that is down-regulated in colon adenomas and adenocarcinomas. Proc Natl Acad Sci USA 90:4166–4170

Shaltout AA, Khuffash FA, Hiilal AA, El Ghanem MM (1989) Pattern of protracted diarrhoea among children in Kuwait. Ann Trop Paediatr 9:30–32

Silberg DG, Wang W, Moseley RH, Traber PG (1995) The down regulated in adenoma (dra) gene encodes an intestine-specific membrane sulfate transport protein. J Biol Chem 270:11897–11902

Tomaszewski LM, Kulesza E, Socha J (1987) Congenital chloride diarrhoea in Poland. Materia Medica Polona 4:271–277

Zlotogora J, Gieselmann V, Bach G (1996) Multiple mutations in a specific gene in a small geographic area: a common phenomenon? Am J Hum Genet 58:241–243