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Diseases caused by mutations in *ORAI1* and *STIM1*

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Abstract

Ca^{2+} release-activated Ca^{2+} (CRAC) channels mediate a specific form of Ca^{2+} influx called store-operated Ca^{2+} entry (SOCE) that contributes to the function of many cell types. CRAC channels are formed by ORAI1 proteins located in the plasma membrane, which form its ion-conducting pore. ORAI1 channels are activated by stromal interaction molecule (STIM) 1 and STIM2 located in the endoplasmic reticulum. Loss- and gain-of-function gene mutations in *ORAI1* and *STIM1* in human patients cause distinct disease syndromes. CRAC channelopathy is caused by loss-of-function mutations in *ORAI1* and *STIM1* that abolish CRAC channel function and SOCE; it is characterized by severe combined immunodeficiency (SCID)-like disease, autoimmunity, muscular hypotonia, and ectodermal dysplasia, with defects in dental enamel. The latter defect emphasizes an important role of CRAC channels in tooth development. By contrast, autosomal dominant gain-of-function mutations in these genes result in constitutive CRAC channel activation, SOCE, and increased intracellular Ca^{2+} levels that are associated with an overlapping spectrum of diseases, including non-syndromic tubular aggregate myopathy (TAM) and York platelet and Stormorken syndromes, two syndromes defined, besides myopathy, by thrombocytopenia, thrombopathy, and bleeding diathesis. The fact that myopathy results from loss- and gain-of-function mutations in *ORAI1* and *STIM1* highlights the importance of CRAC channels for Ca^{2+} homeostasis in skeletal muscle function. The cellular dysfunction and clinical disease spectrum observed in mutant patients provide important information about the molecular regulation of ORAI1 and STIM1 proteins and the role of CRAC channels in human physiology.

Keywords

CRAC channel; channelopathy; STIM1; ORAI1; SOCE; calcium; Ca^{2+}; disease; muscular hypotonia; tubular aggregate myopathy; skeletal muscle; Stormorken syndrome; York platelet syndrome; autoimmunity; platelets; thrombocytopenia; mutation; enamel; ameloblast

CRAC channels composed of ORAI1 subunits mediate Ca^{2+} influx from the extracellular space to the cytoplasm in a large variety of cell types (Fig. 1). They are activated by STIM1 and STIM2 proteins located in the membrane of the endoplasmic reticulum (ER). Both

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STIM proteins sense the Ca^{2+} concentration in ER via an EF hand Ca^{2+} binding domain and bind to ORAI channels upon Ca^{2+} depletion from ER stores. The interaction of ORAI1 and STIM proteins via their respective cytoplasmic domains results in the opening of CRAC channels and store-operated Ca^{2+} entry (SOCE), which is critical for the function of many cells and tissues. The identification of a loss-of-function (LoF) mutation in what was then the novel gene *ORAI1* in CRAC channel-deficient patients was critical for establishing ORAI1 as the long elusive CRAC channel.[1] The phenotypes of these patients and those with null mutations in *STIM1* have subsequently defined the novel disease entity *CRAC channelopathy*, which has helped define the role of CRAC channels in humans (Fig. 2). More recently, a variety of gain-of-function (GoF) mutations in *ORAI1* and *STIM1* were identified in patients afflicted by either non-syndromic TAM or Stormorken syndrome, a rare disorder characterized predominantly by bleeding diathesis with thrombocytopenia, TAM, miosis, and several other symptoms (Fig. 2). The mutations causing Stormorken syndrome and TAM have in common that they result in constitutive CRAC channel activation and Ca^{2+} influx. There is some phenotypic overlap between CRAC channelopathy caused by loss of SOCE, which is dominated by immunodeficiency, autoimmunity, and severe dental enamel defects, and Stormorken syndrome due to constitutive SOCE, which primarily manifests with mild bleeding diathesis. However, both CRAC channelopathy and Stormorken syndrome are associated with distinct forms of myopathies that are characterized by muscular hypotonia and TAM, respectively.

In this review, we provide an overview of the molecular regulation of ORAI1 and STIM1 proteins and discuss the mechanisms by which null and LoF mutations interfere with CRAC channel function. We discuss clinical phenotypes with a particular focus on immunodeficiency, muscular hypotonia, and abnormal dental enamel formation. We also examine the molecular mechanisms by which different GoF mutations in *ORAI1* and *STIM1* result in constitutive CRAC channel activation and describe the clinical phenotypes of Stormorken syndrome and TAM, as well as the cellular pathophysiology underlying these diseases.

Molecular regulation of CRAC channels by ORAI1 and STIM1

SOCE is a conserved Ca^{2+} signaling pathway that is activated after ligand binding to cell surface receptors, e.g. immunoreceptors such as the T cell receptor (TCR) or G-protein coupled receptors, and causes, via the depletion of Ca^{2+} from intracellular stores, activation of CRAC channels (Fig. 1). Stimulation of cell surface receptors results in the activation of phospholipase C and the production of inositol 1,4,5-triphosphate (IP_3), a second messenger that binds to IP_3R receptors located in the membrane of the ER. IP_3Rs are non-selective ion channels whose opening results in Ca^{2+} efflux into the cytosol following the steep Ca^{2+} concentration gradient between the ER ($[Ca^{2+}]_{ER}$ ~0.5–1 mM) and cytoplasm ($[Ca^{2+}]_{Cyt}$ ~0.1 μM). In skeletal muscle, Ca^{2+} efflux from the sarcoplasmic reticulum (SR) is mediated by ryanodine receptor 1 (RYR1), which opens after physical coupling with L-type Ca^{2+} channels in the sarcolemma.

Efflux of Ca^{2+} from either the ER or the SR results in an increase in $[Ca^{2+}]_{Cyt}$, which contributes to activation of many Ca^{2+} dependent signaling molecules and transcriptional

regulators, and in skeletal muscle, Ca^{2+} efflux from the SR is the main source of Ca^{2+} required for muscle fiber contraction. Ca^{2+} release from the ER decreases $[Ca^{2+}]_{ER}$, triggering the activation of STIM1 and STIM2. Both proteins sense the depleted-Ca^{2+} filling state of the ER and can bind to ORAI1, resulting in CRAC channel opening and SOCE. The CRAC channel is the prototypical store-operated Ca^{2+} channel having unique biophysical properties that were defined long before the discovery of ORAI1 and STIM1 as its molecular constituents.[2, 3, 4] These properties include activation upon ER store depletion, high Ca^{2+} selectivity, small unitary conductance, and Ca^{2+}-dependent potentiation and inactivation.[5] The terms “CRAC channel” and “ORAI1” are interchangeably used in this review and refer to the functional channel and the channel-encoding gene/protein, respectively.

Below, we provide a brief introduction to ORAI1 and STIM1 and the mechanisms by which they mediate SOCE, which is necessary to understand the effects of *ORAI1* and *STIM1* automosomal recessive (AR) and autosomal dominant (AD) mutations on CRAC channel function in human patients.

STIM1

STIM1 is a single-pass transmembrane Ca^{2+} binding protein residing in the membrane of the ER (Fig. 1). It has an ER luminal N-terminus and a cytoplasmic C-terminus, with which it responds to depletion of ER Ca^{2+} stores and binds to ORAI Ca^{2+} channels, respectively. STIM2 is a homologue of STIM1, which also induces SOCE upon ER store depletion, but its activation kinetics, affinity for Ca^{2+} and tissue expression differ from that of STIM1. Because mutations in *STIM1*, but not *STIM2*, have been described in human patients, we will focus on STIM1 in this section. Opening of IP_3Rs and RyR channels reduces $[Ca^{2+}]_{ER.}$ Depletion of ER Ca^{2+} can also be achieved by stimulation with pharmacological agents such as ionomycin (a Ca^{2+} ionophore in the ER membrane) or thapsigargin (blocks Ca^{2+} reuptake into the ER via SERCA pumps, which compensates for the slow Ca^{2+} leak from the ER). Reduction of $[Ca^{2+}]_{ER}$ results in dissociation of Ca^{2+} from a canonical EF-hand (cEFh) in the ER luminal N-terminus of STIM1, which acts as a Ca^{2+} sensor. The cEFh is located next to a non-canonical EFh (ncEFh) that does not bind Ca^{2+}, and a sterile alpha motif (SAM). The EFh–SAM domains assume a closed conformation in the Ca^{2+} bound state, i.e. in cells with filled Ca^{2+} ER stores. Upon Ca^{2+} depletion from the ER, the EFh–SAM domain undergoes a conformational change exposing hydrophobic residues and allowing the N-termini of neighboring STIM1 molecules to dimerize.[6, 7] Genetically engineered mutations in acidic residues (D76, D84, or E87) of the cEFh result in constitutive activation of STIM1 and CRAC channels by mimicking the Ca^{2+}-depleted state of the ER.[8, 9, 10] Mutation of D84 in murine STIM1 was shown to cause constitutive Ca^{2+} influx in platelets and a fatal bleeding disorder in mice.[11] Inherited mutations in the EFh of STIM1 in human patients result in constitutive SOCE and cause TAM,[12, 13, 14] as will be discussed in detail below.

Dimerization of luminal EFh–SAM domains promotes the association of cytoplasmic C-termini of neighboring STIM1 molecules.[15] This association is mediated by the first of three cytosolic coiled-coil (CC1) domains (Fig. 1A). Two additional coiled-coil domains, CC2 and CC3, form the bulk of a functional domain that is alternatively called the CRAC

activation domain (CAD),[16] the STIM1–ORAI activating region (SOAR), [17] or the coiled-coil domain b9 (CCb9).[18] Expression of CAD (SOAR, CCb9) as a soluble protein fragment in cells is sufficient to activate CRAC channels and to induce constitutive Ca^{2+} influx via its binding to ORAI1.[16, 17, 18] Besides binding to ORAI1, CAD mediates protein–protein interactions among neighboring STIM1 molecules.[19] In cells with replete Ca^{2+} ER stores, the C-terminus assumes a closed, inactive configuration, which is mediated by intramolecular protein interactions between amino acids in the CC1 domain and residues in CAD.[15, 20, 21] ER store depletion was suggested to promote association of CC1 domains in neighboring STIM1 proteins, weakening the binding of CC1 to CAD and resulting in the unfolding of the STIM1 C-terminus.[15] The extended conformation of the STIM1 C-terminus is critical for its ability to bind to ORAI1 in the plasma membrane (PM) and to activate the CRAC channel because it confers the ability to bridge the gap between the ER and the PM in which ORAI1 is located. In addition, the extended conformation of the STIM1 C-terminus exposes the CAD, thereby allowing it to mediate protein–protein interactions between neighboring STIM1 molecules,[19] and releases the lysine-rich polybasic domain at the C-terminal end of STIM1, which promotes the translocation of STIM1 to ER–PM junctions by interacting with negatively charged phospholipids in the PM. In ER–PM junctions, STIM1 assembles into multiprotein complexes, puncta, into which ORAI1 channels are recruited via interactions of the STIM1 CAD with a CC domain in the C-terminus of ORAI1 and the ORAI1 N-terminus.

ORAI channels

ORAI1 is a tetraspanning plasma membrane protein that forms the ion-conducting pore of the CRAC channel (Fig. 1). It has two structurally conserved homologues, ORAI2 and ORAI3, which can form channels when overexpressed, although the physiological roles of endogenous proteins are not well understood. Since disease-causing mutations have so far been described only in *ORAI1* and most research has been performed on this homologue, we will focus on ORAI1. The gene encoding ORAI1 was identified by forward genetic screens and linkage analysis in human patients with defects in CRAC channel function.[1, 22, 23] It is ubiquitously expressed, consistent with the observation of CRAC channel currents and SOCE in many cell types and tissues.[24] ORAI1 has four alpha-helical transmembrane domains (M1–M4), two extracellular loops, and intracellular N- and C-termini that mediate the interaction with STIM1, STIM2, and potentially other regulatory proteins (reviewed in Ref. 25). M1 lines the ion conduction pathway in the pore of ORAI1 and contains several amino acid residues that define the biophysical properties of the channel. E106[a] is located towards the outer end of the pore and is responsible for Ca^{2+} binding and the high Ca^{2+} selectivity of the CRAC channel.[26, 27] Mutation of E106 to A or Q abolishes CRAC currents, and mutation to D, which preserves the negative charge of E at this position, makes the channel non-selective.[22, 28, 29] R91 is located at the narrowest part of the pore close to its inner mouth, and mutation of R91 to W causes CRAC channelopathy in patients, likely by blocking the ion conduction pathway.[1, 30, 31]

[a]All amino acid residue numbers in this review refer to human proteins unless otherwise noted.

Functional CRAC channels are multimers of ORAI1 proteins. They were initially thought to be tetramers,[32, 33, 34] but this view changed when the crystal structure of *Drosophila* Orai revealed a hexameric channel complex, the central ion pore of which is composed of a ring of six M1 domains, one from each Orai subunit, that span the plasma membrane.[31] Six M2–M3 domains form a concentric ring around the inner circle of M1 domains and are in turn surrounded by an outer ring of M4 helices, which extend into the cytoplasm. The crystal structure confirmed the predicted ring of E106 residues at the outer mouth of the channel pore that constitute the Ca^{2+} selectivity filter, and the pore-lining residues in M1 domains identified previously by other methods.[26, 27] One surprising aspect of the Orai structure was the length of the pore-forming M1 helices and their protrusion into the cytoplasm. The M4 helix of *Drosophila* Orai, which is furthest away from the channel pore, also extends far into the cytoplasm. A conserved hinge region in the middle of M4 positions the cytoplasmic portion of M4 in such a way that it can interact with the extended M4 helix of another Orai subunit. This interaction involves two hydrophobic residues, analogous to L273 and L276 in human ORAI1, whose mutation was shown to disrupt the interaction of ORAI1 with STIM1, and thus CRAC channel activation.[35, 36] Further details of the molecular regulation of CRAC channels and ORAI1 and STIM1 protein function can be found in Refs. [25, 37, 38].

Loss-of-function and null mutations in *ORAI1* and *STIM1*

To date 10 different LoF or null mutations in *ORAI1* and *STIM1* have been described in patients (Table 1 and Fig. 3); all are transmitted in an AR manner and result in strongly impaired or abolished CRAC channel function and SOCE in immune cells (T, B, NK cells) and non-immune cells (e.g. fibroblasts and platelets). The lack of SOCE associated with these mutations suggests that ORAI1 and STIM1 proteins are the main mediators of SOCE in these cells, with little or no contribution from the homologs ORAI2, ORAI3, and STIM2. The clinical phenotype of ORAI1- and STIM1-deficient patients (described in detail below) is uniform and characterized by severe combined immunodeficiency (SCID)-like disease with recurrent and chronic infections, autoimmunity, ectodermal dysplasia, abnormal enamel, and muscular hypotonia (Table 1 and Fig. 3).

LoF mutations in ORAI1

Most LoF mutations in *ORAI1* and *STIM1* are null mutations that abolish protein expression due to frameshifts, splice site defects or substitution of single amino acids in the transmembrane domains of ORAI1 (Table 1 and Fig. 3A). The latter mutations abolish ORAI1 protein expression, likely by interfering with proper folding of the alpha helical transmembrane domains, reducing protein stability. Examples of null mutations are STIM1 E128fsX9 and ORAI1 A88SfsX25. Stimulation of fibroblasts from these patients with thapsigargin, which causes Ca^{2+} depletion from ER stores, failed to induce SOCE.[39, 40] By contrast, a few LoF mutations have been identified that leave ORAI1 and STIM1 protein expression intact. The ORAI1 p.R91W mutation was identified by linkage analysis in an extended consanguineous family and has led to the discovery of *ORAI1* as the gene encoding the CRAC channel; the mutation abolishes CRAC currents and SOCE by impairing ORAI1 channel function.[1, 41] The crystal structure of *Drosophila* Orai showed that K163, the equivalent of R91 in human ORAI1, is located in the elongated pore of the

channel, its side chain protruding into the lumen and constricting the channel's pore diameter. Substitution of R with the large aromatic W side chain at this position blocks the passage of Ca^{2+} ions through the pore.[26, 30, 31] Heterozygous expression of the p.R91W mutation has a dominant negative effect on CRAC channel function, which is likely due to mutant proteins being incorporated into the hexameric channel structure. Together with the neighboring K87 and R83 residues, it was proposed that R91, K87, and R83 participate in anionic gating of the CRAC channel.[31] Mutation of R91 to tryptophan introduces a large aromatic side chain at a narrow part of the channel pore, presumably preventing the channel from opening.

LoF mutations in STIM1

Two missense mutations in *STIM1* result in moderate reduction in protein expression but almost complete loss of SOCE, indicating that the mutations mainly impair STIM1 function (Table 1 and Fig. 3A). The STIM1 p.P165Q mutation is located in the ER luminal SAM domain [42] close to two residues in the hydrophobic core of the SAM domain, L167 and T172. Mutation of both residues was shown to result in constitutive aggregation of EF-SAM domains and thus potentially STIM1 activation.[7] By contrast, P165 is pointed toward the EF-hand domain in the STIM1-NT structure, away from the SAM domain core. The P165Q mutation may interfere with the dimerization ability of EF-SAM domains, thereby impairing STIM1 activation and SOCE. Consistent with this speculation, a structure-based sequence alignment of the STIM1 SAM domain with SAM domains of other proteins shows that P165 is located close (i.e. within 2 amino acids) to residues that mediate protein homomerization.[7] The precise mechanism how the STIM1 p.P165Q mutation abolishes STIM1 function has not been investigated and awaits further investigation.

Another LoF mutation, STIM1 p.R429C, which abolishes SOCE and causes CRAC channelopathy, is located in the CAD (CCb9, SOAR), which has multiple roles in STIM1 function (Table 1; Figs. 3A and 4).[16, 17, 18, 19, 43, 44] STIM1 protein expression in patient B cells and fibroblasts was moderately reduced although SOCE in the patients' cells was abolished.[44, 45] Overexpression of wild-type STIM1 in the patient's cells fully restored SOCE. Defective SOCE in the patient's cells was not specific to the cysteine substitution in STIM1-R429C, and the formation of disulfide bonds as mutation of R429 to other amino acids similarly impaired SOCE. The R429C mutation exerts a dominant negative effect of STIM1 function and SOCE, which was apparent when mutant and wild-type STIM1 were co-overexpressed in the same cell resulting in impaired SOCE. The defects in STIM1 function caused by the R429C mutation are linked to the structural destabilization of the CC3 domain in which R429 is located. C-terminal STIM1 fragments in which R429 was mutated to C, A, or L showed reduced α-helicity and decreased temperature denaturation, indicating that R429 is critical for supporting a stable α-helical fold of CC3. Perturbation of this structure interferes with the ability of CC3 to mediate intra- and intermolecular protein interactions with STIM1 and ORAI1.

Careful molecular analysis of the effects of the R429C mutation revealed multiple important roles of this residue in regulating STIM1 function.[44] First, R429 controls the transition of STIM1 from a quiescent to an active conformational state (Fig. 4). In cells with filled Ca^{2+}

stores, the C-terminus of STIM1 is maintained in a folded, inactive conformation,[15, 21, 46, 47] and undergoes a conformational extension upon store depletion. The molecular mechanisms controlling the conformational extension are incompletely understood, but were proposed to involve hydrophobic[46] and electrostatic interactions[20, 21, 47] between CC1 and CC2 domains of STIM1-CT. The role of CC1 in maintaining STIM1-CT in a closed, inactive conformation has been demonstrated by mutating L251, which is a key residue to keep the STIM1 in an inactive configuration.[15, 46] The C terminus of mutant STIM1 p.R429C assumes an extended conformation, which results in constitutive localization of mutant STIM1 at ER–PM junctions. This translocation is mediated by the exposure of a polybasic domain at the C-terminal end of STIM1 that promotes its recruitment to the PM via interactions with negatively charged PM phospholipids. Deletion of the polybasic domain from mutant STIM1 p.R429C prevented its recruitment to the PM. The R429C mutation thus emphasizes an important role for CC3 in maintaining the inactive conformation of STIM1.[44]

Second, R429 is required for the oligomerization of the cytoplasmic STIM1 C-terminus after store depletion (Fig. 4). This oligomerization mediates the formation of STIM1 clusters, or puncta, at ER–PM junctions, which is a hallmark of SOCE.[8, 10] STIM1 oligomerization and puncta formation is followed by the recruitment of ORAI1 into puncta and subsequent CRAC channel activation.[48] Oligomerization of STIM1 is therefore widely considered to be required for efficient activation of CRAC channels. Considering that functional CRAC channels are hexamers[31] of ORAI1 subunits and that the STIM1:ORAI1 stoichiometry for optimal CRAC channel activation has been estimated to be 1:1[49] or 2:1,[50] 6–12 STIM1 molecules are required to activate the CRAC channel. This and a high degree of cooperativity required for STIM1 redistribution to ER–PM junctions and CRAC channel activation [51] suggest that STIM1 must first oligomerize to activate the channel. Interactions between mutant STIM1 p.R429C proteins were impaired in co-immunoprecipitation and blue native PAGE analysis, suggesting that R429 is critical for STIM1 oligomerization. STIM1 oligomerization can be detected by puncta formation in fluorescence microscopy. This process requires the CAD, as truncated STIM1 proteins lacking CAD failed to properly cluster upon ER store depletion.[19, 52] Mutant STIM1 p.R429C failed to form clusters and abolished FRET between fluorescently labeled STIM1 proteins. Instead, STIM1 p.R429C was constitutively localized in large, plaque-like structures at ER–PM junctions and failed to aggregate into discrete puncta upon store depletion due to its inability to oligomerize. It is noteworthy that the plaque-like distribution of STIM1 p.R429C was due to its constitutively extended C terminus and exposed polybasic domain.

A third essential function of R429 is to mediate binding of the STIM1 CAD to ORAI1, and thus CRAC channel activation (Fig. 4). The exact mechanism by which R429 regulates the binding of STIM1 to ORAI1 remains unclear at present and awaits high-resolution structural data of the STIM1-CC3–ORAI1 complex. It is conceivable that CC3 binds to the N terminus of ORAI1 instead of its C terminus, consistent with the reported binding of STIM1 to the N- and C-termini of ORAI1 for proper CRAC channel gating.[16, 53] Alternatively, CC3 may enhance the interaction of STIM1-CC2 and ORAI1 via intersubunit CC3–CC3′ interactions proposed by Ikura and colleagues.[54] In addition to interfering with the binding of STIM1 to ORAI1 directly, the R429C mutation abrogates SOCE by preventing STIM1

oligomerization, as discussed above. Altogether, the R429C mutation observed in patients with CRAC channelopathy provides compelling evidence that CC3 constitutes a multifunctional domain that controls several STIM1 functions.

A third LoF mutation in *STIM1* was recently described, STIM1 p.R426C.[55] Like R429, which is mutated in patients with full CRAC channelopathy syndrome due to abolished SOCE,[44, 45] R426 is located in CAD (CCb9, SOAR) in the C terminus of STIM1. The effects of the p.R426C mutation on STIM1 protein levels and SOCE have not been investigated in the patients. However, mutation of ectopically expressed STIM1 at position R426 to leucine resulted in impaired colocalization of STIM1 with ORAI1 and activation of CRAC channels in HEK293 cells. This defect is due to the stabilization of the closed structure of the STIM1-CT that prevents STIM1 activation.[46] Like its neighboring residues L416, L423, and R429 within the third CC domain (CC3), R426 appears to regulate the conformational state of STIM1-CT and thus the ability of STIM1 to activate the ORAI1 channel. The dental enamel defect in the patient with STIM1 p.R426C mutation is similar to that observed in patients with LoF or null mutations in STIM1, although the clinical phenotype is more limited and lacks immunodeficiency, autoimmunity and muscular hypotonia. It can be speculated that the STIM1 p.R426C mutation is a hypomorph and only incompletely impairs SOCE.

Clinical phenotype of LoF mutations in ORAI1 and STIM1

Immunodeficiency—The clinical phenotype of patients with null or LoF mutations in *ORAI1* or *STIM1* is dominated by life-threatening immunodeficiency that typically manifests in the first year of life and requires hematopoietic stem cell therapy (HSCT) to control the disease. Patients suffer from a relatively uniform disease syndrome, which is characterized by severe combined immunodeficiency (SCID)-like disease with recurrent and chronic infections, autoimmunity, ectodermal dysplasia, and muscular hypotonia (Table 1 and Fig. 2). The clinical phenotype of patients has been described in detail before.[56, 57, 58] Below we provide a summary, an update on new patients, and describe aspects of the disease not discussed in detail before.

ORAI1- and STIM1-deficient patients suffer from recurrent and severe infections with viral, bacterial and fungal pathogens (Table 1).[39, 40, 59] Almost all patients show increased susceptibility to herpes virus infections including those with cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and varicella zoster virus. One STIM1-deficient patient developed disseminated Kaposi sarcoma (KS) at two years of age due to infection with human herpes virus 8 (HHV8);[59, 60] she died from severe pulmonary infection three months later after the beginning of antitumor therapy. Patients frequently develop upper airway infections including otitis media, pneumonia, and refractory CMV pneumonitis, as well as gastroenteritis, urinary tract infections, meningitis, and sepsis. Several patients have presented with *Candida albicans* infections and two patients developed BCGitis after vaccination with *Bacille-Calmette Guerin* (BCG).[61] The spectrum and severity of infections, as well as their time of onset in the first year of life, are similar to SCID, although the development of T and B cells is largely normal in CRAC-deficient patients.

Of the 16 reported patients with LoF or null mutations in *ORAI1* and *STIM1*, 6 died from infection-related causes including sepsis, pneumonia, and encephalitis. Four patients survived after HSCT, 3 died from comorbid conditions or failed HSCT, and 3 survived without HSCT, presumably due to hypomorphic mutations (Table 1). Of the latter 3 patients, one was homozygous for a STIM1 p.R426C mutation and presented with a dental enamel defect as the leading symptom. Recurrent throat infections in this patient suggest a mild form of immunodeficiency;[55] no data on SOCE and STIM1 expression or an immunological evaluation are available for this patient. Recently Picard and colleagues reported two 21 and 8 years old siblings with STIM1 p.P165Q mutation who survived without HSCT despite apparent immunodeficiency.[42] One sibling presented with recurrent infections, including VZV infection, that were complicated by cellulitis at one year of age, Streptococcus pneumoniae sepsis at 4 years of age, and scabies crustosa at 20 and 21 years of age, which was complicated by Staphylococcus aureus plus S. pyogenes sepsis. His 8-year old sister developed bacterial pneumonia and sepsis in her first year, recurrent upper respiratory tract infections, and chronic dermatitis with superinfection. She is infection free since the start of intravenous Ig therapy at 4 years of age. Although the spectrum and severity of infections is similar to those in other ORAI1- and STIM1-deficient patients, the survival without HSCT led the authors to speculate that the mutant STIM1 p.P165Q protein is a functional hypomorph sufficient to mediate residual SOCE and immune function. This interpretation is consistent with normal numbers of T_{reg} cells and the lack of autoimmune cytopenias in both patients, which is common in other ORAI1- and STIM1-deficient patients. In mice, only complete absence of SOCE following deletion of *Stim1* and *Stim2*, but not either gene alone, impaired T_{reg} cell development and function.[62] It is noteworthy that individuals heterozygous for any of the null or LoF mutations in *ORAI1* or *STIM1* are asymptomatic and do not show any phenotypes associated with CRAC channelopathy. They are healthy despite reduced Ca^{2+} influx as was observed in both parents from patients with STIM1 p.R429C mutation,[45] and parents as well as other relatives of patients with ORAI1 p.R91W mutation.[1] These findings suggest that partial reduction of SOCE and CRAC channel function are well tolerated.

LoF or null mutations in *ORAI1* or *STIM1* cause SCID-like disease because, in contrast to bona fide SCID, the major populations of conventional αβ T cells, B cells, and NK cells are numerically intact. The distribution of $CD4^+$ and $CD8^+$ T cells, $CD16^+CD56^+$ NK cells, and $CD19^+$ or $CD20^+$ B cells in ORAI1- and STIM1-deficient patients is normal.[39, 40] Furthermore, the T cell receptor repertoire in STIM1-deficient patients is comparable to that in healthy controls.[40, 45] Normal T and B cell development and selection are consistent with similar observations in mice with complete or conditional deletion of *Orai1*, *Stim1*, and *Stim1/Stim2* genes, which have largely normal lymphocyte populations[62, 63, 64, 65, 66] and do not show significant differences in positive and negative selection of T cells.[67] Reduced numbers of lymphocyte populations in patients are limited to $Foxp3^+$ T_{reg} cells and iNKT cells, which likely account for some of the observed immune dysregulation, such as autoimmunity and impaired production of antigen-specific antibodies (see below).[45, 62, 67] Reduced numbers of $Foxp3^+$ T_{reg} cells have been observed in 2 unrelated patients with different *STIM1* mutations,[40, 45] and in patients with ORAI1 p.R91W mutation (S.F., unpublished). Nearly absent numbers of iNKT cells have been observed in patients with

STIM1 p.R429C,[45] STIM1 p.P165Q,[42] and ORAI1 p.R91W mutation (S.F., unpublished). It is not clear why the T_{reg} and NKT cell defects are not observed in other patients, but they may have been missed in analyses dating back to the 1990's. In addition, STIM1 p.R429C and ORAI1 p.R91W mutations are dominant negative and may suppress potential compensatory functions of STIM2, ORAI2, and ORAI3, respectively, thereby resulting in a more complete loss of SOCE than results from null mutations. This finding could indicate that small residual SOCE is sufficient for the development of T_{reg} and NKT cells, which is consistent with the absence of these cells only in STIM1/STIM2 double–deficient, but not STIM1-deficient mice.[67]

A consistent observation in virtually all ORAI1- and STIM1-deficient patients is the loss of naive $CD4^+CD45RA^+$ T cells and an increase in memory $CD4^+CD45RO^+$ T cells.[39, 40, 42, 68] A reduction in naive $CD8^+CD45RA^+$ and an increase in terminally differentiated and memory $CD8^+$ T cells were reported in two independent STIM1-deficient patients.[42, 45] Interestingly, one study that examined a STIM1-deficient patient found increased expression of inhibitory receptors associated with T cell exhaustion such as 2B4, PD-1, and Tim-3 on $CD8^+$ T cells.[45] The increased frequencies of effector and memory T cells indicate an activated T cell compartment. This could be due to the chronic viral infections, which is consistent with signs of $CD8^+$ T cell exhaustion and impaired T cell function. Increased expression of exhaustion markers was also observed on $CD8^+$ T cells from mice with T cell–specific deletion of *Stim1* and *Stim2* that had been infected with the Armstrong strain of lymphocytic choriomeningitis virus ($LCMV^{ARM}$).[69] STIM1/STIM2-deficient mice had significantly reduced LCMV-specific memory $CD8^+$ T cells and lacked memory T cell responses to reinfection with LCMV, suggesting that SOCE is critical for the maintenance of antigen-specific memory T cells. This defect was not due to an intrinsic role of SOCE in $CD8^+$ T cells but resulted from impaired $CD4^+$ T cell function in the absence of SOCE, and could be rescued by the presence of wild-type $CD4^+$ T cells. These data from STIM1/STIM2-deficient mice indicate that SOCE is essential for $CD4^+$ and $CD8^+$ T cell–mediated antiviral immunity and memory T cell responses. Importantly, acute infection with $LCMV^{ARM}$ became chronic in STIM1/STIM2-deficient mice in contrast to wild-type mice that cleared the infection,[69] which is consistent with the chronic viral infections observed in ORAI1- and STIM1-deficient patients.

The immunodeficiency in ORAI1- and STIM1-deficient patients is due to impaired function of T cells[61, 65, 70, 71] and NK cells [45, 72] (Table 1), whereas current evidence for impaired B cell and innate immune cell function is limited. Impaired T cell function was first identified in ORAI1-deficient patients by reduced T cell proliferation *in vitro* in response to stimulation with anti-CD3 antibodies, recall antigens such as tetanus toxoid, PPD, and candidin, and mitogens including PHA, ConA, and PMA/ionomycin.[40, 42, 45, 61, 71] In one recently described ORAI1-deficient patient, proliferation to tetanus and diphtheria antigens after a single dose of the diphtheria, tetanus, and pertussis vaccine was present and normal in response to mitogens.[68] A more consistent feature of all ORAI1- and STIM1-deficient T cells is a severe defect in the production of cytokines by $CD4^+$ and $CD8^+$ T cells, including production of IL-2, IL-4, IL-10, IFN-γ, TNF-α, and IL-17A.[45, 73] This defect was not limited to specific T helper (T_H) cell lineages or just cytokines, as hundreds of genes were

found to be dysregulated in $CD8^+$ T cells from an ORAI1-deficient patient [70] and $CD4^+$ T cells from STIM1-deficient mice.[74] The expression of pro-apoptotic factors is dependent on SOCE and decreased in T cells from a patient with ORAI1 p.R91W mutation[70, 74] and STIM1- and ORAI1-deficient mice.[74, 75] While T cell apoptosis after CD3 stimulation was reported to be normal in two STIM1-deficient patients,[40, 45] it was impaired in another STIM1-deficient patient.[42] Repeated stimulation of T cells from an ORAI1-deficient patient with anti-CD3/CD28 *in vitro* also showed a striking resistance to apoptosis,[74] which is consistent with impaired apoptosis in T cells from *Orai1*$^{-/-}$ mice.[75] Impaired T cell apoptosis likely contributes to the lymphoproliferative phenotype of ORAI1- and STIM1-deficient patients.

$CD8^+$ T cells and NK cells from ORAI1- and STIM1-deficient patients have impaired TNF-α and IFN-γ production.[45, 65, 72, 73] These cells also show severe defects in cytotoxic granule exocytosis and in cell-mediated cytotoxicity.[42, 45, 68, 72] A similar defect in cytotoxicity was observed in $CD8^+$ T cells from *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Cd4-Cre* mice[69, 76] and, less pronounced, in $CD8^+$ T cells and NK cells from *Orai1*^{R93W} knock-in mice (S.F., unpublished). These findings suggest that SOCE is an important regulator of cytotoxic T and NK cell function and may thus play a role in anti-tumor and antiviral immune responses. This conclusion is supported by the virus-associated malignancies (EBV-associated B cell lymphoma or human herpes virus (HHV) 8-associated Kaposi sarcoma) in some ORAI1- and STIM1-deficient patients[39, 59, 60] and by impaired antitumor immunity in *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Cd4-Cre* mice, which failed to prevent tumor cell engraftment and growth *in vivo*.[76] Impaired $CD8^+$ T cell– and NK cell–mediated cytotoxicity is likely to play an important role in the patients' susceptibility to CMV and other viral infections.

Humoral immunity is impaired in ORAI1- and STIM1-deficient patients. Their B cells have strongly impaired SOCE,[70, 71] but whether this causes the defect in humoral immunity and contributes to the patients' susceptibility to infections is unclear. The numbers of $CD19^+$ and $CD20^+$ B cells were normal in the originally reported patients,[39, 40, 45] which was confirmed by two recently published studies about patients with a STIM1 p.P165Q mutation [42] and one patient with an ORAI1 p. H165PfsX1 mutation.[68] The percentages of IgD^+CD27^- naive and IgD^-CD27^+ switched memory B cells were normal in the ORAI1-deficient patients. Of the STIM1-deficient patients, one had moderately increased percentages of IgD^+CD27^- naive B cells and reduced IgD^-CD27^+ switched memory B cells, whereas the other patient was normal. Both STIM1- and ORAI1-deficient patients showed reduced percentages of IgD^+CD27^+ unswitched memory B cells; $IgM^+IgD^+CD27^+$ B cells phenotypically resemble splenic marginal zone B cells that are thought to respond independent of T cell help.[77] Marginal zone B cells have been implicated in the immune response against encapsulated bacteria and defects in this subset were found in IRAK-4–, MyD88-, and TIRAP-deficient patients that suffer from infections with encapsulated bacteria.[78] However, it is currently unclear whether the defect in $IgM^+IgD^+CD27^+$ B cells contributes to the high frequency of infections in those patients.[77]

Patients with LoF or null mutations in *ORAI1* and *STIM1* have normal or elevated serum IgM, IgG, and IgA levels. However, many patients lacked specific antibody responses against vaccination antigens such as diphtheria and tetanus toxoid or pathogens such as *C.*

albicans that they had encountered before.[39, 40, 61, 71, 79] A patient with STIM1 p.R429C mutation lacked specific antibodies against *H. influenza* B, pneumococcae, measles, mumps, but had detectable antibody titers against tetanus, rubella, EBV, CMV, and HSV.[45] A recently reported ORAI1-deficient patient had protective antibody titers against 5 of 14 pneumococcal serotypes without immunization.[68] This is in contrast to two recently reported siblings with STIM1 p.P165Q mutation, who showed no response to unconjugated pneumococcal vaccine.[42] It is unclear whether the defect in production of specific antibodies is caused by impaired SOCE in B cells. SOCE was reported to mediate B cell activation [80] and B cell specific deletion of *Stim1* and *Stim2* in mice almost completely suppressed SOCE, which resulted in impaired B cell proliferation and survival upon BCR stimulation *in vitro*.[81] By contrast, these *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Mb1-Cre* mice showed normal primary and memory antigen-specific antibody responses, normal affinity maturation and had similar numbers of germinal center B cells compared to wild type mice suggesting that SOCE is not required for humoral immunity.[81] In addition, *Stim1*$^{-/-}$ mice showed a normal humoral immune response to immunization with keyhole limpet hemocyanin (KLH), and serum titers of anti-KLH antibodies before and after booster immunizations were similar in *Stim1*$^{-/-}$ and wild-type mice.[64] By contrast, mice with T cell–specific deletion of *Stim1* and *Stim2* lacked virus specific antibodies upon infection with LCMV, which likely contributed to their failure to control virus replication and chronification of LCMV infection [69]. Taken together, it is likely that impaired CD4$^+$ T cell function in ORAI1- and STIM1-deficient patients contributes to their compromised humoral immunity.

Defects in innate immune responses may contribute to the immunodeficiency of ORAI1 and STIM1-deficient patients. As discussed above, the cytotoxic function of NK cells is impaired and iNKT cell numbers are reduced in some patients. ORAI1 and STIM1 have also been reported to mediate SOCE in human and mouse DCs, macrophages, and neutrophils, and thus could potentially contribute to innate immune responses to infection.[82, 83, 84, 85, 86] Ca^{2+} signals were shown to promote the maturation of human and mouse DCs *in vitro* resulting in the upregulation of the CD80, CD83, CD86, and HLA-DR.[85, 87] Maturation of human DCs could be inhibited by CRAC channel blockers and siRNA-mediated knockdown of either *STIM1* or *ORAI1* mRNA expression.[85] By contrast, genetic deletion of *Stim1/Stim2* in mice had no effects on the numbers and phenotype of monocytes and DCs *in vivo*, or the differentiation of macrophages and DCs from bone marrow stem cells *in vitro* despite abolished SOCE. Importantly, the function of SOCE-deficient macrophages and DCs was normal; they produced comparable amounts of various cytokines and activated the NLRP3 inflammasome. Stim1/Stim2-deficient macrophages had normal phagocytosis and bone marrow–derived DCs matured normally and activated CD4$^+$ T cells upon coincubation *in vitro*.[88] SOCE has been suggested to play an important role in neutrophil function based on studies in the promyelocytic HL60 leukemia cell line.[83] Evidence from *Stim1*$^{-/-}$ and *Orai1*$^{-/-}$ mice suggests that several functions of neutrophils, including phagocytosis,[84, 86] ROS production,[84] and chemotaxis,[89, 90] are dependent on SOCE. Accordingly, *Stim1*$^{-/-}$ mice were shown to have impaired immunity to bacterial infection[84] but were protected from tissue injury in hepatic ischemia-reperfusion injury[84] and skin inflammation in a murine model of psoriasis.[89] Likewise, *Orai1*$^{-/-}$ mice showed defective neutrophil recruitment to the lung in a hypersensitivity pneumonitis model.[90] This is in contrast to only

moderately impaired SOCE and normal functions of neutrophils, including ROS production, chemotaxis, phagocytosis, and adhesion, from patients with ORAI1 p.R91W and STIM1 p.R429C mutations (R. Elling, P. Henneke, S.F. unpublished). These findings are surprising and suggest that other ORAI and STIM isoforms may contribute to SOCE in human neutrophils and their function.

Autoimmunity—Lymphoproliferation is a common feature of patients with LoF mutations in *STIM1* (Table 1). Most of these patients also develop Coombs-positive autoimmune hemolytic anemia (AIHA) and thrombocytopenia in their first year of life.[40, 45, 59] Thrombocytopenia is caused by autoantibodies against platelets.[40] At least one patient had elevated levels of antinuclear antibodies (ANA).[45] Lymphoproliferation and autoimmunity are less common in patients with LoF mutations in *ORAI1*, and autoimmune thrombocytopenia and neutropenia have been observed in only 1 of 7 patients.[39, 91] The reason for the reduced incidence of autoimmunity in ORAI1-deficient patients is unknown and intriguing, especially since all other disease symptoms in ORAI1- and STIM1-deficient patients are very similar, including the severity of immunodeficiency and the spectrum of infections as well as non-immunological features of the disease.

Autoimmunity in STIM1-deficient patients could be due to impaired negative selection of autoreactive T cells and/or B cells during their development because Ca^{2+} signals contribute to the strength of TCR and BCR signaling and thus potentially influence the selection thresholds in immature T and B cells. This explanation is unlikely however, because of the normal Vβ repertoire of TCRαβ$^+$ T cells in patients with *STIM1* mutations[40, 45] and in mice with conditional deletion of *Stim1* and *Stim2* in T cells.[67] When *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Lck-Cre* mice, whose T cells lack SOCE, were crossed to HY TCR-transgenic (tg) mice they showed only moderate defects in positive and negative selection,[67] suggesting that SOCE does not play an important role in the selection of T cells. A more likely explanation for the patients' autoimmunity is the reduced frequency of CD25$^+$ FOXP3$^+$ T_{reg} cells found in the blood of several STIM1-deficient patients[40, 45] and one patient with ORAI1 p.R91W mutation (S.F., unpublished). The frequency of CD25$^+$FOXP3$^+$ T_{reg} cells in a patient with STIM1 p.R429C mutation was 2.5% of all CD4$^+$ T cells, which is at the lower end of the normal range (1.7–7.7%).[45] The suppressive function of T_{reg} cells isolated from this patient was normal *in vitro* despite absent SOCE. T_{reg} cells are required for maintaining immunological tolerance to self-antigens and prevention of autoimmunity, as is apparent from patients with mutations in *FOXP3* who suffer from IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked) syndrome. In contrast to IPEX patients, autoimmunity in STIM1-deficient patients is mild and limited to autoimmune cytopenias, which may be due to the presence of residual T_{reg} cells and their normal suppressive function,[45] as well as the fact that effector T cell functions are also impaired in patients lacking STIM1.

An important role of SOCE for T_{reg} cell development is confirmed by *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Cd4Cre* mice, which have 5–10-fold reduced frequencies of Foxp3$^+$ T_{reg} cells in the thymus and secondary lymphoid organs.[62, 67] These mice show lympho- and myeloproliferative disease and develop autoantibodies including anti-dsDNA, ANA, and anti-Ro/La. Signs of autoimmunity are present only in mice with deletion of both *Stim1* and *Stim2*, but not mice with individual deletion of either *Stim1* or *Orai1*,[62, 64, 65] whose T cells

have residual SOCE, suggesting that moderate Ca^{2+} influx is sufficient for T_{reg} development in the thymus. Another source of $Foxp3^+$ T_{reg} cells are naive $CD4^+$ T cells, which can differentiate into induced or adaptive T_{reg} cells (iT_{reg} or aT_{reg} cells) *in vitro* under the influence of TGF-β or during mucosal immune responses and chronic infection or inflammation *in vivo*.[92] During chronic pulmonary infection of *Stim1*$^{fl/fl}$*Cd4-Cre* mice with *Mycobacterium tuberculosis* (*Mtb*), we observed reduced numbers of iT_{reg}, but not nT_{reg}, cells in the lungs of mice.[74] This defect was due to an intrinsic role of STIM1 in the differentiation of $CD4^+$ T cells into iT_{reg} cells *in vitro* and *in vivo*. Induced T_{reg} cell development was also suppressed by IFN-γ in a $CD4^+$ T cell extrinsic manner, which was consistent with the high IFN-γ levels in the lungs of *Mtb*-infected STIM1-deficient mice.[74] It is noteworthy that lack of STIM1 did not interfere with the suppressive function of iT_{reg} cells. Taken together, STIM1 and SOCE appear to be important regulators of the differentiation of iT_{reg} cells during chronic infection and likely contribute to regulating the immune response to pathogens like *Mtb*. In the absence of STIM1 and SOCE, reduced iT_{reg} numbers likely exacerbate the pulmonary hyperinflammation in Mtb-infected mice.

Thrombocytopenia and platelet function—Patients with LoF or null mutations in *STIM1* present with intermittent thrombocytopenia, which is due to auto-antibodies against glycoproteins Ib/IX and potentially other surface molecules on platelets.[40] Additional evidence for an autoimmune etiology of the thrombocytopenia comes from the fact that platelet numbers in the patients recovered following glucocorticoid therapy but not in response to platelet transfusions.[40] Except for periods of autoimmune thrombocytopenia, platelet numbers in patients with LoF or null mutations in *ORAI1* and *STIM1* were normal and had no history of bleeding diathesis, except for one patient with STIM1 c.1538–1 G>A mutation who developed a bleeding lesion of her upper lip of uncertain etiology at 2 years of age. SOCE is however an important direct regulator of platelet function, as is evident from the phenotype of patients with GoF mutations in *STIM1* that develop thrombocytopenia, platelet dysfunction and bleeding diathesis as part of Stormorken syndrome[93, 94, 95, 96] and mice lacking *Stim1* or *Orai1* expression, which have impaired platelet function and reduced thrombus formation.[97, 98, 99, 100] We will therefore briefly review the evidence for STIM1 and ORAI1 function in platelets.

A patient with STIM1 p.R429C LoF mutation and recurrent autoimmune thrombocytopenias was investigated for platelet function in more detail.[101] Platelet counts, platelet size, and the bleeding time were all normal, as was platelet aggregation following stimulation *in vitro* with adenosine 5′-diphosphate (ADP), collagen, and ristocetin. Several parameters of platelet activation such as surface expression of GPIb/IX and GPIIb/IIIa, ristocetin-induced binding of Von Willebrand factor, and binding of soluble fibrinogen were also normal. The only defect observed was in the secretion of α- and δ-granules in response to thrombin stimulation measured by surface expression of CD62P and CD63, respectively.[101] SOCE was not measured directly in the patient's platelets but it was abolished in her T cells, B cells, and fibroblasts.[44, 45] Assuming that SOCE is also strongly impaired or lacking in her platelets, as is the case in STIM1-deficient murine platelets,[97, 98] one would have to conclude that STIM1 deficiency only results in a mild defect in platelet activation that does not cause bleeding diathesis.

SOCE is reduced in platelets from mice that lack expression of *Stim1* (*Stim1*$^{-/-}$)[98] or *Orai1* (*Orai1*$^{-/-}$)[99] in all tissues, mice with conditional deletion of *Stim1* in platelets and megakaryocytes (*Stim1*$^{f/f}$ *PF4-Cre*),[97] and mice that express a LoF mutant of *Orai1* (*Orai1*^{R93W}).[100] Platelets from all four strains had impaired platelet function, although the severity of the defects varied from study to study (reviewed in detail in Ref. 102). In general, SOCE-deficient platelets had only minor defects in integrin $\alpha_{IIb}\beta_3$ activation or secretion of alpha granules in response to stimulation with thrombin and collagen analogs.[98, 100] More pronounced defects were observed regarding the ability of SOCE-deficient platelets to switch from a pro-adhesive to a pro-coagulant state, which requires expression of phosphatidylserine (PS) on the outer leaflet of the plasma membrane. PS provides a docking surface for plasma clotting factors, thereby promoting coagulation and the generation of a fibrin plug at sites of vascular injury. In the absence of functional STIM1 or ORAI1, the exposure of PS on the surface of activated platelets was markedly reduced.[97, 100, 103] This defect in PS exposure correlated with impaired collagen-induced pro-coagulant activity of SOCE-deficient platelets *in vitro*.[103]

Stim1$^{-/-}$ or *Orai1*$^{-/-}$ mice showed significantly impaired thrombus formation on collagen-coated surfaces under flow conditions *ex vivo* in two studies,[98, 99] whereas platelets from *Stim1*$^{f/f}$ *PF4-Cre* and *Orai1*^{R93W} mice showed no defects.[97, 100] The cause of this discrepancy is not understood, but may have to do with differences in experimental conditions. Supporting a role of SOCE in arterial thrombus formation *in vivo* is the fact that thrombi formed following laser damage of arterioles in *Stim1*$^{f/f}$ *PF4-Cre* mice were less stable than those in wild-type mice. Unstable thrombi were associated with delayed formation of fibrin at sites of vascular injury, indicating that SOCE in platelets is critical for thrombus stability and growth.[97] *Stim1*$^{-/-}$ or *Orai1*$^{-/-}$ mice were protected from arterial thrombosis when subjected to the middle cerebral artery occlusion (MCAO) model of ischemic stroke,[98, 99] which has been attributed to impaired function of SOCE-deficient platelets. Complicating this interpretation is the fact that *Stim1*$^{-/-}$ or *Orai1*$^{-/-}$ bone marrow chimeric mice used for these studies also lack SOCE in inflammatory immune cells that play a role in arterial thrombosis [104] and ischemia reperfusion injury in stroke.[105] It is important to note, however, that STIM1- or ORAI1-deficient mice, similar to patients with LoF or null mutations, did not have an increased bleeding diathesis as tail bleeding times were normal. The lack of primary platelet defect and bleeding diathesis in ORAI1- and STIM1-deficient patients is in contrast to patients with GoF mutations in STIM1 resulting in Stormorken syndrome, in which mild thrombocytopenia, platelet dysfunction, and bleeding are a major manifestation of disease, as will be discussed below.

Muscular hypotonia—LoF or null mutations in *ORAI1* and *STIM1* resulted in congenital, non-progressive muscular hypotonia in all but one patient (Table 1). It is characterized by reduced muscle strength and endurance, which manifested initially through poor head control and general reduction in muscle tone in newborns.[39, 40, 42, 45, 91] During infancy, myopathy in patients with *ORAI1* mutations manifested through delayed ambulation, generalized muscle weakness, reduced walking distance, difficulty in climbing stairs, positive Gower's sign, ptosis, and hypernasal speech caused by velopharyngeal weakness.[39, 91] Similar global muscular hypotonia was observed in STIM1-deficient

patients.[40, 42, 45] In at least two patients with ORAI1 p.A103E/p.L194P and STIM1 p.P165Q mutations, the myopathy compromised independent ambulation requiring the use of a wheelchair.[39, 42] Another sign of musclular hypotonia observed in several ORAI1- and STIM1-deficient patients is partial iris hypoplasia and/or mydriasis,[39, 40] which was associated with light-insensitive pupils in one patient.[45] It is possible that hypotonia of muscles involved in respiration contributed to chronic pulmonary disease in two ORAI1-deficient patients, although they were successfully treated by HSCT. One of the patients developed chronic pulmonary disease characterized by bronchiectasis, recurrent pneumonia, pulmonary hypertension, and increased CO_2 retention requiring O_2 therapy.[39]

Electromyographic analysis of a patient with ORAI1 p.A88SfsX25 mutation recorded at 10 months of age showed decreased conduction speed,[39] whereas no abnormalities were found in a patient with STIM1 p.E128RfsX9 mutation.[40] A muscle biopsy in a patient with ORAI1 p.R91W mutation taken at the age of 5 years revealed variation in muscle fiber size but no dystrophic changes. Fiber typing with ATPase showed a predominance of type 1 fibers and reduced diameter (atrophy) of type 2 fibers.[39] NADH staining did not reveal any cores or other structural abnormalities, and modified Gomori trichrome staining failed to show rod formation as in congenital nemaline myopathy. Immunofluorescence analysis using an ORAI1-specific antibody confirmed expression of ORAI1 in type 1 and atrophic 2 fibers of the patient. Muscle biopsies of other patients are not available.

Ca^{2+} is essential for the contraction of skeletal muscle fibers and requires the release of Ca^{2+} from the SR. Ca^{2+} release is mediated by ryanodine-receptor (RyR) type 1, which is activated by direct coupling of L-type voltage-gated Ca^{2+} channels in the transverse tubules of skeletal muscle fibers.[106] ORAI1 and STIM1 are robustly expressed in human and mouse skeletal muscle [39, 66, 107, 108] and colocalize with RyR1 at the junction of plasma membrane t-tubules with the terminal cisternae of the SR.[108, 109] Depletion of SR stores in skeletal muscle stimulates SOCE,[110] and it has therefore been speculated that SOCE mediates the refilling of depleted SR Ca^{2+} stores after repeated muscle fiber contractions. However, muscle fibers can contract in the absence of extracellular Ca^{2+} suggesting that they lose little Ca^{2+} and do not require Ca^{2+} influx via SOCE for excitation–contraction (EC) coupling as discussed in more detail by Launikonis *et al*.[111] On the other hand, deletion of *STIM1* or *STIM2* resulted in depletion of Ca^{2+} stores and in decreased fatigue resistance of mouse and human myotubes.[108, 112] In addition, SOCE mediated by ORAI1 and STIM1 was shown to play a role in the differentiation of human myoblasts by regulating the expression of two early markers of myoblast differentiation, myogenin and myocyte enhancer factor-2 (MEF2),[113] and is likely to regulate other Ca^{2+} transcription factors, such as NFAT, that have been implicated in skeletal muscle differentiation.[114]

The myopathy in patients with LoF mutations in *ORAI1* and *STIM1* demonstrates that SOCE is required for skeletal muscle function and is consistent with findings in STIM1- and ORAI1-deficient mice. Skeletal muscle isolated from *Stim1*$^{-/-}$ mice showed reduced muscle fiber diameter, and muscle fibers isolated from *Stim1*$^{-/-}$ mice had reduced SOCE, fatigued rapidly upon repeated stimulation and showed a decrease in tetanic force.[108] This phenotype is reminiscent of mice lacking mitsugumin 29 (mg29), a protein located at the junction between SR and plasma membrane in skeletal muscle; these mice showed impaired SOCE,

accelerated depletion SR Ca^{2+} stores, and impaired SR refilling when muscle fibers were continuously stimulated.[115] A similar phenotype was also observed in transgenic mice with muscle-specific expression of dominant-negative ORAI1 (*Orai1*^{E108Q}) that suppressed SOCE in muscle fibers.[109] The mice had reduced body weight, muscle mass, and fiber cross-sectional area but normal distribution of type 1, 2a, and 2b fibers. Single muscle fibers, excised muscles, and whole mice fatigued more quickly than controls during intense repetitive activity despite normal EC coupling.[109] *Orai1*^{E108Q} mice did not show abnormalities in the ultrastructural characteristics of muscle fiber cells including their mitochondria, in contrast to the swollen and morphologically abnormal mitochondria found by electron microscopy in *Stim1*$^{-/-}$ mice.[108] No histological or ultrastructural changes were observed in skeletal muscle from *Orai1*^{R93W} knock-in mice expressing a LoF mutant of ORAI1 that impairs SOCE, analogous to the ORAI1 p.R91W mutation in human patients associated with muscular hypotonia and atrophy of type 2 fibers.[65] Since *Orai1*^{R93W} mice die a few days post-partum, skeletal muscle function could not be tested, and it is possible that the structural defects observed in *Stim1*$^{-/-}$ and *Orai1*^{E108Q} mice only become apparent in adult mice. Collectively, these findings demonstrate that ORAI1 and STIM1, while not required for short-term EC coupling, promote fatigue resistance in skeletal muscle and the differentiation of myoblasts.

Hypocalcified amelogenesis imperfect—The majority of patients with LoF or null mutations in *ORAI1* or *STIM1* have severe dental enamel defects (Table 1).[1, 39, 40, 45] The phenotype was first described in patients with ORAI1 p.R91W mutation as hypocalcified *amelogenesis imperfecta*.[1] Similar dental phenotypes were subsequently reported in other patients with mutations in *ORAI1* and *STIM1*.[39, 40, 45] The description of the dental phenotype found in these patients is mostly qualitative, and an in-depth analysis of dental tissues has not yet been performed given the main focus of these reports on immune dysfunction. The dental abnormalities in these patients are very similar and characterized by excessive enamel wear, or attrition, related to poor mineralization, and discoloration of the residual enamel, a condition observed in both deciduous (milk teeth) and permanent teeth.[39, 40, 55] In some patients, the enamel is almost completely lost, and the less mineralized, yellowish dentine (the tissue underlying the enamel) is widely exposed by age 6 or earlier (Fig. 5).[39] The enamel phenotype is dramatic, and patients seldom retain sufficient enamel capping on their teeth to enable proper functioning of the masticatory apparatus, requiring extensive dental restoration of entire enamel crowns. Interestingly, the developing teeth appear to achieve normal size and shape, and the problem arises upon eruption of the teeth into the oral cavity. The apparent lack of proper mineralization results in rapid loss of enamel presenting additional clinical problems as hypomineralized enamel is highly sensitive to caries development, which can act as a host environment for bacteria. This puts ORAI1- and STIM1-deficient patients at risk of oral infections in addition to their already impaired immune responses.

A recent study reported isolated dental enamel abnormalities in a patient with a STIM1 p.R426C missense mutation.[55] The patient was 6 years of age at the time of presentation and the dental phenotype was the chief medical complaint. The patient also had nail dysplasia and a history of frequent throat infections, suggesting a milder form of immunodeficiency,

but no immunological evaluation was performed and contact with the patient was lost. The patient lacked other symptoms of CRAC channelopathy. No information regarding the impact of the STIM1 p.R426C mutation on SOCE or STIM1 protein expression in cells from the patient was reported.[55] R426 is located in CAD and it can be speculated that mutation of this residue interferes with STIM1 function and SOCE because ectopic expression of a mutant STIM1 R426L protein in HEK293 cells strongly impaired CRAC channel function by locking the C-terminus of STIM1 in an inactive conformation.[46] R426 is located close to R429, which also regulates the close-to-open transition of the STIM1-CT and mutation of which causes more severe CRAC channelopathy by abolishing SOCE.[44, 45] A potential explanation for the milder clinical phenotype when R426 is mutated compared to R429 is an incomplete defect in CRAC channel function. This would point to a stricter SOCE requirement for ameloblast function and enamel mineralization than is necessary for immunity to infection or skeletal muscle function. In fact, hypocalcified *amelogenesis imperfecta* combined with nail dysplasia and recurrent infections might be a useful biomarker to identify patients with hypomorphic mutations in *ORAI1* and *STIM1* that, short of causing fatal immunodeficiency, may increase the patients' susceptibility to infection. It is noteworthy that individuals heterozygous for LoF or null mutations in *ORAI1* and *STIM1* do not have obvious dental enamel defects or other symptoms of CRAC channelopathy despite reduced SOCE,[1, 45] indicating that residual SOCE is sufficient for enamel mineralization and immune function. However, heterozygous individuals might still be at greater risk of increased enamel wear with age compared to healthy individuals, which remains to be investigated.

Enamel is a highly mineralized tissue containing tightly packed crystals formed by a carbonated variant of calcium hydroxyapatite. Two main stages of enamel development termed *secretory* (forming) stage and *maturation* (mineralization) stage are recognized. During the secretory stage, the volume of tissue is formed guided by the expression of enamel matrix proteins secreted by enamel forming cells (ameloblasts), and only limited mineralization occurs. In the maturation stage, ameloblasts become involved in mineral transport functions and proteolysis as crystals grow in thickness.[116] Ameloblasts at all stages form a tight barrier by being sandwiched between the enamel crystals growing at the apical end, and other cell layers containing blood vessels at the basolateral pole (Fig. 6). Thus ameloblasts from both stages tightly regulate the transport of ions, mainly Ca^{2+} and phosphate, across the cell layer toward the enamel crystals.

Ca^{2+} transport in ameloblasts and the role of SOCE—Ca^{2+} fulfills a wide variety of roles in the formation of enamel most of which are linked to crystal growth in the extracellular domain. Indeed the concentration of free Ca^{2+} is one of the most critical factors affecting the super-saturation of Hap crystals that requires tight stoichiometric control.[117] An important and understudied area in enamel biology concerns the mechanisms involved in the movement of Ca^{2+} across ameloblast cells, which is required for extracellular deposition of Ca^{2+} in the enamel matrix. Originating from the blood, Ca^{2+} can travel 50–100 μm to reach the enamel space.[116] Ameloblasts form a tight cell barrier and actively regulate the passage of Ca^{2+} before it reaches the enamel zone. Whereas Ca^{2+} uptake mechanisms are poorly understood, Ca^{2+} extrusion pathways are somewhat better defined.[118] Ca^{2+} extrusion

is, at least in part, mediated by the solute carrier gene family member *SLC24A4*, which encodes for the $Na^+/K^+/Ca^{2+}$ exchanger NCKX4.[119] Other exchangers (e.g. NCX) as well as PMCAs may also play a role during the secretory stage of enamel development.[120, 121] It is noteworthy that patients with mutations in *SLC24A4* show striking enamel defects highlighting its potential role in enamel mineralization.[122] Intracellular Ca^{2+} stores like the ER were recognized in enamel cells as potentially playing an important role in the proposed *transcytosis model* of Ca^{2+} transport across ameloblast cells.[123, 124] In this model, Ca^{2+} is moved across enamel cells through a transcellular route being contained in the ER rather than being mostly transported through the cytoplasm involving Ca^{2+} binding proteins. This model appears to be supported by the severely hypocalcified *amelogenesis imperfecta* phenotype in patients with *ORAI1* and *STIM1* mutations. It is plausible that ORAI1 mediates uptake of Ca^{2+} into ameloblasts on their basolateral pole to refill ER stores. In genome-wide gene expression studies, we identified increased *Stim1* and *Stim2* mRNA expression in rodent enamel cells.[125] We recently confirmed that STIM1 and ORAI1 protein are present in ameloblast with subcellular distribution patterns that are consistent with their localization in the ER and plasma membrane, respectively.[126] Stimulation of ameloblasts with thapsigargin to deplete ER stores demonstrated SOCE in these cells that could be suppressed with the CRAC channel inhibitor Synta-66.[126] These findings strongly support a role of CRAC channels in dental enamel formation. We and others also detected expression of IP_3Rs, RyRs, and SERCA2 in ameloblasts,[126, 127] which are important components of Ca^{2+} release and uptake into the ER and may act in concert with SOCE to transport Ca^{2+} across ameloblasts and into the enamel matrix.

SOCE in ameloblast signaling and development—Besides being involved in Ca^{2+} transport across ameloblasts and calcification of the enamel matrix, SOCE may be important for signal transduction in ameloblasts and their differentiation.[128] We observed that stimulation of the ameloblast-like LS8 cell line with thapsigargin to induce SOCE resulted in a significant increase in mRNA levels of extracellular matrix proteins such as amelogenin, ameloblastin, and enamelin,[168] which are key constituents of the proteinaceous matrix secreted by ameloblast cells and participate in the organization of the growing enamel crystals.[129] Moreover, thapsigargin stimulation of LS8 cells resulted in activation of NFAT (R.L., unpublished data), a family of transcription factors regulated by Ca^{2+} signals.[130] As NFAT plays an emerging role in bone formation and bone remodeling,[131, 132] SOCE-mediated NFAT activity in enamel cells may become an important are of study.

A critical aspect of enamel biology from a clinical perspective is that human enamel does not have the capacity to self-regenerate or repair, as is the case for instance in bone. This is because ameloblasts undergo apoptosis prior to the eruption of the enamel crowns. In other words, the enamel of erupted teeth is an acellular tissue. For CRAC channel deficient patients this poses long-term health problems. The fact that both primary and permanent teeth were affected in STIM1- and ORAI1-deficient patients is an interesting finding as the enamel crowns of primary (or milk) teeth start to calcify in the developing embryo at around 16 weeks, and at birth, all primary teeth have undergone considerable mineralization.[133] Thus, it appears that SOCE is required in the fetus' developing dentition to enable Ca^{2+} entry into ameloblasts. The need to maintain functional SOCE after birth is evidenced by the

pathological permanent dentition in patients with milder forms of CRAC channelopathy[55] and those surviving immunodeficiency after HSCT, whose later erupting teeth (premolars, canines, and second molars) emerge into the oral cavity but require extensive dental work.

Gain-of-function mutations in *ORAI1* and *STIM1*

Several patients with GoF mutations in *STIM1* and *ORAI1* were recently described providing intriguing information about the pathophysiological consequences of increased SOCE. These mutations result in constitutive or increased activation of CRAC channels and are associated with a spectrum of symptoms affecting different organs and cell types. Although there is some overlap with the features of CRAC channelopathy (Fig. 2 and Table 1), patients with GoF mutations have unique disease phenotypes. Their clinical disease spectrum ranges from non-syndromic tubular aggregate myopathy (TAM) to the more complex York platelet and Stormorken syndromes. The clinical phenotypes of the latter syndromes are very similar and dominated by platelet defects in combination with skeletal myopathy. Both syndromes can be caused by the same GoF mutation in *STIM1;* in addition, another GoF mutation in *STIM1* causes either York platelet syndrome or TAM (Figs 2 and 3; Table 1).[93, 94, 95, 134] All three diseases therefore appear to be part of the same clinical spectrum, with the phenotype and expressivity resulting from the mutation being determined by additional factors such as allelic variation or modifier genes.

Stormorken syndrome and York platelet syndrome

GoF mutation in *STIM1*—Stormorken syndrome is a rare autosomal-dominant disorder first described in 1985[135] although details of its molecular basis were only very recently identified. Three research groups reported independently 21 patients from unrelated families of different ethnic backgrounds, who are heterozygous for the same mutation in STIM1 (Table 1).[93, 94, 95] The transition mutation occurs in exon 7 of *STIM1* (c.910C>T) and results in a single amino acid substitution, STIM1 p.R304W, located in the C-terminus of STIM1 (Fig. 3B and Table 1). The same mutation was identified in patients from 2 families with York platelet syndrome,[134] another rare AD disorder described in 2003. [136, 137, 138, 139] In all three studies describing Stormorken syndrome patients, the p.R304W mutation caused constitutive Ca^{2+} influx without store-depletion in the presence[94, 95] or absence[93] of extracellular Ca^{2+}, and one study demonstrated constitutive CRAC currents (I_{CRAC}) in patient lymphocytes.[95] Heterologous expression of STIM1 p.R304W together with ORAI1 confirmed the constitutive, near maximal activation of I_{CRAC} *in vitro*. In addition to constitutive Ca^{2+} influx, SOCE induced by ER store depletion was moderately increased in two studies that used high (10 mM) extracellular [Ca^{2+}],[94, 95] whereas no further SOCE increase was observed in platelets at low (1 mM) extracellular [Ca^{2+}].[93] Consistent with constitutive activation of mutant STIM1, ectopically expressed YFP-tagged STIM1 p.R304W accumulated in puncta without store depletion, which is in contrast to wild type STIM1 that was uniformly localized in the ER. Together, these data indicate that the STIM1 p.R304W mutation results in constitutive, but incomplete, activation of CRAC channels, as Ca^{2+} influx and I_{CRAC} could be further enhanced by ER store depletion. Similar functional studies were not conducted in patients with York platelet syndrome, but it would be predicted that their cells also show constitutive Ca^{2+} influx and I_{CRAC}.[134]

The mechanism by which the R304 mutation results in STIM1 activation remains to be fully understood, but important clues come from studies on the structure and function of STIM1. The R304 residue is located in the first coiled-coil (CC1) domain of the cytoplasmic STIM1-CT (Fig. 3B). Constitutive activation of STIM1 has been attributed to the effects of R304W mutation on the structure of the CC1 domain and its ability to maintain the STIM1-CT in a closed, inactive conformation. As discussed in detail for the LoF STIM1-R429C mutation above, the STIM1-CT transitions from a closed, inactive to an extended, active conformation upon Ca^{2+} depletion in the ER that enables it to bridge the ~10–25 nm gap between ER and PM membranes[140, 141] and to bind to ORAI1 and PM phospholipids through the exposure of CAD and the polybasic domain, respectively. The molecular mechanisms controlling the transition of STIM1-CT from a closed to open conformation are incompletely understood. They were proposed to involve hydrophobic [46] and ionic interactions[20, 21, 47] between CC1 and CC2 domains of STIM1-CT. A critical residue in CC1 that controls the conformation and activation state of STIM1-CT is L251 because mutation of L251 results in a constitutively open/extended STIM1 conformation.[15, 46] Another important region is an “inhibitory” amphipathic α-helix (aa 310–337) in CC1 that contains four glutamates (aa 318–322), which are thought to interact with basic residues (aa 382–386) in CC2 and thereby act as an electrostatic switch controlling STIM1-CT conformation, restraining CAD function in resting conditions.[21] Complete deletion of the entire α-helix resulted in constitutive STIM1 activation,[142] whereas substitution of the 4 glutamates had complex effects depending on the type of mutation.[21, 46, 54, 143, 144]

The GoF phenotype caused by the STIM1 p.R304W mutation suggests that the proximal end of CC1 is involved in regulating STIM1-CT conformation, although the R304 residue is not itself located within the inhibitory helix (aa 310–340). However, R304 is located in close proximity to CAD in inactive STIM1 based on structural analyses,[144] and R304 was predicted to interact with E318 and Q314 residues within the inhibitory helix of CC1.[144] It is therefore possible that the R304W mutation disrupts the salt bridge and the hydrogen bond between R304 and E318 and Q314, respectively, thus affecting interactions between the inhibitory helix and CAD under resting conditions. Alternatively, it is possible that the R304W mutation, via the larger hydrophobicity of tryptophan (W) relative to arginine (R) and thus increased amphipathic properties of the CC1 domain, stabilizes homotypic interactions of CC domains between STIM1 C-termini in the activated, extended conformation, which are required for higher order oligomerization.[93] This would be consistent with a recent study showing that dimerization of neighboring CC1 domains is sufficient to induce structural changes in the STIM1-CT that result in its extended conformation.[15, 145] The precise mechanisms by which the STIM1-R304W mutation results in constitutive CRAC channel activation awaits further molecular and structural analysis. It is noteworthy that the activating effects of the STIM1 p.R304W mutation are in contrast to those of the LoF STIM1 p.R429C mutation described above. Whereas both mutations result in a constitutively open conformation of the STIM1-CT, only mutation of R304 in the CC1 domain, but not mutation of R429 in CC3, results in constitutive Ca^{2+} influx. This is due to the fact that R429 and CC3 have additional functions in CRAC channel activation by mediating the oligomerization of STIM1 C-termini and STIM1 binding to ORAI1.

Clinical phenotype—Detailed reviews and clinical descriptions of Stormorken syndrome[96, 135, 146, 147] and York platelet syndrome[136, 137, 138, 139] have been published elsewhere. We discuss the clinical phenotypes in the context of a newly reported *STIM1* mutation and data from animal models.

Bleeding diathesis, thrombocytopenia, and abnormal platelet function: Stormorken syndrome is characterized clinically by a range of symptoms that is dominated by mild bleeding tendency due to thrombocytopenia and platelet defects, as well as tubular aggregate myopathy (TAM) with miosis (Table 1 and Fig. 2). Other symptoms that are present in most but not all patients include mild anemia, asplenia, headache, ichthyosis, impaired cognitive function, enophthalmos with hypotelorism and distinct facial features, short stature, low body weight, and hypocalcemia.[93, 94, 95, 135] The clinical phenotype of York platelet syndrome is also dominated by an increased bleeding tendency caused by thrombocytopenia and platelet defects, as well as muscle weakness with skeletal muscle atrophy (Table 1 and Fig. 2).[134, 136, 137, 138, 139] Other symptoms present in Stormorken syndrome such as myalgia, miosis, asplenia, small stature, or cognitive defects have not been reported.

Thrombocytopenia, present in all patients with Stormorken and York platelet syndrome, is associated with an increased bleeding tendency in most patients.[93, 94, 95, 134, 135, 136, 137, 138, 139] Six patients described by Misceo *et al*. initially presented with scalp hematomas, epistaxis, and skin purpura as children. Over their lifetime, most patients displayed epistaxis, hematuria, easy bruising, and hematoma after minor trauma.[93] One patient underwent therapeutic splenectomy at 11 years of age because of a history of purpura while another was treated by nephrectomy because of hematuria during early adulthood. A subarachnoid hemorrhage resulting from a ruptured cerebral aneurysm was the initial manifestation in a 20 year old patient. Two patients reported by Nesin *et al*. also had a history of bleeding diathesis,[95] whereas 3 patients with the same mutation described in another study did not.[94] All patients (except one) with York platelet syndrome suffered from an increased bleeding tendency with epistaxis, easy bruising, or postpartum hemorrhage.[134]

A common feature of Stormorken and York platelet syndrome patients is mild to pronounced thrombocytopenia and thrombocytopathy.[93, 94, 95, 134, 135] Platelets from Stormorken patients were found to be in an activated, procoagulant state without prior stimulation that was characterized by phosphatidylserine (PS) exposure at the plasma membrane.[93] In addition, unstimulated platelets from Stormorken patients had increased levels of CD63 (glycoprotein 53) and CD62p (P-selectin) at their surface and showed alpha granule secretion, which are markers of platelet activation. Other aspects of platelet function were impaired, as collagen-induced ATP secretion and thrombocyte aggregation after ADP and collagen stimulation *in vitro* were impaired in some patients.[94] During normal platelet activation, stimulation with agonists such as thrombin, thromboxane A2, ADP, or collagen induces Ca^{2+} influx in platelets, which mediates a pro-coagulant response with PS exposure and microvesiculation, as well as platelet adhesion.[102] PS exposure enables the binding of coagulation factors to the platelet surface, thereby stimulating the generation of thrombin at sites of vascular injury. Both the adhesive and the pro-coagulant activity of platelets are critical for hemostasis and thrombosis. STIM1 and ORAI1 are essential for Ca^{2+} influx in

platelets and platelet activation, as platelets from STIM1- and ORAI1-deficient mice show impaired activation and PS exposure at their surface, resulting in reduced platelet aggregation *in vitro* and thrombus formation *in vivo*.[97, 98, 99, 100] It could be speculated that constitutive Ca^{2+} influx and platelet activation in Stormorken patients results in an increased incidence of thromboembolisms, but this is not the case. Only one patient presented with pulmonary embolism, thrombosis in his leg and left transverse sinus thrombosis between 36 and 41 years of age, which required anticoagulant (warfarin) treatment.[93] This paradoxical phenotype may be due to abnormally low platelet cohesion measured under shear stress conditions *ex vivo*.[96]

Platelets from patients with York platelet syndrome have been studied in detail[134, 136, 137, 138, 139] and show both functional and structural abnormalities. When analyzed by electron microscopy, many platelets from 3 patients with STIM1 p.R304W mutation were large and agranular or hypogranular and had abnormal features described in York platelet syndrome before.[134] A more detailed description has been provided for platelets from patients with a different mutation, STIM1 p.I115F, whose platelets are normal-sized by EM, with moderately decreased alpha granule content, but contain abnormal electron-dense opaque bodies, which were found to contain a large amount of calcium when analyzed by X-ray diffraction.[134] Functionally, platelets from these patients showed strongly reduced aggregation induced by low and high doses of ADP, had impaired ATP release from dense granules upon stimulation with collagen or epinephrine, and showed reduced expression of platelet activation markers such as CD62p and CD63.[134]

Abnormal platelet numbers, structure and function due to human GoF mutations in *STIM1* are recapitulated in animal models. Nesin *et al*. generated zebra fish that ectopically express the mutant STIM1 p.R304W protein. They found reduced numbers of thrombocytes in zebra fish embryos and spontaneous bleeding, consistent with the hemostasis defect of Stormorken syndrome patients.[95] Furthermore, mice heterozygous for a GoF mutation in the ER luminal EF-hand domain of STIM1 (D84G), which also activates STIM1, also show thrombocytopenia and an activated platelet phenotype that results in an increased bleeding diathesis.[11]

Tubular aggregate myopathy: Tubular aggregates (TA) are arrays of membrane tubules that accumulate in muscle with age and are found as secondary features in several muscle disorders. In inherited forms of myopathy, tubular aggregates cause slowly progressive muscle weakness.[148] TAM is present in most patients with Stormorken syndrome due to STIM1 p.R304W mutation and one feature of a more complex disease spectrum.[93, 94, 95] TA are also present in a non-syndromic form of TAM and in Stormorken-like syndrome (which is limited to TAM and miosis). Skeletal myopathy is also a symptom of York platelet syndrome, with similar clinical and morphological features as TAM in Stormorken syndrome and non-syndromic TAM.[134]

The range of myopathy-related symptoms in patients with Stormorken syndrome includes muscular pain and cramps in the extremities (detected at rest and aggravated by exercise), muscle weakness, decreased endurance, and impaired upward and lateral eye movements.[93, 94] In one patient with mild muscle weakness and tightness, a femoral MRI

showed amyotrophy and lesions with high intensity signal in the hamstrings.[93] A common feature associated with myopathy in Stormorken patients is miosis described to be resistant to dilation with anti-cholinergic drugs (tropicamide) and α1 adrenergic receptor agonists (phenylephrine). All patients have elevated serum creatine kinase levels. Histologically, muscle biopsies taken from quadriceps muscle of different patients showed myopathy with variation in fiber size and inclusions within the cytoplasm or in the periphery of muscle fibers. Inclusions were observable by light microscopy [94] and appeared basophilic in H&E stains, stained red with Gomori trichrome, and were strongly positive for NADH–tetrazolium reductase.[93, 94] They were negative for succinyl dehydrogenase suggesting a reticular rather than mitochondrial origin.[94] Inclusions were observed mostly in type II fibers[93] and were associated with an atrophy of predominantly type II fibers.[94] Electron microscopy confirmed that inclusions were tubular aggregates.[93] By immunohistochemistry, the aggregates stained strongly positive for STIM1[93] or, in another study, for STIM1, STIM2, ORAI1, and ORAI2.[94] The mechanisms underlying the formation of tubular aggregates remain unclear, but several lines of evidence suggest that aggregates originate from the sarcoplasmic reticulum.[148, 149]

The myopathy in York platelet syndrome due to either STIM1 p.I115F or p.R304W mutations is clinically similar to TAM and characterized by congenital muscle weakness predominantly of the proximal arm and leg muscles, an inability to jump or climb stairs, a positive Gower's sign, recurrent falls, ophthalmoplegia and miosis in 2 of 7 patients.[134] Myalgia, cramps, and contractures, such as in TAM, were not reported. All patients have moderately elevated CK levels, and show atrophy and fatty infiltration of muscles in MRI. Histologically, patients with STIM1 p.I115F mutation show variation in myofiber size, predominance of type 1 fibers, and myopathic changes, including increased numbers of myofibers with internalized nuclei. Gomori trichrome stained sections revealed vacuoles lined by granular, red material consistent with rimmed vacuoles that are found in some forms of distal myopathies. Similar fuchsinophilic structures, although granular and not vacuolar in shape, are decribed in TAM. In EM, the rimmed vacuoles appeared to be limited by a distinct membrane and were surrounded by loosely scattered electron dense granules.[134] The authors noted that definite tubulofilamentous inclusions (as in TAM) were not seen, and it remains unclear if the myopathic changes observed in patients with York platelet syndrome due to STIM1 p.I115F mutation are related to those in patients with TAM and Stormorken syndrome. Interestingly, a patient with STIM1 p.R304W mutation in the same study by Markello *et al*. was initially diagnosed with TAM, but no histological or ultrastructural analysis has been reported for this patient.[134]

Non-syndromic TAM and Stormorken-like syndrome

Non-syndromic TAM resembles clinically and histologically Stormorken syndrome but lacks miosis as a clinical symptom. The majority of mutations causing non-syndromic TAM are located in the EF hand Ca^{2+} binding domain of STIM1.[12, 13, 150, 151] In addition, three GoF mutations in *ORAI1* were described that cause non-syndromic TAM with or without miosis.[95, 152] The disease in one group of patients was described as Stormorken-like syndrome, and since it clinically resembles more closely non-syndromic TAM, it will be discussed below.

GoF mutations in *STIM1*—The large majority of AD mutations causing non-syndromic TAM are located in the ER-luminal EF hand of STIM1 (Table 1 and Fig. 3B), which senses a reduction in $[Ca^{2+}]_{ER}$ after store depletion and initiates STIM1 activation through conformational changes in the adjacent EFh-SAM domains. Shortly after the identification of STIM1 as a regulator of SOCE, several aspartate and glutamate residues within the EF hand were shown to coordinate Ca^{2+} binding and thus STIM1 activation. Overexpression of mutant STIM1-D76, -D84 or -E87 proteins resulted in constitutive CRAC channel opening without prior store depletion.[8, 9, 10, 11] In mice, mutation of D84 to G causes constitutive Ca^{2+} influx in T cells and platelets, and results in platelet dysfunction and a bleeding disorder.[11] The STIM1 mutations found in human patients with non-syndromic TAM likewise result in constitutive STIM1 activation and Ca^{2+} influx.[12, 13, 14] Myoblasts generated from a patient with STIM1 p.D84G mutation had elevated cytoplasmic baseline Ca^{2+} levels even in the absence of extracellular Ca^{2+} and showed strong Ca^{2+} influx upon addition of Ca^{2+} to the extracellular medium without prior thapsigargin stimulation.[13] A much weaker influx was observed in control myoblasts, indicating that CRAC channels are constitutively active in patient cells. No myoblasts or cells from patients with other STIM1 mutations were available to measure Ca^{2+} levels directly, but the effects of the mutations on STIM1 function were assessed by overexpression of YFP-tagged mutant proteins in C2C12 myoblasts. All mutants showed spontaneous STIM1 clustering, a hallmark of STIM1 activation, in the absence of store depletion with the exception of a F108L mutation which showed less pronounced constitutive STIM1 clustering.[12, 13] Puncta formation of mutant STIM1 proteins could not be increased further by thapsigargin treatment, suggesting that they are fully constitutively active. A STIM1 p.G81D mutation reported by Walter et al. resulted in constitutive Ca^{2+} influx in the presence of 20 mM or (more weakly) of 2 mM extracellular Ca^{2+} and was associated with increased SOCE after thapsigargin stimulation to deplete ER stores, although both effects were fairly moderate.[14] It is noteworthy that the N terminus of STIM1 contains two EF hands, a canonical EF-hand (cEF, aa 63–96) that binds Ca^{2+} and a non-canonical EF-hand (ncEF, aa 96–128), which does not bind Ca^{2+}.[38] Only 5 of 10 mutations causing TAM are located in the cEF hand and only one of them affects an acidic residue (D84) that coordinates Ca^{2+} binding.[12, 13, 151] The five other mutations (F108I, F108L, H109N, H109R, I115F) are located in the ncEF hand,[12, 13, 150] and likely activate STIM1 by destabilizing the interaction between the paired EF-hands and the SAM domain, which keeps the N-terminus of STIM1 in an inactive conformation in the Ca^{2+} bound state. This is consistent with the effects of targeted mutations introduced into the cEF hand (E87A) and ncEF hand (F108D/G110D) based on NMR spectroscopy structure data, which cause unfolding of the STIM1 luminal domain and constitutive formation of STIM1 puncta, as well as constitutive Ca^{2+} influx.[7]

GoF mutations in *ORAI1*—Several autosomal dominant GoF mutations in *ORAI1* have been reported in patients from 4 independent families that cause constitutive Ca^{2+} influx.[95, 152] Two missense mutations in *ORAI1*, c.292G>A and c.412C>T result in single amino acid substitutions in the first (ORAI1 p.G98S) and second (ORAI1 p.L138F) transmembrane domain of ORAI1 (Fig. 3B).[152] The mutations did not alter ORAI1 protein levels or its expression at the plasma membrane, but resulted in constitutive Ca^{2+} influx in the absence of store depletion in patient myotubes, as well as in HEK293 cells

overexpressing the mutant proteins.[152] Ca^{2+} influx could be suppressed with a number of inhibitors that have been used as CRAC channel blockers, indicating that Ca^{2+} influx was mediated by CRAC channels. G98 is located in the middle of M1 and its side chain faces the lumen of the CRAC channel pore based on its reported susceptibility to Cd^{2+} block.[26] G98 was proposed to function as a gating hinge for the CRAC channel that regulates the opening of the channel gate at the narrowest part of the pore, close to R91 at the inner mouth of the channel.[153] Substitution of G98 with A blocked STIM1-dependent channel activation, whereas G98D, G98P, and G98M mutations caused constitutive channel activation that was both independent of STIM1 and non-selective for Ca^{2+} compared to other cations.[153, 154] Indeed, replacement of G98 with 11 out of the 19 naturally occurring amino acids constitutively opened CRAC channels.[154] Because all 11 amino acids had elongated side chains it has been suggested that they may add tension to the alpha helical structure of M1, pushing the pore into an open configuration. In this study, the G98S mutation observed in patients with TAM resulted in moderate constitutive Ca^{2+} influx, which was, however, lower than that observed with other amino acid substitutions (L, M, N or E).[154] Mutation of G98 has profound effects on the gating of ORAI1 channels. The G98D mutation was able to overcome the block of CRAC channel function by the R91W mutation[1, 28] when both mutations were co-expressed, suggesting that the channel pore is widened and ion selectivity is altered, likely by changing the α-helical structure of M1.[153] In addition, G98M and G98D mutations rendered CRAC channel opening independent of STIM1 binding because they spontaneously activate ORAI1 proteins whose N- or C-termini were truncated and could not conduct Ca^{2+} influx even when coexpressed with STIM1.[154] The L138 residue of ORAI1, mutated in some TAM patients, is located in the middle of M2. Much less is known about the role of residues in this domain. No other mutations, either GoF or LoF, have been described in M2, and the mechanism by which the L138F mutation causes constitutive Ca^{2+} influx remains to be elucidated. Although the alpha helical M2 domain does not line the channel pore, its residues likely interact with amino acid side chains in M1. As the crystal structure of *Drosophila* Orai shows a hexameric channel in which six M2 domains are packed in a concentric circle around six pore-forming M1 helices,[31] interactions of residues in M2 with those in M1 may affect the structure of the pore-lining M1 helix and channel gating. Extrapolating from the *Drosophila* Orai structure, L138 in M2 of human ORAI1 is located in proximity to residues in M1 close to basic residues at the inner mouth of the pore and may thus affect channel gating.

A third mutation in *ORAI1* that causes TAM is located in M4.[95] The ORAI1 p.P245L missense mutation results in enhanced and prolonged SOCE after passive ER store depletion with thapsigargin.[95] In this study, ectopic expression of ORAI1-P245L together with STIM1 in HEK293 cells did not result, however, in constitutive CRAC channel currents, as was observed for the ORAI1 p.G98S and p.L138F mutations and the STIM1 p.R304W mutation described above. Nor did it increase peak CRAC current amplitudes after store depletion. These findings suggest that mutant ORAI1-P245L channels are not constitutively active, and that the unitary conductance of individual channels is not changed. However, the data reported by Nesin *et al*.[95] indicate that the inactivation of the mutant CRAC channel is altered. Whereas fast Ca^{2+}-dependent inactivation (CDI) of CRAC currents was normal, slow CDI of CRAC currents was significantly reduced in cells expressing ORAI1-P245L

when store depletion was induced by EGTA in the patch pipette.[95] These findings indicate that the P245L mutation does not constitutively activate ORAI1 but prolongs the opening time of the channel after STIM1-dependent activation. Slow CDI is thought to be important to limit Ca^{2+} influx through CRAC channels. The prolonged activation of the ORAI1 p.P245L channel likely accounts for the increased intracellular Ca^{2+} concentrations observed in patient lymphocytes after thapsigargin stimulation. P245 is located in M4, and six M4 domains form the outermost concentric ring of membrane helices in the hexameric channel complex.[31] Since M4 domains are furthest away from the channel pore, they have fewer contacts with other parts of the channel compared to M1, M2, and M3 helices, potentially allowing it more mobility. How residues in M4 may regulate channel opening and closing is not well understood. P288 in *Drosophila* Orai, which corresponds to P245 in the human ORAI1 protein, was shown to introduce a bend in M4 near the middle of the transmembrane helix, resulting in a perpendicular orientation of M4 in the outer leaflet of the PM, and a diagonal orientation in the inner leaflet. It is likely that P245 introduces a similar kink in M4 of human ORAI1 protein. Prolines in or close to transmembrane domains of ion channels may function as hinge residues that provide flexibility in the channel structure, and thus regulate channel gating.[155] Proline mutations have been shown to alter the gating of ion channels and to be associated with ion channelopathies.[156, 157, 158] It is worth noting that the molecular mechanisms of slow CDI, which occurs over tens of seconds after CRAC channel activation, are not fully understood. They are thought to involve several mechanisms,[159, 160] one of which involves the refilling of ER Ca^{2+} stores by Ca^{2+} entering the cell through CRAC channels, and subsequent Ca^{2+} uptake into the ER through the SERCA pump. Refilling of ER stores results in Ca^{2+} binding to the EF hand of STIM1 and its inactivation involving its unbinding from ORAI1, dissolution of STIM1 puncta, and STIM1 deoligomerization. The increase in $[Ca^{2+}]_{ER}$ is required, but not sufficient, for STIM1 de-oligomerization,[161, 162] and a localized increase in cytosolic Ca^{2+} levels is required for the dissociation of STIM1 puncta. How cytosolic Ca^{2+} induces STIM1 de-oligomerization is unknown. Slow CRAC channel inactivation was shown to involve posttranslational modifications of ORAI1 and STIM1 and binding of regulatory proteins to them (for a detailed review, see Ref. 25), with additional mechanisms of slow CDI likely to emerge in the future.

An intriguing question is why some GoF mutations cause non-syndromic TAM whereas others result in the more complex Stormorken and York platelet syndromes, although all of the mutations constitutively activate STIM1 and SOCE. The Stormorken mutation (STIM1 p.R304W) located in CC1 clearly has more severe consequences than mutations affecting the EF hand of STIM1 or those that result in constitutive opening of ORAI1 channels. Both types of mutations result in either elevated resting Ca^{2+} levels or spontaneous STIM1 puncta formation. Elevated resting Ca^{2+} levels in platelets from patients with STIM1 p.R304W mutation, measured in Ca^{2+} free buffer, could not be further increased upon store depletion with thapsigargin and re-addition of 1 mM extracellular Ca^{2+} (Ref. 93), suggesting that CRAC channels are maximally activated without stimulation. By contrast, baseline Ca^{2+} levels in Ca^{2+} free buffer were more moderately increased compared to the peak of Ca^{2+} influx after store depletion with thapsigargin in patients with STIM1 p.G81D or p.D84G mutations.[13, 14] It is difficult to compare, however, the effects of the mutations on Ca^{2+}

levels in these studies because of different cell types and methods used. Nesin *et al*. directly compared the effects of STIM1 p.R304W and a EF hand mutation (p.D76A)—albeit not one that was described in patients—on CRAC channel function.[95] Both mutants resulted in constitutively active I_{CRAC} but differed in the extent of fast Ca^{2+}-dependent inactivation (CDI). Whereas overexpressed STIM1 p.R304W did not show fast CDI, STIM1 p.D76A mediated fast CDI similar to wild-type STIM1. These data indicate that CRAC channels stay open longer in cells expressing STIM1 p.R304W than cells with either wild-type STIM1 or STIM1 EF hand mutants, thereby potentially resulting in more severe cellular Ca^{2+} overload and the broader phenotypic spectrum of Stormorken syndrome compared to TAM.

Clinical and histological phenotype—The clinical spectrum of myopathy in patients with STIM1 EF hand mutations is large and ranges from asymptomatic to slowly progressive proximal upper and predominantly lower limb muscle weakness (Table1). Patients report frequent falls and difficulty running and climbing stairs, although they remain ambulatory. Eye movements are mildly to severely impaired in some patients. Miosis, common in patients with Stormorken syndrome, has not been reported. Atrophy of quadriceps muscles has been seen in patients from several families; and contractures of the elbows, wrist and fingers, heels and neck were present in some patients.[13] Walter *et al*. reported a father and son with childhood onset, progressive myopathy caused by a STIM1 p.G81N mutation.[14] The disease was characterized by muscle weakness of upper and lower limbs, with positive Gowers sign, muscle atrophy, contractions, and loss of ambulation at 35 years of age in the father;[14] he also suffered from myalgia and cramp-like pain in his arm and leg muscles. Opthalmoparesis was present in both father and son, and mild miosis was noted in the father. Additional mutations (STIM1 p.N80T, p.L96V, p.F108L), identified by Bohm *et al*., were mainly associated with myalgia,[12] in contrast to mutations reported earlier[13] that manifested predominantly with proximal muscle weakness. Mutation of H109 to R, reported in 2 studies, was found to cause proximal muscle weakness contractures.[13, 150] Intriguingly, mutation of the neighboring residue F108 to either isoleucine or leucine resulted in slightly different symptoms associated with TAM that were dominated by childhood onset proximal muscle weakness (p.F108I) and adult onset myalgia (p.F108L).[12, 13] Even the same mutation (p.H109N) can cause slightly different disease symptoms, with either predominantly post-exercise fatigability and episodic diplopia[12] or lower limb muscle weakness and contractures.[13] All TAM patients with *STIM1* mutations have elevated serum CK levels, and signs of myopathy were detected in all tested individuals by electromyography.[13, 14] Elevated CK was also found in patients with a STIM1 p.H72G mutation, which caused constitutive STIM1 puncta formation like other EF hand mutations, but did not result in clinical symptoms.[13]

The clinical features of patients with ORAI1 p.G98S and p.L138F mutations were similar to those in patients with STIM1 EF hand mutations and included slowly progressive diffuse muscle weakness as well as marked and bilateral ankle joint contractures (Table 1) [152]. Muscles in several patients tested appeared atrophic and showed fat infiltration by CT imaging. Mild hypocalcemia was present in all patients, which is similar to Stormorken syndrome due to STIM1 p.R304W mutations, but in contrast to patients with TAM due to STIM1 EF hand mutations.[93, 94] Hypocalcemia was associated with relatively low normal

levels of parathyroid hormone (PTH) levels. Symptoms characteristic of Stormorken syndrome, including miosis, were not present in these patients, with the possible exception of mild intellectual disability in one patient, and calcifications in the cerebellum, basal ganglia, and cerebral corticomedullary junction in another patient with ORAI1 p.G98S mutation.[152] Patients heterozygous for the ORAI1 p.P245L mutation presented with progressive muscle weakness and exertional cramps. Miosis, present in most patients, was associated with impaired night vision. The patients' pupil sizes were ~1 mm in the dark and reacted to light and accommodation, but were resistant to dilation with tropicamide or phenylephrine.[95, 163] All patients had elevated levels of serum CK but lacked other symptoms present in Stormorken patients, such as thrombocytopenia, bleeding diathesis, and asplenia.[95, 163] While disease in these patients has been described as Stormorken-like syndrome, it more closely resembles non-syndromic TAM, except that patients present with miosis.

Histological and ultrastructural analysis of muscle biopsies revealed very similar myopathic changes independent of the underlying mutations in *ORAI1* and *STIM1*. Like in Stormorken syndrome, TAs were present in muscle fibers of all patients. They appeared as bright red inclusions in Gomori trichrome and dark blue structures in NADH-TR staining.[12, 13] TAs were negative for succinate dehydrogenase (SDH), suggesting an SR-related, but not mitochondrial pathology. Electron microscopy confirmed the presence of large TAs, which upon higher magnification showed single- or double-walled membranes of different diameters in both type 1 and type 2 fibers.[12, 13, 152, 163] In patients with STIM1 p.G81N mutation, TAs were mainly observed in type I fibers.[14] Immunofluorescence analysis showed that TAs contained SERCA1, one of three Ca^{2+} ATPases responsible for reuptake of Ca^{2+} into the SR, as well as the Ca^{2+} release channel RYR1 and STIM1, which were localized at the edge of aggregates.[13] Colocalization of SERCA, STIM1, and ORAI1 was also observed in TAs from patients with ORAI1 p.G98S and p.L138F mutations.[152] The presence of STIM1 in TAs was also found in muscle fibers from Stormorken syndrome patients, suggesting that TAs originate from the SR as has been suggested previously.[148, 149] This is consistent with the presence of different SR proteins involved in the uptake and the storage of Ca^{2+} in TAs.[13, 148] The mechanisms leading to the formation of TAs in skeletal muscle fibers are unclear, but it has been proposed that increased SR Ca^{2+} levels in the SR and/or cytosol of myoblasts, as measured in patients with TAM, may result in altered structure of the SR and formation of TAs.[164] Nesin *et al*. proposed that sustained, unregulated SOCE alters normal protein folding in the SR, thereby initiating the formation of TAs.[95] Alternatively, it is conceivable that tubule aggregation may be initiated by constitutive STIM1 oligomerization resulting from activating STIM1 mutations; however, this would not explain why GoF mutations in *ORAI1* also result in TAs.

The histological features in muscle from patients with TAM are different from those observed in patients with impaired SOCE due to LoF and null mutations in *ORAI1* and *STIM1*. A muscle biopsy from a patient with ORAI1 p.R91W LoF mutation showed atrophy of type 2 muscle fibers and a predominance of type 1 fibers, but lacked other histological signs associated with known myopathies including TAM. Biopsy samples from TAM patients also showed, besides TAs, signs of chronic dystrophic changes such as internal

nuclei, endomysial fibrosis, variation in the size of type 1 and type 2 fibers with a predominance of type 1 fibers, and atrophy of type 2 fibers, indicative of ongoing muscle fiber regeneration.[12, 13, 152, 163] The fact that mutations in *ORAI1* and *STIM1* that either abolish SOCE or result in constitutive SOCE cause severe myopathy emphasizes the important role of this pathway in skeletal muscle Ca^{2+} homeostasis, function, and structural integrity.

GoF mutations in *STIM1* in mice

Mice with genetic deletion of *Orai1* and *Stim1* have contributed immensely to our understanding of CRAC channels and SOCE *in vivo*. By contrast, few models to assess the effects of increased SOCE that could mimic the effects of TAM or Stormorken syndrome are currently available. The exception are *Stim1*^{Sax} mice, which were generated by ENU mutagenesis and carry an activating STIM1 p.D84G mutation[11] identical to the mutation observed in 3 patients with TAM.[13] Mice homozygous for this mutation showed disseminated bleeding and died *in utero* around day E13; heterozygous mice survived but were severely thrombocytopenic due to increased platelet turnover.[11] The fact that homozygous mutant mice died before birth suggests that homozygosity for the GoF mutations in *STIM1* and *ORAI1* that occur in Stormorken syndrome and TAM may likewise be incompatible with life. Platelets of *Stim1*$^{Sax/+}$ mice had increased intracellular Ca^{2+} levels before stimulation and markedly reduced SOCE in response to store depletion. Platelets from *Stim1*$^{Sax/+}$ mice were pre-activated with increased expression of activated integrin $\alpha_{IIb}\beta_3$ in unstimulated cells, but showed impaired $\alpha_{IIb}\beta_3$ and P-selection expression after stimulation with agonists of immunoreceptor tyrosine activation motif (ITAM)-based receptors.[11] The defect in platelet activation resulted in a prolonged bleeding time, but also protection from arterial thrombosis due to delayed time to vascular occlusion. A muscular defect, reminiscent of TAM, was not reported in these mice. In contrast, transgenic overexpression of STIM1 in skeletal muscle of mice showed a significant increase in resting Ca^{2+} levels and SOCE following store depletion.[165] These mice developed fulminant muscle disease characterized by myofiber necrosis, swollen mitochondria, infiltration of inflammatory cells, enhanced interstitial fibrosis, and elevated serum CK levels. This dystrophic-like disease was dependent on SOCE because it could be rescued by crossing mice to *Orai1*^{E108Q} transgenic mice that overexpress a dominant-negative ORAI1 protein.[109] These findings indicate that increased SOCE in skeletal muscle causes a phenotype resembling muscular dystrophy,[165] although the phenotype is different from TAM found in patients with activating mutations in *ORAI1* and *STIM1*. The cause of this discrepancy and how increased or constitutive SOCE causes different forms of myopathy remains to be investigated.

Concluding remarks

Patients with LoF and GoF mutations in *ORAI1* and *STIM1* provide critical information about the role of CRAC channels in human physiology and disease. The phenotype of patients with CRAC channelopathy due to lack of LoF mutations is dominated by immune dysregulation, muscular hypotonia, and defects in ectodermally-derived tissues such as teeth and sweat glands. Conversely, constitutive CRAC channel activation results in a clinical

spectrum of diseases, including non-syndromic TAM, York platelet syndrome, and Stormorken syndrome. Notable areas of overlap between abolished and constitutive CRAC channel function are skeletal muscle and platelet dysfunction (Fig. 2). CRAC channels therefore appear to play an important role in skeletal muscle Ca^{2+} homeostasis. In platelets the role of CRAC channels is less clear, as only some patients with GoF mutations in *STIM1*, namely those with York platelet and Stormorken syndromes, develop a bleeding disorder due to thrombocytopenia and thrombopathy. Patients lacking CRAC channel function also develop thrombocytopenia, but for pathophysiologically different reasons, i.e. autoantibody-mediated platelet destruction. It is noteworthy that studies in ORAI1- and STIM1-deficient mice have revealed an important role for SOCE in platelets and defects in platelet function in the absence of ORAI1 and STIM1, but this phenotype is not prominent in human patients with LoF mutations in *ORAI1* and *STIM1*. Conversely, mice with a GoF mutation in *Stim1* (D84G) show a similar phenotype as patients with York platelet and Stormorken syndromes due to STIM1 p.R304W mutation. Thus, the phenotypes of patients with LoF or GoF mutations in *ORAI1* and *STIM1* are in many respects reproducible in mice and can be studied in more mechanistic detail there. This is true also for the immunodeficiency observed in CRAC channel–deficient patients. Mice with complete or conditional deletion of *Orai1* and *Stim1* in immune cells show impaired T cell function and increased susceptibility to infection, similar to human patients with LoF mutations in *ORAI1* and *STIM1*.

The phenotype of patients with abolished and constitutive CRAC channel function is surprisingly limited to a few affected tissues including skeletal muscle, platelets, immune cells, teeth, and eccrine sweat glands (Fig. 2 and Table 1). This is surprising given the ubiquitous expression of *ORAI1* and *STIM1* and the many cell types in which CRAC channel function and SOCE have been documented. Despite the absence of overt clinical pathologies, CRAC channels are likely to play an important, if partially redundant, role in many tissues besides those affected in patients with LoF or GoF mutations. It is possible that tissues in which CRAC channel function was reported, such as pancreatic acinar or smooth muscle cells, have additional Ca^{2+} influx pathways at their disposal that can compensate for the loss of ORAI1 and STIM1 during ontogeny. The role of CRAC channels in these tissues may become apparent only when their function is inhibited acutely or under conditions when other potentially compensating Ca^{2+} signaling pathways start to fail.

It is noteworthy that GoF mutations in *ORAI1* and *STIM1* cause a similar disease phenotype defined by TAM (Table 1). Some GoF mutations in *STIM1* (p.R304W and I115F) cause additional disease symptoms including bleeding diathesis, asplenia, and other symptoms, as found in York platelet and Stormorken syndromes (Table 1). The cause for the more severe phenotype resulting from the STIM1 p.R304W mutation, compared to other activating mutations in *STIM1* and *ORAI1*, is unclear since they all result in constitutive SOCE. It is conceivable that the extent of constitutive CRAC channel activation might be greater in patients with STIM1 p.R304W mutation, and careful side-by-side analysis of the effects of GoF mutations on CRAC channel function would be necessary to address this issue. Intriguingly, while patients with the activating EF hand mutation STIM1 p.D84G present only with non-syndromic TAM, mice with the same mutation develop a bleeding disorder

with thrombocytopenia that closely resembles York platelet and Stormorken syndromes. The clinical phenotypes of patients arising from various GoF mutations are influenced by additional factors such as allelic variation in *STIM1* and *ORAI1* expression and the effects of modifier genes that modulate CRAC channel function.

Importantly, the phenotype of patients with LoF mutations in *ORAI1* and *STIM1* is almost identical (Table 1). A minor difference is the apparently greater prevalence of autoimmune cytopenias in patients lacking STIM1, whereas only one ORAI1-deficient patient showed autoimmune neutropenia. However, reduced numbers of $Foxp3^+$ T_{reg} cells, which are the likely pathophysiological cause of autoimmunity, have been observed in both STIM1- and ORAI1-deficient patients. Given the small numbers of patients with LoF mutations and their early mortality, it is difficult to say for certain if autoimmunity really is less likely in ORAI1-deficient patients, or if they would develop disease if they did not succumb to immunodeficiency or were treated by HSCT.

Overall, the homogenous phenotypes of patients with LoF and GoF mutations in *ORAI1* and *STIM1* indicate that both molecules operate in the same pathway. Phenotypic variability is likely caused by quantitative effects of the different mutations on SOCE, as well as allelic variation and the effects of modifier genes between individual patients rather than exclusive, non-SOCE related functions of ORAI1 or STIM1. STIM1 in particular has been reported to regulate the function of a variety of other ion channels besides ORAI1, including TRPC channels[166] and the voltage-gated Ca^{2+} channel $Ca_V1.2$.[167, 168] One would therefore expect that mutations in *STIM1*, in particular those resulting in loss of function, result in a more severe disease phenotype than mutations in *ORAI1* due to defects in TRPC activation and inhibition of $Ca_V1.2$ channels, which play an important role in cardiac function. Judging by the reported phenotypes of patients with LoF and GoF mutations in *ORAI1* and *STIM1*, however, this is not the case, suggesting that the main role of STIM1 is regulating the function of ORAI1, and thus CRAC channels.

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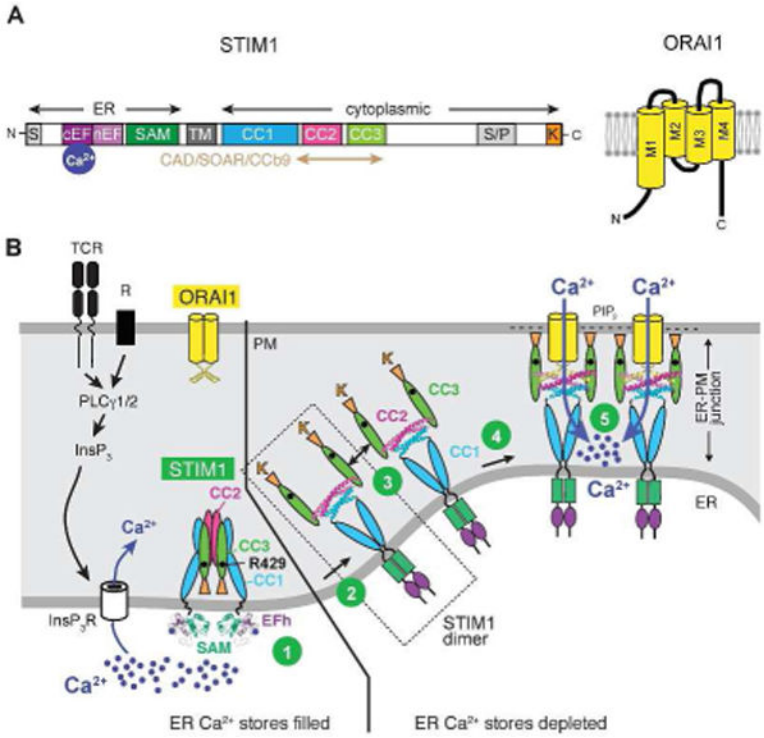

Figure 1.

Model of STIM1 activation and effects of p.R429C mutation. (A) STIM1 and ORAI1 domain organization. ORAI1 is the pore-forming subunit of the CRAC channel in the plasma membrane. It contains 4 alphahelical transmembrane domains (M1–4) and cytoplasmic N- and C-termini that interact with STIM1. M1 lines the ion conducting pore of the channel. STIM1 is a single pass transmembrane protein located in the ER membrane. Its N terminus is located in the ER lumen and contains canonical and non-canonical EF hand (cEFh, nEFh) domains and a sterile alpha motif (SAM). The cytoplasmic C-terminus of STIM1 contains 3 coiled-coil (CC) domains and a lysine-rich (K) domain, which mediate STIM1 binding to ORAI1 and plasma membrane phospholipids, respectively. STIM1 binding to ORAI1 is mediated by the CRAC activation domain (CAD, also called SOAR or CCb9) in STIM1 that encompasses CC2 and CC3. (B) Stepwise activation of ORAI1-CRAC channels by STIM1. In cells with filled ER Ca^{2+} stores, the cytosolic STIM1 domain is in a closed, inactive conformation and forms a dimer with another STIM1 molecule. By contrast, the Ca^{2+}-bound EF-SAM domain of STIM1 located in the ER lumen is monomeric (1). Upon stimulation of cell surface receptors (R) that induce activation of PLCγ1 or PLCγ2 and production of IP_3, Ca^{2+} is released from the ER through IP_3 receptors that are non-selective Ca^{2+} channels. The decreased Ca^{2+} concentration in the ER results in dissociation of Ca^{2+} from the canonical EF hand (cEFh) domain in the N-terminus of STIM1 and dimerization of EF-SAM domains. This causes a change in the conformation of the STIM1 C terminus into an extended, active structure in which the CAD and polybasic domains (K) are exposed (2). In the extended conformation, STIM1 dimers oligomerize mediated by CC domains including CC3 (3). STIM1 is recruited to ER-PM junctions through interactions of the K-rich polybasic domain with membrane phospholipids (4). Oligomerized STIM1 proteins form puncta in ER-PM junctions and bind to ORAI1, thereby recruiting it into ER-PM

junctions and puncta. STIM1 binding results in opening of ORAI1 (CRAC) channels and SOCE (5). Abbreviations: CC1-CC3, coiled-coil; K, lysine-rich polybasic domain; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PM, plasma membrane.

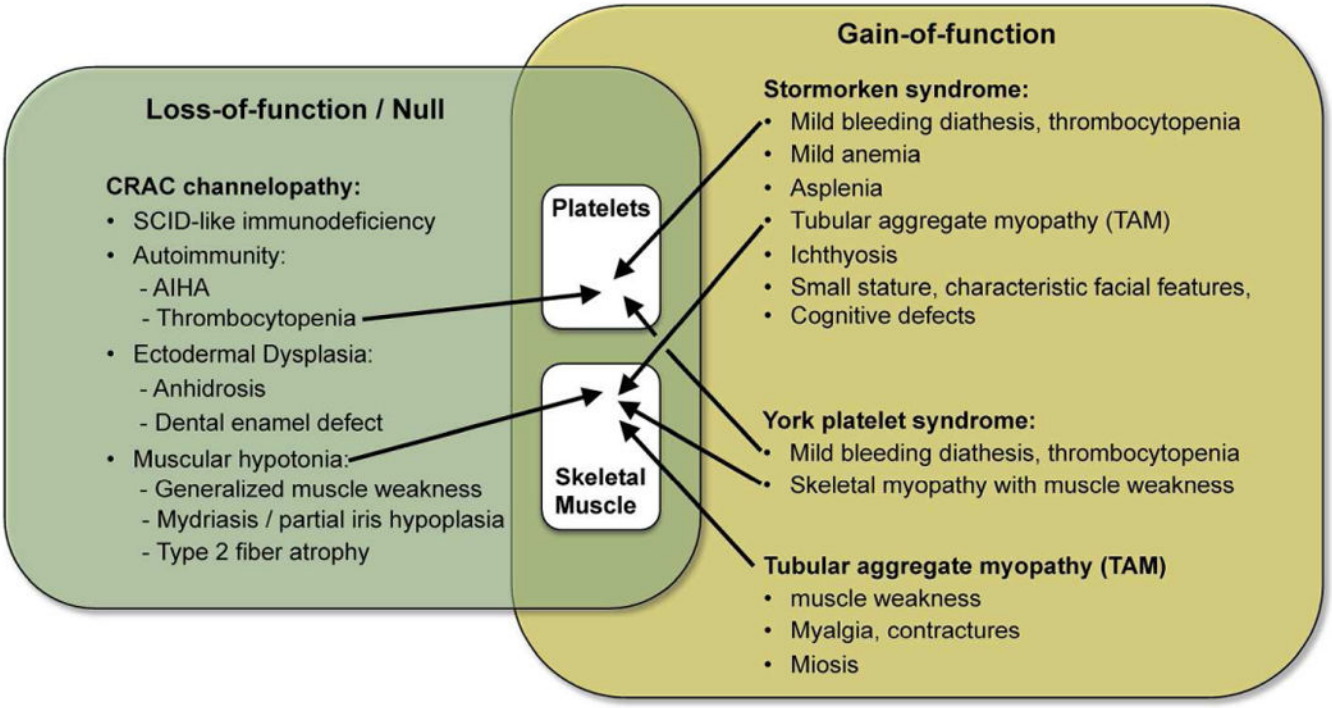

Figure 2.

Disease phenotypes associated with mutations in *ORAI1* and *STIM1*. Null and loss-of-function (LoF) mutations in *ORAI1* and *STIM1* cause CRAC channelopathy, which is defined by (i) SCID-like immunodeficiency with recurrent and chronic infections, (ii) autoimmunity due to autoantibody-mediated hemolytic anemia and thrombocytopenia, (iii) muscular hypotonia and (iv) ectodermal dysplasia characterized by anhidrosis and defects in dental enamel development (left). Gain-of-function (GoF) mutations in *STIM1* and *ORAI1* cause a spectrum of disease entities with partially overlapping symptoms: tubular aggregate myopathy (TAM), York platelet syndrome and Stormorken syndrome. The arrows and white rectangles indicate organs and cell types affected by both LoF and GoF mutations in *ORAI1* and *STIM1* that result in similar disease manifestations although their pathophysiology differs. Abbreviations: AIHA, autoimmune hemolytic anemia; SCID, severe combined immunodeficiency.

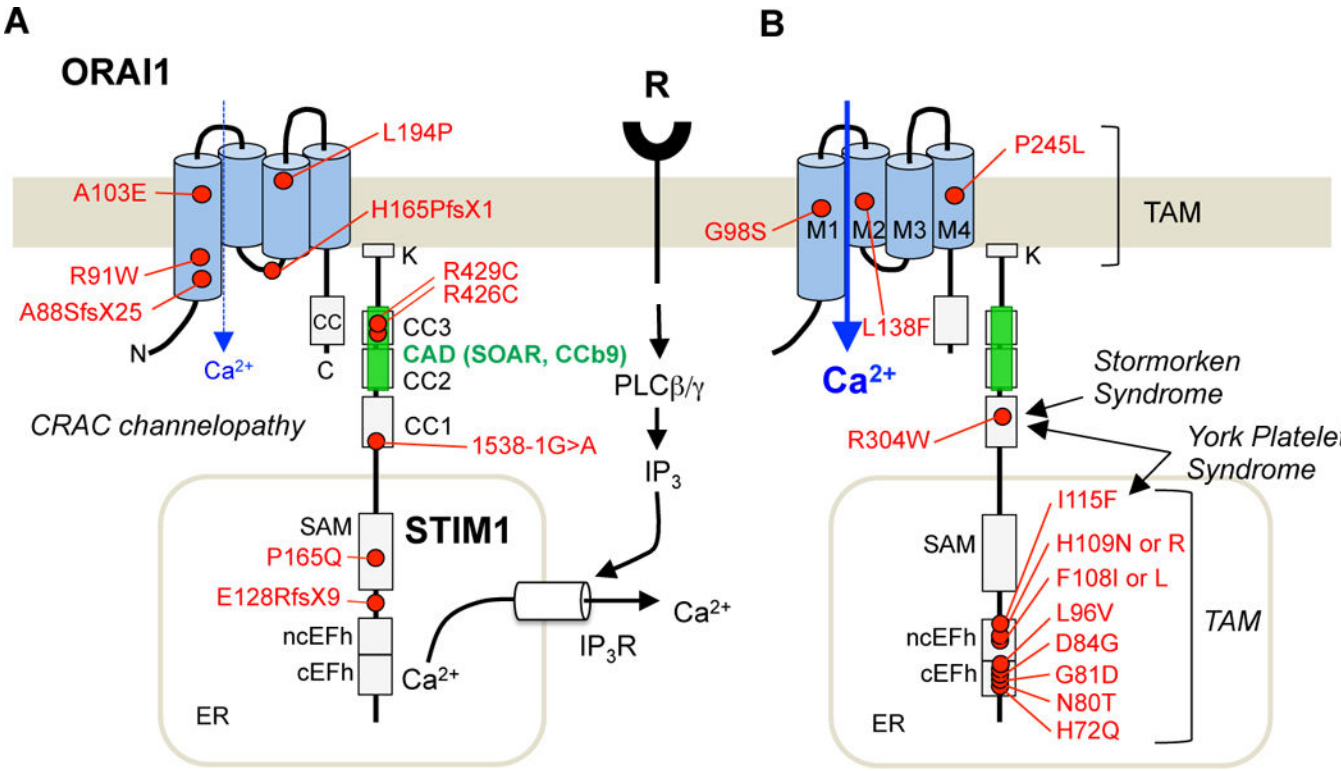

Figure 3.

Loss- and gain-of-function mutations in *ORAI1* and *STIM1*. (A) Loss-of-function (LoF) mutations in *ORAI1* and *STIM1* abolish SOCE and cause CRAC channelopathy. Patients are homozygous either for null mutations that abolish protein expression of ORAI1 (p.A88SfsX25, p.A103E, p.L194P, p.H165PfsX1) and STIM1 (p.E128RfsX9, c. 1538-1G>A) or LoF mutations that disrupt the function of ORAI1 (p.R91W) and STIM1 (p.R429C, p.P165Q, p.R426C?). The ORAI1 p.R91W mutation does not interfere with channel expression but blocks permeation of Ca^{2+} through the CRAC channel pore. The STIM1 p.R429C mutation has complex effects on STIM1 function and impairs CRAC channel activation at multiple steps (as shown in Fig. 4). How STIM1 p.P165Q and p.R426C mutations inhibit SOCE has not been established. (B) Gain-of-function (GoF) mutations in *ORAI1* and *STIM1* result in constitutive Ca^{2+} influx and cause three clinically overlapping disease syndromes. Patients with Stormorken syndrome are heterozygous for a single autosomal dominant STIM1 p.R304W mutation located in CC1. The same mutation was described to cause the clinically very similar York platelet syndrome. Tubular aggregate myopathy (TAM) is caused by a variety of autosomal dominant mutations in the EFh of STIM1 or in transmembrane domains of ORAI1 that result in constitutive CRAC channel activation. Thin, dashed arrows through ORAI1 indicate abrogated SOCE; thick, solid arrows indicate constitutive Ca^{2+} influx. Abbreviations: CCb9, Coiled-coiled fragment b9; fs, frameshift; IP_3, inositol 1,4,5-triphosphate; M, transmembrane domain; ncEFh, non-canonical EF hand; PLC, phospholipase C; SAM, sterile alpha motif; SOAR, STIM-ORAI activation region.

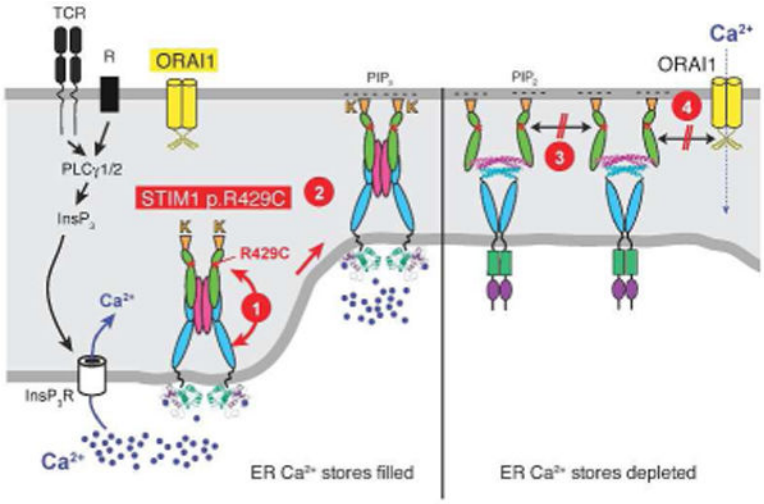

Figure 4.

The STIM1 p.R429C LoF mutation interferes with CRAC channel activation. The STIM1 p.R429C mutation has complex effects on STIM1 function and interferes with CRAC channel activation at multiple steps. The mutation results in constitutive opening of the STIM1-CT independent of the filling state of ER Ca^{2+} stores (1). In the extended conformation, the K-rich polybasic domain is exposed and mutant STIM1 is constitutively recruited to ER-PM junctions (2). Because R429 is required for the CC3 domain-mediated oligomerization of STIM1 dimers, mutant STIM1 cannot form puncta (3). Mutation of R429 abolishes the binding of STIM1 to ORAI1 (4). As a consequence, mutant STIM1 p.R429C fails to open ORAI1 channels and induce SOCE. For details see text. Blue circles represent Ca^{2+} ions. Dashed lines in the PM represent negatively charged phospholipids including PIP_2. Abbreviations: EFh, EF hand; CC1-CC3, coiled-coil; K, lysine-rich polybasic domain; PIP_2, phosphatidylinositol 4,5-bisphosphate; PM, plasma membrane; SAM, sterile alpha motif; STIM1-CT, STIM1 C-terminus.

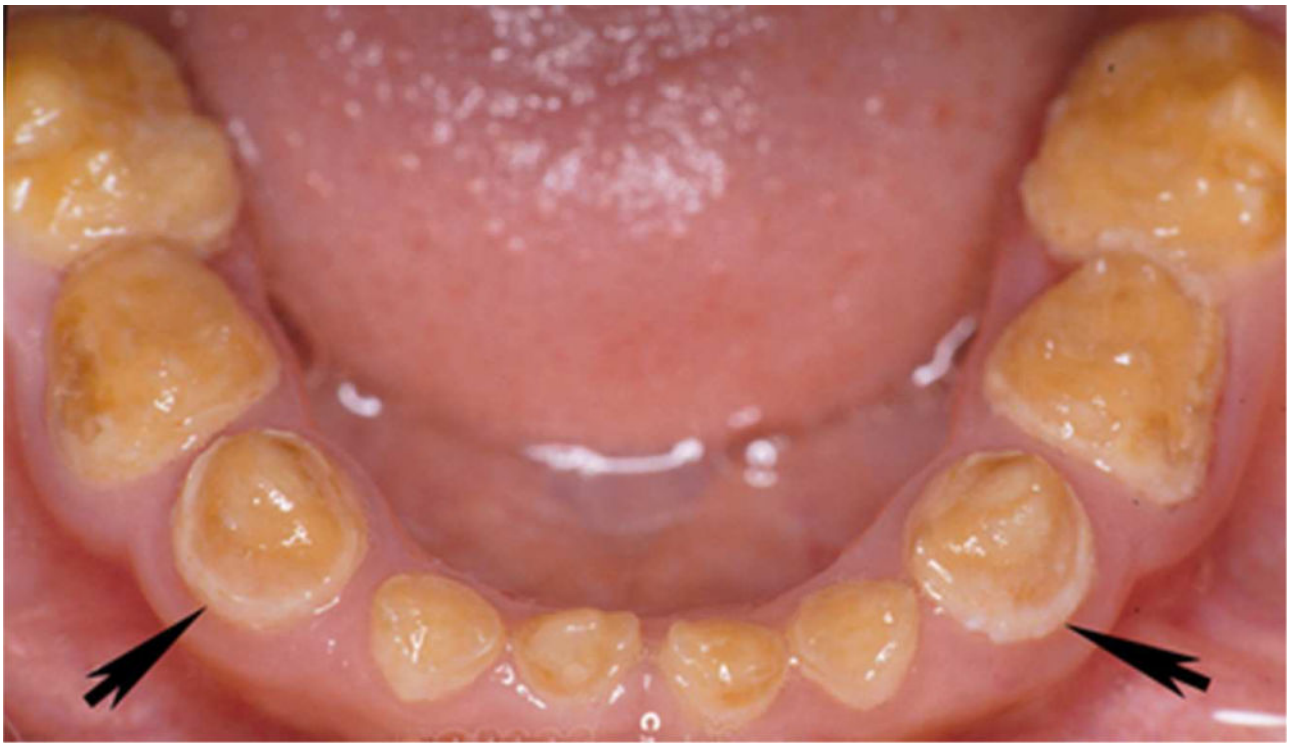

Figure 5.

Hypocalcified *amelogenesis imperfecta* in a patient with *ORAI1* loss-of-function mutation. Photograph of the teeth of a patient with ORAI1 p.R91W mutation that abolishes SOCE (originally reported in Refs.[1, 39]). Shown here is the lower jaw at approximately 6 years of age. All erupted teeth have lost their enamel capping on the occlusal surfaces and only remnants can be seen on the lateral margins of the teeth (arrows). The yellowish appearance of the teeth is related to dentine exposure.

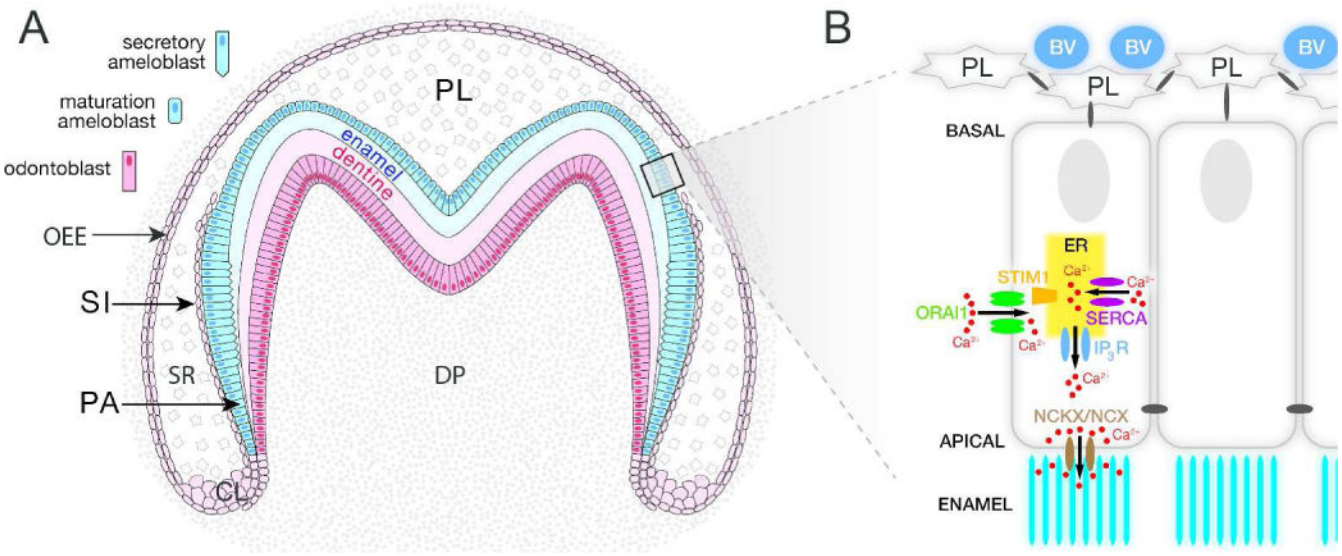

Figure 6.

Schematic representation of a tooth, enamel cells and the Ca^{2+} transport model. (A) Schematic section of a human molar showing the location of enamel forming cells, ameloblasts, in relation to other structures and cells. The main structures found in a tooth are the outer enamel layer, the dentine (formed by odontoblasts) and the innermost dental pulp. Secretory ameloblasts are epithelium-derived cells arising from pre-ameloblasts (PA) at the cervical loop (CL). The cells undergo morphological changes including increases in height, and develop a specialized secretory process at the distal (apical) end of the cell. Secretory ameloblasts reorganize into maturation stage ameloblasts once the thickness (volume) of enamel tissue has been completed. Maturation stage ameloblasts are shorter, loose the secretory process (Tomes' process) and switch their functional properties to become cells specialized in transport. (B) Representative model of maturation stage ameloblasts showing the localization of ORAI1 and STIM1 in the plasma membrane and ER, respectively. This working model of Ca^{2+} transport in ameloblasts builds on the model proposed by Hubbard [124]. It postulates that Ca^{2+} enters through PM Ca^{2+} channels and is taken up into the ER at the basolateral pole of the cell, and released into the cytoplasm via IP_3R or RyR at the apical pole, where it is secreted into the extracellular enamel matrix. The emerging details and molecular components of this Ca^{2+} transport need to be investigated further, but our recent data suggest that STIM1 and ORAI1 mediate Ca^{2+} uptake whereas NCKX/NCX exchangers mediate Ca^{2+} extrusion. Abbreviations: BV, blood vessels; DP (dental pulp); NCKX, Na^+/Ca^{2+}-K^+ exchanger; NCX, Na^+/Ca^{2+} exchanger; OEE (outer enamel epithelium); PL (papillary layer); SERCA, Sarco/endoplasmic reticulum Ca^{2+} ATPase; SI (stratum intermedium); SR (stellate reticulum).

Table 1

Clinical and cellular phenotypes in patients with loss- and gain-of-function mutations in *ORAI1* and *STIM1*

	Gene	Mutations	Prot.	Ca^{2+} influx	Cell types/tissues affected	Clinical phenotype	Lab results	Onset	Outcome	Ref.
Loss-of-function or null mutation (autosomal recessive)	**ORAI1**	p.R91W	Y	None	T, B, NK cells, PMN, platelets, fibroblasts, skeletal muscle, eccrine sweat glands, ameloblasts	**CRAC channelopathy:** • Infections (all patients): BCGitis, rota virus, chronic diarrhea, interstitial pneumonia, gastrointestinal sepsis, recurrent pneumonia, otitis, oral and gastrointestinal candidiasis, pyelonephritis; CMV pneumonitis • Autoimmunity (1/7): neutropenia, thrombocytopenia • Muscular hypotonia (all patients) with atrophy of type 2 fibers (documented in patient with ORAI1 p.R91W) • Ectodermal dysplasia: - Anhydrosis (4/7) - Dental enamel maturation defect (4/7) • Idiopathic encephalopathy, facial dysmorphy, posterior arch closing defect (1/7). EBV^+ lympho-proliferative disease (1/7)	• Lymphocyte counts and populations normal, reduction in naive T cells • Proliferation ↓↓ • Cytokine production ↓↓ • Serum Ig levels normal to ↑ • Specific Ig: none (most patients); protective Ig against 5/14 pneumococcal serotypes without immunization (p.H165PfsX1) • NK cell cytotoxicity ↓↓	< 1y	(i) Death from sepsis 11m. (ii) Survival after HSCT	1, 39, 41, 61, 70, 72, 73, 79
		p.A88SfsX25	N	None				< 1y	(i) Death from pneumonia 5m. (ii) Death from encephalopathy, seizures, fever 11m.	39, 91
		p.A103E/p.L194P (compound het.)	N	None				< 1y	(i) Death 8m. (ii) Initial survival after two HSCT (but post-HSCT EBV^+ lymphoproliferative disease), death from respiratory failure 20y	39, 71
		p.H165PfsX1	N	None				< 1y	(i) Survival after HSCT (sibling survived after HSCT)	68
	STIM1	p.E128RfsX9	N	None	T, B, NK cells, PMN, platelets, fibroblasts, skeletal muscle, eccrine sweat glands, ameloblasts	**CRAC channelopathy:** • Infections (all patients): Pneumonia, otitis, sepsis. Pathogens including herpes viruses (EBV, VZV, CMV, HSV, HHV-8) and bacteria (*E.coli*, *S.pneumoniae)* • Autoimmunity: AIHA and thrombocytopenia (6/7); splenomegaly and lymphadenopathy (7/7), ANA ↑↑ (2/7), eosinophilic colitis (2/7), ekzema (4/7) • Muscular hypotonia (6/7), partial iris hypoplasia / mydriasis (3/7), enuresis (1/7) • Ectodermal dysplasia: -Anhydrosis (6/7)	• Lymphocyte counts and populations largely normal. $CD25^+Foxp3^+$ Treg cells ↓, NKT cells ↓↓ • Proliferation ↓↓ • Serum Ig levels normal to ↑ • Specific Ig: none (p.E128RfsX9); none against H. influenza B, pneumococcae, measles, mumps, but detectable against tetanus, rubella, EBV, CMV, and HSV (p.R429C) • NK T cell cytotoxicity ↓↓	< 1y	(i) Death from complications of HSCT 9y. (ii) Death from encephalitis 18m. (iii) Survival after HSCT.	40
		c.1538-1 G>A	N	None				< 1y	(i) Death from pulmonary infection	59, 60
		p.R429C	Y	None				< 1y	(i) Death from sepsis 21m. (ii) Survival after HSCT.	44, 45, 101
		p.P165Q	Y	None				< 1y	(i) Death from complications of HSCT 9y. (ii) Death from encephalitis 18m. (iii) Survival after HSCT.	40

	Gene	Mutations	Prot.	Ca^{2+} influx	Cell types/tissues affected	Clinical phenotype	Lab results	Onset	Outcome	Ref.
						-Dental enamel maturation defect (6/7) -Nail dysplasia (3/7) • Other: HHV-8^+ Kaposi sarcoma (1/7), EBV^+ lymphoproliferative disease (1/7)	• Platelet degranulation ↓			
		c.1538-1 G>A	N	None				< 1y	(i) Death from pulmonary infection	59, 60
		p.R429C	Y	None				< 1y	(i) Death from sepsis 21m. (ii) Survival after HSCT.	44, 45, 101
		p.P165Q	Y	None				< 1y	unknown	42
		p.R426C	?	n.t.	Ameloblasts	• Dental enamel maturation defect • Mild immunodeficiency? (recurrent throat infections)	n.t.	Child	**i.** Survival w/o HSCT (21y) **ii.** Survival w/o HSCT (8y)	55
Gain-of-function mutation (autosomal dominant)	**ORAI1**	p.P245L	Y	SOCE ↑, impaired I_{CRAC} inactivation	Skeletal muscle	• Stormorken-like syndrome: • Tubular aggregate myopathy (2/2) • Miosis (2/2)	•TA confirmed by histology and EM •CK levels ↑	Adult?	Slowly progressive	95, 162
		p.G98S	Y	Constitutive	Skeletal muscle	**Tubular aggregate myopathy (TAM)** (6/6)	• TA confirmed by histology and EM • CK levels ↑	Child	Slowly progressive	151
		p.L138F	Y					Child		
	STIM1	p.R304W	Y	Constitutive	Platelets, Skeletal muscle	**Stormorken syndrome:** • Bleeding diathesis (8/12), thrombocytopenia (13/13) • Asplenia / hypoplasia (8/13) • Muscle weakness and cramps (11/12) • Myalgia (4/13) • Miosis (13/13) • Headache (6/9) • Ichthyosis (10/10) • Learning defects (6/6) • Short stature (5/9)	• TA confirmed by histology and EM • CK levels ↑ • Hypocalcemia • Mild anemia	Child, early adult	Stable or slowly progressive	93, 94, 95
		p.R304W	n.t.	n.t.	Platelets, Skeletal muscle	**York platelet syndrome (YPS):**	• Platelets: defects in aggregation, activation,	<1y, child	Stable or slowly progressive	133
		p.I115F					• Platelets: defects in aggregation, activation,	<1y, child	Stable or slowly progressive	133

	Gene	Mutations	Prot.	Ca^{2+} influx	Cell types/tissues affected	Clinical phenotype	Lab results	Onset	Outcome	Ref.
						• Bleeding diathesis, thrombocytopenia (6/7) • Spleen hypoplasia (1/7) • Proximal muscle weakness (5/7) • Ophthalmoplegia (2/7) • Miosis (2/7)	ATP release; 'opaque bodies' in EM • Skeletal muscle: -Muscle atrophy, fatty infiltration in MRI -Variation in myofiber size, myopathic changes, 'rimmed vacuoles' in histology • CK levels ↑			
		p.I115F	n.t.	n.t.	Skeletal muscle	**Tubular aggregate myopathy (TAM):** • Progressive muscle weakness (18/23) • Eye movement defects (8/23) • Contractures (13/23) • Myalgia (6/23)	• TA confirmed by histology and EM • CK levels ↑	Child	Slowly progressive	149
		p.H109R								
		p.D84G	Y	Constitutive in p.D84G (others n.t.) (STIM1 clustering)				Child, adol.	Slowly progressive >35y	13
		p.H109N	Y					Child, adol., adult	Slowly progressive >35y	
		p.H109R	Y					Child	Stable or progressive	
		p.H72Q	Y					Asymptomatic		
		p.N80T	Y	n.t. (STIM1 clustering ↑)				Child, adult	Slowly progressive	12
		p.L96V	Y							
		p.F108I	Y							
		p.F108L	Y					Child Child Adult	Myalgia only	
		p.G81D	Y	Moderate constitutive Ca^{2+} influx and SOCE ↑				Child	Slowly progressive	14

Numbers in parenthesis in the "clinical phenotype" column refer to the number of affected patients of all patients examined. The total number of patients varies because not all phenotypes/parameters were measured or reported in all patients. Abbreviations: AD, autosomal dominant; AIHA, autoimmune hemolytic anemia; ANA, anti-nuclear antibody; AR, autosomal recessive; ATP, adenosine triphosphate; BCG, Bacillus Calmette-Guerin; CK, creatine kinase; CMV, cytomegalovirus; EBV, Epstein Barr virus; EM, electron microscopy; fs, frameshift; HHV, human herpes virus; HSCT, hematopoietic stem cell transplantation; HSV, herpes simplex virus; I_{CRAC}, CRAC current; m, month; MRI, magnetic resonance imaging; NK, natural killer; n.t., not tested; PMN, polymorphonuclear cell; SCID, severe combined immunodeficiency; TAM, tubular aggregate myopathy; T_{reg}, regulatory T cell; VZV, varicella zoster virus; y, year.