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The family of human Na,K-ATPase genes

ATP1AL1 gene is transcriptionally competent and probably encodes the related ion transport ATPase

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The multigene family of human Na,K-ATPase is composed of 5 α-subunit genes, 3 of which were shown to encode the functionally active α1, α2 and α3 isoforms of the catalytic subunit. This report describes the isolation, mapping and partial sequencing of the fourth gene (*ATP1AL1*) that was demonstrated here to be functionally active and expressed in human brain and kidney. Limited DNA sequencing of the *ATP1AL1* exons allowed one to suggest that the gene probably encodes a new ion transport ATPase rather than an isoform of the Na,K-ATPase or the closely related H,K-ATPase.

Na^+,K^+-ATPase; Ion transport ATPase; Gene family; Human genome

1. INTRODUCTION

Na^+,K^+-activated adenosine triphosphatase is an integral membrane protein that transduces the energy from the hydrolysis of ATP into a gradient for Na^+ and K^+ across the cell membrane. The enzyme molecule consists of the catalytic α-subunit and glycosylated β-subunit of unknown function. As of now, 5 α-subunit genes and one closely related gene for the catalytic subunit of H,K-ATPase were identified in the human genome [1,2]. Three of the Na,K-ATPase α-subunit genes encoding the α1, α2 and α3 isoform were shown to be expressed in a variety of human and animal tissues (for review see [3]). The functional status of the remaining two genes designated as *ATP1AL1* and *ATP1AL2* [4] was still unknown. Here we report the data on cloning, mapping and partial sequencing of *ATP1AL1*. In addition, we present results indicating that *ATP1AL1* is a new functionally active member of the Na,K-ATPase gene family and expressed in the human brain and kidney.

2. EXPERIMENTAL

Genomic DNA was isolated from the human sperm [8] partially digested with *Sau*3A [6] and fractionated by NaCl gradient centrifugation [5]. Fractions containing 15–23 kb in length were used for cloning in the λEMBL3 vector according to [7]. To make a chromosome walking, high-stringency hybridization was performed at 65°C as described [9]. Phage DNAs of hybridizable clones were isolated according to the procedure of Yamamoto [10] and mapped for *Eco*RI and *Hin*dIII sites, combining the double digestion method [6] and the technique of partial digestion [11]. The exon-containing fragments of the phage inserts were identified after the low-stringency hybridization with pig kidney cDNA [12] at 55°C. The cDNA-hybridizable fragments were subcloned into M13mp18 and sequenced by the method of Sanger [13]. The poly(A^+)-fraction of total cellular RNA was isolated from frozen human tissues as previously described [14]. The random-primed cDNA was generated from 0.5 μg of the poly(A^+)-RNA following the standard technique [6] and one-tenth of the synthesized cDNA was subjected to 25–30 cycles of enzymatic amplification [15] at 94°C, 1 min; 48°C, 1.5 min; and 72°C, 2 min, using the AGATTCCGAGAAGAAGACCA and GCTGGGGCTCAGACTCCCCCGTGAGA oligonucleotides as gene-specific primers. The PCR product was analyzed by electrophoresis on 8% polyacrylamide gels [6], followed by transferring to Hybond N membranes (Amersham, England) and low-stringency hybridization with a pig kidney cDNA probe. The part of the PCR product was cloned to the undephosphorylated *Sma*I-cut M13mp18 and sequenced by the dideoxy method [13].

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3. RESULTS AND DISCUSSION

The *ATP1AL1* gene was described as a member of the human Na,K-ATPase gene family under the names of αNKαSW3.2 [1] and αD [2]. Sverdlov et al. [1], and Shull and Lingrel [2] published the nucleotide sequence of exons 6, 7 [2] and 9 [1] as well as the partial physical map of this gene [2]. The chromosomal location of *ATP1AL1* was determined by somatic cell hybrid mapping studies and assigned to the human chromosome 13 [4]. The objective of this study was to clone and map *ATP1AL1* completely, and to determine whether the gene is transcriptionally competent. To isolate the λ

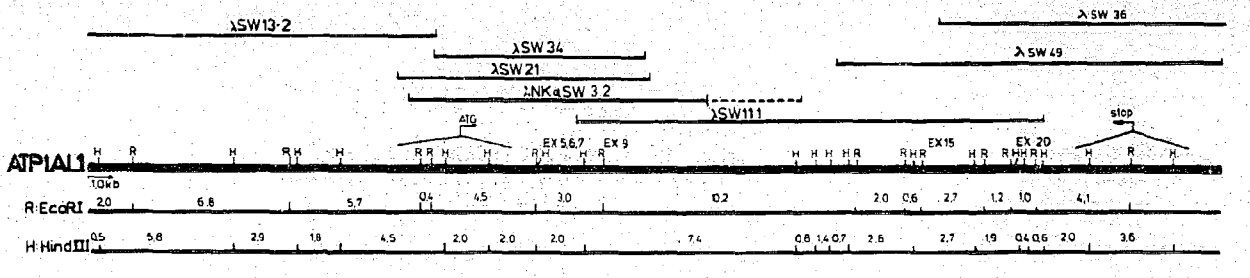

Fig. 1. Restriction enzyme map of the human *ATP1AL1* gene. The gene is represented as a thick line in the middle of the figure, overlapping inserts from the recombinant phages are shown above. Below the gene structure is a map for two restriction endonucleases with fragment sizes indicated in kb.

phage inserts which encompass the entire *ATP1AL1* we performed two steps of bidirectional chromosome walking starting from the end fragments of the primary clone λNKαSW3.2. The set of overlapping clones spanning more than 60 kb were characterized. Hybridization analysis with the pig kidney α1 cDNA probe revealed that the gene itself is 25–30 kb in length. The restriction enzyme map of the *ATP1AL1* depicted in Fig. 1 shows some differences when compared with published data [2]. We failed to find the 2.2 and 0.6 kb fragments which were positioned by Shull and Lingrel between the 2.9 and 12.3 kb cDNA hybridizable fragments. In addition, the locations of the 15th and 20th exons (Fig. 1) indicate that the *ATP1AL1* is longer than was presented earlier [2]. DNA sequence analysis of the λ phage portions that hybridized with pig kidney α1 cDNA allowed us to determine the structure of exons 5, 9 and 20, and part of the 15th exon, as well as exons 6 and 7 which were sequenced by Shull and Lingrel [2]. The deduced amino acid sequences showed 65–75% homology for exons 5, 6 and 20, and 85–90% homology for the highly conservative exons 7 and 9 when compared with the corresponding regions of α1, α2, and α3 isoforms, as well as with the α-subunit of human H,K-ATPase. The degree of homology could be sufficient to consider the putative *ATP1AL1* product as an isoform of the catalytic subunit of either Na,K-ATPase or H,K-ATPase, but several features of the *ATP1AL1* nucleotide sequences caution against this conclusion. Fig. 2A presents the comparison of amino acid sequences encoded by the 5th, 6th and 20th exons of the human genes for α1, α2, α3 isoforms of Na,K-ATPase, α-subunit of H,K-ATPase and *ATP1AL1*. The comparison revealed that the α1, α2, and α3 sequences represent one class of homology exhibiting about 90% identity, while the *ATP1AL1* and H,K-ATPase form a second class which is only 57–69% homologous when compared with human α1. In addition, the exon/intron boundary at the 3′-end of the exon 20 is identical for the H,K-ATPase gene and *ATP1AL1* but differs from the α1, α2, and α3 genes, which have a three nucleotide (one amino acid) deletion at that position (Fig. 2A). On the other hand, it is not very likely that the *ATP1AL1* represents the isoform of the H,K-ATPase because the pairwise comparison of the amino acid sequences encoded by exons 5, 6 and 20

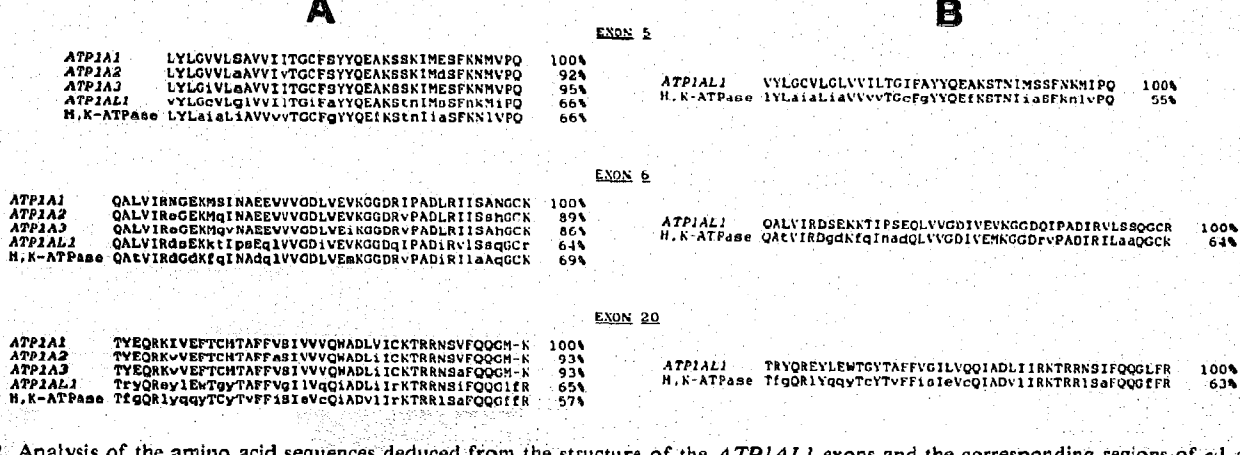

Fig. 2. Analysis of the amino acid sequences deduced from the structure of the *ATP1AL1* exons and the corresponding regions of α1 cDNA, *ATP1A2* (α2 gene), *ATP1A3* (α3 gene), and the human H,K-ATPase gene (data from ref. 17, 18, 19, 16 respectively). Percentage identity is indicated on the right, non-identical residues are indicated in lower case. A: Homology of amino acid sequences encoded by the members of the Na,K-ATPase gene family and the H,K-ATPase gene. B: Comparison of the *ATP1AL1* and human H,K-ATPase amino acid sequences.

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exon 6          ⌄20             ⌄40             ⌄60
AGATTCCGAGAAGAAGACCATCCCTTCAGAGCAGCTGGTGGTGGGGGACATTGTGGAGGT
                ⌄80             ⌄100            ⌄120
CAAAGGAGGAGACCAGATCCCTGCAGACATCAGGGTGCTGTCTTCTCAGGGGTGTCGGgt
                ⌄140            ⌄160            ⌄180
aacgggaagtatccaccccaaggaccatgttccaaacctgctggctctggggtctttccc
                ⌄200            ⌄220            ⌄240
agcatcagtataagaggcagggaatgaggtacccagctgtaaccaccttcaacatttctt
   exon 7       ⌄260            ⌄280
ctagGTGGATAACTCATCTCTCACGGGGGAGTCTGAGCCCCAGC
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Fig. 3. Nucleotide sequence of the *ATP1AL1* fragment flanked by two oligonucleotides which were used for the PCR analysis of the *ATP1AL1* transcript. The intron sequence is shown in lower case. PCR primers are underlined.

(Fig. 2B) exhibits about 60% homology. This level is markedly less than that of the 3 sodium pump α-subunit genes. In addition, a recently published genomic sequence of human H,K-ATPase [16] demonstrated the absence of an intron between the 6th and 7th exon. In contrast, the *ATP1AL1* gene possesses the 126 bp intron at this position (Fig. 3). Thus, the limited sequence information on the *ATP1AL1* structure allowed to suppose that the gene is evolutionary distant from either a Na,K-ATPase or a H,K-ATPase and can represent a gene for the related ion transport ATPase, although the possibility that *ATP1AL1* encodes the marginal forms of a Na,K-ATPase or a H,K-ATPase cannot be excluded.

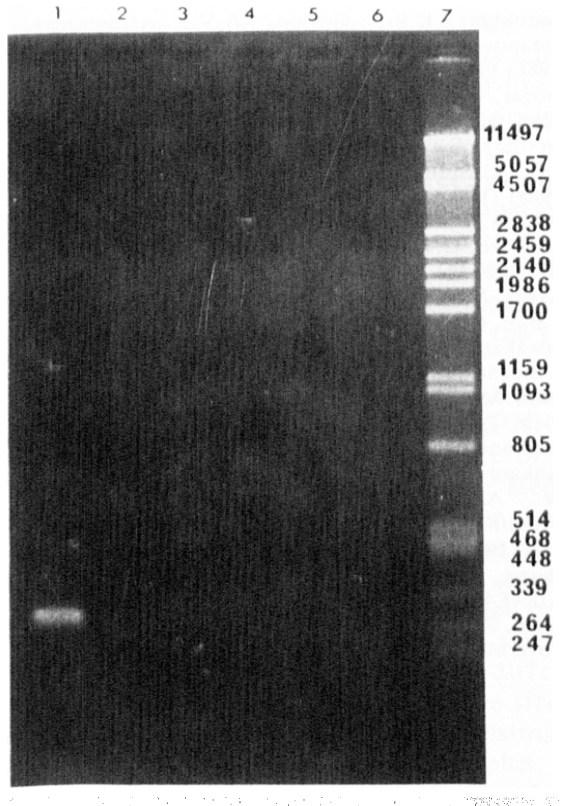

Fig. 4. Test for the specificity of the oligonucleotides which were used to detect the *ATP1AL1* gene transcript. 1 ng of the phage λ and cosmid DNAs from the clones containing exons 6 and 7 of five α-subunit genes were subjected to 25 cycles of PCR followed by gel electrophoresis in 2% agarose. Lanes 1, 2, 3, 4 and 5 represent PCR from the DNA of λSW21 (gene *ATP1AL1*), λRS9-1 (gene *ATP1A1*), cosRC16-10 (gene *ATP1A2*), λRS8.1 (gene *ATP1A3*) and λSAKS35 (gene *ATP1AL2*), respectively. Lane 6, PCR from the 10 ng of the pGC 35 DNA (full-length pig α1 cDNA). Lane 7, size markers (*Pst*I digest of phage λ DNA).

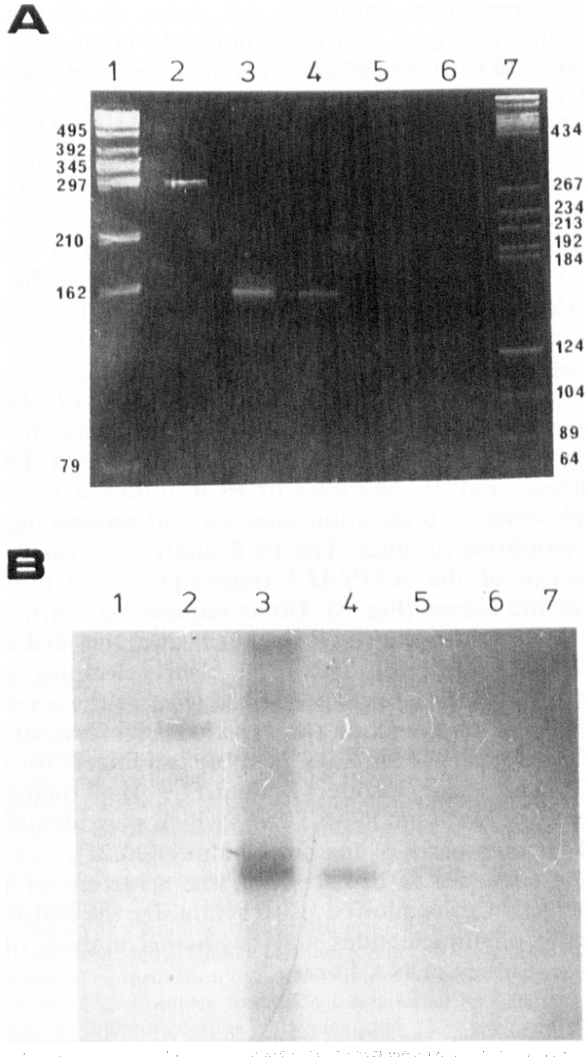

Fig. 5. PCR-based search for the presence of the *ATP1AL1* transcript in poly(A$^+$)-RNA of human tissues. Poly(A$^+$)-RNA was converted to single stranded cDNA and subjected to 25 cycles of PCR with *ATP1AL1*-specific oligonucleotides. The PCR products were electrophoretically analyzed on an 8% polyacrylamide gel (A) followed by Southern blotting and hybridization to the ^{32}P-labeled α1 pig cDNA (B). Lanes 3, 4, 5 and 6, PCR from poly(A$^+$)-RNA of the human brain, kidney, liver, and renal carcinoma, respectively. Lane 2, PCR from the DNA of genomic clone λSW21 (contains intron 6 that increases the size of the fragment). Lanes 1, 7, size markers (*Hin*cII digest of ϕX 174 DNA and *Hae*III digest of pBR 322 DNA, respectively).

It should be noted that the determined nucleotide sequence of the *ATP1AL1* exons as well as the structure of the exon/intron boundaries gave no indication that *ATP1AL1* represents a pseudogene but its functional

status remained unknown. In order to determine whether this gene is transcriptionally competent we decided to use the method of polymerase chain reaction (PCR) for the mRNA detection after the conversion of a RNA preparation to the complementary DNA. The specificity of PCR amplification is based on the specificity of two primers that flank the DNA sequence. In the case of *ATPIAL1* mRNA detection we chose two oligonucleotides from exons 6 and 7 (see Fig. 3). The primers were shown to be very specific for *ATPIAL1* and gave no amplification product when phage λ DNAs containing the corresponding regions of the other α-subunit genes were used as templates in the PCR reaction (Fig. 4). Searching for the *ATPIAL1* transcript in the poly(A^+)-fraction of the human brain, kidney, liver and renal carcinoma RNA included cDNA synthesis and 25–30 cycles of PCR followed by electrophoresis, hybridization analysis and sequencing of the amplified product. The PCR analysis revealed the presence of the *ATPIAL1* transcript in the human brain and kidney (Fig. 5). Direct sequencing confirmed the identity of the PCR product and the deduced *ATPIAL1* transcript. This result clearly demonstrated that the *ATPIAL1* is expressed at least at the level of mRNA and can encode the functional isoform of an ion transport ATPase. The possible candidates for the *ATPIAL1* gene product could be the ouabain-insensitive Na^+- and K^+-ATPases which were identified in differents parts of mammal kidney [20,21].

The sequence information on the structure of the *ATPIAL1* exons allowed us to synthesize the isoform-specific oligonucleotides and begin the analysis of a human kidney cDNA library.

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