Pediatr Blood Cancer 2015;62:2101–2107

Clinical and Genotypic Spectrum of Chronic Granulomatous Disease in 71 Latin American Patients: First Report From the LASID Registry

Edgar Borges de Oliveira-Junior, PhD,[1] Nuria Bengala Zurro, MSc,[1] Carolina Prando, MD, PhD,[2] Otavio Cabral-Marques, PhD,[1] Paulo Vitor Soeiro Pereira, PhD,[1] Lena-Friederick Schimke, MD,[1] Stefanie Klaver, MSc,[1] Marcia Buzolin, MD,[3] Lizbeth Blancas-Galicia, PhD,[4] Leopoldo Santos-Argumedo, PhD,[5] Dino Roberto Pietropaolo-Cienfuegos, MD,[6] Francisco Espinosa-Rosales, MD,[4] Alejandra King, MD,[7] Ricardo Sorensen, MD,[8] Oscar Porras, MD,[9] Persio Roxo-Junior, MD, PhD,[10] Wilma Carvalho Neves Forte, MD,[11] Julio Cesar Orellana, MD,[12] Alejandro Lozano, MD,[13] Miguel Galicchio, MD,[14] Lorena Regairaz, MD,[15] Anete Sevciovic Grumach, MD, PhD,[16] Beatriz Tavares Costa-Carvalho, MD, PhD,[17] Jacinta Bustamante, MD, PhD,[18,19] Liliana Bezrodnik, MD,[20] Matias Oleastro, MD,[21] Silvia Danielian, PhD,[21] and Antonio Condino-Neto, PhD, MD[1]*

Aim. We analyzed data from 71 patients with chronic granulomatous disease (CGD) with a confirmed genetic diagnosis, registered in the online Latin American Society of Primary Immunodeficiencies (LASID) database. ***Results.*** Latin American CGD patients presented with recurrent and severe infections caused by several organisms. The mean age at disease onset was 23.9 months, and the mean age at CGD diagnosis was 52.7 months. Recurrent pneumonia was the most frequent clinical condition (76.8%), followed by lymphadenopathy (59.4%), granulomata (49.3%), skin infections (42%), chronic diarrhea (41.9%), otitis (29%), sepsis (23.2%), abscesses (21.7%), recurrent urinary tract infection (20.3%), and osteomyelitis (15.9%). Adverse reactions to bacillus Calmette-Guérin (BCG) vaccination were identified in 30% of the studied Latin American CGD cases. The genetic diagnoses of the 71 patients revealed 53 patients from 47 families with heterogeneous mutations in the *CYBB* gene (five novel mutations: p.W361G, p.C282X, p.W483R, p.R226X, and p.Q93X), 16 patients with the common deletion c.75_76 del.GT in exon 2 of *NCF1* gene, and two patients with mutations in the *CYBA* gene. ***Conclusion.*** The majority of Latin American CGD patients carry a hemizygous mutation in the *CYBB* gene. They also presented a wide range of clinical manifestations most frequently bacterial and fungal infections of the respiratory tract, skin, and lymph nodes. Thirty percent of the Latin American CGD patients presented adverse reactions to BCG, indicating that this vaccine should be avoided in these patients. Pediatr Blood Cancer 2015;62:2101–2107.

Key words: bacillus Calmette-Guérin vaccine; chronic granulomatous disease; infections; mutations; NADPH oxidase; registry

INTRODUCTION

Chronic granulomatous disease (CGD) is a group of genetic disorders of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex of phagocytes that results in defective microbicidal activity.[1,2] This is due to the defective generation of reactive oxygen species (ROS) in response to physiological stimuli, such as the phagocytosis of microbes.[3–5] As a result of a mutation

Abbreviations: BCG, bacillus Calmette-Guérin; CGD, chronic granulomatous disease; dHPLC, denaturing high-performance liquid chromatography; DHR, dihydrorhodamine; EDTA, ethylenediamine tetraacetic acid; LASID, Latin American Society for Immunodeficiencies; NADPH, nicotinamide adenine dinucleotide phosphate; NBT, nitroblue tetrazolium; PBS, phosphate buffered saline; PHOX, phagocyte oxidase; PIDD, primary immunodeficiency diseases; ROS, reative oxygen species; SSCP, single-strand conformation polymorphism; TB, tuberculosis

[1]Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; [2]Children's Hospital Little Prince, Research Institute Pelé Little Prince, Curitiba, Brazil; [3]Center for Investigation in Pediatrics, State University of Campinas Medical School, Campinas, Brazil; [4]Immunodeficiency Research Unit, National Institute of Pediatrics, Mexico City, Mexico; [5]Centro de Investigacion de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico; [6]Departamento de Alergia e Inmunología, Hospital Infantil de México "Federico Gómez", Ciudad de México, Mexico; [7]Hospital Luis Calvo Mackenna, Santiago, Chile; [8]Department of Pediatrics and JMF Diagnostic Center for PIDD, Louisiana State University Health Sciences Center, New Orleans, Louisiana; [9]Hospital Nacional de Niños, "Dr. Carlos Sáenz Herrera", San Jose, Costa Rica; [10]Department of Pediatrics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil; [11]Departamento de Pediatria, Hospital Santa Casa de Misericórdia, São Paulo, Brazil; [12]Division Alergia e Inmunologia Clinica, Hospital de Ninos de la Santisima Trinidad, Cordoba, Argentina; [13]Department of Allergy and Immunology, Queen Fabiola University Clinic, Catholic University of Cordoba, Cordoba, Argentina; [14]Hospital de Niños Vitor. J. Vilela, Rosario, Argentina; [15]Unidad de Inmunología, Hospital de Niños Sor María Ludovica La Plata, Buenos Aires, Argentina; [16]Department of Medicine, ABC Medical School, Santo André, Brazil; [17]Department of Pediatrics, Federal University of São Paulo, São Paulo, Brazil; [18]Laboratory of Human Genetics of Infectious Diseases, Institut National de la Santé et de la Recherche Médicale, INSERM U1163 Imagine Institute, University Paris Descartes, Paris, France; [19]Center for the Study of Primary Immunodeficiencies, Assistance Publique-Hôpitaux de Paris (AP-HP), Necker-Enfants Malades Hospital, Paris, France; [20]Dr. Ricardo Gutierrez Children's Hospital, Immunology, Buenos Aires, Argentina; [21]Servicio de Inmunología y Reumatología, Hospital Nacional de Pediatría Prof. Dr. Juan P. Garrahan, Buenos Aires, Argentina

Grant sponsor: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP number 2010/51336-0, Brazil); Agence Nationale de la Recherche; Grant number: ANR13-ISV3-0001-01

Conflict of interest: Nothing to declare.

*Correspondence to: Antonio Condino-Neto, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, 1730 Lineu Prestes Avenue, São Paulo, SP 05508-000, Brazil.
E-mail: condino@icb.usp.br

Received 4 March 2015; Accepted 12 June 2015

DOI 10.1002/pbc.25674
Published online 15 July 2015 in Wiley Online Library (wileyonlinelibrary.com).

in one gene of the NAPDH oxidase complex, all myeloid cells have a profound defect in the respiratory burst that normally accompanies phagocytosis.[6] The *CYBB* gene encoding the enzymatic center of the NADPH oxidase complex, gp91^{phox}, is on the X chromosome, and mutations in this gene account for approximately 60% of cases. Autosomal forms of CGD result from mutations in p47^{phox}, p67^{pho}x, p22^{phox}, or p40^{phox}, with the last being the most recently described, whereas p47^{phox} defects account for approximately 30% of the cases.[3–5]

The prevalence of CGD has been estimated to be one per 120,000–250,000 people.[3,4,7,8] Patients with CGD usually present with recurrent severe bacterial and/or fungal infections in infancy or childhood. However, delayed diagnosis in adulthood is also possible.[7–9] Chronic lung disease is a consequence of recurrent infections and is associated with inflammation in patients with CGD.[7–9] Many CGD patients develop chronic inflammatory granulomas, which are a distinctive hallmark of this disorder. Symptomatic disease can include colitis/enteritis or granulomatous obstruction of either the gastric outlet or urinary tract. Due to persistent colitis, some patients with CGD can also present with chronic diarrhea and intestinal bleeding.[10]

Symptoms vary widely from one CGD patient to another, and this may result in substantial diagnostic delay, even in developed countries.[7–12] Limited information is available about the phenotypic and genotypic characteristics of patients with CGD in Latin America. The available literature shows some unusual clinical presentations and outcomes, and it is important to understand how they may differ from those described for patients in developed countries. The objective of this study was to analyze phenotypes and genotypes from patients with a confirmed genetic diagnosis of CGD who are included in the LASID Registry.

PATIENTS AND METHODS

LASID Registry and Study Design

The LASID registry was established to promote the identification and improved management of patients with primary immunodeficiency diseases (PIDD) in Latin America. It was inclusive of all potential doctors caring for patients with congenital disorders of the immune system. This database compiles information about patients with PIDD, including demographic, clinical, and genetic data.[13] This retrospective analysis summarized all available clinical and genetic information for 71 patients with a confirmed genetic diagnosis of CGD who were included in the LASID registry from September 2012 until September 2014. Participating centers throughout Latin America were contacted, and clinicians formally allowed retrieval of data about their patients registered in the LASID Registry database.

Ethics Committee

The LASID Registry protocols were approved by Conselho Nacional de Saúde, Ministry of Health of Brazil, for international studies (CONEP 25000.040727/2008-16, CAAE 0034.1.146.000-08). The protocol is in accordance with the Helsinki convention. Patients or their parents provided written informed consent before the research began.

Criteria for Diagnosis of CGD

Diagnosis of CGD was typically made by examining a clinical history of early-onset recurrent infections and evaluating the neutrophil oxidative burst using one or two tests. The first was the nitroblue tetrazolium (NBT) test, in which normal neutrophils reduce nitroblue tetrazolium to formazan, a dark-blue pigment visible on microscopic inspection. Neutrophils from patients with CGD do not reduce nitroblue tetrazolium.[14] The second was the dihydrorhodamine (DHR) 123 test, which applies flow cytometry to detect the oxidation of DHR 123 in activated neutrophils.[15] Generally, results from CGD patients represent less than 5% of the normal controls. They can differentiate X-linked cases from autosomal recessive (AR) cases in males, when the mother is also evaluated and proved to be an X-linked carrier.[15]

Molecular Genetic Analysis

gDNA samples were prepared by standard methods and *CYBB* exons were amplified by polymerase chain reaction (PCR). The abnormal exons were screened by denaturing high-performance liquid chromatography (dHPLC) or single-strand conformation polymorphism (SSCP) analysis before sequencing as described. [16] Abnormal exons were sequenced to reveal the nature of the mutations. PCR-amplified cDNA samples were analyzed to clarify splicing defects. Our CGD patients were sequenced mainly in Brazil, Argentina, and Mexico. Variations in the methodology occurred according to the available technology in these countries.

Protein Expression (gp91^{phox})

Western blot analysis was conducted to detect the expression of gp91^{phox} in cell extracts.[6] Proteins were solubilized in 1% Triton X-100 buffer [50 mM Tris HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, and 1% Triton X-100] and separated by SDS-PAGE in a 10% polyacrylamide gel; molecular weight markers were included on the gel. Proteins were transferred to an Immobilon-P (EMD Millipore Corporation, Billerica, MA) membrane. Nonspecific binding was blocked by incubation in 1× PBS with 3% nonfat milk powder and 0.01% Tween 20 for 1 hr. To detect gp91^{phox} (SC-54.1), antibodies from Santa Cruz Biotechnology (Dallas, TX) were used. The blot was developed with a chemiluminescence kit (Pierce ECL, ThermoFisher Scientific, Inc., Rockford, IL). Western blot analysis was used to screen all male patients. The results for gp91phox expression were presented as normal (+); diminished (−), or undetectable (0) in Table III.

Statistical Analysis

Statistical analysis of clinical presentations was performed using descriptive statistics and the Mann–Whitney *t*-test or χ^2 test, where a *P*-value of less than 0.05 was considered significant (GraphPad Prism for Mac OS X software, La Jolla).

RESULTS

Patients

This work included 71 CGD patients (58 males and 13 females) with a confirmed genetic diagnosis (among males there were five pairs of brothers and one pair of cousins). Data inclusion occurred between September 2012 and September 2014.

The distribution of patients with respect to their countries was 39% from Brazil, 36% from Argentina, 16% from Mexico, 6% from Chile, and 3% from Colombia. Brazil, Argentina, Mexico, and Colombia have well-established centers for genetic diagnosis of CGD and other PIDD. This is the reason for the predominant contribution of these countries to this and other works related to PIDD in Latin America.[17]

All patients are alive and receive standard prophylaxis with cotrimoxazole and itraconazole. In Mexico and Argentina, CGD patients have access to routine interferon-gamma therapy given subcutaneously three times per week. In Brazil, this medication is not registered by the national regulatory agency, limiting the access to it.

The management of inflammatory bowel disease was mainly based on the use of corticosteroids. Anti-TNF alpha therapy was not used because of the risk of overwhelming infections or tuberculosis, which is endemic in Latin America. Bone marrow transplants were performed in Brazil, Mexico, and Argentina, depending on the availability of a matched donor. Gene therapy for PIDD is not available in Latin America and no patient was referred for this treatment in other centers around the world during the study period.

Clinical Presentations

Within the cohort of patients in this investigation, 26% of the cases had a family history of recurrent or unusual infections, regardless of the affected gene. Recurrent pneumonia (76.8%) was the most frequent clinical condition associated with CGD, from which 13% of the cases presented with concomitant pleural effusion. Other clinical occurrences included lymphadenopathy (59.4%), granulomata (49.3%), skin infections (42%), chronic diarrhea (41.9%), otitis (29%), sepsis (23.2%), abscesses (21.7%), recurrent urinary tract infections (20.3%), and osteomyelitis (15.9%) (Fig. 1).

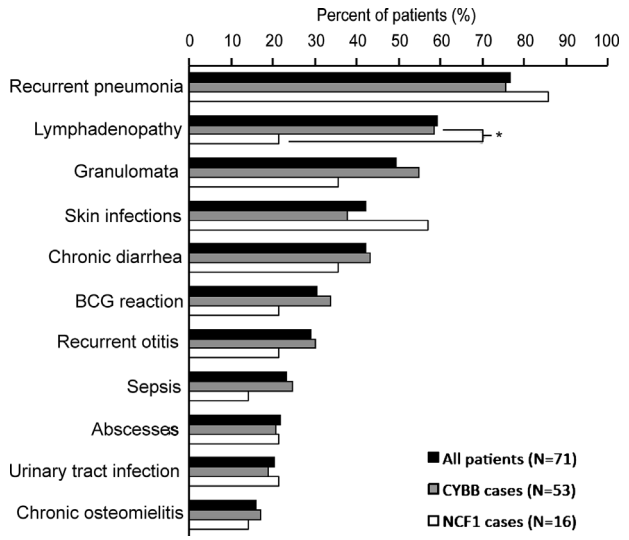

Fig. 1. Clinical phenotypes of Latin American CGD patients. All patients (71 cases, black column); patients with *CYBB* mutations (53 cases, gray column), and patients with *NCF1* mutations (16 cases, white column). Recurrent pneumonia was the most prevalent clinical occurrence in all cases. Lymphadenopathy was more frequent among patients with mutations in *CYBB* gene (58.5%) compared to those with mutations in *NCF1* (21.4%) ($^{*}P = 0.014$, χ^2 test).

CGD patients with *CYBB* mutations presented a higher incidence of lymphadenopathy (58.5%) compared to those with mutations in *NCF1* (21.4%) ($P = 0.014$ by χ^2 test) (Fig. 1). Within cases of skin infections, 26.3% were in the form of cellulitis and 31.6% presented as skin abscesses. All cases of severe skin infections occurred in X-linked CGD cases. Among cases with granulomata, the lung was the most frequently affected organ characterizing chronic lung disease (33.4%), followed by the intestine (chronic inflammatory colitis 19.4%), skin (chronic pyoderma, 16.7%), the liver (16.6%), the urinary tract (5.6%), lymph nodes (5.5%), and bones (2.8%). Within cases of recurrent abscesses, the liver was the most frequently affected organ (84.6%). One X-linked case had a lung abscess and one female patient with a mutation in *NCF1* had a breast abscess.

There was a substantial interval between the manifestation of the first symptoms of CGD and the time of diagnosis. The mean age at the first occurrence of symptoms was 23.9 months (range: 0.13–156.0 months), and the mean age at diagnosis was 52.7 months (range: 1.0–199.0 months). Patients with mutations in the *NCF1* gene were older at disease onset (mean 33.3 months, range: 0.33–84.0 months) and diagnosis (mean 98.4 months, range: 24.0–168.0 months) compared with those with mutations in the *CYBB* gene, $P = 0.0145$ and $P = 0.0028$, respectively (Mann–Whitney test) (Table I).

The Latin American population is mixed, outbred, and with a low rate of consanguineous marriages. It includes descendants from several European, Middle-East, African, and local native populations, with a high rate of interracial marriages, making it difficult to analyze the incidence of infections in particular ethnic groups.

Infecting Pathogens

Recurrent and severe infections were caused by a broad range of etiologic organisms (Table II), including *Staphylococcus aureus, Escherichia coli*, *Aspergillus* spp, *Serratia marcescens*, *Candida* spp, *Klebsiella* spp, *Salmonella* spp, and *Nocardia* spp. Other pathogens isolated from this cohort included *Acinetobacter* spp, *Pseudomonas* spp, H1N1 virus, rotavirus, adenovirus, *Trycophiton*, *Ascaris spp*, and *Giardia spp*. The incidence rate of the several infecting pathogens varied among patient subgoups (Table II).

Adverse Reactions to BCG Vaccination

Approximately 30% of patients had adverse reactions to BCG vaccination, with greater prevalence in the *CYBB* group (85.7%) compared with the *NCF1* group (14.3%) ($P = 0.003$ by χ^2 test). Of these patients, 70% had a scar >5 mm in diameter with satellite

TABLE I. Disease Onset and Diagnosis (Months) in Latin American CGD Patients

	Age at disease onset (months)		Age at diagnosis (months)	
	Mean	Range	Mean	Range
All patients (N = 71)	23.9	0.13–156.0	52.7	1.0–199.0
CYBB patients (N = 53)	22.0	0.13–156.0	43.2	1.0–199.0
NCF1 patients (N = 16)	33.3*	0.33–84.0	98.4**	24.0–168.0

*$P = 0.0145$; **$P = 0.0028$ (Mann–Whitney test).

TABLE II. Infecting Pathogens Spectra in Latin American CGD Patients

	N=71	N=53	N=16
Recurrent severe infections	All patients (%)	*CYBB* patients (%)	*NCF1* patients (%)
Staphylococcus aureus	20.3	22.6	14.3
Escherichia coli	4.3	5.7	0.0
Aspergillus spp	13.0	15.1	7.1
Serratia marcescens	8.7	11.3	0.0
Candida spp	8.7	9.4	7.1
Klebsiella spp	10.1	9.4	14.3
Salmonella spp	7.2	7.5	7.1
Nocardia spp	2.9	3.8	0.0
BCG	30.4	34.0	21.4

lymph node reaction requiring prolonged antibiotic treatment to heal, and 30% had generalized spread of BCG leading to a severe clinical condition. In patients with generalized BCG spread, diagnosis was confirmed by culture. Among the 71 investigated patients, only 5 (7%) had not received the BCG vaccine.

Molecular Genetic Analysis

Molecular genetic analysis revealed 53 (74.6%) patients from 47 families with different mutations in the *CYBB* gene (Fig. 2 and Table III); 16 (22.5%) patients with mutations in the *NCF1* gene showing the common deletion (c.75_76 del.GT, Y26fsX26) that leads to a premature stop codon in exon 2; and two patients (3%) with different missense mutations in the *CYBA* gene, one of whom presented a compound heterozygous mutation (c.268 C>G/p.R90G + c.114delT/p.F38fsX36 and c.352 A>C/p.S118R). A prediction using Polyphen2 indicated the impact of potential damage in all of the missense mutations found in patients with variations in the *CYBB* gene (p.C185R; p.M1V, p.P339H; p.H222L, p.C537R, p.359A, p.W361G, p.E309K, p.H101R, p.W483R, and p.S333P) and in the *CYBA* gene (p.R90G and p.S118R) (Supplemental Figure S1). The most frequent mutations affecting *CYBB* were nonsense (38.3%), followed by missense (27.7%), and splicing defects (21.3%) (Fig. 2 and Table III). In addition, we detected five novel *CYBB* mutations in seven cases: p.W361G, p.C282X, p.W483R, p.R226X, and p.Q93X (Table III).

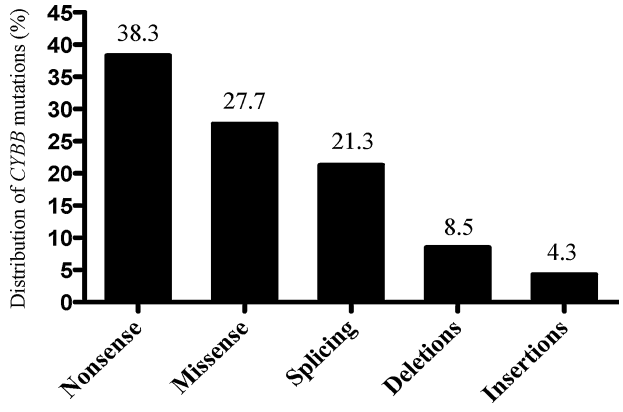

Fig. 2. Heterogenous *CYBB* mutations among 53 patients from 47 families. The most frequent mutations were nonsense (38.3%), followed by missense (27.7%), and splicing defects (21.3%).

DISCUSSION

This analysis of patients from the LASID registry indicates that Latin American patients with CGD had a wide range of clinical manifestations, most often bacterial and fungal infections of the respiratory tract, lymph nodes, and skin. Of these patients, 30% had adverse reactions to BCG vaccination. We must emphasize that, among these 21 BCG reaction patients, 19 had mutations in the *CYBB* gene and only two had mutations in the *NCF1* gene. Our CGD patients had heterogeneous mutations in the *CYBB* gene, a common deletion in the *NCF1* gene, and two different missense substitutions in the *CYBA* gene.

We observed an older average age at disease onset in patients with mutations in the *NCF1* gene. These patients' symptoms were generally milder and more delayed; this resulted in later diagnoses compared with patients with other mutations, such as those in the *CYBB* gene, which is in agreement with a recent study.[18] The most frequent clinical manifestation in this group was recurrent pneumonia (Fig. 1).

The current larger-scale analysis expands upon results from other smaller evaluations. Results from a series of 18 patients from Brazil indicated that the most common manifestations of CGD were lymphadenopathy, hepatosplenomegaly, pneumonia, and abscesses.[19] A second small-scale study of seven patients in Brazil indicated that pneumonia was the most frequent clinical feature of CGD, followed by skin infections, sinusitis, otitis, and liver abscess.[20]

Chronic colitis is a frequent clinical manifestation in patients with CGD, and its symptoms include diarrhea, abdominal pain, and rectal pain.[21,22] Our cohort of Latin American CGD patients presented with chronic diarrhea in 41.9% of the cases as shown in Figure 1. We also report associations with *Giardia* and *Ascaris*, and rotaviruses, agents not usually described in the CGD literature.

The infecting pathogens isolated from CGD patients in this registry analysis were consistent with those from studies of other CGD patients from registries in North America and Europe.[8,18,23–25] Reviews of this disease have indicated that the most commonly encountered pathogens are *S. aureus*, *Pseudomonas* spp, *Nocardia* spp, *Candida*, and *Aspergillus* spp.[8,18,26] Results from individual studies suggest that five main groups of organisms persist inside CGD phagocytes, including *S. aureus*, *B. cepacia* complex, *S. marcescens*, *Nocardia*, and *Aspergillus* spp.[7,8,18] These results have demonstrated that, regardless of geography, studies of CGD reveal varied but similar infectious diseases.

Our study shows differences in the types of pathogens that cause recurrent and severe infections in subgroups of patients with mutations in different genes (Table II). For example, *Klebsiella* infections, which occurred in 10.1% of the 71 patients studied, occurred in 9.4% of the *CYBB* cases, and 14.3% of the *NCF1* cases. There were no *Nocardia* infections in any of the *NCF1* cases, but these infections occurred in 3.8% of the *CYBB* cases, and in one of the *CYBA* cases. *Serratia marcescens* infections occurred in 11.3% of the *CYBB* cases, but in none of the *NCF1* or *CYBA* cases. There were other notable differences between the percentages of infections caused by *S. aureus*, *Aspergillus*, and gram-negative bacteria (Table II). If similar differences in infecting pathogens are

TABLE III. *CYBB* Mutations in Latin American CGD Patients

Paients	Expression	Mutation	Site	cDNA	Protein	Reference for Previous Reports
P1	$X91^0$	Nonsense	exon 7	c.688 C>T	p.R226X	[41]
P2	$X91^0$	Nonsense	exon 7	c.688 C>T	p.R226X	[41]
P3	$X91^0$	Insertion	exon 9	c.903_4 insC	p.T302fsX46	[4]
P4	$X91^0$	Splicing	intron 2	c.141 +5 G>T	exon 2 deletion	[4]
P5	$X91^+$	Missense	exon 6	c.553 T>C	p.C185R	[4]
P6	$X91^-$	Missense	exon 6	c.665 A>T	p.H222L	[4]
P7	$X91^+$	Missense	exon 13	c.1621 T>C	p.C537R	[41]
P8	$X91^0$	Nonsense	exon 3	c.229 C>T	p.R73X	[41]
P9	$X91^0$	Nonsense	exon 5	c.481 C>T	p.R157X	[41]
P10	$X91^0$	Splicing	exon 3	c.264 G>A	exon 3 deletion	[41]
P11	$X91^0$	Splicing	exon 3	c.264 G>A	exon 3 deletion	[41]
P12	$X91^?$	Splicing	intron 10	c.1326 +1 G>A	exon 10 deletion	[41]
P13	$X91^-$	Splicing	intron 9	c.1166 −2 G>A	exon 10 deletion	[20]
P14	$X91^?$	Nonsense	exon 2	c.95 G>A	p.W28X	[41]
P15	$X91^?$	Nonsense	exon 4	c.271 C>T	R91X	[4]
P16	$X91^?$	Insertion	exon 10	c.1255dupA	p.I419NfsX12	[4]
P17	$X91^-$	Missense	exon 9	c.1076 G>C	p.G359A	[4]
P18	$X91^?$	Missense	exon 9	c.1081 T>G	p.W361G	Novel mutation
P19	$X91^0$	Nonsense	exon 5	c.388 C>T	p.R130X	[4]
P20	$X91^?$	Nonsense	exon 2	c.95 G>A	p.W28X	[41]
P21	$X91^-$	Missense	exon 9	c.925 G>A	p.E309K	[4]
P22	$X91^-$	Missense	exon 9	c.925 G>A	p.E309K	[4]
P23	$X91^-$	Missense	exon 9	c.925 G>A	p.E309K	[4]
P24	$X91^0$	Nonsense	exon 8	c.868 C>T	p.R290X	[4]
P25	$X91^0$	Nonsense	exon 5	c.469 C>T	p.R157X	[4]
P26	$X91^0$	Nonsense	exon 7	c.676 C>T	p.R226X	[4]
P27	$X91^0$	Deletion	exon 12	c.1528_29 delTT	p.L510fsX8	[4]
P28	$X91^0$	Deletion	exon 12	c.1528_29 delTT	p.L510fsX8	[4]
P29[a]	$X91^0$	Splicing	intron 6	c.674+5 G>C	exon 6 deletion	[40]
P30	$X91^0$	Splicing	intron 6	c.674+5 G>C	exon 6 deletion	[40]
P31[b]	$X91^0$	Splicing	intron 2	c.142 (-1 G>C)	Skipping of exon 3	[4]
P32	$X91^0$	Splicing	intron 2	c.142 (-1 G>C)	Skipping of exon 3	[4]
P33	$X91^0$	Splicing	intron 12	c.1587 (-2 A>G)	Altern. Splicing	[4]
P34[a]	$X91^0$	Splicing	intron 6	c.674+5 G>C	exon 6 deletion	[40]
P35	$X91^0$	Splicing	intron 6	c.674+5 G>C	exon 6 deletion	[40]
P36	$X91^?$	Splicing	exon 3	c.252 G>A	exon 3 deletion	[4]
P37	$X91^?$	Nonsense	exon 8	c.846 T>A	p.C282X	Novel mutation
P38	$X91^?$	Deletion	exon 9	c.1147_1150delCCTA	p.P383RfsX2	[4]
P39	$X91^0$	Nonsense	exon 8	c.868 C>T	p.R290X	[4]
P40	$X91^?$	Missense	exon 9	c.997 T>C	p.S333P	[4]
P41	$X91^?$	Deletion	exon 6	c.603delC	p.Y201fsX12	[4]
P42[a]	$X91^0$	Missense	exon 4	c.302 A>G	p.H101R	[4]
P43	$X91^0$	Missense	exon 4	c.302 A>G	p.H101R	[4]
P44	$X91^?$	Missense	exon 1	c.1 A>G	p.M1V	[4]
P45	$X91^-$	Missense	exon 9	c.1016 C>A	p.P339H	[4]
P46[a]	$X91^?$	Missense	exon 11	c.1447 T>C	p.W483R	Novel mutation
P47	$X91^?$	Missense	exon 11	c.1447 T>C	p.W483R	Novel mutation
P48	$X91^?$	Nonsense	exon 6	c.618 G>A	p.W206X	[4]
P49[a]	$X91^0$	Nonsense	exon 7	c.676 C>T	p.R226X	Novel mutation
P50	$X91^0$	Nonsense	exon 7	c.676 C>T	p.R226X	Novel mutation
P51	$X91^?$	Nonsense	exon 4	c.277 C>T	p.Q93X	Novel mutation
P52	$X91^0$	Nonsense	exon 2	c.84 G>A	p.W28X	[4]
P53	$X91^?$	Nonsense	exon 9	c.1006 G>T	p.E336X	[4]

[a]P29 and P30 are brothers; P34 and P35 are brothers; P42 and P43 are brothers; P46 and P47 are brothers; P49 and P50 are brothers. [b]P31 and P32 are cousins. For $X91^0$, $X91^-$,$X91^+$, and $X91^?$, the superscript denotes whether the level of gp91-phox protein as assessed by Western blot analysis was undetectable (0), diminished (−), normal (+), or not determined (?).

observed in other groups of *CYBB*, *NCF1*, and *CYBA* cases, these differences may be of interest to clinicians.

Mycobacterial infections are not usually considered part of the typical clinical picture of CGD in Europe and North America, [8,18,24–27] but they more commonly appear in CGD patients in other parts of the world.[28–30] An increasing number of case reports have suggested that mycobacterial disease is an important feature of CGD, especially in countries where the BCG vaccine is routinely administered, or where tuberculosis (TB) is endemic, or both; the results indicated the occurrence of BCG disease in up to 38 of the CGD patients investigated.[29–31] In addition, TB had been reported in 16 patients, including seven patients with both BCG and TB.[29,30] More recent results have indicated the occurrence of BCG disease [24,28,31–34] and diseases associated with environmental mycobacteria [27] in patients with CGD. An important implication for the management of patients with CGD in this registry analysis is the finding that 30% of patients studied had adverse reactions to BCG vaccination. BCG is a mandatory vaccine in Latin America. It is generally given during the neonatal period, before the child is discharged to go home. This practice is potentially harmful for patients with CGD, SCID, or other PIDD affecting the phagocyte or T-cell function.[35] We hope in the future we can develop neonatal screening tests for CGD and other phagocyte defects before giving BCG vaccination. Our study reported no occurrences of TB or other mycobacterial diseases. As new cases continue to be added to the LASID registry, TB may be revealed in the future.

Lymphadenopathy was more frequent in CGD patients with *CYBB* mutations compared with those with *NCF1* mutations in this current study, ($P = 0.014$ by χ^2 test) indicating that the gene mutation influences this clinical presentation. Infection-associated hemophagocytic syndrome in CGD patients triggered by *Leishmania* and complicated by macrophage activation syndrome has been observed in the Mediterranean region and in Mexico.[36,37]

The most common causes of death reported in a large series of patients with CGD were pneumonia and/or sepsis due to *Aspergillus* or *B. cepacia* complex.[8,18] A recent study indicates that fungal infections, not bacterial infections, are determinants of survival and are the major causes of morbidity and mortality in CGD patients, especially in those with mutations in the *CYBB* gene.[18] To date, there have been no deaths to report in this study. All patients are alive and receive prophylactic antibiotics and antifungal treatment.

Results from the present study of Latin American patients with CGD indicate that the disease is associated with heterogeneous mutations in the *CYBB* gene, a common deletion in the *NCF1* gene, and two different missense substitutions in the *CYBA* gene. We also detected five novel *CYBB* mutations in seven cases: p.W361G, p.C282X, p.W483R, p.R226X, and p.Q93X (Table III). The relationship between CGD and mutations in *CYBB* was first reported in 1986.[38] Since then, multiple studies have reported distinct mutations associated with CGD (Table III). Roos et al. have cataloged 681 mutations in *CYBB* associated with CGD.[4] Additional *CYBB* mutations associated with CGD published subsequent to that report include a point mutation, c.493G>T; a double mutation, c.625C>G in exon 6 and c.1510C>T in exon 12, leading to a premature stop codon at Gly165 in $gp91^{phox}$; missense mutations His209Arg/Thr503Ile; splice mutations in the 5' intronic regions of introns 1 and 6; a deletion/insertion, c.1024_1026delCTG/insT, resulting in a frameshift introducing a stop codon at position 346 in $gp91^{phox}$; and insertion of a T at c.1373 leading to a frameshift and a premature stop codon at position 484 in $gp91^{phox}$.[39] Two other published studies have addressed the molecular genetics of CGD in patients from Latin America. Results from a study that included seven unrelated patients with the X-linked form of CGD indicated that all had a mutation affecting the *CYBB* gene (two insertions, one substitution, and four splice site defects). The study also included seven patients with autosomal recessive forms of CGD. All had the GT deletion in exon 2 of the *NCF1* gene.[20] An Argentinian study of 18 patients with X-linked CGD indicated 11 different mutations in the *CYBB* gene (intronic, single-nucleotide substitution resulting in nonsense or missense codons, and one or two nucleotide deletions resulting in frameshifts).[40] We hope to extend these molecular studies to additional CGD patients in Latin America and revisit the data base in the future, aiming to re-analyze the occurrence of mutations in the affected genes in this mixed outbred population.

We conclude that the present study of Latin American patients with CGD is in accordance with previous studies that have shown pulmonary infections to be the most frequent clinical presentation followed by lymphadenopathy, and skin infections. Latin American patients with CGD showed heterogeneous mutations in the *CYBB* and *CYBA* genes or a common deletion in the *NCF1* gene. These patients were infected by a wide range of bacterial and fungal pathogens. This registry has also revealed that approximately 30% of patients expressed BCG-itis or BCG-osis after vaccination. BCG reaction was responsible for the early diagnosis of CGD in several cases and the early age of first clinical manifestation. These results and those from prior studies support the view that BCG vaccination should be avoided in patients with CGD, particularly in patients with the X-linked form of the disease.

ACKNOWLEDGMENTS

The authors thank Conselho Nacional de Pesquisa e Desenvolvimento Científico (CNPq, Brazil), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Brazil), Agence Nationale de la Recherche (ANR13-ISV3-0001-01, France), Baxter, CSL Behring, and Octapharma for supporting the LASID Registry. The authors wish to thank the patients, their families, and physicians for participating and contributing to this study. Editorial assistance of BSG Communications, supported by Baxter Healthcare Corporation, is also acknowledged.

REFERENCES

1. Bridges RA, Berendes H, Good RA. A fatal granulomatous disease of childhood: The clinical pathological, and laboratory features of a new syndrome. AMA J Dis Child 1959;97:387–408.
2. Holmes B, Page AR, Good RA. Studies of the metabolic activity of leukocytes from patients with a genetic abnormality of phagocytic function. J Clin Invest 1967;46:1422–1432.
3. Roos D, Kuhns DB, Maddalena A, Bustamante J, Kannengiesser C, de Boer M, van Leeuwen K, Köker MY, Wolach B, Roesler J, Malech HL, Holland SM, Gallin JI, Stasia MJ. Hematologically important mutations: The autosomal recessive forms of chronic granulomatous disease (second update). Blood Cells Mol Dis 2010;44:291–299.
4. Roos D, Kuhns DB, Maddalena A, Roesler J, Lopez JA, Ariga T, Avcin T, de Boer M, Bustamante J, Condino-Neto A, Di Matteo G, He J, Hill HR, Holland SM, Kannengiesser C, Köker MY, Kondratenko I, van Leeuwen K, Malech HL, Marodi L. Hematologically important mutations: X-linked chronic granulomatous disease (third update). Blood Cells Mol Dis 2010;45:246–265.
5. Rae J, Newburger PE, Dinauer MC, Noack D, Hopkins PJ, Kuruto R, Curnutte JT. X-linked chronic granulomatous disease: Mutations in the CYBB gene encoding the gp91-phox -component of the respiratory burst oxidase. Am J Hum Genet 1998;62:1320–1331.
6. Bustamante J, Arias AA, Vogt G, Picard C, Galicia LB, Prando C, Grant AV, Marchal CC, Hubeau M, Chapgier A, de Beaucoudrey L, Puel A, Feinberg J, Valinetz E, Jannière L, Besse C, Boland A, Brisseau JM, Blanche S, Lortholary O, Fieschi C, Emile JF, Boisson-Dupuis S, Al-Muhsen S, Woda B, Newburger PE, Condino-Neto A, Dinauer MC, Abel L, Casanova JL. Germline CYBB mutations that selectively

affect macrophages in kindreds with X-linked predisposition to tuberculous mycobacterial disease. Nat Immunol 2011;12:213–221.
7. Seger RA. Chronic granulomatous disease: Recent advances in pathophysiology and treatment. Neth J Med 2010;68:334–340.
8. Winkelstein JA, Marino MC, Johnston RB, Jr., Boyle J, Curnutte J, Gallin JI, Malech HL, Holland SM, Ochs H, Quie P, Buckley RH, Foster CB, Chanock SJ, Dickler H. Chronic granulomatous disease: Report on a national registry of 368 patients. Medicine 2000;79:155–169.
9. Song E, Jaishankar GB, Saleh H, Jithpratuck W, Sahni R, Krishnaswamy G. Chronic granulomatous disease: A review of the infectious and inflammatory complications. Clin Mol Allergy 2011;9:10.
10. Dinauer MC. Chronic granulomatous disease and other disorders of phagocyte function. Hematol Am Soc Hematol Educ Program 2005;89–95.
11. Turul-Ozgür T, Türkkani-Asal G, Tezcan I, Köker MY, Metin A, Yel L, Ersoy F, Sanal O. Clinical features of chronic granulomatous disease: A series of 26 patients from a single center. Turk J Pediatr 2010;52:576–581.
12. Movahedi M, Aghamohammadi A, Rezaei N, Shahnavaz N, Jandaghi AB, Farhoudi A, Pourpak Z, Moin M, Gharagozlou M, Mansouri D. Chronic granulomatous disease: A clinical survey of 41 patients from the Iranian primary immunodeficiency registry. Int Arch Allergy Immunol 2004;134:253–259.
13. Errante PR, Franco JL, Espinosa-Rosales FJ, Sorensen R, Condino-Neto A. Advances in primary immunodeficiency diseases in Latin America: Epidemiology, research, and perspectives. Ann N Y Acad Sci 2012;1250:62–72.
14. Ochs HD, Igo RP. The NBT slide test: A simple screening method for detecting chronic granulomatous disease and female carriers. J Pediatr 1973;83:77–82.
15. Jirapongsananuruk O, Malech HL, Kuhns DB, Niemela JE, Brown MR, Anderson-Cohen M, Fleisher TA. Diagnostic paradigm for evaluation of male patients with chronic granulomatous disease, based on the dihydrorhodamine 123 assay. J Allergy Clin Immunol 2003;111:374–379.
16. de Oliveira-Junior EB, Prando C, Lopez JA, Arango JC, Buzolin M, Rehder J, Pedroza LA, Frazão JB, Dantas VM, Roxo-Junior P, Grumach AS, Costa-Carvalho BT, Bustamante J, Condino-Neto A. High-performance liquid chromatography under partially denaturing conditions (dHPLC) is a fast and cost-effective method for screening molecular defects: Four novel mutations found in X-linked chronic granulomatous disease. Scand J Immunol 2012;76:158–166.
17. Cabral-Marques O, Klaver S, Schimke LF, Ascendino EH;, Khan TA, Pereira PVS, Falcai A, Grumach AS, Sorensen R, Condino-Neto A. First report of the Hyper-IgM syndrome registry of the Latin American society for immunodeficiencies: Novel mutations, unique infections, and outcomes. J Clin Immunol 2014;34:146–156.
18. Marciano BE, Spalding C, Fitzgerald A, Mann D, Brown T, Osgood S, Yockey L, Darnell DN, Barnhart L, Daub J, Boris L, Rump AP, Anderson VL, Haney C, Kuhns DB, Rosenzweig SD, Kelly C, Zelazny A, Mason T, DeRavin SS, Kang E, Gallin JI, Malech HL, Olivier KN, Uzel G, Freeman AF, Heller T, Zerbe CS, Holland SM. Common severe infections in chronic granulomatous disease. Clin Infect Dis 2015;60:1176–1183.
19. Carnide EG, Jacob CA, Castro AM, Pastorino AC. Clinical and laboratory aspects of chronic granulomatous disease in description of eighteen patients. Pediatr Allergy Immunol 2005;16:5–9.
20. Agudelo-Flórez P, Prando-Andrade CC, López JA, Costa-Carvalho BT, Quezada A, Espinosa FJ, de Souza Paiva MA, Roxo P, Grumach A, Jacob CA, Carneiro-Sampaio MM, Newburger PE, Condino-Neto A. Chronic granulomatous disease in Latin American patients: Clinical spectrum and molecular genetics. Pediatr Blood Cancer 2006;46:243–252.
21. Goldblatt D. Recent advances in chronic granulomatous disease. J Infect 2014;69:S32–S35.
22. Marciano BE, Rosenzweig SD, Kleiner DE, Anderson VL, Darnell DN, Anaya-O'Brien S, Hilligoss DM, Malech HL, Gallin JI, Holland SM. Gastrointestinal involvement in chronic granulomatous disease. Pediatrics 2004;114:462e8.
23. Liese J, Kloos S, Jendrossek V, Petropoulou T, Wintergerst U, Notheis G, Gahr M, Belohradsky BH. Long-term follow-up and outcome of 39 patients with chronic granulomatous disease. J Pediatr 2000;137:687–693.
24. Van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, Español T, Fischer A, Kurenko-Deptuch M, Mouy R, Petropoulou T, Roesler J, Seger R, Stasia MJ, Valerius NH, Weening RS, Wolach B, Roos D, Kuijpers TW. Chronic granulomatous disease: The European experience. PLoS ONE 2009;4:e5234.
25. Jones LB, McGrogan P, Flood TJ, Gennery AR, Morton L, Thrasher A, Goldblatt D, Parker L, Cant AJ. Special article: Chronic granulomatous disease in the United Kingdom and Ireland: A comprehensive national patient-based registry. Clin Exp Immunol 2008;152:211–218.
26. Segal BH, Veys P, Malech H, Cowan MJ. Chronic granulomatous disease: Lessons from a rare disorder. Biol Blood Marrow Transplant 2011;17:S123–S131.
27. Martire B, Rondelli R, Soresina A, Pignata C, Broccoletti T, Finocchi A, Rossi P, Gattorno M, Rabusin M, Azzari C, Dellepiane RM, Pietrogrande MC, Trizzino A, Di Bartolomeo P, Martino S, Carpino L, Cossu F, Locatelli F, Maccario R, Pierani P. Clinical features, long-term follow-up and outcome of a large cohort of patients with chronic granulomatous disease: An Italian multicenter study. Clin Immunol 2008;126:155–164.
28. Lee PP, Chan KW, Jiang L, Chen T, Li C, Lee TL, Mak PH, Fok SF, Yang X, Lau YL. Susceptibility to mycobacterial infections in children with X-linked chronic granulomatous disease: A review of 17 patients living in a region endemic for tuberculosis. Pediatr Infect Dis J 2008;27:224–230.
29. Bustamante J, Aksu G, Vogt G, de Beaucoudrey L, Genel F, Chapgier A, Filipe-Santos O, Feinberg J, Emile JF, Kutukculer N, Casanova JL. BCG-osis and tuberculosis in a child with chronic granulomatous disease. J Allergy Clin Immunol 2007;120:32–38.
30. Bustamante J, Picard C, Boisson-Dupuis S, Abel L, Casanova JL. Genetic lessons learned from X-linked Mendelian susceptibility to mycobacterial diseases. Ann N Y Acad Sci 2011;1246:92–101.
31. Norouzi S, Aghamohammadi A, Mamishi S, Rosenzweig SD, Rezaei N. Bacillus Calmette-Guérin (BCG) complications associated with primary immunodeficiency diseases. J Infect 2012;64:543–544.
32. Rezai MS, Khotaei G, Mamishi S, Kheirkhah M, Parvaneh N. Disseminated bacillus Calmette-Guerin infection after BCG vaccination. J Trop Pediatr 2008;54:413–416.
33. Kusuhara K, Ohga S, Hoshina T, Saito M, Sasaki Y, Ishimura M, Takada H, Fujita M, Hara T. Disseminated bacillus Calmette-Guérin lymphadenitis in a patient with gp91phox- chronic granulomatous disease 25 years after vaccination. Eur J Pediatr 2009;168:745–747.
34. Doğru D, Kiper N, Ozçelik U, Yalçin E, Tezcan I. Tuberculosis in children with congenital immunodeficiency syndromes. Tuberk Toraks 2010;58:59–63.
35. Marciano BE, Huang CY, Joshi G, Rezaei N, Carvalho BC, Allwood Z, Ikinciogullari A, Reda SM, Gennery A, Thon V, Espinosa-Rosales F, Al-Herz W, Porras O, Shcherbina A, Szaflarska A, Kiliç Ş, Franco JL, Gómez Raccio AC, Roxo P, Jr., Esteves I, Galal N, Grumach AS, Al-Tamemi S, Yildiran A, Orellana JC, Yamada M, Morio T, Liberatore D, Ohtsuka Y, Lau YL, Nishikomori R, Torres-Lozano C, Mazzucchelli JT, Vilela MM, Tavares FS, Cunha L, Pinto JA, Espinosa-Padilla SE, Hernandez-Nieto L, Elfeky RA, Ariga T, Toshio H, Dogu F, Cipe F, Formankova R, Nuñez-Nuñez ME, Bezrodnik L, Marques JG, Pereira MI, Listello V, Slatter MA, Nademi Z, Kowalczyk D, Fleisher TA, Davies G, Neven B, Rosenzweig SD. BCG vaccination in patients with severe combined immunodeficiency: Complications, risks, and vaccination policies. J Allergy Clin Immunol 2014;133:1134–1141.
36. Greenberg DE, Shoffner AR, Zelazny AM, Fenster ME, Zarember KA, Stock F, Ding L, Marshall-Batty KR, Wasserman RL, Welch DF, Kanakabandi K, Sturdevant DE, Virtaneva K, Porcella SF, Murray PR, Malech HL, Holland SM. Recurrent Granulibacter bethesdensis infections and chronic granulomatous disease. Emerg Infect Dis 2010;16:1341–1348.
37. Álvarez-Cardona A, Rodŕguez-Lozano AL, Blancas-Galicia L, Rivas-Larrauri FE, Yamazaki-Nakashimada MA. Intravenous immunoglobulin treatment for macrophage activation syndrome complicating chronic granulomatous disease. J Clin Immunol 2012;32:207–211.
38. Royer-Pokora B, Kunkel LM, Monaco AP, Goff SC, Newburger PE, Baehner RL, Cole FS, Curnutte JT, Orkin SH. Cloning the gene for an inherited human disorder-chronic granulomatous disease-on the basis of its chromosomal location. Nature 1986;322:32–38.
39. Martel C, Mollin M, Beaumel S, Brion JP, Coutton C, Satre V, Vieville G, Callanan M, Lefebvre C, Salmon A, Pagnier A, Plantaz D, Bost-Bru C, Eitenschenck L, Durieu I, Floret D, Galambrun C, Chambost H, Michel G, Stephan JL. Clinical, functional and genetic analysis of twenty-four patients with chronic granulomatous disease—identification of eight novel mutations in CYBB and NCF2 genes. J Clin Immunol 2012;32:942–958.
40. Barese C, Copelli S, Zandomeni R, Oleastro M, Zelazko M, Rivas EM. X-linked chronic granulomatous disease: First report of mutations in patients of Argentina. J Pediatr Hematol Oncol 2004;26:656–660.
41. Heyworth PG, Curnutte JT, Rae J, Noack D, Roos D, van Koppen E, Cross AR. Hematologically importante mutations: X-linked chronic granulomatous disease (second update). Blood Cells Mol Dis 2001;27:16–26.