Human Molecular Genetics, 1999, Vol. 8, No. 10 ***1883–1891***

A family of mammalian anion transporters and their involvement in human genetic diseases

Lorraine A. Everett and Eric D. Green+

Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA

Received May 12, 1999; Accepted June 28, 1999

Tremendous advances in human genetics have been made in recent years, as the fruits of the Human Genome Project are facilitating the identification of genes associated with myriad genetic diseases. Among the many triumphs in positional (and positional candidate) cloning are a number of cases where apparently unrelated diseases have been found to share common genetic origins. A vivid example of this has unfolded in the past few years with the identification of the genes causing diastrophic dysplasia, congenital chloride diarrhoea and Pendred syndrome. While these three disorders are clinically distinct, the associated genes (*DTDST*, *CLD* and *PDS*, respectively) emanate from a well conserved family of genes that all encode anion transporters. Our current knowledge of these diseases coupled with new insights about the implicated genes and proteins illustrates the complex nature of mammalian genomes, especially with respect to the evolutionary subtleties of protein families and tissue-specific gene expression.

INTRODUCTION

The ongoing Human Genome Project has markedly advanced the ability to elucidate the genetic bases of human disease (1,2). Specifically, improved genetic, physical and gene maps of all human chromosomes coupled with ever increasing amounts of human cDNA and genomic sequence data are providing a remarkably strong infrastructure for identifying genes by positional cloning (3) and positional candidate (4) strategies. As a consequence, human disease genes are now being identified at a striking pace (see http://genome.nhgri.nih.gov/clone).

A fundamental feature of positional cloning and positional candidate strategies is the requirement for minimal or no prior insight about the nature of the defective gene or its encoded protein. As a consequence, the characteristics of a newly identified human disease gene may or may not come as a surprise to the discoverer. A corollary to this is that genetic diseases bearing no obvious clinical resemblance to one another can turn out, quite surprisingly, to be caused by defects in closely related genes.

Here we describe an illustrative example of this exact phenomenon, where the genes associated with three human genetic diseases (diastrophic dysplasia, congenital chloride diarrhoea and Pendred syndrome) have been recently identified and found to reside within the same gene family. This family includes representatives from a wide taxonomic span, with all members appearing to encode anion transporters. As a general guide for the information detailed below, a summary of the three genes is provided in Table 1.

THREE DISTINCT DISEASES, THREE SIMILAR PROTEINS

Diastrophic dysplasia

Diastrophic dysplasia (DTD; OMIM 222600, see http://www.ncbi.nlm.nih.gov/Omim) is a rare autosomal recessive chondrodysplasia, with an increased frequency in the Finnish population [where the carrier frequency is estimated at 1 in 70 (5)]. The term ‘diastrophic’, first used by Lamy and Maroteaux in their original description of the disorder (6), is taken from geological vocabulary, where ‘diastrophism’ refers to the twisting movements and deformations of the earth’s crust, a feature that they believed resembled the bony malformations seen in the disease. The clinical manifestations of DTD (described in detail in ref. 7) include short-limbed short stature, kyphoscoliosis, contractures and multiple dislocations of joints, characteristic ‘hitch-hiker’ thumbs (resulting from shortened first metacarpal bones), atypical bilateral talipes equinovarus (club foot) that is refractory to surgical correction and, often, cleft palate. The joint dysplasia causes severe mobility impairment in these patients, requiring repeated corrective orthopaedic surgery, and early secondary osteoarthritic changes also occur. Affected individuals have an increased infant mortality rate due to respiratory insufficiency and neurological complications of their spinal deformities, but subsequent life expectancy is normal.

The DTD gene was initially mapped to a broad interval on chromosome 5 by conventional linkage analysis (8,9). The high incidence of the disease in the isolated Finnish population suggested a founder effect, and thus a novel linkage disequilibrium mapping approach was used to pinpoint the

+To whom correspondence should be addressed. Tel: +1 301 402 0201; Fax: +1 301 402 4735; Email: egreen@nhgri.nih.gov

Table 1. Features of three anion transporter-encoding genes (*DTDST*, *CLD* and *PDS*) implicated in human genetic diseases

	DTDST	*CLD*	*PDS*
Chromosomal location	5q32–q33.1	7q22–q31.1	7q22–q31.1
Genomic size	Unknown	39 kb	59 kb
No. of exons	4 or 5 (alternate splicing)[a]	21	21
mRNA size	8.4 kb	3.7 kb	4.9 kb
Tissue expression	Ubiquitous	Colon, small intestine, prostate	Thyroid, inner ear, kidney
Encoded protein	DTDST	CLD	Pendrin
No. of amino acids	739	764	780
Amino acid identity (%) (compared with pendrin)	32	45	100
Anions transported[b]	Sulphate, chloride, oxalate	Chloride, bicarbonate (or hydroxide), oxalate, sulphate	Iodide, chloride
Associated disease(s)	Diastrophic dysplasia, atelosteogenesis type II, achondrogenesis type IB	Congenital chloride diarrhoea	Pendred syndrome

[a]Derived from data about the mouse gene (101).
[b]Implicated from transport and/or inhibition studies.

gene's location more precisely. Striking linkage disequilibrium was encountered between the disease and polymorphic markers within *CSF1R*, a gene in the region. Luria–Delbrück formulae, initially used for classical studies of bacterial mutation rates (10), were then used to estimate the recombination rate between these genes, with the results indicating that the DTD gene should lie ~60 kb proximal to *CSF1R* (11,12).

Sequencing of a genomic clone derived from this region revealed a segment of significant protein homology to rat sat-1, a known sulphate transporter (13). Remarkably, this putative gene resided within ~6 kb of the predicted location for the DTD gene based on linkage disequilibrium mapping. Northern analyses revealed markedly reduced expression of the gene's 8.4 kb mRNA in most Finnish DTD patients. In five additional non-Finnish patients, one allele was found to contain point mutations; the second allele was assumed to harbour the common Finnish mutation. Together, these findings indicated that defects in this gene (named *DTDST*) were responsible for DTD (12).

DTDST is ubiquitously expressed and its encoded protein has strong homology to sat-1 throughout its sequence (12). Furthermore, fibroblasts from a DTD patient were found to have defective sulphate uptake, thereby confirming the role of the *DTDST*-encoded protein as a sulphate transporter (12). Since previously identified genes involved in other chondrodysplasias encode structural proteins, growth factor receptors or transcription factors (see ref. 14 for a review of the molecular biology of chondrodysplasias), this represented a new type of protein to be implicated in this class of diseases. However, a potential pathological mechanism was instantly recognized in the light of the important role of sulphated proteoglycans in the extracellular matrix of cartilage (Fig. 1) (for reviews see refs 5,15). Previous studies had, in retrospect, showed reduced staining of sulphated proteoglycans in DTD patients (16) and a reduced rate of sulphate incorporation into chondroitin sulphate (17). More recently, the importance of sulphated proteoglycans in cartilage formation has been further substantiated by demonstrating that genes involved in sulphate activation are the cause of other murine and human cartilage disorders (18,19).

Mutations in *DTDST* have also been found to cause atelosteogenesis type 2 (AO-2; OMIM 256050) (20; for reviews see refs 21,22) and the more severe achondrogenesis type 1B (ACG-1B; OMIM 600972) (23; for a review see ref. 24), both severe chondrodysplasias causing perinatal death from pulmonary hypoplasia. These findings suggest a genotype–phenotype correlation for the various *DTDST* mutations.

In the DTD patients examined to date, mutations in the *DTDST* coding region have usually been found in only one allele; typically, in at least one allele (that responsible for the majority of Finnish DTD cases) the coding region is intact. This common Finnish mutation has recently been identified and shown to destroy the splice donor site of a 5′-untranslated exon (25). The effect of this mutation is to severely reduce mRNA levels; one can postulate that residual levels of the encoded protein give rise to the milder DTD phenotype. In ACG-1B, both alleles harbour severe mutations that are assumed to abolish protein function completely (23,26), whereas in AO-2, at least one allele carries a missense mutation that may result in some residual protein function (20,26).

Interestingly, biochemical studies of patients with ACG-1B prior to the identification of the *DTDST* gene revealed the presence of undersulphated proteoglycans (27) and it was in patients with these more severe chondrodysplasias that a precise sulphated proteoglycan biosynthesis defect was observed (20,23,28–30). Finally, the levels of proteoglycan sulphation in patients with DTD, ACG-1B and AO-2 correlate with both clinical severity and the specific mutations (31).

A summary of *DTDST* mutations and their associated phenotypes has been compiled (5), with some additional mutations recently reported (25,31). Since these various related clinical disorders are caused by different mutations in the same gene, the distinction between them is somewhat artificial (i.e. they likely represent a phenotypic spectrum relating to the level of residual protein function). Furthermore,

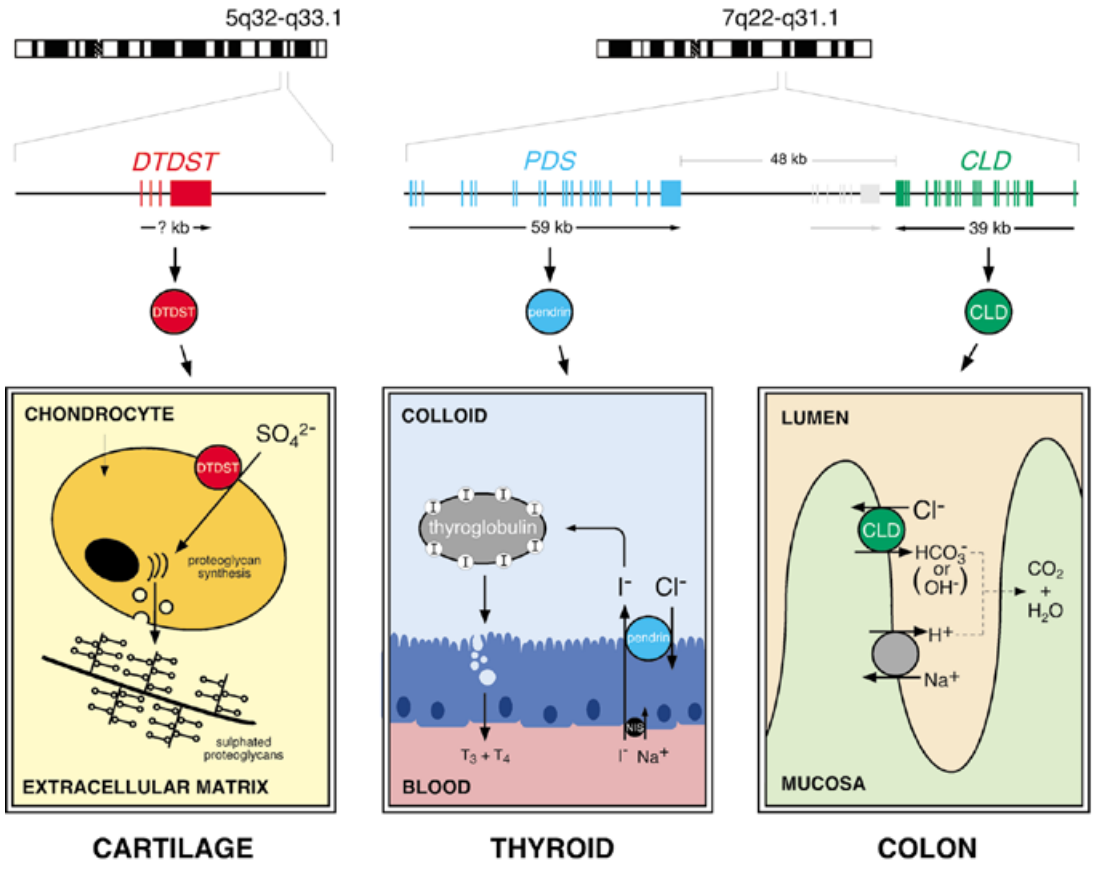

Figure 1. Illustrative comparisons of *DTDST*, *PDS* and *CLD* and the functions of their encoded proteins. The genomic locations and structures of the three genes are depicted. Note that the precise intron–exon organization for *DTDST* has not been established; in contrast, the genomic segment containing *PDS* and *CLD* is completely sequenced (see H_GS113G16, H_RG364P16 and H_RG013F03 at ftp://genome.wustl.edu/pub/gsc1/-sequence/st.louis/human/), revealing the precise spatial relationships of all introns and exons of both genes as well as the presence of a novel gene residing within the intervening segment. While structurally quite similar, each of the encoded proteins (DTDST, pendrin and CLD, respectively) serves different functional roles in distinct tissues, with representative examples shown here: DTDST transporting sulphate in chondrocytes, pendrin transporting iodide in thyrocytes and CLD transporting chloride in colonic mucosa. While these proteins are also synthesized elsewhere, their functional roles in these other tissues are not depicted here. See text for additional details. NIS, sodium–iodide symporter.

in the light of the intrafamilial variability encountered with these disorders (7,32), additional genetic background effects may play a role.

Congenital chloride diarrhoea

Congenital chloride diarrhoea (CLD; OMIM 214700) (33,34) is another rare autosomal recessive disease with an elevated frequency in the Finnish population. Since its first description in 1945 (35,36), ~100 affected individuals have been reported from around the world (33,37,38); however, a higher incidence is seen in Finland (37,39), Poland (40), Kuwait (41) and Saudi Arabia (42). The disease manifests as a lifelong, high volume watery diarrhoea with a markedly elevated chloride content (>90 mmol) (33,37). Infants with the disorder are usually premature due to polyhydramnios caused by fetal diarrhoea. If left untreated, the disorder results in severe dehydration and electrolyte disturbances soon after birth and, if left undiagnosed, is often fatal. Untreated patients that survive the neonatal period and early infancy often show renal impairment as well as retarded growth and psychomotor development. However, with appropriate therapy, patients develop normally and have a normal life expectancy. Long-term treatment involves fluid and electrolyte replacement, given i.v. in the neonatal period and orally in older children and adults.

Early studies indicated that the defect in CLD relates to altered chloride/bicarbonate exchange in the terminal ileum and colon (43–47). Normally, chloride is absorbed from the intestinal lumen by this exchange mechanism; the latter is coupled with absorption of Na^+ by a Na^+/H^+ exchanger (Fig. 1). In turn, the secreted bicarbonate and hydrogen ions form carbon dioxide and water, both of which are then reabsorbed. There is thus a net absorption of sodium and chloride, with water following by osmosis. It is the lack of this process in CLD that leads to increased amounts of sodium and chloride in the intestinal fluid and an osmotic diarrhoea. In addition, the lack of bicarbonate secretion leads to increased acidity of the intestinal contents and, in turn, inhibits the Na^+/H^+ exchange mechanism. Systemically, hypochloraemia and hyponatraemia are seen initially, in addition to a metabolic alkalosis. This leads to hyperaldosteronism and thus renal compensation in the form of increased Na^+ absorption at the expense of K^+ absorption in the distal tubule (as well as in the colon itself), which improves the hyponatraemia but causes hypokalaemia.

Treatment for CLD involves the careful oral administration of supplemental chloride (absorbed by passive diffusion), sodium, potassium and water. Such therapy prevents electrolyte depletion but exacerbates the diarrhoea, since most of the chloride cannot be absorbed. No treatment approach has been found to enhance the net chloride absorption, and the usual antidiarrhoeal drugs [e.g. antimotility agents (45)] and other therapies (48–55) typically fail. One group of pharmacological agents recently found to be effective are the proton pump inhibitors (56), which reduce gastric chloride secretion and thus the chloride content of fluid reaching the colon.

In 1993, the CLD locus was genetically mapped to chromosome 7q after testing for linkage to the *CFTR* (cystic fibrosis) gene, which, due to its involvement in chloride transport, was considered a viable candidate. Serendipitously, linkage was detected to *CFTR*, but the gene itself was excluded and the CLD gene was found to localize proximally on 7q (57). Linkage disequilibrium studies and haplotype analyses were then used to refine the critical region harbouring the CLD gene (39). Fortuitously, this interval contained a known gene named *DRA* (since its expression was down-regulated in colonic adenoma) (58). Its restricted tissue expression pattern, which included the colon, made *DRA* an attractive candidate. Identification of *DRA* mutations in CLD patients demonstrated that it was indeed the CLD gene (59), resulting in its eventual name change to *CLD* (34). Two common mutated alleles are responsible for the disease in the Finnish and Arabic populations, respectively; however a variety of other mutations have now been found in other populations (34). There is no obvious genotype–phenotype correlation for the *CLD* mutations detected to date, although some clustering of mutations in the gene has been noted (60).

The protein encoded by *CLD* was found to be closely related to a family of known sulphate transporters, of which *DTDST* was a member; this was particularly surprising in the light of its expected function as a chloride/bicarbonate transporter. While originally thought to play a role in colonic oncogenesis due to its down-regulation in adenoma (58), the *CLD*-encoded protein was later shown to be a membrane glycoprotein located in the apical brush border membrane of the colonic mucosal epithelial cells (61,62) and to be capable of transporting sulphate and oxalate (63). However, with its implicated role in CLD, speculation grew about the anion transported by this protein *in vivo*. Recently, the *CLD*-encoded protein was shown to function primarily as a Cl^-/OH^- or Cl^-/HCO_3^- antiporter (Fig. 1) (64), consistent with the biochemical defect found in CLD patients. Nonetheless, there is continued speculation as to its possible role in colonic adenocarcinoma (65,66); however, it is also likely that *CLD* expression is simply proportional to the level of cellular differentiation.

Pendred syndrome

Pendred syndrome (PS; OMIM 274600) (67; for reviews see refs 68–70) is a relatively common autosomal recessive disorder characterized by two rather distinct features: prelingual deafness and adenomatous goitre. PS occurs in many different populations and has an estimated incidence of 7.5/100 000 (71), making it one of the most common forms of syndromic deafness.

The deafness in PS is typically profound and sensorineural in nature, although its presentation can be variable and of later onset, with the latter often precipitated by head trauma. Patients typically have structural anomalies of the inner ear, most classically a Mondini malformation (72–74), where the cochlea is missing its apical turn and has an underdeveloped modiolus. This cochlear malformation is often associated with several other characteristic defects, including enlargement of the vestibular aqueducts [which can be present without the classical Mondini defect (75–77)]. These features are suggestive of a developmental arrest at 7 weeks of human embryonic development. In addition, patients with Mondini malformations usually have reduced numbers of hair cells and spiral ganglion cells (78), which probably accounts for the deafness itself.

The goitre is variable in its expression, typically appears around puberty and is associated biochemically with a positive perchlorate discharge test (68,69), whereby an increased amount of unincorporated iodide is released from the thyroid following perchlorate challenge. The latter suggests that the thyroid defect in PS relates to the organification of iodide, either in the transport of iodide into the follicular lumen via the apical membrane of follicular cells or in its binding to thyroglobulin (Fig. 1).

In 1996, the PS gene was mapped to chromosome 7q by performing genome-wide linkage analysis (79) as well as by demonstrating linkage to a known non-syndromic deafness locus previously mapped to 7q (DFNB4) (80,81). Ironically, the original family reported in describing DFNB4 (80) has subsequently been found to have PS (82). Additional genetic mapping allowed refinement of the PS critical region to an ~1.7 cM interval (83,84). The availability of a complete YAC-based physical map of the region (85–88), which contained numerous STSs and a handful of known genes, facilitated the construction of a fully contiguous BAC contig across this critical region (89). The mapped BACs were in turn provided to the Washington University Genome Sequencing Center (see http://genome.wustl.edu/gsc/index.shtml), who were sequencing this region of chromosome 7 as part of the Human Genome Project.

Known genes already mapped to the interval, those localized on the Human Transcript Map (90; see http://www.ncbi.nlm.nih.gov/genemap) and those identified by analysing newly generated genomic sequence data from the region were systematically evaluated as candidates for PS. Ironically, *CLD* itself resides within this region (Fig. 1), although this gene was regarded as an unlikely candidate in the light of its established role in CLD. Following negative mutation screens for seven candidate genes, a new gene emerged from analysis of genomic sequence data by the gene prediction program GRAIL II (91). Interestingly, this predicted gene showed striking similarity to the nearby *CLD* (89). Northern analysis revealed strong expression of this gene in thyroid (and to a much lesser extent in kidney), making it an excellent candidate. Indeed, mutations were promptly identified in all patients tested (89), demonstrating that it was the PS gene (named *PDS*, the original PS locus name).

Numerous *PDS* mutations have now been identified in PS patients (76,77,89,92–95), including small deletions/insertions as well as splice site and missense mutations. It is clear that there are common mutations in certain populations; for

example, four mutations account for 72% of mutant alleles in Northern Europeans. In addition, *PDS* mutations have now been found in families with deafness in the absence of thyroid disease (82,96). This is suggestive of a genotype/phenotype correlation; it may be that residual pendrin function is sufficient to compensate for the mild biochemical thyroid defect but not to prevent the developmental changes in the inner ear and, indeed, this is further corroborated by the fact that many of these families (96) have one allele with a mild (non-conserved) missense mutation. However, since there appears to be as much intrafamilial as interfamilial variation (especially in the severity and age of onset of goitre), differences in disease severity in PS could also be due to genetic background effects and/or environmental factors.

Upon its initial identification, *PDS* was postulated to encode a sulphate transporter based on the similarity of the encoded protein (pendrin) to the family of alleged sulphate transporters that included *DTDST* and *CLD*. However, subsequent functional studies have revealed that pendrin does not transport sulphate, but rather transports iodide and chloride (97). This finding immediately suggests a hypothesis for the role of pendrin in the thyroid (Fig. 1). After entering the thyrocyte from the bloodstream via the sodium–iodide symporter (98), iodide is transported through the apical membrane and into the colloid, where it becomes bound to the tyrosine residues in thyroglobulin. This large protein is subsequently degraded in lysosomes to yield thyroid hormones. Since PS patients inappropriately release iodide from thyrocytes following perchlorate administration (i.e. a positive perchlorate discharge test), normal iodide 'trapping' by thyroglobulin is not occurring. Thus, either the iodide is not properly transported into the follicular lumen or it is not properly incorporated into thyroglobulin. Since pendrin functions as an iodide transporter (and since no apical membrane iodide transporter/channel has been identified to date), it seems most likely that pendrin functions as an apical membrane iodide transporter in the thyrocyte.

In contrast to the emerging model for pendrin function in the thyroid, its role(s) in the inner ear is less clearly defined, although some important clues have recently emerged. RNA *in situ* hybridization studies have revealed that the mouse gene (*Pds*) is expressed throughout the endolymphatic duct and sac and in several other discrete regions of the inner ear (99). A common feature of these *Pds*-expressing cells is their implicated role in fluid homeostasis, in particular fluid resorption. Thus, in PS the absence of normal pendrin may be associated with altered anion transport in the inner ear and a resulting perturbed osmotic state. This may in turn lead to an abnormal hydrostatic effect that results in a widened endolymphatic duct and a malformed cochlea (99). The sensory cell defect could also occur as a consequence of the altered osmotic environment. A different mechanism may account for the deafness in the small subset of patients with later onset deafness occurring after head trauma. In these cases, the enlarged endolymphatic duct may allow abnormal transmission of fluid pressure from the cranial cavity to the inner ear fluids that then ruptures the delicate membrane separating the two fluid chambers of the inner ear (containing endolymph and perilymph), with the endolymph then damaging the sensory cells.

Protein siblings: DTDST, CLD and pendrin

The three closely related anion transporters (DTDST, CLD and pendrin) thus play central roles in the aetiology of several distinct genetic diseases. There are a number of key similarities as well as notable differences among these proteins and their associated genes (Table 1).

At a gene level, the physical organization of *PDS* and *CLD* is strikingly similar. As shown in Figure 1, the two genes reside within an ~150 kb interval on chromosome 7q22–q31.1, are present in a tail-to-tail configuration and contain 21 exons with identical overall intron–exon organizations (89). Another gene, encoding a proline-rich protein, lies within the ~48 kb between *PDS* and *CLD* (L.A. Everett and E.D. Green, unpublished data). These two paralogous genes almost certainly arose from a recent chromosomal duplication event. However, despite their similar genomic structure and close physical proximity, *PDS* and *CLD* have markedly different tissue expression patterns (Table 1). Thus, the regulatory elements controlling their expression must be notably different, a consequence of either sequence divergence of the promoters subsequent to the gene duplication event or the juxtaposition of a different promoter next to one/both of the genes at the time of the duplication. *DTDST* is more distantly related to *PDS* and *CLD*, both with respect to nucleotide identity and genomic structure (Fig. 1 and Table 1).

The three anion transporter proteins are remarkably similar. At the amino acid level, pendrin is 45 and 32% identical to CLD and DTDST, respectively. There appear to be two potential explanations for how such similar proteins could be implicated in such different diseases. Firstly, the proteins have a markedly different tissue distribution (Table 1). *DTDST* is expressed ubiquitously, although the pathology appears to be restricted to cartilage (this may be a consequence of cartilage's particular sensitivity to the levels of proteoglycan sulphation or a reflection of a compensatory mechanism present in other tissues). *CLD* and *PDS* have much more limited tissue expression that mostly coincides with the affected organs seen in CLD (colon) and PS (thyroid and inner ear), respectively. Secondly, the most surprising recent findings are that different primary anions are transported by each of the three proteins, with each anion being readily implicated in the respective disease [sulphate in DTD, chloride in CLD and iodide in PS (Table 1)].

A LARGE FAMILY OF ANION TRANSPORTERS

Computational analyses of the public protein databases reveal the presence of a large family of anion transporters that include DTDST, CLD and pendrin. At present, this family is known to contain >50 proteins that cover a large taxonomic span, from bacteria and yeast to plants, nematodes and mammals. One other mammalian family member, sat-1, has also been characterized. sat-1 is a 703 amino acid protein (at present characterized only in rat) and its gene was initially isolated from rat hepatocytes by functional expression cloning in *Xenopus* oocytes (13). Its 3.7 kb mRNA is highly expressed in kidney and liver and at lower levels in skeletal muscle and brain (13). The sat-1 protein was recently found to localize within the basolateral membrane of the proximal tubule in the kidney (100) and to transport sulphate, oxalate and bicarbonate

(13); however, *SAT1* has not yet been implicated in human disease.

In addition to characterized family members, there are numerous ESTs representing uncharacterized homologues from various organisms, including additional mammalian paralogues. The available EST data coupled with our preliminary characterization of these genes (L.A. Everett, A. Quraishi and E.D. Green, unpublished data) suggest the presence of as many as five new mammalian paralogues [admittedly, some of these uncharacterized ESTs may eventually merge into the same gene(s)].

Figure 2 depicts a preliminary phylogenetic tree of this family of anion transporters. This phylogenetic analysis intentionally included both well characterized proteins and family members for which only partial sequence data are available. Note that each phylogenetic branch (e.g. mammals, plants and yeast) has its own family members, i.e. the divergence of paralogues within each group occurred late in evolution. For the mammalian proteins, this late divergence may explain the non-lethal diseases associated with defective family members (DTDST, CLD and pendrin). A late diverging paralogue is less likely to be essential for general cellular function; rather, it is more likely to serve some phylum-specific role. The latter notion is corroborated by the fact that most of the genes encoding the mammalian family members appear to have highly restricted tissue expression patterns (e.g. *PDS*, *CLD* and *sat-1*).

In addition to the three family members implicated in human diseases and discussed in detail above, there appear to be as many as five additional mammalian anion transporters within this family. From the available EST data, it would appear that most of these (GenBank accession nos AA073427, AA779900 and AA992584) are closely related to the known mammalian proteins, with the corresponding genes diverging from the ancestral *CLD*/*PDS* gene after the ancestral *DTDST*/*SAT1* gene had already branched off. In the light of the distinct anion specificity of the CLD/pendrin branch (i.e. not transporting sulphate), it will be interesting to characterize the anion-transporting function of the other family members residing on this branch of the tree.

Other mammalian paralogues, such as that represented by the human EST AA822817, are far more divergent and may represent an additional group of family members that branched off from the other eukaryotic genes before the divergence of plants and animals. The presence of nine *Drosophila* ESTs with high homology to AA822817 corroborates this notion. It will thus be particularly important to elucidate the expression pattern and function of the AA822817-related gene and the corresponding *Drosophila* orthologues.

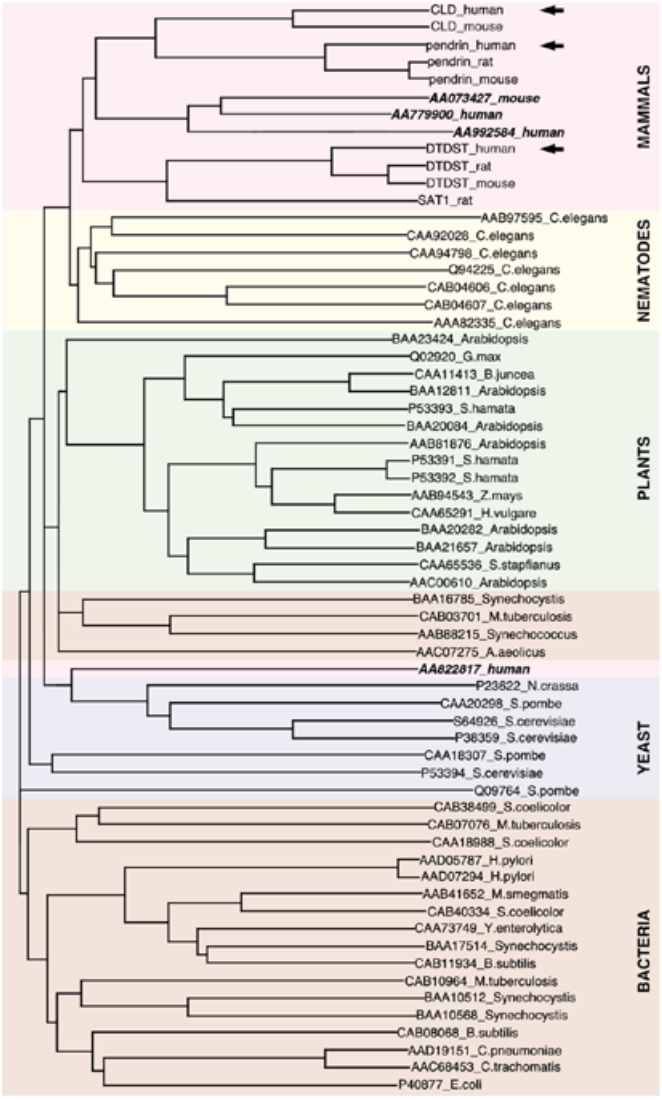

Figure 2. Preliminary phylogenetic tree of the anion transporter family. Family members from each major group of organisms are shaded in a different colour, as indicated. Each protein is labelled with its GenBank accession number followed by the species from which it is derived (except for the mammalian paralogues, where the actual protein name is listed). The three human proteins featured in this review (DTDST, CLD and pendrin) are indicated by arrows. In addition to characterized proteins, an a ttempt has been made to incorporate proteins predicted from genomic sequence data (e.g. from *Caenorhabditis elegans*, *Arabidopsis* and bacteria) as well as incomplete mammalian cDNA sequences. The latter members are labelled by the name of a representative matching EST (as well as italicized in bold). Inclusion of putative members for which incomplete data are available likely introduces some errors into the analysis (e.g. due to incorrectly predicted exons, inaccurate single pass EST sequences and the use of small stretches of protein sequence derived from translated ESTs). Indeed, one other likely human family member, represented by an EST (GenBank accession no. AA547958), was not included in this analysis because sufficient sequence data are not currently available. The depicted tree nicely illustrates the large size of the family and the evolutionary diversity of its members.

SUMMARY

The story that has unravelled in recent years for the anion transporter family described here represents an illustrative prelude to future studies in genetics, as the tens of thousands of mammalian genes are sequenced, characterized and examined for their role in human disease. Already, this family is known to contain a number of mammalian proteins that share common structural features while performing distinct biological functions, with the latter mostly being a consequence of distinct tissue and anion specificity. With three human proteins in this anion transporter family now implicated in genetic diseases, it is intriguing to consider a possible physiological/pathophysiological role(s) for the other mammalian members (Fig. 2). Future efforts will need to be directed at characterizing these other genes, establishing their genomic

location and organization, defining their tissue expression specificity and elucidating the anion-transporting function of their encoded proteins.

ACKNOWLEDGEMENTS

We thank Larry Karniski, Len Kohn, Eric Lander, Inès Royaux and Andy Baxevanis for critical review of the manuscript.

REFERENCES

1. Green, E.D., Cox, D.R. and Myers, R.M. (1998) The human genome project and its impact on the study of human disease. In *The Genetic Basis of Human Cancer*. McGraw-Hill, New York, pp. 33–63.
2. Green, E.D. (2000) The human genome project and its impact on the study of human disease. In *Metabolic and Molecular Bases of Inherited Disease*. McGraw-Hill, New York, in press.
3. Collins, F.S. (1995) Positional cloning moves from perditional to traditional. *Nature Genet.*, **9**, 347–350.
4. Ballabio, A. (1993) The rise and fall of positional cloning? *Nature Genet.*, **3**, 277–279.
5. Hastbacka, J., Superti-Furga, A., Wilcox, W.R., Rimoin, D.L., Cohn, D.H. and Lander, E.S. (1996) Sulfate transport in chondrodysplasia. *Ann. NY Acad. Sci.*, **785**, 131–136.
6. Lamy, M. and Maroteaux, P. (1960) Le nanisme diastrophique. *Presse Med.*, **68**, 1977–1980.
7. Walker, B.A., Scott, C.I., Hall, J.G., Murdoch, J.L. and McKusick, V.A. (1972) Diastrophic dwarfism. *Medicine (Baltimore)*, **51**, 41–59.
8. Hastbacka, J., Kaitila, I., Sistonen, P. and de la Chapelle, A. (1990) Diastrophic dysplasia gene maps to the distal long arm of chromosome 5. *Proc. Natl Acad. Sci. USA*, **87**, 8056–8059.
9. Hastbacka, J., Sistonen, P., Kaitila, I., Weiffenbach, B., Kidd, K.K. and de la Chapelle, A. (1991) A linkage map spanning the locus for diastrophic dysplasia (DTD). *Genomics*, **11**, 968–973.
10. Luria, S.E. and Delbrück, M. (1943) Mutations of bacteria from virus sensitivity to virus resistance. *Genetics*, **28**, 491–511.
11. Hastbacka, J., de la Chapelle, A., Kaitila, I., Sistonen, P., Weaver, A. and Lander, E. (1992) Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. *Nature Genet.*, **2**, 204–211.
12. Hastbacka, J., de la Chapelle, A., Mahtani, M.M., Clines, G., Reeve-Daly, M.P., Daly, M., Hamilton, B.A., Kusumi, K., Trivedi, B. and Weaver, A. (1994) The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. *Cell*, **78**, 1073–1087.
13. Bissig, M., Hagenbuch, B., Stieger, B., Koller, T. and Meier, P.J. (1994) Functional expression cloning of the canalicular sulfate transport system of rat hepatocytes. *J. Biol. Chem.*, **269**, 3017–3021.
14. Rimoin, D.L. (1996) Molecular defects in the chondrodysplasias. *Am. J. Med. Genet.*, **63**, 106–110.
15. Wallis, G.A. (1995) Cartilage disorders. The importance of being sulphated. *Curr. Biol.*, **5**, 225–227.
16. Scheck, M., Parker, J. and Daentl, D. (1978) Hyaline cartilage changes in diastrophic dwarfism. *Virchows Arch. A Pathol. Pathol. Anat.*, **378**, 347–359.
17. Feschenko, S.P., Krasnopolskaia, K.D., Rebrin, I.A. and Rudakov, S.S. (1989) Molecular heterogeneity of proteoglycan aggregates of human hyalin cartilage in normal conditions and in systemic bone dysplasia. *Vopr. Med. Khim.*, **35**, 24–33.
18. Orkin, R.W., Pratt, R.M. and Martin, G.R. (1976) Undersulfated chondroitin sulfate in the cartilage matrix of brachymorphic mice. *Dev. Biol.*, **50**, 82–94.
19. Ul Haque, M.F., King, L.M., Krakow, D., Cantor, R.M., Rusiniak, M.E., Swank, R.T., Superti-Furga, A., Haque, S., Abbas, H., Ahmad, W., Ahmad, M. and Cohn, D.H. (1998) Mutations in orthologous genes in human spondyloepimetaphyseal dysplasia and the brachymorphic mouse. *Nature Genet.*, **20**, 157–162.
20. Hastbacka, J., Superti-Furga, A., Wilcox, W.R., Rimoin, D.L., Cohn, D.H. and Lander, E.S. (1996) Atelosteogenesis type II is caused by mutations in the diastrophic dysplasia sulfate-transporter gene (DTDST): evidence for a phenotypic series involving three chondrodysplasias. *Am. J. Hum. Genet.*, **58**, 255–262.
21. Sillence, D., Worthington, S., Dixon, J., Osborn, R. and Kozlowski, K. (1997) Atelosteogenesis syndromes: a review, with comments on their pathogenesis. *Pediatr. Radiol.*, **27**, 388–396.
22. Newbury-Ecob, R. (1998) Atelosteogenesis type 2. *J. Med. Genet.*, **35**, 49–53.
23. Superti-Furga, A., Hastbacka, J., Wilcox, W.R., Cohn, D.H., van der Harten, H.J., Rossi, A., Blau, N., Rimoin, D.L., Steinmann, B., Lander, E.S. and Gitzelmann, R. (1996) Achondrogenesis type IB is caused by mutations in the diastrophic dysplasia sulphate transporter gene. *Nature Genet.*, **12**, 100–102.
24. Superti-Furga, A. (1996) Achondrogenesis type 1B. *J. Med. Genet.*, **33**, 957–961.
25. Hastbacka, J., Kerrebrock, A., Mokkala, K., Clines, G., Lovett, M., Kaitila, I., de la Chapelle, A. and Lander, E.S. (1999) Identification of the Finnish founder mutation for Diastrophic Dysplasia. *Eur. J. Hum. Genet.*, in press.
26. Superti-Furga, A., Rossi, A., Steinmann, B. and Gitzelmann, R. (1996) A chondrodysplasia family produced by mutations in the diastrophic dysplasia sulfate transporter gene: genotype/phenotype correlations. *Am. J. Med. Genet.*, **63**, 144–147.
27. Superti-Furga, A. (1994) A defect in the metabolic activation of sulfate in a patient with achondrogenesis type IB. *Am. J. Hum. Genet.*, **55**, 1137–1145.
28. Rossi, A., Bonaventure, J., Delezoide, A.L., Cetta, G. and Superti-Furga, A. (1996) Undersulfation of proteoglycans synthesized by chondrocytes from a patient with achondrogenesis type 1B homozygous for an L483P substitution in the diastrophic dysplasia sulfate transporter. *J. Biol. Chem.*, **271**, 18456–18464.
29. Rossi, A., van der Harten, H.J., Beemer, F.A., Kleijer, W.J., Gitzelmann, R., Steinmann, B. and Superti-Furga, A. (1996) Phenotypic and genotypic overlap between atelosteogenesis type 2 and diastrophic dysplasia. *Hum. Genet.*, **98**, 657–661.
30. Rossi, A., Bonaventure, J., Delezoide, A.L., Superti-Furga, A. and Cetta, G. (1997) Undersulfation of cartilage proteoglycans *ex vivo* and increased contribution of amino acid sulfur to sulfation *in vitro* in McAlister dysplasia/atelosteogenesis type 2. *Eur. J. Biochem.*, **248**, 741–747.
31. Rossi, A., Kaitila, I., Wilcox, W.R., Rimoin, D.L., Steinmann, B., Cetta, G. and Superti-Furga, A. (1998) Proteoglycan sulfation in cartilage and cell cultures from patients with sulfate transporter chondrodysplasias: relationship to clinical severity and indications on the role of intracellular sulfate production. *Matrix Biol.*, **17**, 361–369.
32. Hall, B.D. (1996) Diastrophic dysplasia: extreme variability within a sibship. *Am. J. Med. Genet.*, **63**, 28–33.
33. Holmberg, C. (1986) Congenital chloride diarrhoea. *Clin. Gastroenterol.*, **15**, 583–602.
34. Kere, J., Lohi, H. and Hoglund, P. (1999) Congenital chloride diarrhea. *Am. J. Physiol.*, **276**, G7–G13.
35. Gamble, J.L., Fahey, K.R., Appleton, J. and MacLachlan, E. (1945) Congenital alkalosis with diarrhea. *J. Pediatr.*, **26**, 509–518.
36. Darrow, D.C. (1945) Congenital alkalosis with diarrhea. *J. Pediatr.*, **26**, 519–532.
37. Holmberg, C., Perheentupa, J., Launiala, K. and Hallman, N. (1977) Congenital chloride diarrhoea. Clinical analysis of 21 Finnish patients. *Arch. Dis. Child*, **52**, 255–267.
38. Hoglund, P., Auranen, M., Socha, J., Popinska, K., Nazer, H., Rajaram, U., Al Sanie, A., Al-Ghanim, M., Holmberg, C., de la Chapelle, A. and Kere, J. (1998) Genetic background of congenital chloride diarrhea in high-incidence populations: Finland, Poland, and Saudi Arabia and Kuwait. *Am. J. Hum. Genet.*, **63**, 760–768.
39. Hoglund, P., Sistonen, P., Norio, R., Holmberg, C., Dimberg, A., Gustavson, K.H., de la Chapelle, A. and Kere, J. (1995) Fine mapping of the congenital chloride diarrhea gene by linkage disequilibrium. *Am. J. Hum. Genet.*, **57**, 95–102.
40. Tomaszewski, L., Kulesza, E. and Socha, J. (1987) Congenital chloride diarrhoea in Poland. *Mater. Med. Pol.*, **19**, 271–277.
41. Badawi, M.H., Zaki, M., Ismail, E.A. and Majid, M.A. (1998) Congenital chloride diarrhoea in Kuwait: a clinical reappraisal. *J. Trop. Pediatr.*, **44**, 296–299.
42. Kagalwalla, A.F. (1994) Congenital chloride diarrhea. A study in Arab children. *J. Clin. Gastroenterol.*, **19**, 36–40.
43. Turnberg, L.A. (1971) Abnormalities in intestinal electrolyte transport in congenital chloridorrhoea. *Gut*, **12**, 544–551.
44. Bieberdorf, F.A., Gorden, P. and Fordtran, J.S. (1972) Pathogenesis of congenital alkalosis with diarrhea. Implications for the physiology of

normal ileal electrolyte absorption and secretion. *J. Clin. Invest.*, **51**, 1958–1968.
45. Pearson, A.J., Sladen, G.E., Edmonds, C.J., Tavill, A.S., Wills, M.R. and McIntyre, N. (1973) The pathophysiology of congenital chloridorrhoea. *Q. J. Med.*, **42**, 453–466.
46. Lauras, B., Francois, B., Duc, H., Genoud, J., David, M. and Jeune, M. (1973) Contributions a l'etude de la diarrhée chlorée congénitale. *Arch. Fr. Pédiatr.*, **30**, 491–503.
47. Holmberg, C., Perheentupa, J. and Launiala, K. (1975) Colonic electrolyte transport in health and in congenital chloride diarrhea. *J. Clin. Invest.*, **56**, 302–310.
48. Yssing, M. and Friis-Hansen, B. (1966) Congenital alkalosis with diarrhea. *Acta Paediatr. Scand.*, **55**, 341–344.
49. Chaptal, J., Jean, R., Dossa, D., Meylan, F., Morel, G. and Rieu, D. (1967) Diarrhée chlorée congénitale. Etude clinique et biologique d'une observation de l'enfant. *Ann. Pediatr. (Paris)*, **14**, 326–334.
50. Jeune, M., Hermier, M., Hartemann, E., Loras, B., Haour, F., Cotton, J.B. and Collombel, C. (1967) Diarrhée chlorée congénitale avec alcalose métabolique. *Pediatrie*, **22**, 663–683.
51. Clark, E.B. (1977) Effect of acetazolamide on electrolyte balance in congenital chloridorrhea. *J. Pediatr.*, **91**, 148–149.
52. Rask-Madsen, J., Kamper, J., Oddsson, E. and Krag, E. (1976) Congenital chloridorrhoea. A question of reversed brush border transport processes and varying junctional tightness. *Scand. J. Gastroenterol.*, **11**, 377–383.
53. Brocklehurst, J.R. and Walker, A.C. (1978) Cholestyramine in treatment of congenital chloride diarrhoea. *Med. J. Aust.*, **1**, 504–505.
54. Minford, A.M. and Barr, D.G. (1980) Prostaglandin synthetase inhibitor in an infant with congenital chloride diarrhoea. *Arch. Dis. Child*, **55**, 70–72.
55. Mayatepek, E., Seyberth, H.W. and Nutzenadel, W. (1992) Effects of indomethacin in congenital chloride diarrhea. *J. Pediatr. Gastroenterol. Nutr.*, **14**, 319–322.
56. Aichbichler, B.W., Zerr, C.H., Santa, A.C., Porter, J.L. and Fordtran, J.S. (1997) Proton-pump inhibition of gastric chloride secretion in congenital chloridorrhea. *N. Engl. J. Med.*, **336**, 106–109.
57. Kere, J., Sistonen, P., Holmberg, C. and de la Chapelle, A. (1993) The gene for congenital chloride diarrhea maps close to but is distinct from the gene for cystic fibrosis transmembrane conductance regulator. *Proc. Natl Acad. Sci. USA*, **90**, 10686–10689.
58. Schweinfest, C.W., Henderson, K.W., Suster, S., Kondoh, N. and Papas, T.S. (1993) Identification of a colon mucosa gene that is down-regulated in colon adenomas and adenocarcinomas. *Proc. Natl Acad. Sci. USA*, **90**, 4166–4170.
59. Hoglund, P., Haila, S., Socha, J., Tomaszewski, L., Saarialho-Kere, U., Karjalainen-Lindsberg, M.L., Airola, K., Holmberg, C., de la Chapelle, A. and Kere, J. (1996) Mutations of the down-regulated in adenoma (DRA) gene cause congenital chloride diarrhoea. *Nature Genet.*, **14**, 316–319.
60. Hoglund, P., Haila, S., Gustavson, K.H., Taipale, M., Hannula, K., Popinska, K., Holmberg, C., Socha, J., de la Chapelle, A. and Kere, J. (1998) Clustering of private mutations in the congenital chloride diarrhea/down-regulated in adenoma gene. *Hum. Mutat.*, **11**, 321–327.
61. Byeon, M.K., Westerman, M.A., Maroulakou, I.G., Henderson, K.W., Suster, S., Zhang, X.K., Papas, T.S., Vesely, J., Willingham, M.C., Green, J.E. and Schweinfest, C.W. (1996) The down-regulated in adenoma (DRA) gene encodes an intestine-specific membrane glycoprotein. *Oncogene*, **12**, 387–396.
62. Byeon, M.K., Frankel, A., Papas, T.S., Henderson, K.W. and Schweinfest, C.W. (1998) Human DRA functions as a sulfate transporter in Sf9 insect cells. *Protein Expr. Purif.*, **12**, 67–74.
63. Silberg, D.G., Wang, W., Moseley, R.H. and Traber, P.G. (1995) The down regulated in adenoma (dra) gene encodes an intestine-specific membrane sulfate transport protein. *J. Biol. Chem.*, **270**, 11897–11902.
64. Moseley, R.H., Hoglund, P., Wu, G.D., Silberg, D.G., Haila, S., de la Chapelle, A., Holmberg, C. and Kere, J. (1999) Downregulated in adenoma gene encodes a chloride transporter defective in congenital chloride diarrhea. *Am. J. Physiol.*, **276**, G185–G192.
65. Hemminki, A., Hoglund, P., Pukkala, E., Salovaara, R., Jarvinen, H., Norio, R. and Aaltonen, L.A. (1998) Intestinal cancer in patients with a germline mutation in the down-regulated in adenoma (DRA) gene. *Oncogene*, **16**, 681–684.
66. Antalis, T.M., Reeder, J.A., Gotley, D.C., Byeon, M.K., Walsh, M.D., Henderson, K.W., Papas, T.S. and Schweinfest, C.W. (1998) Down-regulation of the down-regulated in adenoma (DRA) gene correlates with colon tumor progression. *Clin. Cancer Res.*, **4**, 1857–1863.
67. Pendred, V. (1896) Deaf-mutism and goitre. *Lancet*, **ii**, 532.
68. Reardon, W. and Trembath, R.C. (1996) Pendred syndrome. *J. Med. Genet.*, **33**, 1037–1040.
69. Reardon, W., Coffey, R. and Trembath, R. (1997) Pendred syndrome—100 years of underascertainment? *Q. J. Med.*, **90**, 443–447.
70. Wilcox, E.R., Everett, L.A., Li, X.C., Lalwani, A.K. and Green, E.D. (1999) The PDS gene, Pendred syndrome, and non-syndromic deafness. *Adv. Otorhinolaryngol.*, in press.
71. Fraser, G.R. (1965) Association of congenital deafness with goitre (Pendred's syndrome): a study of 207 families. *Ann. Hum. Genet.*, **28**, 201–249.
72. Mondini, C. (1791) Anatomia surdi nati sectio. De Boroniensi scientarium et artium instituto atque acedemia commnetarii. *Bononiae*, **7**, 419–431.
73. Johnsen, T., Jorgensen, M.B. and Johnsen, S. (1986) Mondini cochlea in Pendred's syndrome. A histological study. *Acta Otolaryngol. (Stockh.)*, **102**, 239–247.
74. Johnsen, T., Larsen, C., Friis, J. and Hougaard-Jensen, F. (1987) Pendred's syndrome. Acoustic, vestibular and radiological findings in 17 unrelated patients. *J. Laryngol. Otol.*, **101**, 1187–1192.
75. Phelps, P.D., Coffey, R.A., Trembath, R.C., Luxon, L.M., Grossman, A.B., Britton, K.E., Kendall-Taylor, P., Graham, J.M., Cadge, B.C., Stephens, S.G., Pembrey, M.E. and Reardon, W. (1998) Radiological malformations of the ear in Pendred syndrome. *Clin. Radiol.*, **53**, 268–273.
76. Cremers, W.R., Bolder, C., Admiraal, R.J., Everett, L.A., Joosten, F.B., Van Hauwe, P., Green, E.D. and Otten, B.J. (1998) Progressive sensorineural hearing loss and a widened vestibular aqueduct in Pendred syndrome. *Arch. Otolaryngol. Head Neck Surg.*, **124**, 501–505.
77. Cremers, C.W., Admiraal, R.J., Huygen, P.L., Bolder, C., Everett, L.A., Joosten, F.B., Green, E.D., Van Camp, G. and Otten, B.J. (1998) Progressive hearing loss, hypoplasia of the cochlea and widened vestibular aqueducts are very common features in Pendred's syndrome. *Int. J. Pediatr. Otorhinolaryngol.*, **45**, 113–123.
78. Schuknecht, H.F. (1980) Mondini dysplasia; a clinical and pathological study. *Ann. Otol. Rhinol. Laryngol.*, **89** (suppl.), 1–23.
79. Sheffield, V.C., Kraiem, Z., Beck, J.C., Nishimura, D., Stone, E.M., Salameh, M., Sadeh, O. and Glaser, B. (1996) Pendred syndrome maps to chromosome 7q21–34 and is caused by an intrinsic defect in thyroid iodine organification. *Nature Genet.*, **12**, 424–426.
80. Baldwin, C.T., Weiss, S., Farrer, L.A., De Stefano, A.L., Adair, R., Franklyn, B., Kidd, K.K., Korostishevsky, M. and Bonne-Tamir, B. (1995) Linkage of congenital, recessive deafness (DFNB4) to chromosome 7q31 and evidence for genetic heterogeneity in the Middle Eastern Druze population. *Hum. Mol. Genet.*, **4**, 1637–1642.
81. Coyle, B., Coffey, R., Armour, J.A., Gausden, E., Hochberg, Z., Grossman, A., Britton, K., Pembrey, M., Reardon, W. and Trembath, R. (1996) Pendred syndrome (goitre and sensorineural hearing loss) maps to chromosome 7 in the region containing the nonsyndromic deafness gene DFNB4. *Nature Genet.*, **12**, 421–423.
82. Li, X.C., Everett, L.A., Lalwani, A.K., Desmukh, D., Friedman, T.B., Green, E.D. and Wilcox, E.R. (1998) A mutation in PDS causes non-syndromic recessive deafness. *Nature Genet.*, **18**, 215–217.
83. Coucke, P., Van Camp, G., Demirhan, O., Kabakkaya, Y., Balemans, W., Van Hauwe, P., Van Agtmael, T., Smith, R.J., Parving, A., Bolder, C.H., Cremers, C.W. and Willems, P.J. (1997) The gene for Pendred syndrome is located between D7S501 and D7S692 in a 1.7-cM region on chromosome 7q. *Genomics*, **40**, 48–54.
84. Gausden, E., Coyle, B., Armour, J.A., Coffey, R., Grossman, A., Fraser, G.R., Winter, R.M., Pembrey, M.E., Kendall-Taylor, P., Stephens, D., Luxon, L.M., Phelps, P.D., Reardon, W. and Trembath, R. (1997) Pendred syndrome: evidence for genetic homogeneity and further refinement of linkage. *J. Med. Genet.*, **34**, 126–129.
85. Green, E.D., Idol, J.R., Mohr-Tidwell, R.M., Braden, V.V., Peluso, D.C., Fulton, R.S., Massa, H.F., Magness, C.L., Wilson, A.M. and Kimura, J. (1994) Integration of physical, genetic and cytogenetic maps of human chromosome 7: isolation and analysis of yeast artificial chromosome clones for 117 mapped genetic markers. *Hum. Mol. Genet.*, **3**, 489–501.
86. Green, E.D., Braden, V.V., Fulton, R.S., Lim, R., Ueltzen, M.S., Peluso, D.C., Mohr-Tidwell, R.M., Idol, J.R., Smith, L.M. and Chumakov, I. (1995) A human chromosome 7 yeast artificial chromosome (YAC) resource: construction, characterization, and screening. *Genomics*, **25**, 170–183.
87. Bouffard, G.G., Iyer, L.M., Idol, J.R., Braden, V.V., Cunningham, A.F., Weintraub, L.A., Mohr-Tidwell, R.M., Peluso, D.C., Fulton, R.S., Leckie,

M.P. and Green, E.D. (1997) A collection of 1814 human chromosome 7-specific STSs. *Genome Res.*, **7**, 59–64.

88. Bouffard, G.G., Idol, J.R., Braden, V.V., Iyer, L.M., Cunningham, A.F., Weintraub, L.A., Touchman, J.W., Mohr-Tidwell, D.C., Peluso, D.C., Fulton, R.S., Ueltzen, M.S., Weissenbach, J., Magness, C.L. and Green, E.D. (1997) A physical map of human chromosome 7: an integrated YAC contig map with average STS spacing of 79 kb. *Genome Res.*, **7**, 673–692.
89. Everett, L.A., Glaser, B., Beck, J.C., Idol, J.R., Buchs, A., Heyman, M., Adawi, F., Hazani, E., Nassir, E., Baxevanis, A.D., Sheffield, V.C. and Green, E.D. (1997) Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nature Genet.*, **17**, 411–422.
90. Schuler, G.D., Boguski, M.S., Stewart, E.A., Stein, L.D., Gyapay, G., Rice, K., White, R.E., Rodriguez-Tome, P., Aggarwal, A., Bajorek, E., Bentolila, S., Birren, B.B., Castle, A.B., Chiannilkulchai, N., Chu, A., Clee, C., Cowles, S., Day, P.J., Dibling, T., Drouot, N., Dunham, I., Duprat, S., East, C., Edwards, C., Fan, J.-B. *et al*. (1996) A gene map of the human genome. *Science*, **274**, 540–546.
91. Xu, Y., Mural, R., Shah, M. and Uberbacher, E. (1994) Recognizing exons in genomic sequence using GRAIL II. *Genet. Eng. (N.Y.)*, **16**, 241–253.
92. Van Hauwe, P., Everett, L.A., Coucke, P., Scott, D.A., Kraft, M.L., Ris-Stalpers, C., Bolder, C., Otten, B., de Vijlder, J.J.M., Dietrich, N.L., Ramesh, A., Srisailapathy, S.C.R., Parving, A., Cremers, C.W.R.J., Willems, P.J., Smith, R.J.H., Green, E.D. and Van Camp, G. (1998) Two frequent missense mutations in Pendred syndrome. *Hum. Mol. Genet*., **7**, 1099–1104.
93. Coyle, B., Reardon, W., Herbrick, J.A., Tsui, L.C., Gausden, E., Lee, J., Coffey, R., Grueters, A., Grossman, Phelps, P.D., Luxon, L., Kendall-Taylor, P., Scherer, S.W. and Trembath, R.C. (1998) Molecular analysis of the PDS gene in Pendred syndrome. *Hum. Mol. Genet.*, **7**, 1105–1112.
94. Kopp, P., Arseven, O.K., Sabacan, L., Kotlar, T., Dupuis, J., Cavaliere, H., Santos, C.L., Jameson, J.L. and Medeiros-Neto, G. (1999) Phenocopies for deafness and goiter development in a large inbred Brazilian kindred with Pendred's syndrome associated with a novel mutation in the PDS gene. *J. Clin. Endocrinol. Metab*., **84**, 336–341.
95. Coucke, P., Van Hauwe, P., Everett, L.A., Demirhan, O., Kabakkaya, Y., Dietrich, N.L., Smith, R.J.H., Coyle, E., Reardon, W., Trembath, R., Willems, P.J., Green, E.D. and Van Camp, G. (1999) Identification of two different mutations in the PDS gene in an inbred family with Pendred syndrome. *J. Med. Genet.*, **36**, 475–477.
96. Usami, S., Abe, S., Weston, M.D., Shinkawa, H., Van Camp, G. and Kimberling, W.J. (1999) Non-syndromic hearing loss associated with enlarged vestibular aqueduct is caused by PDS mutations. *Hum. Genet.*, **104**, 188–192.
97. Scott, D.A., Wang, R., Kreman, T.M., Sheffield, V.C. and Karniski, L.P. (1999) The Pendred syndrome gene encodes a chloride-iodide transport protein. *Nature Genet.*, **21**, 440–443.
98. Dai, G., Levy, O. and Carrasco, N. (1996) Cloning and characterization of the thyroid iodide transporter. *Nature*, **379**, 458–460.
99. Everett, L.A., Morsli, H., Wu, D.K. and Green, E.D. (1999) Expression pattern of the mouse ortholog of the Pendred syndrome gene (Pds) suggests a key role for pendrin in the inner ear. *Proc. Natl Acad. Sci. USA*, **96**, 9729–9732.
100. Karniski, L.P., Lotscher, M., Fucentese, M., Hilfiker, H., Biber, J. and Murer, H. (1998) Immunolocalization of sat-1 sulfate/oxalate/bicarbonate anion exchanger in the rat kidney. *Am. J. Physiol.*, **275**, F79–F87.
101. Satoh, H., Susaki, M., Shukunami, C., Iyama, K., Negoro, T. and Hiraki, Y. (1998) Functional analysis of diastrophic dysplasia sulfate transporter. Its involvement in growth regulation of chondrocytes mediated by sulfated proteoglycans. *J. Biol. Chem.*, **273**, 12307–12315.