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Inositol 1,4,5-trisphosphate Receptor Mutations associated with Human Disease

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Summary

Calcium release into the cytosol via the inositol 1,4,5-trisphosphate receptor (IP₃R) calcium channel is important for a variety of cellular processes. As a result, impairment or inhibition of this release can result in disease. Recently, mutations in all four domains of the IP₃R have been suggested to cause diseases such as ataxia, cancer, and anhidrosis; however, most of these mutations have not been functionally characterized. In this review we summarize the reported mutations, as well as the associated symptoms. Additionally, we use clues from transgenic animals, receptor stoichiometry, and domain location of mutations to speculate on the effects of individual mutations on receptor structure and function and the overall mechanism of disease.

IP₃R Structure and Function

Agonist binding to plasma membrane receptor tyrosine kinases or G protein-coupled receptors leads to a canonical signal transduction cascade initiated by phospholipase C activation. Cleavage of phosphatidylinositol 4,5-bisphosphate yields membrane-bound diacylglycerol and freely diffusible inositol 1,4,5-trisphosphate (IP₃), which traverses the cytosol to bind tetrameric IP₃ Receptors (IP₃Rs). The IP₃R family is encoded by three distinct *ITPR* genes resulting in IP₃R type 1 (IP₃R1), IP₃R type 2 (IP₃R2), and IP₃R type 3 (IP₃R3). These receptors are located predominately on the endoplasmic reticulum (ER) membrane where binding of IP₃ results in calcium (Ca²⁺) release into the cytosol. The increased free cytosolic Ca²⁺ interacts with a multitude of effectors to control such diverse cellular functions as secretion, gene transcription, cellular metabolism and cell fate (Berridge et al., 2000, Berridge, 1993).

The three monomeric IP₃R isoforms share 60–80% sequence identity and can assemble to form homotetrameric or heterotetrameric Ca²⁺ channels. IP₃R monomers share a basic structure of four distinct regions: an N-terminal suppressor domain (SD) and ligand binding domain (LBD), a central regulatory domain, and a C-terminal six transmembrane (TM) spanning region which encompasses the channel pore of the IP₃R (Nucifora et al., 1996). Domains important for IP₃ binding and channel gating, such as the IP₃ binding core (SD and LBD) and the pore domain (TM), share 90% sequence identity, while the central regulatory/coupling domain is far less conserved. As the LBD is located quite distal to the channel pore, the tertiary structure of the receptor results in these two domains being located 70Å

apart (Fan et al., 2015). Details of interactions between these domains resulting in channel gating remain unresolved (Hamada et al., 2017, Fan et al., 2015). The two main hypotheses of IP₃R channel gating are that IP₃ binding results in conformational changes transmitted from the LBD to the channel domain either through interaction of the N- and C-termini (Fan et al., 2015) or through a long scale conformational change of the whole protein (Hamada et al., 2017). Whether gating is mediated through either of these processes individually, or as a combination of the two processes, remains unresolved.

Several critical residues have been previously identified in IP₃R (Figure 1). Residue Y167 in the SD has been identified as necessary for channel opening, while LBD residues R241, K249, R265, T267, R269, R504K508, R511, Y567, R568, and K569 are critical for IP₃ binding (Yamazaki et al., 2010, Bosanac et al., 2002, Yoshikawa et al., 1996). With the exception of two of these LBD residues (R265 and T267), these sites were also necessary for binding of the IP₃R-binding protein (IRBIT), which competes with IP₃ for binding of the IP₃R resulting in decreased Ca²⁺ release from the store (Sasaki et al., 2015, Ohba et al., 2013, Ando et al., 2003, Ando et al., 2006). The regulatory domain of the receptor houses many residues critical for protein kinase A phosphorylation, caspase and calpain cleavage, and the binding of a variety of proteins including ATP, calmodulin and carbonic anhydrase-related protein VIII(CARP)(Foskett et al., 2007). Also found in the regulatory domain, is residue E2101 (reference sequence P29994.2) previously identified as important for Ca²⁺ modulation of activity, which is important for determining the overall spatiotemporal properties of Ca²⁺ signals (Miyakawa et al., 2001). The C-terminus of the receptor contains the channel pore comprised of the selectivity filter (GGGVD) and 6TM helices which serve to conduct Ca²⁺ from the ER to the cytosol (Fan et al., 2015, Schug et al., 2008, Boehning et al., 2001).

IP₃R mutations are associated with disease

While the physiology controlled by IP₃R-induced Ca²⁺ release has been extensively studied, a burgeoning number of recent reports have identified mutations in the primary amino acid sequence of all isoforms of IP₃Rs that are associated with human disease. These mutations have been identified in all four domains of IP₃R1, as well as in the pore domain of IP₃R2 and the suppressor domain of IP₃R3. However, while the consequences of some of these mutations may be inferred from the expression profile of individual isoforms and predicted effects on primary structure of the IP₃R, given the lack of a full understanding of channel gating and allosteric regulation, the causal relationship between altered IP₃R function and the manifestations of disease are not established for most of mutations. In this short review, we will document the location of the various mutations associated with particular diseases and speculate on potential mechanisms underlying altered function and manifestation of disease.

Effect of Mutations: clues from the localization of IP₃R isoforms and transgenic IP₃R mice

While IP₃Rs are ubiquitously expressed throughout the body, the individual isoforms are differentially expressed such that the central nervous system (CNS) prominently expresses IP₃R1, while IP₃R2 and IP₃R3 are expressed in the periphery. Purkinje cells in the

cerebellum are important for coordination, control, and learning of movement. These cells almost exclusively express IP₃R1 and at levels 10-fold higher than reported for expression in any other cell type (Furuichi et al., 1989). Most genetically engineered *itpr1*-null mice die in utero (Matsumoto et al., 1996). Those mice that are born suffer from severe cerebellar ataxia and seizures and typically die after 3 to 4 weeks (Matsumoto et al., 1996). These results suggest that *itpr1* expression is important for proper brain development and function (Matsumoto et al., 1996).

Similar ataxic phenotypes were observed in Opt and 18 mice which lack exons 43/44 and exon 18 of *itpr1*, respectively (Street et al., 1997, van de Leemput et al., 2007). In the 18 mice, this ataxic phenotype was accompanied by markedly decreased levels of *itpr1* in Purkinje cells (van de Leemput et al., 2007). Mice genetically engineered to be *Itpr1* heterozygous (IP₃R1+/-) had no obvious morphologic differences compared to wild type mice; however, they did exhibit impaired motor coordination when subjected to a rotarod test (Ogura et al., 2001). Otherwise, IP₃R1+/- mice showed no alteration in spontaneous motor activity, muscle strength, or walking patterns compared to wild type mice. In total, the data from these models strongly suggest that IP₃R1 plays an important role in motor coordination (Ogura et al., 2001). Similar motor function deficits were observed in heterozygous staggerer mice, which have a spontaneous ataxia mutation and suffer from loss of cerebellar Purkinje cells with aging (Caston et al., 1995, Doulazmi et al., 1999). These results suggest that *itpr1* expression, and therefore IP₃R1-mediated Ca²⁺ signaling is important for Purkinje cell and cerebellar function. As the majority of IP₃R mutations are found in the IP₃R1 isoform, we would expect most patients to present with a similar phenotype of CNS related symptoms including cerebellar ataxia, intellectual disability, and atrophy.

Whereas *itpr1*-null mice displayed an altered phenotype, mice engineered to be either *itpr2*-null or *itpr3*-null were viable and exhibited more subtle phenotypes (Futatsugi et al., 2005). The lack of distinct, visible abnormalities in these mice is likely due to co-expression of IP₃R2 and IP₃R3 in most cell types. Nevertheless, mice lacking both the *itpr2* and *itpr3* genes suffered from severe deficits in exocrine function including salivary, lacrimal, and pancreatic secretion (Futatsugi et al., 2005). While these mice appeared normal at birth, they gained less body weight compared to wild type littermates when fed dry food and typically died after 4 weeks. When fed wet mash after weaning, *itpr2/itpr3*-null mice survived, though they did remain smaller in size compared to wild type mice. The exocrine phenotype observed was due to impaired Ca²⁺ signaling leading to a loss of secretion from acinar cells in the salivary glands and the pancreas and subsequent lack of proper macronutrient digestion. Thus, these results implicate *itpr2* and *itpr3* in exocrine physiology underlying energy metabolism and animal growth. We would anticipate similar phenotypes in exocrine tissues which contain IP₃R2 or IP₃R3 disease-associated mutations.

Effect of Mutations: Clues from the domain location of IP₃R mutants

While disease-associated mutations have been identified in all four domains of IP₃R (Figure 2), we would predict that the location of IP₃R mutants to particular domains would play a role in overall channel function and patient phenotype. For example, mutations in the N-

terminal SD or LBD would likely result in altered IP₃ binding and a subsequent change in channel function and Ca²⁺ release. Similarly, mutations in the C-terminal channel would likely impact channel oligomerization, as well as the permeability and conductance of the channel leading to decreased channel function and release. While effects of mutations at the N- and C-terminals of the IP₃R can be inferred from the known role of those domains, effects of mutations in the central regulatory domain are not as easily predicted. Due to lack of knowledge of the protein structure, gating, and interactions with other proteins, together with how allosteric modulation alters gating the overall the impact of mutations in this domain on receptor structure and function are uncertain.

Missense, nonsense, insertion/deletion, and splice site mutations of the IP₃R have all been reported to result in disease. The majority of IP₃R mutations have been reported based on association of clinical symptoms and whole exome sequencing with few mutations having been observed or characterized at the protein level. The most commonly diagnosed diseases are those attributed to IP₃R1 mutations. These diseases often target the CNS including spinocerebellar ataxia (SCA), pontine cerebellar hypoplasia (PCH), and Gillespie syndrome (GS; MIM #206700). SCA-associated mutations have been found predominantly in the N-terminal and central regulatory domains of the receptor, while GS and PCH associated mutations have been found predominantly in the C-terminal channel domain. In contrast, mutations in IP₃R2 and IP₃R3 isoforms have been restricted to the C-terminal channel domain and N-terminal suppressor domain, respectively.

Effect of Mutations: Clues from the stoichiometry of IP₃R tetramers

Our group has previously demonstrated using concatenated receptor constructs that the individual isoforms present in an IP₃R heterotetramer help shape the resulting Ca²⁺ signals (Chandrasekhar et al., 2016). These data, in combination with the difference in viability and phenotype observed between *itpr1*-null mice and IP₃R1^{+/-} mice, suggest that the stoichiometry of the IP₃R tetramer in regard to the number of wild type or mutant monomeric subunits may also play a role in determining the structure and function of the tetrameric receptor (Matsumoto et al., 1996, Ogura et al., 2001). As most IP₃R mutations are believed to be dominant-negative, a question that follows is if there is a threshold or number of mutant subunits that a tetramer can tolerate prior to change in receptor function and onset of disease. Thus, the mode of inheritance of each IP₃R mutation likely also plays a role in determining disease phenotype. Multiple modes of inheritance have been observed in IP₃R mutations with most disease-associated IP₃R mutations appearing to be autosomal dominant; however, two reported mutations exhibit autosomal recessive inheritance (Klar et al., 2017, Klar et al., 2014). We would expect that these differences in mode of inheritance would add an additional layer of complexity to predicting the effect of IP₃R mutation on receptor structure and function, as well as mechanism of disease.

IP₃R mutations associated with human disease by domain: mutations in the SD

Of the four IP₃R domains, the SD has the fewest mutations recognized, to date. These mutations include IP₃R3 residues R64 (R64H)¹ and R149 (R149L), which have been

identified as potentially causative of the metastasis or recurrence of head and neck squamous cell carcinoma (MIM #275355) (Hedberg et al., 2016). These are the only two disease-associated mutations associated with the IP₃R3 isoform, while most mutations are identified in the IP₃R1 isoform. That is the isoform where the other two SD mutations were identified – the A95T mutation, a potential candidate mutation for Sézary syndrome (SS; MIM #254400), a leukemic variant cutaneous T-cell lymphoma, and the R36C mutation, identified as the cause of an autosomal dominant form of SCA29 in a mother (*de novo*) and her children (inherited) (Casey et al., 2017, Prasad et al., 2016).

As suggested by the phenotype of the *itpr1*-null and IP₃R1+/- mice models, many IP₃R1-associated diseases result in congenital ataxias. Clinically, these patients exhibit developmental motor delay, hypotonia, tremors, and limb/gait ataxia, often accompanied by small head circumference. Intellectual disability and mild cerebellar hypoplasia/atrophy can also occur; however, depending on the specific mutation, both inter- and intrafamilial variability of symptoms is observed. Broadly, the phenotypes of these diseases fall into two categories – late-onset, slowly progressive ataxia and early-onset, non-progressive ataxia with the locus of these diseases overlapping on chromosome 3 (Dudding et al., 2004). Early-onset, non-progressive ataxia has been diagnosed as many different diseases including spinocerebellar ataxia 29 (SCA29; MIM #117360), congenital non-progressive cerebellar ataxia (CNPCA), autosomal dominant non-progressive congenital ataxia (NPCA), sporadic infantile onset spinocerebellar ataxia, and ataxic cerebral palsy. Found in all four domains of IP₃R1, mutations associated with these diseases are predominantly missense in nature – as is the case for the R36C mutation in the SD. While the family with this mutation presented with the normal early-onset, non-progressive phenotype, they also exhibited atypical symptoms like small pinpoint pupils, dysmorphic facial features, and absence of cerebellar atrophy (Casey et al., 2017). Additionally, the patients reported improvement in symptoms over time, though, they are still considered delayed and ataxic for their age (Casey et al., 2017). Similar amelioration of symptoms has been previously reported in other patients with NPCA; however, reasons for this improvement remain unknown (Dudding et al., 2004).

While functional analysis of most IP₃R disease-associated mutations has not been performed, the effects of the R36C mutation have been tested in the context of a tetramer with all four monomeric subunits containing the mutation (mutant homotetramer). Casey *et al.*, reported that expression of the R36C mutant increases both IP₃ binding affinity and Ca²⁺ release properties of the receptor (Casey et al., 2017). When expressed in an IP₃R-null chicken lymphocyte cell line, cells containing the R36C mutation exhibited elevated peak Ca²⁺ amplitude and total Ca²⁺ signal compared to wild type (WT). Casey *et al.*, referred to this pattern of Ca²⁺ signaling as “sigmoidal” compared to the normal transient Ca²⁺ release signal seen in WT cells (Casey et al., 2017). The overall elevation in Ca²⁺ release observed in these mutated cells suggests that the R36C mutation is a gain-of-function mutation (Casey et al., 2017). As these experiments were performed using mutant homotetramers, and the R36C

¹Footnotes

Mutations reported in the reference sequence of NP_001093422.2 (IP₃R1), NP_002214.2 (IP₃R2), or NP_002215.2 (IP₃R3). If not originally reported in this reference sequence, multiple sequence alignment was utilized to find residue in appropriate reference sequence.

mutation is reported to likely be autosomal dominant, the effect of the stoichiometry of the tetramer on channel function and presentation of disease is unknown. This is because patients would be heterozygous for the mutation resulting in the presence of both WT and mutant monomeric subunits which could form a variety of heterotetramers. Thus, we predict that expression these heterotetramers may result in reduced IP₃ binding affinity and Ca²⁺ release signal compared to the mutant homotetramer, but perhaps increased when compared to the native IP₃R1.

In the context of the primary amino acid sequence, mutation of R36 from a positively charged Arg to a nucleophilic Cys, which could form disulfide bonds with other nearby Cys residues in the SD (residues 1–225), would likely affect both the structure of the SD and its intramolecular interactions with the LBD (Yoshikawa et al., 1996). Residue R36 has been previously identified as one of several SD residues which faces the LBD and is important for decreasing the IP₃ binding affinity for WT IP₃R (Bosanac et al., 2005). This suggests that the R36C mutation likely disrupts intramolecular interactions between the SD and LBD resulting in increased IP₃ binding affinity. While increased IP₃ binding Additionally, Casey *et al.* postulate that R36C disrupts intramolecular interactions between the SD and the LBD resulting in increased efficacy of IP₃ binding to induce opening of the receptor channel which is consistent with inhibited IP₃R channel function when the SD is removed (Casey et al., 2017).

IP₃R mutations associated with human disease by domain: mutations in the LBD

All LBD disease-associated mutations (Table 1) that have been identified thus far are found in the IP₃R1 isoform. Therefore, LBD mutations often result in neurological symptoms. Most IP₃R1 LBD mutations lead to diseases characterized by early-onset, non-progressive ataxia; however, one identified mutation, V494I, has been associated with the late-onset, slowly progressive spinocerebellar atrophy (SCA15; MIM #606658) (Ganesamoorthy et al., 2009). Although generally the result of large, heterozygous deletions of *ITPR1*, SCA-15 associated missense mutations have also been identified in the LBD and regulatory domain (Di Gregorio et al., 2010, Hara et al., 2008, Iwaki et al., 2008, Marelli et al., 2011, Novak et al., 2010, Obayashi et al., 2012, van de Leemput et al., 2007, Yamazaki et al., 2011, Ganesamoorthy et al., 2009). While the suspected mechanism of the autosomal dominant deletions is haploinsufficiency, the mechanism of action of SCA15-associated missense mutations is not as clear (Hara et al., 2008, Yamazaki et al., 2011, Ganesamoorthy et al., 2009). Additionally, why mutation of V494I results in late-onset ataxia, rather than early-onset ataxia, is not well understood.

Most disease-associated mutations in the LBD are missense mutations; however, an insertion (K417_418Ins) and a splicing mutation in exon 14 also both result in early-onset SCA29 (Wang et al., 2017, Zambonin et al., 2017). While the splice mutation is present in 4 individuals of a three-generation, non-consanguineous Chinese family, the K417_418Ins is a sporadic mutation identified in a single individual (Wang et al., 2017, Zambonin et al., 2017). This variability in how mutations were acquired is also found in specific diseases and

residues. NPCA, for example, may be caused by *de novo* heterozygous mutations (A280D and E512K) or inherited mutations (R269W and R241K), while mutations reported in residue R269 have been acquired via both *de novo* and inherited mutations (Barresi et al., 2017, Zambonin et al., 2017, Das et al., 2017). While patients with R269 mutations exhibited similar symptoms including cerebellar hypoplasia/atrophy, both inter- and intrafamilial variability of intellectual disability was observed making it difficult to correlate genotype and phenotype (Barresi et al., 2017, Zambonin et al., 2017, Das et al., 2017). This is made even more difficult by the presence of multiple amino acid changes are reported at this site. Reported mutations R269G and R269W result in smaller and larger changes in residue size, respectively; yet, both mutations result in the same disease (Barresi et al., 2017, Zambonin et al., 2017). Thus, it is likely that the loss of positive charge associated with both mutations, rather than change in size, that affects IP₃R1 function. This would be consistent with the role of residue 269 as one of the residues important for coordinating and binding the negatively charged IP₃ (Yoshikawa et al., 1999, Yoshikawa et al., 1996).

Residue R241 has also been previously identified as an important residue for coordinating and binding IP₃ (Yoshikawa et al., 1999, Yoshikawa et al., 1996). Interestingly, though the mother harboring the R241K mutation suffered from cerebellar atrophy, she was clinically asymptomatic, whereas her daughter with the same mutation exhibited early-onset of both symptoms and cerebellar atrophy (Barresi et al., 2017). This suggests that while most patients with disease-associated mutations in the IP₃R1 LBD had similar presentation of early-onset, non-progressive ataxia, there is potential variability exhibited in the penetrance of disease. Another example of variability associated with LBD mutations includes worsening of symptoms in one individual who expressed the IP₃R1 splice mutation in exon 14 and developed balance problems after 10 years of age (Wang et al., 2017). Additionally, while all patients with IP₃R1 LBD mutations exhibited cerebellar and/or vermis hypoplasia/atrophy, four patients with *de novo* mutations (T267M, T267R, S277I, and T594I) resulting in Sporadic Infantile Onset SCA exhibited atrophy of the pontine tegmentum as well (Sasaki et al., 2015, Ohba et al., 2013).

Based on reported LBD mutations, there appear to be specific residues more prone to mutation than others. Residues T267 (Zambonin et al., 2017, Sasaki et al., 2015, Ohba et al., 2013), R269 (Barresi et al., 2017, Zambonin et al., 2017, Das et al., 2017), and S277 (Sasaki et al., 2015, Fogel et al., 2014) have all been reported in multiple cases of early-onset, non-progressive cerebellar ataxia. Additionally, for residues T267 and R269, the mutations are manifested as multiple amino acid changes. As most residues in the LBD are highly conserved among species, mutation of these residues may affect the ability of IP₃ to bind IP₃R and subsequently lead to decreased Ca²⁺ release. Additionally, the residues of the LBD also form most of the binding domain of IRBIT (Ando et al., 2006, Ando et al., 2003). Thus, mutations in the LBD may prevent IRBIT binding leading to increased Ca²⁺ release. Disruption of these two interactions with opposite affects suggest that disease-associated IP₃R1 mutations in the ligand binding domain may result in increased or decreased Ca²⁺ release.

IP₃R mutations associated with human disease by domain: mutations in the regulatory domain

There is more diversity of diseases associated with IP₃R mutations in the regulatory domain; however, like mutations in the LBD, all disease-associated mutations are found in the IP₃R1 isoform. In addition to early-onset, non-progressive and late-onset, slowly progressive forms of ataxia, GS mutations are also found in the central domain of the receptor. Both the uncertain structure of this domain and the unclear mechanism of channel gating make it more difficult to predict effects of mutations on IP₃R structure and function as mutations in this region may impact transduction of signals from the N-terminal SD and LBD to the C-terminal channel domain. While mutations may directly affect IP₃R function in this manner, they may also alter regulation of the receptor leading to effects on channel function.

IRBIT-binding domain mutations outside the required IP₃-binding Domain

While IP₃R residues 224–576 are required for IP₃ binding, the secondary structure of the LBD and the IRBIT binding domain span residues 224 to 604 of IP₃R1 (Yoshikawa et al., 1996, Ando et al., 2003). Thus, mutations located outside of the required residues for IP₃ binding may also affect IRBIT function. However, unlike mutations located in both the LBD and IRBIT binding domains, we would not expect mutations in the regulatory and the IRBIT binding domains to alter channel function by impacting IP₃ binding directly. These mutations include T594I, which is associated with sporadic infantile-onset SCA (Sasaki et al., 2015), and N602D, which is associated with SCA29 and Ataxic Cerebral Palsy (Huang et al., 2012, Parolin Schnekenberg et al., 2015, Zambonin et al., 2017). Similar to R269W, N602D has also been acquired as both a *de novo* and an inherited mutation (Huang et al., 2012, Parolin Schnekenberg et al., 2015). While all affected members of the family with SCA29-associated N602D mutation suffered from mild cerebellar atrophy with progression of cerebellar vermal atrophy, the individual diagnosed with Ataxic Cerebral Palsy lacked cerebellar hypoplasia/atrophy (Huang et al., 2012, Parolin Schnekenberg et al., 2015). As these cases are otherwise quite similar in presentation, including the presence of intellectual disability in all affected individuals, we would conclude that it is difficult correlate the presence and severity of cerebellar hypoplasia/atrophy with the degree of ataxia or intellectual disability.

Regulatory Domain Mutations in the CARP-binding Domain

Lack of association between presence of cerebellar hypoplasia/atrophy and patient phenotype was also observed in a 4 generation Australian family in which 20 individuals suffered from autosomal dominant NPCA due to a V1553M mutation in IP₃R1 (Dudding et al., 2004, Huang et al., 2012). While most exhibit no change in symptoms, five affected family members reported improvement in ataxic symptoms (Dudding et al., 2004, Huang et al., 2012). A similar amelioration of symptoms has been reported in other patients, similar to those with the R36C mutation and the 13 individuals documented in Zambonin *et al*; however, the mechanism underlying the improvement in their symptoms is unknown (Casey et al., 2017, Zambonin et al., 2017). Interfamilial variability was also observed with the V1553M mutation. Though all members of the Australian family with the V1553M mutation

exhibited intellectual disability, five affected individuals in a four generation Russian family with the same mutation did not (Shadrina et al., 2016, Dudding et al., 2004, Huang et al., 2012). Additionally, whereas amelioration of symptoms was reported in the Australian family, at least one individual in the Russian family reported a mild increase in gait and speech difficulties over time (Shadrina et al., 2016, Dudding et al., 2004, Huang et al., 2012). Worsening of symptoms, despite remaining clinically non-progressive, was also observed in one individual who expressed the IP₃R1 splice mutation in exon 14 and developed balance problems after 10 years of age (Wang et al., 2017).

The V1553M mutation, as well as a *de novo* mutation which results in ataxic cerebral palsy without cerebellar hypoplasia/atrophy (S1493D), are located in the CARP binding domain (residues 1388–1648 in reference sequence NM_001099952.2) (Hirota et al., 2003, Parolin Schneckenberg et al., 2015). CARP, like IRBIT, competes with IP₃ for binding to IP₃R and subsequently results in decreased IP₃R channel activity. Thus, we would expect mutations in this region to alter CARP binding and regulation of IP₃R channel function. This prediction is supported by work done by Kaya *et al.* where mutation of CARP results in lack of inhibition of IP₃R and a subsequent increase in Ca²⁺ release leading to mild intellectual disability (Kaya et al., 2011, Turkmen et al., 2009). This is consistent with the phenotype of some patients whose disease-associated mutations reside in the CARP-binding domain (Kaya et al., 2011, Turkmen et al., 2009).

Autosomal recessive SCA29-associated mutation

While all of the IP₃R mutations discussed thus far have been autosomal dominant, IP₃R1 mutation L1787P is autosomal recessive (Klar et al., 2017). The SCA29-associated mutation was identified in a five-generation consanguineous Pakistani family where six homozygous, affected individuals did not walk, resulting in a quadrupedal gait, and exhibited mild intellectual disability (Klar et al., 2017). While these individuals presented with cerebellar atrophy without pontine abnormalities, five heterozygous, asymptomatic family members exhibited milder cerebellar hypoplasia (Klar et al., 2017). In contrast with SCA15 progression, these heterozygous individuals did not express clinical symptoms with age.

While detailed effects of regulatory domain mutations on channel function are unknown, mutation of a conserved Leu to a Pro is predicted to be structurally destabilizing suggesting individuals homozygous for the L1787P mutation may have almost complete loss of IP₃R1 tetramer function and subsequent loss of neuronal tissue. Klar *et al.* predict that individuals heterozygous for the L1787P mutation are asymptomatic due to compensation by remaining WT IP₃R1 (Klar et al., 2017). We would predict that heterotetramers expressed in these heterozygous individuals would exhibit Ca²⁺ release intermediate to that of the WT homotetramers and mutant homotetramers expressed by the individuals homozygous for the mutation. Thus, cerebellar atrophy observed is likely the result of partial loss of IP₃R1 function, which subsequently leads to a loss of purkinje cells and neuronal tissue in the cerebellum and brainstem due to decreased Ca²⁺ signaling (Klar et al., 2017).

SCA15-associated mutation

Similar to the V494I mutation in the LBD, a single SCA15-associated mutation (P1074L) has been identified in the regulatory domain (Yamazaki et al., 2011). While P1074L is a missense mutation, most cases of SCA15 are caused by complete or partial heterozygous deletions of *ITPR1* resulting in haploinsufficiency (Tada et al., 2016). Associated with late-onset and very slowly progressive cerebellar ataxia, patients with SCA15 generally do not exhibit delayed motor development or intellectual disability as seen with early-onset, non-progressive patients (Tada et al., 2016). Additionally, individuals with the P1074L mutation exhibited tremors and cerebellar atrophy without involvement of the brainstem (Hara et al., 2008).

Of mutations in the regulatory domain, a functional characterization has only been performed on the P1074L mutation. When expressed in a *itpr*-null chicken lymphocyte line, IP₃R1 P1074L mutant tetramers exhibited increased IP₃ ligand binding affinity; however, the presence of the mutation did not alter the Ca²⁺ release compared to WT (Yamazaki et al., 2011). This lack of alteration in Ca²⁺ release differs from the findings associated with functionally characterized mutations located in the SD and channel pore. Additionally, it is once again unclear how and why this single missense mutation results in a different onset and progression of disease when compared to disease-associated mutations in this domain.

GS-associated mutations

Patients with GS exhibit symptoms similar to those observed in SCA29 including early-onset, non-progressive ataxia, developmental delay, hypotonia, variable intellectual disability, and progressive cerebellar atrophy (McEntagart et al., 2016). However, patients with GS are distinguished from those with SCA29 based on the presence of aniridia (McEntagart et al., 2016, Hingorani et al., 2012). Aniridia is a rare, inborn developmental error where the patient exhibits hypoplasia or complete loss of the iris of the eye¹⁴. In GS, this phenotypically presents as iris hypoplasia with “scalloping” of the pupillary edge (Hingorani et al., 2012).

Similar to the large deletions of *ITPR1* that result in SCA15, the majority of mutations in the regulatory domain that result in GS are homozygous or heterozygous biallelic truncating variants (Carvalho et al., 2017, Gerber et al., 2016). Truncations generally contain the SD, LBD, and a portion of the regulatory domain; however, they lack the channel domain. Thus, they are non-functional as they cannot release Ca²⁺ from intracellular stores. The majority of GS-associated missense mutations in IP₃R1 are located in the C-terminal domain, however two missense mutations have been identified in the regulatory domain. Both mutations are found in residue E2061; however, one is inherited (E2061G), while the other is a *de novo* mutation in an individual (E2061Q) (McEntagart et al., 2016). McEntagart *et al.* theorize that these dominant negative mutations resulting in iris hypoplasia are due disruption of functional interactions that are critical to the formation and/or maintenance of the sphincter pupillae muscle or a lower level of residual IP₃R function compared to that in SCA29 (McEntagart et al., 2016).

IP₃R mutations associated with human disease by domain: mutations in the C-terminus.

The C-terminal channel domain contains the most diversity in diseases associated with IP₃R mutations including SCA29, unclassified SCA, SS, PCH, GS, and Aniridia. Mutations, either missense or deletion, are found in the IP₃R1 and IP₃R3 isoforms. Generally, these mutations are found in the TM domains, prior to the start of the cytosolic tail, where they likely disrupt the structure of the channel pore leading to altered channel conductance and permeability. Additionally, mutations in this domain may impair oligomerization of IP₃R monomeric subunits and therefore prevent tetramer formation.

C-terminal Mutations between TM5 and TM6

Two disease-associated mutations were identified between the TM5 and TM6 helices, prior to the selectivity filter. SS-associated mutation S2454F is located in the turret between the outer helix and the pore helix, while mutation of residue T2490 (T2490M) is located in the pore helix (Prasad et al., 2016, Valencia et al., 2015). Classified as an unassigned SCA, this mutation results leads to progressive optic atrophy, ataxia, sensorineural hearing loss, muscle weakness, vertigo, erythrocytosis, and nystagmus, as well as multiple congenital anomalies (Valencia et al., 2015). Both mutations result in primary structure changes from nucleophilic residues to large aromatic or hydrophobic residues that likely disrupt the channel pore.

Mutations in the Selectivity Filter

As previously observed with mutations in the LBD, mutation of specific residues in the C-terminus also result in multiple diseases. For example, mutation of residue G2506 (G2506R) results in multiple *de novo* cases of GS (McEntagart et al., 2016) and SCA29, both *de novo* and inherited (Zamboni et al., 2017). Located in the selectivity filter (GGGVG) mutation of this residue from a small Gly to a large, positively charged Arg is predicted to be strongly destabilizing. Schug *et al.* previously showed that just increasing the size and hindering the mobility of the analogous rat residue (G2545A) resulted in a non-functional receptor (Schug et al., 2008). Functional characterization of other disease-related IP₃R mutations in the selectivity filter (G2498S in NM_002223.3) are consistent with these predictions that mutation of small Gly residues in the selectivity filter to larger residues results in obstruction of Ca²⁺ movement through the channel pore.

Mutations in the 6th TM domain

Several different disease-associated mutations have been identified in 6th TM domain which serves as the pore-lining helix (Fan et al., 2015). Due to this function, mutation of highly conserved residues in this inner helix is likely to alter the structure of the Ca²⁺ channel and impact Ca²⁺ release. For example, the microcephaly with pontine and cerebellar hypoplasia (MICPCH; MIM #300749)-associated T2552P introduces a more hydrophobic residue at this position which may disrupt the α -helical structure of this domain (Hayashi et al., 2017). PCH and MICPCH also exhibit similar symptoms to SCA29 including early-onset motor and cognitive impairments (Namavar et al., 2011b, Hayashi et al., 2017, Moog et al., 2011). While patients may exhibit clinical variability, such as the presence of microcephaly, the

characteristic neuroradiological finding in PCH and MICPCH patients is severe hypoplasia of both the cerebellum and the pons (Namavar et al., 2011a). Thus, while similar to SCA29 in that some SCA29 patients suffer from cerebellar hypoplasia/atrophy, all PCH and MICPCH patients suffer from more extensive hypoplasia/atrophy of the pontocerebellum (Namavar et al., 2011b). Similar hypoplasia/atrophy of the pontine tegmentum was previously observed in sporadic infantile-onset SCA-associated mutations in both the LBD and the regulatory domain; however, the association between increased area of atrophy and IP₃R residues remains unclear (Sasaki et al., 2015).

Another example of a mutation in the 6th TM domain which may alter Ca²⁺ channel structure and function is the GS-associated mutation N2543I in IP₃R1 (Dentici et al., 2017). Dentici *et al.* speculated that, when not mutated, this residue hydrogen bonds with nearby residue L2544 leading the side chain of N2543 to bend toward the pore wall, preventing the pore from being blocked by allowing freedom of rotation for F2546 (Dentici et al., 2017, Fan et al., 2015). However, the hydrophobic N2543I mutation would prevent these actions from taking place and result in the side chain of N2543 restricting the diameter of the pore and blocking passage of Ca²⁺ ions (Dentici et al., 2017).

Mutation of nearby residue F2553 is also associated with GS (G2533L) (Gerber et al., 2016). Schug *et al.* previously showed that functional effects of mutation of this residue and G2547 are conditional upon the specific amino acid substitution. For both residues, mutation to Ala had minimal effects on channel function, whereas mutation to other residues (F2592D and G2586P) inactivated channel function (Schug et al., 2008). Interestingly, though mutation of residue G2547 to Ala (G2547A) did not have a large effect on channel function, this residue has been identified as a SCA29-associated mutation resulting in congenital neuropathy and ataxia (Gonzaga-Jauregui et al., 2015). It is unclear why this mutation results in disease despite indications that channel function is retained.

Mutation of residues I2550 (Zambonin et al., 2017, van Dijk et al., 2017) and V2541 (Hsiao et al., 2017) in *ITPR1* also result in ataxia symptoms. Similar to residues in the LBD and selectivity filter, residue I2550 is also associated with multiple diseases as mutation I2550N is causative for pontocerebellar hypoplasia (PCH), while mutation I2550T is causative for SCA29 (Zambonin et al., 2017, van Dijk et al., 2017). The I2550 residue has been previously identified as potentially structurally significant as it may serve as a constriction site in the channel pore (Baker et al., 2017, Fan et al., 2015, Bhanumathy et al., 2012). Substitution of the highly conserved, hydrophobic Ile, by either Thr or Asn, would likely disrupt the TM domain and affect Ca²⁺ signaling. Mutation V2541A, on the other hand, is linked to an unassigned SCA designated only as an inherited cerebellar ataxia (Hsiao et al., 2017). This is due to the proband exhibiting adult-onset, non-progressive pure cerebellar ataxia, while their offspring exhibits early-onset, non-progressive cerebellar with additional cognitive impairments (Hsiao et al., 2017). Similar differences in parental and offspring onset were observed with the NPCA-associated R241K mutation in the LBD; however, the reason for this change in phenotype between generations remains unknown³⁴.

Mutations in the LNK Domain

The sole deletion mutation in the channel domain, K2563del., resides immediately after the 6th TM domain in the helical linker (LNK) domain which connects the TM channel domain and cytosolic tail (Baker et al., 2017, Fan et al., 2015). As the LNK domain may play an important role in the mechanism of channel gating, disrupting the structure of this region through the GS-associated K2653del. mutation could prevent channel opening. The K2653del. mutation, a heterozygous in-frame *de novo* deletion of a single codon (AAG), was identified in multiple individuals (McEntagart et al., 2016, Gerber et al., 2016, Dentici et al., 2017, Zambonin et al., 2017). Once again, interfamilial variability was observed with this mutation as only one patient with the mutation did not exhibit intellectual disability (Gerber et al., 2016). When stably expressed in an *itpr1*-null chicken lymphocyte cell line, no Ca²⁺ release was observed in response to appropriate stimulus (Gerber et al., 2016). When co-expressed, K2653del. and WT IP₃R1 resulted in inhibition of Ca²⁺ signal amplitude of and a decreased percentage of responding cells; thus, confirming the mutation was dominant-negative (Gerber et al., 2016). However, as the experiment was done in conditions where K2653del. was in excess of WT IP₃R1, questions remain about the number of mutant subunits required to exert the dominant negative effect that was observed.

C-terminal IP₃R2 Mutations

Only two mutations have been identified in IP₃R2, with both being found in the C-terminal channel domain. Residue S2508 in the channel domain of IP₃R2 has been identified as a potential candidate mutation (S2508L) in causing SS, while mutation of residue G2498 to serine (G2498S) has been shown to result in anhidrosis (Prasad et al., 2016, Klar et al., 2014). Anhidrosis – absence of perspiration in the presence of an appropriate stimulus such as heat, exercise, or pharmacological agonists – results in the inability to lower core body temperature and thus leads to increases in heart rate, skin temperature, and ear canal temperature (Fealey and Hebert, 2012, Klar et al., 2014). WES revealed the G2498S missense mutation to be the most likely candidate causative of anhidrosis in five individuals of a consanguineous family (n=10) who exhibited normal morphology and number of sweat glands (Klar et al., 2014). While symptomatic individuals were homozygous for the mutation, heterozygous family members (n=5) were asymptomatic. This led clinicians to suspect an autosomal recessive pattern of inheritance (Klar et al., 2014).

Absence of sweat secretion in the affected individuals suggests that IP₃Rs play an important role in this process. It has been previously shown that intracellular Ca²⁺ release in clear cells, the major secretory cell type of eccrine sweat glands, is a critical even for the process of transporting the required ions and water that compose the isotonic precursor of sweat into the secretory lumen of the gland (Wilke et al., 2007, Cui and Schlessinger, 2015, Wilson and Metzler-Wilson, 2015). The G2498S mutation is located in the selectivity filter (GGGVG) of the pore-forming domain of IP₃R2, the predominant isoform of IP₃R in the sweat gland. IP₃R-null DT40 cells expressing the G2498S mutation failed to release Ca from the store in response to an appropriate stimulation (Klar et al., 2014). This result is consistent with previous work published by Schug *et al.* in which the analogous rat IP₃R1 residue (G2456) was also non-functional when mutated to Ala (Schug et al., 2008). As there was no difference in Castore content between WT and mutant cells, these results suggest that a

small change in the size of the residue at this location may result in loss of IP₃R2 channel function (Klar et al., 2014). Inhibition of Ca²⁺ release via loss of IP₃R2 channel function, as seen in cells expressing the G2498S mutant, would prevent the downstream process of sweat induction from occurring.

While Klar *et al.* characterize channel function in the context of an individual homozygous for the G2498S mutation, channel function in the context of heterozygous individuals is not addressed. One explanation for the asymptomatic phenotype observed in these individuals is compensation of mutant IP₃R2 function by WT IP₃R. Klar *et al.* demonstrated that compensation likely takes place in *itpr2*-null mice in which Ca²⁺ signaling was reduced only 40–50% compared to *itpr2*-expressing mice because IP₃R1 and IP₃R3 were still expressed (Klar et al., 2014). In patient clear cells, immunohistochemical analysis revealed IP₃R2 is the predominant isoform of IP₃R expressed in human eccrine sweat glands with weak expression of IP₃R3 and complete absence of IP₃R1 (Klar et al., 2014). The difference in expression of IP₃R1 and IP₃R3 isoforms between mouse and human sweat glands likely explains why *itpr2*-null mice maintain some level of Ca²⁺ release, while G2498S mutant tetramers have no observable Ca²⁺ release. In the asymptomatic heterozygous individuals, however, it is possible that the weak expression of IP₃R3 and remaining WT IP₃R2 result in sufficient Ca²⁺ release to trigger the downstream processes required for sweat induction. Functionally, we would predict that IP₃R2 mutant heterotetramers, present in the heterozygous individuals may produce a Ca²⁺ release signal between that of a WT IP₃R2 homotetramer and a mutant G2498S homotetramer.

Conclusion

Missense, nonsense, insertion/deletion, and splice site mutations of the IP₃R have been reported to result in predominantly CNS and exocrine related diseases. A caveat of these reports investigating the disease-associated mutations is that the majority have been identified through correlation of clinical symptoms with whole exome sequencing. It is unclear if mutations that result in large deletions/truncations, such as those associated with SCA15 and GS, or even missense mutations are expressed and degraded or even expressed at all. Generally, the SCA15-associated mutations are considered to result in haploinsufficiency; however, questions remain about effect of expression of these truncated domains, particularly because they would likely maintain IP₃ binding capabilities. While few mutations have been observed to be present at the protein level, we generally assume that receptors with small missense or insertion/deletion mutations are expressed. While several disease-associated mutations in IP₃R have been suggested, structural and functional effects of these mutations have not been characterized. Clues from the null mouse models, domain location, and stoichiometry of the receptor may be used predict the mechanism behind effect of mutation on receptor function disease. However, as inter- and intrafamilial variability make it difficult to correlate genotype and phenotype of the individual mutations, detailed structural and functional analysis of mutant homotetramers and heterotetramers, together with generation of animal models may be necessary to fully understand the mechanism and functional effects of these mutations.

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Figure 1. IP₃R Structure.

Ribbon structure of two IP₃R1 subunits as determined by cryo-EM (PDB: 3JAV). The four domains of the IP₃R (suppressor, ligand binding, regulatory, and c-terminal), as well as other important structural landmarks, are indicated.

Figure 2. IP₃R Disease-associated mutations.

The location of IP₃R mutations associated with ataxia (dark blue), head and neck squamous cell carcinoma (black), Sézary Syndrome (purple), Gillespie Syndrome (red), generalized anhidrosis (green), and pontine cerebellar hypoplasia (light blue) are indicated on a linear version of the IP₃R structure. As illustrated, the IRBIT binding domain spans the IP₃R ligand binding domain and a fraction of the regulatory domain, while the CARP binding domain is located solely in the regulatory domain.

Table 1.Molecular characteristics of disease-associated IP₃R mutations.

Mutation ^{#1}	Original Residue (Base Change) & Reference Sequence ^{#2}	Isoform	Domain	Conserved ^{#3}	Diagnosis	Individuals Affected	Reference
R36C	R36C (c.10C>T) in NM_001168272.1	<i>Itpr1</i>	SD	Yes	SCA29	Mother & 2 Children (inherited)	Casey <i>et al.</i> 2017
R64H	R64H (chr6:33623573G>A)	<i>Itpr3</i>	SD	Yes	HNSCC	1 Individual	Hedberg <i>et al.</i> 2016
A95T	A95T ^{#4}	<i>Itpr1</i>	SD	Yes	SS	1 Individual	Prasad <i>et al.</i> 2016
R149L	R149L (chr6:33626523G>T)	<i>Itpr3</i>	SD	Yes	HNSCC	1 Individual	Hedberg <i>et al.</i> 2016
R241K	R241K (c.722G>A) in NM_001168272.1	<i>Itpr1</i>	LBD	Yes	Autosomal dominant NPCA	Mother & Daughter (inherited)	Barresi <i>et al.</i> 2016
T267M	T267M (c.800C>T) in NM_001099952.2	<i>Itpr1</i>	LBD	Yes	Sporadic infantile-onset SCA	1 Sporadic Case	Ohba <i>et al.</i> 2013 Sasaki <i>et al.</i> 2015
					SCA29	2 Sporadic Cases	Zambonin <i>et al.</i> 2017
T267R	T267R (c.800C>G) in NM_001099952.2	<i>Itpr1</i>	LBD	Yes	Sporadic infantile-onset SCA	1 Sporadic Case	Sasaki <i>et al.</i> 2015
R269G	R269G (c.805C>G) in NM_001099952.2	<i>Itpr1</i>	LBD	Yes	SCA29	1 <i>de novo</i> case	Zambonin <i>et al.</i> 2017
R269W	R269W (c.805C>T) in NM_001168272.1 & NM_001099952.2	<i>Itpr1</i>	LBD	Yes	Autosomal dominant NPCA	Mother & 2 Sons (inherited)	Barresi <i>et al.</i> 2016
					SCA29	1 <i>de novo</i> case	Zambonin <i>et al.</i> 2017
					Ataxic Cerebral Palsy	Mother & Daughter (inherited)	Das <i>et al.</i> 2017
S277I	S277I (c.830G>T) in NM_001099952.2	<i>Itpr1</i>	LBD	Yes	SCA15 (with early-onset)	1 Sporadic Case	Fogel <i>et al.</i> 2014
					Sporadic infantile-onset SCA	1 Sporadic Case	Sasaki <i>et al.</i> 2015
					SCA29	1 <i>de novo</i> case	Zambonin <i>et al.</i> 2017
K279E	K279E (c.835A>G) in NM_001099952.2	<i>Itpr1</i>	LBD	No	SCA29	1 <i>de novo</i> case	Zambonin <i>et al.</i> 2017
A280D	A280D (c.839C>A) in NM_001168272.1	<i>Itpr1</i>	LBD	Yes	Autosomal dominant NPCA	1 <i>de novo</i> case	Barresi <i>et al.</i> 2016
Exon 14 Splice Mutation	Exon 14 (c.1207-2A-T)	<i>Itpr1</i>	LBD	Yes	Autosomal dominant CNPCA	4 Related Individuals (inherited)	Wang <i>et al.</i> 2017

Mutation #1	Original Residue (Base Change) & Reference Sequence #2	Isoform	Domain	Conserved #3	Diagnosis	Individuals Affected	Reference
	in <i>itpr1</i>						
K417_418Ins	K417_418Ins (c.1252-1G>T) in NM_001099952.2	<i>Itpr1</i>	LBD	No	SCA29	1 <i>de novo</i> case	Zamboni <i>et al.</i> 2017
V494I	V494I (c.1480G>A) on NG_016144.1	<i>Itpr1</i>	LBD	Yes	SCA15	1 Individual	Ganesamoorthy <i>et al.</i> 2009
E512K	E497K (c.1889G>A) in NM_001168272.1	<i>Itpr1</i>	LBD	No	Autosomal dominant NPCA	1 <i>de novo</i> case	Barresi <i>et al.</i> 2016
T594I	T594I (c.1781C>T) in NM_001099952.2	<i>Itpr1</i>	R/C	Yes	Sporadic infantile-onset SCA	1 Sporadic Case	Sasaki <i>et al.</i> 2015
N602D	N602D (c. 1804A>G) in NM_001099952.2 & (c.1759A>G) in NM_001168272.1	<i>Itpr1</i>	R/C	Yes	Autosomal Dominant CNPCA/SCA29	4 Related Individuals (inherited)	Huang <i>et al.</i> 2012 Zamboni <i>et al.</i> 2017
R728*	R728* (c.2182C>T) in NM_001099952.2	<i>Itpr1</i>	R/C	No	Ataxic Cerebral Palsy	1 <i>de novo</i> case	Parolin Schnekenberg <i>et al.</i> 2015
N984fs	N984fs (c.2952_2953insTATA) in NM_001099952.2	<i>Itpr1</i>	R/C	No	GS + Cardiovascular Symptoms	1 <i>de novo</i> case	Gerber <i>et al.</i> 2016
P1074L	P1059L (c.8581C>T) in NM_002222.5	<i>Itpr1</i>	R/C	No	SCA15	2 Siblings	Carvalho <i>et al.</i> 2017
T1386M	T1386M (c.4157C>T) in NM_001099952.2	<i>Itpr1</i>	R/C	No	SCA29	Multiple Individuals (inherited)	Hara <i>et al.</i> 2008
S1493D	S1487D (c.4459_4460delinsGA) in NM_001168272.1	<i>Itpr1</i>	R/C	Yes	Ataxic Cerebral Palsy	1 <i>de novo</i> case	Zamboni <i>et al.</i> 2017
V1553M	V1553M (c.4657G>A) in NM_001099952.2	<i>Itpr1</i>	R/C	No	Autosomal Dominant CNPCA/SCA29	1 <i>de novo</i> case	Parolin Schnekenberg <i>et al.</i> 2015
Q1558*	Q1558* (c.4672C>T) in NM_001099952.2			No	SCA29	20 Related Individuals (inherited)	Dudding <i>et al.</i> 2004 Huang <i>et al.</i> 2012 Zamboni <i>et al.</i> 2017
L1787P	L1827P (c.5360T>C) in P29994.2	<i>Itpr1</i>	R/C	Yes	SCA29 (Autosomal recessive)	5 Related Individuals (inherited)	Shadrina <i>et al.</i> 2016
E2061G	E2094G (c.6281A>G) in NM_001168272.1	<i>Itpr1</i>	R/C	Yes	GS	1 <i>de novo</i> case	Gerber <i>et al.</i> 2016
						6 Homozygous Cases & 5 Heterozygous Cases (inherited)	Klar <i>et al.</i> 2017
						Mother & Daughter (inherited)	McEntagart <i>et al.</i> 2016

Mutation #1	Original Residue (Base Change) & Reference Sequence #2	Isoform	Domain	Conserved #3	Diagnosis	Individuals Affected	Reference
E2061Q	E2094Q (c.6280G>C) in NM_001168272.1	<i>Itpr1</i>	R/C	Yes	GS	1 <i>de novo</i> case	McEntagant <i>et al.</i> 2016
G2102Valfs5*/A2221Valfs23*	G2102Valfs5*/A2221Valfs23* (c.6366+3A>T/ c.6664+5G>T) in NM_001099952.2	<i>Itpr1</i>	R/C	No	GS	1 <i>de novo</i> case	Gerber <i>et al.</i> 2016
S2454F	S2439F #5	<i>Itpr1</i>	5 th -6 th TM Domains	No	SS	1 Individual	Prasad <i>et al.</i> 2016
T2490M	T2523M (c.7568C>T) #6	<i>Itpr1</i>	5 th -6 th TM Domains	Yes	Unassigned SCA (Progressive optic atrophy, ataxia, etc.)	1 Individual	Valencia <i>et al.</i> 2015
G2506R	G2506R (c.7516G>A) in NM_001099952.2 G2539R (c.7615G>A/C) in NM_001168272.1	<i>Itpr1</i>	Selectivity Filter	Yes	GS (c.7615G>A)	5 <i>de novo</i> cases	McEntagant <i>et al.</i> 2016
G2498S #7	G2498S (c.7492G>A) in NM_002223.2	<i>Itpr2</i>	Selectivity Filter	Yes	Anhidrosis	1 <i>de novo</i> case 1 inherited case	Zambonin <i>et al.</i> 2017
S2508L #8	S2508L (chr12:26553068G>A)	<i>Itpr2</i>	TM	No	SS	1 Individual	Prasad <i>et al.</i> 2016
V2541A	V2574A (c.7721T>C) in NM_001168272	<i>Itpr1</i>	6 th TM Domain	Yes	Molecularly unassigned SCA	Mother & Daughter (inherited)	Hsiao <i>et al.</i> 2017
N2543I	N2576I (c.7727A>T) in NM_001168272.1	<i>Itpr1</i>	6 th TM Domain	Yes	GS	1 <i>de novo</i> case	Dentici <i>et al.</i> 2017
G2547A	G2547A (chr3:4856819G>C)	<i>Itpr1</i>	6 th TM Domain	Yes	SCA29	1 <i>de novo</i> case	Gonzaga-Jauregui <i>et al.</i> 2015
I2550N	I2550N (c.7649T>A) in NM_001099952.2	<i>Itpr1</i>	6 th TM Domain	Yes	PCH	1 <i>de novo</i> case	Van Dijk <i>et al.</i> 2016
I2550T	I2550T (c.7649T>C) in NM_001099952.2	<i>Itpr1</i>	6 th TM Domain	Yes	SCA29	2 sporadic individuals	Zambonin <i>et al.</i> 2017
T2552P	T2585P (c.7753C>A) in NM_001168272.1	<i>Itpr1</i>	6 th TM Domain	Yes	MICPCH	1 <i>de novo</i> case	Hayashi <i>et al.</i> 2017
F2553L	F2553L (c.7659T>G) in NM_001099952.2	<i>Itpr1</i>	6 th TM Domain	Yes	GS	1 <i>de novo</i> case	Gerber <i>et al.</i> 2016

Mutation ^{#1}	Original Residue (Base Change) & Reference Sequence ^{#2}	Isoform	Domain	Conserved ^{#3}	Diagnosis	Individuals Affected	Reference
K2563del.	K2563del. in NM_001099952.2 K2596del. (c.7786_7788delAAG) in NM_001168272.1	<i>Itp1</i>	LNK Domain	Yes	GS	4 <i>de novo</i> cases	McEntagart <i>et al.</i> 2016 Gerber <i>et al.</i> 2016
					SCA29 with Aniridia	1 <i>de novo</i> individual	Zamborini <i>et al.</i> 2017
					GS	1 <i>de novo</i> case	Dentici <i>et al.</i> 2017

Abbreviations: CNPCA: Congenital non-progressive cerebellar ataxia; GS: Gillespie Syndrome; HNSCC: Head & Neck Squamous Cell Carcinoma; LBD: Ligand Binding Domain; LNK: Linker Domain; MICPCH: microcephaly with pontine and cerebellar hypoplasia; NPCA: non-progressive congenital ataxia; PCH: pontocerebellar hypoplasia; R/C: Regulatory and Coupling Domain; SCA: Spinocerebellar Ataxia; SD: Suppressor Domain; SS: Sézary Syndrome; TM: Transmembrane.

Footnotes.

- ^{#1} Mutations reported in the reference sequence of NP_001093422.2 (IP3R1), NP_002214.2 (IP3R2), or NP_002215.2 (IP3R3). If not originally reported in this reference sequence, multiple sequence alignment was utilized to find residue in appropriate reference sequence.
- ^{#2} This indicates the residue, base change, and reference sequence in which the mutation was originally discovered.
- ^{#3} This answers if residue is conserved among human, rat, and mouse sequences of all three isoforms.
- ^{#4} No chromosomal, nucleotide, or protein reference source was reported for this mutation.
- ^{#5} Residue was originally reported as S2439F in *itpr1* without reference sequence. Assumption was made that reference sequence was human (NP_002213.5).
- ^{#6} Reference sequence of residue was not reported. Based on multiple sequence alignment, reference sequence was assumed to be in NM_001168272.1 based on appropriate amino acid residue present.
- ^{#7} Mutation reported in IP3R2 (reference sequence NM_002223.2). Analogous IP3R1 mutation is G2507S in reference sequence NP_001093422.2.
- ^{#8} Mutation reported in IP3R2 (reference sequence NM_002223.2). Analogous IP3R1 mutation is S2517L in reference sequence NP_001093422.2.