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ORAI1 deficiency and lack of store-operated Ca^{2+} entry cause immunodeficiency, myopathy and ectodermal dysplasia

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Abstract

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Background—Defects in the development or activation of T cells result in immunodeficiency associated with severe infections early in life. T cell activation requires Ca^{2+} influx through Ca^{2+}-release activated Ca^{2+} (CRAC) channels encoded by the gene *ORAI1*.

Objective—Investigation of the genetic causes and the clinical phenotype of immunodeficiency in patients with impaired Ca^{2+} influx and CRAC channel function.

Methods—DNA sequence analysis for mutations in the genes *ORAI1, ORAI2, ORAI3, stromal interaction molecules (STIM) 1* and *2* as well as mRNA and protein expression analysis of ORAI1 in immunodeficient patients. Immunohistochemical analysis of ORAI1 tissue distribution in healthy human donors.

Results—We identified mutations in ORAI1 in patients from two unrelated families. One patient is homozygous for a nonsense mutation in *ORAI1* (*ORAI1-A88SfsX25*) and a second patient is compound heterozygous for two missense mutations in *ORAI1 (ORAI1-A103E/L194P)*. All three mutations abolish ORAI1 expression and impair Ca^{2+} influx and CRAC channel function. The clinical syndrome associated with ORAI1 deficiency is characterized by immunodeficiency with a defect in the function but not the development of lymphocytes, congenital myopathy and anhydrotic ectodermal dysplasia (EDA) with a defect in dental enamel calcification. In contrast to the limited clinical phenotype, we found ORAI1 protein expression in a wide variety of cell types and organs.

Conclusion—Ca^{2+} influx through ORAI1 is crucial for lymphocyte function *in vivo*. Despite almost ubiquitous ORAI1 expression, the channel has a non-redundant role in only a few cell-types judging from the limited clinical phenotype in ORAI1 deficient patients.

Keywords

ORAI1; STIM1; CRAC; calcium channel; Ca^{2+}; store-operated Ca^{2+} entry; T cells; immunodeficiency; signal transduction; congenital myopathy; anhydrotic ectodermal dysplasia; dental enamel; amelogenesis imperfecta

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Key messages

- Nonsense and missense mutations in the Ca^{2+} channel gene *ORAI1* abolish ORAI1 protein expression and Ca^{2+} channel function.
- ORAI1 deficiency is defined clinically by immunodeficiency, myopathy and anhydrotic ectodermal dysplasia with a defect in dental enamel calcification.
- ORAI1 is almost ubiquitously expressed in human tissues despite the limited clinical phenotype of ORAI1 deficiency indicating a non-redundant role for ORAI1 in store-operated Ca^{2+} influx in T cells, skeletal muscle and some ectodermal derived tissues.

Introduction

Severe combined immunodeficiency (SCID) is characterized by the absence or significant functional impairment of T, B and/or NK cells [1, 2]. Lymphocyte activation follows immunoreceptor engagement which results in Ca^{2+} signaling, proliferation and cytokine gene expression [3]. In T cells, Ca^{2+} influx occurs following activation of phospholipase C (PLC) γ1 and release of Ca^{2+} from intracellular ER stores. Release of stored Ca^{2+} results in a transient increase in $[Ca^{2+}]_i$ and subsequently activation of the Ca^{2+} release activated Ca^{2+} (CRAC) channel in the plasma membrane [4]. The Ca^{2+} influx resulting from CRAC channel activation is called store-operated Ca^{2+} entry (SOCE) because it depends on the depletion of ER Ca^{2+} stores.

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The CRAC channel constitutes the major Ca^{2+} influx channel in T cells and is encoded by *ORAI1* [3, 4], a tetraspanning plasmamembrane protein that is structurally unrelated to other ion channels except its two paralogues ORAI2 and ORAI3. ORAI1 functions as the pore forming subunit of the CRAC channel [5–7]. A missense mutation in ORAI1 (R91W) abolishes ORAI1 and CRAC channel function and causes SCID characterized by a severe defect in T cell activation [8, 9]. ORAI1-CRAC channels are activated by the ER protein stromal interaction molecule (STIM) 1 which senses the ER Ca^{2+} concentration and, upon release of Ca^{2+} from ER stores, multimerizes and binds to ORAI1 [4]. Lack of STIM1 expression in human patients due to a nonsense mutation in *STIM1* (E136X) severely impairs SOCE and causes immunodeficiency and autoimmunity associated with myopathy and abnormal enamel dentition [10].

In addition to patients with ORAI1-R91W and STIM1-E136X mutations [8, 10], a defect in SOCE and CRAC channel function has been described in patients from two kindreds in which the underlying gene defect remained undefined [11, 12]. We here report three new mutations in *ORAI1* in patients from two of the original kindreds that abolish ORAI1 protein expression and SOCE [11, 12]. These ORAI1 mutations and those in ORAI1 and STIM1 reported before [8, 10] collectively define the clinical phenotype associated with defects in CRAC channel function.

Materials and Methods

Case reports

Case reports of patients P1 – P6 have been published [11–15]. Follow-up data on all patients and clinical descriptions are provided in Table 1 and the online repository.

Cells

SV40-transformed fibroblasts from P4, P6 and a healthy control and Ficoll-Paque (GE Healthcare, Piscataway, NJ) isolated peripheral blood mononuclear cells from P6's parents and controls were grown in RPMI 1640 (Mediatech, Manassas, VA).

Plasmids and transfections

IRES-GFP containing bicistronic vectors for expression of myc-epitope tagged ORAI1, ORAI2, ORAI3 or STIM1 have been described [8, 16]. ORAI1 A88SfsX25, A103E and L194P mutant plasmids were generated by overlap mutagenesis and used for retroviral transduction as described[8]. Transduction efficiencies were evaluated by GFP expression and immunoblotting using anti-myc antibody (clone 9E10, Santa Cruz Biotechnology, Santa Cruz, CA).

Genomic DNA sequencing

Genomic DNA was isolated from cells using standard methods. PCR was conducted using primers flanking exons and splice sites of *ORAI1, ORAI2*, *ORAI3*, *STIM1* and *STIM2* (**Table E2**). PCR products were sequenced directly (Genewiz Inc., South Plainfield, NJ). Sequence alignments were performed using TCoffee software (Swiss Institute of Bioinformatics) and sequence traces visualized using Xplorer software v1.0 (dnaTools). Single nucleotide polymorphism (SNP) searches were performed using dbSNP database (build 129; http://www.ncbi.nlm.nih.gov/SNP/).

Immunohistochemistry and antibodies

For detection of ORAI1 in patient fibroblasts, cell pellets were fixed in 3% phosphate-buffered paraformaldehyde, permeabilized with 1x PBS, 0.5% NP-40, 0.02% sodium azide and incubated with affinity-purified anti- ORAI1 antibodies raised against aa 275–291 of human

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ORAI1. For immunofluorescence, a muscle biopsy sample of P2 was co-incubated with antibodies to anti-ORAI1 and anti-myosin heavy chain fast (clone WB-MHCf, Novocastra, Newcastle upon Tyne, UK) at 1:50 dilution; MHCf was detected by Alexa Fluor 488 goat anti-mouse IgG staining (Invitrogen, Carlsbad, CA). For detection of ORAI1 in tissues from healthy donors, 5-μm sections of paraffin-embedded normal human tissue microarrays (FDA 801, US Biomax Inc., Rockville, MD) were incubated with anti-ORAI1 antibodies and prepared as described [17].

Muscle biopsy

A biopsy of P2's vastus lateralis muscle was frozen in isopentane cooled in liquid nitrogen and 10 μm cryostat sections were stained with standard histological and histochemical techniques [18].

Ca^{2+} measurements

Single-cell Ca^{2+} imaging was performed as described [9]. Traces in figures represent mean $[Ca^{2+}]_i$ of one representative experiment; ~ 30–80 GFP^+ cells per experiment were analyzed. Error bars represent SEM.

Additional methods are available in the online repository accompanying this article.

Results

Homozygous A88SfsX25 ORAI1 nonsense mutation abolishes ORAI1 expression

Ca^{2+} influx and CRAC channel currents were reported to be undetectable in T cells from immunodeficient patient 4 (P4) resulting in severely impaired T cell activation (**Table E1**) [12]. Genomic DNA sequence analysis revealed that P4 is homozygous for a nonsense mutation in exon 1 of *ORAI1* resulting from the insertion of a single adenine between positions 258 and 259 (258–259insA) of the *ORAI1* coding sequence (NM_32790) (Fig 1,***A***). The mutation is not a known SNP and was not observed in two healthy siblings of P4 (B-V-4 and B-V-5 in **Fig E1**) and DNA from 50 control individuals (100 chromosomes). DNA from his parents and his older brother (P3) was not available for analysis. The insertion causes a frame shift starting at amino acid residue 88 and premature termination at position 112 of ORAI1 protein (ORAI1-A88SfsX25) at the end of the first transmembrane (TM) domain (Fig 1, ***A***). No mutations in *ORAI2*, *ORAI3, STIM1* and *STIM2* were found in P4.

Northern blot analysis showed that ORAI1 mRNA transcripts were undetectable in P4 compared to cells from a healthy control (Fig 1, ***B***) most likely due to nonsense-mediated mRNA decay. Fibroblasts from P4 also showed strongly reduced ORAI1 protein expression when cells were analyzed by immunohistochemistry using an anti-ORAI1 antibody (Fig 1, ***C***). Since the antibody is directed against the C-terminus of ORAI1 we tested the possibility that a truncated ORAI1 fragment lacking the C terminus could be expressed. Ectopic expression of an N-terminally myc-tagged version of mutant ORAI1-A88SfsX25 in HEK293 cells, however, only yielded a weak single band observed at ~15 kD in SDS-PAGE, corresponding to the predicted ORAI1 fragment (**Fig E2, *A***). This fragment, if expressed at all endogenously, is very unlikely to be functional as an ion channel because it lacks transmembrane domains required for Ca^{2+} conductance. Finally, reconstitution of patient fibroblasts with wild-type ORAI1, but not empty vector or STIM1, rescued Ca^{2+} influx upon stimulation with thapsigargin, an inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) that induces passive Ca^{2+} store depletion and CRAC channel activation (Fig 1, ***D***). Failure of STIM1 expression to rescue SOCE also argues against residual ORAI1 expression in the cells of P4 because co-expression of STIM1 and ORAI1 was shown to dramatically enhance CRAC

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channel function [19, 20]. Collectively, these data show that the nonsense mutation in *ORAI1* is responsible for the lack of Ca^{2+} influx in P4.

ORAI1 missense mutations A103E and L194P abolish ORAI1 protein expression

T cells from patient 6 (P6) and his deceased brother P5 displayed a severe proliferation defect (**Table E1**) [11]. Ca^{2+} influx in T cells, B cells, platelets and fibroblasts from P6 was reported to be profoundly impaired (**Fig E2, *B***) [11]. Sequence analysis of genomic DNA from P6 demonstrated that he is compound heterozygous for two independent missense mutations in exon 2 of *ORAI1*. The C→A and T→C mutations at positions 308 and 581 of the *ORAI1* coding sequence, respectively, are not known SNPs and were not detected in 50 healthy controls (100 chromosomes) (Fig 2, ***A***). The missense mutations result in the substitution of an alanine with glutamate (A103E) and a leucine with proline (L194P) in the first and third TM domains of ORAI1, respectively (Fig 2, ***A, E***). P6 inherited the mutated alleles from his father (C-I-1 in **Fig E1**: A103E) and his mother (C-I-2 in **Fig E1**: L194P) who are heterozygous for one but not the other mutation. Mutations in other genes including *ORAI2, ORAI3, STIM1* and *STIM2* were not observed in P6.

The mutations had no effect on ORAI1 mRNA transcription (not shown), whereas ORAI1 protein expression was undetectable in fibroblasts from P6 by immunohistochemistry (Fig 2, ***B***) and flow cytometry (not shown) suggesting that both ORAI1 mutations interfere with stable protein expression. To test this hypothesis, we ectopically expressed ORAI1-A103E and ORAI1-L194P separately in HEK293 cells using bicistronic IRES-GFP vectors. Expression of either mutant protein was undetectable compared to wild-type ORAI1 despite normal IRES-mediated GFP protein expression from the same mRNA transcript (Fig 2, ***C***). A dominant negative effect of the mutations on CRAC channel function was ruled out because Ca^{2+} influx in T cells from P6's parents, each heterozygous for one of the mutations, was normal (**Fig E2, *C***) and ectopic expression of ORAI1-A103E or ORAI1-L194P in HEK293 cells had no effect on Ca^{2+} influx (**Fig E2, *D***).

Reconstitution of P6's fibroblasts with wild-type ORAI1, but not STIM1, ORAI2 or empty vector rescued Ca^{2+} influx upon stimulation with thapsigargin (Fig 2, ***D***). Ectopic expression of ORAI3 partially restored SOCE consistent with similar observations in cells of P2 [16]. Taken together, these findings show that two independent missense mutations in *ORAI1* severely compromise stable ORAI1 protein expression abolishing Ca^{2+} influx and causing immunodeficiency.

Myopathy and anhydrotic ectodermal dysplasia (EDA) in ORAI1 deficient patients

The clinical immunodeficiency in patients lacking ORAI1 expression (P3– P6) is very similar to that observed in patients (P1, P2) homozygous for an ORAI1-R91W missense mutation that abolishes ORAI1 function but not its expression (Table 1, Table E1) [8, 14].

All six ORAI1-deficient patients showed non-immunological symptoms as well. Both patients surviving after HSCT (P2, P6) presented with EDA characterized by severely dysplastic dental enamel and pronounced anhydrosis resulting in dry skin and heat intolerance; no hair abnormalities or pigmentation defects were observed (Fig 3, ***A–D***). Hypocalcified amelogenesis imperfecta (type III) in P2 and P6 is due to a failure of dental enamel matrix calcification resulting in use-dependent loss of the unusually soft dental enamel of both deciduous and permanent teeth. All patients (P1–P6) suffered from global muscular hypotonia from birth. In P2 and P6, the myopathy compromises the patients' mobility and results in chronic pulmonary disease due to respiratory muscle insufficiency. Histologically, the myopathy in P2 is characterized by a variation in muscle fiber size with a predominance of type I fibers and atrophic type II fibers (Fig 3, ***E–F***). Other histological abnormalities

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commonly found in myopathies were not observed in P2. Consistent with a role for ORAI1 in myocyte function [21–23], we found sarcolemmal expression of ORAI1 in muscle fibers of wild-type controls and both type I and type II muscle fibers of P2 (Fig 3, ***G–J***).

Almost ubiquitous ORAI1 protein expression

The clinical syndrome associated with ORAI1 deficiency indicates that ORAI1-dependent SOCE is essential for the function and/or development of lymphocytes, skeletal muscle and ectodermal derived tissues. ORAI1 mRNA expression and SOCE, however, have also been demonstrated in many other organs and cell types [16, 24]. No data on the cellular distribution pattern of ORAI1 have been reported. We detected ORAI1 expression in $CD4^+$ and $CD8^+$ T cells and $CD19^+$ B cells consistent with the known role of ORAI1 in lymphocyte function (Fig 4, ***A***). Immunhistochemical analysis showed that ORAI1 is expressed in a subset of cells in primary and secondary lymphoid organs such as thymus, spleen and tonsils (Fig 4, ***B–E*** and data not shown). The strongest expression was observed in lymphoid cells in the periarterial lymphoid sheath (PALS) of spleen (Fig 4, ***E***) and the paracortical zone in tonsils (data not shown) consistent with ORAI1 expression in T cells. Outside the immune system, ORAI1 expression was found in a wide variety of cell types and organs (Fig 4) including those affected in the patients such as eccrine sweat glands (Fig 4, ***F***) and other tissues not apparently affected in the patients such as skin, vascular endothelium, mucosal epithelial cells of the gastrointestinal tract, several endocrine and exocrine glands, hepatocytes, pneumocytes in the lung and kidney tubules (Fig 4). Of note is the almost complete absence of ORAI1 staining in the central nervous system consistent with previously reported low ORAI1 mRNA levels in the brain (http://www.brain-map.org) [16, 25].

Discussion

The ORAI1 deficient patients described here represent all patients known to date with CRAC channel dysfunction due to mutations in ORAI1. Their clinical phenotypes illustrate the *in vivo* role of ORAI1 in human and together with patients lacking STIM1 expression [10] define a new disease syndrome resulting from a defect in SOCE and CRAC channel function [11, 12]. The mutations described in this study cause a human null-phenotype for ORAI1 by interfering with mRNA expression as in the case of the A88SfsX25 nonsense mutation in P4 or protein stability and expression as in the case of the ORAI1-A103E and ORAI1-L194P missense mutations in P6. The A103E substitution introduces a negative charge in close proximity to E106 – a Ca^{2+} binding site in the CRAC channel pore [5–7] – likely resulting in electrostatic repulsion and destabilization of the first transmembrane alpha helix (Fig 2, *A*). Substitution of L194 with proline at the end of the third TM domain is likely to break or kink the TM alpha helix resulting in the destabilization of protein expression [26, 27].

Clinically, ORAI1 deficiency is characterized by a severe defect in adaptive immune responses resulting in life-threatening infections with viral, bacterial and fungal pathogens, congenital myopathy and EDA with defects in enamel dentition and sweat production (Table 1). The immunodeficiency is very similar to SCID but– unlike in the majority of SCID cases – lymphocyte development is unperturbed. Numbers of T cells and T cell subsets, B cells and NK cells in peripheral blood of patients were normal suggesting that lymphocyte differentiation including the selection and maturation of T cells occurs independently of ORAI1 (**Table E1**) [11, 12, 14]. These findings are consistent with normal lymphocyte development observed in STIM1 deficient patients [10] and *Orai1* and *Stim1* deficient mice [28–31]. By contrast, T cell activation is severely compromised in all six patients as skin delayed-type hypersensitivity reactions *in vivo* and proliferative responses to a variety of stimuli *in vitro* are reduced or absent consistent with the severe defect in cytokine production in T cells of P1 and P2 reported previously [13, 14]. T cells from P4 and P6, but not those of P1 and P2, proliferated normally

following PMA and ionomycin treatment. The discrepancy may be due to a potential inhibitory effect of mutant ORAI1-R91W on residual ORAI2 Ca^{2+} channels which have been implicated in Ca^{2+} influx in mouse T cells [31]. Given the important role of Ca^{2+} influx for B and NK cell function [32–35] and ORAI1 expression in both cell types at least in mice [36], the SOCE defect documented in B cells from P2 [37] and P6 (Fig E2) [11] and a potential SOCE defect in NK cells may contribute to the patients' immunodeficiency.

Autoimmunity was observed in only one (P4) out of six ORAI1 deficient patients who presented with neutropenia and thrombocytopenia. By contrast, all STIM1 deficient patients showed autoimmune symptoms and one patient was observed to have reduced numbers of $CD4^+$ $Foxp3^+$ regulatory T cells (T_{reg}) [10]. Numbers of T_{reg} cells in ORAI1 deficient patients could not be evaluated because blood samples preceding HSCT were not available. A plausible explanation for the absence of autoimmunity in most ORAI1 deficient patients is that four of the six patients (P1, P3-P5) died in their first year of life, presumably before the onset of autoimmune disease, and the two surviving patients (P2, P6) received HSCT at 4 months of age preventing autoimmunity. Taken together, our findings demonstrate that ORAI1, SOCE and CRAC channel function are required for T cell activation but largely dispensable for T cell development.

The non-immunological symptoms observed in ORAI1 deficient patients overlap with those found in patients lacking STIM1 strongly indicating that the phenotype is due to a defect in SOCE and CRAC channel function. All ORAI1 deficient patients suffer from congenital myopathy characterized by global muscular hypotonia and, in one patient, atrophy of type II muscle fibers. These observations suggest that SOCE is required for skeletal muscle function and/or differentiation. SOCE in murine skeletal myotubes is mediated by ORAI1 and STIM1 [22, 23] and results in the refilling of sarcoplasmic reticulum Ca^{2+} stores [38, 39] thus ensuring the muscle's ability to undergo repeated cycles of excitation-contraction coupling mediated by Ca^{2+} release from the SR. In addition, SOCE may be critical for skeletal muscle differentiation as it is involved in the expression of two early markers of myoblast differentiation, MEF2 and myogenin [21] and presumably the activation of the Ca^{2+} dependent transcription factor NFAT [40]. Myoblasts of *Stim1* and SOCE deficient mice not only fatigued rapidly but also showed severe morphological abnormalities consistent with a developmental defect [23].

EDA was observed in the two surviving ORAI1-deficient patients (P2, P6) and all STIM1-deficient patients indicating that SOCE is important for eccrine sweat gland function and/or development (skin biopsies from ORAI1 deficient patients to distinguish between these possibilities could not be obtained) and ameloblast function during calcification of the enamel matrix. Encephalopathy was observed only in P3 and P4 but not in the other ORAI1- (and STIM1-) deficient patients. It is therefore unlikely to be a common feature of ORAI1 deficiency but is potentially due to an additional genetic defect in a notably inbred kindred or infection with a neurotrophic pathogen, a relatively common complication in patients with defects in T cell mediated immunity. EDA with immunodeficiency (EDA-ID) caused by mutations in ORAI1 (and STIM1)[10] differs from EDA-ID due to mutations in IKKγ (NEMO)[41–43] and IκBα[44] in that the latter are both characterized by hypodontia, conical teeth in all and sparse scalp hair in some of the patients. In addition, patients with mutations in IκBα and IKKγ, but not ORAI1 (and STIM1), show a hyper-IgM phenotype with decreased serum IgG and increased IgM. While the hypermorphic IκBα mutation impairs T cell proliferation similar to ORAI1 (and STIM1) deficiency, only IκBα but not ORAI1 deficient patients lack γδ and memory αβT cells[44].

The limited clinical spectrum of ORAI1 deficiency contrasts with the almost ubiquitous expression of ORAI1 (Fig. 4) [16] and reports describing SOCE and CRAC channel function in many cell types outside the immune system [24, 45–48]. Explanations for the absence of more

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extensive disease in the ORAI1-deficient patients include that ORAI1 plays a redundant role for SOCE in many tissues and can be functionally replaced by, for instance, ORAI2 or ORAI3 and that SOCE co-exists with non-store operated Ca^{2+} influx. ORAI2 and ORAI3 can form Ca^{2+} channels when co-expressed ectopically with STIM1[49, 50] but direct evidence for a physiological role of endogenous ORAI2 and ORAI3 *in vivo* is still missing.

In summary, the ORAI1 mutations described in this study together with the phenotypes of ORAI1- and STIM1-deficient patients reported earlier[8, 10] define the clinical syndrome associated with defects in CRAC channel function and provide valuable insight into the role of ORAI1 and SOCE in human *in vivo*.

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Abbreviations used in this paper

CRAC	Ca^{2+} release activated Ca^{2+} (channel)
EDA	ectodermal dysplasia with anhydrosis
SCID	severe combined immunodefiency
SOCE	store-operated Ca^{2+} entry
STIM1	stromal interaction molecule 1
TM	transmembrane

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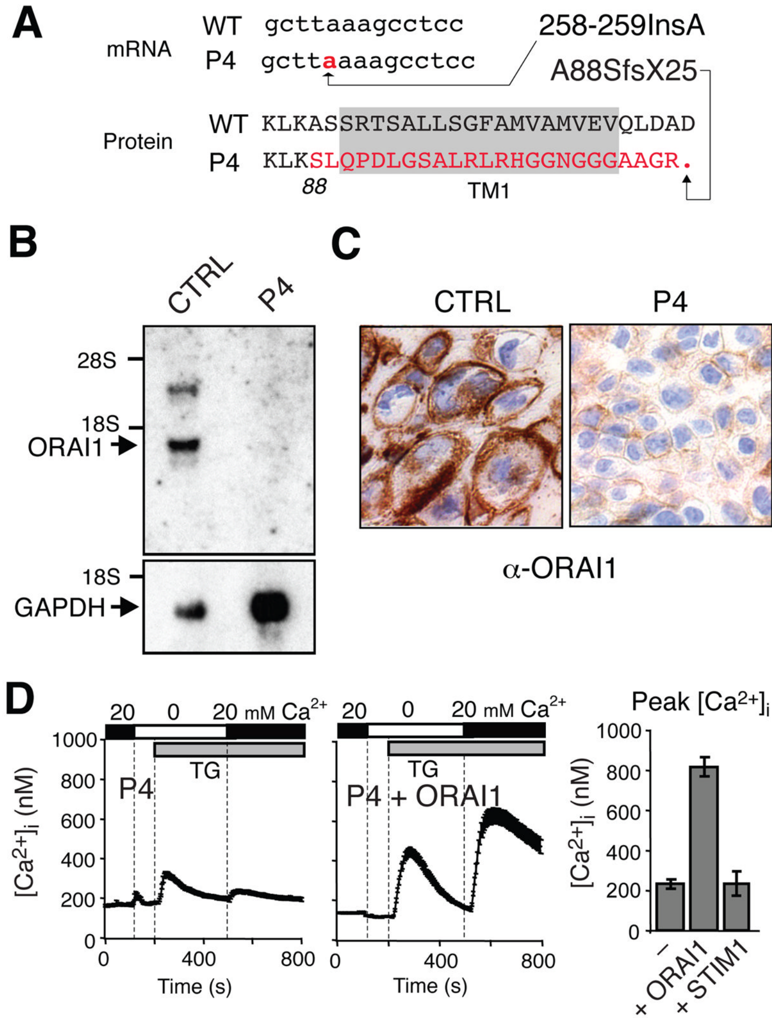

Figure 1. ORAI1 A88SfsX25 nonsense mutation abolishes ORAI1 expression in P4

A, Adenosine insertion (258–259insA) results in frame shift, premature termination (A88SfsX25) and altered amino acid sequence (red) of TM1 in ORAI1 (shaded). **B–C,** Non-detectable ORAI1 mRNA (B) and protein (C) expression in P4 compared to control. **D,** Impaired Ca^{2+} influx in fibroblasts from P4 (left) is restored by expression of ORAI1 (middle). Bar graphs represent averages of peak $[Ca^{2+}]_i$ from 4–6 experiments. TG, thapsigargin.

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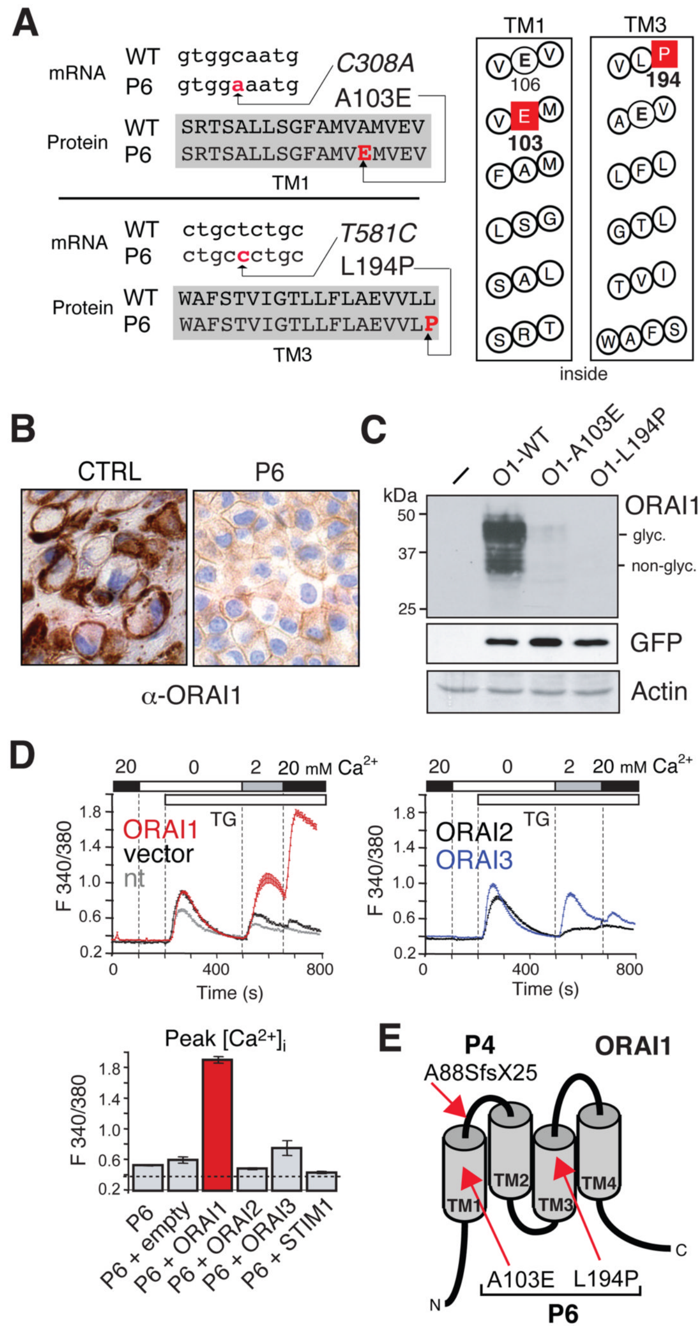

Figure 2. Two missense mutations abolish ORAI1 protein expression in P6

A, *ORAI1* C308A and T581C mutations result in single amino acid substitutions A103E and L194P in TM1 and TM3 (shaded or boxed) of ORAI1, respectively. **B–C,** Undetectable endogenous (B) and ectopic (C) expression of ORAI1 protein in fibroblasts from P6 (B) and HEK293 cells transfected with ORAI1 mutants (C). WT, wild-type; glyc., glycosylated. **D,** Impaired Ca^{2+} influx in nontransfected (nt) fibroblasts from P6 is restored by expression of ORAI1. Bar graphs represent averages of peak Ca^{2+} influx from 4–6 experiments. **E**, Location of *ORAI1* mutations in P4 and P6.

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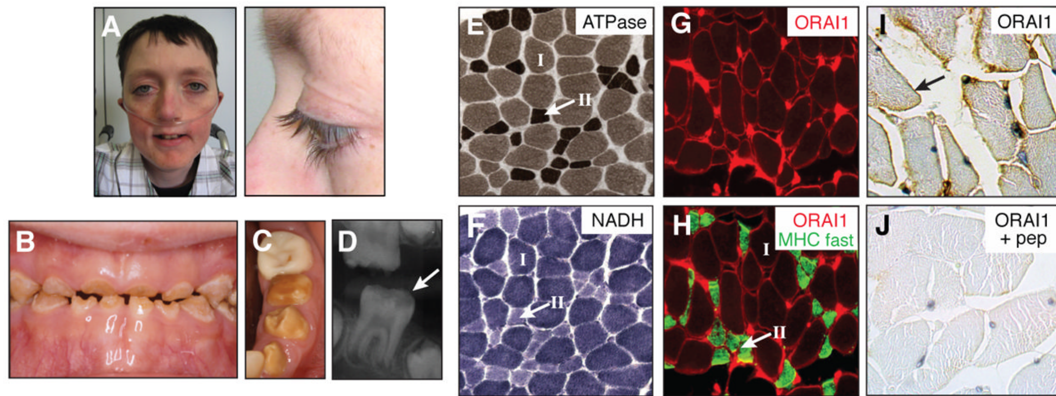

Figure 3. EDA and myopathy in ORAI1 deficient patients

A, Normal hair in P6 (age 16y). **B–D,** Hypocalcified amelogenesis imperfecta with significant loss of enamel substance in deciduous (B, P2 at age 6y) and permanent (C, P2 at age 10.5y; D, P6 at age 9.5y) teeth. **E–H,** Atrophy of type II muscle fibers in P2 (age 5y) by ATPase (E), NADH (F) and αORAI1(red)/αMHC fast (green) staining (G-H). **I–J,** ORAI1 expression in normal human skeletal muscle. Pep, blocking peptide.

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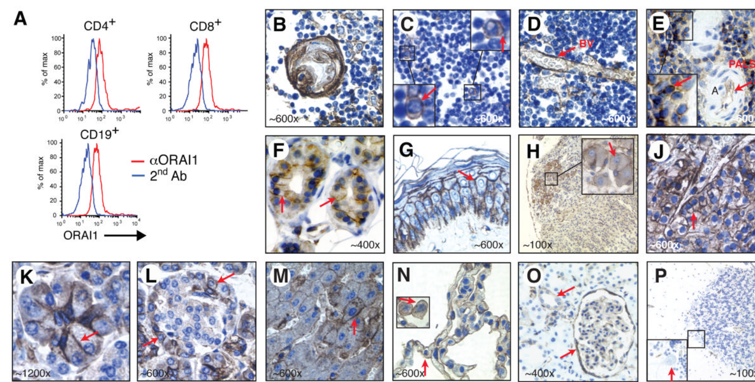

Figure 4. Almost ubiquitous ORAI1 tissue expression

A, ORAI1 expression in human $CD4^+$, $CD8^+$ and $CD19^+$ T and B cells analyzed by flow cytometry. **B–P,** ORAI1 expression in tissues from healthy donors incubated with αORAI1 antibody. Shown are thymus (B–D), spleen (E), eccrine sweat glands (F), skin (G), adrenal gland (H), parathyroid gland (J), exocrine pancreas (K), pancreatic islet (L), liver (M), lung (N), kidney (O), cerebellum (P). Specificity controls are shown in Figure E3. A, artery; BV, blood vessel; PALS, periarterial lymphoid sheath.

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TABLE 1

th ORAI1 mutations

P1 (A-IV-1)*	P2 (A-IV-2)	P3 (B-V-1)	P4 (B-V-3)	P5 (C-II-2)	P6 (C-II-3)
R91W	R91W	nt	A88SfsX25	nt	A103E/L194P
undetectable	undetectable	nt	undetectable	nt	undetectable
ingitis, rotavirus enteritis, al pneumonia, sepsis	No	Chronic diarrhea and candidiasis, pneumonia, pyelonephritis	Chronic diarrhea, chronic candidiasis, pneumonia, pyelonephritis, otitis.	Diarrhea, chlamydia pneumonia, toxoplasma encephalitis	Pneumonia, chronic diarrhea, CMV infection
No	No	No	Neutropenia and thrombocytopenia	No	No
Failure to thrive Small thymus	• Failure to thrive • EDA: -Amelogenesis imperfecta type 3, -Anhydrosis	• Developmental delay • Small thymus	• Developmental delay • Small thymus • Facial dysmorphy • Defect post. arch closing (C6-T6), clubfoot	• Failure to thrive	• Failure to thrive • EDA: - Amelogenesis imperfecta type 3 -Anhydrosis
genital muscular hypotonia Mydriasis	• Congenital muscular hypotonia	• Congenital muscular hypotonia • Encephalopathy	• Congenital muscular hypotonia • Encephalopathy • Spastic tetraparesis • Neonatal hypocalcemia	• Congenital muscular hypotonia	• Congenital muscular hypotonia • Eczema • Neovascularization of cornea
from pneumonia and sepsis	Alive (16y) after HSCT with persisting myo- pathy and EDA	Death at 5m from pneumonia	Death at 11m from progressive encephalo- pathy, fever, seizures	Death at 8m	Alive (16y) after HSCT with persisting myopathy and EDA
(8_9,13_15)	(8_9,13_14)	(12)	(12)	(11)	(11)

CG, Bacille Calmette-Guérin; HSCT, hematopoietic stem cell transplantation; nt, not tested; m, months; P, patient; y, years.