

Mutations in the gene of human type IIb sodium-phosphate cotransporter *SLC34A2*

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Type IIb sodium-phosphate cotransporter (NaPi2b) provides phosphate intake in the cells of some epithelial tissues, osteoblasts and odontoblasts. Abnormal expression of NaPi2b has been detected in some types of epithelial tumors. An alteration in NaPi2b activity, caused by mutations in transporter gene SLC34A2, has been recently revealed in patients with pulmonary alveolar microlithiasis, an autosomal recessively inherited disease, characterized by deposition of calcium-phosphate precipitates in the lungs. In the present study we have combined the information about all mutations found to date in the coding sequence of SLC34A2 and its transcript, compiled their map, and analysed their relevance to the function of NaPi2b.

Key words: SLC34A2 gene, inorganic phosphate transport, pulmonary alveolar microlithiasis, mutations, ovarian cancer.

Introduction. Adequate phosphate absorption is an important process in the maintenance of metabolism at cellular and organism-wide levels. The transport of inorganic phosphate in the cells of the mammals is provided by transporters of three families, NaPi-I, NaPi-II, and NaPi-III, encoded, respectively, by the gene families *SLC17*, *SLC34*, and *SLC20*. A member of the NaPi-II family, type 2b sodium-phosphate cotransporter NaPi2b (synonyms: NaPi-IIb, NPT2b; gene *SLC34A2* localized in chromosome site 4p15), is a tissue-specific transporter which is expressed in a number of mammalian tissues. Expression on mRNA level was shown in lungs, small intestine, kidney, liver, placenta, pancreas, prostate, ovary, thyroid, uterus, salivary gland, testis, mammary gland [1,2], while the expression on protein level was shown only for lungs [1, 3], epididymis [4], mammary gland [5], salivary glands [6], liver [7], bones [8], and teeth [9]. NaPi2b is

a transmembrane glycoprotein of 689 or 690 amino acids residues in length, consisting of eight transmembrane domains. The molecular weight of NaPi2b varies between 77–108 kDa, depending on the glycosylation status [10].

Recently several studies have suggested NaPi2b as a potential molecular marker of several types of cancer. Initially, NaPi2b overexpression had been linked to the papillary thyroid cancer [11]. Gene expression profiling performed with microarray technology and quantitative PCR showed that NaPi2b transcripts are overrepresented in the papillary thyroid cancer cells by 18–49 folds [12]. In the sequel, the transcript of *SLC34A2* was shown to be overrepresented in the human ovarian cancer cells by serial analysis of gene expression (SAGE) [13]. Recently, we have identified NaPi2b as the MX35 antigen, which is overexpressed in 90 % of all epithelial ovarian cancers [14]. In contrast to the ovarian and papillary thyroid cancer cells, in the breast [15] and lung [16] cancer cells the

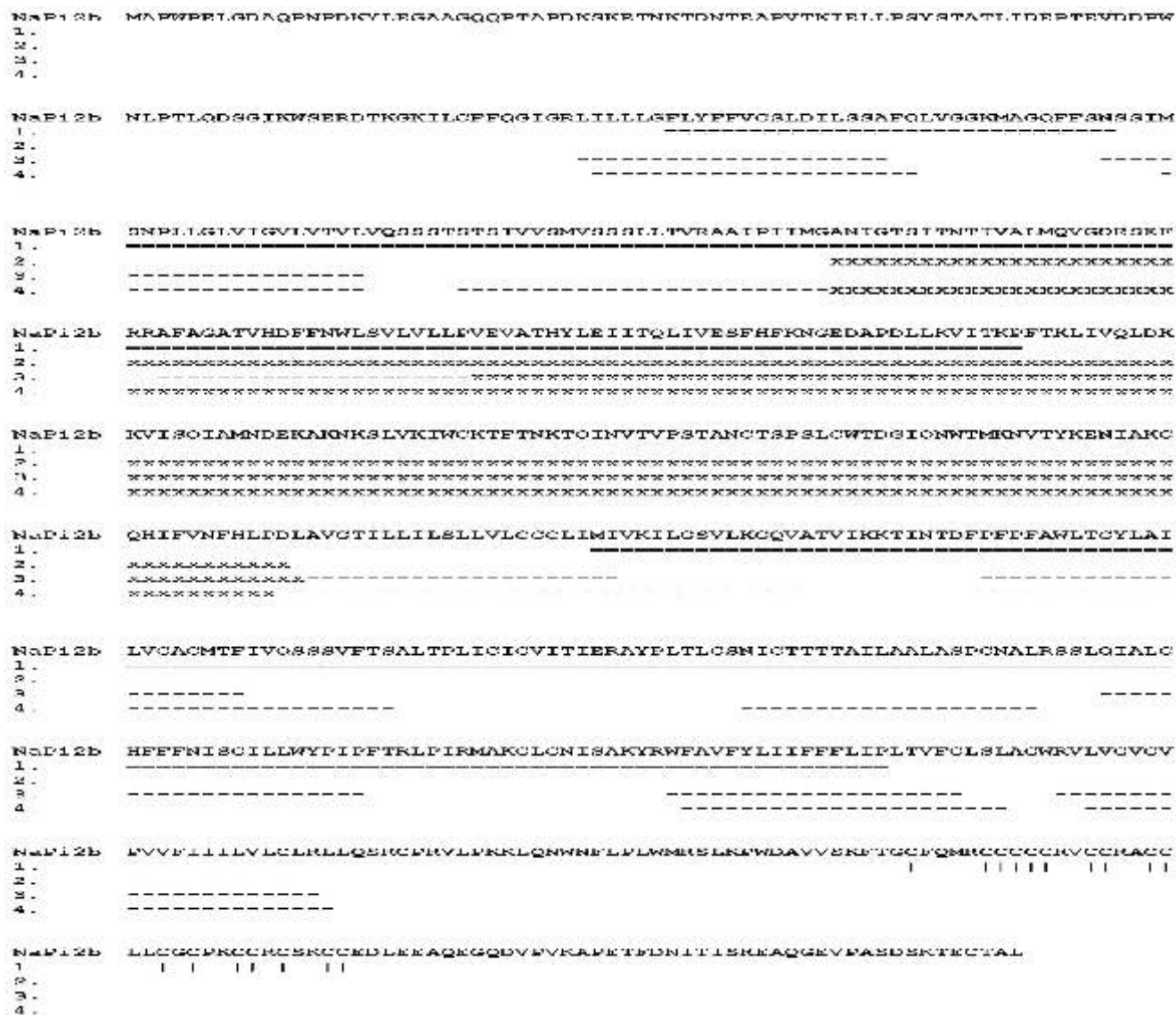


Fig. 1. Cotransporter and transmembrane domains topology of the human NaPi2b. Topology of NaPi2b cotransporter domains according to NCBI Conserved Domain Database (<http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>) (1). Topology of NaPi2b transmembrane domains and the second extracellular loop according to Yin et al. [14] (2). Uniprot KB database prediction service (<http://www.uniprot.org>) (3); Field et al. (4) (= – cotransporter domains; – – the transmembrane domains; ||| – cystein residues in a cystein-rich region; xxx – the second extracellular loop)

level of NaPi2b transcripts is decreased. To date there are very few studies focused on identification of possible mutations in *SLC34A2* gene in malignant cells. According to the literature data only three mutations in the coding sequence of *SLC34A2* have been detected and all of them in the ovarian cancer cell line, OVARC1 [17], implicating their possible role in tumorigenesis.

In two independent studies, the mutations in *SLC34A2* gene were shown to cause an autosomal re-

cessive hereditary disease, pulmonary alveolar microlithiasis (PAM), which is characterized by the deposition of calcium-phosphate microliths in lungs [18, 19]. Moreover, some patients with testicular microlithiasis (TM) were discovered to be heterozygous for mutations in *SLC34A2* [18].

Despite the accumulation of the data concerning the pathologies associated with the mutations in *SLC34A2*, there have been no studies addressing the analysis of the whole array of polymorphisms

occurring in the gene of NaPi2b in health and different pathologies. In order to summarize the current information and evaluate a possible role of the NaPi2b polymorphism in cancer, we have compiled the map of polymorphisms in the coding sequence of NaPi2b gene *SLC34A2* in genomic and cDNA sequences available in different databases.

Models of the NaPi2b transmembrane topology and domain structure. NaPi2b protein has two sodium-phosphate cotransport regions that are conserved among sodium-phosphate cotransporters (Fig. 1): the first region spanning 161 amino acid residues (109–269) and the second, spanning 159 amino acid residues (382–540) (NCBI Conserved Domain Database, <http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>). According to the models of NaPi2b transmembrane topology it has eight transmembrane domains with both N- and C-terminal ends facing the cytoplasm and four extracellular loops with multiple glycosylation sites within the largest (second) one [1, 10, 14, 20, 21]. The position of transmembrane regions and the large extracellular loop are slightly different in these predicted models. The extracellular location of large loop was confirmed in MHA cell surface binding assay [14].

Recent studies have shown that the glycosylation of human NaPi2b is mostly N-linked [14] and six potential sites for N-glycosylation in the large extracellular loop (N²⁹⁴, N³⁰⁷, N³¹², N³²⁰, N³³⁴, N³³⁹) have been predicted [1, 2, 14]. The other member of this family transporters NaPi-IIa (type IIa sodium-phosphate cotransporter, NaPi2a), which is highly homologous to NaPi2b has only two consensus sites for N-glycosylation (N²⁹⁸ and N³²⁸) [1, 2]. These data indicate that posttranslational protein modifications such as glycosylation could be important for transporters functioning.

The cytoplasmic C-tail of NaPi2b contains the polycysteine motif, unique for the type IIb of NaPi cotransporters, which is a potential site for palmitoylation [22]. Studies on metabolic labelling followed by immunoprecipitation confirmed NaPi2b palmitoylation [14]. The C-terminal amino acids of the protein (Thr-Ala-Leu) strongly resemble the PDZ domain-binding motif. In contrast to NaPi-IIa, which was shown to interact with several PDZ domain

containing proteins [23], the binding partners for NaPi2b have not been identified so far. It has been recently demonstrated that the C-terminal leucine of human NaPi2b is necessary for its apical sorting [22].

Human pathologies of phosphate homeostasis associated with NaPi2b. Mutations in the gene of NaPi2b (*SLC34A*) were identified by two independent research groups in patients with PAM, a rare hereditary autosomal recessive disease marked by calcium phosphate precipitate deposition in lungs. Corut et al. [18] implemented the linkage analysis with microsatellite markers and further single strand conformation polymorphism (SSCP) analysis. In this study, six exonic mutations in *SLC34A2* were identified, including a p.G106R substitution, two missense mutations (p.Q76X (C226T), p.V448X (del1342G)), frame shifts at 38th amino acid (p.del114A) and at 443rd amino acid (1328delT), a deletion in promoter exon, and a non-causative substitution in heterozygous state (p.T330M (C989T)). In a parallel study, Huqun and co-authors [19] identified two frame shifting deletion mutations: p.del 461...519 (del 1381–1557) and p.del 591...596 (del 1772–1789) by means of microarray-based SNP mapping technique. Altogether, seven causative mutations in CDS and one causative mutation in the promoter exon of *SLC34A2* for PAM have been reported in these studies.

Mutations in *SLC34A2* gene were also described in patients with TM [18], a more frequent calcium-phosphate accumulation disease, which is often associated with infertility and cancer. The screening of fifteen TM patients' samples by SSCP technique revealed only two of them to carry mutations in heterozygous state. One patient had a synonymous SNP in the CDS (T552C) and the other had one SNP in 3'-UTR (*G27T) of *SLC34A2*. Both mutations were associated with infertility and the latter was also associated with cancer [18]. The authors concluded the rare mutations carried by two patients with TM indicated that *SLC34A2* could be responsible for this pathology, at least in those patients, and noted that others TM patients might have escape detection because the efficiency of SSCP is not 100 % and mutant variants could possibly be located in the gene regions which were not analysed [18].

The authors also hypothesized that phosphate transporter NaPi2b could be responsible for calci-

Table 1
Mutations in NaPi2b sequence

Mutation position		Source	Reference	Entry numbers in Nucleotide (or/and SNP) databases	Context
cDNA (ORF)	Protein				
<i>Non-synonymous SNPs</i>					
T134C	p.V45A	SNP database (genomic DNA)	–	(rs35426730)	Cytoplasmic N-terminus
G316C	p.G106R	PAM, genomic sequence (homozygote)	[17]	–	1 st TMR
C682G	p.V228L	Small intestine cDNA library	[2]	AF146796.1	1 st cotransporter domain
A988G & C989T	p.T330V	OVARC1 cell line cDNA library	[19]	AK075015.1	2 nd extracellular loop
C989T	p.T330M	Healthy donor genomic DNA (single allele)	[17]	–	2 nd extracellular loop
C1525T	p.R509C	SNP database (genomic DNA)	–	(rs34751784)	2 nd cotransporter domain, intracellular region
G1859A	p.C620Y	Small intestine or lung cDNA library	[1]	AF111856	Cystein-rich region
G1901A	p.G634D	A genomic entry and SNP database	–	AC092436.5 (rs6448389)	Cytoplasmic C-terminus
<i>In-frame deletions</i>					
del 1381–1557	p.del 461...519	OVARC1 cell line cDNA library	[19]	AK075046.1	59 aa in 2 nd cotransport, 6 th TMR
del 1772–1789	p.del 591...596	OVARC1 cell line cDNA library	[19]	AK075015.1	6 aa in intracellular C-terminus
<i>Nonsense mutations</i>					
C226T	Truncation Q76X	PAM, genomic sequence (homozygote)	[17]	–	–
del1342G	Truncation V448X	PAM, genomic sequence (homozygote)	[17]	–	–
<i>Frame shifting mutations</i>					
del114A	Frameshift at 38 th aa	PAM, genomic sequence (homozygote)	[17]	–	–
857–871 del ins AA GTT ATC GCT TTT TCA TC	Fframeshift at 268 th aa	PAM, genomic sequence (homozygote)	[18]	–	–
IVS8 + 1G > A	Frameshift after 349 th aa	PAM, genomic sequence (homozygote)	[18]	–	(Substitution in introne, aberrant splicing)
del1328T	Frameshift at 443 rd aa	PAM, genomic sequence (homozygote)	[17]	–	–
<i>Synonymous SNPs</i>					
T552C	(Ile ¹⁸⁴)	TM, genomic sequence (single allele)	[17]	–	The 3 rd TMR
T1089C	(Ala ³⁸³)	SNP database	–	(rs34723230)	The 4 th TMR

fication shown in several other tissues such as kidney, pancreas, prostate, ovary, small intestine, mammary gland, liver, and placenta where *SLC34A2* gene expression is well documented [2, 18].

In this regard it seems very interesting that some tumours also carry occasional calcifications. In the breast and ovarian cancers, calcifications were directly or circumstantially shown to be calcium-phosphate salts [24, 25]. Furthermore, calcium-phosphate precipitates were demonstrated to enhance mitogenesis in the breast cancer cell line cultures [26]. The impairment of calcium-phosphate homeostasis resulting in such calcifications in the ovarian and breast cancer tumours can be presumed to be connected with NaPi2b deregulation [13, 15]. Undoubtedly, the NaPi2b expression and polymorphism in tumours, that carry calcifications, should be further investigated.

Polymorphisms in *SLC34A2* gene: a potential link to deregulation of NaPi2b function. We have performed detailed analysis of all known to date sequence variations in CDS of *SLC34A2* gene and gathered a panel of eight nsSNPs, two sSNPs, two deletions without frame shift, two nonsense mutations and four frame shifting mutations (Table 1). Relying on the data obtained, we have compiled the map of potential mutations in CDS of human phosphate transporter NaPi2b (see supplement 1).

Non-synonymous single nucleotide polymorphisms. Eight nsSNPs in the gene of human NaPi2b have been reported to date.

The p.V45A (T134C) substitution in cytoplasmic N-terminus of NaPi2b according to NCBI SNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) is found in Sub-Saharan African people with allele frequency of 0.025 (120 chromosome samples tested, i. e. found in 3 samples) and was not found either in European (120 chromosomes) or Asian (180 chromosomes) populations. Any phenotypic manifestation of the p.V45A polymorphism has not been described.

The p.G106R substitution, which results in the introduction of a positively charged amino acid in the non-polar length of the first transmembrane region (the first conserved cotransport domain), was identified in homozygote state in PAM patients [18].

The p.V228L substitution in the cDNA clone obtained from an enriched human intestinal cDNA

library [2] is located in the first cotransporter domain. As indicated in Table 2, Val²²⁸ is located in a highly conservative region of the first cotransporter domain.

Two nsSNP have been found in the position of 330th amino acid (Thr³³⁰) of NaPi2b. The p.T330M change-over was identified in heterozygote state in healthy donors by Corut and co-authors [18]. The other substitution at the same site, p.T330V, is present in a cDNA clone obtained from the human ovarian carcinoma cell line OVARC1 cDNA library [17]. This replacement could be derived from p.T330M by consequent change in the second and the third positions of the codon:

T	M	V
acg	aug	gug.

Corut and co-authors have suggested that the p.T330M substitution is likely to inactivate the function of NaPi2b, since it renders the replacement of a non-polar amino acid for polar one [18]. However, since this mutation is located outside of the conserved cotransport domains of NaPi2b and is not strictly conserved in vertebrates, having amino acids other than Thr in two of ten species taken into analysis (Table 2), we suppose that this mutation does not affect the function of the transporter. On the other hand, it is likely to affect the antigenic properties of NaPi2b, because Thr³³⁰ is located in a highly immunogenic region [14, 27]. Moreover, we have recently demonstrated that the mutation p.T330V abolishes recognition of NaPi2b by two monoclonal antibodies, MX35 and anti-NaPi2b L2 (20/3) in Western-blot analysis [28]. Taking into account the fact that two mutations have been independently detected in the same position (Thr³³⁰), it can be assumed to be a mutational hot spot in the *SLC34A* gene.

The p.R509C (C1525T) missense mutation in NCBI SNP database was detected in a single chromosome sample from an African American person from a mixed (Caucasian and Afro-American) set of 74 chromosomes (aggregate allele frequency 0.014). Phenotypic manifestation of the polymorphism is unknown.

The p.C620Y substitution (in a clone obtained from human lung cDNA library [1]) is located in the cysteine cluster within the cytoplasmic C-tail, which is a potential site of fatty acid attachment [22] and is unique

Table 2
Conservation of polymorphic amino acid positions of NaPi2b in vertebrates

Position	Context	Species					
		<i>Pseudopleuronectes americanus</i>	<i>Danio rerio</i>	<i>Canis familiaris</i>	<i>Rattus norvegicus</i>	<i>Mus musculus</i>	<i>Bos taurus</i>
45	Cytoplasmic N-terminus	–	–	V	V	V	I
106	1 st transmembrane region	G	G	G	G	G	V
228	1 st cotransport domain	V	V	V	V	V	V
330	2 nd extracellular loop	E	T	S	T	T	T
509	2 nd cotransporter domain	R	R	R	R	R	R
620	Cystein-rich part in C-terminus	C	C	L	C	C	C
634	Cytoplasmic C-terminus	=	=	G	G	G	G

Position	Context	Species				
		<i>Macaca mulatta</i>	<i>Pongo pygmaeus</i>	<i>Pan troglodytes</i>	<i>Homo sapiens</i> (consensus sequence)	<i>Homo sapiens</i> (polymorphic variants)
45	Cytoplasmic N-terminus	V	V	V	V	A
106	1 st transmembrane region	G	G	G	G	R
228	1 st cotransport domain	V	V	V	V	L
330	2 nd extracellular loop	T	T	T	T	V, M
509	2 nd cotransporter domain	R	R	R	R	C
620	Cystein-rich part in C-terminus	C	C	C	C	Y
634	Cytoplasmic C-terminus	G	G	G	G	D

for NaPi2b in comparison to other type II NaPi cotransporters.

The p.G634D (G1901A) variant is located in the part that is conserved among the terrestrial mammals, but is absent in fishes. According to NCBI SNP database (rs6448389), p.634D (G1901) allelic variant frequency is 0.138–0.188 in European population, 0.043 in Afro-American and it has not been found in Asian and Sub-Saharan African ones.

Taken together, eight non-synonymous SNPs were described in *SLC34A2*, with one of them (p.G106R) having an established phenotypic manifestation (pulmonary alveolar microlithiasis).

Synonymous single nucleotide polymorphisms. Two synonymous SNPs have been described in *SLC34A2* coding sequence, namely T552C (Ile¹⁸⁴) and

T1089C (Ala³⁸³) (Table 1). All two sSNPs are T > C substitutions in the third position of the codons.

The single allele of T552C (Ile¹⁸⁴) sSNP was discovered in testicular microlithiasis patients and in two healthy individuals [18]. Although no causal effect of this mutation was proven, authors [18] hypothesized that this mutation can cause aberrant splicing of the *SLC34A2* transcript by producing an exonic splicing enhancer sequence. Another explanation for the effect of this mutation as the one with incomplete penetrance could be analogous to following. In *MDR1* gene (*Multidrug resistance 1*) coding for a transporter protein, «synonymous» substitutions were recently shown to affect functional activity of the protein, most likely through alteration of protein biosynthesis velocity and folding behaviour [29] due to bias in sy-

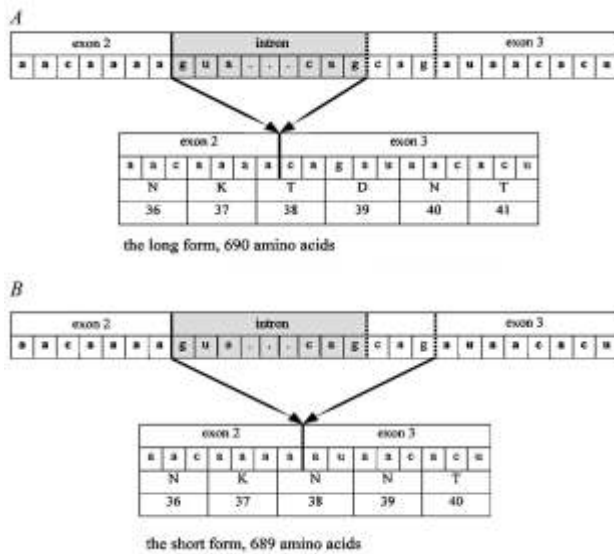


Fig. 2. Alternative splicing of the second and the third exons of human NaPi2b: A – schematic diagram of the long variant (Feild et al., 1999); B – schematic diagram of the short variant (Xu et al., 1999). Pre-mRNA sequences are shown above and mature mRNAs with translated sequences (with amino acid residues numbered) are represented below

nonymous tRNA concentrations. Similarly, the substitution T552C might result in higher susceptibility to environmental and/or genetic factors affecting folding of NaPi2b molecule, declaring itself with incomplete tolerance. However, taking into account that T552C allele have been found in healthy individuals as well the presence of additional factors may be required to facilitate the effect of this substitution on NaPi2b functioning in testicular microlithiasis patients.

Mutations resulting in truncated NaPi2b protein. The mutations causing the truncation of NaPi2b protein were described in PAM patients and comprise a nsSNP (C226T) [18], a nonsense single-nucleotide deletions (1342delG) [18], and four frame-shifting mutations: one intronic SNP causing aberrant splicing (IVS8 + 1G > A) [19], two frame-shifting single-nucleotide deletions (114delA, 1328delT) [18], and one complex exonic deletion-insertion (857–871 del ins AA GTT ATC GCT TTT TCA TC) [19].

Only two of these mutations, the complex deletion-insertion (857–871 del ins) and the mutation affecting splicing (IVS8 + 1G > A) were tested functionally in electrophysiological studies on *Xenopus* oocytes [19]. Both of them cause the synthesis of truncated,

lacking cytoplasmic C-terminal and partially the 2nd cotransport region, functionally inactive proteins [19]. Expression of such proteins may affect transport of inorganic phosphate across the apical membrane of lung alveolar cells in PAM patients resulting in the accumulation of calcium-phosphate deposits in lungs.

In-frame deletions. Two cDNA clones of NaPi2b cotransporter obtained from the ovarian cancer cell line OVARC1 [17] contain deletions that do not shift the reading frame (del 1381–1557 and del 1772–1789). The translated sequences of these cDNA clones lack 59 and 6 amino acids residues correspondingly at the C-terminal of NaPi2b. Notably, that the largest deletion involves the part of the 2nd cotransport domain of NaPi2b and potentially may affect NaPi2b transport function.

The detection of two clones with distinct deletions in the same cell line can be interpreted by presence of two mutated alleles, heterogeneity of the cell line, or by posttranscriptional processing. Both deletions are flanked by short direct repeats, which can be involved in mechanisms of either classical DNA mutating or of the posttranscriptional processing.

Direct repeat are known to participate in the genesis of classical deletions by means of either replication slippage, site-specific recombination or others [30]. The genesis of deletions was also suggested to be mediated by DNA-interacting proteins that perform improper ligation of cut fragments [30]. Analysis performed for the hepta-nucleotide repeats flanking the 59 amino acids residues long deletion in *SLC34A2* CDS showed that they coincide with the recognition site for topoisomerase II and translin (p.del 590–595 aa). Cleavage sites of translin and topoisomerase II were found to flank a host of mutations in human cancer cells, implying that these enzymes could participate in the genesis of chromosomal translocations in human cancers [31]. These data suggest that these mutations are not exclusively random and could occur in ovarian cancer *in vivo* as well.

The other possible origin of these deletions could be posttranscriptional excision, a phenomenon described for human and for cereal plants. During aging, in Alzheimer's and Down's diseases, the short direct repeats were shown to cause deletions in RNA transcripts by means of posttranscriptional processing coined «misreading». Such deletions are one or two nucleotides long and occur within or closely to contiguous bi-

tri-, and tetra-nucleotide repeats [32]. Furthermore, short direct repeats were recently discovered to give rise to posttranscriptional deletions in several genes of cereals [33, 34]. These deletions span sequences from 28 to 225 nucleotides, and are flanked by short direct repeats which are from four to ten nucleotides long. Interestingly, stresses influence switch between different deletions in transcripts of the same gene [34].

Therefore, deletions flanked by short direct repeats can arise *in vivo* at different conditions and pathologies by means of either classical genomic mutational processes or posttranscriptional processing. Studies on occurrence and frequency of these deletions in NaPi2b in ovarian cancer cells *in vivo* along with investigation of mechanisms underlying the origin of such deletions in cancer would promote deeper comprehension of carcinogenesis.

Splice variants. The cDNAs sequences of NaPi2b phosphate transporter that were first published by two independent groups [1, 2] differ in length by three nucleotide residues. The translated sequence of the clone obtained by Feild and co-authors [1] from human lung cDNA (AF111856) is longer than the translated sequence of the clone obtained by Xu and co-authors [2] (AF146796) from human small intestine cDNA library by one amino acid residue (690 compared to 689). In addition, it carries a substitution N38T (aspartate to threonine) and an extra amino acid, asparagine, inserted after the 38th amino acid position.

The analysis of *SLC34A2* genomic sequences shows that these two isoforms are splice variants derived from a common genomic sequence (Fig. 2). The expression of these two variants is enabled by the presence of two contiguous acceptor splicing sites (CAG) in the intron between the exons 2 and 3.

In order to assess the functional consequence of these variations, we performed prediction of post-translational modification sites for these variants. The prediction using NetPhos 2.0 (CBS Prediction Servers [35]) gave a noticeable difference of score for possible phosphorylation of Thr⁴⁰. The score for the long form is 0.164 and for the short form is 0.537. Thus, these isoforms can possibly have different functional features and this fact requires additional studies.

Conclusion. In order to consolidate current information and assess the possible role of NaPi2b

polymorphism in cancer, we have performed analysis of mutations in the *SLC34A2* gene published to date.

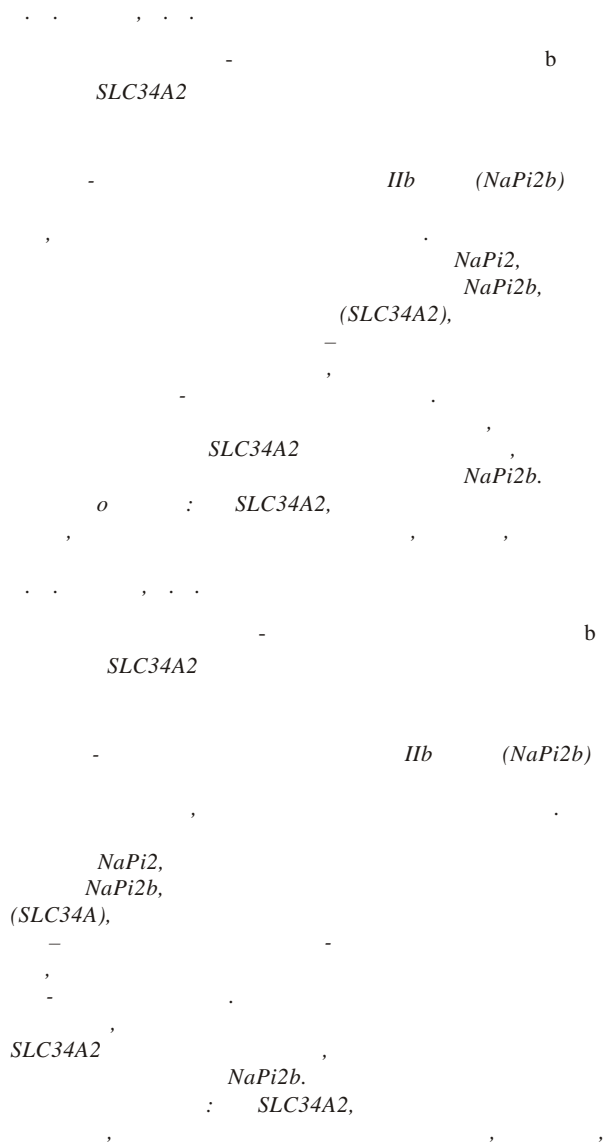
The analysis of existing mutations in *SLC34A2* gene revealed that the most of them are associated with the autosomal recessive inherited disease, pulmonary alveolar microlithiasis and/or testicular microlithiasis, which is characterized by accumulation of calcium-phosphate precipitates in lungs and testis respectively. Totally, eighteen mutations in the coding sequence of human NaPi2b have been described. Seven of them have been revealed in genomic DNA of PAM patients, two in genomic DNA of TM patients and healthy individuals, nine cDNA mutations were retrieved from NCBI data bases including three of them found in ovarian cancer cell line OVARC1. Interesting that almost all mutations (six out of seven) found in PAM patients may cause the expression of truncated from C-terminus form of NaPi2b partially or completely lacking of 2nd cotransporter domain. For two cases it was confirmed experimentally that the truncated forms of NaPi2b are functionally inactive [19]. Taking this into account one can presume that other four mutations in PAM patients may impair NaPi2b transport function as well.

Mutations involving single nucleotide substitutions in genomic DNA of PAM patients hypothetically influence NaPi2b transport activity, however, the significance of single nucleotide substitutions in genomic DNA of TM patients, especially those of them that also occur in healthy individuals – T552C (Ile¹⁸⁴), should be further validated by electrophysiological studies of NaPi2b transport function.

Despite of the knowledge about abnormal NaPi2b expression in some types of epithelial ovarian cancers no information exists to date about mutations in *SLC34A2* gene in malignant cells. The search of NCBI databases allowed us to find three mutations in cDNA of NaPi2b only from ovarian cancer cell line OVCAR1. Analysis of mutations allowed us to suggest that at least one of them – deletion 59 aa may hypothetically affect NaPi2b transport function since it partially covers the 2nd co-transport domain of transporter.

As it was indicated before, according to the literature data precipitates, similar to microliths in lungs of PAM patients, were found in breast and ovarian cancers. This observation could be explained by alterations in NaPi2b activity in malignant cells as

consequence of abnormal expression level or presence of mutations in *SLC34A2* gene as well. Since the calcium-phosphate precipitates were shown to enhance mitogenesis in the breast cancers cell lines one may speculate that among other things impairment of NaPi2b expression and function can lead to accumulations of the precipitates in the tumours that could influence tumour growth and progression. The study of expression profiles and function of wild type and/or mutant forms of NaPi2b could provide the insight into the role of NaPi2b in the pathogenesis of some diseases, including malignancies.



REFERENCES

1. Feild J. A., Zhang L., Brun K. A., Brooks D. P., Edwards R. M. Cloning and functional characterization of a sodium-dependent phosphate transporter expressed in human lung and small intestine // *Biochem. Biophys. Res. Commun.*—1999.—**258**, N 3.—P. 578–582.
2. Xu H., Bai L., Collins J. F., Ghishan F. K. Molecular cloning, functional characterization, tissue distribution, and chromosomal localization of a human, small intestinal sodium-phosphate (Na^+ -Pi) transporter (*SLC34A2*) // *Genomics.*—1999.—**62**, N 2.—P. 281–284.
3. Traebert M., Hattenhauer O., Murer H., Kaissling B., Biber J. Expression of type II Na-P(i) cotransporter in alveolar type II cells // *Am. J. Physiol.*—1999.—**277**, N 5.—P. 868–873.
4. Xu Y., Yeung C. H., Setiawan I., Avram C., Biber J., Wagenfeld A., Lang F., Cooper T. G. Sodium-inorganic phosphate cotransporter NaPi-IIb in the epididymis and its potential role in male fertility studied in a transgenic mouse model // *Biol. Reprod.*—2003.—**69**, N 4.—P. 1135–1141.
5. Miyoshi K., Shillingford J. M., Smith G. H., Grimm S. L., Wagner K. U., Oka T., Rosen J. M., Robinson G. W., Hennighausen L. Signal transducer and activator of transcription (Stat) 5 controls the proliferation and differentiation of mammary alveolar epithelium // *J. Cell Biol.*—2001.—**155**, N 4.—P. 531–542.
6. Homann V., Rosin-Steiner S., Stratmann T., Arnold W. H., Gaengler P., Kinne R. K. Sodium-phosphate cotransporter in human salivary glands: molecular evidence for the involvement of NPT2b in acinar phosphate secretion and ductal phosphate reabsorption // *Arch. Oral Biol.*—2005.—**50**, N 9.—P. 759–768.
7. Frei P., Gao B., Hagenbuch B., Mate A., Biber J., Murer H., Meier P. J., Steiger B. Identification and localization of sodium-phosphate cotransporters in hepatocytes and cholangiocytes of rat liver // *Am. J. Physiol. Gastrointest. Liver Physiol.*—2005.—**288**, N 4.—P. G771–778.
8. Lundquist P. Odontoblast phosphate and calcium transport in dentinogenesis // *Swed. Dent. J. Suppl.*—2002.—N 154.—P. 1–52.
9. Lundquist P., Murer H., Biber J. Type II Na^+ -Pi Cotransporters in osteoblast mineral formation: regulation by inorganic phosphate // *Cell Physiol. Biochem.*—2007.—**19**, N 1–4.—P. 43–56.
10. Murer H., Foster I., Biber J. The sodium phosphate cotransporter family SLC34 // *Pflugers Arch.*—2004.—**447**, N 5.—P. 763–778.
11. Jarzab B., Wiench M., Fajarewicz K., Simek K., Jarzab M., Oczko-Wojciechowska M., Wloch J., Czarniecka A., Chmielik E., Lange D., Pawlaczek A., Szpak S., Gubala E., Swierniak A. Gene expression profile of papillary thyroid cancer: sources of variability and diagnostic implications // *Cancer Res.*—2005.—**65**, N 4.—P. 1587–1597.
12. Ga za-Kulik M., Zebracka J., Szpak-Ulczoek S., Czarniecka A. K., Kukulska A., Guba a E., Stojcev Z., Wiench M. Expression of selected genes involved in transport of ions in papillary thyroid carcinoma [Article in Polish] // *Endokrynologia Polska.*—2006.—**57**, Suppl. A.—P. 26–31.
13. Rangel L. B., Sherman-Baust C. A., Wernyj R. P., Schwartz D. R., Cho K. R., Morin P. J. Characterization of novel human ovarian cancer-specific transcripts (HOSTs) identified by

- serial analysis of gene expression // *Oncogene*.—2003.—**22**, N 46.—P. 7225–7232.
14. Yin B. W., Kiyamova R., Chua R., Caballero O. L., Gout I., Gryshkova V., Bhaskaran N., Souchevnytskyi S., Hellman U., Filonenko V., Jungbluth A. A., Odunsi K., Lloyd K. O., Old L. J., Ritter G. Monoclonal antibody MX35 detects the membrane transporter NaPi2b (*SLC34A2*) in human carcinomas; a new target for cancer immunotherapy // *Cancer Immun.*—2008.—**8**.—P. 3.
 15. Blanchard A., Shiu R., Booth S., Sorensen G., DeCorby N., Nistor A., Wong P., Leygue E., Myal Y. Gene expression profiling of early involuting mammary gland reveals novel genes potentially relevant to human breast cancer // *Front. Biosci.*—2007.—**12**.—P. 2221–2232.
 16. Kopantzev E. P., Monastyrskaya G. S., Vinogradova T. V., Zinovyeva M. V., Kostina M. B., Filyukova O. B., Tonevitsky A. G., Sukhikh G. T., Sverdlov E. D. Differences in gene expression levels between early and later stages of human lung development are opposite to those between normal lung tissue and non-small lung cell carcinoma // *Lung Cancer*.—2008.—**62**, N 1.—P. 23–34.
 17. Otsuki T., Ota T., Nishikawa T., Hayashi K., Suzuki Y., Yamamoto J., Wakamatsu A., Kimura K., Sakamoto K., Hatano N., Kawai Y., Ishii S., Saito K., Kojima S., Sugiyama T., Ono T., Okano K., Yoshikawa Y., Aotsuka S., Sasaki N., Hattori A., Okumura K., Nagai K., Sugano S., Isogai T. Signal sequence and keyword trap *in silico* for selection of full-length human cDNAs encoding secretion or membrane proteins from oligo-capped cDNA libraries // *DNA Res.*—2005.—**12**, N 2.—P. 117–126.
 18. Corut A., Senyigit A., Ugur S. A., Altin S., Ozcelik U., Calisir H., Yildirim Z., Gocmen A., Tolun A. Mutations in *SLC34A2* cause pulmonary alveolar microlithiasis and are possibly associated with testicular microlithiasis // *Am. J. Hum. Genet.*—2006.—**79**, N 4.—P. 650–656.
 19. Huqun, Izumi S., Miyazawa H., Ishii K., Uchiyama B., Ishida T., Tanaka S., Tazawa R., Fukuyama S., Tanaka T., Nagai Y., Yokote A., Takahashi H., Fukushima T., Kobayashi K., Chiba H., Nagata M., Sakamoto S., Nakata K., Takebayashi Y., Shimizu Y., Kaneko K., Shimizu M., Kanazawa M., Abe S., Inoue Y., Takenoshita S., Yoshimura K., Kudo K., Tachibana T., Nukiwa T., Hagiwara K. Mutations in the *SLC34A2* gene are associated with the pulmonary alveolar microlithiasis // *Am. J. Respirat. and Crit. Care Med.*—2007.—**175**, N 3.—P. 263–268.
 20. Werner A., Dehmel L., Nalbant P. Na⁺-dependent phosphate cotransporters: the NaPi protein families // *Exp. Biol.*—1998.—**201**, N 23.—P. 3135–3142.
 21. Kohl B., Wagner C. A., Huelseweh B., Busch A. E., Werner A. The Na⁺-phosphate cotransport system (NaPi-II) with a cleaved protein backbone: implications on function and membrane insertion // *J. Physiol.*—1998.—**508**, N 2.—P. 341–350.
 22. Karim-Jimenez Z., Hernando N., Biber J., Murer H. Requirement of a leucine residue for (apical) membrane expression of type IIb NaPi cotransporter // *Proc. Nat. Acad. Sci. USA.*—2000.—**97**, N 6.—P. 2916–2921.
 23. Gisler S. M., Stagljar I., Traebert M., Bacic D., Biber J., Murer H. Interaction of the type IIa Na/Pi cotransporter with PDZ proteins // *J. Biol. Chem.*—2001.—**276**, N 12.—P. 9206–9213.
 24. Frappart L., Boudeulle M., Boumendil J., Lin H. C., Martignon I., Palayer C., Mallet-Guy Y., Raudrant D., Bremond A., Rochet Y., et al. Structure and composition of microcalcifications in benign and malignant lesions of the breast: study by light microscopy, transmission and scanning electron microscopy, microprobe analysis, and X-ray diffraction // *Hum. Pathol.*—1984.—**15**, N 9.—P. 880–889.
 25. Maki M., Hirota S., Kaneko Y., Morohoshi T. Expression of osteopontin messenger RNA by macrophages in ovarian serous papillary cystadenocarcinoma: a possible association with calcification of psammoma bodies // *Pathol. Int.*—2000.—**50**, N 7.—P. 531–535.
 26. Morgan M. P., Cooke M. M., Christopherson P. A., Westfall P. R., McCarthy G. M. Calcium hydroxyapatite promotes mitogenesis and matrix metalloproteinase expression in human breast cancer cell lines // *Mol. Carcinog.*—2001.—**32**, N 3.—P. 111–117.
 27. Kiyamova R., Gryshkova V., Ovcharenko G., Lituyev D., Malyuchik S., Usenko V., Khozhayenko Y., Gurtovyy V., Yin B., Ritter G., Old L., Filonenko V., Gout I. Development of monoclonal antibodies specific for the human sodium-dependent phosphate cotransporter NaPi2b // *Hybridoma.*—2008.—**27**, N 4.—P. 277–284.
 28. Gryshkova V. S., Lituyev D. S., Filonenko V. V., Kiyamova R. G. Creation of cellular models for the analysis of sodium-dependent phosphate transporter NaPi2b, a potential marker for ovarian cancer // *Biopolymers and Cell.*—2009.—**25**, N 2.—P. 95–100.
 29. Kimchi-Sarfaty C., Oh J. M., Kim I.-W., Sauna Z. E., Calcagno A. M., Ambudkar S. V., Gottesman M. M. A «silent» polymorphism in the *MDR1* gene changes substrate specificity // *Science.*—2007.—**315**, N 5811.—P. 525–528.
 30. Ball E. V., Stenson P. D., Abeyasinghe S. S., Krawczak M., Cooper D. N., Chuzhanova N. A. Microdeletions and microinsertions causing human genetic disease: common mechanisms of mutagenesis and the role of local DNA sequence complexity // *Hum. Mutat.*—2005.—**26**, N 3.—P. 205–213.
 31. Abeyasinghe S. S., Chuzhanova N., Krawczak M., Ball E. V., Cooper D. N. Translocation and gross deletion breakpoints in human inherited disease and cancer I: Nucleotide composition and recombination-associated motifs // *Hum. Mutat.*—2003.—**22**, N 3.—P. 229–244.
 32. Van den Hurk W. H., Willems H. J. J., Bloemen M., Martens G. J. M. Novel frameshift mutations near short simple repeats // *J. Biol. Chem.*—2001.—**276**, N 15.—P. 11496–11498.
 33. Niu X., Zheng W., Lu B. R., Ren G., Huang W., Wang S., Liu J., Tang Z., Luo D., Wang Y., Liu Y. An unusual posttranscriptional processing in two betaine aldehyde dehydrogenase loci of cereal crops directed by short, direct repeats in response to stress conditions // *Plant Physiol.*—2007.—**143**, N 4.—P. 1929–1942.
 34. Fan J., Niu X., Wang Y., Ren G., Zhuo T., Yang Y., Lu B. R., Liu Y. Short, direct repeats (SDRs)-mediated post-transcriptional processing of a transcription factor gene *OsVPI* in rice (*Oryza sativa*) // *J. Exp. Bot.*—2007.—**58**, N 13.—P. 3811–3817.
 35. Blom N., Gammeltoft S., Brunak S. Sequence- and structure-based prediction of eukaryotic protein phosphorylation sites // *J. Mol. Biol.*—1999.—**294**, N 5.—P. 1351–1362.