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A novel mutation in *ORAI1* presenting with combined immunodeficiency and residual T cell function

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Capsule summary

This study identifies a novel *ORAI1* mutation that abolishes protein expression, but permits robust lymphocyte proliferation to mitogens. It is important to consider *ORAI1* deficiency in the setting of a combined immunodeficiency with normal T cell numbers and residual T cell function.

Keywords

ORAI1; combined immunodeficiency; whole genome sequencing

To the Editor

Calcium serves as a second messenger for activating Ca²⁺-dependent enzymes pivotal for lymphocyte activation.^{1,2} T, B, and NK cell receptor stimulation activates phospholipase C γ , resulting in calcium release from the endoplasmic reticulum (ER).¹ Depletion of ER calcium stores triggers calcium influx across the plasma membrane through the calcium release-activated calcium (CRAC) channel, whose pore-forming subunit is encoded by *ORAI1*.^{1,2} *ORAI1* is widely expressed and is important for skeletal muscle development and function,

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eccrine sweat gland function, and dental enamel calcification.³ Human mutations in *ORAI1* are associated with severe combined immunodeficiency (SCID), myopathy and ectodermal dysplasia with anhidrosis.^{3,4} Patients with *ORAI1* defects have normal lymphocyte counts and variable immunoglobulin levels, but defective T cell proliferation in response to mitogen and antigen stimulation.^{3,4} We report a patient with opportunistic infections and hypotonia caused by a novel *ORAI1* mutation that abrogates protein expression, but permits robust lymphocyte proliferation to mitogens.

The patient is an Emirati male born to first-degree consanguineous parents, who presented with refractory CMV pneumonitis and hypotonia since 3 months of age. He had no ectodermal dysplasia or anhidrosis. Immunological evaluation at 7 months of age revealed normal numbers of total T, B, and NK cells (Table 1). He had decreased percentages of CD45RA⁺ naïve CD4⁺ cells and increased percentages of CD45RO⁺ memory CD4⁺ cells. The percentage of IgD⁺CD27⁺ unswitched memory B cells was modestly decreased (Table 1). His serum IgG and IgA were elevated and IgM was normal (Table 1). He had protective antibody titers against 5/14 pneumococcal serotypes without immunization, reflecting natural exposure. Lymphocyte proliferation to mitogens was normal and was present to tetanus and diphtheria antigens after a single dose of the diphtheria, tetanus, and acellular pertussis vaccine (Table 1). NK cell cytotoxicity was absent and CD8⁺ T cell cytotoxicity was severely reduced (Table 1). At 7 months of age, his CMV pneumonitis worsened, prompting initiation of intravenous immunoglobulin and cytomegalovirus intravenous immunoglobulin. At 9 months of age, the patient underwent a myeloablative unrelated donor hematopoietic stem cell transplant (HSCT) with busulfan, cyclophosphamide, and ATG conditioning. He had resolution of his recurrent infections, although his hypotonia persists.

The patient's family had multiple individuals with a history of recurrent infections and hypotonia: a sister, who is alive after HSCT, two deceased siblings, and a deceased female cousin (Supplementary Fig. 1). No laboratory data was available on these other affected individuals. Whole genome sequencing (WGS) was performed on the patient, his affected sister, his healthy sister, and parents; whole exome sequencing (WES) was performed on the parents of his affected cousin. The combined WGS/WES approach identified a single candidate mutation (*ORAI1* c.493_494insC, p.H165Pfs), which was homozygous in the two affected children, heterozygous in both sets of parents, and heterozygous in his healthy sister. This mutation has not been previously reported and was confirmed by Sanger sequencing (Fig. 1A). This frameshift insertion substitutes proline for histidine at a.a 165, followed by a premature stop codon prior to the last two C-terminal domains of the protein (*ORAI1*^{H165Pfs}) (Fig. 1B). To determine if the mutation permitted expression of a truncated form of *ORAI1*, we transiently transfected HEK293T cells with an N-terminal Myc-tagged *ORAI1*^{H165Pfs} construct since an anti-*ORAI1* antibody to the protein's N-terminus was not commercially available. Transfected HEK293T cells failed to express Myc-*ORAI1*^{H165Pfs}, but robustly expressed Myc-tagged WT *ORAI1*, demonstrating that the mutation abolishes protein expression (Fig. 1C). Immunoblotting of lysates from the patient's fibroblasts revealed absent *ORAI1* expression using an anti-*ORAI1* antibody to the protein's C-terminus (Fig. 1C). Fibroblasts from the patient and his affected sister demonstrated residual

store-operated calcium entry (SOCE) (Fig. 1D), which may have contributed to the proband's proliferative response.

The residual SOCE in our patient's fibroblasts indicates the presence an ORAI1-independent mechanism for calcium flux. Previously published patients with *ORAI1* mutations (A88Sfx25, A103E/L194P) abolishing protein expression were found to have normal proliferation to phorbol 12-myristate 13-acetate (PMA) and ionomycin. Conversely, patients with the ORAI1^{R91W} mutation, which permits protein expression and interaction with STIM1, have no proliferative response to PMA and ionomycin stimulation.⁴ The mutant ORAI1^{R91W} protein has been proposed to exert a dominant negative effect on the function of two ORAI1 paralogs, ORAI2 and ORAI3, which hetero-multimerize with ORAI1.^{4,5} In contrast, null *ORAI1* mutations, such as ORAI1^{H165Pfs} in our proband, would not interfere with calcium flux generated by ORAI2 or ORAI3.^{3,4} ORAI2 is widely expressed and ORAI3 is expressed in the spleen, kidney, lung, and fibroblasts.⁵ Reconstitution of ORAI1-deficient T cells with ORAI2 or ORAI3 permits low levels of SOCE.⁵ A compensatory increase in ORAI2 and ORAI3 in our patient's T lymphocytes may have permitted the lymphocyte proliferation to mitogens in the absence of ORAI1, a hypothesis we were unable to test because the patient had undergone HSCT. Differences in genetic background or epigenetic factors may also contribute to our patient's proliferation to mitogens, as has been seen in *Orail*^{-/-} mice. Compared to *Orail*^{-/-} mice on a C57BL/6 background, *Orail*^{-/-} mice on an outbred mixed background have improved survival and less severe skin and hair abnormalities; their naïve T cells have modestly impaired SOCE and normal proliferation to TCR activation, thus highlighting the contribution of genetic or epigenetic factors to phenotypic variations.⁶ WGS did not reveal any variants that would enhance T cell proliferation in our proband. The phenomenon of widely variable presentations in patients with null mutations has been documented in other PIDs,⁷ although the genetic mechanisms for this phenomenon remain unknown.

Despite the robust T cell proliferation to mitogens and a proliferative response to antigens after a single vaccination dose, the history of refractory CMV infections in our patient indicates a clinically significant impairment of the defense against opportunistic infections. ORAI1 has a pivotal role in facilitating cytotoxic granule exocytosis,^{8,9} evidenced by the proband's severely impaired CD8⁺- and NK cell-mediated cytotoxicity, which likely contributed to his susceptibility to CMV.

This case emphasizes the importance of considering *ORAI1* deficiency in patients with combined immunodeficiency in the setting of normal T cell numbers and robust lymphocyte proliferation to mitogen stimulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

IP3	Inositol trisphosphate
ER	endoplasmic reticulum
CRAC channel	calcium release-activated calcium channel
SCID	severe combined immunodeficiency
HSCT	hematopoietic stem cell transplant
WGS	whole genome sequencing
WES	whole exome sequencing
SOCE	store-operated calcium entry
PMA	(phorbol 12-myristate 13-acetate)

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Figure 1. Sanger sequencing and the effect of ORAI1^{H165Pfs} on ORAI1 protein expression and Ca²⁺ flux

(A) Sanger sequencing of the c.493_494insC mutation. (B) Schematic of an ORAI1 monomer showing the patient's mutation in the protein's intracellular loop. (C) Left: Immunoblot of Myc in lysates from HEK293T cells that were untransfected or transfected with a construct encoding either N-terminal Myc-tagged ORAI1 or N-terminal Myc-tagged ORAI1^{H165Pfs}. The predicted molecular weight of ORAI1 is 33 kDa (Myc-ORAI1^{core}), but glycosylation leads to multiple bands at ~37 kDa and ~50 kDa (Myc-ORAI1^{gly}), as has been shown previously.⁵ Right: Immunoblot of ORAI1 in lysates from the patient's fibroblasts using an antibody to the protein's C-terminus. The non-specific band (ns) at ~50 kDa using this C-terminal anti-ORAI1 antibody cannot represent glycosylated ORAI1 because no ORAI1 protein was detected in HEK293T cells transfected with N-terminal Myc-tagged ORAI1^{H165Pfs}. (D) Flow cytometric analysis of Ca²⁺ influx in fibroblasts from the patient, his affected sister, and a control after 4 μM thapsigargin treatment for 300 seconds, followed by the addition of 2 mM Ca²⁺.

Table 1

Immunologic profile of the patient. Values in bold and italics are outside the normal range.

Lymphocyte phenotype (normal range)	
Lymphocyte count, cells/ μ L ⁱ	7,310 (3,400 – 9,000)
CD3 ⁺ , cells/ μ L	4,553 (1,900 – 5,900)
CD3 ⁺ CD4 ⁺	3,972 (1,400 – 4,300)
Naïve CD3 ⁺ CD4 ⁺ CD45RA ⁺	30.1% (50 – 85)
Memory CD3 ⁺ CD4 ⁺ CD45RO ⁺	69.9% (15 – 50)
CD3 ⁺ CD8 ⁺	516 (500 – 1,700)
Naïve CD3 ⁺ CD8 ⁺ CD45RA ⁺	65.8% (50 – 85)
Memory CD3 ⁺ CD8 ⁺ CD45RO ⁺	34.2% (15 – 50)
T cell oligoclonality	Not detectable
CD19 ⁺ , cells/ μ L	2,159 (610 – 2,600)
IgD ⁺ CD27 ⁻ ⁱⁱ	89.6% (65.8 – 91.4)
IgD ⁺ CD27 ⁺	2.1% (3.6 – 18.8)
IgD ⁻ CD27 ⁺	4.2% (0.9 – 14.1)
CD16 ⁺ /CD56 ⁺ , cells/ μ L	312 (160 – 950)
Immunoglobulins, mg/dL (normal range) ⁱⁱⁱ	
IgG	731 (215 – 714)
IgA	433 (8.1 – 68)
IgM	101 (35 – 102)
IgE	6 (0.44 – 16.3)
Lymphocyte proliferation, cpm (control) ^{iv}	
PHA	128,077 (222,208)
Pokeweed mitogen	102,413 (141,446)
Concanavalin A	32,560 (64,276)
Background	403 (775)
Tetanus	8,252 (61,433)
Diphtheria	6,543 (54,298)
Background	2,310 (4,452)
Cytotoxicity studies	
NK cell lytic units	Not detectable (>3.1)
Cytotoxic T lymphocyte lytic units	1.6 (6)

ⁱ Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol* 2003; 112(5):973-80.

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ⁱⁱⁱ Jolliff CR Cost KM, Stivins PC, Grossman PP, Nolte CR, Franco SM, et al. *Clin Chem*. Reference intervals for serum IgG, IgA, IgM, C3, and C4 as determined by rate nephelometry. 1982; 28:126-128.

^{iv} The patient received only one dose of the diphtheria, tetanus, and acellular pertussis vaccine. No further doses were given because he was started on IVIG after this test was performed. Values in parenthesis are from studies done on the same day using a healthy adult control.