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

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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*, and *stromal interaction molecule (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 2 unrelated families. One patient is homozygous for a frameshift nonsense mutation in ORAI1 (ORAI1-A88SfsX25), and a second patient is compound heterozygous for 2 missense mutations in ORAI1 (ORAI1-A103E/L194P). All 3 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 in the development of lymphocytes, congenital myopathy, and anhydrotic ectodermal dysplasia with a defect in dental enamel calcification. In contrast with 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 nonredundant role in only a few cell types judging from the limited clinical phenotype in ORAI1-deficient patients. (J Allergy Clin Immunol 2009;124:1311-8.)

Key words: *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*

Severe combined immunodeficiency (SCID) is characterized by the absence or significant functional impairment of T, B, and/or natural killer (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 after activation of phospholipase C γ1 and release of Ca^{2+} from intracellular endoplasmic reticulum (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.

The CRAC channel constitutes the major Ca^{2+} influx channel in T cells and is encoded by *ORAI1*,[3,4] a tetraspanning plasma-membrane protein that is structurally unrelated to other ion channels except its 2 paralogs 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

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Abbreviations used
CRAC: Ca^{2+} release activated Ca^{2+}
EDA: Ectodermal dysplasia with anhydrosis
HSCT: Hematopoietic stem cell transplantation
NK: Natural killer
SCID: Severe combined immunodeficiency
SNP: Single nucleotide polymorphism
SOCE: Store-operated Ca^{2+} entry
STIM: Stromal interaction molecule

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, on release of Ca^{2+} from ER stores, multimerizes and binds to ORAI1.[4] Lack of STIM1 expression in human patients because of a frameshift nonsense mutation in *STIM1* severely impairs SOCE and causes immunodeficiency and autoimmunity associated with myopathy and abnormal enamel dentition.[10]

In addition to patients with ORAI1-R91W mutation and lack of STIM1 expression,[8,10] defects in SOCE and CRAC channel function have been described in patients from 2 kindreds in which the underlying gene defect remained undefined.[11,12] We here report 3 new mutations in *ORAI1* in patients from 2 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.

METHODS

Case reports

Case reports of patients P1 to P6 have been published.[11-15] Follow-up data on all patients and clinical descriptions are provided in Table I, and Table E1 and Fig E1 in this article's Methods in the Online Repository at www.jacionlinie.org.

Cells

Simian virus (SV)-40-transformed fibroblasts from patients P4 and P6 and a healthy control and Ficoll-Paque (GE Healthcare, Piscataway, NJ)–isolated PBMCs from patient P6's parents and controls were grown in RPMI 1640 (Mediatech, Manassas, Va).

Plasmids and transfections

Internal ribsome entry site (IRES) green fluorescent protein (GFP)–containing bicistronic vectors for expression of myc-epitope tagged ORAI1, ORAI2, ORAI3, and 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, Calif).

Genomic DNA sequencing

Genomic DNA was isolated from cells by using standard methods. PCR was conducted by using primers flanking exons and splice sites of *ORAI1, ORAI2*, *ORAI3*, *STIM1*, and *STIM2* (see this article's Table E2 in the Online Repository at www.jacionline.org). PCR products were sequenced directly (Genewiz Inc, South Plainfield, NJ). Sequence alignments were performed by using TCoffee software (Swiss Institute of Bioinformatics, http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee_cgi/index.cgi) and sequence traces visualized by using Xplorer software v1.0 (dnaTools, Ft. Collins, Colo). Single nucleotide polymorphism (SNP) searches were performed by using the 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% Nonidet P-40, and 0.02% sodium azide; and incubated with affinity-purified anti-ORAI1 antibodies raised against aa 275-291 of human ORAI1. For immunofluorescence, a muscle biopsy sample of patient P2 was coincubated with antibodies to ORAI1 and myosin heavy-chain fast (MHCf; clone WB-MHCf; Novocastra, Newcastle upon Tyne, United Kingdom) at 1:50 dilution; MHCf was detected by Alexa Fluor 488 goat anti-mouse IgG staining (Invitrogen, Carlsbad, Calif). 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 patient P2's vastus lateralis muscle was frozen in isopentane cooled in liquid nitrogen, and 10-μm cryostat sections were stained with standard histologic and histochemical techniques.[18]

Ca^{2+} measurements

Single-cell Ca^{2+} imaging was performed as described.[9] Traces in figures represent the mean $[Ca^{2+}]_i$ of 1 representative experiment; ~30 to 80 GFP^+ cells per experiment were analyzed. Error bars represent SEMs.

See additional Methods in the Online Repository.

RESULTS

Homozygous A88SfsX25 ORAI1 frameshift nonsense mutation abolishes ORAI1 expression

Ca^{2+} influx and CRAC channel currents were reported to be undetectable in T cells from immunodeficient patient P4, resulting in severely impaired T-cell activation (see this article's Table E1 in the Online Repository at www.jacionline.org).[12] Genomic DNA sequence analysis revealed that patient 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 2 healthy siblings of patient P4 (B-V-4 and B-V-5 in this article's Fig E1 in the Online Repository at www.jacionline.org) and DNA from 50 control individuals (100 chromosomes). DNA from his parents and his older brother (patient 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 domain (Fig 1, *A*). No mutations in *ORAI2*, *ORAI3*, *STIM1*, and *STIM2* were found in patient P4.

Northern blot analysis showed that ORAI1 mRNA transcripts were undetectable in patient P4 compared with cells from a healthy control (Fig 1, *B*), most likely because of nonsense-mediated mRNA decay. Fibroblasts from patient P4 also showed strongly reduced ORAI1 protein expression when cells were analyzed by immunohistochemistry using an anti-ORAI1 antibody (Fig 1, *C*). Because 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. However, ectopic expression of an N-terminally myc-tagged version of mutant