ORIGINAL ARTICLE

From Severe Combined Immunodeficiency to Omenn syndrome after hematopoietic stem cell transplantation in a RAG1 deficient family

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Abstract

Background: Mutations in RAG genes cause a spectrum of severe immunodeficiencies ranging from Severe Combined Immunodeficiency (SCID) T-B-NK+ to Omenn syndrome (OS) through intermediate phenotypes, even for the same alteration. Nowadays, hematopoietic stem cell transplantation (HSCT) is the unique curative treatment available.

Methods: We describe three related patients from a Moroccan consanguineous family. Patient 1 developed at 1 month of age moderate eczematous dermatitis with eosinophilia, followed by infections and enteritis. He was transplanted and received reduced intensity conditioning regimen previous to HSCT. His brother, patient 2, was born preterm with a severe neonatal erythroderma, hepatosplenomegaly and lymphadenopathy. Patient 3, cousin of the two siblings, was also born preterm and fulfilled all criteria for classical OS. Immunological evaluation was performed and RAG genes were sequenced.

Results: Immunological data from all three patients were very diversed, from T lymphopenia to marked lymphocytosis, and different degrees of eosinophilia and IgE levels. Non-responder T cells and absent B cells were constant. All patients presented the same homozygous mutation in RAG1 gene (c.631delT). Patient 1 fully recovered both clinically and immunologically after HSCT. Two years later, he lost the accomplished lymphoid chimera and the disease relapsed as a classical OS, leading to patient's death.

Conclusions: This is the first report of a RAG1 deficient patient with a changed clinical and immunological phenotype from SCID to OS after HSCT. The use of a myeloablative conditioning regimen that eliminates reminiscent T cells might have improved patient's outcome and it should be considered in similar cases.

Severe Combined Immunodeficiency (SCID) is a molecularly heterogeneous group of primary immunodeficiencies owing to a congenital disorder of T lymphocyte development (1). T cells are extremely low or absent, and B lymphocytes and NK cells are variably impaired. SCID with depletion of T and B cells (SCID T-B-NK+) can be attributed, amongst others, to defects in the recombinase activating genes (RAG; OMIM: 601457) (2). The products of these genes, the RAG1 and RAG2 proteins, are key players in the V(D)J recombination process leading to the assembly of antigen receptor

genes: T cell receptors (TCR) and B cells receptors (BCR or immunoglobulins, Ig) (3). SCID patients with RAG deficiency present with severe respiratory infections, chronic diarrhoea, oral candidiasis and dermatitis (2). In 1998, RAG defects were also recognized for the first time as a molecular cause for Omenn Syndrome (OS) (OMIM: 603554), a peculiar and even more aggressive form of SCID (4). OS is characterized by early onset erythroderma, hepatosplenomegaly and lymphadenopathy associated with protracted diarrhoea and failure to thrive. Laboratory findings are eosinophilia, low serum immunoglobulins but elevated IgE, lack of circulating B cells and a variable number of mature, activated, oligoclonal and functionally impaired T cells with a skewed Th2 phenotype (5–7). Patients with OS have a residual expression and function of RAG proteins that allows some TCR rearrangement (oligoclonal T cells) (8). Consequently, patients with OS are extremely immunodeficient owing to the lack of a fully diversified repertoire. On the other hand, the expansion of few autoreactive T lymphocyte clones can lead to lymphocytosis and the infiltration of skin and liver.

Classically, RAG defects have been differentiated into three different phenotypes: T-B-NK+ SCID, OS and atypical SCID/Omenn or Omenn-like syndrome, in which clinical and immunological characteristics do not exactly correspond to those found in the previous two diseases (9). Omenn-like features have also been associated with defects in other genes implicated in V(D)J recombination and maturation of lymphoid cells (10).

RAG1 and RAG2 genes are located both in 11p13, separated by 15 kb (11). RAG1 protein has 1.043 aminoacids and 119 kDa of molecular weight, whereas RAG2 has 527 residues and 59 kDa. RAG mutations responsible for SCID are usually insertions, deletions and nonsense mutations that abolish completely RAG function. However, OS is caused by hypomorphic mutations that partially preserve the function of the protein, which are usually missense mutations. It has been previously described that the same RAG mutation can cause both T-B-NK+ SCID and OS (5, 12). Therefore, other elements such as epigenetic and environmental factors must contribute in determining the SCID or OS phenotype (12).

As both humoral and cellular immunity are severely impaired in RAG deficient patients, hematopoietic stem cell transplantation (HSCT) is, for now, the only curative treatment available (13). Owing to the intense severity of these immunodeficiencies, HSCT should be performed as soon as possible to avoid organ damage and irreversible deterioration of the patients. The aim of this study was to compare clinically and immunologically three patients from a RAG1 deficient family, one of them before and after HSCT.

Methods

Patients

Patients 1 and 2, born in 2004 and 2009, respectively, corresponded to the ninth and tenth pregnancy of a couple of cousins, who reported two maternal uncles and two previous sons dead within the first year of life in Morocco of unclear causes. The couple had six other healthy children. The third patient, born in 2010, was the first cousin of the previous cases, and his parents were also consanguineous. Clinical and immunological features are summarized and compared in Table 1, and the family tree is shown in Fig. 1.

Patient 1

A 2.5-month-old boy, born at 40 wk of gestational age, was referred because of moderate eczematous dermatitis since the first month of life (Fig. 2). During the following months, the patient also developed persistent enteritis, failure to thrive, uncommon infections, such as acute pyelonephritis by *Citrobacter koserii* and recurrent trush. The diagnosis of SCID was established and the patient was referred for evaluation of HSCT. At 10 months of age, HSCT from an HLA single mismatched brother (9/10) was performed. Reduced conditioning regimen included fludarabin (150 mg/m^2), rabbit ATG (12.5 mg/kg) and melfalan (140 mg/m^2). Prophylaxis for graft vs. host disease included cyclosporine (2.5 mg/kg/12 h) under pharmacokinetic control (150–200 ng/ml). Haematological and immunological recovery was achieved and the child remained asymptomatic, with normal skin, no diarrhoea and no infections. One year later, he left to Morocco interfering with the usual strict follow-up following HSCT. Twenty-one months after HSCT, he presented with 5 days of fever, lymphadenopathy and hepatoesplenomegaly. He quickly progressed to a respiratory distress and hypoxemia, with a radiological pattern of bilateral alveolar-interstitial infiltrate, suggestive of *Pneumocistis jirovecii* pneumonia. He died despite early advanced management because of the untreatable lung infection and massive organic lymphocytic infiltrates.

Patient 2

He was a moderately preterm infant, born at 31.6 wk of gestational age. Since birth, he showed striking dermatological findings, consisting of severe neonatal erythroderma with bright and thin skin, fissurated areas in scalp and skin flexures and progressive oedema (Fig. 2). He associated hepatosplenomegaly and generalized lymphadenopathy. He was diagnosed with OS, and he was prescribed strict isolation and prophylactic therapy. He quickly developed a refractory protein-loss syndrome complicated with hypernatraemic–hyperkalaemic dehydratation, anuria and pleural effusion. A multiorganic failure occurred at day 40 of life.

Patient 3

He was born in 2010, at 31.4 wk gestation, from a couple of cousins closely related to the former family (all four parents were first degree cousins). Since birth, he presented severe erythroderma, associated with erosions and exudation. He developed progressive hepatoesplenomegaly and generalized lymphadenopathy, and he suffered from recurrent bacteraemia by plasmocoagulase-negative *Staphylococcus*. He was also diagnosed with OS. At 2 months of age, he quickly worsened, developed multiorganic failure and died. A clinical sepsis was suspected as cause of death, despite repeated negative microbiological studies.

Table 1
Clinical and immunological data from patient 1 (onset, recovery and relapse) and patients 2 and 3

Parameters	Patient 1	Patient 1 after HSCT	Patient 1 after HSCT	Patient 1 relapse	Patient 2	Patient 3
Onset age	3 months	12 months (2 months after HSCT)	17 months (7 months after HSCT)	31 months (21 months after HSCT)	At birth (preterm, 31.6 wk)	At birth (preterm, 31.4 wk)
Gender	Male	Male	Male	Male	Male	Male
Clinical features at onset	Moderate eczematous dermatitis and infections: pyelonefritis, persistent oral trush	Healthy	Healthy	Fever, pneumonitis, massive lymphocytic infiltrates	Generalized neonatal erythroderma with desquamation	Generalized neonatal erythroderma with desquamation
Hepatosplenomegaly	Absent	Absent	Absent	Present	Present	Present
Lymphadenopathy	Absent	Absent	Absent	Present	Present	Present
Lymphocyte counts, /mm^3	660 (3900–8600)*	1660 (2300–5600)*	2150 (2300–5600)*	1230 (2300–5600)*	14950 (2070–5380)*	893 (2070–5380)*
T lymphocyte counts, /mm^3; %	178; 27 (2400–5600)*	515; 31 (1400–3600)*	1484; 69 (1400–3600)*	1181; 96 (1400–3600)*	13605; 91 (1720–4430)*	607; 68 (1720–4430)*
T CD4+ T cells, /mm^3	59 (1600–4200)*	183 (700–2000)*	1075 (700–2000)*	726 (700–2000)*	8223 (1320–3620)*	500 (1320–3620)*
T CD8+ T cells, /mm^3	106 (700–1500)*	249 (500–1400)*	344 (500–1400)*	516 (500–1400)*	5382 (290–1470)*	107 (290–1470)*
Memory T cells (CD45RO+), %	>93	NP	NP	NP	>88	>90
T proliferative responses to mitogens	Absent	NP	NP	Absent	Absent	Absent
B lymphocytes counts, /mm^3	Absent (800–2600)*	415 (400–1500)*	108 (400–1500)*	Absent (400–1500)*	Absent (110–740)*	Absent (110–740)*
IgG, mg/dl	Maternal: 115 (170–560)*	IVIG: 1550 (400–1100)*	IVIG: 880 (400–1100)*	IVIG: 823 (400–1100)*	Maternal: 229 (186–728)*	Maternal: 267 (186–728)*
IgA, mg/dl	Undetectable (5–50)*	52 (10–160)*	47 (10–160)*	78 (10–160)*	Undetectable (0.04–1)*	Undetectable (0.04–1)*
IgM, mg/dl	Undetectable (30–100)*	84 (50–180)*	68 (50–180)*	70 (50–180)*	Undetectable (2.1–39.4)*	Undetectable (2.1–39.4)*
IgE, kU/l	Undetectable (<17)*	5 (<59)*	4 (<59)*	364 (<59)*	17 (<17)*	604 (<17)*
NK lymphocyte counts, /mm^3	360 (200–900)*	415 (100–700)*	387 (100–700)*	30 (100–700)*	748 (50–450)*	98 (50–450)*
Eosinophils counts, /mm^3	2600 (<500)*	390 (<500)*	390 (<500)*	690 (<500)*	2875 (<500)*	3973 (<500)*
Diagnosis	Atypical SCID			OS	OS	OS

NP, not performed; HSCT, hematopoietic stem cell transplantation; SCID, Severe Combined Immunodeficiency. *Normal ranges for age.

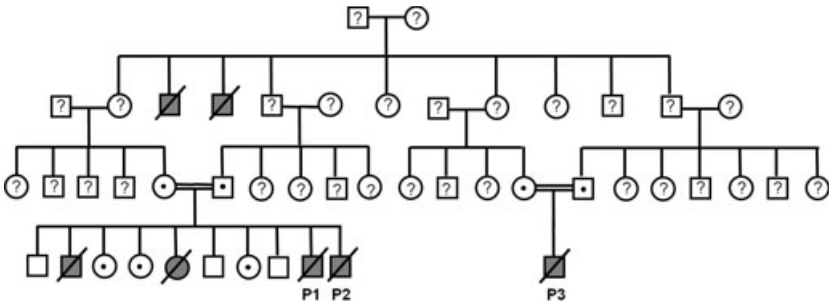

Figure 1 Family tree of this wide consanguineous kindred. Several relatives were inaccessible to study and they are marked with an '?'.

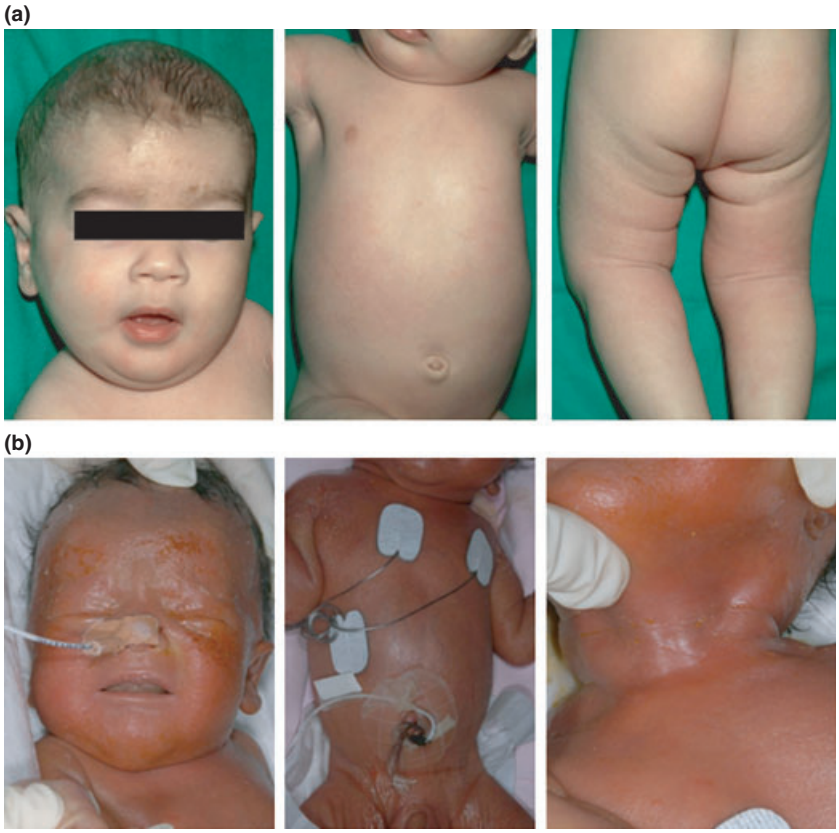

Figure 2 Different degree of skin involvement in two siblings with RAG1 deficiency. (a) Patient 1 at 3 months of age, with moderate eczematous dermatitis, characterized by generalized xerosis. (b) Patient 2 at 4 days of life, with severe erythroderma, bright thin skin, fissurated and exudative areas and oedema.

Cell samples, immunoglobulin levels and lymphocyte populations

All samples were obtained following informed consent and with the approval of the corresponding ethical committees. IgG, IgA and IgM were quantified by nephelometry (IMMAGE 800; Beckman Coulter, Fullerton, CA, USA), whereas IgE by ELISA methodology (ImmunoCap 250; Phadia, Uppsala, Sweden). Whole blood lymphocyte subpopulations were determined using CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 (Beckman Coulter). Activated T cells were detected with CD3-PC5, CD25-FITC and HLA-DR-PE and memory/naive T cells with CD3-PC5, CD45RA-FITC and CD45RO-PE monoclonal antibodies (Beckman Coulter) and analysed by flow cytometry (FC500; Beckman Coulter).

Lymphoproliferative assay

Peripheral mononuclear cells (PBMCs) were stimulated with phytohemaglutinine (PHA), Pokeweed antigen (PWN),

anti-CD3 (OKT3), Staphylococcal enterotoxin A (SEA) and Staphylococcal enterotoxin B (SEB; Sigma, St Louis, MO, USA) in a 3-day culture by standard methods. Lymphoproliferation was determined as incorporation of tritiated thymidine (Perkin Elmer, Boston, MA, USA) and measured in counts per minute (cpm).

Genetics of RAG1 and RAG2 genes

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Crawley, UK). Afterwards, amplifications of the codifying exons of RAG1 and RAG2 genes were performed. PCR products were purified with ExoSAP-IT (USB, Cleveland, OH, USA) and sequence reactions were carried out using BigDye Terminator kit (Applied Biosystems, Foster City, CA, USA). Sequences were then precipitated with ethanol/acetate and analysed in an ABI PRISM 310 Genetic Analyzer (Applied Biosystem).

Analysis of chimerism

Chimerism was studied by short tandem repeats (STRs) in DNA from peripheral blood leucocytes and isolated lymphocytes. STR analyses were performed using fluorescently labelled primer pairs directing the amplification of the polymorphism regions (ABI PRISM Short Tandem Repeat kit; Applied Biosystems). Amplification products were electrophoresed through a capillary system (ABI PRISM 310 Genetic Analyzer; Applied Biosystem) and sized using GENESCAN Analysis software (Applied Biosystem).

Results

Patient 1 was first immunologically evaluated in the third month of life. He showed lymphopenia owing to very low levels of T cells and complete absence of B cells (Table 1). More than 93% of T cells stained with anti-CD25, HLA-DR and CD45RO antibodies, indicating an activated and memory profile. T cell maternal engraftment was ruled out by STR analysis. Further studies of the T cell functionality revealed lack of proliferative responses to all tested mitogens. Immunoglobulin analysis showed residual maternal IgG and undetectable IgA, IgM and IgE levels. Eosinophilia was variable ranging from 0 to 2.600/mm^3 during the same month. Mutational analysis of RAG genes showed a homozygous deletion of Thymine 631 in RAG1 (c.631delT). This mutation had been previously described and characterized (14).

Patient 1 was transplanted. Twenty-five days after HSCT, the lymphoid chimera was fully accomplished (100%) and it remained almost complete (>80%) during the following half year (Fig. 3). Peripheral B cells, IgM and IgA were readily detectable from the second month after HSCT and increased progressively until reaching normal values. IgG was not assessable because of exogenous administration. Regular follow-up showed that the lymphoid chimera became partial (minimum of 51%), but the patient remained asymptomatic. Twenty-one months after HSCT the disease relapsed. At this moment, the patient presented B cells and partial lymphoid chimera, and he had high IgE levels for the first time. But 13 days later, 3 days before he died, B cells were absent and the chimera was fully lost (Fig. 3).

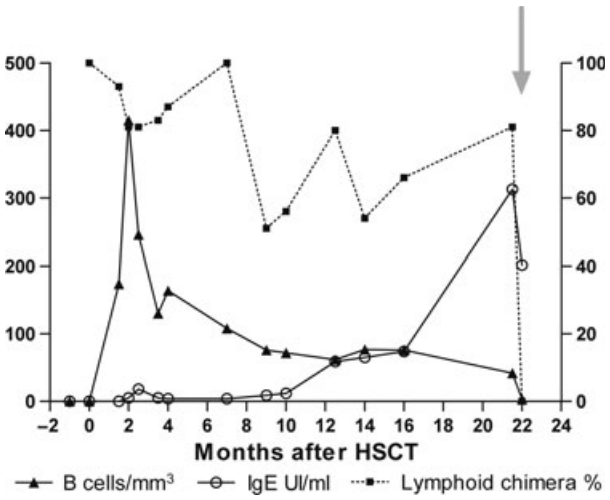

Figure 3 B cells numbers (black triangles), IgE levels (white circles) and % of lymphoid chimera (black squares) determined after HSCT in patient 1. Absence of B cells, elevated IgE and loss of lymphoid chimera were observed simultaneously with the relapse of the disease as OS just before the patient's exitus (grey arrow).

Patient 2 was immunologically evaluated at birth, 2 yr after the death of patient 1. He had marked lymphocytosis, but B cells were absent. His T cells did not proliferate to mitogens. They were stained with anti-CD25, HLA-DR and CD45RO antibodies, showing an activated/memory profile. Immunoglobulin levels were similar to patient 1 at onset, excluding IgE, which was within normal range. Eosinophilia was present and constant.

Patient 3 was also evaluated at birth. He was lymphopenic and no B cells were detected. His T cells were activated and of memory phenotype. In this case, HLA genotype was identical between mother and daughter, thus maternal T cell engraftment was particularly relevant to exclude. It was ruled out by chimerism analysis. IgG was maternal, and IgA and IgM were absent. IgE levels were elevated along with sustained eosinophilia.

As expected, the same deletion c.631delT in RAG1 gene was found in homozygosis in patients 2 and 3. Parents of all three patients, as well as three sisters of patients 1 and 2, were heterozygous carriers of the mutation.

Discussion

We described three patients from an extended consanguineous family with a variable spectrum of clinical presentation, despite sharing the same mutation in RAG1 gene (c.631delT). Persistent enteritis, failure to thrive and opportunistic infections, typical for T-B-NK+ SCID, were the main clinical findings in patient 1, whereas only moderate skin problems were noticed at onset. Severe erythroderma and infiltration of the liver, spleen and lymph nodes dominated the clinical

picture of patients 2 and 3 and determined their outcome. Immunological data also showed a wide diversity, from T lymphopenia to marked lymphocytosis, and different degrees of eosinophilia and IgE levels. In all cases, T cells were patient's own, activated, non-responder to mitogen stimulation and bearing a memory phenotype, suggesting that these T cells came from peripheral expansion. Based on clinical and immunological data, patient 1 was diagnosed with atypical SCID because he presented a T-B-NK + SCID phenotype with eosinophilia, whereas both patients 2 and 3 were diagnosed with OS, despite normal IgE in patient 2. Thus, phenotypical heterogeneity was observed at onset, despite sharing the same genetic alteration.

Interestingly, both patients showing the most severe presentations, patients 2 and 3, were born preterm. The erythroderma was present already at birth. Strikingly, only one OS preterm infant (30 wk of gestational age) has been reported and he also presented at birth with erythroderma (15). We thought that the disease itself could have been the cause of prematurity. Or the other way round, prematurity and skin immaturity could have played a role in the severe phenotypical expression and bad outcome of these two infants.

Nowadays, HSCT is the only curative treatment available for these patients. Patient 1 was transplanted achieving total lymphoid chimera the following month. Although there was a progressive loss of donor's lymphocytes (a partial chimera was established), the patient remained asymptomatic. Twenty-one months after HSCT, he relapsed with a severe infection, lymphadenopathy, hepatosplenomegaly, eosinophilia, increased IgE, coinciding with a progressive lack of B cells and loss of chimera (Fig. 3). At this time, he fulfilled all diagnostic criteria of OS. The patient died 16 days later because of massive lymphocytic infiltrates and infections. The best parameter that could be predictive of the disease relapse was IgE levels, which increased progressively from the first year after HSCT. The extremely aggressive relapse in patient 1 could be related to the conditioning regimen chosen for HSCT. Previous 2004 EBMT guidelines established that SCID patients with one mismatched antigen did not require conditioning regimen. But following more recent publications, we decided to include a reduced conditioning regimen to eliminate possible autoreactive T cells (16). However, the RIC could have allowed the survival of an undetermined amount of patient's T cell precursors and/or autoreactive T cells that progressively could have expanded leading to the relapse into an OS phenotype. In this respect, a more intensive conditioning regimen could have avoided the survival of these cells and consequently, the relapse of the disease.

The phenotypic variability of the described patients is probably related, at least in part, to their particular mutation in the RAG1 gene. The deletion of the Thymine 631 produces an altered frameshift with a predicted stop codon (p.Glu174fs×27). However, an alternative start codon downstream of the deletion (AUG^{183}) has been described. The resultant protein has 860 aminoacids and lacks N-terminal aminoacids, but preserves the entire core region that allows some TCRs rearrangements (14). In normal conditions, natural occurring autoreactive T cells are eliminated in the thymus by negative selection processes. In OS, autoreactive T cells escape this control process and undergo peripheral expansion (17). As TCR rearrangement is a random process, the degree of T cell autoreactivity can be expected to be variable between individuals sharing the same mutation and even in the same patient before and after HSCT. Indeed, after HSCT, the T cell reprogramming starts from zero. This phenomenon could have contributed to the diverse clinical and immunological spectrum in the three patients reported despite identical RAG defect.

Moreover, epigenetic factors, such as DNA and histones modifications, could also be involved in the final phenotype. These epigenetic alterations influence, for example, T cell responses and differentiation after exposure to infectious agents and inflammatory cytokines (18, 19). Therefore, the response after the same aggression not only depends on genetics. Accordingly, the disease relapse in patient 1 could be attributed to both an abnormal response to a pathogen (the relapse occurred coinciding with a pneumonitis of probable infectious origin) and to residual autoreactive T cells.

In summary, the same defect in RAG genes can be responsible for a diverse clinical and immunological spectrum of manifestations from SCID to OS through several forms of atypical SCID/OS. This is particularly relevant in mutations that allow the synthesis of proteins with partially preserved function, such as c.631delT in RAG1, where TCR rearrangements occur and autoreactive T cells are produced. The variable degrees in T cell autoreactivity together with epigenetic and environmental factors will determine the resulting phenotype of OS. After HSCT, patients can relapse with a different clinical and immunological picture because of the reprogramming of the immune system. The use of a more intensive conditioning regimen previous to HSCT to preserve a total chimera could have avoided the relapse of the disease and it should be considered in similar cases.

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