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ORIGINAL ARTICLE

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Identification of the human *CYS1* gene and candidate gene analysis in Boichis disease

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Abstract Recessive mutations cause cystic kidney disease and a variable degree of biliary liver fibrosis in *cpk* mice. Recently, the responsible murine gene (*Cys1*) was identified and expression in renal cilia demonstrated. Here we describe the cDNA cloning of the full-length coding region of the orthologous human *CYS1* gene. *CYS1* is located on Chromosome 2p25. The *CYS1* genomic region comprises three coding exons, which span 22 kb. The transcript harbors an open reading frame of 477 nucleotides encoding a protein with 158 amino acid residues, which is called cystin. Northern analysis identified an expression pattern resembling that of murine *Cys1*. We studied affected individuals of eight families with nephronophthisis and liver fibrosis for evidence of *CYS1* mutations. All three coding exons were amplified by polymerase chain reaction and directly sequenced. Despite the failure to detect a mutation, the human cystin gene remains an interesting candidate for recessive cystic kidney disease.

Keywords *CYS1* · *Cys1* · Cystin · Nephronophthisis · Cilia

Introduction

There has been increasing evidence originating from mouse studies that the function of renal cilia is necessary to maintain normal renal architecture and to prevent the development of cystic kidney disease. The *Tg737* gene was originally identified based on its association with the mouse *Oak Ridge Polycystic Kidney* (*orpk*) insertional mutation (*Tg737*^{orpk}), which causes polycystic kidney disease and other defects [1]. Additional studies including targeted mutation *Tg737*$^{\Delta 2-3\beta Gal}$ showed that mutations in *Tg737* cause a wide spectrum of phenotypes comprising polycystic kidney disease, liver and pancreatic defects, hydrocephalus, skeletal patterning abnormalities, and randomization of left-right asymmetry [2]. *Tg737* encodes the protein polaris, which is present in cells possessing either motile or immotile cilia and sperm [2, 3]. Expression in known motile cilia, such as respiratory, ependymal, and nodal cilia, was demonstrated. In the kidney, expression was confined to glomerular cells and tubular cells carrying monocilia. Polaris appears to be present within the whole axonemal structure of the cilium, but is strongest at the base of the cilium. Insights into the potential function of polaris came from studies of the biflagellate unicellular alga *Chlamydomonas rheinhardtii*. Pazour et al. [4] demonstrated that the intraflagellar transport protein IFT88 and its mouse orthologue polaris are required for assembly of cilia and flagella. *IFT88* mutant algae either lack flagella or show abnormal growth of their flagella. In analogy, murine renal tubular cells carrying *Tg737* mutations have shortened monocilia. Recently, the underlying genetic defect of the congenital polycystic kidney (*cpk*) mouse model was identified [5]. The extensively studied *cpk* phenotype mimics autosomal recessive polycystic kidney disease, including the biliary dysgenesis and congenital hepatic fibrosis observed in humans. The responsible gene *Cys1* is also expressed in renal monocilia [5, 6]. These data indicate that renal ciliary malfunction contributes to the development of cystic kidney disease. Interestingly, the polycystin-2 gene that is responsible for human autosomal dominant polycystic kidney disease type 2 (ADPKD2) also appears to be involved in renal ciliary function. Localization of murine polycystin-2 to renal cilia has been shown, and elevated expression levels in cilia

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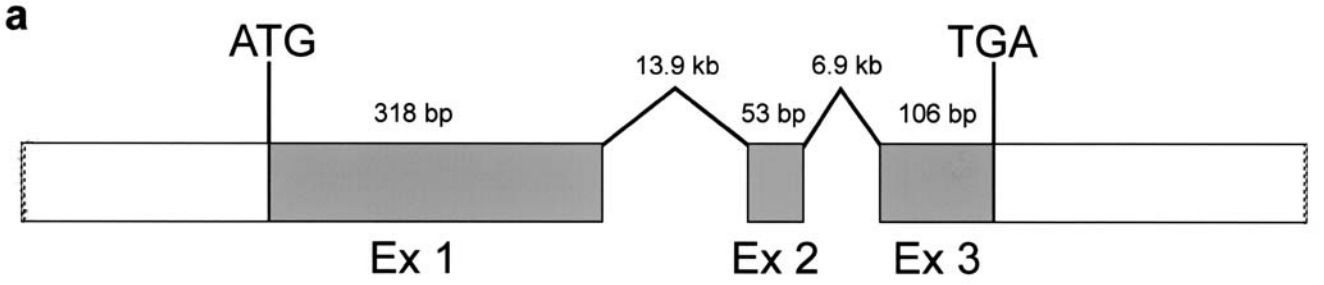

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 301 CCCGCGGGGCCCGGAGCGGC AGCCCTGGAGGGCGGGACCC GCCGGCGGGTGCCGGTGGCG GCGGCCGAGGTCCCGGGGGC AGCCGCGGAGGAGGCGCCCG
 401 GCCGCGACCCCAGCCCCGTG GCGCCCCCCGACGGCAGGGA CGAGACGCTGCGCCTGCTGG ACGAGCTGCTGGCCGAGTCG GCGGCCTGGGGCCCCCCGGA
 501 GCCCGCCCCGCGCCGCCCAG CCCGGCTCCGACCCACCGCG GTCGCGGGGAGCGCGGTGTG CGCAGAGCAGAGCACAGAGG GCCACCCGGGGAGCGGCAAT
 601 GTCTCTGAAGCCCCAGGCAG CGGTCGCAAGAAGCCCGAGA GGCCGGCAGCCATCTCCTAC GACCACTCGGAAGAGGGGCT GATGGCGAGCATCGAGCGGG
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 901 tgctggtgggggcgattagc tgtgtagacccacaggtgcg tgggcctgggccgccggcgc ctcctccccaacgcgggagc ctgcccagttcttctggagc
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Fig. 1a, b Structure of the human cystin gene (*CYS1*). **a** Genomic organization of *CYS1*. The gene consists of three coding exons. The intron-exon structure is shown. **b** The open reading frame of the coding cDNA of *CYS1* comprises 477 base pairs, which encode a 158-amino acid protein. The 5′ untranslated region (UTR), start codon (ATG), stop codon (TGA), and 3′UTR of *CYS1* are shown. Locations of primers that were used to amplify cystin cDNA are *underlined*. The first four primers are forward primers, the last three primers are reverse primers. Primer names in order from 5′ to 3′ end are: Ex1for; Cystin5′forout; Ex1F2; Cystin5′forin; Ex2rev; Cystin3′rev; Ex3rev

of Tg737^{orpk} mice with polycystic kidney disease have been demonstrated [7].

Therefore, we considered human *CYS1* as a promising candidate for human cystic kidney disease. To study *CYS1* we first mapped the gene to Chromosome 2p25. Using a combined approach of an in silico cloning strategy and cDNA amplification we identified *CYS1*, which encodes a 158-amino acid protein. Comparison of human and murine cystin identified highly conserved domains. Northern analysis of human cystin showed an expression pattern resembling that of murine *Cys1*. In view of the phenotype of the *cpk* mouse, *CYS1* appears to be a candidate gene for a recessive disorder characterized by cystic kidney disease and liver fibrosis. Therefore, we studied affected individuals of eight families with Boichis disease (nephronophthisis and liver fibrosis) for evidence of *CYS1* mutations [8]. All three coding exons were amplified from isolated genomic DNA by polymerase chain reaction (PCR) and directly sequenced. Despite the failure to detect mutation in this subset of patients, the human cystin gene remains an interesting candidate for cystic kidney disease.

Materials and methods

Identification of the human CYS1 gene

To identify the human orthologous gene of murine *Cys1*, we applied an in silico cloning strategy. Using the murine *Cys1* coding cDNA sequences (NM_138686, XM_126886, AF390547, AF390548, XM_110716, XM_115698), we obtained by BLAST analysis a human cDNA clone AK091299, which shared a high degree of homology with the murine *Cys1* gene [5, 9]. The sequence of the cDNA clone contained most parts of the human *CYS1* coding region. To obtain the missing part of exon 1 and the 5′ untranslated region (UTR), we first identified by BLAST analysis the human genomic contig containing the *CYS1* gene (*Homo sapiens*, Chromosome 2 working draft sequence, AC104794). Using TWOBLAST analysis with this sequence and the complete murine *Cys1* coding cDNA sequence, we obtained the putative intron-exon structure including the 5′UTR and the missing parts of exon 1 of the human *CYS1* gene. Based on these analyses the full-length coding region of the human cystin cDNA was amplified from a testis cDNA library (Marathon-Ready cDNA, Clontech, Heidelberg, Germany) by nested PCR. Primer locations are given in Fig. 1. First-round amplification (5 min 94°C followed by 35 cycles of 30 s 94°C, 45 s 60°C, 90 s 72°C and a final extension step for 10 min at 72°C) was performed with 20 pmol of each primer (Ex1for 5′-CTC GGG ACG CCT ATG CAG-3′ and Ex3ref 5′- TCA GGC TCC AGA AGA ACT GG-3′), 1.5 mM $MgCl_2$, 1 mM dNTPs, Q-Solution (Qiagen, Hilden, Germany), and 2 µl template cDNA in a final volume of 50 µl. The amplification product (2 µl) was used for nested PCR under identical conditions but with 30 cycles and an annealing temperature of 68°C (Cystin5′forout 5′-GCT CGC ACC CCC GTC CTC–3′ and Cystin3′rev 5′-CGG GAG TGA CTG CGT TTT GGA G-3′). A band with the expected size of 779 bp was isolated by agarose gel electrophoresis and gel extraction (QiaQuick, Qiagen) and subsequently cloned into a TA-vector (pCR2.1, Invitrogen, Karlsruhe, Germany), to obtain pCR2.1-CYS1. Single bacteria clones were analyzed by colony PCR (10 pmol of each primer: Cystin5′forin 5-GGT CGC TTC CCG GAC CTC-3′ and Ex2rev 5′-TGG CCC TCT GTG CTC TGC TC-3′, 1.5 mM $MgCl_2$, Q-Solution, cycles: 94°C 5 min, 30 cycles 94°C 30 s, 65°C 30 s, 72°C 1 min, final extension 72°C 10 min, 477 bp product) and two clones were chosen for sequence

analysis. As a control, second-round PCR was performed in parallel with a nested primer only on the 5′ end (Ex1F2:5′-CCC GGG AAC CCT AGC C-3′) and the Ex3Ref-primer, to obtain a 923-bp product. Following TA-cloning, two clones were sequenced and compared with the clones derived from the nested reaction. The sequences of the overlapping parts were identical. The sequence of the 779-bp nested PCR product was submitted to GenBank under the accession number AF544983. Using TWOBLAST analysis with the human genomic sequence and the complete human *CYS1* cDNA, we confirmed the hypothesized intron-exon structure. For amino acid sequence alignment of the proteins human cystin (CYS1) and murine cystin (Cys1) the program CLUSTAL W was used [10]. We analyzed the putative protein structure of human cystin with several protein motive prediction programs using the PSIPRED server [11] and the ScanProsite tool from the Expasy server (http://www.expasy.ch/), and compared the results with the orthologous murine protein. The potential PEST sequences were found with a PESTfind analysis program (http://www.at.embnet.org/embnet/tools/bio/PESTfind/).

Expression analysis

The tissue-specific expression of the human cystin gene was analyzed by Northern blot hybridization, using the cloned full-length coding region of the human cDNA (an ~ 800-bp *Eco*RI fragment derived from pCR2.1.-Cys1) as a probe. Human multiple tissue Northern blots (Clontech) were hybridized over night at 60°C in Church buffer [500 mM phosphate buffer pH 7.2, 7% SDS (w/v), 1 mM EDTA supplemented with 100 µg/ml salmon sperm DNA]. The cystin-specific probe was radiolabeled with α-^{32}P-dCTP (Amersham, Freiburg, Germany) using a Megaprime DNA labeling kit (Amersham). Filters were washed twice for 5 min at room temperature with 2x SSC/0.1%SDS and twice for 10–15 minutes at 55°C with 0.2xSSC/0.1%SDS. For higher stringency, the last step was repeated for 15–20 min at 60°C. Membranes were exposed to X-ray films (X-OMAT AR, Kodak, Stuttgart, Germany) in the presence of an intensifying screen (Cronex, DuPont, Bad Homburg, Germany) at −70°C for 1–5 days. Equal loading of RNA (approximately 2 µg poly(A)+ RNA per lane, adjusted to obtain a consistent signal for a housekeeping gene across all lanes) was confirmed by the manufacturer of the pre-made blots (Clontech).

Mutational analysis

For mutational analysis of the human cystin gene (*CYS1*), we examined affected individuals from eight families with cystic kidney disease and liver fibrosis (F23, F190, F299, F616, F627, F694, F805, F912). All affected individuals were tested for the presence of homozygous *NPHP1* deletions, as described previously [12]. No homozygous *NPHP1* deletion was demonstrated. In all studied families inheritance was compatible with autosomal recessive transmission. All affected individuals developed renal failure after infancy. Secondary causes for the renal or liver disease were not detected. Renal pathology showed typical findings observed in nephronophthisis, such as irregularly thickened tubular basement membranes, atrophy, and dilatation of tubules [13], in families F23, F190, F299, F616, F694, and F805. Liver biopsy reports were available from families F23, F299, and F805. In family F23 and F 805, affected individuals showed septal liver fibrosis. In addition to this finding in F299, a moderate proliferation of bile ducts was present. In families where no biopsy was performed, the diagnosis rested on clinical presentation (polyuria, polydipsia, progressive renal failure, and liver dysfunction) and typical findings by imaging studies of the kidneys and liver. In some families the hepatic and renal lesions were associated with other anomalies: Cogan syndrome and delay in psychomotor development (F190); bilateral cataract (F805); iris coloboma, optical nerve anomalies, vermis hypoplasia, and delay of psychomotor development (F912). To verify the human genomic sequence, we also studied a sample from a healthy donor. Blood samples were obtained after informed consent was given. Genomic DNA was isolated by standard methods directly from blood samples [14] or after Epstein-Barr virus transformation of peripheral blood lymphocytes [15]. The three coding exons and the corresponding intron-exon boundaries of the *CYS1* gene were amplified from the isolated genomic DNA, and subsequently sequenced using the following primers: exon 1: Ex1F2 (5′-CCC GGG AAC CCT AGC C-3′) and Ex1R (5′-GCA GAG AGG GAG GAA ACC TG-3′); exon 2: Ex2F (5′-AGG CTG CAC TGT TGA CAA TC-3′) and Ex2R (5′-CTG GCT GGA GTC AAC AAA AAG-3′); exon 3: Ex3F (5′-AGG GGA AAT GAG TGT GAA CG-3′) and Ex3R (5′-TCA GGC TCC AGA AGA ACT GG-3′).

PCR was performed in a volume of 50 µl containing 50 pmol of primers, 2 mM each dATP, dGTP, dCTP, and dTTP, 20 mM TRIS-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin (w/v), and 1.5 U of *Thermus aquaticus* DNA polymerase (Invitrogen, Karlsruhe, Germany). Amplification was performed with denaturation at 94°C for 4 min, 32 cycles of 30 s at 94°C, 30 s at 60°C for exon 1, 63°C for exon 2 and 3, respectively, and 60 s at 72°C. Final extension was carried out at 72°C for 10 min. Q-Solution (Qiagen) was added to amplify exon 1. PCR products (exon 1 650 bp, exon 2 168 bp, exon 3 462 bp) were purified by gel extraction after agarose gel electrophoresis or with the QiaQuick PCR-Purification-Kit (Qiagen) and analyzed by direct sequencing with the Big-Dye Terminator Kit (PE Applied Biosystems) on an ABI 377 or ABI 3700 sequencer at the core facility of the University of Freiburg, Medical Center, or by AGOWA GmbH sequencing service (Berlin, Germany). A GTP kit was used to sequence exon 1. All exons were sequenced bidirectionally. Sequence data were compared with the corresponding genomic sequences using TWOBLAST. The quality of the sequence data was judged using Chromas (http://www.technelysium.com.au).

Results

Identification of the human *CYS1* gene

Applying an in silico cloning strategy we identified the human cDNA clone AK091299, which shared more than 80% sequence identity with the murine *Cys1* cDNA. The sequence of the cDNA clone contained most parts of the human *CYS1* coding region gene, starting within exon 1 and extending through exon 2, 3, and the 3′UTR. BLAST analysis showed that the human *CYS1* gene resides on a human genomic contig located on Chromosome 2p25 (*Homo sapiens*, Chromosome 2 working draft sequence, AC104794). Using TWOBLAST analysis between the genomic sequence and the complete murine *Cys1* cDNA sequence, we identified the putative 5′UTR and the missing part of exon 1. Based on these analyses, the full-length coding region of the human cystin cDNA was amplified from a testis cDNA library by nested PCR. The sequence of the complete human cystin cDNA was of the expected 779-bp length, and contained parts of the 5′UTR, exons 1–3, and parts of the 3′UTR (Fig. 1). Using TWOBLAST analysis with the human genomic sequence and the complete human *CYS1* cDNA, we elucidated the intron-exon structure shown in Fig. 1. An ATG codon is positioned within a Kozak consensus sequence [16]. The open reading frame predicts a coding region of 477 bp encoding human cystin, a 158-amino acid protein. All intron-exon boundaries conform to the consensus sequences of the 5′ and 3′ splice sites [17].

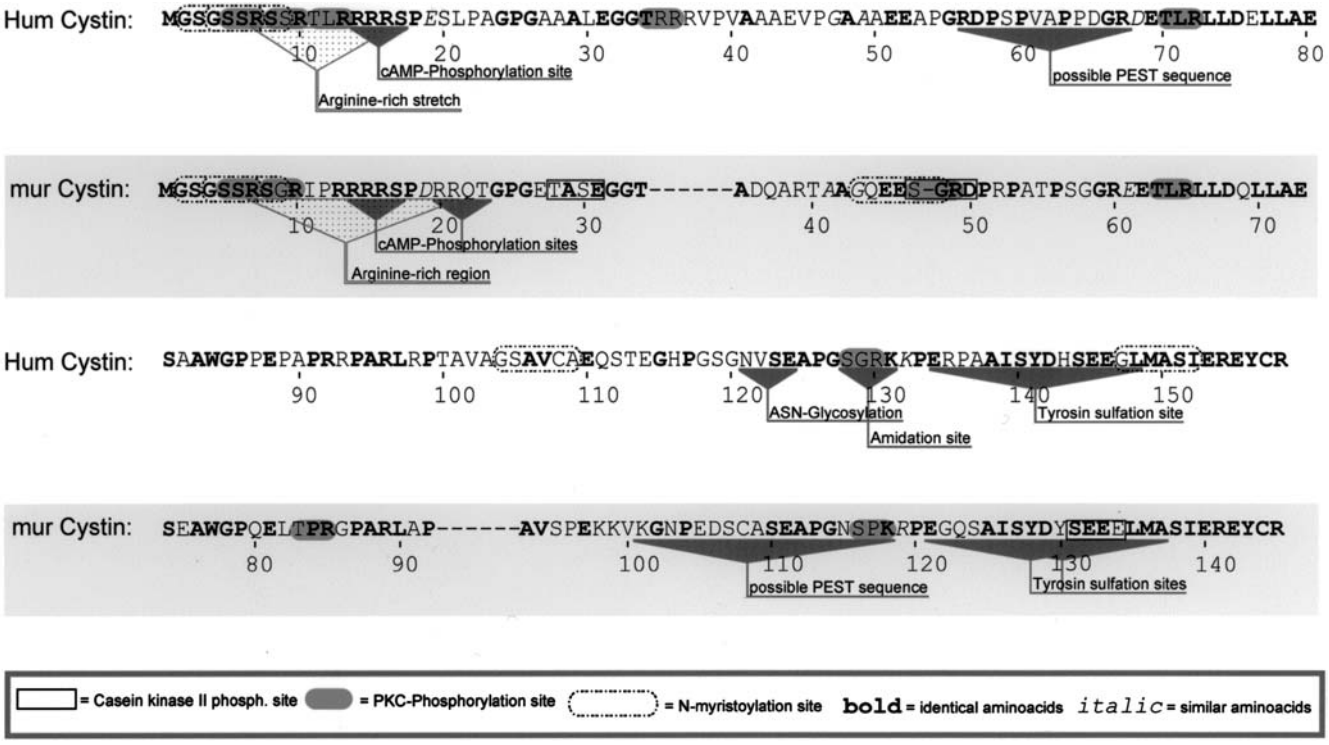

Fig. 2 Protein sequence alignment of human (CYS1) and murine cystin (Cys1). *Bold* and *italic letters* represent identical and conserved amino acids, respectively. Several parts of the sequences are highly conserved. Predicted protein motifs are shown

Sequence comparison of the orthologous proteins human cystin (CYS1) and murine cystin (Cys1) shows 57% identity and 64% similarity (Fig. 2). Like the 145-amino acid murine cystin protein (10% proline), the 158-amino acid human cystin protein (12.7% proline) also exhibits a hydrophilic property. Several domains have been completely or widely conserved between both species: a predicted myristoylation site (amino acids 2–8 murine) and the adjacent polybasic stretch (RRRR); a cAMP phosphorylation site (amino acids 14–17 human and amino acids 14–17 murine); and a possible tyrosine sulfation site (amino acids 134–148 human and amino acids 121–137 murine). Both proteins contain possible PEST domains (score human +5.08 and mouse +6.98), but the localization is not concordant (human amino acids 56–67 and murine amino acids 101–118). The highly conserved middle part (amino acids 46–99 human, amino acids 40–92 murine) and the C-terminal end (amino acids 122–158 human, amino acids 109–145 murine) do not apparently share any predictable protein motifs. Another part of the protein (amino acids 107–122 human, amino acids 92–109 murine) encompassing human exon 2 shows conspicuous divergence from its murine counterpart. Several putative phosphorylation sites within both proteins were predicted with high probability.

Expression analysis

The expression pattern of the human *CYS1* gene was determined by human multiple tissue Northern blot hybridization (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, uterus, small intestine, colon, peripheral blood leukocyte, Fig. 3). Together with the 5′UTR of yet unknown size (>237 nucleotides, estimated from our 5′ primer location), the 477-nucleotide open reading frame and the 2,008-nucleotide 3′UTR (estimated from the AK091299 clone), the length of the complete mRNA from *CYS1* was expected to be approximately 2.7 kb. In addition, the cystin-specific α^{32} P-radiolabeled probe also detected a ~4.4-kb band (Fig. 3) and a weak ~2.- kb band only visible under less stringent washing conditions, which might be due to alternative splicing and/or use of an alternative polyadenylation signal. The highest expression level of the expected 2.7-kb human cystin mRNA was detected in kidney (Fig. 3). A second, weaker band of ~4.4 kb in size was also visible. To a lesser extent, pancreas shares the same distribution of transcripts as kidney. Skeletal muscle, liver, and heart show moderate expression, and prostate, testis, small intestine, and colon show weak expression of both the 2.7-kb and the 4.4-kb transcript. Brain, lung, and uterus exclusively show weak expression of the 2.7-kb gene product, whereas placenta, spleen, thymus, and peripheral blood lymphocytes predominantly express the 4.4-kb transcript.

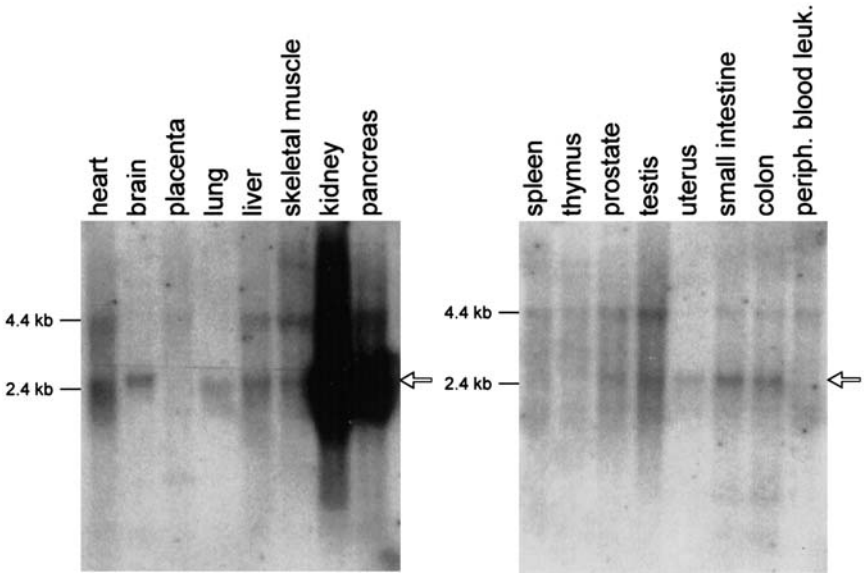

Fig. 3 Human adult tissue Northern blot of the human *CYS1* gene. The *arrow* indicates RNA molecules of a predicted ~2.7-kb size. Strongest expression is present in kidney followed by pancreas. Using a shorter exposure in kidney and pancreas, only single bands of 4.4 kb and 2.7 kb are visible (data not shown). Most tissues show expression of both transcripts. However, in brain, lung, and uterus only the 2.7-kb transcript was present, and in placenta, spleen, thymus, and peripheral blood lymphocytes the 4.4-kb transcript was predominantly detected

Mutational analysis

Sequence analysis of all amplified exons in affected individuals of families (F23, F190, F299, F616, F627, F694, F805, F912) and a healthy control showed no sequence variants. The obtained sequences confirmed the genomic sequence of the human genomic contig located on Chromosome 2p25 (AC104794). A mutation was not detected.

Discussion

To clone the human cystin gene (*CYS1*) we first identified the human genome contig harboring the gene, which is localized on Chromosome 2p25. The gene locus is consistent with the orthologous genomic region of the *Cys1* mouse locus on Chromosome 12 [5]. With the use of cDNA amplification, we obtained the complete coding region of human *CYS1*. The intron-exon structure of the coding region from murine *Cys1* and human orthologue *CYS1* appears to be conserved. In both genes, three exons of similar size encode an open reading frame of 477 bp in human and 435 bp in mouse, respectively. Together with the 5′UTR of yet unknown size (>237 nucleotides, estimated from our 5′ primer location), the 477-nucleotide open reading frame and the 2,008-nucleotide 3′UTR (estimated from the AK091299 clone), the length of the complete mRNA from *CYS1* is at least 2,722 nucleotides. In contrast to the murine counterpart, the 3′UTR of the human *CYS1* gene appears not to contain further untranslated exons. Expression analysis with Northern blot experiments of human *CYS1* demonstrated strongest expression of an expected 2.7-kb transcript in kidney (Fig. 3). Cystin expression was high in pancreas, moderate in heart, liver, and skeletal muscle, and weak in various other tissues. It has previously been shown that the murine 2.2-kb cystin transcript was expressed strongest in kidney and fetal kidney [5]. In addition, Hou et al. [5] reported a ~2.4-kb cystin transcript in human fetal kidney, when they used a murine probe for human Northern blot analysis. Thus, the expression of the human cystin gene (*CYS1*) resembles the tissue-specific expression pattern of the mouse cystin gene (*Cys1*). Furthermore, a larger transcript of 4.4 kb, which contributes to a tissue-specific expression pattern, was identified. Interestingly, in most tissues both the 4.4-kb and the 2.7-kb transcript were present. However, brain, lung, and uterus showed only expression of the 2.7-kb transcript, and placenta, spleen, thymus, and peripheral blood lymphocytes predominantly express the 4.4-kb transcript. The larger transcript is unlikely a result of incomplete splicing, because introns 1 and 2 are too large (~14 kb and ~7 kb, respectively). An alternative explanation is the presence of alternative transcripts with different lengths of the 3'UTR containing untranslated exons, as observed in mice. However, we did not find evidence for untranslated exons 4 and 5 by BLAST analysis, which are present in murine *Cys1* [5]. The existence of longer transcripts containing alternative coding exons cannot be excluded, because we did not perform cDNA cloning on other than testis cDNA library. The observation of tissue-specific transcripts of different size might indicate tissue-specific *CYS1* regulation. An alternative explanation is the presence of a second larger gene with strong similarities to human *CYS1*. However, we did not find evidence for such a gene by BLAST analysis. Interestingly, *CYS1* expression overlaps with *Tg737*, another gene expressed in renal cilia, which is also responsible for cystic kidney disease [1]. *Tg737* encodes polaris, which is found strongest at the basal bodies of renal cilia, where growth of cilia is maintained. Polaris and the orthologous *Chlamydomonas* intraflagellar transport protein IFT88 are required for assembly of cilia and flagella [4]. In contrast, cystin appears to be located throughout the whole renal cilium of mCCD cells [5].

Comparison of murine and human cystin reveals a high degree of conservation between both orthologous proteins. Several domains with known function like the potential N-terminal myristoylation site and the adjacent polybasic stretch have been conserved in both species. N-myristoylation site/polybasic stretch motif is used by proteins such as c-SRC, c-Ki-*ras*, and myristoylated alanine-rich C-kinase substrate (MARCKS) for membrane anchoring [18, 19, 20], which lead Hou et al. [5] to hypothesize that cystin is bound to the axonemal membrane and functions as part of a scaffold that stabilizes microtubule assembly within the ciliary axoneme. Conservation of the N-myristoylation site/polybasic stretch motif in murine and human cystin supports the functional importance of these domains for cystin. Most of the highly conserved middle part and the C-terminal end do apparently not share any predictable protein motifs. These protein motifs are of particular interest, because they appear to be unique for cystin. The function of cystin is still speculative. Further analysis of the novel conserved domains might aid understanding of cystin function within the ciliary axoneme.

Cilia and flagella share similar axonemal structures. Different types of cilia are known. In the first type the axoneme contains nine peripheral microtubule doublets that are arranged around two central microtubules (9+2 structure). Dynein arms are attached on the peripheral microtubules and generate motion by ATP-dependent reactions. These cilia are present in the airways and cover large parts of the epithelial surface to maintain airway clearance. However, the 9+2 structure is also present in ependymal cells lining the brain ventricles, uterus, and fallopian tubes, and in sperm tails. Interestingly, exclusive expression of the 2.7-kb cystin transcript was found in tissues containing this type of cilia. In the second type of cilia only the nine peripheral microtubule doublets are present, and the two central microtubules are absent (9+0 structure). These cilia are present as single cilia (monocilia) on the epithelial surface of the ventral node during embryogenesis. Only recent studies have shown that monocilia of nodal cells are motile and produce an overall leftward flow of extracellular fluid [21]. Disturbances in nodal flow, due to absent nodal cilia in *kif3B*$^{-/-}$ and *Tg737*$^{\Delta 2-3\beta Gal}$ mice, were associated with randomization of left-right asymmetry [2, 21]. Therefore, it has been suggested that the cilia-driven nodal flow creates an asymmetric gradient of a morphogen that starts the left-right signaling cascade. Monocilia can also be found in the nephron, the biliary tract, and the pancreatic ducts. Some authors believe that these cilia are immotile [22]. We recently identified a gene (*DNAH5*) responsible for primary ciliary dyskinesia characterized by defects of respiratory cilia causing chronic upper and lower airway disease, and randomization of left-right asymmetry [23]. *DNAH5* encodes an axonemal heavy dynein motor responsible for the movement of respiratory cilia and nodal monocilia to maintain airway clearance and nodal flow during embryogenesis, respectively. We showed by Northern blot analysis that the expected 14-kb transcript of the dynein motor (*DNAH5*) is second strongest expressed in kidney [23]. Therefore, we believe that some renal monocilia might also be motile like monocilia of the ventral node. However, dysmotility of renal cilia is most likely not the cause of polycystic kidney disease, because only a minority of patients with primary ciliary dyskinesia develops polycystic kidney disease [24]. Another group of cilia belongs to specialized sensory cilia, like the cone receptor of the retina. Recent data suggest that the apical cilium of renal tubular cells has sensory properties. The *Tg737* orthologue is involved in intraflagellar transport in *Caenorhabditis elegans* ciliated sensory neurons and is disrupted in *osm-5* mutant worms [25, 26]. Orthologous genes of the human autosomal dominant polycystic kidney disease genes type 1 (*PKD1*) and 2 (*PKD2*) are also expressed in sensory neurons of *C. elegans* [27]. Renal cilia might measure urinary flow or have specific functions during embryogenesis. To date, the function of renal cilia is still speculative. However, they appear to be essential to prevent polycystic kidney disease. The elucidation of the function of renal cilia might help to find new therapeutic options for cystic kidney disease.

Based on the phenotype of the *cpk* mouse, the human cystin gene is an ideal candidate for a recessive cystic kidney disorder associated with liver fibrosis. Nephronophthisis is a genetic heterogeneous cystic kidney disorder with autosomal recessive transmission that is major genetic cause of chronic renal disease in children [28]. Clinical signs of nephronophthisis include renal symptoms such as polyuria, polydipsia, secondary enuresis, severe anemia, and progressive renal failure. Renal pathology shows the characteristic triad of irregularly thickened tubular basement membranes, atrophy, and dilatation of tubules. Cysts are typically located at the corticomedullary junction. These clinical and pathological findings are the same in juvenile nephronophthisis (NPH1), adolescent nephronophthisis (NPH3), and NPH4 [29, 30, 31]. Extrarenal disease manifestations, such as cerebellar ataxia, skeletal involvement [32], congenital oculomotor apraxia [33], Leber congenital amaurosis [34, 35], and hepatic fibrosis [8] have been described. For candidate gene analysis we studied affected individuals of eight families with Boichis disease (nephronophthisis and liver fibrosis) for evidence of *CYS1* mutations [8]. Sequence analysis of all three coding exons from *CYS1* excluded mutations in the studied individuals. The human cystin gene still remains an interesting candidate for cystic kidney disease. In view of the genomic localization of *CYS1*, the human cystin gene might be an interesting positional candidate for autosomal dominant polycystic kidney disease type 3, where a suggestive LOD score of 2.12 was obtained on Chromosome 2p21.3-q22 [36]. However, because of the phenotype and inheritance pattern of the *cpk* mouse, the human cystin gene is a good functional candidate for human autosomal recessive polycystic disease (ARPKD) not linked to Chromosome 6p [37, 38].

In summary, we localized the human cystin gene (*CYS1*) to Chromosome 2p25, cloned the complete coding cDNA, and studied the expression pattern by Northern analysis. Mutational analysis in affected individuals of eight families with nephronophthisis and liver fibrosis excluded *CYS1* mutations.

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