



HAL
open science

TRPML3 and hearing loss in the varitint-waddler mouse

Margaret Atiba-Davies, Konrad Noben-Trauth

► **To cite this version:**

Margaret Atiba-Davies, Konrad Noben-Trauth. TRPML3 and hearing loss in the varitint-waddler mouse. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2007, 1772 (8), pp.1028. 10.1016/j.bbadis.2007.01.007 . hal-00562759

HAL Id: hal-00562759

<https://hal.science/hal-00562759>

Submitted on 4 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

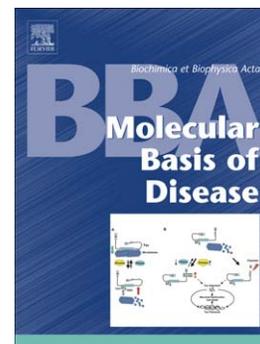
TRPML3 and hearing loss in the varitint-waddler mouse

Margaret Atiba-Davies, Konrad Noben-Trauth

PII: S0925-4439(07)00031-2
DOI: doi: [10.1016/j.bbadis.2007.01.007](https://doi.org/10.1016/j.bbadis.2007.01.007)
Reference: BBADIS 62687

To appear in: *BBA - Molecular Basis of Disease*

Received date: 30 November 2006
Revised date: 16 January 2007
Accepted date: 17 January 2007



Please cite this article as: Margaret Atiba-Davies, Konrad Noben-Trauth, TRPML3 and hearing loss in the varitint-waddler mouse, *BBA - Molecular Basis of Disease* (2007), doi: [10.1016/j.bbadis.2007.01.007](https://doi.org/10.1016/j.bbadis.2007.01.007)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

TRPML3 and hearing loss in the varitint-waddler mouse

Margaret Atiba-Davies and Konrad Noben-Trauth*

Section on Neurogenetics, Laboratory of Molecular Biology,
National Institute on Deafness and Other Communication Disorders,
National Institutes of Health, Rockville, MD 20850, USA

Corresponding author:

Konrad Noben-Trauth

5 Research Court

Rockville, MD 20850

Phone: (301) 402-4223

FAX: (301) 435-4040

email: nobentk@nidcd.nih.gov

Abstract

TRPML3 (also known as mucolipin-3, MCOLN3) belongs to the small family of TRPML ion channel proteins. The mammalian *Trpml3* gene encodes a protein of 553

amino acids with short amino and carboxy termini and a transient receptor potential motif spanning from the third to the sixth trans membrane domain. Dominant mutant alleles of *Trpm13* cause hearing loss, circling behavior, pigmentation defects and embryonic lethality in the varitint-waddler (*Va*) mouse. In the inner ear these mutations cause a reduction or loss of endocochlear potentials, compound action potentials, and auditory-evoked brain stem responses. This hearing phenotype is associated with defects in the cochlea that include disorganization and fusion of stereocilia, distortions at the apical and distal regions of inner and outer hair cells, and loss of pigmented intermediate cells in the stria vascularis. In hair cells the TRPML3 protein is targeted to cytoplasmic vesicles and to the plasma membrane of stereocilia. Both the sub-cellular localization of TRPML3 and the mutant phenotype suggest that TRPML3 is critical for stereocilia bundle formation during development and may function during endocytosis or exocytosis.

Introduction

The physiology of hearing has been studied for many decades and has revealed several attributes that are unique to the inner ear such as the tonotopic organization of the cochlea, the mechanosensitivity of the transducer channels, and cochlear amplification [1]. The molecular characterization of these mechanisms through biochemical means has been hampered by the limiting number of sensory cells in the cochlea but has been facilitated more recently by the systematic application of genetic means in mouse, humans, and zebra fish [2-4].

Sound is heard in the cochlea. Within this coiled compartmentalized cellular structure the stria vascularis, the organ of Corti and the spiral ganglion are the major domains that enable the perception, analysis, and transduction of acoustic stimuli. When sound waves travel through the scala tympany they peak at frequency-specific sites along the basilar membrane causing an upward motion of the organ of Corti. Actin-filled protrusions of the sensory outer hair cells, or stereocilia, are moved by the tectorial membrane overlying the organ of Corti (**Fig. 1**). Deflections of the stereocilia hair bundle stretch molecular springs in the stereocilia, and thereby mechanically gate ion channels located in the plasma membrane of the stereocilia. This results in an influx of K^+ and Ca^{2+} cations, depolarization of the hair cell, and synaptic transmission to the cochlear nerve with neurons located in the spiral ganglion. A major source of the high concentration of K^+ in the endolymph and the establishment of the endocochlear potential are the pigmented intermediate cells located in the stria vascularis, which is a highly stratified epithelium outlining the cochlear lateral wall [5].

The *TRPML* gene family (also known as mucolipins) consists of the three mammalian genes *TRPML1* (*MCOLN1*), *TRPML2* (*MCOLN2*) and *TRPML3* (*MCOLN3*). *TRPML1* was first recognized as the gene mutated in mucopolipidosis type IV (ML-IV) an autosomal recessive lysosomal storage disorder with clinical features that include psychomotor retardation and ophthalmological defects (reviewed in [6]) [7]. Genes homologous to *Trpml3* also have been identified in *Drosophila melanogaster* (CG8743), *Caenorhabditis elegans* (CUP-5) and *Leishmania major* [8-11].

Phenotype of varitint-waddler mice

The varitint-waddler phenotype is the result of two mutations, Va and Va' , that occurred spontaneously on the distal region of mouse chromosome 3. Both mutations exhibit a semi-dominant mode of inheritance with a well-defined set of phenotypes. The Va allele arose in an outcross of (C57black/C57brown)F1 and C57black mice [12] and mice homozygous or heterozygous for Va are deaf, and exhibit circling behaviour indicative of a vestibular defect. They also display a variegated and dilute coat color and homozygotes are embryonic lethal. The second allele, Va' , arose in a colony segregating Va and causes a less severe phenotype. Although homozygotes and heterozygotes are deaf they exhibit normal vestibular behaviour and display much reduced coat color variegation. In addition, Va' homozygotes are generally viable, although the extent of the viability depends on the genetic background [13]. The homozygote phenotype appears to be more severe for both alleles.

Hearing Loss in varitint-waddler

Hearing tests conducted with varitint-waddler mice have demonstrated abnormalities with respect to cochlea functioning. Compound action potentials, which are generated by the auditory nerve exiting the cochlea were absent in both $+/Va'$ and Va'/Va' . In addition the endocochlear potential, which is generated by strial intermediate cells was reduced in some, but not in all mutants. This finding was supported by data obtained from auditory-brain stem response analyses, which measures the activity response of the auditory nerve in response to discrete acoustic stimuli [13]. At postnatal day (P)14 $+/Va'$ heterozygotes showed some responses to sound stimuli, albeit at very high sound intensities (> 80 dB SPL), indicating that some residual hearing existed at this stage in these animals. At P21 response thresholds in Va' heterozygotes had increased to > 100 dB SPL. No response could be elicited from $+/Va$ and Va'/Va' mutants at any age. The congenital hearing loss in Va' mutants also appeared independent of the genetic background. In a series of F2 outcrosses involving C3HeB/FeJ, CZECHII/Ei, BALB/cByJ, A/J, C57BL/6J and DBA/2J strains, F2 $+/Va'$ and Va'/Va' showed no ABR responses [13].

Histological studies on inner ears of $+/Va$ heterozygotes identified several affected structures. The tectorial membrane appeared thicker than in normal ears and failed to contact the hair cells in the organ of Corti and the cell density in the spiral ganglion was decreased [14]. In the organ of Corti sensory hair cells and supporting cells appeared as an undifferentiated cell mass as early as P11. The stria vascularis had an abnormal appearance, showing nuclear and cytoplasmic irregularities within the marginal cell layer. Ultra-structural analyses based on transmission electron microscopy on the Va^j allele refined the cellular defects [15]. In $+/Va^j$ heterozygotes the stria vascularis was normal, but in Va^j/Va^j homozygotes it appeared abnormal with reduced or absent pigmentation, suggesting the absence of the pigmented intermediate cells.

The organ of Corti of 14-day old $+/Va^j$ heterozygotes exhibited a mild phenotype, showing fused stereocilia on mainly inner hair cells. The apical surface of hair cells was also affected showing extrusions and fragmentation of the cuticular plate. Defects were more pronounced in the homozygotes, which showed profound atrophy of the organ of Corti and fusion of stereocilia in all regions of the cochlea duct. At the base of inner and outer hair cells, afferent and efferent synapses were reduced in number or absent. In both $+/Va^j$ and Va^j/Va^j the surrounding supporting cells, Reissner's and tectorial membranes were normal.

The earliest signs of abnormality of the stereocilia hair bundle were observed in Va^j homozygotes at embryonic day 17.5 (E17.5) [16]. At this stage the growing microvilli, at the apical surface of hair cells, from which the mature stereocilia develop, were arranged in an irregular fashion. At P5, hair cell stereocilia had grown to normal height but were disorganized and appeared as clumps atop some hair cells (**Fig. 3**). At P11 disorganization progressed further resulting in fusion, clumping or absence of stereocilia along the length of the organ of Corti.

Mutations in *Trpm13* result in varitint-waddler

A positional cloning approach identified *Trpm13* (*Mcoln3*) as the gene mutated in varitint-waddler [16]. *Trpm13* has an open reading frame of 1,662 base pairs consisting of 12 exons, which encode a 553 amino acid protein, predicted to be 64 kDa in size.

The TRPML3 amino acid sequence is 74% and 77% similar to TRPML1 and TRPML2, respectively, and is 63% similar to *Drosophila* CG8743 and 55% similar to *C. elegans* CUP-5. In addition, the putative channel pore region of TRPML3 is weakly similar to the TRPP2 cation channel (also known as PKD2), which is implicated in autosomal dominant polycystic kidney disease (**Table 1**), suggesting a conserved molecular function. Like all TRP channels TRPML3 is predicted to have six trans membrane domains (6TM), cytoplasmic amino and carboxy termini, an ion transport motif (PF00520), and a transient receptor potential like motif (TRPL, PS50272). The *Va* allele results from a 1225G>C transversion leading to an Ala419Pro substitution in the fifth trans membrane domain, which is a critical component of the pore region of the channel. Although no physiological data exist, it is likely that the Ala419Pro substitution is either a gain-of-function mutation, which severely alters the physiological properties of the channel or a dominant-negative allele, which renders a channel complex dysfunctional. The *Va'* mutation results from a 1085T>C transition that leads to an Ile362Thr substitution in the second extracellular loop. The *Va'* allele arose in a colony segregating the *Va* allele and given that the 1225G>C mutation was also detected in *Va'*, it implies that *Va'* arose in *cis* to *Va*. Owing to the milder phenotypes exhibited by *Va'*, the Ile362Thr substitution may act as an intragenic suppressor of the Ala419Pro allele.

Molecular function of TRPML3

To date there is only limited information about the molecular function of TRPML3. A recent study by Ventakachalam and colleagues showed that TRPML3 forms homo- and heterodimers with both TRPML1 and TRPML2 proteins when transiently co-expressed in HEK293 cells [17]. Under such conditions, TRPML3 homodimers localized predominantly to the endoplasmic reticulum, but formed heterodimers when co-expressed with either TRPML1 or TRPML2 and localized to lysosomes.

The function of TRPML3 in the cochlea has not yet been elucidated. The mutant phenotypes and the immuno-localization studies, however, provide interesting clues about its function. The motif structure of TRPML3 suggests a cell-autonomous

function, implying that TRPML3 acts in strial intermediate cells and inner ear hair cells. In cochlear hair cells, the onset and site of the defects (stereocilia disorganization and fusion) as well as the localization to the plasma membrane of stereocilia [16] are consistent with the view that TRPML3 may function during early embryogenesis. During the elongation of the stereocilia hair bundle, molecular links are established between individual stereocilia, which are modified and become restricted to the top region as the bundle grows and differentiates [18]. TRPML3 may play a role in regulating ion homeostasis along the stereocilia and mutant TRPML3 may thus lead to the selective loss of stereocilia links; hence the disorganized and splayed appearance of stereocilia in *varitint-waddler* mutants [19].

Mutations in another member of the TRPML family, TRPML1, cause the lysosomal storage disorder mucopolipidosis type IV, characterized by the accumulation of large vacuoles in the cytosol. TRPML1 localizes to late endosomes and lysosomes and loss of TRPML1 activity is thought to disrupt biogenesis of lysosomes during the final stages of endocytosis [8]. On the molecular level, TRPML1 has been shown to be permeable to H^+ , leaking H^+ into the cytosol and thereby regulate the pH in lysosomes [20]. Immunostaining with TRPML3 antibodies of cochlea hair cells showed strong labeling of vesicles in the entire cytoplasm including the pericuticular necklace. Areas around the cuticular plate at the apical pole and at sites of synaptic transmission at the basal side are locations of intense exocytotic and endocytotic activities [21]. Thus, given the defects present at the apical and basal sides of hair cells in *Va^l* mutants TRPML3 may play a function during endocytosis or exocytosis.

Refined localization and identification of TRPML3-positive vesicles, as well as measurement of the electrophysiological properties of TRPML3 and the physiology of hair cells in *varitint-waddler* mutant will further elucidate the role of this channel in hearing in the mammalian cochlea.

Acknowledgements

We thank Mark Schneider, Nelson Lopez and Tony Ricci for their insightful comments on the manuscript. MAD and KNT are supported by the intramural program at NIDCD.

References

- [1] A.J. Hudspeth, Sensory transduction in the ear, Principles of Neural Science, 4 ed., McGraw-Hill 2000.
- [2] G.I. Frolenkov, I.A. Belyantseva, T.B. Friedman and A.J. Griffith, Genetic insights into the morphogenesis of inner ear hair cells, *Nat Rev Genet* 5 (2004) 489-98.
- [3] T. Nicolson, The genetics of hearing and balance in zebrafish, *Annu Rev Genet* 39 (2005) 9-22.
- [4] K.P. Steel and C.J. Kros, A genetic approach to understanding auditory function, *Nat Genet* 27 (2001) 143-149.
- [5] P. Wangemann, K⁺ cycling and the endocochlear potential, *Hear Res* 165 (2002) 1-9.
- [6] G. Bach, Mucopolysaccharidosis type IV, *Mol Genet Metab* 73 (2001) 197-203.
- [7] R. Bargal, N. Avidan, E. Ben-Asher, Z. Olender, M. Zeigler, A. Frumkin, A. Raas-Rothschild, G. Glusman, D. Lancet and G. Bach, Identification of the gene causing mucopolysaccharidosis type IV, *Nat Genet* 26 (2000) 118-23.
- [8] G. Bach, Mucopolysaccharidosis type IV: endocytosis and cation channel--a review, *Pflugers Arch* 451 (2005) 313-7.
- [9] M. Chenik, F. Douagi, Y.B. Achour, N.B. Khalef, M. Ouakad, H. Louzir and K. Dellagi, Characterization of two different mucopolysaccharidosis-like genes from *Leishmania major*, *Parasitol Res* 98 (2005) 5-13.
- [10] H. Fares and I. Greenwald, Regulation of endocytosis by CUP-5, the *Caenorhabditis elegans* mucopolysaccharidosis-1 homolog, *Nat Genet* 28 (2001) 64-8.
- [11] F. Qian and K. Noben-Trauth, Cellular and molecular function of mucopolysaccharidosis (TRPML) and polycystin 2 (TRPP2), *Pflugers Arch* 451 (2005) 277-85.
- [12] A.M. Cloudman and L.E. Bunker, The varitint-waddler mouse., *J. Hered.* 36 (1945) 258-263.
- [13] H.J. Kim, T. Jackson and K. Noben-Trauth, Genetic Analyses of the Mouse Deafness Mutations Varitint-Waddler (Va) and Jerker (Espnje), *J Assoc Res Otolaryngol* (2002).
- [14] M.S. Deol, The anomalies of the labyrinth of the mutants varitint-waddler, shaker-2 and jerker in the mouse., *J. Genet.* 52 (1954) 562-588.
- [15] J. Cable and K.P. Steel, Combined cochleo-saccular and neuroepithelial abnormalities in the Varitint-waddler-J (Va^J) mouse, *Hear Res* 123 (1998) 125-36.

- [16] F. Di Palma, I.A. Belyantseva, H.J. Kim, T.F. Vogt, B. Kachar and K. Noben-Trauth, Mutations in *Mcoln3* associated with deafness and pigmentation defects in varitint-waddler (*Va*) mice, *Proc Natl Acad Sci U S A* 99 (2002) 14994-14999.
- [17] K. Venkatachalam, T. Hofmann and C. Montell, Lysosomal localization of TRPML3 depends on TRPML2 and the mucopolidosis-associated protein TRPML1, *J Biol Chem* 281 (2006) 17517-27.
- [18] R.J. Goodyear, W. Marcotti, C.J. Kros and G.P. Richardson, Development and properties of stereociliary link types in hair cells of the mouse cochlea, *J Comp Neurol* 485 (2005) 75-85.
- [19] K.P. Steel, Varitint-waddler: a double whammy for hearing, *Proc Natl Acad Sci U S A* 99 (2002) 14613-5.
- [20] A.A. Soyombo, S. Tjon-Kon-Sang, Y. Rbaibi, E. Bashllari, J. Bisceglia, S. Muallem and K. Kiselyov, TRP-ML1 regulates lysosomal pH and acidic lysosomal lipid hydrolytic activity, *J Biol Chem* 281 (2006) 7294-301.
- [21] B. Kachar, A. Battaglia and J. Fex, Compartmentalized vesicular traffic around the hair cell cuticular plate, *Hear Res* 107 (1997) 102-12.

Fig. 1 Mouse cochlea duct

A photomicrograph of a cross-section through the mouse cochlea duct at the middle turn is shown. The tectorial (tm) and basilar membranes (bm), stria vascularis (sv), organ of Corti (oc), and scala tympani (st) are indicated.

Fig. 2 The varitint-waddler mouse

A representative Va^j/Va^j homozygous varitint-waddler mouse is shown. Note the pigmentation defects seen as white and dilute patches of fur on an agouti background.

Fig. 3 Stereocilia defects in varitint-waddler mouse

Organ of Corti surface preparations from 5-day old neonates stained with phalloidin to visualize the stereocilia hair bundle are shown. Note the disorganized hair bundle in the homozygous mutants (Va^j/Va^j) compared to the normal bundle shape of $+/+$ littermates. Reproduced from Di Palma et al. 2002 [16].

Fig. 4 Schematic diagram of TRPML3

A schematic diagram of the predicted secondary structure with six trans membrane domains, amino and carboxy termini, and the location of the I362T and A419P amino acid substitutions is shown.



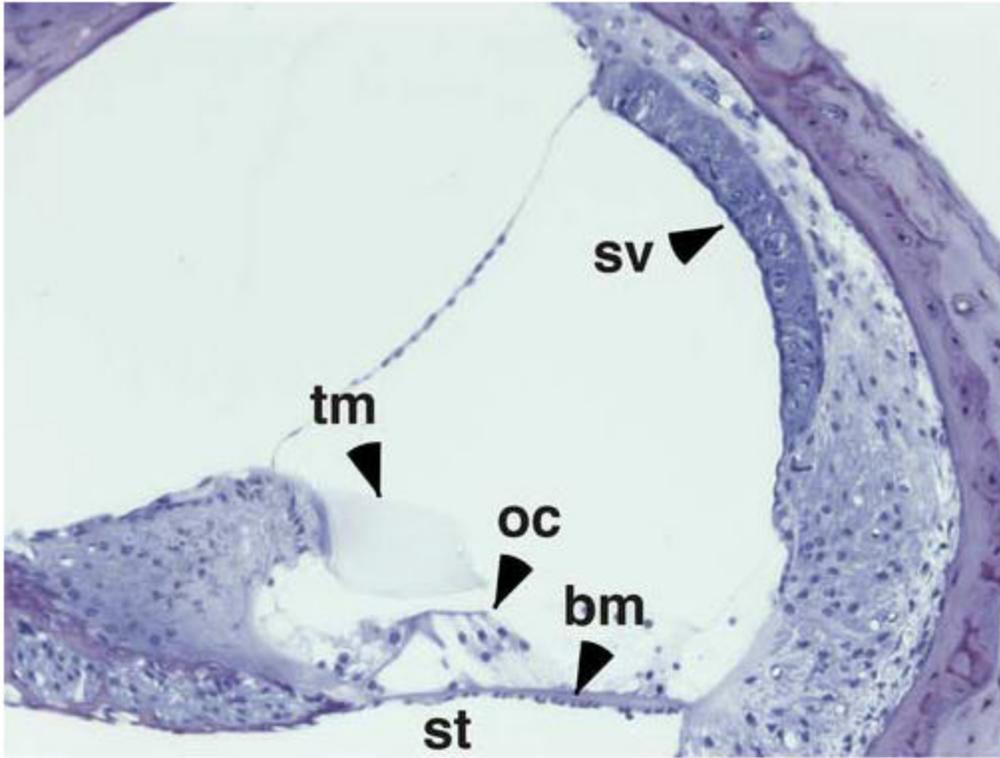
Table 1 - Similarity/Identity matrix of TRPML proteins

	TRPML1	TRPML2	TRPML3	Trpml1	Trpml2	Trpml3	Cup-5	CG8743	TRPP2
TRPML1		50	58	91	48	58	38	38	23
TRPML2	70		59	47	83	58	32	38	24
TRPML3	75	77		57	57	91	35	41	23
Trpml1	94	68	74		48	58	33	38	22
Trpml2	67	91	76	67		59	33	39	23
Trpml3	74	76	96	74	78		34	40	22
cup-5	55	53	57	55	52	57		37	22
CG8743	59	62	61	58	60	61	59		22
TRPP2	44	50	46	44	48	47	43	48	

Percentage of sequence similarity (blue) and identity (yellow) among human (capital), mouse (small), *C. elegans* (Cup-5), *Drosophila* (CG8743) TRPML proteins and mouse TRPP2.

ACCEPTED M.

DT

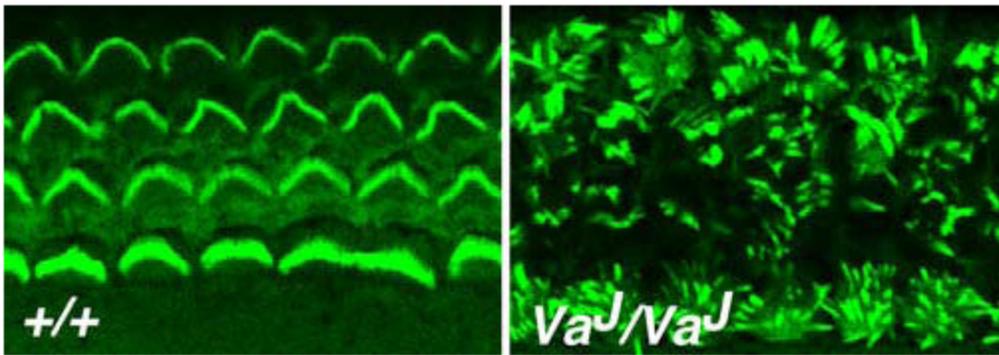


AC

'SCRIPT



CRIPT



ACCEPTED

SCRIPT

