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SHORT COMMUNICATION

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Isolation and chromosomal mapping of a novel human gene showing homology to Na^+/*PO4* cotransporter

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Abstract We isolated a cDNA clone which shows a significant similarity with the renal Na^+/phosphate cotransporter (NPT) from a human intestine mucosa cDNA library. The cDNA is 2626 bases long, with one open reading frame encoding a protein of 497 amino acids. The deduced amino acids sequence shows an overall homology of 48% with the human renal NPT1 protein. This gene is expressed in intestine, colon, liver, and pancreas. Thus, this gene may code for intestinal type NPT or closely related proteins. The chromosomal location of the gene was determined on the chromosome 6p21.3-p22 region by polymerase chain reaction-based analysis with both a human/rodent mono-chromosomal hybrid cell panel and a radiation hybrid mapping panel.

Key words Na^+/Phosphate cotransporter (NPT) · Intestine · Chromosome 6p21.3-p22 · RT-PCR · cDNA library

Introduction

Phosphate homeostasis in humans is maintained by the balance between intake, intestinal absorption, bone deposition and resorption, and renal excretion. The central molecule for the control of kidney excretion is the Na+/phosphate cotransporter (NPT), which is located in the proximal tubule (Biber 1989, Biber et al. 1993). This molecule uses the transmembrane electrochemical potential gradient of sodium to transport phosphate across the cell membrane (Murer et al. 1991). A similar NPT also plays an important role in the absorption of the phosphate in the intestine mucosa (Murer et al. 1994). While human NPT of the kidney has been cloned (Chong et al. 1993) and studied, the molecular structure of human intestinal NPT is yet to be elucidated. Here, we report the isolation of a novel cDNA with significant homology to the renal NPT1 from the human intestinal mucosa cDNA library.

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Results and discussion

A full length-enriched cDNA library was constructed, using the oligo-capping method (Maruyama and Sugano 1994, Suzuki et al. 1997) with mRNA isolated from normal mucosa of ileum. The sequences of the 5′ ends of the cDNA clones from the library were determined for 3150 clones. One clone, kaia2138, showed significant homology to human renal NPT1 cDNA. We determined the entire sequence of this clone. The nucleotide sequence data reported here will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases, with accession number AB020527. The cDNA was 2626 bp in length and has one open reading frame encoding a protein of 497 amino acids. A homology search revealed that it is homologous to the NPT family. Recently, human NPT3 and NPT4 cDNAs, homologous to human NPT1, were isolated from a 1.1-megabase region of the hereditary hemochromatosis locus of human chromosome 6. Their nucleotide sequences have been submitted to GenBank. The multiple alignments of amino acid sequences of the kaia2138 protein and NPTs are shown in Fig. 1. The kaia2138 protein was most homologous to the human NPT3 protein, having 54% identity at the amino acid level. The amino acid sequence of kaia2138 was 48% identical to human NPT1 and 43.5% identical to human NPT4.

The expression level of this gene was not detectable by Northern blot analysis, so we examined the tissue distribution of the transcript in various human adult tissues by reverse transcription-coupled polymerase chain reaction (RT-PCR). Primers used for RT-PCR were to amplify the

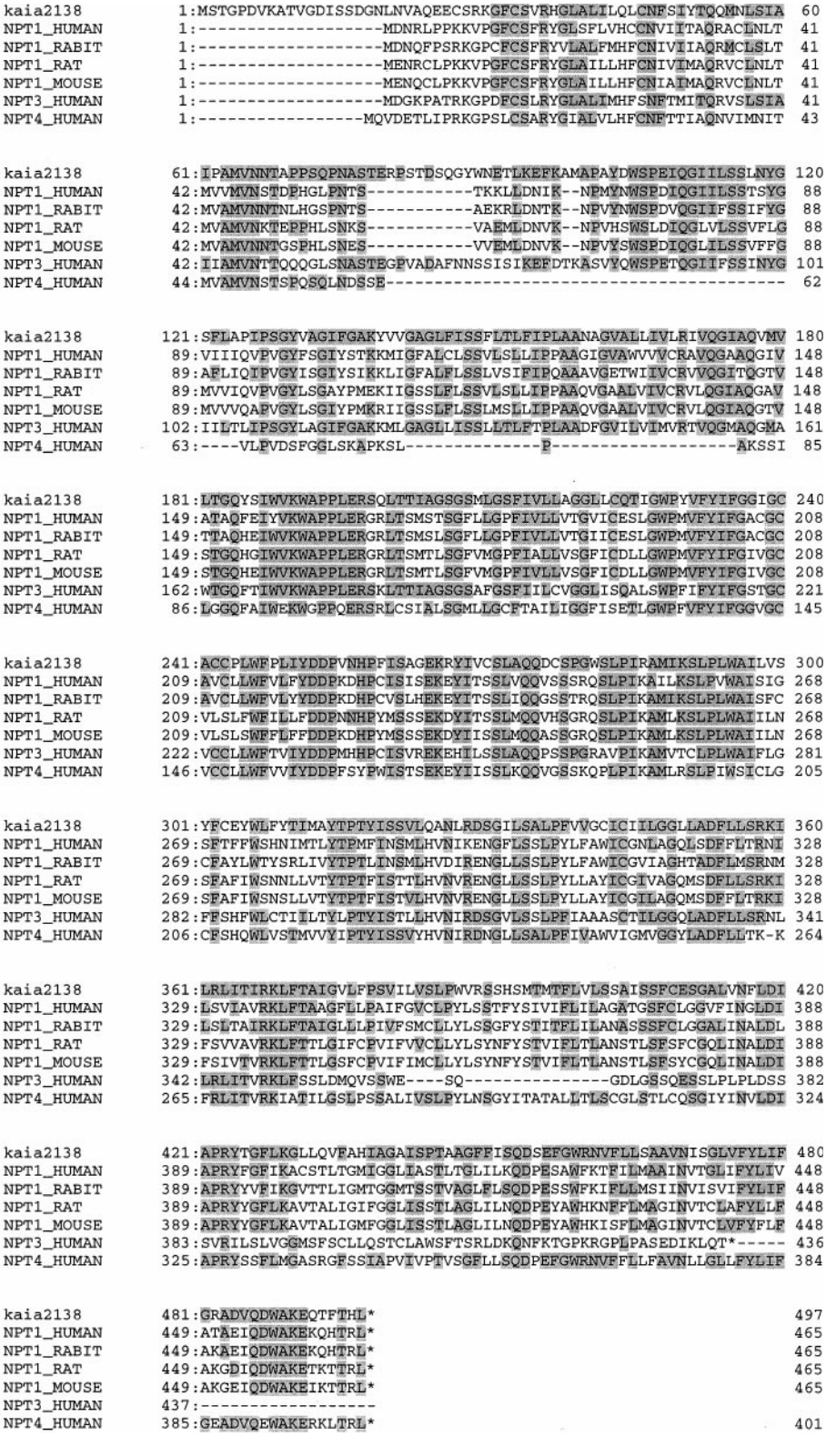

Fig. 1. Multiple alignment of *kaia2138* (accession number AB020527) and Na^+/phosphate cotransporter (NPT) families. The NPT sequences are human NPT1 (NPT1_HUMAN; accession number Q14916), rabbit NPT1 (NPT1_RABIT; accession number Q28722), rat NPT1 (NPT1_RAT; accession number Q62795), mouse NPT1 (NPT1_MOUSE; accession number Q61983), human NPT3 (NPT3_HUMAN; accession number O00624) and human NPT4 (NPT4_HUMAN; accession number O00476). Identities are indicated by *shadowed background*. *Asterisks* denote the terminal codon

407 bp of the 3′-untranslated region of the cDNA (Fig. 2). The *kaia2138* gene was mainly expressed in liver, pancreas, small intestine, and colon.

Chromosomal assignment of the *kaia2138* gene was done by PCR analysis of a human/rodent somatic cell hybrid panel and a radiation hybrid panel, as described previously (Saito et al, 1997). The specific amplified PCR primers were designed at the 3′-untranslated region of the gene (5′-TTCTGGCCATCTTGACTTCTG-3′, 5′-CCAACAGTCAGAGGGGCAAAC-3′; PCR product size was 178bp). First, a specific

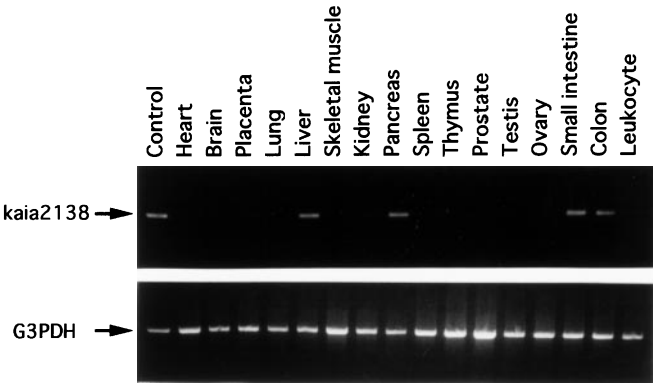

Fig. 2. Reverse transcription-coupled polymerase chain reaction (RT-PCR) analysis of *kaia2138* in multiple human tissues. Primers used for RT-PCR amplify 407 bp of the 3′-non-coding region of the cDNA. The primers were (5′-TACCTGTGCTCCAGAGTTAGC-3′) and (5′-CTTATGATCCTCCATGC-TGC-3′). The cDNA templates for RT-PCR were from the human tissues of poly (A)+ RNAs and purchased from Clontech (Palo Alto, CA, USA). PCR was carried out in a final volume of 50 µl containing 1 × GC buffer I (Takara, Kyoto, Japan), 0.2 µM each primer, 400 µM each dNTP, 5 µl (~1 ng) of template cDNA and 2.5 units of LA-Taq DNA polymerase (Takara, Kyoto, Japan). For the positive control, 50 pg of the kaia2138 clone from a full-length enriched cDNA library was used for the template. The temperature and time schedules were: 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. PCR products were separated on 2.0 % agarose gel. The *lower panel* shows the result with a Human G3PDH Control Amplimer Set (Clontech). The control G3PDH cDNA was also purchased from Clontech and used for PCR (5 µl [~1ng])

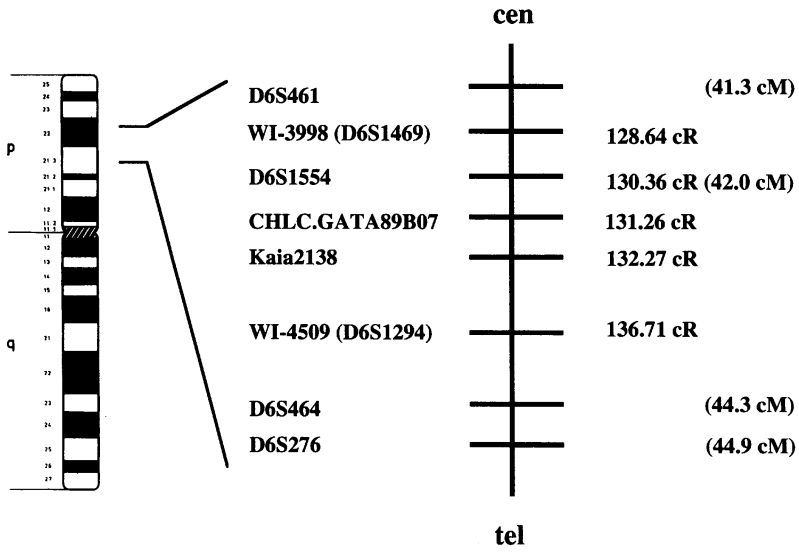

Fig. 3. Chromosomal placement of the *kaia2138* gene at a relative distance to framework markers on the WICGR (Whitehead Institute for Biomedical Research/ MIT Center for Genome Research) radiation hybrid map of the human genome (http://www.genome.wi.mit.edu/). The approximate corresponding cytogenetic location of the gene on the long arm of the telomeric (*tel*) region of human chromosome 6 is indicated. Distances of the markers are in centirays (cR) and centimorgans (cM) from the top of the chromosome 6 linkage group. *Cen*, Centromeric region

amplified product for humans was detected only from the hybrid containing human chromosome 6 (data not shown). Then, we performed further mapping analysis using a PCR-based radiation hybrid panel (Genebridge 4; Research Genetics) with the same primers as those used in the assay for the human/rodent somatic cell hybrid panel. Statistical analysis of the radiation hybrid data was performed using the RHMAPPER software package (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl). The data vector for the gene was 1000000100 0010000000 1100000000 1001010100 1110010000 0001000001 0110000010 0011001100 1111010000 001 and the consequent report indicated that the gene was placed to 1.01 cR proximal from CHLC.GATA89B07 (lod>3.0), which was cytogenetically mapped to the 6p21.3-p22 region (Fig. 3). The human renal *NPT1* gene was also mapped to 6p21.3-p23 (Chong et al. 1993). Furthermore, NPT3 and NPT4 were identified within a 1.1-megabase region of the hereditary hemochromatosis locus (Lauer et al. 1997), which is located at 6p21.3-p22. It is possible that human NPT genes are clustered around the chromosome 6p21-p23 region.

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