

Cloning, Characterization, and Chromosomal Mapping of Human Aquaporin of Collecting Duct

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Abstract

We recently cloned a cDNA of the collecting duct apical membrane water channel of rat kidney, which is important for the formation of concentrated urine (Fushimi, K., S. Uchida, Y. Hara, Y. Hirata, F. Marumo, and S. Sasaki. 1993. *Nature [Lond.]* 361:549–552). Since urine concentrating ability varies among mammalian species, we examined whether an homologous protein is present in human kidney. By screening a human kidney cDNA library, we isolated a cDNA clone, designated human aquaporin of collecting duct (hAQP-CD), that encodes a 271-amino acid protein with 91% identity to rat AQP-CD. mRNA expression of hAQP-CD was predominant in the kidney medulla compared with the cortex, immunohistochemical staining of hAQP-CD was observed only in the collecting duct cells, and the staining was dominant in the apical domain. Functional expression study in *Xenopus oocytes* confirmed that hAQP-CD worked as a water channel. Western blot analysis of human kidney medulla indicated that the molecular mass of hAQP-CD is 29 kD, which is the same mass expected from the amino acid sequence. Chromosomal mapping of the hAQP-CD gene assigned its location to chromosome 12q13. These results could be important for future studies of the pathophysiology of human urinary concentration mechanisms in normal and abnormal states. (*J. Clin. Invest.* 1994. 93:1250–1256.) Key words: vasopressin • urinary concentration • diabetes insipidus • kidney medulla • water channel

Introduction

Among animals, only mammals and birds can produce urine hypertonic to the plasma. Concentrating urine is necessary to prevent water loss, and this is required for living in a terrestrial environment (1). In response to vasopressin, concentrated urine is produced by water reabsorption across the kidney collecting duct. Water is thought to pass through water-permeable pores, i.e., “water channels,” in the apical membrane of this nephron segment (2–7). We recently cloned a cDNA of the apical collecting duct water channel of the rat kidney (8), based on the conserved amino acid sequence in the members of

the major intrinsic protein (MIP)¹ (9) family, including channel integrate protein of 28 kD (CHIP28), a water channel of red blood cells, and renal proximal and descending tubules (10–12). This protein was initially reported as the water channel of collecting duct and subsequently renamed aquaporin of collecting duct (AQP-CD). Rat (r)AQP-CD is a 271-amino acid protein with 43% amino acid identity to CHIP28. It has six putative membrane spanning domains, internal tandem repeat, and conserved NPA box (Asn, Pro, Ala, sequence); all these are characteristics of the MIP family (13, 14). Immunohistochemical study using polyclonal antibody against rAQP-CD showed that this protein was expressed only in the collecting duct, and the staining was strong in the apical and subapical regions. Injection of in vitro transcribed mRNA of rAQP-CD to *Xenopus oocytes* induced a more than eightfold increase in water permeability compared with water-injected oocytes. This induction of water permeability was inhibited by a water channel inhibitor, HgCl₂. All these results suggest that rAQP-CD is the vasopressin-regulated water channel of the kidney collecting duct (8).

There are species differences in urine concentrating abilities of mammals. For example, humans can concentrate urine to 1,400 mOsm/kg, whereas rats concentrate it to 2,600 mOsm/kg, and Australian hopping mice can concentrate it as high as 9,000 mOsm/kg (15). Histological studies have also shown that there are distinct morphological differences among medullary structures of mammals (16). Furthermore, biochemical studies by Morel (17) demonstrated that the response of adenylate cyclase to vasopressin in medullary thick ascending limb is lacking in human and dog kidneys, but it is present in rat, rabbit, and mouse kidneys. Considering these species differences, it is critically important to know if a protein that is homologous to rAQP-CD is present in human kidney collecting duct.

Congenital nephrogenic diabetes insipidus (NDI) is a rare inherited disorder characterized by renal unresponsiveness to vasopressin. Most cases of NDI appear to have an X-linked recessive pattern of inheritance. Very recently several mutations in vasopressin V₂ receptor gene, which is located in chromosome X, have been found in these patients as underlying mechanisms (18–21). However, NDI patients who did not show such an inheritance (22), or whose V₂ receptor did not show any abnormality (20), have been reported, suggesting the presence of other gene abnormalities in some cases of NDI. Since AQP-CD is so critically important for urinary concentra-

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1. Abbreviations used in this paper: AQP-CD, aquaporin of collecting duct; CHIP28, channel integrate protein of 28 kD; FISH, fluorescent in situ hybridization; h, human; MIP, major intrinsic protein of the bovine lens fiber membrane; NDI, nephrogenic diabetes insipidus; r, rat.

tion, it is reasonable to speculate that a defect in this protein would also cause NDI.

In this study we examined whether an homologous protein to rAQP-CD is present in human kidney. By screening the human kidney cDNA library, we isolated a cDNA clone that encodes a protein that is 91% identical to rAQP-CD, and named it human (h)AQP-CD. Studies showed that hAQP-CD was functionally and morphologically a homologous protein to rAQP-CD. Chromosomal mapping of hAQP-CD gene assigned its location to chromosome 12.

Methods

Approximately 3×10^5 plaques of human kidney cDNA library in λ Max1 (Clontech, Palo Alto, CA) were screened using rAQP-CD cDNA as a probe under stringent conditions: hybridization in $6 \times$ SSPE, 50% formamide at 42°C, washing in $2 \times$ SSC, 0.5% SDS at 42°C. Positive clones were excised and converted into pYEUra3 vector by helper phage. Several clones (0.8–1.5 kb) were isolated, and two clones with inserts of 1.5 kb were subcloned into the EcoRI site of the pSPORT vector (GIBCO BRL, Gaithersburg, MD). The pSPORT subclones of BamHI, KpnI, PstI, and SphI restriction fragments were sequenced by the Sanger dideoxynucleotide chain termination method using Sequenase (U.S. Biochem. Corp., Cleveland, OH). T7 and M13 sequencing primers and eight synthetic oligonucleotide primers were used for sequencing both strands of hAQP-CD.

For Northern blot analysis, RNA was extracted from the cortex and medulla of human kidney (nephrectomized for kidney tumor) by acid guanidine phenol chloroform method, and 30 μ g of total RNA per lane was subjected to electrophoresis on agarose gel containing formaldehyde. Equal loading was confirmed by staining with ethidium bromide. After transfer to a nylon membrane, blots were hybridized with the hAQP-CD cDNA labeled with 32 P and autoradiographed for 24 h.

Immunohistochemical study was performed to localize hAQP-CD protein in human kidney. Sequence comparison showed that only one amino acid (Thr265) was different between human and rat 15 COOH-terminal amino acid, where we previously raised the polyclonal antibody against rAQP-CD (Fig. 1). We therefore examined the antibodies for rAQP-CD (8), but the stainings were weak and nonspecific staining was observed. Accordingly, we raised a new polyclonal antibody against a synthetic peptide (VELHSPQSLPRGTKA; Peptide Institute Inc., Minoh, Osaka) corresponding to the 15 COOH-terminal amino acid of hAQP-CD, in rabbits. The same kidney used for Northern analysis was used for immunohistochemical study. The kidney tissues were fixed in 2% paraformaldehyde solution, and thin cryostat sections (4 μ m) were made and mounted on slide glasses. The slides were preincubated with nonimmune goat serum; then they were rinsed and incubated with the rabbit serum (1:500 dilution) at 37°C for 1 h. Next, after being rinsed the slides were incubated for 1 h at 37°C with FITC-conjugated goat anti-rabbit immunoglobulins at 1:100 dilution.

Western blot analysis was performed using the same antibody as used for immunohistochemical studies. Membranes were prepared from human and rat kidneys by homogenization in a Potter Elvehjem apparatus. After homogenization in 10 vol of 0.32 M sucrose, 5 mM Tris-HCl, 2 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride, 1 μ g/ml leupeptin, 1 μ g/ml pepstatin A, two centrifugations (10 min, 1,000 g) were applied. The supernate was then centrifuged at 250,000 g for 30 min, and the pellet was suspended in the same buffer. The samples were solubilized in a sample loading buffer; 3% SDS, 65 mM Tris-HCl, 10% glycerol, 5% 2-mercaptoethanol, and heated at 70°C for 5 min. They were separated by SDS-PAGE using 10% polyacrylamide gels according to Laemmli (23), and were transferred to polyvinyl membranes (Immobilon; Millipore Corp., Bedford, MA). The blots were incubated with antisera (1:100 dilution), and were visualized using a 125 I-protein A (ICN Biochemicals, Costa Mesa, CA). A urine sample from a healthy volunteer was concentrated 10 times by

ultrafiltration (cutoff at 3,000 mol wt, Microcon-3; Amicon Corp., Danvers, MA), and was applied to SDS-PAGE as described above.

Functional expression study in *X. oocytes* was performed as described previously (8). Briefly, in vitro transcribed and capped RNA (cRNA) was injected into oocytes, followed by incubation for 24–48 h at 18°C. Osmotic water permeability was calculated by the volume increase when oocytes were transferred from 200 to 70 mOsm/kg modified Barth's solution, at 20°C. The volume increase was monitored by videomicroscopy and was analyzed by an image analysis system (C-3163; Hamamatsu Photonics, Hamamatsu).

Chromosomal mapping was performed using a fluorescence in situ hybridization (FISH) method. Replicated prometaphase R-banded chromosomes were prepared from human lymphocytes. Two genomic lambda phage clones (~15 kb) were isolated from a human genomic DNA library (Stratagene, La Jolla, CA) using cDNA of hAQP-CD as a probe, and labeled by nick translation using biotin-16-dUTP (GIBCO BRL). In situ hybridization was done as previously described (24) and detected by avidine-FITC conjugate (GIBCO BRL). To the genomic probes, 5–10 times excess amounts of sonicated total human placenta DNA were added for the elimination of repetitive sequences. When using cDNA of hAQP-CD as a probe, the intensity of the FITC was amplified according to the procedure reported by Hori et al. (25). Fluorescent signals on chromosome counterstained with propidium iodide were observed by a Nikon Microphot microscope (FXA-RFL) using filter combinations of B-2A for R-banded and UV-2 for G-banded chromosomes.

Results

Sequencing of cDNA clones obtained from human kidney cDNA library indicated that two clones coded the same sequence of 1.5 kb, and these clones were named hAQP-CD. The longest open reading frame encoded a 271-amino acid protein (28,968 M_r) that was highly homologous to rAQP-CD. In Fig. 1, amino acid sequences of hAQP-CD, rAQP-CD, and human CHIP28 are compared. Sequence identity of hAQP-CD to rAQP-CD is 91%, and to human CHIP28 is 48%. The high homology of hAQP-CD to rAQP-CD indicated that hAQP-CD is a human counterpart of rAQP-CD. The hydropathy profile of hAQP-CD was also very similar to that of rAQP-CD; the profile predicts six membrane-spanning domains (8). The conserved amino acid sequence, named NPA box, and tandem repeat in the sequence, which are characteristics of MIP family (13, 14), were also conserved in hAQP-CD.

One potential N-glycosylation site is present in the sequence of hAQP-CD (Asn123), and one phosphorylation site (26) for protein kinase A (Ser256), which was observed in rAQP-CD, is conserved in hAQP-CD.

Northern blot analysis of mRNA from the cortex and medulla of human kidney showed that the expression is predominant in the medulla (Fig. 2). The major transcript was 4.2 kb, and another small band was detected at 1.6 kb. This pattern was somewhat different from that of rAQP-CD. In rAQP-CD the major band was 1.5 kb, and faint bands at 2.8 and 4.4 kb were observed (8). Since our 1.5-kb cDNA clone of hAQP-CD coded a protein that is homologous to rAQP-CD, and since the predicted size of the protein (28,968 M_r) was observed in Western blot (see below), we speculate that the larger band at 4.2 kb may represent polyadenylation variants.

Immunohistochemical study of the medulla of human kidney showed that the fluorescent staining was localized to only collecting duct cells (Fig. 3a). The staining was strong around the apical and subapical domains at higher magnification (Fig. 3b). Occasionally a faint band was observed on the basolateral

hWCH-CD	1	MWELRSIAFS	RAVFAEFLAT	LLFVFFGLGS	AL	N	WATAPPSVLQ	43
rWCH-CD	1	MWELRSIAFS	RAVLAEFLAT	LLFVFFGLGS	AL	Q	WASPPSVLQ	43
CHIP28	1	MASEFKKKLFW	RAVVAEFLAT	TLFVFISIGS	ALGFKYPVGN	NQTAVQDNVK		51
hWCH-CD	44	IAMAFGLGIG	TLVQALGHIS	GAHINPAVTV	ACLVGCHVSF	LRAAFYVAAQ		93
rWCH-CD	44	IAVAFGLGIG	ILVQALGHVS	GAHINPAVTV	ACLVGCHVSF	LRAAFYVAAQ		93
CHIP28	52	VSLAFGLSIA	TLAQSVGHIS	GAHLNPAVTL	GLLLSCQISI	FRALMYIAQ		101
hWCH-CD	94	LLGAVAGAAL	LHEITPADIR	GDLAVNALS	SSTAGQAVTV	ELFLTQLVL		143
rWCH-CD	94	LLGAVAGAAI	LHEITPVEIR	GDLAVNALHN	NATAGQAVTV	ELFLTMQLVL		143
CHIP28	102	CVGAVATAI	LSGITSSLTG	NSLGRNDLAD	GVNSGQGLGI	EIIGTLQLVL		151
hWCH-CD	144	CIFASTDERR	GENPGTPALS	IGFSVALGHL	LGHYTGCSM	NPARSLAPAV		193
rWCH-CD	144	CIFASTDERR	GDNLGPALS	IGFSVTLGHL	LGITYTGCSM	NPARSLAPAV		193
CHIP28	152	CVLATTDRRR	RDLGGSAPLA	IGLSVALGHL	LAIDYTGCGI	NPARSFGSAV		201
hWCH-CD	194	VTGKFDDHWV	FWIGPLVGAI	LGSLLYNYVL	FPPAKSLSER	LAVLKGLEPD		243
rWCH-CD	194	VTGKFDDHWV	FWIGPLVGAI	IGSLLYNYLL	FPSAKSLQER	LAVLKGLEPD		243
CHIP28	202	ITHNFSNHWI	FWVGF IGGA	LAVLIYDFIL	APRSSDLTDR	VNVWTSQGVE		251
hWCH-CD	244	T DWEEREVR	RRQSVELHSP	QSLPRGTKA				271
rWCH-CD	244	T DWEEREVR	RRQSVELHSP	QSLPRGSKA				271
CHIP28	252	EYDLADDIN	SRVEMKPK					269

Figure 1. The amino acid sequences of hAQP-CD, and its comparison to rAQP-CD and human CHIP28. Conserved residues are shaded.

membrane. In the cortex the staining was also restricted to collecting duct cells, and no signal was detected in proximal tubules and glomerulus (Fig. 3 c). A close inspection indicated that a minority of the cortical collecting duct cells stained negatively, indicating that these cells may be intercalated cells. Intercalated cells are known not to express water channels (3). When the antibody was preincubated with the corresponding peptide immunogen, no specific staining was observed (data not shown). These results were the same as observed in the rat (8).

Western blot analysis was performed to determine the molecular mass of hAQP-CD protein. Using the antibody against hAQP-CD, two bands were observed in the membrane preparation from human kidney medulla: a sharp band at 29 kD, and a diffuse band at 40–50 kD (Fig. 4, lane 4). This staining pattern is quite similar to that of CHIP28, in which a sharp band at 28 kD and a diffuse, higher molecular mass band at 35–55 kD have been observed (12, 27). The latter diffuse band was shown to represent a glycosylated form of the 28-kD protein

(12). As shown in Fig. 5, after N-glycanase digestion of human kidney medulla membranes, the high molecular mass band disappeared, demonstrating that the high molecular mass band represents a glycosylated form of the 29-kD protein. In addition to the 29-kD band, a smaller band (~ 26 kD) was noted after the N-glycanase treatment. The nature of this band is unknown, but may represent a degraded product of the 29-kD protein. Comparison between the cortex and medulla samples clearly indicated the dominant presence of hAQP-CD protein in the medulla (Fig. 4, lanes 3 and 4). Interestingly, the concentrated human urine contained a 29-kD protein and a smaller band indicative of degraded proteins (Fig. 4, lane 5). These results indicate that some of hAQP-CD becomes detached from the apical membrane and is excreted in the urine. Blots stained with preimmune serum did not show any staining (Fig. 4, lanes 1 and 2). Rat kidney medulla membrane fraction was also subjected to the same Western blot analysis (Fig. 4, lane 6). The staining pattern was similar to that of human kidney medulla. The 29-kD protein was observed at the same position in rat medulla, but a diffuse band distributed over a slightly smaller size region (36–45 kD) in the rat, indicating the difference in glycosylation.

To confirm that hAQP-CD works as a water channel, expression of in vitro transcribed mRNA (cRNA) of hAQP-CD in *X. oocytes* was examined. The determined osmotic water permeability coefficient (P_f) when oocytes were exposed to osmotic gradients was six times higher in cRNA-injected (40 ng) oocytes than that of water-injected oocytes (122.2 ± 9.1 vs. $19.8 \pm 1.5 \times 10^{-4}$ cm/s, mean \pm SE; $P < 0.05$). The increase in P_f of hAQP-CD-injected oocytes was partially inhibited by a 5-min pretreatment with 0.3 mM $HgCl_2$, an inhibitor of water channels ($70.4 \pm 2.8 \times 10^{-4}$ cm/s; Fig. 6).

To determine the chromosomal location of hAQP-CD gene, we performed direct R-banded FISH studies on prometaphase chromosome spreads using two different genomic probes (both were ~ 15 kb in size). A total of 41 prometaphases were analyzed for the presence of fluorescent spots of both probes. The hybridization signals showing twin spots on both sister chromatids were observed at chromosome band 12q13. 75% of

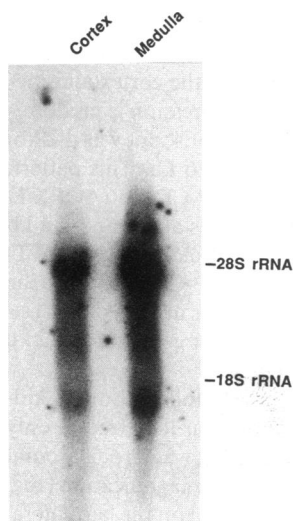


Figure 2. Northern blot analysis of expression of hAQP-CD mRNA in human kidney. Total RNA was extracted from human kidney cortex and medulla, and 30 μ g per lane was subjected to electrophoresis.

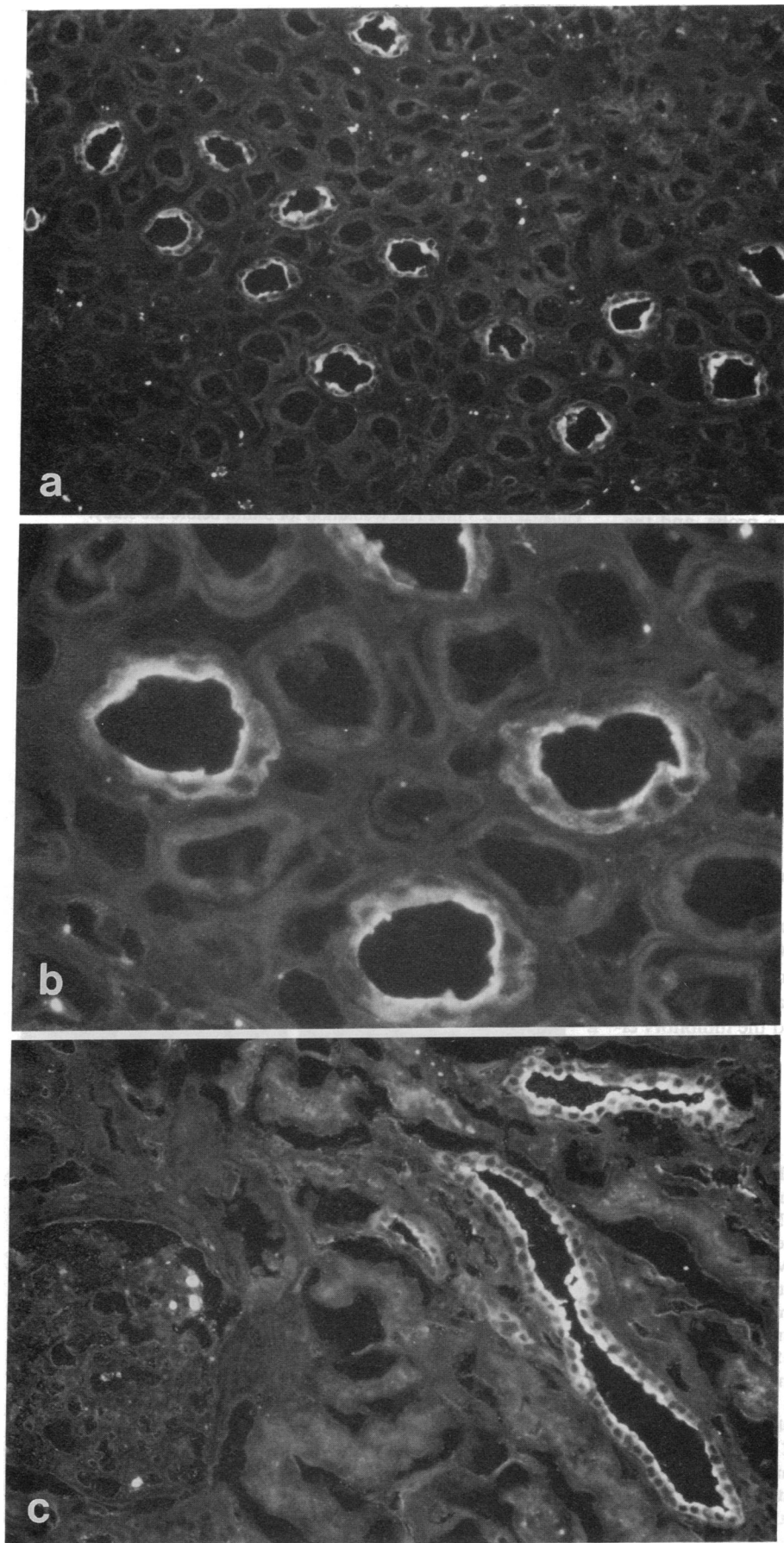


Figure 3. Immunohistochemical localization of hAQP-CD in human kidney. Thin sections of human kidney were incubated with rabbit polyclonal antibody against the COOH terminal of hAQP-CD and visualized by FITC-conjugated goat anti-rabbit immunoglobulins. (a) Medulla, $\times 40$; (b) medulla, $\times 100$; and (c) cortex, $\times 40$.

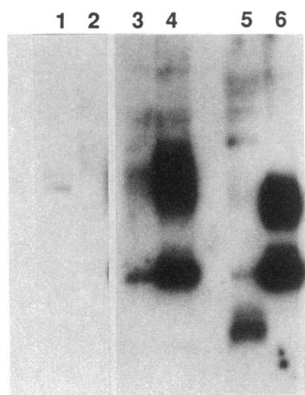


Figure 4. Western blot analysis of human kidney membrane samples. Membrane samples were obtained from the cortex (lanes 1 and 3) and medulla (lanes 2 and 4) of human kidney, and 10 μ g each was applied to SDS-PAGE. A urine sample was enriched by 10 times by ultrafiltration, and 10 μ l was applied (lane 5). After transfer to the membrane, blots were incubated with antiserum (lanes 3–6) or

with preimmune serum (lanes 1 and 2). A rat kidney medulla membrane sample was also applied (10 μ g) in lane 6.

the signals were on the 12q13.11–q13.12 region (Fig. 7). In addition, cDNA of hAQP-CD was used as a probe, and the result was identical.

Discussion

The presence of hAQP-CD, which is highly homologous to rAQP-CD in terms of amino acid sequence, localization of both mRNA and protein, and functional expression in oocytes, strongly suggests that AQP-CD is important for urinary concentration in many mammalian species. Comparison of amino acid sequences among hAQP-CD, rAQP-CD, and human CHIP28 may provide some insight into the functional characteristics of AQP-CD. In the sequence of hAQP-CD, there are four cysteine residues (at 75, 79, 144, and 181) that could be important for water channel function, because HgCl_2 has been known as an inhibitor of water channels (28). All residues except Cys75 are conserved among these three proteins, and Cys75 is conserved in hAQP-CD and rAQP-CD. Cys181 is conserved in these three proteins, but not in other MIP family members. Preston et al. (29) have shown that site-directed mutagenesis of this cysteine to serine abolished the inhibitory ef-

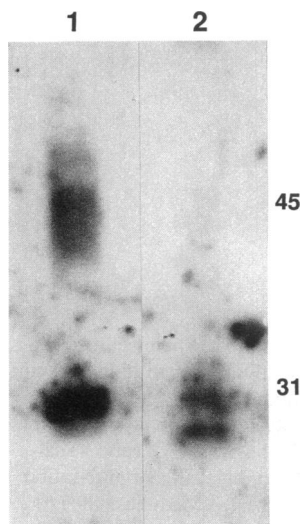


Figure 5. Effect of N-glycanase digestion of human kidney medulla membrane. Membrane aliquots (40 μ g) were denatured in 0.5% SDS, 50 mM β -mercaptoethanol, then incubated for 20 h at 37°C in 75 μ l containing 1 U N-glycanase (Genzyme Corp., Cambridge, MA), 0.2% SDS, 1.3% NP-40, 150 mM sodium phosphate (pH 7.5), and 10 mM 1,10 phenanthroline (39). Samples were analyzed by SDS-PAGE immunoblot. Lanes 1 and 2 contained 15 μ g of human kidney medulla membrane without and with N-glycanase treatment, respectively.

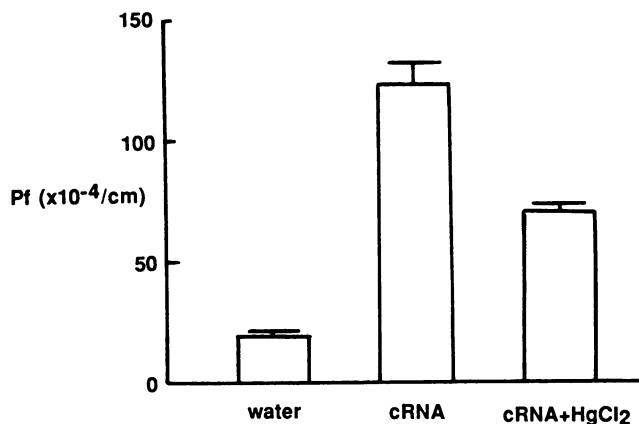


Figure 6. Functional expression of cRNA of AQP-CD in *X. oocytes*. Oocytes were injected with 40 nl water or 40 ng hAQP-CD cRNA, and incubated for 24–48 h at 18°C. Osmotic water permeability (P_f) was determined by measuring the hypotonic volume increase at 20°C. For inhibition by mercurial sulphhydryl agent, oocytes were incubated in 0.3 mM HgCl_2 solution for 5 min before the study.

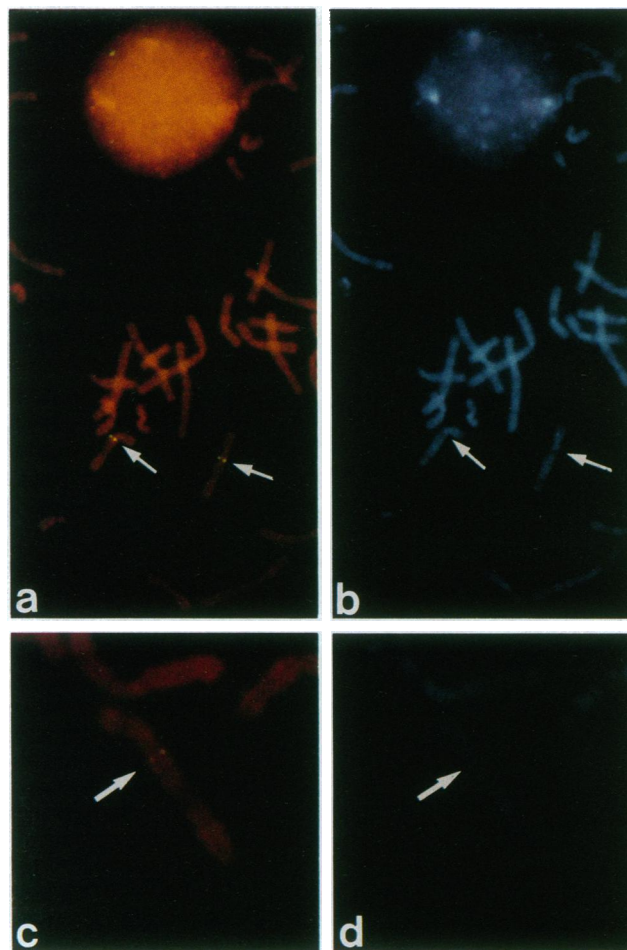


Figure 7. Chromosomal mapping of hAQP-CD gene. Fluorescence in situ hybridization on chromosomes from human cultured lymphocytes with genomic DNA clones (~15 kb) were performed. Fluorescent signals were observed at the 12q13.11–q13.12 region (arrows). (a and c) R-banded chromosomes (Nikon B-2A filter). (b and d) G-banded chromosomes (Nikon UV-2A filter).

fect of mercury. These results taken together indicate that this cysteine residue is critically important for water channel function.

The cytosolic domain of the COOH terminal of AQP-CD (48 amino acids) lacks homology to CHIP28 (12.5%), and may contribute to a specific character of AQP-CD. When we cloned rAQP-CD, we found a consensus sequence for phosphorylation by protein kinase A in the COOH terminal (8). This sequence is conserved in hAQP-CD, but not in CHIP28. It is fascinating to speculate that protein kinase A directly phosphorylates AQP-CD protein to alter the water channel function. This speculation is not unrealistic, because it has been reported that protein kinase A phosphorylates MIP (30–32) and alters the voltage-dependent character of the channel function of MIP (33). If this is the case in AQP-CD, then this process will provide another mechanism other than the shuttle hypothesis, for rapid regulation of water permeability of collecting duct by vasopressin. The shuttle hypothesis explains the rapid regulation of water permeability by exo- and endocytosis of water channel-containing endocytic vesicles to and from the apical membrane (3, 4, 6, 7, 34). A possibility of direct phosphorylation by protein kinase A will be answered in the near future.

Western blot analysis showed that hAQP may be composed of two molecular mass forms: a sharp band of 29 kD and a broad band of ~ 40 kD. A similar study in CHIP28 gave the similar pattern to ours, in which a broad band was shown to be a glycosylated form of 28 kD protein (12). The study of N-glycanase digestion (Fig. 5) clearly showed that the broad high molecular mass band in hAQP-CD is a glycosylated form of the 29-kD protein. The sequence of hAQP-CD also has a potential site for N-glycosylation. The amino acid sequence of hAQP-CD predicts a 28,968-dalton protein, which is consistent with a band at 29 kD. The antibody against hAQP-CD used in this study stained rAQP-CD to the same extent. Comparison of hAQP-CD and rAQP-CD in the same gel (Fig. 4) showed that the molecular mass of AQP-CD is the same in human and rat (29 kD).

It is interesting to observe the presence of the 29-kD intact form of hAQP-CD in the urine of a normal subject. This indicates that hAQP-CD in the apical membrane of collecting duct becomes detached from the membrane and is excreted into the urine. Since there may not be much protease activity and protein-absorbing ability in the collecting duct, ureter, and bladder, it is likely that AQP-CD, once detached from the membrane, will be excreted in an intact form. This result raises a possibility that hAQP-CD can be used as a marker of the damage to collecting duct cells.

There are two types of congenital NDI (35). Type I (MIM 304800) is an X-linked disease whose defect concerns the inability of renal tubule to respond to vasopressin, and in this patient administration of vasopressin is not followed by an increase in urinary cAMP (36). Another one is type II (MIMI 125800), an autosomal dominant disease in which urinary levels of cAMP are elevated in response to vasopressin (37, 38). Recently, the cause of X-linked NDI has been identified as a defect in vasopressin V₂ receptor gene, which locates in the X chromosome (18–21). However, NDI patients who did not show X-linked inheritance (22), or whose V₂ receptor gene was not abnormal (20), have been also reported.

The apical membrane water channel plays a pivotal role in

water absorption in the collecting duct. It can be anticipated that if the function of this protein is disturbed, it will cause NDI. This form of defect predicts a lack of diuretic response to vasopressin accompanied by an intact response of cAMP generation. It might be possible that abnormalities in the hAQP-CD gene exist in type II patients. Thus, the locus of the hAQP-CD gene (chromosome 12q13) might be identical to that of NDI type II. Clearly, this possibility awaits future studies.

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