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(CASE REPORT)

Severe combined immunodeficiency with a homozygous c.464T>C (p.Leu155Pro) mutation in the RAG2 gene in a country without neonatal screening: Report of two clinical cases

Zurisadai Raquel García Osorno [1,*], Augusto Ignacio Siegert Olivares [1], Paola Alejandra Cisneros Conklin [1], Ibeth Judith Reyes Montante[1] and María Andrea Murillo Gallo [2]

[1] *Department of Pediatric, General Hospital with Specialties "Juan María de Salvatierra", La Paz, Baja California Sur, Mexico. Postgraduate Studies Division, Faculty of Medicine, National Autonomous University of Mexico.*
[2] *Department of Research, General Hospital with Specialties "Juan María de Salvatierra", La Paz, Baja California Sur, Mexico. Postgraduate Studies Division, Faculty of Medicine, National Autonomous University of Mexico.*

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Abstract

Introduction: Severe combined immunodeficiency (SCID) is a disorder belonging to the broad spectrum of inborn errors of immunity (IEI), characterized by a predisposition to develop recurrent, severe, difficult-to-treat opportunistic infections. This disorder is associated with abnormalities in genes of the adaptive immune system and should be suspected in patients with infections during the first months of life, mainly due to fungal pathogens and intracellular agents.

Case Presentation: Two cases of SCID diagnosed at the General Hospital with Specialties "Juan María de Salvatierra" in Baja California Sur (BCS), México in 2022 are described. The first patient presented persistent candidiasis stomatitis with no response to oral treatment. The second patient presented mycotic dermatitis and oropharyngeal candidiasis compatible with *Candida albicans* and multiple episodes of multi-treated airway infection with partial remission. The diagnosis was completed with a genetic panel that showed a mutation in RAG2 homozygous variant c.464T>C (p.Leu155Pro) in both patients.

Conclusions: SCID should be considered in patients with recurrent and difficult-to-treat infectious diseases in the first months of life. For its timely detection, implementing neonatal screening quantifying of T-cell receptor excision circles (TRECs) is recommended. This method is not available in Mexico, so it has been relevant to have physicians trained in the timely clinical identification of the disease. Thanks to the prompt referral of both patients to our hospital, we could treat the comorbidities associated with SCID, provide a specific molecular diagnosis, and transfer the patients to a hospital specialized in bone marrow transplantation for definitive treatment.

Keywords: Severe combined immunodeficiency; RAG2; Primary immune deficiency; Inborn errors of immunity; Case report

1. Introduction

SCDI is a disease that is part of the broad spectrum of IEI, characterized by genetic abnormalities caused by mutations in different genes involved in developing T lymphocytes (TL), and B lymphocytes (BL). Currently, 485 genetic defects have been identified [1]. In this work, two cases of patients with SCID diagnosed in BCS, Mexico, in 2022 are described, both whit a rare mutation in the recombination activator gene 2 (RAG2).

* Corresponding author: Zurisadai Raquel García Osorno; ORCiD: 0000-0003-4162-6221; E-mail: zurisadaigarciaosorno@gmail.com

2. Case 1

A 4-month-old male patient with a history of oral ulcer with herpetic characteristics in the first month of life was treated with acyclovir with improvement. In the second month of life, the patient presented an abscess on the right leg, treated with drainage and an unspecified oral antibiotic. Additionally, the patient developed oropharyngeal candidiasis due to *Candida albicans*, isolated in culture from an oropharyngeal exudate sample. Candidiasis was challenging to manage without total remission to initial empirical treatment, evolving to persistent candidal stomatitis after four months, requiring hospitalization for intravenous antifungal treatment and a diagnostic approach. During his hospital stay, treatment was started with intravenous fluconazole, topical miconazole in the chest due to fungal lesions and chloramphenicol due to bacterial conjunctivitis. A quantitative polymerase chain reaction (qPCR) study was performed for SARS-CoV-2 with a negative result. For the diagnostic approach, when SCID was suspected, an ultrasound study was conducted to assess the thymus, which reported the presence of hypoplastic thymic tissue. In addition, flow cytometry was performed, which reported a decrease in helper T lymphocytes (CD4+ TL), an increase in cytotoxic T lymphocytes (CD8+ TL), a decrease in BL and natural killer (NK) cells within normal ranges (Table 1). Subsequently, the primary immunodeficiency panel genetic test was performed, confirming the diagnosis of SCID with heterozygous mutation in ADA c.631C>T (p.Arg211Cys) and mutation in RAG2 homozygous variant c.464T>C (p.Leu155Pro).

The patient remained hospitalized for seven days with the previously mentioned treatment, and after remission of the candidal stomatitis, he was transferred to the pediatric immunology outpatient clinic with treatment based on trimethoprim/sulfamethoxazole (TMP/SMX) and fluconazole at prophylactic doses. The patient was subsequently referred to a hospital specialized in bone marrow transplant for resolution.

3. Case 2

A 7-month-old male patient, started at the third month of life with a fungal dermatitis in the diaper area, with partial improvement after treatment with zinc oxide and miconazole. He also presented oropharyngeal plaques that generated hyporexia and relevant weight loss, for which he received treatment with nystatin. *Candida albicans* was isolated from throat swab culture. At the same time, he presented several episodes of airway infection treated with different oral antibiotic schemes before his admission to the hospital without symptoms reduction, for which the treating physician considered the possibility of some IEI and kept the patient on treatment with TMP/SMX and oral fluconazole at prophylactic doses.

The child was admitted to the hospital in bad health condition. Compensated septic shock secondary to community-acquired pneumonia was diagnosed. Treatment with ceftriaxone and clarithromycin was started. Similarly, high-flow oxygen therapy was initiated; however, 8 hours after admission, he was connected to mechanical ventilation (MV) due to worsening of his condition, requiring invasive ventilatory support for two days.

In admission laboratory studies, the presence of lymphopenia (1,050/mm^3), neutrophilia (13,620/mm^3), elevated procalcitonin (100 ng/mL), and immunoglobulins IgM, IgA and IgG subclasses below normal ranges (Table 2). Immunodeficiency was suspected, and due to the high risk of multiple organ failure, human immunoglobulin (2 g/kg) was administered, and antimicrobial coverage was increased with cefepime and vancomycin. To search the etiological agent causing the respiratory failure, qPCR studies for SARS-CoV-2 and *Mycobacterium tuberculosis* (GeneXpert MTB-RIF) were performed; additionally, serial smear microscopies were performed, and all these studies with negative results. Another serum qPCR study was done for cytomegalovirus (CMV), requested as an approach to IEI, yielded a positive result, with a viral load of 5,833 copies/ml log 3.76 copies/ml. The treatment with valganciclovir was started with a good response. The flow cytometry results showed a decrease in TL, the absence of BL and a decrease in NK cells (Table 3). Prophylaxis with TMP/SMX and fluconazole was continued, and antiviral prophylaxis with acyclovir was added. After not observing the presence of the thymus in the chest X-ray (Figure 1), an ultrasound was performed, confirming its presence with marked hypoplasia. Subsequently, the genetic test of the primary immunodeficiency panel was performed and the result confirmed SCDI with a mutation in RAG2, homozygous variant c.464T>C (p.Leu155Pro). After resolving the opportunistic infections, the patient was referred to a hospital specialized in bone marrow transplant for resolution.

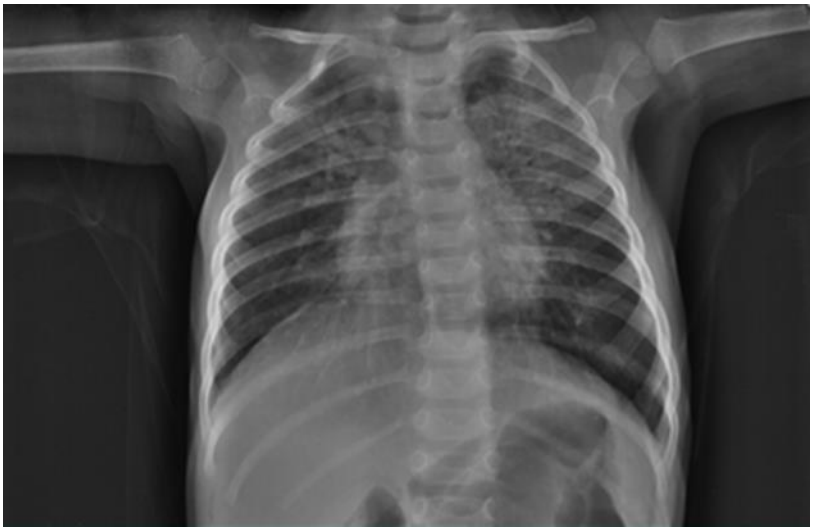

Figure 1 Case 2. Anteroposterior chest X-ray. The presence of a thymic silhouette is not visualized.

Table 1 Case 1. Flow cytometry.

Case 1. Flow cytometry		
Lymphocyte subpopulation	**Results**	**Reference values**
Total leukocytes	7040 thousand/µL	5100 - 18100
Total lymphocytes	3641 thousand/µL	2580 - 7860
Lymphocytes T (CD3+/CD45+)	3401 cells/µL	2500 - 5600
Lymphocytes T (CD3+/CD4+)	437 cells/µL	1800 - 4000
Lymphocytes T (CD3+/CD8+)	2549 cells/µL	590 – 1600
Quotient (CD4/CD8)	0.17	0.97 - 2.82
Lymphocytes CD16/CD56	327 cells/µL	90 - 590
Lymphocytes (CD19)	80 cells/µL	430 - 3000

Table 2 Case 2. Immunoglobulins.

Case 2. Immunoglobulins		
Immunoglobulins	**Results**	**Reference values**
Immunoglobulin M (IgM)	3.4 mg/dL	34 – 126
Immunoglobulin A (IgA)	5.7 mg/dL	11 – 90
Immunoglobulin G Subclass 1	22.9 mg/dL	140 – 620
Immunoglobulin G Subclass 2	10.3 mg/dL	41 – 130
Immunoglobulin G Subclass 3	0.14 mg/dL	11 – 85
Immunoglobulin G Subclass 4	0.35 mg/dL	0 – 0.8
Immunoglobulin G (IgG)	26.7 mg/dL	217 – 904

Table 3 Case 2. Flow cytometry.

Case 2. Flow cytometry		
Lymphocyte subpopulation	**Results**	**Reference values**
Total leukocytes	5020 cells/µL	6700 - 12000
Total lymphocytes	40 cells/µL	3500 - 8800
Lymphocytes T (CD3+/CD45+)	13 cells/µL	1900 – 5900
Lymphocytes T (CD3+/CD4+)	1 cells/µL	1400 - 4300
Lymphocytes T (CD3+/CD8+)	12 cells/µL	500 - 1700
Quotient (CD4/CD8)	0.1	1.20 - 6.20
Lymphocytes CD16/CD56	27 cells/µL	180 - 920
Lymphocytes (CD19)	0.00 cells/µL	610 - 2600

4. Discussion

SCDI is one of the most severe pathologies belonging to the spectrum of IEI, which have in common abnormalities in one or several genes responsible for the correct adaptive immune response. Between the causes for SCID, there are alterations in RAG2, one of the main intracellular signaling genes that participate in the development of antigen-binding regions of cell receptors present on BL (BCR) and TL (TCR) [2]. The primary function of RAG2 is to perform cleavage during V(D)J recombination (variable region (V) gene segments, diversity (D) gene segments, and joining (J) gene segments) through the recombinase activity in the RAG1/ RAG2 complex, this activity, leads to a diverse expression of protein receptors in mature BL and TL capable of recognizing and generating a response to a wide variety of antigens [2].

RAG2 mutations are capable of abolishing or decreasing the formation of receptors for the antigen present in cellular and humoral immunity by altering the V(D)J recombination process, which promotes a state of total suppression of BL, unlike the TL, which may be absent or reduced [3]. Currently, 57 described mutations in RAG2 cause pathologies, of which four are associated with SCID: G35V, R39G, G95R and R229E [2] with an inherited autosomal recessive pattern and represent approximately 50% of the causes of SCID [4]. Additionally, these mutations can be null or hypomorphic, making SCID a genotypically and phenotypically heterogeneous pathology [5]. Null mutations are characterized by the absence of BL and TL, with the presence of NK cells (phenotype T-B-NK+), while in hypomorphic mutations, there is synthesis of RAG proteins with reduced activity, which favors the existence of several subtypes [6]. In the case of our patients, the mutation in RAG2 homozygous variant c.464T>C (p.Leu155Pro) might be a hypomorphic mutation since none of the patients had a T-B-NK+ phenotype.

Regarding the mutation reported in the exposed cases, up to the time of this review, there are only two clinical references published worldwide that report the presence of the c.464T>C (p.Leu155Pro) mutation in the RAG2 gene [7,8]. Interestingly, despite the rarity of the mutation present (c.464T>C (p.Leu155Pro) in the two cases described, both patients were diagnosed in the same year (2022) and reside in the same geographical area. The detection of two patients with this mutation in the same year might result from greater accessibility to genetic tests; besides, it should be investigated whether the environmental and social conditions of Baja California Sur state could influence the SCDI incidence.

Clinically, SCID is characterized by the predisposition to develop recurrent, severe, and difficult-to-treat diseases caused by opportunistic microorganisms, such as *Pneumocystis jirovecii*, *Candida spp.*, *Aspergillus spp.*, Parainfluenza virus, Respiratory syncytial virus, Cytomegalovirus, Herpes simplex virus type 1, Bacillus Calmette-Guérin (BCG), *Pseudomonas spp*, among others [6]. This is due to a deficit of cellular immunity in charge of protecting the organism from fungal pathogens and intracellular etiological agents during the first months of life, while the absent humoral immunity is covered by the transplacental passage of maternal immunoglobulins.

In these two cases presented, recurrent infections were evidenced by the anamnesis and physical examination, sufficient to establish the immediate suspicion of a probable IEI. The diagnosis is mainly clinical in countries where SCID is not part of neonatal screening and in those where genetic tests are not always available for confirmation, so first-

contact physicians must know the signs and symptoms that point to IEI, such as recurrent episodes of sinusitis, otitis, pneumonia, abscesses, thrush, candidiasis, and sepsis, as well as the ineffectiveness of oral antimicrobial treatments, failure to thrive and/or a family history of primary immunodeficiency. Identifying these symptoms could allow a timely detection and referral to specialized centers.

Initial management focuses on treating active infections, isolation measures, as well as the implementation of adequate antibacterial, antiviral, and antifungal prophylaxis. Vaccines with live or attenuated agents should be avoided, and actions that may put the patient at risk of CMV infection, such as breastfeeding (unless there is evidence of a CMV-negative serological status of the mother) and blood products transfusion [9].

The definitive treatment of SCDI is hematopoietic stem cell transplantation (HSCT), preferably within the neonatal period, due to this period, the reconstitution of TL is favored, compared to transplant patients older than 28 days of age [10]. Patients with SCID younger than 3.5 months have demonstrated a survival of 94% at 2 years after transplantation vs a survival of 90% in patients older than 3.5 months without infections, 82% with resolved infections, and 50% with active infections [11]. Additionally, there is evidence of a 100% mortality in patients who do not benefit from a timely approach and treatment within the first two years of life [9].

Some first-world countries have a well-established neonatal screening method for quantifying TRECs as an indicator of T-cell lymphopoiesis. In Mexico, we do not have such neonatal screening within the public health system, so we consider it reasonable to evaluate the benefits that could be obtained after its implementation.

Finally, we expected that this series of cases be a reminder of the existence of IEI that, although rare pathologies, have a high impact on patients, their families, and the public health system. The genetic tests help to confirm the diagnosis, but the clinic should guide the diagnosis and give rise to the initial therapeutic approach and management as soon as possible to avoid complications and increase the chances of success of the definitive treatment in patients.

5. Conclusion

SCDI is a disorder that must be suspected in patients who come to the clinic at an early age with typical signs and symptoms since clinical suspicion will be the cornerstone for a prompt diagnostic and therapeutic approach without a neonatal screening method. We consider it important to have a diagnostic algorithm that allows the detection of patients who may suffer from IEI. The time to diagnosis will directly influence the success of the definitive treatment, which will be inversely proportional to the patient´s age.

Recommendation

The implementation of neonatal screening through the quantification of TRECs is recommended for the timely detection of IEI since it will increase the percentage of successful treatments, favorably impacting the prognosis, reducing the number of complications, and improving the success rate of HSCT. In addition, it will allow the reduction of the costs generated by the treatment of complications secondary to late detection, in turn improving the economy of our health system.

Compliance with ethical standards

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Disclosure of conflict of interest

The manuscript authors: Zurisadai Raquel García Osorno, Augusto Ignacio Siegert Olivares, Paola Alejandra Cisneros Conklin, Ibeth Judith Reyes Montante and María Andrea Murillo declare that they have no conflict of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Tangye SG, Al-Herz W, Bousfiha A, Cunningham-Rundles C, Franco JL, Holland SM, et al. Human inborn errors of immunity: 2022 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol [Internet]. 2022; 42(7):1473–507. Available in: http://dx.doi.org/10.1007/s10875-022-01289-3

[2] Notarangelo LD, Kim M-S, Walter JE, Lee YN. Human RAG mutations: biochemistry and clinical implications. Nat Rev Immunol [Internet]. 2016;16(4):234–46. Available in: http://dx.doi.org/10.1038/nri.2016.28

[3] Schwarz K, Gauss GH, Ludwig L, Pannicke U, Li Z, Lindner D, et al. RAG mutations in human B cell-negative SCID. Science [Internet]. 1996; 274(5284):97–9. Available in: http://dx.doi.org/10.1126/science.274.5284.97

[4] Buckley RH. Molecular Defects in Human Severe Combined Immunodeficiency and Approaches to Immune Reconstitution. Annu Rev Immunol [Internet]. 2004; 22(1):625–55. Available in: http://dx.doi.org/10.1146/annurev.immunol.22.012703.104614

[5] Meshaal SS, El Hawary RE, Abd Elaziz DS, Eldash A, Alkady R, Lotfy S, et al. Phenotypical heterogeneity in RAG-deficient patients from a highly consanguineous population. Clin Exp Immunol [Internet]. 2019; 195(2):202–12. Available in: http://dx.doi.org/10.1111/cei.13222

[6] Niehues T, Perez-Becker R, Schuetz C. More than just SCID—The phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2. Clin Immunol [Internet]. 2010; 135(2):183–92. Available in: http://dx.doi.org/10.1016/j.clim.2010.01.013

[7] Lugo-Reyes SO, Pastor N, González-Serrano E, Yamazaki-Nakashimada MA, Scheffler-Mendoza S, Berron-Ruiz L, et al. Clinical manifestations, mutational analysis, and immunological phenotype in patients with RAG1/2 mutations: First cases series from Mexico and description of two novel mutations. J Clin Immunol [Internet]. 2021;41(6):1291–302. Available in: http://dx.doi.org/10.1007/s10875-021-01052-0

[8] Rigoni R, Fontana E, Dobbs K, Marrella V, Taverniti V, Maina V, et al. Cutaneous barrier leakage and gut inflammation drive skin disease in Omenn syndrome. J Allergy Clin Immunol [Internet]. 2020;146(5):1165-1179.e11. Available in: http://dx.doi.org/10.1016/j.jaci.2020.04.005

[9] Bachiloglu H, Sotomayor R, Poli FC. Severe combined immunodeficiency: The time for newborn screening has come. Rev Chil Pediatr [Internet]. 2019;90(6):581–8. Available in: http://dx.doi.org/10.32641/rchped.v90i6.1310

[10] Myers LA, Patel DD, Puck JM, Buckley RH. Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. Blood [Internet]. 2002;99(3):872–8. Available in: http://dx.doi.org/10.1182/blood.v99.3.872

[11] Shih STF, Keller E, Wiley V, Wong M, Farrar MA, Chambers GM. Economic evaluation of newborn screening for severe combined immunodeficiency. Int J Neonatal Screen [Internet]. 2022;8(3):44. Available in: http://dx.doi.org/10.3390/ijns8030044