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Update on effects of cleaning agents on allergy and asthma

Shivonne Prasad, Joshua C. Lipszyc, and Susan M. Tarlo*

ABSTRACT

Background: Cleaning and disinfecting agents are widely used in modern life, in homes, schools, public places, and workplaces as well as in recreational facilities such as swimming pools. Use has been for sanitizing purposes and to assist in reduction of infection as well as for deodorizing purposes. However, adverse respiratory effects have been associated with use of cleaning products ranging from effects in infancy and early childhood up to adults at home and work.

Methods: This review summarizes recent published literature on the effects of cleaning agents used pre-natally, in childhood and adult life, at home, work, and in swimming pools.

Results: Several studies have indicated that there is an increased risk of developing asthma among adults with frequent exposure to cleaning products at work and in the home. Potential mechanisms include sensitization and respiratory irritant effects. Exposure to irritant chlorine by-products from swimming pools have also been associated with respiratory effects and increased risk of asthma. Potential effects from maternal exposures to cleaning products on infants, and effects on early childhood atopy are less clear.

Conclusions: Exposure to cleaning agents increases relative risks of asthma among workers, and adults using these agents in the home. Risks are also increased with exposure to chlorinated by-products from swimming pools, both in adults and children. Further studies are needed to understand the mechanisms of these associations.

Introduction

Cleaning and disinfecting agents are widely used in modern life, in homes, schools, public places, and workplaces as well as in recreational facilities such as swimming pools. Use has been for sanitizing purposes and to assist in reduction of infection as well as for deodorizing purposes. Products used as part of a hand hygiene program have significantly contributed to reduced transmission of infection in health care but have increased the potential for irritant and contact dermatitis (Kurtz 2016). In addition, these agents can also have other potential unwanted effects. This review will address current knowledge of the effects on allergy and asthma.

Potential effects of cleaning agents on allergy and asthma can range from effects on the unborn baby from maternal exposure, to effects in early childhood, school-age children, and adults. These include possible modulation of TH1/TH2 switching in early childhood resulting in increasing risk of atopy, altered airway epithelial function due to chlorinated compounds from swimming pools, increased airway responsiveness, acute irritant-induced asthma from irritating cleaning chemicals, as well as specific immunologic responses

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to cleaning agents causing allergic rhinitis, conjunctivitis, and asthma.

Pre-natal and early childhood exposures

A Spanish study of 4 prospective birth cohorts, including over 2000 pregnant women (Casas et al. 2013), reported an increased period prevalence of lower respiratory tract infection among the infants up to 12–18 months of age if the mothers had been exposed in pregnancy to sprays (OR = 1.29; 95% confidence interval (CI): 1.04–1.59) or air fresheners (OR = 1.29; 95% CI: 1.03–1.63). Wheezing was increased with spray or solvent use, with similar odds ratios. The associations were seen for sprays and air freshener use in pregnancy even if there was no exposure after birth, but associations were greater when post-natal exposures were included. A Polish study of risk factors for early childhood wheeze and atopic dermatitis (Stelmach et al. 2014) found that more frequent house cleaning was a predictor of atopic dermatitis in the first year of life, odds ratio 1.8 (95% CI: 1.1–2.9), but the mechanism of the association was not clear.

The hygiene hypothesis has suggested that the increased prevalence of allergy and asthma in early childhood may relate to an increase in T-lymphocyte switching from TH1 to TH2 type lymphocytes due to reduced exposure to microorganisms in early childhood, from increased cleanliness in the home, less early childhood exposure to animals and early infection (Strachan 2000). If this hypothesis is correct, it would be expected that increased use of cleaning products in the home during early childhood may result in less exposure to indoor allergens and microorganisms and may be associated with more asthma and allergy. However, the hygiene hypothesis has been questioned and especially the potential role of home cleanliness (Liu 2015; Weber et al. 2015; Ege 2017). Some of the variability in reported studies, illustrated below, might relate to the age of children studied.

Cleaning with bleach was associated with a reduction in surface bacteria, airborne fungal spores, and dust antigens, and also an improvement in asthma quality of life among children with asthma aged 2–17 (Barnes et al. 2008). A Belgian study (Nickmilder et al. 2007), reported that children aged 10–13 living in a home that was cleaned with bleach at least once a week were less likely to have asthma (OR = 0.10; 95% CI: 0.02–0.51), eczema (OR = 0.22; 95% CI: 0.06–0.79), and less risk of being sensitized to indoor aeroallergens (OR = 0.53; 95% CI: 0.27–1.02), especially house dust mite (OR = 0.43; 95% CI: 0.19–0.99). There was no reduction in sensitization to grass pollen, so the apparent benefits in that study may have resulted from reduced exposure to the home allergens and did not suggest a significant effect on T-cell switching. An earlier study (Henderson et al. 2008) showed an association between high domestic chemical exposure in pregnancy and persistent wheezing and reduced FEV_1 and $FEF_{25\%-75\%}$, from birth to age 7 years, but there was no difference in rates of atopy with chemical exposure and the authors concluded the effects may have been from pre-natal respiratory developmental effects or post-natal irritant effects, but were unlikely related to improved hygiene in the home.

In young adults, exposure to household disinfectants has been associated with increased incident asthma (OR = 2.79; 95% CI: 1.14–6.83) (Weinmann et al. 2017). Frequent use of bleach has been reported to increase the risk in women for non-allergic adult-onset asthma (adjusted odds ratio = 3.3; 95% CI: 1.5–7.1) (Matulonga et al. 2016). In addition, a greater progressive decline in both FEV_1 and FVC over a 20 year period has been reported among women in the European Community Respiratory Health Study for those women who undertook cleaning at home or at work at least once a week ($p = 0.004$), compared with women who did not, although there was no difference in decline of FEV_1/FVC ratio (Svanes et al. 2018). No similar pulmonary function effect was seen among men. Doctor-diagnosed asthma was more common among the women cleaning at home or work (9.6% among women not cleaning, 12.3% in women cleaning at home, and 13.7% in women cleaning at work). Rates of doctor-diagnosed asthma among men were lower but also showed a difference for those who cleaned at home: 7% in those who did not clean, or who cleaned at work, and 10.3% among men who cleaned at home (Svanes et al. 2018). Spray cleaners have been associated with increased exhaled nitric oxide and lower FEV_1 (Le Moual et al. 2014), and with asthma in older women (average age 68) (Bedard et al. 2014) as well as with current asthma and poorly-controlled asthma in younger women (Le Moual et al. 2012).

Few studies have assessed short term effects of home cleaning agents among women with asthma and non-asthmatics. In a small prospective study (Bernstein et al. 2009), peak flow readings were associated with increased lower respiratory symptoms in the asthmatics but no differences in serial peak expiratory flow recordings were found with cleaning exposures. In a controlled brief exposure to ammonia at or above irritant thresholds in volunteers with and without asthma who reported sensitivity to household cleaners, there also were no changes in lung function and similar increases in symptoms were reported by both asthmatics and non-asthmatics (Petrova et al. 2008). In a follow-up of the European Community Respiratory Health Survey, use of cleaning sprays at least weekly in the home was associated with the incidence of asthma symptoms and medications (Zock et al. 2007). The incidence of doctor-diagnosed asthma was higher in those who used cleaning sprays at least 4 days a week (Zock et al. 2007).

Occupational exposures among healthcare workers

Healthcare workers (HCW) are over-represented in the workforce among adults with work-related asthma, with 1 study demonstrating that 9% and 37.5% of HCWs assessed had occupational asthma or work-exacerbated asthma, respectively (Knoeller et al. 2013). Moreover, a recent study in a Canadian tertiary clinic that compared 2 time periods (2000–2007 and 2008–2015) reported that cleaning agents as a suspected causative agent for work-related asthma patients increased from 6% to 18% (Gotzev et al. 2016). In a surveillance study assessing 4 states, healthcare was reported as the first or second most prevalent industry for work-related asthma cases between 1993 and 1997 (Pechter et al. 2005). Common cleaning agents that are used by HCWs include quaternary ammonium compounds, glutaraldehyde, bleach, acetic acid, subtilisin, phthalaldehyde, formaldehyde, and chlorhexidine. Cleaning products that contain these agents are often used for cleaning or disinfecting purposes, but may have undesirable respiratory and allergic effects on HCWs. Cleaning products have been shown to remain airborne for several minutes even after cessation of the cleaning task (Bello et al. 2010), which may lead to continued exposure to the individual and others. This was especially found for volatile organic compounds, where background exposure was identified up to 20 minutes following completion of the cleaning task (Bello et al. 2010). The current literature has numerous case reports and studies showing associations between cleaning products and occupational asthma and work-exacerbated asthma through sensitizing or irritant mechanisms.

In a study (Arif and Delclos 2012) that included 3650 HCWs who responded to a questionnaire, the likelihood of developing work-related asthma was 2.64 (95% CI: 0.57–12.14) for those exposed to cleaning products once a week. The odds of developing work-related asthma increased to 5.37 (95% CI: 1.43–20.16) for those exposed to cleaning products at least once a day. Interestingly, this study reported that among HCWs exposed to chloramine for 6 months or longer, there was nearly a fivefold greater likelihood of developing occupational asthma (95% CI: 1.28–18.06). It has been proposed that risk of developing asthma is most significant during disinfection, especially during manual mixing/dilution tasks as the worker may be exposed to peaks of the concentrated cleaning product (Gonzalez et al. 2014). A cross-sectional survey study (Arif et al. 2009) that compared 448 nursing professionals to 3186 other healthcare workers reported a greater frequency of asthma and bronchial hyper-responsiveness among nursing professional who cleaned medical instruments (95% CI: 1.06–2.62) and who were exposed to cleaning disinfectants (95% CI: 1.00–2.94). A recent study (Casey et al. 2017), investigated health effects of surface disinfectant products containing hydrogen peroxide, peracetic acid, and acetic acid. They identified that the prevalence of wheeze and epiphora was significantly higher among HCWs exposed to disinfectant products.

Although studies have revealed strong associations between cleaning products and work-related asthma, it is sometimes difficult to establish temporal causal relations, and even more challenging to identify the respiratory pathophysiological mechanisms underlying patients' asthma. These clinical challenges sometimes accompany specific cleaning agents including, but not limited to, glutaraldehyde, hydrogen peroxide, and peracetic acid. A study (Lipinska-Ojrzanowska et al. 2014) in Poland reported that among 142 hospital cleaners, 59% experienced at least 1 "allergic" symptom (nasal, eye, respiratory, or dermal), including 47.2% of workers with a new onset while cleaning, but none of the

workers had a positive skin prick test or serum IgE antibody test. This may be a reflection of the difficulty demonstrating immunologic hypersensitivity responses to low-molecular weight sensitizers but also indicates the importance of considering possible upper airway irritant mechanisms for asthma-like symptoms in addition to considering sensitizing or irritant lower airway responses. The cleaning agents identified in this study included chloramine, formaldehyde, chlorhexidine, and glutaraldehyde, that are recognized potential sensitizers as well as irritants.

Glutaraldehyde is a common disinfectant used in endoscopy labs to disinfect medical instruments. A surveillance study (Walters et al. 2013) of healthcare workers with occupational asthma reported that the most frequent cleaning agent identified was glutaraldehyde. Another older study (Dimich-Ward et al. 2004) identified that respiratory therapists were at greater risk of developing asthma, wheeze, and episodes of dyspnea upon awakening. They found that sterilization of medical instruments using glutaraldehyde and administration of aerosolized ribavirin was associated with asthma.

A study in France (Dumas et al. 2012) identified that among hospital workers, females were more frequently exposed to cleaning products compared to males on a weekly basis (81% vs. 55%, respectively; $p < 0.001$). The HCWs most frequently identified were cleaners and personal support workers, and some of these HCWs presented with current asthma. Interestingly, this study found no association between exposure to cleaning tasks and asthma for men or women, but specific cleaning products were identified to have an association with current asthma for women. It has been suggested that when environmental and safety measures are appropriately implemented including proper ventilation, the effects of cleaning products on HCWs may be minimized (El-Helaly et al. 2016).

Quaternary ammonium compounds are frequently used as disinfectants and antiseptics. A study in Belgium (Vandenplas et al. 2013) included 44 participants with work-related asthma who were administered a specific inhalation challenge (SIC). Thirty-nine percent of participants had a FEV_1 drop of 20% or greater, and of these, quaternary ammonium compounds were the most frequently identified cleaning agent inducing a positive SIC. This study suggested that quaternary ammonium compounds may represent one of the primary cleaning agents associated with sensitizer-induced occupational asthma.

Other reports have included occupational asthma from a cleaning product containing triclosan (Walters et al. 2017), and asthma that was triggered when a chlorinated cleaning product was mixed with urine but not when the product was used alone, presumably due to the chloramine by-products (Moore et al. 2017).

Risks of asthma among cleaners have been shown to be greater among women and among those with longer exposure and with early life disadvantage, as reviewed recently (Folletti et al. 2017).

A Task Force consensus statement from the European Academy of Allergy, Asthma and Clinical Immunology on asthma among cleaners (Siracusa et al. 2013), and later publications (Tarlo et al. 2018), have provided suggestions as to limiting exposure for these workers.

Effects of swimming pool exposure on allergy and asthma

Swimming in chlorinated pools is a commonly attended activity by both recreational and elite swimmers ranging in age from infancy to adulthood. Research into the effects of lung exposure to chlorine by-products proposes that the increased emergence of allergic diseases in the developed world may relate more to the products that are used to achieve hygiene than the elimination of microbes, as suggested by the hygiene hypothesis (Bernard 2007). As reviewed by Uyan et al. (2009), the "pool chlorine hypothesis" suggests that chlorination products, possibly the world's most ubiquitous cleaning agents, used to sanitize water-based recreational areas are thus implicated in an increased risk of allergy and respiratory disorders.

Chlorine is added to pool water in various forms due to its availability, low cost, biocidal, and deodorant properties. Chemical reactions between chlorine in pool water and organic materials from swimmers create disinfectant by-products (DBPs) which account for the respiratory irritation and odor associated with swimming pools (Uyan et al. 2009). Trichloramine, a common DBP, is a volatile irritant gas. Transfer of this gas from water to the surrounding air is promoted by

the dynamic activities of swimmers (Weng et al. 2011) and is influenced by factors such as ventilation, air recirculation, and water temperature (Uyan et al. 2009). Levels may fluctuate from 0.2 to 0.9 mg/m^3 in indoor pools, depending on occupancy and ventilation, an exposure that is substantially greater when compared to typical air pollutant levels of 0.3 mg/m^3 in non-pool environments (Bernard et al. 2006). Levels also tend to be higher in indoor pool environments than outside swimming pools (Uyan et al. 2009). Trichloramine causes respiratory irritation similar to formaldehyde and chlorine and has been shown to cause epithelial damage in rodents and respiratory tract irritation in lifeguards and other pool attendees (Bernard et al. 2006, 2015).

Data from accidental chlorine exposure has established a harmful role for chlorine DBPs on the respiratory tract. Symptoms ranging from rhinitis, tracheobronchitis, and pneumonitis to pulmonary oedema and bronchiolitis have been reported after acute chlorine exposure. Symptoms tend to remit in most affected individuals, although the development of reactive airways dysfunction syndrome has been reported. In the pediatric population, short term effects may be prolonged and last up to 1 month with increased symptoms in children with respiratory disease (Uyan et al. 2009). A persistence of leukotriene B4, a marker of neutrophilic inflammation, has been demonstrated at several months following exposure despite improvements in symptoms and lung function, suggesting subclinical inflammation (Uyan et al. 2009).

The main mechanism by which non-accidental, lower level exposure to chlorine disinfection by-products (DBPs) may predispose to allergy and respiratory symptoms through swimming relates to their effecting lung epithelium hyperpermeability, conferring vulnerability to allergen sensitization (Bernard et al. 2015). Induction of lung injury and inflammation have also been described in this setting. Font-Ribera et al. (2010) studied respiratory epithelial effects in 48 healthy non-smoking adults who had lung function and biomarkers of airway inflammation, oxidative stress, and epithelial permeability measured before and after a 40-minute swim in a chlorinated pool. Breath levels of trihalomethanes were measured to quantify individual exposure to DBPs. Overall, a slight increase in a marker of epithelial permeability, Clara cell protein 16 (CC16), was found in healthy adults after a swim. No significant changes in inflammatory markers were found or thought to mediate this change. Biomarkers of epithelial permeability have been further studied in school age children (Bernard et al. 2015). A 2015 study of 835 adolescents gathered health information, serum concentrations of CC16, surfactant associated protein D (SP-D), and total and aeroallergen specific IgE (Bernard et al. 2015). The CC16/SP-D ratio was proposed as representing permeability and secretory changes in the epithelium. The study found that a low CC16 and low CC16/SPD index was predicted by early swimming in chlorinated pools and was associated with increased odds for allergic disease including aeroallergen sensitization and rhinitis, supporting the view that early swimming may predispose to atopy and epithelial barrier defects.

Airway inflammation is also postulated to be caused by chlorinated pool attendance. In elite swimmers, several studies have shown increased respiratory symptoms and airway inflammation with chronic chlorine exposure (Uyan et al. 2009). Greater proportions of eosinophils and neutrophils in the sputum of elite swimmers compared to healthy controls, with increase bronchial hyperreactivity have also been demonstrated (Helenius and Haahtela 2000). These effects display attenuation following cessation of the sport. Other work has demonstrated significantly higher exhaled breath condensate leukotriene B4 (LTB4) with fractional exhaled nitric oxide (FeNO) levels similar to age matched individuals in elite swimmers, suggesting that chronic chlorine exposure may lead to the development of a neutrophil-driven inflammation (Uyan et al. 2009). However, in addition to exposure to chlorine, other factors may also be implicated in causing respiratory inflammation in this niche group of individuals. Elite swimmers exhibit low frequency-high tidal volume breathing, engage in long training hours and use increased minute volumes whilst swimming, all mechanisms which may in themselves induce transient epithelial damage and inflammation (Bougault and Boulet 2012).

Beyond experimental studies, researchers have endeavored to answer whether recreational swimming plays a role in the development of asthma and allergies in the pediatric setting. An ecological study looking at geographic swimming pool availability in Europe and childhood asthma showed that the presence of wheeze or ever asthma increased by 0.96%–3.39% with an

increase of 1 indoor chlorinated pool per 100 000 inhabitants for children aged 13–14 years (Nickmilder and Bernard 2007). Similar effects were noted in younger age groups. This group of investigators from Belgium also examined cumulated pool attendance (CPA) with childhood asthma and atopy (Bernard et al. 2006). A comprehensive health questionnaire, exercise induced bronchoconstriction and FeNO testing was conducted in 341 school children aged 10–13 years attending the same school with pool trichloramine levels of 0.3–0.5 mg/m^3. CPA emerged as predictive of doctor-diagnosed asthma and FeNO measurements. However, the probability of developing asthma with increased CPA occurred only in subsets of children with elevated IgE levels >100, particularly in pool attendance at age 6–7 years, suggesting that exposure to pool chlorine may interact with other factors in its association with allergy and asthma.

Exposure of infants to chlorinated pools has come under scrutiny in light of literature suggesting a long-term effect of chlorine exposure. Infants may be particularly susceptible due to attendance to warmer, more polluted pools and their developing lungs (Uyan et al. 2009). In addition, some studies have found that exposure is higher to chloramines in air and water in younger swimmers (Uyan et al. 2009). However, in contrast to previously discussed work that has shown an increased risk of asthma with early age pool attendance in Belgium, follow up of a birth cohort of 2192 children in Germany found that swimming attendance in the first year of life is not associated with atopic disease later in life. Notably, allowable limits for chlorine are lower in Germany than in Belgium (Uyan et al. 2009). An observational study in 430 kindergarten aged children using questionnaire data to assess health, swimming behaviours, and confounders found that attendance at chlorinated pools at any stage prior to age 2 years was associated with an increased risk of bronchiolitis (Voisin et al. 2010). This association was strengthened to an odds ratio of 4.45 and 4.44 for >20 hours cumulative time spent in a chlorinated pool when family history of atopy and day care attendance were excluded. The group suggested an increased risk of asthma and allergies later in childhood in those children who were affected by bronchiolitis and who also had higher cumulative swimming exposure (Voisin et al. 2010).

While an association between pool chlorine exposure and respiratory irritation, epithelial and inflammatory effects has been raised by the literature, meta-analysis data does not support an association with the development of asthma (Valeriani et al. 2017*a*). Seven reports (with 5851 subjects) which looked at the link between exposure to DPBs in indoor swimming pools during childhood and asthma were analyzed. The odds ratio for asthma prevalence in relation to swimming pool attendance was 0.58–2.30, with no significant increase in prevalence to controls (Valeriani et al. 2017*a*, 2017*b*). The finding echoes previous opinion in the literature which suggests that the current evidence of an association between childhood swimming and new-onset asthma is suggestive but not conclusive (Weisel et al. 2009; Valeriani et al. 2017*a*, 2017*b*). Other reviews have found evidence for respiratory effects in elite swimmers more compelling than current data on the effects of low chronic exposure in recreational swimmers (Uyan et al. 2009).

Prospective evaluation and follow-up studies to assess whether a cause–effect relationship exists between recreational swimming and asthma development may be important to inform guidelines that balance caution regarding public health risks with a pragmatic approach to a popular and active pastime. While definitive data is awaited, ongoing regulation of chlorine levels, temperature, and ventilation in indoor swimming pools and an emphasis on personal hygiene in swimmers will be important to decrease the presence of respirable irritants around swimming pools (Uyan et al. 2009).

Conclusions

Several studies have indicated that there is an increased risk of developing asthma among adults with frequent exposure to cleaning products at work and in the home. Risks appear to be greater with sprayed products, likely reflecting greater respiratory exposure. Potential mechanisms include sensitization and potential respiratory irritant effects. Exposure to irritant chlorine by-products from swimming pools have also been associated with respiratory effects and increased risk of asthma. Potential effects from maternal exposures to cleaning products on infants, and effects on early childhood atopy are less clear. There remain several unanswered questions regarding relative risks of specific cleaning chemicals and mechanisms of the observed associations. The relative benefits from a clean indoor environment and reduction of harmful microorganisms need to be taken into consideration and balanced with

the possible risks, as has been suggested for those working with these products.

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

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ABSTRACT

Background: The protein encoded by interleukin-2 receptor common gamma chain (*IL2RG*) is an important signaling component of many interleukin receptors, including those of interleukin-2, -4, -7, and -21, known as the common gamma chain. Mutations in the gene encoding the common gamma chain of the interleukin-2 receptor cause X-linked severe combined immunodeficiency (SCID). In this report, we present an unknown genetic defect of a patient diagnosed with SCID whose genetic analysis was performed 2 decades later.

Methods: Whole genome sequencing and Sanger confirmation were used to identify a novel frameshift mutation in *IL2RG*. Massively parallel sequencing of genes associated with SCID were performed on the patient's mother and sister.

Results: Next generation sequencing techniques identified a heterozygous frame-shift deletion in the gene encoding the common gamma chain of IL2RG in our patient. The patient's mother had a low level mosaicism for the same deletion. The sister had no detectable deletion.

Conclusion: We have identified a novel mutation in *IL2RG* resulting in an X-linked SCID phenotype. The genetic analysis of the patient's mother revealed a mosaicism which was not passed on to his sister. The importance of genetic analysis in family members and SCID patients with an unknown genetic defect should be emphasized for family planning and subsequent genetic counseling.

Statement of novelty: Genetic testing is an extremely important component in evaluating severe combined immunodeficiency as it impacts treatment course and prognosis, and allows for genetic analysis and counselling of family members.

Background

Severe combined immunodeficiency (SCID) represents a rare group of primary immunodeficiency disorders (PIDs) 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 (X-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. More than 300 mutations in the *IL2RG*

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gene have been identified in X-SCID. These mutations are located on the long arm of the X chromosome at position 13.1 (Shearer et al. 2014; Pérez-Simón 2015). The following mutational hot spots in *IL2RG* have been reported, at codons p.Arg224, p.Arg226, p.Arg285, and p.Arg289 (Puck et al. 1997).

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 bidirectional 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. The presentation of maternal engraftment can range from a fine maculopapular erythema to generalized erythroderma and alopecia. Liver, gastrointestinal, and hematologic involvement may be observed (Wahlstrom et al. 2017). A newly diagnosed patient with SCID presenting with detectable T cells is evaluated for chimerism by HLA typing of T cells and non-T cells (Müller et al. 2001). In patients with engraftment, maternal T cells are characterized by phenotype and function in response to mitogen stimulation (Müller et al. 2001). The presence of TME may persist post-transplantation, in which the patient should be assessed for signs of acute GvHD with repeat phenotypic and functional T cell analysis (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 and genetic counselling, and whose evaluation resulted in the identification of a novel mutation.

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. His clinical history was significant for maternal engraftment, failure to thrive and recurrent infections including pneumonitis and *pneumocystis jiroveci*. Pertinent laboratory workup revealed a low mitogenic response and the lymphocyte subsets were irrelevant due to maternal engraftment. His parents were of English decent. He was one of the first patients world-wide to receive a matched unrelated bone marrow transplantation at 1 year of age. His 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 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*, c.245delC causing a frameshift mutation (p.Pro82fs) was identified, as demonstrated in Figure 1. 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. Gene defects account for approximately 85% of SCID cases. Prior to newborn screening and in the absence of family history of SCID, most patients came to medical attention with failure to thrive, recurrent and opportunistic infections, such as our case. Over the years, genetic analysis has become an important component of an evaluation of a patient with SCID as it impacts prenatal diagnosis, treatment course, and prognosis. Further, the importance of genetic analysis in family members of such patients

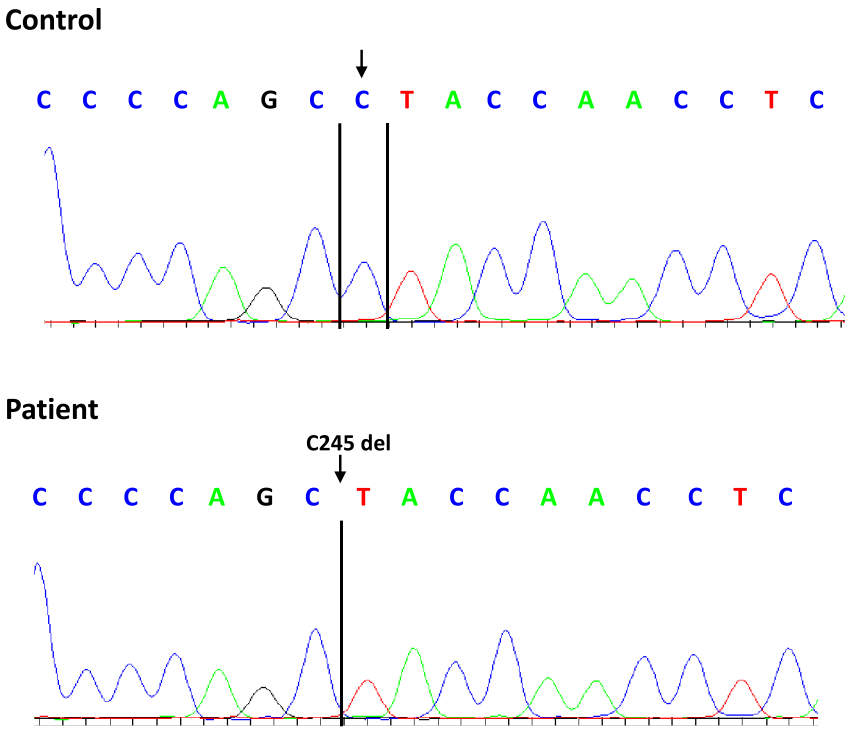

Figure 1: A novel single base deletion in *IL2RG*, c.245delC, was found in our patient resulting in a frameshift mutation (p.Pro82fs).

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 (<1%) in the mother and no deletion in the sister. This extremely low level of mosaicism was the result of a post-zygotic mutation in the mother, hence only some of her cells were affected. 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 describes a novel mutation in *IL2RG*, emphasizing 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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A novel homozygous mutation in CIITA resulting in MHC Class II deficiency in an adult patient

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ABSTRACT

Introduction: Major histocompatibility (MHC) class II deficiency is a rare autosomal recessive primary immunodeficiency with fewer than 200 patients reported worldwide. Patients usually present within their first year of life with severe and recurrent infections, failure to thrive, and chronic diarrhea. The disorder is caused by absent or reduced MHC class II expression on cell surfaces, leading to defective cellular and humoral immune responses. The disease is associated with a poor prognosis, with most patients dying in early childhood due to infectious complications.

Aim: To report the clinical, immunological, and genetic features of an adult patient with MHC class II deficiency who did not undergo hematopoietic stem cell transplant (HSCT). We also explore proposed theories as to why some patients with MHC class II deficiency survive to adulthood, beyond the typical life expectancy.

Results: We present a 23-year-old gentleman who was diagnosed with MHC class II deficiency at the age of 6 months based on a near complete absence of Human Leukocyte Antigen - DR isotype on peripheral blood mononuclear cells and CD4+ lymphopenia. He is one of a few patients with the condition reported in the literature to have survived to adulthood despite not having undergone HSCT. Next generation sequencing revealed a novel homozygous mutation in the *CIITA* gene, 1 of 4 genes involved in the regulation of MHC class II transcription.

Discussion: MHC class II deficiency is considered a single entity phenotypic condition where the main problem lies in reduced or absent MHC class II expression and results in downstream immunologic effects, including CD4+ lymphopenia and impaired antigen specific responses. However, phenotypic differences between patients are emerging as more cases are described in the literature. Our patient, now 23 years old, has survived significantly beyond life expectancy despite not having HSCT.

Statement of novelty: We describe a case of an adult patient diagnosed with MHC class II deficiency due to a novel homozygous intronic splice site variant in the *CIITA* gene.

Background

Major histocompatibility (MHC) class II deficiency is a rare autosomal recessive primary immunodeficiency with fewer than 200 patients reported worldwide (Ben-Mustapha et al. 2013). Most of these reported cases have been observed in North African populations, although some cases have been observed in other populations, especially in those of high consanguinity (Villard et al. 2001; Ouederni et al. 2011; Aluri et al. 2018). Patients usually present within the first year of life with severe and recurrent infections, failure to thrive, and chronic diarrhea.

The disorder is caused by absent or reduced MHC class II expression on cell surfaces. The lack of MHC class II leads to defective antigen presentation, thereby leading to impaired CD4+ T-cell development and

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activation, and impaired T-helper cell-dependent antibody production by B cells. Ultimately, both cellular and humoral immune responses to foreign antigens are affected.

The defect is not in the MHC class II genes themselves but rather in any one of the 4 regulatory genes involved in MHC class II gene transcription: *CIITA* (class II transactivator), *RFXANK* (regulatory factor × associated ankyrin containing protein), *RFX5* (regulatory factor × 5) and *RFXAP* (regulatory factor × associated protein) (Villard et al. 2001; Hanna and Etzioni 2014). Mutations in these regulator genes are respectively classified into what are known as complementation groups A, B, C, D (Villard et al. 2001; Hanna and Etzioni 2014).

Due to the lack of antigen-specific immune responses, patients with MHC class II deficiency are susceptible to severe and recurrent fungal, bacterial, viral and protozoal infections, primarily affecting the respiratory and gastrointestinal tracts (Villard et al. 2001; Hanna and Etzioni 2014). Because the MHC class II molecule plays a critical role in negative thymic selection of CD4+ T-cells, MHC class II deficiency is also associated with a higher risk of autoimmunity manifesting as autoimmune cytopenias and sclerosing cholangitis (Hanna and Etzioni 2014). The disease is associated with a poor prognosis, with most patients dying in early childhood due to infectious complications.

Hematopoietic stem cell transplant (HSCT) is the only therapeutic option for cure, but its success rate is lower than in other primary immunodeficiencies even when an HLA-matched donor is available (Klein et al. 1995; Renella et al. 2006).

There are few reports of patients surviving until adulthood without HSCT (Quan et al. 1999; Wiszniewski et al. 2001; Prod'homme et al. 2003; Ouederni et al. 2011). We report on the experience of a now 23-year-old male patient of Palestinian descent with MHC class II deficiency who was treated in Canada and did not undergo hematopoietic stem cell transplant. Genetic analysis revealed a novel homozygous mutation in the *CIITA* gene. We also review the literature for other adults with the condition and theories as to why some patients may have a longer survival than others.

Case presentation

We present patient DH, now a 23-year-old gentleman who was diagnosed with MHC class II deficiency at the age of 6 months, when he was hospitalized with bilateral interstitial pneumonia presumed to be *Pneumocystis jiroveci* pneumonia. The family history was notable for consanguineous parents of Palestinian descent. The diagnosis was made based on a near complete absence of Human Leukocyte Antigen - DR isotype (HLA-DR) on peripheral blood mononuclear cells, and CD4+ lymphopenia. His family chose not to perform hematopoietic stem cell transplant because his biological sister and cousin, both affected with the same disorder, had died after transplantation. To prevent infections, he was started on monthly infusions of IVIG and antibiotics for prophylaxis against *P. jiroveci* pneumonia.

Concerns since infancy have been failure to thrive, malabsorption, and chronic diarrhea of unclear cause. The patient required a gastric tube for supplementary nutrition at around age 9 until age 20 when the gastric tube was removed at his request. However, dysphagia from increasingly difficult-to-treat oral and esophageal candidiasis made it challenging for him to maintain adequate oral intake. The gastric tube was reinserted after a drop in weight from a highest of 40 kg down to 34 kg.

In terms of infections, in childhood, DH had recurrent ear infections and recurrent gastrointestinal infections with *Clostridium difficile*, *Giardia intestinalis*, and rotavirus. He was frequently hospitalized for febrile neutropenia and pneumonias caused by Coronavirus, Rhinovirus and *Pseudomonas pneumoniae*. By age 8, he developed bronchiectasis. As mentioned, he continues to have chronic and persistent oral and esophageal candidiasis with *Candida glabrata* and *Candida albicans*, which has been difficult to treat due to drug resistance. Up to age 20, his infections responded well to oral and parenteral antibiotics and required only short admissions to hospital.

Over the past year, his health has deteriorated with nearly monthly admissions to hospital for recurrent pneumonias, episodes of febrile neutropenia, and autoimmune hemolytic anemia requiring blood transfusions. He has had recent lung colonization with *P. aeruginosa*. Recently, he has had a trend of increasingly elevated liver enzymes in a cholestatic pattern and

Table 1: **Immunologic investigations.**

	7 mo of age	7 y old	15 y old	23 y old
Lymphocytes (cells/ μL)				
Total CD3	1328	1049	656	619
CD4	**206 (low)**	**151 (low)**	**98 (low)**	**126 (low)**
CD8	1026	779	482	450
CD19+	3361	864	568	242
CD3-/CD56+16+	226	149	89	81
MHC II expression (cells/μL)				
CD19+/HLA-DR+	**0.2% (low)***	ND	ND	ND
CD45+/HLA-DR+	**0.2% (low)***	ND	ND	ND
CD20+/HLA-DR+	ND	**0.1% (2)**	ND	ND
CD3-/HLA-DR	ND	**0.1% (2)**	ND	**0%**
CD3+/HLA-DR+	ND	**0 % (0)**	ND	**0%**
Total HLA DR	ND	**0.1% (2)**	**1**	**0%**
PHA (N>400)	—	—	—	1040 (Ctrl:1699)
Immunoglobulins (g/L)				
IgG	—	—	11.9**	9**
IgM	—	—	1.9 **	1**
IgA	—	—	4.12**	0.1**

*Cell count was not reported.
**While the patient was on IVIG.

hepatomegaly, causing concerns of progressive hepatic dysfunction. Our work-up has not yet been able to elucidate the cause of his liver dysfunction, though *Cryptosporidium* infection and sclerosing cholangitis is often implicated as a cause of progressive liver dysfunction in other reported cases (Hanna and Etzioni 2014).

Immunologic findings for our patient are summarized in Table 1, which mainly shows CD4+ lymphopenia, and a profound deficit of HLA-DR expression on lymphocytes.

He continues to be treated supportively with intravenous immunoglobulin (IVIG) therapy every 3 weeks, trimethoprim-sulfamethoxazole for prophylaxis against *P. jiroveci* pneumonia, and clotrimazole for recurrent oral and esophageal candidiasis. Apart from his long survival with this disorder without HSCT, his clinical features and immunologic findings are consistent with what is described in the literature.

Genetic evaluation

Sequence analysis (Primary Immunodeficiency Panel, Blueprint Genetics) revealed a novel homozygous intronic splice site variant in *CIITA*, resulting in c.3317+2dup. This mutation is predicted to weaken the natural splice donor site leading to aberrant splicing as well as skipping of exon 18.

Discussion

MHC class II deficiency is considered a single entity phenotypic condition in that patients have reduced or absent MHC class II expression, and patients generally share some of the "classic", expected downstream immunologic and clinical consequences of this defect, such as CD4+ lymphopenia, and impaired antigen specific responses. However, phenotypic differences between patients are emerging as more cases are described in the literature. For example, the condition was originally described as a disease that can be lethal in childhood, but there are reported cases of adults in their twenties and thirties (see Table 2), including our patient DH (Quan et al. 1999; Wiszniewski et al. 2001; Towey and Kelly 2002; Prod'homme et al. 2003; Ouederni et al. 2011). There are also reports of patients who present with mild symptoms. These observations have an important implication in that that there may be factors at play in these patients that can prolong survival even without HSCT.

Several theories have been proposed to explain phenotypic differences such as longer survival and milder disease course. One theory is that there is some degree of residual activity of the mutant regulator protein. For example, in a report by Wiszniewski et al. (2001), 3 sisters, the Sa sisters, with mild/asymptomatic disease with a homozygous missense mutation in *CIITA* were

Table 2: **Reports of adults with MHC class II deficiency who did not undergo hematopoietic stem cell transplant.**

Reference	Patient identifier	Gender	Ethnicity	Age of diagnosis (y)	Age at last reported follow-up	Mutation	Clinical features	Immunologic features	Treatment	Other notes
Ouederni et al. 2011	Patient #6	M	Algerian	5	32	Complementation group B: 753delG-25, a 26-bp deletion in the *RFXANK* gene	Protracted diarrhea ENT infections Recurrent pneumonias Failure to thrive Progressive liver disease Autoimmune cytopenia	Not available	IVIG, antibiotics for PJP prophylaxis	Reported to have a Karnofsky Performance Scale Index of 90%.
	Patient #14	M	Algerian	2	23	Complementation group B: 753delG-25, a 26-bp deletion in the *RFXANK* gene	Protracted diarrhea ENT infections Pneumonia Failure to thrive Progressive liver dysfunction	Not available	IVIG, antibiotics for PJP prophylaxis	Reported to have a Karnofsky Performance Scale Index of 90%
Prod'homme et al. 2003	SM	F	Jewish-Egyptian	20 (with symptoms starting at 18 mo that were initially unexplained)	29	Complementation group B: IVS4+5G>A mutation, Splicing defect in *RFXANK* leading to a truncated protein	Recurrent pneumonias Failure to thrive COPD Chronic diarrhea GI infections: *Giardia*, *campylobacter*, *Salmonella* Iron deficiency Malabsorption	Initial immunoglobulins were normal but waned overtime, presence of antibodies to polio and measles and mumps. CD4 lymphopenia	IVIG, total parenteral nutrition	Was not followed for 13 y and from ages 23–29 was free of infections while on IVIG
Wiszniewski et al. 2001	SaE	F	Greek	15	24	All 3 patients are sisters with the same mutation in complementation group A: mutation in the *CIITA* gene with a L469P substitution	Healthy, several episodes of gastroenteritis in infancy, 2 episodes of pneumonia in childhood	Normal immunoglobulins, CD3, CD4, CD8, CD19, CD20, NK cell counts. Serum Abs to *S. pneumoniae* & *H. influenzae* detected. Normal PHA	No treatment; antibiotics in childhood	—
	SaM	F	Greek	12	21		Septicemia (age 3) Pneumonia (age 5) Recurrent respiratory infections since age 9 Asymptomatic for 3 y up to last reported follow-up	Mild CD4+ lymphopenia. Normal CD3, CD8, CD19, CD20 & NK cell counts. Normal IgG, low IgM, low IgA. Absent Ab to *S. pneumoniae*, *H. influenzae* & Candida. Normal PHA	IVIG from age 10 to 15, occasional antibiotics	—
	SaA	F	Greek	11	22		Recurrent respiratory infections Bronchiectasis Recurrent HSV infections Hepatosplenomegaly Lymphadenopathy Atrial Septal Defect Short stature	Normal CD3, CD4, CD8, CD19, CD20, NK cells. Low IgG, IgA. Normal IgM. Normal PHA	IVIG	—
Quan et al. 1999; Towey and Kelly 2002	Fern	M	Not reported	27	33 (died)	Complementation group A: single aa substitution (phenylalanine to serine substitution), located at position 962 within the carboxy terminal region of CIITA, that correlated with lack of class II gene transcription, lack of CIITA translocation in the nucleus	Recurrent bacterial infections (details and other clinical features not reported)	Not reported	Not reported	Became symptomatic in his 30s and succumbed to multiple bacterial infections
Hsieh et al. current article	DH	M	Palestinian	6 mo	23	Complementation group A: homozygous for *CIITA* mutation (splice donor variant c.3317+2 dup)	Failure to thrive, chronic diarrhea, esophageal and oral candidiasis, recurrent pneumonias with bronchiectasis, hepatic dysfunction, autoimmune cytopenias, episodes of febrile neutropenia	CD4 lymphopenia, hypogammaglobulinemia	IVIG, antibiotics for PJP prophylaxis, clotrimazole for oral and esophageal candiasis, gastric tube for supplementary nutrition	Karnofsky Performance Scale Index of 60%.

found to have faint expression of HLA-DP, -DR, and -DQ on B cells and monocytes, leading one to speculate that there may be some residual functional activity in the mutated *CIITA* gene (Wiszniewski et al. 2001). When the mutant gene was transfected in a CIITA-deficient cell line, HLA-DR expression was restored in 30% of cells (Wiszniewski et al. 2001). Interestingly, they also found that the mutated protein is able to translocate into the nucleus whereas other known CIITA mutated proteins cannot (Wiszniewski et al. 2001). As was reported on the Sa sisters, our patient, DH, did not have complete absence of MHC class II molecules. Instead, he had near absent, but detectable expression of HLA-DR on 0.1% of T and B cells meaning there could be residual activity of the defective CIITA regulator protein. We will need to follow-up with functional analyses of DH's mutation to explore how this defective regulatory protein behaves. But, if indeed the mutation that DH has leads to a defective regulatory protein with partial activity, it could be one explanation as to why his sister, who presumably had the same mutation, may have died from complications with HSCT. Engraftment failure due to residual adaptive immunity has been cited as one of the major reasons why HSCT in this population has had only limited success in the past (Klein et al. 1995; Renella et al. 2006; Gennery et al. 2010).

There may be other factor(s) apart from residual activity that explains the differences in immunologic phenotype that we have yet to explain. Ouederni et al. (2011) reported on 35 patients of North African descent with the same genetic mutation, a homozygous I5E6-25_15E6 +1 deletion in *RFXANK*. It is a mutation seen in approximately 70% of patients with MHC class II deficiency and was traced to an ancestor belonging to the Berber civilization who lived 2250 years ago (Ouederni et al. 2011). Despite having the same genetic mutation, there was wide variability in their phenotypes. For example, while many patients had died in childhood due to infectious complications or from complications after HSCT, 4 of 12 patients who did not undergo HSCT had reached puberty, and of these 4 patients, only 1 patient displayed residual MHC class II expression on B cells (Ouederni et al. 2011). For the other 3 patients, is unclear what factors would have influenced their survival.

Another theory that has not been well explored is the possible presence of immune compensatory mechanisms that make up for the loss of MHC class II expression, for example upregulation of MHC I or innate immunity (Prod'homme et al. 2003). Furthermore, there could be external factors such access to medical care, hygienic practices, social support, and other environmental factors (Prod'homme et al. 2003; Ouederni et al. 2011).

In summary, we present a case of a 23-year-old gentleman with MHC class II deficiency with a novel c.3317+2dup homozygous *CIITA* mutation. Based on a review of the literature, there are other cases of MHC class II deficient patients who survive into adulthood, significantly beyond the expected life expectancy even without HSCT. We can only conjecture why there is this variability in immunologic phenotype given the small number of patients reported in the literature, but there are likely to be undefined factors that have a major impact on the severity and course of the disease.

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Chronic granulomas of the skin triggered by rubella virus in a patient with ASXL1 mutation

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Introduction: ASXL1 somatic mutations have been implicated in patients with myelodysplastic syndrome, acute myeloid leukemia, and acquired aplastic anemia. Mutations in other regions of the gene have been implicated in patients with severe developmental issues and malformation disorders. We report here a patient with chronic granulomatous skin lesions, learning difficulties, and mild myelodysplasia with an ASXL1 mutation.

Case Presentation: An 11-year old female of Taiwanese, Scottish and Norwegian descent with a history of learning difficulties, blood dyscrasia, and hypocellular bone marrow was referred to Immunology at 3 years of age with a history of chronic granulomas of the skin. She initially presented at 3 years of age with erythematous macules on the left leg and right arm which progressed to crusted and ulcerated plaques. She was treated with multiple courses of oral antibiotics as well as topical antimicrobial and antifungal therapy. She was immediately referred to Dermatology and underwent multiple punch biopsies which revealed non-specific necrotizing granulomas with ulceration. She was subsequently referred to Immunology for consideration of underlying primary immunodeficiency (PID). Microbiology studies performed on a full skin biopsy of the granulomatous lesion in 2016 were negative for polymerase chain reaction (PCR) of mycobacterial species, and bacterial and fungal cultures. The patient was referred to Hematology/Oncology for persistent macrocytosis (range 94 to 98 fL) unresponsive to folate and vitamin B12 supplementation. As a result of the treatment failure of supplementation, bone marrow biopsies were performed in 2012 and repeated in 2016. Results revealed findings suggestive of a possible myelodysplastic syndrome or an early bone marrow failure syndrome.

Further infectious history revealed 2 episodes of bacterial pneumonia, recurrent acute otitis media, and 1 episode of Ebstein-Barr virus (EBV) infection. Most recently, she developed viral warts on her right middle finger and feet at 10 years of age. The patient was born at term with no significant complications in the neonatal period. There was no history of consanguinity. Family history was significant for rheumatoid arthritis in the mother. All routine childhood immunizations, including live viral vaccines, were administered and tolerated. Physical examination was positive for subtle dysmorphic features. Her weight and height consistently fell between the 3rd to 15th percentiles. However, her head circumference fell between the 50th to 75th percentiles. The skin exam was significant for chronic granulomatous lesions with several ulcerated lesions located on the skin overlying the right upper arm, knees bilaterally, and left anterior thigh and popliteal fossa. Residual atrophic scarring at previous sites of granulomas were present. Examination of the abdomen revealed no evidence of hepatosplenomegaly.

Investigations: The 1, 2, 3 Dihidrorhodamine assay was normal at 3 years of age which ruled out chronic granulomatous disease. A sweat chloride test was negative. CBC was normal including normal lymphocyte and neutrophil counts. She had persistent macrocytosis but no anemia. Immunoglobulin levels including IgG, IgA, IgM, IgE were normal at 4 years of age. Lymphocyte immunophenotyping results at age 4 years

are as follows ($\times 10^9$ cells/L): low CD3 of 0.26 (0.9–4.5), low CD4 of 0.17 (0.5–2.4), and low CD8 of 0.07 (0.3–1.6). B lymphocyte counts and NK cells were normal. Vaccine titres to diptheria and pertussis were normal at 4 years of age, however she had a very high rubella titer of >500 IU/mL. Low quantitative immunoglobulins were noted with an IgG of 3.85g/L (6.20–19.10 g/L) starting at age 6, low IgA of 0.24 g/L (0.3–2.9 g/L) at age 8 and a normal IgM of 0.61 g/L (0.31–1.80 g/L). Urine and blood PCR for rubella virus was negative. Rubella virus was isolated in a skin biopsy suggesting underlying etiology of her chronic skin lesions. Genetic testing including microarray and testing for Fanconi anemia were normal. Cytogenetics revealed a normal karyotype. FISH was normal for 22q11.2deletion. Whole exome sequencing (WES) revealed that she is a compound heterozygote for ASXL1 mutation with both parents demonstrated to each be carriers of a different point mutation in the same gene. The patient is currently followed by Immunology, Hematology/Oncology, Dermatology and Medical Genetics for ongoing management.

Discussion: Granulomas may develop in response to a local antigenic trigger leading to activation of macrophages and T-lymphocytes. Chronic granulomas have been reported in pediatric patients with PID such as T-cell immunodeficiency, and in patients with ataxia telangectasia (AT). AT is caused by a loss of function mutation in the ATM gene on chromosome 11q22 which encodes a protein kinase involved in repair of double stranded DNA breaks. In case reports of patients with AT, prolonged antibiotic treatment for chronic granulomas showed no clinical improvement, and repeated microbial cultures of the lesions and PCR for viruses were negative. Further testing of granulomatous lesions with high throughput sequencing (HTS) detected sequences of rubella virus vaccine. These results indicate that the rubella virus may persist on the skin of individuals with PID. Rubella virus has also been isolated in patients with recombinant-activating gene (RAG) deficiencies.

There are currently no antiviral drugs approved to treat chronic rubella infections. Nitazoxanide (NTZ) has been approved by the Food and Drug Administration for treatment of enteritis secondary to parasites, protozoa, and anaerobic bacteria. It also has broad-spectrum antiviral activities by targeting host functions that are involved in viral replication. A case report of a patient with B-cell and T-cell dysfunction revealed rubella virus (RV) in the epidermis via immunofluorescence staining of a skin biopsy. The patient was treated with 500 mg of NTZ twice daily for 2 months. Repeat immunostaining of skin biopsies taken from the same lesion revealed almost complete elimination of rubella virus antigen from the lesions. The treatment did not, however, result in noticeable clinical improvement of the granuloma despite elimination of the antigen. Further studies on the inhibitory effects of NTZ on RV infection revealed inhibition of production and reduction in the number of RV-positive cells in the NTZ-treated cultures in comparison to controls. NTZ can inhibit replication of different RV strains which have been isolated in patients including RA27/3 vaccine strain. Furthermore, studies on the effect of NTZ on viral replication was observed when the treatment was initiated in the early stage of the replication cycle. Anti-viral mechanisms of NTZ are poorly understood however, it appears to act broadly on cellular pathways involved with RNA synthesis as opposed to specific viral antigens. Based on these findings, NTZ may be effective in preventing spread of the RV which could prevent formation of granulomatous lesions in new locations on the body of infected patients.

Conclusion: Based on the literature describing persistence of RV strains in patients with features of PID and evidence to suggest that treatment with NTZ can eliminate the RV antigen and inhibit replication of the virus, it is important to consider more robust testing such as HTS earlier in the diagnostic work up of patients with chronic granulomas of the skin resistant to treatment. Although NTZ was successful in eliminating the virus in skin biopsies of affected patients, clinically, the granulomas persisted. If the virus is detected earlier in the clinical course via specific testing for RV strains, it is possible to initiate treatment early to prevent long-term atrophic scarring.

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Novel heterozygous PIK3CD mutation presenting with only laboratory markers of combined immunodeficiency

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Introduction: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta (PIK3CD) is a heterodimer of p110δ and p85 subunits, found primarily in leukocytes. It generates phosphatidyl-inositol 3,4,5-trisphosphate (PIP3), a key recruiter of PH domain-containing proteins, including AKT1 and PDPK1, which are the initiators for activating signalling cascades. It is involved in mediating cell growth,

survival, proliferation, motility, morphology; in lymphocytes including T-, B- and NK-cells. Recently an increasing number of patients have been described with heterozygous PIK3CD gain of function mutation, leading to a combined immunodeficiency with both B- and T-cell dysfunction. They suffer recurrent respiratory infections, often associated with bronchiectasis and ear and sinus damage, as well as severe recurrent or persistent infections by herpes viruses, including EBV induced lymphoprolipheration.

Patient Description: We describe here 2 patients—mother and daughter—with heterozygous PIK3CD mutation, both diagnosed following an abnormal newborn screen for severe combined immunodeficiency (SCID) in the child.

Patient 1: A currently 3.5 years old female patient, presented with initial abnormal newborn screen for SCID and T cell lymphopenia. She has had no recurrent infections and had not needed antibiotic therapy, no warts, or fungal infection of nails or thrush. Her initial TREC results on newborn screen was low, 11.3 copy/3 μL. Whole blood TREC 588, which is below the normal. Her laboratory results were remarkable for intermittent leukopenia, attributed to lymphopenia and neutropenia. Lymphocyte immunophenotyping showed low total CD3+, CD4+, and CD8+ levels, normal Nk, and CD19+ B cell counts. Her CD45RA/RO of CD4+ cells demonstrated low number of naiveCD4 cells. In vitro responses to PHA were depressed initially, but gradually increased.

Patient 2: Mother of patient 1, had been evaluated due to the laboratory findings in her daughter and was found to have an individual heterozygous mutation in the gene PIK3CD. She has had a history of eczema and otitis during childhood. Her laboratory work up showed normal complete blood count, normal differential and normal lymphocyte immunophenotyping. However her CD45RA/RO of CD3+ and CD4+ cells demonstrated a lower number of naive cells, and elevated memory population compared to control, similar to her daughter. She also had reduced in vitro responses to PHA as well as low in vitro T cell responses to stimulation with specific antigens.

Signalling evaluation were done on both on peripheral blood lymphocytes as well on flow cytometry assisted selected T-cells (Surface phenotypes were determined by flow cytometry analysis on a Coulter EPICS V Flow Cytometer from Beckman Coulter (Brea, Calif)), following ficoll separation. Both patient`s and control cells were selected, and either left un-stimulated or were stimulated with anti-CD3 UCHT1 antibody (5 μg for 4×106 cells), and incubated for 10 minutes on 37 °C. Following this 1%Triton-X100 lysis buffer was used for cell lysis. Whole-cell lysates were analyzed by Western blotting. All blots were repeated at least twice. The antibodies used for Western blotting were: Anti-pAKT Ser473(Invitrogen), anti- Gα(i) (CellSignaling). The signalling test shows a clear increase in the baseline phosphorylation in patient 1.`s T-cells, as well as both patients show hyperactivation of the catalytic domain in different levels, resulting in increased phosphorylation of AKT on activation.

Discussion: Gain of function mutations affecting the PIK3CD gene, encoding the catalytic subunit of phosphoinositide 3-kinase, were described in the past few years, as an autosomal dominant disease associated with lymphadenopathy, autoimmune cytopenias, splenomegaly, susceptibility to EBV, CMV as well as HSV infection, and an increased risk for lymphoprolipheration leading to Activated PIK3-delta syndrome (APDS). The clinical course of APDS is highly variable, ranging from combined immunodeficiency, with recurrent infections, autoimmune complications, requiring stem cell transplantation, through isolated antibody deficiency, to asymptomatic adults.

The presentation of the patient described here is unique, as no other case with gain of function (GOF) PIK3CD defect had been detected by newborn screen (NBS) for SCID (severe combined immunodeficiency). Surprisingly the clinical course so far had been unremarkable. Further, the mother appears to be completely asymptomatic inspite of having an identical mutation to her daughter.

Nevertheless, the persistent lymphopenia in the proband, of clear GOF origin as demonstrated by AKT phosphorylation, indicate PIK3CD dysfunction. Because of the wide gap between laboratory findings and clinical manifestations, this kindred poses both a diagnostic as well as a of treatment challange.

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A case of *KAT6A* mutation associated with immunodeficiency and granulomatous lymphocytic interstitial lung disease

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Background: The *KAT6A* gene encodes a lysine acetyl transferase which is involved in histone acetylation. It is implicated in the regulation of gene transcription and expression. *KAT6A* is thought to be required for development of the hematopoietic system and thymic tissue, consistent with the observation that *KAT6A* knockout mice are neutropenic (Katsumoto et al. 2006; Sheikh et al. 2017). In the clinical setting, *KAT6A* mutation has been associated with a syndrome of intellectual disability, hypotonia, feeding difficulties, cardiac defects, craniosynostosis, and distinct facial dysmorphisms. Here we present the case of a boy with *KAT6A* deficiency who had additional immunological features not previously reported in patients with *KAT6A* mutations. These features included recurrent infections, hypogammaglobulinemia, splenomegaly and pancytopenia. Moreover, he developed granulomatous lymphocytic interstitial lung disease (GLILD), a condition commonly seen in patients with common variable immunodeficiency (CVID).

Case Presentation: The patient was referred to the Pediatric Immunology Clinic at the age of 11 years, following hospitalization with *Streptococcus pneumoniae* bacteremia and pneumonia. A chest X-ray done at the time of admission showed multifocal infiltrates. A blood culture identified *S. pneumoniae* bacteremia and the patient was treated with appropriate IV antibiotics. Serum IgG at that time was undetectable (<0.5 g/L). The patient had a history of 1 previous admission for pneumonia in which multifocal infiltrates were identified on his Chest X-Ray, and for which he was treated with levofloxacin. He furthermore had a 6-year history of clinically diagnosed pneumonias that occurred approximately once a year and were treated with oral antibiotics in the community. He did not have any history of sinus, ear, or skin infections, nor had he had any other episodes of bacteremia, meningitis, abscesses, dental infections, or osteomyelitis. He had not had fungal or viral infections. He also had a history of splenomegaly and fluctuating mild-to-moderate pancytopenia, followed by Hematology with no specific diagnosis. He had not required any transfusions.

The patient was born via emergency Caesarian section for possible placental abruption and the presence of meconium at 38 + 5 weeks. APGAR scores were 2, 6, and 8 at 1, 5, and 10 minutes respectively and he required positive pressure ventilation and CPAP. MRI at the time was normal but his intellectual delay would later be clinically attributed to Hypoxic Ischemic Encephalopathy. He was noted to have a broad nasal bridge, strabismus, micrognathia, low set ears, a high arch palate and club feet. Initial Genetic and Metabolic workup did not ascertain a diagnosis. Parents are not consanguineous. There is no family history of Immune Deficiency or recurrent infections, or of syndromic features or developmental delay.

Laboratory Evaluation: Upon initial evaluation in the clinic, CBC showed a leukocyte count of 3.6, hemoglobin 113 and platelets 125. Lymphocytes were 1.4 with neutrophils of 2.0. Repeat IgG was 0.34 g/L, IgA <0.07 g/L and IgM 0.41 g/L. Lymphocyte immunophenotyping demonstrated normal T cell counts, but profoundly low B cells with 40×10^6 cells present (lower limit of normal 270×106). Measles, mumps, rubella, and varicella serology were negative despite timely immunization. Neutrophil oxidative burst index testing was normal.

Clinical Evolution: Following the initial consult, he was started on IVIG replacement therapy. Genetic evaluation for X-linked agammaglobulinemia was negative. The patient subsequently presented to hospital with fever and cough and was admitted for treatment of his respiratory infection. He underwent a chest CT scan which showed diffuse pulmonary nodules, mild

interstitial thickening and extensive lymphadenopathy. An echocardiogram was done which demonstrated pulmonary hypertension with RVSP 63 mm Hg and RAP 92 mm Hg. Bronchoscopy was unremarkable and bronchoalveolar lavage was negative for infection or malignancy. A bone marrow aspirate showed no evidence of malignant infiltration. A lymph node biopsy was negative for malignancy. Serum and BAL PCR testing for CMV, EBC, HHV6, and HIV were all negative. Fungal cultures, mycobacteria, legionella, and nocardia were also negative. A lung biopsy specimen showed lympho-histiocytic infiltrate and peribronchial chronic inflammation with non-caseating sarcoid-like granulomas. Also seen were Langerhans giant cells with Shaumann bodies, consistent with granulomatous lymphocytic interstitial lung disease (GLILD). The lung disease was thought to be secondary to his immune deficiency. Treatment was initiated with systemic oral steroids, which greatly alleviated his respiratory symptoms.

Chromosomal microarray was sent and did not reveal a diagnosis. Whole exome sequencing (GeneDX Laboratories) revealed a *de novo* pathogenic heterozygous variant in the *KAT6A* gene, p.R1024X.

Discussion: The *KAT6A* gene encodes a lysine acetyl transferase, which is involved in histone acetylation and regulates gene transcription and expression. It is also known as *MOZ* or *MYST3* and is located on chromosome 8p11. Mutations in *KAT6A* were initially implicated as the cause of recurrent translocations in patients with acute myelogenous leukemia (Tham et al. 2015; Millan et al. 2016). Case reports have identified common dysmorphic and developmental features with autosomal dominant *de novo* mutations in *KAT6A*, which are similar to our case. Features include hypotonia at birth, difficult delivery with low Apgar scores requiring resuscitation, global developmental delay, feeding difficulties, strabismus, micrognathia and broad nasal bridge (Arboleda et al. 2015; Tham et al. 2015). A significant delay in speech has been consistently seen in other reported cases (Tham et al. 2015; Millan et al. 2016).

While this case is morphologically and developmentally in keeping with previously described *KAT6A* mutations, the finding of profound B-lymphopenia and hypogammaglobulinemia is to our knowledge a unique presentation. It is unclear to what extent the protein is involved in immunodeficiency. In rodent studies, *KAT6A* was found to be required for thymus development and the hematopoietic system (Katsumoto et al. 2006; Sheikh et al. 2017). Impaired B cell differentiation was found in knockout mice (Good-Jacobson et al. 2014). These findings are consistent with our case of hypogammaglobulinemia. Recently, a study showed that cyclic neutropenia may also be seen in patients with *KAT6A* mutation and perhaps an under-diagnosed feature (Gauthier-Vasserot et al. 2017). Although Immune Deficiency has not been reported with this diagnosis, recurrent infections have been reported in a few cases (Arboleda et al. 2015; Millan et al. 2016).

To our knowledge, this is also the first case of a patient with *KAT6A* mutation with confirmed GLILD. There have been 2 reported cases where phenotypic features of a *KAT6A* mutation had chronic lung disease5, but not specifically granulomatous disease. GLILD is a well-known complication of Common Variable Immune Deficiency, in which hypogammaglobulinemia is the central feature (Park and Levinson 2010). This would suggest that the immune features in this case may have contributed to the patient's development of GLILD.

Conclusion: Here we report a case of a patient with a novel *KAT6A* mutation, presenting with dysmorphisms and developmental abnormalities typical for this disorder, as well as pancytopenia, profound B-lymphopenia, hypogammaglobulinemia, and granulomatous lymphocytic interstitial lung disease (GLILD). We postulate that this mutation may be responsible for our patient's immune deficiency as well as his lung inflammation. We propose that patients with *KAT6A* mutation be screened for such issues with immunoglobulin quantification, lymphocyte immunophenotyping, and, if an immune defect is detected, surveillance lung imaging. This would allow for early intervention with IVIG replacement which may prevent or delay progression to interstitial lung disease.

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Two cases of somatic p.G13C mutation in *KRAS* causing RAS-associated autoimmune lymphoproliferative disease (RALD)

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Introduction: RAS signaling proteins are small, regulatory guanosine triphosphatases (GTP*ases*) that play a critical role in signal transduction and cell proliferation, differentiation, and apoptosis (Aoki et al. 2008; Calvo et al. 2015; Ragotte et al. 2017). The RAS family of proteins consists of Harvey-RAS (HRAS), Kirsten-RAS (KRAS) and neuroblastoma RAS (NRAS) (Aoki et al. 2013). Germline RAS mutations have been associated with specific autosomal dominant developmental disorders, known as "RASopathies", including Costello *(HRAS)*, Noonan *(PTPN11, KRAS*, and *SOS1)* and cardio-facio-cutaneous syndromes *(KRAS, BRAF, MEK1*, and *MEK2)* (Aoki et al. 2008; Niemela et al. 2011; Takagi et al. 2011; Ragotte et al. 2017). These syndromes carry an increased risk of autoimmunity and malignancy (Calvo et al. 2015). Somatic gain-of-function (GOF) mutations in *RAS* genes are found in >30% of all human cancers (Niemela et al. 2011).

There have been several reports of somatic GOF mutations in the *KRAS* and *NRAS* genes associated with a non-malignant syndrome of autoimmunity and dysregulation of leukocyte homeostasis (Niemela et al. 2011; Ragotte et al. 2017). This syndrome has now been defined as RAS-associated autoimmune lymphoproliferative disease (RALD). RALD typically presents with autoimmunity, lymphadenopathy, splenomegaly, cytopenias, and monocytosis (Ragotte et al. 2017). This presentation can appear similar to autoimmune lymphoproliferative syndrome (ALPS), however, unlike ALPS, patients with RALD lack the characteristic peripherally expanded CD4-/CD8- 'double-negative' T-lymphocytes, do not have a defect in the FAS pathway of apoptosis, and lack the biomarkers typically associated with ALPS (IL-10 and sFASL) (Niemela et al. 2011; Ragotte et al. 2017).

We describe 2 cases of RALD caused by somatic p.G13C mutations in the *KRAS* gene, each presenting with a distinct phenotype, the first of which has been recently published (Ragotte et al. 2017).

Patient 1: A non-dysmorphic Canadian First Nations boy, who was the product of a dichorionic-diamniotic twin pregnancy first presented to the healthcare system at age 4 years with massive cervical lymphadenopathy causing superior vena cava obstruction. An excisional biopsy of a lymph node revealed a marked histiocytic (CD68+, S100+) infiltrate, considered to be in keeping with Rosai-Dorfman disease. He was treated with prednisone, methotrexate and mercaptopurine, however, the lymphadenopathy persisted. Following treatment, he developed pancytopenia and hepatosplenomegaly. A bone marrow biopsy revealed non-specific reactive changes, with no evidence of myelodysplasia, malignancy or hemophagocytosis.

At age 7 years, he presented with a pericardial effusion and cardiac tamponade. Pericardiocentesis revealed serosanguinous fluid with predominant polymorphonuclear cells and lupus erythematosus (LE) cells. Investigations showed antinuclear antibody (ANA) elevated at 1:1280, and positive anti-double stranded DNA (anti-ds-DNA) meeting the clinical diagnostic criteria for SLE. He later developed non-erosive and non-deforming inflammatory arthritis of his wrists, knees, ankles, and fingers. There was a positive family history of autoimmunity with SLE in the maternal aunt and rheumatoid arthritis (RA) in the maternal grandmother.

Immune studies showed hypergammaglobulinemia with IgG 20.7–25 g/L (normal, 5–14.6 g/L), with normal IgA 0.76 g/L (normal 0.4–2.4 g/L), and IgM 0.38 g/L (0.15–1.88 g/L), and no increase in CD4-CD8- double negative T cells, inconsistent with ALPS.

Because of the rare combination of Rosai-Dorfman syndrome and SLE, and unusually severe disease course, whole exome sequencing (WES) was conducted and revealed a mutation in the *KRAS* gene (c.37G>T; p.Gly13Cys). This mutation was unique to the patient, and not present in either parent. Sanger sequencing of the patient's saliva-derived DNA showed a lower peak for the *KRAS* c. 37G>T mutation compared to the whole blood-derived DNA, consistent with somatic mosaicism.

His treatment course over the years has consisted of pulse high-dose corticosteroids, rituximab, intravenous cyclophosphamide and azathioprine. Most recently, his disease has been relatively well controlled on mycophenolate mofetil (MMF), although he continues to have stable mild pancytopenia, hepatosplenomegaly and intermittent arthritis. His growth has also been very restricted, which was thought to be secondary to his underlying condition, in addition to chronic corticosteroid use.

Patient 2: This non-dysmorphic, previously well, Caucasian girl first presented at age 5 years with thrombocytopenia, lymphopenia, neutropenia and monocytosis, with marked splenomegaly. A bone marrow biopsy was judged to be normal with no evidence of malignancy or myelodysplasia.

Immunologic workup revealed hypergammaglobulinemia with IgG 22.4 g/L (normal 5.1–13.6 g/L), IgA 3.03 g/L (normal 0.25–1.9 g/L), and IgM 2.10 g/L (normal 0.31–2.08 g/L). Flow cytometry showed a uniform decrease in T and B cell numbers with normal NK cell numbers. There was no increase in CD4-CD8- T cells, ruling out ALPS. Vaccine titres showed positive diphtheria (1.94IU/mL) and tetanus (4.45IU/mL) antitoxins. WES revealed a pathogenic variant in the *KRAS* gene (c.37G>T; p.Gly13Cys) in the patient, but neither parent. Experiments to formally establish the suspected somatic nature of this mutation are ongoing.

Discussion: We present 2 unusual cases of somatic p.G13C mutations in the *KRAS* gene. This amino acid position is located within the p-loop of the KRAS protein, where it plays an active role in GTP-hydrolysis (Lu et al. 2016). It is hypothesized that the substitution

of glycine with a larger amino acid such as cysteine diminishes KRAS GTPase activity, leading to a GOF phenotype, driving cellular proliferation and inhibiting T-cell apoptosis (Niemela et al. 2011). *KRAS* mutations in this position have been reported in malignancy, RALD and Noonan syndrome (Ragotte et al. 2017). Neither patient had the clinical features of Noonan syndromes, nor evidence of malignancy, making RALD the most appropriate unifying diagnosis.

Calvo et al. (2015) presented 13 patients with RALD, all of whom presented with cytopenias, hypergammaglobulinemia, splenomegaly, B-cell lymphocytosis and persistent relative or absolute monocytosis. Two of this cohort were found to have the same pG13C KRAS mutation.

Niemela et al. (2011) also reported the case of a Caucasian female who presented at 4 years of age with splenomegaly, autoimmune hemolytic anemia and thrombocytopenia, with polyclonal hypergammaglobulinemia and positive serology for several autoantibodies, who was also felt to have RALD.

Both of our patients similarly presented with cytopenias, splenomegaly, hypergammaglobulinemia and monocytosis. Patient 1 had the additional complexity of meeting diagnostic criteria for both Rosai-Dorfman syndrome and SLE, and is the first reported RALD case showing histiocytosis.

Although there is currently no specific molecularly-targeted treatment for RALD, research is currently underway to develop both broad-spectrum KRAS inhibitors and inhibitors that target the specific KRAS mutations found in malignancy, both of which have potential applications in patients with RALD (Ragotte et al. 2017).

Conclusions: RALD should be considered on the differential diagnosis of patients presenting with autoimmune disease, cytopenias, lymphoid organ expansion and monocytosis. In addition, RALD should be considered in cases where patients are suspected to have ALPS, but are lacking the characteristic 'double negative' T cell expansion.

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Chronic mucocutaneous candidiasis (CMCC): a patient presenting with a novel mutation in the IL-17 receptor alpha

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Background: Chronic mucocutaneous candidiasis (CMCC) has traditionally encompassed endocrinopathy, autoimmunity and infection of the skin, nails, oral and genital mucosa typically caused by *C. albicans*; an organism that is found to be commensal in healthy individuals. Most cases thus far were found to have mutations in AIRE or STAT1. In recent years, chronic candida infections which may be a common feature in multiple profound T cell deficiency, can also be identified in a rare group of more selective immune defects. IL-17RA is an example of such a defect which is inherited in an autosomal recessive fashion.

There have been so far a number IL-17RA deficiency reports proposing the association of *S. aureus* skin infections and various degrees of candidiasis. Functionally, IL-17RA is expressed on neutrophils and is activated via the binding of the cytokine IL-17 which appears to contribute to cutaneous immunity to *C. albicans* and *S. aureus* infections. This cytokine is secreted from Th17 cells that are activated in the presence of microbial antigens including *C. albicans* and result in the recruitment of neutrophils to the site of infection (Nahum 2017). Mutations within the IL-17RA could alter effective clearance of these infections thus possibly underlying the phenotype. Most of the mutations reported were homozygous with premature stop codons upstream from the transmembrane domain (Levy et al. 2016). Functionally, these prevent expression of the receptor on the circulating leukocytes.

Methods: We report of patient presenting with the phenotype of chronic candidiasis associated with a deleterious mutation in IL-17 alpha receptor.

Results: Our patient is a 10 year old male, born at term to non-consanguineous parents. The mother is healthy but the father has been diagnosed with Crohn's disease as well as having cutaneous fungal infections with tinea versicolor. The patient presented originally with a history of eczema at the age of 9 months complicated with recurrent superinfection with community acquired methicillin-resistant *S. Aureus*. Other infections in the first few years of life included 1 episode of pneumonia and acute otitis media. He had recurrent oral thrush and diaper rash within the first year of life that did not respond to topical Nystatin. He has till present day, continued on antifungal prophylaxis with Fluconazole and topical Terbinafine, and the thrush and diaper rash had improved. In childhood he had recurrent mouth lesions that were consistent with impetigo and responded well to topical Fucidin. He was also diagnosed with asthma, but had no evidence of endocrinopathy, or other autoimmunity manifestations. Evaluation of the immune system most recently showed a normal IgG level of 8.4 g/L, IgA of 0.8 g/L, and IgM 0.6 g/L. Lymphocyte markers demonstrated normal numbers of CD19(295), CD3 (1361), CD4 (818), and CD8(402) positive cells and NK cells at 160. Specific antibody levels were protective to Rubella, Varicella and Tetanus and a good response to Pneumococcal vaccine. Proliferative responses to phytohemagglutinin were normal.

Genetic testing for STAT1 and AIRE genes were both normal.

Conclusion: We report a child presenting with recurrent *S. aureus* skin infections, atopy and limited oral thrush and diaper rash. He was initially thought to have an AD mutation such as STAT1 given the father's history of autoimmunity. However, mutations of STAT1 and AIRE were negative but whole exome genome sequencing revealed a novel IL17RA mutation. Clinically, this case appears interesting because he presented a diagnostic challenge. While he did have chronic oral thrush and a diaper rash, he also had eczema, skin staph infections and other oral lesions.

Because both *S. Aureus* and Candida are organisms found to be commensal in healthy individuals, these infections can occur after insults to skin such as eczema. One could have argued that the infections in this patient are actually secondary to the initial skin lesions. Moreover, the lack of autoimmune manifestations as well as the limited fungal infection did not support the diagnosis of typical CMCC. Nonetheless, this case highlights the need for a comprehensive genetic analysis in all cases that present with recurrent fungal infections regardless if it is primary or secondary.

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