ORIGINAL ARTICLE

Thr136Ile polymorphism of human vesicular monoamine transporter-1 (SLC18A1 gene) influences its transport activity *in vitro*

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Abstract

OBJECTIVES: Although single nucleotide polymorphisms of the human vesicular monoamine transporter 1 (hVMAT1) gene SLC18A1 have been associated with neuropsychiatric disorders, there is limited information on the function of naturally occurring hVMAT1 variant proteins. This study evaluated transport activity of full length hVMAT1 isoform-a (NP_003044.1) with a threonine (Thr) or isoleucine (Ile) at amino acid 136 and hVMAT1 isoform-b (NP_00135796.1) with a 136-Thr and deletion of 32 amino acids in the central region of the protein. Genetic studies have previously linked the 136-Thr to bipolar disorder.

METHODS: Expression vectors with hVMAT1 DNA coding for isoform variants were transfected into COS-1 cells. Expression of immunoreactive proteins was assessed by Western blotting, and function was assayed by ATP-dependent transport of radiolabeled serotonin and concentration-dependent inhibition by reserpine.

RESULTS: Immunoreactive isoform-a proteins were observed as a major doublet (68–71 Kd) and a minor 39 Kd protein. The major isoform-b protein was 47 Kd with minor 57 and 115 Kd proteins. Isoform-b had no detectable transport activity, despite a large amount of immunoreactive protein. Transport activity of isoform-a with 136-Thr was 20–50% lower than with 136-Ile in time course studies (2.5–5 min) and in additional 5 min assays repeated with 5–6 transfections per variant. Kinetic analyses indicated a lower transport Vmax of isoform-a with 136-Thr but no significant differences in the transport Km or reserpine IC_{50}.

CONCLUSIONS: Deletion of amino acids 307–338 in hVMAT1 isoform-b abolishes transport activity, and a 136-Thr partially reduces activity of isoform-a.

INTRODUCTION

Vesicular monoamine transporters 1 and 2 (VMAT1 and VMAT2) permit vesicular uptake, storage, and regulated release of serotonin, catecholamines and other monoamines. In adult humans and rodents, VMAT1 is the major VMAT in adrenal medulla, and VMAT2 is the major VMAT in brain (Erickson *et al.* 1992; Erickson *et al.* 1996; Liu *et al.* 1992). There is evidence, however, for VMAT 1 gene expression in embryonic rat brain (Hansson *et al.* 1998), rat pineal (Hayashi *et al.* 1999), and several regions of human and mouse brain (Ashe *et al.* 2011; Ibanez-Sandoval *et al.* 2010; Lohoff *et al.* 2006).

Since 2005 four laboratories have detected associations of single nucleotide polymorphisms (SNPs) of the human VMAT 1 (hVMAT1) gene SCL18A1 with neuropsychiatric disorders in populations of European/American, Japanese, and Han Chinese descent (Bly 2005; Chen *et al.* 2007; Lohoff *et al.* 2006; Lohoff *et al.* 2008a; Lohoff *et al.* 2008b; Richards *et al.* 2006). Although three of these SNPs occur in the coding sequence of the gene, there is no information on functional consequences of single amino acid changes in the hVMAT1 protein. Based on amino acid numbers in hVMAT1 protein NP_003044, these SNPs result in a proline or threonine at amino acid 4 (4Pro/Thr), a threonine or serine at amino acid 98 (98Thr/Ser) and a threonine or isoleucine at amino acid 136 (136Thr/Ile).

In addition to SNP variants, there are 4 hVMAT1 mRNA variants. Variants 1 and 2 differ only in the 5' untranslated region and code for the same 525 amino acid protein isoform-a. Variant 3, first identified by the Mammalian Gene Collection Program Team in a brain tumor, has an alternate in-frame exon resulting in isoform-b with 32 amino acids deleted in the central region of the protein (GenBank NM_001142124.1) and (Strausberg *et al.* 2002). Variant 4, also known as VMAT1delta15, lacks an alternate exon in the 3' coding region that results in a frameshift and isoform-c with a shortened and unique C-terminus. Essand and colleagues demonstrated that isoform-c is inactive (Essand *et al.* 2005), but there are no data on the function of isoform-b.

In this study we examined transport activity of hVMAT1 isoforms-a and -b and further investigated effects of SNP rs1390938 coding either an isoleucine or threonine at amino acid 136 in isoform-a. Genetic data previously indicated an association of the 136-Thr with bipolar disorder (Lohoff *et al.* 2006) and an association of heterozygous alleles coding 136-Thr/Ile with anxiety-related traits in females (Lohoff *et al.* 2008a).

MATERIAL AND METHODS

Gene variants and site-directed mutagenesis

The hVMAT1 cDNA Variant 3 in vector pCMV6-XL5 was obtained from Origene (Rockville, MD, #SC325132). The cDNA insert was cut from the plasmid with Not I and transferred into expression vector pCMV6-Neo (Origene), which confers neomycin resistance in mammalian cells. To confirm insert orientation, restriction sites were identified with NEBcutter (http://tools.neb.com/NEBcutter2/index.phpp), and the plasmid was digested with XmnI (New England BioLabs Ipswich, MA). Full-length sequencing of clones with the correct insert (Virginia Commonwealth University DNA Sequencing Core) indicated that the DNA had a cytosine at base 277 coding a 4-Pro. DNA base numbers reflect the reference sequence NM_003053.3. The QuickChange II XL Site Directed Mutagenesis Kit (Stratagene #200521, La Jolla, CA) was used to obtain a 277-adenine coding a 4-Thr. Site directed mutagenesis primers were sense = GGCCATCACCATGCTCCGG**A**CCATTCTG and antisense = CAGAATGG**T**CCGGAGCATGGTGATGGCC.

Variant 2 was obtained from Origene (# SC122643) in the pCMV6-Neo vector. The DNA was amplified in *E.coli* (*end A⁻ and Rec A⁻*) at 30 °C as recommended by Origene, and the plasmid purified (Virginia Commonwealth University Macromolecule Core Facility). Full length sequencing was performed by Operon (Huntsville, AL). Variant 2 from Origene had a 674-thymidine coding a 136-isoleucine in the hVMAT1 protein. Site-directed mutagenesis by the Macromolecule Core generated a modified Variant 2 with a 674-cytocine coding a 136-threonine. Primers were Sense = CTTGGAGGAAGAGA**C**TACCCGGGTCGGGGTTC and anti-sense = GAACCCCGACCCGGGTA**G**TCTCTTCCTCCAAG. Full length sequencing by Operon (Huntsville, AL) verified the mutations and absence of recombination during cloning.

Cells

The COS-1 kidney fibroblast cell line was obtained from American Type Culture Collection. Cells were incubated in DMEM high glucose medium (Invitrogen 11995065) supplemented with 10% heat inactivated fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. Cells were maintained at 37 °C in a 7% CO_2 atmosphere. Cells maintained in a 5% CO_2 atmosphere were grown in ATCC # 30-2002 DMEM, which has a low concentration of sodium bicarbonate (1.5 g/L).

Cell transfection

To examine VMAT1 expression, COS-1 cells were transfected with variant DNA by electroporation as previously described (Ashe *et al.* 2011). Transfected cells were incubated in fresh medium overnight, then grown in medium supplemented with geneticin (500 μg/ml) for three days prior to harvest. Preliminary experiments verified that 3 days with this concentration of geneticin were sufficient to kill wild type COS-1 cells; therefore, only successfully transfected neomycin-resistant cells survived and maintained adherence.

Membrane preparation

On the fourth day after electroporation, COS-1 cells from each T-75 flask were rinsed with PBS, detached and centrifuged at 500 × g for 10 min at °C. The pellet was washed twice in PBS, centrifuged after each wash and resuspended in 250 µl of sucrose-Hepes buffer (SH) containing 0.32 M sucrose and 10 mM Hepes adjusted to pH 7.4 with 1 M KOH and supplemented with proteolytic inhibitors as recommended by Finn *et al.* (Finn *et al.* 1998). The suspension was transferred to a tube on ice and homogenized by sonication (4 pulses, 0.5 sec per pulse at 1 sec intervals and 0.5 Watt output), and centrifuged at 500 × g for 10 min at 4 °C. Total protein in the supernatant was measured with the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA), and aliquots were stored at –70 °C until used for Western blots and assays of VMAT activity.

Transport activity

Each assay tube contained 50 µg of cell protein, 5 mM ATP, and 20 nM radiolabeled serotonin (^{3}H-5HT) = hydroxytryptaminecreatinine sulfate, 5-1,2-[^{3}H] – 27.7–28.25 Ci/mmol (American Radiolabeled Chemicals) in a total volume of 200 µl of SH buffer supplemented with 4 mM $MgSO_4$ and 4 mMKCl. All incubations were conducted at 25 °C for 5 min, except in time course experiments and saturation experiments in which incubation was 1.5 min to approximate an initial transport rate. In saturation experiments, labeled and unlabeled serotonin were mixed in the µM ratio of 1/1.95 and serially diluted to achieve the final concentrations indicated. For inhibition experiments, indicated concentrations of reserpine were added immediately prior to addition of radiolabeled serotonin. The reaction was terminated by addition of 1 ml cold SH buffer, rapid filtration through 0.2 µm Supor 200 membranes (Pall/Gelman #60300) in a Millipore sampling manifold. Reaction tubes were washed with an additional 1 ml of cold SH buffer, which was transferred to the appropriate filter and rapidly filtered as above. Filters were transferred to 10 ml of Scintisafe 50%-Plus scintillation fluid (Fisher Scientific, Fair Lawn, New Jersey), and radioactivity was measured with a Beckman LS6000IC scintillation counter.

Western blotting

Membrane proteins were separated by electrophoresis on 10% polyacrylamide gels and transferred to nitrocellulose. Blots were incubated 60 min at 24 °C in blocking solution containing Tris buffered saline (TBS) and 5% dry milk, and then incubated overnight at 4 °C with primary goat anti-hVMAT1 antibody C-19 (#SC-7718, Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:200 in TBS + 0.1% Tween-20 (TBST). Blots were washed with TBST and incubated for 60 min at 24 °C with 1:10,000 donkey anti-goat IgG-horseradish peroxidase (Santa Cruz). The blots were visualized with enhanced chemiluminesence (Amersham, Biosciences, UK).

Analyses

A split-plot two-way analysis of variance (ANOVA with groups as between-subjects factor and time as the within-subjects factor) and Newman Keuls' post-hoc tests were used for comparisons of VMAT1 activity at various time points. A one-way ANOVA and Newman Keul's post hoc test were used for comparison of activity measured at 5 min. For saturation kinetics and inhibition curves, best fit non-linear regressions were performed on data from 3 transfections of each variant and significant differences were determined from 95% confidence limits of the regressions. ANOVAs and non-linear regressions were performed with GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA).

To facilitate interpretation of results, structures of isoform-a and -b were predicted from the GenBank protein sequences with HMMTOP software (Tusnady & Simon 1998) available at http://www.enzim.hu/hmmtop/html/document.html and drawn with TMRPres2D (Spyropoulos *et al.* 2004); http://biophysics.biol.uoa.gr/TMRPres2D/.

RESULTS

Expression of hVMAT1 variants in transfected cells

Western blotting confirmed expression of hVMAT1 variants in transfected COS-1 cells. An example blot is shown in Figure 1. Loading of 5 µg of total protein was sufficient to observe marked immunoreactive hVMAT1 proteins but no detectable VMAT1 in wild type cells. Immunoreactive isoform-a proteins were observed as a major doublet (68–71 Kd) and a minor 39 Kd protein.

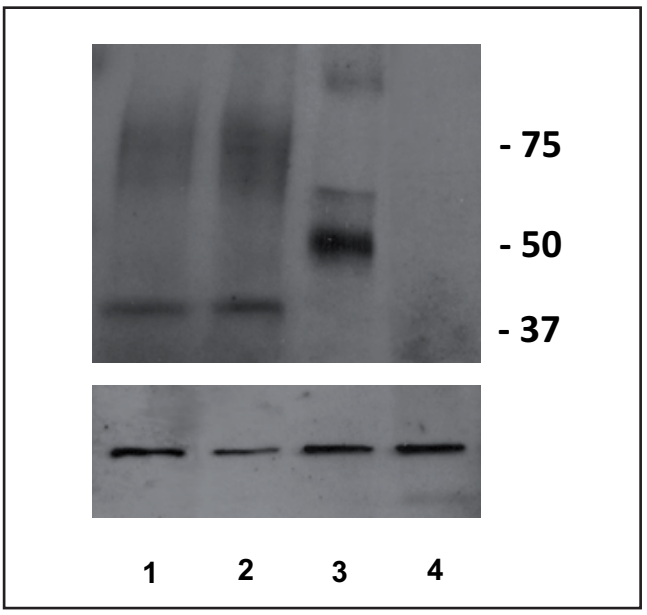

Fig. 1. Immunoreactive hVMAT-1 variant proteins. Total protein (5 µg per lane) was separated by SDS-PAGE on 10% gels. Lane 1 = Isoform-a with 136-Thr. Lane 2 = Isoform-a with 136-ILE. Lane 3 = Isoform-b with 136-Thr. Lane 4 = COS-1 WT. Beta-actin loading controls are shown in the lower blot.

The major isoform-b protein was 47 Kd with minor 57 and 115 Kd proteins.

Based on the amino acid sequences NP_003044.1 and NP_001135796, isoform-a consists of 525 amino acids and has a predicted molecular size of 56 Kd, whereas isoform-b is 493 amino acids with a predicted size of 52.7 Kd. The 68–71 Kd bands of isoform-a protein are consistent with evidence for phosphorylation and/or glycosylation of VMATs that would increase molecular size (Eiden *et al.* 2003). The 47 Kd major isoform-b protein, which is slightly less than its predicted size, and the faint 57 Kd isoform-b protein raise the possibility of decreased post-translational modification of isoform-b. Furthermore, the absence of a 39 Kd protein that was observed only for isoform-a and a 115 Kd band observed only for isoform-b may indicate different processing of the proteins.

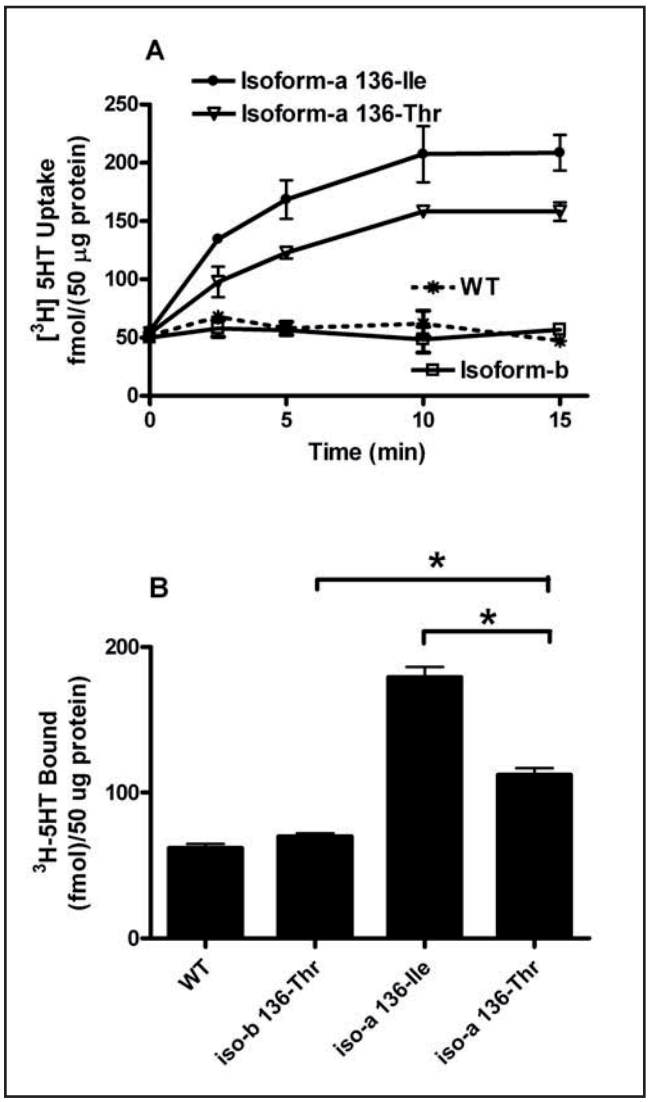

Fig. 2. Time-dependent serotonin transport by hVMAT1 variant proteins. **A.** Transport of radiolabeled serotonin ([^{3}H] 5HT) by membrane proteins from wild type (WT) COS-1 cells or cells transfected with hVMAT1 variant DNA coding isoform-a with a threonine or isoleucine at amino acid 136 or isoform-b with 136-Thr. Data represent the mean ± S.E. of 2 transfections for each variant and duplicates per transfection. Transport by isoform-a proteins was significantly greater than that of WT and isoform-b protein, and transport by isoform-a with 136-Ile was greater than that of isoform-a with 136-Thr at all time points by two-way ANOVA and Newman Keuls' post-hoc test (*p<0.01). **B.** Comparison of serotonin transport at 5 min by membrane proteins from WT and transfected cells. Bars represent mean ± S.E. transport in proteins from 5-6 transfections per variant or 4 replicates of 3 harvests of WT cells. Transport by isoform-a proteins was significantly greater than that of isoform-b or WT protein by one-way ANOVA and Newman Keuls' post-hoc test (*p<0.01).

Time-dependent transport of serotonin by hVMAT1 variants

ATP-dependent transport of radiolabeled serotonin by isoform-a proteins increased over 10 min, but isoform b appeared to lack activity (Figure 2A). Activity at all time points was 20–29% higher for isoform-a with a 136-Ile than with a 136-Thr (p<0.01). Serotonin transport by protein from wild type cells or cells transfected with DNA coding for isoform-b did not increase over time. Additional comparisons of serotonin uptake measured at 5 min on proteins from 5–6 transfections per variant confirmed significantly higher (p<0.01) transport activity with the 136-Ile and demonstrated isoform-b activity was not different (p>0.05) from that of wild type cells (Figure 2B).

Kinetics of serotonin transport and inhibition

At saturating concentrations of serotonin, the transport Vmax was higher for isoform-a with a 136-Ile than with the 136-Thr, but the transport Km did not differ significantly (p>0.05) for the proteins (Figure 3). Concentration-dependent inhibition of serotonin transport by reserpine also did not differ significantly (p>0.05) with the two proteins (Figure 4).

Predicted structures of hVMAT1 isoforms a and b

HMMTOP software predicted 12-transmembrane domains in hVMAT1 isoform-a, 10 transmembrane domains in isoform-b, a reduction in the number of luminal and cytoplasmic domains in isoform-b, and a reduced total number of amino acids in the latter domains (Figure 5). Although the cytoplasmic C- and N-terminals were predicted to remain the same in isoform-a and b, the position of amino acids, such as the 98 and 136 amino acids coded by hVMAT1 SNPs, were predicted to shift in the first luminal loop of isoform-b.

DISCUSSION

Results of the present study suggested hVMAT1 isoform-b is inactive and a 136-Thr in isoform-a reduces transport activity. Although the reduction in activity with the 136-Thr was modest, this finding is interesting in view of genetic data suggesting the 136-Ile is protective in bipolar disorder (Lohoff *et al.* 2006). Reduced storage and release of monoamines in adrenal medulla where VMAT1 is the major VMAT and in selected

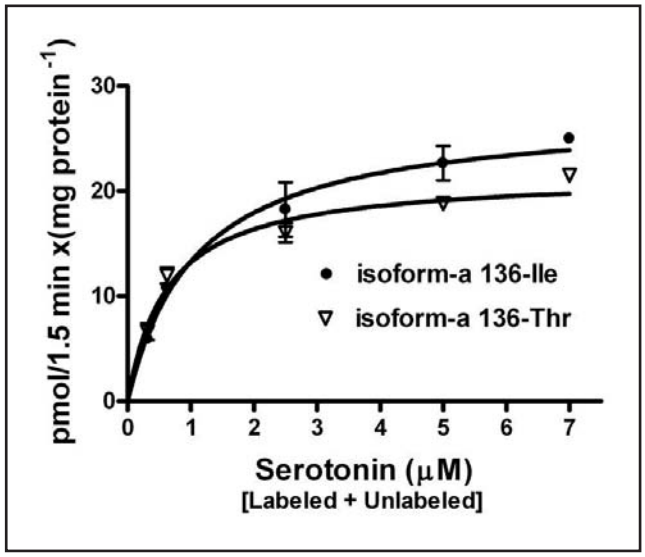

Fig. 3. Saturation kinetics of serotonin transport by hVMAT1 variant proteins. Curves illustrate 1.5 min transport of radiolabeled + unlabeled serotonin (1.0:1.95 μM ratio) by membrane proteins from COS-1 cells transfected with hVMAT1 variant DNA coding isoform-a with a threonine or isoleucine at amino acid 136. Proteins from 3 transfections per variant were pooled, and data points represent the mean ± S.E. of 3 replicates of each pool minus non-specific transport by proteins from wild type cells. Transport Vmax = 27.6 ± 1.6 pmol/1.5 min (mg protein)$^{-1}$ for isoform-a with 136-Ile and 21.5 ± 1.1 for isoform-a with 136-Thr (p<0.05) based on confidence limits of the non-linear regression. Km = 1.07 ± 0.19 μM for isoform-a with 136-Ile and 0.63 ± 0.112 μM for isoform-a with a 136-Thr did not differ significantly.

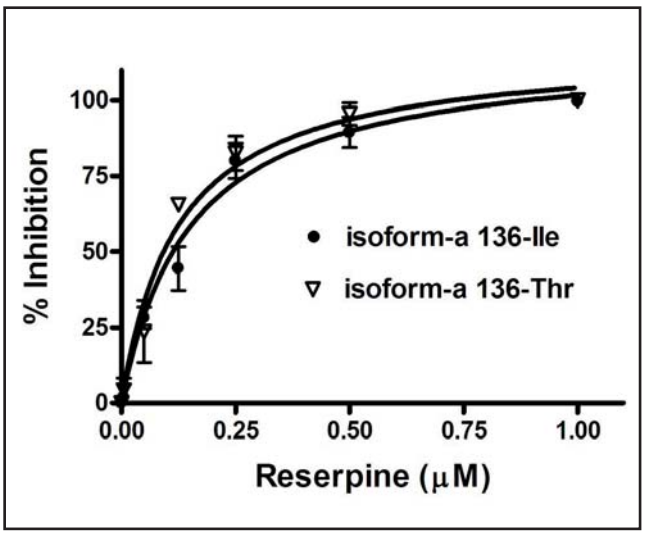

Fig. 4. Reserpine inhibition of serotonin transport by hVMAT1 variant proteins. Membrane proteins from COS-1 cells transfected with hVMAT1 variant DNA coding isoform-a with a threonine or isoleucine at amino acid 136 were incubated for 5 minutes with 20 nM radiolabeled serotonin and either reserpine or reserpine vehicle (0 μM reserpine). Data points represent the mean ± S.E. of 3 transfections per variant protein minus non-specific transport by proteins from wild type cells. Reserpine IC_{50} (mean ± SE) = 152 ± 25 nM for isoform-a with 136-Ile and 124 ± 25 nM for isoform-a with 136-Thr did not differ significantly based on 95% confidence limits of best fit non-linear regressions.

brain regions expressing VMAT1 could alter the balance of monoamine availability both peripherally and centrally. It is conceivable that even a small change in monoamine release could contribute to abnormalities in a psychiatric disorder.

Saturation kinetics indicating a lower Vmax with the 136-Thr and no significant difference in the transport Km are consistent with the 136 amino acid modulating transport by a mechanism unrelated to substrate binding. Nevertheless, these results also are consistent with a model of vesicular transport kinetics in which decreased Vmax is related to a decreased dissociation constant for intra-vesicular substrate binding (Parsons 2000). As shown in Figure 5, amino acid 136 is located in the first intra-vesicular loop of both isoform-a and -b. Brunk *et al.* demonstrated the importance of this luminal loop in the G-protein regulation of VMAT activity but only when cellular compartments were pre-loaded with monoamines (Brunk *et al.* 2006). Whereas decreased transport Vmax can result from damage to protein during isolation (Parsons 2000) or decreased

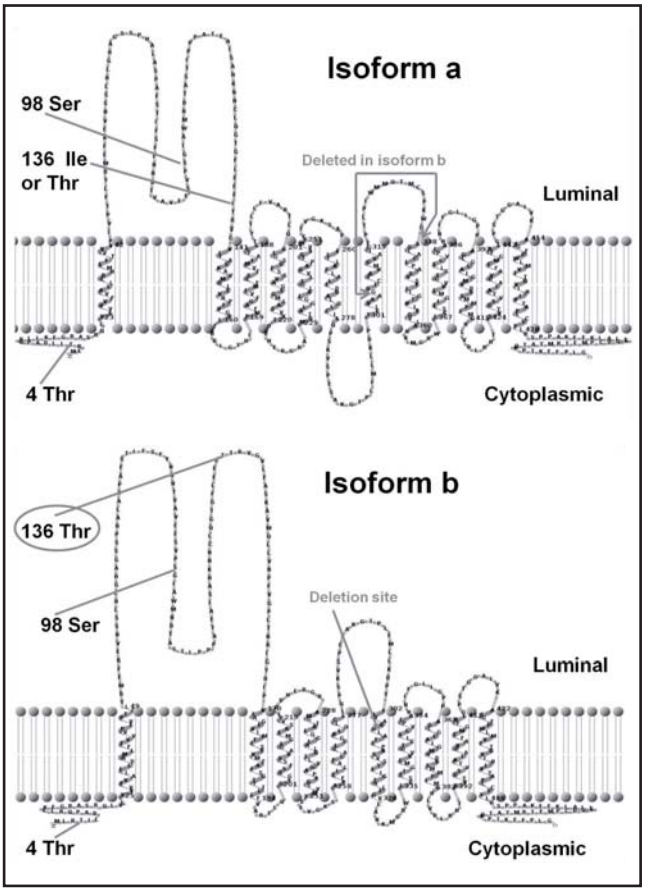

Fig. 5. Predicted structures of hVMAT1 proteins. HMMTOP software (http://www.enzim.hu/hmmtop/html/document.html) was used to generate structures from the translated protein sequences NP_003044.1 (isoform-a) and NP_001135796.1 (isoform-b). Structures were drawn with TMRPres2D (http://biophysics.biol.uoa.gr/TMRPres2D/).

cell expression of a variant DNA, we attempted to mitigate this possibility by evaluating Western blots of protein fractions from multiple transfections of each variant and excluding samples with low hVMAT1 expression.

Considering the numerous changes predicted in the structure of isoform-b compared to full length isoform-a (Figure 5), and potential differences in post-translational modification of the proteins, it is not surprising that isoform-b was inactive. The model of isoform-b presented in Figure 5 predicts altered positions of amino acids comprising the transmembrane domains previously implicated in transport function (Erickson & Varoqui 2000) as well as amino acids located in intravesicular and cytoplasmic domains. Although it is possible that the 136-Thr in isoform-b accounts partially for the reduced activity, it appears unlikely that substitution with 136-Ile would restore function.

In summary, these findings provide new information on hVMAT1 proteins resulting from genetic variation in the hVMAT1 gene. Additional investigation is needed to determine combined effects of various hVMAT1 haplotypes.

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REFERENCES

1 Ashe KM, Chiu WL, Khalifa AM, Nicolas AN, Brown BL, De Martino RR and others (2011). Vesicular monoamine transporter-1 (VMAT-1) mRNA and immunoreactive proteins in mouse brain. Neuro. Endocrinol. Lett. **32**: 253–258.
2 Bly M (2005). Mutation in the vesicular monoamine gene, SLC18A1, asociated with schizophrenia. Schizophr. Res. **78**: 337–338.
3 Brunk I, Blex C, Rachakonda S, Holtje M, Winter S, Pahner I and others (2006). The first luminal domain of vesicular monoamine transporters mediates G-protein-dependent regulation of transmitter uptake. J. Biol. Chem. **281**: 33373–33385.
4 Chen S-F, Chen J-Y, Wang Y-C, Lai I-C, Liou Y-J, Liao D-L and others (2007). Support for association of the A277C single nucleotide polymorphism in human vesicular monoamine transporter 1 gene with schizophrenia. Schizophr. Res. **90**: 363–365.
5 Eiden LE, Schafer MK, Weihe E, Schultz B (2003). The vesicular amine transporter family (Slc18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamine and acetylcholine. Eur. J. Physiol. **447**: 636–640.
6 Erickson JD, Eiden LE, Hoffman BJ (1992). Expression cloning of a reserpine-sensitive vesicular monoamine transporter. Proc. Natl. Acad. Sci. USA **89**: 10993–10997.
7 Erickson JD, Schafer MK, Bonner Ti, Eiden LE, Weihe E (1996). Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. Proc. Natl. Acad. Sci. USA **93**: 5166–5171.
8 Erickson JD, Varoqui H (2000). Molecular analysis of vesicular amine transporter function and targeting to secretory organelles. FASEB J. **14**: 2450–2458.
9 Essand M, Vikman S, Grawe J, Gedda L, Hellberg C, Oberg K and others (2005). Identification and characterization of a novel splicing variant of vesicular monoamine transporter 1. J. Mol. Endocrinol. **35**: 489–501.
10 Finn JP, Merickel A, Edwards RH (1998). Analysis of neurotransmitter transport into secretory vesicles. In: Amara, S.G. (Ed.), Methods in Enzymology, Vol. 296. Neurotransmitter Transporters, New York, NY.Academic Press p. 144–162.
11 Hansson SR, Hoffman BJ, Mezey E (1998). Ontongeny of vesicular monoamine transporter mRNAs VMAT1 and VMAT2.I. The developing rat central nervous system. Dev. Brain Res. **110**: 135–158
12 Hayashi M, Haga M, Yatsushiro S, Yamamoto A, Moriyama Y (1999). Vesicular monoamine transporter 1 is responsible for storage of 5-hydroxytyrptamine in rat pinealocytes. J. Neurochem. **73**: 2538–2545.
13 Ibanez-Sandoval O, Tecuapetla F, Unal B, Shah F, Koos T, Tepper JM (2010). Electrophysiological and morphological characteristics and synaptic connectivity of tyrosine hydroxylase-expressing neurons in adult mouse striatum. J. Neurosci. **30**: 6999–7016
14 Liu Y, Peter D, Roghani A, Schuldiner S, Prive GG, Eisenberg D and others (1992). A cDNA that suppresses MPP+ toxicity encodes a vesicular amine transporter. Cell **70**: 539–551.
15 Lohoff FW, Dahl JP, Ferraro TN, Arnold SE, Gallinat J, Sander T and others (2006). Variations in the Vesicular Monoamine Transporter 1 Gene (VMAT1/SLC18A1) are Associated with Bipolar 1 Disorder. Neuropsychopharmacology **31**: 2739–2747.
16 Lohoff FW, Lautenschlager M, Mohr J, Ferraro TN, Sander T, Gallinat J (2008a). Association between variation in the vesicular monoamine transporter 1 gene on chromosome 8p and anxiety-related personality traits. Neurosci. Lett. **434**: 41–45.
17 Lohoff FW, Weller AE, Bloch PJ, Buono RJ, Doyle GA, Ferraro TN and others (2008b). Association between Polymorphisms in the Vesicular Monoamine Transporter 1 Gene (VMAT1/SLC18A1) on Chromosome 8p and Schizophrenia. Neuropsychobiology **57**: 55–60.
18 Parsons SM (2000). Transport mechanisms in acetylcholine and monoamine storage. FASEB J. **14**: 2423–2434.
19 Richards M, Iijima Y, Kondo H, Shizuno T, Hori H, Arima K and others (2006). Association study of the vesicular monoamine transporter 1 (VMAT1) gene with schizophrenia in a Japanese population. Behav. Brain Funct. **2**(39): 39.
20 Spyropoulos IC, Liakopoulos TD, Bagos PG, Hamodrakas SJ (2004). TMRPres2D: high quality visual representation of transmembrane protein models. Bioinformatics. **20**: 3258–3260.
21 Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS and others (2002). Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc. Natl. Acad. Sci. U. S. A **99**: 16899–16903.
22 Tusnady GE, Simon I (1998). Principles governing amino acid composition of integral membrane proteins: application to topology prediction. J. Mol. Biol. **283**: 489–506.