Inherited CARD9 Deficiency in 2 Unrelated Patients With Invasive *Exophiala* Infection

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(See the editorial commentary by Lionakis and Holland on pages 1205–7.)

Background. *Exophiala* species are mostly responsible for skin infections. Invasive *Exophiala dermatitidis* disease is a rare and frequently fatal infection, with 42 cases reported. About half of these cases had no known risk factors. Similarly, invasive *Exophiala spinifera* disease is extremely rare, with only 3 cases reported, all in patients with no known immunodeficiency. Autosomal recessive CARD9 deficiency has recently been reported in otherwise healthy patients with severe fungal diseases caused by *Candida* species, dermatophytes, or *Phialophora verrucosa*.

Methods. We investigated an 8-year-old girl from a nonconsanguineous Angolan kindred, who was born in France and developed disseminated *E. dermatitidis* disease and a 26 year-old woman from an Iranian consaguineous kindred, who was living in Iran and developed disseminated *E. spinifera* disease. Both patients were otherwise healthy.

Results. We sequenced *CARD9* and found both patients to be homozygous for loss-of-function mutations (R18W and E323del). The first patient had segmental uniparental disomy of chromosome 9, carrying 2 copies of the maternal *CARD9* mutated allele.

Conclusions. These are the first 2 patients with inherited CARD9 deficiency and invasive *Exophiala* disease to be described. CARD9 deficiency should thus be considered in patients with unexplained invasive *Exophiala* species disease, even in the absence of other infections.

Keywords. *Exophiala* species; invasive fungal infection; central nervous system; osteomyelitis; autosomal recessive CARD9 deficiency; parental unidisomy.

Exophiala dermatitidis, previously known as *Wangiella dermatitidis,* is an environmental black yeast [1, 2]. In Western countries, *E. dermatitidis* has been reported to colonize the respiratory tract of about 6% of patients with cystic fibrosis [3] and to be present in the stools of 0.5% of patients with diarrhea [4]. By contrast, invasive *E. dermatitidis* disease (ie, noncontiguous

extracutaneous localization) is rare. Since 1959, only 42 cases have been reported worldwide, and for 23, no risk factors were identified (Table 1) [5–18]. *Exophiala spinifera* is rare, representing 2.7% of the *Exophiala* species among clinical isolates [1, 2]. Only 29 cases of *E. spinifera* diseases have been reported in the literature [19–24]). Only 3 patients, none of whom had any known risk factors, had invasive infection (ie, noncontiguous extra cutaneous localization) [13, 23, 24]. We describe 2 patients with isolated, unexplained invasive fungal disease caused by *E. dermatitidis* or *E. spinifera*. Because autosomal recessive (AR) CARD9 deficiency has recently been reported in 30 otherwise healthy patients from Iran, North Africa, Asia, and Canada with invasive fungal diseases [25–29], we sequenced *CARD9* in the patients.

CASE SUMMARY

The first patient studied was an 8-year-old girl (Figure 1A) born in France to nonconsanguineous parents originating from Angola. At the age of 5 years, she developed liver and brain *E. dermatitidis* infection (Figure 1B and 1C). *E. dermatitidis* grew in cultures and was identified on the basis of its phenotype, and this identification was confirmed by internal transcribed spacer (ITS) sequencing. Her case report is detailed in the Supplementary Materials. The patient was treated by irrigation of the biliary tract with amphotericin B, a high intravenous dose of liposomal amphotericin B, and oral voriconazole. A favorable outcome was achieved, but despite this antifungal maintenance treatment, the patient developed *E. dermatitidis* pachymeningitis (Figure 1D and Figure 2A–D). This patient had never had any other severe infectious disease. In addition, neither her parents nor her 2 older brothers, aged 13 and 16 years, had had any severe infectious disease.

Identification of a Homozygous *CARD9* Missense Mutation

The coding exons of *CARD9* were sequenced by the Sanger method. Patient 1 displayed a homozygous $c.52C > T$ missense mutation in exon 2 of this gene, resulting in the replacement of the arginine in position 18 with a tryptophan residue (R18W), located in the N-terminal caspase-recruitment domain (CARD). The patient's healthy mother and her 2 brothers were heterozygous for the mutation (WT/R18W; Figure 1A). The patient's father could not be tested. An Affymetrix genome-wide human SNP Array 6.0 analysis combined to a CGH array analysis (Supplementary Materials) of the patient's DNA demonstrated that the patient had a segmental uniparental disomy (UPD) of chromosome 9, resulting in the carriage of 2 copies of the maternal *CARD9* allele. The segregation of the mutation was consistent with AR CARD9 deficiency and complete clinical penetrance. The mutation reported here was not found in any of the various public databases or in any of the controls sequenced (Supplementary Materials). This mutation

was predicted to be probably damaging (with the highest possible score of 1), by PolyPhen 2, and as damaging, by SIFT (with a score of 0). Collectively, these data strongly suggested that the patient was homozygous for a rare, deleterious *CARD9* missense mutation.

### The R18W Mutant CARD9 Protein Is Produced but Has Lost Its Function

We evaluated the consequences of the mutation at the CARD9 protein level, by Western blot analysis on whole-cell extracts of HEK-293 T cells transfected with a pcDNA3.1 V5 (C-terminally tagged) plasmid with no insert or carrying the WT or R18W allele of CARD9 (pcDNA3.1 V5 CARD9 WT and pcDNA3.1 V5 CARD9 R18W). Cells transfected with the CARD9 R18W allele had slightly higher CARD9 protein levels than those transfected with the WT allele, and the protein detected was of similar molecular weight (Figure 3A). Flow cytometry analysis of CARD9 protein in monocytes (Supplementary Figure A) and monocyte-derived macrophages (MDMs; Figure 3B) from the patient showed that the levels of this protein were comparable to those in monocytes and MDMs from a healthy control. We evaluated the functional consequence of this mutation by studying interleukin 6 (IL-6) production by whole-blood cells after 24 hours of stimulation with zymosan (an agonist of at least Dectin-1 and Toll-like receptor 2), heat-killed *Saccharomyces cerevisiae*, *Candida albicans*, *E. dermatitidis*, and *Staphylococcus aureus*, vesicular stomatitis virus (VSV), lipopolysaccharide (LPS; a TLR4 agonist), and PMA plus ionomycin. The cells from patient 1 produced much smaller amounts of IL-6 after stimulation with *S. cerevisiae, E. dermatitidis,* and, to a lesser extent, *C. albicans*, than cells from 6 healthy controls or the heterozygous mother tested in parallel (Figure 3C). The responses to zymosan, LPS, *S. aureus*, VSV, and PMA plus ionomycin were normal. We then assessed TNF-α production by monocyte-derived dendritic cells (MDDCs) obtained from the patient and 7 healthy controls and stimulated for 24 hours with curdlan (a dectin-1 agonist) and the same agonists used in the whole-blood assay (Figure 3D). The patient displayed a strong impairment of TNF-α production after stimulation with all fungal ligands used (curdlan, zymosan, heat-killed *S. cerevisiae*, *C. albicans,* and *E. dermatitidis*), whereas TNF-α production was similar to that in healthy controls after stimulation with *S. aureus* and LPS. In addition, 293 HEK cells transfected with the R18W CARD9 allele showed a strongly impaired NF-κB transcriptional activity, as assessed by NF-κB–luciferase reporter assays, compared with cells transfected with the WT CARD9 allele, either at the basal level or after stimulation with curdlan or *E. dermatitidis* (Figure 3E). Finally, we evaluated the proportion of ex vivo interleukin 17A (IL-17A)–producing T cells by flow cytometry, as low proportions of IL-17 T cells have been reported in some CARD9-deficient patients [25–27, 29]. No differences were observed between

Table 1. Demographic and Clinical Characteristics of Patients With Invasive *Exophiala dermatitidis* Infection Described in Previous Studies and the Patient Studied Here

Characteristic	Age, y (Sex)	Location Brain	Location Liver	Location Other	Outcome (Time From Diagnosis to Death, mo)	Reference
Patients without known risk factors						
Country of origin						
South Korea	11 (F)	No	Yes	No	Death (14)	[9]
South Korea	28 (M)	Yes	No	No	Death (0.5)	[10]
Turkey	8 (M)	No	Yes	LN	Death (7)	[17]
Turkey	24 (F)	No	Yes	LN	Cure	[11]
China	3 (M)	Yes	No	No	Death (4)	[12]
China	19 (F)	Yes	No	LN	Death (5)	[13]
China	30 (F)	Yes	No	No	Death (0.1)	[13]
China	3 (F)	Yes	No	No	Death (6)	[13]
China	19 (F)	Yes	No	LN	Death (1)	[8]
China	21 (F)	No	Yes	LN	Cure	[7]
Japan	25 (F)	No	No	Palate, LN	Death (36)	[6]
Japan	30 (F)	Yes	No	LN	Death (3)	[6]
Japan	11 (M)	No	Yes	LN, dig	Death (ND)	[6]
Japan	14 (F)a	No	Yes	No	Death (2)	[6]
Japan	5 (F)a	Yes	No	No	Death (1)	[6]
Japan	NA (F)	Yes	No	No	Death (ND)	[6]
Japan	26 (F)	Yes	No	No	Death (9)	[6]
Japan	19 (M)a	Yes	Yes	LN	Death (2)	[6]
Japan	NA (M)a	Yes	Yes	NA	Death (ND)	[6]
Japan	10 (M)	Yes	No	Lung	Death (11)	[6]
Japan	20 (M)	Yes	No	LN	Death (NA)	[6]
Japan	17 (M)	Yes	Yes	LN	Death (48)	[6]
Pakistan	70 (M)	Yes	No	No	Death (0.5)	[18]
Patients with predisposing factors other than PID						
Predisposing factor						
Contaminated steroid use	77 (F)	Yes	No	No	Death	[5]
Contaminated steroid use	61 (F)	Yes	No	No	Cure	[5]
Contaminated steroid use	71 (F)	Yes	No	No	ND	[5]
Contaminated steroid use	65 (F)	Yes	No	No	ND	[5]
Contaminated steroid use	52 (F)	Yes	No	No	ND	[5]
Bronchiectasis	81 (M)	No	No	Lung	Cure	[15]
Lung cancer, catheter use	58 (F)	No	No	Blood	Cure	[5]
CAPD, catheter use	55 (M)	No	No	Peritoneal	Cure	[5]
CAPD, catheter use	39 (M)	No	No	Peritoneal	Cure	[5]
ALL	62 (F)	No	No	LN	Cure	[5]
Cystic fibrosis	29 (F)	No	No	Lung	Cure	[5]
Cystic fibrosis	6 (F)	No	No	Lung	Cure	[5]
Cystic fibrosis	54 (F)	No	No	Lung	ND	[5]
AIDS, catheter use	3 (M)	No	No	Blood	Cure	[5]
Parenteral nutrition, catheter use	53 (F)	No	No	Blood	Cure	[5]
ALL, catheter use	5 (M)	No	No	Blood	Cure	[5]
Injection drug use	63 (M)	No	No	Endocarditis	Death	[5]
Solid organ transplantation	50 (F)	No	No	Endocarditis	ND	[5]
Patients with PID						
CGD	21 (F)	Yes	No	Lung	Cure	[14]
CARD9 deficiency	6 (F)	Yes	Yes	No	Relapse	This report

Abbreviations: AIDS, acquired immunodeficiency syndrome; ALL, acute lymphoblastic leukemia; CAPD, continuous ambulatory peritoneal dialysis; CGD, chronic granulomatous disease; dig, digestive tract; F, female; LN, lymph node; M, male; mo, months; NA, not available; ND, not determined; PID, primary immunodeficiency disease.

a Multiplex family.
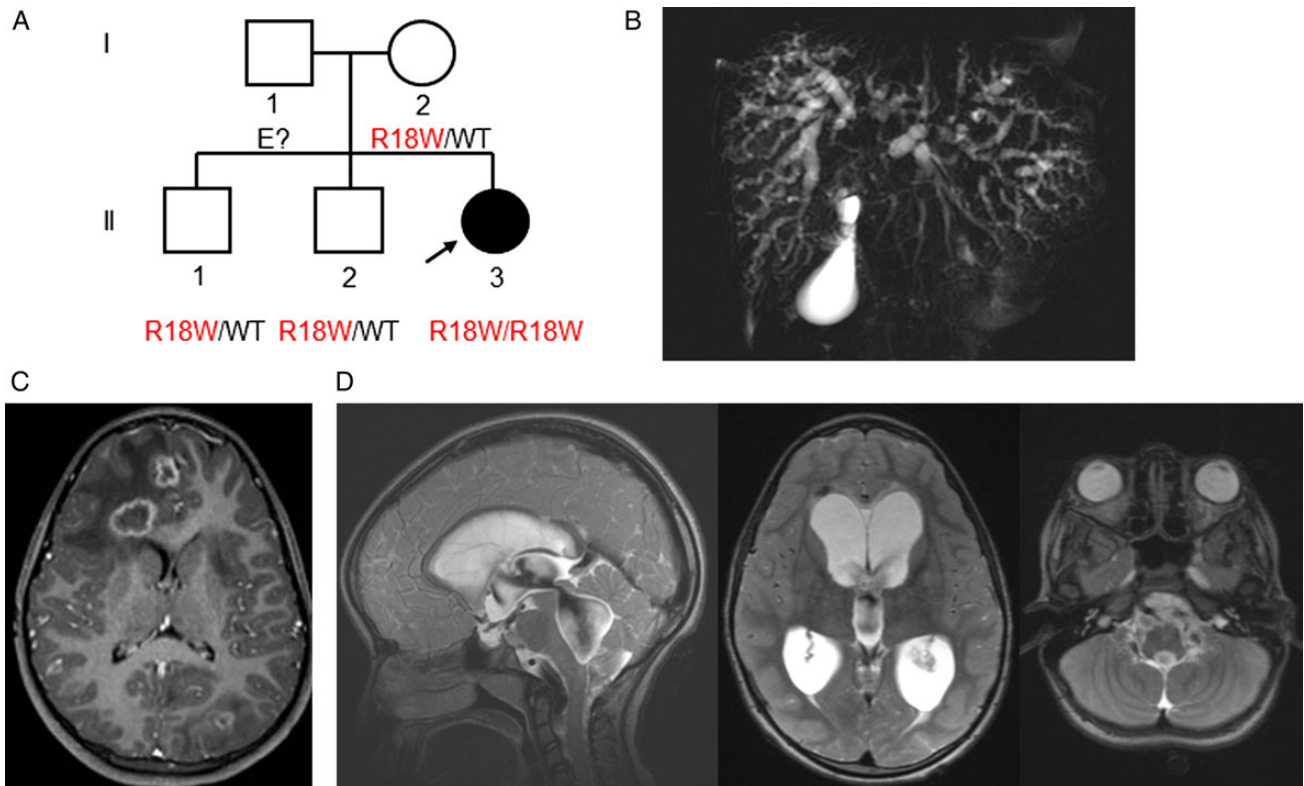
Figure 1. *A*, Pedigree of the patient with *Exophiala dermatitidis* infection and a *CARD9* mutation. Each generation is represented by a Roman numeral, and each individual is represented by an Arabic numeral. The proband with *E. dermatitidis* infection is shown in black and indicated by an arrow. The *CARD9* genotype is indicated below each individual. *B–D*, Radiological features of patient 1. *B*, Cholangio-magnetic resonance imaging showing a dilated and irregular biliary tree with diffuse biliary duct infiltration. *C*, Brain magnetic resonance imaging (MRI) showing 13 cerebral lesions (diameter, 10–16 mm). *D*, Brain MRI showing pachymeningitis and hydrocephalus. Abbreviation: WT, wild-type.

the patient and the 7 healthy controls tested in parallel (Supplementary Figure B). Furthermore, IL-17A production by whole-blood cells after 24 hours of stimulation with PMA and ionomycin, as measured by ELISA, was similar in the patient and the 2 healthy controls tested in parallel (Supplementary Figure B). We therefore conclude that the homozygous R18W mutation led to the production of normal amounts of a loss-of-function CARD9 protein, leading to the impairment of proinflammatory cytokine production by whole-blood cells and MDDCs after stimulation with various fungal ligands and impairment of NF-κB transcriptional activity in transfected HEK cells, whereas IL-17 T-cell production was normal.

### Autosomal Recessive CARD9 Deficiency in Another Patient With Invasive *E. spinifera* Infection

We recently studied a 26-year-old woman (Figure 4*A*) from an Iranian consanguineous kindred living in Iran who presented recurrent fungal infections from the age of 18 years onward, with *E. spinifera* subcutaneous (Figure 4*B*), bone (Figure 4*C*), and lung (Figure 4*D*) infection. *E. spinifera* grew in cultures and was identified on the basis of its phenotype, and this

identification was confirmed by ITS sequencing. The case report is detailed in the Supplementary Materials. The patient's parents and siblings were all healthy and had never had a severe infectious fungal disease. The sequencing of *CARD9* in the patient's DNA showed a homozygous in-frame deletion, *c. GAG967-969del* or p.E323del, located in the coiled-coil domain of CARD9. This deletion was not found in any of the various public databases or in any of the controls sequenced (Supplementary Materials). Collectively, these data strongly suggest that this patient is homozygous for a rare, deleterious *CARD9* deletion mutation. Parental DNA testing was possible only for the father, who was found to be heterozygous for the mutation.

DISCUSSION

We identified a homozygous R18W *CARD9* mutation in a child with cholangitis and central nervous system (CNS) infection caused by *E. dermatitidis*. We showed that this was a loss-of-function mutation and that the child had functional CARD9 deficiency. The mutation was homozygous because of segmental UPD of the maternal chromosome 9q, which harbored the
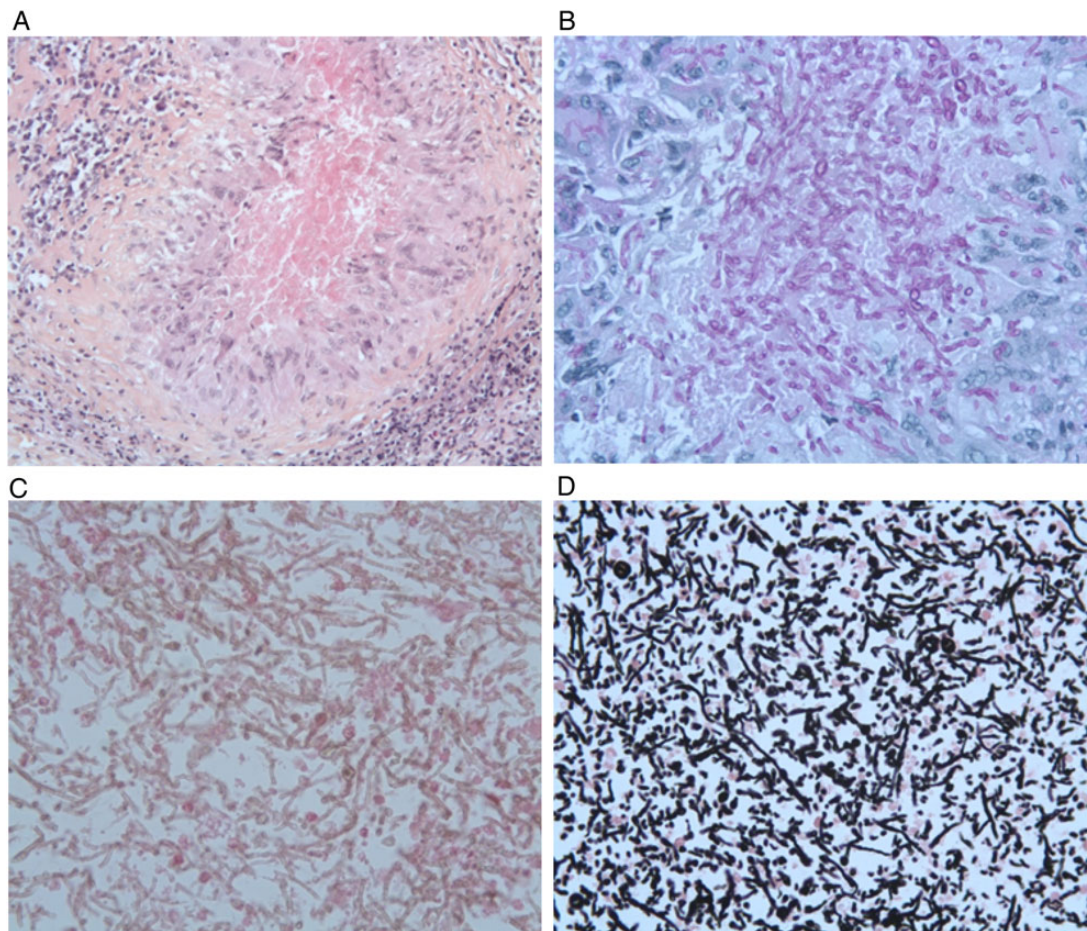
Figure 2. *A–D*, Histological features of the meningeal biopsy specimen. *A*, Hematoxylin-eosin (H-E) staining showing fungi surrounded by an epithelioid and giant cell granuloma (original magnification ×200). *B*, Strong periodic acid-Schiff staining of the fungi within the granuloma (original magnification ×400). *C*, H-E staining revealing a huge infiltration of fungi without granuloma in another area of the biopsy sample (original magnification ×400). *D*, Grocott staining of this large area of fungi (original magnification ×400).

mutant R18W *CARD9* allele. The segmental UPD accounts for the autosomal recessive form of primary immunodeficiency disease (PID) in this patient, with the *CARD9* germline mutation being present in a heterozygous state in the mother. UPD is defined as the inheritance of a pair of duplicated chromosomes from a single parent, and it is associated with 2 main types of developmental risk: the occurrence of imprinting disorders and the inheritance of a recessive trait [30]. Some autosomal recessive PIDs due to UPD have already been reported, such as interferon γR1 deficiency (Mendelian susceptibility to mycobacterial disease), perforin deficiency (familial hemophagocytic lymphohistiocytosis type 2), lysosomal trafficking regulator deficiency (Chediak-Higashi syndrome), or T-lymphocyte–specific protein tyrosine kinase deficiency [31–34]. Patients with these conditions often display other clinical signs unrelated to the mutated PID-causing gene, but due to the extent of the UPD [31]. UPD of chromosome 9 region 9q34 has already been reported for the homozygous sea urchin retroposon family

1 mutation responsible for Leigh syndrome, a mitochondrial subacute necrotizing encephalomyelopathy [35]. Other homozygous variants of the segment of chromosome 9 that the patient inherited from her mother may have clinical impacts that are not immediately evident.

Among the 42 cases reported with invasive *E. dermatitidis* disease, a risk factor was identified for 19, while the other 23 cases remained unexplained (Table 1) [5–13, 16–18]. These 23 patients belonged to 21 families, all from Asia. Twelve patients came from 2 multiplex families from Japan, 3 patients were from South Korea, 5 were from China, 1 was from Pakistan, and 2 were from Turkey [10, 12, 17, 18, 36–40]. Consanguinity was not reported in these families. The clinical outcome was poor, particularly in patients with no known risk factors. In these patients, the disease typically affected the brain and/or liver (Table 1) [5–13, 16, 17], with onset at a median age of 19 years [range, 3–70 years]. For the patients who are known to have an immunodeficiency disease or who are receiving
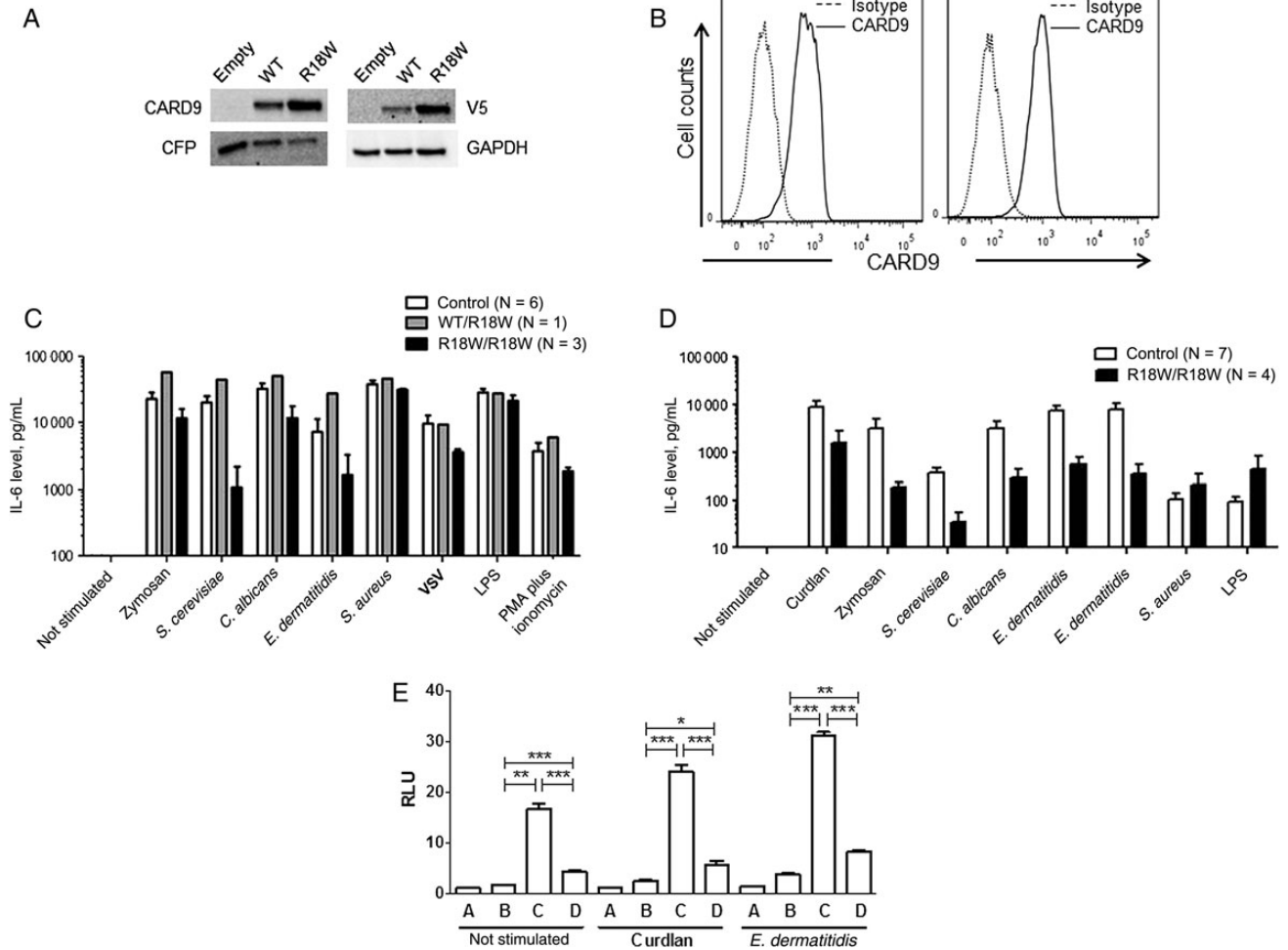
Figure 3. Impact of the *CARD9* R18W mutation on protein level and function. *A*, Immunoblot analysis of CARD9 in whole-cell extracts of HEK-293T cells cotransfected with pcDNA3.1 V5 (C-terminally tagged), either empty or carrying the wild-type (WT) or mutant (R18W) *CARD9* allele, together with a CFP plasmid, as a transfection control. Antibodies against CARD9, V5, CFP, and GAPDH (as a loading control) were used. *B*, Flow cytometry analysis of CARD9 in monocyte-derived macrophages from a control (left panel) or the patient (right panel). *C*, Interleukin 6 (IL-6) production by whole-blood cells, as measured by enzyme-linked immunosorbent assay (ELISA), after 24 hours of stimulation with zymosan (5 µg/mL), heat-killed *Saccharomyces cerevisiae* (10^6 particles/ mL), heat-killed *Candida albicans* (10^6 particles/mL), heat-killed *Exophiala dermatitidis* (10^6 particles/mL), heat-killed *Staphylococcus aureus* (5 × 10^8 particles/mL), vesicular stomatitis virus (VSV), lipopolysaccharide (LPS; 100 ng/mL), and PMA plus ionomycin for 6 controls, the *CARD9*-heterozygous (R18W/ WT) mother, and the *CARD9*-homozygous (R18W/R18W) patient. Results are expressed as mean ± standard error of the mean (SEM) of 3 independent experiments. *D*, Tumor necrosis factor α (TNF-α) production by monocyte-derived dendritic cells, measured by ELISA, after 24 hours of stimulation with curdlan (25 µg/mL), zymosan (25 µg/mL), heat-killed *S. cerevisiae* (10^6 particles/mL), heat-killed *C. albicans* (10^6 particles/mL), heat-killed *E. dermatitidis* (10^7 particles/mL and 10^6 particles/mL), heat-killed *S. aureus* (5 × 10^8 particles/mL), and LPS (100 ng/mL) for 7 controls and the *CARD9*-homozygous (R18W/ R18W) patient. Results are expressed as mean ± SEM of 4 independent experiments. *E*, The CARD9 R18W allele impairs downstream NF-κB activation. NF-κB–luciferase assay in 293 HEK cells transfected with NF-κB–luciferase and pRL-SV40 vectors alone (*A*); with *DECTIN1*, *SYK*, and *BCL10* constructs (*B*); with *DECTIN-1*, *SYK*, *BCL10*, and *CARD9 WT* constructs (*C*); and with *DECTIN-1*, *SYK*, *BCL10*, and *CARD9 R18W* constructs (*D*). Cells were stimulated or not stimulated with 25 µg/mL curdlan or 10^7 particles/mL *E. dermatitidis*. Results are representative of 2 independent experiments performed and are expressed as mean ± SEM of the ratio between *Renilla* luciferase and firefly control luciferase activities adjusted to 1. *$P \leq .05$, **$P \leq .01$, and ***$P \leq .001$ by the Student *t* test. Abbreviation: RLU, relative light units.

immunosuppressive treatment, brain involvement was only reported in the patient with chronic granulomatous disease [14] and in 5 patients with meningitis secondary to the intrathecal injection of contaminated steroids [41]. *E. dermatitidis* has been shown to be neurotropic in a mouse model [42]. However, the

absence of brain involvement in most of the patients with immunodeficiency disease and *E. dermatitidis* infection suggests that predisposing genetic factors are also required for *E. dermatitidis* infection of the CNS in humans. None of the patients with a known immunodeficiency disease had liver involvement.
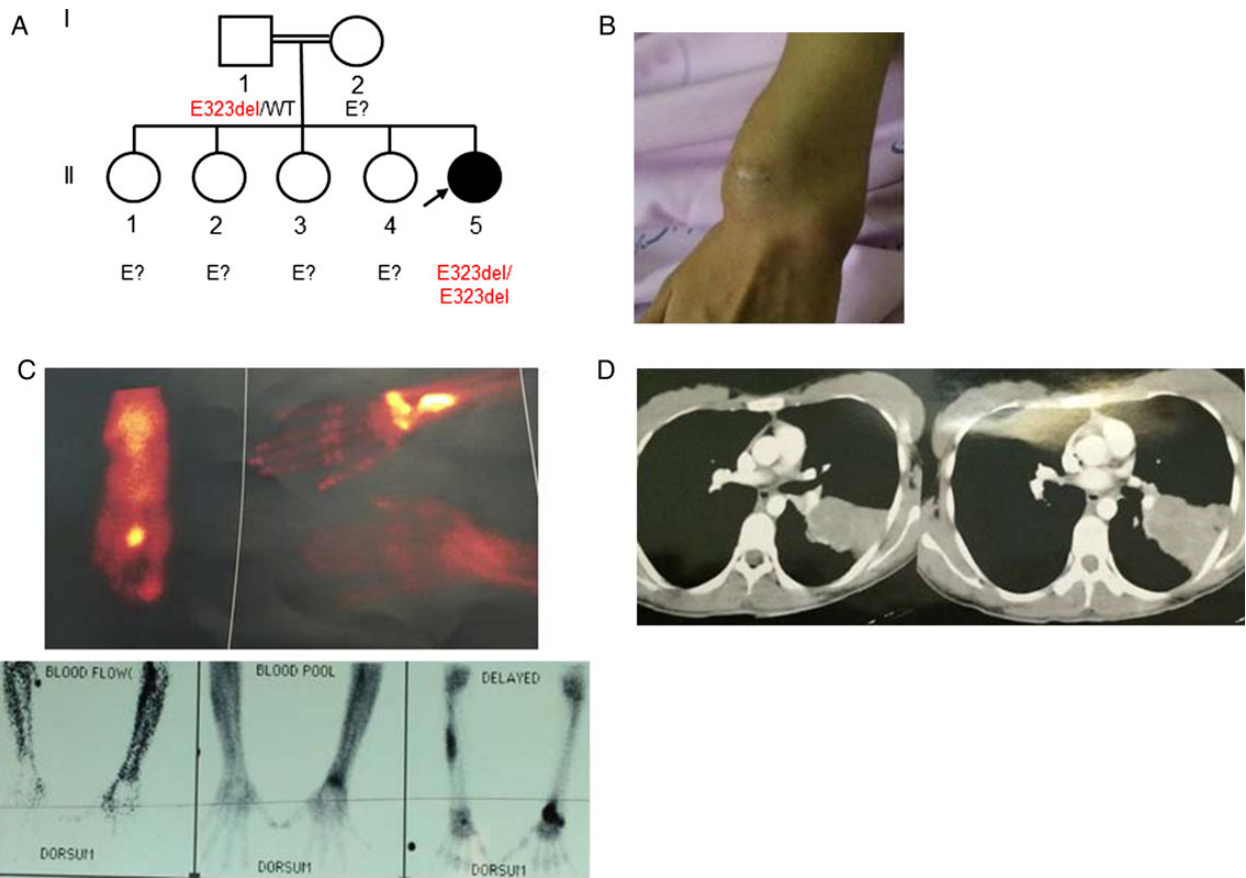
Figure 4. *A*, Pedigree of the patient with *Exophiala spinifera* infection and a *CARD9* mutation. Each generation is represented by a Roman numeral, and each individual is represented by an Arabic numeral. The proband with *E. spinifera* infection is shown in black and indicated by an arrow. The *CARD9* genotype is indicated below the individuals sequenced. *B–D*, Clinical and radiological features of the patient. *B*, Subcutaneous skin infection. *C*, Hyper-fixation revealing osteomyelitis on scintigraphy. *D*, Computed tomography of the chest. Abbreviation: WT, wild type.

Mortality in the group of patients without known risk factors was much higher than that in patients with known risk factors (91% vs 11%).

E. spinifera infections are also very rare. To date, 29 cases of *E. spinifera* infections have been reported in the literature, 3 corresponding to chromoblastomycosis and 26 to phaeohyphomycosis [19–22]. The diseases were cutaneous for all patients, with frequent subcutaneous and bone extension. Among the 26 patients with phaeohyphomycosis, only 3 without known immunodeficiency disease had invasive infections with lymph node involvement or positive blood culture results [13, 23, 24]. *E. spinifera* infections reported in children without any known risk factors usually started as subcutaneous abscesses and frequently disseminated, with a poor outcome [19–24]. In contrast, the 23 *E. spinifera* infections reported in adults were usually localized and occurred in patients with known risk factors, such as systemic lupus erythematous, corticosteroid treatments, solid organ transplant receipt, or cancer [19–22]. Patients with *E. spinifera*–related phaeohyphomycosis were from China (n = 5),

Brazil (n = 2), India (n = 8), El Salvador (n = 1), Pakistan (n = 1), the United States (n = 5), Argentina (n = 1), Japan (n = 1), and France (n = 2). The 3 patients with invasive *E. spinifera* disease were a 9-year-old boy from China who developed fungemia associated with subcutaneous and bone infections, leading to his death [13], and a 12-year-old girl and 41-year-old woman from India and Argentina, respectively, who had lymph node and subcutaneous infections [23, 24].

The similarity of clinical presentation between the 2 patients reported here and the patients with idiopathic *E. dermatitidis* or *E. spinifera* disease reported in the literature suggests that some of these patients may have had CARD9 deficiency. Furthermore, the identification of multiplex families is also consistent with this hypothesis [6]. The identification of CARD9 deficiency in an Angolan patient homozygous for the CARD9 R18W allele and with isolated invasive *E. dermatitidis* disease and in an Iranian patient homozygous for the CARD9 E323del mutation and with recurrent invasive *E. spinifera* disease broadens the spectrum of invasive fungal diseases known to be

associated with CARD9 deficiency. The previously reported patients with other *CARD9* mutations displayed other invasive fungal infections. Three patients from a consanguineous Iranian kindred homozygous for the Q295X CARD9 allele [26], 1 Korean girl compound heterozygous for the CARD9 G72S and R373S alleles [27], and 1 French-Canadian male patient homozygous for the CARD9 Y91H allele [28] had *Candida* species infections of the CNS (all had meningitis/meningoencephalitis and 2, the Korean girl and an Iranian girl, also had brain abscesses). In addition, 6 members of the Iranian kindred were reported to have chronic mucocutaneous candidiasis, and 3 were found to have superficial dermatophytosis [26]. Two related Moroccan patients homozygous for the CARD9 R101C allele and 15 North African patients (11 from 5 Algerian kindreds and 4 patients from 2 Tunisian kindreds) homozygous for the CARD9 Q289X allele developed deep dermatophytosis [25]. Finally, 4 unrelated Chinese patients, 1 with compound heterozygous mutations (*c.191-192insTGCT* encoding L64fsX59 and *c.472C > T* encoding Q158X) and 3 with the same homozygous frameshift mutation (*c.819-820insG* encoding D274fsX60) were reported to have subcutaneous phaeohyphomycosis caused by *Phialophora verrucosa* [29].

The identification and characterization of 32 patients from 17 families in 8 countries and with 11 different morbid alleles indicate that CARD9 deficiency is associated with invasive fungal infection due to at least 4 genera (*Candida* species*, Exophiala* species, *P. verrucosa*, *Trichophyton* species), with a tropism for the CNS [25–29] (this report). CARD9 deficiency may thus be associated with a specific predisposition to fungal infections of the CNS. As an initial hypothesis, we suggest that CARD9-deficient patients may display an impairment of fungal elimination by monocytes, macrophages, and/or microglial cells at the blood-brain barrier [43]. On the other hand, CARD9-deficient cells have been shown to require fungal opsonization by serum for the efficient killing of fungi [27]. The limited access of plasma proteins to the CNS results in fungi not being opsonized, potentially accounting for the impairment of fungal killing specifically in the CNS of CARD9-deficient patients [27]. No other severe infections have been reported in these patients, with a particular absence of reported infections due to *Aspergillus* species, bacteria, and parasites. It is also intriguing that the CARD9-deficient patients identified to date, including in this report, have each displayed a single type of invasive fungal disease [25–29].

The diverse and expanding clinical presentations of CARD9 deficiency suggest that this protein may control multiple molecular pathways in multiple cell types involved in immunity to various fungi. Interestingly, like patients homozygous for the R101C or Q289X allele [25], the patient homozygous for the R18W *CARD9* allele displayed impaired production of both IL-6 by whole-blood cells and TNF-α by MDDCs in response to stimulation with fungal ligands (heat-killed *C. albicans* and *S. cerevisiae,* curdlan, and zymosan). In addition, whole-blood

cells and MDDCs also displayed impaired responses to *E. dermatitidis*, potentially accounting for the susceptibility of the patient. Similarly, macrophages and dendritic cells from patients with subcutaneous phaeohyphomycosis and the L64fsX59/Q158X or the D274fsX60 *CARD9* alleles displayed impaired IL-6, TNF-α, interleukin 1β (IL-1β), and interleukin 23 production in response to *P. verrucosa* stimulation [29], and peripheral blood mononuclear cells from the patient with *Candida dubliniensis* meningoencephalitis and the G72S/R373P *CARD9* alleles showed impaired IL-6 and IL-1β production in response to *C. albicans*. All these *CARD9* alleles are deleterious for at least some leukocyte and dendritic cell responses. In addition, there seems to be a correlation between the in vitro defect and the clinical presentation. However, we hypothesize that *CARD9* mutations confer a predisposition to infections caused by dermatophytes, *Candida* species, *P. verrucosa,* or *Exophiala* species, with the phenotype depending on the nature and amount of the infecting fungus.

The R18W mutation affects the CARD domain of the protein, which is known to create heterodimers with the CARD domain of BcL10. It may therefore impair this interaction, decreasing downstream NF-κB activation and target gene transcription [44]. In particular, patients with CARD9 deficiency have been reported to have low proportions of IL-17 T cells, probably resulting from impaired IL-17–inducing cytokine production [25–27, 29]. However, patient 1 (patient 2 could not be tested) had a normal proportion of these cells, consistent with her lack of mucocutaneous candidiasis [45–47]. Similar observations have recently been reported for another patient homozygous for the Y91H CARD9 allele with recurrent *C. albicans* meningoencephalitis [28]. These findings suggest that poor IL-17 T-cell development is not an intrinsic feature, or at least not a general feature, of human CARD9 deficiency. Finally, there seems to be no correlation between the type of mutation and the type of fungal disease. Indeed, we recently identified a patient with CNS candidiasis bearing the homozygous Q289X *CARD9* mutation (Lanternier et al, unpublished data), an allele previously identified in patients with deep dermatophytosis [25]. Our findings indicate that CARD9 deficiency should be considered as a possible diagnosis in children and young adults with disseminated, isolated, unexplained *Exophiala* disease and in cases of fungal infection of the CNS. More generally, this study suggests that idiopathic invasive fungal infections should lead to a search for underlying inborn errors of immunity [48, 49].

Supplementary Data

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