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IL-7 receptor deficient SCID with a unique intronic mutation and post-transplant autoimmunity due to chronic GVHD

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Abstract

Severe combined immunodeficiency (SCID) may result from a variety of genetic defects that impair the development of T cells. Signaling mediated by the cytokine interleukin-7 is essential for the differentiation of T cells from lymphoid progenitors, and mutations of either the interleukin-7 receptor α chain (IL-7R α) or its associated cytokine receptor chain, the common γ chain (γ c), result in SCID. Here we report a case of SCID due to heterozygous mutations of the *IL7R* gene encoding IL-R α . A previously unrecognized mutation found within intron 3 created a new exon between exons 3 and 4 in the mRNA transcribed from this allele, producing a truncated, unstable mRNA. This mutation illustrates the necessity of evaluating both coding and non-coding regions of genes when searching for pathogenic mutations. Following hematopoietic stem cell transplantation of our patient, immune reconstitution was accompanied by two unusual complications, immune-mediated myositis and myasthenia gravis.

Keywords

Severe combined immunodeficiency; SCID; myasthenia gravis; myositis; hematopoietic stem cell transplantation; HSCT; interleukin-7 receptor; human mutation; intronic mutation; mechanisms of mutation

Introduction

Severe combined immunodeficiency (SCID) describes a group of life-threatening primary immunodeficiencies that have in common a failure of T lymphocyte production. Patients with SCID are therefore susceptible to infections from common bacteria, viruses, fungi, and opportunistic organisms; their long-term survival requires immune reconstitution, such as by successful hematopoietic stem cell transplantation (HSCT) or enzyme-replacement therapy (in the case of adenosine deaminase deficiency). T cell development and proliferation depend upon

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cytokine signaling, and SCID results from mutations of the genes encoding the common gamma chain (γ_c) of the receptors for interleukins (IL)-2, -4, -7, -9, -15, and -21; the Jak3 signaling kinase; or the IL-7 receptor α chain (IL-7R α) [1]. Mutations in the X-linked *IL2RG* gene encoding γ_c affect males and cause roughly half of all cases of SCID; mutations of *IL7R*, encoded on chromosome 5p13, account for at least 10% of cases of SCID and occur in both males and females [1,2].

In this report, we present a patient with SCID with B cells (T-B+ SCID) due to compound heterozygous mutations of *IL7R*, one reported previously, and one which was undetectable by the standard sequencing of exons and their adjacent splice signals in genomic DNA. We also discuss complications that followed HSCT including recurrent acute myositis and myasthenia gravis.

Patient and Methods

Patient History

This term male infant was born non-consanguineous parents of Portuguese descent who gave informed consent to participate in this study. There was no family history of susceptibility to infections. The infant developed *Salmonella* diarrhea age 4 months, and at 5 months, had failure to thrive and pneumonia attributed to reflux and treated with ranitidine and metoclopramide. At 7 months, a cough was followed by somnolence and hypoxemia requiring intubation, mechanical ventilation and emergent transport to Children's Hospital Boston, where physical exam revealed absent palpable lymph nodes. Laboratory tests (Table 1) revealed lymphocytopenia and other diagnostic features for T-B+ SCID. NK cells were essentially absent. Silver staining of a tracheal aspirate demonstrated *Pneumocystis jiroveci*. Treatment with trimethoprim/sulfamethoxazole and intravenous immune globulin led to improvement. He was extubated after 17 days.

Lacking an HLA-matched relative, the patient received a maternal, haploidentical, peripherally mobilized, T-cell-depleted HSCT (8×10^6 CD34+ cells/kg with 7×10^4 T cells/kg) with no pre-transplant conditioning or post-transplant prophylaxis for graft vs. host disease (GVHD). After 5.5 months, engraftment failure was diagnosed and he was given a second maternal T-depleted HSCT (2×10^6 CD34+ cells/kg with 5×10^4 T cells/kg), this time following myeloablative conditioning with cyclophosphamide and busulfan. There was no prophylaxis for GVHD. The patient showed engraftment with >98% donor T cells at 140 days, and 96% donor B cells after 8, and 84% after 15 months. T cell proliferation to *in vitro* mitogenic stimulation was normal at 7 months, and the patient weathered rotavirus gastroenteritis uneventfully at that time.

After 15 months, however, he had refusal to walk 2 weeks following a mild cold. He again developed respiratory failure necessitating mechanical ventilation. His serum creatine kinase (CK) peaked at 10,700 units/L. Muscle biopsy revealed acute myositis with infiltrating T cells and other predominantly mononuclear cells (Fig. 1). At that time, he had normal T cell counts and a normally diverse T cell antigen receptor V β repertoire by spectratyping (not shown).

He received intravenous methylprednisolone and was extubated in 8 days. Cyclosporine A was added, but when immunosuppressive therapy was weaned, he had new onset of ptosis, weakness, difficulty swallowing, and respiratory failure, this time without CK elevation. He again required mechanical ventilation and responded to a high-dose pulse of methylprednisolone and cyclosporine A. Total anti-acetylcholine receptor antibodies (Ab) were 0.8 nmol/L (normal 0 - 0.4 nmol/L), with receptor blocking Ab 29% (normal 0 - 15%), and receptor modulating Ab 46% (normal 0 - 20%). Electromyography and Tensilon testing were diagnostic for myasthenia gravis. Pyridostigmine treatment led to improvement. The patient is currently 32 months past

his second HSCT with excellent engraftment of donor T and B cells, but still requiring immunosuppression for myasthenia.

IL7R Sequencing

Nucleotide and amino acid numbering of *IL7R* is from NIH RefSeq entries NM_002185.2 and NP_002176.2, respectively (Fig. 2, A and B). After patient, parental and control DNAs were isolated from whole blood or B cell lines, amplification of *IL7R* exons was performed with PuReTaq Ready-To-Go PCR beads. PCR primers used were: Exon 1: 5'-tgtcttcagattcttttaaagtggcccttagtc, 5'-ttgagaatactgaggtcttataaaccacccatag; Exon 2: 5'-aataatcagtgccacttttaattgggatgc, 5'-gtgttgggcaacagatttttggtgaga; Exon 3: 5'-cagccaaaatacctatgaaaatccgttacg, 5'-cctgaatttctagcctactggcttgccttat; Exon 4: 5'-gacagaggggacccctgaggaca, 5'-tgaccttttgaggccctgaatacacag; Exon 5: 5'-tatcacattcacaatgtaaaatggcgtcttt, 5'-agccgcacttgcctgcatacctg; Exon 6: 5'-tgggcctgtgcacccaagtcaatg, 5'-ggaaaaagccctagagggaagggaacacc-3'; Exon 7: 5'-ggtcaccacctaattgtgttagagccaagacta, 5'-ggagggggcagcgtgctgtttgta; Exon 8: 5'-cctggggctggaggagacag, 5'-aagaatggggcagtcctcagtgaaagaa. Annealing temperatures were 57.3°C for exons 1, 4, 6, 7, and 8 and 56°C for exons 2, 3, and 5. Thirty-five cycles were performed of: 96°C for 10s, $T_{\text{anneal}} + 5^{\circ}\text{C}$ for 10 s, and 72°C for 2.5 m. Products were treated with ExoSAP-IT (USB), and sequencing was carried out with Prism Big Dye® terminators (PE Applied Biosystems, Foster City, CA), using primers: Exon 1: sense 5'-gggaggtgaaaattgcagtgagccgagat, anti-sense 5'-ttgagaatactgaggtcttataaaccacccatag; Exon 2: sense 5'-gcccttgggcttttcttctgaatactac; Exon 3: sense 5'-ttccataattttataaatatgtcttgacta, anti-sense 5'-ccattcatgtaggtttgcacaacactatc; Exon 4: anti-sense 5'-tggcctcaatctatattgttatccaactc; Exon 5: sense 5'-ctgtccctaatttctgttgactcctttacg; Exon 6: anti-sense 5'-agatagggatactgggcactaaattcgtgaaa; Exon 7: anti-sense 5'-gaatcaaataccaaaagggtgaggttcaactgt; Exon 8: sense 5'-tggagggcacagccagtggt, sense 5'-agcacgctgccccctccatttct, anti-sense 5'-cacatggctcaggaactgcaattagactc.

After 35 sequencing extension cycles, products were purified using Performa® DTR filtration cartridges and analyzed with an ABI 3100 sequencer.

RT-PCR and Cloning of *IL7R* cDNA

RNA was isolated from patient B cells transformed with Epstein Barr virus and parental T cells using RNA Stat-60 Reagent (Iso-Tex Diagnostics). *IL7R* cDNA was made with SuperScript™III One-Step RT-PCR System (Invitrogen) with Platinum® *Taq* High Fidelity for 20 cycles of amplification with gene-specific sense and anti-sense primers, 5'-tccctccctccctctcttactctca and 5'-cctgccttctcactctcaatcattt, respectively, at an annealing temperature of 58.1°C. Further nested PCR was performed with primers 5'-atgacaattctaggtacaacttttggcatgg and 5'-ctgaatcattgggtcaccttaaaccttggtg for 20 cycles of 96°C for 10s, 63.1°C for 10s, 72°C for 2 m. The cDNA was purified with a S.N.A.P. Gel Purification Kit and cloned with a TOPO® TA Cloning Kit (both from Invitrogen). Colonies were amplified with PuReTaq Ready-To-Go beads and sequenced as above using the following primers: sense 5'-atgacaattctaggtacaacttttggcatgg and 5'-tgtatcggggaaggagccaatgactttg; and anti-sense 5'-tggggttttctaattttgtcttctctgtg and 5'-gaagcctttaaatagtgatcagggatgga.

Intron 3 PCR and Sequence

Intron 3 DNA was amplified and sequenced as above with sense primer 5'-cctacagtggggccctctggag and anti-sense primer 5'-acagccatgtgcctaattgtcttcttactac. Long PCR from exons 3 to 6 of genomic *IL7R* DNA was done with a Takara LA PCR v2.1 kit. The products were cloned as above into GeneHog electrocompetent cells (Invitrogen).

Splicing analysis

The EMBL-EBI Alternative Splicing Workbench (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=7>) was used to calculate splicing scores.

Results

The patient's and paternal genomic DNA had a heterozygous mutation in the coding region of *IL7R*, cDNA 353 G>A, resulting in substitution of tyrosine for the cysteine residue 118 (C118Y) (Fig. 2C, left). This mutation was reported previously in two Spanish brothers with SCID [3] and a Brazilian boy with Omenn syndrome [4] all of whom were homozygous. Genomic DNA from the mother of our patient did not contain any mutations in coding or splice regions.

To pursue our patient's second mutation, we hypothesized that in the absence of a coding abnormality a defective maternally inherited allele would have unstable mRNA and therefore no protein expression. We compared the relative abundance of *IL7R* mRNA transcripts from the two alleles of both patient and mother. We were able to distinguish each subject's alleles by using coding single nucleotide polymorphisms (SNPs) for which they were heterozygous. Only 1 of 6 cDNA clones from a patient-derived B cell line, and 1 of 31 cDNA clones from maternal T cells, represented the *IL7R* allelic haplotype that the mother donated to her son. Moreover, both of these poorly expressed cDNA clones had a 104 bp insertion following exon 3 that matched a region within the *IL7R* intron 3 of genomic DNA (shaded region in Fig. 2C, right). This cDNA insertion contained an early termination codon. To determine whether a mutation in intron 3 caused this aberrant cDNA, we sequenced *IL7R* intron 3 from the patient's and mother's genomic DNA. Both had a heterozygous mutation of guanine to adenine 288 nucleotides beyond the 3' end of exon 3, cDNA 379 (+288) g>a (Fig. 2C, Fig. 3).

To determine if the aberrant cDNA clone came from the same allele that contained the intronic mutation, we cloned and sequenced a long genomic PCR fragment from the patient, spanning the intron 3 (+288) variant and a frequent polymorphic SNP in exon 6, 731 C>T, for which both patient and mother were heterozygous (not shown). This exon 6 SNP was thymidine in both patient and maternal cDNA clones containing the intron 3 insertion, thereby proving that the intron 3 mutation was in the maternally inherited *IL7R* gene.

To rule out that the intronic mutation 379 (+288) g>a was a previously unreported frequent polymorphism, *IL7R* intron 3 was sequenced in 50 unrelated genomic DNA samples (100 alleles); none had this change. Noting that 379 (+288) g>a occurs at the third base following the 3' end of the 104 bp intronic insert in the cDNA, AGgt(g>a)tcc (Fig. 3), we used an alternative splicing analysis program, which predicted this g>a change to strengthen a cryptic donor splice site, from a weak, low-likelihood score of 4.4 to a somewhat stronger score of 4.9. Taken together, our results showed that an intronic substitution specific to our patient caused insertion of a new 104 bp pathologic exon into the *IL7R* mRNA, producing a premature termination codon, and unstable mRNA (Fig. 2).

Discussion

Although successfully rescued from SCID by HSCT, our patient suffered 2 severe, uncommon complications of chronic GVHD: recurrent acute myositis and myasthenia gravis (MG). MG has been associated with particular haplotypes of HLA class II, including DR3 [5], and class I, including A1 and B8 [6]. However, neither the patient nor his mother, the donor, had these HLA haplotypes. Although each of the patient's complications has been reported in SCID patients post-HSCT, we could find only 2 reports in which both disorders co-existed in a patient with chronic GVHD [7,8]. One recovered, a child with aplastic anemia post HSCT from an

HLA-matched sibling, but GVHD-associated myositis, like ours, requires prolonged immunosuppression, with glucocorticoids or calcineurin antagonists [9].

Persistence of host B cells is a common finding post-HSCT in patients with T-B+ SCID, including those with *IL7R* mutations [3]. Some *IL7R*-deficient SCID patients who retain only autologous B cells are nonetheless able to make specific antibodies and avoid lifelong IVIG treatment, presumably because IL-7 signals are not required for development of functional human B cells as long as allogeneic T helper cells are restored. Whether autoimmune disease in these patients could result from mixed chimerism, as was suggested in a mouse model of chronic GVHD [10] is as yet unknown. However, our patient's successful B cell engraftment indicates that autoantibodies and immune dysregulation are not limited to patients who have donor T cells but retain autologous B cells.

Over a dozen genes have been implicated in SCID, and the biological effects of their mutations can be grouped into four broad categories: defects in cytokine receptor pathways (γ c, Jak3, *IL7Ra*), accumulation of toxic metabolites (ADA, PNP), T cell receptor signaling defects (CD45 and the CD3 γ , ϵ , δ , and ζ chains), and defects in DNA recombination (recombinase activating genes RAG1/2, Artemis, Cernunnos, and DNA Ligase 4) [2,11]. At least 10% of SCID cases still have no identified mutations. However, most gene sequencing is limited to coding and immediately flanking splice regions from genomic DNA, and this approach would miss intronic mutations such as that in our patient.

Our patient had a previously unreported mutation in intron 3 of *IL7R* that introduced into the mRNA a 104 bp insertion containing an early termination codon. This mutation was greatly under-represented in panels of cDNA clones from both patient and mother, indicating that mRNA containing the 104 bp insertion was unstable, consistent with nonsense mediated decay. Intronic mutations that result in incorporation of new exons into transcripts have been described in a number of human genetic diseases, including β thalassemia [12], ornithine aminotransferase deficiency [13], cystic fibrosis [14,15], β -glucuronidase deficiency [16,17], neurofibromatosis type I [18], ataxia-telangiectasia [19], and Fabry disease [20]. Searching within introns could be fruitful in other cases of a suspected compound heterozygous mutation where only one mutant allele has been found. Analysis of cDNA would require banking of mRNA or cells if a molecular diagnosis is not easily made.

The discovery of the mutations in *IL7R* causing SCID in this patient has also made possible prenatal testing of the parents' subsequent at-risk pregnancy. Fetal DNA obtained from an amniocentesis contained the paternal 353 A>G C118 Y mutation, but not the maternal 379 (+288) g>a mutation, predicting that this infant would not have *IL7R* SCID. Indeed, postnatal lymphocyte counts and T cell function were normal, a confirmation that the baby is unaffected.

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Hematoxylin & Eosin

20x

CD3 (T cells)

20x

CD8 (Cytotoxic T cells)

20x

CD45 (all lymphocytes)

40x

Figure 1.

Muscle histology from post-transplant SCID patient, showing infiltration around muscle bundles with primarily mononuclear cells (hematoxylin and eosin stain, top left). Immunohistochemical analysis shows CD3⁺ T cells (upper right), CD8⁺ T cells (lower left), and CD45⁺ lymphocytes in higher power (lower right).

A

IL7R genomic DNA
23.2 Kb

B

IL7R cDNA
1.7 Kb

C

IL7R Mutations
Inherited by
Proband

Deleterious missense mutation

Figure 2.

IL7R locus and mutations. **A.** Genomic structure of the *IL7R* gene on human chromosome 5p13. **B.** Normal *IL7R* cDNA, showing exons and protein domains: Sig, signal sequence; C, extracellular conserved cysteine; W, WSXWS motif, TM, transmembrane domain, B1-box1, S-serine containing domain, T-tyrosine containing domain; 3' UTR, 3' untranslated region. The cDNA number of the first base of each exon is shown. **C.** *IL7R* Mutations inherited by proband. Paternal Mutation (left), cDNA 353G>A, is a GC dinucleotide mutation due to a C to T transition on the anti-coding strand, causing an amino acid change from C to Y. Maternal Mutation (right), is a g to a transition 288 bp beyond intron 3. The mutation is preceded by a Ggt, part of a canonical splice donor sequence. The mutation improves splice donor function, resulting in inclusion of a new 104 bp exon (hatch marks) following exon 3 in the cDNA. This new exon contains a premature stop (arrow, TGA) and produces unstable mRNA.

Figure 3.
IL7R genomic DNA sequence of patient (above) and control (below) showing Exon 3(+288) g>a heterozygous mutation in the patient. AGgtatcc is a 3' splice site used to form a pathologic new exon in the patient's mRNA.

Table 1

Immunologic findings at initial presentation, age 7 months.

White blood cells	12.17 × 10 ³ /μL	
Differential	77% neutrophils, 10% bands, 8% lymphocytes, 3% eosinophils	
Absolute lymphocyte count	970/μL (normal 3400 – 9000)	
IgG	<10 mg/dL	
IgA	<7 mg/dL	
IgM	37 mg/dL	
IgE	<4 IU/mL	
ADA enzyme activity	96.6 nmol/h/mg (normal 63 ± 41)	
PNP	1990 nmol/h/mg (normal 1336 ± 441)	
CD3 ⁺ T cells % (absolute number)	0 (0) ²	
CD19 ⁺ B cells % (absolute number)	95 (921, normal 700-2500)	
CD3 ⁺ CD16 ⁺ / CD56 ⁺ NK cells % (absolute number)	4 (39, normal 100-1000) ³	

Lymphocyte proliferation	Patient cpm +/- std	Control cpm +/- std
Background	498 ± 43	193 ± 57
Concanavalin A	263 ± 74	70,149 ± 3,279
Phytohemagglutinin	203 ± 27	113,339 ± 5,784
Pokeweed mitogen	342 ± 47	32,387 ± 1,847

¹ One lytic unit is defined as the effector to target cell ratio that achieves 10% cytotoxicity.

² Cell% (cells/μL).

³ While *IL7R* SCID patients typically have NK cells, genotype-phenotype relationships are not always predictable.