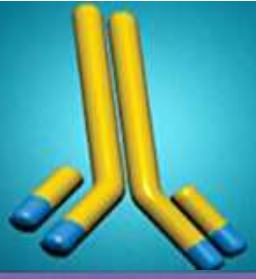
IGJ | Immunology and Genetics Journal



Research Center for Immunodeficiency

Research Center for Immunodeficiencies

IGJ

Immunology and Genetics Journal

The official journal of RCID: (Research Center for Immunodeficiencies)
and IPIN (Iranian Primary Immunodeficiencies Network)

- Recent advances and current status of primary immunodeficiency disease in Iran
- Genetic association in familial common variable immunodeficiency (CVID) and IgA deficiency (IgAD)
- Correlation analysis of mutation severity and BTK-expression and clinical manifestations in the patients with X-linked agammaglobulinemia
- Chediak-Higashi Syndrome Presented with Recurrent Episodes of Diarrhea: A Case Report

Volume 1, 2018

http://www.igjournal.ir

Immunology and Genetics Journal

The official journal of the RCID (Research Center for Immunodeficiencies)
The IPIN (Iranian Primary Immunodeficiencies Network)

Editors in Chief:

Asghar Aghamohammadi, Iran
Nima Rezaei, Iran

Associate Editors:

Hassan Abolhassani, Iran
Reza Yazdani, Iran

Editorial Boards:

Aliakbar Amirzargar, Iran
Gholamreza Azizi, Iran
Ahmed Aziz Bousfiha, Morocco
Talal Chatila, USA
Max D. Cooper, USA
Raif S. Geha, USA
Andrew Gennery, UK
Abbas Ghaderi, Iran
Mahmoud Jeddi Tehrani, Iran
Hirokazu Kanegane, Japan
Hosseinali Khazaei, Iran
Yu-Lung LAU, Hong Kong
Abbas Mirshafiei, Iran
Javad Mohammadi, Iran
Mohammad Hossein Nicknam, Iran
Hans D. Ochs, USA
Nima Parvaneh, Iran
Alessandro Plebani, Italy
Hojatollah Rabbani, Iran
Ismail Reisli, Turkey
Reinhold E. Schmidt, Germany
Fazel Shokri, Iran
E. Richard Stiehm, USA

RCID (*Research Center for Immunodeficiencies*)

Publishing Editor:

Radan English Edit

Table of Contents

Editorial

Immunology and Genetics Journal is a peer-reviewed journal published every four months that presents original articles on the molecular, cellular, and genetic bases of immunological disorders. In addition to original articles, the journal publishes interesting review articles and case reports. Authors will receive both reviews of their submissions and the editors' decision within six to eight weeks of receipt of their manuscripts by the journal office.

Prospective contributors are encouraged to check recent articles published in immunology and genetics on topics related to their manuscript to have a better understanding of the articles of interest to the readership. We believe that all this will represent a significant improvement for both the readership and the authors. We hope that you will appreciate our efforts and that the Immunology and Genetics Journal becomes a useful support to your daily practice as well as a tool for your educational activities.

This is the official journal of the RCID (Research Center for Immunodeficiencies), the main referral center for primary immunodeficiencies in Iran. We would like to thank our editors, members of the Editorial Boards, and the publisher for the advice, dedication, and skill that they, collectively, have lent this task. It is hoped that the superb contributions to the first issue of this journal will encourage prospective authors to submit their best work to the Immunology and Genetics Journal.

Sincerely,

Asghar Aghamohammadi, MD. Ph.D., Co-editor in chief

Nima Rezaei, MD. Ph.D., Co-editor in chief

Hassan Abolhassani, MD. PhD, Associate Editor

Reza Yazdani, PhD, Associate Editor

Immunology and Genetics Journal VOL 1, N 1, Sept 2018.

Immunology and Genetics Journal (2018) 1:5-27
Doi: 10.22034/igj.2018.69304

Review

Recent Advances and Current Status of Primary Immunodeficiency Disease in Iran

Hassan Abolhassani, Nima Rezaei, Asghar Aghamohammadi*

Received: 19 April 2018 /Accepted: 22 August 2018 /Published online: 22 September 2018

Abstract

Although comprehension of the molecular basis of primary immunodeficiency diseases (PID) provides unique insight into the functioning of the immune system, translational research is also needed to provide better care to affected individuals. Many institutions and academic departments have been established to provide training and encourage collaborative research on the immune system and related disorders. In Iran, one of the frontiers of PIDs in the Middle Eastern region, considerable progress in basic and clinical immunology has been achieved during the last three decades. During this period, massive improvements have revolutionized the management of PIDs in the country, from educational and research related aspects to diagnostic procedures and treatments available to Iranian PID patients. In this review, we seek to elucidate the current status of PIDs in Iran from different angles and to speculate upon the opportunities that the future may bring.

Keywords Iran, Immunodeficiency diseases, Registry, Research, Education, National network

* **Corresponding author:** Asghar Aghamohammadi
aghamohammadi@tums.ac.ir

Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Introduction

Primary immune deficiency (PID) diseases are characterized by a wide spectrum of inherited disorders caused by intrinsic defects in one or more components of the immune system (1-3). Individuals affected with a PID present with increased susceptibility to infections in the vast majority of cases; however sometimes they are associated with autoimmunity, immune dysregulation, allergic diseases, auto-inflammatory disorders, and malignant tumors (4-7). Infections in PIDs can occur repeatedly and severely. Atypically they can locally or systematically damage different organs and reduce quality of life (8, 9). PIDs were previously thought to be rare and exclusively

present in infants and young children who manifest severe clinical infections. However, observations report the boundaries of such complicated disorders with widely divergent pathologies among adolescents and adults and varying phenotypes and symptoms along with a broad spectrum, from very mild to potentially life-threatening.

The first case of PID was described in 1952 (10). Subsequently, through continued progress in both basic and clinical immunology, the availability of different methods such as complementing traditional linkage analysis and homozygosity mapping, and after successful completion of the Human Genome Project (HGP) and the availability of next generation sequencing as a more efficient and invaluable method, more than 350 PIDs have been described with about 20 new entities per year (1). The overall frequency of PIDs has been estimated at about 1:10,000 individuals; however, this rate is an underestimation particularly in countries with a higher rate of consanguineous marriages (11). Early diagnosis and adequate therapies are the keys to survival and a better quality of life, while delays in diagnosis and/or inadequate management may lead to permanent organ damage and a shortened lifespan (9, 12, 13). Understanding the genetic and mechanistic basis of PIDs provides a unique insight into the functioning of the immune system. Such progress leads to translational research to provide better care for affected individuals. Focused approach on the immunologic and genetic bases of PIDs provides a unique opportunity for research into immune disorders, particularly those severely attacking host defense mechanisms. A number of medical schools and organizations in the world have focused on basic and clinical-based research and developed into specialized centers of clinical immunology for research on the immune system and related disorders. The evidence demonstrates that our country has made appropriate progress in basic and clinical immunology in the past 30 years. In this review, we try to describe the current status of PIDs in Iran and the country's strengths and weaknesses as well as challenges and opportunities in facing them.

A brief history of clinical immunology and PID in the world

The history of clinical immunology refers to the study of resistance to smallpox and measles that was performed by the 9th-century Persian physician, Zakariya al-Razi (880-932 A.D). In (1843–1910), the germ theory of contagious disease was described by the German physician Robert Koch, and later, Louis Pasteur discovered how to make vaccines from attenuated microbes. Pasteur developed the earliest vaccines to prevent against fowl cholera, anthrax, and rabies, while Koch demonstrated that tuberculin sensitivity can be transferred passively through cells but not by serum. Elie Metchnikoff (1884) described phagocytosis as phagocyte cells digesting foreign materials to destroy them and to protect the host against infectious agents (2). In 1890, Behring and Kitasato showed that the transfer of antibodies from animals immunized against diphtheria to animals suffering from it could cure the infected animals. Later in 1900, Paul Ehrlich suggested the side-chain theory

and hypothesized that side chain receptors on cells are bound to a given pathogen. In the early twentieth century, much attention was focused on the various types of antibodies as well as their use in diagnosis and treatment. Afterwards, Dr. John Enders and Dr. Hugh Ward described "opsonization" and demonstrated that the optimal process needs the antibodies (3). Although several patients with immunodeficiency disorders such as complement deficiency (1919) (4), neutropenia (1922) (5), ataxia telangiectasia (AT) (1926) (6), or Wiskott-Aldrich syndrome (WAS) (1937) (7) had been reported by signs/symptoms in the early 1950s, the birth of the PID field is related to 1952, when the first case of agammaglobulinemia was reported by Dr. Ogden Bruton (8). Since that time more than 350 different PIDs have been identified (1, 9). The discovery of PIDs and the characterization of these diseases led to crucial contributions to understanding the functional organization of the immune system and molecular biology. Thus, the study of PIDs has contributed to progress in immunological and molecular diagnostic techniques (10). As a result of these advances and major biotechnology breakthroughs, new screening methods as well as therapeutic strategies have been devised, leading to the better care of individuals affected by PIDs.

PIDs in Iran: history at a glance

Progress and activities in the field of PIDs have developed during last three decades, and as one of the frontiers of PIDs diagnosis and treatment in the Middle Eastern region, Iran has achieved much progress in the fields of both clinical and molecular immunology during the last three decades in the four periods described below (**Table 1**).

Improvements in treating PIDs were initiated in Iran in the 1970s, when the Division of Clinical Immunology and Allergy at the Children's Medical Center in Tehran was established. Then, in 1988, a training program for fellowship in the field of clinical immunology and allergy established, followed by a unit for patients requiring treatment with intravenous immunoglobulin infusion. The third period of progress began in 1998 with the development of a national registry for PID. Finally, in 2009, the Research Center for Immunodeficiencies (RCID) was established and subsequent events were synchronized by this research center. Each event is described in more detail in the following sections.

Table1. Progress and activities in the field of PIDs in Iran over the last three decades

Period	Years	Events
First period	1978–1988	Professor Abolhasan Farhoudi returns to Iran after allergy training in the United Kingdom. Clinics for patients affected with PIDs are established.
Third period	1997–2009	Training program for clinical fellowship in the field of Allergy and Immunology is established. Clinics for PID patients requiring intravenous immunoglobulin infusion are established.
Third period	1997–2009	Iranian Primary Immunodeficiency Registry (IPIDR) is established. Iranian Primary Immunodeficiency Association (IPIA) is established.
Fourth period	2009-2018	Research Center for Immunodeficiencies (RCID) is established. Training program for Ph.D. by research student in the field of PID is established. Training program for clinical researcher in the field of PID is established. Iranian PID network is established. Immunology and Genetics Journal is established.

Establishment of clinics for patients affected with PIDs

Among the countries located in the Middle Eastern region, Iran has established itself as a front runner in treating PID patients during the last three decades. Improvements began in the 1970s when Professor Abolhasan Farhoudi (11), who trained in pediatric immunology and allergy in the United Kingdom, returned to Iran and established the Division of Clinical Immunology and Allergy as well as the Immunology Laboratory in the Children's Medical Center affiliated with Tehran University of Medical Sciences (TUMS) (12, 13).

Establishment of clinics for PID patients needing treatment with intravenous immunoglobulin infusionMost patients with a PID require regular immunoglobulin replacement therapy (14). Intravenous immunoglobulin (IVIG), a blood product obtained from human serum, is the treatment of choice for the majority of patients with antibody deficiencies, and it has been used since the 1970s. IVIG is used at a replacement dose of 400–600 mg/kg given approximately every 3 to 4 weeks (15, 16). Deciding on IVIG replacement in the management of patients associated with hypogammaglobulinemia is critical. IVIG therapy is vital in reducing the burdens of PIDs, including the affected patient’s quality of life (17) and mortality due to life-threatening invasive infections and complications (18). Timely and regular administration of IVIG at the correct dosage can also prevent the development of many end organ damages (such as bronchiectasis), which cause significant morbidity and increased mortality for PID patients (19). Our preliminary results indicated that the incidences of pneumonia and hospitalization in patients with agammaglobulinemia were significantly decreased after IVIG administration. The data showed the importance of early diagnosis and appropriate treatment with IVIG in this group of patients (20, 21). Because of the risk of adverse reactions, IVIG infusion should be administered under the supervision of trained physicians and nurses who are aware of the possible complications (14, 22, 23). In 1995, the Immunoglobulin Infusion Unit was established in the Children's Medical Center. This unit serves four times a week; all patients with hypogammaglobulinemia, including those with antibody deficiency or combined immunodeficiency, who receive IVIG in this unit are monitored by trained nurses and clinical fellows. Since the establishment of this unit, regular monthly follow-up of patients who require IVIG has been performed and the efficacy of this treatment was studied. Furthermore, adverse reactions of this treatment were regularly recorded. A recent report on a total of 3004 infusions during a 13-year period showed that less than 10% of patients receiving infusions faced adverse reactions, and most of those cases were as mild as chills and a low fever. Only 3 severe reactions were ever recorded during this period. To the best of our knowledge, there are currently at least 4 special units for IVIG administration in the country and other patients also receive their medications. In line with the improvement seen in specialized centers, there are three IVIG brands available with costs of approximately $80 per gram. The plasma

fractionated for the production of IVIG used in Iran is obtained from the blood of Iranian donors, which commonly contains antibodies against the endemic pathogens of the country. IVIG is administered mainly to patients suffering from different forms of antibody production impairment, including common variable immunodeficiency (CVID), combined immunodeficiency (CID), X-linked agammaglobulinemia (XLA), undefined hypogammaglobulinemias, AT, WAS, and hyper IgM syndrome (HIgM). The number of patients affected by these conditions is estimated to be 4000, of which currently about 40% are covered.

Students' research group for immunodeficiencies

In 1997, some academic members of TUMS and medical students started to investigate the frequency of PID in Iran. In recent years, the number of interested researchers has risen substantially, resulting in an increase in the complexity of the group, and this situation has brought about the requirement for a clearer definition of the group's purposes and activities. Meanwhile, an informal research group with a specific interest in the field of PID got the opportunity to design several national and international research projects with outstanding scientific output in this field. In 2009, a proposal for the establishment of Research Center for Immunodeficiencies (RCID, http://rcid.tums.ac.ir) was submitted to TUMS by this group and was accepted by the Ministry of Health Organization. The RCID is located in the Pediatrics Center of Excellence, Children's Medical Center Hospital in Tehran, Iran and is directed by Professor Asghar Aghamohammadi.

Iranian PID association for diagnostic and therapeutic aims

In more developed countries, thousands of people with PID still do not have access to their treatment of choice due to misdiagnosis of their underlying condition. Early diagnosis can be lifesaving and prevent permanent organ damage. Therefore, it is expected that by increasing the current level of knowledge of PIDs among first line physicians, the number of PID patients identified will rise consistently. To achieve the aforementioned goals, physicians and researches should work in close harmony with non-governmental organizations (NGOs) to convince the authorities and for-profit organizations to sponsor training programs, treatment, and research in the field of PIDs. A successful attempt was made in 1998 to support PID patients by establishing the Iranian Primary Immunodeficiency Association (IPIA) as a national non-profit organization. The IPIA held a significant number of meetings and reunions with authorities as consultants of the related ministries in an attempt to inform them of the dangers of delayed diagnosis of PIDs. This NGO has highlighted the importance of research for PID patients during its lifetime. IPIA modified and translated into Persian a poster showing 10 Warning Signs of PIDs and distributed it to all medical university hospitals in hopes that it will help increase awareness of PIDs among medical personnel and improve the diagnosis and

treatment of PID patients. Full details of the application and all supporting documentation can be found on the website for the International Patient Association for Primary Immunodeficiencies (IPOPI). The IPIA, which has been recognized as a global organization working to improve diagnosis and management of PID through research, advocacy, and education, was accepted as a member of IPOPI in 2002. (www.ipopi.org).

Iranian PID registry (IPIDR)

Epidemiological studies have shown wide geographical and racial variations in terms of prevalence and patterns of PIDs. Many countries worldwide have developed registries to estimate the prevalence and characteristics of different PID phenotypes among their populations (24). In order to determine the frequency and characteristic features of various PIDs in Iran, the IPIDR was established in August 1999 (25). The main goals of this national registry were to determine the frequency of different types of PIDs in Iran, follow the importance of treatment procedures of patients, encourage physicians to record secondary complications and their consequences experienced by patients, and to subsequently enhance advanced molecular/clinical research on PIDs in our country (26). The patient registration process in IPIDR initially consisted of different steps. First, a preliminary one-page questionnaire was sent to all participating centers. Then, after confirmation of definite diagnoses by clinical immunologists, the centers were asked to send complementary information. Recently, an online registry system has become available to all participating centers which facilitates and accelerates data entry. Each center is provided with a specific password and can update its own data by visiting the registry website at http://rcid.tums.ac.ir. Currently, data is collected from 42 different centers and distributed in 25 major cities of Iran where patients with PID are treated and immunologic laboratories are available for the diagnosis of PID patients. Patients from peripheral states are usually referred to central centers in order to be managed under advanced immunologic evaluation and genetic analysis. IPIDR is currently the only center from Iran accepted as a documenting center of the European Society for Immunodeficiencies (ESID, https://esid.org/Working-Parties/Registry-Working-Party/Documenting-centers/Iran-Iranian-Primary-Immunodeficiency-Registry-IPIDR). It is also a well-known Jeffrey Modell foundation collaborating on the global study of PID; it is the only Iranian center among 358 institutions from 86 countries spanning 6 continents, www.info4pi.org.

Establishment of the Research Center for Immunodeficiencies (RCID)

In 2009, a proposal to establish the Research Center for Immunodeficiencies (RCID, http://rcid.tums.ac.ir) was submitted to Tehran University of Medical Sciences (TUMS) by Dr. Aghamohammadi, Dr. Nima Rezaei, and Dr. Nima Parvaneh and was accepted by TUMS and the Ministry of Health Organization. The RCID is located in the Pediatrics Center of Excellence, Children's Medical Center Hospital in Tehran, Iran and is directed by Professor Asghar

Aghamohammadi. It is the first established specific PID research center in Iran. It has published more than 300 publications since its establishment, and three members of this research center (Dr. Aghamohammadi, Dr. Nima Rezaei, and Dr. Hassan Abolhassani) have been honored as the top 1% of the most cited scientists in the category of immunology according to Thomson Scientific's Essential Science Indicators (ESI).

Education and meetings on PID

World PID Week in Iran

World PID Week (WPIW, http://worldpiweek.org/) is part of the global campaign aimed at improving knowledge, diagnosis, and treatment of PID through the participating of different centers around the world in this theme. It has been taking place in Iran since 2011 after WPIW events entitled "Awareness Raising in Iran" were organized and held on the 22nd of April 2011. In these annual meetings, participants mostly comprise general practitioners and pediatricians, nurses and paramedic staff, and patients and their families. Discussions have been centered on "PID warning signs" and experiences sharing among groups of general practitioners, expert pediatricians, and clinical immunologists. The second annual WPIW included Immunodeficiency Day in Iran on the 22nd of April 2013, organized by the RCID and Children's Medical Center of TUMS. Case discussions, an expert PID meeting, a junior scientists' meeting, a panel discussion, and interviews with the media were all part of this event. From 2014 through 2018, the RCID has harmonized its international conferences with WPIW by inviting all pediatricians as well as basic and clinical immunology scientists to join and commemorate this global event. Expert PID meetings, PID morning reports and case discussions, special panels and expert discussions with the subject of each year's PID slang suggested by the Jeffrey Modell Foundation were planned and held.

J-Project meetings

The mission of the J-Project is to be the forum in Eastern European countries for increasing awareness and improving diagnostic facilities and the complex management of PIDs. The main aims of the J-Project are to organize professional meetings on PID and related diseases in several developing countries that have a low number of registered PID patients and limited budgets, discuss diagnostic and therapeutic practices and problems, define specific areas to be improved and to generate support by other developed Western countries, institutions, companies, and foundations. Other goals of the J-Project include updating national PID registries, establishing PID professional working groups, and forming a group for PID patients. Iran has been selected as the pioneer country in Central Asia, and the RCID has aimed to spread its valuable knowledge in Persian-speaking countries including Afghanistan and Tajikistan through its project titled J-Persia.

Continuing medical education programs for targeted physicians (Awareness of PID)

Among all healthcare providers, general practitioners and pediatricians are the most likely to

visit PID patients at the onset of the disease, so their up-to-date knowledge in this field could prevent most delayed diagnoses, disease complications, and life-threatening challenges. As the most common type of clinical presentations is an infection, PIDs are very likely to be missed by first line physicians, especially general practitioners. There are only about 20 PIDs the diagnosis of which can help save lives. This indicates the importance of education in preventing life-threatening side effects. In 2001, the Center for Disease Control and Prevention (CDC) began a program aimed at improving the health outcomes of PID patients. It was concluded that educational efforts have top priority because of the role of education in every aspect of improving the health outcomes of PID patients. According to a 2011 article on an Iranian physician's awareness of PID, about half of general practitioners and one third of pediatric specialists lacked basic knowledge about PIDs. Hence, educating primary care physicians must be considered to achieve early clinical recognition. This can be achieved through continuing medical education (CME) programs containing special lessons such as approaches to recurrent infections (27), the effect of early diagnosis and appropriate treatment on morbidity and mortality of PIDs, the identification of most common PID diseases, the evaluation of the usefulness and accuracy of family history, the recognition of early clinical signs and symptoms, and the role of initial laboratory tests in diagnosing PIDs (18, 28). In the past 5 years, 6 different local CME projects have been held in different states with higher PID prevalence rates. Recently, non-infectious complications of PIDs, including autoimmunity, allergic diseases, syndromic features, malignancies, and angioedema, were integrated into the CME programs.

Establishment of a program to train Ph.D. candidates by a research student in the field of PID

The education and training of doctoral students are highly important activities of a research center. The program of training special Ph.D. students in the field of PID aims to train scholars who will go on to conduct original research as faculty members of leading global institutions. The RCID is trying to improve healthcare services and train skilled researchers in the field of immunodeficiency. In 2012, with the goals of having a Ph.D. by research course for specialists who are surrounded by scientific literature in the PID-specific field and who know about advanced research methods and accessing the latest basics of education, the RCID launched a training program promoting knowledge in the field of PIDs. The RCID is now educating 2 post-doctoral and 2 Ph.D. students of clinical immunology. Three individuals have graduated with a Ph.D. by research, and more than 20 medical students are collaborating on PID projects.

Establishment of a program to train clinician-researchers in the field of PID

The clinician-researcher program focuses on clinical and research knowledge and skills, targeted simultaneously in a particular domain of medical science. Volunteers are selected on the basis of their potential abilities in leadership, innovation, analytic and critical thinking, quality and quantity of

research output, national interest and commitment, university and research center needs, quality of the intended project, and available facilities. All rules of specialty or sub-specialty courses as well as Ph.D. by research courses are applied in the clinician-researcher curriculum.

Establishment of a specialized PID referral laboratory

The list of specific immunologic laboratories in different provinces of Iran was acquired and analyzed with the help of Iran's Ministry of Health, the portal for Iran's medical research (http://labs.research.ac.ir). The search method was based on two keywords, "pathobiology" and "immunology", under the name field of the list of specific laboratories. Overall, a total of 250 laboratories all around the country were assigned to specific immunological tests (3.6 labs per 1 million people). Well more than one third of these are centered in Tehran (seven labs per 1 million people), and 85% of provinces provide more than one lab per 1 million people (**Table 2**). Although most of these labs can provide the basic tests necessary for making a clinical diagnosis of PID according to the ESID criteria, the RCID has decided to establish a central lab professionally designed for molecular and advanced experiments which need more skilled and research-based technology, which is currently located in the Children's Medical Center affiliated with TUMS.

Table 2. Number of specific immunologic labs per million population in different provinces of Iran

Province	Capital	Area km^2	Population	Labs	Labs/One Million Population	Density (population/km^2)
Alborz	Karaj	5833	1375450	17	12.4	235.8
Golestan	Gorgan	20195	1637063	17	10.4	81.1
Qom	Qom	11526	1064456	8	7.5	92.4
Tehran	Tehran	18814	13530742	94	6.9	645.8
Bushehr	Bushehr	22743	887115	6	6.8	35.9
Gilan	Rasht	14042	2410523	16	6.6	171.7
Kerman	Kerman	180836	2660927	17	6.4	13.5
Khorasan, South	Birjand	69555	640218	4	6.2	7.3
Lorestan	Khorramabad	28294	1758628	9	5.1	62.2
Mazandaran	Sari	23701	2940831	14	4.8	118.9
Kermanshah	Kermanshah	24998	1938060	9	4.6	77.5
Khorasan, North	Bojnourd	28434	820918	3	3.7	27.7
Semnan	Semnan	97491	590512	2	3.4	6
Ardabil	Ardabil	17800	1257624	4	3.2	70.7
Markazi	Arak	29130	1361394	4	2.9	46.7
Fars	Shiraz	122608	4385869	9	2.1	35.8
Azerbaijan, West	Urmia	37437	2949426	6	2.0	78.8
Yazd	Yazd	129285	992318	2	2.0	7.4
Ilam	Ilam	20133	545093	1	1.8	27.1
Hamadan	Hamadan	19368	1790770	3	1.7	91
Kohgiluyeh and Boyer-Ahmad	Yasuj	15504	695099	1	1.4	44.8

Sistan and Baluchestan	Zahedan	181785	2410076	3	1.2	12.6
Zanjan	Zanjan	21773	970946	1	1.0	44.6
Hormozgān	Bandar Abbas	70669	1410667	1	0.7	18.6
Isfahan	Isfahan	107029	4590595	3	0.7	41.6
Kurdistan	Sanandaj	29137	1574118	1	0.6	54
Khuzestan	Ahvaz	64055	4345607	2	0.5	67.8
Azerbaijan, East	Tabriz	45650	3620183	NI	NI	76.7
Chahar Mahaal and Bakhtiari	Shahrekord	16332	842002	NI	NI	51.6
Khorasan, Razavi	Mashhad	144681	5620770	NI	NI	36
Qazvin	Qazvin	15549	1166861	NI	NI	75
Iran (Total)	**Tehran**	**1628554**	**71767413**	**257**	**3.6**	**44**

lishment of Iranian PID network

One delay in the timely diagnosis and treatment of PIDs in Iran is caused by deficient physician-physician interactions. It has been demonstrated that poor communication among physicians could significantly impede the timely diagnosis and treatment of patients and waste time and resources (29). In addition, the absence of appropriate diagnostic laboratories in endemic regions in Iran leaves some patients undiagnosed for years, despite recognizable symptoms. Thus, the Iranian PID Network (IPIN), a multidisciplinary organization dedicated to PID disorders, was established in 2016. All clinical immunologists, subspecialists, scientists, and medical practitioners who are working on PID were invited to be members of IPIN. This network has several aims, including to increase physicians' awareness, increase collaboration between centers for clinical education, develop consensus statements and clinical guidelines for the diagnosis and therapy of PID patients, balance the quality of care for PID patients, and facilitate the registration of diagnosed patients (http://ipin.tums.ac.ir).

Research Projects

Targeted research at the national level based on community requests

In the last decade, many projects focusing on PIDs have been designed and completed based on the frequency of the disease in the country. A number of descriptive studies on series of children and adults with specific disorders from this region were reported initially. More clinical and laboratory characteristics of patients with high regional prevalence such as CVID (30-32), XLA (33-35), HIgM (36, 37), selective IgA deficiency (SIgAD) (38-40), CID (41-43), AT (44, 45), chronic mucocutaneous candidiasis (CMCC) (46, 47), and Chediak–Higashi syndrome (48), were well described in detail. Moreover, the characteristics of less common diseases, such as severe congenital neutropenia (SCN) (49-51), cyclic neutropenia (52, 53), hyper-IgE syndrome (HIES) (54, 55),

leukocyte adhesion defects (LADs) (56-58), Griscelli syndrome type 2 (59), and Shwachman-Diamond syndrome (SDS) have also been acceptably investigated.

International Collaborative Projects

International cooperative studies have had a significant effect on the quality of current projects in the field of PID (60, 61). Universities/Institutes that have published in collaboration with centers in Iran are the Karolinska University Hospital (Sweden); Hannover Medical School, Freiburg University Hospital Department of Pediatric Oncology, Hematology and Immunology, Heinrich Heine University Medical Center, Dusseldorf (Germany); National Institute of Health, University of Washington, Division of Immunology, Boston Children's Hospital, Harvard Medical (USA); University College of London and University of Sheffield(UK),Toyama Medical and Pharmaceutical University (Japan); University of Brescia (Italy); and Inserm Institutet and Unit of Pediatric Immunology-Hematology, Necker-Enfants Malades Hospital, Assistance Publique Hoˆpitaux de Paris (France).

Discovery of new genes and new PID diseases

Although comments and assistance from internationally well-known PID experts have led to effective projects, contributing to international projects, especially those that culminated in the discovery of specific phenotypes and genetic defects, has greatly refined science in this field. Findings on mutations in the *HAX1* gene (severe congenital neutropenia), the *G6PC3* gene (congenital neutropenia), the *ELA2* gene (severe congenital neutropenia), the *JAGN1* gene (severe congenital neutropenia), the *CARD9* gene (susceptibility to fungal infections), the *IFNGR2* gene (susceptibility to mycobacterial disease), and *DOCK8* (susceptibility to HIES), which were described as a new PID disease in the last IUIS classification, are additional achievements stemming from international collaborations. New investigations have also revealed the roles of the *STK4*, *LRBA*, and *CD70* genes in CVID and autoimmune disorders (62-69).

Scientific Outputs

Published articles

Since 2000, approximately 300 papers have been published in the field of PIDs, representing Iran's significant advancement in this field (42, 70-73). At the beginning of the third millennium, only 1 to 2 papers were published annually from Iran; this number reached 40 in 2008, around 60 by 2018, and still rising (**Figure 1**). More than 80% of these publications are based on research performed by TUMS scientists, but Shaheed Beheshti University and Shiraz University of Medical Sciences rank second and third in respect to PIDs publications. This accelerated rate of investigation and research keeps Iran among the 14 leading countries in the field of PID (**Figure 1**). During the last decade, the participation of Iranian scientists in international congresses was also noticeable; more than 250 abstracts in the field of PIDs were presented by

Iranian experts, either orally or as a poster, in international congresses.

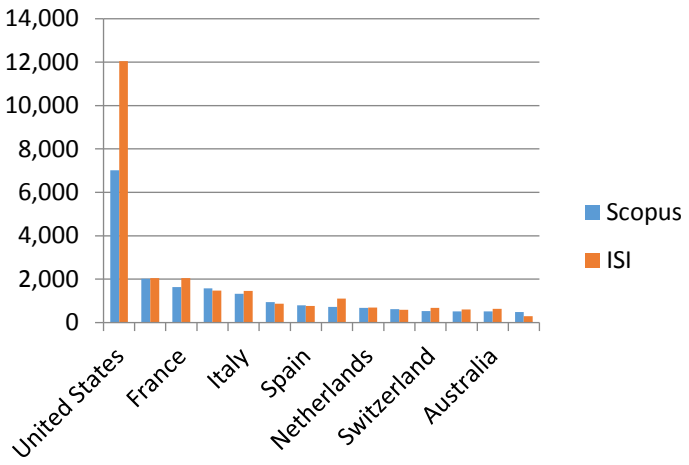


Figure 1. International ranking of first 14 countries on scientific publications in the field of primary immunodeficiency according to the indexed articles in the Thomson Reuters (formerly ISI) Web of Knowledge and Scopus.

Published PID Textbooks

To date, 8 textbooks (in Persian and English) on PID have been published by member of RCID in order to improve the knowledge of healthcare providers, students, patients and their families, and the public. "Primary Immunodeficiency Disorders in Iran" (edited by A. Farhoudi, 2002) is one of the first published books and resulted from collaboration with other clinical immunologists. "Immune System and Microorganisms" (edited by N. Rezaei, A. Aghamohammadi, Z. Pourpak, and M. Mahmoudi), affiliated with TUMS, is a remarkable book published by the United Nations Educational, Scientific and Cultural Organization (UNESCO) Chair in Health Education, in 2005, and was dedicated to all PID patients and their families. This book helps to bridge the gap between physicians and families and is an important tool for improving the quality of clinical management of patients with PID. Three other published books are titled: "Primary Immunodeficiency Disorders in Iran" (edited by A. Aghamohammadi, Z. Pourpak, N. Rezaei, A. Farhoudi, and M. Moin), "Treatment in Primary Antibody Deficiencies" (edited by A. Aghamohammadi, N. Parvaneh, and M. Yeganeh), and "Diagnosis and Treatment in Primary Immunodeficiency Disorders" (Edited by A. Aghamohammadi, HA Khazaei, and N. Rezaei). As references for the course of clinical immunology, three books titled "Primary Immunodeficiency Diseases: Definition, Diagnosis, and Management" (two editions, both edited by N. Rezaei, A. Aghamohammadi, and LD Notarangelo), “Clinical Cases in Primary Immunodeficiency Diseases: A Problem Solving Approach" (edited by A. Aghamohammadi and N. Rezaei), and" Cancer Immunology: A Translational Medicine Context" were published by Springer and included the contributions of international senior and junior scientists in this field from more than 30 universities worldwide.

Immunology and Genetics Journal

Immunology and Genetics Journal is a peer-reviewed journal published every four months that publishes original articles on the molecular, cellular and genetic bases of immunological disorders. In addition to original articles, the journal publishes interesting reviews and case reports. This publication is the official journal of the RCID, and contributor authors will receive both reviews of heir submissions and the editors' decision within six to eight weeks of receipt of their manuscripts by the journal office (http://www.igjournal.ir).

Improvements in diagnosis and treatment

Diagnosis of PID patients in Iran

The high prevalence of PID can be explained by the high rate of consanguineous marriages in the Middle East (ME) compared with Western countries (74, 75). Indeed, many defective genes with autosomal recessive patterns of inheritance that underlie PIDs were first described in patients originating from this region (76, 77). Hence, the abovementioned activities in recent years have led to considerable improvements in the diagnosis of PIDs, considering that more than half of the currently diagnosed Iranian PID patients were recognized in the last 5 years. The estimated diagnostic rate has increased from 7 patients per year in the 1980s to 30 patients per year during the early 1990s, 58 patients per year from 2000 to 2006, and 104 per year from 2006 till March of 2012. This rapid progress, which recently brought the diagnostic rate of PIDs to 350 patients per year, is critical for improving patients' quality of life and chances for survival (78). In recent years, PIDs have been diagnosed at earlier ages, reducing delays in diagnosis from 7 years in the 1980s to 2.5 years in the 1990s and to 6 months by the year 2000. The current diagnostic delay is as little as 3 months. Three reports on the national registry of Iranian PID patients in 2002 (79), 2006 (80), and 2014 (81) have played important roles in determining the prevalence of various types of PID in Iran. A total of 1,661 PID patients (1,028 male and 633 female) were registered in the IPIDR before 2014; that number increased to more than 3000 in 2018. The registry reports declared that the number of patients included 930 PID patients who were diagnosed during a 30-year period ending in March of 2006, and the remaining PID cases were diagnosed and registered in the IPIDR afterwards. The cumulative incidence of PIDs in Iran during the past 10 years is estimated to be around 30 cases per 1,000,000 population. The majority of registered cases were diagnosed predominantly with antibody deficiencies (35.7%) with CVID comprising 60% of this group as the most common PID. Based on the underestimated frequency of PID (a prevalence of 1:10,000 population), there should be at least 7,500 patients with the diagnosis of PID in Iran, but only 1,661 patients have been reported (82), and fewer than 3,500 patients have been registered so far. Moreover, less than 30% of diagnosed patients have a defined molecular diagnosis (mainly patients with combined immunodefiency and autoinflammatory disorders). Many factors can influence this difference, such as a lack of awareness among the

normal population as well as widespread ignorance among first-line general practitioners about the importance of PIDs and their related complications, a lack of subspecialists in major cities which further complicates referral and follow-up systems, the absence of newborn screening and special laboratories for specific immunologic tests and molecular identification which results in ineffective tests, and finally, the early deaths of PID patients due to disease severity or secondary infections which often lead to misdiagnosis or misclassification of their conditions under disease groups other than PID (29). Despite the new reliable techniques which have facilitated the diagnosis of PIDs, delays in diagnosing PIDs is still ponderable. Examples of this phenomenon can be observed in other countries; for instance, in Japan and England, better education results in early diagnosis of many PID patients and effective treatment for them. In India or China, however, which have much larger populations and presumably more PID patients, both the number and the rate of diagnosed patients are not considerable. This correlation between education and diagnosis brings to mind that low prevalence of PIDs in some countries could be a secondary outcome of low education and diagnostic facilities, necessitating more reliable investigations.

Treatment of PID patients in Iran

Beside therapeutic and prophylactic antibiotics for infections, the most common treatment options for PID patients are immunoglobulin replacement therapy, interferon gamma (IFN-γ) therapy, granulocyte colony stimulating factor (G-CSF) injection, and hematopoietic stem cell transplantation (HSCT). Of the estimated 7,500 Iranian patients requiring these therapies, 1,282 (17.09%) are diagnosed and receiving appropriate treatment. The information regarding these treatment procedures in Iran is summarized in **Table 3**. Another technique that recently became available for the treatment of PIDs is gene therapy. This option is currently not available in Iran, although many patients could benefit from it. Another commonly employed therapy is HSCT, a useful procedure for the treatment of a variety of PIDs, including (but not limited to) CID, WAS, AT, CD40/CD40 ligand defect, neutropenia, chronic granulomatous disease (CGD), and LADs. In many instances, HSCT increases PID patients' quality and quantity of life by dramatically decreasing their various complications and, sometimes (typically in younger patients), nearly reconstructing their defective immune system. HSCT was introduced in Iran in 1991, and there are currently near 10 centers capable of running this procedure across the country. However, there are currently only two active centers for transplantation in PID cases, both affiliated with TUMS (Hematology-Oncology and Stem Cell Transplantation Research Centers in "Dr. Shariati" and "Children's Medical Center" hospitals). These centers were established officially in 1993 and 2016, respectively, and have been the greatest Iranian contributors, both scientifically and practically, to HSCT procedures. The cost of HSCT in Iran is remarkably low in comparison with European countries and the U.S., equaling between $16,000 and $40,000. Of this amount, less than $3,000 is paid by the patient, and most are supported by the Iranian Ministry of Health and

other organizations, including several NGOs. In non-PID patients, there have been nearly HSCT 4000 operations up to now, and an average of 400 are carried out every year, generally with acceptable outcomes (in more than 70% of all cases). In contrast, of the around 2,250 PID patients requiring HSCT, less than 100 patients have been transplanted, and only 32 (1.4%) are reportedly diagnosed and have undergone therapy. According to this report, 12 were diagnosed with LADs, 5 with SCID, 3 with CHS, 1 with SCN, 6 with WAS, 3 with Griscelli syndrome, 1 with primary CD4 deficiency, and another with Familial Erythrophagocytic Lymphohistiocytosis (83, 84). The third major drug for PID patients is G-CSF, and it is regularly administered to all patients suffering pathologic immunodeficiency associated with neutropenia. Important PIDs treated by G-CSF therapy include congenital and severe congenital neutropenia, cyclic neutropenia, and Kostmann syndrome. Many patients undergoing chemotherapy and HSCT or those affected by secondary neutropenia require G-CSF therapy as well. For most patients, G-CSF is administered on a daily dosage of 5-20 µg/kg of body weight by subcutaneous injection, but for some, the dosage might vary widely. This therapy is effective for increasing blood neutrophil levels but has several side effects, including skin reactions, osteoporosis, arthralgia, and alopecia. The administered G-CSF for these PID patients is a recombinant drug, and the price of each premade syringe (containing 300 µg) is between $45 for the major domestic brand and about $115 for imported types. Almost a quarter of the estimated 500 Iranian PID patients requiring G-CSF therapy have been diagnosed and are currently receiving therapy. Finally, IFN-γ is the treatment of choice for many primary phagocytic disorders, the most common of which in Iran is CGD. IFN-γ acts on macrophages and other cells and activates them in response to infection, causing an increase in the macrophage killing and antigen presenting abilities. As a potent macrophage activator, this drug has side effects such as fever, weight loss, fatigue, and gastrointestinal complications. The average required dose is 50 µg/m^2 of body surface for those with a body surface of more than 0.5 m^2 and 1.5 µg/m^2 of body surface for those with a lesser body surface. The drug is usually administrated by subcutaneous injection 3 times a week. IFN-γ exists in the form of 0.5 ml vials, each containing 100 µg of IFN-γ and costing about $95 in Iran. Of the estimated 500 CGD patients in Iran, about 250 have to date been reportedly diagnosed and are receiving IFN-γ therapy, and the coverage of these patients is ~50% accordingly. Both G-CSF and IFN-γ using patients' expenses are covered mostly by the Ministry of Health and various insurance companies.

Table 3. General status of different PID treatment procedures in Iran.

Parameters	IVIG	HSCT	IFN-γ	G-CSF
Number of covered patients	970	<100	173	75
Percentage of coverage (%)	11.32	<0.5	45.2	24.5
Cost of treatment ($)	78.9 /g	15,800 -39,600/n	94.7/100 µg	44.2- 116.9/300 µg

HSCT: Hematopoietic stem cell transplantation, G-CSF: Granulocyte colony stimulating factor, IVIG: Intravenous immunoglobulin, IFN-γ: Interferon gamma

Plan for the Future

Despite current achievements in the field of PID in Iran, there are still strong ambitions for the future. Although the RCID is already a leading center for diagnostics and treatment in Iran, a priority area for the country's main cities undergoing rapid expansion is mandatory. Our aim is to continue building our influence and reach to turn the RCID into a major national and international location for translational medicine research and become a key player in the life sciences expansion in the field of PID. Therefore, we hope in the near future to establish the Iranian Society for Immunodeficiencies (ISID) and later the Iranian Academy of Clinical Immunology. Current pilot studies into newborn screening for PID should be translated to the public routine, and the genetic diagnosis of patients will be used to expand prenatal diagnosis clinics for PID family members. Weaknesses in the donation, detection, and time-span for performing HSCT will be targeted by developing programs, and CME programs will be continued in other states of Iran. We hope to collaborate on more professional research with integration between basic scientists and clinical researchers and more collaboration with international PIDs research centers. National guidelines for the diagnosis and management of PIDs will be continually revised by expert panels, and the current patient support organization in the country will be strengthened (85, 86).

Conclusion

The anticipated road map for improving PID diagnosis and treatment in Iran requires the focus to initially be on abating current problems; these issues are categorized in 4 main areas: awareness/education, diagnosis/prevention, treatment, and infrastructural facilities, especially for research. All four areas are described as follows. Both the general population and the medical community are involved. Awareness of PIDs is lacking among the general population and healthcare providers and physicians such that primary symptoms are ignored and there is low compliance by parents, low rate of suspicion to early symptoms of PID and misunderstanding of PID by first line physicians due to a lack of education, and a lack of training programs in clinical immunology for medical and nursing schools. All these factors can lead to shortages, exposing the essential requirement of a vast investment in this area. Changing from curative- to prophylactic-based policies in the field of PID is a somewhat time-consuming process for which a variety of factors must be established. From one perspective, the need for accurate screening tests for the identification of PIDs and the development of genetic laboratories as part of prenatal, newborn, and carrier screening programs can be sensed so far. Moreover, designing specific programs for those who are planning consanguineous marriages indicates the role of education in PID prevention, as mentioned before. In order to achieve these goals, a developed referral system is needed to further

reduce the delay time of diagnosis and complications prevention. The cost and availability of life-saving treatments such as IVIG and HSCT are now major issues in the course of treatment, requiring more assistance from insurance companies and patient support organizations. The development of national guidelines for the provision of equal access to treatment seems to be vital. Currently, research in the field of PIDs suffers from the shortage of specified centers focusing on PIDs and is limited to the centers previously named. Although encouraging the current research centers to develop research groups for PIDs are important steps in this process, the establishment of a defined program to train new researchers and scientists should not be neglected. Recent developments in the area of molecular, cellular, and clinical characteristics of genetically determined PIDs have paved the way for accelerated improvement in identifying the genetic bases of newly defined PIDs.

Conflict of interest

The authors declare no conflicts of interest.

References

1.Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol. 2018 Jan;38(1):96-128.

2.Tauber AI, Chernyak L. The birth of immunology. II. Metchnikoff and his critics. Cellular immunology. 1989 Jul;121(2):447-73.

3.von Behring E, Kitasato S. [The mechanism of diphtheria immunity and tetanus immunity in animals. 1890]. Molecular immunology. 1991 Dec;28(12):1317, 9-20.

4.Alper CA, Rosen FS, editors. Inherited deficiencies of complement proteins in man. Springer seminars in immunopathology; 1984: Springer.

5.Schultz W. Ueber eigenartige halserkrankungen. Dtsch Med Wochenschr. 1922;48(1495):7.

6.Syllaba L, Henner K. Contribution an l'independence de l'athetose double idiopathique et congenitale. Rev Neurol. 1926;1(541-562):147.

7.Wiskott A. Familiarer, angeborener morbus werlhofii? Monatsschr Kinderheilkd. 1937;68:212-6

8.Bruton OC. Agammaglobulinemia. Pediatrics. 1952 Jun;9(6):722-8.

9.Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. Journal of clinical immunology. 2015 Nov;35(8):696-726.

10.Rezaei N, Aghamohammadi A, Notarangelo LD. Primary immunodeficiency diseases: Definition, diagnosis, and management: Springer Science & Business Media; 2008.

11.Rezaei N. Obituary: Abolhassan Farhoudi (1924-2006). Iranian Journal of Allergy, Asthma and Immunology. 2006;5(1):1-2.

12.Farhoudi A. Cell-mediated immunodeficiency after BCG vaccination. Developments in biological standardization. 1986;58 (Pt A):347-9.

13.Farhoudi AH. [Recurrent pneumococcal meningitis associated with C3 deficiency]. Presse medicale (Paris, France : 1983). 1988 Apr 16;17(14):696.

14.Dashti-Khavidaki S, Aghamohammadi A, Farshadi F, Movahedi M, Parvaneh N, Pouladi N, et al. Adverse reactions of prophylactic intravenous immunoglobulin; a 13-year experience with 3004 infusions in Iranian patients with primary immunodeficiency diseases. Journal of investigational allergology & clinical immunology. 2009;19(2):139-45.

15.Aghamohammadi A, Moin M, Farhoudi A, Rezaei N, Pourpak Z, Movahedi M, et al. Efficacy of intravenous immunoglobulin on the prevention of pneumonia in patients with agammaglobulinemia. FEMS immunology and medical microbiology. 2004 Mar 8;40(2):113-8.

16.Rezaei N, Abolhassani H, Aghamohammadi A, Ochs HD. Indications and safety of intravenous and subcutaneous immunoglobulin therapy. Expert review of clinical immunology. 2011 May;7(3):301-16.

17.Mozaffari H, Pourpak Z, Pourseyed S, Moin M, Farhoodi A, Aghamohammadi A, et al. Health-related quality of life in primary immune deficient patients. Iranian journal of allergy, asthma, and immunology. 2006 Mar;5(1):23.

18.Aghamohammadi A, Pouladi N, Parvaneh N, Yeganeh M, Movahedi M, Gharagolou M, et al. Mortality and morbidity in common variable immunodeficiency. Journal of tropical pediatrics. 2007 Feb;53(1):32-8.

19.Mir Saeid Ghazi B, Aghamohammadi A, Kouhi A, Farhoudi A, Moin M, Rezaei N, et al. Mortality in primary immunodeficient patients, registered in Iranian primary immunodeficiency registry. Iranian journal of allergy, asthma, and immunology. 2004 Mar;3(1):31-6.

20.Pourpak Z, Aghamohammadi A, Sedighipour L, Farhoudi A, Movahedi M, Gharagozlou M, et al. Effect of regular intravenous immunoglobulin therapy on prevention of pneumonia in patients with common variable immunodeficiency. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi. 2006 Apr;39(2):114-20.

21.Salehzadeh M, Aghamohammadi A, Rezaei N. Evaluation of immunoglobulin levels and infection rate in patients with common variable immunodeficiency after immunoglobulin replacement therapy. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi. 2010 Feb;43(1):11-7.

22.Abolhassani H, Sadaghiani MS, Aghamohammadi A, Ochs HD, Rezaei N. Home-based subcutaneous immunoglobulin versus hospital-based intravenous immunoglobulin in treatment of primary antibody deficiencies: systematic review and meta analysis. Journal of clinical immunology. 2012 Dec;32(6):1180-92.

23.Aghamohammadi A, Farhoudi A, Moin M, Pourpak Z, Rezaei N, Nikzad M, et al. Adverse effects of intravenous immunoglobulin therapy in patients with antibody deficiency. Iranian journal of allergy, asthma, and immunology. 2003 Sep;2(3):121-6.

24.Abolhassani H, Aghamohammadi A, Abolhassani F, Eftekhar H, Heidarnia M, Rezaei N. Health policy for common variable

immunodeficiency: burden of the disease. Journal of investigational allergology & clinical immunology. 2011;21(6):454-8.

25.Rezaei N, Aghamohammadi A, Moin M, Pourpak Z, Movahedi M, Gharagozlou M, et al. Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian Primary Immunodeficiency Registry. Journal of clinical immunology. 2006 Nov;26(6):519-32.

26.Farhoudi A, Aghamohammadi A, Moin M, Rezaei N, Pourpak Z, Movahedi M, et al. Distribution of primary immunodeficiency disorders diagnosed in the Children's Medical Center in Iran. Journal of investigational allergology & clinical immunology. 2005;15(3):177-82.

27.Aghamohammadi A, Abolhassani H, Mohammadinejad P, Rezaei N. The approach to children with recurrent infections. Iranian journal of allergy, asthma, and immunology. 2012 Jun;11(2):89-109.

28.Karimi A, Isaiyan A, Aghamohammadi A, Moin M, Zandiyeh F, Dini AT,et al. Immunological evaluation of children with recurrent ear, nose, and throat (ENT) infections. Iranian journal of pediatrics. 2007;17(Suppl 1):5-13.

29.McPhee SJ, Lo B, Saika GY, Meltzer R. How good is communication between primary care physicians and subspecialty consultants? Archives of internal medicine. 1984 Jun;144(6):1265-8.

30.Yazdani R, Heydari A, Azizi G, Abolhassani H, Aghamohammadi A. Asthma and Allergic Diseases in a Selected Group of Patients With Common Variable Immunodeficiency. Journal of investigational allergology & clinical immunology. 2016;26(3):209-11.

31.Abolhassani H, Farrokhi AS, Pourhamdi S, Mohammadinejad P, Sadeghi B, Moazzeni S-M, et al. Expression of activation-induced cytidine deaminase gene in B lymphocytes of patients with common variable immunodeficiency. Iranian journal of pediatrics. 2013;23(4):451.

32.Abolhassani H, Sagvand BT, Shokuhfar T, Mirminachi B, Rezaei N, Aghamohammadi A. A review on guidelines for management and treatment of common variable immunodeficiency. Expert review of clinical immunology. 2014.

33.Ghazi BMS, Aghamohammadi A, Kouhi A, Farhoudi A, Moin M, Rezaei N, et al. Mortality in primary immunodeficient patients, registered in Iranian primary immunodeficiency registry. Iranian Journal of Allergy, Asthma and Immunology. 2004;3(1):31-5.

34.Aghamohammadi A, Fiorini M, Moin M, Parvaneh N, Teimourian S, Yeganeh M, et al. Clinical, immunological and molecular characteristics of 37 Iranian patients with X-linked agammaglobulinemia. International archives of allergy and immunology. 2006;141(4):408-14.

35.Moin M, Aghamohammadi A, Farhoudi A, Pourpak Z, Rezaei N, Movahedi M, et al. X-linked agammaglobulinemia: a survey of 33 Iranian patients. Immunological investigations. 2004;33(1):81-93.

36.Mohammadzadeh I, Shahbaznejad L, Wang N, Farhadi E, Aghamohammadi A, Hammarström L, et al. A novel CD40 ligand mutation in a patient with

pneumonia, neutropenia, and hyperimmunoglobulin M phenotype. Journal of investigational allergology & clinical immunology. 2012;23(1):50-1.

37.Atarod L, Aghamohammadi A, Moin M, Kanegane H, Rezaei N, Kalantari KR, et al. Successful management of neutropenia in a patient with CD40 ligand deficiency by immunoglobulin replacement therapy. Iranian Journal of Allergy, Asthma and Immunology. 2007;6(1):37-41.

38.Aghamohammadi A, Mohammadi J, Parvaneh N, Rezaei N, Moin M, Espanol T, et al. Progression of selective IgA deficiency to common variable immunodeficiency. International archives of allergy and immunology. 2008;147(2):87-92.

39.Nikfarjam J, Shahrabi M, Pourpak Z, Nikfarjam L, Kouhkan A, Aghamohammadi A, et al. Oral manifestations in selective IgA deficiency. International journal of dental hygiene. 2004;2(1):19-25.

40.Soheili H, Abolhassani H, Arandi N, Khazaei HA, Shahinpour S, Hirbod-Mobarakeh A, et al. Evaluation of natural regulatory T cells in subjects with selective IgA deficiency: from senior idea to novel opportunities. International archives of allergy and immunology. 2012;160(2):208-14.

41.Farhoudi A, Chavoshzadeh Z, Ghazi BMS, Aghamohammadi A, Gharagozlou M. Recurrent infections and bilateral uveitis in a patient with CD8 deficiency. receptor. 2005;3:5.

42.Yeganeh M, Heidarzade M, Pourpak Z, Parvaneh N, Rezaei N, Gharagozlou M, et al. Severe combined immunodeficiency: a cohort of 40 patients. Pediatric Allergy and Immunology. 2008;19(4):303-6.

43.Norouzi S, Aghamohammadi A, Mamishi S, Rosenzweig SD, Rezaei N. Bacillus Calmette-Guérin (BCG) complications associated with primary immunodeficiency diseases. Journal of Infection. 2012;64(6):543-54.

44.Moin M, Aghamohammadi A, Kouhi A, Tavassoli S, Rezaei N, Ghaffari S-R, et al. Ataxia-telangiectasia in Iran: clinical and laboratory features of 104 patients. Pediatric neurology. 2007;37(1):21-8.

45.Isaian A, Bogdanova NV, Houshmand M, Movahadi M, Agamohammadi A, Rezaei N, et al. BAK, BAX, and NBK/BIK proapoptotic gene alterations in Iranian patients with Ataxia Telangiectasia. Journal of clinical immunology. 2010;30(1):132-7.

46.Moghtaderi M, Kashef S, Rezaei N. Interstitial lung disease in a patient with chronic granulomatous disease. Iranian journal of pediatrics. 2012 Mar;22(1):129-33.

47.Movahedi M, Aghamohammadi A, Rezaei N, Shahnavaz N, Babaei Jandaghi A, Farhoudi A, et al. Chronic granulomatous disease: a clinical survey of 41patients from the Iranian primary immunodeficiency registry. International archives of allergy and immunology. 2004;134(3):253-9.

48.Farhoudi A, Chavoshzadeh Z, Pourpak Z, Izadyar M, Gharagozlou M, Movahedi M, et al. Report of six cases of chediak-higashi syndrome with regard to clinical and laboratory findings. Iranian journal of allergy, asthma, and immunology. 2003 Dec;2(4):189-92.

49.Eghbali A, Eshghi P, Malek F, Rezaei N. Cardiac and renal malformations in a patient with

sepsis and severe congenital neutropenia. Iranian journal of pediatrics. 2010;20(2):225.

50.Rezaei N, Farhoudi A, Ramyar A, Pourpak Z, Aghamohammadi A, Mohammadpour B, et al. Congenital neutropenia and primary immunodeficiency disorders: a survey of 26 Iranian patients. Journal of pediatric hematology/oncology. 2005;27(7):351-6.

51.Rezaei N, Farhoudi A, Pourpak Z, Aghamohammadi A, Moin M, Movahedi M, et al. Neutropenia in Iranian patients with primary immunodeficiency disorders. Haematologica. 2005;90(4):554-6.

52.Rezaei N, Farhoudi A, Pourpak Z, Aghamohammadi A, Ramyar A, Moin M, et al. Clinical and laboratory findings in Iranian children with cyclic neutropenia. Iranian Journal of Allergy, Asthma and Immunology. 2004;3(1):37-40.

53. Rezaei N, Farhoudi A, Pourpak Z, Aghamohammadi A, Moin M, Gharagozlou M, et al. Neutropenia in patients with primary antibody deficiency disorders. Iranian Journal of Allergy, Asthma and Immunology. 2004;3(2):77-82.

54. Rezaei N, Aghamohammadi A. Hyper-IgE syndrome. Journal of postgraduate medicine. 2010 Apr-Jun;56(2):63-4.

55. Moin M, Farhoudi A, Movahedi M, Rezaei N, Pourpak Z, Yeganeh M, et al. The clinical and laboratory survey of Iranian patients with hyper-IgE syndrome. Scandinavian journal of infectious diseases. 2006;38(10):898-903.

56. Behmanesh F. Leukocyte Adhesion Deficiency; Case Report. Iranian journal of pediatrics. 2007;17(Suppl 2):311-3.

57. Movahedi M, Entezari N, Pourpak Z, Mamishi S, Chavoshzadeh Z, Gharagozlou M, et al. Clinical and laboratory findings in Iranian patients with leukocyte adhesion deficiency (study of 15 cases). Journal of clinical immunology. 2007;27(3):302-7.

58. Parvaneh N, Mamishi S, Rezaei A, Rezaei N, Tamizifar B, Parvaneh L, et al. Characterization of 11 new cases of leukocyte adhesion deficiency type 1 with seven novel mutations in the ITGB2 gene. Journal of clinical immunology. 2010;30(5):756-60.

59. Ashrafi MR, Mohseni M, Yazdani S, Alizadeh H, Ramyar A, Aghamohammadi A, et al. Bilateral basal ganglia involvement in a patient with Griscelli syndrome. European Journal of Paediatric Neurology. 2006;10(4):207-9.

60. Aghamohammadi A, Kanegane H, Moein M, Farhoudi A, Pourpak Z, Movahedi M, et al. Identification of anSH2D1A mutation in a hypogammaglobulinemic male patient with a diagnosis of common variable immunodeficiency. International journal of hematology. 2003;78(1):45-7.

61. Farhadi E, Nemati S, Amirzargar A, Hirbod-Mobarakeh A, Nabavi M, Soltani S, et al. AICDA single nucleotide polymorphism in common variable immunodeficiency and selective IgA deficiency. Allergologia et immunopathologia. 2014;42(5):422-6.

62.Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schäffer AA, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia(Kostmann disease). Nature genetics. 2007;39(1):86-92.

63. Boztug K, Appaswamy G, Ashikov A, Schäffer AA, Salzer U, Diestelhorst J, et al. A syndrome with congenital neutropenia and mutations in G6PC3. New England Journal of Medicine. 2009;360(1):32-43.

64. Salipante SJ, Benson KF, Luty J, Hadavi V, Kariminejad R, Kariminejad MH, et al. Double de novo mutations of ELA2 in cyclic and severe congenital neutropenia. Human mutation. 2007;28(9):874-81.

65. Glocker E-O, Hennigs A, Nabavi M, Schäffer AA, Woellner C, Salzer U, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. New England Journal of Medicine. 2009;361(18):1727-35.

66. Vogt G, Chapgier A, Yang K, Chuzhanova N, Feinberg J, Fieschi C, et al. Gains of glycosylation comprise an unexpectedly large group of pathogenic mutations. Nature genetics. 2005;37(7):692-700.

67. Saghafi S, Pourpak Z, Nussbaumer F, Fazlollahi MR, Houshmand M, Hamidieh AA, et al. DOCK8 deficiency in six Iranian patients. Clinical Case Reports. 2016;4(6):593-600.

68. Abdollahpour H, Appaswamy G, Kotlarz D, Diestelhorst J, Beier R, Schäffer AA, et al. The phenotype of human STK4 deficiency. Blood. 2012;119(15):3450-7.

69. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z, et al. Spectrum of phenotypes associated with mutations in LRBA. Journal of clinical immunology. 2016;36(1):33-45.

70. Rezaei N, Aghamohammadi A, Mahmoudi M, Shakiba Y, Kardar GA, Mahmoudi M, et al. Association of IL-4 and IL-10 gene promoter polymorphisms with common variable immunodeficiency.Immunobiology. 2010;215(1):81-7.

71. Javad M, Zahra P, Sara J, Shiva S, Kazem Z, Akbar PA, et al. Human leukocyte antigens (HLA) associated with selective IgA deficiency in Iran and Sweden. Iranian Journal of Allergy, Asthma and Immunology. 2008;7(4):209-14.

72. Kiani-amin M, Daneshi M, Ayazi P, Mohammadian S, Rezaei N. Serum immunoglobulin levels in splenectomized and non-splenectomized patients with major Beta-thalassemia. Iranian journal of pediatrics. 2011;21(1):95.

73. Rezaei N, Abolhassani H, Kasraian A, Mohammadinejad P, Sadeghi B, Aghamohammadi, A. Family study of pediatric patients with primary antibody deficiencies. Iranian Journal of Allergy, Asthma and Immunology. 2013;12(4):377.

74. Rezaei N, Mohammadinejad P, Aghamohammadi A. The demographics of primary immunodeficiency diseases across the unique ethnic groups in Iran, and approaches to diagnosis and treatment. Annals of the New York Academy of Sciences. 2011;1238(1):24-32.

75. Rezaei N, Pourpak Z, Aghamohammadi A, Farhoudi A, Movahedi M, Gharagozlou M, et al. Consanguinity in primary immunodeficiency disorders; the report from Iranian Primary Immunodeficiency Registry. American Journal of Reproductive Immunology. 2006;56(2):145-51.

76. Farhoudi A, Aghamohammadi A, Moin M, Rezaei N, Pourpak Z, Movahedi M, et al.

Distribution of primary immunodeficiency disorders diagnosed in the Children's Medical Center in Iran. Journal of Investigational Allergology and Clinical Immunology. 2005;15(3):177.

77. Aghamohammadi A, Moin M, Rezaei N. History of primary immunodeficiency diseases in Iran. Iranian journal of pediatrics. 2010;20(1):16-34.

78. Mozaffari H, Pourpak Z, Pourseyed S, Moin M, Farhoodi A, Aghamohammadi A, et al. Health-related quality of life in primary immune deficient patients. Iranian Journal of Allergy, Asthma and Immunology. 2006;5(1):23-7.

79. Aghamohammadi A, Moein M, Farhoudi A, Pourpak Z, Rezaei N, Abolmaali K, et al. Primary immunodeficiency in Iran: first report of the National Registry of PID in Children and Adults. Journal of clinical immunology. 2002;22(6):375-80.

80. Rezaei N, Aghamohammadi A, Moin M, Pourpak Z, Movahedi M, Gharagozlou M, et al. Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian Primary Immunodeficiency Registry. Journal of clinical immunology. 2006;26(6):519-32.

81. Aghamohammadi A, Mohammadinejad P, Abolhassani H, Mirminachi B, Movahedi M, Gharagozlou M, et al. Primary immunodeficiency disorders in Iran: update and new insights from the third report of the national registry. Journal of clinical immunology. 2014;34(4):478-90.

82. Abolhassani H, Aghamohammadi A, Abolhassani F, Eftekhar H, Heidarnia M, Rezaei N. 3 Health Policy for Common Variable Immunodeficiency: Burden of the Disease. Journal of Investigational Allergology and Clinical Immunology. 2011;21(6):454.

83. Ghavamzadeh A, Alimoghaddam K, Ghaffari F, Derakhshandeh R, Jalali A, Jahani M. Twenty years of experience on stem cell transplantation in Iran. Iranian Red Crescent Medical Journal. 2013;15(2):93.

84. Hamidieh A, Behfar M, Babaki A, Jalali A, Hosseini A, Jahani M, et al. Hematopoietic SCT in Iranian children 1991–2012. Bone marrow transplantation. 2015;50(4):517-22.

85. Nourijelyani K, Aghamohammadi A, Sadaghiani MS, Behniafard N, Abolhassani H, Pourjabar S, et al. Physicians awareness on primary immunodeficiency disorders in Iran. Iranian Journal of Allergy,Asthma and Immunology.2012; 11(1):57-64.

86. Isaian A, Moin M, Pourpak Z, Rezaei N, Aghamohammadi A, Movahedi M, et al. DNA banking of primary immunodeficiency disorders in Iran. Iranian Journal of Allergy, Asthma and Immunology. 2006;5(4):201-2.

Immunology and Genetics Journal (2018) 1:28-37
Doi: 10.22034/igj.2018.69305

Original Articles

Genetic Association in Familial Common Variable Immunodeficiency (CVID) and IgA Deficiency (IgAD)

Javad Mohammadi[1]*, Lennart Hammarström[2]

Received: 17 April 2018 /Accepted: 24 August 2018 /Published online: 22 September 2018

Background/objectives Common variable immunodeficiency (CVID) is a heterogeneous syndrome described by defective antibody production and occurrence of multiple clinical manifestations including autoimmune, lymphoproliferative, and granulomatous diseases. Genes within the major histocompatibility complex (MHC) region have previously been reported to be involved in the pathogenesis of the disease.

Methods To elucidate the human leukocyte antigen (HLA) association, PCR was performed to clarify type HLA B, DR, and DQ alleles in a large sample of Iranian and Swedish CVID patients.

Results No HLA association was observed between Iranian patients with "sporadic" CVID (n=50) and controls. A slight HLA association (B8, DR3, DQ2) was found in Swedish CVID patients (n=95). However, the latter was entirely due to an association in the familial form of the disease. Using 13 informative multiplex families with patients affected by CVID and IgA deficiency (IgAD), shared haplotypes such as HLA-B8-DR3-DQ2; HLA-DR1-DQ5; HLA- DR4- DQ3, and HLA-DR7-DQ2 were observed.

Conclusions Based on our results, we hypothesize that only the familial form of CVID/IgAD may have a common HLA-associated genetic background, whereas "sporadic" cases show no HLA association.

Keywords Common variable immunodeficiency; IgA deficiency HLA; sporadic

* **Corresponding author**: Javad Mohammadi

drjmohamadi@yahoo.com

1 Department of Life Science, Faculty of New Science and Technology, University of Tehran, Tehran, Iran.

2 Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, SE-141 86 Stockholm, Sweden

Introduction

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency syndrome. It is identified by decreased IgA, IgG levels and, in some patients, reduced IgM levels. These patients have various clinical manifestations, including frequent respiratory and gastrointestinal tract infections, autoimmune and allergic diseases (for review, see [1]).

Selective IgA deficiency (IgAD) is the most common primary immunodeficiency disorder in Caucasians. It is characterized by a reduced serum IgA level (<0.07 g/l) and normal serum levels of IgM and IgG. The majority of these individuals do not manifest disease, whereas some suffer from recurrent infections at mucosal sites, allergies, and autoimmune manifestations (2).

CVID shares many clinical features with IgAD (3), and progression from IgAD to CVID has been reported in several cases (reviewed in [4]). Recessive and dominant modes of inheritance have both been suggested, and IgAD and CVID have occasionally been observed in different members of the same family, suggesting that the same genetic defect may underlie both diseases. In support of this notion, a similar genetic (HLA) predisposition has been found in both CVID and IgAD (5, 6). However, the causal gene defects leading to IgAD and CVID remain unknown. It is likely that the disorders are due to variances in etiologies, and to identify the susceptibility gene(s) for development of the disease, homogenous groups of patients must be investigated.

The aim of this study was to investigate whether a subgroup of CVID patients could be identified using HLA as a selection marker.

Materials and methods

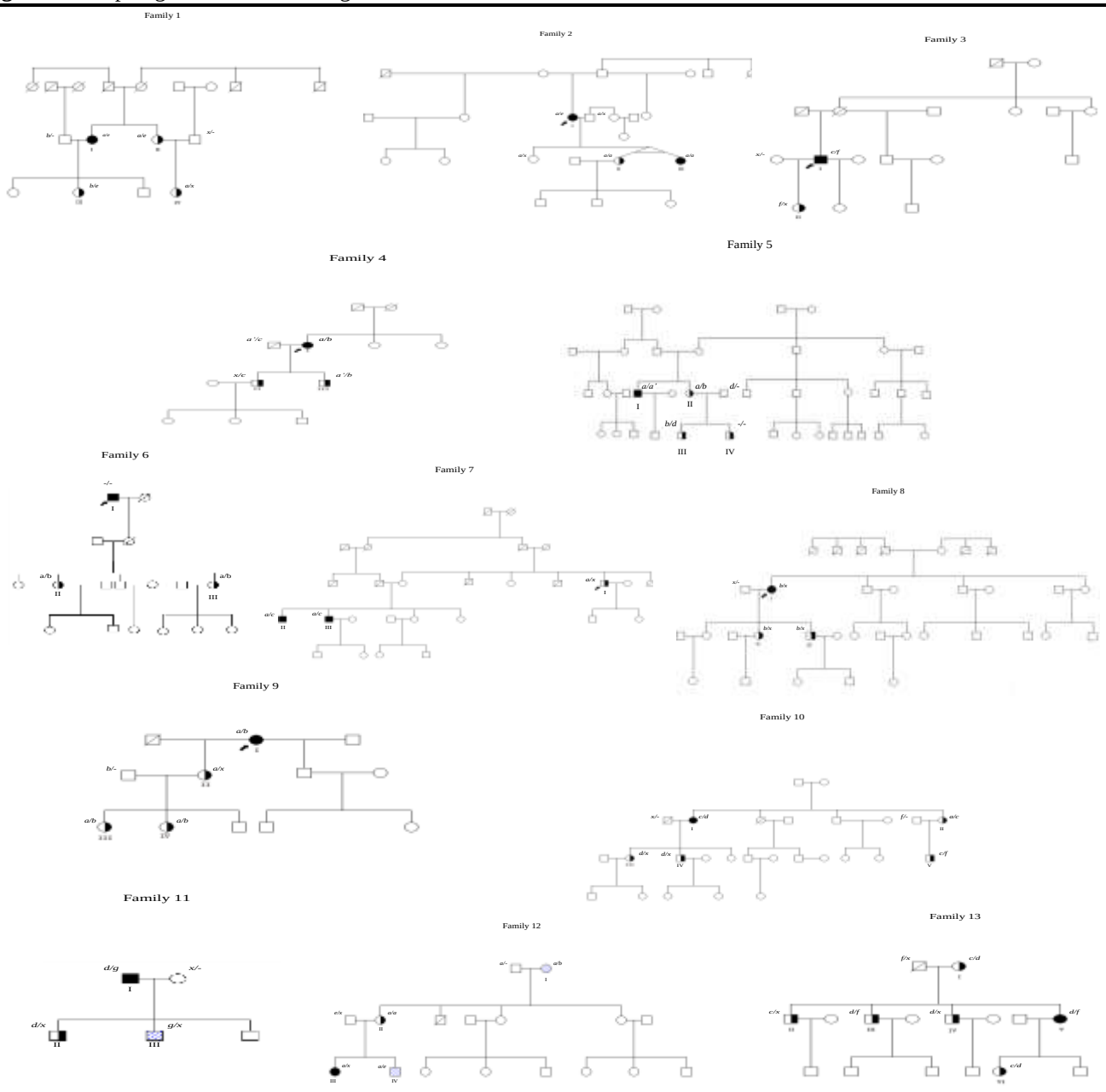
CVID patients

Three groups of Caucasian CVID patients were studied: Iranian, Swedish, and German. Fifty Iranian CVID patients with no family history of immunodeficiency (up to now sporadic) and who were referred to the Immunology, Asthma, and Allergy Research Institute in Tehran, were included in the study as were Iranian, ethnically matched controls (84 typed for HLA B and 180 typed for HLA DR and DQ). All patients and controls were unrelated Iranian Caucasians. The pedigrees of CVID and IgAD families are demonstrated in **Figure 1**.

For comparison, 95 CVID patients, diagnosed at the Immunodeficiency Unit at the Karolinska University Hospital Huddinge in Stockholm, were also included in the study as were tissue typing data on 41021 Swedish ethnically matched controls (41,021 typed for HLA B, 11,678 for HLA DR, and 672 for HLA DQ) from the volunteer bone marrow donor registry (Tobias registry, www.tobiasregistret.se). Most cases were sporadic (n=84), but 11 had family members with immunodeficiencies (multiplex families comprising.

13 subjects with CVID and 21 with IgAD). In addition, three German multiplex families with 3 members with CVID, 7 with IgAD, and 3 with dysgammaglobulinemia were included in this study.

Figure 1. The pedigrees of CVID and IgAD families



In family I, affected members including I (CVID), II (IgAD) and IV (IgAD) all share the haplotype *a*. In family 2, two haploidentical siblings-CVID (III) and IgAD (II) have inherited the same haplotype (*a*) in a homozygous form from their parents. Their mother (proband) suffers from CVID (I). The patients' father, however, HLA identical to the affected siblings, was not affected; Arrow = Proband. In family 3, the IgAD daughter inherited the same haplotype (*f*) from her mother with CVID. In family 4, the concurrence of CVID and IgAD in a mother and her sons, respectively is shown. Patients I and III shared haplotype *a'*. IgAD patient II has a different haplotype from his mother. In the latter patient, a recombination event may have happened between the DQ5 and DR regions. In family 5, the IgAD mother gave the haplotype *b* to her IgAD children. However, her haploidentical (*a*) brother with CVID did not have antibody deficient children. In family 6, two haploidentical sisters (II, III) are IgAD. Their grandfather suffered from CVID (I). In family 7, two haploidentical brothers with CVID have an IgAD uncle with the same haplotype (*a*). In family 8, two siblings with IgAD have inherited the same haplotype (*b*) from their mother who suffers from CVID.

In family 9, three generations with CVID and IgAD possessed the same haplotype (*a*). Also, two sisters with IgAD (III, IV) carried haplotype *b*, similar to their grandmother (I).
In family number 10, two IgAD siblings have inherited the same haplotype (*d*) from their mother with CVID. Their IgAD aunt (II) transmitted the same haplotype (*c*) to her son (V) that is identical to her sister (patient I). In family 11, the IgAD patient inherited the haplotype *d* from his father with CVID. His brother, who has IgG subclass deficiency and IgA deficiency, inherited haplotype *g* from his father as well. The shaded box or circle = patients in families who have IgG subclass deficiency with IgA deficiency and no recurrent infections. In family 12, all immunodeficient individuals (I, II, III, IV) in three generations possessed identical haplotype (*a*). Individual number I and her grandson (IV) both have low levels of IgG subclass and IgA. The mother with IgAD is homozygous for the haplotype *a*. In family 13, the grandmother with IgAD (I) distributed identical haplotype (*d*) to her three affected children, two with IgAD (III, IV) and one with CVID (V). Also, the CVID patient transmitted haplotype *d* to her daughter with IgAD (VI). On the other hand the IgAD son (II) inherited the haplotype *c* from his mother with IgAD (I).
• Solid box or circle = Common variable immunodeficiency, Semisolid box or circle = IgA deficiency. Haplotype HLA B8, DR3, DQ2 = a-, Haplotype HLA DR3, DQ2 = a'-, Haplotype HLA DR1, DQ5 = b, Haplotype HLA DR4, DQ3 = c, Haplotype HLA DR7, DQ2 = d, Haplotype HLA DR7, DQ3 = e, Haplotype HLA DR15, DQ6 = f, Haplotype HLA B35, DR14, DQ5 = g, other haplotypes = x, Haplotype same with x in two alleles = x', Not determined haplotypes = -.

IgAD patients

To determine the HLA association between CVID and IgAD, data on 386 Swedish sporadic and 56 Swedish familial IgAD patients and 29 Iranian sporadic patients with IgAD were also analyzed in this study. All Iranian and 299 (49 familial and 250 sporadic) Swedish individuals with IgAD who were included in the present study have been described previously (7).

Serum immunoglobulin levels

Serum levels of IgG, IgA and IgM were measured by nephelometry. The diagnosis of CVID and IgAD was made using ESID criteria (www.esid.org).

HLA typing

Samples were genotyped at the HLA B, DR, and DQ loci using PCR-SSP (8). The kits utilized in the present study included the HLA-B low resolution (L21, M26, J82, N80, N02, R56, X13, X82, Y52), the HLA-DQ-DR SSP Combi Tray (K88, R60, V95, M01, M84), and the HLA DQB1*06 high resolution (L 46) from Olerup SSP AB, Saltsjöbaden, Sweden.

Statistical analyses

The analysis was performed by the Stata statistical program, and the frequencies of the HLA alleles and haplotypes were compared using Hardy-Weinberg equilibrium, 2x2 contingency tables, chi-square analysis, and Fisher's exact test and the Bonferroni method. IgAD and CVID were associated with multiple alleles or haplotypes in the MHC region; thus, the relative predisposition effects (RPEs) method (9) was used to determine several associations.

Results

No HLA association was found in Iranian patients with sporadic CVID as compared with controls. The comparison of HLA alleles between Iranian CVID patients and Iranian IgAD patients showed no similarity between their MHC class I and II alleles (all $p<0.05$). A slight, negative association with HLA B15 ($p=0.038$) was found in the Swedish CVID patients (combined sporadic and familial).

In Swedish sporadic CVID patients (data not shown), a positive association with HLA DR9 ($p=0.03$) was observed, and 16 out of 84 (19%) carried the HLA B8, DR3, DQ2 haplotype, one in a homozygous form (**Table 1**). Four (4.2%) Swedish

CVID patients carried the HLA DQ2 allele in a homozygous form; however, this frequency was not significantly different from the controls.

Interestingly, in the Swedish familial CVID patients, a significant increase in the frequency of the HLA B8, DR3, and DQ2 alleles was observed (**Table 2**) as were the complete HLA B8, DR3, DQ2 and the HLA DR7, DQ2 haplotypes in a heterozygous form (**Table 1**).

Analysis of the HLA alleles shared between Swedish CVID (combined familial and sporadic) and IgAD (combined familial and sporadic) patients showed a similarity in HLA DR7 only ($p>0.05$). In Swedish individuals with sporadic IgAD (n=386), a strong association with the HLA B8, B12, B13, B14, DR1, DR3, DR5, DR6, DR7, DR9, DQ2, DQ5, and DQ6 (non-DQ0602) alleles as well as a negative association with the HLA B7, B15, DR2 and DQ0602 alleles was found (Table 3). In this group of patients, an increased frequency of subjects homozygous and heterozygous for the complete HLA B8, DR3, DQ2; HLA DR1, DQ5; HLA DR7, DQ2 and the HLA DR13, DQ6 haplotypes was also noted (**Table 4**). In subjects positive for the DR7, DQ2 haplotype, the most frequent inferred class I allele was B44 (n=16) followed by B13 (n=15).

In individuals with familial IgAD, a strong association with the HLA B8, DR3, DR7, and DQ2 alleles was also seen (**Table 3**). In the latter IgAD group, heterozygosity for the HLA B8, DR3, DQ2 haplotype and homozygosity and heterozygosity (a borderline association) for the HLA DR7, DQ2 haplotype constituted risk factors for the development of IgAD (**Table 4**). In addition, among individuals with familial IgAD positive for the HLA DR7, DQ2 haplotype, the most frequent inferred class I allele was B44 (n=3).

Assessment of the HLA antigens in Swedish familial CVID and familial IgAD (patients with family members with immunodeficiency) showed a sharing of the HLA B8, DR3, DR7, and DQ2 alleles (all $p>0.05$, **Table 5**). Furthermore, a sharing of the B15 ($p>0.05$) and the DR7 alleles ($p>0.05$) in Swedish sporadic CVID and Swedish sporadic IgAD was also found.

Table 1. Associations of the HLA B8, DR3, DQ2 and the HLA DR7, DQ2 haplotypes in Swedish patients with familial and sporadic CVID compared to controls

Haplotype	Form	Familial (*n*=11) [a]	*P* value	Sporadic (*n*=84)	*P* value	Controls [a]
B8, DR3, DQ2	Homozygous	1 (9%)	NS [b]	1 (1.2%)	NS	283 (1.3%)
	Heterozygous	6 (27.3%) [c]	0.002	18 (810.7%)	NS	3581 (8.5%)
DR7, DQ2	Homozygous	0 (0%)	NS	2 (2.4%)	NS	1 (0.5%)
	Heterozygous	3 (13.6%)	0.01	6 (3.6%)	NS	55 (4.1%)

[a] Number of patients and controls (for the HLA B8, DR3, DQ2 =21108 and for the HLA DR7, DQ2=672)
[a] NS=Not significant
[c] The percentage of heterozygous individuals based on number of haplotypes

Table 2. HLA associations (allele frequency) in Swedish familial CVID patients compared to controls

HLA	CVID (*n*=22) [a]	Controls [a, b]	*P* value [c]	OR (CI)
B8	8 (36.4%)	9448 (11.5%)	0.006	4.4 (1.6-11.2)
DR3	9 (40.9%)	2355 (10.1%)	3.3×10^{-5}	6.2 (2.3-15.6)
DQ2	11 (50%)	259 (19.3%)	0.006	4.2 (1.6-10.8)

[a] Allele frequency
[b] HLA B= 82042, DR n=23356, DQ n=1344
[c] The Bonferroni method was used for correction of the *p* value

Table 3. Allele frequency in Swedish individuals with IgAD (familial and sporadic) compared to controls

Allele	Familial	*P* value [b], OR	Sporadic	*P* value, OR	Controls [c]
	Case (n=112) [a]		Case (n=772)		
B7	10	NS	51	0.004, 0.55	12152
B8	34	1.4×10^{-8}, 3.4	199	1.9×10^{-36}, 2.8	9448
B12	16	NS	115	0.0007, 1.6	10600
B13	3	NS	25	3.2×10^{-7}, 3.1	1270
B14	4	NS	32	8.9×10^{-9}, 3.1	1590
B15	11	NS	26	4.1×10^{-5}, 0.38	9285
DR1	11	NS	111	2.4×10^{-9}, 2.1	2602
DR2	5	NS	26	0.004, 0.4	3508
DR3	43	1.8×10^{-21}, 5.6	218	1.5×10^{-57}, 3.5	2355
DR5	7	NS	44	0.03, 1.8	1805
DR6	13	NS	133	0.004, 1.5	3724
DR7	14	0.03, 2.6	108	4.1×10^{-30}, 3.8	1853
DR9	1	NS	13	0.02, 2.7	363
DQ2	57	1.9×10^{-13}, 4.3	312	2.2×10^{-25}, 2.9	259
DQ5	19	NS	143	7.8×10^{-5}, 1.8	222
DQ (non-0602)	7	NS	103	3×10^{-4}, 1.9	180
DQ0602	5	NS	18	1.3×10^{-9}, 0.11	198

[a] Number of familial patients=56; sporadic patients =386

[b] The Bonferroni method was used for correction of the *p* value

[c] Number of controls for the HLA B=82042, DR=23356, and DQ=1344

Table 4. Associations of the HLA B8, DR3, DQ2, the HLA DR7, DQ2, and the HLA DR1, DQ5 haplotypes among Swedish patients with familial and sporadic IgAD

Haplotype	Form	Familial (*n*=56) [a]	*P* value	Sporadic (*n*=386) [a]	*P* value	Controls [b]
B8, DR3, DQ2	Homozygous	2 (3.6%)	NS	24 (6.2%)	1.2×10^{-15}	283 (1.3%)
	Heterozygous	29 (25.9%) [c]	4.5×10^{-11}	158 (20.5%)	1.6×10^{-35}	3581 (8.5%)
DR7, DQ2	Homozygous	2 (3.6%)	0.0001	6 (1.6%)	0.004	1 (0.1%)
	Heterozygous	8 (7.1%)	0.058	76 (9.8%)	6.8×10^{-16}	60 (4.5%)
DR1, DQ5	Homozygous	1 (1.8%)	NS	14 (3.6%)	0.002	7 (1.4%)
	Heterozygous	9 (8%)	NS	81 (10.5%)	0.0002	121 (9%)
DR13, DQ6	Homozygous	0	NS	8 (2.1%)	0.008	3 (0.4%)
	Heterozygous	6 (5.4%)	NS	82 (10.6%)	0.005	124 (9.2%)

[a] Number of patients

[b] Number of controls for the HLA B8, DR3, DQ2 haplotype=21108 and for the HLA DR7, DQ2 and the HLA DR1, DQ5 haplotypes=672

[c] The percentage of heterozygous individuals are based on number of haplotypes

Table 5. Similarities in the HLA B, DR and DQ loci in Swedish familial CVID as compared to Swedish familial IgAD patients [a]

HLA	CVID (*n*=22) [b]	IgAD (*n*=112) [b]	*P* value
B8	8 (36.4%)	34 (30.1%)	>0.05
DR3	9 (40.9%)	43 (38.4%)	>0.05
DR7	3 (13.6%)	14 (12.5%)	>0.05
DQ2	11 (50%)	57 (50.9%)	>0.05

[a] No significant difference in the HLA B8, DR3, DQ2 between the two patient groups was observed.

[b] Allele frequency

Multiplex families

Assessment of the haplotypes in 13 multiplex families (families with both CVID and IgAD, families 1-10 were Swedish and 11-13 were German) with 46 affected individuals showed that 24 out of 46 (52.1%) carried the HLA DR3, DQ2 haplotype (haplotype *a*), 4 of them in a homozygous form. Thirteen out of 46 (28.3%) carried the HLA DR1, DQ5 haplotype (haplotype *b*), 11 out of 46 (23.9%) carried the HLA DR4, DQ3 haplotype (haplotype *c*) and 10 out of 46 (21.7%) carried the HLA DR7, DQ2 haplotype (haplotype *d*) (**Table 6**). Among the 13 multiplex families, three individuals with IgAD and two CVID patients carried the HLA DQB1* 0602 alleles which have been suggested to be protective for the development of IgAD (10).

Table 6. Family data of the multiplex families (CVID patients with IgAD relatives)

			Haplotype 1			Haplotype 2		
Family	**ID**	**Disorder**	**HLA B**	**HLA DR**	**HLA DQ**	**HLA B**	**HLA DR**	**HLADQ**
1	I	CVID	8	3	2	57	7	3
1	II	IgAD	8	3	2	57	7	3
1	III	IgAD	18	1	3	57	7	3
1	IV	IgAD	8	3	2	7	4	3
2	I	CVID	8	3	2	18	4	3
2	II	IgAD	8	3	2	8	3	2
2	III	CVID	8	3	2	8	3	2
3	I	CVID	7	15	6*	55	4	3
3	II	IgAD	7	15	6*	51	13	6
4	I	CVID	8	3	2	7	1	5
4	II	IgAD	7	4	3	7	14	5
4	III	IgAD	40	3	2	35	1	5
5	I	CVID	8	3	2	44	3	2
5	II	IgAD	8	3	2	15	1	5
5	III	IgAD	53	7	2	15	1	5
6	I	CVID	- [a]	-	-	-	-	-
6	II	IgAD	8	3	2	14	1	5
6	III	IgAD	8	3	2	14	1	5
7	I	IgAD	8	3	2	7	12	3
7	II	CVID	8	3	2	47	4	3
7	III	CVID	8	3	2	47	4	3
8	I	CVID	7	13	6	44	1	5
8	II	IgAD	35	14	5	44	1	5
8	III	IgAD	35	14	5	44	1	5
9	I	CVID	8	3	2	27	1	5
9	II	IgAD	8	3	2	27	1	2
9	III	IgAD	8	3	2	27	1	5
9	IV	IgAD	8	3	2	27	1	5
10	I	CVID	27	4	3	44	7	2
10	II	IgAD	35	8	4	44	7	2
10	III	IgAD	35	8	4	44	7	2
10	IV	IgAD	8	3	2	47	3	3
10	V	IgAD	47	4	3	7	15	6*
11	I	CVID	13	7	2	35	14	5
11	II	IgAD	13	7	2	51	13	6
11	III	Dys	51	13	6	35	14	5

12	I	Dys	8	3	2	55	1	5
12	II	IgAD	8	3	2	8	3	2
12	III	Dys	8	3	2	57	7	3
12	IV	CVID	8	3	2	51	12	3
13	I	IgAD	35	4	3	18	7	2
13	II	IgAD	35	4	3	44	13	6
13	III	IgAD	7	15	6*	18	7	2
13	IV	CVID	7	15	6*	18	7	2
13	V	IgAD	44	13	6	18	7	2
13	VI	IgAD	37	4	3	18	7	2

[a]- = Not determined

Discussion

No association was found between HLA antigens and sporadic CVID in Iran, as has also been observed in previous studies in other ethnic populations (3, 11-13). Despite the high prevalence (66.2%) of consanguineous marriages among these families, no familial case was seen (14), suggesting that they may represent a subgroup of the disorder. Furthermore, Iranian individuals with IgAD showed an over-representation of the HLA B14, DR1, and DQ5 alleles (7). However, only 4.1% (2 out of 49) of the Iranian CVID patients carried the HLA B14, DR1, DQ5 haplotype, all in a heterozygous form, a frequency similar to that of the controls (1.2%). The data thus suggested that different predisposing genetic factors in the MHC class I and II regions are involved in the development of sporadic IgAD and CVID in Iran.

The weak association between Swedish sporadic CVID and the HLA DR9 supported the notion of involvement of non-MHC region (more than MHC region) in the development of sporadic CVID.

In Swedish patients with familial CVID, a strong association with the HLA B8, DR3, DQ2 alleles was noted (Table 2), suggesting a different genetic predisposition than the sporadic form of the disease, and the heterozygosity for the HLA B8, DR3, DQ2 and the HLA DR7, DQ2 haplotypes constitutes risk factors for the development of CVID in the familial form (Table 1).

Investigators have previously suggested an additive effect of susceptibility loci with individuals homozygous for the MCH class II loci for the development of CVID (3, 12). However, according to our assessment, the frequencies of the CVID subjects (combined familial and sporadic forms) homozygous for MHC class II alleles were similar to those of the controls (data not shown).

In the present study, which used an additional 136 Swedish sporadic IgAD subjects, in addition to the authors' previous findings (7), a strong association with the HLA DR5, DR6, DR9 and DQ6 (DQ non-0602) alleles was noted; however, the frequency of B40 did not reach a significant level (Table 3). In sporadic IgAD, all risk haplotypes in addition to a new risk haplotype - the HLA DR13, DQ6 - were increased in frequency (Table 4). The number of limited HLA markers shared between the sporadic and familial form of IgAD suggested that these two forms of immunodeficiencies may represent genetically different disorders.

Based on previous observations, only a few individuals with CVID share a common underlying genetic defect with IgAD (3, 11). In a study of multiplex families by Volanakis *et al.* (11), 77% (24 out of 31) of patients with CVID and IgAD carried the whole, or part of, the ancestral HLA B8, DR3 and/or HLA DR7, DQ2 haplotypes.

Subsequently, we investigated Swedish familial CVID and familial IgAD, and a similarity in the HLA B8, DR3, DR7 and DQ2 loci between the two immunodeficiency disorders was observed (Table 5). Strikingly, in the familial form of CVID and IgAD, the HLA DQ2 allele was the most frequent allele (50% and 50.9% respectively) (Table 5), suggesting an influence of this locus for the development of IgAD and CVID in familial cases.

In our sporadic CVID and IgAD patient groups, the weak HLA similarities suggest that different genetic susceptibility elements are involved in the pathogenesis of the two immunodeficiencies in the sporadic form.

Another important observation in the present study was that a few HLA haplotypes were frequently noted in multiplex families. Among these patients, at least one copy of the HLA B8, DR3, DQ2 (haplotype *a*); DR1, DQ5 (haplotype *b*); DR4, DQ3 (haplotype *c*); or DR7, DQ2 (haplotype *d*) haplotypes was observed in 93.3% of cases as has been previously reported (11). No differences were found between CVID and IgAD in terms of the distribution of these haplotypes. These findings provide further support to the hypothesis that IgAD and CVID are associated with particular HLA haplotypes in the familial form (11, 12).

No differences were observed in the distribution of the selected haplotypes in families with autosomal dominant or autosomal recessive inheritance.

In the present study, an increased frequency of familial IgAD with autosomal dominant inheritance (22 out of 28, 78.6%) was observed as compared to familial CVID subjects (3 out of 12, 25%) (p=0.002), suggesting a dominant role for the selected haplotypes in the development of familial IgAD with autosomal dominant mode of inheritance as compared to familial CVID.

Conclusion

The current analysis of the alleles within the MHC class I and class II regions in a large sample size comprised of CVID and IgAD subjects provides evidence that the majority of CVID and IgAD patients (sporadic form) possess different predisposing gene(s) in the MHC region, and that a causal relationship exists between CVID and IgAD in multiplex families. Consequently, a genetic heterogeneity model would suggest that CVID is not a single disease, but rather represents several etiologically and phenotypically distinct diseases presenting a similar clinical picture.

Conflict of interest

The authors declare no conflict of interest.

References

1. Hammarstrom L, Smith CI. Genetic approach to common variable immunodeficiency and IgA deficiency (Ochs, H., Puck, J. Primary immunodeficiency diseases a molecular and genetic

approach) Oxford pp. 313-325: Oxford university press; 2007.

2. Burrows PD, Cooper MD. IgA deficiency. Adv Immunol. 1997;65:245-76.

3. Schroeder HW, Jr., Schroeder HW, 3rd, Sheikh SM. The complex genetics of common variable immunodeficiency. J Investig Med. 2004 Mar;52(2):90-103.

4. Aghamohammadi A, Mohammadi J, Parvaneh N, Rezaei N, Moin M, Espanol T, et al. Progression of selective IgA deficiency to common variable immunodeficiency. Int Arch Allergy Immunol. 2008 Jun 3;147(2):87-92.

5. Oen K, Petty RE, Schroeder ML. Immunoglobulin A deficiency: genetic studies. Tissue Antigens. 1982 Mar;19(3):174-82.

6. Hammarstrom L, Smith CI. HLA-A, B, C and DR antigens in immunoglobulin A deficiency. Tissue Antigens. 1983 Jan;21(1):75-9.

7. Mohammadi J, Pourpak Z, Jarefors S, Saghafi S, Zendehdel K, Pourfathollah AA, et al. Human Leukocyte Antigens (HLA) Associated with Selective IgA Deficiency in Iran and Sweden. Iran J Allergy Asthma Immunol. 2008 Dec;7(4):209-14.

8. Olerup O, Aldener A, Fogdell A. HLA-DQB1 and -DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. Tissue Antigens. 1993 Mar;41(3):119-34.

9. Payami H, Joe S, Farid NR, Stenszky V, Chan SH, Yeo PP, et al. Relative predispositional effects (RPEs) of marker alleles with disease: HLA-DR alleles and Graves disease. Am J Hum Genet. 1989 Oct;45(4):541-6.

10. Olerup O, Smith CI, Bjorkander J, Hammarstrom L. Shared HLA class II-associated genetic susceptibility and resistance, related to the HLA-DQB1 gene, in IgA deficiency and common variable immunodeficiency. Proc Natl Acad Sci U S A. 1992 Nov 15;89(22):10653-7.

11. Volanakis JE, Zhu ZB, Schaffer FM, Macon KJ, Palermos J, Barger BO, et al. Major histocompatibility complex class III genes and susceptibility to immunoglobulin A deficiency and common variable immunodeficiency. J Clin Invest. 1992 Jun;89(6):1914-22.

12. de la Concha EG, Fernandez-Arquero M, Martinez A, Vidal F, Vigil P, Conejero L, et al. HLA class II homozygosity confers susceptibility to common variable immunodeficiency (CVID). Clin Exp Immunol. 1999 Jun;116(3):516-20.

13. Howe HS, So AK, Farrant J, Webster AD. Common variable immunodeficiency is associated with polymorphic markers in the human major histocompatibility complex. Clin Exp Immunol. 1991 Mar;83(3):387-90.

14. Aghamohammadi A, Farhoudi A, Moin M, Rezaei N, Kouhi A, Pourpak Z, et al. Clinical and immunological features of 65 Iranian patients with common variable immunodeficiency. Clin Diagn Lab Immunol. 2005 Jul;12(7):825-32.

Immunology and Genetics Journal (2018) 1:38-46
Doi: 10.22034/igj.2018.69266

Original Articles

Correlation Analysis of Mutation Severity and BTK-expression and Clinical Manifestations in the Patients with X-linked Agammaglobulinemia

Fatemeh Kiaee[1], Saeed Nasseri[2], Mahsa Sohani[1], Samaneh Delavari[1], Sima Habibi[1], Sepideh Shahkarami[1, 3]*

Received: 13 April 2018 /Accepted: 20 August 2018 /Published online: 22 September 2018

Abstract

Backgrounds/Objectives X-linked agammaglobulinemia (XLA) is a primary immunodeficiency disorder caused by mutations in the Bruton tyrosine kinase (*BTK*) gene. It is characterized by severely reduced numbers of peripheral B cells and a significant deficiency in all serum immunoglobulins. In the present study, the impact of mutation severity on the clinical and immunological characteristics of XLA patients was evaluated.

Methods Mutation analysis was performed in 19 XLA patients by PCR assay to identify variations in the *BTK* gene. Subsequently, the western blotting technique was applied for measuring *BTK* expression and function. A genotype-phenotype correlation was investigated regarding the impact of mutation severity on clinical and immunological parameters.

Results Mutation detection in the *BTK* gene revealed missense mutations in 9 patients, nonsense mutations in 3 cases, splicing site defects in 5 patients, and small in-frame deletions in 2 patients; 31% of patients displayed normal *BTK* expression. A significant correlation was found between types of *BTK* mutation and *BTK* expression.

Discussion Generally, genotype-phenotype correlation studies on XLA disease seem to be very controversial. The results of the correlation analysis in the present study could indicate that evolution of the disorder is not completely similar in all cases, even with the same mutation.

Keywords XLA, Correlation analysis, Mutation severity, Clinical and immunological characteristics

*** Corresponding author**: Sepideh Shahkarami
Shahkarami_s@yahoo.com

1 Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

2 Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.

3 Medical Genetics Network (MeGeNe), Universal Scientific Education and Research Network (USERN), Tehran, Iran.

Introduction

X-linked agammaglobulinemia (XLA) is one of the commonest primary immunodeficiencies (PIDs) characterized by strongly reduced numbers of peripheral B-cells, significant hypogammaglobulinemia, and recurrent bacterial infections, particularly in the respiratory and gastrointestinal tracts and the central nervous system (CNS) (1, 2). The prognosis of XLA has been profoundly improved by earlier diagnoses and more significantly by immunoglobulin replacement therapy which increases normal concentrations of immunoglobulin (3).

Bruton's tyrosine kinase (*BTK*), a responsible gene for XLA, is a member of the Tec family of non-receptor tyrosine kinases (nRTKs). It is involved in the pre–B-cell immunoglobulin receptor signaling pathway and composed of five distinct domains, the pleckstrin homology (PH), Tec homology (TH), Src homology 3 (SH3), SH2, and the kinase domain SH1 (4). The *BTK* gene is located on the X chromosome at Xq21.3–Xq22 (1, 5, 6). Up to now, over 1100 unique mutations in the *BTK* gene (BTKbase) have been reported (7, 8), resulting in the defective expression and function of the *BTK* protein. A lack of *BTK* protein, or nonfunctional protein, has been considered as the most reliable evidence confirming XLA disorder (9, 10). However, affected patients show heterogeneity in the expression of the *BTK* protein as well as clinical manifestations (8, 11). Various investigations have shown the absence of any significant genotype/phenotype correlation in XLA disease (12-15). Nevertheless, some have indicated that well-defined mutations may profoundly influence *BTK* expression as well as the clinical outcome of this disorder (11, 16, 17).

In the present study, efforts were made to determine whether the type of variation in the *BTK* gene is associated with the expression of the *BTK* protein and if the mutation severity is associated with the clinical outcome of XLA patients.

Materials and Methods

Patients

Nineteen male patients with defined X-linked agammaglobulinemia were enrolled in the present study. The diagnosis criteria were those of the European Society of Immune Deficiencies (ESID, (https://esid.org/Working-Parties/Registry/Diagnosis-criteria)) and included circulating B cells lower than 2%, normal T cell counts, and decreased serum IgG level (according to age) with evidence of recurrent infections before the age of 5 years. Blood samples were collected with the ethical approval of Tehran University of Medical Sciences (TUMS). Demographic, clinical, and laboratory data was obtained from patients' hospital records. Written informed consent was obtained from all participants and their parents.

BTK gene mutations were grouped into "severe" or "mild" (11, 16, 18). Premature stop codons, gross deletions, frameshifts, and splicing-site mutations that affect the splice consensus invariant sequences were considered as ''severe''. Mild mutations

considered the missense mutations in non-conserved subdomains and those in non-invariant splicing sites. Disease severity was defined as the age of onset (≤6 month) and the presence of severe recurrent infections.

DNA extraction and polymerase chain reaction

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the conventional phenol-chloroform method and was quantified using a NanoDrop 1000TMS spectrophotometer (OD 260 nm/OD 280 nm). PCR amplification of the *BTK* gene was performed as previously described (19). Amplicons were sent for direct sequencing in accordance with the manufacturer's instructions. Analysis of sequences was carried out using chromas software and NCBI-Blast.

Western immunoblotting assay

PBMCs were lysed and used for western blot analysis. Western immunoblotting was performed with primary antibodies against *BTK* and β-actin proteins (Sigma). Immunoreactivity was detected using an enhanced chemiluminescence western blotting detection kit (Amersham, Piscataway, NJ). Anti β-actin antibody (Sigma) was recruited as the internal control.

Statistical analysis

Statistical analysis was performed using the SPSS software version 21.0. The diagnostic delay was defined as the time between onset of clinical symptoms and diagnosis. The time between diagnosis time and the last patient visit was considered as the follow-up time. The correlation between the mutation type and *BTK* expression was determined using the Fisher exact test. The association between the clinical/immunological findings and affected domains and the mutation severity was examined using the chi-square and Mann-Whitney tests. A p-value of <0.05 was considered to be statistically significant in all tests.

Results

Demographic and clinical characteristics

A total of 19 Iranian male XLA patients from 13 unrelated families were evaluated in the present study. The main demographic and biological features of all patients at the time of diagnosis are summarized in **Table 1**. The median age at the time of diagnosis was 5 years. The median age of the cases at disease onset was 1 year. All patients are alive and were followed up for a median period of 17 years. Six out of 19 (13%) cases were from a consanguineous marriage, and 8 (42%) patients had a family history of XLA. P10 is the uncle of P13, and (P8 and P12) were brothers as well as (P6 and P7) and (P15 and P19). At the time of diagnosis, upper and lower respiratory tract infections were the commonest symptom (83.3%), followed by otitis media (61%), recurrent diarrhea (44.4%), rheumatic manifestations (44.4%), dermatologic manifestations (33.3%), and neurologic manifestations (33.3%).

Table 1. Demographic and biological characteristics of XLA patients

Variables	ALL patients	Mild mutation	Severe mutation	*P* Value
Number of patients	19	10	9	-
Age at onset, year (IQR)	1.0 (0.7-5)	1.3 (0.6-5.5)	1.0 (0.4-4.5)	0.49
Age at delayed diagnosis, year (IQR)	3.4 (2-6.6)	3.0 (1.3-6.7)	4.0 (2.2-6.8)	0.4
Diagnostic age, year (IQR)	5.0 (4-9)	4.0 (2.6-9.5)	7.6 (5.9-9.5)	0.15
IgG, mg/dl (IQR)	120 (39-221)	78 (2.5-129)	203 (116-385)	0.063
IgA, mg/dl (IQR)	3.0 (0-9.25)	1.0 (0-10)	5.0 (0-7.5)	0.79
IgM, mg/dl (IQR)	18 (4-29.25)	10 (0.5-53)	19 (15.5-27.5)	0.48
B cells (%) (IQR)	0.0 (0-2)	2.0 (0-2)	0.0 (0-0.75)	0.13
BTK expression+	6/19	6/10	0/9	0.011
CD3+ (%) (IQR)	92 (78-94)	92 (64.5-93.5)	92 (80.5-94.5)	0.6
CD4+ (%) (IQR)	44 (32-54)	42 (29.5-54)	44 (28-52.5)	0.78
CD8+ (%) (IQR)	39 (28-47)	36 (20.5-47)	43 (27.5-47)	0.72
Clinical outcome (Severe/Mild)	10/9	6/4	4/5	0.49
Influenced domain (SH2/ SH1-TK/PH)	1/8/10	1/5/4	0/3/6	0.49

Mutation analysis

All patients had confirmed mutations in the *BTK* gene, including 5 missense variations in 9 patients, 3 nonsense mutations in 3 patients, 4 splicing site defects in 5 cases, and 2 small deletions in 2 patients. The mentioned variations were positioned on the SH1/TK domain of the *BTK* protein in 8 out of 19 cases (42.1%), in the PH domain in 10 cases (52.6%), and in the SH2 domain in 1 case (5.2%). Fourteen identified types of mutations were predicted and assigned into two groups of mild (n = 6) or severe (n = 8). The frequency of each type of mutations is shown in **Figure 1**.

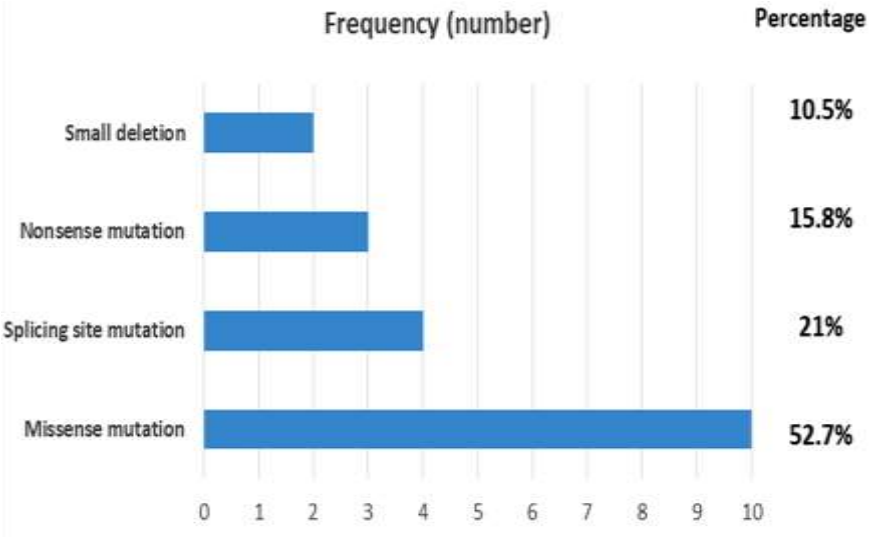


Figure 1. The frequency of different types of mutations (number and percentage) in XLA patients

Correlation of mutational type and clinical/immunological features

A summary of detected mutations and their predicted severity is given in **Table 2**. There was no

significant difference between the groups with mild and severe mutations in terms of demographic data such as age at onset, diagnosis, and delayed diagnosis or immunological data including immunoglobulin levels, B and T cell percentages.

The protein expression level was evaluated in all 19 patients. The results indicated that 6 out of 19 patients had normal levels of *BTK* expression, while no expression was found in 13 patients (data not shown). Statistical analysis showed that there was a significant correlation between the severity of the mutations and *BTK* expression (-p-value <0.05). It was found that all severe predicted mutations had an absence or reduced level of protein production. Six (31.5%) patients (P1, P6, P7, P15, P16, and P19) harboring a mild mutation in the *BTK* gene displayed normal expression of *BTK* as compared with healthy controls. However, the normal expression level of *BTK* was not observed in 4 out of 10 patients with mild mutations.

The data showed that the influenced domain does not have a significant association with mutation severity, nor with the *BTK* expression level. In addition, statistical analysis indicated that there was not a particular domain in which mutations occurred with higher statistical significance. No significant correlation was observed between the patients' clinical outcome and types of *BTK* gene variations in the 19 studied cases (**Table 3**).

Table 2. Summary of detected mutations and their predicted severity in individuals diagnosed with XLA

Patient	Family history	Domain	Mutation	Protein variation	Variation type	Predicted severity
1	-	SH2	c.906_908 delAGG	P.G303del	Small in-frame deletion	Mild
2	-	PH	c.178_180 delAAG	p.K60del	Small in-frame deletion	Mild
3	-	SH1/TK	ivs15-13 delTTG	exon16 skiping	Frameshift nonsense	Severe
4	-	PH	c.110 T<C	p.L37P	Non-frameshift missense	Severe
5	-	SH1/TK	c.1978 C<T	p.L616F	Non-frameshift missense	Mild
6	+	PH	c.214 C<T	p.r28C	Non-frameshift missense	Mild
7	+	PH	c.214 C<T	p.r28C	Non-frameshift missense	Mild
8	+	PH	c.31+5G<C	Splicing defect	Splice-site	Severe
9	-	SH1/TK	ivs14-1G>A	Splicing defect	Splice-site	Severe
10	+	SH1/TK	c.1922G<A	p.R641H	Non-frameshift missense	Mild
11	-	PH	ivs1+5G<C	Splicing defect	Splice-site	Severe
12	+	PH	ivs1+5 G<C	Splicing defect	Splice-site	Severe
13	+	SH1/TK	c.1922G<A	p.R641H	Non-frameshift missense	Mild
14	-	PH	c.349 delA	p.N72fs.120X	Frameshift nonsense	Severe
15	+	SH1/TK	c.1651T<C	p.Y551H	Non-frameshift missense	Mild
16	-	PH	c.214 C<T	p.r28C	Non-frameshift missense	Mild
17	-	SH1/TK	c.1896 G<A	p.W588X	Non-frameshift nonsense	Severe
18	-	PH1	ivs3+2 T>C	Splicing defect	Splice-site	Severe
19	+	SH1/TK	c.1651T<C	p.Y551H	Non-frameshift missense	Mild

Table 3. Correlation of clinical features with mutation severity in 19 XLA patients

Clinical manifestation	Total (n=19)	Severe	Mild	*P* value
Rheumatic manifestation (Number)	8	4	4	1
Otitis (Number)	11	6	5	1
Dermatologic manifestation (Number)	6	3	3	1
Neurologic manifestation (Number)	6	4	2	0.6
Pneumonia (Number)	12	6	6	1
Sinusitis (Number)	13	6	7	1
Diarrhea (Number)	8	6	2	0.15

Discussion

The current study evaluated the correlation of *BTK* gene alterations with the expression level of *BTK* and the clinical/immunological findings of 19 XLA patients. The data showed that all patients with severe mutations represented a severe reduction in *BTK* protein expression, while some patients with mild mutations were associated with normal protein expression. However, heterogeneity was observed in the serum level of immunoglobulins, CD-markers, clinical manifestations, and age at onset in the patients, demonstrating that none of them reflected the consequence of the type of mutation. It was also shown that mutations in all affected domains could cause the disease without any preferences.

In general, genotype-phenotype correlation studies seem to be controversial as the clinical manifestations in XLA disease are mostly variable. As previously mentioned, *BTK* belongs to the Tec family of non-receptor protein tyrosine kinases. The Tec family includes Tec, Itk/Emt/Tsk, Bmx, and Txk/Rlk. We have previously demonstrated that severe mutations do not always lead to severe phenotypes. We have shown that Tec can influence the clinical phenotype of XLA patients by substituting for *BTK*. In fact, the presence of single nucleotide polymorphisms in Tec was in part attributed to a tendency toward mild XLA phenotype despite the severe mutations (20). Consistent with our previous data, we demonstrated that the severe mutations were accompanied by mild clinical phenotypes in some cases. This may necessitate further consideration of gene modifiers affecting XLA severity (16). In line with the our previous report, the present study also indicated that severe genotypes such as splicing and frameshift mutations attributed to splice site defects and premature stop codons, respectively (20). This notion further implicates a direct relevance between the mutational defect and the resultant protein.

To confirm XLA disease, direct and indirect investigation of *BTK* mutations is essential (8, 21-23); however, *BTK* gene mutations are not always reflective of the level of expression or functional activity of the protein. Three patients in the present study represented the same non-frame missense mutation (R28C substitution) and almost normal *BTK* expression. This data was in agreement with the study by Kanegane *et al.* which showed that the mentioned alteration may result in normal *BTK* expression (24). In a previous report, we could identify one patient with an almost 40% reduction

in protein expression of this mutant by flowcytometry and western immunoblotting methods. This discrepancy is probably due to the unknown individual and environmental factors which could affect protein expression (20). However, the precise underlying reason still remains elusive. Interestingly, there were two cases with the same Y551H mutation. Lyn mediated trans-phosphorylation of *BTK* at tyrosine 551 induces auto-phosphorylation of the protein at Y-223 with the ultimate increment in *BTK* enzymatic activity (25). We have previously demonstrated a patient with Y551H mutation and increased *BTK* protein expression, while the protein expression was almost normal in the two patients in the present study; this result reinforces the individual variations in the protein's integrity.

An interesting point in the present study is related to patients who had the same mutations but different clinical manifestations. P10 and P13 were relatives with the same mutation (c.1922G>A) in their SH1/TK domain of *BTK*. However, clinical presentations were more severe in P10 than in his nephew. P10 represented Hodgkin's lymphoma along with other XLA manifestations, including pneumonia, sinusitis, and bronchiectasis as well as rheumatic, dermatologic, and neurologic manifestations. Similarly, while P15 was suffering from Kawasaki disease, his brother (P19) did not manifest the same complication. These divergences may suggest other ambient factors and genetic modifiers that alter the course of the disease.

The current study has also identified a previously described mutation in a patient with a mild phenotype of an in-frame deletion of arginine (P.G303del) affecting two consecutive glycines [20]. An interesting observation regarding this mutation is the deletion of the first glycine and the presence of the second one with a modified codon at the context of the protein.

No significant correlation was found between age at onset of symptoms, the percentage of peripheral B-cells or plasma IgM levels, and *BTK* gene variations, which could be probably explained by the small sample size.

Conclusion

Finding a genotype-phenotype correlation among XLA patients may not be well established at the level of clinical outcome of the disease. It seems that environmental and individual factors as well as other unidentified modifiers may profoundly affect disease severity, especially in cases with identical gene mutations and variable clinical representations.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors are grateful to the entire staff of the Immunodeficiency Research Center.

References

1. Ochs HD, Smith CI. X-linked agammaglobulinemia. A clinical and molecular analysis. Medicine. 1996 Nov;75(6):287-99.

2. Yazdani R, Abolhassani H, Asgardoon M, Shaghaghi M, Modaresi M, Azizi G, et al. Infectious and Noninfectious Pulmonary

Complications in Patients With Primary Immunodeficiency Disorders. J Investig Allergol Clin Immunol. 2017;27(4):213-24.

3. Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. The Journal of pediatrics. 2002 Oct;141(4):566-71.

4. Yu L, Mohamed AJ, Vargas L, Berglof A, Finn G, Lu KP, et al. Regulation of Bruton tyrosine kinase by the peptidylprolyl isomerase Pin1. J Biol Chem. 2006;281(26):18201-7.

5. Sideras P, Smith CI. Molecular and cellular aspects of X-linked agammaglobulinemia. Advances in immunology. 1995;59:135-223.

6. Kwan SP, Kunkel L, Bruns G, Wedgwood RJ, Latt S, Rosen FS. Mapping of the X-linked agammaglobulinemia locus by use of restriction fragment-length polymorphism. The Journal of clinical investigation. 1986 Feb;77(2):649-52.

7. Abolhassani H, Vitali M, Lougaris V, Giliani S, Parvaneh N, Parvaneh L, et al. Cohort of Iranian Patients with Congenital Agammaglobulinemia: Mutation Analysis and Novel Gene Defects. Expert review of clinical immunology. 2016;12(4):479-86.

8. Lopez-Granados E, Perez de Diego R, Ferreira Cerdan A, Fontan Casariego G, Garcia Rodriguez MC. A genotype-phenotype correlation study in a group of 54 patients with X-linked agammaglobulinemia. The Journal of allergy and clinical immunology. 2005 Sep;116(3):690-7.

9. Gaspar HB, Lester T, Levinsky RJ, Kinnon C. Bruton's tyrosine kinase expression and activity in X-linked agammaglobulinaemia (XLA): the use of protein analysis as a diagnostic indicator of XLA. Clinical and experimental immunology. 1998 Feb;111(2):334-8.

10. Futatani T, Miyawaki T, Tsukada S, Hashimoto S, Kunikata T, Arai S, et al. Deficient expression of Bruton's tyrosine kinase in monocytes from X-linked agammaglobulinemia as evaluated by a flow cytometric analysis and its clinical application to carrier detection. Blood. 1998 Jan 15;91(2):595-602.

11. Conley ME, Broides A, Hernandez-Trujillo V, Howard V, Kanegane H, Miyawaki T, et al. Genetic analysis of patients with defects in early B-cell development. Immunological reviews. 2005 Feb;203:216-34.

12. Gaspar HB, Bradley LA, Katz F, Lovering RC, Roifman CM, Morgan G, et al. Mutation analysis in Bruton's tyrosine kinase, the X-linked agammaglobulinaemia gene, including identification of an insertional hotspot. Human molecular genetics. 1995 Apr;4(4):755-7.

13. Jin H, Webster AD, Vihinen M, Sideras P, Vorechovsky I, Hammarstrom L, et al. Identification of Btk mutations in 20 unrelated patients with X-linked agammaglobulinaemia (XLA). Human molecular genetics. 1995 Apr;4(4):693-700.

14. Holinski-Feder E, Weiss M, Brandau O, Jedele KB, Nore B, Backesjo CM, et al. Mutation screening of the BTK gene in 56 families with X-linked agammaglobulinemia (XLA): 47 unique mutations without correlation to clinical course. Pediatrics. 1998 Feb;101(2):276-84.

15. Kobayashi S, Iwata T, Saito M, Iwasaki R, Matsumoto H, Naritaka S, et al. Mutations of the

Btk gene in 12 unrelated families with X-linked agammaglobulinemia in Japan. Human genetics. 1996 Apr;97(4):424-30.

16. Broides A, Yang W, Conley ME. Genotype/phenotype correlations in X-linked agammaglobulinemia. Clinical immunology (Orlando, Fla). 2006 Feb-Mar;118(2-3):195-200.

17. Aghamohammadi A, Fiorini M, Moin M, Parvaneh N, Teimourian S, Yeganeh M, et al. Clinical, immunological and molecular characteristics of 37 Iranian patients with X-linked agammaglobulinemia. International archives of allergy and immunology. 2006;141(4):408-14.

18. Lee PP, Chen TX, Jiang LP, Chan KW, Yang W, Lee BW, et al. Clinical characteristics and genotype-phenotype correlation in 62 patients with X-linked agammaglobulinemia. Journal of clinical immunology. 2010 Jan;30(1):121-31.

19. Vorechovsky I, Vihinen M, de Saint Basile G, Honsova S, Hammarstrom L, Muller S, et al. DNA-based mutation analysis of Bruton's tyrosine kinase gene in patients with X-linked agammaglobulinaemia. Human molecular genetics. 1995 Jan;4(1):51-8.

20. Teimourian S, Nasseri S, Pouladi N, Yeganeh M, Aghamohammadi A. Genotype-phenotype correlation in Bruton's tyrosine kinase deficiency. Journal of pediatric hematology/oncology. 2008 Sep;30(9):679-83.

21. Jefferies CA, Doyle S, Brunner C, Dunne A, Brint E, Wietek C, et al. Bruton's tyrosine kinase is a Toll/interleukin-1 receptor domain-binding protein that participates in nuclear factor kappaB activation by Toll-like receptor 4. J Biol Chem. 2003 Jul 11;278(28):26258-64.

22. Aghamohammadi A, Parvaneh N, Kanegana H, Moin M, Amirzargar AA, Farhoudi A, et al. Screening of the Bruton Tyrosine Kinase (BTK) Gene Mutations in 13 Iranian Patients with Presumed X-Linked Agammaglobulinemia. Iranian journal of allergy, asthma, and immunology. 2004 Dec;3(4):175-9.

23. Nasseri S, Sorouri R, Pourpak Z, Yeganeh M, Aghamohammadi A, Fiorini M, et al. Molecular characterization of Bruton's tyrosine kinase deficiency in 12 Iranian patients with presumed X-linked agammaglobulinemia. Journal of investigational allergology & clinical immunology. 2011;21(7):572-4.

24. Kanegane H, Futatani T, Wang Y, Nomura K, Shinozaki K, Matsukura H, et al. Clinical and mutational characteristics of X-linked agammaglobulinemia and its carrier identified by flow cytometric assessment combined with genetic analysis. The Journal of allergy and clinical immunology. 2001 Dec;108(6):1012-20.

25. Rawlings DJ, Scharenberg AM, Park H, Wahl MI, Lin S, Kato RM, et al. Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. Science. 1996 Feb 9;271(5250):822-5.

Immunology and Genetics Journal (2018) 1:47-54
Doi: 10.22034/igj.2018.69344

Case Report

Chediak-Higashi Syndrome Presented with Recurrent Episodes of Diarrhea: A Case Report

Seyedeh Nina Masoom*, Arash Havaei

Received: 18 April 2018 /Accepted: 22 August 2018 /Published online: 22 September 2018

Abstract

Chediak-Higashi syndrome (CHS) is an inherited primary immunodeficiency with an autosomal recessive pattern which is usually identified by partial albinism and frequent pyogenic infections. Herein, we report the interesting case of childhood onset with the main presentation of chronic diarrhea which was treated with dexamethasone and various antibiotics for a chronic fever. The patient was given etoposide once a week and intravenous immunoglobulin monthly thereafter, which caused partial shrinkage in the size of the liver and spleen and improved the patient's clinical condition. Since CHS is invariably lethal after entering the accelerated phase and early diagnosis may facilitate bone marrow transplantation as the only curative treatment, careful examination in unusual patients without multiple recurrent infections or diagnosed hemophagocytic lymphohistiocytosis should be considered.

* **Corresponding author**: Seyedeh Nina Masoom
nina.masoom@gmail.com

Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Science, Tehran, Iran

Keywords Primary immunodeficiency, Clinical presentation, Chediak-Higashi syndrome

Introduction

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder characterized clinically by partial albinism and frequent pyogenic infections (1). The presence of abnormally large granules in the leucocytes and other granule-containing cells is a pathognomonic criterion, and absolute diagnosis is based on the morphology of the peripheral blood and bone marrow containing these enlarged granules. Other features of CHS are neutropenia, thrombocytopenia, nystagmus, photophobia, recurrent fever, and periodontitis (2, 3).

Most patients with CHS eventually enter an accelerated phase after variable periods of recurrent infection, although some rare cases present initially with an accelerated phase which is characterized by high fever, hepatosplenomegaly, anemia, jaundice, and lymphohistiocytic infiltration of the liver, spleen, and lymph nodes (4, 5). The accelerated phase makes the prognosis of the disease very poor, mainly because of associated infections and coagulopathies.

The only lifesaving therapeutic intervention for CHS is an allogenic bone marrow transplantation to correct the immunologic and hematologic manifestations of CHS, although it appears to be efficient only if the bone marrow transplantation is performed prior to the accelerated phase or during remission (4, 6). Herein is described an interesting case of Chediak-Higashi syndrome from 2 years ago which presented with chronic diarrhea.

Case presentation

A male child aged three and a half years, the product of a normal pregnancy born at 37 weeks gestation due to PROM, was referred to Children`s Medical Center because of prolonged diarrhea and high fever. The parents were first cousins and the patient had no other siblings. The child was found to have silver-blonde hair with multiple hypopigmented areas on his face, forehead, and trunk (**Figure 1**). He had no clinical problem during his first year of life, but at one year of age, following the appearance of multiple infected papules on his buttocks and continuing perianal abscess, he was admitted for abscess drainage. Afterwards, he was frequently admitted due to multiple episodes of diarrhea, fever, upper respiratory tract infection, and once for pneumonia. At 20 months of age, the child underwent adenectomy for tonsillar hypertrophy and congestion. Four months prior to his admission to Children`s Medical Center, he had been admitted to another hospital with an enlarged painless right post-auricular and submandibular mass, abdominal distention, diarrhea, and fever; at that time, he was found to have pancytopenia, elevated liver function tests, and lipid profiles. The patient had been diagnosed with Hemophagocytic Lymphohistiocytosis (HLH) and underwent immunochemotherapy on the HLH-94 protocol. The child received high-dose dexamethasone, etoposide (VP16) every week and intravenous immunoglobulin (IVIG) for four months. His condition improved until he was admitted to our center with high fever, poor feeding, and diarrhea. On examination, the child was fair skinned with silver blonde hair, but no ocular albinism was noted. He had bilateral neck swelling with a prominent enlarged right post-auricular and submandibular mass. Hepatomegaly (5 cm below the right subcostal margin) and splenomegaly (6 cm below the left subcostal margin) were noted. The cardiovascular, respiratory, and nervous systems as well as chest X-ray were found to be normal. With these clinical findings, lymphoma was suggested for the patient.

Hematological laboratory findings revealed leucopenia (WBC of 3.0×10^9/liter, with a differential count of 89% neutrophils, 10% lymphocytes, and 1% monocytes), anemia (hemoglobin 6.3 gr/dL), and

thrombocytopenia (platelet count 28 × 10^9/liter). Liver function tests showed increased levels of total bilirubin (17.4 mg/dL), direct bilirubin (9.9mg/dL), aspartate transaminase (AST, 181 U/liter), alanine transaminase (ALT, 182 U/liter). His triglycerides (281 mg/dl), cholesterol (150 mg/dl), and fibrinogen (70 mg/dl) were evaluated. An abdominal CT scan and ultrasound examination showed hepatosplenomegaly and multiple para-aortic lymphadenopathies. The direct smear stool examination reported many cryptosporidium oocysts. The post-auricular mass was found to be an enlarged lymph node which was resected, and its biopsy showed partially affected architecture by proliferated lymphoid cells. The bone marrow aspiration smear was hypercellular with megakaryocytic erythropoiesis. The myeloid series, especially monocytes and myelocytes, showed single to multiple eosinophilic inclusion bodies surrounded by a clear halo, representing intracytoplasmic giant granules. A peripheral blood smear showed giant granulation of neutrophils and granulocytes (**Figure 2**). Direct microscopy of the patient`s hair revealed an atypical granular distribution of pigmented clumps in his hair shafts (**Figure 3**). Based on these clinical presentations, hematologic and pathologic findings, the patient was diagnosed with the accelerated phase of CHS.

The child was treated with dexamethasone and various antibiotics for the fever. He was given VP16 once a week and IVIG monthly thereafter, which caused partial shrinkage in the size of the liver and spleen and improved the patient's clinical condition. Since there was no appropriate allogenic candidate, bone marrow transplantation was not performed.

Figure 1. The child was found to have silver-blonde hair with multiple hypo-pigmented areas on his face, forehead, and trunk.

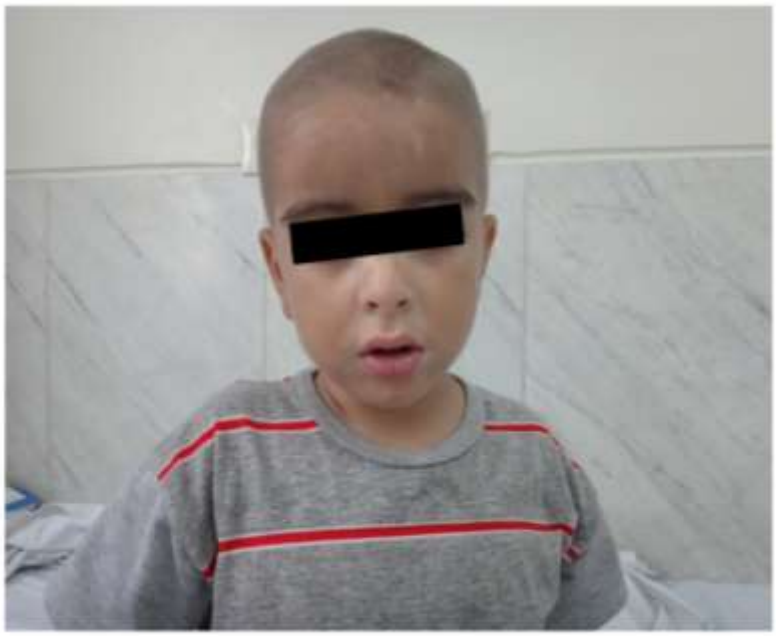


Figure 2. Neutrophil with large granules 2, 1000X, o

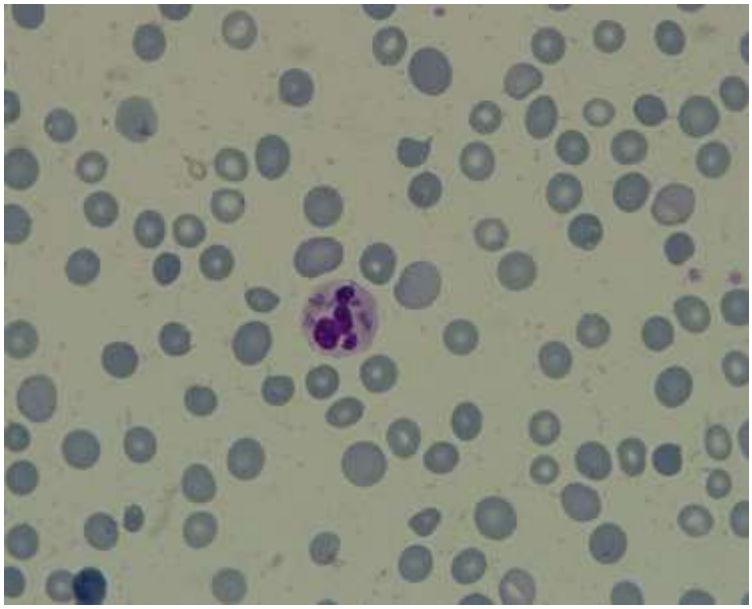


Figure 3. Direct microscopy of patient`s hair revealed the atypical granular distribution of pigmented clumps in the hair shaft.

Discussion

Chediak-Higashi Syndrome (CHS) is a rare autosomal recessive disorder typically affecting patients during infancy and early childhood characterized by various findings including partial oculocutaneous albinism, prolonged bleeding times, recurrent pyogenic infections, abnormal large granules in leukocytes and other granule containing cells, impaired natural killer cell function, and

peripheral neuropathy (7). Patients are often the result of a consanguineous marriage (8-10), as in the present case, but may be from unrelated parents (11).

CHS was first described by Beguez-Cesar in 1943 (12). In 1952, Chediak described the full clinical and hematological features in four members of a Cuban family (13), all children, who showed similar presentations, including pale hair, photophobia, lymphadenopathy and frequent infection, and they died in an early stage of life. Enlarged inclusion like cytoplasmic granules was seen in the granulocytes of the peripheral blood smear and bone marrow of these patients (14). In 1954, Higashi showed that these granules are peroxidase positive and contain lysosomal enzymes (15).

The product of the gene responsible for the defect, named in 1996 as the *CHS1/LYST* gene located on the 1q42 chromosome, is a vesicle trafficking regulatory protein. An imperfection in this protein leads to the aberrant fusion of vesicles and the failure to transport lysosomes to the appropriate site of action (16). This will cause the formation of mega-granules in all types of cells in CHS patients, including abnormally large granules in leukocytes and other granule containing cells like melanocytes, platelets, neural cells, renal tubular cells, and fibroblasts (1, 17). Enlarged granules are usually found in all granule-containing cells in Chediak-Higashi syndrome, whereas in pseudo-Chediak-Higashi syndrome, the granules are only seen in granulocyte lineage cells. The effect of this abnormality is different in different types of cells depending on the function of the granules (18). For example, an irregular distribution of melanosomes in the hair follicles leads to small aggregates of clumped pigmentation which cause the grayish-silver appearance of a CHS patient's hair. Patients with CHS exhibit alterations in the number and function of neutrophils, including neutropenia, impaired chemotaxis, and phagolysosomal fusion, resulting in recurrent bacterial infections in early stages of life.[6] Pyogenic infections occur frequently in patients with CHS and can be severe and life threatening. The most common sites of infection are the skin, mucous membranes, lungs, and respiratory tract, and the common organisms involved are usually Staphylococcus aureus, *Streptococcus pyogenes,* and Pneumococcus species. Cutaneous infections range from superficial pyoderma to deep abscess and ulcerations as seen in our patient.

Only a few patients survive severe infections in early childhood to reach their teenage life. About 50-80% of patients enter into an "accelerated phase" manifested by fever, jaundice, hepatosplenomegaly, lymphadenopathy, and widespread lymphohistiocytic infiltrations in virtually all organ systems with hemophagocytosis, leading to pancytopenia, hypertriglyceridemia, and bleeding disorders secondary to low platelet and fibrinogen levels.[1, 2] This accelerated lymphoma-like phase is thought to be related to viral infections, particularly the Epstein-Barr virus, and may occur shortly after birth or even several years later; it invariably leads to death if untreated. Treatment with high dose glucocorticoids combined with etoposide (VP16), intrathecal methotrexate, and splenectomy may result in transient remission in the

accelerated phase, but subsequent relapses have been noted to cause the patient to become less responsive to treatment (4, 6).

In approximately half of all CHS patients, neurological manifestations appear in lymphoproliferative lymphoma-like phases such as seizures, mental retardation, and long tract signs. Those patients who survive the infections usually develop a progressive sensory muscular peripheral neuropathy which leads them to become wheelchair bound in their young adulthood. Probably due to an early detection, our case did not show neurological changes. The patient was born with pale silvery-hair but normal eyes and no photosensitivity. He had no clinical problems until one year of age, when he was first admitted with subcutaneous abscess, recurrent fever, and diarrhea. Diarrhea was the common clinical presentation of our patient for more than two years and recurrently happened in association with a high-grade fever. The probability of CHS was first considered when the patient presented with lymphadenopathy, jaundice, fever, and hepatosplenomegaly resembling the accelerated phase of CHS. The presence of abnormal large granules in the granulocytes and lymphocytes of the patient's peripheral blood smear was the first diagnostic evidence which confirmed the diagnosis by direct microscopy of the hair and findings of giant melanosomes in the hair follicles.

The treatment options available for CHS patients are controversial. Parental vitamin C administered in the stable phase of CHS is believed to rule in normalizing neutrophil bactericidal activity, but it has little therapeutic effect during the accelerated phase. Symptomatic treatment includes antibiotic therapy for acute bacterial infections and blood product replacement for bleeding complications. Allogenic bone marrow transplantation has been shown to correct the hematologic and immunologic complications of CHS and has been proposed as the only possible curative treatment if performed early, before the onset of accelerated phase. However, it has not been shown to reverse or prevent a further neurological deficit.

Since the CHS disease is invariably lethal after entering the accelerated phase and early diagnosis may facilitate BMT as the only curative treatment, we suggest that careful examination of a well-prepared peripheral blood film is an essential investigation in all young children presenting with multiple recurrent infections or diagnosed with hemophagocytic lymphohistiocytosis.

Conflict of interest

The authors declare no conflicts of interest.

References

1.Blume RS, Wolff SM. The Chediak-Higashi syndrome: studies in four patients and a review of the literature. Medicine. 1972 Jul;51(4):247-80.

2.Rubin CM, Burke BA, McKenna RW, McClain KL, White JG, Nesbit ME, Jr., et al. The accelerated phase of Chediak-Higashi syndrome. An expression of the virus-associated hemophagocytic syndrome? Cancer. 1985 Aug 1;56(3):524-30.

3. Barak Y, Nir E. Chediak-Higashi syndrome. The

American journal of pediatric hematology/oncology. 1987 Spring;9(1):42-55.

4. Kanjanapongkul S. Chediak-Higashi syndrome: report of a case with uncommon presentation and review literature. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2006 Apr;89(4):541-4.

5. Ahluwalia J, Pattari S, Trehan A, Marwaha RK, Garewal G. Accelerated phase at initial presentation: an uncommon occurrence in Chediak-Higashi syndrome. Pediatric hematology and oncology. 2003 Oct-Nov;20(7):563-7.

6. Haddad E, Le Deist F, Blanche S, Benkerrou M, Rohrlich P, Vilmer E, et al. Treatment of Chediak-Higashi syndrome by allogenic bone marrow transplantation: report of 10 cases. Blood. 1995 Jun 1;85(11):3328-33.

7. Root RK, Rosenthal AS, Balestra DJ. Abnormal bactericidal, metabolic, and lysosomal functions of Chediak-Higashi Syndrome leukocytes. The Journal of clinical investigation. 1972 Mar;51(3):649-65.

8. Al-Herz W, Naguib KK, Notarangelo LD, Geha RS, Alwadaani A. Parental consanguinity and the risk of primary immunodeficiency disorders: report from the Kuwait National Primary Immunodeficiency Disorders Registry. International archives of allergy and immunology. 2011;154(1):76-80.

9. Rezaei N, Pourpak Z, Aghamohammadi A, Farhoudi A, Movahedi M, Gharagozlou M, et al. Consanguinity in primary immunodeficiency disorders; the report from Iranian Primary Immunodeficiency Registry. American journal of reproductive immunology. 2006 Aug;56(2):145-51.

10. Al-Nasser AA, Harfi HA, Sabbah RS, Malik SM. Chediak-Higashi syndrome: Report on five Saudi Arab children and review of the literature. Annals of Saudi medicine. 1993 Jul;13(4):321-7.

11. Pettit RE, Berdal KG. Chediak-Higashi syndrome. Neurologic appearance. Archives of neurology. 1984 Sep;41(9):1001-2.

12. Béguez César A. Neutropenia Crónica Maligna Familiarcon Granulaciones Atípicas de los Leucocitos. Bol Soc Cub Pediatr. 1943;15:900-22.

13. Chédiak M. Nouvelle anomalie leucocytaire de caractère constitutionel et familial. Rev hématologie. 1952;7:362-7.

14. Sato A. Chediak and Higashi's disease: probable identity of a new leucocytal anomaly (Chediak) and congenital gigantism of peroxidase granules (Higashi). The Tohoku journal of experimental medicine. 1955 Feb 25;61(2-3):201-10.

15. Higashi O. Congenital abnormity of peroxidase granules; a case of congenital gigantism of peroxidase granules, preliminary report. The Tohoku journal of experimental medicine. 1953 Oct 25;58(3-4):246.

16. Perou CM, Leslie JD, Green W, Li L, Ward DM, Kaplan J. The Beige/Chediak-Higashi syndrome gene encodes a widely expressed cytosolic protein. The Journal of biological chemistry. 1997 Nov 21;272(47):29790-4.

17. Burkhardt JK, Wiebel FA, Hester S, Argon Y. The giant organelles in beige and Chediak-Higashi fibroblasts are derived from late endosomes and mature lysosomes. The Journal of experimental

medicine. 1993 Dec 1;178(6):1845-56.

18. Higashi O, Kagaya H, Hayashi T, Sutoh T. Alkaline phosphatase in congenital gigantism of peroxidase granules. The Tohoku journal of experimental medicine. 1956 Feb 25;63(2-3):133-6.