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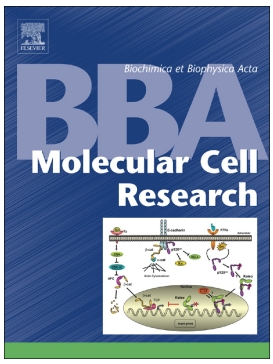

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PII: S0167-4889(24)00052-1

DOI: https://doi.org/10.1016/j.bbamcr.2024.119709

Reference: BBAMCR 119709

To appear in: *BBA - Molecular Cell Research*

Received date: 5 February 2024

Revised date: 15 March 2024

Accepted date: 18 March 2024

Please cite this article as: R. Roelens, A.N.F. Peigneur, T. Voets, et al., Neurodevelopmental disorders caused by variants in TRPM3, *BBA - Molecular Cell Research* (2024), https://doi.org/10.1016/j.bbamcr.2024.119709

Neurodevelopmental disorders caused by variants in TRPM3

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Keywords: TRP channel, TRPM3 variants, neurodevelopmental disorders, DEE

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ABSTRACT

Developmental and epileptic encephalopathies (DEE) are a broad and varied group of disorders that affect the brain and are characterized by epilepsy and comorbid intellectual disability (ID). These conditions have a broad spectrum of symptoms and can be caused by various underlying factors, including genetic mutations, infections, and other medical conditions. The exact cause of DEE remains largely unknown in the majority of cases. However, in around 25% of patients, rare nonsynonymous coding variants in genes encoding ion channels, cell-surface receptors, and other neuronally expressed proteins are identified. This review focuses on a subgroup of DEE patients carrying variations in the gene encoding the Transient Receptor Potential Melastatin 3 (*TRPM3*) ion channel, where recent data indicate that gain-of-function of TRPM3 channel activity underlies a spectrum of dominant neurodevelopmental disorders.

Ion channels in DEE

Developmental and epileptic encephalopathies (DEE) encompass a diverse range of neurodevelopmental conditions marked by seizures in early life, significant epileptic activity, and atypical neurocognitive development (Syrbe, 2022). They are recognized by the International League Against Epilepsy (ILAE) as an independent group from epileptic encephalopathies, diverging in the independent development of both the epileptic and the neurodevelopmental comorbidity (Scheffer et al., 2017). The etiology of the disease is often unknown but genetic testing yields in nearly 50% of the cases a diagnosis (Fernández et al., 2019). Furthermore, in one in four individuals, the molecular origin of DEE are *de novo* missense point mutations in genes encoding ion channels, cell-surface receptors, or other neuronally expressed proteins (Hamdan et al., 2017).

There are several types of DEE with regards to the patient phenotype and the genetic roots of the disease. Focusing on ion channels associated with DEE, the type of DEE developed by the patient can be correlated with the missense mutation of the affected ion channel (either gain- or loss-of-function). For example, Dravet syndrome has an almost monogenic association with *SCN1A* (Catterall, 2018; Sadleir et al., 2017). *SCN1A* encodes for the neuronal voltage-gated sodium channel alpha-subunit 1 ($Na_v1.1$) (Specchio & Curatolo, 2021). It is suspected that a heterozygous loss of function mutation is the main cause of neuronal pathology (Catterall, 2018; Sun et al., 2016). The following haploinsufficiency then causes a decrease in inhibitory postsynaptic currents (IPSCs) in GABAergic interneurons, driving the low epileptic threshold, as seen in an *in vivo* mouse model and in an *in vitro* model of patient-derived iPSCs (Han et al., 2012; Sun et al., 2016; Tai et al., 2014). However, DEE patients harboring a gain-of-function variant of SCN1A causing altered channel gating, resulting in hyperexcitability-evoked seizures, have also been identified (Brunklaus et al., 2022; Spampanato et al., 2001). The mutants cluster in a different region of the protein compared to the Dravet-associated variants (Brunklaus et al., 2022). This diverging underlying mechanism emphasizes the importance of personalized medicine, as sodium channel blockers only exacerbate the symptoms of Dravet syndrome (Sisodiya, 2021), while it can provide a therapeutic opportunity in gain-of-function SCN1A variants.

Other DEE types, such as West syndrome, Ohtahara syndrome, and Lennox-Gastaut syndrome, have a more complex genotype-phenotype correlation, with multiple different ion channels causing similar phenotypes (Talwar & Hammer, 2021; Wolff et al., 2019). Reviews by Scheffer (2020) and Syrbe (2022) give a comprehensive overview of recurrent examples of monogenic DEE (mutations in a single gene) (Scheffer & Liao, 2020; Syrbe, 2022). Both reviews reiterate the emphasis on the need for personalized precision therapy, which in turn calls for a thorough understanding of the genetic and cellular causes of the pathology.

Currently, many genetically unresolved DEE cases remain, and modeling predicts that there are around 1,000 more genes associated with developmental disorders yet to be identified (Scheffer & Liao, 2020). Dyment et al. (2020) were the first to identify a novel subgroup of patients with DEE who carry *de novo* missense mutations in the *TRPM3* gene, which codes for a Ca^{2+} -permeable cation channel. Several subsequent reports followed (de Sainte Agathe et al., 2020; Dyment et al., 2019; Gauthier et al., 2021; Kang et al., 2021; Lines et al., 2022), confirming the direct genetic link between TRPM3 and neurodevelopmental disease. In this review, we provide an overview of TRPM3 biology and of known TRPM3 variants causing DEE. Additionally, we discuss possible mechanisms whereby TRPM3 gain-of-function variants could cause neurological disease.

Introduction to TRPM3

Gene and structure

TRPM3 is a member of the superfamily of TRP channels, related to the gene product of the *Drosophila transient receptor potential* (*trp*) gene. The superfamily of TRP channels encompasses 28 members in mammals, which can be classified into 6 subfamilies based on sequence homology. TRPM3 distinguishes itself from other TRP channels in that it undergoes extensive alternative splicing (Oberwinkler & Philipp, 2014), resulting in a large number of isoforms. Importantly, different splice isoforms can have substantially different functional properties. For instance, alternative splicing in the pore region affects ion permeability and pharmacology (Held et al., 2022), and the only channel isoforms that include the optional exon 17 are subject to regulation by the $G_{\beta\gamma}$ subunit of trimeric G proteins. The vast number of possible isoforms poses difficulties for amino acid numbering, which has resulted inconsistent numbering of disease-associated variants in the literature. Burglen et al. (2023) proposed to use a

reference transcript of TRPM3, which comprises the longest functional splice variant that includes all exons that are frequently used in human brain tissue and covers all known human disease-associated TRPM3 variants (Burglen et al., 2023). In this manuscript, we use this variant as the basis for amino acid numbering.

Recently, structures of a mouse TRPM3 isoform (mTRPM3α2) have been obtained via cryo-EM, and these structures will be used as model in this review to indicate the position of disease-associated variants (C. Zhao & MacKinnon, 2023).

Functional properties and pharmacology of TRPM3

TRPM3 is a multimodal calcium-permeable cation channel, which can be activated by physical stimuli including heat and voltage, as well as by various chemical ligands (Grimm et al., 2003; Vangeel et al., 2020; Vriens et al., 2011). Exon 17 has been shown to interact with the $G_{\beta\gamma}$ subunit of trimeric G proteins, which functions as an inhibitory regulator of TRPM3 with a half inhibition constant (IC_{50}) of ca. 240 nM (Behrendt et al., 2020; Chen Zhao et al., 2023; Quallo et al., 2017).

TRPM3 activity can be induced by a number of ligands, with the neurosteroid pregnenolone sulfate (PS) as the most potent known endogenous agonist (EC_{50} = ~ 12-32 µM)) (Majeed et al., 2010; Oberwinkler & Philipp, 2014; Vriens et al., 2014a; Wagner et al., 2008a)). Furthermore, several synthetic compounds increase TRPM3 channel activity, most notably the small molecule CIM0216 (Held et al., 2015a).

Additionally, specific ligand combinations, such as co-application of PS or other steroids (DHEA-S, estradiol, progesterone, and testosterone) with the antifungal clotrimazole (Clt), induce an alternative ion permeation, which was identified by electrophysiological assays as an inwardly rectifying current component at negative voltages (Vriens et al., 2014a) (Persoons et al., 2021). The alternative ion permeation pathway differs from the canonical current through the main pore in its resistance to Ca^{2+} desensitization and La^{3+} block, low permeability to Ca^{2+} ions, and insensitivity to mutations in the pore-forming region (Held et al., 2018; Vriens et al., 2014a). Although the exact location of the non-canonical pore is not yet fully determined, mutations in transmembrane region 4 (TM4) specifically affect currents through the alternative permeation pathway (Held et al., 2016; Vriens et al., 2014a). Additionally, the synthetic TRPM3 agonist CIM0216 can induce the opening of both the canonical and non-

canonical pore, making it an ultrapotent TRPM3 agonist (Held et al., 2015a; Kahler et al., 2023).

Conversely, different TRPM3 antagonists have been described. The best-studied TRPM3 inhibitor so far is the anticonvulsant primidone, which exhibits a half-maximal inhibitory concentration (IC_{50}) of 0.60 ± 0.07 µM. Primidone gets metabolized to phenobarbital (IC_{50} = 4.4 µM) and phenylethylmalonamide (PEMA, no inhibitory effect on TRPM3 at 10 µM) (Becker et al., 2023). Screening of a plant-based compound library revealed that flavanones selectively inhibit TRPM3 channel activity, with isosakuranetin (IC_{50} = ~ 50 ± 6 nM) (Straub, Mohr, et al., 2013) as themost potent inhibitor of TRPM3 so far, leading to its broad use in TRPM3 *in vitro* and *in vivo* studies (Straub, Krügel, et al., 2013). Noteworthy, alternative splicing of the *TRPM3* gene influences its sensitivity towards pharmacological modulation. The utilization of different 5' splice sites within exon 24 results in channel isoforms featuring either a shorter or an extended pore loop, which resulted in differentiating cation selectivity, sensitivity to PS and inhibition by compounds such as isosakuranetin or primidone (Held et al., 2015b, 2022; Held & Tóth, 2021; Oberwinkler et al., 2005).

Expression and (patho)physiological role

TRPM3 has a specific expression pattern including cells in the brain, eye, kidney, sensory neurons of the peripheral system, pancreatic beta-cells, pituitary gland, and adipose tissue (Burglen et al., 2023; Grimm et al., 2003; Oberwinkler & Philipp, 2014). TRPM3 channel activity has been measured in pancreatic beta cells and in various neuronal cells of the peripheral and central nervous system (Thiel et al., 2013; Vriens & Voets, 2019; Zamudio-Bulcock et al., 2011a). In the context of this review, we will focus on the role of TRPM3 in the peripheral (PNS) and central nervous system (CNS) during development and at the adult stage. Molecular and functional expression of TRPM3 has been described in dorsal root ganglia (DRG) and trigeminal ganglia (TG) neurons of mouse, rat and human (Vangeel et al., 2020; Vriens et al., 2011). Similar expression levels were noted in human stem cell-derived sensory neurons, providing an *in vitro* model for TRPM3 regulation in a human cellular context (Vangeel et al., 2020).

The functionality of TRPM3 in the PNS was demonstrated by the robust responses in human and mouse sensory neurons upon PS stimulation, which can be inhibited by TRPM3 antagonists and are absent in TRPM3-deficient mice (Vangeel et al., 2020;

Vriens et al., 2011). In sensory neurons, TRPM3 acts as one of three nociceptors involved in the detection of acute noxious heat, together with TRPA1 and TRPV1, and triggers protective reflexes (Hill & Bautista, 2018; Vandewauw et al., 2018; Vriens et al., 2011). Additionally, TRPM3 has been shown to play a role in various pathological pain models. In mice, the channel is upregulated in sensory neurons innervating inflamed tissue, and TRPM3-deficient mice do not develop inflammatory heat hyperalgesia (Mulier et al., 2020; Vanneste et al., 2021; Vriens et al., 2011). The channel has also been shown to play a role in spontaneous pain after chronic nerve injury (Su et al., 2021), and in oxaliplatin-induced peripheral neuropathic pain (Aloi et al., 2023). Recent reports also identified TRPM3 as a potential candidate for the development of migraine pain (Krivoshein et al., 2022). In summary, seeing its role in different contexts of chronic pain, TRPM3 represents a novel potential target for new pain therapies.

Expression of TRPM3 in the CNS has been reported since the first functional descriptions of the ion channel (Grimm et al., 2003; Lee et al., 2003; Oberwinkler et al., 2005). Abundant mRNA expression in neuronal and non-neuronal cell types of both the adult and developing brain have been described, in particular highlighting distinct cellular clusters in the developing and adult cerebellum (Burglen et al., 2023; Held & Tóth, 2021). During development, cells of the rhombic lip were found to form the cluster with the highest expression level (Burglen et al., 2023). This germinal zone of the cerebellum gives rise to all glutamatergic neurons (Beckinghausen & Sillitoe, 2019a; Englund et al., 2006; Wingate, 2001). TRPM3 expression in further differentiated cells of the rhombic lip, such as excitatory cerebellar and unipolar brush cell interneurons and Purkinje cells, was also described (Aldinger et al., 2021; Burglen et al., 2023). Overall, these data point towards an potential role of TRPM3 during cerebellar development, especially in excitatory cerebellar neurons. However, functional annotations of TRPM3 in these tissues encompassing the CNS are still lacking. Exceptions are primary oligodendrocytes, glutamatergic synapses in neonatal cerebellar Purkinje cells of rats, and epithelial cells of the choroid plexus, where channel-specific pharmacological modulators were used to showcase TRPM3-activity (Hoffmann et al., 2010; Millar et al., 2007; Zamudio-Bulcock et al., 2011a).

TRPM3 in DEE

Clinical disease manifestation in patients

Recently, attention has been drawn to the involvement of TRPM3 in developmental and epileptic encephalopathies, where patients exhibit various symptoms, including severe epileptic seizures and developmental delays. Research into the specific mutations of *TRPM3* associated with DEE has begun to shed light on the gene's significance in this group of disorders, providing a new avenue for understanding the molecular underpinnings of these complex conditions. The diverse clinical manifestations linked with TRPM3 underscore its multifaceted role within the central nervous system and its importance in human health.

The initial discovery of a connection between TRPM3 and DEE emerged in 2019, in a study reporting two *de novo* variants of *TRPM3* in eight individuals diagnosed with DEE. These patients predominantly exhibited moderate to severe developmental delays or intellectual disabilities, along with hypotonia or abnormalities in muscle tone. Notably, seven of these patients were confirmed to have epilepsy. Four displayed autism-like behaviors, one demonstrated a notable reduction in heat sensitivity, and another showed an increased pain threshold (Dyment et al., 2019).

Subsequent to this publication, additional studies have corroborated the association between TRPM3 variants with DEE. These reports involve patients carrying *de novo* mutations in *TRPM3* (Burglen et al., 2023; de Sainte Agathe et al., 2020; Gauthier et al., 2021; Kang et al., 2021; Lines et al., 2022; Sundaramurthi et al., 2024), with one rare exception where the condition was inherited from father to son (Burglen et al., 2023). Commonly, symptoms are observed before the child's first birthday, the most frequent symptoms being intellectual disability (seen in 95% of patients), hypotonia (79%), delayed walking (62%), and seizures (55%). Decreased pain sensitivity and reduced heat sensitivity has been mentioned in some of the patients (Burglen et al., 2023; Dyment et al., 2019; Sundaramurthi et al., 2024). Conversely, one particular case showed both higher pain sensitivity and heat intolerance (de Sainte Agathe et al., 2020). Note that several patients are dealing with a limited vocabulary, which makes it difficult to objectively address possible alterations in pain sensitivity or heat detection. Finally, skeletal anomalies are a recurrent feature in patients with TRPM3-related DEE (Burglen et al., 2023).

TRPM3 RNA levels are low in skeletal muscle (Burglen et al., 2023), which suggests that the frequent hypotonia stem from impaired neuronal control of muscle function, possibly linked to cerebellar abnormalities. In fact, a specific cerebellar phenotype characterized by ataxia, severe hypotonia, nystagmus or abnormal eye movements, and cerebellar atrophy has been proposed as a distinctive feature of TRPM3-linked DEE (Burglen et al., 2023). In a recent study, the *de novo* missense variant of TRPM3, N1126D, was found in a patient diagnosed with cerebral palsy (CP), a group of disorders characterized by impaired movement and posture (Sundaramurthi et al., 2024). CP not only involves motor disturbances but also encompasses a broad spectrum of additional complications, including challenges in sensory perception, cognitive function, communication, behavior, and a predisposition to epilepsy and musculoskeletal issues (Rosenbaum et al., 2007). Note that the same N1126D variant was also identified in a patient presenting with hypotonia, autism and severe intellectual disability by Burglen et al. (2023). The notion that TRPM3 gain of function can be a cause of CP adds another layer to our understanding of the diverse impacts of TRPM3 mutations in neurological disorders.

Functional effects of DEE-associated TRPM3 variants on channel function

Since the discovery of the *de novo* heterozygous missense variants of TRPM3 in patients, important progress has been made in uncovering the molecular etiology of the disease. Following the identification of the first DEE patients carrying *de novo* variants V1002M and P1102Q (Dyment et al., 2019), two reports presented the functional consequences of these variants on channel activity following heterologous expression in HEK293 cells and *Xenopus* oocytes (Van Hoeymissen et al., 2020; S. Zhao et al., 2020). Consistently, both studies revealed that these variants lead to a robust gain of channel function, including increased basal activity and enhanced responses to stimuli such as PS or heat. Importantly, gain of function was also observed in cells co-expressing equal amounts of wild type and variant TRPM3, mimicking the heterozygous condition in the patients.

Following these first studies, a growing number of additional variants are being reported in patients diagnosed with DEE/neurodevelopmental delay (Figure 1A, Figure 2 and Table 1). Interestingly, the V1002M variant is by far the most recurrent, with over 50% of identified patients harboring this missense mutation. At this point, functional characterization has been carried out for 9 disease-associated variants, revealing two

consistent features when co-expressed with the wild type TRPM3 subunit. First, co-expression of disease-associated variants causes an increased basal channel activity, leading to significantly elevated basal calcium levels. Second, there is an enhanced sensitivity toward PS-stimulation (Burglen et al., 2023). In addition, some but not all variants display additional alterations in other aspects of TRPM3 gating and pharmacology, such as increased sensitivity to heat (P1102Q), large inwardly rectifying currents in response to PS (V1002M; L769V), or reduced sensitivity to inhibition by primidone on μ-opioid receptor activation (V1002M) (Becker et al., 2023; Burglen et al., 2023; Van Hoeymissen et al., 2020). Overall, these alterations allow classification of the disease-associated variants as dominant gain-of-function.

The molecular mechanisms whereby known disease-associated TRPM3 variants increase channel activity are currently largely unknown, but some hypotheses can be built based on their localization within the channel structure. The D614V variant is located in the N-terminus on the outside of the cytosolic domain, just adjacent to exon 17, which forms the part of the channel that directly interacts with $G_{\beta\gamma}$ subunits (Badheka et al., 2017; Behrendt et al., 2020; Dembla et al., 2017; Quallo et al., 2017). This raises the possibility that channels containing the D614V variant may be differentially affected by inhibitory GPCR signaling, which may contribute to increased channel activity.

Disease-associated variants affecting V1002 (V1002M, V1002G, and V1002L) and G1007S map to the intracellular end of TM4 and the TM4-TM5 linker, a crucial domain in most members of the voltage-gated cation channel superfamily, involved in linking voltage sensor movements or ligand binding to opening of the central pore. Notably, the TRPM3 cryo-EM structures (Chen Zhao et al., 2023) indicate that several other disease-associated variants in the cytosolic N-terminal (L769V) and proximal C-terminal (N1126D and S1133P) domains are located in close apposition to V1002, G1007 and the TM4-TM5 linker (Burglen et al., 2023), making this key gating-domain a hotspot for disease-variants. In the case of TRPM3, this region has also been shown to be a crucial determinant for opening of the alternative ion permeation pathway pore (Held et al., 2018; Vriens et al., 2014b). In this respect, it is interesting to note that channels containing the V1002M or L769V variants show enhanced activation of inward currents in response to PS, indicative of opening of the alternative ion permeation pathway (Van Hoeymissen et al., 2020; Burglen et al., 2023).

The P1102Q variant maps to the pore-forming domain at the extracellular side of the TM6 helix (Burglen et al., 2023; Chen Zhao et al., 2023), but it is currently unclear how this variant causes a gain of channel function. In the available cryo-EM structures, P1102 is surrounded by flat lipid densities, possibly cholesterol. Since cholesterol and many cholesterol-derived steroids modulate TRPM3 gating (Naylor et al., 2010; Wagner et al., 2008b; Persoons et al., 2021), is can be speculated that mutation of the proline will affect the their binding to the channel. In this respect, it is interesting to note that P1102Q leads to enhanced responses to PS, DHEA-sulfate and estradiol, whereas responses to progesterone and testosterone are suppressed (Persoons et al., 2021). Further research, including a better delineation of the binding site for PS and related TRPM3 agonists, is required to better understand the consequence of disease-associated variants on channel activity.

How does gain of TRPM3 channel function lead to brain disease?

From the above, it is apparent that disease-associated variants generally lead to a gain-of-channel function, which is expected to lead to profound alterations in excitability and/or calcium signaling in cells expressing TRPM3. The broad expression of TRPM3 in various cell types in the brain and periphery, and the large spectrum of neurological, neurodevelopmental and non-neurological symptoms observed in the patients, indicate that the etiology of TRPM3-associated disease is complex and multifactorial. We are currently only scratching the surface of how the different mutations lead to recurrent disease manifestations such as intellectual disability, epilepsy, hypotonia, altered pain sensitivity or skeletal malformations. Based on our current, limited insights, the following contributing mechanisms can be envisaged.

Epileptic seizures typically arise from an imbalance between excitatory and inhibitory neuronal activity. In this respect, TRPM3 is highly expressed in various excitatory neurons in the developing and adult human brain, including glutamatergic neurons in the cerebral cortex and hippocampus (Hoffmann et al., 2010; Zamudio-Bulcock et al., 2011b), where increased glutamate due to exaggerated TRPM3 channel function may contribute to seizure induction.

Additionally, TRPM3 is highly expressed in ependymal cells of the choroid plexus (Aldinger et al., 2021; Burglen et al., 2023; Haoui et al., 2021), which regulate the production and ccomposition of the cerebrospinal fluid (CSF). While the role of TRPM3 in choroid plexus physiology is currently unknown, it is likely that increased calcium

levels due to TRPM3 gain-of-function can affect processes that regulate CSF composition or toxin clearance, which can in turn affect neurodevelopment and neurodegenerative processes (Hutton et al., 2022).

High levels of TRPM3 are also found in different cell types in the developing and adult cerebellum (Aldinger et al., 2021; Englund et al., 2006; Wingate, 2001). Hyperexcitability and cellular calcium overload-induced cell death caused by gain-of-function in TRPM3 activity in the cerebellum may contribute to the hypotonia and progressive cerebellar neurodegeneration seen in patients (Burglen et al., 2023).

The altered pain sensitivity reported in a subset of patients may be directly related to the well-described role of TRPM3 in somatosensory neurons, where increased TRPM3 activity may not only lead to enhance responses to thermal stimuli, but possibly also to prolonged neuronal desensitization and even cell death. However, it cannot be excluded that altered pain processing in the CNS contributes to abnormal pain sensitivity.

Clearly, further research, possibly involving animal models, iPSC-derived stem cells from patients, and more detailed characterization of the growing cohort of patients is likely to give more detailed insights into the neurodevelopmental and pathological processes that occur in patients.

Conclusions and future perspectives

TRPM3-associated neurodevelopmental disease has recently emerged as a new disease entity caused by gain-of-function of the cation channel TRPM3. Patients present with a spectrum of neurological symptoms, indicating that multiple cell types and brain regions can be affected.

Whereas the precise physiological roles of TRPM3 in the brain are not well understood, TRPM3-deficient mice show normal brain development and do not have any obvious brain pathology. This suggests that pharmacological inhibition of TRPM3 function may be safe, and could be explored as a potential therapy for patients. Currently, In this respect, primidone, an FDA-approved medication for the treatment of seizures and essential tremor, offers interesting opportunities. Indeed, primidone acts as an inhibitor of TRPM3 at concentrations that are reached in the plasma and CSF of patients taking the drug for antiepileptic or anti-tremor therapy (Krügel et al., 2017). Currently, pharmacological management of TRPM3-related DEE patients diagnosed with

seizures may involve the use of non-specific anti-epileptics such as levetiracetam, clobazam, diazepam, and valproic acid (Dyment et al., 2019; Lines et al., 2022) This notion motivated the use of primidone as a potential treatment for patients with TRPM3-related neurodevelopmental disease, and a first retrospective study showed promising results in two patients with treatment-resistant DEE with continuous spike-and-wave activation in sleep (DEE-SWAS) (Becker et al., 2023). Developmental deterioration was halted, psychomotor abilities improved, and SWAS was no longer seen on EEG-recordings (Becker et al., 2023). Although no final conclusions can be drawn from this first report due to the small cohort, it provides some hopeful prospects for patients with a gain-of-function mutation in TRPM3.

However, it is important to recognize that a primidone is not selective for TRPM3, has significant side-effects, and that one of its main metabolites, phenobarbital, activates $GABA_A$ receptors, which may contribute to the antiseizure effects observed in the patients (Becker et al., 2023) Moreover, the long-term effects of primidone treatment on disease progression are currently fully unknown. More selective TRPM3 antagonists, along with the establishment of animal models carrying disease-associated mutations in TRPM3, would represent invaluable tools to better understand disease progression, and may eventually lead to the development of effective and safe treatments for patients. Further insights into the function of TRPM3 in the brain may also reveal whether non-genetic increases in channel activity, for instance due to increased levels of agonist or high temperature, contributes to acquired brain pathologies such as sporadic epilepsy or neurodegeneration.

Moreover, recent studies have suggested that TRPM3 may also have implications for mental health, particularly in mood and anxiety disorders (Thippeswamy & Davies, 2021). Along with many other processes influenced by TRPM3 activity, these roles highlight broad implications of TRPM3 in various physiological processes ranging from metabolic regulation and sensory perception to neural communication and mental health. Understanding the full spectrum of the biological roles of TRPM3 not only provides insight into fundamental physiological processes but also offers potential avenues for therapeutic interventions in a variety of health conditions.

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Acknowledgements

The authors thank all members of the Laboratory of Endometrium, Endometriosis and reproductive Medicine (LEERM) and all members of the Laboratory of Ion Channel Research (LICR), Leuven for their scientific discussions and contributions.

Funding

We thank the Research Foundation-Flanders FWO, (G.0D1417N, G.084515N, G.0A6719N) and the Research Council of the KU Leuven (C14/18/106) for funding the project of JV.

Figures and Tables

Figure 1

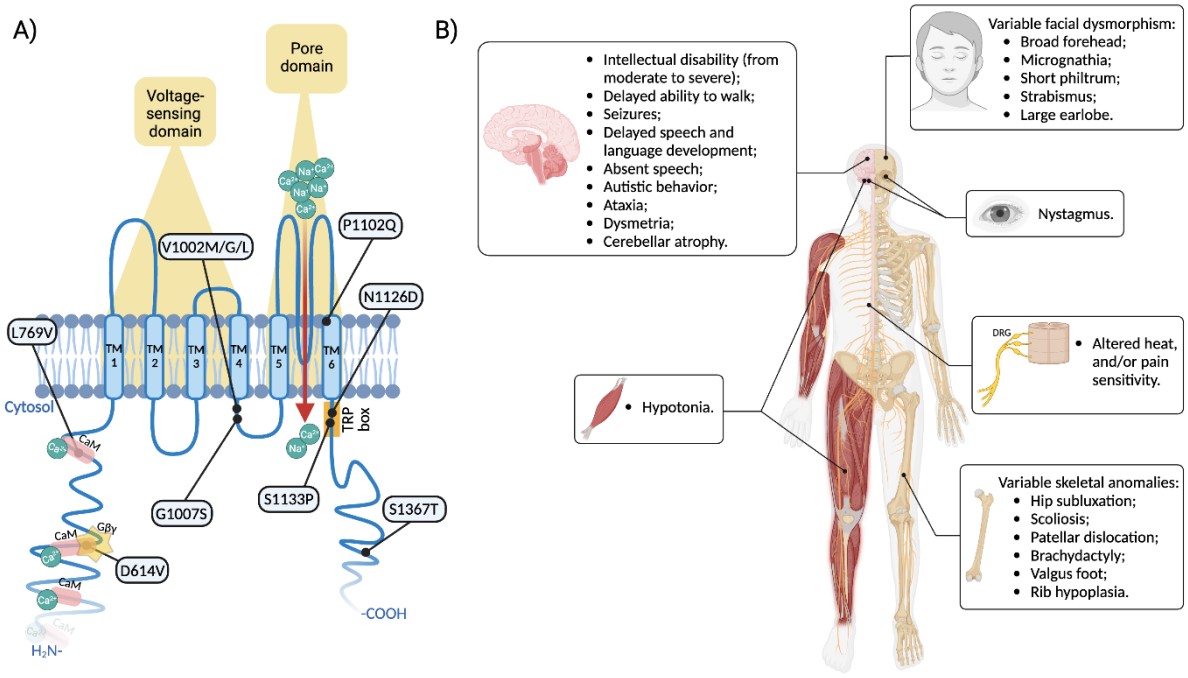


Figure 1: *De novo* Mutations in TRPM3 and Associated Clinical Features. (A) TRPM3 protein with the N-terminus (H_2N-) and C-terminus (-COOH). The blue cylinders represent the transmembrane (TM) domains of the TRPM3 protein, with the voltage-sensing domain situated from TM1 to TM4 and the pore-forming domain located between TM5 and 6. Position of Calmodulin (CaM) motifs and the binding site of the $G_{\beta\gamma}$-subunit are indicated. Specific mutations identified in patients are marked along the protein structure. (B) Phenotypic spectrum found in patients with *de novo* substitutions of *TRPM3* gene.

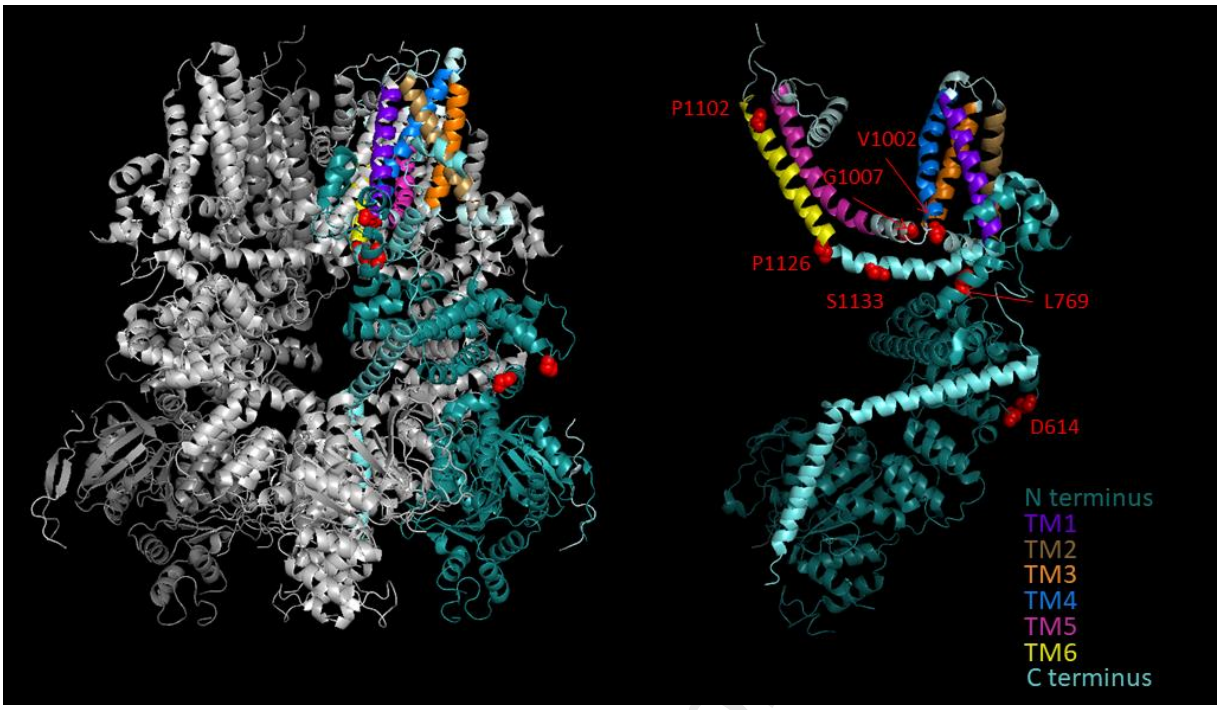
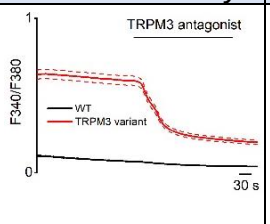
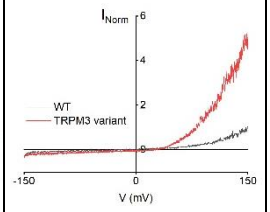
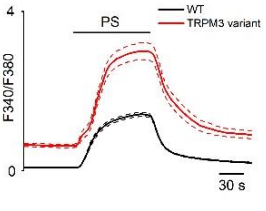
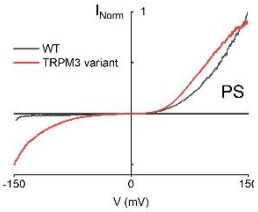


Figure 2

Cryo-EM structure of TRPM3, showing the tetrameric channel (left) and a single subunit (right). Different domains of the channel are shown in the indicated color-code, and disease-associated variants are indicated in red. Based on PDB: 8ED7

Table 1

	Basal activity		PS stimulation	
	Microfluorimetry	Patch clamp	Microfluorimetry	Patch clamp
TRPM3 Variant				
D614V	***	nt	**	nt
L769V	***	nt	*	nt
V1002M	***	*	***	***
V1002G	***	nt	*	nt
V1002L	***	nt	*	nt
G1007S	***	nt	*	nt
P1102Q	*	*	*	*
N1126D	*	nt	**	nt
S1133P	**	***	**	***

* ≤ 1.4 x WT *** ≤ 2.5 x WT

** ≤ 2 x WT Nt: not tested

Table 1. Overview of the characterized *TRPM3* variants resulting in gain-of-function variants. The basal activity and response towards PS stimulation was investigated using calcium microfluorimetric experiments and whole-cell patch-clamp experiments. Illustrative panels show Fura-2 microfluorimetric experiments in basal conditions and after stimulation by PS (40µM) for WT (black) and a TRPM3 variants (red). Fura-2-based calcium imaging in transfected HEK293T cells was used to evaluate basal channel activity. To measure the basal activity, application of a TRPM3 antagonist caused a reduction in ratio F340/F380 value, which was more pronounced in cells transfected with a TRPM3 variant (red) compared to WT (black). Whole cell patch clamp experiments further confirmed the increased basal activity as illustrated by current - voltage relationships of WT (black) and a TRPM3-variant (red) transfected cells at basal conditions. Values were normalized to WT current at +150 mV.

Responses to PS (40) stimulation were increased in TRPM3-variant expressing cells (red) compared to WT (black) in Fura-2 microfluorimetric experiments and whole cell patch clamp experiments.