When Acetylcholine Transporter Goes Awry: The Consequences of VAChT Mutations

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Vesicular Acetylcholine Transporter (VAChT) is a kind of membrane protein that is usually found in SVs of cholinergic nerve system[1]. VAChT is 500-amino acid polypeptides consisting of 12 trans-membrane helices (TMs) (Figure 1), with an N-C terminal in the cytosol, and a large *N*-glycosylated loop between the first and second TMs. It is similar to VMATs (VMAT1 and VMAT2). These two have 60% amino acid similarity and they are 40% identical to VAChT (Figure 2). VAChT protein is coded by the SLC18A3 segment and this segment is expressed by the SLC18 family. SLC18 itself is a member of MFS. VAChT is the part of the drug H^+ antiporter-1 family (DHA1, TCID 2.A.1.2)[2-4].

As for how of its name, VAChT performs the function as an active transporter (Figure 3) for the acetylcholine into the SVs[1,4]. VAChT expressions also regulate the amount of ACh in the SVs[5]. It means that the decreased VAChT expression will directly reduce the level of ACh in SVs. VAChT exchanges two protons per substrate molecule with very similar initial velocity kinetics and pH dependencies[1]. It relates to the activity of VAChT that is able to change tonicity and morphological in SVs[6].

A research was conducted by Rodrigues, et al. in 2013 to understand the affection of reduced VAChT and neurotransmitter content to the distribution and shape of SVs in mouse NMJ. The research concludes that VAChT expression plays a role in the recycling and mobilization of specific pools of SV in NMJ. Normal expression and activity of VAChT are important for maintaining tonicity and morphology of SVs in nerve terminals from diaphragm NMJ[7]. But, this research could not prove the relationship of the reduced VAChT to the SVs' shape-changing.

Diseases and Disorders Related to VACht

As we know that acetylcholine performs the function of wiring the neuromuscular junction. ACh is one of neurotransmitters where its primary function is to deliver information between the NMJ. Some researches show that ACh relate to learning and memorizing process[8]. ACh itself plays a vital role in neurons developing process[9-11]. This is important to know the function of ACh as how of this protein is regulated by VAChT, before we study the disease relates to VAChT disorder. But, scientists found no specific disease relates to the mutation of SLC18 family member[4].

Later in 2016, Sugita et al. showed that over-expressing VAChT increases ACh accumulation in matured synaptic vesicles. They noticed that the increased amount of ACh in NMJs contributes to the age- and disease-related motor deficits such as ALS. In

the development of NMJs, ACh acts as an anti-synaptogenic molecule by decreasing the stability of AChRs, and through this activity, it is believed to function together with the neural-derived factor, z-agrin, in sculpting the NMJ. Thus, an increase in the amount of synaptic ACh together with less functional z-agrin in adult NMJs would result in structural changes due to increased destabilization of AChRs. Sugita et al. confirmed that dysregulated ACh level causes pathophysiological changes in NMJs and contributes to the motoric functional loss during aging and the progression of ALS[12]. This result was reinforced by the next research conducted by Vaughan, et.al in 2019[13].

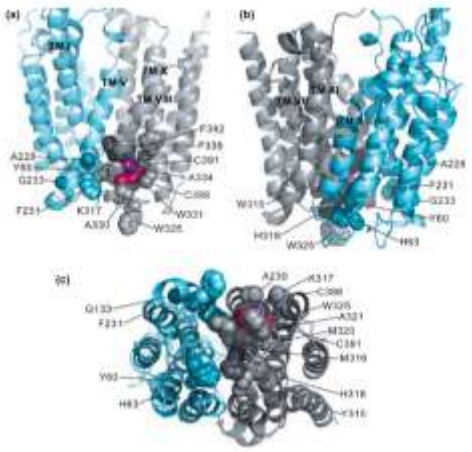

Figure 1. Homology model of human vesicular acetylcholine transporter (hVAChT) showing mutated residues. Non-transmembrane helix (TM) regions are clipped off. The first bundle of TMs is cyan and the second bundle is gray. The cytoplasm is at the top in (a) and (b). (c) An observer inside the vesicular lumen would see this view. Figures were rendered using PyMOL[14].

Figure 2. VMAT and VAChT's multiple sequence alignment. These multiple sequences show that VAChTs' His replaces VMATs' Tyr in TM8[2].

Another research said that VAChT defect may contribute to CMS. Most cases of CMS are due to defect in postsynaptic proteins, some 10% is due to a defect in synaptic proteins, and fewer than 5% are due to defect in pre-synaptic proteins. Meanwhile, the CMS that was caused by the mutation of VAChT appears in a rare frequency. This research was done on two brothers from a nonconsanguineous Yemeni Jewish family. The first brother died at the age of 5 days due to respiratory failure, meanwhile, the younger brother was 4,5 years when the research conducted. The 4,5 years brother use a mechanical ventilator since he was born. It gives a conclusion that the complete functional loss of VAChT disrupts cholinergic neurotransmission, resulting in severe hypotonia and respiratory failure[15].

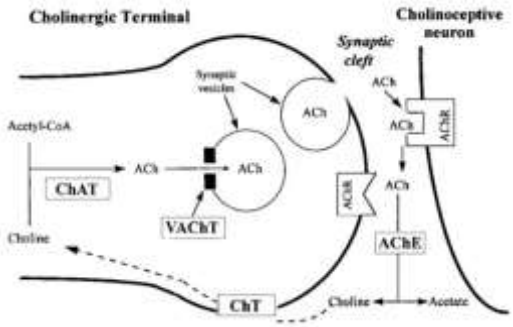

Figure 3. The illustration of how the ACh is synthesized in a cholinergic terminal and synaptic cleft. The ACh is catalyzed by ChAT and then carried by VAChT to cross the synapse terminal[16].

Another report said that recessive mutations in SLC18A3 results in mild pre-synaptic CMS suffered by 2 patients come from 2 different families. The first patient, a 14-year-old boy has heterozygote genomic deletion including SLC18A3 and for SLC18A3 p.Gly186Ala. This patient undergoes ptosis, ophthalmoplegia, and mild facial weakness, as well as mild cognitive deficits. The second patient, a 6-year-old girl has homozygous for SLC18A3 p.Asp398His (Figure 5). The second patient presents hypotonia, feeding difficulties, apneic episodes, ptosis, and ophthalmoplegia[17].

In 2018, Schwartz, et al. explained how chromosomal deletion can unmask recessive mutation. They showed that deletion in segment 10q11.2 associated with SLC18A3 lead to the CMS. They reported for the first time hemizygous mutation in CHAT in a patient with CMS and a 10q11.2 deletion inherited from a healthy parent (Figure 6). They also informed that detecting a 10q11.2 deletion should lead to looking for CMS symptoms, such as hypomimia and ptosis since birth, or recurrent respiratory distress episodes[18].

The latest research that was conducted in 2019 gave information that SLC18A3 mutation causes lethal disease in the fetus. Two fetuses showed fetal akinesia, arthrogryposis, edema, and partial cleft palate. That research confessed for the first time SLC18A13 variants were known to associate with a severe prenatal phenotype compatible with FADS. They ended up listing SLC18A3 to the growing up genes that should be investigated in cases of fetal arthrogryposis of unknown origin[19].

To the case of pathophysiological changes in NMJs that are caused by over-expressing VAChT, some research usually used vesamicol ($_{L}$-trans-2-(4-phenyl[3,4-3H]piperidino)cyclohexanol). Vesamicol works by inhibiting VAChT. Vesamicol binds to the cytosolic side of VAChT[4]. But, unfortunately, scientist and pharmacologist do not yet find the right drugs or treatment procedures for curing the case of CMS that caused by the mutation of SLC18A3 gene or VAChT[15].

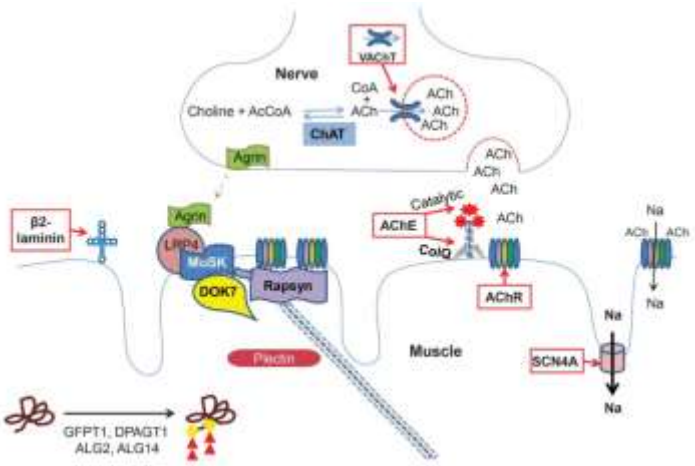

Figure 4. The schematic diagram of NMJ and all of the proteins relate to the CMS[15].

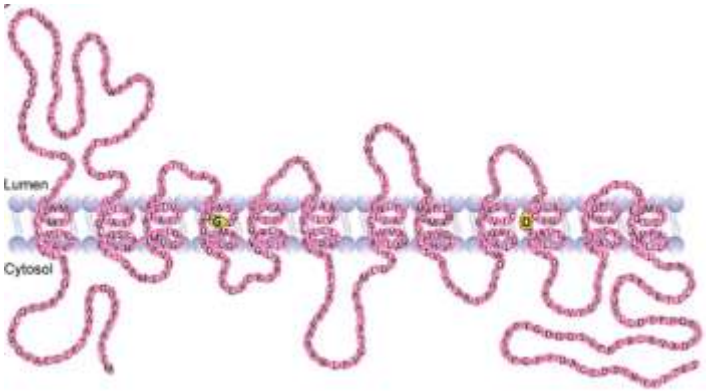

Figure 5. Schematic diagram showing the 12 trans-membrane domains as owned by VAChT. The Gly186Ala variant identified in patient 1 is shown in yellow in the fourth trans-membrane domain. The Asp398His variant identified in patient 2 is shown in yellow in the 10th trans-membrane domain[17].

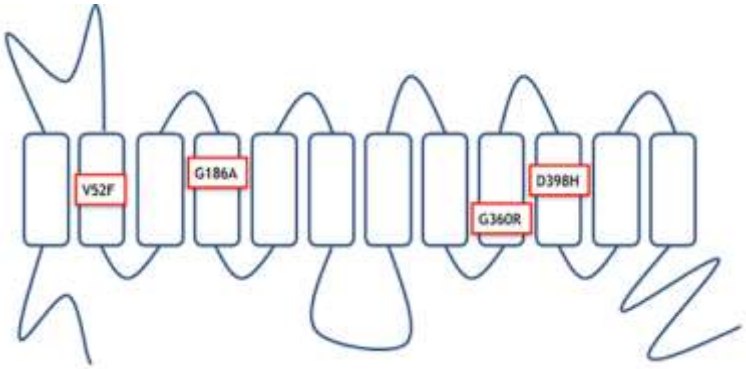

Figure 6. Schematic drawing of the VAChT protein with its 12 trans-membrane helixes, and localization of the four CMS-associated miss-sense mutations known to this day, including the new mutation that we report (V52F)[18].

References:

1. Parsons SM. Transport mechanisms in acetylcholine and monoamine storage. *FASEB J*. 2000;14(15):2423–2434. doi:10.1096/fj.00–0203rev
2. Vardy E, Arkin IT, Gottschalk KE, Kaback HR, Schuldiner S. Structural conservation in the major facilitator superfamily as revealed by comparative modeling. *Protein Sci*. 2004;13(7):1832–1840. doi:10.1110/ps.04657704
3. Yaffe D, Radestock S, Shuster Y, Forrest LR, Schuldiner S. Identification of molecular hinge points mediating alternating access in the vesicular monoamine transporter VMAT2. *Proc Natl Acad Sci U S A*. 2013;110(15):2–11. doi:10.1073/pnas.1220497110
4. Manuscript A. SLC18: Vesicular neurotransmitter transporters for monoamines and acetylcholine. *PMC J*. 2014;34(0):360–372. doi:10.1016/j.mam.2012.07.005.SLC18
5. Ferreira LT, Santos MS, Kolmakova NG, et al. Structural requirements for steady-state localization of the vesicular acetylcholine transporter. 2005:957–969. doi:10.1111/j.1471–4159.2005.03244.x
6. Herman MA, Ackermann F, Trimbuch T, Rosenmund C. Vesicular glutamate transporter expression level affects synaptic vesicle release probability at hippocampal synapses in culture. *J Neurosci*. 2014;34(35):11781–11791. doi:10.1523/JNEUROSCI.1444–14.2014
7. Fonseca MDC, Camargo W, Lima P, Martinelli P. Reduced Expression of the Vesicular Acetylcholine Transporter and Neurotransmitter Content Affects Synaptic Vesicle Distribution and Shape in Reduced Expression of the Vesicular Acetylcholine Transporter and Neurotransmitter Content Affects Synaptic Vesic. 2014;(June). doi:10.1371/journal.pone.0078342
8. Hasselmo ME. The Role of Acetylcholine in Learning and Memory Michael. *Natl Inst Heal*. 2009;16(6):710–715. doi:10.1016/j.conb.2006.09.002.The
9. Brandon EP, Lin W, D'Amour KA, et al. Aberrant patterning of neuromuscular synapses in choline acetyltransferase-deficient mice. *J Neurosci*. 2003;23(2):539–549. doi:10.1523/jneurosci.23–02–00539.2003
10. Castro BM De, Jaeger X De, Martins-silva C, et al. The Vesicular Acetylcholine Transporter Is Required for Neuromuscular Development and Function □. 2009;29(19):5238–5250. doi:10.1128/MCB.00245–09
11. Misgeld T, Burgess RW, Lewis RM, Cunningham JM, Lichtman JW, Sanes JR. Roles of Neurotransmitter in Synapse Formation. *Neuron*. 2002;36(4):635–648. doi:10.1016/s0896–6273(02)01020–6
12. Sugita S, Fleming LL, Wood C, Vaughan SK, Gomes MPSM, Camargo W. VAChT overexpression increases acetylcholine at the synaptic cleft and accelerates aging of neuromuscular junctions. *Skelet Muscle*. 2016;(October). doi:10.1186/s13395–016–0105–7
13. Vaughan SK, Sutherland NM, Valdez G. Attenuating Cholinergic Transmission Increases the Number of Satellite Cells and Preserves Muscle Mass in Old Age. *Front Aging Neurosci*. 2019;11(September):1–12. doi:10.3389/fnagi.2019.00262
14. Khare P, Mulakaluri A, Parsons SM. Search for The Acetylcholine and Vesamicol Binding Sites in Vesicular Acetylcholine Transporter: The Region Around The Lumenal End of The Transport Channel. *J Neurochem*. 2010;(115):984–993.

doi:10.1111/j.1471-4159.2010.06990.x
15. Aran A, Renbaum P, Oliphant S, et al. Vesicular acetylcholine transporter defect underlies devastating congenital myasthenia syndrome. *Am Acad Neurol*. 2017;88(March 14):1021-1028.
16. Yao W, Godfrey DA. Vesicular acetylcholine transporter in the rat cochlear nucleus: An immunohistochemical study. *J Histochem Cytochem*. 1999;47(1):83-90. doi:10.1177/002215549904700109
17. O'Grady GL, Verschuuren C, Yuen M, et al. Variants in SLC18A3, vesicular acetylcholine transporter, cause congenital myasthenic syndrome. *Neurology*. 2016;87(14):1442-1448. doi:10.1212/WNL.0000000000003179
18. Schwartz M, Sternberg D, Whalen S, et al. How chromosomal deletions can unmask recessive mutations? Deletions in 10q11.2 associated with CHAT or SLC18A3 mutations lead to congenital myasthenic syndrome. *Am J Med Genet Part A*. 2018;176(1):151-155. doi:10.1002/ajmg.a.38515
19. Hakonen AH, Polvi A, Saloranta C, et al. SLC18A3 variants lead to fetal akinesia deformation sequence early in pregnancy. *Am J Med Genet Part A*. 2019;179(7):1362-1365. doi:10.1002/ajmg.a.61186