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*Review*

# Transmembrane 163 (TMEM163) Protein: A New Member of the Zinc Efflux Transporter Family

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**Abstract:** A growing body of evidence continues to demonstrate the vital roles that zinc and its transporters play on human health. The solute carrier (SLC) 30 and 39 families, with ten and fourteen members, respectively, control zinc transport in cells. TMEM163, a recently characterized zinc transporter, has similar characteristics in both structure and function to the SLC30 family. This review examines recent data that reveal TMEM163 to be a zinc efflux transporter and a new member of the cation diffusion facilitator (CDF) family of mammalian zinc transporter (ZNT) proteins. It also discusses reports that implicate TMEM163 in various human diseases.

**Keywords:** zinc, cation diffusion facilitator, zinc transporter, SV31

## 1. Introduction

Zinc is a micronutrient involved in many fundamental roles that are vital for routine bodily functions. It is estimated that one in ten proteins have zinc-binding motifs (Harmaza & Slobozhanina, 2014). Additionally, every one of the main six classes of enzymes (hydrolases, isomerases, ligases, lyases, oxidoreductases, and transferases) contains zinc-dependent proteins (Harmaza & Slobozhanina, 2014; Wessels, Maywald, & Rink, 2017), totaling around 800 proteins (Harmaza & Slobozhanina, 2014). At the molecular level, zinc binds to proteins (Bonaventura, Benedetti, Albarède, & Miossec, 2015; Fukada, Yamasaki, Nishida, Murakami, & Hirano, 2011; Harmaza & Slobozhanina, 2014) in various capacities such as protein structural integrity, catalysis, and regulatory activity of DNA function (Harmaza & Slobozhanina, 2014; Vallee & Falchuk, 1993; Wessels et al., 2017). The widespread responsibilities and dependence on zinc by various proteins demonstrate the broad usefulness of this trace metal.

Mammals, especially in humans, also require zinc for proper immune responses. Cells functioning in innate and adaptive immune system both rely on proper zinc levels in order to develop and carry out their duties (Bonaventura et al., 2015; Wessels et al., 2017). Evidence also demonstrates that zinc can benefit those suffering from viral, bacterial, and even parasitic infections (Wessels et al., 2017). In addition to helping to destroy endocytosed pathogens, zinc can be used in ridding cells of reactive oxygen species (ROS) (Wessels et al., 2017). However, for some cell types, there is evidence suggesting excessive zinc concentrations can lead to cell death due to ROS formation (Harmaza & Slobozhanina, 2014) by the mitochondria (Sensi, Yin, Carriedo, Rao, & Weiss, 1999). The strict control of ROS production demonstrates another crucial, although indirect role for zinc, especially that ROS imbalance creates oxidative stress within the cells leading to disease (Gammoh & Rink, 2017; Radi, 2018).

Zinc dyshomeostasis is a known factor in many human health problems. In Diabetes mellitus (DM), there is evidence associating zinc imbalance occurring at the physiological and cellular levels (Chabosseau & Rutter, 2016; Gammoh & Rink, 2017). For example, insulin needs two zinc ions to

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stabilize its granules, but the role of zinc in DM are more complicated than a simple zinc imbalance or abnormal metabolism (Chasapis, Loutsidou, Spiliopoulou, & Stefanidou, 2012), some of which will be discussed later in this review. Zinc deficiency also has consequences on the immune system. One such example is that zinc deficiency negatively affects the function of immune cells as evidenced by differential gene expression levels in these cells versus control (Harmaza & Slobozhanina, 2014). Additionally, zinc deficiency has been shown to be factor in chronic diseases like cirrhosis (Harmaza & Slobozhanina, 2014) and asthma (Bonaventura et al., 2015). While cases of zinc deficiency in human population are often more mild to moderate in their severity, it is relatively widespread and affects one in four people (Bonaventura et al., 2015; Gammoh & Rink, 2017). With zinc deficiency afflicting many people worldwide and a wide variety of health issues stemming from with it, nutritional supplementation appears to be quite important. Nevertheless, zinc supplementation alone is not enough to solve the many issues in human health (Bonaventura et al., 2015) because zinc imbalance in the human body could also mean increased tissue or cellular zinc levels that result in cytotoxicity. Thus, it is necessary to further understand zinc homeostasis with respect to the living cell.

## 2. Zinc Transport

Further insight into human diseases linked with zinc imbalances can be obtained by studying how zinc is transported into and out of cells. The solute carrier (SLC)-30 and the SLC39 families comprise the two major groups of cellular zinc transporters (Baltaci & Yuce, 2018). As such, these families play vital roles in regulating zinc homeostasis. Due to their considerable control of zinc levels, it is not surprising that a large number of diseases can result from or are associated with members from these two families lacking proper function or having different expression levels (Baltaci & Yuce, 2018; Huang & Tepaamorndech, 2013; Jeong & Eide, 2013).

### *2.1. The SLC30 Family or ZNT Efflux Transporters*

The SLC30 family of proteins or ZNTs belong to the CDF superfamily (Hara et al., 2017; Huang & Tepaamorndech, 2013). These are zinc transporters found across a wide variety of species from bacteria to humans and are comprised of six transmembrane domains (TMDs) with intracellular amino and relatively longer carboxyl terminal regions (Huang & Tepaamorndech, 2013; Lichten & Cousins, 2009). There are currently ten ZNTs that have been discovered (Baltaci & Yuce, 2018; Hara et al., 2017; Huang & Tepaamorndech, 2013) with ZNT1-4 having been identified via experimentation with zinc-resistant cells or cloning, while ZNT5-10 were found via analysis of sequence homology with previously discovered ZNTs (Huang & Tepaamorndech, 2013). As a whole, the ZNTs function almost exclusively as zinc effluxers, and as such, they export zinc from the cytosol to either the outside of the cell, or into vesicles or organelles (Baltaci & Yuce, 2018; Hara et al., 2017; Huang & Tepaamorndech, 2013). It is interesting to note that a truncated isoform of ZNT5 (Valentine et al., 2007) has been reported to act as zinc influxer, while a variant of ZNT8 (Nicolson et al., 2009) implicated in DM appears to show a similar influx activity when heterologously expressed in cells. While such bidirectional activity may suggest novel function for these members of the ZNT family, this characteristic has been called into question (Weijers, 2010) and would need further validation. Thus, members of the SLC30 family should be recognized as mainly effluxers until more extensive evidence showing bidirectional function is observed otherwise.

ZNTs localize to the different areas in the cell (Figure 1). Some common subcellular localization of ZNTs include the plasma membrane, synaptic vesicles, and the Golgi apparatus (Baltaci & Yuce, 2018; Hara et al., 2017; Huang & Tepaamorndech, 2013; Cotrim, Jarrott, Martin, & Drew, 2019). A central feature of ZNTs is that they transport zinc as a dimer. Noteworthy is that certain ZNT subunits heterodimerize with each other to carry out their functions, although some ZNT subunits can only form functional dimers while others could form more than one heterodimer (Golan, Berman, & Assaraf, 2015; Huang & Tepaamorndech, 2013; Lasry et al., 2014). Strong evidence shows that ZNT1-4 (Lasry et al., 2014), ZNT7 (Lasry et al., 2014), and ZNT8 (Murgia et al., 2009) form homodimers, but that ZNT1-4 are able to form heterodimers with each other (Golan et al., 2015). ZNT5 may homodimerize as well as

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heterodimerize with ZNT6 (Fukunaka et al., 2009; Lasry et al., 2014). ZNT3 and ZNT10 are also known to form heterodimers with each other (Patrushev, Seidel-Rogol, & Salazar, 2012). As heterodimers, some ZNTs can even change from their normal subcellular localizations (Golan et al., 2015), demonstrating the nature of zinc transport being far more intricate than merely effluxing zinc from the cytosol out of the cells or into specific organelles or compartments. Research is lacking, however, into the exact nature of these subunit interactions and the express purposes of creating functionally redundant ZNT heterodimers in various cell types. Further investigation into ZNT dimerization, differential subcellular localizations, and the homeostatic consequences of these interactions should be priorities in future studies of the SLC30 family.

### *2.2. The SLC39 Family or ZIP Influx Transporters*

The SLC39 of proteins or ZIPs confer similarities to Zrt- and Irt-like proteins that import zinc in S. cerevisiae and iron in A. thaliana, respectively (Lichten & Cousins, 2009). They function as zinc influxers, and therefore work to bring zinc into the cytosol from vesicles and organelles or from the extracellular space (Jeong & Eide, 2013). In terms of their predicted structures, ZIPs have eight TMDs with extracellular amino and carboxyl termini (Jeong & Eide, 2013). There are currently fourteen known ZIPs (Baltaci & Yuce, 2018; Jeong & Eide, 2013; Lichten & Cousins, 2009). Similar to the ZNTs, ZIPs have a wide variety of subcellular localization (Figure 1). More than half of ZIPs, such as ZIP1-6, 8, 10, and 14, localize to the plasma membrane (Hara et al., 2017; Jeong & Eide, 2013), while the others localize to a variety of intracellular compartments such as lysosomes, the endoplasmic reticulum, and the Golgi apparatus (Baltaci & Yuce, 2018; Hara et al., 2017; Jeong & Eide, 2013). Together with the ZNTs, much of the strict control of cellular zinc concentration is maintained by these two SLC families.

### *2.3. ZNTs and ZIPs in human diseases*

Many diseases and health issues have been linked with the improper function or altered expression of ZNT and ZIP proteins (Figure 2). Indeed, various ZNTs have been implicated in neurodegenerative diseases and cancers. Both a loss of function (Lehvy et al., 2019) and an over-expression of ZNT1 (Singh et al., 2016), for example, are associated with cancer, but the former findings may be able to predict patient survivability (Lehvy et al., 2019). Mutations in ZNT2 cause zinc concentrations in breast milk to be deficient, which leads to zinc deficiency in children if their primary source of the micronutrient comes from breast milk (Kelleher et al., 2019). ZNT3 has lowered expression levels in the post-mortem brains of Alzheimer's disease (AD) patients (Baltaci & Yuce, 2018; Beyer et al., 2009) and in a mouse model of Mucolipidosis type IV (MLIV) disease (Chacon, Rosas, & Cuajungco, 2019). On the other hand, ZnT3 levels appear to be elevated in the cerebellum of a mouse model of AD (Zheng et al., 2010). Meanwhile, increased expression of ZNT6 (Huang & Tepaamorndech, 2013) and decreased expression of ZNT10 (Bosomworth, Adlard, Ford, & Valentine, 2013) have been associated with AD pathology in the hippocampus and frontal cortex, respectively. ZNT10 mutations are also association with Parkinson's Disease (PD) (Quadri et al., 2012). Meanwhile, ZNT5 and ZNT6 expression levels are downregulated, while ZNT9 and ZNT10 expression levels are increased in prostate cancer (Singh et al., 2016). A mutation in ZNT9 was recently found to be responsible for cerebro-renal syndrome disease [1]. ZNT8 has been widely reported to be involved in DM (Chimienti et al., 2006; Dwivedi et al., 2019; Nicolson et al., 2009; Yi, Huang, & Zhou, 2016); however, its role in Type II Diabetes (T2D), particularly for a loss-of-function ZNT8 variant (Dwivedi et al., 2019), conflicts with evidence supporting treatment of T2D by increasing ZNT8 function (Chimienti et al., 2006).

Abnormal expression of ZIPs is linked with certain cancer pathology (Baltaci & Yuce, 2018; Harmaza & Slobozhanina, 2014; Liu et al., 2020). For example, ZIP6, ZIP7, ZIP8, and ZIP10 are implicated in brain cancer. ZIP1, ZIP4 and ZIP6 are associated with prostate cancer implicates (Baltaci & Yuce, 2018; Harmaza & Slobozhanina, 2014). ZIP4 and ZIP6 expression are also correlated with pancreatic cancer (Harmaza & Slobozhanina, 2014). It is not clear why ZIP6 is associated not only with three of the cancers previously mentioned but that it is also linked to cervical (Baltaci & Yuce, 2018) and breast (Levenson & Somers, 2008) cancers. Further research is needed to understand the role that ZIP6 may or may not play in cancer. Meanwhile, there is also evidence that ZIP1 and ZIP3 may play a role

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in neurodegeneration (Harmaza & Slobozhanina, 2014). The increased expression of ZIP9 is connected to radiation-induced skin fibrosis (Qiu et al., 2020). Lastly, abnormal ZIP14 expression is implicated in asthma (Harmaza & Slobozhanina, 2014) and high insulin secretion (Baltaci & Yuce, 2018; Maxel et al., 2019).

The list of zinc transporters that appear to be correlated with various human diseases mentioned in the current review is not exhaustive. However, it demonstrates that zinc plays many roles in influencing normal and diseased states, and thus, further investigation of these zinc transporters is of significant value in order to devise or discover a form of therapeutics against many of debilitating diseases in humans.

## 3. TMEM163

### *3.1. Characteristics and Functions*

Transmembrane 163 protein (TMEM163), recently characterized as a zinc efflux protein (Sanchez, Ali, Escobar, & Cuajungco, 2019), is also known as synaptic vesicle 31 (SV31) (Burré, Zimmermann, & Volknandt, 2007). TMEM163 is a 31.5 kDa protein that binds divalent cations such as zinc, nickel, and copper, although the protein does not bind as strongly for the latter two metals (Barth, Zimmermann, & Volknandt, 2011). It has a predicted six TMDs with intracellular amino and carboxyl termini, and forms a functional homodimer (Waberer et al., 2017). TMEM163 has high relative transcript expression in the lungs, followed by the brain (cortex and cerebellum), and then the testis (Cuajungco et al., 2014). However, the reported transcript expression patterns of TMEM163 in human tissues appear to vary depending on the housekeeping gene used to quantify relative mRNA levels – our laboratory used 18S rRNA while another group used GAPDH (Chakraborty et al., 2020). Nevertheless, the tissue expression of TMEM163 is consistent with tissues that also express other ZNT proteins (Cuajungco & Kiselyov, 2017). It is worth noting that the relative mRNA tissue expression of mouse Tmem163 parallels that of its human counterpart (Sanchez et al., 2019), despite the initial claims that the gene is only exclusively expressed in the mouse brain (Barth et al., 2011; Burré et al., 2007). At the cellular level, TMEM163 localizes to the plasma membrane and membrane-bound compartments such as lysosomes, endosomes, and synaptic vesicles (Burré et al., 2007; Cuajungco et al., 2014). As a new member of the CDF superfamily, and specifically the SLC30 family, phylogenetic analysis showed that TMEM163 is evolutionary related to ZNT9 (Sanchez et al., 2019). In terms of amino acid sequence identity and similarity, however, TMEM163 appears to be closely related to ZNT4 (Table 1). It is worth noting that both proteins are detected within the lysosomes [2, 3]. Interestingly, ZNT8 isoforms hare phylogenetically similar, but their amino acid sequence percent identity and similarity scores are also low upon our analysis. Nevertheless, TMEM163 is a zinc efflux transporter that should be re-classified as a ZNT11 protein that is encoded by the *SLC30A11* gene (Sanchez et al., 2019).

### *3.2. Protein Interactome*

TMEM163 was shown to interact and partially colocalize with TRPML1, a non-selective cation channel found within lysosomes (Cuajungco et al., 2014) The two proteins share a similarly predicted six TM domain and are both expressed in the human brain (Cuajungco et al., 2014; Fine, Schimiege, & Li, 2018). It is important to note that loss-of-function mutations or deletions within TRPML1 are responsible for MLIV, a rare neurodevelopmental and neurodegenerative disorder (Wakabayashi, Gustafson, Sidransky, & Goldin, 2011). Intriguingly, the functional relevance of TMEM163-TRPML1 interaction suggests that both proteins affect zinc homeostasis in cells (Cuajungco et al., 2014). This is particularly pertinent in the context of disease due to the previous observation by our laboratory that zinc levels in MLIV patient fibroblasts are elevated (Cuajungco et al., 2014) and in post-mortem brains of MLIV mouse model (Eichelsdoerfer, Evans, Slaugenhaupt, & Cuajungco, 2010). Fluorescence microscopy following knock down of TRPML1 in cultured cells also showed high lysosomal zinc levels (Eichelsdoerfer et al., 2010; Kukic, Jeffrey,

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Coblentz, Shannon, & Kiselyov, 2013), but spectrofluorometric analysis did not necessarily reveal significant increase of zinc concentration in cells with reduced TRPML1 expression (Cuajungco et al., 2014). Notwithstanding, the subcellular localization of TMEM163, its zinc transport function, and its interaction with TRPML1 all suggest that it may play a role in MLIV pathology.

There is recent evidence showing that TMEM163 has a vital role in the expression and function of P2X receptors (Salm et al., 2020). P2X receptors are ATP-gated ion channels that serves to transmit pain signaling, and thus are a prime drug target for pain relief (Khakh, 2001). In a recent paper, it was revealed that TMEM163 modulates the activity P2X3 receptor (Salm et al., 2020) and while this function helps to show an increasing importance of TMEM163, a second finding in this study relates to the SLC30 family. It was found that TMEM163 was able to alter the expression of the P2X3R protein (Salm et al., 2020), which could further solidify the phylogenetic relationship between ZNT9 and TMEM163 proteins (Sanchez et al., 2019), since ZNT9 has been demonstrated to influence and regulate specific transcriptional targets (Chen, Kim, & Stallcup, 2005). It would be interesting to further prove the hypothesis that ZNT9 and TMEM163 share a similar function in cells that impact normal and pathological states in human health.

## 4. Genome-wide Association Study (GWAS) Implicating TMEM163 in Human Diseases

A growing number of GWAS reports has recently implicated TMEM163 as potentially causative factor in several human disorders. We summarize below the findings that link TMEM163 in PD and DM.

### *4.1. Diabetes Mellitus*

One GWAS had a relatively fair number of Indian subjects with over 12,000 people in total. The study found significant association with two single nucleotide polymorphisms (SNPs) within the TMEM163 gene with T2D: rs998451 and rs6723108. Specifically, it was hypothesized that TMEM163 may influence the secretion of insulin, due to the two SNPs being associated with lower fasting plasma insulin levels in the subjects (Tabassum et al., 2013). Interestingly, a replication study using a population of 1209 people from Northwest India showed no significant association with T2D and the two SNPs rs998451 and rs6723108. However, as the authors state, since India has many different ethnic groups, treating the country as a whole may lead to incorrect conclusions and further studies would have to be done (Sharma et al., 2017). With this in mind, testing future populations and taking into account ethnicity should be a goal of future GWAS and especially in places with high ethnic diversity. More recently, however, a follow-up study done by another group revealed that one of the TMEM163 SNPs may indeed be linked to T2D (Chakraborty et al., 2020). First, the group showed that knock down of mouse Tmem163 in MIN6 cells produced elevated intracellular zinc levels (Chakraborty et al., 2020), which is consistent with our laboratory's earlier findings knocking down TMEM163 in human cells (Cuajungco et al., 2014). The group also found that the reduced Tmem163 expression resulted in decreased insulin secretion and differential expression of glucose metabolism genes (Chakraborty et al., 2020). To further support their hypothesis that TMEM163 plays a role in T2D, the authors showed that the relative TMEM163 mRNA expression levels in human pancreatic tissue were higher compared to other tissues (Chakraborty et al., 2020); however, as mentioned earlier, the apparent difference in relative levels is due to the housekeeping gene that was used to compare relative quantification (Cuajungco et al., 2014). Nevertheless, the authors reported that a novel variant of TMEM163, believed to cause a partial loss of function, was found in 33% of study participants with T2D (Chakraborty et al., 2020). This TMEM163 variant was found to be associated with a high glycemic index and that fasting plasma insulin level is decreased among the participants (24 patients with T2D and 24 control subjects); however, the association was not found to be significant upon further analysis (Chakraborty et al., 2020). As previously mentioned, ZNT8 is implicated in DM and that both ZNT8 and TMEM163 are expressed in pancreatic cells. It is thus quite conceivable that both proteins may play a redundant role in pancreatic zinc homeostasis and insulin packaging. Further studies on whether both ZNT8 and TMEM163 influence glucose and insulin metabolism are warranted in light of these recent observations.

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In another DM investigation, albeit with a Han Chinese population and with respect to gestational diabetes mellitus (GDM), no association was found with the TMEM163 SNP rs998451 present in the population they studied. The study had a sample size of 367 control pregnant women and 334 pregnant women with GDM (Tan, Hu, You, Yang, & Wang, 2019), which was not a very large sample size. Finally, another GWAS with Mongolian Chinese population found an association with the TMEM163 SNP rs6723108 and T2D. The study consisted of 497 subjects with T2D and 469 control subjects, making it once again a relatively small GWAS report. Of interest is the fact that nearly every participant of the study has the TMEM163 SNP rs6723108 variant. However, the reason and consequences of this risk allele being at relatively high frequency remain unknown (Bai et al., 2015).

*4.2. Parkinson's Disease (PD)*

Once again using GWAS, a meta-analysis consisting of around 14,000 patients, and just under 100,000 controls showed that the SNP rs6430538 (near ACMSD and TMEM163) showed genome-wide significance with Parkinson's disease among Caucasians (Nalls et al., 2014). With such a large sample size, this finding may implicate TMEM163 in PD, though whether or not this SNP truly corresponds with TMEM163 is still unknown, and little research has been done into this specific SNP's function and what has been done focused on ACMSD (Thirtamara-Rajamani et al., 2017). It was of note, however, that as mentioned in Thirtamara et al., 2017, the SNP may be able to affect the promoters of both genes. Thus, it would be valuable for functional studies into this SNP on TMEM163 and PD to be carried out.

Very recently, using 743 nonconsanguineous Chinese patients with early onset PD, multiple novel TMEM163 variants demonstrated association with PD. Notably, one variant in particular, c.98C>G, was found to be significantly associated with PD (Li et al., 2020). There were a few limitations of this study, such as the lack of age and sex-matched controls and opting rather for gnomAD v2.1.1 data from East Asians. Additionally, the sample size, as noted by the authors was not large, and as such further studies into variants of interest should continue with larger populations.

A GWAS with a Southern Spanish population that consisted of 240 patients with PD and 192 control subjects failed to find genome-wide significance with the rs6430538 variant and PD (Bandrés-Ciga et al., 2016). Since the sample size was not large and the fact some association was found at an uncorrected p-value led the authors to believe that at larger sample sizes, genome-wide significance may be found with this SNP. In this vein, a replication study with another Southern Spanish population was done with a larger number of people (750 patients with PD and around 1100 control subjects) by a different set of researchers. The new population's data did demonstrate an association of significance between the SNP and PD. However, this study also did a meta-analysis which combined data with the previously mentioned Southern Spanish population, and with this combined data, no genome wide significance was found. There once again, however, appeared to be some significance with the rs6430538 locus and PD when looking at the uncorrected p-value (Tejera-Parrado et al., 2019). While the sample size grew larger with respect to the first study, the lack of true genomic significance in their meta-analysis leaves the true significance of this SNP, at least in this population, unknown.

Two independent GWAS with East Asian populations attempted to determine if the rs6430538 SNP had any association in people with the aforementioned ethnic background. The first, with a Taiwanese population, had around 600 patients with PD and 600 control subjects and found no association between the SNP and PD. They also mention the importance in how the allelic frequency in their population greatly differed in some cases with Caucasian populations, and thus, while this SNP may not be significant for this people of East Asian origin, it does not rule out possible significance for others of different genetic backgrounds (Chang, Chen, Chen, Fung, & Wu, 2019). The second GWAS had a Han Chinese population numbering around 1000 patients with sporadic PD and around 1000 control subjects. Once again, no association was found in this

population, though it was urged that further studies are carried out in East Asian populations to further prove this (Wang et al., 2019).

Though not unexpected, different populations show conflicting results, and this demonstrates how variable populations are and how specific SNPs may only confer some function in some population. It is crucial to point out how some studies such as, Tan et al., (2019) and Bai et al., (2015) are not as convincing as others due to the low sample size, and thus, future GWAS should make sure to use larger populations. Additionally, more types of populations should be looked into as specific Indian, Chinese, and Caucasian populations made up the majority. Ethnic groups should be also be taken more into account as was said in Sharma et al., 2017 due to populations in certain parts of the world being more ethnically diverse. Thus, inaccurate data may arise if such a point is not heeded.

## 5. Conclusions

Over the past decade, the elucidation of TMEM163 has been slow, but progressive. While the current research suggesting TMEM163 to be reclassified as ZNT11 is becoming stronger, further research must be carried out. Specifically, determination of more interaction partners and especially with ZNTs would bolster this hypothesis due to the heterodimeric nature of certain SLC30 family members. Far more GWAS studies with larger populations and accounting for ethnic diversity should also be done. Additionally, based on the current evidence, the functional importance of the SNPs must be researched as the current literature is lacking. With the mounting evidence of TMEM163's role in disease as well as its possible membership to the ZNTs, the role of zinc and its transporters continues to be vital in understanding human health.

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Figures and Legends

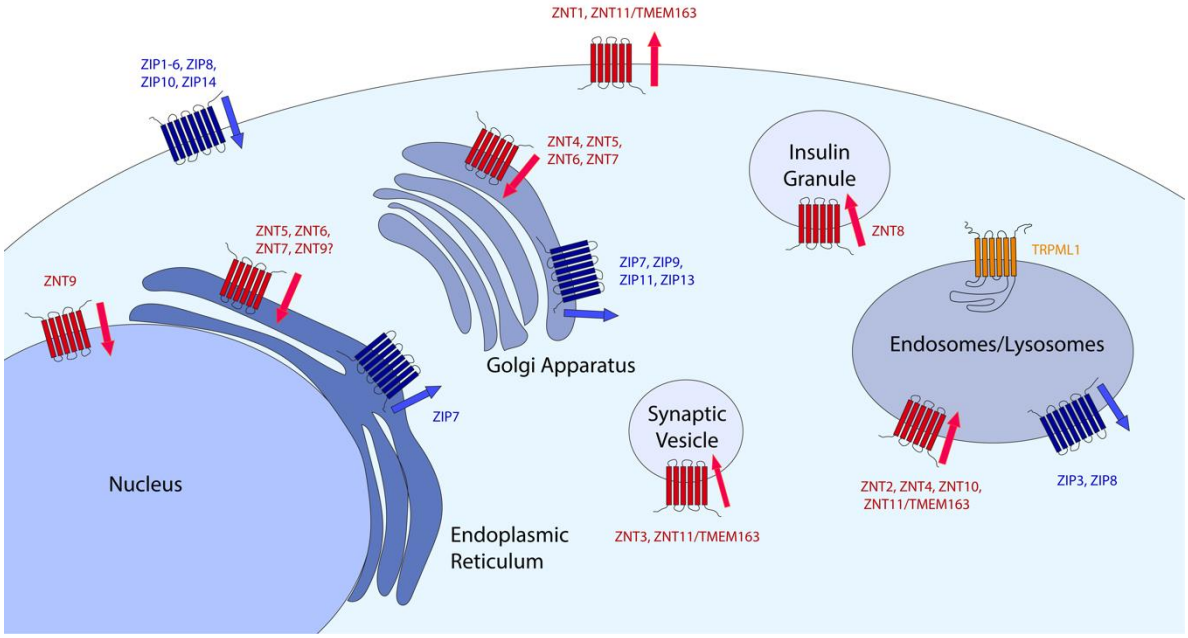


**Figure 1**. Localizations of membrane-spanning zinc transporter proteins. Members of the SLC30 family (red) act as zinc effluxers, while members of the SLC39 family (blue) act as zinc effluxers. TRPML1 ion channel localization is also shown (orange).

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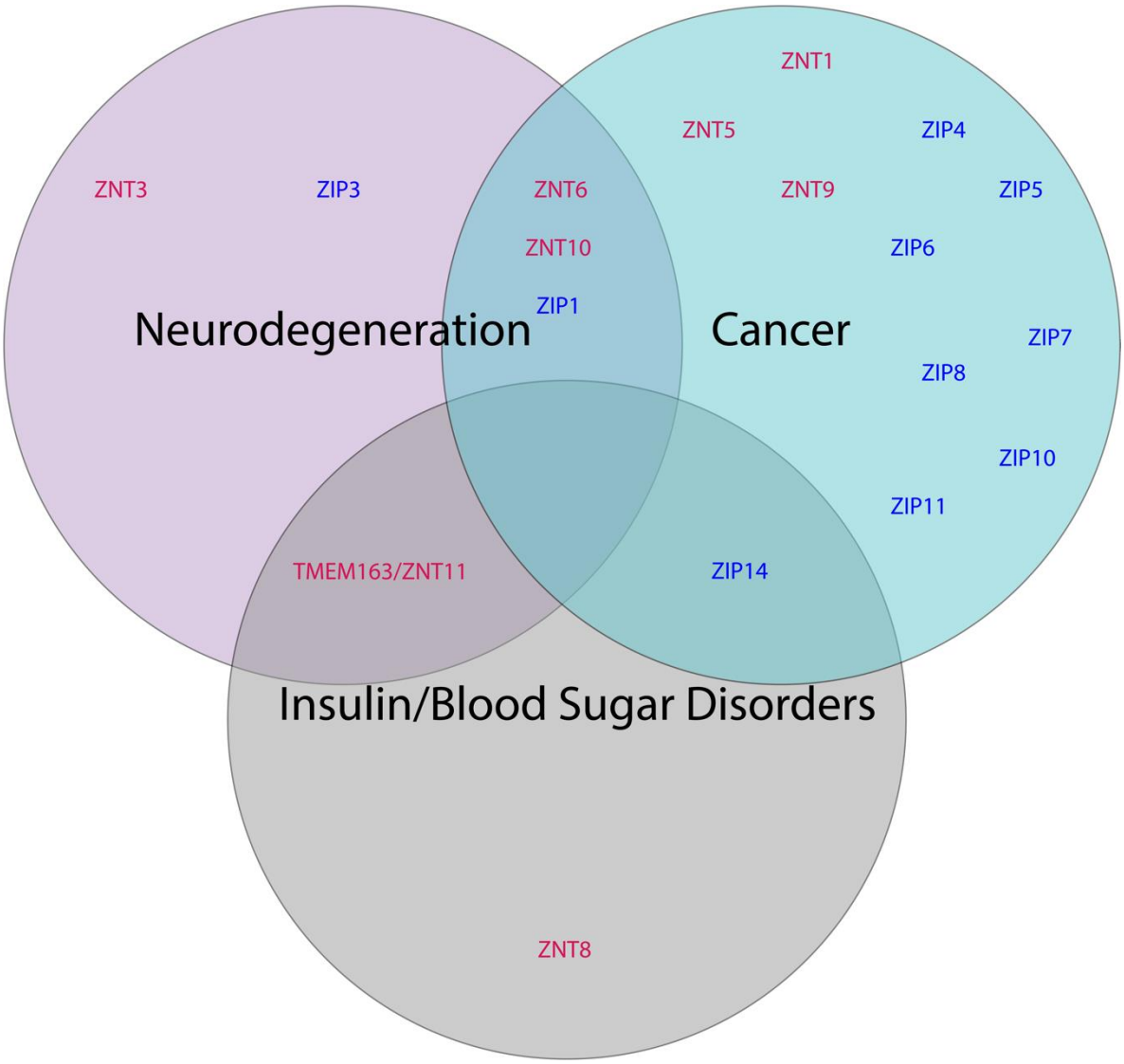


**Figure 2**. Associations between altered ZNT/ZIP expression and notable human diseases.

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**Table 1**: TMEM163 amino acid sequence identity and similarity in comparison to members of the SLC30 family of proteins. Comparisons were done using the online Sequence Identity and Similarity (SIAS) software (www.imed.med.ucm.es/tools/sias.html). The three SLC30 family members with high percentages for either identity or similarity are in bold text.

| SLC30 Member | TMEM163 Identity | TMEM163 Similarity |
|---|---|---|
| ZNT1 | 4.84% | 11.76% |
| ZNT2 | 7.26% | 15.57% |
| ZNT3 | **9.34%** | 15.91% |
| ZNT4 | **11.76%** | **20.41%** |
| ZNT5 | **8.65%** | **16.26%** |
| ZNT6 | 7.26% | 14.18% |
| ZNT7 | 4.49% | 12.45% |
| ZNT8 | 7.26% | **18.33%** |
| ZNT9 | 3.8% | 10.72% |
| ZNT10 | 6.92% | 13.49% |