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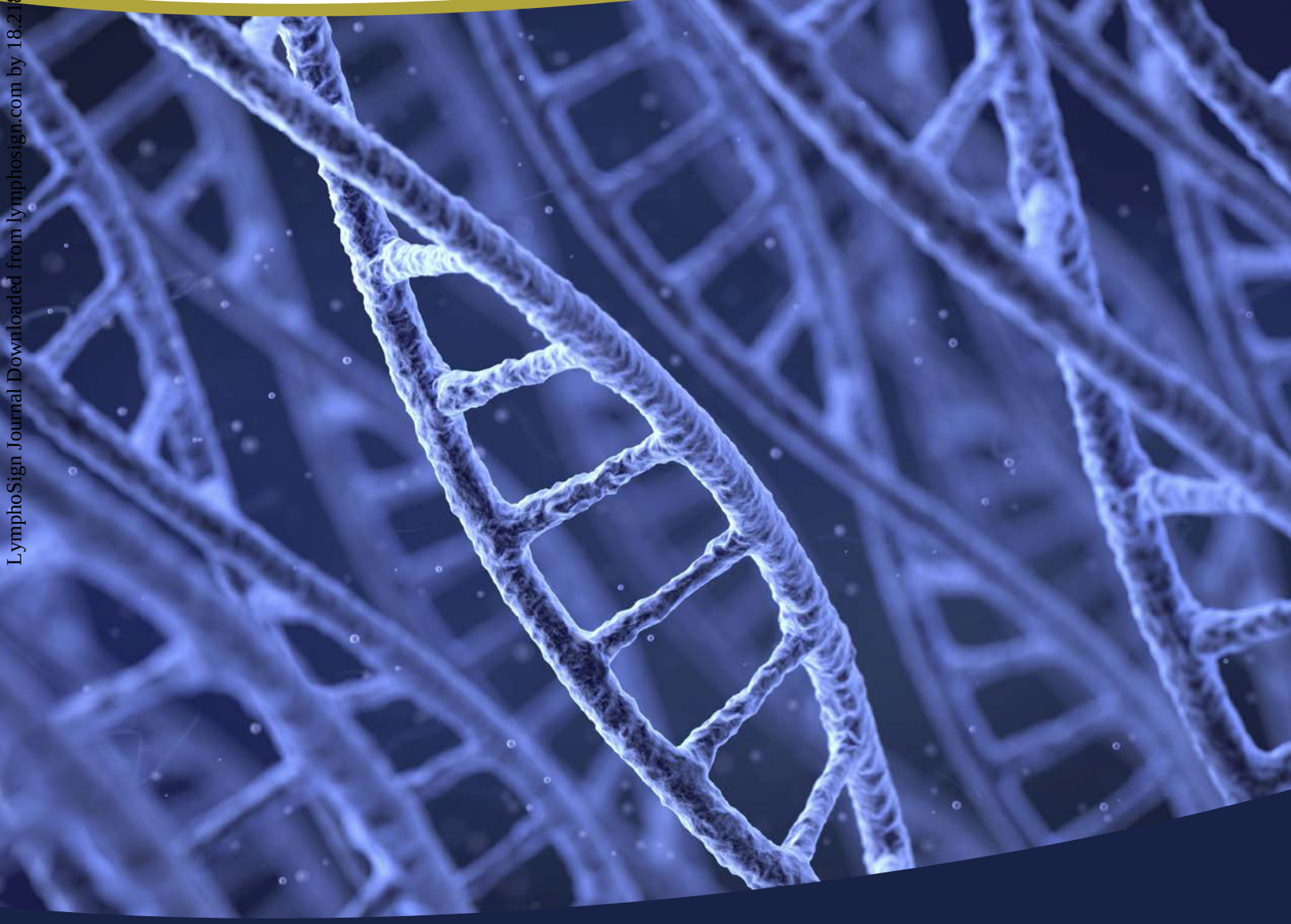

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Histamine, histamine receptors, and anti-histamines in the context of allergic responses

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ABSTRACT

Histamine is a bioactive amine which is considered a key player in the allergic response. Thus, histamine receptor blockers (antihistamines) play an important role in the treatment of a number atopic diseases such as allergic rhinitis, conjunctivitis, and acute and chronic forms of urticaria. Histamine is produced by immune cells but also by bacteria in the gut. Beyond its role in the acute allergic response, histamine exerts numerous effects by binding to its 4 pleiotropic G-protein coupled histamine receptors. Here, we describe the roles of these histamine receptors and antihistamines in the human system, clinical applications, side effects, and novel concepts for the usage of antihistamines with different specificity based on guidelines and recommendations.

Statement of novelty: This review provides an overview of histamine receptors and links it to clinical relevance of antagonizing their action in clinical routine.

Introduction

Histamine is a bioactive amine that acts as a signalling molecule and neurotransmitter. It exerts its diverse biological effects through the activation of 4 types of membrane bound receptors from the aminergic G-protein coupled receptor family: H_1, H_2, H_3, and H_4 (named according to their order of discovery). Due to their pleiotropic expression, these receptors can exert multiple clinical effects, but can also explain the side effects of therapeutics used to modify their action. Following the identification of histamine and H_1 receptors , the therapeutic use of first generation H_1 blockers (which lacked specificity) revealed a number of histamine related effects that were not inhibited. These cardiovascular, gastric (leading to peptic ulcer treatment discovery), neural, as well as various immune cell related mechanisms eventually led to the identification of additional histamine receptors as well and new molecules with agonist and antagonist effects. More recently, low-affinity intracellular non-H_1, -H_2, -H_3, or -H_4 receptors have been described in cell nuclei and microsomes, although the biological functions of these receptors are still somewhat unclear. This review focuses on the role of histamine receptors and anti-histamines in the context of allergic diseases.

Histamine in the context of atopic disease

Receptor structure

In general terms, the 4 histamine receptors are heptahelical G-protein coupled receptors (GPCR), encompassing a diverse group of membrane receptors

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composed of a single polypeptide that is folded into a globular shape, forming a 7-transmembrane structure. The extracellular loops are responsible for signalling molecule binding (N-terminal) (Bockaert and Pin 1999). Yamashita et al. (1991) cloned the structure of bovine H_1 receptor, which is characterized by a large (212 amino acids) 3rd intracellular loop and a relatively short (17 amino acids) intracellular C-terminal tail. The third and fifth transmembrane domains of H_1 and H_2 receptors contribute to histamine binding (Birdsall 1991; Timmerman 1992). The full length human H_1, H_2, H_3, and H_4 receptors are composed of 487, 359, 445, and 390 amino acids, respectively (Simons 2004). H_4 receptor has 40% homology with the H_3 receptor, and was identified based on differences in tissue distribution and binding affinity (Nakamura et al. 2000). Liu et al. (2001) identified 35% homology between the H_4 receptor and the H_3 receptor (Table 1).

Histamine receptor expression and function

The H_1 receptor is widely distributed throughout the body, with well-documented expression in the central nervous system (CNS), smooth muscle, sensory nerves, heart, adrenal medulla, as well as immune, endothelial, and epithelial cells. It mediates most of the postsynaptic effects of histamine within the CNS. Through binding of the transmembrane domains 3 and 5 of H_1 receptor, histamine stimulates smooth muscle contraction in the respiratory and gastrointestinal tract, stimulates sensory nerves leading to pruritus and sneezing, and increases vascular permeability (through prostacyclin, platelet activating factor, von Willebrand factor, and nitric oxide; NO) leading to oedema. H_1 receptor intracellular signals are transmitted through Ca^{2+}, cGMP, phospholipase D, phospholipase A2, and NF-κB activation. Simultaneous activation of H_1 and H_2 receptors via histamine can lead to clinical symptoms of anaphylaxis such as hypotension, tachycardia, flushing, and headache. With its diverse distribution and various effector activities, the H_1 receptor is involved in allergic rhinitis, asthma, atopic dermatitis, conjunctivitis, and urticaria development.

The H_2 receptor is widely expressed and can be found in gastric mucosal cells, heart, CNS, immune cells, and smooth muscles of the airway, vasculature, and uterus. It consists of a special short intracellular loop and long C-terminal tail. H_2 receptor activation leads to activation of cAMP-dependent and -independent pathways (adenylate cyclase, c-Fos, c-Jun, PKC, p70S6K). Differential levels of cellular expression result in the stimulation of hydrochloric acid secretion from acid secreting parietal cells of the gastric mucosa, smooth

Table 1: **Histamine receptors.**

Receptor subtype	G protein coupling	Signal transduction	Selective agonists	Antagonists/inverse agonists
H_1	Gq/11	PLP-C activation, through increase in IP3 and DAG leads to Ca influx and PKC activation; PLP-A2 activation, leads to increased arachniodic acid level, NOS activation, PLP-D activation	Histaprodiphen	See Table 2
H_2	Gs	PKA activation through cAMP increase	Amthamine Dimaprit Impromidine	Ranitidine Cimetidine Famotidine Nizatidine
H_3	Gi/o	Adenylate cyclase inhibition, MAPK pathway activation, PLP-A2 activation, leads to increase in arachniodic acid levels; Na/H channel blockage, decrease of intracellular Ca	Alpha methyl histamine Imetit Immepip	Thioperamid Pitolisant MK-0249 JNJ-17216498
H_4	Gi/o	Adenylate cyclase inhibition, MAPK pathway activation	Clobenopropit (partial agonist) Imetit Immepip 4-methylhistamine	2-aminopyrimidine JNJ-7777120 VUF-6002 CZC-13788 PF-2988403 A-940894 A-987306

cAMP, cyclic AMP; DAG, diacylglycerol; IP3, inositol triphosphate; PLP, Phospholipase; PKA, protein kinase A; MAPK, MAP kinase; NOS, nitric oxide synthase.

muscle relaxation of the vasculature and airways, increased cardiac rate and contractility, and immunomodulatory effects through basophil suppression (Novak et al. 2012). H_2 receptors also mediate suppression of Th1 and Th2 responses (Jutel et al. 2001). Moreover, H_2 receptors play a central role in intestinal immune homeostasis by modifying innate immune responses to bacterial components. H_2 receptors mediate invariant natural killer T cell (iNKT) dependent lung inflammation in a mouse model of asthma; consequently, H_2 receptor null mice show iNKT dependent lung inflammation (Ferstl et al. 2017). Comparison of H_2 receptor gene expression among healthy individuals and patients with inflammatory bowel disease suggest the possibility of down regulated innate immune responses by H_2 receptor signaling in the intestinal tract (Smolinska et al. 2016).

The H_3 receptor is predominantly expressed in the CNS (basal ganglia, hippocampus and cortical areas), but can also be found in the peripheral nervous system, airways, the cardiovascular system, the gastrointestinal tract and on mast cells. Several isoforms are linked to different signaling pathways (Ishikawa et al. 2010) which may suggest selective regulation of diverse cerebral functions. (García-Gálvez et al. 2018). Acting through the presynaptic H_3 receptor, histamine regulates its own release (negative feedback) as well as the release of other neurotransmitters such as noradrenaline, dopamine, serotonin, acetylcholine, and gamma-amino-butyric acid. In the lower airways, H_3 receptors are located on postganglionic cholinergic nerves and counteracts bronchoconstriction, through stimulation of phospholipase A2 and subsequent enhanced release of endothelium-derived relaxing factor in guinea pigs (Barnes 1991; Burgaud and Oudart 1993; Nieto-Alamilla et al. 2016). In the upper airways, histamine may play a role in nasal congestion through its activity at H_3 receptors. The intracellular pathways related to H_3 receptor activation are enhanced Ca^{2+} influx, MAP kinase, and inhibition of cAMP.

The H_4 receptor shows the highest level of expression in bone marrow and peripheral blood leukocytes but is also found in spleen, thymus, lung, gastrointestinal tract, liver, peripheral nerves, and central neurones in the cerebellum and hippocampus (Cogé et al. 2001). The H_4 receptor is predominantly detected on hematopoietic cells, including mast cells, eosinophils, basophils, dendritic cells, monocytes, NK, and T cells (Th17). H_4 receptor activation induces calcium mobilisation through cAMP in mast cells and promotes mast cells migration towards histamine. Moreover, the receptor plays a significant role in regulating dendritic and T cell function. The H_4 receptor has also been implicated in the regulation of other non-hematopoietic systems such as the dermis and epidermis (resulting in stronger expression), especially in keratinocytes, intra-articular synovial cells, and chondrocytes.

The H_4 receptor has been considered a promising therapeutic target in atopic dermatitis, asthma, and chronic arthritis. This relates not only to its direct activating function of effector cells of the hematopoietic system, but also to its expression on regulatory T cells. H_4 receptor agonists have been shown to suppress CCL2 mRNA expression in monocyte-derived Langerhans cells, and induce the migration of Langerhans cells from the epidermis (Gschwandtner et al. 2010). The application of H_4 receptor antagonists in studies showed significant inhibition of CCL17 and CCL22 production by monocytes of patients with atopic dermatitis, with only marginal effects in healthy individuals (Miyano et al. 2016). Since the application of H_1 and H_4 receptor antagonists decreases pruritus in atopic dermatitis, the H_4 receptor has also been linked to pruritus development (Ohsawa and Hirasawa 2012; Köchling et al. 2017).

Pharmacological blockade of histamine function via antihistamines

Antihistamines were synthesized as blocking compounds against the actions of histamine; they stabilize the inactive conformation of the heptahelical H_1 receptors via cross-linkage. Due to differences in their chemical structures, the site that antihistamines occupy on the H_1 receptor is different from the histamine binding site, therefore, antihistamines are classified as inverse agonists rather than competitive antagonists (Church 2011) (Figure 1). Both histamine and antihistamines act by receptor stabilization, either in the active or inactive state, shifting the natural balance of the receptor state (Table 2).

Pharmacokinetics and side effects of approved antihistamines

The most important cause of side effects of first generation H_1 antihistamines relates to their ability to cross the blood-brain barrier. They have low selectivity and lipophilic properties because of their basic structure of either aromatic or heterocyclic rings. This leads to decreased hepatic degradation and increase

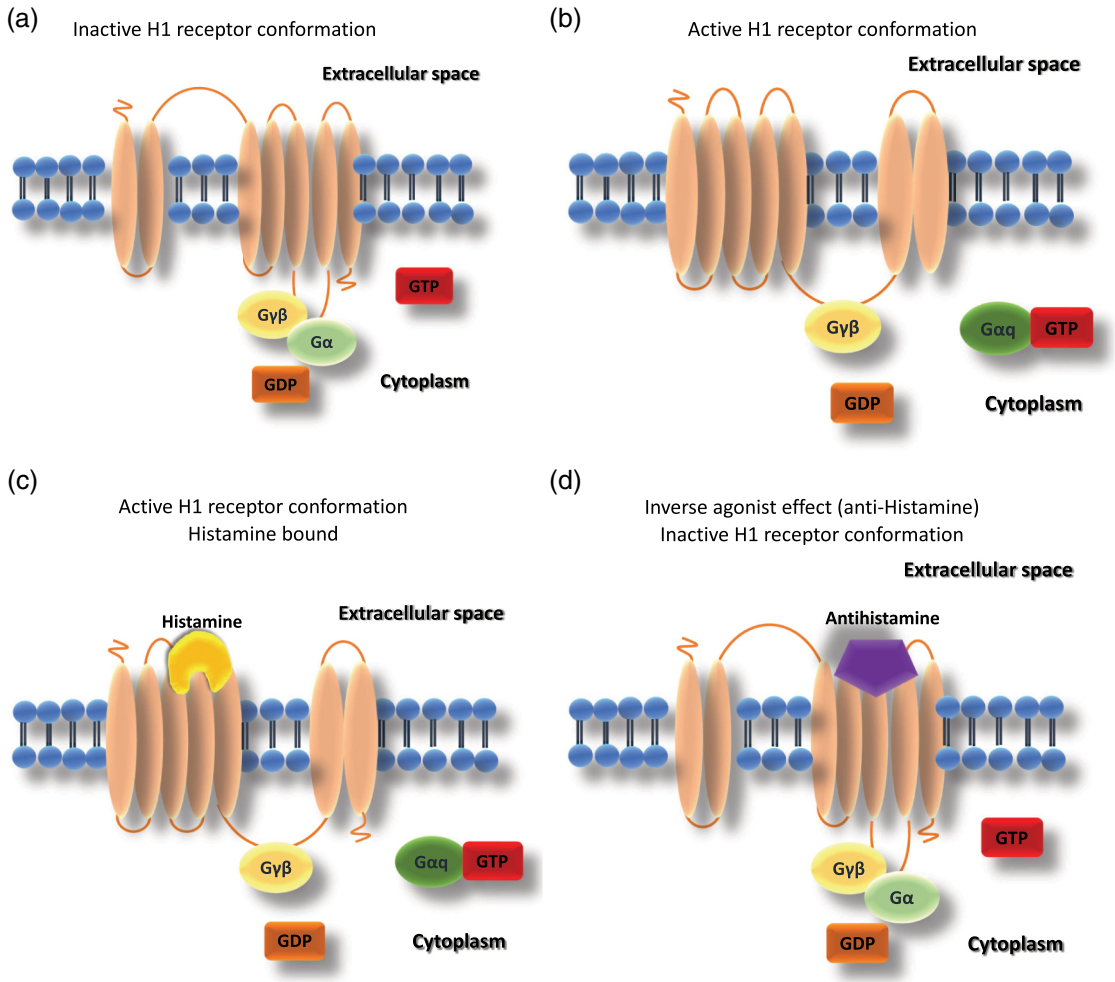

Figure 1: Histamine receptor structure and function.

hepatobiliary circulation. Newer generation antihistamines express high H_1 receptor selectivity, leading to less or no cholinergic, adrenergic or serotoninergic side effects (Church 2011; Simons and Akdis 2013).

Bioavailability

Following oral administration, antihistamines reach their peak concentration within 0.5–3 hours. Bioavailability of antihistamines is regulated by the abundance of drug transporters in the intestine. P-glycoprotein expression (P-gp) is a drug efflux pump which is expressed in a polarized fashion and found on epithelial and mucosal cells (such as the luminal surface of the intestine), on cerebral endothelial cells of the blood-brain barrier, the liver, and the kidneys. Rodent derived data suggests that antihistamines, which act as substrates of the P-gp transporter, display little to no CNS effects. This may relate to the fact that P-gp actively pumps substrates out of the brain, thereby limiting their effects in the CNS. The importance of P-gp in this context has been confirmed in particular for second generation antihistamines. P-gp$^{-/-}$ mice showed significantly greater enrichment of nonsedative second generation antihistamines (such as cetirizine, loratadine, and desloratadine) in the brain, as compared to wild type mice. On the other hand, brain penetration of the sedative first generation antihistamines hydroxyzine, diphenhydramine, and triprolidine did not differ between wild type and P-gp$^{-/-}$ mice (Chen et al. 2003; Polli et al. 2003). This apparent difference between second and first generation antihistamines is in keeping with the knowledge that only the former are P-gp substrates. P-gp inhibition and induction has the potential to disrupt antihistamine absorption and elimination. Co-administration of P-gp inhibitors such as amiodarone, propranolol, ketoconazole, and cyclosporine, carries the potential risk of increasing antihistamine exposure due to inhibition of P-gp activity in the

Table 2: **H_1 receptor inverse agonists.**

	First generation		Second generation	
Family	FDA approved	Not FDA approved	FDA approved	Not FDA approved
Alkylamines	Brompheniramine, chlorpheniramine, dexchlorpheniramine, pheniramine (opth.), triprolidine →	Dimethindene	Acrivastine	—
Ethanolamines	Carbinoxamine, clemastine, dimenhydrinate, diphenhydramine, doxylamine	Phenyltoloxamine (CA, in cough med. with codein)	NA	NA
Ethylenediamines	Antazoline, pyrilamine, tripelennamine	NA	NA	NA
Phenothiazines	Methdilazine, promethazine	NA	NA	NA
Piperidine	Azatadine, cyproheptadine, diphenylpyraline, ketotifen	NA	Bepotastine (opth.), desloratadine, fexofenadine, levocabastine, loratadine, alcaftadine	Astemizole, bilastine (CA), ebastine, mizolastine, rupatadine (CA), terfenadine
Piperazines	Buclizine, cyclizine, hydroxyzine, meclizine →	Oxatomide	Cetirizine, levocetirizine	—
Other	Doxepin	—	Azelastine, emedastine, epinastine, olopatadine	—

Note: CA, approved in Canada; Opth., ophthalmic drop/solution; →, derivative.

gastrointestinal tract (Shimizu et al. 2006). Some second generation antihistamines (for example, loratadine and rupatadine) are extensively metabolized by cytochrome P450 in the liver. Thus, cytochrome P450 inhibitors, such as macrolide antibiotics and imidazole antifungals (ketoconazole), may increase the plasma concentrations of antihistamines, especially in the elderly (Kaliner 2002; del Cuvillo et al. 2006). There is a substantial overlap between drugs that interfere with cytochrome P450 3A4 (CYP3A4) and P-gp. Importantly, dual inhibitors of P-gp and CYP3A4 do not necessarily have the same inhibitory potency towards P-gp and CYP3A4. Potential drug interactions should always be evaluated in the context of characteristics of both the individual drug and patient. Potential drug-drug interactions may be handled by additional monitoring, dose adjustment, staggered administration, paused treatment and search for alternative treatments.

Selectivity

Antihistamines have organ-receptor specific affinity and efficacy. Depending on the receptor location and the histamine/antihistamine action, site effects can be organ specific. Consequently, blockade of the central nervous H_1 receptor leads to somnolence, fatigue, increased appetite, disturbed circadian sleep-wake cycle and altered rapid eye movement sleep, decreased cognitive functions (impaired memory and learning) and may lead to aggressive behaviour and seizures. The interaction between H_1 blockers and the H_1 receptor in the brain was directly demonstrated by positron-emission tomography studies. Antihistamines can be classified based on these studies of receptor occupancy: Sedative, where receptor CNS occupancy is 50%–100%; less-sedative, where receptor occupancy is 20%–50%; and non-sedative, where receptor occupancy is 0%–20%. The limitations of these studies is the different and limited number of time points receptor occupancy analysis was performed, as well as the lack of quantitative dose/serum concentration/T_{max} in relation to the receptor occupancy (Kanamitsu et al. 2017). To assess treatment safety, proportional impairment ratio was implemented to describe the correlation between the brain H_1 receptor occupancy and the somnolence

observed. Two studies revealed that the potential of antihistamines to impair cognitive function and performance was different both between generations of antihistamines, as well as between antihistamines of the same generations (Shamsi and Hindmarch 2000; McDonald et al. 2008). In the first generation group, hydroxyzine, chlorpheniramine, ketotifen, and diphenhydramine had the highest proportional impairment ratio.

First generation oral antihistamines have no site specific receptor selectivity. Thus, in addition to peripheral and central nervous side effects they have the potential to exert anti-alpha-adrenergic, anti-serotoninergic and anti-muscarinergic side effects. These side effects may present with dry mouth, photophobia, mydriasis, tachycardia, constipation/urinary retention, agitation, confusion. This has not been reported for second generation antihistamines.

Another important aspect is the increased risk of cardiac side effects of any H_1 antihistamine that prolongs the QT interval (measurement made on an electrocardiogram) in patients with pre-existing heart disease, cardiac arrhythmias, or electrolyte imbalance. During the pre-clinical and early clinical development of new drugs, regulatory agencies worldwide now aim to identify all new medications, including H_1 antihistamines, that may block the rapid delayed rectifier current and thus interfere with cardiac electrical cycle repolarization (IKr current, which conducts potassium ions out of the cardiac myocytes and is critical in timing of the repolarization), prolong the QT interval, and potentially cause polymorphic ventricular arrhythmias. Second generation H_1 antihistamines such as cetirizine, desloratadine, fexofenadine, and loratadine appear to be relatively free of cardiac toxic effects as compared with astemizole and terfenadine (Woosley 1996; Simons and Simons 1999; De Bruin et al. 2002; Yap and Camm 2002; Simons and Akdis 2013).

Antihistamines in atopic disease

Chronic urticaria

According to the European Academy of Allergy and Clinical Immunology (EAACI)/Global Allergy and the Asthma European Network (GA^2LEN)/the European Dermatology Forum (EDF)/the World Allergy Organization (WAO) Guideline, chronic urticaria is defined as a "condition characterized by the development of wheals (hives), angioedema or both in the case of these symptoms lasting more than 6 weeks" (Dressler et al. 2018). Histamine is the central mediator in urticaria and promotes the wheal and flare reaction via an increase of vascular permeability and vasodilation. A previous GA^2LEN position paper suggests not to use first generation sedating antihistamines in allergy due to side effects (Church et al. 2010). Non-sedating H_1 antihistamines are recommended as baseline treatment, to be started with a standard dose and increased up to 4-fold of the recommended dose. Omalizumab is recommended as add-on treatment in the case of unresponsiveness for high dose of H_1 antihistamines (Dressler et al. 2018).

Allergic rhinitis

Allergic rhinitis (AR) is defined as an IgE-mediated inflammatory response of the nasal mucosa after exposure to allergens. Symptoms involve anterior or posterior rhinorrhea, sneezing, nasal obstruction and (or) itching of the nose (Bousquet et al. 2008). Many reports indicate that managing allergic rhinitis is beneficial for better asthma control. Therefore, as a state-of-the-art review for the specialist and the general practitioner as well as other health care professionals, Bousquet et al. (2001) published the Allergic Rhinitis and its Impact on Asthma (ARIA) document. ARIA guidelines were subsequently updated in 2008 (Bousquet et al. 2008), while a 2016 revision was limited to only 6 clinical questions (Brożek et al. 2017).

The ARIA 2008 Update recommended intranasal glucocorticosteroids as the most effective drugs for adult and pediatric patients with AR. Topical H_1 antihistamines and second generation oral H_1 antihistamines are recommended. However, with regards to the effectiveness of second generation H_1 antihistamines, first generation oral H_1 antihistamines are less effective and therefore not recommended (Bousquet et al. 2008). The ARIA 2016 revision comments on combination therapies and a leukotriene receptor antagonist (LTRA) as treatment options. Either an intranasal corticosteroid may be applied or co-administered with an oral H_1 antihistamine. An intranasal H_1 antihistamine is available as intranasal application alone, while a combination of an intranasal H_1 antihistamine and an intranasal corticosteroid is more effective than an intranasal H_1 antihistamine alone. The use of an oral or intranasal H_1 antihistamine depends on the patient's preferences and local availability. For patients with persistent AR, an oral H_1 antihistamine is recommended rather than a LTRA (Brożek et al. 2017).

Allergic conjunctivitis

The symptoms of environmental (seasonal and perennial) allergic conjunctivitis are caused by IgE mediated hypersensitivity and local mast cell degranulation, as well as release of inflammatory mediators and histamine. Besides allergen avoidance, both topical and oral antihistamines are the recommended treatment of choice for symptomatic management of allergic conjunctivitis, alone or in combination with mast cell stabilisers and topical anti-inflammatory medications (non-steroidal and steroidal). Topical antihistamines used alone have a short (up to 4 hours) effect, indicating frequent 4×/day use, and do not have anti-inflammatory effects, which is important in the prevention and management of chronic inflammatory changes (La Rosa et al. 2013).

Asthma

Asthma is defined as a heterogeneous disease, usually characterized by chronic airway inflammation by Global Initiative for Asthma (GINA) (GINA 2018). A definition by the British Thoracic Society (BTS)/ Scottish Intercollegiate Guideline Network (SIGN) also included airway hyperresponsiveness and airway inflammation (SIGN 2016). H_1 antihistamines are not referred to in either. Oral H_1 antihistamines are recommended only in asthma patients with concomitant AR (Brożek et al. 2017).

Food allergy

Food allergy is defined as adverse reaction to food mediated by an immunologic mechanism, involving specific IgE (IgE-mediated), cell-mediated mechanisms (non-IgE-mediated), or both IgE- and cell-mediated mechanisms (mixed IgE- and non-IgE-mediated) by EAACI Food Allergy and Anaphylaxis Guidelines (Muraro et al. 2014). Currently, avoidance of allergenic foods and the use of rescue medication in case of an allergic reaction is still the treatment of choice (Muraro et al. 2017; Pajno et al. 2018). H_1 antihistamines should be used as rescue medication in emergency cases such as anaphylaxis, but notably not initial medications (Simons et al. 2014). According to EAACI treatment guidelines, 1st-line medication for treatment of anaphylaxis is an intramuscular epinephrine injection which could be repeated within 10 minutes if indicated, 2nd-line are inhaled beta-2 agonists for wheezing and inhaled adrenaline for stridor, and 3rd-line are H_1 antihistamines, H_2 antihistamines, and glucocorticoids (Muraro et al. 2014).

Novel applications of antihistamines

Recent progress in identifying new H_4 antagonists resulted in derivates which are structurally related to 2 chemicals: 2-aminopyrimidine and indole carboxamides. Indole carboxamide analogs of compound 1 (JNJ7777120) was first identified as a H_4 receptor antagonist by Jablonowski et al. (2003) JNJ7777120 inhibited histamine-induced eosinophil shape change and upregulation of adhesion molecules (Ling et al. 2004). A combination therapy with H_1 receptor antagonist (olopatadine) and H_4 receptor antagonist (JNJ777120) was effective for atopic dermatitis in mice (Matsushita et al. 2012), and in a second approach scratching behavior improved due to the same combination of H_1 and H_4 receptor antagonist treatment in a chronic dermatitis mouse model (Ohsawa and Hirasawa 2012). Currently, approximately 47 compounds are described as H_4 receptor antagonists (Tichenor et al. 2015). The expression of the H_4 receptor is limited to the area of inflammatory and immune responses on bone marrow and hematopoietic cells. Thus, the function of H_4 antagonists may also involve allergic disease in humans, although this has not yet been clarified (Thurmond 2015). Some H_4 receptor antagonists have reached clinical trials for the treatment of asthma, atopic dermatitis, and allergic rhinitis. JNJ39758979 reduced histamine-induced pruritus despite not demonstrating reduction in wheal or flare area (Kollmeier et al. 2014). The safety and efficacy of JNJ39758979 was assessed in adult Japanese patients with moderate atopic dermatitis. Assessment of efficacy showed improvement of several pruritus assessments (Murata et al. 2015). ZPL3893787, on the other hand, has completed Phase I in healthy volunteers with encouraging safety and pharmacokinetic profile. UR-63325 has entered into clinical studies for the treatment of allergic respiratory diseases and underwent Phase II study with additional benefit compared with H_1 antagonism in reducing nasal blockage (Kiss and Keseru 2014).

Frequently used non-sedating H_1 antihistamines

Cetirizine and levocetirizine

Cetirizine is a racemic mixture composed of R-levocetirizine and S-dextrocetirizine, a member of the piperazine family. It is among the early second generation H_1 antihistamines (SGAHs) developed to provide selective H_1 receptor inhibition with little CNS binding. It has

favourable pharmacological properties in that it does not undergo interconversion and therefore maintains configuration stability in the body, and has high binding to serum albumin and the lowest apparent volume of distribution (Tillement 2000). As well, ceterizine exhibits low brain uptake, low affinity for lean tissues such as the myocardium (thus low cardiotoxicity), and low/lack of sedative effects. Cetirizine has also been shown to be absorbed extensively and rapidly from the gut (Wood et al. 1987), leading to high bioavailability and rapid onset of drug action (Greisner 2004). Unlike many other SGAHs, cetirizine does not undergo hepatic metabolism to any appreciable extent, but is excreted mostly unchanged in the urine, equally well in both healthy volunteers and patients with chronic liver disease (Horsmans et al. 1993). Dose adjustment is not needed in case of abnormal liver function but is needed in renal dysfunction (Dávila et al. 2013). The lack of hepatic metabolism demonstrates a low potential for drug-drug interactions, which avoids any exaggerated pharmacological or toxicological effects with drugs that are subject to metabolism by P450 enzymes and to transmembrane transport (Boobis et al. 2009). Onset of action of wheal and flare inhibition is 1 hour and duration of time is 24 hours (Simons 2004).

With regards to safety, cetirizine has very low to absent cardiotoxic potential. Importantly, no deleterious effects in pregnancy or lactation have been reported with cetirizine. The Food and Drug Administration (FDA) has classified cetirizine as a pregnancy category B drug, i.e., without known harm to an animal foetus and no human studies available.

Although research employing subjective assessments of the CNS effects of cetirizine has produced conflicting data, studies involving objective assessments, e.g., driving and psychomotor performance tests, have unanimously demonstrated that therapeutic doses of cetirizine do not generally produce more psychomotor impairment than placebo. Similarly, the adverse effects profile of cetirizine has been shown to be comparable with that of astemizole, ebastine, fexofenadine, loratadine, mizolastine, or terfenadine; with the adverse effects of cetirizine generally being of mild-to-moderate intensity. Importantly, the longest clinical trial with any antihistamine launched in the 20th century, the ETAC study, in children as young as 18–24 months at randomization, demonstrated that continuous cetirizine treatment over 18 months had no adverse effects on neurological and behavioural events, nor on natural development milestones, e.g., no influence on height, body mass, gross and fine motor skills, speech and language skills, as well as haematology, and biochemistry tests. The proportion of treatment-emergent adverse events in the ETAC study, as described by the investigators, was similar between the cetirizine and placebo group.

Levocetirizine, is the active enantiomer of cetirizine. Levocetirizine is poorly metabolized because of the lack of CYP2D6 (Baltes et al. 2001). There is only a need for dose adjustment in the event of serious renal failure (Dávila et al. 2013). The time it takes to reach the peak plasma concentration is 0.9 hour and the terminal elimination half-life is 7.9 hours (Baltes et al. 2001). Symptoms score for allergic rhinitis improved within 2 hours after dosing (Horak et al. 2010). Based on suppression tests of wheal and flare, time of action is also 2 hours and duration of action is 24 hours (Simons 2004). Levocetirizine is a designated FDA Pregnancy Category B (animal studies negative, human data not available, or animal studies positive, human data negative).

Long-term safety of the second generation H_1 antihistamines, desloratadine and loratadine, has been documented in randomized controlled trials lasting 6–18 months in adults and in children as young as 1–2 years old (Simons et al. 2003; Simons and Early Prevention of Asthma in Atopic Children (EPAAC) Study Group 2007; Klimek 2009).

Loratadine and desloratadine

Loratadine is a second generation, oral histamine H1 receptor antagonist of the piperidine family, metabolized by the hepatic cytochrome P450 system to the active, desloratadine metabolite. Desloratadine is further processed to 3-hydroxydesloratadine, which undergoes further glucuronidation in the liver. Studies have demonstrated its efficacy for seasonal allergic rhinitis (SAR) via a reduction of symptoms over placebo. Time to onset of action has been reported widely from 75 to 180 minutes in clinical situations (Simons 2004; Tenn et al. 2018) and the duration of action is 24 hours (Simons 2004). Dose adjustment is only needed in case of serious renal failure. Minimal or no adverse effects are reported with 10 mg of loratadine. At higher doses it might cause dose-related CNS effects. Up to 30-fold overdoses of loratadine have not been causally

associated with serious adverse events or fatality. A study by Wu et al. (2012) comparing the efficacy of treatment for persistent allergic rhinitis (PAR) in children 2–12 years old of loratadine syrup formulation and cyproheptadine solution described a significantly reduced total symptom score with an improvement in side effects involving the central nervous system in the loratadine group. In the Preventia I study, well tolerated long term safety effects were described in the 12–30 months of age group during Phase I studies. 12 months medication administration and Phase II 12 months follow up period (Grimfeld et al. 2004).

Loratadine is a designated FDA Pregnancy Category B. Previous studies indicated loratadine as a possible factor for the increased incidence of hypospadias in pregnancy (Pedersen et al. 2006). But according to a systematic review and meta-analysis, this risk could not be detected (Schwarz et al. 2008).

Desloratadine is a metabolite of loratadine (as well as rupatadine) and is well metabolized by the hepatic cytochrome P450 system. The enzyme pathway involved in the liver metabolism of desloratadine to 3-hydroxydesloratadine has not yet been reported. It undergoes less first-pass metabolism than loratadine; therefore, the variability in systemic exposure to desloratadine is potentially decreased. Elimination occurs via both the renal and faecal pathways (Affrime et al. 2002). The bioavailability of desloratadine is minimally affected by drugs interfering with transporter molecules; of the second generation antihistamines, desloratadine has the greatest binding affinity for the H_1 receptor (Anthes et al. 2002). In an in vitro study investigating inverse agonism, desloratadine appeared to be more potent than fexofenadine and cetirizine, a result explained by the high correlation between H_1 receptor affinity and inverse agonist activity (Bakker et al. 2000). Peak plasma concentrations of desloratadine at recommended doses for antihistaminic activity are 10-fold lower than the concentrations at which in vitro functional antimuscarinic activities are observed. Desloratadine does not induce any clinically relevant antimuscarinic effects at therapeutic doses. No interaction with cardiac potassium channels has been reported with desloratadine. Furthermore, desloratadine does not impair cognitive or psychomotor performance or potentiate the deleterious effects of alcohol (ethanol) on psychomotor performance, at up to 9 times its standard dose, no sedating effects have been observed (Simons 1999; Kay and Quig 2001; Scharf and Berkowitz 2007). No known clinically relevant drug-drug interactions have been observed and dose adjustment is only needed in serious renal failure (Dávila et al. 2013). Onset of action is 2 hours on the wheal and flare response and the duration of action is more than 24 hours (Simons 2004; Antonijoan et al. 2017).

Desloratadine is a designated FDA Pregnancy Category C (animal studies not available or positive, human data not available). In the US, desloratadine is approved for the treatment of SAR in adults and children aged ≥2 years, and PAR and chronic idiopathic urticaria in adults and children aged ≥6 months (prescribing information).

Rupatadine is almost completely metabolised when administered orally. Two of its main metabolites, desloratadine and 3-hydroxylated desloratadine, retain antihistaminic properties which may contribute to the overall efficacy of the drug. Although rupatadine is 98%–99% bound to human plasma proteins, it is well distributed in other tissues, indicating that this high degree of binding does not cause the compound to be retained in the circulating blood, allowing it to reach its target receptors.

Rupatadine is extensively metabolised in the liver and CYP3A4 was identified as the primary isoenzyme responsible for its metabolism. Thus, rupatadine should be used with caution when administered in combination with CYP3A4 inhibitors and should be avoided (like ketoconazole, azithromycin, fluconazole, diltiazem). Caution should be taken when rupatadine is co-administered with drugs with narrow therapeutic windows, since knowledge of the effect on other drugs is limited. No major sedating effect was described (Shamizadeh et al. 2014; Potter et al. 2016). Rupatadine has no or low side effect potential on cardiac repolarization (De Bruin et al. 2002; Yap and Camm 2002; Täubel et al. 2018); however, careful surveillance and risk-minimalization is required in patients in patients with a history of QTc prolongation and (or) torsade de pointes, including congenital long QT syndromes, or a history of other cardiac arrhythmias. There has been 1 torsade de pointes reported (Fité-Mora 2009) with rupatadine during post-market use, thus it should not be recommended to be used Rupatadine should not be administered concomitantly with erythromycin, ketoconazole or grapefruit.

Rupatadine is a generally well tolerated, rapid onset, effective second-generation H_1 antihistamine for the symptomatic relief of allergic rhinitis (Guadano 2004; Fantine 2008). It is known as dual blocker, since it blocks both the action of histamine as well of other inflammatory mediators, such as platelet-activating factor. It is indicated for use in allergic rhinitis and chronic idiopathic urticaria in patients aged 2 years or more. The onset of time is 2 hours and duration of action is 24 hours (Simons 2004; Antonijoan et al. 2017). The rapid absorption of rupatadine correlates with the onset of the antihistamine and PAF actions as assessed by wheal and flare inhibition, which occurs within 1–2 hours post dose.

No adequate studies on pregnant women exists, prescribing information recommends avoiding use in pregnant women. In preclinical studies, foetal toxicity (growth delay, incomplete ossification, minor skeletal findings) was reported in rats at materno-toxic dose levels only (25 and 120 mg/kg/day). In rabbits, no evidence of developmental toxicity was noted at doses up to 100 mg/kg.

In the CNS, rupatadine behaves similarly to second generation antihistamines. Rupatadine displays psychomotor impairment activity only at the highest dose (80 mg) 4× highest therapeutic dose (Barbanoj et al. 2004). No anticholinergic effects were observed in several preclinical models at doses of up to 7 mg/kg (>40 times the human expected dose) or at single doses in the range of 10–80 mg in humans (Shamizadeh et al. 2014). A recent multicenter placebo controlled study assessing the oral solution of rupatadine in children ages 2–11 years with persistent allergic rhinitis demonstrated efficacy in both nasal and non-nasal symptom reduction, and improved quality of life in comparison to placebo (Juniper et al. 1998; Potter et al. 2013; Santamaría et al. 2018).

Bilastine

Bilastine is a new H_1 receptor inverse agonist, belonging to the same non sedating piperidine family as loratadine, desloratadine, and fexofenadine. In vitro studies have shown that bilastine has a high specific affinity for the H_1 receptor but no or very low affinity for other tested receptors. This affinity for the H_1 receptor is 3 and 6 times higher than for cetirizine and fexofenadine, respectively. The effectiveness of bilastine is comparable to that of desloratadine. Besides its H_1 antagonism, bilastine has also demonstrated anti-inflammatory properties, including inhibiting the release of proinflammatory cytokines such as interleukin (IL)-4 and tumor necrosis factor-α. Furthermore, in vivo studies in rats demonstrated a significant reduction in histamine-stimulated endothelial permeability, microvascular extravasation, and inhibition of smooth muscular contraction and bronchospasm 11 times that of cetirizine. Laboratory studies also demonstrated that bilastine may reduce passive cutaneous anaphylaxis induced by homologous serum and monoclonal antibodies, along with IgG/IgE dependent active cutaneous anaphylaxis. Studies in humans have also shown its ability to rapidly inhibit the erythematous area in response to histamine.

In a comparison performed in the Vienna Challenge Chamber or with natural exposure, bilastine proved to be more effective than fexofenadine and as effective as cetirizine in subjects with grass AR (Horak et al. 2010). With regard to pharmacokinetics, in the fasting state, bilastine is absorbed quickly and does not undergo significant metabolization. Bilastine has no impact on the cytochrome P450 enzyme system of the liver, and there is no evidence of interaction with other drugs except for an increased uptake of bilastine when taken concomitantly with ketoconazole, erythromycin, or diltiazem. Approximately 95% is excreted intact in feces (67%) and urine (33%). Therefore, in case of renal and liver failure, dose adjustments is not necessary, however the use of bilastine and a concomitant drug with a pharmacokinetics through the P-gp system should be avoided in case of renal failure (Dávila et al. 2013). Bilastine presents a favorable safety profile with regard to CNS and cardiovascular effects. Its safety profile in terms of adverse effects was very similar to that of placebo in all phase I, II, and III clinical trials. Its distribution in the brain is undetectable. Bilastine (20 mg), unlike cetirizine, does not increase the effects of alcohol on the CNS, does not increase the CNS depressant effect of lorazepam, and is similar to placebo in the driving test. No prolongation of the QTc interval (even at doses higher than the therapeutic dose) has been seen. Bilastine is well tolerated at all doses in the healthy population. The incidence of side effects was similar to that of placebo and lower than that of cetirizine with regard to fatigue and somnolence. The flare and wheal inhibition occurred at 30 and 60 minutes, respectively and duration of action is more than 24 hours (Antonijoan et al. 2017).

Fexofenadine

Fexofenadine is an active metabolite of terfenadine. Fexofenadine is now widely used as an alternative drug for terfenadine which had a risk of inducing severe ventricular arrhythmia. It is very poorly metabolized by cytochrome P450 and occurs primarily in the gastrointestinal tract. Some transporters involving P-gp, organic anion transporting polypeptide (OATP) 2B1, and multidrug resistance-associated protein 2 (MRP2) contribute to the uptake of fexofenadine (Akamine and Miura 2018; Akamine et al. 2019). Fexofenadine does not require dose adjustment (Dávila et al. 2013). It's T_{max}, onset of action, and duration of action is 2.6, 2, and 24 hours, respectively (Simons 2004). Fexofenadine is a designated FDA Pregnancy Category C. Reduction in pup weight and survival were observed and human data was not available with fexofenadine (Kar et al. 2012).

Discussion

Atopic diseases are a great burden to patients, from the young to the elderly. The prevalence of these conditions are increasing and expected to further increase in the future. Increased awareness by general practitioners, both of the conditions and therapeutic options, is will help to recognise and properly treat them. Here, we provide a current update and review of possible antihistamine effects and side effects, to aid practitioners in their treatment choice.

The different histamine receptors are distributed uniquely in the human body, with specific tissue localisation explaining their various functions and effects. From the H1 receptors' diverse distribution and various effector activity, including in allergic rhinitis, asthma, atopic dermatitis, conjunctivitis and urticaria development, through to the H_2 receptors' effect on gastric mucosa, smooth muscle relaxation of the vasculature and the airways, cardiac rate and contractility, and immunomodulatory effects by basophil suppression. As well, the various isoforms of H_3 receptors which are linked to different signaling pathways leading to selective regulation of diverse cerebral functions, and the H4 receptors' direct activating function of effector cells of the haematopoietic system but also to the expression on regulatory T cells. These are all explored in order to better understand their mechanism, effect, and role in the development of atopic diseases and developing new therapeutic agents for the management of atopic dermatitis, allergies, and asthma. The improving knowledge of receptor action at the cellular level helps to develop antihistamines with less side effects and more selectivity. Understanding the structure related side effects of first generation antihistamines, leading to sedation, helped in the development of the less sedating compounds.

Bioavailability of antihistamines is regulated by the abundance of drug transporters in the intestine. The significance of P-gp expression is in its limiting effects on the CNS, by actively pumping out the substrate, in this case, second generation non sedative antihistamines that may pass through the blood brain barrier. Thus, P-gp inhibition and induction has the potential to disrupt antihistamine absorption and elimination. Its importance is highlighted by the potential side effects of co-administration of P-gp inhibitors (e.g., amiodarone, propranolol, ketoconazole, and cyclosporine). Some second generation antihistamines, such as loratadine and rupatadine, are extensively metabolized by cytochrome P450 in the liver. Therefore, co-adminitration of CYP450 inhibitors, such as macrolide antibiotics and imidazole antifungals (ketoconazole) may increase the plasma concentrations of these antihistamines, especially in the elderly. Potential drug-drug interactions may be handled by additional monitoring, dose adjustment, staggered administration, paused treatment and search for alternative treatment.

Antihistamines have organ-receptor specific affinity and efficacy. Depending on the receptor location and the histamine/antihistamine action, site effects can be organ-specific. First generation oral antihistamines do not have site specific receptor selectivity, thus, in addition to peripheral and central nervous side effects they have the potential to exert anti-alpha-adrenergic, anti-serotoninergic, and anti-muscarinergic side effects. These important side effects present with dry mouth, photophobia, mydriasis, tachycardia, constipation/urinary retention, agitation, and confusion. This has not been reported for second generation antihistamines. Another important aspect is the increased risk of cardiac side effects of any H1 antihistamine that prolongs the QT interval in patients with pre-existing organic heart disease, cardiac arrhythmias, or electrolyte imbalance, however second-generation H1-antihistamines appear to be relatively free of cardiac toxic effects.

In the therapy of atopic diseases, like chronic urticaria, non sedating H_1 antihistamines are recommended as baseline treatment, to be started with a standard dose and increased up to 4-fold of the recommended dose. In allergic rhinitis many reports indicate that intranasal glucocorticosteroids combined with topical H_1 antihistamines or second generation oral H_1 antihistamines is the most recommended effective drugs therapy for adult and children patients with AR. However patient preferences and co-morbidities largely affect the medication and the administration route (therapy of choice). Oral antihistamine therapy is not recommended as part of the therapeutical regimen for asthma; oral H_1 antihistamines are recommended in asthma patients only in case of concomitant AR. Food Allergy and Anaphylaxis Guidelines do not recommend the use of antihistaminesas part of the initial medication choice. The first line medication of anaphylaxis is intramuscular epinephrine injection, which could be repeated if indicated in 5–10 minutes, 2nd-line are inhaled beta-2 agonists for wheezing and inhaled adrenaline for stridor, and 3rd-line are second generation H_1 antihistamines, H_2 antihistamines, and glucocorticoids. H_1 antihistamines should be used as rescue medication in emergency case such as anaphylaxis but notably not initial medications.

In this article, we provide a review of the histamine receptor, their function and role in disease, the most important antihistamines currently available, as well as their pharmacological effects and side effect potential. This knowledge will help practitioners use antihistamines in the right context when treating atopic diseases. The general recommendation is to avoid first generation sedation antihistamines with very few exception. Second generation non sedating antihistamine are generally considered very safe medications. Nevertheless, careful assessment of potential side effects is important before starting long term or high dose treatment for each antihistamine medication including concomittant illnesses and therapies (medication, herbal remedies, OTC medications) to achieve the best individualized therapy.

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Original Article

Successful hematopoietic stem cell transplantation in a patient with a novel mutation in coronin 1A

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ABSTRACT

Introduction: Coronin 1A is part of a family of highly conserved actin regulatory proteins with key roles in T cell homeostasis and T cell receptor signaling. Null mutations in coronin 1A result in severe combined immunodeficiency, whereas hypomorphic mutations have been associated with a somewhat milder immunological phenotype. Nevertheless, all patients described so far have markedly reduced naïve peripheral T cells, impaired T cell responses to mitogens, and limited T cell receptor diversity. Interestingly, despite poor thymic output, thymus architecture appears normal. To date, only 2 cases of hematopoietic stem cell transplantation (HSCT) have been reported in coronin 1A deficiency.

Aim: To describe the identification, transplantation course, and long term outcome of a Canadian Inuit patient diagnosed with coronin 1A deficiency.

Methods: Patient chart review was performed in accordance with institutional research ethics approval. A combination of immunological investigations and molecular genetic analyses were utilized to identify a novel mutation in the tryptophan-aspartate repeat region of coronin 1A. Based on the patient's profound T cell dysfunction, the decision was made to proceed with HSCT.

Results: The patient presented with a history of recurrent urinary tract infections, otitis media, and developmental delay involving poor axial and peripheral muscle tone. Axillary lymphadenopathy was noted and subsequent thymus biopsy revealed aberrant CD7+ T cell deficiency. Lymphocyte responses to mitogens and T cell receptor excision circle levels were markedly reduced, consistent with the diagnosis of severe combined immunodeficiency. Whole exome sequencing and Sanger confirmation revealed a novel mutation in coronin 1A. HSCT using a HLA-matched unrelated donor resulted in long term engraftment and solid immune reconstitution.

Conclusion: Very few patients with coronin 1A deficiency have been described to date, making it difficult to evaluate its natural history and management. Here, we describe the presentation, identification, transplantation, and outcome in our patient.

Statement of novelty: We describe the successful hematopoietic stem cell transplantation course and outcome in a patient with a novel mutation in coronin 1A.

Introduction

Coronin 1A is a key regulatory protein involved in cytoskeletal remodeling and calcium signaling (Rybakin and Clemen 2005; Mueller et al. 2008), and is expressed in a number of hematopoietic cells, including lymphocytes, macrophages, and NK cells (Oku et al. 2003). Part of a larger family of highly conserved actin

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regulatory proteins (Xavier et al. 2008), the structure of coronin 1A comprises: (*i*) an amino tryptophan-aspartate (WD) repeat-containing region forming a beta propeller—a motif involved in plasma membrane binding; (*ii*) a unique region; and (*iii*) a leucine zipper coiled-coil domain that is required for oligomerization and cytoskeletal association (Gatfield et al. 2005; Kammerer et al. 2005).

The 46 amino acid, 57 kDa actin binding protein is encoded by the *CORO1A* gene, located on chromosome 16p11.2 (Suzuki et al. 1995). Essential roles for coronin 1A have been reported in cell survival, migration, phagocytosis, vesicular trafficking, and signal transduction (Rybakin and Clemen 2005; Foger et al. 2006; Mueller et al. 2008, 2011). In murine studies, loss of coronin 1A expression is associated with T lymphocytopenia, due in part to aberrant T cell receptor mediated proliferation and failure of T cells to progress through the cell cycle (Foger et al. 2006; Haraldsson et al. 2008; Mueller et al. 2008; Mugnier et al. 2008; Shiow et al. 2008). Dysfunctional CD4+ T helper cell and associated IgG responses have also been reported (Tchang et al. 2013), while B and NK cells remain at normal levels. In humans, null mutations in *CORO1A* resulting in complete absence of coronin 1A expression are associated with a severe combined immunodeficiency phenotype (Shiow et al. 2008), while hypomorphic mutations lead to somewhat milder immunological manifestations (Moshous et al. 2013; Yee et al. 2016). In all cases described so far, patients experienced recurrent infections, had markedly reduced naïve peripheral T cells, and impaired T cell responses to mitogens (Moshous et al. 2013; Mace and Orange 2014). All but 3 patients showed failure to control Epstein-Barr virus (EBV) predisposing to B cell lymphoproliferation and lymphoma (Yee et al. 2016; Dinur Schejter et al. 2019). Interestingly, the thymus of coronin 1A deficient patients appears normal, indicating that loss of naïve T cells may be due to defects in cell survival. Variability in humoral function, B and NK cell counts, as well as T cell responses to mitogens and antigens have been described among affected individuals.

To date, only 2 patients with coronin 1A deficiency are reported to have received hematopoietic stem cell transplantation (HSCT). The first had a human leukocyte antigen (HLA)-matched unrelated donor transplant at the age of 4 years (Shiow et al. 2009). Although the procedure was successful, a full account of long term outcome has not been provided. The second patient received 6/6 HLA-matched parental bone marrow, however, died at 4 months post-transplant due to respiratory failure (Moshous et al. 2013).

Here, we describe a novel mutation in coronin 1A in a patient of Canadian Inuit origin. The patient successfully underwent HSCT using a HLA-matched unrelated donor and achieved long term engraftment and solid immune reconstitution.

Methods

Patient

Patient chart review was performed following informed consent, and in accordance with a research ethics approved protocols.

Serum concentration of immunoglobulin and specific antibodies

Serum concentrations of immunoglobulins were measured by nephelometry. Levels of serum antibodies to tetanus were measured by ELISA.

T and B cell proliferative response

Lymphocyte proliferative responses to mitogens including phytohemagglutinin (PHA) and anti-CD3. All assays were performed in triplicate and were compared with simultaneously stimulated normal controls.

Exome sequencing and variant calling

DNA from blood was submitted to The Centre for Applied Genomics (TCAG), Toronto, ON, Canada for exome library preparation and sequencing. DNA was quantified by Qubit DNA HS assay (Life Technologies, Carlsbad, CA, USA) and 100 ng of input DNA was used for library preparation using the Ion AmpliSeq Exome Kit (Life Technologies) according to the manufacturer's recommendations. The Ampliseq Exome library was immobilized on Ion PI™ Ion Sphere™ particles using the Ion PI Template OT2 200 Kit v3. Sequencing was performed with the Ion PI Sequencing 200 Kit v3 and Ion PI Chip v2 in the Ion Proton™ semiconductor sequencing system following the manufacturer's recommendation.

Alignment and variant calling were performed using Torrent Suite (v4.0) on the Ion Proton Server, using the Ion Proton ampliseq germline low stringency setting and the hg19 reference genome. The variants

were annotated using an in-house annotation pipeline (Stavropoulos et al. 2016) based on Annovar (November 2014 version) (Wang et al. 2010) and RefSeq gene models (downloaded from UCSC 1 August 2015).

Sequencing analysis

Patient's genomic DNA was extracted from peripheral blood lymphocytes using the Geneaid Genomic DNA Mini Kit. Genomic DNA was amplified by PCR with specific primers designed upstream and downstream of the coronin 1A gene. Sequencing was done using GenomeLab Dye Terminator Cycle Sequencing (DTCS) Quick Start Kit (Beckman Coulter) and analyzed on CEQ 8000 Genetic Analysis System (Beckman Coulter).

Western blotting

Whole-cell lysates were prepared in a 1% Triton X-100 buffer and analyzed by Western blotting. Anti-coronin 1A antibodies were purchased from Creative Diagnostics Inc. and Giα3 were purchased from Santa Cruz Biotechnology Inc.

Results

Case presentation

The female patient was born at term to a single mother of a 4 year old male, and is a product of consanguineous Canadian Inuit parents. Serology during pregnancy was negative for hepatitis B and HIV. Group B *Streptococcus* was positive and during labor she received 3 doses of penicillin G. There were no labor or postnatal complications and her birth weight was 3882 g. As per routine practice in her community, she received the live-attenuated BCG vaccination shortly after birth.

At 1 month of age, the patient had vomiting episodes which raised the possibility of pyloric stenosis. At 2 months of age, she suffered a right upper lobe pneumonia and the nasopharyngeal swab was positive for parainfluenza at the time. At 6 months of age, she had recurrent urinary tract infections and urine cultures grew *Escherichia coli* and *Klebsiella*. A renal ultrasound was normal.

Developmental assessment at 6 months revealed a delay as she was not sitting up on her own. Her muscle tone was decreased axially and peripherally. At 6 months of age, she was admitted for diarrhea that lasted for 3 weeks. *Clostridium difficile* was detected in the stool and she was given a 10 day course of metronidazole. She also suffered chronic suppurative otitis media bilaterally since the age of 3 months.

X-ray revealed some atelectasis and chronic changes in the upper right lobe. Blood counts revealed anemia with a hemoglobin level of 104 g/L, a low white cell count of 3.9 cells/μL and low lymphocyte count of 0.5×10^9 cells/L.

At the age of 7 months, she was transferred to a tertiary care center and was noticed to have axillary lymphadenopathy. A lymph node biopsy showed confluent well-formed non-necrotizing granulomas, while architecture and cellularity were normal. Ziehl–Neelsen staining revealed abundant acid-fast bacilli and cultures grew *Mycobacterium bovis*. Bronchoalveolar lavage fluid microbiological cultures were negative including mycobacteria. She was started on appropriate anti-mycobacterials. Circulating lymphocytes were low with only 135 CD4+ and 15 CD8+ cells/μL. Responses to mitogens were markedly reduced and T cell receptor excision circle (TREC) levels were only 2.

Immune evaluation

At 7 months of age the patient had profound lymphopenia. Flow cytometry revealed 202 CD3+, 135 CD4+ and 34 CD8+ T cells/μL, 194 CD19+ B cells/μL and normal numbers of circulating CD16/56+ NK cells/μL. Most CD3+ and CD4+ T cells were also CD45RO+, suggesting poor thymus output. In vitro responses to PHA and anti-CD3 were borderline reduced (Table 1).

In contrast, immunoglobulin levels were normal for age and antibody levels to Tetanus toxoid was protective, suggesting some partial T cell function.

To ascertain the diagnosis, a thymus biopsy was performed which revealed an architecturally normal thymus with medullary Hassall's corpuscles and normal corticomedullary demarcation. However, an overall aberrant deficiency of CD7+ T cells was noted (Figure 1).

Analysis of CD4+ T cell repertoire demonstrated reduced representation of $V_\beta1$, $V_\beta4$, $V_\beta5.1$, $V_\beta5.2$, $V_\beta5.3$, $V_\beta9$, and $V_\beta18$, whereas $V_\beta2$, $V_\beta7.1$, and $V_\beta23.3$ were over represented. Similarly, CD8+ T cell repertoire was skewed showing under representation of $V_\beta1$, $V_\beta4$,

Table 1: **Immune characteristics of coronin 1A deficient patient.**

	Pre-BMT (age 7 mo)	Post-BMT (age 7 y)	Normal range
Lymphocyte count [× 10^9/L, (NR)]	2.2 (4–10.5)	1.05 (1.5–7)	—
Markers [cells/μL, (NR)]			
CD3+	153 (2300–6500)	1432 (900–4500)	—
CD4+	123 (1500–5000)	963 (500–2400)	—
CD8+	10 (500–1600)	635 (300–1800)	—
CD19+	131 (600–300)	214 (200–1600)	—
CD16/56+	103 (100–1300)	142 (100–1000)	—
TRECs (copies/0.5 μg/L DNA)	2	640	>400
Mitogenic response	(Stimulation index/control)		
PHA	46/417	543/417	>300
Antigenic response			
Candida	ND	ND	—
Immunoglobulins [g/L, (NR)]			
IgG	5.03 (2.7–9.2)	7.5 (5.4–13.6)	—
IgM	0.61 (0.27–0.80)	0.5 (0.5–1.9)	—
IgA	0.20 (0.10–0.55)	0.7 (0.5–2.2)	—
Specific antibodies			
Anti-tetanus (IU/mL)	0.10	7.0	>0.01

Note: NR, normal range.

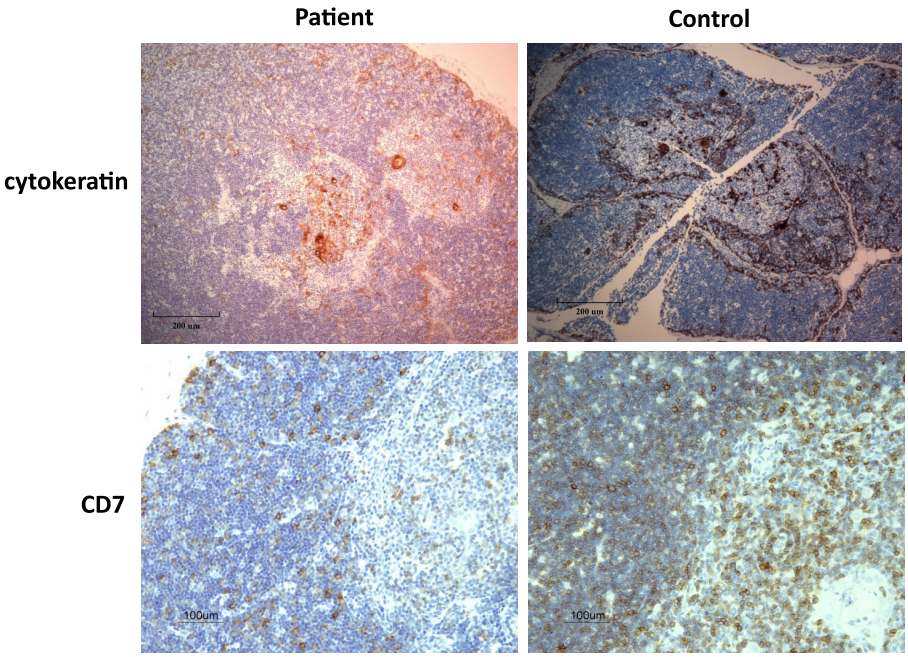

Figure 1: Thymus pathology. Pan cytokeratin staining shows normal thymic architecture with medullary Hassall's corpuscles, and a well demarcated medullary junction (upper panels). Aberrant CD7 deficiency in T cells in the cortex and medulla were identified (lower panels).

V_β5.1, V_β5.3, V_β7.1, V_β8, V_β9, V_β11, V_β13.1, V_β13.6, V_β14, V_β17, V_β21.3, and V_β22 (Figure 2).

Retrospective measurement of TREC from the patient's Guthrie dried blood spot revealed just 1 copy/3 μL, a positive screen for SCID.

Genetic analysis

Whole exome sequencing identified a novel homozygous missense mutation in *CORO1A*, c. 602G>A (p.R201H), and was confirmed by Sanger sequencing (Figure 3). The mutation is localized to the WD repeat-containing region of coronin 1A (Figure 4).

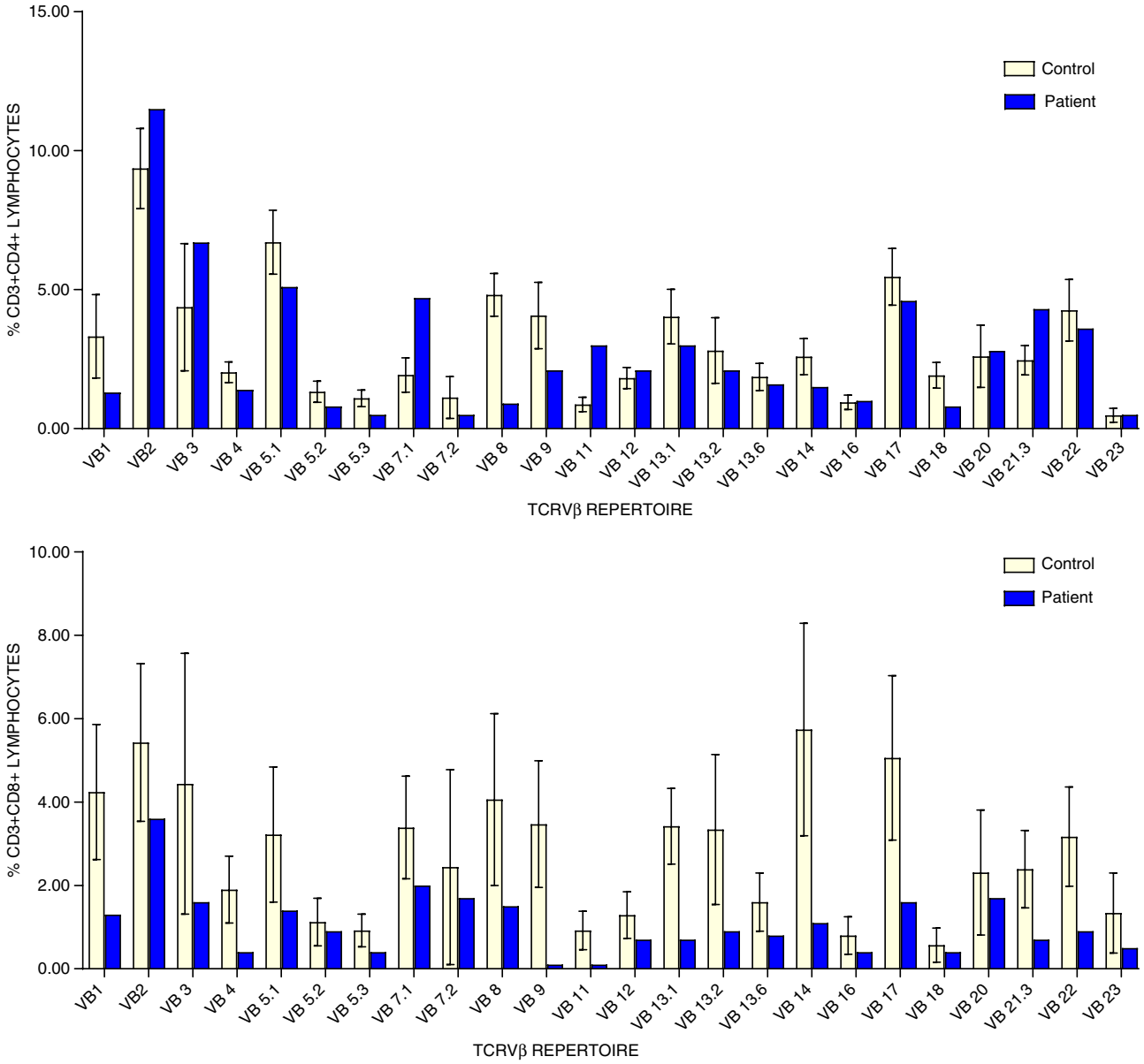

Figure 2: TCRVβ repertoire prior to HSCT. Assessment of CD4+ T cell repertoire revealed reduced representation of $V_\beta1$, $V_\beta4$, $V_\beta5.1$, $V_\beta5.2$, $V_\beta5.3$, $V_\beta9$, and $V_\beta18$, whereas $V_\beta2$, $V_\beta7.1$, and $V_\beta23.3$ were over represented. Similarly, $CD8^+$ T cell repertoire was skewed showing under representation of $V_\beta1$, $V_\beta4$, $V_\beta5.1$, $V_\beta5.3$, $V_\beta7.1$, $V_\beta8$, $V_\beta9$, $V_\beta11$, $V_\beta13.1$, $V_\beta13.6$, $V_\beta14$, $V_\beta17$, $V_\beta21.3$, and $V_\beta22$.

The patient's mother was found to be a heterozygous carrier. Genetic analysis of the patient's father was not available.

Coronin 1A protein expression

Western blotting confirmed the complete lack of coronin 1A protein expression (Figure 5).

HSCT course, engraftment, and outcome

At 13 months of age, the patient received a 9/10 HLA-matched unrelated donor transplant after myeloablative conditioning with busulfan, cyclophosphamide, and alemtuzumab. Neutrophil engraftment occurred on day 17 post-transplant. She had 1 infectious episode with *Pseudomonas aeruginosa* bacteremia at day 4 post-transplant, which responded rapidly to antibiotic treatment. Cyclosporine and methyl prednisone prophylaxis prevented acute graft versus host disease. However, at 1.5 years post-transplant she had an episode of fever associated with generalized rash, hemolytic anemia, and pancytopenia. Bone marrow biopsy showed normal cellularity. She was successfully treated

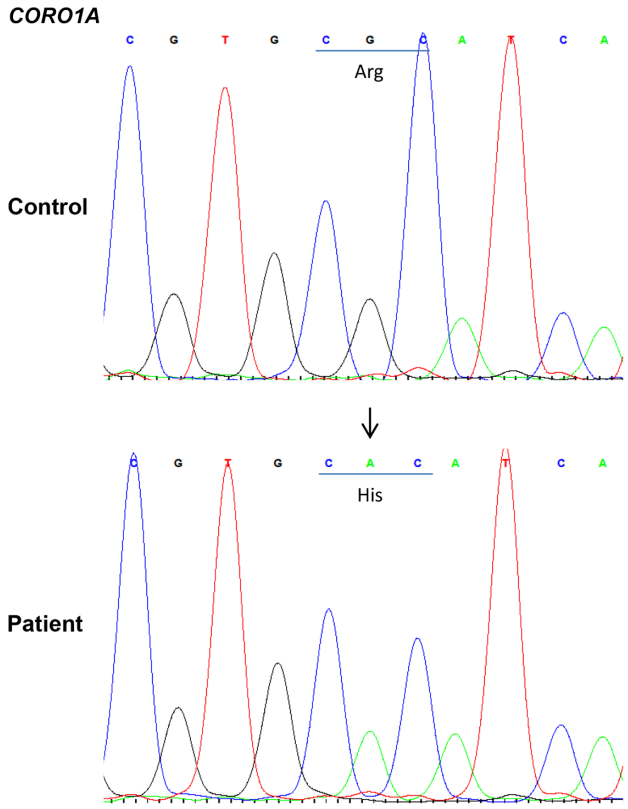

Figure 3: Homozygous missense mutation in coronin 1A. Electropherogram of the wild-type control (upper panel) sequence and novel homozygous missense mutation detected in the patient, c.602G>A (lower panel), in the *CORO1A* gene.

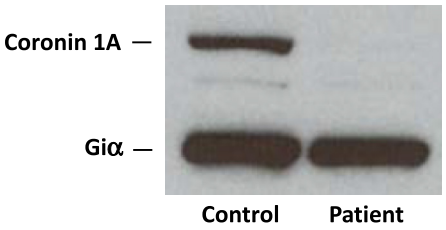

Figure 5: Assessment of coronin 1A protein expression in patient-derived cell lysates. Western blot analysis reveals the complete absence of coronin 1A protein in cellular lysates of our patient (right) compared to control (left).

with steroids and cyclosporine, but subsequently developed repeated episodes of hemolytic anemia and partial scalp alopecia which again responded well to immunosuppressive medications.

By 2 years post-transplant her immune evaluation demonstrated full engraftment (86% donor cells) as well as solid immune reconstitution with normal TREC levels and normal responses to mitogens and antigens. She continued anti-mycobacterials (isoniazid, rifampin) throughout the transplant course without evidence of BCG disease. These were discontinued around age 3. She did not develop evidence of graft versus host disease. Currently, at the age of 7 years, her engraftment and immune function remain solid (Table 1).

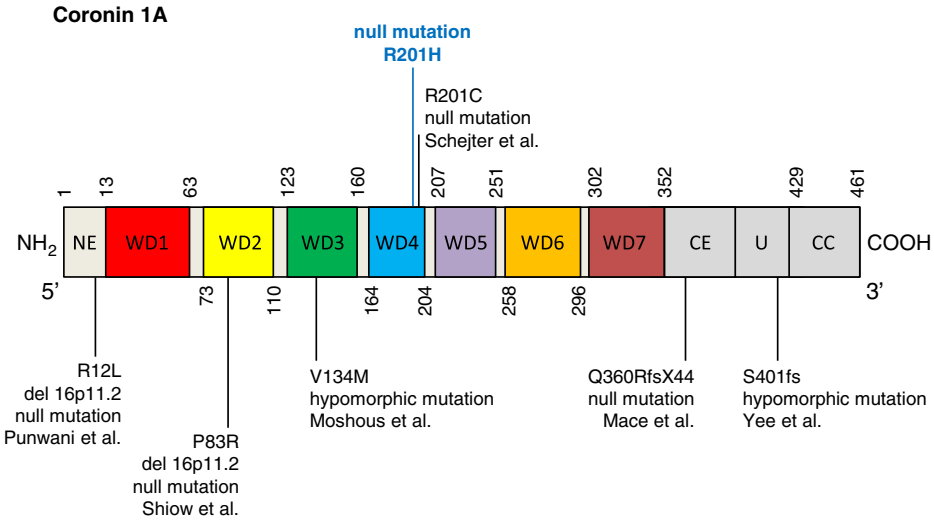

Figure 4: R201H null mutation in coronin 1A. Depiction of the coronin 1A structure, characterized by an amino-terminal (NE) region, a tryptophan-aspartate (WD) repeat containing region, a unique (U) region, and a coiled-coil (CC) domain. The p.R201H mutation identified in this patient is localized to WD4, which together with the other WD repeats, is believed to aid in the formation of protein complexes by functioning as a scaffold protein.

Discussion

Very few patients with coronin 1A deficiency have been described so far, making it difficult to evaluate its natural history and management. The challenge stems from the observation that the clinical features as well as the degree of immune dysfunction are highly variable in these patients.

Our patient presented with a phenotype of severe combined immunodeficiency; immune evaluation revealed low circulating T lymphocytes, skewed T cell repertoire, markedly reduced lymphocyte proliferative responses to mitogens, and TREC level (an indicator of thymic output) of only 2. The thymus, while architecturally normal, was deficient in CD7+ T cells. In an effort to identify the underlying cause of immunodeficiency, we performed whole exome sequencing and identified a novel homozygous missense mutation in *CORO1A* (c.602G>A), resulting in p.R201H within the WD repeat containing region.

In cases where profound T cell dysfunction has been documented, it is plausible to consider curative HSCT. We thus proceeded with a 9/10 HLA-matched unrelated donor transplant when the patient was 13 months of age. At 2- and 7-years post-transplant, her immune evaluations revealed full engraftment, robust immune reconstitution, as well as normal TREC levels and T cell responses to mitogens and antigens.

To date, there have been reports of 10 patients (from 6 kindreds) with coronin 1A deficiency (Shiow et al. 2008; Moshous et al. 2013; Stray-Pedersen et al. 2014; Punwani et al. 2015; Yee et al. 2016; Dinur Schejter et al. 2019). Here, we describe an 11th patient with a novel mutation in the WD4 repeat which is necessary for formation of the beta propeller motif. Mutations affecting the N-terminal extension (R12L/del116p11) (Punwani et al. 2015), WD repeat regions—WD2 (P83R/del116p11) (Shiow et al. 2008), WD4 (R201C) (Dinur Schejter et al. 2019), and C-terminal extension (Q360RfsX44) (Stray-Pedersen et al. 2014) have each been associated with complete loss of coronin 1A protein expression, either through truncation codons leading to nonsense mediated decay of mRNA or possibly loss of the stabilizing coiled-coil domain. In cases where residual albeit low levels of coronin 1A were detected (V134M affecting WD3, and S401fs targeting the CE region), a somewhat milder immunological phenotype was described. Nevertheless, attempts to assign genotype–phenotype correlations are complicated by the relatively small number of affected individuals, variability observed in time of disease onset, as well as novel manifestations that continue to broaden the spectrum of disease. With the increased use of next generation sequencing techniques, it is likely that the identification of further coronin 1A deficient patients will help to establish effective evidence-based treatment recommendations.

We have shown here that HSCT using a HLA-matched unrelated bone marrow donor is a curative therapeutic option for coronin 1A deficiency, and results in long term engraftment and solid immune reconstitution. This report also revealed for the first time a novel mutation identified in a patient of Canadian Inuit origin.

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Novel heterozygous *NFKB1* mutation in a pediatric patient with cytopenias, splenomegaly, and lymphadenopathy

Lucy Duan* and Stephen Feanny

ABSTRACT

Background: The nuclear factor κ-light-chain enhancer of activated B cells (NF-κB) signaling pathway is a critical regulator of many important adaptive and innate immune responses. The NF-κB transcription factor family consists of 5 structurally related core proteins, one of which is NFKB1. Mutations in the *NFKB1* gene has been reported in patients with common variable immune deficiency (CVID) as well as with a large spectrum of clinical features including recurrent viral, bacterial, and fungal infections, autoimmunity, inflammation, and malignancy.

Aim: We describe the clinical characteristics of a pediatric patient with a novel mutation in *NFKB1*.

Methods: Patient informed consent was obtained in accordance with approved protocols from the Research Ethics Board at the Hospital for Sick Children. Gene panel testing was employed to identify the immune aberration.

Results: Our patient, a previously well 18-month-old boy of Philippines descent, presented with multi-lineage cytopenias (thrombocytopenia, hemolytic anemia, neutropenia), splenomegaly, and lymphadenopathy. He did not have prior history of recurrent infections. Immunological work-up showed normal numbers of T and B cells, normal quantitative immunoglobulins, and adequate vaccination titres. Gene panel testing revealed a novel heterozygous missense variant c.425T>C (p. Ile142Thr) in the *NFKB1* gene. Due to persistent cytopenias unresponsive to steroids and IVIG, he was started on Sirolimus with improvement in symptoms.

Conclusion: *NFKB1* encodes for p105, which is processed to generate the active p50 transcription factor that can interact with different proteins to activate or inhibit downstream signaling. Our patient was found to have a missense mutation in the Rel homology domain (RHD) of p50, which has distinct functions including DNA binding, protein dimerization, and inhibitory protein binding. The clinical presentation described here broadens the scope of characteristics associated with heterozygous *NFKB1* mutations.

Statement of novelty: We report a novel heterozygous missense variant c.425T>C (p. Ile142Thr) in the NFKB1 gene in a pediatric patient with cytopenias, lymphadenopathy, and splenomegaly. To the best of our knowledge, this variant has not been previously reported.

Introduction

The nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) signaling pathway is a critical regulator of many important cellular processes including the innate and adaptive immune responses, cell proliferation and apoptosis, inflammation, and ectodermal development (Karin and Lin 2002; Vallabhapurapu and Karin 2009). Defects in the NF-κB pathway are associated with a large spectrum of human disease presentations

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manifesting as increased infections, autoimmunity, inflammation, ectodermal dysplasia, and malignancy (Zhang et al. 2017; Scott and Roifman 2019).

The NF-κB transcription factor family consists of 5 structurally related core proteins, NFKB1 (the mature p50 and its precursor p105), NFKB2 (the mature p52 and its precursor p100), RelA, RelB, and c-Rel, which can bind with each other and dimerize to drive or inhibit gene expression at a number of different sites (Siebenlist et al. 1994; Gilmore 2006). Each protein has a conserved N-terminal Rel homology domain (RHD) that allows NF-κB proteins to interact with other proteins (Zhang et al. 2017). In unstimulated cells, NF-κB largely remains in the cytoplasm because of a set of inhibitor proteins comprising the inhibitor of κB (IκB) family (Hayden and Ghosh 2008).

There are 2 well characterized NF-κB signaling pathways, the canonical and non-canonical pathways. The canonical NF-κB pathway is triggered by numerous stimuli including microbial pattern recognition receptors, inflammatory cytokine receptors, or antigen receptors, and leads to phosphorylation of IκBα by the inhibitor of κB kinase (IKK) complex which includes IKKα, IKKβ, and the regulatory protein NF-κB essential modulator (NEMO). Phosphorylation leads to the degradation of IκBα and the subsequent release and translocation of NF-κB into the nucleus (Zhang et al. 2017). The non-canonical NF-κB pathway is activated through binding of a more limited number of receptors including the B-cell activating factor (BAFF) receptor, lymphotoxin β receptor, or CD40 receptor (Shih et al. 2011). Activation leads of this pathway leads to phosphorylation and degradation of different IκB proteins and release of NF-κB into the nucleus. The 2 cascades are likely closely interconnected (Oeckinghaus et al. 2011; Scott and Roifman 2019).

The *NFKB1* gene encodes for p105, which is processed to generate the active p50 transcription factor, and can dimerize with different proteins leading to different functions. It can heterodimerize with RelA or c-Rel to activate canonical NF-κB signaling, or form homodimers to inhibit pro-inflammatory gene expression (Kaustio et al. 2017). In recent years, loss of function heterozygous mutations in *NFKB1* have been increasingly reported in patients with a previous diagnosis of common variable immunodeficiency (CVID) and others presenting with a wide spectrum of clinical features including infections, auto-inflammation, and malignancy.

We report a novel mutation of *NFKB1* in a pediatric patient with cytopenias, lymphadenopathy, and splenomegaly. To the best of our knowledge, this variant has not been reported in previously described *NFKB1* cases (Boztug et al. 2016; Maffucci et al. 2016; Schipp et al. 2016; Kaustio et al. 2017; Lougaris et al. 2017; Dieli-Crimi et al. 2018; Fliegauf and Grimbacher 2018; Tuijnenburg et al. 2018).

Methods

Informed consent was obtained in accordance with requirements of the Research Ethics Board at Hospital for Sick Children, Toronto, ON, Canada. Data was gathered both prospectively and retrospectively from available medical records. Genetic testing was performed through Blueprint Genetics, a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory (#99D2092375). The Primary Immunodeficiency Plus Panel (test code IM0301), a 274 gene panel assessing non-coding variants as well as deletion/duplication analysis, was used.

Case presentation

Patient

Our patient, an 18-month-old male, initially presented to a community hospital with petechiae and bruising on his flank, and was found to have thrombocytopenia, DAT+ hemolytic anemia, and neutropenia. He had a history of a respiratory illness 2 weeks prior to presentation. The patient was born at term by spontaneous vaginal delivery to an uncomplicated pregnancy. There were no maternal illnesses, and serology and ultrasounds were normal. He had normal birth length and weight parameters with no dysmorphic features.

The patient was transferred to the Hospital for Sick Children, Toronto, ON, Canada, for further investigations. Following a marrow bone biopsy which was normal (80% cellularity), he started treatment with intravenous immunoglobulin (IVIG) and steroids, with minimal response in his pancytopenia. Abdominal imaging including an ultrasound and magnetic resonance imaging (MRI) demonstrated splenomegaly and intra-abdominal lymphadenopathy. He continued to

have ongoing pancytopenia with pronounced thrombocytopenia, and was started on sirolimus (mammalian target of rapamycin [mTOR] inhibitor) 3 months following initial presentation. Following the start of sirolimus, he had improvement in his cytopenias, splenomegaly, and lymphadenopathy. He remained on sirolimus for about 6 months, at which point he was weaned off but unfortunately re-developed thrombocytopenia and neutropenia, and required restarting of sirolimus.

The patient did not have a history of recurrent bacterial, viral, fungal, or opportunistic infections. He did not have any significant atopic disease or skin or nail abnormalities, and no arthritis, mouth ulcers, diarrhea, or change in stool habits. He did not have any constitutional symptoms such as fever, weight loss, or decreased energy. His vaccinations were up to date and he was developmentally appropriate for his age. Family history was significant for systemic lupus erythematosus in his paternal grandmother, and 2 paternal grandaunts who both died of renal failure. He had an older sister who was healthy. His parents were of Philippines descent with no known consanguinity.

On physical examination, there were no dysmorphic features. His skin exam was significant for mild bruising and petechiae on his right abdomen. He did not have any palpable lymphadenopathy or evidence of organomegaly. The remainder of his cardiovascular, respiratory, musculoskeletal, and neurological exams were unremarkable.

General investigations

At initial presentation, the patient's complete blood count demonstrated total white blood cell count of 6.9×10^9/L, hemoglobin 90 g/L, platelet count 30×10^9/L, neutrophils 0.21×10^9/L, and lymphocytes 5.72×10^9/L. DAT was positive with reticulocytosis. Electrolytes, renal function, and liver enzymes were normal. He had a normal vitamin B12 level and normal numbers of peripheral double negative T cells. Extensive infectious disease investigations showed negative testing for hepatitis A, B, C, HIV, EBV, CMV, HSV, and HHV6. Metabolic work-up for an underlying metabolic disorder was unremarkable.

Immunological investigations

The patient's immunological investigations are summarized in Table 1. Humoral evaluation demonstrated elevated IgG of 12 g/L with normal IgA, IgM, and IgE.

Table 1: **Immunological investigations at time of initial presentation.**

Laboratory parameters	Patient	Normal range
White blood cell count ($\times 10^9$/L)	6.9	5.0–12.0
Hemoglobin (g/L)	90	110–140
Platelets ($\times 10^9$/L)	30	150–400
Neutrophils ($\times 10^9$/L)	0.21	1.50–8.50
Lymphocytes ($\times 10^9$/L)	5.72	2.0–8.0
Lymphocyte subsets (cells/μL)		
CD4	1184	900–5500
CD8	1363	400–2300
CD19	1132	600–3100
CD16+56	196	100–1400
PHA stimulation index	171	>200
Quantitative immunoglobulins		
IgG (g/L)	12.5	3.2–11.5
IgM (g/L)	0.9	0.0–0.9
IgA (g/L)	0.7	0.4–1.5
IgE (IU/mL)	<25	<60
Vaccination titres (IgG)		
Measles (mIU/mL)	295.73 positive	—
Mumps (IU/mL)	2.91 negative	—
Rubella (IU/mL)	43.9 positive	—
Varicella	Positive	—
Tetanus (IU/mL)	0.49	—
Isohemagluttinin anti B	4	—
CH50 (kU/L)	>60	>29

Vaccination titres demonstrated positive reactivity to measles, rubella, and tetanus and negative to mumps. T cell evaluation showed normal numbers of T, B, and NK cells with low T cell proliferation, however PHA test was performed while patient was on sirolimus, and shortly after being on IVIG. The patient has not been off of sirolimus for a long enough period for repeat of the PHA to be repeated.

Genetic testing

The patient's clinical features of multi-lineage cytopenias, lymphadenopathy, and splenomegaly was concerning for an underlying immunodeficiency or immune dysregulation disorder. Initial targeted Sanger gene sequencing of *FAS* and *PIK3CD* genes were normal. Panel genetic testing was then conducted utilizing a commercially available Primary Immunodeficiency panel. He was found to have a novel heterozygous missense variant in the *NFKB1* gene (NM_003998.3), c.425T>C, resulting in p. Ile142Thr (Figure 1). There is a strong association between the gene and this patient's phenotype, and variant is predicted to be damaging by in silico tools. This genetic variant has not been observed in the reference population cohorts of the

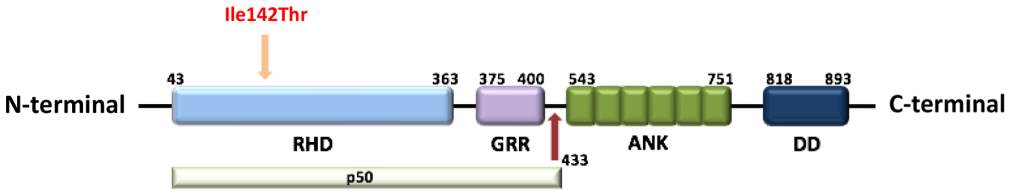

Figure 1: Novel heterozygous mutation targeting the RHD domain of p50/105. Schematic representation of the p105 precursor protein, composed of the Rel homology domain (RHD), a glycine rich region (GRR), C-terminal Ankyrin repeats (ANK), and death domain (DD). Mature p50 comprises the N-terminal portion of p105. The novel heterozygous missense mutation described in our patient, c.425T>C resulting in p. Ile142Thr in the RHD of p50/105, is shown in red.

Genome Aggregation Database (gnomAD, $n > 120\,000$ exomes and >15 000 genomes), nor reported in disease-related variation databases such as ClinVar or the Human Gene Mutation Database (HGMD).

Discussion

We describe a young pediatric patient who first presented with severe thrombocytopenia, neutropenia, splenomegaly, and lymphadenopathy at the age of 18 months. He was found to have a novel missense mutation in the *NFKB1* gene that lead to a change from isoleucine to threonine at the 142 amino acid position in the Rel homology domain (RHD) of p50. This variant has not yet been reported in the literature (Table 2) and likely explains his clinical presentation and has possible future implications for the development of other symptoms in the future.

The RHD is composed of 300 amino acids and has 3 distinct functions including DNA binding, protein dimerization, and inhibitory protein binding (Hayden et al. 2012). Mutations in the RHD has been reported in a number of families with variable clinical phenotype. In a Dutch-Australian family with CVID, a splice-site mutation was found to cause in-frame skipping of exon 8 which lead to degradation of the altered protein (Fliegauf and Grimbacher 2018). Similarly, 2 other families with heterozygous frameshift mutations in RHD were found to have decreased expression of p50. Haploinsufficiency of NFKB1 in these 3 families presented with a heterogeneous spectrum of illness including hypogammaglobulinemia, recurrent sinopulmonary infections, autoimmunity, skin lesions, colitis, lymphoproliferation, and malignancy (Fliegauf and Grimbacher 2018). Interestingly, 1 of the patients in the New Zealand family with a heterozygous frameshift mutation (c.465dupA) in exon 7, presented with similar symptoms as our patient, consisting of thrombocytopenia, hemolytic anemia, and neutropenia from the young age of 2 years in the absence of other symptoms (Fliegauf and Grimbacher 2018).

Multiple recent studies have identified variants in *NFKB1* as a monogenic cause of immunodeficiency/immune dysregulation (Table 2) (Maffucci et al. 2016; Schipp et al. 2016; Kaustio et al. 2017; Lougaris et al. 2017; Dieli-Crimi et al. 2018; Fliegauf and Grimbacher 2018; Tuijnenburg et al. 2018). Patients display a wide range of phenotypic heterogeneity including recurrent sinopulmonary infections, viral and fungal infections, autoimmunity, lymphoproliferation, and malignancy. Differences in gene dosage, modifier genes, and post-transcriptional elements may account for the wide spectrum of penetrance and expressivity.

Heterozygous mutations in 3 Finnish families have been reported in patients with antibody deficiency, recurrent infections, and auto-inflammation (arthritis, mouth ulcers, hyper-inflammatory response to surgery) (Kaustio et al. 2017). Two families had mutations in the RHD of p50 and 1 had a mutation in the Ankyrin repeats of p105. The *NFKB1* variants were associated with different changes in downstream signaling including reduction in p50 and p105 protein quantity, enhanced degradation of p105, and reduced nuclear entry of p50 and decreased transcriptional activity (Kaustio et al. 2017). There was also increased secretion of pro-inflammatory cytokines interleukin-1-β (IL-1β) and tumor necrosis factor (TNF) in 1 of the kindreds (Kaustio et al. 2017). All patients developed symptoms in adulthood and no patients had a similar presentation to our patient from such a young age.

Table 2: **Summary of reported NFKB1 variants and main clinical features.**

Authors	Reported mutation(s)	Clinical features
Fliegauf and Grimbacher 2018	• c.730+4A>G • c.835+2T>G • c.465dupA	• Recurrent sinopulmonary infections • Autoimmunity • Cytopenias • Colitis • Lymphoproliferation—lymphadenopathy, splenomegaly • Malignancy
Boztug et al. 2016	• c.491delG	• Recurrent sinopulmonary infections • Bacterial parapharyngeal abscess • EBV-associated lymphoproliferative disease
Lougaris et al. 2017	• c.730+4A>G • c.1517delC	• Recurrent sinopulmonary infections • Autoimmunity
Schipp et al. 2016	• c.137delA • c.469C>T	• Recurrent sinopulmonary infections • Chronic lung disease and bronchiectasis • Cytopenias • Lymphoproliferation—lymphadenopathy, splenomegaly
Maffucci et al. 2016	• c.1301-1G>A • c.259-4A>G • c.957T>A • c.1375delT	• Recurrent sinopulmonary infections • Autoimmunity • Cytopenias • Viral and opportunistic infections
Kaustio et al. 2017	• c.667A>G • c.1659C>G • c.936C>T	• Recurrent sinopulmonary infections • Autoimmunity: arthritis • Mucosal aphthae
Dieli-Crimi et al. 2018	• c.1149delT	• Recurrent sinopulmonary infections • Colitis • Cytopenias
Tuijnenburg et al. 2018	• c.850C>T • c.1539_1543del • c.160-1G>A • c.1621_1622del • c.843C>G • c.293T>A • c.260T>G • c.83512T>C • c.1423del • c.187delG • c.830dup • c.904dup • c.295C>T • c.1005delG	• Recurrent sinopulmonary infections • Lymphoproliferation—lymphadenopathy, splenomegaly • Autoimmunity • Cytopenias • Colitis • Malignancy

In a cohort of 846 unrelated patients with primary immunodeficiency, including 390 patients with CVID, 16 patients were identified to have pathogenic mutations in *NFKB1* (Tuijnenburg et al. 2018). All 16 patients were within the CVID subgroup, and mutations in *NFKB1* accounted for 4% of all patients with CVID, which was the most common monogenic defect in this group of patients with CVID. Individual *NFKB1* variants were classified as high-effect variants (large deletion, nonsense, frameshift, and splice site variants) or moderate effect (missense substitutions). Interestingly, a large number of missense *NFKB1* variants were identified (52), the majority of which were predicted to not be associated with pathogenicity. Three variants were unique in the cohort of patients with CVID and classified as likely pathogenic. Consistent with other reports of patients with mutations in *NFKB1*, there was a high degree of variability in both the penetrance and expressivity associated with *NFKB1* mutations (Tuijnenburg et al. 2018).

Although haploinsufficiency of *NFKB1* has been predominantly described as a defect of B cells, there are reported cases of diminished T cell function, EBV-associated lymphoproliferation, and viral and fungal infections, suggesting that it may also affect T cell function (Boztug et al. 2016; Schipp et al. 2016). One patient

first presented with autoimmune hemolytic anemia at 14 years of age, then developed lymphadenopathy, hepatosplenomegaly, and frequent viral, bacterial, and fungal infections (Schipp et al. 2016). She had hypogammaglobulinemia, decreased class-switched B cells, naïve CD4 and regulator T cells, and increased double negative T cells. She was found to have a frameshift mutation in RHD which lead to decreased protein expression (Schipp et al. 2016). In our patient, T cell numbers were normal but the T cell proliferation response was low, although it was performed while the patient was on sirolimus and shortly after IVIG treatment. This will need to be repeated in the future, however at this time, he does not have evidence of any severe viral, fungal, or opportunistic infections suggestion of aberrant T cell function.

NFKB1 knockout mice have been shown to develop multi-organ autoimmunity, as well as aberrant immune responses involving B cell dysfunction and abnormal maturation of T regulatory cells. Increased production of pro-inflammatory cytokines including interferon-γ (IFNγ) and tumor necrosis factor (TNF) has also been reported. These mice have increased susceptibility to infection and gut inflammation, which has also been seen in patients with *NFKB1* haploinsufficiency (Stanic et al. 2004; de Valle et al. 2016; Schipp et al. 2016; Kaustio et al. 2017; Dieli-Crimi et al. 2018). To further delineate the biological implications of this novel mutation in our patient, additional functional studies are warranted to ascertain the degree of p50 protein expression, activation of downstream signaling elements such as phosphorylation, and production of cytokines.

Our patient was started on sirolimus for his cytopenias, lymphadenopathy, and splenomegaly. He had improvement in his symptoms following the start of treatment and recurrence when it was discontinued. Sirolimus is an mTOR inhibitor which inhibits cytokine receptor-dependent signal transduction, blocking the activation of T and B lymphocytes (Sehgal 2003). It can be taken orally, is safe, and has been used in a prospective trial of patients with refractory multi-lineage cytopenias from different underlying diagnoses including autoimmune lymphoproliferative syndrome (ALPS), CVID, systemic lupus erythematosus (SLE), and single lineage cytopenias (Bride et al. 2015). Current treatment options for patients with *NFKB1* mutations and CVID clinical presentation including symptomatic treatment with immunoglobulin replacement, immunosuppression, and (or) antibiotics. Better delineation of the pathogenic mechanisms associated with *NFKB1* variants may inform more targeted treatment strategies.

Conclusion

We report a novel mutation in the RHD domain of *NFKB1* in a pediatric patient with early onset cytopenias, splenomegaly, and lymphadenopathy. This case demonstrates the importance of considering primary immunodeficiency in patients with unexplained autoimmune cytopenias, and further contributes to the growing clinical phenotype of heterozygous *NFKB1* mutations.

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Chronic mucocutaneous candidiasis associated with a novel frameshift mutation in IL-17 receptor alpha

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ABSTRACT

Background: Chronic mucocutaneous candidiasis (CMCC) has traditionally encompassed endocrinopathy, autoimmunity, and infection of the skin, nails, oral and genital mucosa. It is typically caused by *Candida albicans*, an organism that is found to be commensal in healthy individuals. To date, most patients with CMCC have mutations in *AIRE* or *STAT1*. While chronic *Candida* spp. infection is a feature of multiple profound T cell deficiencies, it has also been identified in rare cases involving selective immune defects, including interleukin-17 receptor A (IL-17RA) deficiency. An association between *Staphylococcus aureus* infections and candidiasis due to IL-17RA deficiency has recently been proposed.

Aim: We sought to identify the genetic defect in a patient presenting with recurrent oral thrush and *S. aureus* infections, but otherwise unremarkable immune workup.

Methods: Whole exome sequencing and Sanger confirmation was performed, and protein expression analysis utilized to assess the impact of the genetic aberration. A comprehensive immune workup was completed to characterize any possible deficits in his immune system.

Results: Next generation sequencing techniques identified a homozygous mutation in *IL17RA*, c.1696insAG, resulting in the frameshift mutation p.Q566fs. Western blot analysis confirmed the loss of IL-17RA expression.

Conclusion: We describe here a novel frameshift mutation in *IL17RA*. Clinically, the patient was a diagnostic challenge as he did not present with a classic CMCC phenotype. This case emphasizes the importance of genetic analysis in patients presenting with recurrent infections.

Statement of novelty: We identify a novel frameshift mutation in *IL17RA* in a patient presenting with recurrent bacterial and fungal mucocutaneous infections.

Introduction

Chronic mucocutaneous candidiasis (CMCC) is characterized by chronic, non-invasive *Candida* spp. infections of the skin, nails, and mucus membranes (Ahonen et al. 1990; Puel et al. 2011). Interleukin (IL)-17-producing T cells have been demonstrated to play a critical role in fighting off bacterial and fungal infections (Puel et al. 2012). The cytokine IL-17 can be further characterized into 6 different types, IL-17 A-F, which can bind to 5 unique receptors, termed IL-17R A-E. The IL-17RA is the common subunit for all 5 members of the IL-17 receptor family (Okada et al. 2016).

Genetic aberrations which can disrupt the differentiation of lymphocytes into Th17 effector cells, or production and binding of IL-17, can result in a CMCC phenotype. These include upstream players responsible for the production of IL-17 producing cells, such as

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the adaptor protein caspase recruitment domain-containing protein 9 (CARD9) or transcription factors like signal transducer and activator of transcription 1 and 3 (STAT1 and STAT3) (Glocker et al. 2009; Minegishi et al. 2009; Liu et al. 2011). Furthermore, mutations in the autoimmune regulator (*AIRE*) gene have previously been associated with neutralizing autoantibodies against IL-17A, IL-17F and (or) IL-22, as well as syndromic CMCC due to impaired IL-17 mediated immunity (Puel et al. 2010). Downstream of IL-17RA, patients with mutations in the intracellular signaling SEF/EL-17R (SEFIR) domain of the adaptor molecule ACT1 (Zhang et al. 2014), which is crucial for IL-17 signaling, also suffer from CMCC (Boisson et al. 2013). Collectively, these findings suggest that CMCC is caused by mutations which impair the IL-17 pathway.

Given that neutralizing antibodies against IL-17 cytokines can result in CMCC phenotypes (Kisand et al. 2010; Sarkadi et al. 2014), it is thus plausible that genetic aberrations affecting IL-17 cytokines and receptors could also cause this syndrome (Puel et al. 2010). IL-17 is secreted from TH17 cells in the presence of microbial antigens, resulting in the recruitment of IL-17RA expressing neutrophils to the site of infection (Nahum 2017). This process is thought to contribute to cutaneous immunity against *C. albicans* and *S. aureus* infections. The clinical and immunological features of a cohort of 21 patients with autosomal recessive mutations in *IL17RA* was recently described (Levy et al. 2016). Affected individuals were most commonly of Middle Eastern, Japanese or South American ancestry. Interestingly, they did not present with classic CMCC—only a minority of patients had nail candidiasis. However, all patients suffered oral thrush and scalp or skin *Candida* spp. infections, as well as *S. aureus* skin infections. The staphylococcal infections presented in the form of folliculitis, furunculosis and pustulosis. There have also been reports of sinopulmonary infections as well as pulmonary tuberculosis (Levy et al. 2016; Okada et al. 2016).

We report here a patient who presented with recurrent oral thrush, a history of eczema complicated with recurrent superinfection of *S. aureus* and 2 episodes of sinopulmonary infections. When utilizing whole exome sequencing, we identified a novel homozygous mutation in *IL17RA*.

Methods

Ethics

Patient informed consent was obtained, and information collected prospectively and retrospectively from medical records (REB Protocol no. 100005598, The Hospital for Sick Children).

Serum concentration of immunoglobulin and specific antibodies

Levels serum concentrations of immunoglobulins (IgG, IgA and IgM) were measured by nephelometry and IgE concentrations determined by RIA using the IgE PRIST kit (Pharmacia Diagnostics, Dorval, QC, Canada). Serum antibodies to tetanus toxoid were measured by ELISA (Binding Site, Birmingham, UK). Serum antibodies to measles, mumps and rubella were measured using ELISA kits available from Euroimmun (Gross Groenau, Germany). Isohemagglutinin titres were determined by 2-fold serial dilution with erythrocytes, and are reported as antiglobulin phase, the dilution at which agglutination occurs.

T and B cell proliferative response

Lymphocyte proliferative responses to the mitogen phytohemagglutinin (PHA) was performed in triplicate and compared with simultaneously stimulated normal controls, as previously described (Arpaia et al. 1994).

Exome sequencing and variant calling

Exome library preparation and sequencing was performed by The Centre for Applied Genomics (TCAG), Toronto, ON, Canada. DNA was quantified by Qubit DNA HS assay (Life Technologies, Carlsbad, CA, USA) and 100 ng of input DNA used for library preparation using the Ion AmpliSeq Exome Kit (Life Technologies). The Ampliseq Exome library was immobilized on Ion PI™ Ion Sphere™ particles using the Ion PI Template OT2 200 Kit v3. Sequencing was performed with the Ion PI Sequencing 200 Kit v3 and Ion PI Chip v2 in the Ion Proton™ semiconductor sequencing system in accordance with the manufacturer's instructions. Alignment and variant calling were performed using Torrent Suite (v4.0) on the Ion Proton Server, using the Ion Proton ampliseq germline low stringency setting and the hg19 reference genome. The variants were annotated using an in-house annotation pipeline (Stavropoulos et al. 2016).

Sanger sequencing analysis

Genomic DNA was extracted from isolated peripheral blood lymphocytes using the Geneaid Genomic DNA Mini Kit. Genomic DNA was amplified by PCR with specific primers designed upstream and downstream of the IL17RA gene. 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).

Western blotting

Whole blood was collected by venipuncture and peripheral blood mononuclear cells isolated by Ficoll separation. Whole cell lysates were prepared in 1% TritonX-100 buffer and protein expression of IL-17RA analyzed by Western blotting. Primary anti-IL17RA and goat anti-rat secondary antibodies were purchased from Abcam.

Results

Case report

Our patient is a 10-year-old male, born at term to non-consanguineous parents. The mother is healthy but the father was 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 respond to topical anti-fungal medication. Although at 3 years of age he had recurrent oral thrush and diaper rash that did not respond to topical Nystatin. At this point he had developed Candida of the nails and scalp. At this point he was started on oral fluconazole prophylaxis of 3–6 mg/kg. The patient also suffered from recurrent mouth lesions that were consistent with impetigo and responded well to topical antibiotics. He was also diagnosed with asthma, but had no evidence of endocrinopathy, or other autoimmune manifestations. He was taken off anti-fungal prophylaxis at age 9 and has not had recurrence of fungal infections since being taken off for the last 1.5 years.

Immune evaluation

Despite recurrent infections, evaluation of the immune system (Table 1) was unremarkable. Serum IgG, IgA, and IgM levels were normal, as were lymphocyte subsets. Specific antibody levels were protective to Rubella, Varicella and Tetanus as well as a good response to pneumococcal vaccine. Proliferative responses phytohemagglutinin were normal.

Table 1: **Immune work up of patient with IL-17RA deficiency.**

Laboratory parameters	Patient	Reference range
White blood cell count ($\times 10^9$/L)	5.7	5–12
Neutrophils ($\times 10^9$/L)	2.55	1.5–8.5
Lymphocytes ($\times 10^9$/L)	2.61	2–8
Eosinophils ($\times 10^9$/L)	0.09	0.02–0.5
Lymphocyte subsets (cells/μL)		
CD3+	2856	700–4200
CD19+	1099	200–1600
CD3+/CD4+	1799	300–2000
CD3+/CD8+	916	300–1800
NK (CD16+/56+)	667	90–900
PHA stimulation index	>50%	>50% of patient control
Immunoglobulins		
IgG (g/L)	7.2	5.0–14.6
IgM (g/L)	1.0	0.2–2.5
IgA (g/L)	0.6	0.3–2.0
IgE (IU/mL)	9	<90
Vaccination titres (IgG)		
Measles (IU/mL)	817 (positive)	Positive
Mumps (RU/mL)	26 (positive)	Positive
Rubella (IU/mL)	60 (positive)	Positive
Varicella	Positive	Positive
Tetanus (IU/mL)	0.37	>0.1
Isohemagglutinins		
Anti B	32	>32
Anti A	Not tested	—

Genetic evaluation

Genetic testing of *STAT1* and *AIRE* genes were negative. Subsequent whole exome sequencing and Sanger confirmation identified a novel homozygous mutation in *IL17RA*. The patient had a 2 base insertion in *IL17RA* (NM_014339.6), c.1696insAG causing a frameshift mutation (p.Q566fs) (Figure 1). The mutation was found in the SEFIR domain (amino acid residues 351–597) of IL-17RA (Figure 2) and has not previously been reported in the literature.

Quantification of IL-17RA protein levels

Western blot analysis of IL-17RA expression revealed loss of protein expression, as compared to healthy control (Figure 3).

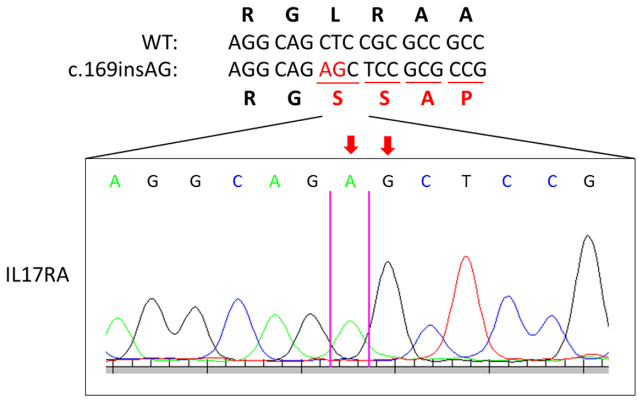

Figure 1: Novel frameshift mutation in *IL17RA*. Electropherogram showing the novel mutation in *IL17RA* (NM_014339.6), c.1696insAG, causing a frameshift mutation p.Q566fs. Insertion of bases A and G are identified with red arrows. Wild type (WT) and novel c.169insAG sequences and resultant amino acids are shown.

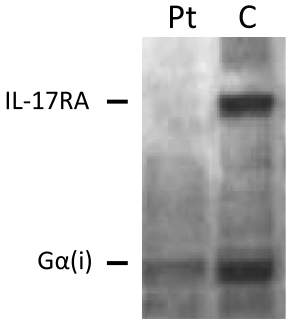

Figure 3: IL-17RA expression in patient PBMCs. Loss of IL-17RA protein expression is shown relative to healthy control, demonstrating a null phenotype.

Discussion

We report here a child presenting with recurrent *S. aureus* skin infections, atopy, and limited oral thrush and diaper rash. He was initially thought to have an autosomal dominant mutation given his father's history of autoimmunity; however, genetic analysis of *STAT1* and *AIRE* were negative. Further assessment by whole exome genome sequencing revealed a novel homozygous mutation in *IL17RA*, encoding a crucial receptor involved in IL-17 signaling.

Patients affected with *IL17RA* mutations are categorized under the umbrella of CMCC, however, they do not present with classic CMCC—only a minority have skin and nail candidiasis (Levy et al. 2016; Okada et al. 2016). Our patient's recurrent *S. aureus* infections were initially thought to be secondary to a leaky skin barrier due to his atopic dermatitis. Thus, his diagnosis was delayed until years later when next generation sequencing technologies became more readily accessible.

Clinically, genetic aberrations in *IL17RA* are associated with susceptibility to oral thrush and scalp or skin *Candida* spp. infections, as well as *S. aureus* skin infections. The staphylococcal infections present in the form of folliculitis, furunculosis, and pustulosis. There have also been reports of sinopulmonary infections (Levy et al. 2016; Okada et al. 2016). Together, our patient's clinical picture fits with the reported phenotypes in the literature.

IL17RA encodes IL-17RA, a type 1 membrane glycoprotein that forms one half of a heterodimer required for IL-17A and IL-17F signaling (Gaffen 2009). The mutation identified in our patient is localized to the SEFIR domain, which is essential for interaction with ACT1 and allows for IL-17RA dependent downstream effects (Maitra et al. 2007; Ho et al. 2010). Structure-function studies have demonstrated that the SEFIR motif is critical for IL-17A dependant activation of pathways, including MAPK, NF-κB, and C/EBP (Maitra et al. 2007). Levy et al. (2016) previously reported on a kindred with a frameshift mutation (p.N440Rfs*50) affecting the same SEFIR domain as our patient. The reported patients' clinical picture resembled our patient as they also suffered from eczema

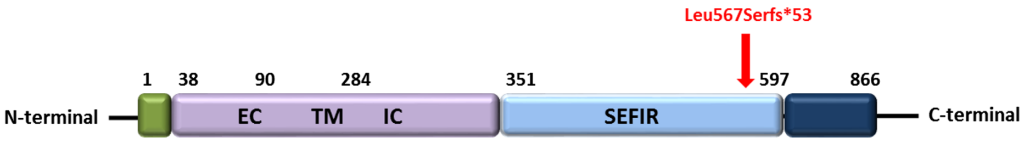

Figure 2: Schematic diagram of IL-17RA structure. The extracellular (EC), transmembrane (TM), intracellular (IC) and SEFIR (similar expression to fibroblast growth factor genes/IL17R) domains are shown, as well as the location of the mutation.

and had skin pustulosis and folliculitis. The null mutation was associated with abolished lymphocyte responses to IL-17, as well as susceptibility to recurrent staphylococcal skin infections and bacterial respiratory infections, consistent with our patient.

IL-17A plays an important role in the clearance of *S. aureus* (Ishigame et al. 2009; Cho et al. 2010). Using murine models of IL-17A deficiency, Ishigame et al. (2009) showed increased susceptibility of *Il17a*$^{-/-}$ mice to opportunistic *S. aureus* infection compared to wild-type mice. However, intravenous administration of the bacterium revealed no difference in levels of bacterial dissemination, implying a role for IL-17A (and thus IL-17RA) in controlling local but not systemic *S. aureus* infections. In our patient, the localized infection at the skin and mucosal membranes may be partially accounted for by the dependence of keratinocytes and bronchial epithelial cells on this cytokine's protective effects. Further, IL-17A (alongside IL-17F) regulates immune function through stimulation of, among others, antimicrobial peptide production (Ouyang et al. 2008). The absence of functional IL-17RA results in abolished responses to the cytokine IL-17A (Minegishi et al. 2009). Mechanistically, this supports the chronic *S. aureus* skin infections that our patient suffered from.

Clinically, this case presented a diagnostic challenge. While the patient suffered from chronic oral thrush and a diaper rash, he also had eczema, skin staphylococcal infections and other oral lesions. Because both *S. aureus* and *Candida* spp. are typically commensal organisms found in healthy individuals, these infections can occur after insults to the skin, such as eczema. Therefore, it is possible that the infections in our patient were 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 associated with *AIRE* and *STAT1* mutations. However, this patient did ultimately have concerning features for immunodeficiency which warranted completing whole genome sequencing in an attempt to identify a genetic culprit. Specifically, in our patient with the phenotype of more than 1 recurrent and persistent infection of both *Candida* spp. and *S. aureus*, requiring multiple treatments of both oral and topical anti-microbial medications, and ultimately anti-fungal prophylaxis for 8 years, further genetic investigations were necessary when common culprit mutations for CMCC (*AIRE* and *STAT1*) were not identified. Together, this report highlights the need for comprehensive genetic analysis in all cases that present with recurrent fungal infections, regardless of whether the cause is thought to be primary or secondary.

In summary, we have described here a patient with recurrent CMCC and *S. aureus* infections due to a novel frameshift mutation in *IL17RA* affecting the SEFIR domain. Functionally, the absence of IL-17RA receptor expression confirms that this is a loss of function mutation.

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A clinical trial protocol to evaluate the safety and pharmacokinetics of subcutaneously administered immunoglobulin in patients with primary immunodeficiency

Linda Vong*

ABSTRACT

This protocol is excerpted from recent clinical trials used to study the pharmacokinetics, safety, and tolerability of subcutaneously administered immunoglobulin (SCIG) in subjects with primary immunodeficiency. The primary objective is to determine the weekly dose of SCIG product that produces a steady-state area under the concentration-time curve of total immunoglobulin G level that is non-inferior to that of regularly administered intravenous immunoglobulin (IVIG). We include details of the target population, eligibility criteria, treatment phases, key assessments and procedures, and study analyses. Given that IVIG may be problematic in patients with poor venous access or those who develop systemic adverse effects, among others, the development of SCIG for use in the home setting provides an alternative treatment technique for adults and children with primary immunodeficiency.

Statement of novelty: This protocol describes the main topics found in prospective clinical studies evaluating the safety and pharmacokinetics of SCIG in subjects with primary immunodeficiency.

Introduction

Primary immunodeficiency

Primary immunodeficiency diseases (PIDs) are a group of rare, chronic disorders caused by either de novo or hereditary mutations in genes of the immune system. Such changes may alter the development or function of individual immune cell types, affect the response to an immune trigger, or broadly affect a common cellular process required for immunological competence. Clinically, patients with PID experience frequent bacterial, fungal, protozoal, and viral infections (Picard et al. 2015). B cell deficiencies, also known as humoral or antibody deficiencies, comprise the largest group of PIDs and are characterized by insufficient or absent levels of protective antibodies. While the underlying cause is often due to mutations within genes regulating B cell differentiation, in many instances, the molecular basis remains unknown. Hypogammaglobulinemia and agammaglobulinemia are common features of X-linked agammaglobulinemia (XLA) (Bruton 1952; Bonilla and Geha 2003) and common variable immunodeficiency (CVID) (Resnick and Cunningham-Rundles 2012).

It is notable that immunoglobulin (Ig) G replacement therapy, first introduced in 1952 with the infusion of serum globulin fraction, has proven efficacious in reducing the frequency of infections among these patients (Roifman and Gelfand 1988). To date, the therapeutic management of PID has been carried out

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using various IgG preparations via intramuscular (IM), intravenous (IV), and subcutaneous (SC) routes.

Immunoglobulin replacement therapy

Infusion of IV IgG (IVIG) is widespread, however, in a subset of patients with poor venous access or those who develop systemic adverse events (AE), such as headaches, fever, chills, and myalgia (Ochs et al. 2006), delivery of IgG by SC infusion (SCIG) represents an alternative that is safe and has good efficacy (Stiehm et al. 1998; Radinsky and Bonagura 2003). Whereas IVIG is associated with marked variability in IgG levels between infusions (coinciding with a rise in IgG immediately following infusion and a decline after redistribution to the extravascular space), SCIG is absorbed and redistributed slowly, resulting in higher and more consistent trough levels (Orange et al. 2012; Berger and Allen 2015). It is generally accepted that SCIG causes few, if any, adverse systemic reactions and such home-based therapy may afford patients greater independence, freedom from reliance on trained personnel and specialized facilities, as well as improved quality-of-life.

Study rationale

The primary objective is to determine a weekly dose of SCIG product (referred to herein as SCIG-x) that produces a steady-state area under the concentration-time curve (AUC) of total IgG level that is non-inferior to that of a regularly administered IVIG product (referred to as IVIG-x). Given that the bioavailability of SCIG is reportedly 30% lower compared to administration of IVIG, due in part to extracellular matrix binding and breakdown by the tissue enzymes (Wang et al. 2008; Berger et al. 2013), an IVIG to SCIG dose adjustment of 1.37 will be used. This is based on historical data assessing bioequivalence of these routes of infusion (Wasserman et al. 2010, 2011; Berger et al. 2011), and addresses the requirement of the US Food and Drug Administration (US FDA) that doses of SCIG be adjusted to provide equivalent AUC of total IgG compared to that of previous IVIG therapy. The dose adjustment factor of 1.37 will be evaluated in interim pharmacokinetic (PK) analysis and modified if necessary.

Study objective

The objective of this study is to evaluate the pharmacokinetics, safety, and tolerability of an SCIG in subjects with PID.

Primary pharmacokinetic objective

To determine a dose of weekly SCIG-x that produces a steady-state AUC of total IgG by SC administration that is not inferior to that of a regularly administered IV dose of IVIG-x in subjects with PID.

Secondary pharmacokinetic objective

To determine whether SCIG-x maintains steady-state trough total IgG levels that are comparable to the mean trough total IgG levels of IVIG-x in subjects with PID.

Safety objective

To assess the safety and tolerability of the SCIG-x formulation.

Exploratory objectives

To evaluate:

- Maximum concentration (C_{max}) and time to reach C_{max} (T_{max})
- Trough levels of IgG subclasses and measles antibody subclasses
- Antibody titres of *Streptococcus pneumoniae*, *Hemophilus influenzae*, and *Clostridium tetani*
- Rate of serious bacterial infections
- All types of infections as well as validated infections
- Number of days on antibiotics (prophylactic or for treatment of infection)
- Number of hospitalizations due to infections
- Number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment

Investigational plan

The study is a prospective, multi-center, open-label, single-sequence study. To account for possible dropouts, 50 subjects with well-defined PID and antibody deficiency will be enrolled to ensure approximately 30 adult subjects and 10 pediatric subjects (age 2–16 years) complete treatment with SCIG-x. This number of subjects would provide enough data to assess the primary objective. All subjects will be followed for 6 months. Pharmacokinetic (PK) samples will be collected to assess total IgG profiles.

Study duration

The study period is expected to be up to 50 weeks, including 7–28 days for screening. There are

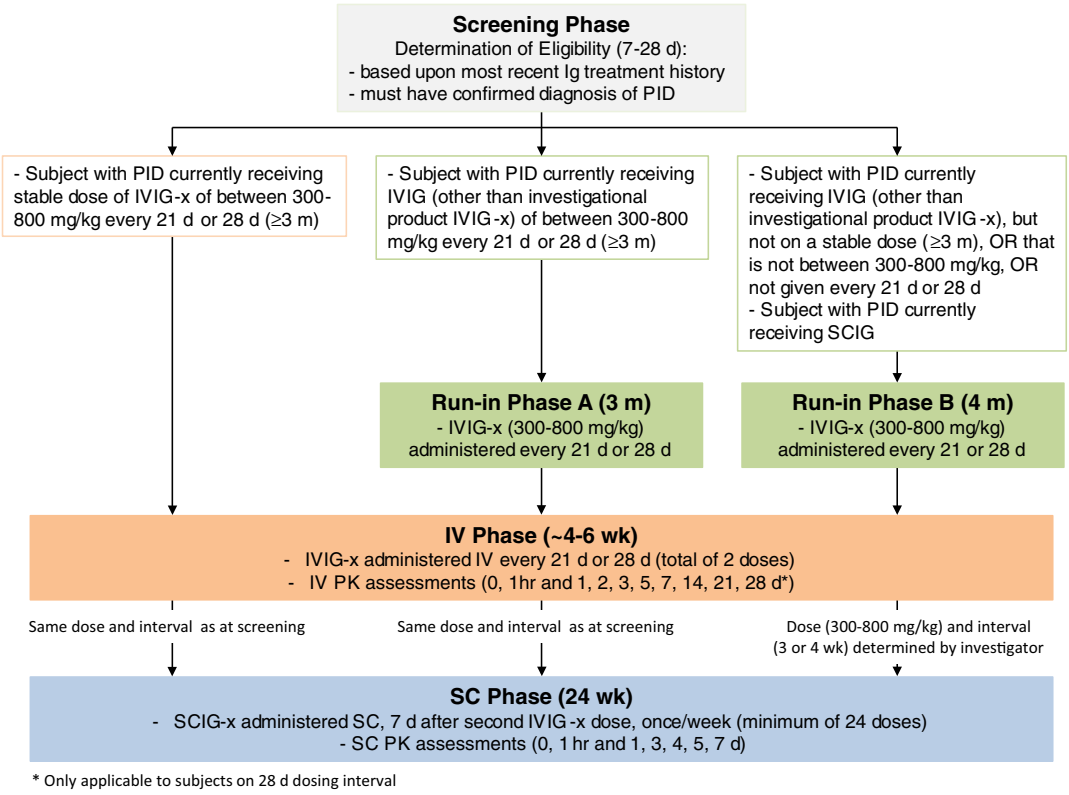

Figure 1: Study design, including details of IV and SC dosing.

3 treatment phases (Figure 1): run-in phase (3–4 m), IV phase (4–5 weeks), and SC phase (24 weeks). A follow-up visit will be scheduled 21 or 28 days after the last study infusion.

Study design

All subjects will be screened for between 7 and 28 days before enrollment into the study and, if eligible, will enter the run-in or IV phase (IV administration of IVIG-x) based upon the most recent IgG treatment history. Subjects will subsequently receive SC administration of SCIG-x during the SC phase, using a dose adjustment factor of 1.37.

Enrolled subjects who currently receive a stable IV dose (≥3 m) of the study drug IVIG-x (300–800 mg, every 3 or 4 weeks) will bypass the run-in phase and proceed directly to the IV phase. Their first infusion of IVIG-x will coincide with their next scheduled IV infusion, in accordance with their regular dosing interval.

Run-in phase

Subjects will be assigned entry into run-in phase A if they were previously receiving IVIG therapy (300–800 mg/kg, every 3 or 4 weeks) but not IVIG-x (i.e., a different commercially available drug). The subjects in this category will receive IVIG-x, at an equivalent dose and interval prior to study entry, for a total duration of 3 m.

Subjects will be assigned entry into run-in phase B if they were previously receiving either SCIG or IVIG therapy, but not at a stable IV dose for 3 m prior to screening, or where the dose is not 300–800 mg/kg, or where the infusions were not at an interval of 3 or 4 weeks. The subjects in this category will receive IVIG-x, at an equivalent dose and interval prior to study entry, for a total duration of 4 m.

IV phase

All subjects will receive 2 in-clinic IV doses of IVIG-x at their regular dosing interval (every 3 or 4 weeks). PK samples will be collected pre-dose and post-dose (0 and 1 hours, and 1, 2, 3, 5, 7, 14, and 21 days. Subjects on a 4 week interval will also have samples collected at 28 days).

SC phase

Seven days after the second IV infusion of IVIG-x, subjects will begin weekly SC infusions of the

investigational product SCIG-x. Weekly infusions will take place for 6 m, with PK measurements performed after 3 m (SC infusion #13; 0 and 1 hours, and 1, 3, 4, 5, 7 days). The SC dose will be calculated using the IV to SC dose adjustment factor of 1.37:

$$\text{SC dose} = [\text{IV dose(mg/kg)}/\text{dosing interval (week)}] \times 1.37$$

Ten infusions will take place in-clinic (SC infusion #1, 2, 3, 5, 9, 13, 14, 17, 21). All others will occur in the home setting. A final in-clinic visit will take place at week 25.

Interim PK analysis

Interim PK analysis will be performed to evaluate whether the dose adjustment factor is adequate to provide steady-state AUC of total IgG levels that is not inferior to levels achieved during the prior IV dose. Analysis will be based on the first 6 subjects (age ≥12–75 years) to complete PK assessments during both the IV phase and SC phase (i.e., after SC infusion #14). Standard PK analysis methods will be used: if the geometric least-squares mean (LSM) ratio of SC AUC of total IgG vs. IV AUC of total IgG ≥0.9, no changes will be made to the dose adjustment factor. If the ratio is <0.9 and mean trough concentrations of IgG falls below the target level during administration of SCIG-x, the dose adjustment factor will be increased with consideration given to mean IgG trough levels obtained during SCIG-x administration. A revised dose adjustment factor will be communicated to sites and implemented at the next in-clinic infusion. All subjects will be required to complete 24 weeks of SC infusions with the revised dose adjustment factor, with the first 6 subjects undergoing PK analysis after 12 weeks (as above).

Investigators and study centres

Study centres will enroll up to 50 subjects.

Randomization and stratification

Not applicable.

Blinding

Not applicable.

Selection of the study population

Subjects with a confirmed diagnosis of PID requiring IgG replacement will be selected for screening. Those who fail to meet eligibility criteria may be rescreened once.

Inclusion criteria

1. Male or female, between ages of 2–75 years (inclusive) at the time of screening.
2. Confirmed clinical diagnosis of PID (IUIS classification) with agammaglobulinemia or hypogammaglobulinemia requiring IgG replacement therapy.
3. Subject has not had documented serious bacterial infection (SBI) within the 3 months prior to screening.
4. Subject has received licensed IgG replacement therapy (IV or SC) for at least 3 consecutive months prior to this study. Those on IVIG must currently receive a dosage of 300–800 mg/kg per infusion.
5. At least 1 documented IgG trough level of ≥500 mg/dL within the last 3 months while on current IgG replacement therapy.
6. Screening trough IgG level must be ≥500 mg/dL. If below this threshold, the subject will be logged as a screen failure. The subject may be screened a second time following dose adjustment and maintenance of stable dosing for a period of at least 3 consecutive months.
7. Authorization to access personal health information, including medical records.
8. Subject, parent, or guardian has signed an informed consent. If applicable, pediatric subjects will require assent forms as appropriate per study documentation and approved by the local jurisdiction (Research Ethics Board/Institutional Review Board).
9. Subject/caregiver is willing to comply with all aspects of the protocol.

Exclusion criteria

1. Subject has previously had a serious adverse reaction to Ig or severe anaphylactic reaction to blood or any blood-derived product.
2. Subject has a history of disorders where SC therapy may be contraindicated, including blistering skin disease, clinically significant thrombocytopenia, bleeding disorder, recurrent skin infections, or diffuse rash.
3. Subject has been diagnosed with isolated IgG subclass deficiency, isolated specific antibody deficiency, or transient hypogammaglobulinemia of infancy.

4. Subject has isolated IgA deficiency (with or without antibodies to IgA).
5. Females who are pregnant, breast feeding, or planning a pregnancy during the course of the study. Subjects who become pregnant during the study will be withdrawn from the study.
6. Subject has significant proteinuria (dipstick proteinuria is ≥3, urinary protein loss >1–2 g/24 hours, nephrotic syndrome), protein losing enteropathy, history of acute renal failure, severe renal impairment (blood urea nitrogen or creatinine >2.5 times the upper normal limit), receiving dialysis, nephrotic syndrome, or lymphangiectasia.
7. Subject has levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.5 times the upper limit of normal range for the testing laboratory.
8. Subject has hemoglobin levels <9 g/dL.
9. Subject has a history (incident in the year prior to screening, or 2 episodes during lifetime) or current diagnosis of thromboembolism or deep venous thrombosis, or any other significant acute or chronic disease that, in the opinion of the investigator, may place the subject at undue medical risk or interfere with the subjects' ability to complete the study.
10. Subject is currently receiving anti-coagulant therapy which would make SC route of administration inadvisable.
11. Subject currently has a known hyperviscosity syndrome.
12. Subject has secondary immunodeficiency or human immunodeficiency virus (HIV) infections/acquired immune deficiency syndrome (AIDS).
13. Subject has a known history, or is positive at enrolment, for current hepatitis B virus (HBV) or hepatitis C virus (HCV) infection.
14. Subject (≤18 years of age) has non-controlled arterial hypertension ≥90th percentile systolic or diastolic blood pressure for age and height; or, if subject is an adult, has non-controlled arterial hypertension (systolic blood pressure >160 mm Hg and (or) diastolic blood pressure >100 mm Hg).
15. Subject is receiving the following medication: (*i*) long-term systemic steroids (dose >1 mg/kg/day of prednisone or equivalent for >30 days); (*ii*) immunosuppressive drugs or chemotherapy; and (*iii*) immunomodulators.
16. Subject has known prescription drug or substance abuse.
17. Subject has participated in another clinical study (interventional) within 3 weeks prior to study enrollment.

Withdrawal of subjects

Subjects can withdraw from the study at their own request, or at the request of their legally acceptable representative, without prejudice. In all cases, the reason for withdrawal will be recorded. The investigator may withdraw a subject from the study drug or the study for the following reasons:

- If, in the investigator's opinion, continuation in the study poses a risk or would be detrimental to the well-being of the subject (for example, following occurrence of severe or serious AE, or medical condition)
- Subject develops an SBI during the IV phase of the study, prior to the first dose of SCIG-x
- Subject does not comply with the protocol
- Pregnancy
- At the specific request of the sponsor

Premature termination of study/closure of centre

During the study, the Sponsor, REB/IRB and (or) regulatory authorities have the right to terminate the study or close the study centre at any time. The REB/IRB must be informed. In the event of premature study termination or site closure, all materials must be returned to the sponsor with exception of documents that have to remain stored on site. A study centre may be closed due to:

- Insufficient enrolment
- Non-compliance with the study protocol
- Unexpected and significant or unacceptable risks for study subjects, usually due to non-compliance with ICH guideline for Good Clinical Practice (GCP)

Study conduct

Treatments regimens

Subjects will be assigned entry into run-in phase A, run-in phase B, or IV phase, based on the subject's current antibody replacement regimen (refer to Study Design, Figure 1). Subjects entering run-in phase A or run-in phase B will be administered 300–800 mg/kg IVIG-x (every 21 or 28 days, depending on current dose interval) for 3 or 4 m, respectively. Subjects entering

directly into the IV phase will be administered 2 doses of IVIG-x (300–800 mg/kg of body weight every 21 or 28 days). All subjects will proceed to the SC phase of the study, and receive weekly doses SCIG-x according to the IV to SC dose adjustment calculation formula. The dosing of IVIG-x and SCIG-x will be dependent on the treatment each subject receives at the time of screening. This will remain unless there is a medically justified need to change it or if there is a change made to the dose adjustment factor.

Treatment assignment

A subject will be enrolled into the study once written informed consent/assent has been obtained and inclusion/exclusion criteria is fulfilled. Assignment of the treatment phase (run-in phase A, run-in phase B, or IV phase) will be based on the subject's current dose of antibody replacement product and interval.

Subject identification

Subjects will be assigned a numerical subject identifier in the format xxx-yyyy. This is based on a 3-digit study centre number (xxx, assigned by the sponsor) and a sequential 4-digit subject number designated by the investigator at the screening visit (yyyy). Once a subject identifiers has been assigned it will not be reused at any centre.

Physical examination

A full physical examination will be performed during the screening visit, first SCIG-x infusion (SC infusion 1) and the final visit/termination visit. This will include evaluation of all body systems as considered normal standard of care for the centre. Abbreviated physical examinations will be performed during all other visits, and will target symptoms as well as examination of heart, lung, ears/nose/throat, inspection of previous injection sites.

Medical history and demography

Medical records will be reviewed in detail to confirm documented diagnosis of PID, including current and previous IgG treatments. Demographics including age, gender and history of PID diagnostic tests will be recorded in case report forms (CRF).

Subject diary

Subjects will be provided an infusion diary prior to the first SC infusion and given careful instructions to record items daily. Entries made since the previous clinic visit will be reviewed at every in-clinic visit. The following will be entered into the diary:

- Local infusion site reactions
- Concomitant medications, prescription and non-prescription
- Details of study drug infusion (number and location of sites, date and (or) time of start/end, dose and (or) volume of each dose, duration and rate)
- Number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment
- Adverse events (AE) that occurred following the previous study infusion

Dosage

The amount of IVIG-x and SCIG-x to be administered will be calculated based on each subject's current IgG replacement regimen (refer to Figure 1). For SC infusions, the weekly dose will be one quarter (for subjects on 4 week dosing regimens) or one third (for subjects on 3 week dosing regimens) of the IV dose of IVIG-x, multiplied by the dose adjustment factor of 1.37. The subject's weight will be recorded at screening and prior to each infusion to determine if adjustment of the dose is necessary.

Subcutaneous administration

The subject and investigator will determine the number of injections sites, rate of infusion, and the time of day that the SC infusions will take place, with emphasis on adhering to the dosing regimen without deviation. The subject will receive the first 3 infusion in clinic under supervision to ensure adequate training before self-administration in the home setting is allowed.

The same anatomical region may be used for SC infusions, and regions may be rotated throughout the study. A maximum of 8 infusion sites will be used per infusion, with no less than 2 in. between sites and target infusion rates not exceeding 25 mL/hour/site. Target infusion rates should not be changed once the rate is reached, unless there are tolerability issues.

Visit schedule

The schedule of study procedures is summarized in Table 1. Each study visit will be allowed a window of ±1 day. All IVIG procedures will take place in the clinic setting. During the first 3 SC phase infusions, subjects will be trained to self-administer the SCIG product in

Table 1: **Schedule of study procedures.**

			IV phase		SC phase									
Clinic Visits	Screening (7–28 d)	Run in (if needed) IVIG-x (3–4 m)	IV 1	IV 2	SC 1	SC 2 and 3	SC 5	SC 9	SC 13	SC 14	SC 17	SC 21	Final visit/early termination	Extension visit (monthly)
Study procedures														
Informed consent	X	—	—	—	—	—	—	—	—	—	—	—	—	—
Medical history, eligibility, demographics	X	—	—	—	—	—	—	—	—	—	—	—	—	—
Full physical exam	X	—	—	—	X	—	—	—	—	—	—	—	X	—
Abbreviated physical exam	—	—	—	—	—	X	X	X	X	X	X	X	—	—
Chest X-ray[a]	X	—	—	—	—	—	—	—	—	—		—	—	—
Vital signs[b]	X	X	X	X	X	X	X	X	X	X	X	X	—	X
Body weight[c]	X	X	X	—	X	—	X	X	X	—	X	X	—	X
Height	X	—	X	—	—	—	—	X	—	—	X	X	—	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology[d]	X	—	X	—	X	—	—	X	—	—	X	—	X	X
Blood chemistry[e]	X	—	X	—	X	—	—	X	—	—	X	—	X	X
Special tests[f]	X	—	X	—	X	—	—	X	—	—	X	—	X	X
Urinalysis[g]	X	—	X	—	X	—	—	X	—	—	X	—	X	X
Serum pregnancy test	X	—	—	—	—	—	—	—	—	—	—	—	X	—
Urine pregnancy test	—	—	X	—	—	—	—	—	—	—	—	—	—	—
Trough total IgG[h]	X	X	X	X	—	—	X	X	X	X	X	X	X	X
IgG subclass and specific antibody titres[i]	—	—	X	X	—	—	—	X	—	—	X	—	X	X
Measles antibody titre	—	—	—	X	—	—	—	—	—	—	—	—	X	—
Virology safety retain[j]	—	—	—	—	X	—	—	—	—	—	—	—	—	—
IVIG-x (IV infusion)	—	X	X	X	—	—	—	—	—	—	—	—	—	—
SCIG-x (SC infusion)	—	—	—	—	X	X	X	X	X	X	X	X	X	X
PK analysis[k]	—	—	X	—	—	—	—	—	X	—	—	—	—	—
Adverse events (incl. SBI)	—	X	X	X	X	X	X	X	X	X	X	X	X	X
Days lost from work/school/ kindergarten/daycare due to infection and treatment	—	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary review	—	—	—	—	X	X	X	X	X	X	X	X	X	X

[a]If baseline chest X-ray not done within last 6 m.
[b]Systolic blood pressure, diastolic blood pressure, heart rate, temperature, and respiration rate.
[c]For dose calculations.
[d]To be obtained prior to infusion; hemoglobin, hematocrit, platelets, red blood cell count and morphology, white blood cell count, and differential counts.
[e]To be obtained prior to infusion; total bilirubin, indirect bilirubin, creatinine, BUN, ALT, ALP, AST, alkaline phosphatase, LDH, glucose, sodium, potassium, chloride, CO_2, calcium, bicarbonate.
[f]To be obtained prior to infusion; DAT, serum-free hemoglobin, haptoglobin.
[g]To be obtained prior to infusion; pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (including microscropic examination of urine sediment if abnormal).
[h]To be obtained prior to infusion.
[i]To be obtained prior to infusion; IgG subclasses IgG1, IgG2, IgG3, IgG4 and antibody titres for *S. pneumoniae*, *H. influenzae*, and *C. tetani*.
[j]Only test if there is clinical suspicion of HAV, HBV, HCV, HIV, or B19V infection.
[k]IV phase: PK samples will be taken before infusion, immediately following completion of infusion, and 1 h, 1, 2, 3, 5, 7, 14, 21, and 28 d post-infusion (for 28 d infusion intervals). SC phase: PK samples will be taken before infusion, immediately following completion of infusion, and 1, 3, 4, 5, and 7 d post-infusion.

the clinic. Subsequent infusions will be done in the home setting with the exception of infusions 5, 9, 13, 14, 17, and 21.

Screening: day 28 or day 21 to day 7

- Obtain written informed consent (and assent if applicable) prior to accessing personal health information
- Confirm eligibility by checking whether subject meets inclusion/exclusion criteria
- Record demography and medical history, including age of PID diagnosis, previous/current IgG therapy
- Full physical examination, body weight, height, vital signs (SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; T, temperature; RR, respiration rate)
- Chest X-ray (if not performed within 6 m prior to screening)
- Prior and concomitant medications
- Blood and urine for clinical laboratory assessments:
  - Hematology; including complete blood count, white blood differential and platelet count
  - Blood chemistry; including total bilirubin, creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, lactate dehydrogenase (LDH), glucose, sodium, potassium, chloride, CO_2, calcium
  - Special tests; direct antiglobulin (DAT), serum free hemoglobin, haptoglobin
  - Urinalysis; including microscopic examination of urine sediment
  - Pregnancy test for females of child-bearing potential
- Documented total IgG trough level ≥500 mg/dL

If eligibility is confirmed, subjects will commence their first run-in phase visit or IV phase visit at their next scheduled IgG infusion, in accordance with their regular dosing interval.

Run-in phase (if required)

- Record vital signs
- Body weight
- Record concomitant medications
- Take blood sample for pre-dose trough total IgG
- Administer study product IVIG-x
- Record AE (subjects with SBI will be withdrawn from the study)
- Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment

IV phase, baseline visit 1 and PK assessment

- Record vital signs
- Body weight (for dose calculation) and height
- Take samples prior to infusion (within 0.5 hours) for hematology, clinical chemistry, special tests, trough total IgG, IgG subclass, antibody titer levels for *S. pneumoniae*, *H. influenzae*, and *C. tetani*
- Urinalysis sample
- Pregnancy test for females of child-bearing potential
- Record concomitant medications
- Administer study product IVIG-x
- Document date and time of start and end of infusion, amount and rate of dose infused
- Record AE
- Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment
- Take samples for PK assessment of total IgG levels at the following time points:
  - Prior to infusion (within 0.5 hours)
  - Immediately following completion of infusion
  - Post infusion—1 hour (±2 hours), 1 day (±2 hours), 2 days (±2 hours), 3 days (±4 hours), 5 days (±4 hours), 7 days (±1 day), 14 days (±1 day), 21 days (±1 day; final sample for subjects on a 21 days interval and will also be used as pre-dose blood sample for next visit), 28 days (±1 day; only for subjects on a 28 days interval and will also be used as pre-dose blood sample for next visit)

IV phase, visit 2

- Record vital signs
- Concomitant medications
- Take sample prior to infusion for trough total IgG, IgG subclass and antibody titer levels for *S. pneumoniae*, *H. influenzae*, and *C. tetani* (same as post-infusion 21 or 28 days PK sample)
- Blood drawn pre-infusion for measles antibody titer
- Administer study product IVIG-x

- Record AE (subjects with SBI will be withdrawn from the study)
- Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment

SC phase, infusion week 1

- Full physical examination, body weight (for dose calculation), height, record vital signs
- Concomitant medications
- Blood and urine for clinical laboratory assessments:
  - Hematology; including complete blood count, white blood differential and platelet count
  - Blood chemistry; including total bilirubin, creatinine, BUN, ALT, AST, alkaline phosphatase, LDH, glucose, sodium, potassium, chloride, CO_2, calcium
  - Special tests; direct antiglobulin (DAT), serum free hemoglobin, haptoglobin
  - Urinalysis; including microscopic examination of urine sediment
  - Virology; retain samples for hepatitis A virus (HAV), HBV, HIV, B19V
- SC infusion and subject diary training (refer to section on "Subject diary"; includes but not limited to sites of administration, volume of infusion, concomitant medications, AE's, number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment)
- Administer study product SCIG-x
- Record AE (subjects with SBI will be withdrawn from the study)
- Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment

SC phase, infusion weeks 2 and 3

- Abbreviated physical examination (with inspection of prior injection sites), record vital signs
- Record concomitant medications
- SC infusion training
- Administer study product SCIG-x
- Review infusion details and diary
- Record AE (including SBI)
- Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment

SC phase, infusion weeks 4, 6, 7, 8, 10, 11, 12, 15, 16, 18, 19, 20, 22, 23, 24 (home setting)

- Subject/caregiver administers study product SCIG-x
- Complete subject diary

SC phase, infusion weeks 5, 9, 14, 17, 21

- Abbreviated physical examination (with inspection of prior injection sites), body weight (for dose calculation, excluding visit 14), height, vital signs
- Record concomitant medications
- Take sample prior to infusion for trough total IgG
- Additional assessments prior to infusion (for infusions 9 and 17 only):
  - Hematology; including complete blood count, white blood differential and platelet count
  - Blood chemistry; including total bilirubin, creatinine, BUN, ALT, AST, alkaline phosphatase, LDH, glucose, sodium, potassium, chloride, CO_2, calcium
  - Special tests; direct antiglobulin (DAT), serum free hemoglobin, haptoglobin
  - Urinalysis; including microscopic examination of urine sediment
  - IgG subclass and antibody titer levels for *S. pneumoniae*, *H. influenzae*, and *C. tetani* (within 0.5 hours before SCIG-x infusion)
- Subject/caregiver administers study product SCIG-x
- Review infusion details and diary
- Record AE (including SBI)
- Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment

SC phase, infusion week 13 and PK assessment

- Abbreviated physical examination (with inspection of prior injection sites), body weight (for dose calculation), record vital signs
- Record concomitant medications
- Take sample prior to infusion for trough total IgG
- Subject/caregiver administers study product SCIG-x
- Review infusion details and diary
- Record AE (including SBI)
- Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment

- Take samples for PK assessment of IgG levels at the following time points (exact time of sample collection to be recorded; only for subjects age >5 years):
  - Prior to infusion (within 0.5 hours; same as pre dose trough IgG sample)
  - Immediately following completion of infusion
  - Post infusion—1 day (±4 hours), 3 days (±4 hours), 4 days (±4 hours), 5 days (±4 hours), 7 days (±1 day; within 0.5 hours before infusion 14)

Completion of 24 weeks of SC infusions before PK analysis is complete

- Continue to receive weekly SC infusion of SCIG-x in the home setting
- Complete subject diary
- Return to clinic every 4 weeks:
  - Abbreviated physical examination (with inspection of prior injection sites), body weight (for dose calculations), height, vital signs
  - Record concomitant medications
  - Take sample prior to infusion for trough total IgG
  - Caregiver/subject to administer SCIG-x
  - Review infusion details and diary
  - Record AE (including SBI)
  - Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment
- At 8 week intervals:
  - Hematology; including complete blood count, white blood differential and platelet count
  - Blood chemistry; including total bilirubin, creatinine, BUN, ALT, AST, alkaline phosphatase, LDH, glucose, sodium, potassium, chloride, CO_2, calcium
  - DAT, serum free hemoglobin, haptoglobin
  - Urinalysis; including microscopic examination of urine sediment
  - IgG subclass and antibody titer levels for *S. pneumoniae*, *H. influenzae*, and *C. tetani* (within 0.5 hours before SCIG-x infusion)

Final study visit/early termination visit

- Full physical examination, record vital signs
- Record concomitant medications
- Blood and urine for clinical laboratory assessments:
  - Trough total IgG, IgG subclass, and antibody titer levels for measles, *S. pneumoniae*, *H. influenzae*, and *C. tetani*
  - Serum pregnancy test (females of child-bearing potential)
  - Hematology; including complete blood count, white blood differential and platelet count
  - Blood chemistry; including total bilirubin, creatinine, BUN, ALT, AST, alkaline phosphatase, LDH, glucose, sodium, potassium, chloride, CO_2, calcium
  - Special tests; Direct antiglobulin (DAT), serum free hemoglobin, haptoglobin
  - Urinalysis; including microscopic examination of urine sediment
- Record AE (subjects with SBI will be withdrawn from the study)
- Record number of days of missed work/school/kindergarten/daycare/daily activities due to infections and related treatment
- Review subject diary

Assessment of pharmacokinetics

Primary PK objective

Total IgG concentrations will be tabulated for IV and SC modes of administration, with individual as well as mean total IgG concentrations vs. time curves established. AUC, C_{max}, and T_{max} will be determined. To meet the primary PK endpoint, non-inferiority of the steady-state IgG AUC between SC dose ($AUC_{0\text{-}t,SC}$; established from SC infusion 13) of SCIG-x and IV dose ($AUC_{0\text{-}t,IV}$; established from IV infusion 1) of IVIG-x will be tested using established protocols for bioequivalence, and the 90% confidence interval of the geometric LSM ratio of SC AUC and IV AUC compared.

Secondary PK objective

Steady-state mean trough concentrations of total IgG will be determined from the average of C_{trough} measurements at SC infusions 13, 14, 17, and 21 (for SC administration), and IV infusion 1 and immediately prior to IV infusion 2 (for IV administration).

Exploratory PK objectives

Trough levels of IgG subclasses IgG1, IgG2, IgG3, IgG4, as well as antibody titres against *S. pneumoniae*,

H. influenzae, and *C. tetani* will be determined for IV and SC methods of administration.

Assessment of efficacy

Exploratory variables assessing the efficacy of SCIG-x study drug include:

- Rate of SBIs: bacteremia/sepsis, bacterial meningitis, osteomyelitis/septic arthritis, bacterial pneumonia, visceral abscess
- Rate of infections: serious and non-serious (including acute sinusitis, worsening of chronic sinusitis, otitis media, pneumonia, bronchitis and pathogen-associated diarrhea), as well as infections validated by positive radiograph, fever, culture, or diagnostic testing
- Days of missed work/school/kindergarten/daycare/daily activities due to infections and associated treatments
- Days on antibiotics (prophylactic as well as for treatment of infection)
- Number of hospitalizations due to infections

Assessment of safety

Safety analysis will include summaries of:

- Adverse events (encompassing adverse reactions, suspected adverse reactions, unexpected AE, serious AE), including determination of severity (mild, moderate, severe) and causality (certain, probably, possible, not related), to be presented as the number of adverse events and number and percentage of subjects with adverse events
- Subjects who discontinued due to death, adverse events or serious adverse events
- Adverse events taking place within 72 hours of completion of study drug administration
- Local infusion site reactions

Safety parameters

Results of all laboratory tests and vital signs will be summarized:

- Vital signs (SBP, DBP, HR, T, RR)
- Routine blood samples
    - Hematology: hemoglobin, hematocrit, platelets, red blood cell count and morphology, white blood cell count, and differential counts
    - Blood chemistry: total bilirubin, indirect bilirubin, creatinine, BUN, ALT, ALP, AST, alkaline phosphatase, LDH, glucose, sodium, potassium, chloride, CO_2, calcium, bicarbonate
- Urinalysis: pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (including microscropic examination of urine sediment if abnormal)
- Virology: HAV, HBV, HCV, HIV, B19V (these virus safety retain samples will be stored for possible future testing)
- Special tests: DAT, serum-free hemoglobin, haptoglobin

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