Journal of Microbiology, Immunology and Infection 56 (2023) 130—138

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Clinical features and outcomes of patients with chronic granulomatous disease in Taiwan

Ting-Sheng Lin [a], Jyh-Hong Lee [b], Li-Chieh Wang [b], Yao-Hsu Yang [b], Yu-Lung Lau [c], Wen-I Lee [d], Yu-Tsan Lin [b], Bor-Luen Chiang [e], Hsin-Hui Yu [b,*]

[a] *Department of Pediatrics, Min-Sheng General Hospital, Taoyuan, Taiwan*
[b] *Department of Pediatrics, National Taiwan University Children's Hospital, Taipei, Taiwan*
[c] *LKS Faculty of Medicine, Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong*
[d] *Primary Immunodeficiency Care and Research (PICAR) Institute, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[e] *Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan*

Received 20 November 2021; received in revised form 30 April 2022; accepted 16 June 2022
Available online 6 July 2022

KEYWORDS
Chronic granulomatous disease;
Hematopoietic stem cell transplantation;
Interferon-gamma;
Primary immunodeficiencies

Abstract *Background:* Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disease characterized by defective neutrophil killing of microbial pathogens and recurrent infections. We aimed to investigate the clinical, genetic features, treatment, and outcomes in patients with CGD.
Methods: Pediatric patients diagnosed with CGD from a medical center in Taiwan were enrolled from January 1999 to Oct 2021.
Results: Nine pediatric patients with CGD were enrolled: six X-linked (XL) CGD with *CYBB* gene mutations, three autosomal recessive (AR) CGD with two *NCF1* and one *CYBA* gene mutations. The median age of onset and age of diagnosis was 0.92 and 2.64 years, respectively. Patients with XL-CGD had a younger age of onset (4.6 months vs. 1.83 years, $P = 0.06$) and age of diagnosis (1.71 vs. 8.86 years, $P = 0.024$) than AR-CGD patients. The most common sites of infections were skin and soft tissue abscesses. The most common pathogens were *Staphylococcus, Serratia*, and *Salmonella* spp. Prophylactic antibiotics, anti-fungal agents, and interferon-gamma (IFN-γ) were given in 9 (100%), 7 (77.8%), and 8 (88.9%) patients, respectively. The mean duration of IFN-γ usage was 5.15 years. One male patient with XL-CGD was successfully treated with hematopoietic stem cell transplantation

* Corresponding author.
E-mail addresses: marcmarc089@gmail.com (T.-S. Lin), leonid@ntu.edu.tw (J.-H. Lee), lcwang5@ntu.edu.tw (L.-C. Wang), yan0126@ms15.hinet.net (Y.-H. Yang), lauylung@hku.hk (Y.-L. Lau), wen2707@gmail.com (W.-I. Lee), yutsanlin@ntu.edu.tw (Y.-T. Lin), gicmbor@ntu.edu.tw (B.-L. Chiang), yuhsinhui0121@ntu.edu.tw (H.-H. Yu).

https://doi.org/10.1016/j.jmii.2022.06.005

at 2.2 years. The mortality rate was 11.1%, and the estimated overall survival at 20 years was 66.7%.
Conclusion: Staphylococcus aureus, Serratia marcescens, and *Salmonella* infections are important in Taiwanese CGD patients. Patients with XL-CGD have early disease onset. IFN-γ prophylaxis and prophylactic anti-microbial agents might have an effect on alleviating the infection episodes in CGD patients.

Introduction

Chronic granulomatous disease (CGD) is a primary immune deficiency disease that was first described in the 1950s.[1] The incidence of CGD is estimated at approximately 1 in 200,000—300,000 live births worldwide.[2—4] CGD patients present recurrent life-threatening bacterial and fungal infections, granuloma formation, or inflammatory bowel disease-like colitis. CGD is caused by defects in genes encoding the subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (phox) complex.[5] Mutations in the cytochrome B-245 beta chain (*CYBB*)/gp91^{phox} (X-linked, XL) account for 65% of CGD patients. The autosomal recessive (AR)-CGD is caused by mutations in neutrophil cytosolic factor 1 (*NCF1)*/p47^{phox} (25%), cytochrome B-245 alpha chain (*CYBA*)/p22^{phox} (5—10%), (*NCF2)*/p67^{phox} (5—10%), (*NCF4)*/p40^{phox} (rare), or cytochrome B-245 chaperone 1 (*CYBC1*)/Eros (rare).[6,7]

NADPH oxidase is a multi-component enzyme consisting of a membrane-bound heterodimer of gp91^{phox} and p22^{phox}, the chaperone protein Eros, together with cytoplasmic subunits p47^{phox}, p40^{phox}, p67^{phox}, and cofactor Rac 2 GTPase. NADPH oxidase transfers electrons to molecular oxygen to generate reactive oxygen species (ROS) in the phagosome. ROS production in phagocytes, which is also known as a "respiratory burst" reaction, contributes to microbial killing.[7,8] Phagocytes from patients with CGD are deficient in bacterial killing, neutrophil extracellular trap formation, autophagy, and apoptosis, but the hyperactivation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and inflammasome signaling lead to long-lasting inflammation.[9]

There is a wide range of clinical variability in CGD patients. Few large-scale studies on the genetic and clinical features and prognosis of CGD in Han Chinese have been reported.[10,11] We analyzed the clinical, genetic features, treatment, and outcomes of pediatric patients with CGD with 20 years of follow-up in a single medical center in Taiwan.

Methods

Study subjects

We retrospectively identified and analyzed data from nine patients diagnosed with CGD at a medical center for children in Taiwan from 1999 to 2020. Demographic data, clinical data, laboratory features at the time of disease diagnosis and at follow-up, genetic mutation data, treatment course, and outcomes were collected. This study was approved by the Institutional Ethics Committee (No. 201812007RIND).

Neutrophil function

Production of neutrophil-derived reactive oxygen species by NADPH oxidase in phagocytes of patients and healthy controls was measured using Nitro blue tetrazolium (NBT) or a 2′7′-dichlorofluorescein diacetate (DCF) assay and stimulation for 15 min using phorbol myristate acetate (PMA) (10 μg/mL). A diagnosis of CGD was based on abnormal respiratory burst responses (<20% of levels of healthy controls) detected by NBT or DCF assay at least twice.

Expression of gp91^{phox} protein

The expression of gp91^{phox} protein of patients and controls was determined by flow cytometry-based surface staining with the monoclonal antibody (mAb) against gp91^{phox} (Santa Cruz biotechnology, TX, USA).[4] Neutrophils and monocytes were stained with fluorescent FITC-conjugated anti-gp91^{phox} mAb or isotype control antibody. The experiments were performed in a FACSCalibur flow cytometer, and the analysis was performed with FlowJo Software (Ashland, OR, USA). The results were shown as undetectable (X91°), low (X91^{-}), or normal (X91^{+}).

Genetic diagnosis

Genomic DNA of the patients and parents was extracted from peripheral blood. PCR amplification was performed with 100 ng of template genomic DNA from patients and other family members for each exon using previously reported primers and Taq polymerase (Qiagen, Venlo, Netherlands).[12] PCR products were sequenced in both directions using an ABI 3730XL sequencer (Applied Biosystems). The mode of inheritance was determined based on family history, granulocyte function test, and genetic mutations in the patient, parents, and siblings (when available).

Statistical analysis

The results were presented as medians (interquartile range, IQR) or medians (range) for continuous variables

and percentages for nominal variables. Proportions were compared using Fisher's exact test, and continuous variables between two groups were compared using the Mann—Whitney U test. The infection incidence rate per patient-year of follow-up for major infection episodes that need hospitalization was calculated. Survival was analyzed by the Kaplan—Meier method, with and log-rank tests performed for comparison. A two-tailed p-value of less than 0.05 was considered statistically significant. All data were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA, USA) and R software.

Results

We enrolled nine pediatric patients with CGD in this study, with male predominance (7/9, 77.8%). One (11.1%) patient with XL-CGD had a family history of CGD. The median age at onset, age of diagnosis, and current age were 0.92 (range 15 days to 4 years), 2.64 (range 0.3—17.7 years), and 12.34 (range 4.2—28.4 years) years, respectively. The median age of onset for XL-CGD patients (0.38 years) was younger than that for AR-CGD patients (1.83 years) ($P = 0.06$). The median age of diagnosis for XL-CGD patients (1.71 years) was significantly younger than that for AR-CGD patients (8.86 years) ($P = 0.024$). The median follow-up time of the cohort was 7.6 (range 2.47—25.49) years (Table 1).

The most common initial symptoms were fever, lymph node enlargement, and abdominal pain. Patients suffered from infections, including: the skin/soft tissue abscess (7/9, 77.8%), bloodstream (6/9, 66.7%), oral/tonsillitis (6/9, 66.7%), and lung infections (5/9, 55.6%). There was no significant difference in infection patterns between patients with XL-CGD and AR-CGD (Table 1). The top five ranked sites of identified bacterial and fungal infections were the skin/soft tissue abscesses (17/72, 23.6%), perianal abscesses (9/72, 12.5%), bloodstream (6/72, 8.3%), lungs (6/72, 8.3%), lymph nodes (5/72, 6.9%), and gastrointestinal tract (5/72, 6.9%) (Table 1, Supplementary Table 1, and Fig. 2).

Staphylococcus spp. (17/72, 23.6%), *Serratia marcescens* (10/72, 13.9%), *Salmonella* (9/72, 12.5%), *Aspergillus* (4/72, 5.6%), *Klebsiella pneumoniae* (4/72, 5.6%) were top five ranked pathogens in 72 major infections episodes during the entire clinical course of follow-up (Fig. 1 and Supplementary Table 1). The most common pathogens in specific sites of infections were: *Staphylococcus (Staphylococcus aureus, S. epidermidis,* and *S. capitis)* in skin/soft tissue abscesses and lymphadenitis, *K. pneumoniae* in perianal abscess, *Salmonella* in bloodstream and GI tract,

Table 1 Clinical features, treatment and outcome of pediatric patients with CGD.

Clinical features	CGD (N = 9)	XL-CGD (N = 6)	AR-CGD (N = 3)	P value[a]
Male	7 (77.8%)	6 (100%)	1 (33.3%)	0.083
Age of onset (yrs)	0.92 (0.33—1.33)	0.38 (0.15—0.8)	1.83 (1.38—2.92)	0.060
Diagnosis age (yrs)	2.64 (1.08—4.94)	1.71 (0.6—2.56)	8.86 (6.9—13.27)	**0.024**
Current age (yrs)	12.34 (6.91—24.00)	7.92 (5.58—11.49)	26.41 (25.21—27.42)	**0.024**
Follow-up time (yrs)	7.6 (4.72—22.17)	5.36 (3.34—7.20)	24.43 (23.3—24.96)	**<0.0001**
Family history	1 (11.1%)	1 (16.67%)	0 (0%)	>0.99
Infection				
Skin/soft tissue abscess	7 (77.8%)	4 (66.7%)	3 (100%)	0.5
Bloodstream	6 (66.7%)	3 (50%)	3 (100%)	0.46
Oral/tonsillitis	6 (66.7%)	4 (66.7%)	2 (66.7%)	>0.99
Lung	5 (55.6%)	3 (50%)	2 (66.7%)	>0.99
Any Salmonella infections	5 (55.6%)	3 (50%)	2 (66.7%)	>0.99
Lymphadenitis	3 (33.3%)	1 (16.7%)	2 (66.7%)	0.23
Perianal abscess	3 (33.3%)	3 (50%)	0 (0%)	0.46
Salmonella enteritis	2 (22.2%)	0 (0%)	2 (66.7%)	0.08
Liver abscess	2 (22.2%)	1 (16.7%)	1 (33.3%)	>0.99
Treatment				
Prophylactic antibiotics	9 (100%)	6 (100%)	3 (100%)	>0.99
Prophylactic anti-fungal	7 (77.8%)	5 (83.3%)	2 (66.7%)	>0.99
Anti-mycobacterial	4 (44.4%)	3 (50%)	1 (33.3%)	>0.99
Prophylactic IFN- γ	8 (88.9%)	6 (100%)	2 (66.7%)	0.33
IFN- γ starting age (yrs)	3.56 (2.28—6.92)	2.55 (2.0—3.56)	8.38 (8.15—8.62)	0.095
Duration of IFN- γ (yrs)	5.15 (2.42—9.2)	3.67 (1.19—5.58)	12.9 (11.35—14.45)	0.071
HSCT	1 (11.1%)	1 (16.67%)	0 (0%)	>0.99
Comorbidities and outcome				
ESRD	1 (11.1%)	0 (0%)	1 (33.3%)	0.33
Inflammatory enterocolitis	2 (22.2%)	2 (33.3%)	0 (0%)	0.50
Death	1 (11.1%)	1 (16.7%)	0 (0%)	>0.99

[a] Comparison between XL-CGD and AR-CGD.
Abbreviations: years, yrs; Interferon-gamma, IFN-γ; hematopoietic stem cell transplantation, HSCT; end-stage renal disease, ESRD.
Expressed as median (25—75th percentile) or number (%).

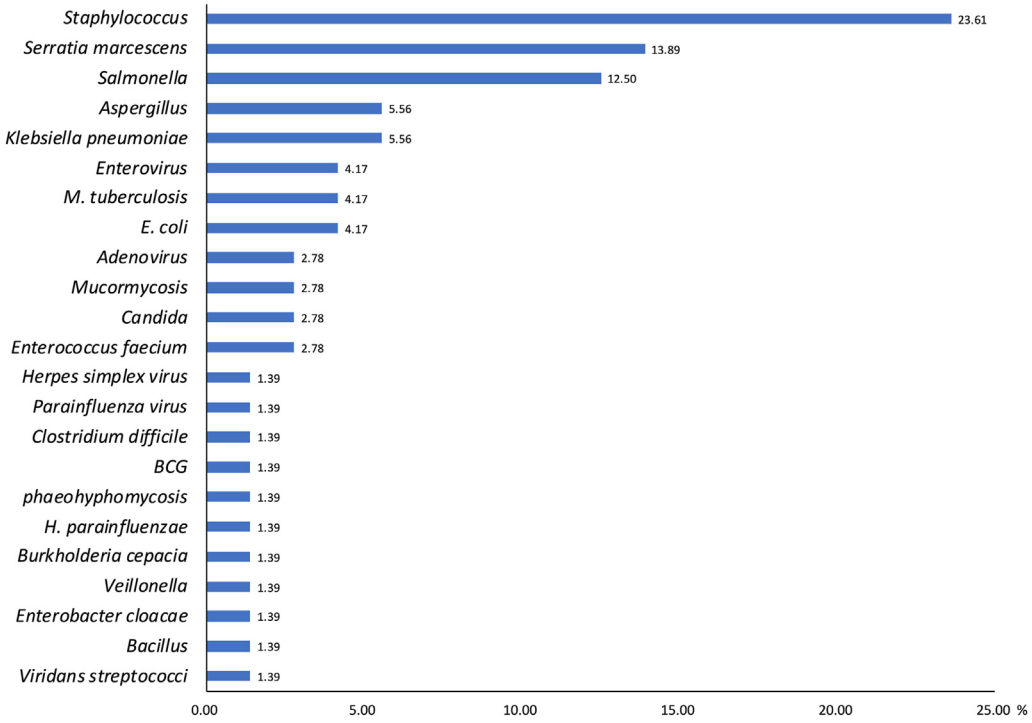

Figure 1. Pathogens identified in 72 infection events in patients with CGD.

Mycobacterium tuberculosis (M. tb) in the lungs (Fig. 2 and Supplementary Table 1). Other pathogens for infections included *M. bovis Calmette-Guérin bacillus (BCG)* (lymphadenitis), mucormycosis (paranasal sinusitis), and phaeohyphomycosis (mediastinal abscess) (Supplementary Table 1). *Aspergillus flavus* (spondylodiscitis and sinusitis), *Aspergillus fumigatus* (osteomyelitis and empyema), *Candida parapsilosis* (oral mucosa), and *Candida lusitaniae* (tongue) are the most frequently isolated fungi in the current study.

We identified six patients with XL-CGD, with one missense, two essential splicing, two deletion, one nonsense mutations in the *CYBB* gene. *CYBB* c.406_424del19 (p.Pro136Leufs*19) and c.1028C > A (p.Thr343Lys) gene mutations have not been reported. AR-CGD etiologies included one compound heterozygous mutation in the *CYBA* gene (c.70G > A, IVS3-2 A > G) and two *NCF1* hot spot c.75_76delGT mutations (Table 2). We evaluated two XL-CGD patients (P4 and P5) for monocytes and neutrophils $gp91^{phox}$ expression: both were $X91^{-}$ (low) (Fig. 3 and Table 2). Respiratory burst responses of phagocytes did not show a significant difference between patients with XL-CGD and AR-CGD, between patients with low $gp91^{phox}$ protein expression ($X91^{-}$) and absent $gp91^{phox}$ protein expression ($X91^{\circ}$), or patients with low NADPH oxidase phox protein expression ($X91^{-}$) and absent protein expression ($X91^{\circ}$, $A47^{\circ}$, $A22^{\circ}$) (Supplementary Fig. 1).

The median major infection episodes that need hospitalization were seven times (range 4—24) in all patients with CGD during follow-up. The median frequency of infection episodes that need hospitalization was 1.09 (IQR 0.9—2.07, range 0.27—10) episodes/year. The median major infections per year in patients with lower and higher respiratory burst responses was 1.49 (0.79—2.55) and 1.08 (0.68—1.21), respectively. There was no significant difference of major infections per year between patients with lower and higher respiratory burst responses (P = 0.63) (Supplementary Fig. 2).

All patients with CGD received the prophylactic antibiotic trimethoprim/sulfamethoxazole (TMP/SMX) (2.5 mg/kg twice daily). Seven (77.8%) patients received prophylactic itraconazole (5 mg/kg or 100 mg daily) to prevent fungal infections. Eight (88.9%) patients received IFN-γ (50 μg/m^2/dose, Imukin® subcutaneous injections three times a week) prophylaxis with a median treatment duration of 5.15 years (range 0.27—16 years) (Table 1). After IFN- γ prophylaxis, the median frequencies of infection-related hospitalizations decreased from 1.72 (IQR 0.97—2.64) times per year to 0.61 (IQR 0.38—0.97) times per year (P = 0.11). However, one patient had poor compliance to IFN- γ treatment due to adverse events after injection, including fever, myalgia, and general discomfort.

Two patients (P3 and P5) with XL-CGD developed inflammatory enterocolitis, which was treated with oral corticosteroids and mesalamine as immunomodulators which has been shown to be beneficial in CGD-associated inflammatory bowel disease.[13] One male patient (P7) with *NCF1* gene mutation had end-stage renal disease with hemodialysis after prolonged amphotericin-B treatment and an episode of hemolytic uremic syndrome seven years after CGD diagnosis.[14] One male CGD patient with a *CYBB* mutation (P2) received hematopoietic stem cell transplantation (HSCT) using peripheral blood stem cells from a matched unrelated donor successfully at the age of 2 years and 2 months, which was complicated with grade I graft-versus-host disease on the skin. One male patient (P6)

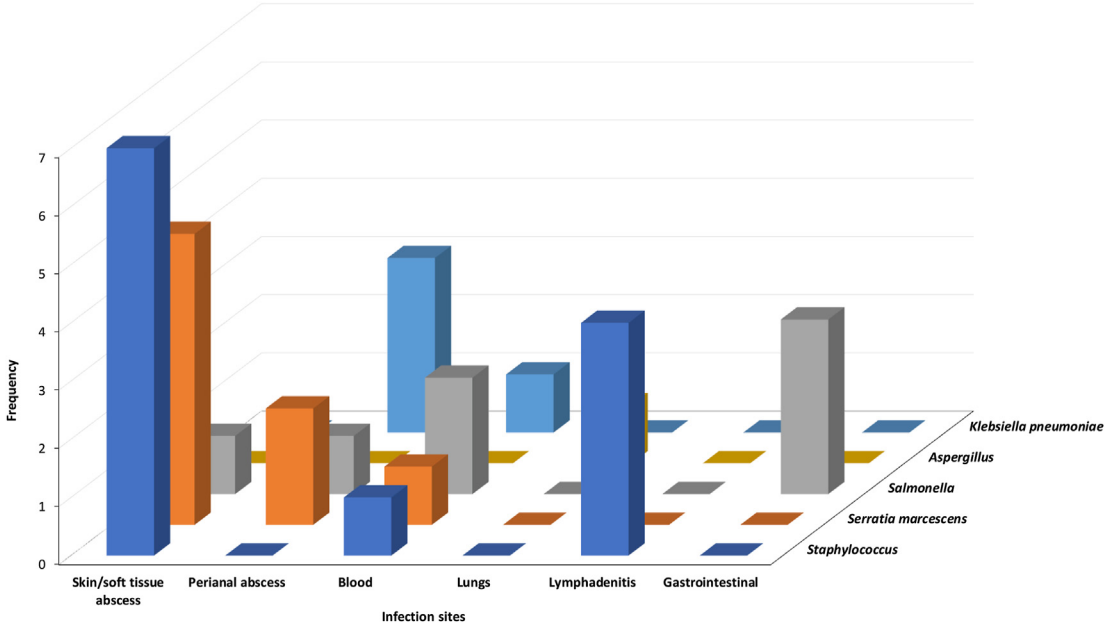

Figure 2. Numbers of top five ranked pathogens identified according to site of infection in patients with CGD.

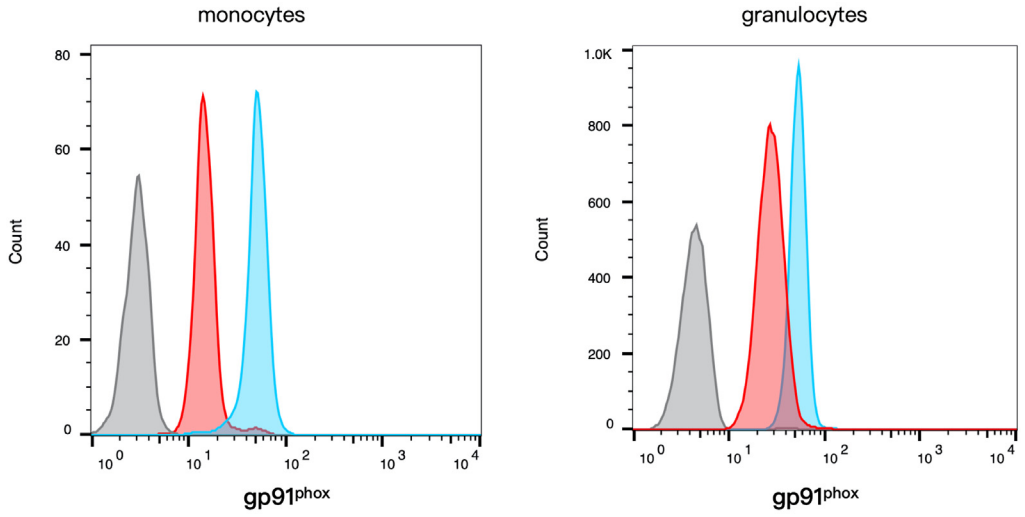

Figure 3. Flow cytometry analysis of surface protein expression of gp91^{phox} on monocytes and granulocytes of a XL-CGD patient and a control. Solid histograms represent fluorescence using isotype control antibody of a control (gray), anti-gp9^{phox} antibody of our patient P4 (red), and anti-gp91^{phox} antibody of a healthy control (blue).

with XL-CGD died at the age of 17.5 years due to intracranial hemorrhage. The estimated 10-year and 20-year survival probabilities were 100% and 66.7% (95% CI 30—100%), respectively (Fig. 4A). Median survival for XL-CGD and AR-CGD were 17.5 years and undefined, respectively (Log-rank $P = 0.083$) (Fig. 4B).

Discussion

We reported clinical and genetic features of nine patients with CGD with long-term follow-up. Most previously published manuscripts were sporadic case reports or case series since 1988 in Taiwan.[12,14—18] Improved awareness, ROS production detected by flow cytometry, and genetic diagnosis have led to earlier diagnosis of CGD. The frequency of consanguineous marriages is low, accounting for the finding that XL-CGD is the most frequent (66.7%) pattern of inheritance for CGD in Taiwan. Previous studies have shown that about 21—40% of patients with CGD have a family history of CGD.[11,19] Only one patient (11.1%) had a positive family history of CGD in our cohort.

We reported causal mutations in *CYBB* (six mutations), *NCF1* (one mutation), and *CYBA* (one mutation) genes, similar to the previous report by Lee WI et al.[12] We found

Table 2 The Genetic mutations in patients with CGD.

No	Sex	Gene	Exon/intron	Nucleotide change	Codon change	Type of mutation	Carrier	CGD type[a]	Respiratory burst (%)[b]	Pathogenic	Ref
P1	M	*CYBB*	Intron 1	c.45+5G>A	Predicted aberrant splicing	Splice	M	$X91^0$	16.7	Pathogenic	39
P2	M	*CYBB*	Exon 5	c.406_424del19	p.Pro136Leufs*19	Deletion, fs with premature stop codon	M	NR	11.8	Pathogenic	-
P3	M	*CYBB*	Exon 7	c.676C>T	p.Arg226X	nonsense	M	$X91^0$	9.3	Pathogenic	40,41
P4	M	*CYBB*	Exon 8	c.897A>G	p. Lys299Lys, Predicated aberrant splicing	Splice	M, S	$X91^-$	10.7	Pathogenic	40
P5	M	*CYBB*	Exon 9	c.1028C>A	p.Thr343Lys	missense	M	$X91^-$	18.9	Likely pathogenic	
P6	M	*CYBB*	Exon 13	c.1679delG	p.Gly560Glufs*16	Deletion, fs	M	$X91^-$	0.7	Pathogenic	16
P7	M	*NCF1*	Exon 2	c.75_76delGT	p.Tyr26Hisfs*26	Deletion, fs with premature stop codon	—	$A47^0$	8.6	Pathogenic	14
P8	F	*NCF1*	Exon 2	c.75_76delGT	p.Tyr26Hisfs*26	Deletion, fs with premature stop codon	—	$A47^0$	14.5	Pathogenic	42
P9	F	*CYBA*	Exon 2, Intron 3	c.70G>A, IVS3-2A>G	p.Gly24Arg, aberrant splicing with exon 4	Missense, splice	M, F	$A22^0$	16.4	Pathogenic	15,41

[a] $gp91^{phox}$ expression was detected by flow cytometry (P4, P5) or was reported in previous studies. The results were reported for "X" (X-linked) or "A" (autosomal recessive inheritance); "91" ($gp91^{phox}$), "47" ($p47^{phox}$), "22" ($p22^{phox}$) indicating the protein affected; and the superscript symbols indicates "0" (undetectable), "-" (low), or "+" (normal).

[b] Respiratory burst activity was evaluated by NBT test (P6) or DCF assay (P1–P5, P7–P9). The results were expressed as the percentage of normal controls.

Abbreviations: mother, M; sister, S; father, F; frameshift, fs; not reported, NR.

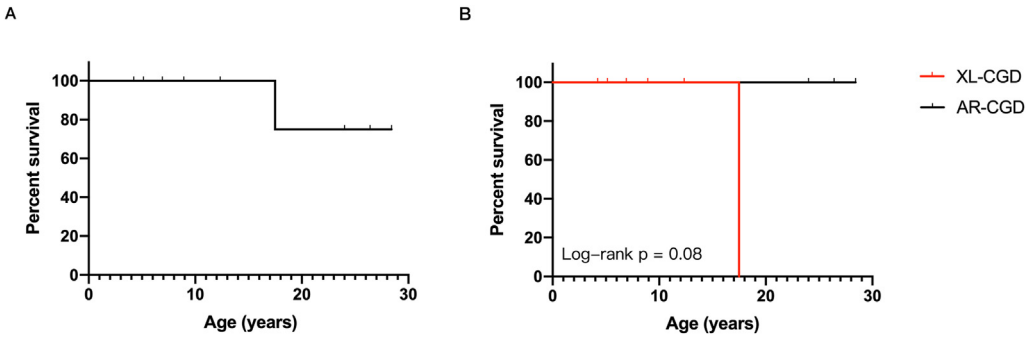

Figure 4. Outcome of pediatric patients with CGD. A. Kaplan—Meier survival curve for nine patients with CGD. B. Kaplan—Meier survival curve for patients with CGD, comparing XL-CGD and AR-CGD. Median survival for XL-CGD and AR-CGD was 17.49 years and undefined, respectively.

two novel mutations for CGD in our cohort: *CYBB* c.1028C > A (p.Thr343Lys, missense) and c.406_424del19 (p.Pro136Leufs∗19, deletion and frameshift mutation). Over 90% of patients with *NCF1* gene mutations carry the deletion c.75_76delGT (ΔGT). The recombination between *NCF1* wild-type gene and its highly homologous pseudogenes with ΔGT accounts for the majority of this hot spot mutation in p47-phox deficient CGD.[20] Previous studies have shown the expression levels of NOX2 protein components correlates with the severity of ROS production defects,[3,21] which was not observed in our study probably due to small sample size. Patients with mutations in $p47^{phox}$ gene and most missense mutations in $gp91^{phox}$ gene had more residual ROS production than patients with nonsense, frameshift, splice, or deletion mutations in $gp91^{phox}$ gene.[3] Moreover, residual ROS production negatively correlates with severity of illness and positively correlates with survival in patients with CGD, regardless of specific gene affected.[3]

The clinical features of our patients with CGD have similarities to other cohorts reported worldwide.[4,11,22] The age of onset and age of diagnosis for patients with XL-CGD were younger than that for patients with AR-CGD.[4] The common infections include skin/soft tissue abscess (77.8%), bloodstream (66.7%), oral/tonsillitis (66.7%), and lung infections (55.6%) in our study. The lungs (66—97%), skin/soft tissues (43%—80%), and lymph nodes (35%—72%) are common sites of infections in the previous studies.[11,19] *S. aureus, Burkholderia cepacia complex, S. marcescens, Nocardia species, and Aspergillus species* are the most common pathogens in CGD in the United States.[7,23,24] The most common site of *Aspergillus* infections in CGD are the lungs (pneumonia) and the skeleton (osteomyelitis).[25] *Salmonella* spp and *M. tb* are also major pathogens in CGD in other non-U.S. countries globally, which is compatible with our study.[19]

Taiwan is endemic for *M. tb*, and the incidence was 74.6 per 100,000 population.[26] Systemic BCG infection and BCG sepsis have been previously described in patients with XL-CGD.[27] BCG infections (especially BCG lymphadenitis) were the most common initial infection in AR-CGD patients.[27] According to the BCG vaccination program in Taiwan, children were recommended to receive routine BCG vaccination at one day old in the past, and it has been changed to give BCG vaccination at five months of age since 2016. Although BCG vaccination is contraindicated for CGD patients or infants with a family history of CGD, all patients in our cohort received BCG vaccination before the diagnosis of CGD. Our male patient with XL-CGD infected with BCG lymphadenitis was successfully treated with prolonged combination isoniazid, rifampicin, and regular IFN-γ therapy.

Salmonella spp. are the organisms isolated most commonly from the blood of patients with CGD in our study and in North America.[19] Approximately 5% of individuals with a gastrointestinal illness caused by non-typhoidal *Salmonella* (NTS) develop bacteremia. NTS bacteremia is more likely to occur in immunocompromised patients,[28] as *Salmonella* group E1 bacteremia in one patient of XL-CGD (P3). According to studies from Taiwan's National Health Insurance Database, the incidence rate of non-typhoid *Salmonella* infection-associated hospitalizations are high in infants (524.7/100,000 person-years) and children aged 1—9 years (110.8/100,000 person-years), which is higher than that in Spain and Poland.[29—31] The higher prevalence of *Salmonella* infections in our patients with CGD might be influenced by the weather, environmental factors, and high population density.[31]

Prophylaxis with trimethoprim-sulfamethoxazole (TMP/SMX) and antifungal itraconazole is the cornerstone for prevention of bacterial and fungal infections. Prophylaxis with TMP/SMX prolonged life-threatening infection-free intervals from one episode every ten months to one episode every 40 months in patients with CGD.[32] Margolis et al. demonstrated that prophylaxis with TMP/SMX decreased the incidence of bacterial infections from 7.1 to 2.4 per 100 patient-months in patients with AR-CGD and from 15.8 to 6.9 infections per 100 patient-months in patients with X-linked CGD.[33] Prophylaxis with itraconazole prolonged the interval of first invasive mold infections from 4 years to 10 years in patients with CGD, and decreased mortality.[34]

We demonstrated that add-on IFN-γ prophylaxis therapy might have an effect on alleviating the infection episodes in CGD patients under antibiotics and antifungal prophylaxis. An Italian multicenter study comparing prophylaxis with TMP/SMX and itraconazole versus the addition of IFN-γ showed no significant difference in the rate of infections.[35] The major reasons for poor compliance were flu-like symptoms (fever, headache, myalgia, fatigue) and local infection—site reactions after prolonged IFN-γ therapy in our cohort.

Allogeneic HSCT can be curative for CGD. According to the European Society for Immunodeficiencies (ESID) and the European Blood and Marrow Transplantation (EBMT) guidelines 2017, HSCT is indicated in patients with XL- or AR-CGD with a matched donor or mismatched unrelated donor plus one clinical or social complication (https://www.ebmt.org/ebmt/documents/esid-ebmt-hsct-guidelines-2017). Since levels of residual superoxide production have correlated well with overall survival in CGD patients, HSCT has been recommended in patients with XL-CGD.[36,37] Due to current therapies and recent advances in HSCT, the overall survival for CGD after HSCT has reached 78.6—100% in recent studies.[7] On the other hand, it is now expected that about 90% of patients with CGD will survive into adulthood with or without HSCT, given an improved diagnosis and prophylaxis therapy.[38] In a recent study from the United States Immunodeficiency Network, allogeneic HSCT was associated with reduced infection and improved functional performance in CGD patients, but not with a change in overall survival.[36] One male patient with XL-CGD (P2) in our cohort received HSCT successfully. Other patients did not receive HSCT because of end stage renal disease (ESRD), good conditions with milder infections under prophylaxis therapy, or family issues. The mortality rate was 11.1% in our cohort. The 20-year survival probability was 66.7% in our cohort, which was similar to36 or better than recent studies.[4,11]

In conclusion, *S. aureus, S. marcescens,* and *Salmonella* infections are important in Taiwanese CGD patients. Patients with XL-CGD have early disease onset. IFN-γ prophylaxis and prophylactic anti-microbial agents might have an effect on alleviating the infection episodes in CGD patients. There remain significant challenges from life-long risk of infections, inflammatory complications, and organ damage in CGD patients.

Authorship contributions

TSL designed the project, collected and analyzed the data, and drafted the manuscript. HHY conceived and designed the project, collected the data, drafted and revised the manuscript, and approved the final manuscript for submission. JHL, LCW, YTL, and YHY collected the data. YLL and WIL provided the genetic diagnosis. HHY and BLC revised the manuscript and applied for funding. All co-authors have reviewed this manuscript and have contributed in a substantive and intellectual manner to this work.

Funding

This study was supported by the National Taiwan University Hospital (111-A152). The National Taiwan University Hospital did not have a role in deciding the study design; the collection, analysis, and interpretation of data; the writing of the report; or the decision to submit the manuscript for publication.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgements

Not applicable.

References

1. Janeway CA, Craig J, Davidson M, Downey W, Gitlin D. Hypergammaglobulinemia associated with severe recurrent and chronic nonspecific infection. *Am J Dis Child* 1954;**88**:388—92.
2. van den Berg JM, van Koppen E, Åhlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. *PLoS One* 2009;**4**:e5234.
3. 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**:2600—10.
4. Blancas-Galicia L, Santos-Chavez E, Deswarte C, Mignac Q, Medina-Vera I, Leon-Lara X, et al. Genetic, immunological, and clinical features of the first Mexican cohort of patients with chronic granulomatous disease. *J Clin Immunol* 2020;**40**:475—93.
5. Roos D, de Boer M. Molecular diagnosis of chronic granulomatous disease. *Clin Exp Immunol* 2014;**175**:139—49.
6. Rider N, Jameson M, Creech C. Chronic granulomatous disease: epidemiology, pathophysiology, and genetic basis of disease. *J Pediatric Infect Dis Soc* 2018;**7**:S2—5.
7. Yu HH, Yang YH, Chiang BL. Chronic granulomatous disease: a comprehensive review. *Clin Rev Allergy Immunol* 2021;**61**:101—13.
8. Thomas DC, Charbonnier L-M, Schejtman A, Aldhekri H, Coomber EL, Dufficy ER, et al. EROS/CYBC1 mutations: Decreased NADPH oxidase function and chronic granulomatous disease. *J Allergy Clin Immunol* 2019;**143**:782—785.e1.
9. Anjani G, Vignesh P, Joshi V, Shandilya JK, Bhattarai D, Sharma J, et al. Recent advances in chronic granulomatous disease. *Genes Dis* 2020;**7**:84—92.
10. Lee PP, Chan KW, Jiang L, Chen T, Li C, Lee TL, et al. 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—30.
11. 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**:378—86.
12. Lee WI, Huang JL, Jaing TH, Shyur SD, Yang KD, Chien YH, et al. Distribution, clinical features and treatment in Taiwanese patients with symptomatic primary immunodeficiency diseases (PIDs) in a nationwide population-based study during 1985—2010. *Immunobiology* 2011;**216**:1286—94.
13. Angelino G, De Angelis P, Faraci S, Rea F, Romeo EF, Torroni F, et al. Inflammatory bowel disease in chronic granulomatous disease: an emerging problem over a twenty years' experience. *Pediatr Allergy Immunol* 2017;**28**:801—9.
14. Chang HM, Yu HH, Yang YH, Lee WI, Lee JH, Wang LC, et al. Successful treatment of Aspergillus flavus spondylodiscitis with epidural abscess in a patient with chronic granulomatous disease. *Pediatr Infect Dis J* 2012;**31**:100—1.
15. Chang YH, Yu HH, Lau YL, Chan KW, Chiang BL. A new autosomal recessive, heterozygous pair of mutations of CYBA in a patient with chronic granulomatous disease. *Ann Allergy Asthma Immunol* 2010;**105**:183—5.
16. Ma HR, Mu SC, Yang YH, Chen CM, Chiang BL. Therapeutic effect of interferon-gamma for prevention of severe infection in X-linked chronic granulomatous disease. *J Formos Med Assoc* 2003;**102**:189—92.

17. Lin CJ, Wang SC, Ku CL, Kao JK, Chen M, Liu CS. Successful unrelated cord blood stem cell transplantation in an X-linked chronic granulomatous disease patient with disseminated BCG-induced infection. *Pediatr Neonatol* 2015;**56**:346—50.
18. Wang SM, Shieh CC, Liu CC. Successful treatment of Paecilomyces variotii splenic abscesses: a rare complication in a previously unrecognized chronic granulomatous disease child. *Diagn Microbiol Infect Dis* 2005;**53**:149—52.
19. Bennett N, Maglione PJ, Wright BL, Zerbe C. Infectious complications in patients with chronic granulomatous disease. *J Pediatric Infect Dis Soc* 2018;**7**:S12—7.
20. Chanock SJ, Roesler J, Zhan S, Hopkins P, Lee P, Barrett DT, et al. Genomic structure of the human p47-phox (NCF1) gene. *Blood Cells Mol Dis* 2000;**26**:37—46.
21. Lin SJ, Huang YF, Chen JY, Heyworth PG, Noack D, Wang JY, et al. Molecular quality control machinery contributes to the leukocyte NADPH oxidase deficiency in chronic granulomatous disease. *Biochim Biophys Acta* 2002;**1586**:275—86.
22. 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**:e5234.
23. Winkelstein JA, Marino MC, Johnston Jr RB, 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.
24. Marciano BE, Spalding C, Fitzgerald A, Mann D, Brown T, Osgood S, et al. Common severe infections in chronic granulomatous disease. *Clin Infect Dis* 2015;**60**:1176—83.
25. King J, Henriet SSV, Warris A. Aspergillosis in chronic granulomatous disease. *J Fungi (Basel)* 2016;**2**:15.
26. Hsueh PR, Liu YC, So J, Liu CY, Yang PC, Luh KT. Mycobacterium tuberculosis in Taiwan. *J Infect* 2006;**52**:77—85.
27. Ishikawa T, Okai M, Mochizuki E, Uchiyama T, Onodera M, Kawai T. Bacillus calmette-guérin (BCG) infections at high frequency in both AR-CGD and X-CGD patients following BCG vaccination. *Clin Infect Dis* 2021;**73**:e2538—44.
28. Hohmann EL. Nontyphoidal salmonellosis. *Clin Infect Dis* 2001; **32**:263—9.
29. Gil Prieto R, Alejandre CG, Meca AA, Barrera VH, de Miguel AG. Epidemiology of hospital-treated Salmonella infection; data from a national cohort over a ten-year period. *J Infect* 2009; **58**:175—81.
30. Trevejo RT, Courtney JG, Starr M, Vugia DJ. Epidemiology of salmonellosis in California, 1990-1999: morbidity, mortality, and hospitalization costs. *Am J Epidemiol* 2003;**157**:48—57.
31. Chen PL, Li CY, Hsieh TH, Chang CM, Lee HC, Lee NY, et al. Epidemiology, disease spectrum and economic burden of nontyphoidal Salmonella infections in Taiwan, 2006—2008. *Epidemiol Infect* 2012;**140**:2256—63.
32. Gallin JI, Buescher ES, Seligmann BE, Nath J, Gaither T, Katz P. NIH conference. Recent advances in chronic granulomatous disease. *Ann Intern Med* 1983;**99**:657—74.
33. Margolis DM, Melnick DA, Alling DW, Gallin JI. Trimethoprim-sulfamethoxazole prophylaxis in the management of chronic granulomatous disease. *J Infect Dis* 1990;**162**:723—6.
34. Blumental S, Mouy R, Mahlaoui N, Bougnoux ME, Debré M, Beauté J, et al. Invasive mold infections in chronic granulomatous disease: a 25-year retrospective survey. *Clin Infect Dis* 2011;**53**:e159—69.
35. Martire B, Rondelli R, Soresina A, Pignata C, Broccoletti T, Finocchi A, et al. 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—64.
36. Yonkof JR, Gupta A, Fu P, Garabedian E, Dalal J, the United States Immunodeficiency Network C. Role of allogeneic hematopoietic stem cell transplant for chronic granulomatous disease (CGD): a report of the United States immunodeficiency Network. *J Clin Immunol* 2019;**39**:448—58.
37. Åhlin A, Fasth A. Chronic granulomatous disease - conventional treatment vs. hematopoietic stem cell transplantation: an update. *Curr Opin Hematol* 2015;**22**:41—5.
38. Connelly JA, Marsh R, Parikh S, Talano JA. Allogeneic hematopoietic cell transplantation for chronic granulomatous disease: controversies and state of the art. *J Pediatric Infect Dis Soc* 2018;**7**:S31. s9.
39. Roos D, de Boer M, Kuribayashi F, Meischl C, Weening RS, Segal AW, et al. Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. *Blood* 1996;**87**:1663—81.
40. Kannengiesser C, Gérard B, El Benna J, Henri D, Kroviarski Y, Chollet-Martin S, et al. Molecular epidemiology of chronic granulomatous disease in a series of 80 kindreds: identification of 31 novel mutations. *Hum Mutat* 2008;**29**:E132—49.
41. 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**:246—65.
42. Roesler J, Curnutte JT, Rae J, Barrett D, Patino P, Chanock SJ, et al. Recombination events between the p47-phox gene and its highly homologous pseudogenes are the main cause of autosomal recessive chronic granulomatous disease. *Blood* 2000;**95**:2150—6.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2022.06.005.