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Molecular Identification of Bacterial DNA in the Chorioretinal Scars of Chronic Granulomatous Disease

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Abstract

Purpose—Chronic granulomatous disease (CGD) is an inherited disorder characterized by defects in phagocyte-derived nicotinamide adenine dinucleotide phosphate oxidase. It is typically diagnosed in childhood and leads to severe, recurrent bacterial or fungal infections. Chorioretinal lesions are the most common ocular manifestation. We sought to determine whether there are infectious agents in CGD-associated chorioretinopathy.

Methods—Medical records and ocular histopathology from CGD cases from January 1983 to January 2012 at the National Institutes of Health were retrospectively reviewed. Chorioretinal cells from normal and lesional tissues of the same eye were microdissected. Primers for *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia sp.*, and a panbacterial 16S ribosomal DNA were used for polymerase chain reaction.

Results—Seventeen CGD patients had ocular tissues (16 autopsied cases and 1 chorioretinal biopsy) examined. Of these 17, 8 demonstrated CGD-associated chorioretinal lesions in at least one eye on histopathology. Of these 8, 7 showed amplification of 16S ribosomal DNA within the lesion; of these 7, two also amplified *S. epidermidis* and one *P. aeruginosa*. One had no bacterial DNA amplified. Importantly, no microbial DNA was amplified from the normal, non-lesional ocular tissues of these 8 cases. Furthermore, only 1 of the 9 eyes without chorioretinopathy had amplified *Burkholderia* DNA, that patient had a history of *Burkholderia* infection.

Conclusions—We detected bacterial DNA in 7 of 8 (88 %) cases with CGD-associated chorioretinopathy and only in 1 normal ocular tissue of 17 CGD cases. Bacterial infection may play a role in the pathogenesis of CGD-associated chorioretinal lesions.

Keywords

Chronic granulomatous disease; chorioretinitis; bacterial infection; polymerase chain reaction

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Conflict of interest

The authors declare that they have no conflict of interest.

Introduction

Chronic granulomatous disease (CGD) is a rare genetic disorder characterized by defective phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity; it occurs in about 1:200,000 live births in the United States [1]. Most CGD patients are diagnosed in childhood, typically before age 5 [1]. It was originally named “a fatal granulomatous disease of childhood” in the 1950s [2, 3]. Due to impaired production of superoxide and related reactive oxygen intermediates by phagocyte-derived NADPH oxidase, CGD patients are susceptible to recurrent life-threatening infections by a relatively narrow spectrum of bacteria and fungi, including *Staphylococcus aureus*, *Burkholderia cepacia* (formerly *Pseudomonas cepacia*), *Serratia marcescens*, *Nocardia* species, and *Aspergillus* species [4, 5]. Although mutations in any of the five structural genes encoding the subunits of the phagocyte NADPH oxidase cause CGD, those caused by defective gp91^{phox} (*CYBB*), account for 65% of CGD cases. The rest are autosomal recessive caused by defects in p47^{phox} (*NCF1*, 25%), p67^{phox} (*NCF2*, <5%), p22^{phox} (*CYBA*, <5%), or p40^{phox} (*NCF4*, one case) [6–10]. The autosomal recessive forms of CGD usually have a milder course and later onset than the X-linked form [1, 11].

The most frequent sites of CGD-associated infection are the lung, skin, lymph nodes, and liver. Ocular lesions are less frequent and have been reported as conjunctivitis, keratitis, uveitis, chorioretinal lesions, and retinal/subretinal masses [12–23]. Among ocular presentations, sharply demarcated chorioretinal lesions are the most commonly reported, with a wide range of incidence from 12.5% to 100 % [18, 20, 21, 23]. However, in a more recent and larger scale study by van den Berg et al., symptomatic chorioretinitis was found only in 8 of 429 (1.9 %) CGD patients [11]. Pathological features of post-mortem CGD cases include extensive chorioretinal scars and choroidal inflammatory cellular infiltration [24, 25]. Other histopathological studies found inflammatory cells in the retina and/or choroid [16, 22, 26]. We have reported bacterial DNA from chorioretinal lesions in the eye of a patient with CGD [27]. In this study, we retrospectively reviewed the clinical and histopathological records of all CGD patients with ocular pathology at the National Institutes of Health over the past 29 years for evidence of bacteria in the CGD-associated chorioretinal lesions.

Methods

Patients and Specimens

This study was approved by the National Eye Institute Institutional Review Board for human subjects and was performed in accordance with the 1964 Declaration of Helsinki and its later amendments. Archival ocular specimens from 17 CGD patients (31 autopsied eyes and 1 chorioretinal biopsy) followed at the National Institutes of Health from January 1983 to January 2012 were reviewed retrospectively. All patients had the diagnosis of CGD confirmed by either pathologic nitroblue tetrazolium reduction or dihydrorhodamine oxidation. The specific genetic defect was determined by immunoblotting, gene sequencing, or both.

The following information was collected: demographics (age and gender), specific gene defect, history of systemic infections, history of infectious microorganisms, ocular examination, ocular histopathological features, other systemic findings, corticosteroid treatment, and history of bone marrow transplantation.

The 31 autopsied eyes (16 cases, 1 case had only 1 eye for autopsy) underwent macroscopic and microscopic evaluation. Each eye was cut through the macula along the papillary-optic nerve head plane. In cases of chorioretinal lesions, a segment through the lesion was

obtained. The grossed eyes were then processed for paraffin embedding and routine sectioning, as was the biopsied chorioretinal tissue [16]. Slides were stained with hematoxylin & eosin, Periodic acid-Schiff, and/or Brown and Brenn (Gram).

Two autopsied cases with Cobblestone degeneration and without CGD were included as negative controls.

Microdissection and Polymerase Chain Reaction

Microdissection was performed manually on uncovered, stained glass slides. The slides were prepared from formalin-fixed, paraffin-embedded tissue cut at 4–6 μm , and then stored at room temperature [28–30]. Equal numbers of cells from the chorioretinal scars and normal chorioretinal tissues of the same specimen were microdissected. Additionally, cells from the chorioretinal scars in the 2 eyes with cobblestone degeneration were only microdissected and served as negative controls.

The microdissected cells were immediately digested with Proteinase K buffer. Polymerase chain reaction (PCR) followed by gel electrophoresis was used to detect the amplification of bacterial DNA. The oligonucleotide primers were synthesized by Integrated DNA Technologies, Inc. (IA, USA). Primers for *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *B. pseudomallei*, *B. mallei*, *B. thailandensis*, *B. cepacia*, and bacterial 16S ribosomal DNA (rDNA) were ^{32}P labeled and used for PCR amplification (Table 1). Bacterial 16S rDNA primers were tested on the 17 CGD cases (Table 2) and the 2 cobblestone degeneration cases. Furthermore, ocular tissues of the patients with a clinical history of either *Staphylococcus* or *Burkholderia* infection were also tested with primers for *S. aureus*, *B. pseudomallei*, *B. mallei*, *B. thailandensis*, and *B. cepacia*, accordingly. Specifically, ocular DNA from Patients #2, 8, 15 and 16, who had a clinical history of *Burkholderia* infection, was amplified with primers for *B. pseudomallei*, *B. mallei*, *B. thailandensis* (SCU2), and *B. cepacia* (PSR); ocular DNA from Patients #2, 3, 5, 10, 11, 13 and 17, who had clinical histories of *S. aureus* infection, was amplified with primer (SA). In addition, ocular tissues from 12 cases (Patients #2, 3, 5–8, 10, 11, 13 and 15–17) with a clinical history of *Staphylococcus* and/or *Burkholderia* infections were also subjected to additional PCR amplification. All 12 cases were tested using primers for *S. epidermidis* (icaA-1) and *P. aeruginosa* (PS21). NCBI/BLAST nucleotide database shows that primers used to test for *S. epidermidis* (icaA-1) also amplify *S. haemolyticus*. Primers used for *P. aeruginosa* (PS21) also amplify *Acidovorax ebreus*.

PCR amplification was performed in a Peltier thermal cycler PTC 200 (MJ research, Inc., Watertown, MA, USA) in a 10 μl -volume Eppendorf tube, containing 2 μl of extracted sample DNA, 1 μl of 10 $\times$ Taq buffer, 1 unit of Taq DNA polymerase, 1 μl of MgCl_2 (25 mM), 2 μl of a four-deoxynucleoside triphosphate mix (10 mM each), and 2 μl of each primer (sense and antisense). The PCR amplification protocol was based on a previous publication with some modifications [28–30]. Initial denaturation was performed at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at a temperature which was dependent on the primer for 1 minute and extension at 72°C for 1 minute. The final extension was at 72°C for 10 minutes, after which the products were cooled to 4°C before they were removed. The amplification products were analyzed by 15% polyacrylamide gel electrophoresis in TBE buffer. Following gel electrophoresis and overnight autoradiography, the PCR products were visualized using a molecular imager (Bio-Rad Laboratories, CA, USA).

Statistical Analysis

Demographic data were compared using the Student's t-test. Categorical data (e.g. genotype, ocular phenotype) were compared using the Fisher exact test. The analysis was performed using the statistical software "GraphPad Prism 5" (GraphPad Software, CA, USA). A *p* value less than 0.05 was considered statistically significant.

Results

All 17 patients were male, 15 (88%) had X-linked CGD and 2 (12%) had AR CGD. The detailed medical information of each patient is listed in Table 2. All patients had a history of multiple infections in various organs, with a variety of microorganisms isolated. The median age of CGD diagnosis was 9 months (range: 2 months–8 years) and the median age of death was 20 years (range: 16–50 years). There was a significantly higher incidence of chorioretinopathy in patients who had undergone bone marrow transplantation (Table 3). No significant differences were found in age of CGD diagnosis, genotype, or chronic corticosteroid use between patients with and without chorioretinopathy (Table 3).

Of the 17 CGD patients studied, 5 had positive clinical ophthalmic histories (Table 4): three (Patients #1, 2 and 4) had recorded active chorioretinopathy (uveitis or episcleritis) in addition to chorioretinal scars; two (Patients #7 and 8) had inactive chorioretinal scars only (Fig. 1). Two patients (Patients #5 and 6) had ocular examination without chorioretinal lesions (Table 4). The median age at diagnosis of chorioretinal scars was 8 years (range: 4–18 years).

During macroscopic evaluation of the 17 cases (31 autopsied eyes and 1 chorioretinal biopsy), 8 cases comprised of 10 specimens (9 eyes and 1 biopsy) had chorioretinal lesions. Of those cases, 4 had bilateral involvement (Patients #1, 2, 4 and 8) and 4 unilateral. All 10 eyes (Patients #1–8) with typical macroscopic ocular CGD lesions had CGD-associated chorioretinopathy by microscopic evaluation (Table 4). The chorioretinal biopsy case (Patient #1) showed lymphoplasmacytic inflammatory cells and vascular inflammatory granulation [27]. The other 9 autopsied eyes of the 7 cases (Patients #2–8) had well demarcated chorioretinal scars of atrophic retina and abnormal retinal pigment epithelial cells (Fig. 2a), some of which had chronic inflammatory cells in the choroid (Fig. 2b). Gram stain was negative in all cases.

In the 8 cases with chorioretinal lesions, 7 had focal mild to moderate inflammatory infiltration of lymphocytes and macrophages. The infiltrated tissues were microdissected and subjected to PCR amplification. All 7 (Patients #1–7) had amplification of 16S rDNA, a region of prokaryotic DNA found in all bacteria and archaea (Fig. 3). In addition, two cases (Patients #3 and 6) were positive for *S. epidermidis* and one case (Patient #2) was positive for *P. aeruginosa*. Due to the limited quantity and quality of microdissected material from paraffin-embedded archived slides, bacterial sequence analysis could not be performed. No microbial DNA was detected in the normal ocular tissues of any of these 8 cases. In the other 9 autopsied CGD cases without chorioretinal lesions, 1 case (Patient #15) had *Burkholderia* DNA amplified from ocular tissue using primer pairs of SCU. No microbial DNA was detected in the cobblestone degeneration tissue of the 2 cases without CGD. The odds ratio for detecting bacterial genes in cases with CGD-associated chorioretinopathy was 112 times higher than that without chorioretinopathy (95% confidence interval = 6–2059, *p* = 0.0001).

Discussion

We analyzed the ocular histopathology of 17 CGD patients, and examined the relationship between CGD-associated chorioretinopathy and presence of microbial DNA. CGD-associated chorioretinopathy typically manifests as chorioretinal scarring or proliferative (infectious/inflammatory) lesions. Microdissection and PCR of the chorioretinal lesions revealed bacterial DNA in 88% (7/8) of patients with chorioretinal lesions. By contrast, bacterial DNA was not detected in the uninvolved areas of affected eyes, and was identified in only 1 of 9 normal ocular cases. This yields a specificity of 92% (16/17), suggesting that our positive DNA samples are unlikely to be due to field contamination.

Although CGD is a rare, inherited, primary immune deficiency disorder, the infections in CGD may involve any organ of the body, including the eye. Chorioretinal lesions have been reported as the most common presentation of CGD-associated ocular disease [18, 20, 23]. Typically, fundus examination reveals multiple unilateral or bilateral atrophic chorioretinal scars, in addition to pigment clumping and occasional optic nerve pallor [19, 23]. In our case series, 47% (8/17) of autopsied or biopsied CGD patients had chorioretinal scars or active inflammation in at least one eye. Although most CGD-associated chorioretinal lesions have been reported in X-linked CGD patients [20], we found similar chorioretinal lesions in both X-linked (7/15) and autosomal recessive (1/2) CGD patients.

Bacterial DNA was identified within the chorioretinal lesions and not detected in the normal areas of the affected eyes. Meanwhile, 1 of the 9 normal cases showed amplification of *Burkholderia* DNA. However, no bacterial DNA was detected in the non-infectious chorioretinal scars of the cases with cobblestone degeneration. Bacterial infection could be one of the inciting causes of CGD-associated chorioretinal scarring, resulting in bare sclerae with total loss of retinal and most choroidal tissue. In normal situation, the eye is protected by the blood–ocular barrier and resistant to microorganism infection. However, when the barrier is disrupted during infection and inflammation, bacteria pass through the ocular blood flow and cause intraocular infection and tissue damage. Because the phagocyte-derived NADPH oxidase is impaired in CGD patients, infection of the choroid and retina might lead to chronic inflammation and scarring. The organisms may initiate an inflammatory response and ultimately result in scar formation when healing occurs. It remains perplexing that despite the fact that these lesions (except Patient #1) appeared clinically inactive and stable for many years, and that the patients had received broad spectrum antibiotics and aggressive immune suppression, bacterial DNA was identified in those areas with microscopic low-grade chronic inflammation. On the one hand, it is plausible that bacterial infection may initiate CGD chorioretinitis but that it is eventually controlled clinically, without obvious benefit from directed antibiotics, and in spite of immune suppression. On the other hand, it is possible that the bacteria did not directly cause the ocular lesions, but rather their DNA was incidentally engulfed into a non-infectious inflammatory mass. Moreover, the detection of bacterial DNA in the normal eye of one CGD patient may represent different stages of disease progression. It could either represent a stage of bacterial dissemination inside the blood vessel only, without ocular lesions yet in the choroid and retina, or an early stage of bacterial infection prior to the formation of ocular morphological changes. Therefore, it could be possible to identify bacterial footprints in normal ocular tissue of a CGD patient who suffers from severe bacterial infection (Patient #15).

Most CGD-associated chorioretinal lesions are clinically inactive and stable and do not affect visual acuity [19, 23, 24]. However, when an intraocular lesion is severe, involves the macula, or causes hemorrhage or retinal detachment, patients may experience visual loss. Kim et al. reported two CGD cases with progressive and vision-threatening complications:

one with extensive vitreous hemorrhage, and the other with inflammatory chorioretinal lesions [18]. Matsuura et al. described a case of chronic granulomatous uveitis in which good vision was maintained despite relapses of intraocular inflammation [17]. Additionally, Valluri et al. documented two cases of progressive chorioretinal lesions with extensive granulomatous inflammation of the choroid and sclerae [26]. We found CGD patients with both p47^{phox} deficient (Patient #1) and X-linked (Patient #2) forms of CGD with recurrent uveitis in addition to inactive chorioretinal scars. Active uveitis might be due to intraocular microbial infection coexisting with inactive chorioretinal scars. Our odds ratio of 112 (confidence interval: 6–2059) for bacterial DNA in chorioretinal scars suggests that bacterial infection is highly associated with CGD chorioretinopathy.

Conclusions

Patients with CGD should undergo regular ophthalmic examinations to rule out potentially vision-threatening ocular involvement. Considering the presence of bacterial DNA in CGD-associated chorioretinal lesions, antibiotics could be added in treatment of patients with active intraocular inflammation, whereas for patients with chronic chorioretinal scars, close follow-up by an ophthalmologist is highly recommended. The potential role of antibiotics and anti-inflammatory treatment, whether it might shorten the active stage of intraocular inflammation, or further reduce the low incidence of vision-threatening complications, remains to be prospectively studied. Moreover, when CGD patients with active intraocular inflammation that masquerades as malignancy do not respond to antimicrobial and/or anti-inflammatory therapies, biopsy of the lesion(s) should be considered. In conclusion, chorioretinopathy is relatively common in CGD and may be due to direct infection leading to recurrent uveitis or inactive chorioretinal scarring.

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Fig. 1. Fundus photograph of chronic granulomatous disease-associated chorioretinal lesions (Patient 2). Two discrete yellowish-white inactive chorioretinal scars are present in the inferior temporal midperipheral retina of the right eye. Hyperpigmentation is noted in some areas of the scar margins

Fig. 2. Photomicrograph of chronic granulomatous disease-associated chorioretinopathy (R, retina; C, choroid). **a**, Patient 5, Abrupt chorioretinal scars (asterisk) with an atrophic retina (R) is noted. **b**, Patient 6, Mild chronic inflammatory infiltrates (asterisk) in the choroid (C) are observed (stain, hematoxylin and eosin, original magnification, A. 100, B. 200)

Fig. 3.

Polymerase chain reaction of 16S ribosomal DNA (rDNA) in chronic granulomatous disease (CGD)-associated chorioretinal scars (Patient 7). Using a 16S rDNA primer pair, bacterial 16S rDNA is detected in the cells within the chorioretinal scar (lane 3) of the eye with CGD-associated chorioretinopathy, but is not detected in the normal retina (lane 2). No bacterial 16S rDNA is detected in the normal retina of the contralateral eye (lane 1) using the same primer pair (N, negative control; P, positive control)

Table 1

Oligonucleotide Primers Information

PCR primer	Primer pair	Sequences (5' to 3')	Annealing temp (°C)	Reaction cycles
<i>Staphylococcus epidermidis</i> (<i>icaA-1</i> gene)	icaA-1 forward	GGA AGT TCT GAT AAT ACT GCT G	56	40
	icaA-1 reverse	GAT GCT TGT TTG ATT CCC TC		
<i>Staphylococcus aureus</i> (<i>nuc</i> gene)	SAF	GCG ATT GAT GGT GAT ACG GTT	55	40
	SAR	AGC CAA GCC TTG ACG AAC TAA AGC		
<i>Pseudomonas aeruginosa</i> (<i>PS21</i> gene)	PS21.fwd	AAG CAG GCC AGC GTG TCT A	61	40
	PS21.rev	AAA ACG TAG CAA GCA GTG		
<i>Burkholderia pseudomallei</i> , <i>Burkholderia mallei</i> , <i>Burkholderia thailandensis</i> (<i>BpSCU2</i> gene)	SCU2F	CTC GAG CTC GTG AAG ATG AT	60	45
	SCU2R	ACG CGT GCG ATC TTG TAA TC		
<i>Burkholderia cepacia</i> (<i>16S rRNA</i> gene)	PSR1	TTT CGA GCA CTC CCG CCT CTC AG	57	40
	PSR2	AAC TAG TTG TTG GGG ATT CAT TTC		
Bacterial 16S rDNA	27F	AGA GTT TGA TCC TGG CTC AG	54.5	60
	907R	CCC CGT CAA TTC ATT TGA GTT T		

PCR Polymerase chain reaction; rDNA ribosomal DNA

Table 2

Clinical Features of 17 Patients with Chronic Granulomatous Disease

Patient	Age at death (years)	Age at CGD diagnosis	Gene affected	Infection history	Microorganism history	Other systemic CGD manifestations	Steroids chronic use	BMT	Reference
1 ^a	19	8 years	p47 ^{phox}	Skin, Soft tissue	<i>Serratia marcescens</i>	No	Yes	No	[31]
2	18	2 years	gp91 ^{phox}	Liver abscess, Multiple Pneumonias	MRSA, <i>Aspergillus</i> , <i>Burkholderia</i>	Granulomatous colitis with fistulae	Yes	No	[32, 33]
3	16	7 months	gp91 ^{phox}	Liver abscess, Multiple pneumonias, Osteomyelitis	MRSA, <i>Aspergillus tanneri</i>	No	No	No	[31]
4	26	6 months	gp91 ^{phox}	Multiple pneumonias, Osteomyelitis, Orbital cellulitis	<i>Aspergillus</i> , <i>Serratia marcescens</i> , <i>Nocardia</i>	β-thalassemia minor, Recurrent pancreatitis, End-stage renal disease	Yes	No	[31]
5	20	2 months	gp91 ^{phox}	Recurrent lymphadenitis, Osteomyelitis, Liver abscess, Pneumonia	<i>Aspergillus nidulans</i> , MRSA	Gastrointestinal involvement	Yes	No	[31, 33, 34]
6	50	3 years	gp91 ^{phox}	Multiple pneumonias	<i>Aspergillus fumigatus</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>	Chronic obstructive pulmonary disease, Esophageal strictures, Portal hypertension	Yes	No	[31, 34]
7	19	3 years	gp91 ^{phox}	Multiple pneumonias, Liver abscess, Skin abscess, Osteomyelitis	<i>Aspergillus</i> , <i>Pyrenochaeta roseni</i> , <i>Staphylococcus aureus</i> , <i>Serratia marcescens</i> , <i>Nocardia</i>	No	No	Yes	
8	18	4 years	gp91 ^{phox}	Liver abscess, Multiple pneumonias, Osteomyelitis, Lymphadenitis	<i>Aspergillus Burkholderia</i> , <i>Serratia marcescens</i> , <i>Nocardia</i> , <i>Staphylococcus aureus</i>	No	No	No	[35]
9	28	9 months	p47 ^{phox}	Multiple pneumonias	<i>Pseudomonas aeruginosa</i>	Renal transplant graft failure	Yes	No	[31]
10	30	2 years	gp91 ^{phox}	Liver abscess, Multiple pneumonias, Osteomyelitis	MRSA, <i>Aspergillus</i>	Recurrent perirectal abscess, Gastroesophageal reflux disease	Yes	No	[31]
11	19	2 months	gp91 ^{phox}	Pneumonia, Liver abscess	<i>Aspergillus tanneri</i> , <i>Serratia marcescens</i> , MRSA	McLeod syndrome, Ureteral stricture	No	No	
12	19	7 months	gp91 ^{phox}	Multiple pneumonias	<i>Aspergillus</i> , <i>Serratia marcescens</i> , <i>Nocardia</i>	Juvenile rheumatoid arthritis, Chronic renal tubular acidosis, Growth failure	Yes	Yes	[31, 34]
13	39	2 years	gp91 ^{phox}	Liver abscess, Pneumonia, Skin, Osteomyelitis	<i>Nocardia</i> , <i>Pseudomonas aeruginosa</i> , <i>Serratia marcescens</i>	No	Yes	Yes	[31]
14	19	1.5 years	gp91 ^{phox}	No severe infections	<i>Candida</i>	Severe esophageal strictures, Urethral strictures	Yes	No	[31]

Patient	Age at death (years)	Age at CGD diagnosis	Gene affected	Infection history	Microorganism history	Other systemic CGD manifestations	Steroids chronic use	BMT	Reference
15 ^b	29	6 months	gp91 ^{phox}	Liver abscess, Multiple pneumonias, Recurrent lymphadenitis	<i>Aspergillus</i> , <i>Burkholderia</i>	No	No	No	[31]
16	32	6 months	gp91 ^{phox}	Multiple pneumonias, Multiple liver abscess, Lymphadenitis	<i>Aspergillus</i> , <i>Serratia</i> , <i>Burkholderia</i>	Portal hypertension, Obstructive lung disease, Recurrent pancreatitis, Chronic renal failure	Yes	Yes	[31, 34]
17	24	6 months	gp91 ^{phox}	Liver abscess, Multiple pneumonias	MRSA, <i>Aspergillus</i> , <i>Nocardia</i> , <i>Paeecilomyces</i>	Granulomatous colitis and cystitis	Yes	No	[31, 33, 34]

^aChorioretinal biopsy case

^b*Burkholderia* DNA was detected in the normal ocular tissue

CGD chronic granulomatous disease; *BMT* bone marrow transplantation; *MRSA* methicillin resistant *Staphylococcus aureus*

Table 3

Comparison of Clinical Features of 17 Chronic Granulomatous Disease Patients with and without Chorioretinopathy

	CGD without chorioretinopathy (n=9)		CGD with chorioretinopathy (n=8)		Total (n=17)	p value
Age of CGD diagnosis (median, range)	7 months (range, 2 months–2 years)		2.5 years (range, 2 months–8 years)		9 months (range, 2 months–8 years)	0.07
Age of death (median, range)	19 years (16–50 years)		29 years (19–39 years)		19 years (16–50 years)	0.63
Genotype (gp91 ^{phox} /p47 ^{phox})	8/1		7/1		15/2	1.00
Systemic corticosteroids use (Yes/No)	7/2		5/3		12/5	0.62
BMT (Yes/No)	0/9		4/4		4/13	0.03
PCR (positive/negative)	Normal area: 1/8 Lesion area: -		Normal area: 0/8 Lesion area: 7/1		Normal area: 1/16 Lesion area: 7/1	0.0001

CGD chronic granulomatous disease; BMT bone marrow transplantation; PCR polymerase chain reaction

Table 4
Clinical and Pathological Findings of 8 Patients with Chronic Granulomatous Disease-Associated Chorioretinal Lesions

Patient	Clinical eye examination	Macroscopic findings	Microscopic findings	Microdissection and PCR		Reference
				Lesion	Normal tissue	
1 ^{a,b}	Uveitis, subretinal mass, chorioretinal scars, O.U.	Subretinal mass (biopsy from O.S.)	Lymphoplasmacytic infiltrates, vascular inflammatory granulation	16S rDNA +	-	[16]
2 ^b	Uveitis, chorioretinal scars, O.U.	Chorioretinal scars, O.U.	Chorioretinal scars, RPE hyper/hypopigmentation, hyper/hypotrophy, trace-mild perivascular lymphocytes/macrophages	16S rDNA + <i>Pseudomonas aeruginosa</i> +	-	[27]
3	NA	Chorioretinal scars, O.D. (examined O.U.)	Chorioretinal scars, photoreceptor/RPE atrophy, Mild choroidal lympho-monocytic infiltrates	16S rDNA + <i>Staphylococcus epidermidis</i> +	-	
4 ^b	Episcleritis, chorioretinal scars, optic nerve pallor, O.U.	Chorioretinal scars, O.D. (only O.D. for autopsy)	Chorioretinal scars, RPE hyperplasia/hypertrophy, mild inflammatory infiltrates around scars	16S rDNA +	-	
5	Retinitis pigmentosa, no chorioretinal lesions mentioned	Chorioretinal scars, O.D. (examined O.U.)	Chorioretinal scars, RPE hyperplasia/hypertrophy, focal inflammatory infiltrates in choroid	16S rDNA +	-	
6	Glaucoma, no chorioretinal scars mentioned	Chorioretinal scars, O.D. (examined O.U.)	Chorioretinal scars, RPE hypertrophy/migration, mild choroidal inflammatory infiltrates	16S rDNA + <i>Staphylococcus epidermidis</i> +	-	
7	Chorioretinal scars, retinitis pigmentosa, O.D.	Chorioretinal scars, O.D. (examined O.U.)	Chorioretinal scars, RPE hypertrophy/migration, mild scattered choroidal inflammatory infiltrates	16S rDNA +	-	
8	Chorioretinal scars, O.U.	Chorioretinal scars, O.U.	Chorioretinal scars, hypopigmentation, atrophy of all retinal layers in the scars	16S rDNA -	-	

^aChorioretinal biopsy case

^bPatient was treated with topical corticosteroids

PCR polymerase chain reaction; O.U. bilateral eyes; O.D. right eye; O.S. left eye; rDNA ribosomal DNA; NA not available