Mutations in *aquaporin-1* in Phenotypically Normal Humans Without Functional CHIP Water Channels

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The gene *aquaporin-1* encodes channel-forming integral protein (CHIP), a member of a large family of water transporters found throughout nature. Three rare individuals were identified who do not express CHIP-associated Colton blood group antigens and whose red cells exhibit low osmotic water permeabilities. Genomic DNA analyses demonstrated that two individuals were homozygous for different nonsense mutations (exon deletion or frameshift), and the third had a missense mutation encoding a nonfunctioning CHIP molecule. Surprisingly, none of the three suffers any apparent clinical consequence, which raises questions about the physiological importance of CHIP and implies that other mechanisms may compensate for its absence.

The high water permeability of red cells and certain epithelia is due to the presence of water-selective membrane channels (*1*). The first characterized molecular water channel was CHIP, an integral protein purified from membranes of mammalian red cells and kidneys (*2*), and the complementary DNA (cDNA) was isolated from a human bone marrow library (*3*). The function of CHIP was demonstrated by expression of the complementary RNA (cRNA) in *Xenopus* oocytes and verified by reconstitution of the purified protein in proteoliposomes (*4*). CHIP is presumed to be a major water transporter of mammalian tissues, as it is abundant in red cells, renal proximal tubules, descending thin limbs of Henle, and several other water-permeable epithelia (*2, 5*). CHIP is encoded by *aquaporin-1*, a single-copy gene on human chromosome 7p14 that is processed without alternative mRNA splicing (*6*). Homologous proteins from diverse tissues and species have subsequently been demonstrated to transport water and are now referred to as the aquaporins (*7*). The gene *aquaporin-2* encodes the renal collecting duct water channel and is mutated in a severe form of nephrogenic diabetes insipidus (*8*). Other aquaporins have been linked to the aging of bovine lens, abnormal neural development in *Drosophila*, and the response of plants to nematodal infestations and water deprivation (*9*). Because of the abundance and distribution of CHIP, it was predicted that mutations in *aquaporin-1* would also result in a severe or lethal phenotype (*7*).

The Colton blood group antigens result from an Ala-Val polymorphism at residue 45, located on the first extracellular loop of CHIP (*10*). Worldwide blood group referencing has led to the identification of only five kindreds whose red cells do not express the Colton antigens, Co(a−b−), and structural alterations were expected near the site of the Colton polymorphism. We obtained blood samples and urine sediment from one member of three of these kindreds (referred to here as probands 1, 2, and 3). These Co(a−b−) individuals are unrelated women of Northern European ancestry (*11*), and none has experienced hematological, renal, ocular, respiratory, gastrointestinal, reproductive, or neurological dysfunction.

The Co(a−b−) red cells of these individuals appeared morphologically normal, and the membranes appeared normal on SDS–polyacrylamide electrophoresis gels stained with Coomassie blue (Fig. 1). The 28-kD CHIP polypeptide and the 40- to 60-kD glycosylated CHIP have been consistently detected on immunoblots of red cell membranes from more than 100 normal humans (*2, 12*) and in red cell membranes from rat and diverse mammalian species (*13*). Nevertheless, CHIP was not detected in membranes from probands 1 and 2 on immunoblots (Fig. 1). Prolonged radiographic exposure revealed that membranes from proband 3 had a barely detectable signal, equivalent to <1% of the normal intensity. Renal tubule CHIP was investigated by immunoblot analysis of urinary sediment (*14*); CHIP polypeptides and proteolytic fragments were observed in urine samples from normal controls but not from the probands (Fig. 1).

To determine if other mechanisms compensated for the absence of CHIP in the Co(a−b−) red cells, we measured their osmotic water permeability, P_f. At 37°C, the P_f of each Co(a−b−) sample was reduced by ~80% when compared to that of red cells from 11 normal adults (Table 1). Similar studies, performed at 8° to 39°C, permitted calculation of the Arrhenius activation energy, E_a. Consistent with the absence of water channels, the average E_a value for the Co(a−b−) red cells was ~9.5 kcal/mol, whereas the average for control red cells was similar to the E_a of water diffusing in bulk solution, <5 kcal/mol (*1*). Mercuric chloride is known to inhibit CHIP water channels by interacting with Cys^{189} (*15*), and incubation of red cells in 1 mM $HgCl_2$ greatly reduced the P_f of control samples but affected the patient samples only marginally.

The absence of CHIP from Co(a−b−) red cells and urine sediment suggested that these individuals have mutations in *aquaporin-1*. DNA sequences corresponding to introns and untranslated flanking regions surrounding the four exons were used to design oligonucleotide primers (*6, 10*). Amplification by the polymerase chain reaction (PCR) of genomic DNA from each proband (*16*) yielded identical products from exons II to IV, and the sequences of these PCR products were identical to the known sequences of human *aquaporin-1* (*3, 6*). Attempts to amplify exon I from the genomic DNA of proband 1 were unsuccessful. A Southern (DNA) blot of genomic DNA from proband 1 incubated with the full-length coding region of *aquaporin-1* re-

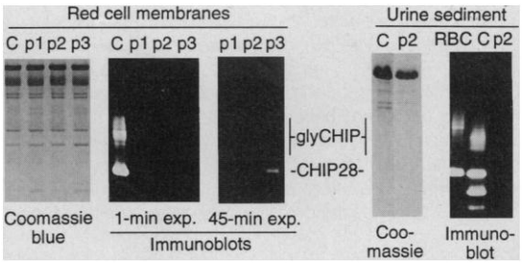

Fig. 1. SDS–polyacrylamide gel electrophoresis of red cell membranes (left) and urine sediment (right) from Co(a−b−) individuals. The membranes were visualized by Coomassie blue staining or by immunoblot analysis with an antibody specific for the proteolysis-resistant NH_2-terminus of CHIP (*14*). Lanes contain red cell membranes (7 μg of protein) from a normal, age- and gender-matched Co(a+b−) control (C) or probands of three Co(a−b−) kindreds (p1, p2, p3). Control red cell membranes (RBC; 0.2 μg of protein) were compared to urine sediment (~25 μg of protein) from the control and proband 2. Similar results were obtained with urine sediment from probands 1 and 3, and the presence of red cells in urine was excluded by immunoblot with antibody to spectrin.

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vealed the absence of a 1.88-kb fragment and the presence of a weakly hybridizing band of 1.27 kb (Fig. 2A), implying that most of exon I had been deleted. Exon I amplification products of the correct size were obtained from genomic DNA of probands 2 and 3. Nucleotide sequencing revealed that proband 2 had a single-base insertion at position 307, producing a frameshift mutation starting after Gly^{104} (Fig. 2B), whereas proband 3 had a C → T substitution at position 113, resulting in a Pro → Leu substitution at residue 38 (P38L) (Fig. 2C). Indicative of homozygosity for these mutations, the exon I amplification products from probands 2 and 3 contained unique restriction enzyme sites that led to complete cleavage of the DNA (*17*). Therefore, the sites of these three mutations within the CHIP polypeptide are consistent with translational failure in proband 1, major disruption of the CHIP structure in proband 2, and possible disruption in proband 3 (Fig. 2D).

Mutations identified in *aquaporin-2* from individuals with nephrogenic diabetes insipidus were recently shown to be inactivating when expressed in *Xenopus* oocytes (*8*). We similarly assessed the functional effects of the missense mutation of proband 3 by injecting *Xenopus* oocytes with water containing no cRNA, mutant P38L cRNA, or normal CHIP cRNA. Two days after injection, osmotic swelling experiments (Fig. 3) demonstrated that oocytes expressing the P38L mutant protein exhibited higher osmotic water permeabilities than water-injected oocytes (P_f = 33.0 ± 8.3 × 10^{-4} cm/s, n = 8, compared to 10.2 ± 4.1 × 10^{-4} cm/s, n = 8), but oocytes expressing CHIP displayed much higher permeabilities (P_f = 262 ± 35 × 10^{-4} cm/s, n = 8). Two days after injection, the oocytes contained similar amounts of normal and mutant CHIP polypeptides, but high mannose and complex glycosylated CHIP subunits were not detected in oocytes expressing P38L (Fig. 3, inset) and the amount of P38L in the plasma membrane was reduced. At 3 days, the total amount of P38L protein in the oocytes was substantially reduced (*14*), suggesting that protein instability may explain the small amount of CHIP in the red cells of proband-3. Moreover, Pro and Gly are present in this region of most aquaporin homologs (*18*); this implies that there is a structural requirement for these residues at the end of the first bilayer-spanning α helix.

Our studies document that rare humans missing antigens from a minor blood group system are also missing red cell CHIP because of mutations in *aquaporin-1*. Developmental patterns of CHIP expression are

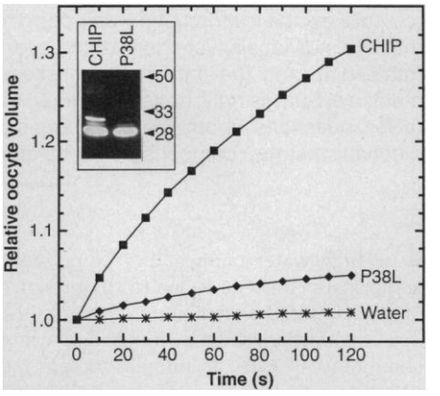

Fig. 3. Osmotic swelling of *Xenopus* oocytes expressing CHIP or the mutant P38L. Oocytes were injected with 50 nl of water without cRNA, with 10 ng of P38L cRNA, or with 10 ng of CHIP cRNA. After 48 hours, the oocytes were transferred from 200 to 70 mosmol per liter of modified Barth's buffer, and changes in volume were measured by videomicroscopy (*15*). Shown are representative tracings of volume versus time for individual oocytes. (Inset) Immunoblot of membrane proteins from three oocytes expressing CHIP or P38L after incubation with affinity-purified antibody specific for the 4-kD COOH-terminus of CHIP (*14*).

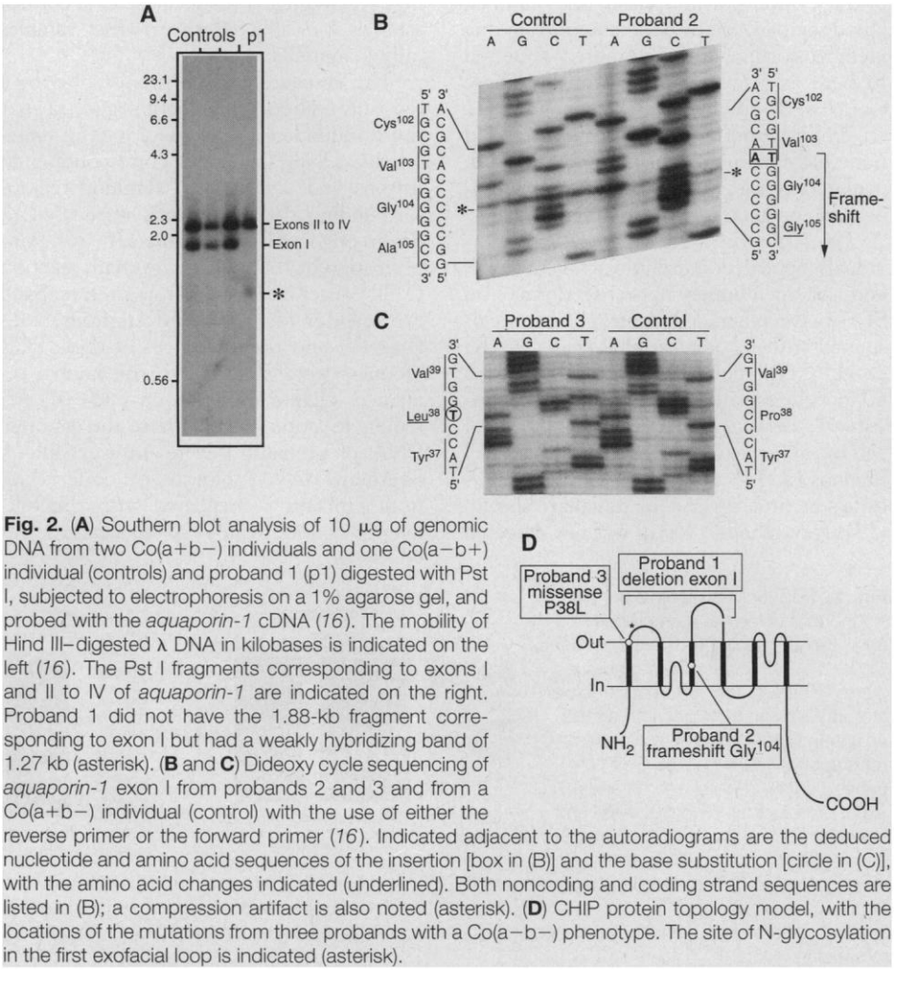

Fig. 2. (**A**) Southern blot analysis of 10 μg of genomic DNA from two Co(a+b−) individuals and one Co(a−b+) individual (controls) and proband 1 (p1) digested with Pst I, subjected to electrophoresis on a 1% agarose gel, and probed with the *aquaporin-1* cDNA (*16*). The mobility of Hind III–digested λ DNA in kilobases is indicated on the left (*16*). The Pst I fragments corresponding to exons I and II to IV of *aquaporin-1* are indicated on the right. Proband 1 did not have the 1.88-kb fragment corresponding to exon I but had a weakly hybridizing band of 1.27 kb (asterisk). (**B** and **C**) Dideoxy cycle sequencing of *aquaporin-1* exon I from probands 2 and 3 and from a Co(a+b−) individual (control) with the use of either the reverse primer or the forward primer (*16*). Indicated adjacent to the autoradiograms are the deduced nucleotide and amino acid sequences of the insertion [box in (B)] and the base substitution [circle in (C)], with the amino acid changes indicated (underlined). Both noncoding and coding strand sequences are listed in (B); a compression artifact is also noted (asterisk). (**D**) CHIP protein topology model, with the locations of the mutations from three probands with a Co(a−b−) phenotype. The site of N-glycosylation in the first exofacial loop is indicated (asterisk).

Table 1. We determined the osmotic water permeability of red cells, P_f, by measuring the change in light scattering after abrupt doubling of the extracellular osmolality with a mixture of phosphate-buffered saline and sucrose in a stopped-flow apparatus (*13*). Tracings from multiple determinations were fit to single exponential curves and iteratively solved with software (MathCAD, MathSoft, Cambridge, Massachusetts). Where indicated, red cells were incubated for 5 min in 1 mM $HgCl_2$. The Arrhenius activation energy, E_a, was calculated from values for P_f determined between 8° and 39°C in the absence of $HgCl_2$. The statistical analysis of values determined from red cells of normal adult controls and probands of Co(a−b−) kindreds is as follows: P_f at 37°C (−$HgCl_2$), P < 0.001; P_f at 37°C (+$HgCl_2$), not significant; E_a, P < 0.02.

Sample	P_f (cm/s) at 37°C		E_a (kcal/ mol)
	−$HgCl_2$	+$HgCl_2$ (1 mM)	
Proband 1	0.006	0.004	8.4
Proband 2	0.010	0.005	8.8
Proband 3	0.006	0.005	11.2
Controls (n = 11)	0.037 ± 0.007	0.009 ± 0.005	4.6 ± 0.8

complex (*19*), and expression of the protein in the rat red cells and renal tubules does not occur until birth (*13*); however, the total absence of CHIP has not previously been described in prenatal or postnatal humans (*2*, *12*). It is surprising that none of the Co(a−b−) patients suffers from any apparent anemia, and the biological need for water channels in red cell membranes remains unexplained (*1*).

Our studies also dictate the need to rethink the postulated roles of CHIP in nonerythroid tissues (*7*). An average adult human produces ~200 liters of glomerular filtrate daily, of which ~90% was thought to be reabsorbed through the abundant CHIP water channels in the proximal tubules and descending thin limbs of Henle (*5*). CHIP was also thought to be essential for secretion of cerebrospinal fluid, aqueous humor, reproductive fluids, and bile, as well as for maintenance of interstitial fluid and prevention of perialveolar edema (*5*, *20*), processes that appear to be normal in the Co(a−b−) individuals. Further clinical evaluations may reveal other physiological roles for CHIP and elucidate mechanisms that compensate for the absence of CHIP. The existence of subclinical defects in renal concentration, neurological status, or age-dependent problems in the Co(a−b−) individuals must be considered.

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14. Urine sediment was collected after a 5,000*g* spin for 10 min at 4°C; red cell membranes were prepared by hypotonic lysis [V. Bennett, *Methods Enzymol.* **96**, 313 (1983)]; total oocyte membranes (*15*) and oocyte plasma membranes were also prepared by hypotonic lysis [D. A. Wall and S. Patel, *J. Membr. Biol.* **107**, 189 (1989)]. Membranes were subjected to electrophoresis on 12% SDS-polyacrylamide gels, transferred to nitrocellulose (Bio-Rad), incubated with affinity-purified antibodies to CHIP at 0.05 μg/ml (*2*), and visualized by the enhanced chemiluminescence detection system (Amersham).
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16. Genomic DNA was isolated from peripheral blood lymphocytes of normal healthy volunteers and from Co(a−b−) individuals with the use of the QIAamp blood kit (Qiagen, Chatsworth, CA). Southern analysis of genomic DNA was performed as described (*6*), except that the blot was probed with denatured *aquaporin-1* cDNA (10^6 dpm/ml) corresponding to nucleotides 15 to 822 (*3*). The *aquaporin-1* exons were amplified with primers complementary to the 5′ and 3′ untranslated sequences and intronic sequences flanking each of the four exons (*10*). PCR samples (50 μl) containing 100 to 200 ng of genomic DNA, 25 pmol of each primer, and 0.7 to 1.0 mM $MgCl_2$ were denatured for 5 min at 95°C. Two units of AmpliTaq DNA polymerase (Perkin-Elmer) were added, and 35 cycles of amplification were performed (1 min at 94°C, 1 min at 50° to 60°C, and 1 min at 72°C). PCR products were gel-purified for sequencing or restriction analysis.
17. The single-base insertion in exon I of proband 2 corresponded to the loss of a Pfl MI restriction site and the appearance of a Bst XI site; the single-base substitution in exon I of proband 3 corresponded to the loss of a Msp I site. Similar results were obtained with different sets of nonoverlapping amplification primers.
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Reduced Rate of Disease Development After HIV-2 Infection as Compared to HIV-1

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Human immunodeficiency virus type–2 (HIV-2) is a close relative of the prototype acquired immunodeficiency syndrome (AIDS) virus, HIV-1. HIV-2 is biologically similar to HIV-1, but information is lacking concerning clinical outcomes of HIV-2–infected individuals. From 1985 to 1993, a prospective clinical study was conducted in women with HIV-2 and HIV-1 infection to determine and compare rates of disease development. HIV-1–infected women had a 67% probability of AIDS-free survival 5 years after seroconversion in contrast with 100% for HIV-2–infected women. In addition to having significantly less HIV-related disease outcome in HIV-2 enrollees compared to HIV-1 enrollees, the rate of developing abnormal $CD4^+$ lymphocyte counts with HIV-2 infection was also significantly reduced. This natural history study demonstrates that HIV-2 has a reduced virulence compared to HIV-1.

Evidence of a second human immunodeficiency virus was first demonstrated in a study of Senegalese women in 1985 (*1*). HIV-2 was subsequently found to be highly prevalent in many countries in West Africa (*2*) and has been present in populations in this region as early as the 1960s (*3*). Nonetheless, fewer AIDS cases have been reported in West African countries, where HIV-2 is the predominant AIDS virus, than in Central or East Africa, where HIV-1 infection predominates (*4*). The population of HIV-2–seropositive Senegalese sex workers, in whom HIV-2 was initially described, was clinically surveyed and found to be relatively free from HIV-associated signs or symptoms when compared with prostitutes surveyed in HIV-1–prevalent areas in Africa (*5*). Given the observed differences in the prevalences of AIDS and in the geographical distribution of HIV-2 as compared to

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