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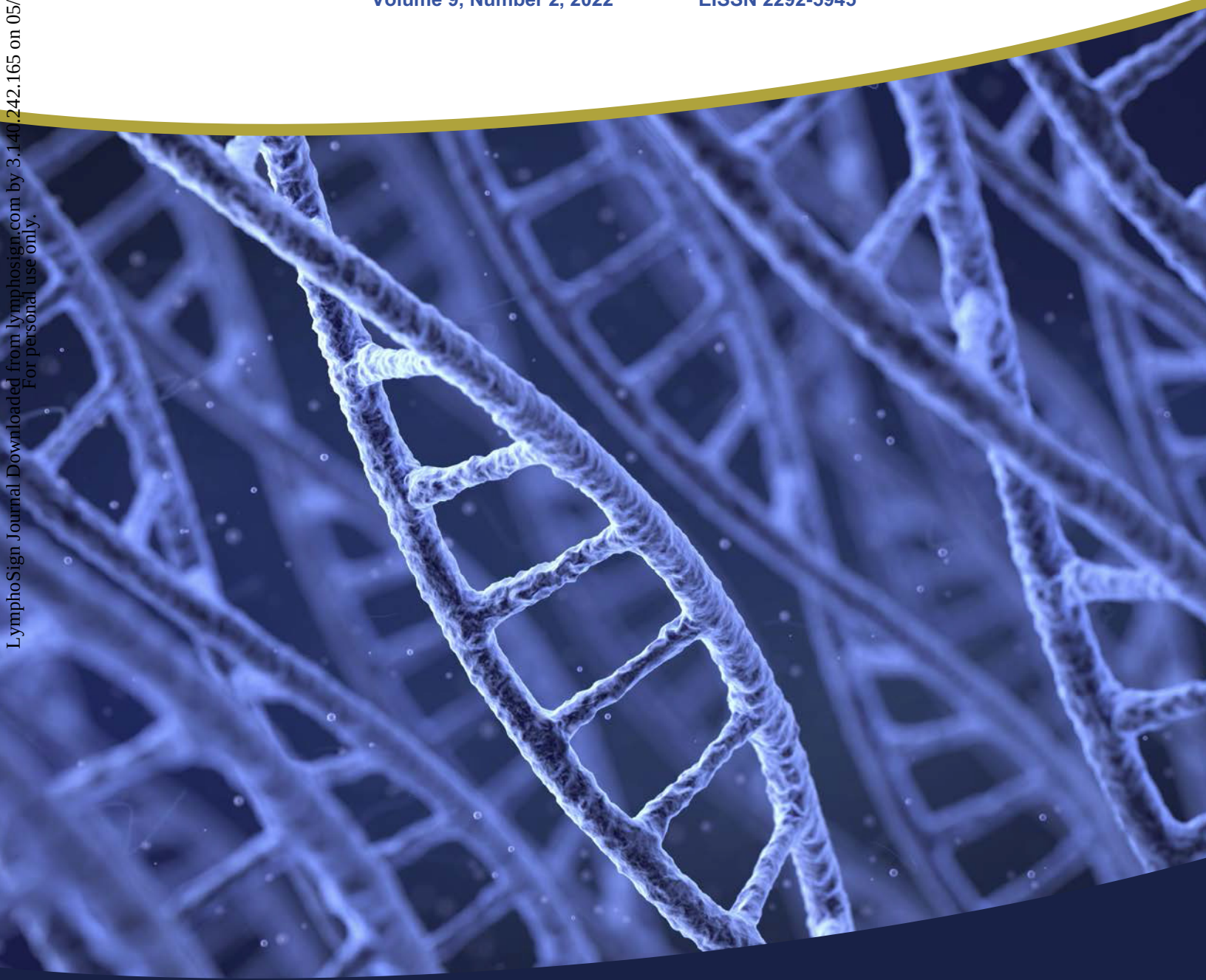

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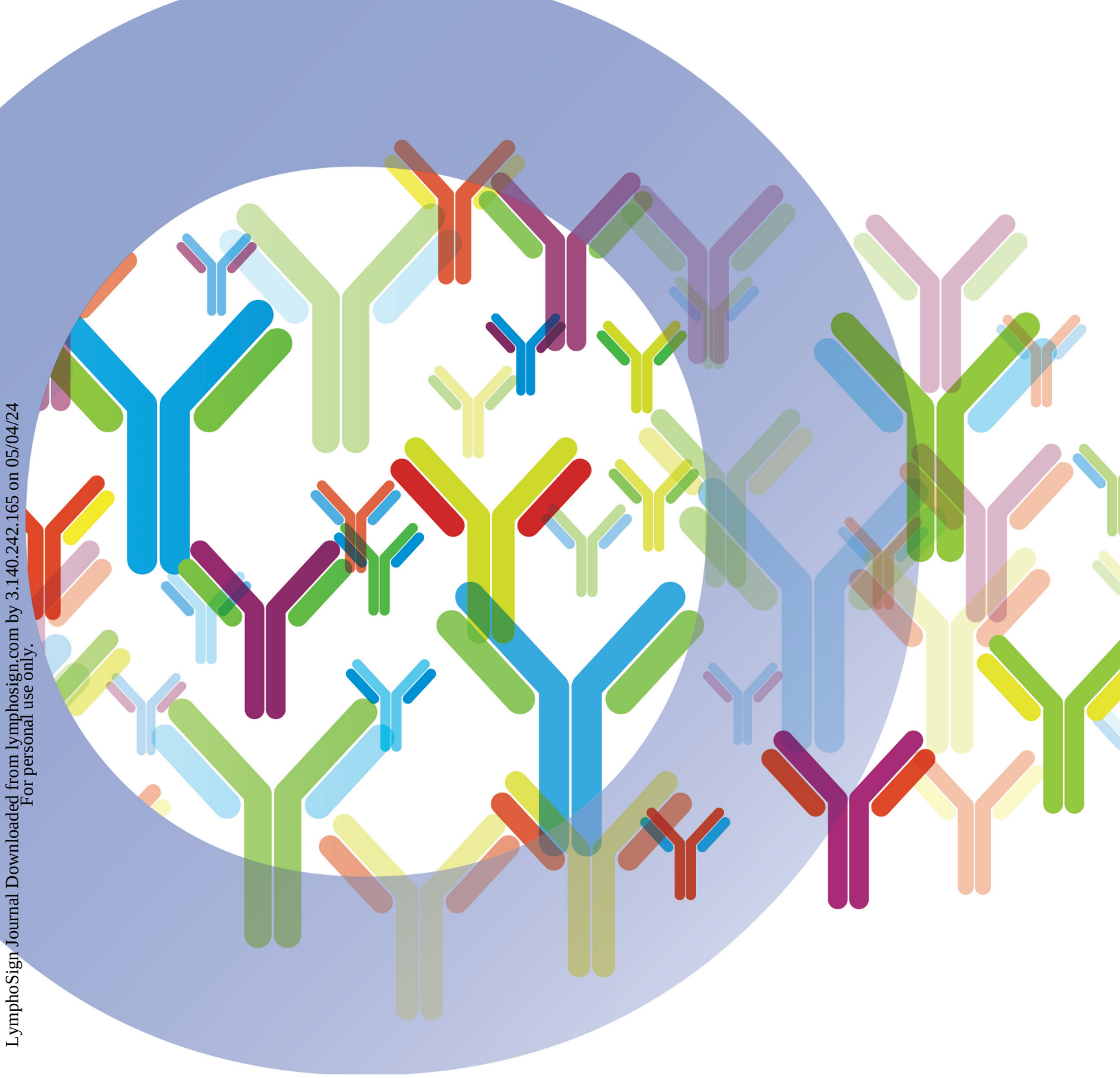

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COVID-19 outcomes in immunocompromised individuals: seroconversion and vaccine effectiveness

Linda Vong, PhD[a*] and Chaim M. Roifman, CM, MD, FRCPC, FCACB[a,b]

As we round the corner on the 6th coronavirus disease 2019 (COVID-19) wave amid gradual lifting of social distancing and masking restrictions, it is timely to review the guidance measures for individuals with compromised immune systems, particularly those with primary immunodeficiency (PID) (Roifman 2020). The clinical spectrum of COVID-19 varies from those who are asymptomatic, experience mild symptoms such as fever, cough, and dyspnea, to more severe outcomes including acute respiratory distress, pneumonia, renal failure, and death. Comorbidities predisposing to complications and severe illness include older age (>65 y), males, obesity, cancer, serious cardiovascular disease, chronic obstructive pulmonary disease, type II diabetes mellitus, and immunodeficiency (including PID) (Chen et al. 2020; Huang et al. 2020; Li et al. 2020).

Over the past 2 years since the pandemic took hold, those with PID have been advised to practice rigorous social distancing, hand hygiene, and if appropriate — COVID-19 vaccination, given the possibility of more severe outcomes (Roifman and Vong 2021*b*, 2021*a*). During this period, studies examining the effectiveness of the COVID-19 vaccine and longevity of antibody titers have been instrumental in guiding the need for additional 'boosters', allowing for additional protection due to insufficient seroconversion — the development of specific antibodies following vaccination (or exposure to an infectious agent), or waning immunity. In Canada, individuals with moderate to severe PID have been advised to a receive 3-dose primary series with the Pfizer-BioNTech mRNA vaccine (or a 2-dose primary series using the Moderna adenoviral vector-based vaccine), and 2 booster doses — up to 5 doses in total.

Early clinical trials demonstrated the safety and efficacy of mRNA and adenoviral-vector based COVID-19 vaccines, including against symptomatic SARS-CoV-2 infection and more severe outcomes (Baden et al. 2021; Voysey et al. 2021). Seroconversion was shown to be high regardless of the type of vaccine administered (Eyre et al. 2021; Wang et al. 2021). Further, several studies supported the use of neutralizing antibody titers as surrogate markers of protection (Khoury et al. 2021; Garcia-Beltran et al. 2021). However, such data rarely extrapolate to subpopulations with immune deficits especially given that immunocompromised individuals were excluded from evaluations of vaccine effectiveness. In Canada, 4–5 times more immunocompromised individuals, including those on immunosuppressive medications, solid organ transplant recipients, malignancy, and PID, have been hospitalized or admitted to hospital with COVID-19 than the general population (NACI 2021).

More recently, case series and reports of disease course in those with PID indicate variable outcomes, from those who remain asymptomatic to severe

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complications. Nevertheless, seroconversion rates remain lower than the general population suggesting the need for continued caution.

There remains limited data on efficacy following the 3-dose vaccine primary series in PID, with most studies reporting outcomes following a 2-dose schedule. This likely reflects the initial lack of access to sufficient doses/boosters of COVID-19 vaccines globally and the lag time during transition from a 2-dose to 3-dose schedule. In a U.S. study, among 100 immunocompromised individuals who received a 2-dose mRNA vaccine, half were able to produce an antibody-mediated (humoral) immune response while 69% raised a cell-mediated (interferon-γ release) response (Ramanathan et al. 2021). Separately, an Italian study reported that 7 out of 34 (20%) patients with common variable immunodeficiency (CVID) with no prior exposure to SARS-COV-2 were able to produce IgG and IgA antibody responses to the COVID-19 vaccine spike protein, while 6 out of 7 who had previously been infected showed boosted antibody responses after vaccination. Spike protein antibodies were absent in patients with X-linked agammaglobulinemia, although reassuringly, in 5 out of 6 of this cohort, vaccination specific T cell responses were still detectable (Salinas et al. 2021). Similarly, an Israeli study of 26 patients with PID reported that 18 were able to develop specific antibody responses following the Pfizer-BioNtech COVID-19 vaccine, while 19 raised specific T cell responses (Hagin et al. 2021). Overall, a recent meta-analysis assessing seroconversion rates among immunocompromised individuals reported significantly lower rates between the first and second dose of the vaccine (Lee et al. 2022). A third dose boosted antibody levels, although some may not respond to a fourth dose.

Real-world data describing the severity and level of protection following vaccination against COVID-19 in patients with PID continue to emerge, and paint a conflicting scenario. It is noteworthy that, given the assumption of severe outcomes, many with PID have remained vigilant in sheltering from exposure, resulting in relatively low infection rates. Further, the presence of COVID antibodies in antibody replacement products likely confer some additional level of protection (Karbiener et al. 2021; Romero et al. 2021). Some regions have documented only mild COVID-19 symptoms (Drabe et al. 2021; Goudouris et al. 2021; Deyà-Martínez et al. 2021), perhaps due to the innate inability of those with PID to mobilize inflammatory responses. Marcus and colleagues reported minimal impact of COVID-19 in a cohort of 19 patients with PID from Israel — none were hospitalized (Marcus et al. 2021). However, with a high proportion of asymptomatic cases and most on antibody replacement therapy, this cohort is hard to assess. Our own experience with a cohort of pediatric patients (including those with humoral immunodeficiency, combined immunodeficiency and phagocytic defects) revealed similarly mild COVID-19 disease course (fever, sore throat, nasal congestion, and rhinitis) which resolved without complications (Roifman et al. unpublished observations).

In contrast, globally, overall rates of hospital admissions stand at closer to 50% of reported PID cases of COVID-19 (Fill et al. 2020; Van Damme et al. 2020; Soresina et al. 2020; Ahanchian et al. 2020; Jin et al. 2020; Quinti et al. 2020; Delavari et al. 2020; Ho et al. 2021; Mullur et al. 2021; Meyts et al. 2021; Esenboga et al. 2021; Shields et al. 2021; Castano-Jaramillo et al. 2021; Goudouris et al. 2021). Shields and colleagues reported the outcome of 60 patients with PID, with just over half requiring hospital admission and overall higher case fatality ratios compared to control (Shields et al. 2021). A study by Delavari and colleagues reported 10-fold higher mortality rate in those with PID compared to the general population, despite only 1.23-fold higher COVID-19 infection (Delavari et al. 2020). What is clear is that a more severe clinical course is documented in patients with defects in type I interferon signaling (Bastard et al. 2020; van der Made et al. 2020; Zhang et al. 2020), including those with defects in IFNAR1, IFNAR2, STAT1, STAT2, TICAM1, TRAF3, TBK1, TLR3, and IRF3.

It would be of paramount importance to obtain stratified data on the impact of COVID-19 over the 2-year period of the pandemic. Currently, patients with PID are more protected now with an effective vaccine, effective anti-viral treatments, and gradual enrichment of immunoglobulin products with neutralizing anti-SARS-CoV-2 antibodies.

We propose to differentiate between PID patients who responded well to vaccination with sustained antibody levels and/or those receiving immunoglobulin replacement, from non-responders, especially if they have significant T cell deficiency or an interferon-γ pathway defect.

We also believe that after a prolonged period of home schooling for children with PID, further extension of this practice may be more harmful than exposure to the virus. We recommend that with the advice and guidance of an immunologist, many of these children could relatively safely attend physical school. Yet, we still advise they adhere to masking at school, hand hygiene, and distancing as much as possible. The introduction of new preventative and therapeutic medications renders this transition to normal life easier.

In summary, despite gradual lifting of COVID-19 mandates, continued evidence of impaired immunity and COVID-19 disease course in individuals with PID indicate that this population should continue to remain cautious — but with gradual and selective relaxation of practices.

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CTLA4 haploinsufficiency caused by a novel heterozygous splice site mutation

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ABSTRACT

Background: Cytotoxic T lymphocyte-associated antigen-4 (CTLA4) haploinsufficiency is characterized by a variety of phenotypes, ranging from autoimmune disorders, enteropathy, fatal combined immunodeficiency, as well as lymphoproliferation and malignancy.

Aim: To broaden the genotypic spectrum and clinical presentations of patients with CTLA4 variants.

Methods: We evaluated a female patient with autoimmunity and lymphopenia. Immune workup and whole exome sequencing (WES) were performed.

Results: The proband presented at 11 years of age with hypothyroidism and later developed Evans syndrome, alopecia, eczema, and lymphocytic interstitial pneumonia. Immune evaluation revealed T, B, and NK lymphopenia with normal humoral immunity. Following a negative genetic panel for autoimmune lymphoproliferative syndrome (ALPS), WES analysis identified a novel heterozygous intronic variant predicted in-silico to cause skipping of exon 2 of the *CTLA4* gene.

Conclusion: A novel heterozygous mutation in *CTLA4* caused variable presentations of immune dysregulation, one of the hallmarks of CTLA4 haploinsufficiency.

Statement of Novelty: We herein report a novel mutation in *CTLA4* resulting in various features of autoimmunity.

Background

Cytotoxic T lymphocyte-associated antigen-4 (CTLA4) is a transmembrane receptor that acts as a checkpoint to dampen T cell-mediated responses and maintain immune self-tolerance. It serves as the opposing face of the CD28 signaling pathway, the latter of which drives T cell activation, differentiation and effector responses, as well as T follicular helper cell and regulatory T cell (Treg) generation and survival. CTLA4 is localized to the plasma membrane and various intracellular compartments in Tregs, and can be upregulated by conventional T cells. Binding of CTLA4 with the shared CD28 costimulatory receptor ligands, CD80 and CD86, found on antigen presenting cells, leads to ligand internalization/degradation and subsequent impairment of archetypal CD28-dependent signaling and T cell responses. Importantly, the ability to capture these ligands is dependent on sufficient CTLA4 cell surface expression. Defects in CTLA4 expression or internalization pathways result in immune dysregulation (Tivol et al. 1995; Lo et al. 2015).

The critical role of CTLA4 in maintaining immune tolerance is supported by findings in murine models showing fatal autoimmunity, including lymphoproliferation and multiorgan tissue damage, within the first few weeks of life (Tivol et al. 1995; Waterhouse et al. 1995). In humans, heterozygous (autosomal dominant) pathogenic mutations in the *CTLA4* gene were shown to

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cause quantitative reductions in CTLA4 expression, leading to loss of peripheral tolerance, infiltrative autoimmune disease, enteropathy, immune dysregulation, as well as immunodeficiency and malignancy such as lymphoma and gastric cancer (Kuehn et al. 2014; Schubert et al. 2014; Hayakawa et al. 2016; Schwab et al. 2018). Interestingly, the presentation of CTLA4 deficiency varies widely, even within kindreds bearing the same mutation. Schubert et al (2014) described a family with 11 members carrying the same heterozygous mutation in *CTLA4*; 5 suffered from hypogammaglobulinemia, autoimmune cytopenia, recurrent respiratory infections and lung disease, while the other 6 remained asymptomatic.

Treatment of CTLA4 haploinsufficiency includes immunosuppressants, immunomodulatory drugs such as Rapamycin, biologics including Rituximab, immunoglobulin replacement therapy, splenectomy, and hematopoietic stem cell transplantation (HSCT) in rare cases. Therapy is mostly based on clinicians' judgement and takes into consideration specific organ involvement and immunological assessment (Schwab et al. 2018). Importantly, treatment with Abatacept, a CTLA4 fusion protein, was reported to be beneficial in CTLA4 haploinsufficiency patients with autoimmune cytopenias, as well as lung, gut, and central nervous system involvement (Schwab et al. 2018; Egg et al. 2021).

There are currently 45 known genes associated with immune dysregulation. For patients with common variable immunodeficiency (CVID; diagnosis based on clinical picture and immune laboratory values) or autoimmune lymphoproliferative syndrome (ALPS), pursuing a genetic diagnosis of CTLA4 haploinsufficiency has a direct impact on affected individuals due to available treatment with Abatacept.

Here, we describe a novel splice site *CTLA4* mutation, resulting in a phenotype of various autoimmune manifestations and lymphocytic interstitial pneumonia with almost no history of infections.

Case Presentation

Clinical case

The proband is a 24-year-old female, born to non-consanguineous parents of East Asian descent. Family history was significant for the patient's father who passed away from metastatic adrenal carcinoma; however, there is no other family history suggestive of immune deficiency, immune dysregulation, nor other malignancies.

She first presented with Hashimoto's thyroiditis at 11 years, followed by Coombs positive autoimmune hemolytic anemia (AIHA) and thrombocytopenia over the next two years. She required blood transfusions and was treated with a single infusion of intravenous immunoglobulins as well as a course of Prednisone. With regards to infections, four years after her initial presentation she developed Salmonella bacteremia and osteomyelitis of the left distal femur while being on Prednisone, with no other bacterial, viral or opportunistic infections since then. At 17 years of age, she was admitted for fever and diffuse lymphadenopathy. Her work up revealed multiple pulmonary nodules on MRI, later diagnosed as lymphocytic interstitial pneumonia and follicular bronchiolitis. Other immune dysregulation features included alopecia areata, mild eczema managed topically, and photosensitive skin rash.

Over the years she received the diagnosis of ALPS-like syndrome until genetic investigation revealed her diagnosis. For the cytopenias, she continued treatment with steroids and Mycophenolate Mofetil with partial response to this date.

Immune evaluation

The patient's immune work up (Table 1) was remarkable for leukopenia 3–3.2 (normal: $4.37–9.68 \times 10^9$/L), neutropenia 0.78–1.6 (normal: $2.00–7.15 \times 10^9$/L), persistent lymphopenia 0.8–1.14 (normal: $1.16–3.18 \times 10^9$/L), hemoglobin 80–140 (normal: 106–135 g/L), and platelets 9–220 (normal: $186–353 \times 10^9$/L). Immunoglobulins were within the normal range and specific vaccine titers were protective. Lymphocyte subsets showed CD4, CD8, B cell, and NK cell lymphopenia. T cell function was normal, including normal PHA stimulation index and robust response to antigen stimulation to Candida, varicella-zoster, herpes simplex and CMV. CD45 RA/RO showed increased amount of memory T cells compared to control (not shown). Autoantibodies including RF, anti-dsDNA, anti-ENA, and ANCA were negative although ANA was briefly positive (1:160) with normal C3 and C4.

Genetic investigations

Initial genetic analysis with an ALPS panel was returned negative. Research whole exome sequencing (WES) analysis subsequently revealed a novel

Table 1: **Laboratory evaluation of proband between 14-20 years.**

	Patient's results	Normal Range
WBC ($\times 10^9$/L)	3–3.2	4.37–9.68
Neutrophils ($\times 10^9$/L)	0.78–1.6	2.00–7.15
Lymphocytes ($\times 10^9$/L)	0.8–1.14	1.16–3.18
Hemoglobin (g/L)	80–140	106–135
Platelets ($\times 10^9$/L)	9–220	186–353
CD4 (cells/mL)	400–500	610–1446
CD8 (cells/mL)	250–350	282–749
NK cells (cells/mL)	65	87–504
CD19 (cells/mL)	52–78	173–685
Double negative T cells	2.4–3.9%	0.2%–2%
PHA Stimulation Index	595	>50% of control or >300

heterozygous variant in *CTLA4* (NM_005214.5), c.457+5delG, which was not previously found in large population databases including gnomAD nor dbSNP. The variant is an intronic change located 5 bases distal to the exon in a splice site, predicted in-silico to cause skipping of exon 2 of the *CTLA4* gene and loss-of-function. The variant was subsequently confirmed with a clinical PID panel (Prevention Genetics).

Discussion

CTLA4 haploinsufficiency, first described in 2014, is caused by heterozygous variants in *CTLA4* and characterized by a variety of clinical manifestations including hypogammaglobulinemia, T cell lymphopenia, autoimmune cytopenias, and lymphocytic infiltration of non-lymphoid organs as well as malignancy. Mutations described have thus far included nonsense, missense, frame-shift and splice site mutations and were shown to cause reduction in CTLA4 mRNA and protein expression (Schubert et al. 2014; Sun et al. 2014; Hayakawa et al. 2016; Mahat et al. 2021). Importantly, human biallelic CTLA4 deficiency has not yet been described, and likely indicates incompatibility with life.

The *CTLA4* gene contains 4 exons encoding the signal peptide (exon 1), ligand binding and dimerization domains (exon 2), transmembrane domain (exon 3), and cytoplasmic tail (exon 4). Genetic aberrations targeting the signal peptide have been shown to abolish CTLA4 protein expression (Schubert et al. 2014), while those affecting exon 2 impair protein dimerization and interactions with the CD80 and CD86 co-stimulatory ligands (Schubert et al. 2014; Schwab et al. 2018). Mutations in exon 3 impair ligand binding and uptake through the CTLA4 transendocytosis pathway (Schubert et al. 2014; Schwab et al. 2018).

Although protein and mRNA expression of CTLA4 in Treg cells were not performed in our patient, in-silico predictions indicate that the c.457+5delG variant most probably leads to a splice anomaly and reduced CTLA4 expression. The clinical presentation of this patient was relatively similar to previously reported cases of autosomal dominant CTLA4 haploinsufficiency. Furthermore, CTLA4 was the only candidate gene identified on WES to explain this patient's clinical manifestations. In addition, this variant was not reported in large population databases, increasing our confidence in this variant as accountable for the immune dysregulation seen in our patient.

In most of the reported cases, patients were diagnosed initially with CVID, while further genetic analysis showed variants in *CTLA4* (Schubert et al. 2014; Sun et al. 2014; Mahat et al. 2021). Clinically, our patient was followed for years with a diagnosis of ALPS-like syndrome and treated empirically with immunosuppression and immune modulators according to symptoms. ALPS is a rare condition, characterized by lymphoproliferation, autoimmune manifestations, and susceptibility to malignancy. ALPS and CVID may overlap in some of the clinical and immunological features such as autoimmunity, lymphoproliferation, and susceptibility to malignancy. Often times patients receive a diagnosis of ALPS or CVID without additional genetic investigation due to low yield of genetic testing, furthermore, requesting genetic testing as part of the immunological workup was not recommended in previous years, especially in cases without a clear family history of

immunodeficiency (Cunningham-Rundles 2010; Rosenzweig et al. 2016). In this case, further genetic analysis was able to elucidate the underlying diagnosis and provide specific treatment option with Abatacept.

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Novel heterozygous *FOXN1* mutation identified following newborn screening for severe combined immunodeficiency is associated with improving immune parameters

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ABSTRACT

Background: Forkhead-box protein N1 (FOXN1) plays a critical role in the proper development and function of thymic epithelial cells, required for T cell ontogeny. Homozygous variants in the *FOXN1* gene, encoding FOXN1, cause severe combined immunodeficiency (SCID), whereas heterozygous mutations are associated with variable presentations and over time, improving T cell function.

Aim: To highlight the importance of broader genetic investigations to attain a definitive molecular diagnosis following abnormal newborn screening for SCID.

Methods: Case report of a patient with immunodeficiency due to a novel de novo *FOXN1* mutation.

Results: The patient was identified following abnormal newborn screening for SCID in which T cell receptor excision circles were absent/very low. Initial immune investigations revealed severe T cell lymphopenia and poor lymphocyte function and she was diagnosed with T-B+NK+SCID. During work-up for hematopoietic stem cell transplantation, extensive genetic investigations identified a novel heterozygous mutation in *FOXN1*. A more conservative management approach was taken, and over the following months, the patient's immune parameters improved.

Conclusion: Newborn screening for SCID has facilitated the detection of SCID, as well as other T cell immunodeficiencies, before infectious complications and organ damage occur. Heterozygous mutations in *FOXN1* are associated with more variable presentations including improving immune indices with age. Here, results of genetic investigations were essential for informing the management of this case.

Statement of Novelty: We report a novel heterozygous mutation in *FOXN1*, presenting initially as T-B+NK+ SCID with gradual improvement of immune parameters over time.

Introduction

The Forkhead-box (FOX) superfamily of transcription factors play important roles in the homeostasis, function, and aging of a variety of organs and tissues – including the immune system. FOX protein N1 (FOXN1), encoded by the *FOXN1* gene, is an essential regulator of thymic epithelial cell differentiation

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required for T cell development, and is also expressed in skin and hair follicles (Vaidya et al. 2016). Potential roles in hematopoiesis within the bone marrow have been reported (Zook et al. 2013).

The thymus provides a unique environment for the differentiation, selection, and maturation of bone marrow-derived T cell progenitors into fully functional T cells. This process relies on the ordered architecture of the thymus, consisting of a cortical area rich in cortical thymic epithelial cells (cTECs) and lymphoid cells, a medullary area containing medullary thymic epithelial cells (mTECs), Hassall's corpuscles, macrophages, dendritic cells and B lymphocytes, as well as a transitional corticomedullary junction. Importantly, activation of FOXN1 and subsequent transcriptional regulation of downstream genes are needed for the proper differentiation, maturation, and function of cTECs and mTECs during prenatal thymus organogenesis, as well as homeostasis of the postnatal thymus (Corbeaux et al. 2010; Vaidya et al. 2016). Thus, loss-of-function of this so-called master regulator results in ablated thymic and T cell development, and the phenotype of severe combined immunodeficiency (SCID).

Bi-directional cues between the thymic stroma and T cells are essential for the normal development and function of both entities (van Ewijk et al. 1994). Defective thymic architecture has been documented in instances where T cells exhibit stunted development (Palmer et al. 1993), whereas reconstitution of the thymic microenvironment has been reported with the administration of T cell-committed precursors (Roberts et al. 2009). Importantly, animal models have been an essential tool for dissecting the role of FOXN1 during early thymus development (Bosticardo et al. 2019). Mice deficient in *Foxn1* ('nude' spontaneous mutation) are athymic, lack T cells, and exhibit abnormal hair growth (Nehls et al. 1994). Among the panel of genes regulated by foxn1 are *Ccl25*, required for homing of immature thymocytes to the thymus (Liu and Leung, 2006), *Dll4*, involved in the Notch pathway-directed sorting of cells towards T cell lineage (Koch et al. 2008), and *Cxcl12*, needed for expansion of T cell precursors in the thymus (Ara et al. 2003). Other genes induced by foxn1 required for thymic epithelial cell and thymocyte exchange are *Psmb11*, *Prss16*, and *Cd83* (Bosticardo et al. 2019).

In humans, null mutations in *FOXN1* are characterized by severe T cell immunodeficiency (T-B+NK+ phenotype), congenital alopecia, and nail dystrophy (OMIM #601705) (Amorosi et al. 2008; Frank et al. 1999; Pignata et al. 1996). Affected individuals suffer recurrent infections, failure to thrive, and oral candidiasis. While some attempts to correct the deficiency have been made with hematopoietic stem cell transplantation (HSCT), overall, post-transplant outcomes for individuals with defects in *FOXN1* indicate that thymus transplants are a more effective and curative option (Bosticardo et al. 2019; Chou et al. 2014).

Monoallelic mutations in *FOXN1* are more frequently associated with variable clinical features. A recent longitudinal analysis of 25 children and 22 adults with heterozygous *FOXN1* variants described the majority being clinically well except for viral respiratory infections (Bosticardo et al. 2019). Common non-infectious manifestations included eczema and nail dystrophy. Of the children, 21 were identified following an abnormal newborn screen (NBS) result and were associated with T cell lymphopenia. For most of these cases, CD4+ T cells eventually recovered.

Here, we report on our experience with an infant who was diagnosed with T-B+NK+ severe combined immunodeficiency (SCID) following an abnormal NBS result. While undergoing work up for HSCT, extensive genetic investigations including whole exome sequencing (WES) and whole genome sequencing (WGS) identified a novel heterozygous variant in *FOXN1*, which subsequently changed the management plan for this patient.

Case presentation

The patient is currently a 19-month-old female, the first child to non-consanguineous parents of Asian descent. The pregnancy was uncomplicated and she was born at 39+2 weeks gestation by spontaneous vaginal delivery. Her mother had 2 prior early miscarriages and her father has 2 healthy older children from a previous marriage. The patient came to the attention of the Immunology team following an abnormal NBS result for SCID. The initial T cell receptor excision circle (TREC) level was 0 (cut-off: 75), and the repeat TREC was 7. Immunodeficiencies associated with ADA, PNP, ZAP70, and IKBKB were ruled out during initial screening tests, and assessment for microdeletions in 22q11.6 was returned negative.

Initial Evaluations

Initial immune evaluation revealed T cell lymphopenia of both CD4+ cells (43 cells/μL, normal: 2330-3617 cells/μL) and CD8+ cells (5 cells/μL, normal: 712-1361 cells/μL). B cells (431 cells/μL, normal: 315-1383 cells/μL) and NK cells (622 cells/μL, normal: 201-870 cells/μL) were within normal range. Output of naïve T cells was low at 9% (CD4+/CD45RA+), while memory T cells predominated at 84% (CD4+/CD45RO+). Recent thymic emigrants were low at 9% (normal: 64–94%). Her lymphocyte proliferation responses to the mitogen phytohemagglutinin (PHA) were very low at 12 (stimulation index, SI normal >400) (Table 1). Ultrasound of the neck revealed normal size lymph nodes in both jugular chains; however, there was no convincing evidence of thymic tissue.

Genetics

Genetic work-up to identify a causative molecular defect underlying the patient's immunodeficiency was started. An initial SCID gene panel (Newborn Screening Ontario; 20 genes) was negative. Subsequently, a more extensive PID panel (Blueprint Genetics) was sent which identified, at 2 months of age, a novel heterozygous frameshift mutation in *FOXN1* (NM_003593.2), c.1465del, resulting in p. Gln489Argfs*61. This variant was absent from large population databases (gnomAD). To ensure that no other genetic aberrations were present, trio WGS of the patient and her parents were sent. While no further changes were found (including non-coding or copy number variants), the mutation was identified as a de novo variant targeting exon 7 — a region where many pathogenic variants reside.

Management

Our patient's initial presentation was in keeping with T-B+NK+ SCID, and she was managed accordingly with antibiotic prophylaxis and immunoglobulin replacement therapy (from 4 weeks of age) while workup for HSCT was initiated. After the pathogenic mutation in *FOXN1* was identified, in line with our center's experience with patients harboring heterozygous variants in *FOXN1*, we instead changed our approach to 'watchful waiting' to see if her immune parameters would improve.

In the subsequent months, the patient's lymphocyte function recovered (PHA stimulation index increased

Table 1: **Immune evaluation.**

Parameter	Age 3 days	Age 2 months	Age 4 months	Age 8 months	Age 14 months	Age 18 months
WBC (10^9/L)	5.35 (8.16–14.56)	2.67 (5.75–13.50)	2.92 (5.75–13.50)	2.38 (5.75–13.50)	8.04 (5.75–13.50)	4.65 (5.75–13.50)
Neutrophils ($\times10^9$/L)	2.58 (1.73–6.75)	1.04 (0.69–5.82)	1.11 (0.69–5.82)	0.68 (0.69–5.82)	3.32 (1.45–6.75)	1.51 (1.45–6.75)
Lymphocytes ($\times10^9$/L)	0.75 (1.75–8.00)	0.91 (1.96–8.94)	1.20 (1.96–8.94)	1.19 (1.96–8.94)	3.98 (1.90–6.30)	2.61 (1.90–6.30)
CD3 (cells/μL)	46 (3180–5401)	92 (3,180–5,401)	174 (2,284–4,776)	267 (2,284–4,776)	674 (2,542–4,933)	537 (2,542–4,933)
CD4 (cells/μL)	43 (2330–3617)	88 (2,330–3,617)	159 (1,523–3,472)	237 (1,523–3,472)	578 (1,573–2,949)	464 (1,573–2,949)
CD8 (cells/μL)	5 (712–1361)	4 (712–1,361)	15 (524–1,583)	30 (524–1,583)	83 (656–1,432)	61 (656–1,432)
CD19+ B cells (cells/μL)	622 (201–870)	163 (315–1,383)	625 (776–2,238)	526 (776–2,238)	951(733–1,338)	931 (733–1,338)
CD16+CD56+ NK cells (cells/μL)	431 (315–1383)	558 (201–870)	531 (230–801)	458 (230–801)	2,238 (186–724)	1,335 (186–724)
CD4/CD8 Ratio	8.70 (1.93–4.19)	21.80 (1.93–4.19)	10.91 (1.48–3.77)	8.01 (1.48–3.77)	6.96(1.34–3.04)	7.58 (1.34–3.04)
PHA Stimulation Index	11.8 (>400)	318.1 (>400)	—	52.9 (>400)	481.7 (>400)	
CD4+/CD45RA+ (%)	—	9.00	25.50	34.40	—	31.10
CD4+/CD45RO+ (%)	—	84.00	59.00	50.90	—	65.30

gradually to 481.7), and her T cell counts continue to show improvement although are still low (Table 1). CD45RA/RO assessment revealed improving naïve T cells (31.10%) versus memory T cells (65.30%). Recent thymic emigrants are steadily increasing 21% (normal: 40–100%). Assessment of TCRVβ repertoire revealed only slight under-representation of CD4+ Vβ8 and Vβ21.3 clones, and over-representation of CD8+ Vβ3 and Vβ14 clones (Figure 1). At 8 months of age, antibody replacement therapy was stopped and her immunoglobulin levels are currently within normal range (IgG: 5.1 g/L (normal: 3.2–11.5 g/L); IgA: 0.2 g/L (normal: 0–0.9 g/L); IgM: 0.5 g/L (normal: 0.5–1.9 g/L)). She has received her routine non-live vaccines and is responding well (anti-tetanus toxoid IgG >5 IU/mL).

To date, the patient remains clinically well and has not had any significant infections. There is presently no indication for HSCT and she continues to be followed closely.

Discussion

The implementation of SCID NBS has transformed our ability to identify and treat infants with SCID before infectious complications and end-organ damage occurs (Kwan et al. 2014). Screening utilizes measurement of TRECs, which are a by-product of T cell receptor recombination. Thus, very low/absent TRECs in neonates are an indication of defects in T cell development and prompts the diagnostic evaluation for SCID (Biggs

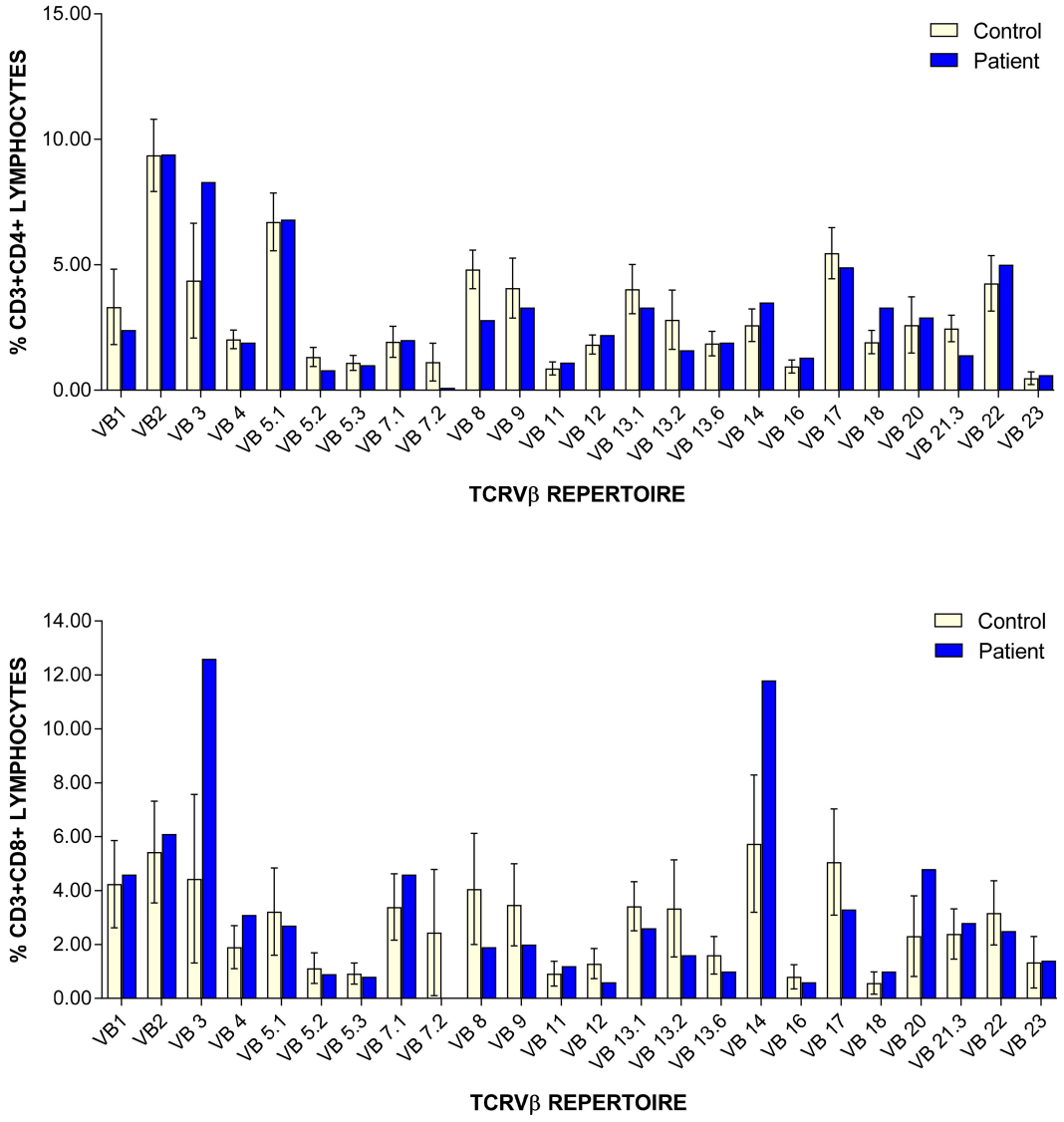

Figure 1: TCRVbeta repertoire.

et al. 2017). By extension, less profound immunodeficiencies that would not have been detected by past practices until individuals presented clinically are also being flagged for follow-up (Amatuni et al. 2019; Mandola et al. 2019; Scott et al. 2021).

Our patient was initially diagnosed with T-B+NK+ SCID following abnormal SCID NBS with two absent/low TREC determinations. While she underwent workup for HSCT, extensive genetic investigations identified a single novel de novo heterozygous mutation in *FOXN1*. Our center's experience with heterozygous *FOXN1* mutations (Scott et al. 2021) as well as those reported in the literature, changed the intended management plan to a more conservative stance given the likelihood that the patient's immune parameters would improve with age. Moreover, HSCT is unlikely to correct the lymphopenia associated with a poorly developed thymus (Bosticardo et al. 2019; Chou et al. 2014). Our case is unique in that the genetic work-up involved gene panel sequencing, WES, and WGS to rule out the possibility of another underlying genetic etiology, affirming our approach to closely monitor the patient for improvement.

FOXN1 is a 648-amino acid protein with an N-terminal region of residues 1–270, a Forkhead domain located centrally between amino acids 271 and 366, and a C-terminal region comprising residues 367–648 (Figure 2). Within the C-terminal region is an evolutionarily conserved transcriptional activation domain. The N-terminal region has been implicated in thymic epithelial cell differentiation while the C-terminal region is required for high-affinity DNA binding (Newman et al. 2020). The *FOXN1* frameshift mutation identified in our patient, c.1465del (p.Gln489Argfs*61), targets exon 7 and causes a premature stop at position 61 within the C-terminal domain. This is predicted to result in loss of normal protein function due to truncation of the protein or nonsense-mediated mRNA decay. The clinical picture of our patient, with CD4+ and CD8+ T cell lymphopenia and unaffected B and NK cell counts is not dissimilar to other reports of patients with mutations within the C-terminal domain, nor of other domains within FOXN1 (Bosticardo et al. 2019). Thus, it remains to be seen whether a genotype-phenotype correlation exists.

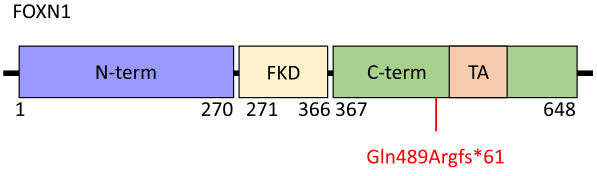

Figure 2: Schematic diagram of FOXN1 and the mutation identified in our patient.

Athymia has been reported in individuals with pathogenic homozygous *FOXN1* mutations (Chen et al. 2009) as well as heterozygous variants (Bosticardo et al. 2019). Of the 13 infants reported by Bosticardo and colleagues, only 3 had a normal-sized thymic shadow while the remaining were absent or reduced in size. In our patient, ultrasound did not detect evidence of thymic tissue. Nevertheless, the presence of near normal TCRV beta repertoire indicates that the thymus is likely small or perhaps ectopically placed (Shah et al. 2001).

In summary, this report highlights the importance of robust genetic investigations to delineate the underlying cause of immunodeficiencies detected by SCID NBS. We continue to follow a conservative approach with this patient, particularly as more reports of disease course and outcomes of individuals affected by heterozygous *FOXN1* mutations come to light.

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Original Article

An unusual presentation of DiGeorge syndrome

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ABSTRACT

Introduction: DiGeorge syndrome is a heterogenous disorder with various clinical presentations. Common features include thymic hypoplasia, T cell lymphopenia, conotruncal heart defects, facial dysmorphism, cleft palate, developmental delay, and hypoparathyroidism. The severity of this condition varies, however typical presentation includes congenital heart defects and characteristic facial features. Isolated hypocalcemia in DiGeorge syndrome is rarely seen in neonates but rather as the sole manifestation in older teenagers or adults.

Aim: To report a case of an atypical presentation of DiGeorge syndrome.

Results: We report here a case of an infant who was diagnosed with DiGeorge syndrome, with seizures being the only clinical manifestation displayed by the patient. He was found to have low T cell receptor excision circle levels on a newborn screen (NBS) for severe combined immunodeficiency (SCID). He did not have facial dysmorphism nor cardiac defect.

Conclusion: Our case shows that severe hypocalcemia can be the only presenting symptom in DiGeorge syndrome. Based on this case, we recommend physicians test for calcium levels and PTH at the first encounter with a patient who screened positive during NBS for SCID.

Statement of Novelty: We describe an infant with DiGeorge syndrome who presented with severe hypocalcemia.

Introduction

DiGeorge syndrome (DGS, OMIM #188400) is one of a group of phenotypically similar disorders caused by microdeletions of chromosome 22q11.2 and rarely by microdeletions in 10p, 18q21.33, or 4q21.3-q25 (Morrow et al. 2018; Greenberg et al. 1988; Fukushima et al. 1992). Genes mapping to the deleted 22q11.2 region include *TBX1* (Chieffo et al. 1997), *COMT* (Shashi et al. 2006), *ZNF74* (Aubry et al. 1993), and *DGCR6* (Demczuk et al. 1996). The related syndromes include velocardiofacial syndrome (Shprintzen syndrome) (Shprintzen et al. 1978), and conotruncal anomaly face (CTAF) syndrome (Burn et al. 1993). About 10% of cases are transmitted in an autosomal dominant fashion but most are generated *de novo* (McDonald-McGinn et al. 2001). The group of disorders are currently referred to by some as 22q11.2 deletion syndrome but the more traditional term DGS is still used, especially when the immune system is abnormal (Burn 1999).

The syndrome is heterogenous and includes most frequently thymic hypoplasia, congenital heart defects (ventricular septal defect, Truncus arteriosus, Tetralogy of Fallot), facial anomalies (including cleft palate, epicanthus, telecanthus, wide and prominent nasal bridge, bulbous nose and low set ears), and

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developmental delay (Botto et al. 2003; Goodship et al. 1998). Hypoparathyroidism, hearing impairment, and absent dentition are less common but still a frequent feature of the syndrome. The 22q11.2 microdeletion causes a fault in the development of cervical neural migration in the derivatives of the pharyngeal arches, thus explaining the poor development of the thymus and parathyroid glands (Molsted et al. 2010).

The extent of immunodeficiency, dysmorphic features, and hypoparathyroidism is highly variable. Yet in most instances patients have a combination of features which alerts healthcare providers to the possible diagnosis of DGS.

We report here a rare case of an infant who was found to have low TRECs in a newborn screen (NBS) for severe combined immunodeficiency (SCID). He had no facial dysmorphism nor cardiac lesion. He was admitted for seizures at 3 weeks of age.

Case presentation

The patient, a product of non-consanguineous parents, was delivered at 37 weeks gestation via elective c-section due to maternal gestational diabetes and hypertension. The mother was treated with Labetolol and low dose aspirin since week 34 of gestation. The delivery was uncomplicated but the patient experienced hypoglycemia after birth which resolved within days, as well as a rise in bilirubin and jaundice which resolved in a week.

The patient has 2 healthy siblings. There is no history of immunodeficiency or severe infections in family members. NBS for SCID revealed low T cell receptor excision circle (TREC) levels in the patient's blood sample (53, cut-off: 75). This result triggered a retrieval visit with a NBS genetic counselor and Immunology staff (Reid et al. 2017).

On examination, the baby appeared alert with proper muscle tone and was positive for moro, stepping, and grasp reflex. He had no notable facial abnormalities with the exception of mild ear lobe folding. No heart murmurs were noted on auscultation. The rest of the physical assessment was normal.

The parents reported he was feeding well with a combination of breastmilk and formula. According to NBS protocol, we sent out the following tests: repeat TREC, CBC and differential, immunophenotyping, 22q11.2 FISH, lymphocyte proliferation to phytohemagglutinin (PHA), and CMV status in baby and mom. A genetic panel for primary immunodeficiency as well as samples for research (DNA for future whole exome sequencing if needed) were also obtained. Lymphocyte count was within normal limits but immunophenotyping revealed low CD3+, CD4+, and CD8+ cells with increased CD19+ B cells and CD16+CD56+ NK cells. However, T cell function appeared normal with a PHA response within normal range (Table 1).

Table 1: **Patient immune and blood chemistry evaluation.**

Immune Parameters	Values	Reference Range
WBC ($\times 10^9$ g/L)	8.23	5.75–13.50
Neutrophils ($\times 10^9$ g/L)	2.35	0.69–5.82
Eosinophils ($\times 10^9$ g/L)	1.23	0–0.79
Lymphocytes ($\times 10^9$ g/L)	2.59	1.96–8.94
CD3+ (%)	25.8	62.7–81.6
CD4+ (%)	20.1	42.8–65.7
CD8+ (%)	4	15–23
CD19+ (%)	42.1	7.4–21.3
CD16+ D56+ (%)	28.4	4.2–14.8
Lymphocyte proliferation to PHA (stimulation index)	645	>350
TREC level	53	75
Ionized Calcium (mmol/L)	0.82	1.22–1.37

He continued to feed well and gain weight until he was 5 weeks old when he was admitted to hospital with status epilepticus secondary to profound hypocalcemia. One day before admission his FISH analysis was reported confirming a deletion in 22q11.2. He gradually improved in hospital with calcium supplementation and anti-seizure medications including Keppra. He had no seizure activity and a normal EEG after cessation of Keppra. However, this was re-started following MRI findings of multiple anomalies consistent with DGS including diffuse polymicrogyria, heterotopia, and abnormal semicircle canals, as well as a left frontal subdural hematoma.

Discussion

Newborn screening for SCID revolutionized the field of primary immunodeficiency (Kwan et al. 2014). Using TRECs as a surrogate indicator of extremely low circulating lymphocytes, mainly naïve (new thymic emigrants), has allowed for effective detection of typical SCID

(Puck 2012). Still, atypical SCID such as Zap-70 deficiency or leaky SCIDs may be missed by this tool (Grazioli et al. 2014; Kwan et al. 2014; Kwan and Puck 2015). In contrast, other non-SCID immunodeficiencies have been picked up as incidental findings by this method. While the list of these conditions continues to grow, most commonly detected are cases with DiGeorge syndrome, Ataxia Telangiectasia, and FOXN1-related lymphopenia (Scott et al. 2021; Mandola et al. 2019; Barry et al. 2017). However, the majority of NBS-positive cases appear insignificant (prematurity) or secondary to maternal or perinatal events (such as maternal or newborn treatment with immunosuppressive medications or perinatal stress events) (Mandola et al. 2019).

The TREC levels in typical SCID are traditionally low or undetectable. That was not the case in the patient reported here, where levels were only somewhat lower than the cut-off level. This and the lower than normal level of CD3+ cells could have been associated with a putative T cell immunodeficiency or secondary to the stress experienced by this baby. Moreover, the normal response to the mitogen PHA eliminated most cases of profound T cell immunodeficiency.

As part of our routine first visit evaluation (Biggs et al. 2017; Reid et al. 2017), we requested FISH analysis with the intent to rule out DGS, one of the causes of neonatal lymphopenia. While in retrospective analysis, an acceptable option for diagnosis, the finding of microdeletion of 22q11.2 was surprising given this case had no obvious dysmorphic facial features nor cardiac defects.

Our patient did not display any clinical symptoms until age 3 weeks when seizures began. Hypocalcemia was confirmed in hospital, 20 hr after the genetic FISH analysis was released. Indeed, sudden seizures were the only clinical manifestation displayed by the patient.

Frequently in newborns and infants, hypocalcemia may be present in combination with other features associated with DGS (Barry et al. 2017). Rarely, hypocalcemia presents as the sole manifestation in older teenagers of adults (Cabrer et al. 2018; Zammit et al. 2013; Maalouf et al. 2004). Few case reports in adults identified retrospectively facial anomalies typical of DGS (Vogels et al. 2014). Our case demonstrates that severe hypocalcemia can be the only clinical manifestation of DGS and subsequently we recommend testing for calcium levels and PTH at the first encounter with a positive NBS for SCID.

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Primary Immunodeficiency

There are more than 400 genetic defects and disorders of the immune system that are recognized as Primary Immunodeficiency. Approximately 29,000 Canadians suffer from forms ranging widely in severity and symptoms. Over 70% are undiagnosed.

Red Flags for **Primary Immunodeficiency**

- Repeated invasive infection (two or more pneumonias, recurrent septicemia, abscesses, meningitis).[1]
- Infections with unusual or opportunistic pathogens (PJP).[1]
- Poor response to prolonged or multiple antibiotic therapies.[1]
- Chronic diarrhea with or without evidence of colitis.[1]
- Chronic failure to gain weight and grow.[2]
- Persistent (or recurrent) unusual (atypical) or resistant to treatment oral lesions (thrush) or skin rash (erythroderma, telangiectasias, recurrent pustules/nodules/plaques).[1]
- Structurally abnormal hair (kinky, silvery) nails (dystrophic) or teeth (pointy).[2]
- Low serum IgG, chronic lymphopenia, neutropenia or thrombocytopenia.[1]
- Absent lymph nodes and tonsils or chronic enlargement of lymphoid tissues.[1]
- A family history of Primary Immunodeficiency, autoimmunity or leukemia/lymphoma. [1]

References:
[1] All age groups
[2] Infancy and childhood

Early diagnosis and treatment are vital in saving lives. Treatment can improve or prevent long term organ damage. Each Red Flag alone should alert healthcare providers to the possibility of Primary Immunodeficiency and require further testing and investigation. Two or more Red Flags should trigger an urgent referral to an Immunologist.

Immunodeficiency Canada
Immunodéficience Canada

Providing patient support, education and research to cure Primary Immunodeficiency

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