

Chronic Granulomatous Disease in Latin American Patients: Clinical Spectrum and Molecular Genetics

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Background. Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by early onset of recurrent and severe infections. The molecular defects causing CGD are heterogeneous and lead to absence, low expression, or malfunctioning of one of the phagocyte NADPH oxidase components. The aim of this study was to analyze the clinical features and to investigate the molecular genetic defects of Latin American patients with CGD.

Procedures. The study included 14 patients. The diagnosis was based on a history of recurrent severe infections, impaired respiratory burst, and the demonstration of an underlying mutation by single strand conformation polymorphism (SSCP) or RT-PCR analysis, followed by genomic DNA or cDNA sequencing. **Results.** Seven unrelated patients were found to have the X-linked form of CGD (X-CGD). Heterogeneous mutations affected the *CYBB* gene: two insertions, one substitution, and four splice site

defects; two of them are novel. Seven patients presented with one of the autosomal recessive forms of CGD (A47-CGD); all had the most common mutation, a Δ GT deletion in exon 2 of the *NCF1* gene. Pneumonia was the most frequent clinical feature, followed by pyoderma, sinusitis, otitis, and liver abscess. Patients with X-CGD were more likely to have initial infections before age 2 years and to have inflammatory obstructive granulomas later. None of the patients had severe adverse reactions to BCG immunization. **Conclusions.** X-CGD patients from Latin America showed a high degree of molecular heterogeneity, including two novel mutations. Their clinical characteristics included early onset of infections and eventual obstructive granulomas. A47-CGD represented 50% of the reported cases, a higher prevalence than reported in other series.

Key words: BCG; chronic granulomatous disease; mutations; neutrophils; phagocytes; primary immunodeficiencies

INTRODUCTION

Chronic granulomatous disease (CGD) is a primary immunodeficiency, first characterized in 1957 as a clinical entity affecting male infants and originally termed “fatal granulomatous of childhood” [1,2]. The estimate inci-

dence of this disease in the northern hemisphere is 1/250,000 live births per year [3]. CGD is characterized by severe recurrent infections affecting mainly the natural barriers such as the respiratory tract and lymph nodes, and eventually inner structures, such as the liver, spleen, bones, and brain [1,2]. The infections are generally caused

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by catalase positive bacteria—such as *Staphylococcus aureus* and gram-negative bacilli—or fungal species including *Aspergillus* and *Candida* [4]. In principle, patients with CGD should not receive BCG immunization due to the risk of severe adverse effects [5].

The molecular defects causing CGD generally relate to the absence, low expression, or malfunctioning of one component of the phagocyte NADPH oxidase responsible for the generation of microbicidal reactive oxygen species [3]. The X-linked form of the disease (OMIM 306400) is caused by defects in the heavy chain of the cytochrome b_{588} component (gp91-*phox*) and accounts for 56% of the cases [6]. The autosomal recessive forms are caused by defects in one of the cytosolic components of the NADPH oxidase: p47-*phox* (OMIM 233700) or p67-*phox* (OMIM 233710), accounting respectively for 33% and 5% of the cases [7]; or the cytochrome b_{588} light chain component p22-*phox* (OMIM 233690), representing 6% of CGD cases [6,8]. A related immunodeficiency has been associated with a defect in the gene for Rac2 [9]. No patients have been reported with defects in the p40-*phox*, rap1A, rac1, or GDI components of the oxidase.

Genetic defects of patients with CGD described to date include approximately 410 reported mutations in the four affected genes [9]. The diversity of these mutations and affected genes explains, at least in part, the clinical and genetic heterogeneity of CGD [10,11] and suggests that the worldwide incidence of CGD is a consequence of many independent mutational events.

Few studies analyzing clinical or genetic features of CGD patients have been performed in the southern hemisphere [12,13], where the population has a diverse genetic background and children are exposed to a different environment, including routine immunization with BCG. The present study sought to analyze the clinical features and to investigate the molecular genetic defects leading to CGD in Latin American patients.

METHODS

Patients

Fourteen patients with a clinical history of recurrent severe infections (including pneumonia, lymphadenitis, liver abscesses, or pyodermitis) were included. A detailed clinical and family history, and physical examination were performed. Patients and their families received explanations about the research plan and written informed consent was obtained. The Medical School Ethics Committee approved the research plan and the experimental procedures according to the Ministry of Health of Brazil (Resolution 196/96). Alternatively, a physician certified by the Latin American Group of Primary Immunodeficiencies, using Pan-American Group of Primary Immunodeficiencies protocols [14], evaluated patients at distant locations or in other Latin American

countries for inclusion in this research protocol. All patients were born at term to healthy non-consanguineous parents after uneventful pregnancies.

Patient A1. Female, from Caucasian and African background, Brazilian, born in 1981. She presented at age 10 years with a history of three episodes of skin infection and protracted pneumonia. During a follow-up of 9 years, she had a Bartolin gland infection and another pneumonia, both treated without hospitalization. She receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and itraconazole.

Patient A2. Male, from Caucasian and African background, Brazilian, born in 1990; brother of patient A1. He presented with a history of recurrent skin infections after age 10 years, including pneumonia at age 11 years. He receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and itraconazole.

Patient B1. Female, from Native American and Caucasian ethnic background, Mexican, born in 1991. She presented with recurrent pneumonia since age 4 years. During a follow-up of 8 years, she had no severe infections since starting prophylaxis with sulfamethoxazole/trimethoprim, itraconazole, and recombinant human interferon gamma.

Patient B2. Female, from Native American and Caucasian ethnic background, Mexican, born in 1992; sister of patient B1. She presented with a history of recurrent pneumonia since age 7 years, as well as chronic lymphadenitis. Like her sister, patient B1, she has had no severe infections since initiation of prophylaxis with sulfamethoxazole/trimethoprim, itraconazole, and recombinant human interferon gamma.

Patient C. Female, African Brazilian, born in 1997. She presented at age 3 years with pneumonia due to multi-resistant *Staphylococcus aureus*, then developed liver abscess at age 9 years. She receives antimicrobial treatment with sulfamethoxazole/trimethoprim and itraconazole, but has had continued recurrent infections.

Patient D. Male, Caucasian, Brazilian, born in 1970. He presented with recurrent pulmonary infections starting in his first year of life. He has recurrent skin infections, otitis, sinusitis, and liver abscesses. He receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and itraconazole.

Patient E. Female, from African and Caucasian background, Brazilian, born in 1994. She started with recurrent respiratory infections at age 2 years. From 4 to 8 years of age, she was hospitalized four times to treat pneumonia. She has chronic otitis and sinusitis, recurrent tonsillitis and aphthous ulcers. She receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and itraconazole.

Patient F. Male, Caucasian, Brazilian, born in 1990. He suffered several infections, starting at age 18 months with cytomegalovirus infection. He has had recurrent

pneumonia, tonsillitis, otitis, sinusitis, skin infection, and lymphadenitis. When he was 12 years old he developed granulomas that resulted in gastric obstruction; later he developed granulomas obstructing the airway and urinary tract. Granulomas were treated with prednisone 1 mg/kg/day for 3 months, and he receives prophylactic sulfamethoxazole/trimethoprim and itraconazole. Interferon- γ therapy was tried several times, but discontinued due to marked lymphopenia. His family history includes male relatives with recurrent infections.

Patient G. Male, Caucasian, Chilean, born in 1998. He presented with cervical lymphadenitis caused by *Staphylococcus aureus* when he was 6 months old. At age 18 months he developed skin infection and recurrent diarrhea, then pneumonia with *Staphylococcus aureus* and *Pseudomonas* species isolated from his upper right lobe. He developed left upper lobe bronchiectasis and *Nocardia* pneumonia in November, 2004. He receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and itraconazole.

Patient H. Male, Caucasian, Chilean, born in 1995. He presented with skin and liver abscesses during his first year of life, and pulmonary abscess at age 2 years, 6 months; all abscesses were caused by *Staphylococcus aureus*. He receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim.

Patient I. Male, Caucasian, Brazilian, born in 1997. He presented with recurrent infections since age 6 months, starting with pneumonia, followed by bone infection and abscesses in the axilla and liver. He also developed cutaneous and mucosal infections caused by *Candida* species. He has a family history of male relatives with recurrent infections. He receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and itraconazole.

Patient J. Male, from African and Caucasian background, Brazilian, born in 1997. He presented with bullous impetigo at 7 days of life, then pneumonia at age 5 years, followed by diarrhea, skin infection, septic arthritis, otitis, and cervical abscess. When he was 6 years old he had cervical and perirectal abscesses, pneumonia, and meningoencephalitis caused by *Aspergillus fumigatus*, and at age 7 years he developed inflammatory granuloma of the bowel, treated with prednisone 1 mg/kg/day for 3 months. He receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and itraconazole.

Patient K. Male, African Brazilian, born in 1993. He presented with pneumonia at age 2 years, and later developed recurrent pneumonia and chronic lung disease, including bronchiectasis and fibrosis; otitis, sinusitis, skin infections, and diarrhea caused by *Salmonella sp.* He also has erythrocyte glucose-6-phosphate dehydrogenase deficiency (African variant). His family history includes male relatives with recurrent infections. He receives antimicrobial prophylaxis with sulfamethoxazole/tri-

methoprim and itraconazole. This case has been reported in detail elsewhere [15].

Patient L. Male, from African and Caucasian background, Brazilian, born in 1989. He presented with a skin infection on his second day of life; in addition, a BCG vaccination took 5 months to heal. He also had pneumonia, chronic otitis, sinusitis, cervical lymphadenitis, and *Staphylococcus aureus* liver abscesses. He receives antimicrobial prophylaxis with sulfamethoxazole/trimethoprim and itraconazole.

NBT Test and Superoxide Release Assay

The biochemical diagnosis of CGD was established according to the Pan American Group for Immunodeficiency criteria [14,16]. Neutrophils and monocytes were isolated by centrifugation of blood over a Ficoll-Hypaque density gradient [17]. A defective respiratory burst was demonstrated by the nitroblue tetrazolium (NBT) slide test or by the superoxide release assay.

The NBT slide test is based on the reduction of NBT to formazan by activated leukocytes [16], and was performed as previously published [12]. In concurrent normal controls, more than 95% of 200 neutrophils stimulated with PMA (30 nM) must be able to reduce NBT. Absent reaction or <5% positive cells is diagnostic of CGD [14]. "Variant" CGD patients may show >95% positive cells, but with very little formazan production. The NBT test also detects female carriers for X-linked CGD, in whom random X inactivation generally leads to NBT reduction by >5% but <95% of phagocytes.

Quantitative superoxide release by neutrophils and monocytes was measured as the superoxide dismutase-inhibitable reduction of cytochrome c as previously described [18,19]. Results were expressed as nmol of superoxide released by 10^6 cells per hour. Leukocytes from patients with CGD show less than 10% of control values.

Genotype Determinations

DNA and RNA samples were extracted from neutrophils, monocytes, and EBV-transformed B-lymphocytes from confirmed CGD patients and healthy controls by DNAzol[®] (Life Technologies, Gibco, Carlsbad, CA) and by the guanidine HCl method for RNA, as previously described [20]. EBV-transformed B cell lines, which reproduce the biochemical and molecular defects of CGD patients [21], provide an alternative to repeated blood collections from patients and serve as an abundant source of nucleic acids for molecular studies. To prepare such cell lines, mononuclear cells were cultured with supernatants from B95-8, an EBV-producer cell line, as previously described [18,22]. Cellular viability was monitored and the cultures were maintained during the studies. Aliquots

of EBV-transformed B cells were frozen in liquid nitrogen for archiving and future studies.

Molecular studies were initiated in male patients with positive maternal NBT tests by searching for defects in the *CYBB* gene responsible for X-CGD. In the case of the female patients, considering the higher prevalence of defects in *p47-phox*, molecular genetic studies were initiated by testing for the Δ GT deletion at the beginning of exon 2 of the *NCF1* gene. This approach, based on nucleic acid analysis, allowed us to omit identification of the defective NADPH oxidase component by western blotting, which was not always possible with leukocytes from patients at distant locations.

In order to identify the molecular genetic defect responsible for the CGD phenotype, we investigated the underlying mutation by single strand conformation polymorphism (SSCP) analysis of genomic DNA amplified by nested PCR and by sequencing of any affected gene region, both according to previously published procedures [12]. Primer sequences were designed for amplification of all 13 exons of *gp91-phox* [12] and of *NCF1* exon 2 (Gen Bank NM 000265, nucleotide 48–451 region; forward primer cgccttctgggctttgagaag and reverse primer tcttccatctttgggcatca).

The SSCP assay detects changes in the electrophoretic mobility of single stranded DNA fragments that keep their native molecular conformation; it is sensitive even to single nucleotide mutations. The polyacrylamide gels were stained with silver and the electrophoretic mobility of the PCR-amplified products from CGD patients and healthy controls were compared.

Samples of PCR-amplified genomic DNA or RT-PCR amplified cDNA were purified using the “Concert Rapid PCR Purification System” (Life Technologies, Gibco) and sequenced using the DNA sequencing Kit, Big Dye Terminator Cycle Sequencing Ready Reaction for ABI 377 PE/Applied Biosystems (Foster City, CA), as previously published [23].

The sequences obtained from CGD patients and healthy controls were compared to GenBank data (*CYBB* and *NCF1* accession numbers NM_000397 and NM_000265, respectively) and submitted for BLAST analysis. Description of sequence changes was made in accordance with the nomenclature of the Human Genome Variation Society (www.hgvs.org/mutnomen/). The nucleotides were numbered according to the cDNA sequence used by Heyworth et al. [24].

RESULTS

Fourteen Latin American patients (10 Brazilians, 2 Chileans, and 2 Mexicans) were referred to our laboratory. They were nine males and five females from heterogeneous ethnic backgrounds. All patients presented histories of recurrent infections at the natural barriers of the body. *Staphylococcus aureus* was isolated in four

patients, *Pseudomonas* species in one patient, and *Aspergillus fumigatus* and *Candida* species in two other patients. Prophylactic treatment included sulfamethoxazole/trimethoprim (all patients) and itraconazole (except three patients); two patients received recombinant human interferon gamma on a regular basis.

All patients had NBT slide tests showing less than 5% positive leukocytes; eight patients also demonstrated impaired superoxide release by granulocytes and/or monocytes (less than 10% compared to healthy controls). The mothers of seven male patients had NBT slide tests compatible with the carrier state of X-linked CGD.

All of the X-CGD patients presented with their first infections before the age of 2 years. A single A47-CGD patient (D) had infection during the first year of life. All patients had recurrent pneumonia; pyoderma was the second most frequent infection for both groups (X-CGD $n = 5$; A47-CGD $n = 3$). Other infections included otitis ($n = 4$), sinusitis ($n = 3$), and liver abscess ($n = 3$) in X-CGD patients, and sinusitis ($n = 2$) and liver abscess ($n = 2$) in A47-CGD patients.

Two X-CGD patients (F and J) had the non-infectious complication of inflammatory granulomas with obstructive symptoms, treated with prednisone 1 mg/kg/day for 3 months. None of the patients had a history of severe adverse effects to BCG immunization, although one (patient L with X-CGD) had delayed healing of a BCG vaccination site. Three X-CGD patients reported a family history of recurrent infections (F, I, and K).

All of the patients with autosomal recessive CGD (five females—A1, B1, B2, C, and E and two males—A2 and D) exhibited the same mutation, a Δ GT deletion at the beginning of exon 2 of the *NCF1* gene, which encodes the *p47-phox* protein (Fig. 1).

Sequence analysis of the *CYBB* gene in patient H revealed an insertion c.1267_1268insA in exon 10 leading to a frameshift mutation (Fig. 2). This mutation is novel. We found two single base-pair substitutions that lead to nonsense mutations: in patient J, a c.95 G > A substitution in exon 2 which predicts a stop codon W28X (Fig. 3) and in patient I a c.229 C > T substitution in exon 3 which predicts a stop codon R73X (Fig. 4). Both mutations were confirmed by reverse primer sequencing. Four cases were associated with different splice site mutations. Two unrelated patients (K and F), showed an exon 3 deletion in *gp91-phox* cDNA [15]; in genomic DNA, both showed a substitution c.264 G > A at the 5' end of exon 3 (Fig. 5). Two other splice site mutations were identified, both resulting in exon 10 deletion. One patient (G) had a c.1326+1 G > A substitution in intron 10, with the mutation also detectable in his heterozygous mother (Fig. 6). The other patient (L) demonstrated a c.1164–2 A > G substitution in intron 9 (Fig. 7); this mutation is also novel. All *CYBB* mutations were compared to the X-CGD mutations database [24] and are summarized in Table I.

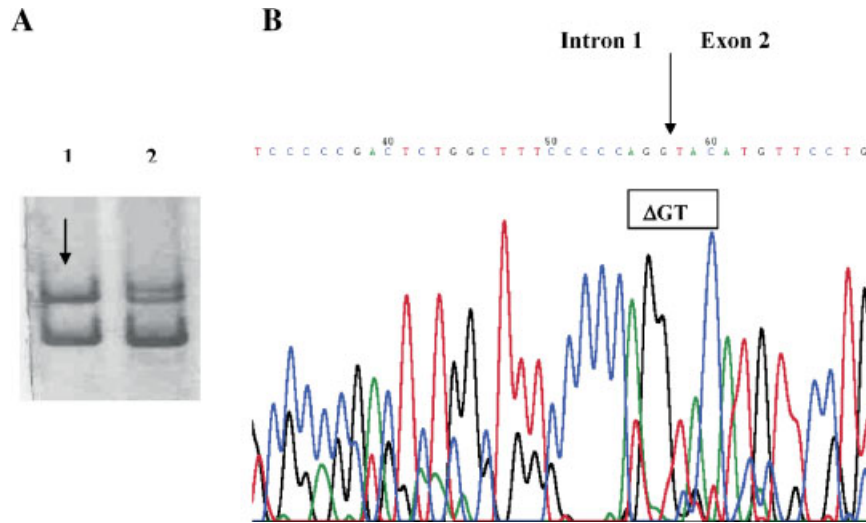


Fig. 1. *NCF1* genotype of patients A1, A2, B1, B2, C, D, E. **Panel A:** SSCP analysis of exon 2 amplified from genomic DNA. Arrow shows the altered migration pattern. **Lane 1:** Patient A1; **lane 2:** normal control. **Panel B:** DNA sequence shows a Δ GT deletion at the beginning of exon 2 in genomic DNA from patient A1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

DISCUSSION

This study analyzed clinical features and molecular genetic defects of Latin American patients with CGD, a primary immunodeficiency in which phagocytic cells are unable to generate reactive oxygen intermediates and hence fail to kill microorganisms. The identification of the genetic molecular defect in each CGD patient allows appropriate genetic counseling for patients and relatives. In addition, genotype–phenotype correlations can help to advise families and physicians on the prognosis of CGD patients.

Molecular defects leading to X-linked CGD have been identified in the coding region, introns, and (rarely) in the 5' flanking regulatory region of the *CYBB* gene [4,25,26]. Over 300 *CYBB* mutations resulting in X-linked CGD have been registered in an international database (<http://www.uta.fi/imtbioinfo/CYBBbase>) [24]. The mutations causing X-linked CGD include large multigenic or smaller deletions, insertions, missense or nonsense substitutions, and splicing defects. The mutations are distributed evenly among the exons and gene boundaries [4,25,26] and most patients have mutations unique to their kindred.

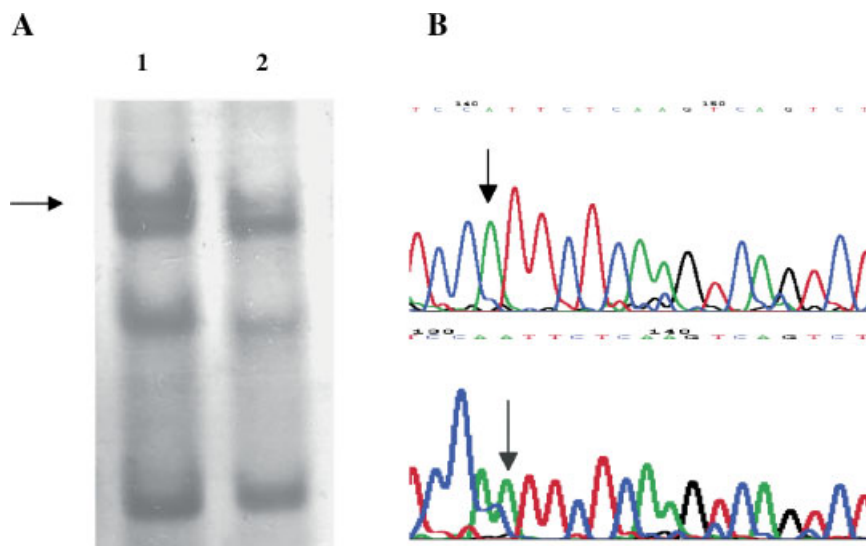


Fig. 2. *CYBB* genotype of patient H. **Panel A:** SSCP analysis of exon 2 amplified from genomic DNA. Arrow shows the altered migration pattern. **Lane 1:** Patient H; **lane 2:** normal control. **Panel B:** DNA sequence of exon 10 shows an insertion c.1267_1268 insA in patient H (lower tracing) compared to a normal control (upper tracing). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

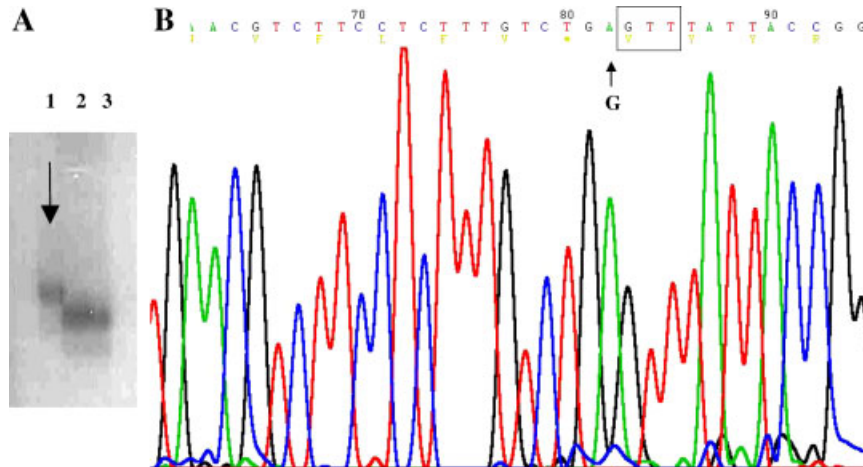


Fig. 3. *CYBB* genotype of patient J. **Panel A:** SSCP analysis of exon 2 amplified from genomic DNA. Arrow shows the altered migration pattern. **Lane 1:** Patient J; **lanes 2 and 3:** normal controls. **Panel B:** DNA sequence shows a hemizygous G > A mutation leading to the nonsense substitution W 28X. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The seven *CYBB* mutations identified in this study include several different mechanisms that interfere with gp91-*phox* expression. An insertion c.1267_1268insA in exon 10 leads to a frameshift mutation and eventual downstream termination. This mutation is novel. Overall, mutations of this type constitute 24% of *CYBB* gene defects resulting in X-linked CGD [25]. We found two single base-pair substitutions that lead to nonsense mutations. The frequency of nonsense mutations is around 23% for X-linked CGD [25].

In four cases, splice site mutations were identified. The same mutation was found at the 5' end of exon 3 in two cases (K and F). This mutation is itself silent as a coding change, but it disrupts the donor splice site of intron 3, changing the CpG sequence to CpA. Most single nucleotide substitution mutations involve CpG sequences, which are considered mutational hot spots. Ten similar mutations have been documented to date [24]. In addition,

we identified two other splice site mutations which lead to exon 10 deletions. Mutations near splice sites leading to defects in RNA processing have been observed in 39 out of 251 X-CGD cases in the largest studies [3,4,11,25].

The gp91-*phox* mutations demonstrated in these Latin American patients show a high degree of molecular heterogeneity, as reported in other ethnic groups. All of the specific mutations predict structural defects that alter the expression and function of the gene product; two of them are novel. Most mutations are distributed throughout the 13 exons or at exon/intron boundaries. In this study the most common location was the splice site. The absence of a large portion of the mRNA might generate an unstable transcript, which would be degraded after its synthesis. Patient K also presents an association of glucose-6-phosphate deficiency in addition to X-linked CGD [15]. In previous studies from Latin America, Patino et al. [12] and Barese et al. [13] also identified

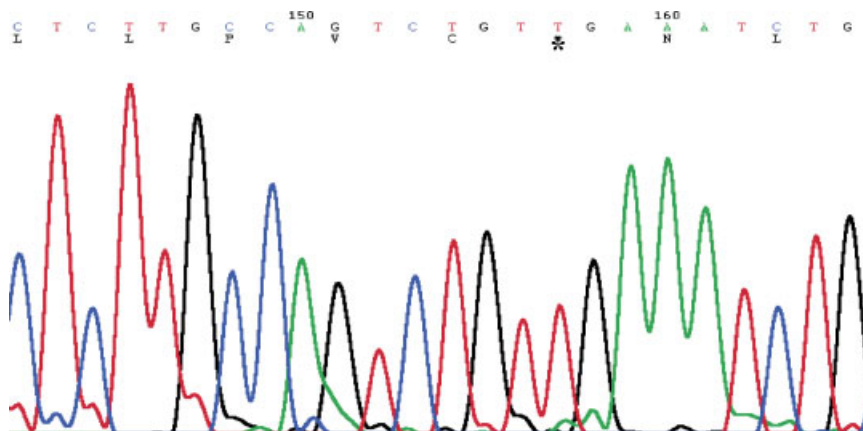


Fig. 4. *CYBB* genotype of patient I. DNA sequence analysis of patient I shows a C > T mutation in exon 3 leading to the nonsense substitution R 73X. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

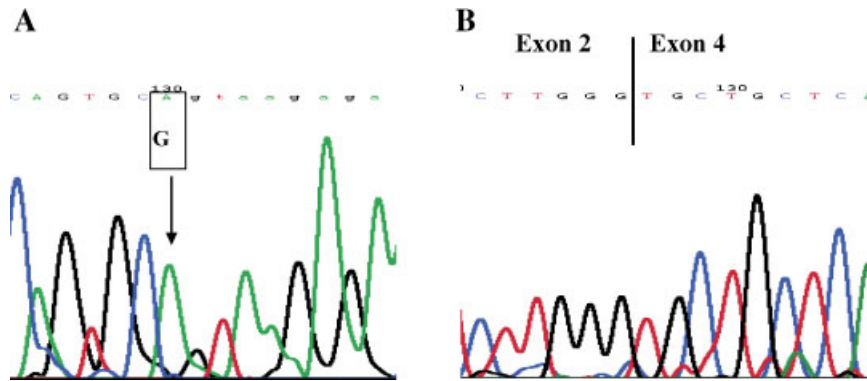


Fig. 5. *CYBB* genotype of patients K and F. **Panel A:** DNA sequence shows a hemizygous G > A transition in the splice site. **Panel B:** cDNA sequence shows a deletion of exon 3. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

heterogeneous and novel X-linked CGD mutations in kindreds from Colombia, Brazil, and Argentina.

In contrast, all A47-CGD patients were genetically homogenous, with the common dinucleotide GT deletion in exon 2. This deletion results in a frame shift with a

premature stop codon following amino acid residue 50 and is the same mutation previously observed in 74 of 82 CGD patients with p47-*phox* deficiency [24]. The presence of a neighboring, highly homologous pseudo-gene, that eliminates the normal gene or converts it to the

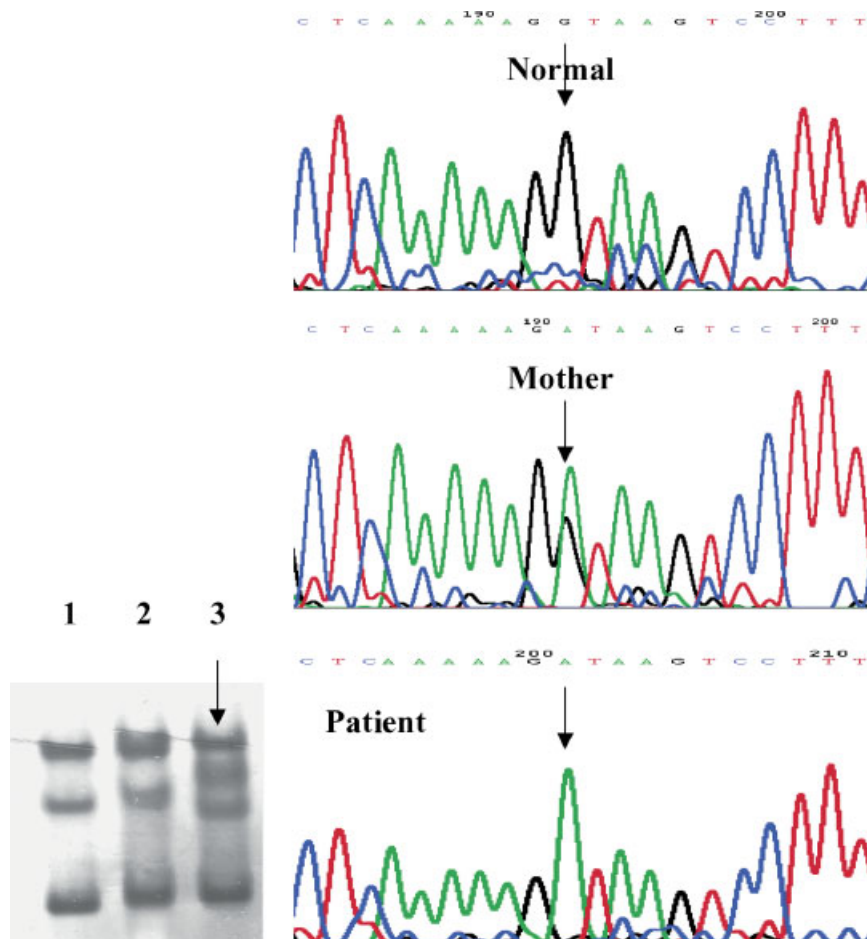


Fig. 6. *CYBB* sequence analysis of patient G. **Left panel:** The arrow in SSCP shows the altered migration pattern. **Lanes 1 and 2:** Normal controls. **Lane 3:** Patient G. **Right panel:** DNA sequence shows a hemizygous G > A transition in the splice site of intron 10 in the patient (bottom tracing), while his mother (middle tracing) is heterozygous for this mutation. The top tracing is a normal control. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

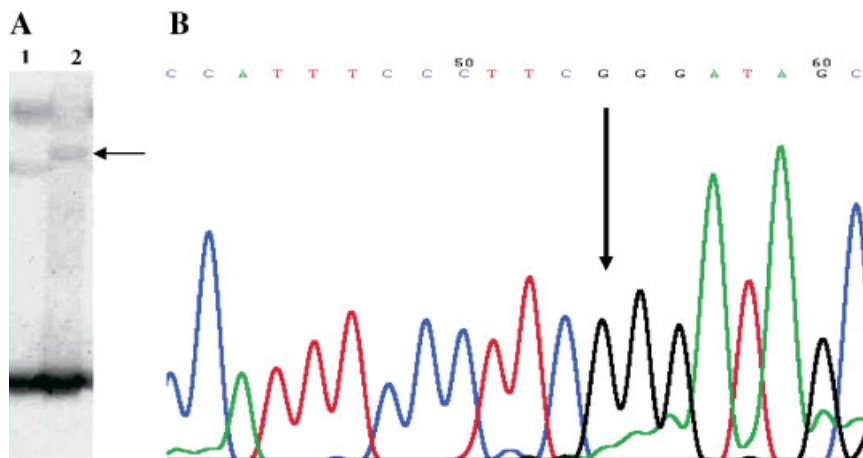


Fig. 7. *CYBB* genotype of patient L. **Panel A:** SSCP analysis of exon 10 and flanking intronic sequence amplified from genomic DNA. Arrow shows the altered migration pattern. **Lane 1:** Normal control. **Lane 2:** patient L. **Panel B:** DNA sequence shows a hemizygous A > G transition in the splice site of intron 9. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

abnormal sequence during meiotic recombination, probably explains the frequency of GT deletion in unrelated patients [27].

The current group of patients showed high degree ethnic background heterogeneity. Most of the patients experienced infections at the natural barriers of the organism early in life. Recurrent pneumonia was the most frequent infection and resulted in chronic lung disease in one patient. Liver abscess, another common complication of CGD, occurred in two A47-CGD and in three X-CGD patients. As in series of CGD patients from other geographic regions [3,28–31], our X-CGD patients presented with their first infections before the age of 2 years, as did one patient with A47-CGD.

As the length and quality of life has improved for CGD patients, more non-infectious complications have been reported [32]. Inflammatory granulomas obstructing urinary or gastric tract have occurred [33–37], and are more common in X-CGD [3]. In the present study, two X-CGD patients experienced inflammatory granulomas, treated with prednisone 1 mg/kg/day, as described in previously-reported cases [33,36–41]. For these patients it was necessary to maintain medication for 3 months due to recurrence of urinary or airway tract granuloma. The pathogenesis of granulomas in CGD remains uncertain.

They may be the result of persistent inflammatory responses due to failure to inactivate chemotactic factors by myeloperoxidase [42] or due to reduced clearance of antigenic material [36]. No infections occurred during glucocorticoid therapy.

Antimicrobial prophylaxis included the regular use of sulfamethoxazole/trimethoprim (all patients) and itraconazole (all but three patients). Only two patients received interferon-gamma therapy, which may be attributed to high cost and practical difficulty of obtaining this medication in Latin America.

BCG immunization is routinely offered to children in Brazil, México, and Chile, and severe complications, primarily disseminated infection, have been reported in CGD patients [5,43–49]. However, in our study none of the patients had a severe complication of BCG. Indeed, in vitro experiments have shown that CGD-neutrophils are not less bactericidal to *M. tuberculosis* compared to normal neutrophils, suggesting that this agent is killed by additional mechanisms other than the respiratory burst [50]. In addition, Denis demonstrated that killing of *M. avium* correlates with nitric oxide, but not with superoxide, production by macrophages [51]. In contrast, Lamhamedi-Cherradi et al. [52], demonstrated that intracellular BCG was not killed by CGD phagocytes,

TABLE I. Mutations in *CYBB* Gene Identified in Latin American CGD Patients

Patient	Mutation	Position	Nucleotide change	Product	Reference
H	Frameshift	Exon 10	c.1267_1268insA	Termination site exon 10	New report
J	Nonsense	Exon 2	c.95 G > A	Termination site W28X	Heyworth et al. [24]
I	Nonsense	Exon 3	c.229 C > T	Termination site R73X	Heyworth et al. [24]
K	Splicing	Exon 3	c.264 G > A	Splicing product exon 3 deletion	Heyworth et al. [24]
F	Splicing	Exon 3	c.264 G > A	Splicing product exon 3 deletion	Heyworth et al. [24]
G	Splicing	Intron 10	c.1326 + 1 G > A	Splicing product exon 10 deletion	Heyworth et al. [24]
L	Splicing	Intron 9	c.1164–2 A > G	Splicing product exon 10 deletion	New report

suggesting an essential role of the respiratory burst for BCG killing in human phagocytes. Most likely, the many genes regulating the immune response, mycobacteria species, and age-related immune development all contribute to the balance between host defense and mycobacterial invasion.

We conclude that the present cohort of Latin American patients with A47-CGD possess the common GT deletion in exon 2 of *NCF1* gene, while in X-CGD we observed a high degree of molecular heterogeneity, consisting of two frameshift, two nonsense and two splice site mutations, including two novel *CYBB* gene mutations. Clinically, the X-CGD patients presented with earlier onset of infections and more frequent inflammatory granulomas. All patients developed infections at natural barrier organs, mainly the lungs and skin. No patient had a severe adverse complication from BCG immunization.

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