

Title: Persistent *rotavirus* diarrhea post-transplant in a novel *JAK3*-SCID patient after vaccination.

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Abbreviations: SCID - severe combined immunodeficiency; NBS - newborn screening; TRECs - T cell receptor excision circles; HSCT - hematopoietic stem cell transplantation; *JAK3* - Janus kinase 3; TPN - total parental nutrition.

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To the Editor:

Severe combined immunodeficiency (SCID) encompasses a phenotypic and genetic heterogeneous group of disorders, characterized by significantly impaired cellular and humoral immunity (1). Without reconstitution of the immune system, the disease is usually fatal within the first two years of life (1). Early diagnosis and treatment of SCID significantly improves clinical outcome, indicating the importance of newborn screening (NBS) for SCID (2, 3). Currently, population-wide NBS for SCID is only conducted in the USA (2, 3). Until recently, genetic defects were found in approximately 85% of clinically diagnosed SCID patients, of which almost half carried mutations in the gene encoding the common gamma chain located on the X chromosome (X-linked SCID) (1). However, unbiased evaluation by NBS programs in the USA shows that the genetic distribution of SCID might be different than previously thought, with a substantially larger proportion of autosomal recessive forms of SCID (2, 3).

This case report demonstrates the detrimental consequences of an oral live attenuated *rotavirus* vaccine in SCID patients, pre- as well as post-transplantation. This emphasizes the need for worldwide implementation of NBS for SCID, which could have prevented the administration of live vaccines to the herein described patient. Furthermore, we identified a novel homozygous *JAK3* mutation as the cause of the $T^+B^+NK^-$ SCID phenotype.

Patient description

A boy from Turkish descent was referred to our hospital at seven months of age for recurrent infections and failure to thrive. He was born at term as the first child of healthy consanguineous parents (Figure 1A). He did not attend day care. At the age of three months, he developed recurrent upper and lower respiratory tract infections. At time of referral, he had persistent tachypnea due to *Respiratory Syncytial Virus* bronchiolitis. In addition, the boy suffered from recurrent urinary tract infections (*Escherichia coli*, *Enterococcus faecalis*) and chronic diarrhea with persistent detection of *rotavirus* antigen on stool. The chronic *rotavirus* diarrhea was believed to be induced by previous administration of an oral live attenuated *rotavirus* vaccine, which is given at the age of two months in Belgium. Testing for HIV was negative (Abbott ARCHITECT HIV Ag/Ab Combo Assay). Family history was negative for early childhood deaths. Laboratory assessment is shown in Table 1. Noteworthy, T cells existed almost exclusively of memory cells. Peripheral lymphocytes did not proliferate upon mitogenic stimulation, indicating a marked functional T cell defect. T cell receptor excision

circles (TRECs) could not be detected in his blood, demonstrating lack of thymic output. Consistently, radiography revealed that thymus tissue was absent. Further testing showed that approximately 29% of leukocytes were of maternal origin.

Based on the clinical and immunological presentation, the diagnosis of $T^+B^+NK^-$ SCID was made. Three weeks after diagnosis, the boy underwent haploidentical hematopoietic stem cell transplantation (HSCT), with his mother as donor, without pre-transplant conditioning. HSCT was successful and donor engraftment proceeded as follows: donor chimerism of 17% after 5 weeks, 25% after 6 months, and 39% after 12 months. Strikingly, the gastrointestinal *rotavirus* infection persisted for eight months after HSCT causing intractable diarrhea and excessive weight loss requiring total parenteral nutrition (TPN). Finally, when *rotavirus* could no longer be isolated from his stool, the diarrhea gradually resolved and TPN was stopped. In addition to long-lasting *rotavirus* diarrhea, his immunoglobulin levels remain significantly reduced until today. Moreover, peripheral B cell phenotyping continues to be aberrant with less than 1% memory B cells. The boy is currently 2,5 years old and is doing well under antibiotic prophylaxis and immunoglobulin replacement therapy.

Genetic analysis

Based on parental consanguinity, homozygosity mapping was performed using a SNP-based array (HumanCytoSNP-12 BeadChip platform, Illumina), to identify homozygous regions larger than 1 Mb. For this patient, several SCID genes were located in homozygous regions, of which *Janus kinase 3 (JAK3)* best matched the patient's phenotype. Subsequent Sanger sequencing of the coding region of *JAK3* revealed a homozygous C to T transition in exon 2 (NM_000215.3:c.115C>T), resulting in a nonsense mutation and a premature stop codon (p.Q39*) (Alamut Visual version 2.4, Interactive Biosoftware). Both parents were heterozygous carriers of the *JAK3* mutation (Figure 1B). To our knowledge, this mutation has not been reported in literature so far (Figure 1C).

Discussion

We identified a novel homozygous *JAK3* mutation (c.115C>T, p.Q39*) in a male SCID patient born from consanguineous parents of Turkish descent. The nonsense mutation, leading to a transcript with a premature stop codon close to the N-terminus is expected to be unstable and to be rapidly degraded by nonsense-mediated mRNA decay (4). Indeed, other truncating *JAK3* mutations downstream of exon 2 have been shown to cause loss-of-function

due to complete absence of the JAK3 transcript and protein (5, 6). Overall, we can conclude that the newly discovered p.Q39* *JAK3* mutation is causal for the SCID phenotype in this patient. JAK3 mediates signal transduction downstream of the common gamma chain, essential for lymphocyte development (5). JAK3 deficiency accounts for approximately 7-14% of SCID patients, and is phenotypically indistinguishable of X-linked SCID (1, 5). Mutations in the *JAK3* gene appear to arise sporadically, and most patients are compound heterozygous. Homozygous *JAK3* mutations are generally the result of parental consanguinity, like in this case (5).

Chronic *rotavirus* infection in SCID patients who were given a live *rotavirus* vaccine prior to diagnosis, has been repeatedly documented in literature (7). In contrast to the herein described boy, patients usually recover from their preexisting *rotavirus* infection shortly after transplantation (7). We believe that the prolonged time for clearing the preexisting *rotavirus* infection is at least partly due to the remaining predominance of non-functional host B cells (8). Donor B cell reconstitution in *JAK3*-SCID patients is variable and most patients continue to have compromised humoral immune responses post-transplantation (5, 8, 9). This patient received HSCT from a haploidentical parent without conditioning, in which case donor B cell reconstitution is expected to be very low (5, 9). We found that donor B cell chimerism in this patient was only 6% 21 months after HSCT. Clinically, this is reflected in a persistent hypogammaglobulinemia and aberrant peripheral B cell phenotyping, with a need for long-term immunoglobulin replacement therapy (9).

This case report illustrates the potentially devastating consequences of live vaccines on undiagnosed SCID patients. Live (viral and mycobacterial) vaccines are generally contraindicated in immunocompromised individuals (10). However, since *rotavirus* vaccination is recommended to be started at the age of two months, many SCID patients have already received it at time of diagnosis (7). TREC-based NBS for SCID has been found efficient for early diagnosis of the disease, and could thus prevent administration of live vaccines to those infants (2, 3). NBS for SCID has become nationally recommended in the USA in 2010 (2). It is not yet carried out in Europe, although trials for implementing population-wide NBS for SCID are currently ongoing in several European countries (3).

Conflict of Interest

None of the authors has any potential financial conflict of interest related to this manuscript.

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Table 1. Laboratory assessment at time of diagnosis.

	Patient	Reference values
White blood cells	12150/ μ L	7000-15000/ μ L
Neutrophils	7320/ μ L	1500-7000/ μ L
Total lymphocytes	2640/ μ L	3000-9000/ μ L
T lymphocytes (CD3 ⁺)	1320/ μ L	2400-6900/ μ L
T helper cells (CD3 ⁺ CD4 ⁺)	449/ μ L	1400-5100/ μ L
- naive (CD45RA ⁺)	3%	N.A.
- memory (CD45RO ⁺)	100%	N.A.
Cytotoxic T cells (CD3 ⁺ CD8 ⁺)	871/ μ L	600-2200/ μ L
- naive (CD45RA ⁺)	8%	N.A.
- memory (CD45RO ⁺)	90%	N.A.
B lymphocytes (CD19 ⁺)	1190/ μ L	700-2500/ μ L
- naive (IgD ⁺ CD27 ⁻)	99%	N.A.
- memory (IgD ⁻ CD27 ⁺)	< 1%	N.A.
NK cells (CD3 ⁻ CD16 ⁺ CD56 ⁺)	26/ μ L	100-1400/ μ L
IgG	0.2 g/L	3.4-7.6 g/L
IgM	0.3 g/L	0.23-0.5 g/L
IgA	0.027 g/L	0.17-0.5 g/L
Albumin	41 g/L	34-48 g/L
Total protein	59 g/L	60-76 g/L
LTT PHA 5 μ g/mL	5.5 x 10 ⁻³ cpm	>370 x 10 ⁻³ cpm
LTT PHA 1 μ g/mL	3.7 x 10 ⁻³ cpm	>320 x 10 ⁻³ cpm
LTT PWM dilution 1/100	5 x 10 ⁻³ cpm	>90 x 10 ⁻³ cpm
LTT PWM dilution 1/500	3.9 x 10 ⁻³ cpm	>90 x 10 ⁻³ cpm
TRECs	Absent	Present

Blood cell counts are shown in absolute numbers. Ig, immunoglobulin. LTT, lymphocyte transformation test (test for lymphocyte proliferation). PHA, phytohaemagglutinin. PWM, pokeweed mitogen. TRECs, T cell receptor excision circles. N.A., not available.

