

SHORT COMMUNICATION

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Isolation and chromosomal mapping of a novel human gene showing homology to Na⁺/P₀4 cotransporter

Received: December 4, 1998 / Accepted: January 20, 1999

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 so-

dium 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.

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

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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

kaia2138	1: MSTGPDVKATVGDISSDGNLVAQEECSRKGFCSVRHGLALILQLCNESTIYTCOMNLSIA	60
NPT1_HUMAN	1: -----MDNRLPPKKVPGFCSFRYGLSFLVHCNVIITIAQRACLNLIT	41
NPT1_RABIT	1: -----MDNQFPSRKGPCFCSFRYVLAALFMHFCNIVITIAQRACLNLIT	41
NPT1_RAT	1: -----MENRCLPKKVPGFCSFRYGLAILLHFNCNIVITIAQRACLNLIT	41
NPT1_MOUSE	1: -----MENQCLPKKVPGFCSFRYGLAILLHFNCNIVITIAQRACLNLIT	41
NPT3_HUMAN	1: -----MDGKPTRKGPDPFCSLRVGLALIMHFSNFTMITQRVLSLSIA	41
NPT4_HUMAN	1: -----MQVDETLPKRGPSLCSARYGIALVLFHCNFTTIAQNVIMNIT	43
kaia2138	61: IPAMVNNITAPPSQPNASTERESTDSQGYWNETLKEFKAMAPAYDWSPEIQGIIILSSLVNG	120
NPT1_HUMAN	42: MVVMVNSTDEHGLPNTS-----TKKLDNIR--NPMYNWSPDIQGIILSSSTSYG	88
NPT1_RABIT	42: MVMAMVNNITNLHGSPNTS-----AEKRLDNTK--NPVYNWSPDVOGIIIFSSIFYG	88
NPT1_RAT	42: MVMAMVNKTEPHLSNKS-----VAEMLDNVK--NEVHSWSLDIQGLIIFSSVFLG	88
NPT1_MOUSE	42: MVMAMVNTGSPHLSNES-----VVEMLDNVK--NPVYSWSPDIQGLIILSSVFFG	88
NPT3_HUMAN	42: IIAMVNTTQQQGLSNASTEGVADAFNNSISIKFEDTKASVQWSPETQGIIFSSINYG	101
NPT4_HUMAN	44: MVMAMVNSTEQSQLDSE-----	62
kaia2138	121: SFLAPITPSGYVAGIFGAKYVVVAGLFISSFLTLFIPLAANAGVALLVLRIVQGIACVMV	180
NPT1_HUMAN	89: VIIIQVPEVGYFSGIYSTKMKIGFALCLSSVLSLIPPAAGIGVAVVVVCRVQGAQGIIV	148
NPT1_RABIT	89: AFLIQIPVGYISGIYSIKKLIQFALFLSSLSVIFIPQAAAVGETWIVICRVVQGITQGTV	148
NPT1_RAT	89: MVVIQVPEVGYLSGAYPMEKIISSFLSSVLSLIPPAACVGAALVIVCRVLOGIAQGYV	148
NPT1_MOUSE	89: MVVVQAPEVGYLSGIYPMKRIIGSSFLSSLSLIPPAACVGAALVIVCRVLOGIAQGYV	148
NPT3_HUMAN	102: IIITLIPSGYLAGIFGAKKMLGAGLIISSLTLFTPLAADFGVILVIMVTRVQGAQGMMA	161
NPT4_HUMAN	63: ---VLEVDSPFGGLSKAPKSL-----P-----AKSSI	85
kaia2138	181: LTCQYSIWKWAPPLERSQLTTIAGSGSMLGSPFIVLLAGGLLCQTIGWVYVYIFGGIGC	240
NPT1_HUMAN	149: ATAQFEIYVKWAPPLERGRITSMSTSGFLGPFIVLLVTVGICESLGWPMVFYIFGACGC	208
NPT1_RABIT	149: TTAQHEIWKWAPPLERGRITSMLSLGLFPGPFIVLLVTVGICESLGWPMVFYIFGACGC	208
NPT1_RAT	149: STGQHCIIWKWAPPLERGRITSMTSLGPFVMPPTIALLVSGFICDGLLWPMVFYIFGIVGC	208
NPT1_MOUSE	149: STGQHEIWKWAPPLERGRITSMTSLGPFVMPPTIVLLVSGFICDGLLWPMVFYIFGIVGC	208
NPT3_HUMAN	162: WTGQFTIWKWAPPLERSKLTITAGSGSAGSFIFILCVGGLISQALSWEFIFYIFGSTGC	221
NPT4_HUMAN	86: LGGQFAIWEKGGPQERSRLCSIALSGMLLGCFTAILIGGFISETLGWVFVYIFGGVGC	145
kaia2138	241: ACCPLWFPLIYDDPVNHPFISAGEKRYIVCSLAQQDCSEGWSLPIRAMIKSLPLWAILVS	300
NPT1_HUMAN	209: AVCLLWFVLFYDDPKDHCISISEKEYITSSLVQOVSSSRQSLPIKAILKSLPVWVAISIG	268
NPT1_RABIT	209: AVCLLWFVLFYDDPKDHCIVSLHEKEYITSSLSIQGSSSTRQSLPIKAILKSLPLWVAISFC	268
NPT1_RAT	209: VLSLFWFIFLFDDEPNHFMSSSEKDYITSSLMQOVHSGRQSLPIKAILKSLPLWVAIILN	268
NPT1_MOUSE	209: VLSLWFVLFYDDPKDHPHFMSSSEKDYITSSLMQOASSGRQSLPIKAILKSLPLWVAIILN	268
NPT3_HUMAN	222: VCCLLWFTVIYDDPMHHCISVREKEHILSSLAQQPSSPGRVPIKAMVTCLPLWVAIFLG	281
NPT4_HUMAN	146: VCCLLWFVVIYDDPFYPIWISTSEKEYITSSLKQOVSSSKQPLPIKAMLRSLPIWISICLG	205
kaia2138	301: YFCEYWLFTIMAYTPTIYISSVLQANLRDGSILSALPFVWGCICIILGGLLADPFLLSRKI	360
NPT1_HUMAN	269: SFTFFWNSHIMTYTEMPINSMHVNIKENGLSSLEPLFAWICGNLAGLSDFFLTRNRI	328
NPT1_RABIT	269: CFAYLNTYSRLIVYPTLINSMLHVDIRENGLSSLEPLFAWICGNLAGHTADFLMSRNM	328
NPT1_RAT	269: SFAFIWNSNLLVYPTPTISTTLHVNIRENGLSSLEPYLLAYICGIVAGQMSDFFLSRKI	328
NPT1_MOUSE	269: SFAFIWNSNLLVYPTPTISTTLHVNIRENGLSSLEPYLLAYICGIVAGQMSDFFLTRKI	328
NPT3_HUMAN	282: FESHFWLCTIILTYLPTIYSTLLHVNIRDGVLSSLEPFAIAAASCTILGGOLADPFLSRNL	341
NPT4_HUMAN	206: CFESHQVLVSTMVVYIPTIYISSVYHVNIRDNGLSALPFIWAVWIGMVGGLADPFLTK-K	264
kaia2138	361: LRLITIRKLFATAIGVLPSPVILVSLPWVRSRHSMTMTFVLVLSAISSPFCESGALVNFLLDI	420
NPT1_HUMAN	329: LSVLAVRKLFATAAGFLLEAIFGVCLPYLSSSTFYISIVIFLILAGATGSCFLGQVFINGLDI	388
NPT1_RABIT	329: LSSETAIRKLFATAIGLLPIVFSMCLLYLSSGFYSTITFELILANASSPFCGLGALINALLDI	388
NPT1_RAT	329: FVVAVRKLFTTLGIFCVFVIVVCLLYLSYNFYSTVIFLTLANSTLSEFSCGQLINALDI	388
NPT1_MOUSE	329: FVIVTVRKLFTTLGIFCVFVIFMCLLYLSYNFYSTVIFLTLANSTLSESYCGQLINALDI	388
NPT3_HUMAN	342: LRLITVRKLFSSLDQVSSWE----SQ-----GDLCSSQESSLPLPLDSS	382
NPT4_HUMAN	265: FRLITVRKIATTLGSLPSSALIVSLPYLNSGYITATALLTSCGLSTLQCSGIYINVLDI	324
kaia2138	421: APRYTGFLKGLLQVFAHTAGATSPATAAGFFISQDSEFGWRNVFLLSAAVNISGLVFYLI	480
NPT1_HUMAN	389: APRYTGFIRKACSTLTGMIQGLTASTLTGLLLKQDPESAWFKFTILMAAINVTGLIFYLIV	448
NPT1_RABIT	389: APRYYVFIKGVTTLIGMTGGMTSSTVAGLELSDQDPSSWFKIFLMSIINVISVIFYLIV	448
NPT1_RAT	389: APRYYGFILKAVTALIGIFGLISSTLAGLILNQDPEYAWHKNFELMAGINVTCLAFYLF	448
NPT1_MOUSE	389: APRYYGFILKAVTALIGMFGGLISSTLAGLILNQDPEYAWHKISFLMAGINVTCLVYFYLE	448
NPT3_HUMAN	383: SVRILSLVGGMSFSCLLQSTCLAWSFTSRLLDKQNFKTGPKRGPPLPASEDIKLT*-----	436
NPT4_HUMAN	325: APRYSSFLMGASRGFSSITAPVIVPTVSGFLLSDQPEFGWRNVFLLFAVNLLGLLFLYLI	384
kaia2138	481: GRADVQDWAKEQTFTHL*	497
NPT1_HUMAN	449: ATAEIQDWAKERQHTRL*	465
NPT1_RABIT	449: AKAEIQDWAKERQHTRL*	465
NPT1_RAT	449: AKGDIQDWAKETKTRL*	465
NPT1_MOUSE	449: AKGEIQDWAKEIKTRL*	465
NPT3_HUMAN	437: -----	465
NPT4_HUMAN	385: GEADVQEWAKERKTRL*	401

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 μ l containing 1 \times GC buffer I (Takara, Kyoto, Japan), 0.2 μ M each primer, 400 μ M each dNTP, 5 μ l (~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 μ l [~1ng])

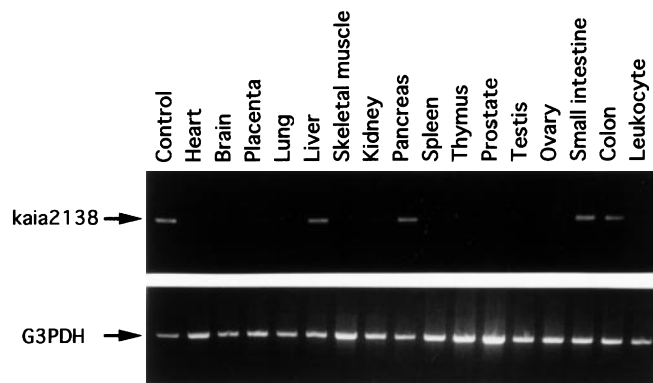
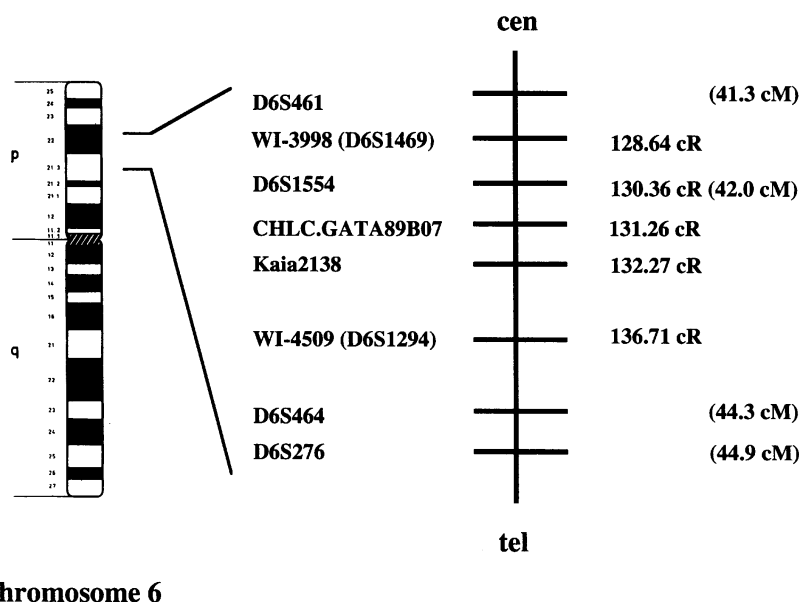


Fig. 3. Chromosomal placement of the *kaia2138* gene at a relative distance to framework markers on the WICGR (Whitehead Institute for Biomedical Research/MIT Center for Genome Research) radiation hybrid map of the human genome (<http://www.genome.wi.mit.edu>). The approximate corresponding cytogenetic 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 111010000 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.

References

- Biber J (1989) Cellular aspects of proximal tubular phosphate reabsorption. *Kidney Int* 36: 360-369
- Biber J, Custer M, Werner A, Kaissling B, Murer H (1993) Localization of NaPi-1, a Na/Pi cotransporter, in rabbit kidney proximal tubules. II. Localization by immunohistochemistry. *Pflugers Arch* 424: 210-215
- Chong SS, Kristjansson K, Zoghbi HY, Hughes MR (1993) Molecular cloning of the cDNA encoding a human renal sodium phosphate transport protein and its assignment to chromosome 6p21.3-p23. *Genomics* 18: 355-359
- Lauer P, Meyer NC, Prass CE, Starnes SM, Wolff RK, Gnirke A (1997) Clone-contig and STS maps of the hereditary hemochromatosis region on human chromosome 6p21.3-22. *Genome Res* 7: 457-470
- Maruyama K, Sugano S (1994) Oligo-capping: a simple method to replace the cap structure of eukaryotic mRNAs with oligo-ribonucleotides. *Gene* 138: 171-174
- Murer H, Werner A, Reshkin S, Wuarin F, Biber J (1991) Cellular mechanisms in proximal tubular reabsorption of inorganic phosphate. *Am. J Physiol* 260: C885-899
- Murer H, Markovich D, Biber J (1994) Renal and small intestinal sodium-dependent symporters of phosphate and sulphate. *J Exp Biol* 196: 167-181
- Saito T, Seki N, Ishii H, Ohira M, Hayashi A, Kozuma S, Hori T (1997) Complementary DNA cloning and chromosomal mapping of a novel phosphatidylinositol kinase gene. *DNA Res* 4: 301-305
- Suzuki Y, Yoshitomo-Nakagawa K, Maruyama K, Suyama A, Sugano S (1997) Construction and characterization of a full length and 5'-end-enriched cDNA library. *Gene* 200: 149-156