LymphoSign Journal

The journal of inherited immune disorders

Volume 4, Number 3, 2017 EISSN 2292-5945

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Your health is our mission, Green Cross Care

- More than 40 years of providing safe biotherapeutic products
- Focused on immunology and orphan drugs

녹십자 GREEN CROSS Green Cross BT Biotherapeutics

녹십자 GREEN CROSS GREEN CROSS

LymphoSign Journal

Volume 4, Number 3, 2017 EISSN 2292-5945

TABLE OF CONTENTS

Review

87 **Chronic mucocutaneous candidiasis: a spectrum of genetic disorders**
Amit Nahum

Novel Mutation

101 **The novel p.Gly306Asp perforin mutation causes familial hemophagocytic lymphohistiocytosis type 2 (FHL-2) probably due to a critical role of Gly306 in the pore-forming perforin domain**
Ramón Urrea Moreno, Itziar Astigarraga, Ana Fernández-Teijeiro, Carmen Rodríguez-Sainz, María Alonso-Martinez, Lydia Martín-Martín, Susana García-Obregón, and Juana Gil-Herrera

Original Article

113 **Hemophagocytic lymphohistiocytosis associated with ataxia telangiectasia**
Mohammad Alsalamah and Chaim M. Roifman

Protocol, Practice, and Policy

117 **National Immunoglobulin replacement Expert Committee recommendations**
Stephen Betschel, Peter Dent, Elie Haddad, Alison Haynes, Thomas Issekutz, Bruce Mazer, Brenda Reid, Chaim M. Roifman, Bob Schellenberg, Gordon Sussman, Stuart Turvey, and Susan Waserman

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

LymphoSign Journal (ISSN electronic 2292-5945) is published quarterly in March, June, September, and December by LymphoSign Journal Limited Partnership, Toronto, Ontario, Canada; Tel.: 416-964-2246; Fax: 416-964-6594.

All statements and opinions in *LymphoSign Journal* are solely those of the individual authors and contributors and not of the Publisher. The Publisher accepts no responsibility or liability for damages arising from any error or omission or from the use of any information or advice contained in the Journal. Acceptance of an advertisement, announcement, or other material does not imply endorsement by either the Journal's Editors or the Publisher.

© 2017 LymphoSign Journal Limited Partnership or its licensors

No part of *LymphoSign Journal* may be reproduced, stored, or transmitted in any form or by any means, without the written permission of the Publisher, except as stated below.

Under the Canadian Copyright Act, individuals may download or print single copies of articles for personal research or study. Any person may reproduce short excerpts from articles in the Journal for any purpose that respects the moral rights of authors, provided that the source is fully acknowledged. As a courtesy, the consent of authors of such material should be obtained directly from the author.

Authorization to reproduce items for other than personal research or study, as stated above, may be obtained by contacting the Editorial Office at editorialoffice@lymphosign.com.

The Publisher also extends certain additional rights to authors. The above rights do not extend to copying or reproduction for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. For such copying or reproduction, arrangements must be made with LymphoSign Journal Limited Partnership, Toronto, Ontario, Canada; Tel.: 416-964-2246; Fax: 416-964-3827.

Service information

Scope

LymphoSign Journal publishes novel clinical, translational. and basic research in the fields of immunology, gastroenterology, neurology, dermatology, rheumatology, hematology, and infectious disease. The aim of the journal is to provide a forum for clinicians and scientists to discuss clinical observations, therapies, and insights into underlying disease mechanisms in immune disorders including immune deficiencies, auto-inflammatory disorders, allergy, bone marrow failure, and lymphoid malignancies. Special consideration will be given, but is not limited to, articles exploring adaptive and innate immunity, mucosal immunity, signal transduction, lymphocyte development and cell death, genomic medicine, gene regulation, DNA repair, and cell cytoskeletal networks. *LymphoSign Journal* publishes peer-reviewed original research articles, reviews, clinical trials, case reports, novel mutations, and imaging, as well as practice guidelines, algorithms, and protocols.

Subscriptions

Requests for subscriptions, renewals, and single or back issues, and all remittances should be addressed to: Subscription Office, Canadian Science Publishing, PO Box 361, Birmingham, AL 35283-0399, USA; Toll-free: 1-800-852-7404; Outside North America: 205-995-1567; Fax: 205-995-1588; Email: nrcresearchpress-csp@subscriptionoffice.com. 2017 subscriptions rates are as follows: Institution: electronic $525.00, Personal: electronic $150.00.

Submissions

To submit, use the journal's online submission system at http://mc06.manuscriptcentral.com/lpsn. The Instructions to Authors are available at http://lymphosign.com/page/lpsn-authors. All correspondence concerning submissions should be addressed to the Editorial Office at editorialoffice@lymphosign.com.

Offprints and reprints

Offprint and reprint requests should be directed to the Editorial Office at editorialoffice@lymphosign.com.

Permissions

For permission to reprint materials, contact the Editorial Office at editorialoffice@lymphosign.com.

Advertising sales

Advertising inquiries should be directed to the Editorial Office at editorialoffice@lymphosign.com. Acceptance of or the publishing of advertisements does not imply endorsement by either the Journal's Editors or the Publisher.

Publishing services

Publishing services are provided by Canadian Science Publishing, 65 Auriga Dr., Suite 203, Ottawa, ON K2E 7W6; Tel: 613-656-9846; www.cdnsciencepub.com. Scientific Publishing Editor: Lianne Johnsen, lianne.johnsen@cdnsciencepub.com; Manager, Production: Judy Hum-Delaney, judy.hum-delaney@cdnsciencepub.com; Manager, Publishing Services: Judy Busnarda, judy.busnarda@cdnsciencepub.com.

Indexing

CrossRef, Google Scholar, HINARI, J-Gate, OCLC, ReadCube, and Ulrich's

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

LymphoSign Journal

Volume 4, Number 3, 2017 EISSN 2292-5945

Editor-in-Chief

Chaim M. Roifman, MD — University of Toronto, Toronto, Canada

Associate Editor

José Regueiro, PhD — University Complutense, Madrid, Spain

Editorial Board

Upton Allen, MD — University of Toronto, Toronto, Canada
Amnon Altman, PhD — La Jolla Institute for Allergy and Immunology, La Jolla, U.S.A.
Alessandro Auiti, MD, PhD — San Raffaele Telethon Institute for Gene Therapy, Milan, Italy
John Cambier, PhD — University of Colorado, Aurora, U.S.A.
Marina Cavazzana, MD, PhD — Institut Des Maladies Genetiques, Paris, France
Talal Chatila, MD — Harvard University, Boston, U.S.A.
Max Cooper, MD — Emory University, Atlanta, U.S.A.
Charlotte Cunningham-Rundles, MD, PhD — The Mount Sinai Hospital Medical School, New York, U.S.A.
Stephan Ehl, MD — Centrum fur Chronische Immundefizienz, Freiburg, Germany
Alain Fischer, MD, PhD — Hôpital Necker-Enfants Malades, Paris, France
Raif Geha, MD — Harvard University, Boston, U.S.A.
Sergio Grinstein, PhD — University of Toronto, Toronto, Canada
Eyal Grunebaum, MD — University of Toronto, Toronto, Canada
Jonathan Heeney, PhD, DVM — University of Cambridge, Cambridge, U.K.
Neena Kapoor, MD — University of Southern California, Los Angeles, U.S.A.
Bruce Mazer, MD — McGill University Health Center, Montreal, Canada
Aleixo Muise, MD — University of Toronto, Toronto, Canada
Tomas Mustelin, MD, PhD — Sandford Burnham Medical Research, California, U.S.A.
Luigi Notarangelo, MD — Harvard University, Boston, U.S.A.
Yair Reisner, PhD — Weizmann Institute, Rechovot, Israel
Philip Sherman, MD — University of Toronto, Toronto, Canada
Yossi Shiloh, PhD — Tel Aviv University, Tel Aviv, Israel
Scott Snapper, MD, PhD — Harvard University, Boston, U.S.A.
Stuart Tangye, PhD — Garvan Institute of Medical Research, Sydney, Australia
Cox Terhorst, PhD — Harvard University, Boston, U.S.A.
Juan Carlo Zúñiga-Pflücker, PhD — University of Toronto, Toronto, Canada

Editors

Original Articles Editor
Tim Niehues, MD — Klinikum Krefeld Academic Hospital, Krefeld, Germany

Classification Editors
Arnon Broides, MD — Ben-Gurion University, Beer Sheva, Israel
Amit Nahum, MD, PhD — Soroka University Medical Center, Beer Sheva, Israel

Imaging Editor
David Manson, MD — The Hospital for Sick Children, Toronto, Canada

Review Editors
Alison Haynes, MD — Janeway Children's Hospital, St. Johns, Newfoundland, Canada
Ilan Dalal, MD — Tel Aviv University, Tel Aviv, Israel

Ethics and Business Editor
Rona Zeeli, LLB — Toronto, Canada

Scientific Content Editors
Harjit Dadi, PhD — University of Toronto, Canada
Nigel Sharfe, PhD — University of Toronto, Canada

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Putting patients first in immunotherapy.

At Octapharma, we are driven by a desire to make a difference in patients' lives. That's why we are committed to the research and development of **new immunotherapy solutions**.

Your immunotherapy partner for over 30 years
octapharma.ca

octapharma®
For the safe and optimal use of human proteins

Chronic mucocutaneous candidiasis: a spectrum of genetic disorders

Amit Nahum[a,b]*

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

ABSTRACT

Chronic mucocutaneous candidiasis (CMCC) encompasses a heterogeneous group of syndromes associated with persistent or recurrent *Candida* infections of the skin, nails, and mucous membranes. While chronic candidiasis can present by itself or as part of a complex, including endocrinopathy, autoimmune manifestations, bone marrow failure and neoplastic diseases, it is often regarded as a warning sign for immunodeficiency. Here, we review the processes involved in host-microbial recognition of *Candida* and highlight underlying genetic causes of CMCC—including those that are monogenic (such as mutations in AIRE and STAT1) as well as polymorphisms that increase susceptibility to candidal infection.

Statement of novelty: This review provides an overview of the pathophysiology of *Candida* fungal infection as well as genetic defects that have been identified to cause CMCC.

Introduction

Chronic mucocutaneous candidiasis (CMCC) encompasses a heterogeneous group of syndromes associated with persistent or recurrent *Candida* infections of the skin, nails, and mucous membranes (Kirkpatrick 1994). While chronic candidiasis can present by itself or as part of a complex, including endocrinopathy, autoimmune manifestations, bone marrow failure and neoplastic diseases, it is often regarded as a warning sign for immunodeficiency. Indeed, much current knowledge is derived from study of families and individuals with immune dysfunction.

Although the exact mechanisms underlying CMCC are still being unraveled, advancements made in whole genome sequencing techniques have been key to furthering our understanding of the pathophysiology as well genetic causes of this disorder. Here, we review the pathophysiology of *Candida* fungal infection as well as genetic defects that have been identified to cause CMCC.

Candida infection in healthy and immunocompromised individuals

Candida spp. are part of the commensal microflora and often found on mucosal surfaces including the mouth, intestine, vagina, and skin of healthy individuals. These microorganisms contribute to the development of the host immune system, however, can act as opportunistic pathogens when there is microbial imbalance, for example, due to immune suppression associated with antibiotic or steroid treatment. *C. albicans* is among the most prevalent and well-studied microorganism identified in CMCC.

Most healthy women will experience transient vaginal candidiasis at some point in their lifetime (Sobel 2007).

[a]Pediatrics Department A, Saban Pediatric Medical Center, Soroka University Medical Center, and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel; [b]The Primary Immunodeficiency Research Laboratory, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

*Corresponding author: Amit Nahum/amitna@clalit.org.il

Submitted 4 August 2017
Accepted 5 September 2017
Available online 7 September 2017

LymphoSign Journal 4:87–99 (2017)
dx.doi.org/10.14785/lymphosign-2017-0011

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

In contrast, *Candida* infection in an immunocompromised individual can cause severe systemic infection, candidemia, which in turn can be fatal in a considerable number of patients (Das et al. 2011). Almost all oncologic patients with neutropenia experience oropharyngeal candidiasis (Viscoli et al. 1999), and in patients undergoing bone marrow transplantation, as many as 40% suffer systemic infections. Of these, 4% are infected with *Candida* and the associated mortality rate may be as high as 42% (Ortega et al. 2005).

Another risk factor for candidemia is prolonged hospitalization; such is the case for patients in intensive care (Das et al. 2011). Surgical procedures and insertion of medical devices are also associated with higher incidence of candidemia and is augmented by the ability of *Candida* to create biofilms on medical devices such as central vein catheters (Donlan and Costerton 2002). Neonates with central catheters experience high rates of bloodstream infections, with *Candida* being the third most common microbial source (Chitnis et al. 2012).

It is important to note that in most patients with candidiasis, no single genetic cause can be found. Rather, susceptibility to infection is due a combination of genetic variants (polymorphisms) and environmental factors, as mentioned above. In contrast, the underlying causes of CMCC may be monogenic (Table 1), such as single gene mutations in autoimmune regulator (*AIRE*), signal transducer and activator of transcription-1 (*STAT1*) and -3 (*STAT3*), or the result of polymorphisms in genes encoding Dectin-1, NACHT LRR and PYD-containing protein 3 (NLRP3), protein tyrosine phosphatase non-receptor type-22 (PTPN22), and Toll-like receptors (TLRs) which contribute to disease susceptibility (Table 2). Candidiasis in these circumstances can be a dominating, constitutive feature or an associated, inconsistent sign.

Detection of *Candida* by immune cells

The cell membrane of *Candida* consists of 2 distinct layers; an inner layer composed of polysaccharides such as chitin, 1,3-beta-glucans and 1,6-beta-glucans, and an outer layer structured mainly of proteins that are mannosylated with mannan side-chains. These components serve as ligands for receptors of the innate immune system, such as TLRs and C-type lectins (CLRs) that are found on the surface of immune cells (Smeekens et al. 2013*a*).

Pattern recognition receptors are requisite for host microbial recognition of *Candida* (Figure 1). Whereas TLR2 recognizes phospholipo-mannans (Jouault et al. 2003), TLR4 recognizes O-linked mannans (Netea et al. 2006; Ferwerda et al. 2009). CLRs such as Dectin-2 recognize mannose residues (McGreal et al. 2006) while Dectin-1 is activated by β-glucan (Brown and Gordon 2001; Rogers et al. 2005). Upon ligation, these receptors

Table 1: **Monogenic defects associated chronic mucocutaneous candidiasis.**

Gene	Inheritance	Phenotype and immune characteristics	Reference
AIRE	AD	Autoantibodies to IL-17A/F and IL-22	Nagamine et al. 1997; Meager et al. 2006; Puel et al. 2010
CARD9	AR	Reduced Th17 responses	Glocker et al. 2009
STAT3	AD	HIES, Absent Th17 cells	Holland et al. 2007
TYK2	AR	HIES, T cell defects	Minegishi et al. 2006
DOCK8	AR	HIES, T cell defects, Th17 polarization	Engelhardt et al. 2009
STAT1	AD	Reduced Th17 responses, IFN-γ Gain-of-function mutations—isolated CMCC CMCC IPEX-like (autoimmunity, recurrent infections) Loss-of-function mutations—progressive combined immunodeficiency	van de Veerdonk et al. 2011; Uzel et al. 2013; Sharfe et al. 2014
CD25	AR	Combined immunodeficiency, immune dysregulation.	Sharfe et al. 1997
IL-17F	AD	CMCC	Puel et al. 2011
IL-17RA	AR	CMCC and *S. aureus* infection	Puel et al. 2011
IL-17RC	AR	Candidiasis (isolated)	Ling et al. 2015
ACT1	AR	Candidiasis (oral), recurrent blepharitis (*S. aureus*)	Boisson et al. 2013
IL-12Rβ1	—	Candidiasis, reduced IFN-γ	Ouederni et al. 2014
RORC	—	Candidiasis and mycobacteria infections	Okada et al. 2015

Note: AD, autosomal dominant; AR, autosomal recessive; CMCC, chronic mucocutaneous candidiasis; HIES, hyper IgE syndrome.

Table 2: **Susceptibility genes associated with chronic mucocutaneous candidiasis.**

Gene	Phenotype and immune characteristics	References
Dectin-1	CMCC, reduced IL-1β and Th17 mediated responses	Plantinga et al. 2009
MBL2	Recurrent vulvo-vaginal candidiasis	Babula et al. 2003; Giraldo et al. 2007
NLRP3	Recurrent vulvo-vaginal candidiasis, reduced IL-1β	Lev-Sagie et al. 2009
TLR1	Reduced IL-1β, IL-6, IL-8	Plantinga et al. 2012
TLR2	Reduced IFN-γ, IL-8	Woehrle et al. 2008
TLR3	CMCC, bacterial, viral infections, autoimmunity L412F variant showing reduced biological activity Reduced IFN-γ, IL-6	Nahum et al. 2011, 2012
TLR4	Candidemia, elevated IL-10	Van der Graaf et al. 2006
PTPN22	Autoimmunity, CMCC	Nahum et al. 2008
IL-4	Recurrent vulvo-vaginal candidiasis and systemic candidemia	Babula et al. 2003; Choi et al. 2003
IL-12β	Candidemia bloodstream, reduced IFN-γ	Johnson et al. 2012
IL-10	Candidemia bloodstream, elevated IL-10 levels	Johnson et al. 2012

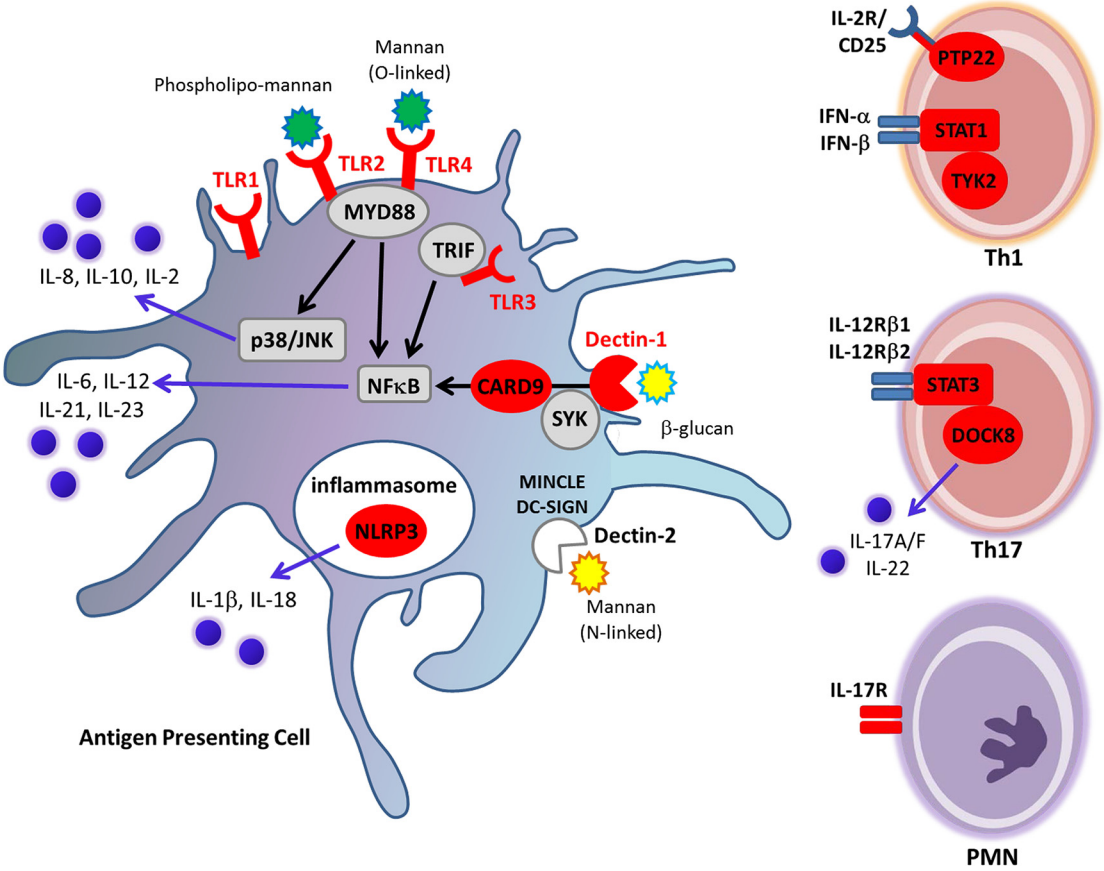

Figure 1: Overview of immune response elements involved in host defense against *Candida albicans*. Cell wall components of *C. albicans* (such as mannans and β-glucan) are recognized by Toll-like receptors (TLRs) and C-type lectins (CLRs) present on antigen presenting cells and T cells. Receptor-ligand engagement initiates an inflammatory response through activation of several transduction pathways. The adaptive immune response is triggered by Th1 to Th17 cell polarization, which in turn initiates secretion cytokines such as IL-17 and IL-22 to recruit polymorphonuclear leukocytes (PMN) to the site of infection. Defects in molecules associated with CMCC are marked in red.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

signal through different downstream adaptor molecules and kinases, resulting in secretion of pro-inflammatory cytokines. At the same time, host recognition of the pathogen ensures phagocytosis and killing of the pathogen and presentation to other immune cells.

The prompt and correct recognition by these receptors also define and shape the next phase of the immune response by adaptive immune components (Akira et al. 2006). Recently, it was shown that exposure of *Candida* to healthy individuals induces expression of type I interferon (IFN) family cytokines, skewing the immune response towards Th1 responses. This resulted in a unique cytokine profile that was different from that induced by bacterial pathogens (Smeekens et al. 2013*b*).

Th1 and Th17 cell-mediated responses are central for controlling *Candida* infections. Their precise role was elucidated through different genetic defects and autoimmune phenomenon resulting in reduced interleukin (IL)-17F, IL-17A, and IL-22 activity alongside the occurrence of fungal infections. The differentiation of naïve T cells to Th17 cells is dependent upon a certain cytokine milieu. Upon recognition of fungal cell wall elements by pattern recognition receptors on activated T cells and antigen presenting cells, cytokines such as IL-1β, IL-6, IL-21, IL-23 and transforming growth factor (TGF)-β are secreted. These lead to activation of transcription factors STAT-1, STAT-3 and retinoic acid orphan receptor-γt (RORγt), resulting in polarization to Th17 cells which in turn exert their effects by secreting IL-17A, IL-17F, IL-22 (Ivanov et al. 2006; Yang et al. 2007). These cytokines further induce secretion of chemokines that attract neutrophils to sites of infection, and act in concert with TNF-α and IL-22 to induce anti-microbial peptide (AMP) from endothelial cells (Iwakura et al. 2011; Hernández-Santos and Gaffen 2012; McGeachy and McSorley 2012; Rutz et al. 2013).

Together, these components are necessary to control and overcome *Candida* infections. Aberration or immune dysregulation during any of these steps is thought to underlie CMCC.

Monogenic defects causing CMCC

Several monogenic defects are known to cause CMCC. These are described in the next paragraphs.

Autoimmune regulator (AIRE) deficiency

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) associated CMCC was the first to be described and deciphered genetically. APECED is caused by mutations in the *AIRE* gene, resulting in loss-of-function of the encoded AIRE transcription factor (Nagamine et al. 1997; Björses et al. 1998). It is transmitted in an autosomal recessive manner, and is found mainly in certain populations; 1:25 000 in Finns, 1:14 500 in Sardinians and 1:9000 in Iranian Jews. While more than 50 mutations have been described to date, the most common mutation is the R257X variant identified in the Finnish population. It is noteworthy, however, that the majority of patients with CMCC outside of these ethnic groups do not carry mutations in *AIRE*.

APECED is characterized by the classic triad of chronic candidiasis (appearing in almost all patients), adrenal failure which is present in 84% of patients, and hypoparathyroidism which presents in 88% of patients. Hypogonadism is also very common (mainly ovarian failure, and to lesser extent male hypogondasim). Such individuals may also present with other endocrynopathies; hypothyroidism and diabetes mellitus are found in as many as 1/3 of patients (Perheentupa 2006). Skin is often involved and appears dystrophic, and common skin manifestations include vitiligo and alopecia, which is found in as many as 30% of patients. Teeth enamel is also frequently affected. Physicians should stay alert for occurrence of keratoconjunctivitis which could lead to blindness. Other less common manifestations are chronic diarrhea as well as bouts of rash and fever (Perheentupa 2006). Hepatitis, ranging from asymptomatic elevated liver enzymes to severe necrotizing hepatitis, has been reported in 12 of 68 patients and pernicious anemia in 13 of 68 patients (Ahonen et al. 1990).

The AIRE transcription factor is responsible for regulating the expression of autoantigen receptors on thymocytes. During lymphocyte maturation in the thymus, the random rearrangement of antigen-specific receptor genes produces a vast and diverse repertoire of receptors, some of which mistakenly recognize "self" as "non-self". To prevent autoimmunity, these cells undergo clonal deletion, a process that is closely regulated by AIRE in thymus medullary epithelial cells. Thus, T cells recognizing these antigens with high affinity are clonally deleted.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

AIRE deficiency resulting in loss-of-function thus allows T cell expressing autoantigen receptors to expand to the periphery and induce autoimmunity.

While the mechanisms underlying the autoimmune phenomena and endocrinopathies associated with CMCC could be explained by defects in AIRE itself, the link between the IL-17 pathway and chronic candidiasis (which is frequently the presenting symptom and almost invariably present) was only recently elucidated.

The first clue came from reports of high titers of neutralizing antibodies to type I IFNs (Meager et al. 2006). In a study involving Finnish and Norwegian populations, almost all patients with APECED exhibited autoantibodies to IFN-α subtypes as well as IFN-ω. More recently, neutralizing antibodies against IL-17A, IL-17F, and IL-22 were also detected (Kisand et al. 2010; Puel et al. 2010). These cytokines are critical for the inflammatory response against *Candida*, and the presence of such autoantibodies likely explains candidiasis in these patients. Interestingly, susceptibility to candidal infection may result from both abnormal adaptive and innate immunity. Further studies are needed to fully decipher the pathophysiology of AIRE deficiency in CMCC, given the interaction of AIRE with the beta-glucan receptor Dectin-1 pathway components such as caspase recruitment domain-containing protein 9 (CARD9) and Syk (Pedroza et al. 2012). TNF-α production following stimulation of the Dectin-1 pathway is reduced in patients with AIRE deficiency. In addition, it appears that organ involvement and severity of disease is also affected by genetic background and modifying genes such as HLA (Betterle et al. 1998; Karvonen et al. 2000).

Signal transducer and activator of transcription-1 (STAT1) dysfunction

The signaling molecule STAT1 mediates cellular responses following type I and type II IFN receptor ligation, as well as IL-23 and IL-12 receptor engagement. Monoallelic mutations in STAT1 have been identified in patients with CMCC (van de Veerdonk et al. 2011), although the clinical manifestations were limited to the skin, nails, and mucous membranes, as well as increased incidence of hypothyroidism. The reported STAT1 gain-of-function mutations occurred in the coiled-coil domain, resulting in reduced production of IL-17, IL-22, and IFNγ, thereby linking these defects with aberrant Th17 cell activity. It was proposed that overexpression and activity of type I, type II, and type III IFNs inhibited Th17 differentiation (Liu et al. 2011; Maródi et al. 2012). However, this is in counter to recent findings highlighting the central role of IFNs in the protective host response to *Candida* (Smeekens et al. 2013*a*).

We recently described a group of CMCC patients with loss-of-function STAT1 mutations (Sharfe et al. 2014). These patients were phenotypically different from previous reports, where alongside chronic candidiasis, individuals also suffered recurrent infections with bacterial and viral pathogens (cytomegalovirus, John Cunningham virus) as well as severe autoimmune phenomena reminiscent of a combined immunodeficiency phenotype. Immunologic laboratory evaluation revealed progressive loss of T and B cell function.

It is important to note that mutations in STAT1 have been reported in several other phenotypically different immunodeficiencies, such as the Mendelian susceptibility to mycobacterial diseases (MSMD) (Boisson-Dupuis et al. 2012). A possible explanation for the diverse phenotypes include mutations which target different regions of STAT1, thereby affecting other cellular functions. Another explanation may be that defects in other modifying genes are present, resulting in different phenotypes.

Caspase recruitment domain-containing protein 9 (CARD9) deficiency

Mutations in the signal transduction molecule CARD9 have also been identified as a cause of CMCC. CARD9 signals downstream of Dectin-1 as well as Dectin-2 and Mincle (Gross et al. 2006; Robinson et al. 2009). The mode of inheritance appears to be autosomal dominant, with the phenotype consisting mainly of recurrent chronic candidiasis as well as systemic infections(Glocker et al. 2009; Lanternier et al. 2012).

Interleukin-17 pathway defects

Mutations in the cytokine IL-17F and cytokine receptor IL-17RA causing CMCC have been reported, highlighting the role of the IL-17 pathway in mucocutaneous immunity against *Candida*. One patient born to consanguineous parents presented with candida dermatitis, and was found to have a homozygous mutation in IL-17RA (Q248X) which was transmitted in an autosomal recessive manner. The patient's peripheral mononuclear cells and fibroblasts lacked IL-17RA expression, resulting in

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

absent responses to IL-17A/F (Puel et al. 2010). Levy and colleagues later reported a case series of 21 patients with IL-17RA mutations. While some had CMCC, others also suffered recurrent bacterial infections (Lévy et al. 2016). A separate kindred was reported to have a heterozygous mutation in IL-17F, showing impaired cytokine secretion (Puel et al. 2010). The inheritance was suggested to be autosomal dominant, although there were other asymptomatic family members with the same mutation, indicating a model of incomplete penetrance.

Biallelic mutations in ACT1, an adaptor protein downstream of the IL-17 receptor which activates nuclear factor κB (NFκB), MAPK, and C/EBP pathways, has been reported in a kindred of 2 brothers who presented with chronic oral candidiasis and recurrent blepharitis with *S. aureus* (Boisson et al. 2013). Mutations in IL-17RC, another member of the IL-17 pathway, resulted in IL-17RC deficiency in 3 unrelated kindreds involving an Argentinian and 2 Turkish patients. All had CMCC with no other infections, no autoimmune phenomena and a normal B and T cell immune workup (Ling et al. 2015). This same pathway is affected by biallelic mutations in RORC, resulting in deficiency of IL-17 producing T cells as well as defective INF-γ responses to mycobacteria. These patients from 3 kindreds presented with susceptibility to mycobacterial infections—this was fatal in 1 child with disseminated BCG infection. Most of the patients also had chronic candidiasis (Okada et al. 2015).

Signal transducer and activator of transcription-3 (STAT3) dysfunction

Some monogenic diseases may feature CMCC as a component of the clinical manifestations. In such cases, CMCC is not a predominant feature and may not always be present. Autosomal dominant hyper IgE syndrome (AD-HIES) has been shown to result in CMCC. The main clinical features include eczema, recurrent skin infections, cold abscesses, as well as recurrent severe pneumonia with pneumatocele formation, which is typical of *S. aureus* infections. Among these infections, persistent mucosal and skin candidiasis and other fungal infections are often seen. Patients may also have defects in bone formation resulting in skeletal defects, as well as coarse facial features.

AD-HIES is caused by mutations in STAT3 (Holland et al. 2007; Minegishi et al. 2007), a signaling molecule downstream of the IL-23 receptor. The defect in STAT3 results in reduction in Th17 cell numbers and absent IL-17 secretion (de Beaucoudrey et al. 2008).

Dedicator of cytokinesis 8 (DOCK8) deficiency

CMCC has been attributed to mutations in DOCK8, a member of the DOCK180-related protein family which is also involved in Th17 polarization (Engelhardt et al. 2009). The autosomal recessive form of HIES caused by defects in DOCK8 is phenotypically different, with patients showing highly elevated levels of IgE and eosinophilia. In this disorder, CMCC is not one of the main features but, rather, part of the severe combined immunodeficiency state with viral and bacterial infections (Zhang et al. 2009). Patients experience severe recurrent sinopulmonary infections, recurrent viral infections especially of the skin (herpes simplex, herpes zoster, molluscum contagiosum, and human papilloma virus), alongside atopic disease such as atopic dermatitis and food allergies. Hepatic disorders and high incidence of neoplastic disease, vulvar, facial, and anal squamous cell dysplasia and carcinomas, as well as T cell lymphoma-leukemia are also common. The overall immunologic profile of these patients, which include lymphopenia and reduced lymphocyte proliferation, is typical of combined immunodeficiency.

Tyrosine Kinase 2 (TYK2) deficiency

CMCC associated with HIES is caused by mutations in TYK2 (Minegishi et al. 2006), which encodes for a tyrosine kinase from the Janus kinase (JAK) family. The patient reported had characteristic features of both autosomal recessive HIES and atypical mycobacteriosis and chronic candidiasis.

CD25 deficiency

CD25 (or IL-2Rα) deficiency causes a combined immunodeficiency phenotype. Patients present with severe chronic candidiasis, bacterial and viral infections, as well as autoimmune phenomena (Sharfe et al. 1997). This autosomal recessive defect results in severe reduction in T cell numbers, with B cells showing normal development. Due to lack of regulatory mechanisms and abnormal proliferation, these patients experience lymphocytic infiltration of tissues, lung, liver, bone and skin causing inflammation and severe damage.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Patients with CD25 deficiency suffer from chronic candidiasis as well as invasive candidemia (Ouederni et al. 2014). Several genetic variants were also associated with CMCC, some with isolated candidiasis while others as part of a complex syndrome.

Protein tyrosine phosphatase non-receptor type-22 (PTPN22) mutations

Mutations in *PTPN22*, encoding Lyp, are associated with wide variety of autoimmune diseases. In particular, the R620W variant is found in significantly high prevalence in a group of CMCC patients (Nahum et al. 2008). The substrates for Lyp phosphatase activity include Lck, Fyn, Zap70 and the TCR zeta chain, and Lyp is also known to interact with Csk, an Src negative regulatory kinase. Whereas Lyp overexpression downregulates TCR signaling, Lyp deficiency has been shown to enhance T cell activation. The R620W variant is a gain-of-function mutation, resulting in defects in central as well as peripheral tolerance (Gregorieff et al. 1998; Gjörloff-Wingren et al. 1999; Vang et al. 2005), as seen in rheumatoid arthritis, systemic lupus erythromatosis, and adrenal insufficiency (Bottini et al. 2004; Wu et al. 2005; Michou et al. 2007). While the underlying mechanism is not completely understood, it may well be related to disruption of regulatory mechanisms which are known from other genetic defects to promote colonization with *Candida*.

Susceptibility genes associated with CMCC

Polymorphisms in genes that result in innate immune related defects are also responsible or associated with chronic candidiasis.

Such is the case of Dectin-1 single nucleotide polymorphisms (Ferwerda et al. 2009). Dectin-1 is an important sensor of *Candida*, which recognizes the microbial cell wall constituent β-glucan. The Y238X variant, found in as much as 8% of European and 40% of sub-Saharan African populations, has been suggested to confer susceptibility to mucosal candidiasis but not systemic candidemia (Rosentul et al. 2011). Reduced secretion of IL-1β and attenuation of Th17 responses have been reported for this variant (Plantinga et al. 2009).

Polymorphisms or variants in TLR receptors were also reported as susceptibility genes for chronic candidiasis. The TLR2 R753Q variant was shown in 1 study to be associated with candidiasis through reduced IFN-γ and IL-8 secretion (Woehrle et al. 2008). The TLR4 variants, D299G and Y399I, have also been reported as risk factors for invasive blood stream candidemia (Van der Graaf et al. 2006). We have previously shown that a variant of TLR3, L412F, confers susceptibility to CMCC (Nahum et al. 2011). Responses to stimulation with *Candida* as well as TLR3 ligands were significantly abrogated, resulting in reduced secretion IFN-γ (Nahum et al. 2012).

Recently, polymorphisms in TLR1 were shown to contribute to high risk of candidemia, likely through effects on IL-8 and IFN-γ levels (Plantinga et al. 2012). Recurrent vulvo-vaginal candidiasis is a common finding in individuals with polymorphisms in the inflammasome protein NLRP3 (Lev-Sagie et al. 2009), as well another innate receptor gene, MBL2, which encodes for mannose binding lectin (Babula et al. 2003; Giraldo et al. 2007). Recent studies suggest an important role for the inflammasome product IL-1β in eliciting a proper T cell mediated responses to Candida (Zielinski et al. 2012).

Chronic disseminated candidiasis has also been reported in association with changes in IL-4 (Choi et al. 2003), and persistent candidemia shown with variants of IL-12b and IL-10 (Johnson et al. 2012).

Concluding remarks

Chronic candidiasis is a feature present in a broad range of diseases, from isolated candidiasis involving skin and mucous membrane, to syndromes encompassing severe immunodeficiency and autoimmunity. A careful immune workup is therefore necessary, and should include thorough genetic and functional studies. Issues to consider include different treatment modalities (as is the case for STAT1 loss-of-function mutations, where bone marrow transplantation may be of benefit but is complicated by the many phenotypes associated with this gene) and the possibility that prolonged and repeated courses of anti-fungal treatment may result in microbial resistance.

It is important to note that although monogenic immunodeficiency disorders are interesting and insightful with regard to mechanisms of infection; in the vast majority of *Candida* infections, the cause is multifactorial. These include susceptibility genes found in large numbers of the general population in combination with factors such as altered local microbial flora (resulting from use of antibiotics, immunomodulators, and chemotherapy).

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Together, these stress the need for improved understanding of the mechanisms underlying susceptibility to *Candida* infection, which may pave the way for new and innovative treatment strategies. Of equal importance is the implementation of large population-based genetic studies combined with methods from the fields of functional genomics and mathematical-biological methods. Targeting future treatments towards key molecules may prove to be beneficial to a large number of patients suffering from *Candida* infections such as those with hematological malignancies and other immunocompromised patients.

REFERENCES

Ahonen, P., Myllärniemi, S., Sipilä, I., and Perheentupa, J. 1990. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N. Engl. J. Med. **322**(26): 1829–1836. PMID: 2348835. doi: 10.1056/NEJM199006283222601.

Akira, S., Uematsu, S., and Takeuchi, O. 2006. Pathogen recognition and innate immunity. Cell. **124**(4): 783–801. PMID: 16497588. doi: 10.1016/j.cell.2006.02.015.

Babula, O., Lazdane, G., Kroica, J., Ledger, W.J., and Witkin, S.S. 2003. Relation between recurrent vulvovaginal candidiasis, vaginal concentrations of mannose-binding lectin, and a mannose-binding lectin gene polymorphism in Latvian women. Clin. Infect. Dis. **37**(5):733–737. PMID: 28877549. doi: 10.1086/377234.

Betterle, C., Greggio, N.A., and Volpato, M. 1998. Clinical review 93: Autoimmune polyglandular syndrome type 1. J. Clin. Endocrinol. Metab. **83**(4):1049–1055. PMID: 9543115. doi: 10.1210/jcem.83.4.4682.

Björses, P., Aaltonen, J., Horelli-Kuitunen, N., Yaspo, M.L., and Peltonen, L. 1998. Gene defect behind APECED: A new clue to autoimmunity. Hum. Mol. Genet. **7**(10):1547–1553. PMID: 9735375.

Boisson, B., Wang, C., Pedergnana, V., Wu, L., Cypowyj, S., Rybojad, M., Belkadi, A., Picard, C., Abel, L., Fieschi, C., Puel, A., Li, X., and Casanova, J.L. 2013. An ACT1 mutation selectively abolishes interleukin-17 responses in humans with chronic mucocutaneous candidiasis. Immunity. **39**(4):676–686. PMID: 24120361. doi: 10.1016/j.immuni.2013.09.002

Boisson-Dupuis, S., Kong, X.-F., Okada, S., Cypowyj, S., Puel, A., Abel, L., and Casanova, J.L. 2012. Inborn errors of human STAT1: Allelic heterogeneity governs the diversity of immunological and infectious phenotypes. Curr. Opin. Immunol. **24**(4):364–378. PMID: 22651901. doi: 10.1016/j.coi.2012.04.011.

Bottini, N., Musumeci, L., Alonso, A., Rahmouni, S., Nika, K., Rostamkhani, M., MacMurray, J., Meloni, G.F., Lucarelli, P., Pellecchia, M., Eisenbarth, G.S., Comings, D., and Mustelin, T. 2004. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat. Genet. **36**(4):337–338. PMID: 15004560. doi: 10.1038/ng1323.

Brown, G.D., and Gordon, S. 2001. Immune recognition: A new receptor for β-glucans. Nature. **413**(6851): 36–37. PMID: 11544516. doi: 10.1038/35092620.

Chitnis, A.S., Magill, S.S., Edwards, J.R., Chiller, T.M., Fridkin, S.K., and Lessa, F.C. 2012. Trends in Candida central line-associated bloodstream infections among NICUs, 1999–2009. Pediatrics. **130**(1): e46–e52. PMID: 22711720. doi: 10.1542/peds.2011-3620.

Choi, E.H., Foster, C.B., Taylor, J.G., Erichsen, H.C., Chen, R.A., Walsh, T.J., Anttila, V.J., Ruutu, T., Palotie, A., and Chanock, S.J. 2003. Association between chronic disseminated candidiasis in adult acute leukemia and common IL4 promoter haplotypes. J. Infect. Dis. **187**(7):1153–1156. PMID: 12660931. doi: 10.1086/368345.

Das, I., Nightingale, P., Patel, M., and Jumaa, P. 2011. Epidemiology, clinical characteristics, and outcome of candidemia: Experience in a tertiary referral center in the UK. Int. J. Infect. Dis. **15**(11):e759–e763. PMID: 28864395. doi: 10.1016/j.ijid.2011.06.006.

de Beaucoudrey, L., Puel, A., Filipe-Santos, O., Cobat, A., Ghandil, P., Chrabieh, M., Feinberg, J., von Bernuth, H., Samarina, A., Jannière, L., Fieschi, C., Stéphan, J.L., Boileau, C., Lyonnet, S., Jondeau, G., Cormier-Daire, V., Le Merrer, M., Hoarau, C., Lebranchu, Y., Lortholary, O., Chandesris, M.O., Tron, F., Gambineri, E., Bianchi, L., Rodriguez-Gallego, C., Zitnik, S.E., Vasconcelos, J., Guedes, M., Vitor, A.B., Marodi, L., Chapel, H., Reid, B., Roifman, C., Nadal, D., Reichenbach, J., Caragol, I., Garty, B.Z., Dogu, F., Camcioglu, Y., Gülle, S., Sanal, O., Fischer, A., Abel, L., Stockinger, B., Picard, C., and Casanova, J.L. 2008. Mutations in STAT3 and IL12RB1 impair the development of human IL-17–producing T cells. J. Exp. Med. **205**(7):1543–1550. PMID: 18591412. doi: 10.1084/jem.20080321.

Donlan, R.M., and Costerton, J.W. 2002. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. **15**(2):167–193. PMID: 11932229.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Engelhardt, K.R., McGhee, S., Winkler, S., Sassi, A., Woellner, C., Lopez-Herrera, G., Chen, A., Kim, H.S., Lloret, M.G., Schulze, I., Ehl, S., Thiel, J., Pfeifer, D., Veelken, H., Niehues, T., Siepermann, K., Weinspach, S., Reisli, I., Keles, S., Genel, F., Kutukculer, N., Camcioğlu, Y., Somer, A., Karakoc-Aydiner, E., Barlan, I., Gennery, A., Metin, A., Degerliyurt, A., Pietrogrande, M.C., Yeganeh, M., Baz, Z., Al-Tamemi, S., Klein, C., Puck, J.M., Holland, S.M., McCabe, E.R., Grimbacher, B., and Chatila, T.A. 2009. Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. J. Allergy Clin. Immunol. **124**(6): 1289–1302.e4. PMID: 20004785. doi: 10.1016/j.jaci.2009.10.038.

Ferwerda, B., Ferwerda, G., Plantinga, T.S., Willment, J.A., van Spriel, A.B., Venselaar, H., Elbers, C.C., Johnson, M.D., Cambi, A., Huysamen, C., Jacobs, L., Jansen, T., Verheijen, K., Masthoff, L., Morré, S.A., Vriend, G., Williams, D.L., Perfect, J.R., Joosten, L.A., Wijmenga, C., van der Meer, J.W., Adema, G.J., Kullberg, B.J., Brown, G.D., and Netea, M.G. 2009. Human dectin-1 deficiency and mucocutaneous fungal infections. N. Engl. J. Med. **361**(18):1760–1767. PMID: 19864674. doi: 10.1056/NEJMoa0901053.

Giraldo, P.C., Babula, O., Gonçalves, A.K.S., Linhares, I.M., Amaral, R.L., Ledger, W.J., and Witkin, S.S. 2007. Mannose-binding lectin gene polymorphism, vulvovaginal candidiasis, and bacterial vaginosis. Obstet. Gynecol. **109**(5):1123–1128. PMID: 17470593. doi: 10.1097/01.AOG.0000260386.17555.a5.

Gjörloff-Wingren, A., Saxena, M., Williams, S., Hammi, D., and Mustelin, T. 1999. Characterization of TCR-induced receptor-proximal signaling events negatively regulated by the protein tyrosine phosphatase PEP. Eur. J. Immunol. **29**(12):3845–3854. PMID: 10601992. doi: 10.1002/(SICI)1521-4141(199912)29:12<3845::AID-IMMU3845>3.0.CO;2-U.

Glocker, E.-O., Hennigs, A., Nabavi, M., Schäffer, A.A., Woellner, C., Salzer, U., Pfeifer, D., Veelken, H., Warnatz, K., Tahami, F., Jamal, S., Manguiat, A., Rezaei, N., Amirzargar, A.A., Plebani, A., Hannesschläger, N., Gross, O., Ruland, J., and Grimbacher, B. 2009. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. N. Engl. J. Med. **361**(18):1727–1735. PMID: 19864672. doi: 10.1056/NEJMoa0810719.

Gregorieff, A., Cloutier, J.F., and Veillette, A. 1998. Sequence requirements for association of protein-tyrosine phosphatase PEP with the Src homology 3 domain of inhibitory tyrosine protein kinase p50(csk). J. Biol. Chem. **273**(21):13217–13222. PMID: 9582365.

Gross, O., Gewies, A., Finger, K., Schäfer, M., Sparwasser, T., Peschel, C., Förster, I., and Ruland, J. 2006. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. Nature. **442**(7103): 651–656. PMID: 16862125. doi: 10.1038/nature04926.

Hernández-Santos, N., and Gaffen, S.L. 2012. Th17 cells in immunity to Candida albicans. Cell Host Microbe. **11**(5):425–435. PMID: 22607796. doi: 10.1016/j.chom.2012.04.008.

Holland, S.M., DeLeo, F.R., Elloumi, H.Z., Hsu, A.P., Uzel, G., Brodsky, N., Freeman, A.F., Demidowich, A., Davis, J., Turner, M.L., Anderson, V.L., Darnell, D.N., Welch, P.A., Kuhns, D.B., Frucht, D.M., Malech, H.L., Gallin, J.I., Kobayashi, S.D., Whitney, A.R., Voyich, J.M., Musser, J.M., Woellner, C., Schäffer, A.A., Puck, J.M., and Grimbacher, B. 2007. *STAT3* mutations in the hyper-IgE syndrome. N. Engl. J. Med. **357**(16):1608–1619. PMID: 17881745. doi: 10.1056/NEJMoa073687.

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. 2006. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. **126**(6):1121–1133. PMID: 16990136. doi: 10.1016/j.cell.2006.07.035.

Iwakura, Y., Ishigame, H., Saijo, S., and Nakae, S. 2011. Functional specialization of interleukin-17 family members. Immunity. **34**(2):149–62. PMID: 21349428. doi: 10.1016/j.immuni.2011.02.012.

Johnson, M.D., Plantinga, T.S., van de Vosse, E., Velez Edwards, D.R., Smith, P.B., Alexander, B.D., Yang, J.C., Kremer, D., Laird, G.M., Oosting, M., Joosten, L.A., van der Meer, J.W., van Dissel, J.T., Walsh, T.J., Perfect, J.R., Kullberg, B.J., Scott, W.K., and Netea, M.G. 2012. Cytokine gene polymorphisms and the outcome of invasive candidiasis: A prospective cohort study. Clin. Infect. Dis. **54**(4):502–510. PMID: 28877549. doi: 10.1093/cid/cir827.

Jouault, T., Ibata-Ombetta, S., Takeuchi, O., Trinel, P., Sacchetti, P., Lefebvre, P., Akira, S., and Poulain, D. 2003. Candida albicans phospholipomannan is sensed through toll-like receptors. J. Infect. Dis. **188**(1): 165–172. PMID: 12825186. doi: 10.1086/375784.

Karvonen, M., Viik-Kajander, M., Moltchanova, E., Libman, I., LaPorte, R., and Tuomilehto, J. 2000. Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. Diabetes Care. **23**(10):1516–1526. PMID: 11023146.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Kirkpatrick, C.H. 1994. Chronic mucocutaneous candidiasis. J. Am. Acad. Dermatol. **31**(3):S14–S17. PMID: 8077500. doi: 10.1016/S0190-9622(08)81260-1.

Kisand, K., Bøe Wolff, A.S., Podkrajšek, K.T., Tserel, L., Link, M., Kisand, K.V., Ersvaer, E., Perheentupa, J., Erichsen, M.M., Bratanic, N., Meloni, A., Cetani, F., Perniola, R., Ergun-Longmire, B., Maclaren, N., Krohn, K.J., Pura, M., Schalke, B., Ströbel, P., Leite, M.I., Battelino, T., Husebye, E.S., Peterson, P., Willcox, N., and Meager, A. 2010. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J. Exp. Med. **207**(2):299–308. PMID: 20123959. doi: 10.1084/jem.20091669.

Lanternier, F., Pathan, S., Vincent, Q., Liu, L., Cypowij, S., Prando, C., Migaud, M., Taibi, L., Ammar-Khodja, A., Stambouli, O.B., Guellil, B., Jacobs, F., Goffard, J.-C., Shepers, K., del Marmol, V., Bachelez, H., Michel, L., Lefranc, G., Fraitag, S., Bougnoux, M.-E., and Boudia, A. 2012. Human invasive dermatophytic disease is caused by inborn errors of CARD9. J. Clin. Immunol. **32**:1–379. doi: 10.1007/s10875-012-9756-2.

Lev-Sagie, A., Prus, D., Linhares, I.M., Lavy, Y., Ledger, W.J., and Witkin, S.S. 2009. Polymorphism in a gene coding for the inflammasome component NALP3 and recurrent vulvovaginal candidiasis in women with vulvar vestibulitis syndrome. Am. J. Obstet. Gynecol. **200**(3):303.e1–303.e6. doi: 10.1016/j.ajog.2008.10.039.

Lévy, R., Okada, S., Béziat, V., Moriya, K., Liu, C., Chai, L.Y.A., Migaud, M., Hauck, F., Al Ali, A., Cyrus, C., Vatte, C., Patiroglu, T., Unal, E., Ferneiny, M., Hyakuna, N., Nepesov, S., Oleastro, M., Ikinciogullari, A., Dogu, F., Asano, T., Ohara, O., Yun, L., Della Mina, E., Bronnimann, D., Itan, Y., Gothe, F., Bustamante, J., Boisson-Dupuis, S., Tahuil, N., Aytekin, C., Salhi, A., Al Muhsen, S., Kobayashi, M., Toubiana, J., Abel, L., Li, X., Camcioglu, Y., Celmeli, F., Klein, C., AlKhater, S.A., Casanova, J.L., and Puel, A. 2016. Genetic, immunological, and clinical features of patients with bacterial and fungal infections due to inherited IL-17RA deficiency. Proc. Natl. Acad. Sci. USA. **113**(51):E8277–E8285. PMID: 27930337. doi: 10.1073/pnas.1618300114.

Ling, Y., Cypowyj, S., Aytekin, C., Galicchio, M., Camcioglu, Y., Nepesov, S., Ikinciogullari, A., Dogu, F., Belkadi, A., Levy, R., Migaud, M., Boisson, B., Bolze, A., Itan, Y., Goudin, N., Cottineau, J., Picard, C., Abel, L., Bustamante, J., Casanova, J.L., and Puel, A. 2015. Inherited IL-17RC deficiency in patients with chronic mucocutaneous candidiasis. J. Exp. Med. **212**(5):619–631. PMID: 25918342. doi: 10.1084/jem.20141065.

Liu, L., Okada, S., Kong, X.-F., Kreins, A.Y., Cypowyj, S., Abhyankar, A., Toubiana, J., Itan, Y., Audry, M., Nitschke, P., Masson, C., Toth, B., Flatot, J., Migaud, M., Chrabieh, M., Kochetkov, T., Bolze, A., Borghesi, A., Toulon, A., Hiller, J., Eyerich, S., Eyerich, K., Gulácsy, V., Chernyshova, L., Chernyshov, V., Bondarenko, A., Grimaldo, R.M., Blancas-Galicia, L., Beas, I.M., Roesler, J., Magdorf, K., Engelhard, D., Thumerelle, C., Burgel, P.R., Hoernes, M., Drexel, B., Seger, R., Kusuma, T., Jansson, A.F., Sawalle-Belohradsky, J., Belohradsky, B., Jouanguy, E., Bustamante, J., Bué, M., Karin, N., Wildbaum, G., Bodemer, C., Lortholary, O., Fischer, A., Blanche, S., Al-Muhsen, S., Reichenbach, J., Kobayashi, M., Rosales, F.E., Lozano, C.T., Kilic, S.S., Oleastro, M., Etzioni, A., Traidl-Hoffmann, C., Renner, E.D., Abel, L., Picard, C., Maródi, L., Boisson-Dupuis, S., Puel, A., and Casanova, J.L. 2011. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. J. Exp. Med. **208**(8):1635–1648. PMID: 21727188. doi: 10.1084/jem.20110958.

Maródi, L., Cypowyj, S., Tóth, B., Chernyshova, L., Puel, A., and Casanova, J.L. 2012. Molecular mechanisms of mucocutaneous immunity against Candida and Staphylococcus species. J. Allergy Clin. Immunol. **130**(5):1019–1027. PMID: 23040277. doi: 10.1016/j.jaci.2012.09.011.

McGeachy, M.J., and McSorley, S.J. 2012. Microbial-induced Th17: Superhero or supervillain? J Immunol. **189**(7):3285–3291. PMID: 22997231. doi: 10.4049/jimmunol.1201834.

McGreal, E.P., Rosas, M., Brown, G.D., Zamze, S., Wong, S.Y.C., Gordon, S., Martinez-Pomares, L., and Taylor, P.R. 2006. The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. Glycobiology. **16**(5):422–430. PMID: 16423983. doi: 10.1093/glycob/cwj077.

Meager, A., Visvalingam, K., Peterson, P., Möll, K., Murumägi, A., Krohn, K., Eskelin, P., Perheentupa, J., Husebye, E., Kadota, Y., Willcox, N., and Willcox, N. 2006. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med. **3**(7):e289. PMID: 16784312. doi: 10.1371/journal.pmed.0030289.

Michou, L., Lasbleiz, S., Rat, A.-C., Migliorini, P., Balsa, A., Westhovens, R., Barrera, P., Alves, H., Pierlot, C.,

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Glikmans, E., Garnier, S., Dausset, J., Vaz, C., Fernandes, M., Petit-Teixeira, E., Lemaire, I., Pascual-Salcedo, D., Bombardieri, S., Dequeker, J., Radstake, T.R., Van Riel, P., van de Putte, L., Lopes-Vaz, A., Prum, B., Bardin, T., Dieudé, P., Cornélis, F., and European Consortium on Rheumatoid Arthritis Families. 2007. Linkage proof for PTPN22, a rheumatoid arthritis susceptibility gene and a human autoimmunity gene. Proc. Natl. Acad. Sci. USA. **104**(5):1649–1654. PMID: 17237219. doi: 10.1073/pnas.0610250104.

Minegishi, Y., Saito, M., Morio, T., Watanabe, K., Agematsu, K., Tsuchiya, S., Takada, H., Hara, T., Kawamura, N., Ariga, T., Kaneko, H., Kondo, N., Tsuge, I., Yachie, A., Sakiyama, Y., Iwata, T., Bessho, F., Ohishi, T., Joh, K., Imai, K., Kogawa, K., Shinohara, M., Fujieda, M., Wakiguchi, H., Pasic, S., Abinun, M., Ochs, H.D., Renner, E.D., Jansson, A., Belohradsky, B.H., Metin, A., Shimizu, N., Mizutani, S., Miyawaki, T., Nonoyama, S., Karasuyama, H., and Karasuyama, H. 2006. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. Immunity. **25**(5):745–755. PMID: 17088085. doi: 10.1016/j.immuni.2006.09.009.

Minegishi, Y., Saito, M., Tsuchiya, S., Tsuge, I., Takada, H., Hara, T., Kawamura, N., Ariga, T., Pasic, S., Stojkovic, O., Metin, A., and Karasuyama, H. 2007. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature. **448**(7157):1058–1062. PMID: 17676033. doi: 10.1038/nature06096.

Nagamine, K., Peterson, P., Scott, H.S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K.J., Lalioti, M.D., Mullis, P.E., Antonarakis, S.E., Kawasaki, K., Asakawa, S., Ito, F., and Shimizu, N. 1997. Positional cloning of the APECED gene. Nat. Genet. **17**(4): 393–398. PMID: 9398839. doi: 10.1038/ng1297-393.

Nahum, A., Bates, A., Sharfe, N., and Roifman, C.M. 2008. Association of the lymphoid protein tyrosine phosphatase, R620W variant, with chronic mucocutaneous candidiasis. J. Allergy Clin. Immunol. **122**(6): 1220–1222. PMID: 19084113. doi: 10.1016/j.jaci.2008.10.027.

Nahum, A., Dadi, H., Bates, A., and Roifman, C.M. 2011. The L412F variant of Toll-like receptor 3 (TLR3) is associated with cutaneous candidiasis, increased susceptibility to cytomegalovirus, and autoimmunity. J. Allergy Clin. Immunol. **127**(2): 528–531. PMID: 21093032. doi: 10.1016/j.jaci.2010.09.031.

Nahum, A., Dadi, H., Bates, A., and Roifman, C.M. 2012. The biological significance of TLR3 variant, L412F, in conferring susceptibility to cutaneous candidiasis, CMV and autoimmunity. Autoimmun. Rev. **11**(5):341–347. PMID: 22024499. doi: 10.1016/j.autrev.2011.10.007.

Netea, M.G., Gow, N.A.R., Munro, C.A., Bates, S., Collins, C., Ferwerda, G., Hobson, R.P., Bertram, G., Hughes, H.B., Jansen, T., Jacobs, L., Buurman, E.T., Gijzen, K., Williams, D.L., Torensma, R., McKinnon, A., MacCallum, D.M., Odds, F.C., Van der Meer, J.W., Brown, A.J., and Kullberg, B.J. 2006. Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. J. Clin. Invest. **116**(6): 1642–1650. PMID: 16710478. doi: 10.1172/JCI27114.

Okada, S., Markle, J.G., Deenick, E.K., Mele, F., Averbuch, D., Lagos, M., Alzahrani, M., Al-Muhsen, S., Halwani, R., Ma, C.S., Wong, N., Soudais, C., Henderson, L.A., Marzouqa, H., Shamma, J., Gonzalez, M., Martinez-Barricarte, R., Okada, C., Avery, D.T., Latorre, D., Deswarte, C., Jabot-Hanin, F., Torrado, E., Fountain, J., Belkadi, A., Itan, Y., Boisson, B., Migaud, M., Arlehamn, C.S.L., Sette, A., Breton, S., McCluskey, J., Rossjohn, J., de Villartay, J.P., Moshous, D., Hambleton, S., Latour, S., Arkwright, P.D., Picard, C., Lantz, O., Engelhard, D., Kobayashi, M., Abel, L., Cooper, A.M., Notarangelo, L.D., Boisson-Dupuis, S., Puel, A., Sallusto, F., Bustamante, J., Tangye, S.G., and Casanova, J.L. 2015. IMMUNODEFICIENCIES. Impairment of immunity to Candida and Mycobacterium in humans with bi-allelic RORC mutations. Science. **349**(6248): 606–613. PMID: 26160376. doi: 10.1126/science.aaa4282.

Ortega, M., Rovira, M., Almela, M., Marco, F., de la Bellacasa, J.P., Martínez, J.A., Carreras, E., and Mensa, J. 2005. Bacterial and fungal bloodstream isolates from 796 hematopoietic stem cell transplant recipients between 1991 and 2000. Ann. Hematol. **84**(1):40–46. PMID: 15480665. doi: 10.1007/s00277-004-0909-0.

Ouederni, M., Sanal, O., Ikinciogullari, A., Tezcan, I., Dogu, F., Sologuren, I., Pedraza-Sánchez, S., Keser, M., Tanir, G., Nieuwhof, C., Colino, E., Kumararatne, D., Levy, J., Kutukculer, N., Aytekin, C., Herrera-Ramos, E., Bhatti, M., Karaca, N., Barbouche, R., Broides, A., Goudouris, E., Franco, J.L., Parvaneh, N., Reisli, I., Strickler, A., Shcherbina, A., Somer, A., Segal, A., Angel-Moreno, A., Lezana-Fernandez, J.L., Bejaoui, M., Bobadilla-Del Valle, M., Kachboura, S.,

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Sentongo, T., Ben-Mustapha, I., Bustamante, J., Picard, C., Puel, A., Boisson-Dupuis, S., Abel, L., Casanova, J.L., and Rodríguez-Gallego, C. 2014. Clinical features of Candidiasis in patients with inherited interleukin 12 receptor β1 deficiency. Clin. Infect. Dis. **58**(2): 204–213. PMID: 28877549. doi: 10.1093/cid/cit722.

Pedroza, L.A., Kumar, V., Sanborn, K.B., Mace, E.M., Niinikoski, H., Nadeau, K., Vasconcelos Dde, M., Perez, E., Jyonouchi, S., Jyonouchi, H., Banerjee, P.P., Ruuskanen, O., Condino-Neto, A., and Orange, J.S. 2012. Autoimmune regulator (AIRE) contributes to Dectin-1-induced TNF-α production and complexes with caspase recruitment domain-containing protein 9 (CARD9), spleen tyrosine kinase (Syk), and Dectin-1. J. Allergy Clin. Immunol. **129**(2): 464–472, 472.e1–472.e3. PMID: 21962774. doi: 10.1016/j.jaci.2011.08.027.

Perheentupa, J. 2006. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J. Clin. Endocrinol. Metab. **91**(8):2843–2850. PMID: 16684821. doi: 10.1210/jc.2005-2611.

Plantinga, T.S., Johnson, M.D., Scott, W.K., van de Vosse, E., Velez Edwards, D.R., Smith, P.B., Alexander, B.D., Yang, J.C., Kremer, D., Laird, G.M., Oosting, M., Joosten, L.A., van der Meer, J.W., van Dissel, J.T., Walsh, T.J., Perfect, J.R., Kullberg, B.J., and Netea, M.G. 2012. Toll-like receptor 1 polymorphisms increase susceptibility to candidemia. J. Infect. Dis. **205**(6):934–943. PMID: 22301633. doi: 10.1093/infdis/jir867.

Plantinga, T.S., van der Velden, W.J.F.M., Ferwerda, B., van Spriel, A.B., Adema, G., Feuth, T., Donnelly, J.P., Brown, G.D., Kullberg, B.J., Blijlevens, N.M., and Netea, M.G. 2009. Early stop polymorphism in human DECTIN-1 is associated with increased candida colonization in hematopoietic stem cell transplant recipients. Clin. Infect. Dis. **49**(5):724–732. PMID: 19614557. doi: 10.1086/604714.

Puel, A., Cypowyj, S., Bustamante, J., Wright, J.F., Liu, L., Lim, H.K., Migaud, M., Israel, L., Chrabieh, M., Audry, M., Gumbleton, M., Toulon, A., Bodemer, C., El-Baghdadi, J., Whitters, M., Paradis, T., Brooks, J., Collins, M., Wolfman, N.M., Al-Muhsen, S., Galicchio, M., Abel, L., Picard, C., and Casanova, J.L. 2011. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science. **332**(6025):65–68. PMID: 21350122. doi: 10.1126/science.1200439.

Puel, A., Döffinger, R., Natividad, A., Chrabieh, M., Barcenas-Morales, G., Picard, C., Cobat, A., Ouachée-Chardin, M., Toulon, A., Bustamante, J., Al-Muhsen, S., Al-Owain, M., Arkwright, P.D., Costigan, C., McConnell, V., Cant, A.J., Abinun, M., Polak, M., Bougnères, P.F., Kumararatne, D., Marodi, L., Nahum, A., Roifman, C., Blanche, S., Fischer, A., Bodemer, C., Abel, L., Lilic, D., and Casanova, J.L. 2010. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J. Exp. Med. **207**(2):291–297. PMID: 20123958. doi: 10.1084/jem.20091983.

Robinson, M.J., Osorio, F., Rosas, M., Freitas, R.P., Schweighoffer, E., Gross, O., Verbeek, J.S., Ruland, J., Tybulewicz, V., Brown, G.D., Moita, L.F., Taylor, P.R., and Reis e Sousa, C. 2009. Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. J. Exp. Med. **206**(9):2037–2051. PMID: 19703985. doi: 10.1084/jem.20082818.

Rogers, N.C., Slack, E.C., Edwards, A.D., Nolte, M.A., Schulz, O., Schweighoffer, E., Williams, D.L., Gordon, S., Tybulewicz, V.L., Brown, G.D., and Reis e Sousa, C. 2005. Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. Immunity. **22**(4):507–517. PMID: 15845454. doi: 10.1016/j.immuni.2005.03.004.

Rosentul, D.C., Plantinga, T.S., Oosting, M., Scott, W.K., Velez Edwards, D.R., Smith, P.B., Alexander, B.D., Yang, J.C., Laird, G.M., Joosten, L.A., van der Meer, J.W., Perfect, J.R., Kullberg, B.J., Netea, M.G., and Johnson, M.D. 2011. Genetic variation in the Dectin-1/CARD9 recognition pathway and susceptibility to candidemia. J. Infect. Dis. **204**(7): 1138–1145. PMID: 21881131. doi: 10.1093/infdis/jir458.

Rutz, S., Eidenschenk, C., and Ouyang, W. 2013. IL-22, not simply a Th17 cytokine. Immunol. Rev. **252**(1): 116–132. PMID: 23405899. doi: 10.1111/imr.12027.

Sharfe, N., Dadi, H.K., Shahar, M., and Roifman, C.M. 1997. Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. Proc. Natl. Acad. Sci. USA. **94**(7):3168–3171. PMID: 9096364.

Sharfe, N., Nahum, A., Newell, A., Dadi, H., Ngan, B., Pereira, S.L., Herbrick, J.A., and Roifman, C.M. 2014. Fatal combined immunodeficiency associated with heterozygous mutation in STAT1. J. Allergy. Clin. Immunoly. **133**(3):807–817. PMID: 24239102. doi: 10.1016/j.jaci.2013.09.032.

Smeekens, S.P., Ng, A., Kumar, V., Johnson, M.D., Plantinga, T.S., van Diemen, C., Arts, P., Verwiel, E.T., Gresnigt, M.S., Fransen, K., van Sommeren, S.,

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Oosting, M., Cheng, S.C., Joosten, L.A., Hoischen, A., Kullberg, B.J., Scott, W.K., Perfect, J.R., van der Meer, J.W., Wijmenga, C., Netea, M.G., and Xavier, R.J. 2013*a*. Functional genomics identifies type I interferon pathway as central for host defense against Candida albicans. Nat. Commun. **4**:1342. PMID: 23299892. doi: 10.1038/ncomms2343

Smeekens, S.P., van de Veerdonk, F.L., Kullberg, B.J., and Netea, M.G. 2013*b*. Genetic susceptibility to Candida infections. EMBO Mol. Med. **5**(6):805–813. PMID: 23629947. doi: 10.1002/emmm.201201678.

Sobel, J.D. 2007. Vulvovaginal candidosis. Lancet. **369**(9577):1961–1971. PMID: 17560449. doi: 10.1016/S0140-6736(07)60917-9.

Uzel, G., Sampaio, E.P., Lawrence, M.G., Hsu, A.P., Hackett, M., Dorsey, M.J., Noel, R.J., Verbsky, J.W., Freeman, A.F., Janssen, E., Bonilla, F.A., Pechacek, J., Chandrasekaran, P., Browne, S.K., Agharahimi, A., Gharib, A.M., Mannurita, S.C., Yim, J.J., Gambineri, E., Torgerson, T., Tran, D.Q., Milner, J.D., and Holland, S.M. 2013. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation–polyendocrinopathy–enteropathy–X-linked–like syndrome. J. Allergy Clin. Immunol. **131**(6): 1611–1623. PMID: 23534974. doi: 10.1016/j.jaci.2012.11.054.

van de Veerdonk, F.L., Plantinga, T.S., Hoischen, A., Smeekens, S.P., Joosten, L.A., Gilissen, C., Arts, P., Rosentul, D.C., Carmichael, A.J., Smits-van der Graaf, C.A., Kullberg, B.J., van der Meer, J.W., Lilic, D., Veltman, J.A., and Netea, M.G. 2011. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N. Engl. J. Med. **365**(1):54–61. PMID: 21714643. doi: 10.1056/NEJMoa1100102.

Van der Graaf, C.A.A., Netea, M.G., Morré, S.A., Den Heijer, M., Verweij, P.E., Van der Meer, J.W.M., and Kullberg, B.J. 2006. Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for Candida bloodstream infection. Eur. Cytokine Netw. **17**(1):29–34. PMID: 16613760.

Vang, T., Congia, M., Macis, M.D., Musumeci, L., Orrú, V., Zavattari, P., Nika, K., Tautz, L., Taskén, K., Cucca, F., Mustelin, T., and Bottini, N. 2005. Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. Nat. Genet. **37**(12): 1317–1319. PMID: 16273109. doi: 10.1038/ng1673.

Viscoli, C., Girmenia, C., Marinus, A., Collette, L., Martino, P., Vandercam, B., Doyen, C., Lebeau, B., Spence, D., Krcmery, V., De Pauw, B., and Meunier, F. 1999. Candidemia in cancer patients: A prospective, multicenter surveillance study by the Invasive Fungal Infection Group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). Clin. Infect. Dis. **28**(5):1071–1079. PMID: 10452637. doi: 10.1086/514731.

Woehrle, T., Du, W., Goetz, A., Hsu, H.-Y., Joos, T.O., Weiss, M., Bauer, U., Brueckner, U.B., and Marion Schneider, E. 2008. Pathogen specific cytokine release reveals an effect of TLR2 Arg753Gln during Candida sepsis in humans. Cytokine. **41**(3):322–329. PMID: 18249133. doi: 10.1016/j.cyto.2007.12.006.

Wu, H., Cantor, R.M., Graham, D.S.C., Lingren, C.M., Farwell, L., Jager, P.L., Bottini, N., Grossman, J.M., Wallace, D.J., Hahn, B.H., Julkunen, H., Hebert, L.A., Rovin, B.H., Birmingham, D.J., Rioux, J.D., Yu, C.Y., Kere, J., Vyse, T.J., and Tsao, B.P. 2005. Association analysis of the R620W polymorphism of protein tyrosine phosphatase PTPN22 in systemic lupus erythematosus families: Increased T allele frequency in systemic lupus erythematosus patients with autoimmune thyroid disease. Arthritis. Rheum. **52**(8):2396–2402. PMID: 16052563. doi: 10.1002/art.21223.

Yang, X.O., Panopoulos, A.D., Nurieva, R., Chang, S.H., Wang, D., Watowich, S.S., and Dong, C. 2007. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J. Biol. Chem. **282**(13): 9358–9363. PMID: 17277312. doi: 10.1074/jbc.C600321200.

Zhang, Q., Davis, J.C., Lamborn, I.T., Freeman, A.F., Jing, H., Favreau, A.J., Matthews, H.F., Davis, J., Turner, M.L., Uzel, G., Holland, S.M., and Su, H.C. 2009. Combined immunodeficiency associated with DOCK8 mutations. N. Engl. J. Med. **361**(21): 2046–2055. PMID: 19776401. doi: 10.1056/NEJMoa0905506.

Zielinski, C.E., Mele, F., Aschenbrenner, D., Jarrossay, D., Ronchi, F., Gattorno, M., Monticelli, S., and Lanzavecchia A Sallusto, F. 2012. Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. Nature. **484**(7395): 514–518. PMID: 22466287. doi: 10.1038/nature10957.

Immunodeficiency Canada

Created in 1999, Immunodeficiency Canada is a national organization working closely with diagnostic and treatment centres across Canada. It plays a vital role in being one of the first organizations in Canada to finance and encourage research in the field of primary immunodeficiency, as well as, providing education for patients and medical professionals. Programs and services are provided through the support of generous donors.

Vision
To cure Primary Immunodeficiency (PI)

Mission
To improve the lives of people with Primary Immunodeficiency (PI) by promoting early diagnosis and effective treatments through leadership in research, education and advocacy in partnership with health-care professionals, volunteers, industry and government

The Immunodeficiency Canada addresses its mission through four core programs:

- Network
- Education
- Research
- Patient Support

Programs and Services
- Emergency Financial Assistance
- Patient Support
- Primary Immunodeficiency Information
- Publications
- Resources
- Social Support

For Healthcare Professionals
Chaim Roifman Scholar Award for research

Supported by Baxter, CSL Behring, Grifols, Octapharma and Immunodeficiency Canada

- for Senior Investigators
- for Young Investigators

Conferences, seminars and symposiums
Immunodeficiency Canada-Grifols Fellow Training Program
Immunodeficiency Canada Network

- Consultations
- Education
- Immunologists network grants
- PI video conferences
- Healthcare facility sites and affiliates

Medical Advisory Board
Physicians Login (for diagnostic tools, case studies and resources)
PI Nurses Network
PI Social Workers Network

Board of Directors
Chaim Roifman MD FRCPC, Chair & Scientific Director
John F. Perl, President
Joseph Vital, Treasurer
Peter Clark
Thomas Moran
Barry Reichmann

Chief Executive Officer
Richard B. Thompson, CFRE

Corporate Advisory Board
Joel Abelson
Darin Brock
Vicki Modell
Paul R. Perreault, Sr.

Medical Advisory Board
Adelle R. Atkinson MD FRCPC, Toronto
Stephen Betschel MD FRCPC, Toronto
Eyal Grunebaum MD, Toronto
Elie Haddad MD PHD, Montreal
Kyla Hildebrand MD FRCPC, Vancouver
Thomas B. Issekutz MD FRCPC, Halifax
Amin Kanani MD, FRCPC, Vancouver
Fotini Kavadas MD, FRCPC, Winnipeg
Bruce Mazer MD FRCPC, Montreal
Stuart Turvey, MD, FRCPC, Vancouver

Additional Resources
Canadian, International resources and links to condition-specific information can be found at **www.immunodeficiency.ca**

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Immunodeficiency Canada
Immunodéficience Canada

Providing patient support, education and research to cure Primary Immunodeficiency

Suite 1604, 415 Yonge Street, Toronto, Ontario M5B 2E7
Tel 416-964-3434 **Fax** 416-964-6594
contactus@immunodeficiency.ca
charitable # 87276 0897 RR0001

www.immunodeficiency.ca

The novel p.Gly306Asp perforin mutation causes familial hemophagocytic lymphohistiocytosis type 2 (FHL-2) probably due to a critical role of Gly306 in the pore-forming perforin domain

Ramón Urrea Moreno[a], Itziar Astigarraga[b,c,d], Ana Fernández-Teijeiro[e], Carmen Rodríguez-Sainz[a], María Alonso-Martinez[a], Lydia Martín-Martín[a], Susana García-Obregón[c], and Juana Gil-Herrera[a*]

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

ABSTRACT

Background: Three substitutions at either Gly305 or Gly306 within the membrane attack/complex perforin domain (MACPF) of perforin have been previously identified in a number of patients with hemophagocytic lymphohistiocytosis (HLH). However, their pathogenic impact remains unclear since all the cases reported so far carried heterozygous genotypes and showed very heterogeneous clinical presentations. Here, we report a new substitution (p.Gly306Asp) and use *in silico* tools to elucidate the pathogenic mechanisms and severity associated with human Gly306 and Gly305 mutations.

Methods: The immunological workup included perforin expression and perforin gene (*PRF1*) mutation analysis. Computer algorithms based on conservation, secondary, and tertiary protein structure analyses were applied to assess the role of the mutations in disease pathogenesis.

Results: In our patient, we found a previously undescribed homozygous c. 917G>A (p.Gly306Asp) mutation in the *PRF1* gene that was associated with null perforin expression in her natural killer lymphocytes. Sequence alignments revealed that Gly306 and Gly305 are highly conserved positions among vertebrate perforins, as well as in other related pore-forming proteins such as bacterial cytolysins. Further *in silico* analyses consistently predicted mutations in these 2 positions to be pathogenic due to diminished stability of the perforin molecule.

Conclusion: Age of HLH onset, severity of the disease and undetectable perforin in our p.Gly306Asp homozygous patient along with the *in silico* results unmask this novel mutation as highly detrimental. Our results highlight the need of combining all clinical features, *in vitro* phenotypes and computer based approaches to classify human perforin mutations accurately.

Statement of novelty: The study of these *PRF1* mutations points to an important role of the 2 glycine amino acids (Gly305 and Gly306) in the molecular stability of perforin, which may also be likely in other pore-forming proteins. Our *in silico* results conclude that the pathogenicity of mutations in highly conserved Gly305 and Gly306 is likely to be associated with a serious destabilization of the native perforin conformation.

[a]Hospital General Universitario and Health Research Institute "Gregorio Marañón", Madrid, Spain; [b]Paediatric Service, Hospital Universitario Cruces, Barakaldo, Spain; [c]Paediatric Oncology Group, BioCruces Health Research Institute, Barakaldo, Spain; [d]Department of Paediatric, Faculty of Medicine and Dentistry, University of the Basque Country, UPV/EHU, Leioa, Bizkaia, Spain; [e]Pediatric Onco-Hematology Unit, Hospital Virgen de la Macarena, Sevilla, Spain

*Corresponding author: Dr. Juana Gil-Herrera/juana.gil@salud.madrid.org

Submitted 23 May 2017
Accepted 27 July 2017
Available online 3 August 2017

LymphoSign Journal 4:101–112 (2017)
dx.doi.org/10.14785/lymphosign-2017-0006

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Introduction

Familial hemophagocytic lymphohistiocytosis (FHL) is a primary immunodeficiency due to defects in several genes involved in the cytotoxic pathway (Janka 2012; Sieni et al. 2014). FHL usually presents during early childhood and is fatal without hematopoietic cell transplantation (HCT); however, atypical, late-onset FHL cases are increasingly being described (Clementi et al. 2002; Molleran Lee et al. 2004; Mancebo et al. 2006; Zur Stadt et al. 2006; Nagafuji et al. 2007; Ueda et al. 2007; Zhang et al. 2011; Sanchez et al. 2012; Sieni et al. 2012; Wang et al. 2012; Dias et al. 2013; Mhatre et al. 2014; Tesi et al. 2015; Barmettler et al. 2016; Gao et al. 2016).

Type 2 FHL (FHL-2) is caused by mutations in the *PRF1* gene (Stepp et al. 1999; Voskoboinik et al. 2010; Voskoboinik et al. 2015). *PRF1* encodes perforin, a protein present in the secretory granules of cytotoxic T and natural killer (NK) lymphocytes, which is essential for cytotoxic immune responses and homeostasis (Voskoboinik et al. 2010; Thiery and Lieberman 2014; Voskoboinik et al. 2015). Most of the mutations reported in FHL-2 patients are located in the membrane attack complex/perforin (MACPF) domain (An et al. 2013; Voskoboinik et al. 2015). The MACPF domain is critical for pore formation, specifically the oligomerization of perforin molecules after their preactivation by binding to the target cell membrane (Baran et al. 2009; Stewart et al. 2015; Yagi et al. 2015). Despite recent advances regarding perforin structure (Law et al. 2010; Yagi et al. 2015), little is known about the precise mechanism of pore formation by perforin, and the molecular mechanisms underlying the pathogenicity of MACPF mutations remain poorly understood.

We describe an early-onset FHL-2 case showing the novel MACPF homozygous missense mutation c.917G>A which leads to the amino acid change p.Gly306Asp. Different amino acid substitutions that are located at the same position (c.916G>T leading to p.Gly306Cys (Grossman et al. 2005; Abdalgani et al. 2015), c.916G>A leading to p.Gly306Ser (Nagafuji et al. 2007; Wang et al. 2012; Gao et al. 2016)) or neighbouring glycine (c.914C>A, leading to p.Gly305Asp, (Clementi et al. 2005)) have been previously reported in very heterogeneous FHL-2 patients. We performed perforin immunophenotyping and *in silico* analysis to ascertain the relevance of the novel p.Gly306Asp as well as the above mentioned mutations in HLH pathogenesis.

Functional and clinical presentation

Case report: A 19-month-old girl, the first child of a consanguineous couple with no previous familial history of hemophagocytic lympohistiocytosis (HLH), was first admitted to hospital because of fever due to unknown origin. Progressive anemia (haemoglobin 7.9 g/L), thrombopenia (8000/µl), hypertrigliceridemia (720 mg/dL), elevated ferritin (3373 ng/mL), hypofibrinoginemia (120 mg/dL) and hepatosplenomegaly, along with hemophagocytosis in bone marrow led to diagnosis of HLH, and she was started on the HLH-2004 therapeutic protocol (Henter et al. 2007). According to the immunological results, she was classified as FHL-2 and after HLH-2004 induction therapy and conditioning, she underwent cord blood HCT (1.36×10^5 $CD34^+$ cells). Continuing maintenance treatment was required post-HCT and the patient unfortunately died at 150 days post-transplant because of graft failure and HLH disease reactivation.

Perforin expression was assessed by flow cytometry using anti-T cell receptor FITC, anti-CD8 PerCP, and anti-CD56 APC (BD Biosciences, San Jose, CA) antibodies for surface staining and anti-perforin PE (clone DG9, Pharmingen, BD Bioscience) or anti-granzyme B PE (Immunotech) antibodies for intracellular staining, as previously described (Urrea Moreno et al. 2009). Samples were acquired in a FACSCalibur cytometer and analysed with FlowJo 7.6.1 software. Figure 1A shows that perforin staining within patient's circulating NK lymphocytes (corresponding to 16% of total lymphocytes) was as negative as the fluorescence intensity of isotypic control. On the other hand, granzyme-B expression—analyzed as a positive intracellular staining—was increased in the patient when compared to the patient´s mother and a healthy individual tested the same day of the analysis (Figure 1B).

The mutational analysis was performed on genomic DNA from peripheral blood. Coding region of *PRF1* (exons 2 and 3) was amplified by PCR (Gene Amp PCR System 9700, PE Applied Biosystems, Warrington, UK) and sequenced (ABI PRISM 310 Genetic Analyzer from Applied Biosystems) as described (Urrea Moreno et al. 2009). Chromatograms were created with Geneious Pro 5.5.7 and sequences were compared with

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

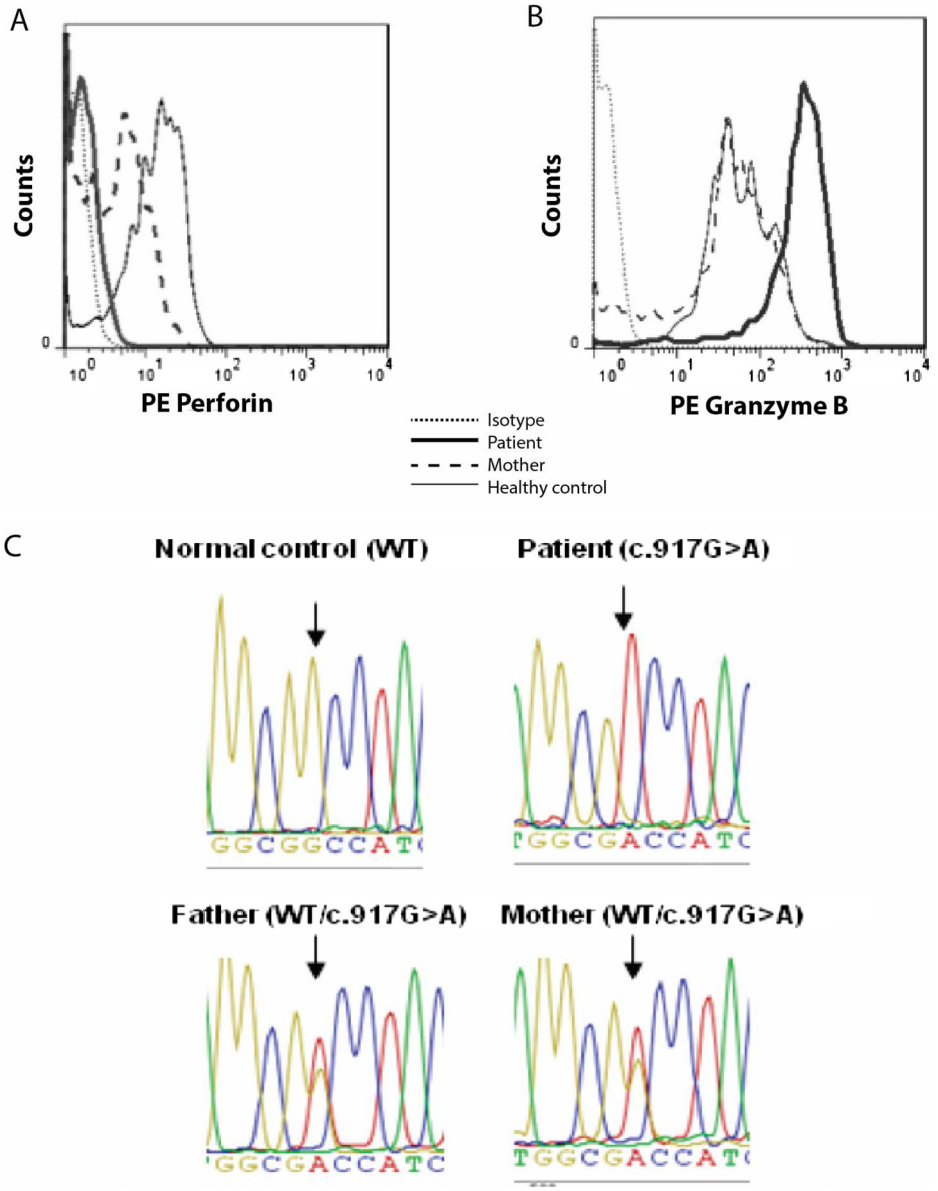

Figure 1: Intracellular protein expression and PRF1 sequencing. Flow cytometry was used to detect perforin (A) and granzyme-B (B) in NK-cells from patient, her mother, and a healthy control. Both histograms are gated on $CD56^{+}TCRab^{-}$ cells. The counts of positive gated events represented on the Y axis included at least 500 to a maximum of 1600 cells. (C) DNA electropherograms. Arrows indicate the position at the wild-type PRF1 gene sequence in exon 3, and the homozygous missense mutation at the corresponding position in the patient as well as the heterozygous change in her parents.

reference *PRF1* (RefSeq: NG_009615.1). Figure 1C shows the homozygous mutation c. 917G>A in exon 3 of *PRF1* that was found in the patient; this nucleotide change results in the non-conservative substitution of the non-polar amino acid glycine at position 306 of wild-type perforin protein with the negatively charged aspartic acid (p.Gly306Asp) in MACPF domain. The parents were both found to be heterozygous carriers of the mutated allele (Figure 1C), correlating with the decreased perforin expression found in the mother (Figure 1A).

Figure 2 shows a molecular model of perforin and of perfringolysin (PFO), a bacterial cholesterol dependant cytolysin (CDC), to illustrate the structural homology of their 2 pore-forming domains. 3D models were performed with Yasara View, v16.7.22 (http://yasara.org/) (Krieger and Vriend 2014). Gly306 and the preceding

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

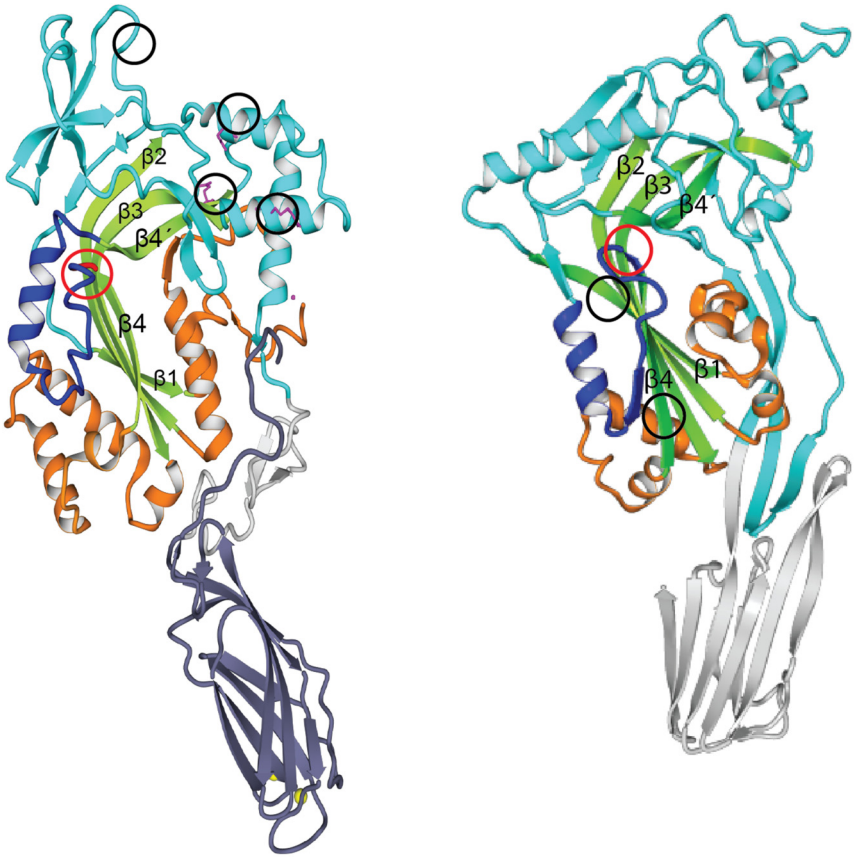

Figure 2: Molecular models of perforin and PFO. Ribbon representations of perforin molecule on the left, based on the murine perforin crystal structure (PDB identifier: 3nsj) and the cytolysin PFO (PDB identifier:1pfo) on the right, are shown. Different subregions of pore forming domains were coloured as follows: in green, the antiparallel central β sheet (conformed by β1, β2, β3, β4, and β4′); in dark blue, the "U" shaped helical loop connecting β4 and β4′ regions, with Gly305 and Gly306 in perforin, or the homologous glycines in PFO circled in red; in orange, clusters of transmembrane helices CH1 and CH2; in cyan, remaining MACPF regions. Circled in black on each protein are the location of residues potentially involved in perforin or PFO oligomerization.

Gly305 are located at the beginning of a helical loop region that connects β4 and β4′ strands of the central beta sheet in both MACPFs and CDCs (Lukoyanova et al. 2016; Dudkina et al. 2016). As shown in Figure 2, no proximity exists between the helical loop in perforin and the putative residues involved in its oligomerization, as opposed to what has been previously described in PFO (Ramachandran et al. 2004).

For amino acid conservation analysis, representative sequences were obtained from the NCBI database (www.ncbi.nlm.nih.gov). Alignments were performed with PROMALS3D (http://prodata.swmed.edu/promals3d/promals3d.php), and edited with Geneious Pro 5.5.7 using Blosum45 matrix. Figure 3 shows that both Gly305 and Gly306 in human perforin molecule or corresponding glycines in other vertebrate perforins, as well as other MACPF domains from different proteins, or even in pore-forming domains of bacterial cholesterol CDCs, constitute a highly conserved motif in all these proteins despite the low global sequence homology.

This strikingly high conservation led us to hypothesize that substitutions in any of these 2 glycines would be functionally detrimental for perforin, and therefore pathogenic. To predict the pathogenicity of mutations, we used Polyphen-2 server, version 2.2.2 (http://genetics.bwh.harvard.edu/pph2/), that collects information available on conservation, secondary and tertiary structure of the molecule, as well as functional sites

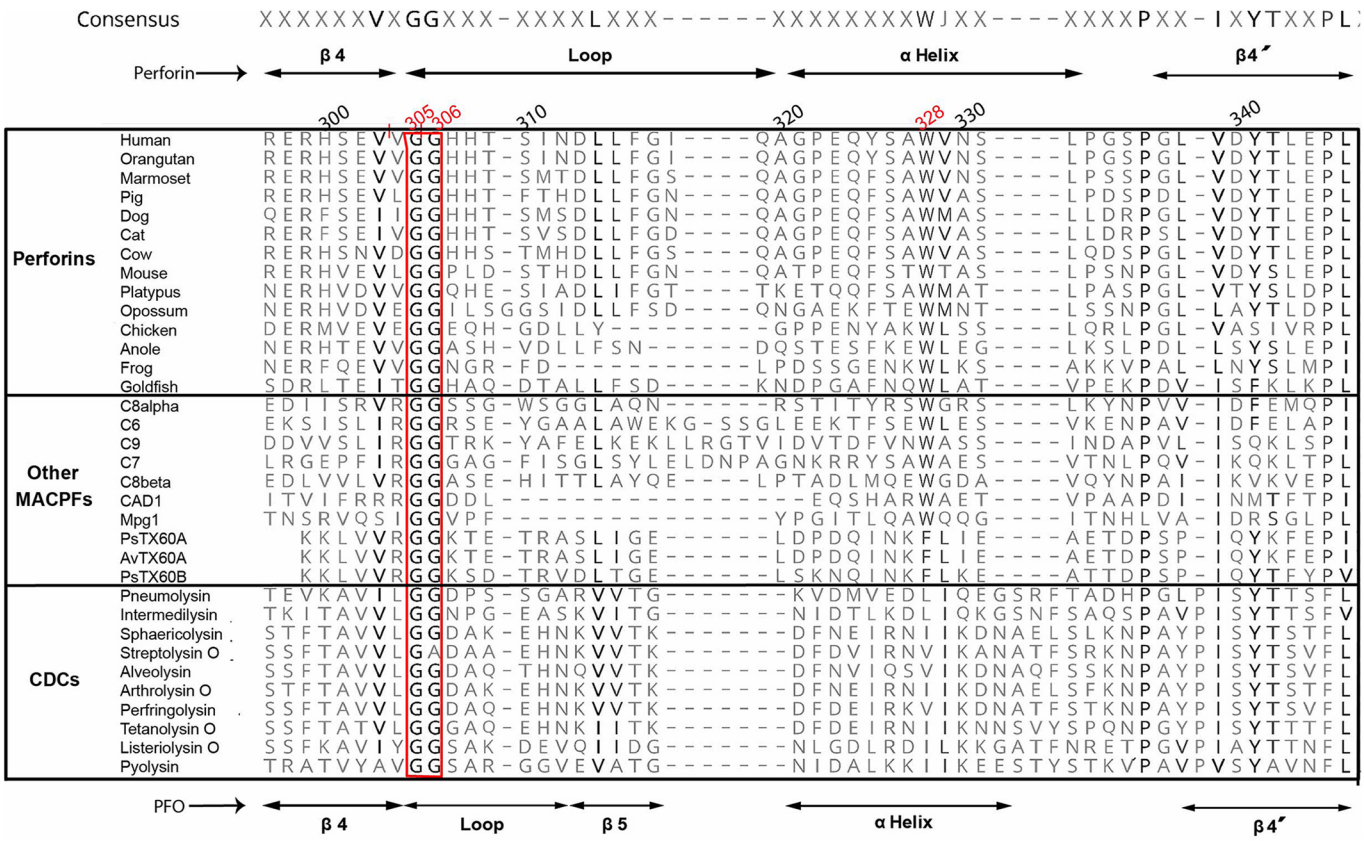

Figure 3: Conservation of the glycine pair across vertebrate perforins, other protein containing MACPFs and CDCs. Shown are sequences corresponding to the region including β4, the helical loop and β4′. The glycine motif is enclosed in the red column. Consensus sequence is shown at the top of the alignment and it is based on the rule of 50% identity. Amino acid numbering corresponds to human perforin, with Gly305, Gly306, and Trp328 indicated in red. Secondary structures corresponding to MACPFs (as represented by perforin) are indicated at the top of the alignment; secondary structures corresponding to CDCs (as represented by PFO) are indicated at the bottom of the alignment. Name of the species (vertebrate perforins) or proteins (other MACPFs, CDCs) is shown preceding the corresponding sequence. Higher degree of amino acid conservation is denoted by higher grey intensity.

hypothetically altered by a new residue (Adzhubei et al. 2010). As shown in Table 1, p.Gly306Asp, as well as p.Gly305Asp (Clementi et al. 2005), Gly306Cys (Grossman et al. 2005; Abdalgani et al. 2015) and p.Gly306Ser (Nagafuji et al. 2007; Wang et al. 2012; Gao et al. 2016) were undoubtedly predicted as pathogenic by the *HuVar* strategy of Polyphen-2.

We next studied the impact of the above mutations on perforin thermodynamic stability, or the change in folding free energy upon mutation (i.e., differences in Gibb's free energy between the folded and unfolded state of mutant compared to wild-type molecules, also ΔΔG or ddG). For this, we generated a molecular model of human perforin, using BuildModel and RepairPDB commands within FoldX v4 (http://foldxsuite.crg.eu) (Guerois et al. 2002), employing perforin X-ray crystal structure (PDB identifier:3nsj) (Law et al. 2010) as a template. Independent algorithms used for stability calculations were FoldX itself, SDM (http://mordred.bioc.cam.ac.uk/~sdm/sdm.php) (Worth et al. 2011), PoPMuSiC (http://dezyme.com/) (Dehouck et al. 2009) and Eris (http://troll.med.unc.edu/eris) (Yin et al. 2007). In support of our hypothesis, all 4 algorithms predicted that p.Gly306Asp, as well as the other 3 FHL-2 mutations reported in these glycines, i.e., p.Gly305Asp, p.Gly306Cys and p.Gly306Ser result in severe destabilization of the perforin molecule (Table 1), albeit to a lesser extent in p.Gly306Ser according to Eris prediction. As shown in Figure 4, Asp306 creates important steric clashes with Gly305 and Trp328, and perturbs the hydrogen bond (H-bond) between wild-type (WT)-Gly305 and WT-Trp328. The latter residue extends from the neighboring helix and

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

***Table 1: In silico* analysis of FHL-2 associated mutations in Gly306 and Gly305.**

	Pathogenic impact		Stability impact								
	Polyphen-2		FoldX		SDM		PoPMuSiC		Eris		
Mutant	pph2 prob.	Pred.	Score	Pred.	Score	Pred.	Score	Pred.	Score	Pred.	Reference
p.Gly306Asp	0.999	PD	9.8	SD	–4.3	HD	1.71	D	6.3	D	
p.Gly306Cys	0.999	PD	6.5	SD	–3.1	HD	1.36	D	NA	NA	Grossman et al. 2005; Abdalgani et al. 2015
p.Gly306Ser	0.999	PD	6.3	SD	–4.3	HD	1.85	D	3.6	D	Nagafuji et al. 2007; Wang et al. 2012; Gao et al. 2016
p.Gly305Asp	0.997	PD	20	SD	–3.2	HD	2.62	D	>10	D	Clementi et al. 2005

Note: Shown is the impact predicted by Polyphen-2 and several stability based in silico methods. *pph2 prob.*: posterior probability for being damaging; it ranges from 0 (benign) to 0.999 (most probably damaging, PD). *Score*: it represents the change in folding free energy upon mutation ("ddG", Kcal/mol); *Pred*: predicted outcome of mutations, defined as "destabilizing" (D) scores from 0 to 5, versus "severely destabilizing" (SD) scores >5, in FoldX; "destabilizing" (D) scores from –0.5 to –2, versus "highly destabilizing" (HD) scores <–2 in SDM; or simply "destabilizing" (D) scores > 0 in PoPMuSiC and Eris. *NA*: not available. Options were set as follows: for FoldX, pH = 7.4, temperature = 310K, ion strength = 0.05 M, vdwDesign = 2; For SDM and PoPMuSiC, default settings; for Eris, fixed backbone and no backbone pre-relaxation.

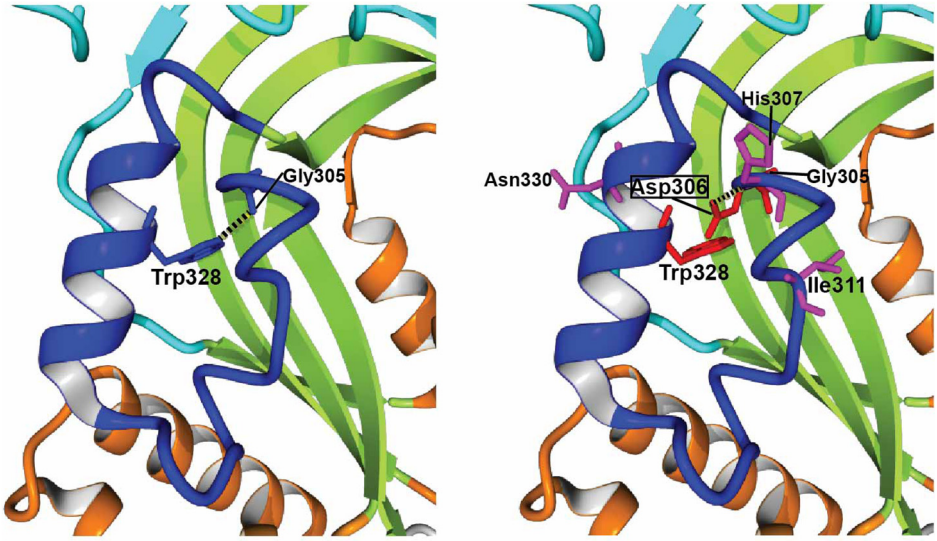

Figure 4: FoldX analysis of p.Gly306Asp perforin mutation. Shown is a zoomed view of the helical loop region of our WT (left) and p.Gly306Asp (right) human perforin models. H-bonds are represented as black dots; in the p.Gly306Asp model (right) the H-bond from WT perforin disappears and a new H-bond is created by the mutant. Those residues involved in steric clashes are indicated in red. Other residues affected by the mutation are coloured in magenta. Graphics performed with Yasara View v16.7.22.

is highly conserved both in vertebrate perforins and in other MACPF proteins (Figure 3), which suggests that the H-bond with WT-Gly305 or equivalent glycine plays a critical role in stability. Moreover, p.Gly306Asp originates a new H-bond between Asp306 and His307, which is absent in the WT structure. With these and other mutation-specific alterations not mentioned above, it is very likely that these 3 substitutions, and probably nearly all Gly306 variants, would be deleterious for perforin folding and (or) function.

Discussion

Intracellular perforin staining was studied as part of the current laboratory diagnostic workup in HLH patients (Arico et al. 2002; Henter et al. 2007;

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Bryceson et al. 2012; Imashuku 2014). We found null perforin expression and overexpressed granzyme-B; this granzyme pattern most likely indicates hyperactivation, given it has been previously described in the HLH setting (Molleran Lee et al. 2004; Mellor-Heineke et al. 2013). In our analysis, both the healthy donor and the patient's mother (healthy heterozygous p.Gly306Asp carrier) had no inflammatory conditions and showed normal granzyme-B expression.

Null perforin expression in our homozygous patient and the decreased, intermediate amount of perforin in her heterozygous mother along with the c. 917G>A segregation in this family suggested the inability of p.Gly306Asp perforin to fold properly. Impaired folding would cause reduced intracellular levels and (or) conformational changes that could inhibit DG9 antibody binding. Two other mutations affecting the same amino acid position (p.Gly306Cys (Grossman et al. 2005; Abdalgani et al. 2015) and p.Gly306Ser (Nagafuji et al. 2007; Wang et al. 2012; Gao et al. 2016)) are also associated with null or very limited perforin expression in cytotoxic lymphocytes. Although diminished expression suggests misfolding for all of these mutants, a defect in perforin content could also be attributed to the altered immunoregulatory state of the active HLH setting (Voskoboinik et al. 2010). Unfortunately, we have not tested intracellular perforin in highly effective recombinant expression systems and (or) under temperatures permissive for folding as it has been done in the neighbouring mutation p.Gly305Asp (Risma et al. 2006; Chia et al. 2009; Urrea Moreno et al. 2009).

Our results show an extremely high conservation of the Gly305/Gly306 pair through MACPF/CDC superfamily, which denotes a critical role for these residues. Previous studies attributed these 2 glycines the role of a "hinge point" which allow the conformational changes leading to membrane spanning (Rosado et al. 2007; Gilbert et al. 2013; Dudkina et al. 2016; Lukoyanova et al. 2016). Although this hinge point has also been involved in the oligomerization process of the cytolysin PFO (Ramachandran et al. 2004; Rosado et al. 2007), this seems unlikely in perforin, where residues critically involved in oligomerization (Baran et al. 2009; Dudkina et al. 2016) are neither proximal nor directly connected to these glycines (Figure 2). However, since the hinge points of each subunit come into close proximity during pore formation, mutations in Gly305 and Gly306 could hypothetically perturb the association of monomers within the pore and therefore alter its properties and (or) hinder its formation.

In addition to the high conservation of both Gly305 and Gly306, Polyphen-2 predicted a severe pathogenic impact of the novel p.Gly306Asp, and of p.Gly306Cys, p.Gly306Ser and adjacent p.Gly305Asp. The 4 mutations result in high thermodynamic destabilization, as predicted by the 4 algorithms that we have used. The impact on stability would primarily explain HLH pathogenicity associated to these mutations. Decreased stability is a major factor underlying the dysfunction of disease-associated missense mutations (Wang and Moult 2001; Yue et al. 2005; Pey et al. 2007; Casadio et al. 2011; Stefl et al. 2013). A recent and comprehensive *in silico* study of human perforin molecule, using FoldX and PoPMUSIC as predictive algorithms, identified nearly half of all missense FHL-2 perforin mutations as significantly destabilizing (An et al. 2013). Gly305Asp and Gly306Cys were also included in this work, and their predicted impact was nearly identical to what we have found with our human perforin model.

Protein destabilization results in misfolding and premature degradation, and correlates positively with the degree of protein dysfunction and clinical severity (Pey et al. 2007; Tokuriki et al. 2008; Tokuriki and Tawfik 2009; McKeone et al. 2014). Interestingly, the Arginine mutation of Gly357 in the complement factor C7—a glycine corresponding to Gly305 in perforin, is one of the most frequent mutations associated with C7 deficiency, and was linked to serious misfolding and defective secretion (Fernie et al. 1997; Barroso et al. 2004; Rameix-Welti et al. 2007; Barroso et al. 2010).

Perforin immunophenotyping and the predictions *in silico* suggest serious misfolding in the novel p.Gly306Asp, as well as the p.Gly306Cys, p.Gly306Ser and the p.Gly305Asp mutants. As discussed earlier, it may be possible that residual perforin expression and secretion of those mutants could still exist when using recombinant mutants in *in vitro* expression systems. However, even if secreted, it is likely that the mutants would display aberrant pore formation, since the Gly305/306 pair is presumably essential for this postsynaptic process.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Our *in silico* methods homogeneously predicted a severe clinical impact of Gly305/Gly306 FHL-2 mutations. This correlates with the early onset FHL-2 in our patient with homozygous p.Gly306Asp mutation, along with the very early onset and undetectable NK-cell mediated killing FHL-2 in 2 triplets with p.Gly306Cys mutation (Grossman et al. 2005). However, less severity could be attributed to p.Gly306Ser, found in 2 late onset FHL-2 patients and even in a healthy sibling; all of them carrying compound heterozygous *PRF1* genotypes (Nagafuji et al. 2007; Gao et al. 2016). In fact, despite its impaired maturation, likely related to misfolding, p.Gly306Ser mutant seems to retain cytotoxic function (Nagafuji et al. 2007). Finally, p.Gly305Asp was described along with the p.Arg356Trp in an 18 year-old HLH patient (Clementi et al. 2005). Since p.Arg356Trp is considered a hypomorphic mutant (Zhang et al. 2011; Huang et al. 2014), this may explain the delayed age of HLH onset in this patient.

In summary, HLH in our patient was caused by a unique, homozygous mutation p.Gly306Asp in a critical residue located at MACPF domain of perforin. Age of onset and nearly absent perforin expression pointed to p.Gly306Asp being a severe mutant, which is further supported by our *in silico* studies. *In vitro* studies with recombinant expressed mutants would be needed to definitively classify p.Gly306Ser and related FHL-2 mutations, and to determine the precise contribution of the proposed misfolding (pre-synaptic) and (or) the inhibition of pore formation (post-synaptic) as the molecular factors underlying their pathogenicity (Voskoboinik et al. 2010).

Funding

This work was supported by a grant from the Spanish NIH (ISCIII FIS PI12/02761).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Professor Andrew Herr for reviewing the manuscript and for his useful advice on discussing the *in silico* results. We are also grateful to Jane Kinnear for technical help.

REFERENCES

Abdalgani, M., Filipovich, A.H., Choo, S., Zhang, K., Gifford, C., Villanueva, J., Bleesing, J.J., and Marsh, R.A. 2015. Accuracy of flow cytometric perforin screening for detecting patients with FHL due to PRF1 mutations. Blood. **126**(15):1858–1860. PMID: 26450956. doi: 10.1182/blood-2015-06-648659.

Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R. 2010. A method and server for predicting damaging missense mutations. Nat. Methods. **7**(4):248–249. PMID: 20354512. doi: 10.1038/nmeth0410-248.

An, O., Gursoy, A., Gurgey, A., and Keskin, O. 2013. Structural and functional analysis of perforin mutations in association with clinical data of familial hemophagocytic lymphohistiocytosis type 2 (FHL2) patients. Protein Sci. **22**(6):823–839. PMID: 23592409. doi: 10.1002/pro.2265.

Arico, M., Allen, M., Brusa, S., Clementi, R., Pende, D., Maccario, R., Moretta, L., and Danesino, C. 2002. Haemophagocytic lymphohistiocytosis: Proposal of a diagnostic algorithm based on perforin expression. Br. J. Haematol. **119**(1):180–188. PMID: 12358924. doi: 10.1046/j.1365-2141.2002.03773.x.

Baran, K., Dunstone, M., Chia, J., Ciccone, A., Browne, K.A., Clarke, C.J., Lukoyanova, N., Saibil, H., Whisstock, J.C., Voskoboinik, I., and Trapani, J.A. 2009. The molecular basis for perforin oligomerization and transmembrane pore assembly. Immunity. **30**(5):684–695. PMID: 19446473. doi: 10.1016/j.immuni.2009.03.016.

Barmettler, S., Nowak, R.J., Parker, T., and Price, C. 2016. Previously undiagnosed fatal familial haemophagocytic lymphohistiocytosis in a 24-year-old woman. BMJ Case Rep. 2016. doi: 10.1136/bcr-2015-213698.

Barroso, S., Lopez-Trascasa, M., Merino, D., Alvarez, A.J., Nunez-Roldan, A., and Sanchez, B. 2010. C7 deficiency and meningococcal infection susceptibility in two spanish families. Scand. J. Immunol. **72**(1):38–43. PMID: 20591074. doi: 10.1111/j.1365-3083.2010.02403.x.

Barroso, S., Sanchez, B., Alvarez, A.J., Lopez-Trascasa, M., Lanuza, A., Luque, R., Wichmann, I., and Nunez-Roldan, A. 2004. Complement component C7 deficiency in two Spanish families. Immunology. **113**(4):518–523. PMID: 15554930. doi: 10.1111/j.1365-2567.2004.01997.x.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Bryceson, Y.T., Pende, D., Maul-Pavicic, A., Gilmour, K.C., Ufheil, H., Vraetz, T., Chiang, S.C., Marcenaro, S., Meazza, R., Bondzio, I., Walshe, D., Janka, G., Lehmberg, K., Beutel, K., zur Stadt, U., Binder, N., Arico, M., Moretta, L., Henter, J.I., and Ehl, S. 2012. A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. Blood. **119**(12):2754–2763. PMID: 22294731. doi: 10.1182/blood-2011-08-374199.

Casadio, R., Vassura, M., Tiwari, S., Fariselli, P., and Luigi Martelli, P. 2011. Correlating disease-related mutations to their effect on protein stability: A large-scale analysis of the human proteome. Hum. Mutat. **32**(10):1161–1170. PMID: 21853506. doi: 10.1002/humu.21555.

Chia, J., Yeo, K.P., Whisstock, J.C., Dunstone, M.A., Trapani, J.A., and Voskoboinik, I. 2009. Temperature sensitivity of human perforin mutants unmasks subtotal loss of cytotoxicity, delayed FHL, and a predisposition to cancer. Proc. Natl. Acad. Sci. USA. **106**(24):9809–9814. PMID: 19487666. doi: 10.1073/pnas.0903815106.

Clementi, R., Emmi, L., Maccario, R., Liotta, F., Moretta, L., Danesino, C., and Arico, M. 2002. Adult onset and atypical presentation of hemophagocytic lymphohistiocytosis in siblings carrying *PRF1* mutations. Blood. **100**(6):2266. PMID: 12229880. doi: 10.1182/blood-2002-04-1030.

Clementi, R., Locatelli, F., Dupre, L., Garaventa, A., Emmi, L., Bregni, M., Cefalo, G., Moretta, A., Danesino, C., Comis, M., Pession, A., Ramenghi, U., Maccario, R., Arico, M., and Roncarolo, M.G. 2005. A proportion of patients with lymphoma may harbor mutations of the perforin gene. Blood. **105**(11):4424–4428. PMID: 15728124. doi: 10.1182/blood-2004-04-1477.

Dehouck, Y., Grosfils, A., Folch, B., Gilis, D., Bogaerts, P., and Rooman, M. 2009. Fast and accurate predictions of protein stability changes upon mutations using statistical potentials and neural networks: PoPMuSiC-2.0. Bioinformatics. **25**(19):2537–2543. PMID: 19654118. doi: 10.1093/bioinformatics/btp445.

Dias, C., McDonald, A., Sincan, M., Rupps, R., Markello, T., Salvarinova, R., Santos, R.F., Menghrajani, K., Ahaghotu, C., Sutherland, D.P., Fortuno, E.S., III, Kollmann, T.R., Demos, M., Friedman, J.M., Speert, D.P., Gahl, W.A., and Boerkoel, C.F. 2013. Recurrent subacute post-viral onset of ataxia associated with a *PRF1* mutation. Eur. J. Hum. Genet. **21**:1232–1239. doi: 10.1038/ejhg.2013.20.

Dudkina, N.V., Spicer, B.A., Reboul, C.F., Conroy, P.J., Lukoyanova, N., Elmlund, H., Law, R.H., Ekkel, S.M., Kondos, S.C., Goode, R.J., Ramm, G., Whisstock, J.C., Saibil, H.R., and Dunstone, M.A. 2016. Structure of the poly-C9 component of the complement membrane attack complex. Nat. Commun. **7**:10588. doi: 10.1038/ncomms10588.

Fernie, B.A., Orren, A., Sheehan, G., Schlesinger, M., and Hobart, M.J. 1997. Molecular bases of C7 deficiency: Three different defects. J. Immunol. **159**(2):1019–1026. PMID: 9218625.

Gao, L., Dang, X., Huang, L., Zhu, L., Fang, M., Zhang, J., Xu, X., Li, T., Zhao, L., Wei, J., and Zhou, J. 2016. Search for the potential "second-hit" mechanism underlying the onset of familial hemophagocytic lymphohistiocytosis type 2 by whole-exome sequencing analysis. Transl. Res. **170**:26–39. PMID: 26739415. doi: 10.1016/j.trsl.2015.12.004.

Gilbert, R.J., Mikelj, M., Dalla Serra, M., Froelich, C.J., and Anderluh, G. 2013. Effects of MACPF/CDC proteins on lipid membranes. Cell Mol. Life Sci. **70**(12):2083–2098. PMID: 22983385. doi: 10.1007/s00018-012-1153-8.

Grossman, W.J., Radhi, M., Schauer, D., Gerday, E., Grose, C., and Goldman, F.D. 2005. Development of hemophagocytic lymphohistiocytosis in triplets infected with HHV-8. Blood. **106**(4):1203–1206. PMID: 15840696. doi: 10.1182/blood-2005-03-0950.

Guerois, R., Nielsen, J.E., and Serrano, L. 2002. Predicting changes in the stability of proteins and protein complexes: A study of more than 1000 mutations. J. Mol. Biol. **320**(2):369–387. PMID: 12079393. doi: 10.1016/S0022-2836(02)00442-4.

Henter, J.I., Horne, A., Arico, M., Egeler, R.M., Filipovich, A.H., Imashuku, S., Ladisch, S., McClain, K., Webb, D., Winiarski, J., and Janka, G. 2007. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr. Blood Cancer. **48**(2):124–131. PMID: 16937360. doi: 10.1002/pbc.21039.

Huang, Z., Wang, Y., Xie, Z., and Shen, K. 2014. Remission and relapse of hemophagocytic lymphohistiocytosis in a patient harboring a PRF1 homozygous mutation: A case report. J. Pediatr. Hematol. Oncol. **36**(1):e5–e8. PMID: 23073044. doi: 10.1097/MPH.0b013e318271c963.

Imashuku, S. 2014. Hemophagocytic lymphohistiocytosis: Recent progress in the pathogenesis, diagnosis and treatment. World J. Hematol. **3**(3):14. doi: 10.5315/WJH.v3.i3.71.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Janka, G.E. 2012. Familial and acquired hemophagocytic lymphohistiocytosis. Annu. Rev. Med. **63**:233–246. PMID: 22248322. doi: 10.1146/annurev-med-041610-134208.

Krieger, E., and Vriend, G. 2014. YASARA View—Molecular graphics for all devices—From smartphones to workstations. Bioinformatics. **30**(20):2981–2982. PMID: 24996895. doi: 10.1093/bioinformatics/btu426.

Law, R.H., Lukoyanova, N., Voskoboinik, I., Caradoc-Davies, T.T., Baran, K., Dunstone, M.A., D'Angelo, M.E., Orlova, E.V., Coulibaly, F., Verschoor, S., Browne, K.A., Ciccone, A., Kuiper, M.J., Bird, P.I., Trapani, J.A., Saibil, H.R., and Whisstock, J.C. 2010. The structural basis for membrane binding and pore formation by lymphocyte perforin. Nature. **468**(7322):447–451. PMID: 21037563. doi: 10.1038/nature09518.

Lukoyanova, N., Hoogenboom, B.W., and Saibil, H.R. 2016. The membrane attack complex, perforin and cholesterol-dependent cytolysin superfamily of pore-forming proteins. J. Cell Sci. **129**(11):2125–2133. PMID: 27179071. doi: 10.1242/jcs.182741.

Mancebo, E., Allende, L.M., Guzman, M., Paz-Artal, E., Gil, J., Urrea-Moreno, R., Fernandez-Cruz, E., Gaya, A., Calvo, J., Arbos, A., Duran, M.A., Canet, R., Balanzat, J., Udina, M.A., and Vercher, F.J. 2006. Familial hemophagocytic lymphohistiocytosis in an adult patient homozygous for A91V in the perforin gene, with tuberculosis infection. Haematologica. **91**(9):1257–1260. PMID: 16956828.

McKeone, R., Wikstrom, M., Kiel, C., and Rakoczy, P.E. 2014. Assessing the correlation between mutant rhodopsin stability and the severity of retinitis pigmentosa. Mol. Vis. **20**:183–199. PMID: 24520188.

Mellor-Heineke, S., Villanueva, J., Jordan, M.B., Marsh, R., Zhang, K., Bleesing, J.J., Filipovich, A.H., and Risma, K.A. 2013. Elevated granzyme B in cytotoxic lymphocytes is a signature of immune activation in hemophagocytic lymphohistiocytosis. Front. Immunol. 472. doi: 10.3389/fimmu.2013.00072.

Mhatre, S., Madkaikar, M., Jijina, F., and Ghosh, K. 2014. Unusual clinical presentations of familial hemophagocytic lymphohistiocytosis type-2. J. Pediatr. Hematol. Oncol. **36**(8):e524–e527. PMID: 24390453. doi: 10.1097/MPH.0000000000000102.

Molleran Lee, S., Villanueva, J., Sumegi, J., Zhang, K., Kogawa, K., Davis, J., and Filipovich, A.H. 2004. Characterisation of diverse *PRF1* mutations leading to decreased natural killer cell activity in North American families with haemophagocytic lymphohistiocytosis. J. Med. Genet. **41**(2):137–144. PMID: 14757862. doi: 10.1136/jmg.2003.011528.

Nagafuji, K., Nonami, A., Kumano, T., Kikushige, Y., Yoshimoto, G., Takenaka, K., Shimoda, K., Ohga, S., Yasukawa, M., Horiuchi, H., Ishii, E., and Harada, M. 2007. Perforin gene mutations in adult-onset hemophagocytic lymphohistiocytosis. Haematologica. **92**(7):978–981. PMID: 17606450. doi: 10.3324/haematol.11233.

Pey, A.L., Stricher, F., Serrano, L., and Martinez, A. 2007. Predicted effects of missense mutations on native-state stability account for phenotypic outcome in phenylketonuria, a paradigm of misfolding diseases. Am. J. Hum. Genet. **81**(5):1006–1024. PMID: 17924342. doi: 10.1086/521879.

Ramachandran, R., Tweten, R.K., and Johnson, A.E. 2004. Membrane-dependent conformational changes initiate cholesterol-dependent cytolysin oligomerization and intersubunit beta-strand alignment. Nat. Struct. Mol. Biol. **11**(8):697–705. PMID: 15235590. doi: 10.1038/nsmb793.

Rameix-Welti, M.A., Regnier, C.H., Bienaime, F., Blouin, J., Schifferli, J., Fridman, W.H., Sautes-Fridman, C., and Fremeaux-Bacchi, V. 2007. Hereditary complement C7 deficiency in nine families: Subtotal C7 deficiency revisited. Eur. J. Immunol. **37**(5):1377–1385. PMID: 17407100. doi: 10.1002/eji.200636812.

Risma, K.A., Frayer, R.W., Filipovich, A.H., and Sumegi, J. 2006. Aberrant maturation of mutant perforin underlies the clinical diversity of hemophagocytic lymphohistiocytosis. J. Clin. Invest. **116**(1):182–192. PMID: 16374518. doi: 10.1172/JCI26217.

Rosado, C.J., Buckle, A.M., Law, R.H., Butcher, R.E., Kan, W.T., Bird, C.H., Ung, K., Browne, K.A., Baran, K., Bashtannyk-Puhalovich, T.A., Faux, N.G., Wong, W., Porter, C.J., Pike, R.N., Ellisdon, A.M., Pearce, M.C., Bottomley, S.P., Emsley, J., Smith, A.I., Rossjohn, J., Hartland, E.L., Voskoboinik, I., Trapani, J.A., Bird, P.I., Dunstone, M.A., and Whisstock, J.C. 2007. A common fold mediates vertebrate defense and bacterial attack. Science. **317**(5844):1548–1551. PMID: 17717151. doi: 10.1126/science.1144706.

Sanchez, I.P., Leal-Esteban, L.C., Alvarez-Alvarez, J.A., Perez-Romero, C.A., Orrego, J.C., Serna, M.L., Coll, Y., Caicedo, Y., Pardo-Diaz, E., Zimmer, J., Bleesing, J.J., Franco, J.L., and Trujillo-Vargas, C.M. 2012. Analyses of the PRF1 gene in individuals with hemophagocytic lymphohystiocytosis reveal the common haplotype R54C/A91V in Colombian

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

unrelated families associated with late onset disease. J. Clin. Immunol. **32**(4):670–680. PMID: 22437823. doi: 10.1007/s10875-012-9680-5.

Sieni, E., Cetica, V., Hackmann, Y., Coniglio, M.L., Da Ros, M., Ciambotti, B., Pende, D., Griffiths, G., and Arico, M. 2014. Familial hemophagocytic lymphohistiocytosis: When rare diseases shed light on immune system functioning. Front. Immunol. **5**:167. doi: 10.3389/fimmu.2014.00167.

Sieni, E., Cetica, V., Piccin, A., Gherlinzoni, F., Sasso, F.C., Rabusin, M., Attard, L., Bosi, A., Pende, D., Moretta, L., and Arico, M. 2012. Familial hemophagocytic lymphohistiocytosis may present during adulthood: Clinical and genetic features of a small series. PLoS ONE. **7**(9): e44649. PMID: 22970278. doi: 10.1371/journal.pone.0044649.

Stefl, S., Nishi, H., Petukh, M., Panchenko, A.R., and Alexov, E. 2013. Molecular mechanisms of disease-causing missense mutations. J. Mol. Biol. **425**(21):3919–3936. PMID: 23871686. doi: 10.1016/j.jmb.2013.07.014.

Stepp, S.E., Dufourcq-Lagelouse, R., Le Deist, F., Bhawan, S., Certain, S., Mathew, P.A., Henter, J.I., Bennett, M., Fischer, A., de Saint Basile, G., and Kumar, V. 1999. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science. **286**(5446):1957–1959. PMID: 10583959. doi: 10.1126/science.286.5446.1957.

Stewart, S.E., Bird, C.H., Tabor, R.F., D'Angelo, M.E., Piantavigna, S., Whisstock, J.C., Trapani, J.A., Martin, L.L., and Bird, P.I. 2015. Analysis of perforin assembly by quartz crystal microbalance reveals a role for cholesterol and calcium-independent membrane binding. J. Biol. Chem. **290**(52):31101–31112. PMID: 26542805. doi: 10.1074/jbc.M115.683078.

Tesi, B., Chiang, S.C., El-Ghoneimy, D., Hussein, A.A., Langenskiold, C., Wali, R., Fadoo, Z., Silva, J.P., Lecumberri, R., Unal, S., Nordenskjold, M., Bryceson, Y.T., Henter, J.I., and Meeths, M. 2015. Spectrum of atypical clinical presentations in patients with biallelic *PRF1* missense mutations. Pediatr. Blood Cancer. **62**(12):2094–2100. PMID: 26184781. doi: 10.1002/pbc.25646.

Thiery, J., and Lieberman, J. 2014. Perforin: A key pore-forming protein for immune control of viruses and cancer. Subcell Biochem. 80197–80220. doi: 10.1007/978-94-017-8881-6_10.

Tokuriki, N., and Tawfik, D.S. 2009. Stability effects of mutations and protein evolvability. Curr. Opin. Struct. Biol. **19**(5):596–604. PMID: 19765975. doi: 10.1016/j.sbi.2009.08.003.

Tokuriki, N., Stricher, F., Serrano, L., and Tawfik, D.S. 2008. How protein stability and new functions trade off. PLoS Comput. Biol. **4**(2):e1000002. PMID: 18463696. doi: 10.1371/journal.pcbi.1000002.

Ueda, I., Kurokawa, Y., Koike, K., Ito, S., Sakata, A., Matsumora, T., Fukushima, T., Morimoto, A., Ishii, E., and Imashuku, S. 2007. Late-onset cases of familial hemophagocytic lymphohistiocytosis with missense perforin gene mutations. Am. J. Hematol. **82**(6):427–432. PMID: 17266056. doi: 10.1002/ajh.20878.

Urrea Moreno, R., Gil, J., Rodriguez-Sainz, C., Cela, E., LaFay, V., Oloizia, B., Herr, A.B., Sumegi, J., Jordan, M.B., and Risma, K.A. 2009. Functional assessment of perforin C2 domain mutations illustrates the critical role for calcium-dependent lipid binding in perforin cytotoxic function. Blood. **113**(2):338–346. PMID: 18927437. doi: 10.1182/blood-2008-08-172924.

Voskoboinik, I., Dunstone, M.A., Baran, K., Whisstock, J.C., and Trapani, J.A. 2010. Perforin: Structure, function, and role in human immunopathology. Immunol. Rev. **235**(1):35–54. PMID: 20536554. doi: 10.1111/j.0105-2896.2010.00896.x.

Voskoboinik, I., Whisstock, J.C., and Trapani, J.A. 2015. Perforin and granzymes: Function, dysfunction and human pathology. Nat. Rev. Immunol. **15**(6):388–400. PMID: 25998963. doi: 10.1038/nri3839.

Wang, Y., Wang, Z., Chen, H., and Wang, X. 2012. Adult onset of primary hemophagocytic syndrome in subjects carrying PRF1 mutations. Ann. Hematol. **91**:1489–1490. doi: 10.1007/s00277-012-1409-2.

Wang, Z., and Moult, J. 2001. SNPs, protein structure, and disease. Hum. Mutat. **17**(4):263–270. PMID: 11295823. doi: 10.1002/humu.22.

Worth, C.L., Preissner, R., and Blundell, T.L. 2011. SDM—A server for predicting effects of mutations on protein stability and malfunction. Nucleic Acids Res. **39**(Web Server issue):W215–W222. PMID: 21593128. doi: 10.1093/nar/gkr363.

Yagi, H., Conroy, P.J., Leung, E.W., Law, R.H., Trapani, J.A., Voskoboinik, I., Whisstock, J.C., and Norton, R.S. 2015. Structural basis for Ca^{2+}-mediated interaction of the Perforin C2 Domain with Lipid membranes. J. Biol. Chem. **290**(42):25213–25226. PMID: 26306037. doi: 10.1074/jbc.M115.668384.

Yin, S., Ding, F., and Dokholyan, N.V. 2007. Eris: An automated estimator of protein stability. Nat. Methods. **4**(6):466–467. PMID: 17538626. doi: 10.1038/nmeth0607-466.

Yue, P., Li, Z., and Moult, J. 2005. Loss of protein structure stability as a major causative factor in monogenic

disease. J. Mol. Biol. **353**(2):459–473. PMID: 16169011. doi: 10.1016/j.jmb.2005.08.020.

Zhang, K., Jordan, M.B., Marsh, R.A., Johnson, J.A., Kissell, D., Meller, J., Villanueva, J., Risma, K.A., Wei, Q., Klein, P.S., and Filipovich, A.H. 2011. Hypomorphic mutations in *PRF1*, *MUNC13-4*, and *STXBP2* are associated with adult-onset familial HLH. Blood. **118**(22):5794–5798. doi: 10.1182/blood-2011-07-370148.

Zur Stadt, U., Beutel, K., Kolberg, S., Schneppenheim, R., Kabisch, H., Janka, G., and Hennies, H.C. 2006. Mutation spectrum in children with primary hemophagocytic lymphohistiocytosis: Molecular and functional analyses of PRF1, UNC13D, STX11, and RAB27A. Hum. Mutat. **27**(1):62–68. PMID: 16278825. doi: 10.1002/humu.20274.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Original Article

Hemophagocytic lymphohistiocytosis associated with ataxia telangiectasia

Mohammad Alsalamah[a] and Chaim M. Roifman[a,b]*

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening inflammatory condition believed to be caused by uncontrolled activation of macrophages and histiocytes. HLH may be triggered by infections or associated with malignancy, metabolic disorders and drug toxicity, or alternatively, by a variety of genetic defects. While this disorder has been reported to be associated with a growing number of primary immunodeficiencies, especially those with significant T cell and (or) NK cell dysfunction, it has never been reported in ataxia telangiectasia (AT). AT is characterized by truncal ataxia, dilatation of blood vessels, immunodeficiency and a high predisposition to cancer. Almost all cases of AT have at least 1 or a combination of more than 1 of the following features: low immunoglobulin levels, inability to produce specific antibodies in response to vaccination, T cell lymphopenia and (or) T cell dysfunction. In this report, we describe the first case of a fatal episode of HLH in a patient with AT. The overlapping laboratory anomalies of HLH and lymphoid malignancy poses a challenge for accurate diagnosis, and awareness of the phenomenon by clinicians may result in earlier treatment and resolution of inflammation.

Statement of novelty: HLH can affect various types of immunodeficiency but has never been reported in patients with AT. Here, we report the first case of a fatal episode of HLH in a patient with AT.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening inflammatory condition believed to be caused by uncontrolled activation of macrophages and histocytes (Janka 2007). HLH may be triggered by infections or associated with malignancy, metabolic disorders and drug toxicity (Henter et al. 1991; Janka et al. 1998), or alternatively, by a variety of genetic defects.

The familial form of HLH presents frequently in infants born to consanguineous parents and may involve a history of other affected family members. Most patients in this group carry lesions in genes that are responsible for the cytotoxic function of T cells and NK cells. Commonly, genetic mutations in *PRF-1*, *UNC13D*, or *STXII*, encoding for cytolytic granule trafficking and exocytosis, are detected in these patients (Goransdotter Ericson et al. 2001; Feldmann et al. 2003; zur Stadt et al. 2005).

HLH was also reported to be associated with a growing number of primary immunodeficiencies, especially those with significant T cell and (or) NK cell dysfunction (Egeler et al. 1996; Dalal et al. 2015).

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by truncal ataxia, dilatation of blood vessels, immunodeficiency and a high predisposition to cancer (Boder and Sedgwick 1958; Micol et al. 2011).

[a]Division of Immunology and Allergy, Department of Pediatrics, The Hospital for Sick Children and the University of Toronto, Toronto, ON; [b]The Canadian Centre for Primary Immunodeficiency and The Jeffrey Modell Research Laboratory for the Diagnosis of Primary Immunodeficiency, The Hospital for Sick Children, Toronto, ON

*Corresponding author: Chaim M. Roifman/chaim.roifman@sickkids.ca

Submitted 7 July 2017
Accepted 27 August 2017
Available online 29 August 2017

LymphoSign Journal 4:113–116 (2017)
dx.doi.org/10.14785/lymphosign-2017-0007

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Patients present early in life with hypotonia and delayed developmental milestones, and are frequently diagnosed with cerebral palsy. Later in childhood, ataxia becomes evident as well as telangiectasia in the eyes and ear lobes. Patients may suffer recurrent pulmonary infections and malignancy. Immune abnormalities include immunoglobulin deficiencies, T cell lymphopenia and dysfunction, and a dysplastic thymus (Nowak-Wegrzyn et al. 2004; Driessen et al. 2013).

Typical laboratory features consist of increased alpha-fetoprotein, increased chromosomal breakage and increased sensitivity to ionizing radiation (Canman and Lim 1998; Suzuki et al. 1999; Wu et al. 2000; Stray-Pedersen et al. 2007). AT is caused by mutations in the *ATM* gene, which is involved in DNA repair during double-stranded breaks (Savitsky et al. 1995; Staples et al. 2008).

HLH can affect various types of immunodeficiency but has never been reported in patients with AT. Here, we report the first case of a fatal episode of HLH in a patient with AT.

Case report

The patient was born at term to consanguineous parents of Italian descent. Development was delayed and a neurological assessment revealed hypotonia and poor head control. Sitting and standing were also markedly delayed and he was given a diagnosis of mild cerebral palsy. He was unstable when he walked and his gait was wide. At the age of 4 years, he was diagnosed with ataxia and was prescribed physiotherapy.

AT was suspected at the age of 6 years when he suffered from dysarthria, ataxia and swallowing difficulties, and was found to have dilated blood vessels in his eyes and ear lobes. Diagnosis was confirmed by the finding of markedly elevated alpha-fetoprotein as well as increased chromosomal breakage.

He continued to deteriorate neurologically and was wheelchair bound by the age of 9 years. At the age of 7 years, he suffered a bout of pneumonia and subsequently developed chronic bronchitis. At the age of 11 years, he developed persistent fevers with no signs of a source of infection. He gradually stopped eating and was admitted for evaluation for a possible malignancy. He was found to have an enlarged liver and spleen as well as lymphadenopathy. Laboratory evaluation revealed increased sedimentation rate, increased lactate dehydrogenase and ferritin, low platelets of $65 \times 10^3/\mu L$ and reduced neutrophil count at $1.2 \times 10^3/\mu L$. This combination of clinical manifestations and laboratory abnormalities raised the possibility of a lymphoid malignancy, especially in a patient with AT.

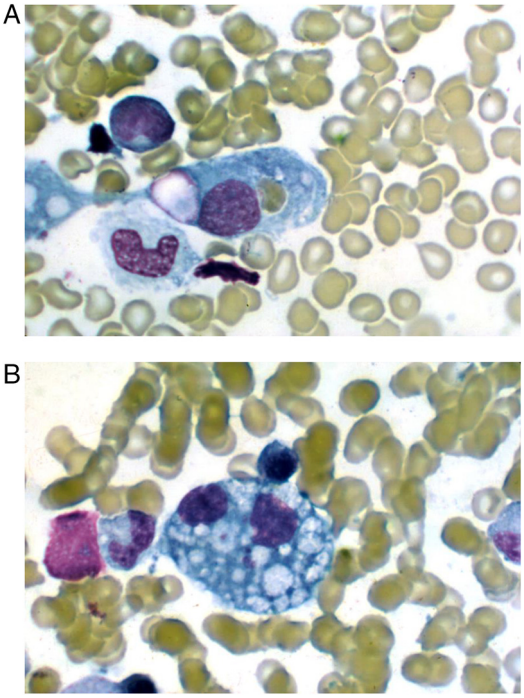

Figure 1: Hemophagocytic macrophages identified in our patient with ataxia telangiectasia. Hemophagocytosis, the engulfment of host blood cells by macrophages, was visualized in bone marrow aspirates stained with Wright-Giemsa (A, B).

A bone marrow biopsy was therefore performed. While no malignant cells were detected, a large number of hemophagocytic macrophages were identified (Figure 1). This finding supported the diagnosis of HLH. In fact, the clinical features of splenomegaly and laboratory aberrations, including 2 lineage cytopenia, hypertriglyceridemia, increased ferritin as well as typical histopathological changes, meet the criteria for the diagnosis of HLH (Table 1).

The patient was initially treated with broad spectrum antibiotics with little effect, and was subsequently switched to dexamethasone once HLH was suspected. Unfortunately, within 4 days he developed multi-organ failure leading to death.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Table 1: **Immune characteristics of hemophagocytic lymphohistiocytosis in ataxia telangiectasia.**

	Patient	Normal ranges
Ferritin	2402	30–177 µg/L
Fibrinogen	1.5	1.9–4.3 g/L
Triglycerides	14	0.4–1.3 mmol/L
WBC	2.5	$4–10 \times 10^9$/L
Neutrophil count	1.2	$2.5–6.0 \times 10^9$/L
Platelets	65	$150–400 \times 10^9$/L
Hemoglobin	76	120–160 g/L
Lymphocyte count	1.0	1.5–4.2
Immunophenotyping		
OKT3	35%	65%
OKT4	19%	45%
OKT8	11%	20%
PHA (stimulation index)	<50%	>50% of control
Serum immunoglobulins		
IgG (mg/L)	1080	700–1560
IgA (mg/L)	125	53–204
IgM (mg/L)	200	31–179
IgE (IU/mL)	53	<35
Isohemagglutinins		
Anti-B (IU/mL)	1:8	>1:16
Anti-tetanus toxoid (IU/mL)	<0.01	>0.1

Discussion

The symptoms of HLH consist of persistent fever, hepatosplenomegaly, multi-lineage cytopenia, morbiliforme rash, encephalopathy and seizures (Henter et al. 2007). Indeed, our patient presented with most of these typical features. Without treatment, familial HLH is fatal in more than 85% of cases within 1 year of diagnosis (Janka 1983). Even specific therapy cannot rescue up to 45% of cases. Treatment is aimed at suppressing inflammation by using immunosuppressive agents such as dexamethasone, etoposide and cyclosporine, and when appropriate, bone marrow transplantation (Henter et al. 2002). In spite of immunosuppression therapy, our patient deteriorated rapidly and died of multi-organ failure.

It is surprising that HLH was not reported previously in patients with AT. Almost all AT cases have at least 1 or a combination of more than 1 of the following features: low immunoglobulin levels, inability to produce specific antibodies in response to vaccination, T cell lymphopenia and (or) T cell dysfunction. Some patients present with profound T cell deficiency and are detected by newborn screening for SCID (Puck 2012). This may possibly be explained by the dysfunction of ATM in repairing rearrangement of the T cell receptor gene.

This case demonstrates that some overlapping laboratory anomalies of HLH and lymphoid malignancy may pose a challenge for an accurate diagnosis. It is therefore possible that, if suspected, HLH may be a more common manifestation in AT than expected. This knowledge may alert clinicians, resulting in earlier treatment and resolution of inflammation.

REFERENCES

Boder, E., and Sedgwick, R.P. 1958. Ataxia-telangiectasia; a familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. Pediatrics. **21**:526–554. PMID: 13542097.

Canman, C.E., and Lim, D.S. 1998. The role of ATM in DNA damage responses and cancer. Oncogene. **17**:3301–3308. PMID: 9916992. doi: 10.1038/sj.onc.1202577.

Dalal, B.I., Vakil, A.P., Khare, N.S., Wang, S.Y., Richards, M.J., and Chen, L.Y. 2015. Abnormalities of the lymphocyte subsets and their immunophenotype, and their prognostic significance in adult patients with hemophagocytic lymphohistiocytosis. Ann. Hematol. **94**:1111–1117. PMID: 25800135. doi: 10.1007/s00277-015-2350-y.

Driessen, G.J., Ijspeert, H., Weemaes, C.M., Haraldsson, A., Trip, M., Warris, A., van der Flier, M., Wulffraat, N., Verhagen, M.M., Taylor, M.A., van Zelm, M.C., van Dongen, J.J., van Deuren, M., and van der Burg, M. 2013. Antibody deficiency in patients with ataxia telangiectasia is caused by disturbed B- and T-cell homeostasis and reduced immune repertoire diversity. J. Allergy Clin. Immunol. **131**:1367–1375.e9. PMID: 23566627. doi: 10.1016/j.jaci.2013.01.053.

Egeler, R.M., Shapiro, R., Loechelt, B., and Filipovich, A. 1996. Characteristic immune abnormalities in hemophagocytic lymphohistiocytosis. J. Pediatr. Hematol. Oncol. **18**:340–345. PMID: 8888739. doi: 10.1097/00043426-199611000-00002.

Feldmann, J., Callebaut, I., Raposo, G., Certain, S., Bacq, D., Dumont, C., Lambert, N., Ouachée-Chardin, M., Chedeville, G., Tamary, H., Minard-Colin, V., Vilmer, E., Blanche, S., Le Deist, F., Fischer, A., and de Saint Basile, G. 2003. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell. **115**:461–473.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

PMID: 14622600. doi: 10.1016/S0092-8674(03)00855-9.

Goransdotter Ericson, K., Fadeel, B., Nilsson-Ardnor, S., Soderhall, C., Samuelsson, A., Janka, G., Schneider, M., Gurgey, A., Yalman, N., Revesz, T., Egeler, R., Jahnukainen, K., Storm-Mathiesen, I., Haraldsson, A., Poole, J., de Saint Basile, G., Nordenskjold, M., and Henter, J. 2001. Spectrum of perforin gene mutations in familial hemophagocytic lymphohistiocytosis. Am. J. Hum. Genet. **68**:590–597. PMID: 11179007. doi: 10.1086/318796.

Henter, J.I., Elinder, G., Soder, O., and Ost, A. 1991. Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis. Acta Paediatr. Scand. **80**:428–435. PMID: 2058392. doi: 10.1111/j.1651-2227.1991.tb11878.x.

Henter, J.I., Horne, A., Arico, M., Egeler, R.M., Filipovich, A.H., Imashuku, S., Ladisch, S., McClain, K., Webb, D., Winiarski, J., and Janka, G. 2007. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr. Blood Cancer. **48**:124–131. PMID: 16937360. doi: 10.1002/pbc.21039.

Henter, J.I., Samuelsson-Horne, A., Arico, M., Egeler, R.M., Elinder, G., Filipovich, A.H., Gadner, H., Imashuku, S., Komp, D., Ladisch, S., Webb, D., and Janka, G. 2002. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. Blood. **100**:2367–2373. PMID: 12239144. doi: 10.1182/blood-2002-01-0172.

Janka, G., Imashuku, S., Elinder, G., Schneider, M., and Henter, J.I. 1998. Infection- and malignancy-associated hemophagocytic syndromes: Secondary hemophagocytic lymphohistiocytosis. Hematol. Oncol. Clin. North Am. **12**:435–444. PMID: 9561911. doi: 10.1016/S0889-8588(05)70521-9.

Janka, G.E. 1983. Familial hemophagocytic lymphohistiocytosis. Eur. J. Pediatr. **140**:221–230. PMID: 6354720. doi: 10.1007/BF00443367.

Janka, G.E. 2007. Familial and acquired hemophagocytic lymphohistiocytosis. Eur. J. Pediatr. **166**:95–109. PMID: 17151879. doi: 10.1007/s00431-006-0258-1.

Micol, R., Ben Slama, L., Suarez, F., Le Mignot, L., Beauté, J., Mahlaoui, N., Dubois d'Enghien, C., Laugé, A., Hall, J., Couturier, J., Vallée, L., Delobel, B., Rivier, F., Nguyen, K., Billette de Villemeur, T., Stephan, J.L., Bordigoni, P., Bertrand, Y., Aladjidi, N., Pedespan, J.M., Thomas, C., Pellier, I., Koenig, M., Hermine, O., Picard, C., Moshous, D., Neven, B., Lanternier, F., Blanche, S., Tardieu, M., Debré, M., Fischer, A., and Stoppa-Lyonnet, D. 2011. Morbidity and mortality from ataxia-telangiectasia are associated with ATM genotype. J. Allergy Clin. Immunol. **128**:382–389.e1. PMID: 21665257. doi: 10.1016/j.jaci.2011.03.052.

Nowak-Wegrzyn, A., Crawford, T.O., Winkelstein, J.A., Carson, K.A., and Lederman, H.M. 2004. Immunodeficiency and infections in ataxia-telangiectasia. J. Pediatr. **144**:505–511. PMID: 15069401. doi: 10.1016/j.jpeds.2003.12.046.

Puck, J.M. 2012. Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: The winner is T-cell receptor excision circles. J. Allergy Clin. Immunol. **129**:607–616. PMID: 22285280. doi: 10.1016/j.jaci.2012.01.032.

Savitsky, K., Sfez, S., Tagle, D.A., Ziv, Y., Sartiel, A., Collins, F.S., Shiloh, Y., and Rotman, G. 1995. The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. Hum. Mol. Genet. **4**:2025–2032. PMID: 8589678. doi: 10.1093/hmg/4.11.2025.

Staples, E.R., McDermott, E.M., Reiman, A., Byrd, P.J., Ritchie, S., Taylor, A.M., and Davies, E.G. 2008. Immunodeficiency in ataxia telangiectasia is correlated strongly with the presence of two null mutations in the ataxia telangiectasia mutated gene. Clin. Exp. Immunol. **153**:214–220. PMID: 18505428. doi: 10.1111/j.1365-2249.2008.03684.x.

Stray-Pedersen, A., Borresen-Dale, A.L., Paus, E., Lindman, C.R., Burgers, T., and Abrahamsen, T.G. 2007. Alpha fetoprotein is increasing with age in ataxia–telangiectasia. Eur. J. Paediatr. Neurol. **11**:375–380. PMID: 17540590. doi: 10.1016/j.ejpn.2007.04.001.

Suzuki, K., Kodama, S., and Watanabe, M. 1999. Recruitment of ATM protein to double strand DNA irradiated with ionizing radiation. J. Biol. Chem. **274**:25571–25575. PMID: 10464290. doi: 10.1074/jbc.274.36.25571.

Wu, X., Ranganathan, V., Weisman, D.S., Heine, W.F., Ciccone, D.N., O'Neill, T.B., Crick, K.E., Pierce, K.A., Lane, W.S., Rathbun, G., Livingston, D.M., and Weaver, D.T. 2000. ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response. Nature. **405**:477–482. PMID: 10839545. doi: 10.1038/35013089.

zur Stadt, U., Schmidt, S., Kasper, B., Beutel, K., Diler, A.S., Henter, J.I., Kabisch, H., Schneppenheim, R., Nurnberg, P., Janka, G., and Hennies, H.C. 2005. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum. Mol. Genet. **14**:827–834. PMID: 15703195. doi: 10.1093/hmg/ddi076.

National Immunoglobulin replacement Expert Committee recommendations

ABSTRACT

The use of immunoglobulin therapy has grown steadily over the past 3 decades, mainly due to the increased awareness and expansion of indications. This limited resource is now shared between patients with immunodeficiency disorders who need it as replacement therapy, and individuals who suffer a variety of autoimmune or inflammatory disorders. While alternative therapies exist for the latter diseases, immunodeficient patients are dependent upon this treatment for life. Due to the long-term cost burden of this treatment on healthcare systems, healthcare providers have attempted to moderate its use but are frequently seeking evidence from experts in the field. This raised the critical need for recommendations from experts. To this end, a group a Canadian immunologists representing all regions of the country have formed a panel, the National Immunoglobulin replacement Expert Committee (NIGEC), and formulated a set of unanimously agreed upon recommendations for the use of immunoglobulin replacement in primary immunodeficiency.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

Consensus among experts

1. Children and adults with a suspected immunodeficiency should be referred to an immunologist with expertise in the field of primary immunodeficiency ("expert" in PID).
2. Ideally, this should be carried out in an academic centre with the capability of performing specialized diagnostic tests for immunodeficiency. Management should be performed by a specialized team including physicians, nurses, and allied health care providers.
3. Ideally, replacement with IVIG or SCIG, changes in dosage, changes in product or its mode of delivery should be initiated directly by "experts" or in consultation with an "expert".
4. Diagnosis of antibody deficiency should be evaluated and ideally confirmed using an appropriate combination of the following tests:
   A. Serum immunoglobulin levels.
   B. Antibody formation in response to vaccination and the ability to sustain protective levels.
   C. Immunophenotyping including enumeration of memory and naïve B and T cells.
   D. In-vitro mitogenic responses.
   E. Assessment of TRECs and TCR-Vβ (where available).
   F. Genetic analysis.
5. IVIG and SCIG are similarly efficacious in preventing infections and can therefore be used interchangeably.
6. IVIG and SCIG deliver IgG differently resulting in different pharmacokinetic properties. An "expert" should tailor therapy to what is most suitable for each patient.
7. After a thorough discussion with patients on the benefits and challenges associated with IVIG or SCIG, their preference should be considered when deciding therapy.
8. We recommend the establishment of a national database based on post marketing surveillance to capture adverse reactions to all immunoglobulin products.
9. Immunoglobulin should not be dispensed as a generic product. Patients requiring ongoing infusions should be maintained on a consistent product that is best tolerated by the patient.
10. Consistent national practices should be developed for monitoring patients that are receiving SCIG self-infusions.

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

11. We recommend flexible systems of transition from one modality of immunoglobulin administration to another (from IVIG to SCIG or the reverse), to accommodate changing patient needs across the lifespan.
12. Replacement should be at a dose of 400–600 mg/kg given once every 3–4 weeks for IVIG, or 100–150 mg/kg per week for SCIG, doses or frequency to be adjusted by experts according to desired trough levels and individual patient clinical needs. Infrequently, patients who metabolize exogenous IgG faster, leading to a shorter half-life, might require more frequent administration.
13. Serum IgG trough levels should exceed 500 mg/dL, and ideally more than 700 mg/dL. Levels should be kept in the normal range per age or increased to obtain optimal clinical response.
14. When serum IgG trough levels drop below protective levels while on SCIG replacement, it is recommended to either transiently switch to IVIG or increase frequency and or dosage of SCIG.

Stephen Betschel[a]
Peter Dent[b]
Elie Haddad[c]
Alison Haynes[d]
Thomas Issekutz[e]
Bruce Mazer[f]
Brenda Reid[g]
Chaim M. Roifman[g,h]*
Bob Schellenberg[i]
Gordon Sussman[j]
Stuart Turvey[k]
Susan Waserman[l]

a. Division of Clinical Immunology and Allergy, St. Michael's Hospital, University of Toronto, Toronto, ON
b. Department of Pediatrics, McMaster Children's Hospital, Hamilton, ON
c. Division of Clinical Immunology & Rheumatology, Pediatric Department, CHU Sainte-Justine, University of Montreal, Montreal, QC
d. Department of Pediatrics, The Janeway Children's Hospital, Memorial University of Newfoundland, St. John's, NL
e. Division of Immunology, IWK Health Centre, Dalhousie University, Halifax, NS
f. Division of Pediatric Allergy and Immunology, Department of Pediatrics, Montreal Children's Hospital, McGill University Health Center, Montreal, QC
g. Division of Immunology and Allergy, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, ON
h. Canadian Centre for Primary Immunodeficiency, The Hospital for Sick Children, University of Toronto, Toronto, ON
i. Division of Allergy and Clinical Immunology, Department of Medicine, University of British Columbia, Vancouver, BC
j. Clinical Immunology and Allergy, Faculty of Medicine, University of Toronto, Toronto, ON
k. Division of Clinical Immunology and Allergy, Department of Pediatrics, BC Children's Hospital, University of British Columbia, Vancouver, BC
l. Division of Clinical Immunology and Allergy, McMaster University, Hamilton, ON

*Corresponding author: Chaim M. Roifman/chaim.roifman@sickkids.ca

dx.doi.org/10.14785/lymphosign-2017-0008

Submitted 31 July 2017
Accepted 17 August 2017
Available online 17 August 2017

LymphoSign Journal

Working together
for patients with PI

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

LymphoSign Journal Downloaded from lymphosign.com by 18.216.57.207 on 05/17/24

75 YEARS OF IMPROVING THE HEALTH & WELL-BEING OF PEOPLE WORLDWIDE

At Grifols, we are proud of our pioneering spirit and history of innovation. In 1940, we founded a company that has helped transform hematology.
Today, we are a global leader providing live-saving protein therapies, diagnostic solutions, and other tools that hospitals, pharmacies, and healthcare professionals need to deliver expert medical care.

GRIFOLS
pioneering spirit

Learn more about Grifols at
www.grifols.com