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The journal of inherited immune disorders

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Eosinophilic esophagitis: a review

Elisa Ochfeld[a] and Melanie Makhija[b]*

ABSTRACT

Eosinophilic esophagitis is an antigen-mediated chronic disease that has been increasing in prevalence for the last few decades. A plethora of research has been done recently studying the pathophysiology and potential treatments for the disease. This review paper highlights the epidemiology, diagnosis, pathophysiology, and treatment of eosinophilic esophagitis including new diagnostic and treatment modalities for the disorder.

Statement of novelty: We review the epidemiology, diagnosis, pathophysiology, and treatment of eosinophilic esophagitis, and include new diagnostic and treatment modalities for the disorder.

Introduction

Eosinophilic esophagitis (EoE) is a chronic antigen-mediated esophageal disease, which is characterized by symptoms of esophageal dysfunction and histologically is identified by eosinophil predominant inflammation. Landres et al. (1978) first described EoE in 1978 in a case report. EoE was distinguished from gastroesophageal reflux disease (GERD) in the 1990s, after the pathologic differences of eosinophil presence were identified and validated (Attwood et al. 1993). Cases prior to this were often misidentified as severe, treatment-refractory GERD, with complications such as strictures, mucosal tears, perforations, and esophageal rings. In the 1990s, it was also first noted that the symptoms and histologic changes of the esophagus related to EoE could be improved with the use of elemental formula, and thus that the pathology could be related to the ingestion of certain foods (Franciosi et al. 2009). Since the 1990s, there has been a significant increase in research and therefore understanding of EoE.

Epidemiology

EoE can occur at any age. Most cases occur in children, adolescents, and adults under the age of 50 (Dellon 2014). There is a male predominance and an increased prominence in Caucasians compared to other ethnicities. Males are affected 3–4 times more than females, and the reasons for this male predominance have yet to be identified. In 1 study, when adjusting for race as a confounding variable, the socioeconomic status and geographic characteristics of EoE patients were not found to be different than those of the general gastrointestinal (GI) clinic population (Franciosi et al. 2009). In this study of 335 children in the greater Philadelphia area with EoE, 83.6% were Caucasian, compared with 70.9% of GI control subjects, 64.9% of allergy control subjects, and 73% of the greater Philadelphia population. About 75.8% of subjects were male, compared to 48% of GI control subjects, 60.4% of allergy control subjects, and 48% of the greater Philadelphia population (Franciosi et al. 2009). EoE has been identified in all continents with the exception

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of Africa (Dellon 2014; Akhondi 2017), and is reported more frequently in Western countries than in Asia (Dellon 2014). There are also environmental and geographic patterns. EoE varies by climate zone, with cold and arid climates having higher rates of EoE (Hurrell et al. 2012). Other studies show that EoE is more common in rural areas with low population density (Spergel et al. 2011; Jensen et al. 2014).

There is a strong association between EoE and atopic disease, and it is more commonly diagnosed in those with a strong personal or family history of atopy. An estimated 26%–86% of adults and 42%–93% of children diagnosed with EoE also have another allergic condition (Ishihara et al. 2016). Family history of EoE is present in 7% of cases (Akhondi 2017). If 1 child has a diagnosis of EoE, their sibling has a 50% increase of being affected compared to the general population. Family studies performed by Alexander et al. (2014) reveal high heritability in nuclear families (72.0%). When analyzing monozygotic and dizygotic twins, a 58% proband concordance was found in identical twins whereas dizygotic twins have a 36% concordance compared to non-twin siblings, indicating that shared environmental factors strongly contribute as well (Alexander et al. 2014). Risk also increases with the severity of the sibling's disease (for example, advanced stenotic disease) (Akhondi 2017).

Prevalence helps to measure burden of disease and varies based on the population that is studied. In EoE, prevalence is also affected by the timing of the study and is dependent on if it was conducted before or after the discovery of proton pump inhibitor (PPI)-responsive esophageal eosinophilia (PPI-REE) (Liacouras et al. 2011; Dellon et al. 2013*a*). EoE prevalence is estimated to be highest in the US, Western Europe, and Australia, compared to Japan or China (Dellon 2014; Dellon et al. 2014). In a large US study with data from 2009 to 2011, prevalence of EoE was estimated at 56.7/100 000 persons (Dellon et al. 2014). An epidemiologic review of EoE from 2014 demonstrated prevalence in the general population to be approximately 0.5–1 per 1000 (Dellon 2014), with most estimates in the US ranging from 40 to 90 cases per 100 000 (Dellon 2014). These estimates of prevalence in the US were found to be consistent with prevalence data from Australia (89/100 000), Switzerland (43/100 000), Spain (45/100 000), and Canada (34/100 000) (Dellon 2014). The prevalence of EoE seems to increase with age until a peak occurs at age 35–45, and then prevalence decreases, which is not an expected observation for a chronic and non-fatal disease (Dellon 2014; Dellon et al. 2014).

When looking at the specific population of patients undergoing endoscopy, the prevalence of EoE is quite high. A prospective study that collected esophageal biopsies in adult patients undergoing outpatient upper endoscopy for any indication found that 6.5% of patients had a diagnosis of EoE (Veerappan et al. 2009). In this study, EoE patients were more likely to be male (80% vs. 48% of those without EoE), under 50 years of age (72% vs. 48.9%), have asthma (32% vs. 10.8%), present with food impaction (32% vs. 8.9%), have dysphagia (64% vs. 38.1%) and were more likely to have classic endoscopic findings of EoE (rings, furrows, plaques, strictures, etc.) (Veerappan et al. 2009). In patients undergoing endoscopy for symptoms of dysphagia, the prevalence is higher at 12%–23% (Prasad et al. 2007; Mackenzie et al. 2008; Ricker et al. 2011; Sperry et al. 2011; Dellon et al. 2013*b*). In 1 study, the prevalence of EoE in patients undergoing endoscopy after a PPI trial for symptoms of dysphagia was found to be 23% (Dellon et al. 2013*b*), whereas 14% of patients had PPI-REE (Dellon et al. 2013*b*). In another study of 222 patients with dysphagia undergoing endoscopy with biopsy, 15% were found to have EoE (Prasad et al. 2007). In patients presenting to the emergency department with food impaction, EoE is the most common etiology identified, with 46%–63% of these patients ultimately receiving the diagnosis of EoE (Sperry et al. 2011). Physicians should have a high degree of suspicion for EoE in patients undergoing endoscopy for any reason, and should strongly consider obtaining biopsies to properly evaluate for this condition. Currently, the guidelines in place recommend obtaining biopsies for all patients presenting with unexplained dysphagia, even if the esophagus on upper endoscopy looks grossly normal or a cause of dysphagia other than EoE is identified (Liacouras et al. 2011; Dellon et al. 2013*a*).

The incidence of EoE is approximately 1/10 000 new cases per year, and has been increasing for the last 10–20 years (Dellon 2014). Increasing EoE incidence has been identified in the United States, Switzerland, and the Netherlands (Okada et al. 2010; Hruz et al. 2011). The number of new cases could be rising for a multitude of reasons. All allergic diseases including

asthma, atopic dermatitis, allergic rhinitis, and food allergies have been increasing in recent decades (Okada et al. 2010). The incidence of EoE, as an allergen/immune related disorder, would be expected to rise in parallel with these other allergic diseases. Another explanation for the rise of EoE incidence and prevalence is increasing recognition. Medical practitioners are more aware of this diagnosis and thus more biopsies during endoscopy are performed to assess for the condition. This, however, only partially explains the increase.

There are many hypotheses that aim to explain the increasing incidence of EoE, including changes in food allergens, aeroallergens, increase in PPI use, decrease in *Helicobacter pylori* colonization, and early life exposures (Dellon 2014). Certain foods have long been understood to be triggers of EoE, but it is unknown why foods that were tolerated for so long are now common offending agents. Potential contributors include changes in agricultural practice such as increasing pesticide, antibiotic, and hormone use. With regard to aeroallergens, EoE is more commonly diagnosed in the summer and fall, seasons with higher aeroallergen activity (Prasad et al. 2009). Another factor could be the decrease in *H. pylori* colonization, occurring since the 1990s. In a large cross-sectional analysis using over 165 000 patients, there was an inverse relationship between *H. pylori* colonization and EoE diagnosis on biopsy (Dellon et al. 2011). Additionally, the use of PPIs has increased over the last 30 years and may play a role in EoE incidence. PPI use can increase the permeability of the upper GI tract, creating new routes of antigen exposure and may be associated with the development of new food specific antibodies (Mullin et al. 2008; Merwat and Spechler 2009). However, it is also well known that PPI use can significantly reduce eosinophils in PPI responsive disease. A PPI trial is now the first line in the treatment algorithm for EoE (Figure 1).

Early life exposures may also be important in the development of EoE, and may be contributing to the increasing incidence. One study showed that infants who received antibiotics were 6 times more likely to develop pediatric EoE than controls with no antibiotic exposure in infancy (confidence interval: 1.7–20.8) (Jensen et al. 2013). This study also showed an increased risk of EoE in those delivered by cesarean section, premature infants and those who were non-exclusively breast-fed (Jensen et al. 2013). More research is required to fully elucidate and differentiate the variables thought to be contributing to rising rates of EoE.

Pathophysiology

Evidence suggests a strong familial association in EoE although inheritance seems to be non-Mendelian (Noel et al. 2004). Several candidate genes that likely contribute to the development of EoE have been identified using genome-wide association studies (GWAS) and candidate-gene identification. These genes include thymic stromal lymphopoietin (*TSLP*), periostin (*POSTN*), calpain 14 (*CAPN14*), desmoglein 1 (*DSG1*), *EMSY*, *LRRC32*, *STAT6*, *ANKRD27*, and *FLG* (O'Shea et al. 2017).

The EoE transcriptome is a term that was coined by Blanchard et al. (2006). This is a group of 574 dysregulated genes, which help differentiate EoE patients from healthy controls and from patients with non-eosinophilic esophagitis. The EoE transcriptome is highly conserved across age, gender, atopy, and non-familial relationship. A screening tool developed using 94 genes from the transcriptome may become a diagnostic tool to help differentiate patients with EoE from those with other forms of esophagitis, as well as helping distinguish patients with active EoE from those in remission (Wen et al. 2013). The largest numbers of transcriptional changes in the EoE transcriptome occur at 1q21, a region that contains genes involved in squamous epithelia cell differentiation including Filaggrin. These genes are down regulated in EoE consistent with impaired barrier function (Sherrill et al. 2014; O'Shea et al. 2017; Rochman et al. 2017). The Chemokine ligand 26 (*CCL26*) or eotaxin-3 gene is the most highly up-regulated gene (53-fold) in the EoE transcriptome (Blanchard et al. 2006). *CCL26* is thought to be the main driver for eosinophil recruitment into the esophagus. Levels of *CCL26* correlate significantly with esophageal eosinophil and mast cell levels (O'Shea et al. 2017). Another up-regulated gene in the transcriptome is *POSTN*. *POSTN* is involved in regulating cell migration and adhesion. *POSTN* promotes allergic inflammatory responses in the lung and esophagus. It may also induce expression of *TSLP* (Blanchard et al. 2008; Masuoka et al. 2012). Other important genes include *STAT6*, a gene important for Th2 development; *LRRC32*, a TGFβ binding protein; and *EMSY*, which is involved in transcriptional regulation (O'Shea et al. 2017).

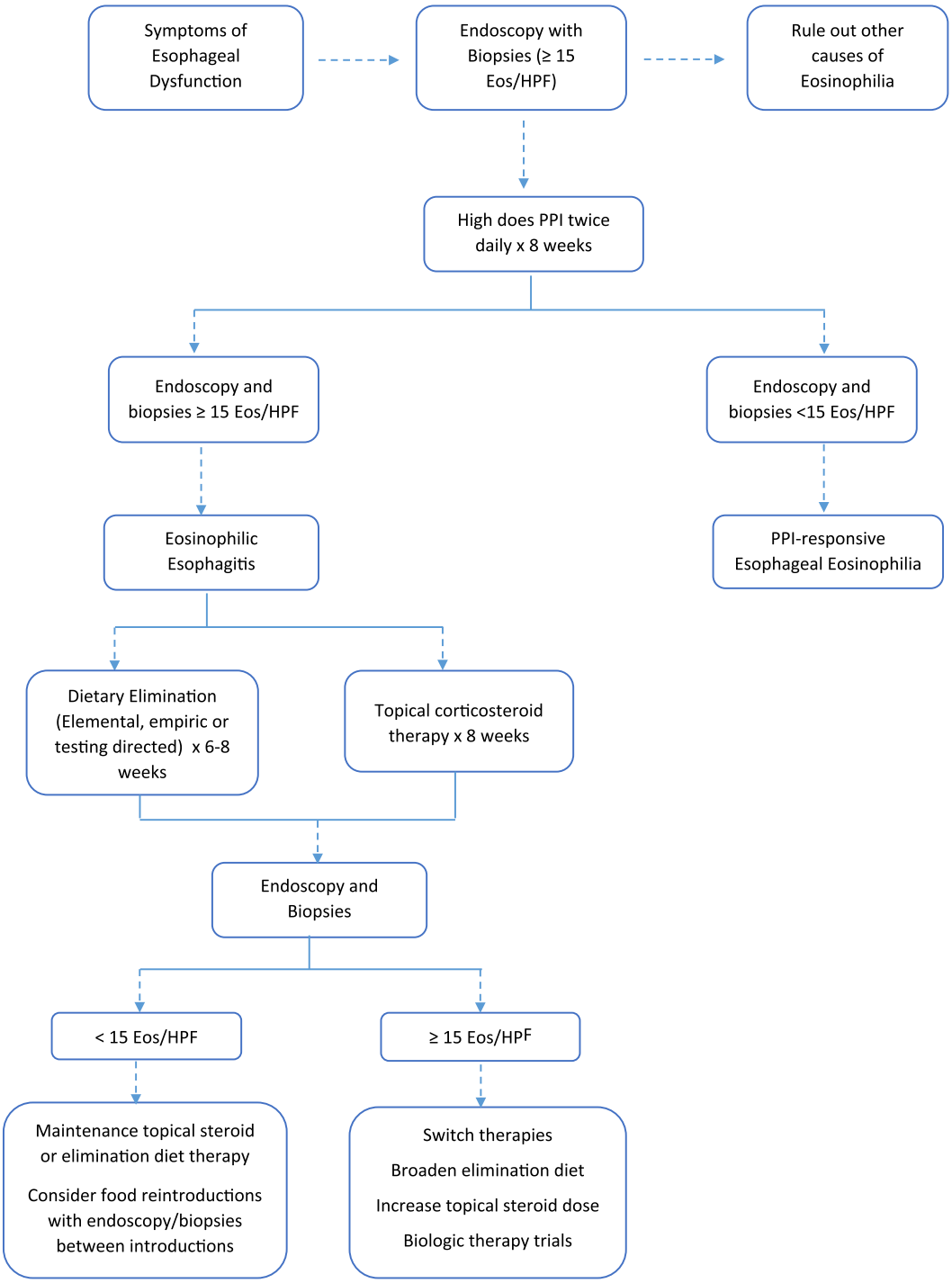

Figure 1: Diagnostic and treatment algorithm for the management of eosinophilic esophagitis.

Together these genes promote development of eosinophilic inflammation of the esophagus.

A GWAS study of 351 patients with EoE and 3104 controls was genotyped for 550 000 variants. A single locus on chromosome 5q22 spanning *TSLP* was associated with EoE susceptibility (Rothenberg et al. 2010). *TSLP* encodes a Th2 promoting cytokine involved in allergic disease. It is released by activated epithelial cells and promotes Th2 differentiation (Gudbjartsson et al. 2009). *CAPN14* is a gene that encodes a proteolytic enzyme specific to the esophagus, which is induced by interleukin (IL)-13 (Litosh et al. 2017). Unlike *TSLP*, which is associated with multiple allergic diseases,

CAPN14 may be specific to the esophagus and EOE. *CAPN14* is overexpressed in esophageal epithelial cells resulting in diminished barrier function (Litosh et al. 2017). Another important gene is *DSG1*, which encodes a transmembrane protein that is down regulated in esophageal mucosa of patients with active disease. The protein's main role is to maintain epithelial integrity through calcium-dependent intercellular adhesion. Down-regulation of *DSG1* is mediated by IL-13. Although the role of impaired barrier function in EoE is not completely understood, barrier abnormalities may provide an entry pathway for allergens and induce systemic allergen sensitization (O'Regan et al. 2008).

Th2 cytokines important in the development of EoE include IL-5 and IL-13. IL-5 drives mucosal esophageal eosinophilia and may potentiate tissue remodeling (O'Shea et al. 2017). IL-13 is secreted mainly by activated Th2 cells and is critical for eosinophil survival, activation, and recruitment. IL-13 contributes to eosinophilic chemotaxis and induces eotaxin-3. IL-13 also induces tissue remodeling and disruption of the epithelial barrier. In EoE, the esophagus expresses elevated levels of IL-13 (O'Shea et al. 2017). Evidence suggests that IgE does not have a prominent role in the pathogenesis of EoE. A clinical trial looking at omalizumab, a humanized monoclonal anti-IgE antibody, as a treatment for EoE showed no histological or symptomatic improvement in those on active treatment compared to controls (Clayton et al. 2014). Immunoglobulin (Ig) G_4 in tissue may play a role in the pathogenesis of EoE. In this same trial, mucosal biopsies of adults with active EoE revealed a 45-fold increase in IgG_4 staining compared to controls (Clayton et al. 2014). The role of IgG_4 in EoE continues to be studied.

Microbial imbalance may also contribute to EoE. Early life exposures such as cesarean section delivery and antibiotic use in infancy may increase the risk of EoE later in life (Radano et al. 2014). This may be related to microbial imbalance and Th2 skewing of the immune system. A prospective study of children and adults with EoE found that subjects with EoE had a higher bacterial load in esophageal mucosal secretions compared to controls regardless of treatment stage (Harris et al. 2015). In addition, several centers have noted an inverse relationship between *H. pylori* infection and EoE. This may have to do with skewing the immune system towards a Th1 milieu in *H. pylori* infection and Th2 environment when *H. pylori* is lacking (Dellon et al. 2011; von Arnim et al. 2016; O'Shea et al. 2017; Sonnenberg et al. 2017). The exact pathogenic mechanism that microbes may contribute to the development of EoE remains to be determined.

Clinical presentation

There are no pathognomonic presenting features of EoE. The most common presenting symptom in adults is solid food dysphagia (Kapel et al. 2008; Liacouras et al. 2011; Akhondi 2017). Other common presenting symptoms in adults include food impaction, atypical chest pain, emesis, and epigastric pain. In children, the common presenting symptoms are abdominal pain, food refusal, recurrent emesis, poor growth, and weight loss (Akhondi 2017). Infants and toddlers often present with feeding difficulties, food refusal, and failure to thrive (Aceves et al. 2009; Mukkada et al. 2010; Liacouras et al. 2011). School-aged children often present with emesis or abdominal pain (O'Regan et al. 2008; Liacouras et al. 2011). Dysphagia is the most common presenting symptom in adolescents (Liacouras et al. 2011). Another frequent presenting symptom is food impaction requiring endoscopic removal, which occurs in 33%–54% of adults with EoE (Aceves et al. 2009; Liacouras et al. 2011). History is often extremely useful, and attention should be dedicated to the patient's swallowing, chewing, and eating habits. Often these patients have adapted mechanisms such as eating abnormally slowly, taking small bites, cutting food into small pieces, and drinking excessive amounts of water with meals to reduce dysphagia symptoms. Patients may also cope by avoiding textured or bulky foods. In many cases of EoE in both adults and children, the physical exam is within normal limits until late in the disease process.

History of atopy is critical, and can help point the clinician to a diagnosis of EoE in both adults and children. Approximately 75% of people with EoE have a history of atopy (Akhondi 2017), and thus any co-morbid allergic diseases should be identified. In pediatric patients, it is crucial to evaluate the growth chart, as growth failure and weight loss are common presenting symptoms for EoE. There are no oral or pharyngeal manifestations of EoE that are visible on examination (Liacouras et al. 2011).

PPI-REE is an important subgroup of EoE. Patients with PPI-REE tend to have symptoms of esophageal dysfunction and have had GERD diagnostically excluded, but demonstrate clinicopathologic response to PPIs, with improvement in symptoms and eosinophilia of the esophagus after PPI therapy (Liacouras et al. 2011; Molina-Infante et al. 2011). It is not clear exactly why PPIs assist with the condition in these individuals. Proposed mechanisms include PPI-induced healing of the epithelial barrier, decreased eosinophil lifespan, and anti-inflammatory properties of PPIs (Kedika et al. 2009; Liacouras et al. 2011). Many prospective studies have shown that 30%–40% of adults with EoE in fact have PPI-REE (Molina-Infante and Katzka 2014).

A thorough allergic evaluation is necessary in patients undergoing a work-up or who have received a diagnosis of EoE. Using data from multiple studies, it is estimated that 28%–86% of adults and 42%–93% of pediatric patients with EoE have another allergic disease (Roy-Ghanta et al. 2008; Spergel et al. 2009; Erwin et al. 2010; Liacouras et al. 2011). Comorbid atopic conditions include asthma, IgE-mediated food allergies, atopic dermatitis, and allergic rhinitis. Many patients with EoE have a sensitization to food allergens and (or) aeroallergens based on skin prick testing or specific IgE testing (Liacouras et al. 2011). It is estimated that the rate of IgE mediated food hypersensitivity in patients with EoE ranges from 15% to 43% (Liacouras et al. 2011). Higher rates of food-induced anaphylaxis can occur in patients with EoE (Sugnanam et al. 2007; Liacouras et al. 2011). Serum food-specific IgE levels and skin prick testing for foods may be helpful to identify comorbid disease. These tests may also be useful as patients with sensitization may develop IgE-mediated allergy to foods they were previously consuming if a prolonged elimination occurs as part of their treatment. Interestingly, cow's milk protein is the food implicated in all of the case reports to date of patients developing IgE-mediated allergy after elimination diet for EoE (Hill et al. 2015; Alsalamah et al. 2016; Soller et al. 2017). Cow's milk protein is the most common trigger of EoE (Kagalwalla et al. 2011), therefore, it is the most common food eliminated from the diet of patients with EoE. Other than frequency of elimination, the reasons for this association between elimination of cow's milk protein for EoE and the development of IgE mediated allergy are not fully understood. Environmental triggers have been found in experimental models of EoE (Mishra et al. 2001; Rayapudi et al. 2010). As many studies have documented aeroallergen sensitization and seasonal variability in patients with EoE, EoE patients should also be evaluated for aeroallergen sensitization (Liacouras et al. 2011).

Esophageal eosinophilia can be present in disease pathologies other than EoE. It is important to have a differential diagnosis, to distinguish EoE from other illnesses. Other diseases that can cause esophageal eosinophilia include GERD, infectious processes, and systemic diseases. GERD should respond to high-dose PPI therapy. PH monitoring can also be performed to rule out GERD. Parasites and fungal infections that cause elevated eosinophil counts will often be positive on specific laboratory tests and respond to tailored treatment, and eosinophils will often be found beyond the esophagus and in the periphery. Hyper-eosinophilic syndromes will have systemic findings and peripheral eosinophilia. Systemic diseases will have other organ system involvement, including Crohn's disease, celiac disease, vasculitis, connective tissue disorders, and other rheumatologic and dermatologic conditions. Drug reactions should show improvement of symptoms and histology after the offending medication is removed (Akhondi 2017).

Diagnosis

EoE is a clinicopathologic condition that requires symptoms of esophageal dysfunction, and an esophageal biopsy showing eosinophil-predominant inflammation in 1 or more biopsy specimens (with a minimum threshold of 15 eosinophils per at least 1 high power field) (Liacouras et al. 2011; Akhondi 2017). The disease must be isolated to the esophagus. EoE is a diagnosis of exclusion, and therefore other causes of esophageal eosinophilia must be excluded before the diagnosis can be made.

Patients often have years of persistent or intermittent symptoms prior to an official diagnosis (Akhondi 2017). It is crucial to perform a detailed history and physical examination, and if EoE is suspected, proceed with endoscopy with biopsy. Laboratory testing is often performed but is not required for diagnosis, and is frequently not useful in making the diagnosis.

With regard to laboratory studies, patients with EoE can have elevated inflammatory markers and an

elevated serum IgE, though these findings are non-specific. A complete blood count will typically show a normal peripheral eosinophil count (Akhondi 2017). However, some studies have shown increased peripheral eosinophilia in patients with EoE. In 1 study, 40%–50% of patients had an increase in circulating peripheral eosinophils (over 300–350 per mm^3) (Aceves et al. 2007; Dellon et al. 2009; Liacouras et al. 2011). Total serum IgE levels have been found to be increased in 50%–60% of patients with EoE however there is little evidence to support measuring IgE as a marker of disease or inflammation in these patients (Roy-Ghanta et al. 2008; Erwin et al. 2010; Liacouras et al. 2011). Patients with EoE can have increased levels of IL-5, IL-13, and IL-15, though there is not enough evidence to endorse measuring these levels arbitrarily in the work-up or management of EoE (Liacouras et al. 2011). Currently, there is not enough evidence to support the use of any single inflammatory marker as an indicator of disease in patients with EoE (Liacouras et al. 2011).

Endoscopy with esophageal biopsy is the only reliable diagnostic test for EoE (Liacouras et al. 2011). On endoscopy, there are many features that can be visible in EoE, including linear longitudinal furrows (seen in 48% of patients in 1 study), stacked circular rings (44%), attenuation of the sub-epithelial pattern (41%), eosinophil micro-abscesses or whitish plaques (27%), strictures (21%), and esophageal narrowing (9%) (Akhondi 2017; Bonis and Furuta 2017). In extreme cases, mucosal lacerations or perforations of the esophagus are identified (Akhondi 2017; Bonis and Furuta 2017). One can also find fixed esophageal rings (corrugated rings or trachealization), transient esophageal rings (feline folds or felinization), edema, diffuse esophageal narrowing, a narrow caliber esophagus or evidence of mucosal fragility (crepe-paper appearance) (Liacouras et al. 2011). The sensitivity of these endoscopic findings is low, but their specificity is high. In 1 study, it was found that the specificity for a diagnosis of EoE was 91% for the presence of esophageal rings, 94% for whitish plaques, and 95% for linear furrows and strictures (Akhondi 2017). However, because all of these endoscopic features can be seen in other disorders as well as EoE, none are pathognomonic for the condition (Liacouras et al. 2011). There are 2 subtypes of EoE, which are characterized based on endoscopic findings: inflammatory EoE and fibrostenotic EoE. Inflammatory EoE is more common in pediatric patients, and will often show transient esophageal rings, furrows, and plaques. Fibrostenotic EoE more commonly presents with fixed rings and strictures (Akhondi 2017).

Biopsy in EoE reveals eosinophil-predominant inflammation in at least 1 biopsy site, with a minimum threshold of 15 eosinophils per at least 1 high power field. There is not enough evidence to confirm that elevated levels of eosinophils per high power field correlate with higher levels of disease severity (Pentiuk et al. 2009; Liacouras et al. 2011). Eosinophils should be confined to the esophagus, and not extend to the stomach or more distal portions of the GI tract. To establish the diagnosis, patients should undergo a PPI trial for 8 weeks prior to biopsy, to evaluate if the eosinophilia is persistent despite PPI therapy (and thus ruling out GERD and PPI-REE).

Esophageal biopsy should be performed by obtaining 2–4 biopsies from the distal esophagus and 2–4 biopsies from the proximal and midsection of the esophagus (Akhondi 2017). EoE distribution is often not consistent and is variable between esophageal segments, thus multiple biopsies from each site are necessary to make the diagnosis. It is common to obtain gastric antrum and duodenal biopsies, to rule out eosinophilia in these locations (Liacouras et al. 2011; Akhondi 2017). Per the diagnostic EoE guidelines updated in 2011, it is recommended that gastric and duodenal biopsy specimens be obtained in all children being evaluated for EoE (due to more difficult symptom identification), and for clinicians to use their discretion in adult patients (Liacouras et al. 2011).

Pathologists assessing the biopsy specimens should report all abnormalities visualized, including peak eosinophil values (from the area with the highest density of eosinophils), eosinophilic micro-abscesses, extracellular eosinophil granules, surface layering of eosinophils, basal cell hyperplasia, dilated intercellular spaces, and lamina propria fibrosis (Liacouras et al. 2011). It is critical to acknowledge that many patients with EoE have normal endoscopic findings, and thus EoE diagnosis is confirmed by biopsy histopathology and symptomatology. In 1 study, 9.8% of 102 patients with a normal endoscopic evaluation had histologic evidence of EoE (Prasad et al. 2007; Liacouras et al. 2011). The sensitivity of biopsy increases as the number of biopsy sites increases. One study showed that by using

the threshold of 15 eosinophils per high power field for diagnosis, there was a diagnostic sensitivity of 84%, 97%, and 100% for obtaining 2, 3, and 6 biopsy specimens respectively (Shah et al. 2009; Liacouras et al. 2011). This supports obtaining more biopsy specimens to promote diagnostic accuracy. Another consideration regarding diagnosis of EoE is the limitation of using a high-powered field (HPF) approach to diagnosis. This is because there is lack of standardization regarding the size of an HPF, and thus the histologic findings may vary based on the interpreter/pathologist. There is also debate as to the utility of barium contrast radiography in EoE. While it may be helpful in patients with strictures, 1 study showed that barium contrast radiography was normal in 12/17 children with EoE (Binkovitz et al. 2010; Liacouras et al. 2011). Barium contrast studies are not part of the routine diagnostic workup for EoE.

Diagnostic modalities for esophageal disorders including EoE, other than endoscopy, are being studied. One of these modalities is the esophageal string test (EST). For the EST, the patient swallows a capsule with nylon string attached to one end. Luminal secretions from the proximal string are evaluated for eosinophil-derived proteins, including eosinophil-derived neurotoxin, eosinophil cationic protein, eosinophil peroxidase, major basic protein 1, and charcot-leyden crystal protein (Furuta et al. 2013). In 1 study, ESTs were performed in 41 children with active EoE, 8 patients with treated EoE in remission, 4 with GERD, and 15 normal controls (Furuta et al. 2013). EST secretions and endoscopic biopsy samples were evaluated. It was found that EST measured eosinophil-derived protein biomarkers significantly distinguished children with active EoE from those with EoE in remission, those with GERD and controls. Additionally, the level of eosinophil-derived proteins found on EST significantly correlated with peak and mean eosinophils per HPF in the esophagus (Furuta et al. 2013).

Another diagnostic modality being studied is the Cytosponge. The Cytosponge is an ingestible gelatin capsule with compressed mesh attached to a string. In 1 study, 20 adults with EoE were assessed with the Cytosponge and then with endoscopy and biopsies (Katzka et al. 2015). All of the subjects in this study had dysphagia; 15 had strictures and 13 had active disease. Cytosponge evaluation was able to correctly identify 11/13 patients with active EoE. 57% specificity was found, with the Cytosponge testing correctly identifying 4 of the 7 patients without active disease. All patients preferred Cytosponge as a diagnostic modality, compared to endoscopy with biopsy (Katzka et al. 2015). More information and larger controlled studies are required to further elucidate whether these newer methods are reliable diagnostic tools for EoE.

Treatment

The goal of EoE treatment is to improve both clinical symptoms and number of eosinophils obtained on histologic specimens to obtain complete remission of the disease. Symptomatic treatment is not enough to define regression of disease. A multi-center series of 269 EoE patients found significant discrepancy between the presence of symptoms and histological response to drug therapy (Safroneeva et al. 2016). Endoscopy and histologic follow up is recommended, however, the 15 eosinophils/HPF cut-off is sometimes arbitrary and clinical judgment needs to be used to interpret borderline eosinophil counts.

The first line of therapy for all EoE is PPI treatment for at least 8 weeks. A subgroup of patients who present with clinical and histological findings of EoE will respond to PPI therapy with complete resolution of their disease (Liacouras et al. 2011). In addition to PPI therapy, current treatment options for EoE include dietary elimination therapy, swallowed (topical) corticosteroids, and esophageal dilations. Possible biologic therapies are being studied (Table 1).

The 3 main types of dietary therapy include elemental diet, allergy test-directed elimination, and empiric

Table 1: **Current and emerging therapies for the treatment of eosinophilic esophagitis.**

Current therapies	
Topical corticosteroids	Budesonide
	Fluticasone propionate
Dietary therapies	Elemental diet
	Elimination diets
Esophageal dilation	—
Emerging therapies	
Anti-IL-5 antibody	Mepolizumab
	Reslizumab
Anti-IL-13 antibody	QAX576
Anti-IL-4Rα antibody	Duplizumab
CRTH2 inhibitor	OC000459
Anti-IL-5Rα	Benralizumab

elimination diets. Elemental diet is the most effective form of therapy with remission rates of >90% having been described in both children and adults (Markowitz et al. 2003; Peterson et al. 2013; Arias et al. 2014). This diet involves eliminating all foods and consuming only an amino acid-based, elemental formula (amino acids, carbohydrates, and medium chain triglycerides). Barriers to this dietary therapy include poor taste, monotony, and high cost along with the social isolation that is felt from not eating regular foods. Occasionally, 1 or 2 solid foods are added for this reason. This diet is useful in children who have failure to thrive and feeding difficulties or when patients are refractory to other therapies. Gastric tube placement may be necessary in order for patients to receive adequate nutrition. Very few adults accept this form of therapy (Peterson et al. 2013).

Empiric elimination diets involve elimination of common IgE-mediated allergic foods. There is some variation in terms of which foods are eliminated depending on location. The first empiric elimination diet studied was the 6-food elimination diet (SFED). This diet includes removal of cow's milk, egg, wheat, soy, nuts (peanut and tree nuts), and seafood (fish and shellfish) from the patients' diet. The first SFED study demonstrated histological remission in 74% of children (Kagalwalla et al. 2006). Other studies in both children and adults treated with the SFED have demonstrated remission rates of 72%–74%. Subsequent food reintroduction after SFED has identified cow's milk, wheat, egg, and soy as the 4 foods most likely to induce disease recurrence (Kagalwalla et al. 2011; Henderson et al. 2012; Spergel et al. 2012*a*). Given this knowledge, a multi-center prospective study of a 4-food elimination diet (4-FED) was done. In this study, subjects eliminated cow's milk, egg, soy, and wheat from their diet. This study demonstrated an efficacy of 64% in children with clinical and visual improvement on endoscopy in 91% and 93%, respectively (Kagalwalla et al. 2017). Since cow's milk has been found to be the most common trigger food, and much easier to avoid than multiple foods, a cow's milk only elimination diet has been studied and seems to be effective in 50%–65% of patients (Kagalwalla et al. 2012; Kruszewski et al. 2016). Cow's milk elimination is complicated as conformational epitopes of cow's milk protein change with heating. A small retrospective study of 15 patients with cow's milk protein-induced EoE found that 11 (73%) maintained histologic remission despite consuming baked milk products regularly for 6 weeks. Further prospective study is needed to validate whether this can be generalized (Leung et al. 2013).

The last form of dietary therapy is test-directed elimination. This consists of avoidance of foods that test positive on allergy testing. A 2006 study found that using a combination of skin prick and atopic patch test results to identify foods for elimination demonstrated remission in 72% of children (Spergel et al. 2007). Subsequent studies in both children and adults have failed to replicate these results (Moawad et al. 2016; Philpott et al. 2016).

The goal of dietary therapy is to gradually reintroduce foods one at a time with histological and clinical assessment in between introductions, with a long-term goal of avoiding the minimal amount of foods to keep the disease in remission (Kagalwalla et al. 2011). All dietary therapies are performed for at least 6–8 weeks after which another endoscopy with biopsies is performed. If histological and clinical remission has been induced, foods will be reintroduced either one at a time or in groups (i.e., fruit, meats, etc.). Each food reintroduction lasts 6–8 weeks followed by repeat endoscopy with biopsies, as well as clinical assessment of symptoms between introductions (Kagalwalla et al. 2011).

Topical steroids are the other first-line option for the treatment of EoE. They have been shown to induce histological remission and symptom improvement. After 8 weeks of topical steroid therapy, a repeat endoscopy with biopsies is performed to assess response to therapy. The topical steroid formulations used were originally designed for patients with asthma and were not intended for esophageal disease (Moawad et al. 2016). Budesonide and fluticasone are the 2 medications that have been studied for EoE. Swallowed fluticasone in doses of 440 μg twice-daily to 880 μg twice-daily has been studied in 3 placebo controlled, randomized controlled trials. Response rates range from 50% to 65% in children and adults (Konikoff et al. 2006; Alexander et al. 2012; Butz et al. 2014). Higher doses have been shown to increase risk of esophageal candidiasis. Randomized controlled trials of budesonide, given as a viscous suspension or nebulized, have shown histological remission rates of 27%–87% depending on the dose and method used. In a study of 20 children treated twice-daily with budesonide (in a slurry form using a sugar substitute to constitute the mixture),

80% had complete histological remission (Gupta et al. 2015). Other forms of budesonide have also been tried including an effervescent tablet (83% histological response) and oral suspension (70% histological response) (Miehlke et al. 2016; Moawad et al. 2016). Maintenance therapy is recommended for patients on swallowed steroid therapy. Half of the induction dose of topical steroid is commonly used as maintenance, however, further study is needed to understand the appropriate dose needed to keep patients in remission. Long-term administration of topical steroids appears to be safe and well tolerated (Moawad et al. 2016).

Esophageal dilation can be used to improve clinical symptoms in patients with narrowed esophagus or a stricture. Dilations are used mostly in adults. A barium swallow may be useful in guiding whether or not to proceed with dilation in patients who are not responding to pharmacologic or dietary therapy. Dilation does not change the histology of the disease. Dilation can be performed using 1 of 3 techniques: bougie, wire-guided, and controlled radial expansion balloons. The goal is to achieve an esophageal diameter of at least 15 mm. Clinical efficacy seems to last 1–2 years (Moawad et al. 2013). Initial concerns of increased risk of dilations in EoE patients have been disproven. A meta-analysis reviewed 860 subjects and found a risk of hemorrhage of 0.1% and esophageal perforation of 0.3% (Moawad et al. 2013).

There have been multiple different biologic treatments that have been postulated to help in EoE. Omalizumab, an anti-IgE monoclonal antibody, was trialed in adolescent EoE subjects and showed decreased IgE levels in esophageal tissue, however, a prospective trial of omalizumab in EoE was ineffective in decreasing symptoms or esophageal eosinophilia (Loizou et al. 2015). Mepolizumab, an anti-IL-5 monoclonal antibody, showed promise in an open-label study of EoE patients. This study demonstrated a decrease in blood and esophageal eosinophilia as well as improved clinical outcomes with mepolizumab (Stein et al. 2006). A subsequent randomized, double-blind, placebo controlled (RDBPC) trial of mepolizumab in adult patients showed histological improvement, however, patients had minimal symptom improvement (Straumann et al. 2010). In a RDBPC trial of 59 pediatric subjects, mepolizumab decreased eosinophil counts in 89% of subjects. Interestingly, mast cell numbers also decreased in 77% of treated subjects, although, once again no symptomatic improvement was seen (Assa'ad et al. 2011; Otani et al. 2013). In the pediatric mepolizumab trial, subjects had mild symptom severity at baseline. Similarly, a large RDBPC trial of reslizumab, another anti-IL-5 monoclonal antibody, was conducted in 226 children and adolescents with EoE. This study found that treatment markedly decreased esophageal eosinophilia but symptomatic improvement was similar in both the active treatment and placebo groups (Spergel et al. 2012*b*). Most patients did not exhibit complete eosinophil resolution after therapy. The dissociation between esophageal eosinophil count improvement and symptom improvement suggests that other pro-inflammatory cells may contribute to EoE pathogenesis (Sriaroon and Ballow 2016).

QAX576, an anti-IL-13 monoclonal antibody, has been studied as a potential treatment for adults with EoE. A 60% reduction in esophageal eosinophils was seen with some symptomatic improvement including improved dysphagia. Genetic markers of EoE were modified with treatment including eotaxin-3, *POSTN* and mast cell markers. QAX576 continues to be studied (Rothenberg et al. 2015). Duplizumab is a monoclonal antibody that targets the IL-4 receptor α subunit as well as the IL-13 receptor system. Duplizumab has been effective in asthma and atopic dermatitis (Spergel et al. 2012*b*; Boguniewicz 2017). An RDBPC trial is underway in adults with EoE (Spergel et al. 2012*b*). A CRTH2 antagonist, OC000459, was used to treat severe, refractory EoE in a small trial. In this trial there was a significant decrease in esophageal eosinophilic inflammation without complete resolution (Straumann et al. 2013).

Prognosis

EoE is a chronic and non-fatal disease. Endoscopic signs and histologic esophageal eosinophilia will persist if the condition is not adequately treated, and if treatment is stopped, the symptoms and signs (including histologic features) will recur in the majority of patients (Helou et al. 2008). The ultimate consequence of esophageal remodeling and fibrosis is stiffening and dysmotility. The natural history of untreated EoE in adults is progressive fibrostenosis. It has been found that esophageal rigidity may begin in childhood (O'Shea et al. 2017). While the esophageal signs and symptoms are chronic and progressive without treatment, there are no cases of EoE that have progressed

to a systemic hyper-eosinophilic syndrome, or even spread beyond the esophagus to other areas of the GI tract. EoE does not increase cancer risk.

Conclusion

EoE is a chronic, immune-mediated disease, which is increasing in prevalence. A tremendous increase in research and therefore in the understanding of the pathogenesis and treatment of this disease process has occurred in the last few decades and continues. Emerging biologic therapies continue to be studied as our knowledge of pathogenesis of the disease process increases. In the mean-time, multiple treatment modalities including dietary therapy, topical corticosteroid therapy, and mechanical dilation continue to be used. The hope is that with better understanding of the disease process, targeted therapy will eventually make this an easier disease to manage.

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Severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation is caused by mutation in the *NHEJ1* gene

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Background: Severe combined immunodeficiency (SCID) with microcephaly, growth retardation, and sensitivity to ionizing radiation is a novel autosomal recessive primary immunodeficiency (OMIM #611291). This form of SCID is caused by autosomal recessive mutations in the non-homologous end-joining factor 1 (*NHEJ1*) gene on chromosome 2q35, which encodes cernunnos protein. Since this syndrome was first described in 2006, only 5 patients with a similar phenotype have been reported from 4 unrelated families.

The *NHEJ1* gene is a repair gene in the non-homologous end-joining (NHEJ) pathway. The NHEJ pathway repairs DNA double strand breaks. Failure of this pathway can have deleterious results and can impact development, genetic stability, and cause immunodeficiency. *NHEJ1* is primarily expressed in the cerebellum and cerebral cortex, and may play a role in brain development (Figure 1).

We report a 20-month-old female who was found to have an autosomal recessive compound heterozygous mutation in *NHEJ1*. Two novel pathogenic variants in *NHEJ1* were identified, including c.350delT and c.355T>G. The c.350delT mutation causes a frameshift, replacing the phenylalanine residue at codon position 117 to a serine residue, ultimately leading to a premature stop codon at position 9 of the new reading frame (p.F117SfsX9). This pathogenic variant is predicted to affect normal protein function. The c.355T>G mutation substitutes the amino acid tryptophan for glycine at position 119 (p.W119G) and is predicted to damage protein structure. Our patient's specific mutation is novel, but her clinical presentation is consistent with the phenotypic features found in the very small patient population with *NHEJ1* mutations.

The clinical presentation and disease trajectory of *NHEJ1*-SCID is similar in the population of patients reported. To date, all 5 patients with this form of SCID had failure to thrive, microcephaly, and recurrent infections. Two unrelated individuals died of septic shock at age 18 and 4 years old. All patients had severe T and B cell lymphocytopenia and hypogammaglobinemia, with low IgG, IgA, but normal or increased IgM. Natural Killer cell numbers were normal in all 5 individuals. The patients' fibroblasts showed elevated sensitivity to ionizing radiation. Like our patient, 2 individuals had autoimmune features including autoimmune anemia and thrombocytopenia. Ahnesorge et al. (2006) identified a mutation in the *NHEJ1* gene, in a patient previously reported in 2003 to have SCID and increased sensitivity to ionizing radiation, but without microcephaly.

As patients with *NHEJ1*-SCID have been shown to have severe infections, with potential for mortality, proper management and follow-up is crucial. Management strategies for other forms of SCID include IgG substitution, antibiotics, immune modulation, and hematopoietic stem cell transplantation; however, evidence-based strategies for this novel form of SCID are lacking. Sensitivity to ionizing radiation is a prominent feature and adds complexity to patient management (Dai et al. 2003; Ahnesorg et al. 2006; Buck et al. 2006; Callebaut et al. 2006; Cantagrel et al. 2007).

Methods: The patient's initial immunology assessment was at age 15 months. During this time, our patient was evaluated for a genetic cause with whole

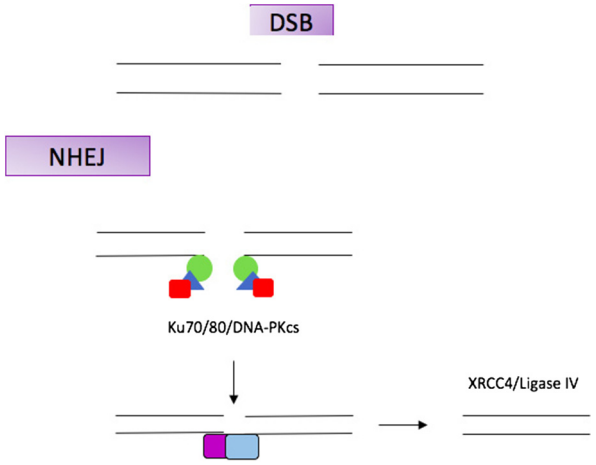

Figure 1: Illustration of NHEJ pathway. (*i*) DNA double strand break (DSB) induced. (*ii*) Ku heterodimer (Ku70 and Ku80) binds to the ends of the broken DNA and acts as a scaffold to recruit core NHEJ factors. (*iii*) DNA-PKcs, XRCC4, and Ligase IV are independently recruited to the Ku-DNA complex. (*iv*) The NHEJ factors interact to create a stable complex at the break (Davis and Chen 2013).

exome sequencing. The results were diagnostic of SCID caused by 2 novel pathogenic variants in the *NEHJ1* gene, with autosomal recessive inheritance. As this is a recent diagnosis, the patient is currently being followed by Immunology and the Bone Marrow Transplant team.

Results:

Clinical features: This is a now 20-month-old female who was born at term weighing 2.4 kg. She had a history of intrauterine growth retardation and failure to thrive. Antenatal ultrasounds documented microcephaly. Amniocentesis was unremarkable and chromosomal microarray was normal. She was initially assessed at 15 months by Immunology, prior to diagnosis of SCID, during hospitalization for autoimmune hemolytic anemia. On admission to hospital, head circumference, height, and weight were >2 standard deviations below the mean for age. On initial consultation, she had no unusual or recurrent infections. She had several mild, self-resolving viral illnesses each lasting 2–3 days. She had no clinical history of sinopulmonary infections and had never received antibiotics. Her vaccinations were up to date per provincial schedule and she tolerated live vaccines, including rotavirus, varicella, and MMR without adverse reaction. Her umbilical cord fell off within 1 week. She met developmental milestones as expected. She is the first and only child to nonconsanguineous parents. The father is of Middle Eastern descent and the mother is Chinese. There was no pertinent family history.

Following her diagnosis, she has been followed jointly by Immunology and the Bone Marrow Transplant team. She is currently receiving intravenous immunoglobulin monthly, Trimethoprim/Sulfamethoxazole prophylaxis, and Mycophenolate Mofetil (MMF) for autoimmune cytopenias. She has remained infection free. Growth continues to be proportional and under the third percentile for age. Development continues to be appropriate. Options for hematopoietic stem cell transplantation are currently under discussion.

Immunological features: When first assessed at 15 months, CBC revealed a white blood cell count of 6.8, neutrophils were low at 0.84, and lymphocytes normal at 4.99. Hemoglobin was low at 76, and platelets normal at 314. Serum immunoglobulin levels were abnormal: IgG was very low at 0.3 g/L, IgA undetectable, and IgM was normal at 1.07 g/L.

T and B lymphocyte subsets were abnormal: CD3 normal, CD8 normal, CD4 low at 0.63, CD19 low at 0.28. CD4 to CD8 ratio reduced at 0.41, and NK cells normal at 0.59.

Mitogen stimulations were within normal limits:

1. PHA—proliferation count of 7541 (4484–37 943)
2. PWM—proliferation count of 1508 (422–21 922)
3. SAC—proliferation count 1187 (413–14 027)

Conclusions: We report a 20-month-old female with an autosomal recessive *NHEJ1* (c.350delT and c.355T>G) gene mutation. To our knowledge, there are 5 patients reported with this rare condition. Our patient's features of profound T and B cell lymphocytopenia, hypogammaglobinemia, autoimmune hemolytic anemia, and microcephaly are consistent with those described in other patients with *NHEJ1*-SCID. Our patient's clinical presentation is unique, however, as she has been free of recurrent or unusual infections, and her development has been normal. Out of the very small patient population with *NHEJ1*-SCID, 2 patients have died as a result of septic shock. Due to the high risk of mortality, hematopoietic stem cell transplantation is an important treatment consideration. In this unique patient population, the process is complicated by sensitivity to ionizing radiation, propensity for chromosomal breakage secondary to ineffective cernunnos, and finding an appropriate donor. As this is a very rare and novel condition, reporting our patient is beneficial to

the Immunology community. Further research is needed to fully understand the impact of defective cernunnos on brain development and disease trajectory.

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Maintenance of T cell receptor excision circles (TREC) levels in zeta chain-associated protein kinase of 70 kD (ZAP70) deficiency

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Background: ZAP70 is a tyrosine kinase whose primary role is in signaling downstream of the T cell receptor (Arpaia et al. 1994). It is activated after recruitment to phosphorylated motifs on CD3ζ and phosphorylation by Lck, a Src family kinase (Wange et al. 1995). Once activated, ZAP70 phosphorylates numerous proteins including LAT (linker of activated T cells), SLP-76 and Cbl (Roifman et al. 2010). The downstream actions of ZAP70 are multipronged—it helps mediate intracellular calcium signaling, helps maintain the cytoskeleton framework of the immunologic synapse, as well as having roles in T cell development and regulation (Arpaia et al. 1994; Au-Yeung et al. 2009). Clinical manifestations of ZAP70 deficiency include CD8 lymphopenia, impaired T cell function and severe combined immunodeficiency, with varying degrees of autoimmunity (Arpaia et al. 1994; Shirkani et al. 2017).

As CD4 T cell numbers are preserved in ZAP70 deficiency, and the surmised primary role of ZAP70 is downstream to T cell receptor (TCR) development, it was plausible to assume that TREC levels—a byproduct of T cell receptor development, would be normal. However, previous studies have shown that TREC levels in ZAP70 deficiency may actually be low in patients diagnosed after 6 months of age, suggesting that ZAP70 has an important role in pre-T cell receptor development (Roifman et al. 2010, 2012; Liu et al. 2017). Yet, this observation was not always consistent. We report here 4 cases of ZAP70 deficiency that had TREC levels above established newborn screening

cutoffs for severe combined immunodeficiency (SCID), but gradually declined in a variable rate.

Methods: Consent was obtained for a retrospective case series of 4 patients with ZAP70 deficiency, as well as research testing on clinical samples. Whole blood and dried blood spot TREC levels were measured via real-time polymerase chain reaction (PCR).

Results:

Clinical manifestations: Patient 1 was diagnosed after a history of chronic diarrhea, recurrent lower respiratory tract infections, and a respiratory deterioration at 7 months of age requiring extra-corporeal life support. A bronchoscopy was positive for multiple organisms including *Pneumocystis jirovecii*, *Parainfluenza type 3*, *Candida albicans*, and *Klebsiella pneumoniae*. He was given an emergency, unconditioned 10/10 matched sibling donor hematopoietic stem cell transplant (HSCT) (Kim et al. 2013), and then eventual re-transplant after engraftment issues. Patient 2, patient 1's sibling, was diagnosed postnatally, and had prompt institution of isolation precautions and antimicrobial prophylaxis, with eventual HSCT at 5 months of age. Patient 3 was relatively thriving until respiratory failure at 5 months of age, with bronchoscopy positive for *Pneumocystis jirovecii*, *parainfluenza type 1*, and *Enterobacter* sp. He received a 10/10 matched sibling donor HSCT at 5 months of age. Patient 4 was diagnosed at 9 months of age, after a history of refractory diaper dermatitis, oral thrush, and recurrent lower respiratory tract infections with pathogens including RSV and *bocavirus*. He received at 10/10 matched unrelated transplant at 11 months of age.

Laboratory investigations: Results of laboratory investigations are shown in Table 1. All patients had mutation IVS12-11G>A, c.[1624-11G>A]; c.[1624-11G>A] in ZAP70. Newborn screen (NBS) TREC levels were done at 0–7 days of life while all whole blood TREC levels were done periodically up to HSCT. Local cutoff values for abnormal newborn screening are flagged for those <75 copies/3 μL DNA. All patients had NBS levels far exceeding this level (van der Spek et al. 2015). Over a course of up to 1 year, TREC levels declined and some reached abnormal levels.

Conclusions: We report here 4 cases of ZAP70 deficiency with sustained TREC levels at birth and in the first year of life. Both NBS and subsequent whole blood TREC levels in patients with ZAP70 deficiency are in the normal range at birth and in young infants, but tend to decline over time. This indicates that thymopoiesis in ZAP70 deficiency is not durable. This finding has important implications for both newborn screening programs for SCID, as well as the pathophysiology of ZAP70 deficiency.

Table 1: **Laboratory investigations of 4 patients with ZAP70 deficiency and preserved TREC levels.**

Lab	Patient 1	Patient 2	Patient 3	Patient 4
WBC (× 10^9 cells/L)	8.1	27	7.8	9.9
Lymphocyte (× 10^9 cells/L)	2.11	2.46	4.61	4.01
Neutrophil (× 10^9 cells/L)	4.7	21	2.04	2
CD19 (cells/μL)	774	711	782	1230
CD3 (cells/μL)	1071	1050	2987	3930
CD4 (cells/μL)	1044	984	2945	3900
CD8 (cells/μL)	27	68	46	32
NK (cells/μL)	183	304	607	738
IgG (g/L)	1.8	4.7	5.3	3.6
IgA (g/L)	0.4	0.1	0.5	0.9
IgM (g/L)	0.4	0.4	1.1	0.5
PHA (% control)	0.2	—	0.2	0.17
PMA + ionomycin (% control)	85	—	—	—
TCR Vβ repertoire CD4 cells	—	No clonal expansion or missing family	TCR Vβ13.6 and TCR Vβ12 clonal expansion	No clonal expansion or missing family
TCR Vβ repertoire CD8 cells	—	Insufficient numbers to run test	Insufficient numbers to run test	Mild expansion of TCR Vβ13.1, TCR Vβ2 clones
CD3 Ra/Ro	—	2/95	11/66	17.67/70
CD4 Ra/Ro	—	0.3/9.8	3.8/61	12.55/66.73
CD8 Ra/Ro	—	3/84.1	0.15/0.96	0.15/0.48

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Moesin deficiency: on the spectrum of combined immunodeficiency

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Background: Ezrin, radixin, and moesin (ERM) proteins are structural components of the intracellular cortex and are expressed in a variety of cell types. The function of these proteins is to maintain cell shape, microvilli formation, organize cell membranes for migration (pseudopod/uropod formation), immune synapse (IS) formation, phagocytosis, and apoptosis (Niggli and Rossy 2008). The activation of ERM proteins by phosphorylation leads to intramolecular conformational changes which unmasks sites for interaction with other proteins critically involved in shape regulation—including actin filaments, transmembrane proteins (membrane PIP, L-selectin, CD43(IS), CD44, and ICAM-1), and scaffolding proteins (EBP50 for CFTR, PDGFR, β2AR). Dysregulation of ERM proteins, particularly moesin, disrupts human lymphoid and neutrophil cells (Niggli and Rossy 2008; Liu et al. 2015; Lagresle-Peyrou et al. 2016). Moesin is ubiquitously expressed in lungs, spleen, kidney, endothelial cells of vessels, and is the predominant ERM protein in lymphocytes and neutrophils (Liu et al. 2015). Given the role and unique expression of this ERM protein, it is clear that moesin has important non-redundant roles in immune function.

Methods: We report on a patient who was evaluated in the IDEA Complex Immunology Clinic at The Hospital for Sick Children, and was enrolled in the Primary Immunodeficiency Registry and Tissue Bank under REB protocol number 1000005598.

Results: Our patient is a 54-year-old male, from a non-consanguineous family, who presented with 3 major invasive bacterial infections: bacterial meningitis at age 5 years, pneumonia, ARDS and septic shock at age 46 years (the pathogen was not identified), as well as cellulitis of the face due to Staphylococcal infection at age 50 years. He suffers from recurrent oral ulcers, recurrent sinusitis, and recurrent leg cellulitis with ulceration. He has a family history of a large amount of infections and a maternal uncle who died from Legionella sepsis at age 40 years. His immune evaluation revealed chronic anemia, profound lymphopenia, abnormal flow cytometry with low CD3+ T cells including both CD4+ and CD8+, as well as low CD19+ and NK cells. He had abnormal phytohemagglutinin response. He was found to have normal albumin as well as immunoglobulin levels (IgG was normal low at 7.8), reactive specific antibody titers, NOBI, CH50, and chromosomal microarray as well. Genetic analysis revealed a novel mutation in the moesin gene (*MSN*).

Discussion: Moesin deficiency has only recently been described and this is the second report. Patients with moesin deficiency can present during infancy or childhood with a severe form of the disease or with a milder form during adulthood (Lagresle-Peyrou et al. 2016). The clinical manifestation was consistent with the sole previous report.

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Newborn screening for SCID detects infants with no primary immunodeficiency

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Background: Severe combined immunodeficiency (SCID) is a life-threatening disease with an asymptomatic period and a curative treatment that is time-sensitive (Pai et al. 2014). Newborn Screening (NBS) for SCID was first introduced as a pilot study in 2008, using the measurement of T cell receptor excision circles (TRECs) as an index of T cell receptor development (Kwan et al. 2014). This test is inexpensive and can be readily performed on Guthrie cards, with acceptable sensitivity and specificity (Chan and Puck 2005; McGhee et al. 2005; Morinishi et al. 2009). For these reasons, SCID met the criteria to be a candidate for NBS (Wilson and Jungner 1968).

After the initial success of NBS in Wisconsin, many states in the USA also implemented SCID NBS (Kwan et al. 2014). In 2013, Immunodeficiency Canada

spearheaded the campaign to introduce SCID NBS in Ontario, as part of the provincial NBS program. To date, the program has been successful in identifying otherwise undiagnosed patients with SCID. However, as with most screening tests, there is potential for both false-negative and false-positive results.

Causes of false-negative results include primary immunodeficiency disease (PID) associated with significant residual autologous T cells (combined immunodeficiency). These conditions may not have critical lymphopenia at birth, have dysfunctional T cells with only a missing subset (such as MHC class I or II), or those affecting T cell maturation after the VDJ recombination process (ZAP70). False-negative results have been discussed in the past, with some approaches suggested that may improve the screening program which still remains inherently devoid of solutions (Roifman et al. 2012; Grazioli et al. 2014).

True false-positive screen tests consist of positive screens which could not subsequently be confirmed by a second TREC test. This can occur because of laboratory error or inadequate sample. These causes are out of the scope of this report. Approaches to increase the sensitivity of TREC-based NBS may result in increased detection of non-SCID cases. This may bring about undue stress for families as well as increase healthcare utilization (Gurian et al. 2006).

Here, we report our 4-year experience in the largest quaternary referral centre with the identification of NBS-positive cases that turned out to have no evidence of PID.

Methods: We retrospectively analyzed all SCID NBS-positive results that were evaluated at The Hospital for Sick Children, Ontario. TREC levels were measured via polymerase chain reaction on Guthrie spot cards. Positive newborn screens were defined as those with undetectable TREC levels. In 2014, the threshold was changed to those with TRECs <75 copies/3 μL DNA.

Results: Since August 2013, 63 patients have been evaluated at The Hospital for Sick Children for SCID NBS-positive results. Five of the 63 patients were subsequently diagnosed with SCID (1 patient with ADA deficiency, 1 patient with Coronin 1A deficiency, 1 with classic SCID, and 2 patients with Omenn's Syndrome with unknown genetic mutation). Four of the 63 had non-SCID PID (2 patients with a mutation in 22q11, 1 patient with Ataxia-Telangiectasia, and 1 with pending genetic work-up). The remaining 54 infants were deemed to have no PID. Details of these patients are outlined in Table 1.

Table 1: **Likely causes of false-positive SCID NBS results.**

Causes	Number of patients[a]
30 weeks gestation, with significant perinatal stress[b]	22
<30 weeks gestation	10
Neonatal immunosuppressive medications	8
Post-thymectomy	7
Maternal factors[c]	8
Unknown	7

[a]Some patients had more than 1 likely cause and were tallied more than once.
[b]Perinatal stress is defined as abnormal fetal monitoring requiring intervention, respiratory distress at birth, shock, encephalopathy, congenital diaphragmatic hernia, IUGR, necrotizing enterocolitis, hypoglycemia requiring intervention, fetal hydrops, twin-twin transfusion, or cardiac conditions.
[c]Maternal factors are defined as those receiving immunosuppressive medications and confirmed active infection (by microbiological work-up) during delivery.

Discussion: We present here multiple causes for NBS-positive results. The main causes in our centre are prematurity, use of immunosuppressive agents in mother or infant, incidental thymectomy for cardiac surgery, and acute stress (Table 1). In some cases causes were not identified.

Conclusions: This evidence is of great clinical importance as it provides physicians and genetic counselors with the data that most NBS-positive cases are detected in infants with no PID.

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Interleukin-2 receptor common gamma chain (IL2RG) defects present a diagnostic challenge

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Background: Severe combined immunodeficiency (SCID) represents a rare group of primary immunodeficiency disorders (PID) characterized by a reduced number of T lymphocytes in association with a functional or quantitative defect in B lymphocytes, natural killer cells, or both. Patients with SCID may have

known or yet unidentified genetic alterations explaining their phenotype. Mutations in the *IL2RG* gene, which encodes the common gamma chain of the interleukin-2 receptor, cause X-linked SCID (XL-SCID) as well as X-linked combined immunodeficiency and remain the most common cause of SCID. This protein is an important signaling component of many interleukin receptors, including those of interleukin-2, -4, -7, and -21, and has therefore been commonly known as the common gamma chain (Shearer et al. 2014).

Transplacental maternal engraftment (TME) is defined as the presence of maternal T cells in peripheral blood before bone marrow transplantation. The human placenta allows for bi-directional passage of nucleated cells between mother and fetus, and in healthy infants the immune system eradicates maternal cells. Patients with SCID lack the functional immunity required to reject circulating maternal T cells, resulting in persistent TME in up to 40% of these patients (Fischer et al. 1997; Liu et al. 2016). Although TME can be asymptomatic, some infants with SCID and TME can have clinical symptoms of graft-versus-host disease (GvHD) at diagnosis (Wahlstrom et al. 2017). TME has also been an impediment to proper infant immune evaluation as well as genetic analysis. Genetic testing is extremely important in SCID as early diagnosis allows for life-saving interventions such as bone marrow transplantation, which results in a higher survival rate when administered during the first 3 months of life (Kwan et al. 2014; Wahlstrom et al. 2015). In addition, proper molecular diagnosis aids in the important task of family genetic counseling.

Here, we present a patient with TME that posed a challenge to both genetic diagnosis as well as genetic counselling.

Methods:

Patient: Following informed consent, patient information was collected from medical records in accordance with REB Protocol No. 1000005598.

Sanger sequencing: Genomic DNA was extracted from peripheral blood lymphocytes using the Geneaid Genomic DNA Mini Kit. Genomic DNA was amplified by PCR with specific primers. Sequencing was performed using GenomeLab Dye Terminator Cycle Sequencing (DTCS) Quick Start Kit (Beckman Coulter) and analyzed on CEQ 8000 Genetic Analysis System (Beckman Coulter).

Next generation sequencing: Massively parallel sequencing was performed on a panel of 20 SCID genes (*ADA, AK2, CARD11, CD247, CD3D, CD3E, DCLRE1C, IL2RG, IL7R, JAK3, LIG4, NHEJ1, PNP, PRKDC, PTPRC, RAC2, RAG1, RAG2, RMRP, ZAP70*), following standard procedures for DNA sample preparation. Libraries were constructed using the Kapa Hyper Prep kit, and targeted capture of coding exons as well as splice junctions performed with Nimblegen's SeqCap EZ Choice. Sequencing was done with 150 bp paired-end reads on an Illumina MiSeq.

Case Presentation: Our patient, now 25 years old, was diagnosed with SCID at 5 months of age with the presence of TME at time of diagnosis. He was one of the first patients world-wide to receive a matched unrelated bone marrow transplantation at 1 year of age and conditioning consisted of Busulfan and Cyclophosphamide. He had an uneventful transplant course with the exception of mild cutaneous GvHD. His engraftment was full and rapid with no complications. He continues to do extremely well 2 decades later, with no episodes of infections, autoimmunity or atopy. His engraftment remains solid and immune reconstitution is complete.

Genetic analysis: Sanger sequencing of the patient's peripheral blood mononuclear cells performed in the early 90s detected no abnormalities in the *IL2RG*, *ADA*, and *RAG1/2* genes, likely because of TME. Several years later, a more extensive panel of SCID-causing genes was sequenced using patient-derived EBV-transformed cell lines, a DNA source not impacted by TME. This time, a novel single base deletion in *IL2RG* causing a frameshift mutation was identified. However, because transformed lines are notorious for EBV-induced genetic aberrations this finding could not have been used as a definitive diagnosis. Sanger sequencing of maternal cells was normal for *IL2RG*, suggesting this might have been either a de-novo mutation or false-positive result. The next option for diagnosis was to obtain fibroblasts via a skin biopsy, or epithelial cells from a buccal smear.

Next generation sequencing (Stavropoulos et al. 2016) performed on a buccal smear showed that approximately 81% of the sequence contained the deletion in *IL2RG* and 19% of the sequence was wild-type. This result could be consistent with the fact that buccal-derived cells can be contaminated with engrafted donor cells, likely stemming from lymphocytes.

Sequencing performed in our patient's sister did not detect the single base deletion in *IL2RG*. A similar analysis on the patient's mother yielded 1 read out of 164 that showed the same deletion. This is an extremely

low level of mosaicism and could be easily missed by performing traditional sequencing.

Discussion: Patients with SCID may have unknown genetic mutations explaining their phenotype. The importance of genetic analysis in family members of such patients needs to be emphasized for family planning and subsequent genetic counselling. Our patient underwent next generation sequencing (SCID panel) 2 decades following his SCID diagnosis, confirming a suspected mutation in *IL2RG*. Subsequently, his mother and sister underwent molecular genetic testing which revealed mosaicism in the mother and no deletion in the sister. As the sister had no deletion detected, her risk of being mosaic was extremely low and she is therefore unlikely to pass on the mutation to her children.

Our patient poses no risk to passing on his mutation to his male children given his X-linked condition, but his risk of passing on the mutation to his female children is 100%. Thus, none of the patient's offspring will develop SCID, but female descendants will be carriers of the pathogenic variant.

This case report emphasizes the complexity of genetic analysis in SCID patients and their family members, and the importance of pursuing a molecular diagnosis. Next generation sequencing appears superior to traditional methods in providing answers for family planning and subsequent genetic counselling.

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Coronin 1A deficiency: first presentation as a positive newborn screen for severe combined immunodeficiency

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Background: Coronin 1A belongs to a large family of highly conserved actin regulatory proteins (Xavier et al. 2008). This protein has a clear role in T cell homeostasis both in mice and humans (Shiow et al. 2008), although the exact mechanism is yet to be clarified. A role for Coronin 1A was also observed in macrophages (Jayachandran et al. 2007), NK (Mace and Orange 2014), and neuronal cells (Martorella et al. 2017).

Human Coronin 1A deficiency was first described in a patient who presented with severe combined immunodeficiency (SCID). The patient received a hematopoietic stem cell transplant (HSCT) at the age of 4 years, with full lymphoid and myeloid donor chimerism (Shiow et al. 2008, 2009), but details of her long term follow up are not available. Later on, several reported cases extended the spectrum of disease associated with mutations in Coronin 1A (Moshous et al. 2013; Mace and Orange 2014; Stray-Pedersen et al. 2014): Hypomorphic mutations in 3 siblings were associated with a predisposition for EBV-mediated B cell lymphoproliferation at an early age (Moshous et al. 2013). The older sibling was reported to be in stable remission more than 10 years post-treatment of his EBV-associated lymphoma with no HSCT. The 2 younger siblings died during induction of chemotherapy and 4 months post-HSCT, respectively. An additional kindred with a mutation resulting in complete loss of protein expression (Stray-Pedersen et al. 2014), presented with a late onset disease at 7 years of age, with epidermodysplasia-verruciformis-human-papilloma-virus (EV-HPV), molluscum contagiosum and mucocutaneous herpetic ulcers, as well as granulomatous tuberculoid leprosy. The first patient developed EBV-associated lymphomas at 15 years of age and died despite aggressive treatment, including a haploidentical HSCT from her mother. Her younger brother is reported to be planned for HSCT. Abnormalities in NK cell cytotoxic function in one of these patients were identified (Mace and Orange 2014). Interestingly, both asymptomatic carrier parents were found to have immune abnormalities, including CD4+ and NK cell lymphopenia.

We report here a case of coronin 1A deficiency detected by newborn screening for SCID. To the best of our knowledge, this is the first reported case of Coronin 1A detected after birth by T cell receptor excision circle (TREC)-based newborn screening.

Methods: Patient information was collected prospectively and retrospectively from medical records.

Exome Sequencing and Variant Calling as well as Western blotting were done according to standard protocols. Sequencing analysis by polymerase chain reaction (PCR) with specific primers designed upstream and downstream of the Coronin 1A gene.

Case Presentation: The patient is a 14-month-old female, born at term to a single mother of African descent. Perinatal history was unremarkable. There is no known consanguinity in the family. The patient was found to have low TREC values in a newborn screening program. An initial TREC value was 42 copies/3 μL. A repeat test from the same dried blood sample was

abnormal at 17 copies/3 µL (cutoff values >75 copies/3 µL). Screening for TBX deletion and purine profile was normal.

Immune evaluation: Since birth, the patient has had persistent lymphopenia as well as neutropenia. Immune work up further revealed a reduction in B and T cell counts, with a relatively more profound CD8+ cell lymphopenia. Over time, the patient developed a mild reduction in NK cell counts as well. Her T cell responses to mitogens were normal. A humoral work up showed hypogammaglobulinemia with good specific response to tetanus vaccine.

Whole exome sequencing (Stavropoulos et al. 2016, Wang et al. 2010) identified a novel homozygous mutation in Coronin 1A. The mother was found to be a heterozygous carrier.

Discussion: We report here the first Coronin 1A deficiency patient detected by newborn screening for SCID. This patient had leukopenia and neutropenia, but is currently doing clinically well at 14 months of age with no severe or recurrent infections. Previous reports show great variability in clinical presentations, ranging from patients presenting as SCID (Shiow et al. 2008), through EBV-related lymphoproliferation at a young age (Moshous et al. 2013), and a yet later onset of disease, at 7 years of age (Stray-Pedersen et al. 2014). All patients reported thus far presented with T cell lymphopenia (Shiow et al. 2008; Moshous et al. 2013; Stray-Pedersen et al. 2014) and a severe reduction in CD45Ra+ naïve T cells (Moshous et al. 2013; Moshous and de Villartay 2014; Stray-Pedersen et al. 2014), suggesting a role for Coronin 1A in mature T cell survival. However, B and NK cell counts, T cell responses to mitogens and antigens, as well as humoral function are all variable among patients (Shiow et al. 2008; Moshous et al. 2013; Mace and Orange 2014; Stray-Pedersen et al. 2014). One patient (Mace and Orange 2014) was also found to have abnormalities in NK cytotoxic function.

Summary: We hereby report the first case of Coronin 1A deficiency presenting in a well newborn as part of the newborn screening program. Coronin 1A deficiency is a rare combined immunodeficiency, and the few cases reported in the literature had a variable although detrimental clinical course. As our patient is currently well, we are confronted with one of the challenges posed by early diagnosis of rare diseases, i.e., the inability of predicting prognosis, and thus the difficulty in recommending a morbidity and mortality associated treatment, such as HSCT.

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Primary Immunodeficiency

There are more than 250 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]

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[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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