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ORIGINAL RESEARCH

First Report of the Hyper-IgM Syndrome Registry of the Latin American Society for Immunodeficiencies: Novel Mutations, Unique Infections, and Outcomes

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Abstract Hyper-IgM (HIGM) syndrome is a heterogeneous group of disorders characterized by normal or elevated serum IgM levels associated with absent or decreased IgG, IgA and IgE. Here we summarize data from the HIGM syndrome Registry of the Latin American Society for Immunodeficiencies (LASID). Of the 58 patients from 51 families reported to the registry with the clinical phenotype of HIGM syndrome, molecular defects were identified in 37

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patients thus far. We retrospectively analyzed the clinical, immunological and molecular data from these 37 patients. CD40 ligand (CD40L) deficiency was found in 35 patients from 25 families and activation-induced cytidine deaminase (AID) deficiency in 2 unrelated patients. Five previously unreported mutations were identified in the *CD40L* gene (*CD40LG*). Respiratory tract infections, mainly pneumonia, were the most frequent clinical manifestation. Previously undescribed fungal and opportunistic infections were observed in CD40L-deficient patients but not in the two patients with AID deficiency. These include the first cases of pneumonia caused by *Mycoplasma pneumoniae*, *Serratia marcescens* or *Aspergillus sp.* and diarrhea caused by *Microsporidium sp.* or *Isospora belli.* Except for four CD40L-deficient patients who died from complications of presumptive central nervous system infections or sepsis, all patients reported in this study are alive. Four CD40L-deficient patients underwent successful bone marrow transplantation. This report characterizes the clinical and genetic spectrum of HIGM syndrome in Latin America and expands the understanding of the genotype and phenotype of this syndrome in tropical areas.

Keywords Latin American Society for Immunodeficiencies · Hyper-IgM syndrome · CD40L deficiency · AID deficiency, infections, mutations

Introduction

Hyper-immunoglobulin M (HIGM) syndrome is a heterogeneous group of primary immunodeficiency disorders characterized by recurrent infections and defects of immunoglobulin class switch recombination (CSR) associated with elevated or normal levels of serum IgM and low levels of serum IgG, IgA, and IgE [1]. HIGM syndrome was first clinically described more than 50 years ago, [2–4] and was molecularly defined in 1993, when 5 groups reported mutations in the *CD40L* gene (*CD40LG*) [5–9], located on the X chromosome. Subsequently, a second X-linked molecular defect caused by mutations in the nuclear factor-κB (*NF-κB*) essential modulator gene (*NEMO*) [10, 11], and autosomal recessive HIGM syndromes caused by mutations in *CD40* or by B cell–intrinsic defects of CSR due to mutations in activation-induced cytidine deaminase (*AICDA*) [12] or uracil-DNA glycosylase (*UNG*) [13] have been described. More recently mutations in the post-

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meiotic segregation 2 gene (*PMS2*) [14] have been identified as cause of autosomal recessive HIGM. Among these molecular defects, CD40L and AID deficiencies are the most common forms of HIGM syndrome estimated to represent 75 % and 20 % of cases [15], respectively.

In general, patients with B cell–intrinsic defects are susceptible to extracellular bacterial and rarely viral infections while CD40L- and CD40-deficient patients are in addition susceptible to fungal, opportunistic and intracellular bacterial infections. This difference can be explained by the broader function of CD40L-CD40 interaction in the regulation of B cell [16], dendritic cell (DCs) and T cell immune responses [17, 18].

Following the characterization of the molecular basis of CD40L deficiency 20 years ago, over 200 cases of HIGM syndrome from Europe and North America (http://bioinf.uta.fi/base_root) and approximately 100 cases from a single Center [15], as well as additional cases from the United States [19] have been described. In these geographic regions, the clinical and genetic spectrum of HIGM syndromes are well defined. However, the characterization of these syndromes in Latin America remains to be determined.

Here we describe the genetic and clinical characteristics of CD40L-deficient and AID-deficient patients from Latin America as an ongoing effort of the Latin American Society for Immunodeficiencies (LASID) Registry, aiming to improve diagnosis and treatment of these disorders locally. The LASID Registry contains demographic, genetic, clinical and immunologic information from 58 Latin American patients with HIGM syndrome. We have recently described the occurrence of previously unreported microorganisms causing infections in CD40L deficiency such as *Paracoccidioides brasiliensis*, *Klebsiella sp.* and *Acinetobacter sp.*, and human papilloma virus [20]. This report expands the genetic and clinical spectrum of CD40L deficiency globally with the report of five novel mutations in the *CD40LG* gene and previously undescribed infectious agents associated with CD40L deficiency, such as the occurrence of pneumonia caused by *Mycoplasma pneumoniae*, *Serratia marcescens* or *Aspergillus sp.* and diahrrea caused by *Microsporidium sp.* microsporidiosis or *Isospora belli.*

Methods

Data Collection and Construction of Registry

The patients were registered by physicians from different Latin American countries including Argentina, Brazil, Chile, Costa Rica, Mexico, and Peru who care for and followed patients with HIGM syndrome. In June 2011, a 4-page questionnaire asking for detailed information about demographic, clinical, immunological and molecular characteristics was sent to physicians who are members of LASID and to investigators who perform molecular genetic analysis of patients with HIGM syndrome in Latin America.

Molecular Genetic Diagnosis

Investigators from countries where molecular genetic analyses were feasible started to investigate every male patient with HIGM syndrome for mutations in the *CD40LG* because mutations in this gene lead to the most common form of HIGM syndrome [15, 21]. Analysis of CD40L expression by activated $CD4^+$ T cells was performed by flow cytometry using specific monoclonal antibodies (mAbs) (clones available upon request). Because CD40-Ig fusion protein became only recently available in one center in Brazil, the functional assay to evaluate the CD40L-CD40 binding was performed in only a few patients (P12-P17) as previously described [22]. Because AID deficiency is the most common autosomal defect underlying this syndrome, male patients with no abnormality in the molecular genetic analysis of *CD40LG* and female patients were analyzed for mutations in *AID*.

Exons and flanking intron sequences of *CD40LG* and *AID* were amplified by PCR, the PCR products were purified and sequenced (primers and conditions used in each country are available upon request). Mutations are described according to the suggested nomenclature of the Human Genome Variation Society [23, 24].

Diagnostic Criteria

Male patients with the clinical diagnosis of HIGM syndrome were considered to have CD40L deficiency if reduced CD40L protein expression and/or binding of CD40-Ig fusion protein by activated $CD4^+$ T cells was identified and a mutation within the *CD40LG* could be ascertained by gene sequencing. On the other hand, patients were considered AID-deficient if they had an autosomal pattern of inheritance and a mutated *AICDA* gene. Patients P1-P11 [20], P20 and P21 [25], and P26–P31 [26] have been reported previously.

Results

Patient Demographic and Baseline Characteristics

The six participating countries reported a total of 58 patients from 51 families with clinical features of HIGM syndrome. The gender ratio was 48 male/10 female. The distribution of patients by countries is shown in Fig. 1. Of these 58 patients, genetic defects were identified in 37. The detailed clinical and laboratory data for these 37 patients were analyzed for this paper.

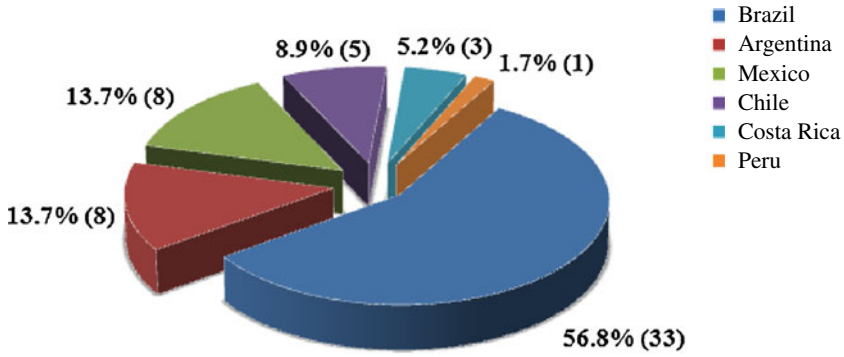

Fig. 1 Distribution of patients with HIGM among the Latin American countries

In this group of molecularly-defined patients, 35 from 25 non-related families were CD40L-deficient and 2 unrelated (1 male and 1 female) patients were AID-deficient. Seventeen patients (all males with CD40L-deficiency) are from families with a positive history of infections affecting other male members. Patient P36, with AID deficiency is from a consanguineous family. The patients' baseline characteristics including the mean age at the first clinical manifestation, age at diagnosis, serum immunologlobulin levels, etc. are described in Table 1.

Infections and Associated Pathogens

Recurrent severe and unusual infections including those caused by non-opportunistic and opportunistic microorganisms were the hallmark clinical manifestation in the CD40L-deficient cohort. Among them, pneumonia was the most common (28 patients) (Table 1). The most frequent cause of pneumonia (Table 2) was *Pneumocytis jirovecii* (P1-P3, P6, P8, P10, P14, P32 and P34), followed by cytomegalovirus (P16), *Pseudomonas aeruginosa* (P24), *Streptococcus pneumonia* (P26), parainfluenza virus type II (P27) and unknown pathogens. In two patients pneumonia was caused by *Aspergillus sp.* (P14, P25) (Fig. 2a and b), and in one each *by Mycoplasma pneumoniae* (P21) and *Serratia marcescens* (P31), which are etiologic agents not previously reported in CD40L-deficient patients. Following pneumonia, upper respiratory tract infections (URTIs) (22 patients) and chronic diarrhea (16 patients) were the most frequent clinical manifestations. In the latter category, *Giardia lamblia* was the most commonly isolated pathogen (P16, P20, P21, P24 and P28) followed by *Cryptosporidium parvum* (P3, P8 and P14) and by the fungus *Microsporidium sp.* (P25) or the protozoa *Isospora belli* (P16 and P17), the latter two being etiological agents not previously described in CD40L deficiency. It is noteworthy that in P25 at age 10 years severe microsporidiosis occurred simultaneously with aspergillosis and in P16 at age 15 years recurrent diarrhea caused by *Isospora belli* led to cachexia with body weight of 22 kg—body mass index (BMI) of 10.4 kg/m^2 - (Fig. 2c).

Table 1 Patients' baseline, laboratory data and Clinical manifestations

	–		CD40L-deficient patients (n=35)	AID-deficient patients (n=2)
Baseline characteristics	Mean age at first clinical manifestation (range)		0.7 years (0.1–4)	4.5 years [4, 5]
	Mean age at diagnosis of HIGM syndrome (range)		2.3 years (0.3–13.6)	24.5 years [9, 40]
	Alive		31	2
Laboratory data	Immunoglobulin levels (before IVIG[51])	IgG (< P3)	35 (100 %)	2 (100 %)
		IgA (< P3)	20 (57 %)	2 (100 %)
		IgM (> P97)	20 (57 %)	2 (100 %)
	Neutropenia		17 (48 %)	–
Clinical manifestations	Pneumonia		28 (80 %)	2 (100 %)
	Upper resp. tract infections		22 (63 %)	2 (100 %)
	Chronic diarrhea		16 (46 %)	2 (100 %)
	Urinary tract infections		3 (9 %)	1 (50 %)
	Sepsis		3 (9 %)	1 (50 %)
	Hepatitis		2 (6 %)	–
	Meningitis/encephalitis		2 (6 %)	–

[a] Reference values of immunoglobulin levels are according to Guerra et al. [51]. *IVIG* intravenous immunoglobulin therapy; *P* percentile

Table 2 Isolated pathogens from Latin American patients with HIGM syndrome

Patients	Fungi	Extracellular bacteria		Intracellular bacteria	Virus	Protozoa
		Gram-positive	Gram-negative			
CD40L-deficient (*n*=35)	*P. jirovecii* (*n*=9; 26 %) *C. albicans* (*n*=6; 17 %) *Aspergillus sp.* (*n*=2; 6 %) *Microsporidium sp.* (*n*=1; 3 %) *P. brasiliensis* (*n*=1; 3 %) *H. capsulatum* (*n*=1; 3 %) *C. neoformans* (*n*=1; 3 %)	*S. pneumoniae* (*n*=1; 3 %) *S. aureus* (*n*=1; 3 %)	*P. aeruginosa* (*n*=5; 14 %) *K. pneumoniae* (*n*=3; 9 %) *Actinobacter sp.* (*n*=1; 3 %) *S. marcenscens* (*n*=1; 3 %) *E. coli* (*n*=2; 6 %) *E. cloacae* (*n*=1; 3 %) *B. pertussis* (*n*=1; 3 %)	*Mycoplasma pneumoniae* (*n*=1; 3 %) *Mycobacterium tuberculosis* (*n*=3; 9 %)	CMV (*n*=1; 3 %) Hepatitis B virus (*n*=1; 3 %) Herpes simplex (*n*=1; 3 %) Molluscum contagiosum (*n*=1; 3 %) HPV (*n*=1; 3 %) Parainfluenza virus type II (*n*=1; 3 %)	*G. lamblia* (*n*=5 14 %) *C. parvum* (*n*=3; 9 %) *Isospora sp* (*n*=2; 6 %)

CMV cytomegalovirus; *HPV* human papillomavirus

In addition to pneumonia caused by *P. jirovecii* and *Aspergillus sp.* and diarrhea caused by *Microsporidium sp.,* the CD40L-deficient patients frequently developed other fungal infections such as paracoccidioidomycosis (P10), severe candidiasis which manifested itself in the form of esophagitis (P5), candidiasis of the scalp (P9), cellulitis and urinary tract infection (P29), and oral (P7 and P21), genital (P18) and perianal (P6) candidiasis.

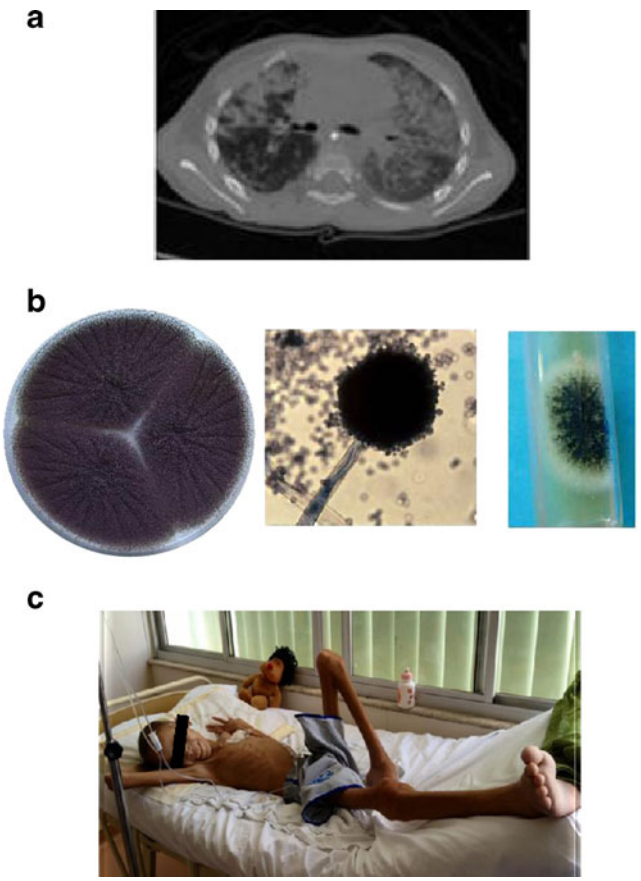

Fig. 2 Pulmonary aspergillosis and *Isospora belli* infection in CD40L-deficiency. **a** Computed tomography (CT) scan of the chest showing Aspergillus lung infection in P14. **b** Images showing growth of *Aspergillus niger* from sputum cultures of patient P25. **c** Cachectic state of patient P16 after recurrent diarrhea caused by *Isospora belli*

Other bacterial infections such as pulmonary (P10 and P19) or disseminated *Mycobacterium tuberculosis* (P25), sepsis caused by *P. aeruginosa* (P15) or caused simultaneously by *K. pneumonia* and *Actinobacter sp.* (P9), and cellulitis caused by *Klebsiella pneumonia* or *E. coli* (P29) and urinary tract infection (UTI) caused by *Escherichia coli* (P2) were observed in CD40L-deficienct patients. In addition, viral infections caused by human papilloma virus (HPV) (P6), herpes simplex (P6), molluscum contagiosum (P24) and hepatitis B virus (P26) were reported.

The two AID-deficient patients developed recurrent pneumonia, URTIs and chronic diarrhea, while one (P37) reported, in addition, UTI and sepsis. The etiologic agents in these two patients were not identified.

Lymphoid Hyperplasia

Lymphoid hyperplasia was observed in four CD40L-deficient patients characterized by enlarged tonsils (P2 and P10), lymph nodes (P4 and P10), spleen (P4 and P10) and thymus (P13). The latter finding has not been described previously in CD40L-deficient patients and was identified as idiopathic true massive thymic hyperplasia by chest computed tomography when P13 was 7 years old, followed by thymectomy and biopsy (data not shown).

Both AID-deficient patients, P36 and P37 at age 5 and 17 years respectively, presented with lymphoid hyperplasia limited to the tonsils.

Autoimmunity and Inflammatory Disorders

Autoimmunity and inflammatory disorders were infrequent. One CD40L-deficient patient (P27) developed hypothyroidism and two (P13 and P25) had autoimmune hemolytic anemia while

none of the two AID-deficient patients reported inflammatory disorders or clinical autoimmune manifestations.

Molecular Genetic Characterization

Each country in which molecular analysis was feasible investigated every male patient for CD40L deficiency. This approach led to the identification of 35 CD40L-deficient patients. The results of mutation analyses are summarized in Table 3 and Fig. 3. Of those, five novel mutations abolishing CD40L protein expression on activated $CD4^+$ T cells were identified: the nonsense mutation c.159C > T affecting codon 35 in exon 1 (p.Q35X), located in the transmembrane domain (TM) in P32; the frameshift insertion c.633_634insAGCC (p.L193fsX201) in exon 5 (P19) and the frameshift deletion c.430_431delAT (p.H125fsX128) in exon 4 (P34) affect the tumor necrosis factor homologous (TNFH) domain. The other two novel mutations were found within the *CD40LG* promoter (a gross deletion, P14) and in the polyadenylation signal sequence (a gross insertion, P15) completely abrogating *CD40LG* transcription or resulting in unstable mRNA. The details of the mutations identified in the *CD40LG* of P14 and P15 will be published elsewhere. The mutations observed in the remaining CD40L-deficient patients (Table 3) have been previously described [9, 15, 20, 22, 25–29].

Two patients (P36 and P37) were found to be homozygous for the previously reported [30] missense mutation (p.C87S) of AICDA that affects exon 3 of the cytidine deaminase domain of the AID protein (Table 3 and Fig. 3).

Treatment and Outcome

All Latin American patients with HIGM syndrome are receiving IVIG replacement therapy resulting in a substantial decrease in the number of infections. The infections, (including 4 deaths) that occurred while receiving IVIG therapy were more frequent in CD40L-deficient patients than in AID-deficient patients. Three CD40L-deficient patients died due to complications resulting from presumptive central nervous system (CNS) infections: P9 died at age 8 years due to progressive multifocal leukoencephalopathy, P20 at 9 years due to progressive panencephalitis, and P32 at 7 years due to encephalopathy. P16, when in a cachectic state, died at age 15 years of sepsis. The survival curve for our cohort of CD40L-deficient patients is shown in Fig. 4.

Sixteen CD40L-deficient patients developed neutropenia (Table 1) which was mostly intermittent and associated with oral, rectal or esophageal ulcers. Neutropenia was effectively treated with granulocyte colony stimulating factor (G-CSF), except P4 who showed only partial response to this treatment. Several CD40L-deficient patients had neutropenia at the time or just before developing fungal infections: P14 and P25 (Aspergillosis), P25 (Microsporidiosis), P3, P5, P29 (candidiasis), P3 and P14 (*P. jiroveci* pneumonia). Twenty seven CD40L-deficient patients are currently receiving antibiotic prophylaxis for *P. jirovecii* or had been on prophylaxis at an earlier age.

Four CD40L-deficient patients underwent successful allogenic hematopoietic stem cell transplantation (HSCT). Patients P5 and P12 received bone marrow and patients P22 and P23 were given peripheral blood stem cells. Graft-versus-host disease (GVHD) was observed in three patients: P5 developed acute GVHD grade II, P22 cutaneous acute GVHD grade II and P23 cutaneous hyperacute GVHD grade III. In addition, P5 developed systemic CMV infection and autoimmune hemolytic anemia following HSCT and were successfully treated with ganciclovir, methylprednisolone, Rituximab and IVIG. GVHD resolved and all four patients are alive and well at last follow-up (0.5 to 3 years post HSCT) without immunosuppressive therapy.

Discussion

This is the first report of HIGM syndrome across Latin America. This registry study expands the spectrum of microorganisms reported in this disease. Severe and unusual infections caused by microorganisms not previously associated with CD40L deficiency such as *Aspergillus sp.*, *Mycoplasma pneumoniae, Serratia marcescens, Microsporidium sp.*, and *Isospora belli* were identified in Latin American patients. In addition, infections caused by other not previously described etiological agents such as *Paracoccidioides brasiliensis, Klebsiella pneumonia, Acinetobacter sp.,* and *human papilloma virus (HPV)* were recently identified in Brazilian patients [20].

The clinical and molecular characterizations of HIGM syndromes in North America and Europe, particularly those caused by CD40L and AID deficiencies [15, 19, 21, 31, 32], have been well established following the discovery of the specific genetic defects underlying the different entities designated as HIGM syndromes [5–9, 12, 13, 33]. In this communication, LASID, which was given the mandate to advance and improve the diagnosis and management of PIDs in Latin America [34, 35], reports the results of its HIGM syndrome registry. In addition to the clinical and immunologic features of the HIGM syndromes described by North American and European investigators [15, 19, 31–33, 36, 37], our report of Latin American patients, which is a genetically heterogenous group of subjects, expands the spectrum of microorganisms previously reported in HIGM syndromes.

Noteworthy, P24 with gastrointestinal infection caused by mycrosporidiosis, a clinical complication frequently observed in patients with acquired immunodeficiency syndrome (AIDS) [38], is to our knowledge, the first case of a well-defined primary immunodeficiency disorder suffering from

Table 3 Molecular genetic analysis of CD40L-deficient and AID-deficient patients

Pt	Origin	Type of mutation	cDNA mutation[a] (*CD40LG*)	Predicted effect on protein	Protein expression by flow cytometry		Ref.
					CD40L mAb	CD40-Ig	
P1	Brazil	Missense	c. 433 T>G	p.V126G	−	N/E	20
P2	Brazil	Missense	c.476 G>C	p.W140C	−	N/E	20
P3	Brazil	Missense	c.496 C>A	p.T147N	+	−	22
P4[c1]	Brazil	Nonsense	c.475 G>A	p. W140X	−	N/E	9
P5[c1]	Brazil	Nonsense	c.475 G>A	p. W140X	−	N/E	9
P6[c2]	Brazil	FS del	c.213_216delATAG	p.I53fsX65	−	N/E	28
P7[c2]	Brazil	FS del	c.213_216delATAG	p.I53fsX65	−	N/E	28
P8	Brazil	FS del	c.213_216delATAG	p.I53fsX65	−	N/E	28
P9[c]	Brazil	Splice site	c.345_402del	exon 3 skipping	−	N/E	22
P10	Brazil	Splice site	c.345_402del	exon 3 skipping	−	N/E	22
P11	Brazil	FS ins	c.551_552insAA	p.R165fsX190	−	N/E	20
P12[b1]	Brazil	Splice site	c. 213-1G>T	exon 2 skipping	−	−	22
P13[b1]	Brazil	Splice site	c. 213-1G>T	exon 2 skipping	−	−	22
P14	Brazil	Deletion	deletion in promoter[b]	−	−	−	Novel
P15	Brazil	insertion	insertion in poly(A)signal[b]	−	−	−	Novel
P16[b2]	Brazil	Missense	c.817C>T	p.T254M	−	−	22
P17[b2]	Brazil	Missense	c.817C>T	p.T254M	−	−	22
P18	Brazil	Nonsense	c.710 C>A	p.C218X	+	−	22
P19	Brazil	Insertion	c.633_634insAGCC	p.L193fsX201	−	−	Novel
P20[b3 c]	Argentina	FS del	c.500delG	p.M148X153	−	N/E	25
P21[b3]	Argentina	FS del	c.500delG	p.M148X153	−	N/E	25
P22[b4]	Argentina	Missense	c.424 C>A	p.A123E	−	N/E	29
P23[b4]	Argentina	Missense	c.424 C>A	p.A123E	−	N/E	29
P24	Argentina	Nonsense	c.576 C>T	p.Q174X	−	N/E	15
P25	Argentina	Missense	c.813 T>A	p.F253I	−	N/E	25
P26	Mexico	Nonsense	c.714 C>T	p.Q220X		N/E	15
P27	Mexico	Splice site	c.465+1 G>A	Exon 4 skipping	−	N/E	15
P28	Mexico	Missense	c.429 C>T	p.H125Y	−	N/E	26
P29[b5]	Mexico	Missense	c.424 C>A	p.A123E	−	N/E	29
P30[b5]	Mexico	Missense	c.424 C>A	p.A123E	−	N/E	29
P31	Mexico	Nonsense	c.750C>T	p.Q232X	−	N/E	27
P32[c]	Costa Rica	Nonsense	c.159C>T	p.Q35X	−	N/E	Novel
P33	Costa Rica	Nonsense	c.475G>A	p.W140X	−	N/E	9
P34	Costa Rica	FS del	c.430_431delAT	p.H125fsX128	−	N/E	Novel
P35	Chile	Missense	c.473 T>C	p.W140R	−	N/E	28
Pt	Origin		cDNA mutation (*AICDA*)	Predicted effect on protein	Gene expression by real-time PCR		Ref.
P36	Brazil	Missense	c.260 C>G	p.C87S	D		30
P37	Brazil	Missense	c.260 C>G	p.C87S	D		30

The small letters b and c associated with superscript numbers indicate brothers and cousins, respectively, from the same family. Nucleotide number is based on the sequence data from http://bioinf.uta.fi/CD40Lbase/to describe CD40L mutations and from http://bioinf.uta.fi/AICDAbase/index.php?content=index/IDbases to describe AID mutations

FS del frame shift deletion; *FS ins* frame shift insertion; *N/E* not evaluated

[a] The nomenclature for the description of mutations are according to guidelines of Human Genome Variation Society [23, 24]

[b] detailed molecular genetic alterations will be published elsewhere

[c] patient died

+present; -absent

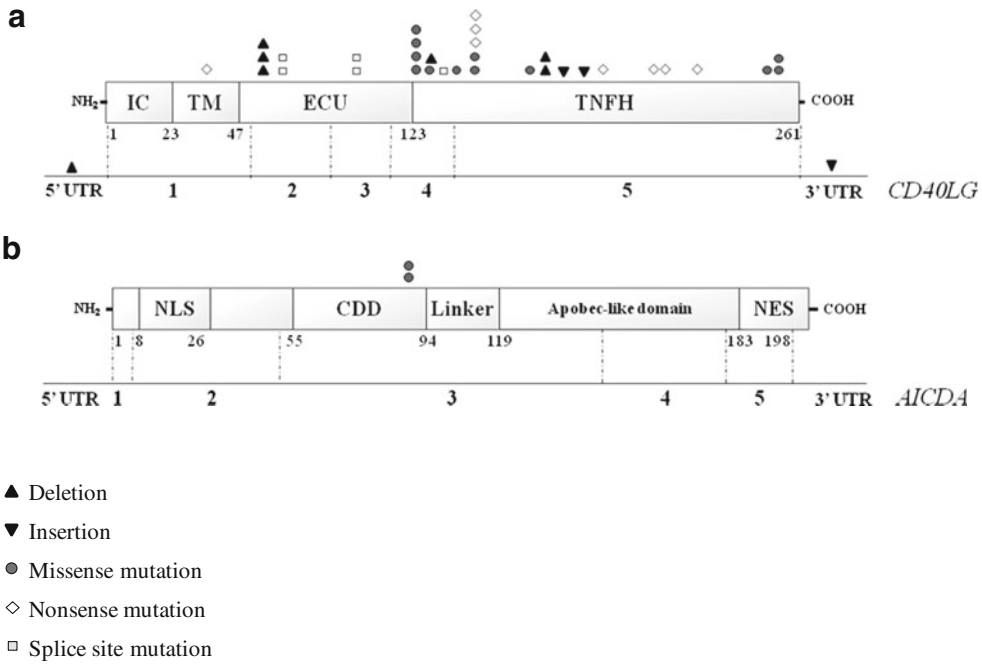

Fig. 3 Mutations in CD40L and AID. Schematic representation of **a** the *CD40LG* and **b** the *AICDA* coding regions and protein domains. Both genes have 5 exons. The type and position of each mutation observed in Latin American patients are shown. *IC* intracellular tail; *TM* transmembrane domain; *ECU* extracellular unique domain; *TNFH* TNF homology domain; *NLS* nuclear localization signal; *CDD* Cytidine deaminase domain; *NES* nuclear export signal; *UTR* untranslated region

microsporidiosis. On the other hand, infections caused by *Aspergillus sp., Mycoplasma sp., Serratia marcescens, and Isospora belli* have been well documented in patients with other PIDs [39–46]. This observations in Latin American patients are important findings which emphasize that patients worldwide with defects in CD40L-CD40 interaction should be monitored for these pathogens.

The high susceptibility to a broad spectrum of pathogens in CD40L deficiency can be explained only in part to be due to neutropenia. CD40L-deficient patients are susceptible to opportunistic infections with high mortality rates even when neutrophil counts are normal and patients are on G-CSF and IVIG treatment [20, 31]. The explanation for this global susceptibility to infections may be the pleiotropic role of CD40L-CD40 interaction in the regulation of immune

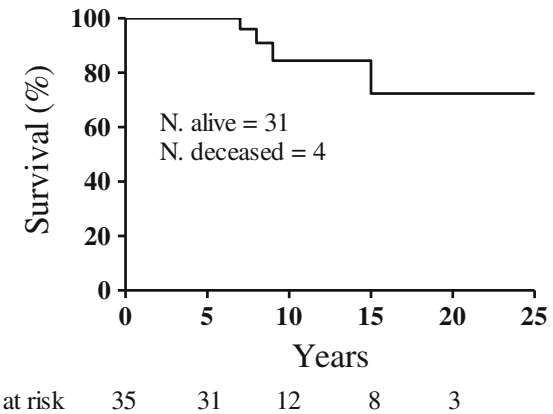

Fig. 4 Kaplan-Meier graphs showing the survival rate of CD40L-deficient patients. The graph was made using the GraphPad PRISM 4.03 software (GraphPad Software, San Diego, Calif)

responses. In addition to transitory neutropenia, abnormal B cell differentiation and reduced antibody responses, these patients have defective T [18, 47] and dendritic cell (DC) activation [17, 48] which contributes to the immunopathologic mechanisms in CD40L and CD40 deficiencies. Compared with HIGM syndromes caused by B cell–intrinsic defects affecting CSR and somatic hypermutation (eg. AID and UNG deficiencies), patients suffering from CD40L deficiency, with few exceptions [49], manifest the first symptoms of PID at an earlier age, are sicker and more likely to develop severe infections even while on IVIG therapy [31, 32]. This was also the case in our cohort, although only two of our patients had AID deficiency. P36 and P37 did not receive IVIG therapy until the age of 9 and 40 years, respectively, when the diagnosis of AID deficiency was established, while on the other hand, multiple deaths occurred during the first months of life in untreated presumably X-linked HIGM patients reported by families of CD40L-deficient patients included in this study [20].

The clinical manifestations reported by the two Latin American patients with AID deficiency (P36 and P37) are not different from those described by others as recurrent pneumonia, gastrointestinal infections and lymphoid hyperplasia without the occurrence of opportunistic infections [12, 21, 32]. Neither of our two patients, however, developed autoimmune or related inflammatory diseases which have been reported by Quartier et al. in 21 % of their cohort of AID-deficient patients [21] but not by Minegishi et al. [32] and Revy et al. [12]. On the other hand, in accordance with previously studies [19, 31] autoimmune or related inflammatory diseases were observed in approximately 8 % of our CD40L-deficient patients.

The molecular diagnosis could be established in 37 of the 58 patients reported to the registry with symptoms of HIGM syndrome, 35 with CD40L and two with AID deficiencies. Twenty five different mutations (24 in *CD40LG* and one in *AICDA*) including five novel mutations that abolish CD40L protein expression were identified. Mutation analysis of the most common HIGM syndromes—and a limited number of other PIDs caused by single gene defects—is presently available in only a small number of Latin American countries and in those, is limited to a few states/provinces of Brazil, Argentina, Chile and Mexico, while it is not available in other Latin American countries. For this reason it is still difficult to determine the actual prevalence and genetic basis of HIGM syndromes in Latin America. On the other hand, in the United States, where multiple diagnostic centers are set up to perform sequence analysis for genes associated with HIGM, the estimated minimal incidence of CD40L deficiency is 1/1,035,000 and the incidence of AID deficiency is estimated to be not greater than 2/10,000,000 per year of birth [32]. To overcome this internal problem in Latin America, State and Provincial governments have to be encouraged to support the establishment of new centers dedicated to the study of PID. Established centers have to expand already existing international collaborative studies within Latin America, and with North America and Europe.

Another limitation in Latin American countries is the availability of transplantation centers, which accounts for the small percentage of CD40L-deficient patients who underwent HSCT. This reflects social economic factors because HSCT is one example of high-cost and highly specialized medical care that requires significant infrastructure and a network of specialists from all fields of medicine. HSCT is most frequently performed in U.S and Europe where developed countries have higher gross national incomes and higher governmental health care expenditures. While the total number of performed HSCT is more than 300 per 10 million inhabitants in U.S and Europe, it ranges from zero to 49 per 10 million inhabitants in Latin American countries [50]. However, the economic improvement in some Latin American countries points to the potential for better transplant options over the coming years. For instance, in Brazil, where the highest number of CD40L-deficient patients were diagnosed, the number of HSCT donors available for all diseases demanding this treatment has recently improved (from 12 thousand in 2000 to 3 million in 2013) according to statistical data from the Ministry of Health (http://www.inca.gov.br). Associated with these data, the increasing availability of molecular diagnostic centers in Brazil and other Latin American countries will contribute to this improvement.

A phenotype/genotype correlation could not be established in our cohort nor in the previously described cohorts of North American and European patients with HIGM syndromes [12, 19, 21, 31, 32]. Such a correlation is difficult due to the small number of patients. Additionally, while AID-deficient patients have a relative phenotypic homogeneity [21, 32], this is not the case for CD40L-deficient patients who may develop different clinical phenotypes while carrying the same mutation [20], More clinical and genetic studies are required to eventually establish a phenotype/genotype correlation.

In conclusion, this first report of the LASID registry highlights the similarities and differences in the clinical and genetic spectrum of HIGM syndromes in Latin America versus other reported cohorts from North America, Europe, and elsewhere and it suggests that patients with HIGM syndrome due to mutations in *CD40LG* are susceptible to a broader species of opportunistic pathogens than previously recognized.

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References

1. Notarangelo LD, Duse M, Ugazio AG. Immunodeficiency with hyper-IgM (HIM). Immunodefic Rev. 1992;3(2):101–21.
2. Burtin P. An example of atypical agammaglobulinemia (a case of severe hypogammaglobulinemia with increase of the beta-2 macroglobulin. Rev Fr Etud Clin Biol. 1961;6:286–9.
3. Israel-Asselain R, Burtin P, Chebat J. A new biological disorder: agammaglobulinemia with beta2-macroglobulinemia (a case). Bull Mem Soc Med Hop Paris. 1960;76:519–23.
4. Rosen FS, Kevy SV, Merler E, Janeway CA, Gitlin D. Dysgammaglobulinaemia and recurrent bacterial infection. Lancet. 1961;1(7179):700.
5. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science. 1993;259(5097):990–3.
6. Aruffo A, Farrington M, Hollenbaugh D, Li X, Milatovich A, Nonoyama S, et al. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. Cell. 1993;72(2):291–300.
7. DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint BG. CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM. Nature. 1993;361(6412):541–3.
8. Fuleihan R, Ramesh N, Loh R, Jabara H, Rosen RS, Chatila T, et al. Defective expression of the CD40 ligand in X chromosome-linked immunoglobulin deficiency with normal or elevated IgM. Proc Natl Acad Sci U S A. 1993;90(6):2170–3.

9. Korthauer U, Graf D, Mages HW, Briere F, Padayachee M, Malcolm S, et al. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. Nature. 1993;361(6412):539–41.
10. Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, et al. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. Nat Genet. 2001;27(3):277–85.
11. Jain A, Ma CA, Liu S, Brown M, Cohen J, Strober W. Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia. Nat Immunol. 2001;2(3):223–8.
12. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell. 2000;102(5):565–75.
13. Imai K, Slupphaug G, Lee WI, Revy P, Nonoyama S, Catalan N, et al. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat Immunol. 2003;4(10):1023–8.
14. Peron S, Metin A, Gardes P, Alyanakian MA, Sheridan E, Kratz CP, et al. Human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination. J Exp Med. 2008;205(11):2465–72.
15. Lee WI, Torgerson TR, Schumacher MJ, Yel L, Zhu Q, Ochs HD. Molecular analysis of a large cohort of patients with the hyper immunoglobulin M (IgM) syndrome. Blood. 2005;105(5):1881–90.
16. Notarangelo LD, Lanzi G, Peron S, Durandy A. Defects of class-switch recombination. J Allergy Clin Immunol. 2006;117(4):855–64.
17. Cabral-Marques O, Arslanian C, Ramos RN, Morato M, Schimke L, Soeiro Pereira PV, et al. Dendritic cells from X-linked hyper-IgM patients present impaired responses to Candida albicans and Paracoccidioides brasiliensis. J Allergy Clin Immunol. 2012;129(3):778–86.
18. Jain A, Atkinson TP, Lipsky PE, Slater JE, Nelson DL, Strober W. Defects of T-cell effector function and post-thymic maturation in X-linked hyper-IgM syndrome. J Clin Invest. 1999;103(8):1151–8.
19. Winkelstein JA, Marino MC, Ochs H, Fuleihan R, Scholl PR, Geha R, et al. The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. Medicine (Baltimore). 2003;82(6):373–84.
20. Cabral-Marques O, Schimke LF, Pereira PV, Falcai A, de Oliveira JB, Hackett MJ, et al. Expanding the clinical and genetic spectrum of human CD40L deficiency: the occurrence of paracoccidioidomycosis and other unusual infections in Brazilian patients. J Clin Immunol. 2012;32(2):212–20.
21. Quartier P, Bustamante J, Sanal O, Plebani A, Debre M, Deville A, et al. Clinical, immunologic and genetic analysis of 29 patients with autosomal recessive hyper-IgM syndrome due to Activation-Induced Cytidine Deaminase deficiency. Clin Immunol. 2004;110(1):22–9.
22. Seyama K, Nonoyama S, Gangsaas I, Hollenbaugh D, Pabst HF, Aruffo A, et al. Mutations of the CD40 ligand gene and its effect on CD40 ligand expression in patients with X-linked hyper IgM syndrome. Blood. 1998;92(7):2421–34.
23. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat. 2000;15(1):7–12.
24. Taschner PE, den Dunnen JT. Describing structural changes by extending HGVS sequence variation nomenclature. Hum Mutat. 2011;32(5):507–11.
25. Danielian S, Oleastro M, Eva Rivas M, Cantisano C, Zelazko M. Clinical follow-up of 11 Argentinian CD40L-deficient patients with 7 unique mutations including the so-called "milder" mutants. J Clin Immunol. 2007;27(4):455–9.
26. Vargas-Hernandez A, Berron-Ruiz L, Staines-Boone T, Zarate-Hernandez M, Cordova-Calderon W, Espinosa-Rosales F, et al. Clinical and genetic analysis of patients with X-linked hyper-IgM syndrome. Clin Genet. 2012 Sep 10.
27. Lin Q, Rohrer J, Allen RC, Larche M, Greene JM, Shigeoka AO, et al. A single strand conformation polymorphism study of CD40 ligand. Efficient mutation analysis and carrier detection for X-linked hyper IgM syndrome. J Clin Invest. 1996;97(1):196–201.
28. Macchi P, Villa A, Strina D, Sacco MG, Morali F, Brugnoni D, et al. Characterization of nine novel mutations in the CD40 ligand gene in patients with X-linked hyper IgM syndrome of various ancestry. Am J Hum Genet. 1995;56(4):898–906.
29. Prasad ML, Velickovic M, Weston SA, Benson EM. Mutational screening of the CD40 ligand (CD40L) gene in patients with X linked hyper-IgM syndrome (XHIM) and determination of carrier status in female relatives. J Clin Pathol. 2005;58(1):90–2.
30. Mu Y, Prochnow C, Pham P, Chen XS, Goodman MF. A structural basis for the biochemical behavior of activation-induced deoxycytidine deaminase class-switch recombination-defective hyper-IgM-2 mutants. J Biol Chem. 2012;287(33):28007–16.
31. Levy J, Espanol-Boren T, Thomas C, Fischer A, Tovo P, Bordigoni P, et al. Clinical spectrum of X-linked hyper-IgM syndrome. J Pediatr. 1997;131(1 Pt 1):47–54.
32. Minegishi Y, Lavoie A, Cunningham-Rundles C, Bedard PM, Hebert J, Cote L, et al. Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome. Clin Immunol. 2000;97(3):203–10.
33. Ferrari S, Giliani S, Insalaco A, Al-Ghonaium A, Soresina AR, Loubser M, et al. Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. Proc Natl Acad Sci U S A. 2001;98(22):12614–9.
34. Condino-Neto A, Franco JL, Espinosa-Rosales FJ, Leiva LE, King A, Porras O, et al. Advancing the management of primary immunodeficiency diseases in Latin America: Latin American Society for Immunodeficiencies (LASID) Initiatives. Allergol Immunopathol (Madr). 2012;40(3):187–93.
35. Condino-Neto A, Franco JL, Trujillo-Vargas C, Espinosa-Rosales FJ, Leiva LE, Rodriguez-Quiroz F, et al. Critical issues and needs in management of primary immunodeficiency diseases in Latin America. Allergol Immunopathol (Madr). 2011;39(1):45–51.
36. Durandy A, Revy P, Fischer A. Human models of inherited immunoglobulin class switch recombination and somatic hypermutation defects (hyper-IgM syndromes). Adv Immunol. 2004;82:295–330.
37. Erdos M, Lakos G, Derfalvi B, Notarangelo LD, Durandy A, Marodi L. Molecular genetic analysis of Hungarian patients with the hyper-immunoglobulin M syndrome. Mol Immunol. 2008;45(1):278–82.
38. Krones E, Hogenauer C. Diarrhea in the immunocompromised patient. Gastroenterol Clin North Am. 2012;41(3):677–701.
39. Ben-Ari J, Wolach O, Gavrieli R, Wolach B. Infections associated with chronic granulomatous disease: linking genetics to phenotypic expression. Expert Rev Anti Infect Ther. 2012;10(8):881–94.
40. Foy HM, Ochs H, Davis SD, Kenny GE, Luce RR. Mycoplasma pneumoniae infections in patients with immunodeficiency syndromes: report of four cases. J Infect Dis. 1973;127(4):388–93.
41. Gelfand EW. Unique susceptibility of patients with antibody deficiency to mycoplasma infection. Clin Infect Dis. 1993;17 Suppl 1:S250–3.
42. Henriet SS, Verweij PE, Warris A. Aspergillus nidulans and chronic granulomatous disease: a unique host-pathogen interaction. J Infect Dis. 2012;206(7):1128–37.
43. Marcinkowski M, Bauer K, Stoltenburg-Didinger G, Vogel M, Versmold H. Fatal aspergillosis with brain abscesses in a neonate with DiGeorge syndrome. Pediatr Infect Dis J. 2000;19(12):1214–6.
44. Silva GB, Fernandes KP, Segundo GR. Common variable immunodeficiency and isosporiasis: first report case. Rev Soc Bras Med Trop. 45(6):768–9.

45. Wolach B, Eliakim A, Gottesman G, Yellin A. Pulmonary aspergillosis in a child with hyperimmunoglobulin E syndrome. Clin Infect Dis. 1998;26(1):204–5.
46. Yoshihara T, Morimoto A, Nakauchi S, Fujii N, Tsunamoto K, Misawa A, et al. Successful transplantation of haploidentical CD34+ selected bone marrow cells for an infantile case of severe combined immunodeficiency with aspergillus pneumonia. Pediatr Hematol Oncol. 2002;19(6):439–43.
47. Subauste CS, Wessendarp M, Sorensen RU, Leiva LE. CD40-CD40 ligand interaction is central to cell-mediated immunity against Toxoplasma gondii: patients with hyper IgM syndrome have a defective type 1 immune response that can be restored by soluble CD40 ligand trimer. J Immunol. 1999;162(11):6690–700.
48. Fontana S, Moratto D, Mangal S, De Francesco M, Vermi W, Ferrari S, et al. Functional defects of dendritic cells in patients with CD40 deficiency. Blood. 2003;102(12):4099–106.
49. Seyama K, Kobayashi R, Hasle H, Apter AJ, Rutledge JC, Rosen D, et al. Parvovirus B19-induced anemia as the presenting manifestation of X-linked hyper-IgM syndrome. J Infect Dis. 1998;178(2):318–24.
50. Gratwohl A, Baldomero H, Aljurf M, Pasquini MC, Bouzas LF, Yoshimi A, et al. Hematopoietic stem cell transplantation: a global perspective. JAMA. 2010;303(16):1617–24.
51. Guerra-Maranhao MC, Costa-Carvalho BT, Nudelman V, Barros-Nunes P, Carneiro-Sampaio MM, Arslanian C, et al. Response to polysaccharide antigens in patients with ataxia-telangiectasia. J Pediatr (Rio J). 2006;82(2):132–6.