Inherited CARD9 deficiency in otherwise healthy children and adults with *Candida* species–induced meningoencephalitis, colitis, or both

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Background: Invasive infections of the central nervous system (CNS) or digestive tract caused by commensal fungi of the genus *Candida* are rare and life-threatening. The known risk factors include acquired and inherited immunodeficiencies, with patients often displaying a history of multiple infections. Cases of meningoencephalitis, colitis, or both caused by *Candida* species remain unexplained.

Objective: We studied 5 previously healthy children and adults with unexplained invasive disease of the CNS, digestive tract, or both caused by *Candida* species. The patients were aged 39, 7, 17, 37, and 26 years at the time of infection and were unrelated, but each was born to consanguineous parents of Turkish (2 patients), Iranian, Moroccan, or Pakistani origin. Meningoencephalitis was reported in 3 patients, meningoencephalitis associated with colitis was reported in a fourth patient, and the fifth patient had colitis only.

Methods: Inherited caspase recruitment domain family, member 9 (CARD9) deficiency was recently reported in

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otherwise healthy patients with other forms of severe disease caused by *Candida*, *Trichophyton*, *Phialophora*, and *Exophiala* species, including meningoencephalitis but not colitis caused by *Candida* and *Exophiala* species. Therefore we sequenced *CARD9* in the 5 patients.

Results: All patients were found to be homozygous for rare and deleterious mutant *CARD9* alleles: R70W and Q289* for the 3 patients with *Candida albicans*–induced meningoencephalitis, R35Q for the patient with meningoencephalitis and colitis caused by *Candida glabrata*, and Q295* for the patient with *Candida albicans*–induced colitis. Regardless of their levels of mutant CARD9 protein, the patients' monocyte-derived dendritic cells responded poorly to CARD9-dependent fungal agonists (curdlan, heat-killed *C albicans*, *Saccharomyces cerevisiae*, and *Exophiala dermatitidis*).

Conclusion: Invasive infections of the CNS or digestive tract caused by *Candida* species in previously healthy children and even adults might be caused by inherited CARD9 deficiency. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Inborn error of immunity, primary immunodeficiency, invasive fungal diseases, inherited CARD9 deficiency, central nervous system, colitis,* Candida *species, human*

Candida species are commensal yeasts colonizing the skin and digestive tracts of most healthy subjects. However, they can cause mucocutaneous candidiasis, which when chronic (ie, chronic mucocutaneous candidiasis [CMC]) is commonly observed in patients with broad and profound acquired or inherited T-cell disorders. These patients typically display various other infections.[1,2] Syndromic CMC, in which CMC is a key element of the description of the clinical entity (eg, autosomal recessive autoimmune polyendocrinopathy type 1), and isolated CMC, which generally affects otherwise healthy subjects (eg, autosomal dominant CMC disease), have both been reported to result from primary immunodeficiencies impairing IL-17 immunity.[2-11] *Candida* species might also cause severe invasive infections. Candidemia, the most frequent clinical form of invasive candidiasis,[12,13] is the fourth most frequent cause of bloodstream infection in hospitals and is classically reported in patients with neutropenia, patients with a central catheter receiving broad-spectrum antibiotics and/or parenteral nutrition, or both. In contrast, *Candida* species infection of the central nervous system (CNS) is rare. *Candida* species–induced meningitis is reported principally in preterm neonates (0.59% of neonates weighing <1000 g at birth have *Candida* species–induced meningitis), possibly because of immaturity of the blood-brain barrier.[14] Neurosurgery, abdominal surgery, intravenous catheter use, intravenous drug use, HIV infection, immunosuppressive treatments, and cancer have been associated with the few reported cases of *Candida* species infection of the CNS.[15-18] *Candida* species CNS infections have also been observed in a few patients with inherited chronic granulomatous disease.[19,20] Finally, 39 patients with *Candida* species CNS infection and no known underlying risk factors have been reported,[21-48] although chronic granulomatous disease was not excluded in most of these cases. In these patients the median age at CNS infection was 27 years (1-54 years), 62% were male, and 55% died of infection. Interestingly, *Candida* species–induced meningitis has been reported in 5 patients with inherited caspase recruitment domain family, member 9 (CARD9) deficiency,[49-51] whereas 2 other patients with CARD9 deficiency had brain abscesses, which were caused by *Exophiala* species in 1 patient[52] and possibly by *Trichophyton* species in the other.[15] *Candida* species causes colitis even less frequently than meningoencephalitis, with only 25 cases reported in the context of neutropenia, cancer, lymphoma, systemic lupus erythematosus, AIDS, corticosteroid treatment, or preterm neonates.[53-61] Therefore we sequenced *CARD9* in 5 unrelated patients: 4 with *Candida* species infections of the CNS, one of whom had proved *Candida* species–induced colitis, with the final patient having proved *Candida* species–induced colitis solely.

Abbreviations used

CARD9:	Caspase recruitment domain family, member 9
CMC:	Chronic mucocutaneous candidiasis
CNS:	Central nervous system
CSF:	Cerebrospinal fluid
CT:	Computed tomography
DHR:	Dihydrorhodamine
MDDC:	Monocyte-derived dendritic cell
MRI:	Magnetic resonance imaging
NF-κB:	Nuclear factor κB
NK:	Natural killer
PMA:	Phorbol 12-myristate 13-acetate
VSV:	Vesicular stomatitis virus
WT:	Wild-type

METHODS

Details of the materials and methods used in this study are provided in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Case reports

We describe 5 patients with proved *Candida* species infections of the CNS, the digestive tract, or both from 5 unrelated consanguineous families originating from Turkey (n = 2), Iran Morocco, and Pakistan (n = 1 each, Table I).

Kindred A

A 42-year-old woman (P1, A.II.2; Fig 1, *A*) living in France and born to consanguineous Turkish parents presented at 39 years of age with *Candida albicans*–induced meningitis and brain abscesses. She had recurrent vulvovaginal candidiasis, with episodes occurring about 5 times per year since the age of 36 years. She presented with headache, persistent fever, and vomiting. She then displayed an altered mental state, right-arm paresis, and facial palsy. Brain magnetic resonance imaging (MRI) provided evidence of an infiltrative frontal lesion with a mass effect and contrast enhancement with ventricular dilation (Fig 2, *A*). Lumbar puncture revealed the presence of 1100 leukocytes/mm^3 (80% lymphocytes and 16% eosinophils) in the cerebrospinal fluid (CSF), together with increased protein levels up to 1.53 g/L and hypoglycorrhachia of 1.5 mmol/L. CSF pressure was high at up to 25 cm H_2O. Three lumbar punctures were performed, and *C albicans* grew from the CSF samples collected. Brain biopsy showed yeasts and numerous pseudohyphae in giant cell granulomas with necrosis (Fig 3, *A-D*). A culture of the biopsy sample was positive for *C albicans*. Abdominal and thoracic computed tomography (CT) and transesophageal echocardiography provided no evidence

TABLE I. Characteristics of the 5 patients with invasive fungal infection and homozygous *CARD9* mutations

Patient ID	Age at onset (y)	Age at last follow-up (y)	Sex	Country of origin	Organ involvement	Associated CMC	Fungus	Status	*CARD9* mutation
P1	39	42	F	Turkey	CNS	Yes	*C albicans*	Alive	R70W/R70W
P2	7	8	F	Turkey	CNS	Yes	*C albicans*	Alive	R70W/R70W
P3	17	28	M	Iran	CNS, sinus, digestive tract	No	*C glabrata*	Alive	R35Q/R35Q
P4	37	37	F	Morocco	CNS	Yes	*C albicans*	Alive	Q289*/Q289*
P5	26	34	M	Pakistan	Digestive tract	No	*C albicans*	Alive	Q295*/Q295*

F, Female; *M*, male.

for the dissemination of *C albicans* infection. Immunologic explorations showed normal $CD4^+$ T, $CD8^+$ T, and natural killer (NK) lymphocyte counts and B-cell lymphopenia at 4% (56 cells/μL). T-lymphocyte proliferations were normal in response to PHA and antigens (tuberculin and candidin). Leukocyte oxidative burst, as assessed by using dihydrorhodamine (DHR) tests, was normal, and IgG, IgA, and IgM levels were also normal. The infection was cured by 2 months of combined intravenous antifungal therapy combining liposomal amphotericin B and 5-fluorocytosine, which was subsequently replaced with oral fluconazole. A cerebral shunt was performed to treat intracranial hypertension. Two years later, fluconazole treatment is continuing, and the patient is alive without sequel. Neither her parents nor her siblings and children have had any severe infectious disease.

Kindred B

P2 is an 8-year-old girl born to a Turkish kindred (P2, kindred B, B.II.1; Fig 1, *A*) living in Belgium. She has had chronic thrush and onychomycosis since the age of 5 years. At the age of 7 years, she had fever for several weeks, with headache and vomiting. CSF analysis provided evidence of *C albicans*–induced meningitis but with 920 cells/mm^3, 20% of which were eosinophils (fluconazole minimum inhibitory concentration = 0.5 mg/L). Brain MRI revealed the presence of 2 lesions of 11 and 6 mm in diameter, respectively. Medullary MRI revealed several enhancing lesions. *C albicans* grew from nail and buccal samples. The patient was treated with liposomal amphotericin B for 2 weeks and then fluconazole (12 mg/kg/d), with a positive outcome. Soon after fluconazole treatment, a relapse occurred, with fever, headache, and vomiting. CSF culture was sterile but with 1520 cells/mm^3, mostly eosinophils (60%). Symptoms improved with liposomal amphotericin B treatment, which was replaced after 5 months with fluconazole because of renal failure related to liposomal amphotericin B. Lesions were controlled 6 months after starting fluconazole, as shown by means of MRI. Brain lesions were in regression, whereas medullar lesions remained unchanged. The patient's parents did not have any severe infectious disease.

Kindred C

P3 is a 28-year-old man from a consanguineous Iranian kindred (P3, kindred C, C.II.2; Fig 1, *A*) living in Iran. He had a history of left hemiplegia at the age of 17 years. MRI and CT scans revealed a brain abscess (Fig 2, *B*). *Candida* species grew from the surgical biopsy specimen obtained. The patient was subsequently discharged on oral fluconazole treatment. At the age of 20 years, he had fever and right ptosis. Cerebral CT scanning showed soft tissue opacities and calcifications in the sphenoids, ethmoids, and left maxillary and frontal sinuses, with 2 regions of bone erosion in the median wall of the right orbit adjacent to the right orbital apex. There was a 6 × 4–mm region of high-density soft tissue that extended to the frontal sinus. Bone attenuation was observed at the superolateral left orbital rim and the posterolateral wall of the left sphenoid tissue, suggesting fungal sinonasal infection with orbital and intracranial extension. The patient underwent sinus surgery, and the cultures obtained from the surgical specimen were positive for *Candida* species. The patient was discharged on oral itraconazole treatment. At the age of 22 years, he had bloody diarrhea complicated with anemia. Colonoscopy revealed extensive linear ulcers throughout the colon, a low level of vascular development, low levels of haustration, and many sessile and ulcerative polyps, with a larger number of small polyps and ulcerations in the terminal ileum (Fig 2, *C*). Two gut biopsies were carried out on the terminal ileum. Histopathologic analysis of the biopsy specimens revealed a diffuse inflammatory lesion characterized by infiltration of macrophages and eosinophils associated with the presence of round yeasts, which was identified by using periodic acid–Schiff and Gomori-Grocott staining and measured up to 4 μm in diameter without pseudohyphae (Fig 3, *E* and *F*). Anti–*Candida* species immunohistochemistry results were positive (Fig 3, *G*). Collectively, morphologic and immunohistochemical data suggested invasive colonic *Candida* species infection. *Candida glabrata* grew from a cultured biopsy specimen. Immunologic explorations were carried out as follows: neutrophils; $CD4^+$ T, $CD8^+$ T, B, and NK lymphocyte blood counts; DHR test results; and IgG, IgA and IgM plasma levels were normal. IgE levels were high at 1.7 mg/mL. Eosinophil counts were also high at up to 1500/mm^3. Neither the patient's parents nor his siblings had any severe infectious disease.

Kindred D

P4 is a 37-year-old woman from a consanguineous Moroccan kindred (P4, kindred D, D.II.1; Fig 1, *A*). Three years before presentation, she had thrush, with recurrent episodes occurring about 3 times per year. At the age of 37 years, she suddenly had severe headache, vomiting, and right hemiparesis. Fundus examination provided evidence of papillary edema. Cerebral MRI showed a 30 × 40–mm left temporoparietal lesion (Fig 2, *D*) with several small peripheral nodules displaying peripheral enhancement after contrast medium injection and a large perilesional edema with a mass effect on the left ventricle. *C albicans* grew from a CNS biopsy specimen. Histologic examination revealed the presence of a lymphoplasmocytic infiltrate around the vessels, and Gomori-Grocott staining revealed the presence of pseudohyphae. Abdominal and thoracic CT and transesophageal echocardiography provided no evidence for the dissemination of *C albicans* infection. Immunologic explorations were carried out as follows: neutrophils; $CD4^+$ T,

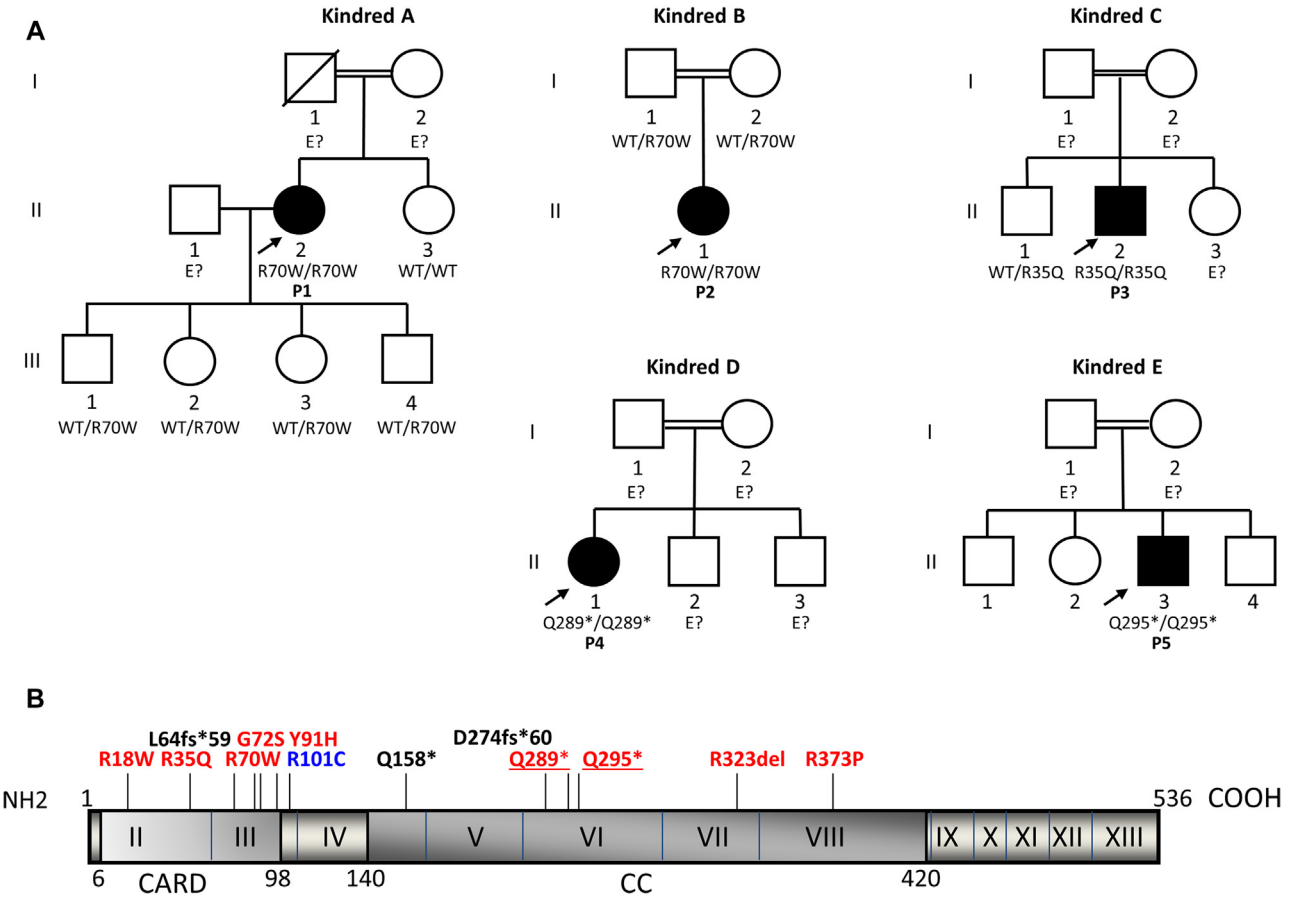

FIG 1. A, Pedigrees of 5 kindreds with invasive fungal infection and *CARD9* mutations. Each kindred is designated by a letter (A-E), each generation is designated by a Roman numeral (I-III), and each subject is designated by an Arabic numeral (1-4). Patients with invasive fungal infections are indicated in black. The probands are indicated by *arrows*. The genotype of *CARD9* is indicated below each subject. *E?*, No DNA was available. **B,** Schematic diagram of the human *CARD9* (isoform 1) gene and its mutations. The human *CARD9* isoform 1 gene is shown, with its known pathogenic mutations. Coding exons are numbered with Roman numerals. Regions corresponding to the CARD and coiled-coil *(CC)* domains are indicated. Mutations associated with invasive fungal (*Candida* and *Exophiala* species) infections are indicated in *red*. Mutations associated with invasive fungal (*Candida* species) infections or deep dermatophytosis are *underlined*. Mutations previously reported in patients with deep dermatophytosis are indicated in *blue* or *underlined*, and mutations associated with subcutaneous phaeohyphomycosis are indicated in *black*.

$CD8^+$ T, B, and NK lymphocyte counts; DHR test results; and IgG, IgA, and IgM plasma levels were normal. Serologic test results for HIV were negative, and the patient did not have diabetes mellitus. The infection was cured after treatment for 15 days with a combination of liposomal amphotericin B and 5-fluorocytosine, followed by fluconazole treatment, which is still underway after 10 months. The patient's father had a history of recurrent skin dermatophytosis. Neither her mother nor her siblings had any severe infectious disease.

Kindred E

A 34-year-old man (P5, kindred E, E.II.3; Fig 1, *A*) from a consanguineous Pakistani kindred had been living in the United Kingdom for the last 8 years. While he was living in Pakistan, he was given a diagnosis of cervical lymphadenitis ascribed to tuberculosis, although not microbiologically proved, and had a full course of antituberculous chemotherapy. Several months after completing his antituberculous treatment, he noticed bloody diarrhea, lost weight, and became anemic. On initial assessment at the age of 26 years, the patient was found to have oral and esophageal candidiasis and florid right-sided colitis with multiple pseudopolyps. Biopsy specimens showed multiple fungal organisms. Chest radiography and abdominal CT scans showed no sign suggestive of tuberculosis, intra-abdominal lymphadenopathy, or hepatosplenomegaly, and colic biopsy results were consistently negative for tuberculosis. Initially, a diagnosis of histoplasmosis was retained. Therefore the patient was treated with fluconazole for 2 years, followed by itraconazole. His symptoms improved. However, colonoscopy showed persistent infection, and symptoms recurred when the treatment was stopped. The patient was HIV seronegative, with normal lymphocyte subsets. At the age of 29 years, he was found to be anemic, with iron deficiency. Immunoglobulin levels (IgG, IgA,

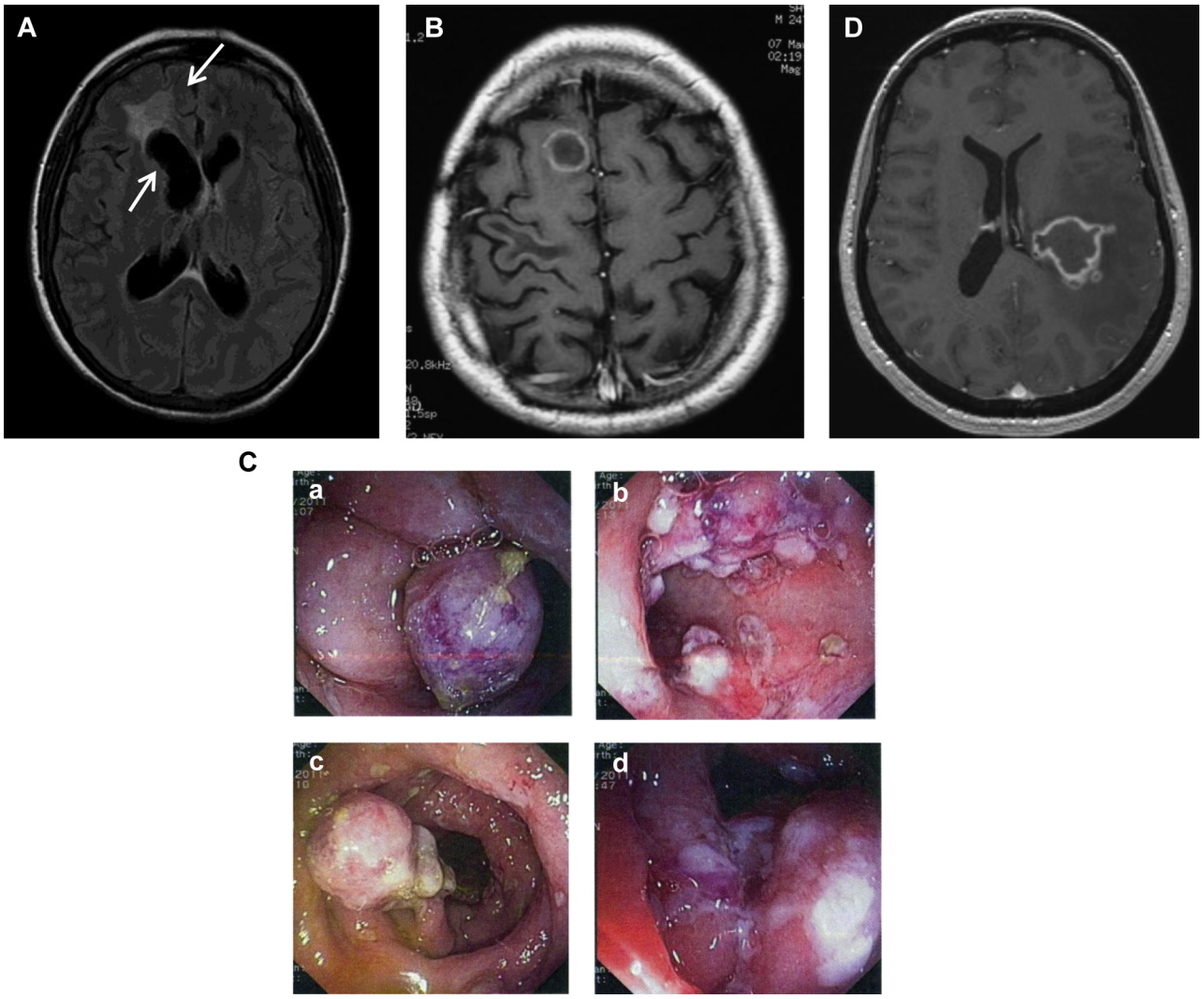

FIG 2. Clinical, pathologic, and radiologic features of patients. **A** and **B,** Brain abscess of P1 (Fig 2, *A*) and brain CT scan of P3 (Fig 2, *B*). **C,** *a-d*, Colonoscopy results for P3. **D,** Brain MRI of P4.

and IgM) were within normal ranges, but IgE levels increased at 4979 IU/mL. After a diagnosis of possible histoplasmosis, the patient was treated with a short induction course of liposomal amphotericin B, followed by posaconazole. His symptoms improved, but after a colonoscopy performed in September 2009, obvious histologic signs of ongoing infection were observed on colonic samples, and *C albicans*, resistant to triazoles but susceptible to amphotericin B and caspofungin, grew from cultured samples. The tissue samples had positive test results for *Candida* species by using PCR. Results of histoplasmosis complement fixation, precipitin, and immunodiffusion antibody tests all remained negative, and results of the serum Histoplasma Antigen EIA performed in Indianapolis by the laboratory of Dr Joe Wheat were also negative, as was the test for cryptococcal serum antigen. This patient had no history of other recurrent infections, and there was no family history of susceptibility to any particular infection.

Identification of homozygous *CARD9* mutations

We investigated the above 5 patients from unrelated consanguineous kindreds. They displayed CNS candidiasis and *Candida* species–induced colitis but no detectable immunodeficiency in the first round of routine immunologic tests (HIV serology, neutrophil counts, and T-cell, B-cell, and NK cell lymphocyte counts). We sequenced *CARD9* exons and found homozygous *CARD9* mutations in all 5 patients. P1 and P2 had a homozygous *CARD9* missense mutation, *c.208C>T* in exon 3, replacing the arginine residue in position 70 with a tryptophan (R70W) within the CARD domain of the CARD9 protein (Fig 1, *B*). P1 has 4 healthy children, all of whom were heterozygous for the mutation. The parents of P2 are heterozygous for the mutation. P3 had a homozygous *CARD9* missense mutation, *c.104G>A* in exon 2, replacing the arginine residue in position 35 with a glutamine (R35Q), within the CARD domain of the CARD9 protein (Fig 1, *B*). His healthy brother is heterozygous for the mutation (wild-type [WT]/R35Q). P4 and P5 had homozygous *c.865C>T* and c.*883C>T* mutations in exon 6, resulting in premature termination codons at positions 289 (Q289*) and 295 (Q295*), respectively, in the region encoding the coiled-coil domain of CARD9 (Fig 1, *B*). Genotypes were not available for the other members of the family.

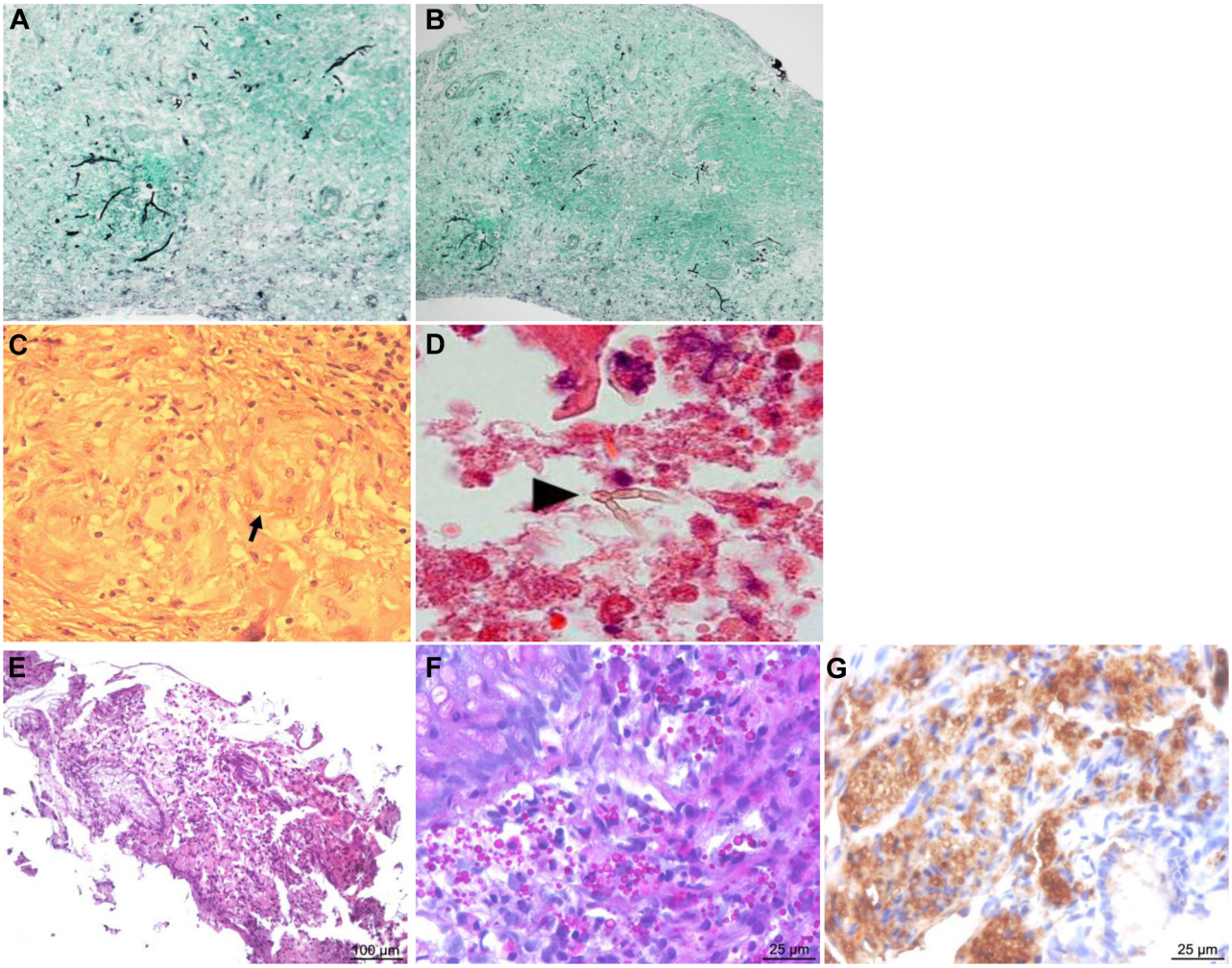

FIG 3. Histology. **A-D,** Histologic results for the brain biopsy carried out on P1. *Arrows* indicate fungal agents. Fig 3, *A* and *B*, Epithelioid granuloma containing pseudohyphae and displaying Gomori-Grocott staining. Fig 3, *C*, Epithelioid granuloma containing pseudohyphae and stained with hematoxylin-eosin-safran. Fig 3, *D*, Periodic acid-Schiff stain. **E-G,** Histologic results for the ileum mucosa biopsy specimen of patient P3. Fig 3, *E* and *F*, Round yeasts measuring up to 4 μm in diameter identified by using periodic acid–Schiff staining. Fig 3, *G*, Anti–*Candida* species immunohistochemistry.

The segregation of the 4 mutations in the 5 kindreds was consistent with autosomal recessive CARD9 deficiency with complete clinical penetrance. None of the mutations reported here was found in any of the various public databases checked (HGMD, Ensembl, and 1000 Genomes or our in-house whole-exome sequencing database [>2000 exomes]). We also sequenced 1052 control subjects from the Human Genome Diversity Cell Line Panel and 30, 90, and 83 Iranian, Turkish, and Algerian healthy control subjects, respectively, in whom we found none of the 4 variants described here. These data ruled out the possibility of R35Q, R70W, Q289*, or Q295*, being irrelevant polymorphisms. The 2 missense mutations were predicted to be probably damaging by using PolyPhen 2 (with the highest possible score of 1) and damaging by using SIFT (scores of 0 for R35Q and 0.02 for R70W). In addition, the Q289* and the Q295* mutations have already been reported and shown to be rare and deleterious *CARD9* alleles.[15,49] Collectively, these data strongly suggest that all 5 patients tested are homozygous for rare and deleterious mutant *CARD9* alleles.

Effect of *CARD9* mutation on protein level and function

We investigated the consequences of these mutations for protein levels by carrying out an immunoblot analysis for CARD9 on whole-cell extracts from HEK-293T cells transfected with a pcDNA3.1 V5 (C terminal–tagged) plasmid with no insert or carrying the WT (pcDNA3.1 V5 CARD9 WT) or one of the 4 mutant alleles of *CARD9* (pcDNA3.1 V5 CARD9 R35Q, pcDNA3.1 V5 CARD9 R70W, pcDNA3.1 V5 CARD9 Q289*, or pcDNA3.1 V5 CARD9 Q295*). In cells transfected with the *CARD9* R35Q allele, CARD9 protein levels and molecular weights were similar to those in cells transfected with the WT allele, whereas cells transfected with the *CARD9* R70W allele had reproducibly lower levels of a protein of normal size, and cells transfected with the *CARD9* Q289* and Q295* alleles had normal levels of a protein truncated by about 25 kDa, as previously reported (Fig 4, *A*).[15] Flow cytometric analysis of CARD9 protein levels carried out only on monocyte-derived dendritic cells (MDDCs) from P1 (R70W/R70W) showed this

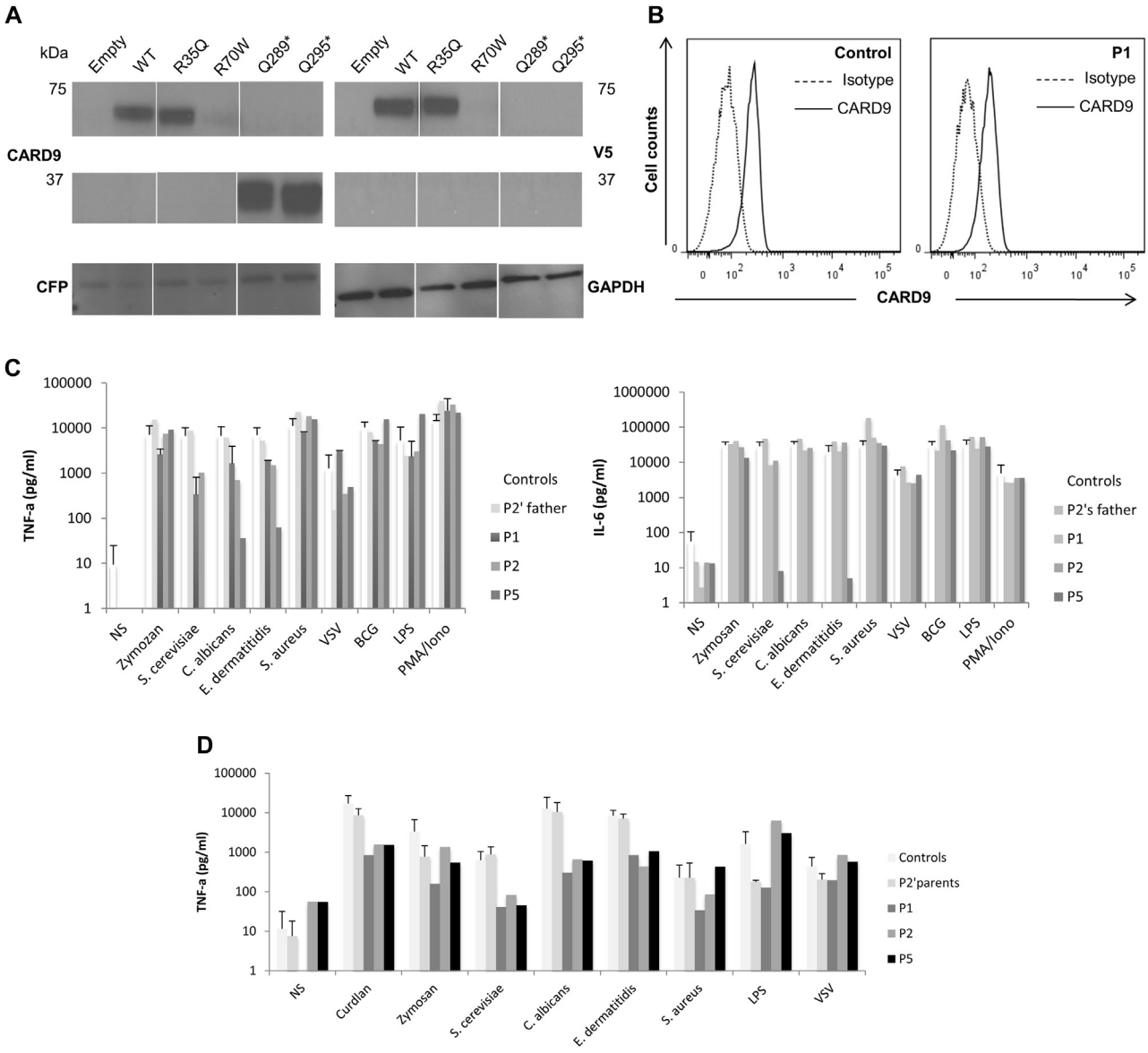

FIG 4. Effect of *CARD9* mutations on CARD9 protein levels and function. **A** and **B,** Effect of *CARD9* mutations on CARD9 protein levels. Fig 4, *A*, Immunoblot analysis of CARD9 in whole-cell extracts of HEK-293T cells cotransfected with pcDNA3.1 V5 (C-terminally tagged), either empty or carrying the WT or mutant (R35Q, R70W, Q289*, and Q295*) *CARD9* alleles, together with a cyan fluorescent protein plasmid as a transfection control. Antibodies against CARD9, V5, cyan fluorescent protein, and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; as a loading control) were used. Fig 4, *B*, Flow cytometric analysis of CARD9 expression in MDDCs from patient P1 and a control subject. **C** and **D,** Effect of *CARD9* mutations on CARD9 protein function. Fig 4, *C*, TNF-α *(left)* and IL-6 *(right)* production by whole blood cells after 24 hours of stimulation with zymosan, heat-killed *S cerevisiae*, *C albicans*, *E dermatitidis*, *S aureus*, VSV, BCG, LPS, and PMA plus ionomycin for P1, P2, and P5; P2's father; and 7 control subjects. Fig 4, *D*, TNF-α production after 24 hours of stimulation of MDDCs with curdlan, zymosan, *S cerevisiae*, *C albicans*, *E dermatitidis*, *S aureus*, LPS, and VSV for P1, P2, and P5; P2's parents; and 6 healthy control subjects tested in parallel. *NS*, Not stimulated.

protein to be slightly less abundant than in MDDCs from a healthy control subject (63% of MDDCs were CARD9 positive in P1, whereas 87% of MDDCs were CARD9 positive in the control subject, as tested in parallel; Fig 4, *B*). Collectively, these data are consistent with the previously characterized pathogenic mutations of *CARD9*[15,49-52,62]; nonsense mutations prevented the production of full-length CARD9, whereas missense mutations did not necessarily do so.

We then evaluated the functional consequences of the mutations by studying the production of TNF-α and IL-6 using whole blood cells after 24 or 48 hours of stimulation with zymosan (an agonist of Dectin-1 and Toll-like receptor 2), heat-killed *Saccharomyces cerevisiae*, *C albicans*, *Exophiala dermatitidis*, *Staphylococcus aureus*, vesicular stomatitis virus (VSV), BCG, LPS (Toll-like receptor 4 agonist), and phorbol 12-myristate 13-acetate (PMA) plus ionomycin. P1, P2 (both

homozygous for the CARD9 R70W allele), and, more strikingly, P5 (Q295*/Q295*) had impaired TNF-α production after 24 hours of stimulation with *S cerevisiae*, *C albicans*, and *E dermatitidis* in comparison with the 7 healthy control subjects tested in parallel or P2's father (R70W/WT; Fig 4, *C*, left). In addition, P1 and P2 had reduced levels of IL-6 production after 24 hours of stimulation with *S cerevisiae* but subnormal to normal levels of IL-6 production in response to *C albicans* and *E dermatitidis*. By contrast, P5 showed a marked impairment of IL-6 production in response to *S cerevisiae*, *C albicans*, and *E dermatitidis* in comparison with the 7 healthy control subjects tested in parallel or P2's father (R70W/WT; Fig 4, *C*, right). Production of both TNF-α and IL-6 in response to zymosan, *S aureus*, VSV, BCG, LPS, and PMA plus ionomycin was comparable in the 3 patients tested (P1, P2, and P5) and in healthy control subjects. Moreover, IL-6 production tested after 48 hours (see Fig E1 in this article's Online Repository at www.jacionline.org) on whole blood from P1 only was strongly impaired after stimulation with heat-killed *S cerevisiae*, *C albicans*, and, to a lesser extent, zymosan but was normal after stimulation with LPS, *S aureus*, VSV, BCG, or PMA/ionomycin. Unfortunately, P3 (R35Q/R35Q) and P4 (Q289*/Q289*) could not be tested.

We then assessed TNF-α production by MDDCs from P1, P2, and P5; P2's father and mother; and 6 healthy control subjects stimulated for 24 hours with curdlan and the same agonists as used in the whole blood assay (Fig 4, *D*). The patients displayed a strong impairment of TNF-α production in response to stimulation with all fungal ligands used (curdlan, heat-killed *S cerevisiae*, *C albicans*, *E dermatitidis*, and, to a lesser extent, zymosan), whereas it was within the range of healthy control subjects after stimulation with *S aureus*, LPS, and VSV.

In addition, 293 HEK cells transfected with the R35Q and the R70W CARD9 alleles displayed impaired nuclear factor κB (NF-κB) transcriptional activity, as shown by NF-κB–luciferase reporter assays, comparing these cells with cells transfected with the WT CARD9 allele at the basal level and after stimulation with curdlan, *S cerevisiae*, or *C albicans* (Fig 5). Surprisingly, under these conditions, the 2 nonsense mutations (Q289* and Q295*) were associated with constitutive NF-κB–luciferase activity. These results can be accounted for by the production in this overexpression system of normal amounts of truncated CARD9-truncated proteins able to interact through their intact coiled-coil domain with the downstream partner BCL10.

Finally, we evaluated the proportion of *ex vivo* IL-17A–producing T cells using flow cytometry because low proportions of IL-17 T cells have been reported in some,[15,49,50,62] but not all,[51,52] CARD9-deficient patients. Under these conditions, no differences were observed between the patients tested (P1, P2, and P5), P2's parents (CARD9 R70W/WT), and the 10 healthy control subjects tested in parallel (Fig 6, *A*). Moreover, IL-17A production by whole blood cells after 24 hours of stimulation with PMA and ionomycin, as measured by using ELISA, was similar for the 3 patients tested, P2's father, and 7 healthy control subjects tested in parallel (Fig 6, *B*). Therefore we conclude that the homozygous R35Q and R70W mutations led to the production of normal or small amounts of loss-of-function CARD9 proteins, whereas the Q289* and Q295* mutations led to the absence of a normal CARD9 protein. This resulted in the impairment of proinflammatory cytokine production by CARD9-deficient whole blood cells and particularly by MDDCs specifically in response to various fungal ligands, as well as an impairment of NF-κB transcriptional activity in transfected HEK cells, whereas IL-17 T-cell production was normal.

DISCUSSION

Four of the 5 patients described here displayed *Candida* species infections of the CNS, one of which was associated with *Candida* species–induced colitis, whereas the fifth patient had *Candida* species–induced colitis solely. Our findings demonstrate that CARD9 deficiency is a genetic cause of rare forms of invasive candidiasis and that CARD9 plays an essential role against *Candida* species infection in the brain and colon. This is concordant with the recently reported role of Card9 in mouse antifungal immunity of the gut.[63] Indeed, Card9-deficient mice were shown to display particularly strong fungal colonization of the digestive tract with fewer than normal numbers of colonic IL-17–producing T cells and innate lymphoid cells, strongly suggesting a critical role for CARD9 in gut IL-17 immune responses and in fungal control. The 5 unrelated patients described here are homozygous for 4 different *CARD9* mutations, 2 of which have never before been described. In addition to the Q295* homozygous nonsense mutation previously reported in a large multiplex consanguineous Iranian family,[49] a homozygous missense (R101C) mutation found in a consanguineous Moroccan family,[15] a homozygous nonsense (Q289*) mutation found in 5 Algerian and 2 Tunisian kindreds[15] and an Egyptian patient,[64] compound heterozygous missense mutations (G72S and R373P) reported in a child of Korean origin,[50] a homozygous missense mutation (Y91H) reported in a French-Canadian patient,[51] a homozygous missense mutation (R18W) found in a patient of Angolan origin,[52] a homozygous in-frame deletion (E323del) in an Iranian patient,[52] compound heterozygous nonsense mutations (L64fs*59 and Q158*) reported in a Chinese patient,[62] and a homozygous frameshift mutation (D274fs*60) reported in 3 unrelated Chinese patients,[62] we identified 2 new homozygous *CARD9* missense mutations, R35Q and R70W, in an Iranian kindred and 2 unrelated Turkish kindreds. We also report a patient from Morocco with the previously described Q289* *CARD9* allele[15,64] and a patient from Pakistan with the previously described Q295* mutation.[49] In all kindreds studied, none of the heterozygous subjects were symptomatic, whereas all homozygotes were symptomatic, which is consistent with an autosomal recessive mode of inheritance with complete clinical penetrance (albeit often late in adulthood, up to 39 years). In total, 38 patients from 23 families in 9 countries have been identified with CARD9 deficiency caused by 13 different alleles, mostly with invasive fungal infections: deep dermatophytosis in 17 patients,[15] superficial or extensive dermatophytosis in 4 patients,[49,64] CMC in 15 patients (present report),[15,49] CNS infection with *Candida* species in 9 patients (present report),[49-51] *Candida* species–induced colitis in 2 patients (present report), *Exophiala* species–induced CNS and liver disease in 1 patient, lung and bone disease in another patient,[52] and *Phialophora verrucosa*–induced subcutaneous disease in 4 patients.[62] These mutations are all loss of function, with a partial or complete defect, as suggested based on whole blood TNF-α or IL-6 production on stimulation, with fungal agonists being more impaired in P5 (Q295*) than in P1 or P2 (R70W). However, this difference was no longer observed when using MDDCs, suggesting some cell-specific compensatory mechanisms. None of these patients had other fungal, parasitic, bacterial, or viral

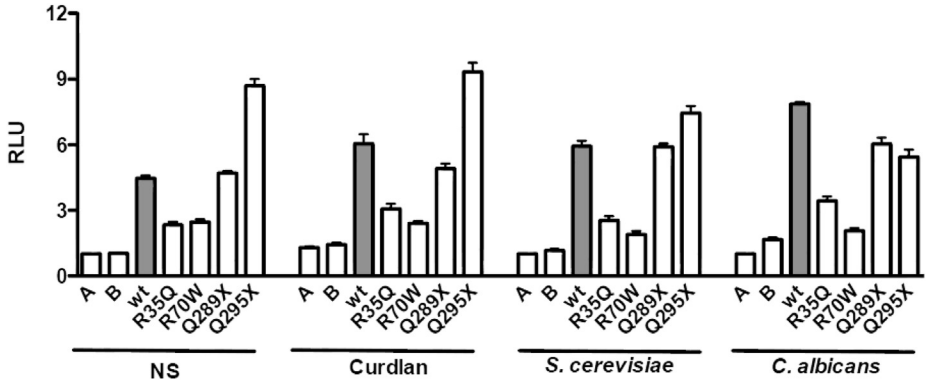

FIG 5. NF-κB transcriptional activity based on results of NF-κB–luciferase assay in 293 HEK cells transfected with NF-κB–luciferase and pRL-SV40 vectors alone *(A)*; DECTIN1, SYK, and BCL10 constructs *(B)*; DECTIN-1, SYK, BCL10, and CARD9 WT constructs; and DECTIN-1, SYK, BCL10, and CARD9 mutant constructs (R35Q, R70W, Q289*, or Q295*). Cells were left unstimulated or were stimulated with 25 μg/mL curdlan or 10^7 particles/mL of *S cerevisiae* or *C albicans*. Results are representative of 2 independent experiments and are expressed as means ± SEMs of the ratio of *Renilla* luciferase and firefly control luciferase activities. *RLU*, Relative light units. *NS*, Not stimulated.

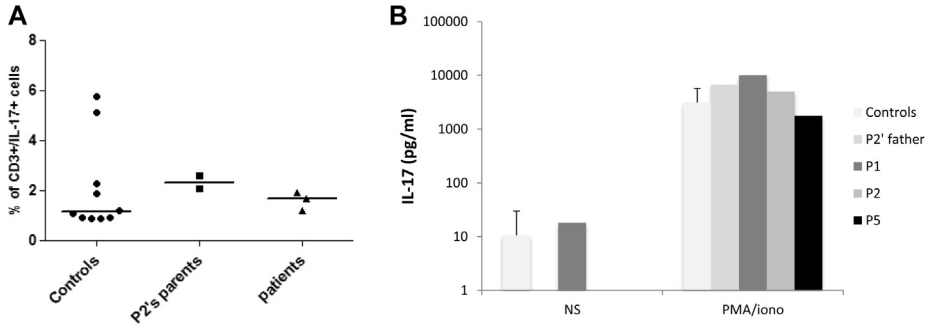

FIG 6. IL-17 production. **A,** Percentage of $CD3^+/IL\text{-}17^+$ cells of 10 control subjects, P2's parents (CARD9 R70W/WT), and P1, P2, and P5 measured by means of flow cytometry after 12 hours of stimulation with PMA/ionomycin. **B,** IL-17A production by whole blood of 7 control subjects; P2's father; and P1, P2, and P5 measured after 24 hours of stimulation with PMA/ionomycin by using ELISA. *NS*, Not stimulated.

infections, whereas CARD9-deficient mice are susceptible not only to *Candida* species but also to *Mycobacterium tuberculosis* and *Listeria monocytogenes*.[65-67] Intriguingly, individual CARD9-deficient patients seem to be prone to a single type of invasive fungal disease.[68] Patients with invasive dermatophytic disease do not have invasive candidiasis and *vice versa*. Moreover, none of these patients were reported to have *Aspergillus* species infections, which is concordant with a CARD9-independent neutrophil killing of *Aspergillus* species.[50]

In any case our report shows that *Candida* species infection of the CNS is a major clinical phenotype in CARD9-deficient patients. Patients with *Candida* species infections of the CNS should be explored for possible CARD9 deficiency, even if they are previously healthy adults. The same principle applies to rare patients with unexplained histologically documented colitis caused by *Candida* species. Finally, these studies add further weight to the idea that life-threatening infectious diseases, whether in children or adults, striking otherwise healthy subjects in the course of primary infection can result from single-gene inborn errors of immunity.[69,70]

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Clinical implications: CARD9 deficiency might predispose to meningoencephalitis, colitis, or both caused by *Candida* species. Therefore CARD9 deficiency should be investigated in patients with *Candida* species–related meningoencephalitis, colitis, or both without any known risk factors for fungal infection.

REFERENCES

1. Lanternier F, Cypowyj S, Picard C, Bustamante J, Lortholary O, Casanova JL, et al. Primary immunodeficiencies underlying fungal infections. Curr Opin Pediatr 2013;25:736-47.
2. Puel A, Cypowyj S, Marodi L, Abel L, Picard C, Casanova JL. Inborn errors of human IL-17 immunity underlie chronic mucocutaneous candidiasis. Curr Opin Allergy Clin Immunol 2012;12:616-22.
3. Liu L, Okada S, Kong XF, Kreins AY, Cypowyj S, Abhyankar A, et al. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. J Exp Med 2011;208:1635-48.
4. Puel A, Doffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 2010;207:291-7.

5. Puel A, Picard C, Cypowyj S, Lilic D, Abel L, Casanova JL. Inborn errors of mucocutaneous immunity to Candida albicans in humans: a role for IL-17 cytokines? Curr Opin Immunol 2010;22:467-74.
6. Puel A, Cypowyj S, Bustamante J, Wright JF, Liu L, Lim HK, et al. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science 2011;332:65-8.
7. Boisson B, Wang C, Pedergnana V, Wu L, Cypowyj S, Rybojad M, et al. An ACT1 mutation selectively abolishes interleukin-17 responses in humans with chronic mucocutaneous candidiasis. Immunity 2013;39:676-86.
8. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 2007;448:1058-62.
9. de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M, et al. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. J Exp Med 2008;205:1543-50.
10. Chandesris MO, Melki I, Natividad A, Puel A, Fieschi C, Yun L, et al. Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. Medicine (Baltimore) 2012;91:e1-19.
11. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 2007;357:1608-19.
12. Lamagni TL, Evans BG, Shigematsu M, Johnson EM. Emerging trends in the epidemiology of invasive mycoses in England and Wales (1990-9). Epidemiol Infect 2001;126:397-414.
13. Lortholary O, Renaudat C, Sitbon K, Madec Y, Denoeud-Ndam L, Wolff M, et al. Worrisome trends in incidence and mortality of candidemia in intensive care units (Paris area, 2002-2010). Intensive Care Med 2014;40:1303-12.
14. Benjamin DK Jr, Stoll BJ, Fanaroff AA, McDonald SA, Oh W, Higgins RD, et al. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics 2006;117:84-92.
15. Lanternier F, Pathan S, Vincent QB, Liu L, Cypowyj S, Prando C, et al. Deep dermatophytosis and inherited CARD9 deficiency. N Engl J Med 2013;369:1704-14.
16. Nguyen MH, Yu VL. Meningitis caused by *Candida* species: an emerging problem in neurosurgical patients. Clin Infect Dis 1995;21:323-7.
17. O'Brien D, Stevens NT, Lim CH, O'Brien DF, Smyth E, Fitzpatrick F, et al. *Candida* infection of the central nervous system following neurosurgery: a 12-year review. Acta Neurochir (Wien) 2011;153:1347-50.
18. Casado JL, Quereda C, Corral I. Candidal meningitis in HIV-infected patients. AIDS Patient Care STDS 1998;12:681-6.
19. Agus S, Spektor S, Israel Z. CNS granulomatosis in a child with chronic granulomatous disease. Br J Neurosurg 2000;14:59-61.
20. Fleischmann J, Church JA, Lehrer RI. Primary Candida meningitis and chronic granulomatous disease. Am J Med Sci 1986;291:334-41.
21. Belisle LG. [Day treatment at the internal clinic of the department of child psychiatry of the Hopital Sainte Justine]. Rev Neuropsychiatr Infant 1968;16:763-71.
22. Emdin W, Finlayson MH. Moniliasis of the central nervous system in a child with recovery. S Afr Med J 1954;28:868-71.
23. Fine JM, Franklin DA, Lieberthal AS. Mycotic meningitis due to *Candida albicans*; a four year recovery. Neurology 1955;5:438-43.
24. Halpert B, Wilkins H. Mycotic meningitis due to *Candida*. J Am Med Assoc 1946;130:932-4.
25. Morris AA, Kalz GG, Lotspeich ES. Ependymitis and meningitis due to *Candida (Monilia) albicans*. Arch Neurol Psychiatry 1945;54:361-6.
26. Zimmerman SL, Frutchey L, Gibbes JH. Meningitis due to *Candida (Monilia) albicans* with recovery. J Am Med Assoc 1947;135:145-7.
27. Craig WM, Gates EM. Metastatic mycotic abscesses of the brain. Arch Neurol Psychiatry 1949;62:314-21.
28. Janke D. [Serology of moniliasis]. Arch Klin Exp Dermatol 1957;206:608-14.
29. Vorreith M, Bares L, Benes V, Vancurik J. [Candidiasis of the central nervous system diagnosed by a bioptic test]. Cas Lek Cesk 1961;100:966-71.
30. Black JT. Cerebral candidiasis: case report of brain abscess secondary to *Candida albicans*, and review of literature. J Neurol Neurosurg Psychiatry 1970;33:864-70.
31. White BE. Cerebral candidiasis. N Engl J Med 1972;286:321.
32. Edelson RN, McNatt EN, Porro RS. *Candida* meningitis; with cerebral arteritis. N Y State J Med 1975;75:900-4.
33. Holyst J, Majewski A, Tyszkiewicz S. Massive cerebellar abscess due to *Candida albicans*. Neurochirurgia (Stuttg) 1976;19:126-9.
34. Popow C, Haschke F, Maida E, Kristoferitsch W, Schilling R, Götz M, et al. [Combined Tb and Candida meningitis in an 8-year old boy (author's translation)]. Klin Padiatr 1981;193:401-3.
35. Kauffman CA, Shea MJ, Frame PT. Invasive fungal infections in patients with chronic mucocutaneous candidiasis. Arch Intern Med 1981;141:1076-9.
36. Wietholter H, Thron A, Scholz E, Dichgans J. Systemic *Candida albicans* infection with cerebral abscess and granulomas. Clin Neuropathol 1984;3:37-41.
37. Vilella JM, Jacquemin JL, Brion S, Robert R, Debiais P, Roualdes G, et al. [*Candida albicans* meningoencephalitis in an apparently non-immunosuppressed patient]. Presse Med 1985;14:980.
38. Ikeda K, Yamashita J, Fujisawa H, Fujita S. Cerebral granuloma and meningitis caused by *Candida albicans*: useful monitoring of mannan antigen in cerebrospinal fluid. Neurosurgery 1990;26:860-3.
39. Vasylyk VU, Kokoshko VP, Kudla VM, Stempinskii EI. [Candidal meningoencephalitis diagnosed as tuberculous meningoencephalitis]. Probl Tuberk 1992;(3-4):62-3.
40. Jamjoom A, al-Abedeen Jamjoom Z, al-Hedaithy S, Jamali A, Naim Ur R, Malabarey T. Ventriculitis and hydrocephalus caused by *Candida albicans* successfully treated by antimycotic therapy and cerebrospinal fluid shunting. Br J Neurosurg 1992;6:501-4.
41. Lisch S, Steudel WI. [Unusual course of candidiasis of the central nervous system]. Dtsch Med Wochenschr 1994;119:13-8.
42. Zheng L, Okabe S, Hino K, Kohno T, Kamata K. [Intracranial fungal granuloma with CSF space dissemination: a case report]. No Shinkei Geka 1996;24:389-92.
43. Mason TB 2nd, Chiriboga CA, Cargan AL, Hauger SB, La Russa PS, Glick RS, et al. Postinflammatory hydrocephalus and intracranial mass lesion from *Candida* in an immunocompetent child. J Child Neurol 1996;11:336-41.
44. Hanci M, Kafadar A, Sarioglu A, Islak C, Oz B. Cerebral candidiasis presenting as a mass lesion. Zentralbl Neurochir 1998;59:129-31.
45. Akyuz M, Karpuzoglu G, Acikbas C, Tuncer R. *Candida albicans* granuloma imitate clivus chordoma. Acta Neurochir (Wien) 2002;144:505-6.
46. Choudhari YK, Choudhari KA. Life-threatening fungal meningitis-ventriculitis secondary to vaginal candidiasis. Br J Hosp Med (Lond) 2007;68:274-5.
47. Mathai AM, Khadilkar UN. Candidosis of brain. Indian J Pathol Microbiol 2007;50:838.
48. Borha A, Parienti JJ, Emery E, Coskun O, Khouri S, Derlon JM. [*Candida albicans* cerebral granuloma in an immunocompetent patient. A case report]. Neurochirurgie 2009;55:57-62.
49. Glocker EO, Hennigs A, Nabavi M, Schäffer AA, Woellner C, Salzer U, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. N Engl J Med 2009;361:1727-35.
50. Drewniak A, Gazendam RP, Tool AT, van Houdt M, Jansen MH, van Hamme JL, et al. Invasive fungal infection and impaired neutrophil killing in human CARD9 deficiency. Blood 2013;121:2385-92.
51. Gavino C, Cotter A, Lichtenstein D, Lejtenyi D, Fortin C, Legault C, et al. CARD9 deficiency and spontaneous central nervous system candidiasis: complete clinical remission with GM-CSF therapy. Clin Infect Dis 2014;59:81-4.
52. Lanternier F, Barbati E, Meinzer U, Liu L, Pedergnana V, Migaud M, et al. Inherited CARD9 deficiency in 2 unrelated patients with invasive *Exophiala* infection. J Infect Dis 2014 [Epub ahead of print] http://dx.doi.org/10.1093/infdis/jiu412.
53. Praneenararat S. The first reported case of colonic infection caused by *Candida tropicalis* and a review of the literature. Case Rep Gastroenterol 2014;8:199-205.
54. Kudo T, Aoyagi Y, Fujii T, Ohtsuka Y, Nagata S, Shimizu T. Development of *Candida albicans* colitis in a child undergoing steroid therapy for ulcerative colitis. J Pediatr Gastroenterol Nutr 2010;51:96-9.
55. Kitagawa KH, Kalb RE. Efalizumab treatment associated with *Candida* colitis. J Am Acad Dermatol 2008;59(suppl):S120-1.
56. Cimbaluk D, Scudiere J, Butsch J, Jakate S. Invasive candidal enterocolitis followed shortly by fatal cerebral hemorrhage in immunocompromised patients. J Clin Gastroenterol 2005;39:795-7.
57. Kouklakis G, Dokas S, Molyvas E, Vakianis P, Efthymiou A. *Candida* colitis in a middle-aged male receiving permanent haemodialysis. Eur J Gastroenterol Hepatol 2001;13:735-6.
58. Jayagopal S, Cervia JS. Colitis due to *Candida albicans* in a patient with AIDS. Clin Infect Dis 1992;15:555.
59. Prescott RJ, Harris M, Banerjee SS. Fungal infections of the small and large intestine. J Clin Pathol 1992;45:806-11.
60. Nadorra RL, Nakazato Y, Landing BH. Pathologic features of gastrointestinal tract lesions in childhood-onset systemic lupus erythematosus: study of 26 patients, with review of the literature. Pediatr Pathol 1987;7:245-59.
61. Adderson EE, Pappin A, Pavia AT. Spontaneous intestinal perforation in premature infants: a distinct clinical entity associated with systemic candidiasis. J Pediatr Surg 1998;33:1463-7.
62. Wang X, Wang W, Lin Z, Wang X, Li T, Yu J, et al. CARD9 mutations linked to subcutaneous phaeohyphomycosis and TH17 cell deficiencies. J Allergy Clin Immunol 2014;133:905-8.e3.
63. Sokol H, Conway KL, Zhang M, Choi M, Morin B, Cao Z, et al. Card9 mediates intestinal epithelial cell restitution, t-helper 17 responses, and control of bacterial infection in mice. Gastroenterology 2013;145:591-601.e3.

64. Jachiet M, Lanternier F, Rybojad M, Bagot M, Ibrahim L, Casanova JL, et al. Posaconazole treatment of extensive skin and nail dermatophytosis due to autosomal recessive deficiency of CARD9. JAMA Dermatol 2014 [Epub ahead of print] http://dx.doi.org/10.1001/jamadermatol.2014.2154.
65. Gross O, Gewies A, Finger K, Schäfer M, Sparwasser T, Peschel C, et al. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. Nature 2006;442:651-6.
66. Dorhoi A, Desel C, Yeremeev V, Pradl L, Brinkmann V, Mollenkopf HJ, et al. The adaptor molecule CARD9 is essential for tuberculosis control. J Exp Med 2010; 207:777-92.
67. Hsu YM, Zhang Y, You Y, Wang D, Li H, Duramad O, et al. The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. Nat Immunol 2007;8:198-205.
68. Lionakis MS, Holland SM. Human invasive mycoses: immunogenetics on the rise. J Infect Dis 2014 [Epub ahead of print] http://dx.doi.org/10.1093/infdis/jiu411.
69. Alcais A, Quintana-Murci L, Thaler DS, Schurr E, Abel L, Casanova JL. Life-threatening infectious diseases of childhood: single-gene inborn errors of immunity? Ann N Y Acad Sci 2010;1214:18-33.
70. Casanova JL, Abel L. The genetic theory of infectious diseases: a brief history and selected illustrations. Annu Rev Genomics Hum Genet 2013;14:215-43.

METHODS

Patients

We recruited 5 patients with a history of proven meningoencephalitis, colitis, or both caused by *Candida* species (Table I) and no known underlying associated condition. Diagnosis was based on the revised European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group Consensus Group (EORTC/MSG) criteria.[E1] This study was conducted in accordance with the Helsinki Declaration. All patients and their relatives provided written informed consent for participation in the study.

Molecular genetics

Genomic DNA was isolated from whole blood cells. *CARD9* was amplified with specific primers (PCR amplification conditions and primer sequences are available on request). PCR products were sequenced with the Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif) and analyzed on an ABI Prism 3700 apparatus (Applied Biosystems).[E2]

Control subjects

We sequenced exons 2, 3, and 6 of the *CARD9* gene for all 1050 healthy unrelated control subjects from the Human Genome Diversity Cell Line Panel originating from 52 different ethnic groups initially sampled for population genetics studies[E3] and 30 subjects from Iran, 90 from Turkey, and a total of 83 from Algeria.

Whole blood cell and MDDC stimulation

Whole blood was diluted 1:2 and incubated for 24 or 48 hours with RPMI medium alone, zymosan (5 μg/mL), heat-killed *C albicans* (10^6 particles/mL), heat-killed *S cerevisiae* (10^6 particles/mL), heat-killed *E dermatitidis* (10^6 particles/mL), LPS (100 ng/mL), heat-killed *S aureus* (10^7 particles/mL), PMA plus ionomycin (0.2 μg/mL and 2×10^{-4} μg/mL, respectively), VSV (10^6/mL) or BCG for P1, P2, and P5, as well as P2's father and 7 healthy control subjects. Production of IL-6 or TNF-α was assessed by determining the levels of these cytokines in supernatants by means of ELISA, according to the kit manufacturer's instructions (Sanquin, Amsterdam, The Netherlands). Human PBMCs were isolated from whole blood by using Ficoll-Hypaque density gradient centrifugation (Amersham Pharmacia Biotech, Sweden). MDDCs were derived from PBMCs after positive selection of $CD14^+$ cells with CD14 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) by means of differentiation in the presence of GM-CSF (50 ng/mL) and IL-13 (20 ng/mL). After 6 days, 30,000 cells per well were plated in 96-well plates and stimulated for 24 hours with curdlan (25 μg/mL), zymosan (25 μg/mL), heat-killed *S cerevisiae* (10^6 or 10^7 particles/mL), heat-killed *C albicans* (10^6 or 10^7 particles/mL), heat-killed *E dermatitidis* (10^7 particles/mL), heat-killed *S aureus* (2×10^8 particles/mL), and LPS (100 ng/mL) for P1, P2, and P5; P2's father and mother; and 6 healthy control subjects tested in parallel. TNF-α production was evaluated by means of ELISA, according to the manufacturer's instructions.

Flow cytometry

Antibody against human CARD9 (5281; Epitomics, Burlingame, Calif), an isotype control antibody, and a secondary goat anti-rabbit–Alexa Fluor 488 antibody (3064-1, Epitomics) were used in accordance with the manufacturers' protocols.

Cell transfections

Full-length WT *CARD9* cDNA was inserted into a pcDNA3.1 expression vector, such that the protein was produced with a V5 tag at the C-terminus. The c.*G104A* (p.R35Q), c.*C208T* (p.R70W), c.*C865T* (p.Q289*), and c.*C883T* (p.Q295*) mutations were introduced into the cDNA with the QuikChange II XL site–directed mutagenesis kit from Stratagene (La Jolla, Calif; 200522-5), according to the manufacturer's instructions. Plasmids containing the WT or mutated *CARD9* sequences were then amplified and purified with the QIAprep Spin Miniprep Kit from Qiagen (Hilden, Germany; 27106). The resulting plasmids were used to transfect HEK-293T cells in 6 cm-diameter plates, with a calcium phosphate transfection kit (278001; Invitrogen, Carlsbad, Calif) and a cyan fluorescent protein plasmid, according to the manufacturer's instructions.

Western blotting

Total extracts of HEK-293 T cells were prepared 48 hours after transfection. Proteins were separated by means of electrophoresis and transferred to a membrane, which was then probed with anti-V5 (Invitrogen 46-0708), anti–CARD9 H-90 (Sc-99054), anti–cyan fluorescent protein, or anti–glyceraldehyde-3-phosphate dehydrogenase (Sc-25778) antibodies.

NF-κB–luciferase reporter assay

We plated 10^5 HEK-293 cells in Dulbecco modified Eagle medium supplemented with 10% FBS in 96-well plates. These cells were incubated for 6 hours and then transfected in the presence of Lipofectamine LTX with PLUS Reagent (Invitrogen), according to the manufacturer's protocol. The cells were transfected with 6 ng of *DECTIN1*-, *SYK*-, and *BCL10*-expressing pcDNA3 constructs with or without *CARD9* WT or *CARD9* R35Q, R70W, Q289*, or Q295* constructs and with 100 ng of NF-κB–firefly luciferase vector and 40 ng of pRL-SV40 reporter vector used as an internal control and expressing the *Renilla* gene under control of the SV40 promoter. Cells were then left unstimulated or stimulated with 25 μg/mL curdlan or 10^7 particles/mL *S cerevisiae* or *C albicans*. After 24 hours, cells were lysed and both firefly and *Renilla* luciferase activities were determined with the Dual-Glo Luciferase Assay System (Promega, Madison, Wis). Results are expressed as means ± SEMs of the ratio of firefly and *Renilla* luciferase activities adjusted to 1. We used the Student *t* test to determine the significance of differences. Statistics were calculated with GraphPad Prism version 5 software (GraphPad Software, La Jolla, Calif).

IL-17 production

We evaluated IL-17A production using whole blood *ex vivo* after 24 hours of stimulation with PMA/ionomycin using ELISA, according to the manufacturer's recommendations for P1, P2, and P5; P2's father; and 7 healthy control subjects. We also determined the percentages of $CD3^+$/$IL\text{-}17A^+$ cells using flow cytometry after 12 hours of stimulation with PMA/ionomycin, as previously described,[E4] for P1, P2, and P5, as well as P2's father and 10 healthy control subjects.

REFERENCES

E1. Wang X, Wang W, Lin Z, Wang X, Li T, Yu J, et al. CARD9 mutations linked to subcutaneous phaeohyphomycosis and TH17 cell deficiencies. J Allergy Clin Immunol 2014;133:905-8.e3.
E2. Glocker EO, Hennigs A, Nabavi M, Schäffer AA, Woellner C, Salzer U, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. N Engl J Med 2009;361:1727-35.
E3. Sokol H, Conway KL, Zhang M, Choi M, Morin B, Cao Z, et al. Card9 mediates intestinal epithelial cell restitution, t-helper 17 responses, and control of bacterial infection in mice. Gastroenterology 2013;145:591-601.e3.
E4. de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M, et al. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. J Exp Med 2008;205:1543-50.

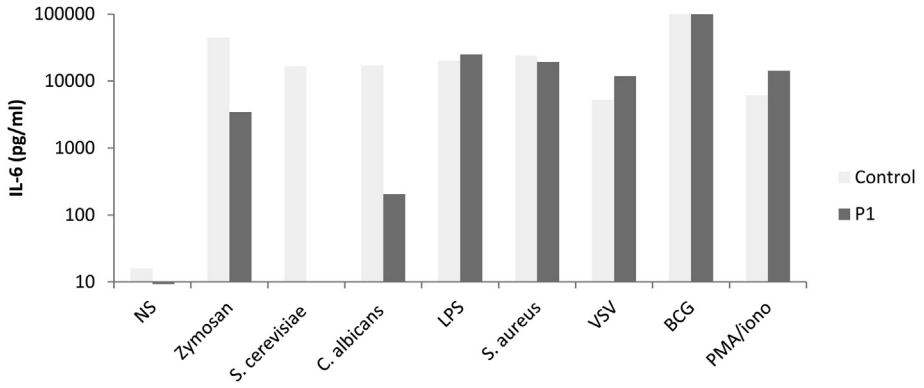

FIG E1. IL-6 production by whole blood cells after 48 hours of stimulation with zymosan, heat-killed *C albicans*, *S cerevisiae*, LPS, heat-killed *S aureus*, VSV, BCG, and PMA plus ionomycin. *NS*, Not stimulated.