Amsterdam UMC

Amsterdam UMC

Genetic Characteristics, Infectious, and Noninfectious Manifestations of 32 Patients with Chronic Granulomatous Disease

Aygun, Deniz; Koker, Mustafa Yavuz; Nepesov, Serdar; Koker, Nezihe; Koker, Nezihe; van Leeuwen, Karin; de Boer, Martin; Klyklm, Ayca; Ozsoy, Sevil; Cokugras, Haluk; Cokugras, Haluk; Kuijpers, Taco; Kuijpers, Taco; Roos, Dirk; Camcloglu, Ylldlz; Camcloglu, Ylldlz

Published in:
International archives of allergy and immunology

DOI:
10.1159/000507366

Published: 01/06/2020

Document Version
Publisher's PDF, also known as Version of record

Document license
Taverne

Link to publication

Citation for pulished version (APA):
Aygun, D., Koker, M. Y., Nepesov, S., Koker, N., Koker, N., van Leeuwen, K., de Boer, M., Klyklm, A., Ozsoy, S., Cokugras, H., Cokugras, H., Kuijpers, T., Kuijpers, T., Roos, D., Camcloglu, Y., & Camcloglu, Y. (2020). Genetic Characteristics, Infectious, and Noninfectious Manifestations of 32 Patients with Chronic Granulomatous Disease. *International archives of allergy and immunology*, *181*(7), 540-550. https://doi.org/10.1159/000507366

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Amsterdam UMC Medical Library know, stating your reasons. In case of a legitimate complaint, the Medical Library will make the material inaccessible and/or remove it from the website. Contact address: outputregistratie@amsterdamumc.nl

Download date: 09. Nov. 2026

International Archives of Allergy and Immunology

Clinical Immunology – Research Article

Int Arch Allergy Immunol 2020;181:540–550
DOI: 10.1159/000507366

Received: January 3, 2020
Accepted: March 17, 2020
Published online: June 8, 2020

Genetic Characteristics, Infectious, and Noninfectious Manifestations of 32 Patients with Chronic Granulomatous Disease

Deniz Aygun[a] Mustafa Yavuz Koker[b] Serdar Nepesov[c] Nezihe Koker[b, d] Karin van Leeuwen[d] Martin de Boer[d] Ayca Kıykım[c] Sevil Ozsoy[b] Haluk Cokugras[a, c] Taco Kuijpers[d, e] Dirk Roos[d] Yıldız Camcıoglu[a, c]

[a]Department of Pediatric Infectious Disease, Istanbul University-Cerrahpasa, Cerrahpasa Medical School, Istanbul, Turkey; [b]Department of Immunology, Faculty of Medicine, University of Erciyes, Kayseri, Turkey; [c]Department of Pediatric Allergy and Immunology, Istanbul University-Cerrahpasa, Cerrahpasa Medical School, Istanbul, Turkey; [d]Sanquin Research, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; [e]Emma Children's Hospital, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

Keywords
Chronic granulomatous disease · Neutrophil · Nicotinamide adenine dinucleotide phosphate oxidase · Dihydrorhodamine-1,2,3 assay

Abstract
Background: Chronic granulomatous disease (CGD) is a rare genetic disorder characterized by failure of phagocytic leukocytes to destroy certain microbes. We present a study on CGD patients enrolled at a single medical center concerning the infectious and noninfectious complications and genetic properties of the disease. ***Methods:*** Icotinamide adenine dinucleotide phosphate oxidase activity and the expression of flavocytochrome b_{558} were measured by flow cytometry, and clinical outcomes of the patients were listed in relation to the genetic results. ***Results:*** The clinical and genetic findings of 32 pediatric cases with CGD from 23 families were enrolled. Pneumonia and anemia were the most common infectious and noninfectious symptoms. Genetic analysis showed that 10 families (43.5%) carried *CYBB* variants and 13 families (56.5%) have autosomal recessive (AR) CGD, in which 6 families (26%) carried *NCF1* variants, 4 (17.4%) carried *CYBA* variants, and 3 (13%) carried *NCF2* variants. The median age of clinical onset was 3.3 and 48 months for patients with X-linked CGD (X-CGD) and AR-CGD, respectively. The onset of symptoms before age 1 year was 94% in X-CGD, 28.5% in AR-CGD, and 12.5% in patients with oxidase residual activity. Moreover, a de novo germline mutation at c.1415delG in *CYBB* (OMIM#300481) and a novel c.251_263del13bp in *CYBA* (OMIM#608508) were also investigated. ***Conclusions:*** Ihydrorhodamine-1,2,3 assay could not detect carrier mother in de novo case with *CYBB* variant. Most X-CGD patients have the onset of symptoms before age 1 year. Additionally, residual oxidase activity in AR-CGD causes a delay in onset, diagnosis, and prophylaxis. The protective role of residual activity is limited while the infection is ongoing and becoming serious.

© 2020 S. Karger AG, Basel

D.A. and M.Y.K. contributed equally to this work.
Edited by: H.-U. Simon, Bern.

karger@karger.com
www.karger.com/iaa

© 2020 S. Karger AG, Basel

Deniz Aygun
Department of Pediatric Infectious Disease
Istanbul University-Cerrahpasa, Cerrahpasa Medical School
Istanbul 34098 (Turkey)
fdenizaygun@gmail.com

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

Introduction

Chronic granulomatous disease (CGD) is a rare genetic disorder characterized by the failure of phagocytic leukocytes to produce reactive oxygen species [1–3]. CGD is caused by defects in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, affecting the respiratory burst in neutrophils and macrophages [2]. Mutations in any of the genes encoding structural proteins of the NADPH oxidase result in CGD. Mutations in the *CYBB* gene, which is X-linked and encodes gp91^{phox}, are responsible for approximately two-thirds of CGD cases in Western countries. Autosomal recessive (AR) forms result from defects in p47^{phox}, p67^{phox}, p40^{phox}, or p22^{phox} encoded by the *NCF1, NCF2, NCF4,* and *CYBA* genes, respectively [4–6]. Mediterranean countries like Turkey and Tunis have higher percentages of autosomal cases due to consanguineous marriages [7–10].

Functional analysis with dihydrorhodamine-1,2,3 (DHR) can diagnose CGD and is useful for differentiation between the X-linked and the AR mode of inheritance [7]. A mosaic population of oxidase-positive and oxidase-negative neutrophils suggests an X-linked CGD (X-CGD) carrier state in females [2, 7, 10, 11]. Occasionally, de novo germline mutations in *CYBB* result in male X-CGD patients without their mother being a carrier [2].

The main clinical manifestations of CGD are severe and recurrent infections with bacteria such as *Staphylococcus aureus*, *Burkholderia*, *Nocardia*, *Serratia*, and *Salmonella* species. Patients are also predisposed to *Mycobacterium tuberculosis* infections and life-threatening invasive fungal infections caused by *Aspergillus* species [2, 12]. Pneumonia, lymphadenitis, and cutaneous, hepatic, and perianal abscesses are among the most common types of infections in patients with CGD. CGD affects between 1/200,000 and 1/250,000 live births, although the real incidence might be higher as a result of underdiagnosis of milder phenotypes [2, 13].

In the current report, we present a single-center study with 32 CGD patients from 23 families with respect to clinical outcome and genetic background of the disease. We compared X-CGD and AR-CGD patients for age at first symptoms, age at diagnosis, and diagnostic delay and also investigated the effect of residual NADPH oxidase activity on these parameters, since this activity has been proven to strongly affect clinical severity and survival of CGD patients [7, 14].

Patients and Methods

This is a retrospective single-center study with ethics approval, conducted at the Medical Faculty of Istanbul Cerrahpasa University, Turkey, between 2007 and 2019. This center, a reference hospital for approximately 8 million people, is located in the European part of Istanbul. Detailed data were analyzed from 5 female and 27 male patients diagnosed with CGD in 23 families. The age at onset of clinical symptoms was between 1 and 176 months.

Laboratory Analysis of Neutrophils

Total leukocytes were isolated from 100 to 200 μL of blood from patients and relatives by means of lysis of the erythrocytes in the pellet fraction with a nonfixing lysis solution, as described by Köker et al. [7]. The capacity of the neutrophils to generate reactive oxygen species was tested with the DHR assay [7]. Myeloperoxidase (MPO) deficiency, which gives a DHR result consistent with CGD, was ruled out in all patients by MPO expression analysis with anti-MPO antibody (Beckman Coulter, Brea, CA, USA) by flow cytometry [1].

The DHR assay was performed as described by Köker et al. [7, 10]. In this test, isolated neutrophils were incubated with DHR, stimulated with phorbol 12-myristate 13-acetate, and analyzed by means of flow cytometry. The results are shown as the stimulation index (SI) [7]. Expression of flavocytochrome b_{558}, the membrane unit of NADPH oxidase consisting of a heterodimer of gp91^{phox} and p22^{phox}, was checked in all patient samples by gp91^{phox}/p22^{phox} antibody clone 44.1 (Santa Cruz Biotechnology; Santa Cruz, CA, USA), which is negative if either p22^{phox} or gp91^{phox} is absent [7].

Genetic Analysis

Genomic DNA was isolated from total blood leukocytes by standard procedures and analyzed for mutations in *CYBB*, *CYBA*, *NCF1*, and *NCF2* by means of PCR amplification of each exon with its intronic boundaries, followed by sequence analysis [15]. Gene scan analysis for *NCF1* exon-2 GT deletion was performed according to Dekker et al. [16]. Laboratory and genetic diagnoses of CGD patients were carried out in the Immune Deficiency Research Laboratory at the University of Erciyes, Kayseri, Turkey, by Dr. M.Y. Köker. Additional laboratory tests for the investigation of undetected cases with next generation sequencing (NGS) system was carried out at the Sanquin research laboratory, Amsterdam.

Statistical Analysis

SPSS program (version 21.0; IBM Co., SPSS Inc.) was used for statistical analysis. The χ^2 test was used to compare the differences. Continuous variables within 2 groups were compared using the independent *t* test. All tests were 2 tailed, and $p < 0.05$ was considered statistically significant, and the data were represented by whiskers and boxes.

Results

Diagnostic Laboratory Results

Thirty-two CGD patients (27 male, 5 female; Table 1) who were followed in the Clinics of Pediatric Immunology and Infectious Disease at Cerrahpaşa Medical Fac-

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

Table 1. Characteristics and laboratory results in patients with X-CGD and AR-CGD

Index patient/ Family	Sex	Cons	DHR assay		Expression Cyto-b_{558} (gp91/p22phox)	Gene	Mutation	Nucleotide change	aa change
			SI	carrier mother					
P1/F1	M	+	1	+	−	*CYBB*	Splice site	c.141+2T>C	p.Leu16_Gly47del
P2–6/F2&	M	+	1	+	−	*CYBB*	Nonsense	c.388C>T	p.Arg130Ter
P7–8/F3	M	−	1	+	−	*CYBB*	Nonsense	c.676C>T	p.Arg226Ter
P9/F4	M	−	1	+	−	*CYBB*	Missense	c.1012C>G	p.His338Asp
P10–11/F5	M	−	1	+	−	*CYBB*	Missense	c.1031C>T	p.Ser344Phe
P12–13/F6	M	−	1.2	+	−	*CYBB*	Missense	c.1235G>A	p.Gly412Glu
P14/F7	M	−	1	−	−	*CYBB*	De novo deletion	c.1415delG	p.Gly472AlafsTer30
P15/F8	M	−	1	+	−	*CYBB*	Deletion	CYBB del	Not relevant
P16–17/F9	M	−	1	+	−	*CYBB*	Deletion	Exon 4–6 del	Not relevant
P18/F10	M	−	1.3	+	−	*CYBB*	Missense	c.356A>G	p.His119Arg
P19/F11	M	+	4^R	−	+	*NCF1*	Deletion	c.75_76delGT	p.Tyr26HisfsTer26
P20/F12	M	+	6^R	−	+	*NCF1*	Deletion	c.75_76delGT	p.Tyr26HisfsTer26
P21–P22/F13	M	+	8^R	−	+	*NCF1*	Deletion	c.75_76delGT	p.Tyr26HisfsTer26
P23/F14	F	+	1	−	+	*NCF2*	Missense/splice	c.279C>G + intron4+1G>C	p.Asp93Glu
P24/F15	M	+	1	−	+	*NCF2*	Missense/splice	c.279C>G + intron4+1G>C	p.Asp93Glu
P25/F16	F	+	1	−	−	*CYBA*	Splice	c.58+2T>G	not relevant
P26/F17	F	−	1	−	−	*CYBA*	Splice	c288-15C>G	not relevant
P27/F18	F	+	10^R	−	−	*CYBA*	Missense	c.70G>A	p.Gly24Arg
P28/F19	M	−	3.5^R	−	−	*CYBA*	Nonsense/deletion[a]	c.27G>A/c.251_263del13bp	p.Trp9Ter
P29/F20	M	+	5^R	−	+	*NCF1*	Deletion	c.75_76delGT	p.Tyr26HisfsTer26
P30/F21	M	+	3^R	−	+	*NCF1*	Splice	c.574G>A	Deletion exon 6–7
P31/F22	F	+	1	−	+	*NCF2*	Insertion	c.767_768dupA	p.Glu257LysfsTer15
P32/F23	M	+	5^R	−	+	*NCF1*	Deletion	c.75_76delGT	p.Tyr26HisfsTer26

CGD, chronic granulomatous disease; X-CGD, X-linked CGD; AR-CGD, autosomal recessive CGD; DHR, dihydrorhodamine-1,2,3; NADPH, nicotinamide adenine dinucleotide phosphate. [a] Novel. R, residual NADPH oxidase activity; &, Syrian Arabic family; M, male; F1, female; SI, the ratio of the mean fluorescence intensity of the stimulated cells and that of the unstimulated cells in the index patient; Cons, consanguinity.

ulty Hospital were evaluated for consanguinity, laboratory and molecular results, and infectious and noninfectious clinical outcomes. Twenty-seven patients were from Turkish origin and 5 patients (with X-CGD) from a Syrian Arabic family. Fourteen patients (2 X-CGD and all but 2 of out of AR-CGD) were born from consanguineous couples (Table 1).

The clinical diagnosis was confirmed by quantification of the patients' neutrophil oxidative burst by the DHR assay (Fig. 1a). X-CGD was diagnosed in 18 patients from 10 families, with a mean DHR SI of 1.05 (normal: 60- to 100-fold) [7]. Four families had 2 children with X-CGD (Table 1). Nine mothers from 10 families showed bimodal PMN subpopulations in the DHR assay, indicative of X-linked carrier state. A de novo germline mutation c.1415delG in *CYBB* was found in P14, predicting p.Gly472AlafsTer30 in $gp91^{phox}$ (Fig. 1b). The mother of P14 did not have a bimodal X-CGD carrier pattern in the DHR assay, and NGS analysis showed P14's parents lacked the c.1415delG mutation. The neutrophils of all X-CGD patients lacked $gp91^{phox}/p22^{phox}$ expression as detected with an antibody against $gp91^{phox}/p22^{phox}$ (Fig. 1a; Table 1).

The remaining 14 patients from 13 families were suspected to have AR-CGD, with DHR SI between 1 and 10, and all but 2 of these patients had consanguineous parents (Table 1). In addition, their mothers had no bimodal neutrophil population in the DHR assay. Also, these patients were checked with the specific antibody for $gp91^{phox}/p22^{phox}$; in 4 of them, the reaction was negative (Table 1). These 4 patients had mutations in *CYBA*, encoding $p22^{phox}$. A splice site variant was found in patient P25 and P26; a homozygous missense mutation at c.70G > A, predicting p.Gly24Arg in patient P27; and a compound heterozygous mutation at c.27G > A and novel (ENST00000261623.8) c.251_263del13bp in patient P28 were investigated with NGS analysis (Ion Torrent sequencing platform) (Table 1).

In 10 patients from the group with suspected AR-CGD, $gp91^{phox}/p22^{phox}$ membrane protein expression

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

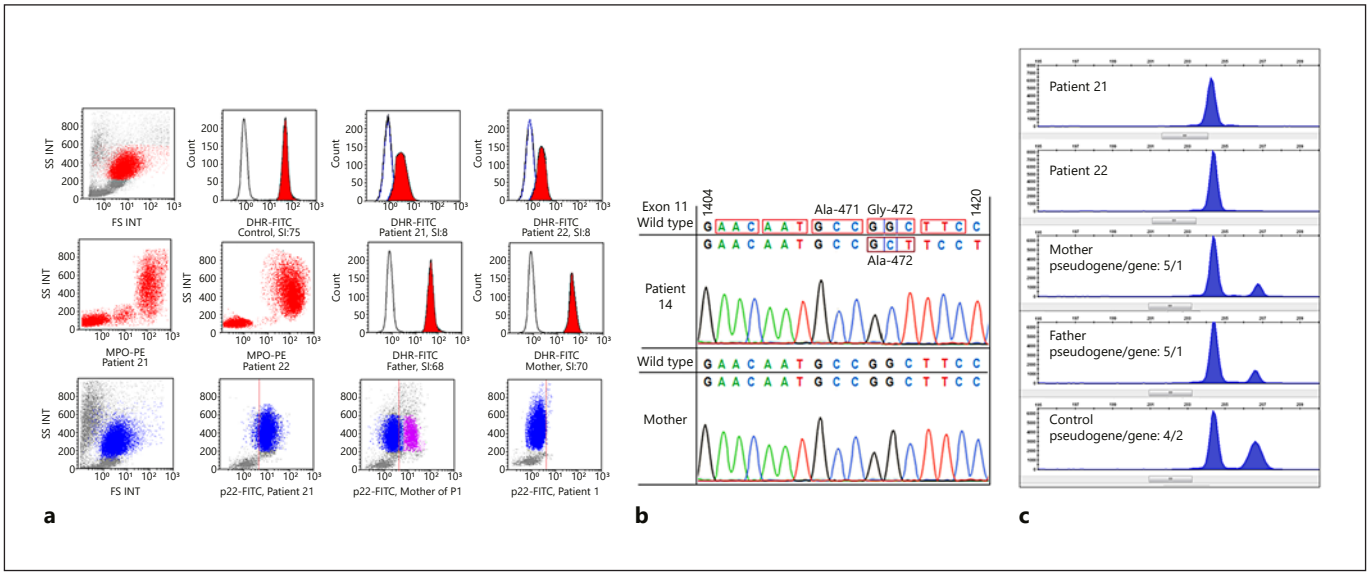


Fig. 1. a Flow cytometry results of patients (P21, P22); DHR assay (upper figs) and MPO expression (middle figs, left) of family 13 with p47^{phox} defect, including P21, P22, and parents. Flavocytochrome b_{558} expression (lower figs) with specific antibody for p22^{phox} component; normal in P21 with p47^{phox} defect, lack in P1 with X-CGD, and bimodal in the carrier mother of P1. **b** Sequence analysis results of P14 for de novo mutation in *CYBB*; mother is not a carrier of this mutation. A germline mutation was found at c.1415delG position in *CYBB*, predicting p.Gly472AlafsTer30 in gp91^{phox}. **c** GeneScan result of F13; patients including P21 and P22 have lost wild-type *NCF1* genes and contain only *NCF1* pseudogenes (with GT deletion in exon-2). The ratio of pseudogene/gene was 5/1 in carrier parents and 2/1 in a normal individual. CGD, chronic granulomatous disease; DHR, dihydrorhodamine-1,2,3; MPO, myeloperoxidase.

was intact. First, we checked with GeneScan analysis for *NCF1* exon-2 GT deletion by determining the pseudogene/gene ratio. In 6 of these patients, we did indeed find a 2/1 (pseudogene/gene) ratio in controls, one pseudogene peak in the patients, and a 5/1 peak ratio in their parents by GeneScan analysis (Fig. 1c). This result confirmed homozygous ΔGT in both alleles of exon 2 at c.75_76delGT in *NCF1*. In the remaining 3 patients, we found a mutation in *NCF1* in P30 at c.574G > A, predicting p.Gly192Ser, and 2 previously identified mutations in *NCF2* by NGS analysis (Table 1) [5, 7].

There was residual oxidase activity (SI >3) in all patients with a p47^{phox} defect and in 2 patients (P27-P28) with a p22^{phox} defect, with mean SI = 5.56 in the DHR assay. The remaining patients with X-CGD and p67^{phox} defect and 2 patients (P25-P26) with p22^{phox} defect had oxidase null activity, with mean SI = 1.05 in the DHR assay (Table 1).

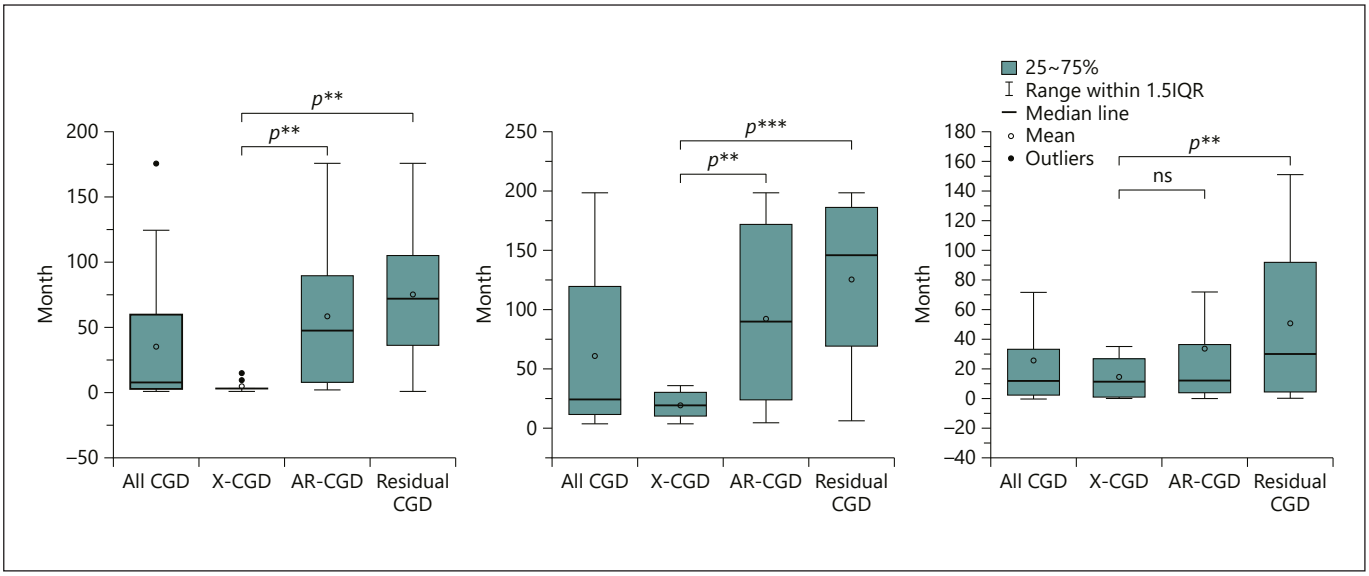
The Onset of Symptoms, Age of Diagnosis, and Diagnostic Delay

The median ages (index patients) at the onset of symptoms (in all patients, X-CGD, and AR-CGD forms) were 8, 3.3, and 48 months (range 1–176 months), respectively. The median ages at diagnosis were 24, 19.5, and 90 months (range 3–198 months), for all patients, X-CGD, and AR-CGD forms, respectively. The median diagnostic delays were 12, 11.5, and 12 months (range 0–150 months), respectively (Fig. 2). There was a statistically significant difference in terms of age at the onset of symptoms and diagnosis between the X-linked and AR forms of the disease ($p < 0.010$, $p < 0.009$) (Fig. 2).

Additionally, one-third of the patients had residual oxidase activity. The median age at the onset of symptoms and diagnosis were 72 and 146 months, respectively, and diagnostic delay was 30 months for these patients. When we compare these data with X-CGD, there was a statistically significant difference in terms of age at the onset of symptoms and at diagnosis in relation to the presence of residual oxidase activity ($p < 0.003$ and $p < 0.001$, respectively). There was also a statistically significant difference in terms of diagnostic delay of the disease between these 2 groups ($p < 0.01$) (Fig. 2). The onset of symptoms before 1 year of age was 94% (17/18) for patients with X-CGD, 28.5% (4/14) for patients with AR-CGD, and 12.5% (1/8) for patients with oxidase residual activity (Table 2).

Color version available online

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026



Color version available online

Fig. 2. Age at onset of symptoms (left), age at diagnosis (middle), and diagnostic delay (right) in patients with CGD in terms of CGD subtype and residual oxidase activity of index patients. CGD, chronic granulomatous disease; X-CGD, chronic granulomatous disease; AR-CGD, chronic granulomatous disease.

Clinical Manifestations

Infections

The most common presenting symptoms were pneumonia (n = 10, 31.3%), followed by lymphadenitis (n = 8, 25%), subcutaneous abscess (n = 3, 9.4%), and septicemia (n = 3, 9.4%) (Table 2). An infection was considered when hospital admission and antimicrobial treatment was required. During the follow-up of the patients, pneumonia was the most common infectious manifestation (n = 24, 75%), followed by lymphadenitis (n = 23, 71.9%) and subcutaneous abscess (n = 14, 43.8%) (Table 3). The most common causative agents for infections during the follow-up were *S. aureus* (n = 10, 32.2%), *Aspergillus fumigatus* (n = 5, 16.1%), *M. tuberculosis* (n = 4, 12.5%), and agents listed in Table 2. Bilateral diffuse nodules, suggestive of invasive pulmonary aspergillosis, were present in computed tomography of 3 patients (P15, P16, and P26), and septated hyaline hyphae were observed in the respiratory specimen culture that yielded *A. fumigatus*.

All patients were vaccinated with BCG vaccine according to the vaccine schedule of the nation, except a second child (P17) in a family, diagnosed with CGD at 1 month of age, in which BCG vaccination was canceled. There were 4 patients with tuberculosis (TB), 1 of them with pulmonary (P29) and 3 with miliary TB (P16, P19, and P20). *M. tuberculosis* was isolated in all patients with TB. BCGitis developed in 6 patients (P10, P13, P15, P18, P28, and P29); *Mycobacterium bovis*, the vaccine strain, was isolated only in P10 and P28.

Recurrent diarrhea was present in 8 patients (Table 2). *Salmonella enteritis* was determined in 3 blood cultures of a 7-year-old girl (P27) admitted with prolonged fever and septic shock (Table 2).

Noninfectious Complications

The most common noninfectious findings were anemia (22, 68.8%) and failure to thrive (19, 59.4%), and other findings are listed in Table 2. Anemia and failure to thrive were observed more in the X-linked form than in the AR form ($p < 0.001$ and $p < 0.016$, respectively). Two patients, one with X-CGD (P9) and one with AR-CGD (P30) had inflammatory bowel disease (IBD).

The mean immunoglobulin (Ig) G levels were 1,292 ± 616 (range 505–3,434) mg/dL, mean IgM levels 134 ± 68 (range 20–312) mg/dL, mean IgA levels 123 ± 130 (5–462) mg/dL, and mean IgE levels 393 ± 858 (10–3,425) mg/dL. Ten patients (32.2%) had increased serum IgG levels compared to healthy subjects in the same age group. Five patients (16.1%) had increased serum IgE levels. One of the patients (P14) with X- CGD had isolated IgA deficiency. Five X-CGD patients and 3 AR-CGD patients had hypereosinophilia in the absence of parasitic infections. The detailed clinical manifestations of patients are listed in Table 2.

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

Table 2. Phenotypes, presenting symptoms, infections, and clinical manifestations of all patients

Patients	CGD subtypes	Age at onset, months	Presenting symptom	Infection[§]	Microorganism	Noninfectious manifestation	Outcome
P1/F1	X91^{O}	4	Lymphadenitis	Lymphadenitis Pneumonia Perianal abscess	*Klebsiella pneumoniae*	Failure to thrive Hepatosplenomegaly Lymphadenopathy	Death at age 9 years
				Osteomyelitis Pericarditis Pericardial abscess	*Streptococcus mitis*	↑ Serum IgG level	
P2/F2 index	X91^{O}	2.5	Pneumonia	Pneumonia Perianal abscess Skin abscess Gastroenteritis	*Staphylococcus aureus*	Anemia Failure to thrive Hepatosplenomegaly Lymphadenopathy ↑ Serum IgE and IgG levels[a] Aphthous stomatitis	Alive
P3/F2	X91^{O}	2.5	Pneumonia	Pneumonia Lymphadenitis Skin abscess Otitis media Cat scratch disease	*Staphylococcus aureus*	Anemia Failure to thrive ↑ Serum IgG level	Alive
P4/F2	X91^{O}	3	Gastroenteritis	Pneumonia Skin abscess Septicemia Gastroenteritis	*Staphylococcus aureus*	Anemia Hypereosinophilia ↑ Serum IgE and IgG levels Aphthous stomatitis	Alive
P5/F2	X91^{O}	2.5	Septicemia	Pneumonia Lymphadenitis Septicemia Gastroenteritis Otitis media	*Staphylococcus aureus*	Anemia Failure to thrive Aphthous stomatitis	Alive
P6/F2	X91^{O}	3	Septicemia	Skin abscess Septicemia Otitis media	*Staphylococcus aureus*	Anemia Failure to thrive Hypereosinophilia Aphthous stomatitis	Alive
P7/F3 index	X91^{O}	3	Lymphadenitis	Lymphadenitis		Anemia	HSCT
				Skin abscess Otitis media		Failure to thrive Hepatosplenomegaly Lymphadenopathy	Alive
P8/F3	X91^{O}	2.5	Lymphadenitis	Lymphadenitis Skin abscess Pneumonia		Anemia Failure to thrive Hepatosplenomegaly Lymphadenopathy	HSCT, alive
P9/F4	X91^{O}	2	Gastroenteritis	Gastroenteritis Pneumonia Lymphadenitis Perianal abscess Otitis media Tooth abscess	*Klebsiella pneumoniae*	Anemia Failure to thrive Lymphadenopathy IBD Aphthous stomatitis	Alive
P10/F5 index	X91^{O}	4	Subcutaneous abscess	Pneumonia Lymphadenitis	*Staphylococcus aureus*	Anemia Failure to thrive	HSCT, alive
				Skin abscess BCGitis	*M. bovis*	Hepatosplenomegaly Lymphadenopathy	
P11/F5	X91^{O}	3	Pneumonia	Pneumonia		Anemia	Death at age 7 years
				Lymphadenitis Perianal abscess Pericarditis Otitis media		Failure to thrive	

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

Table 2 (continued)

Patients	CGD subtypes	Age at onset, months	Presenting symptom	Infection[§]	Microorganism	Noninfectious manifestation	Outcome
P12/F6 index	X91^{O}	10	Liver abscess	Pneumonia	*Candida albicans*	Anemia	Alive
				Lymphadenitis		Failure to thrive	
				Skin abscess		Hepatosplenomegaly	
				Liver abscess		Lymphadenopathy Hypereosinophilia ↑ Serum IgG level Aphthous stomatitis	
P13/F6	X91^{O}	1.5	Lymphadenitis	Pneumonia Lymphadenitis BCGitis		Anemia Failure to thrive Lymphadenopathy	Alive
P14/F7	X91^{O}	1	Subcutaneous abscess	Pneumonia		Anemia	Alive
				Lymphadenitis		Failure to thrive	
				Skin abscess		Lymphadenopathy Isolated IgA deficiency	
				Perianal abscess		↑ Serum IgG level	
				Gastroenteritis Tooth abscess		Aphthous stomatitis	
P15/F8	X91^{O}	3.5	Osteomyelitis	Osteomyelitis	*Serratia marcescens*	Anemia	HSCT, alive
				Pneumonia	*Aspergillus fumigatus*	Cow's milk allergy	
				Lung abscess	*Candida albicans*	Lymphadenopathy	
				Lymphadenitis Septicemia Gastroenteritis Otitis media BCGitis		Aphthous stomatitis	
P16/F9 index	X91^{O}	15	Pneumonia	Pneumonia Lymphadenitis	*Aspergillus fumigatus*	Anemia Hypereosinophilia	Alive
				Otitis media	*Mycobacterium tuberculosis*	↑ Serum IgE and IgG levels Aphthous stomatitis	
P17/F9	X91^{O}	1	Lymphadenitis	Lymphadenitis	*Aspergillus flavus* in lymph node	Anemia	Alive
P18/F10	X91^{O}	2.5	Lymphadenitis	Lymphadenitis BCGitis		Anemia Failure to thrive Lymphadenopathy Hypereosinophilia	Alive
P19/F11[&]	AR *p47-phox*	60	Liver abscess	Liver abscess (3 times)	*Staphylococcus aureus* in liver	Lymphadenopathy	Alive
				Lymphadenitis Skin abscess Otitis media	*Mycobacterium tuberculosis*	Hypereosinophilia ↑ Serum IgE and IgG levels	
P20F12[&]	AR *p47-phox*	24	Pneumonia	Pneumonia	*Mycobacterium tuberculosis*	Failure to thrive	Alive
				Skin abscess	*Haemophilus influenzae type B*		
P21/F13 index[&]	AR *p47-phox*	120	Lymphadenitis	Lymphadenitis Otitis media		Lymphadenopathy	Alive

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

Table 2 (continued)

Patients	CGD subtypes	Age at onset, months	Presenting symptom	Infection[§]	Microorganism	Noninfectious manifestation	Outcome
P22/F13[&]	AR *p47-phox*	120	Lymphadenitis	Lymphadenitis Tooth abscess		Lymphadenopathy	Alive
P23/F14	AR *p67-phox*	12	Pneumonia	Pneumonia Lymphadenitis Otitis media	*Staphylococcus aureus*	Anemia Hepatosplenomegaly Hypereosinophilia	Alive
P24/F15	AR *p67-phox*	2	Perianal abscess	Perianal abscess	*Staphylococcus aureus*	Anemia	Death at age 8 years
				Septicemia	*Candida albicans*	Aphthous stomatitis	
					Escherichia coli		
P25/F16	AR *p22-phox*	6	Osteomyelitis	Pneumonia Lung abscess Lymphadenitis Skin abscess Otitis media Osteomyelitis	*Staphylococcus aureus*	Anemia Urolithiasis	Alive
P26/F17	AR *p22-phox*	8	Subcutaneous abscess	Pneumonia Lung abscess Skin abscess Lymphadenitis Otitis media	*Aspergillus fumigatus*	Lymphadenopathy Aphthous stomatitis	Alive
P27/F18[&]	AR *p22-phox*	90	Septicemia	Septicemia Pneumonia Gastroenteritis	*Salmonella enteritis*	Anemia ↑ Serum IgG level	Alive
P28/F19[&]	AR *p22-phox*	3	Perianal abscess	Pneumonia	*Aspergillus fumigatus* in lymph node	Anemia	Alive
				Lymphadenitis Perianal abscess BCGitis	*M. bovis*	Lymphadenopathy Hypereosinophilia	
P29/F20[&]	AR *p47-phox*	48	Pneumonia	Pneumonia BCGitis	*Mycobacterium tuberculosis*	Failure to thrive ↑ Serum IgE levels	Alive
P30/F21[&]	AR *p47-phox*	176	Pneumonia	Pneumonia Liver abscess Gastroenteritis Perianal abscess	*Candida guilliermondii*	Failure to thrive Lymphadenopathy Hepatosplenomegaly IBD	Alive
P31/F22	AR *p67-phox*	125	Pneumonia	Pneumonia	*Aspergillus fumigatus* in skin abscess	Failure to thrive	Alive
				Lung abscess Skin abscess Liver abscess	*Arcanobacterium haemolyticum*	Hepatomegaly ↑ Serum IgG level	
P32/F23[&]	AR *p47-phox*	84	Pneumonia	Pneumonia Lymphadenitis		Failure to thrive Lymphadenopathy	Alive

X91[o], X-CGD; AR, AR-CGD; § infection during the follow-up of patients; & NADPH oxidase residual activity; index patient, expressed with bold label; HSCT, hematopoietic stem cell transplantation. CGD, chronic granulomatous disease; Ig, immunoglobulin; IBD, inflammatory bowel disease, X-CGD, X-linked CGD; AR-CGD, autosomal recessive CGD. [a] Cutoff values for increased serum IgG and IgE levels in Turkish pediatric population: IgG range 505–3,434 mg/dL and IgE range 10–3,425 mg/dL.

Treatment and Outcome

Prophylactic antimicrobials like trimethoprim-sulfamethoxazole at a dose of 6–8 mg/kg, twice daily, and itraconazole in a single dose of 10 mg/kg were given to all patients at the time of diagnosis. In the last 5 years, 4 patients (P7, P8, P10, and P15) with X- CGD underwent allogeneic stem cell transplantation from HLA-matched related donors by reduced intensity conditioning, and

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

Table 3. Comparison of number of infections and noninfectious manifestations according to the CGD phenotypes

Findings	All patients		X-CGD	AR-CGD	*p* value
	N = 32	%	*n* = 18	*n* = 14	
Infections[a]					
Pneumonia	24	75.0	14	10	0.681
Lymphadenitis	23	71.9	15	8	0.102
Skin abscess	14	43.8	9	5	0.419
Otitis media	13	40.6	8	5	0.618
Gastroenteritis	8	25.0	6	2	0.217
Perianal abscess	8	25.0	5	3	0.681
Septicemia	6	18.8	4	2	0.568
Lung abscess	4	12.5	1	3	0.178
Liver abscess	4	12.5	1	3	0.178
Tooth abscess	3	9.4	2	1	0.702
Noninfectious manifestations					
Anemia	22	68.8	17	5	0.001
Failure to thrive	19	59.4	14	5	0.016
Lymphadenopathy	19	56.3	12	7	0.341
Aphthous stomatitis	11	34.4	9	2	0.035
↑ Serum IgG levels	10	31.3	7	3	0.290
Hepatosplenomegaly	8	25.0	6	2	0.217
Hypereosinophilia	8	25.0	5	3	0.681
↑ Serum IgE levels	5	15.6	3	2	0.854

CGD, chronic granulomatous disease; AR-CGD, autosomal recessive CGD; X-CGD, X-linked CGD; Ig, immunoglobulin. [a] Percentages of infections during the follow-up of patients are listed. An infection was considered when hospital admission and antimicrobial treatment were required.

they are all well for 3 years. The normal neutrophil function and respiratory burst were also confirmed by the DHR assay at the 6-month posttransplantation visit of patients.

Three patients died during follow-up. Patient P1 with X-CGD presented with cough and developed symptoms of tamponade, and urgent pericardiectomy was performed, but he died because of cardiac arrest. Patient P11 with X-CGD died at age 7 years due to pneumonia and pericarditis. Patient P24 with $p67^{phox}$ defect died because of septicemia at 8 years of age; *Candida* and *E. coli* were isolated in the blood culture (Table 2).

Discussion

Here, we describe clinical and laboratory outcome of 32 CGD patients from 23 families. Ten families (43.5%) carried *CYBB* variants X-CGD and 13 families (56.5%) have AR-CGD, carrying *NCF1*, *CYBA*, or *NCF2* variants (Table 1). Our results show the predominance of AR-CGD in the Turkish families investigated. In European series and in China, 68–89% of CGD cases have been reported to be X-linked [8, 17]. However, AR-CGD is the predominant form in Turkey and other Mediterranean regions because of the high rate of consanguineous marriages [7–10]. Our result is consistent with these results and similar to the previous research in Turkey [7]. Approximately 10% of X-CGD may be caused by new germline mutations, [1] and among our X-CGD patients, only P14 has a de novo mutation in *CYBB* (Fig. 1b). Thus, 1 (F7) out of 10 mothers from X-CGD families did not have a carrier pattern in the DHR assay and in the genetic analysis (Table 1). Therefore, care should be taken during the evaluation of the DHR assay for a carrier pattern: a negative DHR pattern should always be confirmed by genetic analysis. Also, clinicians should be aware of consanguinity in the family. They should investigate parents as well as patients and be aware of additional patients in the family: autosomal CGD can be without symptoms during the first years of life.

The median age of all index patients at the onset of symptoms was similar to reports of Meshaal et al. [18] and

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

Rawat et al. [19]. The median age at diagnosis was 2 years for index patients, which was higher than the report from China (median 1 year old) but lower than the reports from India (3 years) and Iran (5.5 years) [19–21]. Clinicians should always be aware of late disease manifestations, especially in AR-CGD.

Patients with CGD are prone to mycobacterial infections, and TB was recorded in 32–44% of CGD patients in large multicenter studies [21, 22]. *M. tuberculosis* was demonstrated in only 4 patients (12.5%) in our center. Although 31/32 patients were vaccinated with BCG, BCGitis developed only in 6 patients (19.4%), similar to the rate of BCGitis (22.4%) in a previous multicenter study from Turkey [7].

IBD has been reported in 27–44% of patients with CGD, especially in the X-linked form [23]. Marciano et al. [23] demonstrated endoscopy-proven IBD in 32.8% of CGD patients. Only 2 patients (P9, X-CGD and P30, AR-CGD) developed IBD in the present study. Still, clinicians should be aware that patients with IBD might suffer from CGD if additional symptoms (bacterial or fungal infections, intestinal, pulmonary, or urinary duct obstruction) point in that direction.

Anemia and failure to thrive are a multifactorial process and commonly reported in chronic manifestations of CGD due to this gastrointestinal involvement [7, 18]. Both findings are signs of persistent inflammation and present in many chronic diseases. Macrophage stimulations determine the severity of inflammation. Inflammation and infection increase hepcidin synthesis, which blocks iron absorption from the diet and its further storage [24]. So, reduced absorption of iron from the gastrointestinal tract is the possible cause of anemia, which can also explain the cause of failure to thrive. Anemia was also more common in patients with X-CGD than AR-CGD ($p < 0.001$).

Oxidase residual activity can be seen not only in the $p47^{phox}$ defect but also in other CGD subtypes, depending on the mutation [7]. We found a higher residual oxidase activity, approximately 5–7% of normal, in a patient (P27) with a homozygous missense mutation in *CYBA* at c.70G > A, predicting p.Gly24Arg. Previously Yamada et al. [25] and Wolach et al. [26] described this mutation, and Köker et al. [7] mentioned this mild phenotype of CGD in 2 different Turkish families. The median age of patients with AR-CGD cases at the onset of symptoms was 48 months (Fig. 2), but the median age was 90 months in patient P27 with this c.70G>A mutation in *CYBA*. It is the most prevalent missense mutation related with $p22^{phox}$ defect and AR-CGD [5]. Depending on the diagnostic facilities, we expect that the number of patients with this mild phenotype of CGD will increase in many countries. Although our patient P27 has residual oxidase activity, prolonged improper treatment at the undiagnosed period of CGD might be the underlying cause for the presentation with septicemia (Table 2). Additionally, P19 with residual oxidase activity presented with liver abscess at 60 months of age, and also 4 other patients with residual oxidase activity presented with pneumonia during their undiagnosed period without prophylaxis (Table 2). Despite the antimicrobial prophylaxis, patients with CGD still develop serious infections that can be life-threatening. This can also be due to improper usage of prophylactic antimicrobial agents, environmental conditions, and the quality of healthcare services.

In conclusion, we describe our clinical experience with CGD from a single medical center. The onset of symptoms was earlier in X-CGD cases and later in cases with residual NADPH oxidase activity ($p < 0.01$). Residual oxidase activity may cause delayed diagnosis and prophylaxis, but its role is limited while the infection is ongoing and becoming serious. So, early laboratory diagnosis of patients, especially in the first year of life in X-CGD, proper and early prophylaxis with antimicrobials, and special treatment during serious infections are essential for the good management of CGD patients.

Acknowledgment

We thank BAP of Erciyes University in project TSA-2019-8322.

Statement of Ethics

This protocol was approved by the Ethical Committee of the University of Erciyes; all subjects provided written informed consent in accordance with the Helsinki Declaration.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

D.A., S.N., A.K., Y.C., and H.Ç. collected the patients' clinical data; N.K. and S.O. made functional laboratory analysis; M.Y.K., N.K., K.L., and M.B. made genetic tests; and D.R., T.K., Y.C., and M.Y.K. wrote the draft of the manuscript.

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026

References

1 Roos D, Holland SM, Kuijpers TW. Chronic granulomatous disease. In: Ochs HD, Smith CIE, Puck JM, editors. Primary immunodeficiency diseases, a molecular and genetic approach. 3rd ed. New York: Oxford University Press; 2014. p. 689–722.
2 Winkelstein JA, Marino MC, Johnston RB Jr, Boyle J, Curnutte J, Gallin JI, et al. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine. 2000; 79(3):155–69.
3 de Oliveira-Junior EB, Bustamante J, Newburger PE, Condino-Neto A. The human NADPH oxidase: primary and secondary defects impairing the respiratory burst function and the microbicidal ability of phagocytes. Scand J Immunol. 2011;73(5):420–7.
4 Roos D, Kuhns DB, Maddalena A, Roesler J, Lopez JA, Ariga T, et al. Hematologically important mutations: X-linked chronic granulomatous disease (third update). Blood Cells Mol Dis. 2010;45(3):246–65.
5 Roos D, Kuhns DB, Maddalena A, Bustamante J, Kannengiesser C, de Boer M, et al. Hematologically important mutations: the autosomal recessive forms of chronic granulomatous disease (second update). Blood Cells Mol Dis. 2010;44(4):291–9.
6 Segal BH, Leto TL, Gallin JI, Malech HL, Holland SM. Genetic, biochemical, and clinical features of chronic granulomatous disease. Medicine. 2000;79(3):170–200.
7 Köker MY, Camcıoğlu Y, van Leeuwen K, Kılıç SŞ, Barlan I, Yılmaz M, et al. Clinical, functional, and genetic characterization of chronic granulomatous disease in 89 Turkish patients. J Allergy Clin Immunol. 2013; 132(5):1156–63.e5.
8 Van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. PLoS One. 2009; 4(4):e5234.
9 Fattahi F, Badalzadeh M, Sedighipour L, Movahedi M, Fazlollahi MR, Mansouri SD, et al. Inheritance pattern and clinical aspects of 93 Iranian patients with chronic granulomatous disease. J Clin Immunol. 2011; 31(5):792–801.
10 Köker MY, Sanal O, de Boer M, Tezcan I, Metin A, Tan C, et al. Skewing of X-chromosome inactivation in three generations of carriers with X-linked chronic granulomatous disease within one family. Eur J Clin Invest. 2006; 36(4):257–64.
11 Roesler J, Hecht M, Freihorst J, Lohmann-Matthes ML, Emmendörffer A. Diagnosis of chronic granulomatous disease and of its mode of inheritance by dihydrorhodamine 123 and flow microcytofluorometry. Eur J Pediatr. 1991;150(3):161–5.
12 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(1):10.
13 Seger RA. Modern management of chronic granulomatous disease. Br J Haematol. 2008; 140(3):255–66.
14 Kuhns DB, Alvord WG, Heller T, Feld JJ, Pike KM, Marciano BE, et al. Residual NADPH oxidase and survival in chronic granulomatous disease. N Engl J Med. 2010;363(27): 2600–10.
15 Roos D, de Boer M, Kuribayashi F, Meischl C, Weening R, Segal A, et al. Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. Blood. 1996; 87(5):1663–81.
16 Dekker J, de Boer M, Roos D. Gene-scan method for the recognition of carriers and patients with p47(phox)-deficient autosomal recessive chronic granulomatous disease. Exp Hematol. 2001 Nov;29(11):1319–25.
17 Gao LW, Yin QQ, Tong YJ, Gui JG, Liu XY, Feng XL, et al. Clinical and genetic characteristics of Chinese pediatric patients with chronic granulomatous disease. Pediatr Allergy Immunol. 2019;30(3):378–86.
18 Meshaal S, El Hawary R, Abd Elaziz D, Alkady R, Galal N, Boutros J, et al. Chronic granulomatous disease: review of a cohort of Egyptian patients. Allergol Immunopathol. 2015; 43(3):279–85.
19 Rawat A, Singh S, Suri D, Gupta A, Saikia B, Minz RW, et al. Chronic granulomatous disease: two decades of experience from a tertiary care centre in North West India. J Clin Immunol. 2014;34(1):58–67.
20 Wu J, Wang WF, Zhang YD, Chen TX. Clinical features and genetic analysis of 48 patients with chronic granulomatous disease in a single center study from Shanghai, China (2005–2015): new studies and a literature Review. J Immunol Res. 2017;2017:8745254.
21 Movahedi M, Aghamohammadi A, Rezaei N, Shahnavaz N, Jandaghi AB, Farhoudi A, et al. Chronic granulomatous disease: a clinical survey of 41 patients from the Iranian primary immunodeficiency registry. Int Arch Allergy Immunol. 2004;134(3):253–9.
22 Conti F, Lugo-Reyes SO, Blancas Galicia L, He J, Aksu G, Borges de Oliveira E Jr, et al. Mycobacterial disease in patients with chronic granulomatous disease: a retrospective analysis of 71 cases. J Allergy Clin Immunol. 2016;138(1):241–8.e3.
23 Marciano BE, Rosenzweig SD, Kleiner DE, Anderson VL, Darnell DN, Anaya-O'Brien S, et al. Gastrointestinal involvement in chronic granulomatous disease. Pediatrics. 2004; 114(2):462–8.
24 Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood. 2008;112(10):4292–7.
25 Yamada M, Ariga T, Kawamura N, Ohtsu M, Imajoh-Ohmi S, Ohshika E, et al. Genetic studies of three Japanese patients with p22-phox-deficient chronic granulomatous disease: detection of a possible common mutant CYBA allele in Japan and a genotype-phenotype correlation in these patients. Br J Haematol. 2000;108(3):511–7.
26 Wolach B, Gavrieli R, de Boer M, Gottesman G, Ben-Ari J, Rottem M, et al. Chronic granulomatous disease in Israel: clinical, functional and molecular studies of 38 patients. Clin Immunol. 2008;129(1):103–14.

Downloaded from http://karger.com/iaa/article-pdf/181/7/540/2971605/000507366.pdf by Universiteits Bibliotheek Amsterdam user on 19 January 2026