

Chronic Granulomatous Disease on Jeju Island, Korea

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Chronic granulomatous disease (CGD) is a rare inherited disorder of a defective NADPH oxidase enzyme, resulting in very low or no production of superoxide and subsequent reactive oxygen species. Consequently, patients with CGD are highly susceptible to severe bacterial and fungal infections. CGD is a genetically heterogeneous disease caused by defects in any one of the genes encoding the NADPH oxidase components. CGD generally affects about 3-4 per 1,000,000 individuals; thus, it is surprising that the prevalence of CGD on Jeju Island is 34.3 per 1,000,000 individuals. At present, 20 patients with CGD from 14 unrelated families on Jeju Island have been identified; nine males and 11 females. All patients with CGD tested on Jeju Island had an identical and homozygous mutation (c.7C>T in *CYBA*, p.Q3X in p22^{phox}). Therefore, all patients were autosomal recessive form of CGD. This strongly suggests that the unique and identical mutation in *CYBA* may be inherited from a common proband. Using mutation-specific primers to detect the mutated allele in *CYBA*, the frequency of subjects carrying a mutated allele was 1.3% of enrolled subjects from Seogwipo City. Further studies are necessary to elucidate how frequently this mutant allele occurs in the population on Jeju Island. Additionally, it is important to construct a national registry system to understand the pathophysiology of CGD and develop a strategy for long-term therapy.

Key words: Chronic granulomatous disease, NADPH oxidase, *CYBA*, p22^{phox}

Introduction

Chronic granulomatous disease (CGD) is a rare inherited disorder of a defective NADPH oxidase enzyme characterized by recurrent bacterial and fungal infections.¹⁾ The functional activity of NADPH oxidase is remarkably diminished or completely absent in patients with CGD, resulting in very low or no production of superoxide and subsequent reactive oxygen species (ROS), which are important for killing invading microorganisms.²⁾

The clinical manifestations of patients with CGD are mainly pneumonia, pyoderma, lymphadenitis, liver abscess, inflammation of the gastrointestinal tract, and osteomyelitis. Infections are primarily due to bacteria such as *Staphylococcus aureus*, *Escheri-*

chia coli, non-typhoid *Salmonella* species, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Burkholderia cepacia*, as well as the fungal genus *Aspergillus*.^{1,3-6)} Isolation of the causative pathogens is essential for appropriate antimicrobial therapy, but it is difficult to identify the causative pathogen in most clinical CGD cases.^{5,6)}

NADPH oxidase is a multi-component complex composed of a plasma membrane-bound flavocytochrome b₅₅₈ subunit and cytosolic proteins p47^{phox} (*phox* for *phagocytic oxidase*), p67^{phox}, p40^{phox}, and two small GTPase, Rac2 and Rap1A.⁶⁾ The flavocytochrome b₅₅₈ subunit is a heterodimer comprised of gp91^{phox} (also called NOX2, NOX for NADPH oxidase) and p22^{phox} encoded by *CYBB* and *CYBA* respectively, whereas the three components p47^{phox}, p67^{phox}, and p40^{phox} of the cytosolic subunit are encoded

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by *NCF1*, *NCF2*, and *NCF4*, respectively.²⁾

The X-linked form of CGD is caused by *CYBB* mutations and accounts for approximately 70% of cases. The autosomal recessive forms are caused by mutations in *CYBA*, *NCF1*, and *NCF2*, accounting for about 5%, 20%, and 5% of cases, respectively.⁷⁻⁹⁾ A mutation in only *NCF4* has been reported.¹⁰⁾

According to a survey by the Korean College of Pediatric Clinical Immunology, most regions of Korea have a similar prevalence of CGD, but that of Jeju Island is highest.¹¹⁾ This review describes the current characteristics of CGD on Jeju Island and future research necessary to resolve the problem.

Characteristics of patients with CGD on Jeju Island

1. Epidemiology

The incidence of CGD is about 3–4 per 1,000,000 individuals.²⁾ The prevalence of CGD in European studies is 2–5 per 1,000,000 individuals⁴⁾ and the prevalence of CGD in Korea is 3.4 per 1,000,000 individuals.¹¹⁾ The variability in the incidence of CGD is related to ethnic heterogeneity.

The prevalence of CGD on Jeju Island has been increasing gradually. In the first survey of CGD in Korea,¹²⁾ seven patients with CGD (total of 33 patients) on Jeju Island were reported, a prevalence of 13.0 per 1,000,000 individuals (statistics from Jeju Special Self-Governing Province, 1999). The prevalence of CGD on Jeju Island in 2005 was 20.7 per 1,000,000 individuals, which was ~10–50 times higher than that in other regions of Korea.¹¹⁾

At present, 20 patients with CGD from 14 unrelated families on Jeju Island have been identified; five were from Jeju City and 15 from Seogwipo City. There were nine males and 11 females, and five families (36%) had multiple affected siblings. Therefore, the overall prevalence of CGD on Jeju Island is 34.3 per 1,000,000 individuals, with a prevalence of 11.7 per 1,000,000 individuals in Jeju City, and a surprising 96.3 per 1,000,000 individuals in Seogwipo City (statistics from Jeju Special Self-Governing Province, 2011).

We hypothesized that the high prevalence of CGD on Jeju Island is associated with the same mutation inherited from a common ancestor because there is an increase in the prevalence of autosomal recessive disorders in regions with a higher incidence of consanguinity in the population. As Jeju Island has long been geologically isolated, unique marriage patterns similar to consanguineous marriage may have occurred.¹³⁾

Data from other regions and ethnic groups may deviate from the actual prevalence. According to studies carried out to date,

the most common form of CGD is X-linked, which has a different, more severe, clinical phenotype than the autosomal recessive form of CGD.³⁾ Therefore, many patients with X-linked CGD may not have been included due to early death. Hence, the different distribution of CGD forms on Jeju Island may be attributed to its high prevalence.

We attempted to identify as many patients as possible, but we may have underestimated the actual prevalence of CGD on Jeju Island because this was a single-center study. The clinical manifestations of CGD in residents of Jeju Island seem to be mild in that 30% of living patients are ≥ 20 years of age.¹³⁾ Therefore, some CGD patients may not have been included in this study because they have not attended a tertiary hospital as their symptoms are mild.

2. Diagnosis and Genotype

The dihydrorhodamine-1,2,3 (DHR) flow cytometric assay is effective for diagnosing CGD, and the patterns of the assay correlate with the molecular defect identified.^{14,15)} However, the various molecular defects are difficult to discriminate by measuring NADPH oxidase activity in neutrophils.

In our study, most CGD patients on Jeju Island showed an almost undetectable level of ROS production, and their parents with a normal phenotype showed slightly impaired ROS production compared with that of healthy controls (Fig. 1).¹³⁾ The extent of ROS impairment in mothers of patients with CGD on Jeju Island was not as great as that of mothers of patients with X-linked CGD.^{14,15)} Similar patterns in the DHR assay indicate that the absence of p22^{phox} results in a significantly reduced level of respiratory burst activity, whereas reduced but detectable levels of respiratory burst activity are observed in patients with CGD with a diminished level of p22^{phox}.¹⁵⁾

More than two thirds of all cases of CGD are X-linked recessive and result from defects in gp91^{phox} subunit; the remaining cases are autosomal recessive and caused by defects in p22^{phox}, p67^{phox}, p47^{phox}, and p40^{phox} subunits, respectively.²⁾ Western blot analyses demonstrated that expression of p67^{phox}, p47^{phox}, and p40^{phox} was normal, but expression of gp91^{phox} and p22^{phox} was absent in patients with CGD on Jeju Island (Fig. 2).¹³⁾ Patients with gp91^{phox}-deficient CGD did not have detectable p22^{phox} within phagocytes, and patients with p22^{phox}-deficient CGD did not have detectable gp91^{phox} protein.¹⁶⁾ In other reports of p22^{phox}-deficient CGD,^{17,18)} gp91^{phox} expression was not detected in all patients with p22^{phox}-deficient CGD, suggesting that p22^{phox} and gp91^{phox} require each other for mutual stability and are essential for superoxide generation by the NADPH oxidase system.^{19,20)} Female patients with CGD

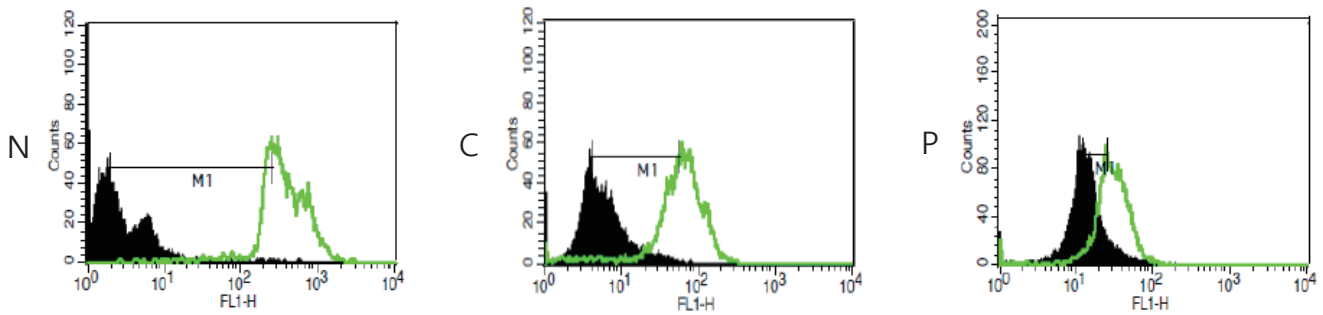


Fig. 1. Dihydrorhodamine-1,2,3 (DHR) flow cytometric analysis of granulocyte oxidative activity with phorbol myristic acid (PMA) stimulation. The flow cytometry results of two reactions are shown overlapping: before PMA stimulation (black) and after PMA stimulation (green). A slightly decreased response after PMA stimulation was found in a parent of a patient with chronic granulomatous disease (CGD) compared with that in a healthy control. A lack of phagocyte oxidative activity after stimulation with PMA was found in a patient with CGD. N, healthy control; C, parent of $p22^{phox}$ -deficient CGD patient; P, $p22^{phox}$ -deficient CGD patient.

who are deficient in flavocytochrome b_{558} likely have a primary defect in *CYBA*.¹⁷⁾ We conducted mutation analysis of *CYBB* was screened by single-stranded conformation polymorphism (SSCP), but all seven male patients with CGD on Jeju Island did not show any abnormal migrating band in SSCP analysis. Sequencing of 5' flanking region and all 13 exons in *CYBA* also did not show any mutation in *CYBA*.

Collectively, we ruled out the possibility that the inheritance of CGD on Jeju Island is X-linked or some other autosomal recessive form of CGD. All patients with CGD on Jeju Island were presumed to be the $A22^0$ (A indicates autosomal recessive, superscript ⁰ indicates complete absence of the affected subunit) phenotype.

Sequencing of *CYBA* genomic DNA revealed that all patients with CGD on Jeju Island have a single-base substitution of C to T in *CYBA* exon 1 (c.7C>T), which was expected to result in a nonsense mutation (p.Q3X) in $p22^{phox}$. The genomic DNA sequencing results of the parents showed a double signal (c.7C/T) at the same position, indicating that they were heterozygous for the *CYBA* mutation (Fig. 3).¹³⁾

All patients with CGD tested on Jeju Island had an identical and homozygous mutation in *CYBA*. This mutation has been reported previously in Japanese patients.¹⁷⁾ In addition, six different single nucleotide polymorphic substitutions within *CYBA* were detected in all patients in the same manner.¹³⁾ These results strongly suggest that a unique and identical mutation in *CYBA* may be inherited from a common proband.

3. Identification of subjects carrying the *CYBA* mutated allele

We developed a highly sensitive assay using mutation-specific primers to detect the unique mutated allele (c.7T) of *CYBA*. Samples

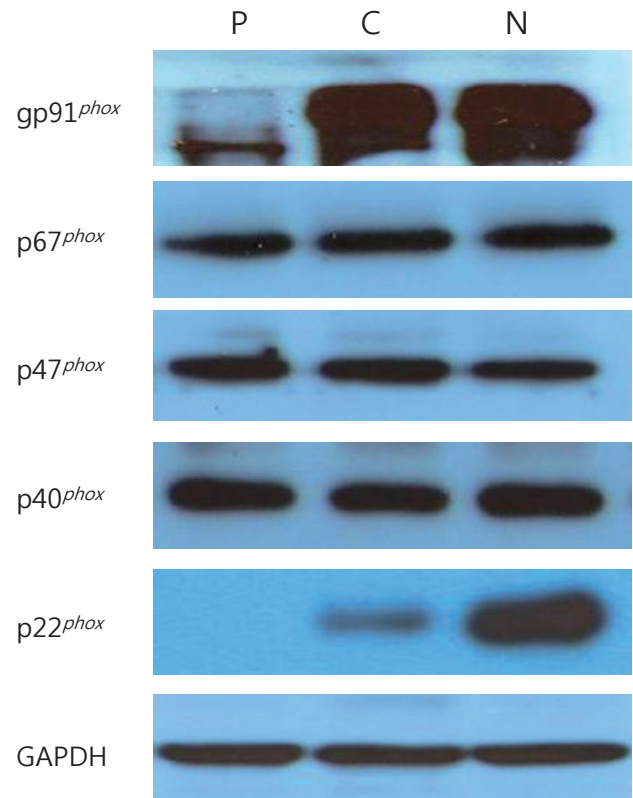


Fig. 2. Western blot analysis of NADPH oxidase components in a chronic granulomatous disease (CGD) family. Western blot analysis demonstrates the absence of $p22^{phox}$ and $gp91^{phox}$ expression in a patient with CGD, but that expression of $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ was normal. No abnormal expression of any NADPH oxidase component was observed in the parent of the $p22^{phox}$ -deficient patient with CGD. N, healthy control; C, parent of $p22^{phox}$ -deficient CGD patient; P, $p22^{phox}$ -deficient CGD patient.

were screened by nested polymerase chain reaction (PCR), and positive samples were subjected to DNA sequencing. All positive samples detected by nested PCR contained one mutated allele (c.7C/T) (Fig. 4).²¹⁾

We investigated the frequency of subjects carrying the unique

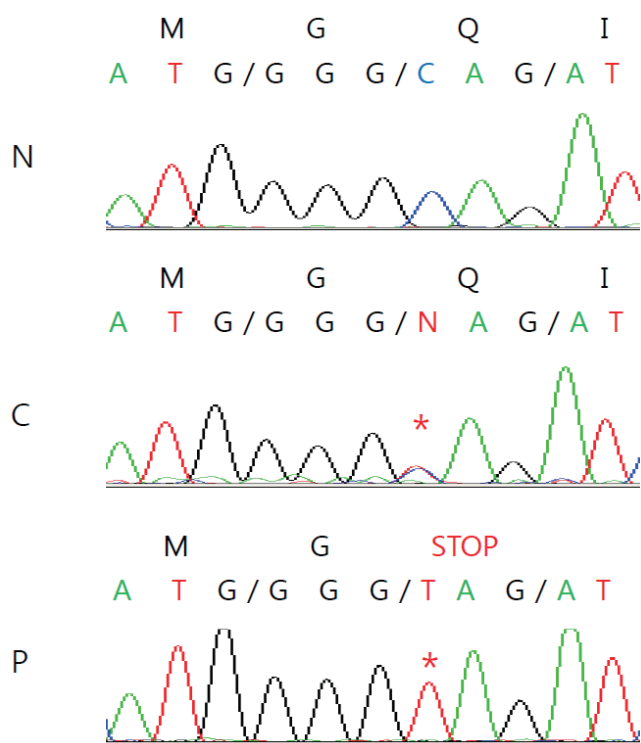


Fig. 3. Sequence analysis of the *CYBA* gene from a family with chronic granulomatous disease (CGD). Patients have a single base substitution of C to T in exon 1 (c.7C>T), which was expected to result in a nonsense mutation (p.Q3X). The sequencing result for the parent of the p22^{phox}-deficient patient with CGD showed a double signal at the same position, indicating that they were heterozygous for the mutation. N, healthy control; C, parent of p22^{phox}-deficient CGD patient; P, p22^{phox}-deficient CGD patient. *site of mutation.

mutated allele in *CYBA* (in preparation). Circa 700 subjects from Seogwipo City (~0.5% of the population of Seogwipo City) were enrolled, 1.3% of whom had the *CYBA* mutated allele. A significant difference was observed between the expected and calculated numbers in the population of Seogwipo City (~0.02% of the population) by Hardy-Weinberg equilibrium. However, this was a pilot study that had several limitations such as small sample size, sampling bias, and sequencing a part of samples for test the specificity of mutation-specific primers. Further studies are necessary to elucidate the frequency at which this mutant allele occurs in the population of Jeju Island.

Future study

1. Relationship of p22^{phox} with the p22^{phox}-deficient CGD phenotype

NADPH oxidase is expressed only in phagocytes. Recently, various homologues of the catalytic subunit gp91^{phox} were identified, including NOX1, NOX3, NOX4, NOX5, DUOX1, and DUOX2. These homologues are referred to as the NOX family of NADPH oxidase.^{22,23} The physiological functions of the NOX family include host defense, post-translational processing of proteins, cellular signaling, regulation of gene expression, and cell differentiation.²²

All NOX family proteins, except NOX5, require p22^{phox}.²³ The

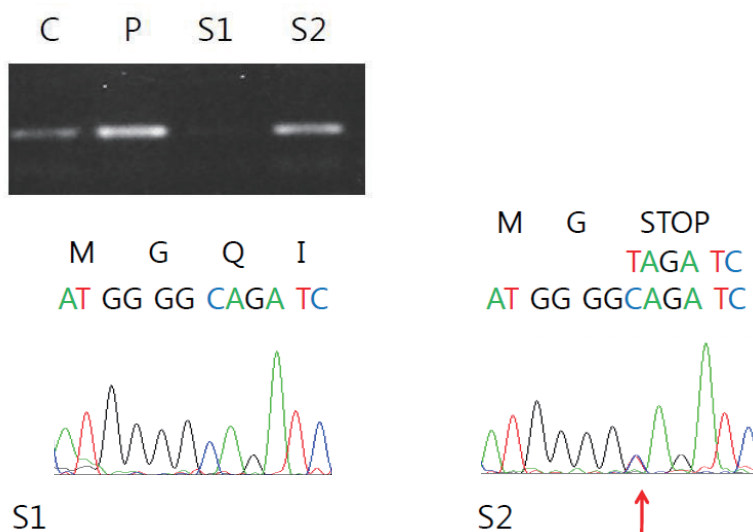


Fig. 4. Detection of subjects carrying the mutated allele in the *CYBA* gene. All subjects were screened by nested polymerase chain reaction (PCR), and positive samples were subjected to DNA sequencing. All positive samples detected by nested PCR contained one mutated allele by sequencing. Subject with the mutated allele showed a double signal at the position (c.7C/T). C, known parent of p22^{phox}-deficient CGD patient; P, p22^{phox}-deficient CGD patient; S1, subject without the mutated allele; S2, subject with the mutated allele.

NOX proteins and p22^{phox} are stable only as a heterodimer, as monomers are degraded by proteosomes.²²⁾ In line with this, NOX2 (gp91^{phox})-deficient patients with CGD do not have detectable p22^{phox} protein within phagocytes, and p22^{phox}-deficient patients with CGD do not have detectable NOX2 protein.¹⁶⁾ Several studies have investigated the subcellular distribution of p22^{phox}; p22^{phox} co-localizes with NOX2 in phagocytes, NOX2 in coronary endothelial cells, NOX1 in vascular smooth muscle cells, and with NOX1 and NOX4 in transfected cells.²²⁾

Considering the data on the role of p22^{phox} in the NOX family, the prognosis of p22^{phox}-deficient patients with CGD may not be better than that of those with X-linked CGD. A few reports have demonstrated that p22^{phox}-deficient CGD with the A22⁰ notation is of severity similar to that of X-linked CGD.^{24,25)} Of the 55 different mutations in p22^{phox}, only one family with A22⁺ has been described (superscript⁺ indicates normal expression of the mutated subunit).²⁶⁾ However, patients with CGD on Jeju Island did not show obviously different clinical manifestations from X-linked CGD or other autosomal recessive forms of CGD; the mortality rate was 25% at the 10-year follow up, and 30% of the living patients are \geq 20 years of age. This may explain that the number of patients is so small, the duration of follow-up was relatively short, and tools for evaluation of prognosis and phenotype are limited. Further studies are necessary to elucidate whether p22^{phox} is indispensable in vivo for the other NOX proteins (except NOX2) and why Jeju Island CGD patients have a different prognosis than p22^{phox}-deficient CGD patients in other regions and of other ethnicities.

2. Frequency of the unique *CYBA* mutated allele in the population of Jeju Island

We described previously that the prevalence of CGD on Jeju Island is increasing gradually and may be associated with the same mutation inherited from a common ancestor. This is because the prevalence of autosomal recessive disorders is elevated in regions with a higher incidence of consanguinity in the population. However, none of the parents of CGD patients on Jeju Island are related. Regarding the possibility that the nonsense mutation (c.7C>T, p.Q3X) in p22^{phox} may be a hot-spot or a common mutation in the population, we investigated the frequency of subjects carrying the mutated allele in Seogwipo City; 1.3% of enrolled subjects possessed the mutated *CYBA* allele. Because the frequency of subjects carrying a particular mutated allele in an as geographically separated region as Jeju Island has not been reported, the figure is impossible to estimate. However, the carriage rate is as high as that of other high-risk genetic diseases that require prenatal

diagnosis. Further study is necessary to elucidate the frequency at which this mutant allele occurs in the population of Jeju Island. Our findings suggest that early diagnosis facilitates treatment of patients with CGD, and that introduction of preventive measures will reduce the prevalence of CGD.

3. Necessity for a national registry system for primary immunodeficiency (PID) or CGD

Unlike the situation in North America and Western Europe, p22^{phox}-deficient CGD is the predominant autosomal recessive form in Japan; p22^{phox}-deficient, p67^{phox}-deficient, and p47^{phox}-deficient CGD account for 8.7%, 6.6%, and 6% of the total, respectively.²⁷⁾ We presume that the distribution of CGD forms in Korea may be similar to that of Japan.

The first epidemiological study based on the national PID registry in Korea was performed in 2005. Patient data were collected from 23 major hospitals. Among 152 patients with PID, 44 cases (28.9% of PID) of CGD were reported in this registry.¹¹⁾ Because these data did not include a mutation-based diagnosis, it is difficult to evaluate the distribution of the CGD forms in Korea. Only two studies have reported a genetic analysis of X-linked CGD in Korea.^{28,29)} Hence, construction of a national registry system for CGD is necessary to understand the pathophysiology of CGD and develop a long-term therapeutic strategy.

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