Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary appendix

Supplement to the manuscript: « Deep dermatophytosis and inherited CARD9 deficiency » by Lanternier F, Pathan S, Vincent QB *et al.*

Contents

1. Patients and case reports

Patients

This study was conducted in accordance with the Helsinki Declaration. The research protocol was approved by the French Ethics committee and registered under number ID-RCB 2010 A00636-33. The patients were included in accordance with French National Health Agency (AFSSAPS) rules, with registration under number B100712-40. All patients provided written informed consent for participation in the study.

Seventeen otherwise healthy patients with a history of deep dermatophytosis and no known acquired immunodeficiency (HIV or solid organ transplantation) were recruited from six hospitals (Necker-Enfants Malades, Paris, France; Mustapha Hospital, Algiers, Algeria; Tlemcen Hospital, Tlemcen, Algeria; Sidi Bel-Abbes Hospital, Sidi Bel-Abbes, Algeria; Erasme Hospital, Brussels, Belgium; Farhat Hached Hospital, Sousse, Tunisia). Diagnosis was based on medical and family history, clinical signs, histological and mycological results. All patients displayed first symptoms in childhood or early adulthood (at ages ranging from 2 to 21 years). They did not suffer from any other severe and/or recurrent infections (bacterial, mycobacterial or viral). Following the 2008 EORTC/MSG criteria,[1] we defined deep dermatophytosis as an infection caused by dermatophyte fungi characterized by (i) the presence of Grocott-positive hyphae compatible with dermatophytes in lymph nodes, and/or (ii) lymph node culture positive for dermatophytes, and/or (iii) the presence of Grocott-positive hyphae compatible with dermatophytes in the dermis or the hypodermis on skin biopsy and/or (iv) the presence of Grocott-positive hyphae compatible with dermatophytes in deep organs (bone, digestive tract, central nervous system). All the patients described here met the criteria for idiopathic deep dermatophytosis. The deep dermatophytosis of these patients was clearly an invasive fungal disease, as we documented typical pathological

lesions, with fungal elements in multinucleate giant cells in the mid and deep dermis (Fig. 2A). Skin culture and PCR were positive for dermatophytes (*T. rubrum* or *T. violaceum*) and histological results were unequivocal, resulting in the confirmation of infection according to the EORTC/MSG definition consensus.[1] Dermatophytic invasion of the lymph nodes and organs has been described before, in well documented case reports.[2, 3] One of the patients (Patient 2) described here had a probable brain infection. This patient died and no post mortem histological examination could be performed. Another extra-dermatological lesion was also observed, in the form of granulomatous lymphadenopathies with hyphae in four patients (Patients 1, 2, 3, and 17). Overall, the patients with CARD9 deficiency described here clearly had deep dermatophytosis. The patients with *CARD9* mutations and deep dermatophytosis reported here had clinical characteristics similar to the patients with deep dermatophytosis with unknown genetic etiology reported in previous studies.[4-10] Disease started in childhood (11 years in previous studies vs. 8 years in our series) with severe and recurrent tinea capitis (51.7% vs. 82%) or tinea corporis (41% vs. 59%). The frequency of onychomycosis as a presenting symptom was higher in our study than in previous publications (87% vs. 7%).[11] Dissemination was frequent, with lymph node (58% vs. 59% in our series) or central nervous system (9% vs. 6%) involvement.[11] Bone involvement was less common, with five cases reported in total. [2, 3, this report] Histological findings were also similar, including the formation of tuberculoid granulomas containing hyphae. *T. violaceum* was the most prevalent species isolated, both in previous studies (29%) and in our series (77%). *T. violaceum* is also the species most frequently isolated from patients with dermatophytosis in North Africa (44% in Libya).[12] Two discrepancies between our findings for these patients and published results were identified: a higher frequency of oral candidiasis (35% in the cases reported here, whereas no cases were reported among the other patients), probably reflecting an acquisition bias, and lower mortality (29% in our series vs. 44%), probably reflecting the

use of new antifungal treatments, such as itraconazole and voriconazole in the most recently diagnosed patients.

Case reports:

Kindred A: Proband Patient 1 (A.II.1, Figure 1) was born to consanguineous Algerian parents from Tlemcen and developed deep dermatophytosis at the age of six years. He developed deep dermatophytosis with *tinea corporis*, *tinea capitis* and onychomycosis with pachyonychia (Figure S4.1, A-D).[13] Non-insulin-dependent diabetes mellitus was diagnosed at the age of 50 years. At this age, blood counts revealed eosinophilia (counts of up to 1700/mm^3) and cultures of skin samples from multiple sites were positive for *Trichophyton violaceum*. When the patient was 52 years old, the erythematous lesions on the skin extended, with severe itching, and a large, soft, subcutaneous lobular tumor (10 x 15 cm) appeared in the patient's left armpit (Figure S4.1.D). Patient 1 also presented large axillary, submaxillary (4 cm) and mesenteric lymphadenopathies. Histological examination of the axillary lymphadenopathy revealed the presence of hyphae within a necrotic granuloma. Treatment with a combination of griseofulvin and econazole was not effective. Patient 1 is now 75 years old and has been treated with itraconazole since September 2010, resulting in a decrease in the size of the lesions. No other severe infections were reported.

One cousin of Patient 1, (Patient 2, A.III.6) presented deep dermatophytosis, beginning with onychomycosis at the age of two years.[14,15] He then developed recurrent tinea capitis and corporis and lymphadenopathy at the age of eight years and mycologically confirmed oral thrush at the age of nine years. At 25 years of age, Patient 2 presented erythroderma, cutaneous nodules (several of which were ulcerated), alopecia and onychodystrophy. He also had iliac and inguinal, axillary and cervical fistulizing lymphadenopathies (Fig. S4.1E). *T.*

violaceum was isolated from skin and lymph nodes. Histological examination of skin samples revealed dermis and hypodermis infiltration with necrotizing granuloma and hyphae. Eosinophilia (2700/mm^3) was detected at the age of 25 years. Patient 2 subsequently developed seizures, leading to the detection of three cerebral abscesses on CT scan (Fig. S4.1F). Despite itraconazole treatment, the cerebral and skin lesions worsened and the patient died from septicemia at the age of 29 years.[14,15] After death, there was no biopsy or microbiology of cerebral/skin lesions.

Kindred B: Patient P3 (B.II.6, Figure 1) is a woman from a consanguineous Algerian family from Tlemcen. Her deep dermatophytosis began with recurrent *tinea capitis* and extensive *tinea corporis* at the age of nine years.[16] She was immunized with BCG with no adverse effects. Following a course of treatment with systemic corticosteroids for uncontrolled t*inea corporis* and severe pruritus at the age of 12 years, she developed a severe skin infection with general thickening, lichenification, squamous areas, pruritus, multiple erythematous nodules, palmo-plantar keratotic lesions (Fig. S4.1G), severe nail involvement with onychogryphosis, and scaly scalp. Patient 3 also developed multiple lymphadenopathies with fistula formation (Fig. S4.1H), worsening between the ages of 12 and 17 years. *T. rubrum* grew from cultures of skin, scalp, nails, and lymph nodes taken from the patient at the age of 17 years. Histological examination of lymph nodes showed necrotizing granuloma with eosinophils and hyphae, stained with periodic acid-Schiff reagent, in giant multinucleate cells. Griseofulvin treatment, at a dose of 1 g/day, was introduced when the patient was 17 years old. This treatment was continued for two years and resulted in a clear improvement. The treatment was then stopped, but deep dermatophytosis relapsed, with extensive *tinea corporis*. At the age of 35 years, Patient 3 developed insulin-dependent diabetes mellitus. She is now 40 years old and has normal counts of T CD4$^+$, T CD8$^+$, B, and NK lymphocytes. Itraconazole treatment was initiated in September 2010 and again led to a reduction of the lesions and itching. No

other severe infections were reported. Neither the patient's parents nor her siblings have ever suffered from a dermatophytic infection.

Kindred C: The proband, Patient 4 (C.II.1, Figure 1), is from a consanguineous Algerian family from Algiers. His deep dermatophytosis has been described elsewhere.[9] He was immunized with BCG with no adverse effects. He began to suffer from recurrent *tinea capitis* (Fig. S4.1I) and *tinea corporis* at the age of eight years. He then developed extensive foot and hand onychomycosis and glabrous skin lesions with lichenification (Fig. S4.1I-L). He also had recurrent oral candidiasis. *T. violaceum* was cultured from skin lesions after seven months of growth, probably because the patient received a prolonged course of antifungal treatment. IgE levels were high (1300 IU/ml). Griseofulvin treatment was initiated when the patient was 17 years old and led to some improvement. However, chronic residual lesions remained and relapses occurred whenever the treatment was stopped. Patient 4 is now 56 years old, is still treated with griseofulvin and has residual skin lesions. T $CD4^+$, T $CD8^+$, B, and NK lymphocyte subsets were normal. No other severe infections were reported.

His brother, Patient 5 (C.II.5, Figure 1) first showed signs of the disease at the age of eight years, with extensive *tinea capitis* and onychomycosis.[17] He then presented extensive keratotic and ichthyotic lesions, disseminated papules, nodules, alopecia, pachyonychia, and onycholysis. He had several subcutaneous abscesses and all his peripheral enlarged lymph nodes have fistulized. At the age of 15 years, Patient 5 had an eosinophil count of 2600/mm^3 and high IgE levels (1300 IU/ml). *T. violaceum* was isolated from skin lesions (after five months of growth). Patient 5 also had recurrent thrush caused by *C. albicans*. Griseofulvin treatment was initiated when the patient was 15 years old. No other severe infections were reported. Despite initial improvement, the patient's disease worsened and he died at 34 years of age with disseminated deep dermatophytosis which was no longer controllable.[17] However, no post-mortem examination could be carried out to determine the definitive cause of death.

The sister of these two patients (Patient 6, C.II.11, Figure 1) is currently 41 years old. At the age of eight years, she presented with chronic onychomycosis of all nails due to *T. violaceum*. She was treated with griseofulvin and presented no other infection. Neither the parents nor the other siblings had any dermatophytic infections.

Kindred D: The proband, Patient 7 (D.II.6, Figure 1), from a consanguineous Algerian family from Constantine, presented with ulcerative and nodular lesions of the left thigh (Fig. S4.1M) and scalp (Fig. S4.1N) at the age of 19 years. He has been immunized with BCG with no adverse effect. He developed recurrent tinea capitis, onychomycosis of the hands and feet and enlargement of the cervical lymph nodes, at the age of 39 years. Skin biopsy at the age of 20 years provided evidence of a hyperkeratotic epithelium with granuloma. Tuberculosis was initially suspected and the patient was given antimycobacterial therapy for nine months, without improvement. A second skin biopsy at the age of 40 years revealed the presence of hyphae within the granuloma. Skin scrapings also demonstrated the presence of hyphae. However, no dermatophytes could be grown from the lesions in culture. At the age of 40 years, Patient 7 was treated with griseofulvin and fluconazole, with temporary improvement, and relapse occurred when the antifungal drugs were withdrawn. The patient is now 43 years old, treated with griseofulvin and fluconazole and has residual, but stable skin lesions. No other severe infections were reported.

His brother (Patient 8, D.II.7, Figure 1), who is now 40 years old, has had deep dermatophytosis since the age of 21 years, with extensive ulcerating skin lesions on the face, scalp and perineum. He was immunized with BCG with no adverse effect. He was treated with griseofulvin between the ages of 21 and 27 years and then with fluconazole. The scalp and face lesions resolved and the perineal lesions improved. When fluconazole treatment was stopped, the perineal lesions enlarged to 20 centimeters in diameter and major locoregional extension of the disease led to perineal involvement and a stenotic anus. There was no

mucosal invasive disease (Fig. 2.C). Surgery, with colostomy, was therefore required and terbinafine treatment was initiated in September 2010. P8 also developed extensive tinea versicolor, onychomycosis and inguinal lymph node enlargement. Hyphae were found within necrotizing granulomas in skin lesions. A clear improvement was observed after one month of treatment with itraconazole and terbinafine (Fig. S4.1O). No other severe infections were reported.

The brother of Patients 7 and 8 (Patient 9, D.II.4, Figure 1) died from deep dermatophytosis, with pseudotumoral and ulcerating lesions on his face, at the age of 28 years. Neither the parents of these patients nor the other siblings suffered from dermatophytic infection.

Kindred E: The proband, Patient 10 (E.II.7, Figure 1), from another unrelated consanguineous family from Tlemcen in Algeria, developed recurrent tinea in childhood and was subsequently diagnosed with deep dermatophytosis. At the age of 27 years, he presented with erythematosquamous warty lesions with onychomycosis and giant palmo-plantar horns (8 cm high) with onychogryphosis (Fig. S4.1P-S).[18] He had eosinophilia (550/mm^3) at the age of 29 years. *T. violaceum* was isolated from the skin and nails. Skin biopsy showed acanthosis and hyperkeratosis of the epidermis. The stratum corneum and dermis were invaded by hyphae and lymphoid granulomas. The patient improved on griseofulvin, but a relapse occurred and he died from septicemia at the age of 39 years. Unfortunately, the microbiological etiology of sepsis was not documented. It probably resulted from either fungal disease or bacterial sepsis secondary to the bacterial superinfection of skin lesions. No other severe infections were reported.

His sister (Patient 11, E.II.10, Figure 1), who is currently 37 years old, has suffered from chronic onychomycosis since childhood. Neither their parents nor any other siblings have been reported to suffer from dermatophytic infection.

Kindred F: The proband, P12 (F.II.6, Figure 1), was born in Belgium, but is from a consanguineous family originating from Morocco. He presented with recurrent thrush and tinea during childhood. At the age of 16 years, squamous hyperkeratosic skin lesions appeared on his left foot. These lesions worsened at the age of 35 years, with vegetative and ulcerating lesions extending to feet, calves and the left thigh (Fig. 2.B, S4.1T). Lesions were associated with left inguinal lymphadenopathy, squamous pigmented lesions of the groin and left foot onychomycosis. X rays suggested osteolysis of the left first and second toes and MRI revealed soft tissue infiltration (27 mm) (Fig. S4.1U-W). Hypereosinophilia (up to 1500/mm^3) was observed from the age of 35 years onwards and IgE levels were high, at up to 1741 kIU/ml. T CD4$^+$, T CD8$^+$, B, and NK lymphocyte subsets were normal. A skin biopsy carried out when P12 was 35 years old showed an inflammatory infiltrate extending throughout the dermis, comprising numerous granulomas associated with eosinophils, plasma cells and lymphocytes. These granulomas were observed mostly in the mid and deep dermis and consisted of giant multinucleate cells containing PAS-positive intracytoplasmic hyphae and spores (Fig. 2.A, Fig. S4.2A-C). Fontana Masson staining was negative, excluding the presence of a black fungus. Skin culture and molecular analyses were positive for *T. rubrum*.[19] The lesions improved, but a relapse occurred, despite the sequential administration of terbinafine, voriconazole, posaconazole, liposomal amphotericin B and combined treatment with terbinafine, voriconazole and interferon-γ. The second toe on the patient's left foot required amputation. Patient 12 had normal phagocytic oxidative burst function as assessed by dihydrorhodamine assay. Patient 12 is now 40 years old and his disease has relapsed despite voriconazole treatment. No other severe infections were reported.

The sister of Patient 12 (Patient 13, F.II.3) is 49 years old. She was born in Morocco and had recurrent severe tinea during childhood. As an adult, she has suffered from hand and foot

onychomycosis. Neither the parents nor the other siblings have been reported to suffer from dermatophytic infections.

Kindred G: The proband (Patient 15, G.III.1) is from a Tunisian family (Sousse). He presented tinea corporis at 12 years of age. At 16 years of age, skin lesions extended, with nodules, and onychomycosis appeared, affecting all nails. *T. rubrum* was isolated from nails and skin. Treatment with fluconazole followed by itraconazole stabilized the lesions and no regression was observed. Histological examination of the skin showed granuloma with PAS-positive hyphae.

His sister (Patient 16, G.III.4) presented tinea capitis at five years of age, and tinea corporis and onychomycosis of all nails at eight years of age. Since the age of 12 years, she has presented fistulized skin nodules and axillary lymph node enlargement. *T. violaceum* and *T. rubrum* were identified from nails and skin. Skin biopsy provided evidence of dermal and hypodermal invasion with hyphae. Griseofulvine, ketoconazole and fluconazole stabilized the lesions.

The father of these patients (Patient 14, G.II.6) presented tinea capitis at the age of six years, followed by tinea corporis and onychomycosis of all nails. He died bed-ridden at the age of 91 years.

Kindred H: The proband (Patient 17, H.II.1), from an unrelated family from Kairouan, Tunisia, presented severe tinea capitis at the age of six years. At the age of 40 years, he developed extensive tinea corporis and numerous skin nodules, onychomycosis affecting all nails and inguinal, cervical and axillary lymph node enlargement. *T. rubrum* was isolated from nails and *T. violaceum* was isolated from hair and skin. Histological examination of skin nodules provided evidence of dermal infiltration with epithelioid and giant cells, with necrosis. PAS and Grocott staining revealed the presence of hyphae within the granuloma.

Histological examination of the axillary lymph nodes showed a large granuloma with giant multinucleate cells and necrosis. PAS staining demonstrated the presence of hyphae within the granuloma. Hypereosinophilia was detected (up to 2700/mm^3) and the patient's IgE level was 51190 IU/ml. Patient 17 required treatment with griseofulvine, fluconazole, terbinafine and itraconazole. He is currently being treated with voriconazole and his condition has stabilized.

2. Methods

Controls

We sequenced the exons 3 and 6 of *CARD9* gene for all the 1,050 healthy unrelated control individuals from the Human Genome Diversity Cell Line Panel (HGDP-CEPH), originating from 52 different ethnic groups initially sampled for population genetics studies,[20] and 138 individuals from Morocco, 100 from Tunisia and a total of 83 individuals from Algeria.

Primers used

For exon 2 and 3 amplification:

Forward primer: 5'GTCTGAGAAGGAGTGGGAGC 3'

Reverse primer: 5'GCTGTGGCAGGAGCTCAGG 3'

Tm = 61°C

For exon 3 sequencing:

Forward primer: 5' GGCACACCTCATCTGCATGC 3'

Reverse primer: 5' GCTGTGGCAGGAGCTCAGG 3'

For exon 6 amplification and sequencing:

Forward primer: 5' GCAAGCACACGCTGAAGCTC 3'

Reverse primer: 5’ CACTGTGCCTCCAGGAGTGG 3’

Tm = 59°C

Cell transfections

Wild-type (*WT*) *CARD9* full length cDNA was inserted into a pcDNA3.1 expression vector V5-tagged at the C-terminus. The c.*C301T* (p.R101C) and the c.*C865T* (p.Q289X) mutations were introduced using the Quickchange II XL site-directed mutagenesis kit from Stratagene (200522-5), according to the manufacturer instructions. Plasmids containing the *CARD9 WT* or mutated sequences were then amplified and purified using QIAprep Spin Miniprep Kit from Qiagen (27106). The resulting plasmids were used to co-transfect HEK-293T cells in 6 centimeter plates using Calcium-Phosphate kit (InVitrogen 278001) together with a CFP plasmid, according to the manufacturer’s instructions.

Western blot

Total protein extracts of HEK-293T cells were prepared 48 hours after transfection. Proteins were separated by electrophoresis and the membrane were blotted with anti-V5 antibody (InVitrogen 46-0708), anti-CARD9 H-90 (Sc-99054), anti CFP or GAPDH (Sc-25778). For MDDC immunoblot, 20 µg of whole MDDC total cell extracts were separated on a 10% SDS-PAGE gel and then immunoblotted using the H-90 anti-CARD9 (Sc-99054) and GAPDH (Sc-25778) antibodies.

Histological analysis

Skin samples were immediately fixed in 10% neutral-buffered formalin, and embedded in paraffin; four-micrometer sections were cut and stained with Hematoxylin and Eosin, Periodic Acid-Schiff and Fontana Masson to highlight tissue lesions, fungi and melanin, respectively. Immunohistochemistry analysis was carried out using a fully automated IHC Leica BOND-III

(Leica Biosystems), and the following primary antibodies: polyclonal anti-*Candida spp*. (991018V, dilution: 1:500, Biogenesis), monoclonal anti-*Aspergillus spp*. (Mab-WF-AF-1/M3564, dilution 1:30, Dako), monoclonal anti-*Rhizomucor* (Mab-WSSA-RA-1/M3565, dilution 1:40, Dako) and monoclonal anti-Dermatophytes (XCMA01-1000, dilution 1:100, Xceltis GmbH).

Founder effect analysis

Founder-effect analysis was carried out on a subset of seven available, apparently unrelated patients (five from Algeria, including three from Tlemcen (Patients 1, 3, 11), one from Algiers (Patient 4), one from Constantine (Patient 8) and two from Sousse, Tunisia (Patients 15, and 17)) bearing the same homozygous Q289X CARD9 mutation. Genotypes were obtained for > 250,000 single-nucleotide polymorphisms (SNPs) on the Affymetrix GeneChip Human Mapping 250K SNP Array. SNPs with a 100% call rate were scanned for continuous stretches of homozygosity upstream and downstream from the *CARD9* locus on chromosome 9q34. Pairwise comparisons revealed the limits of the longest shared haplotype and the positions of subsequent recombination break points. The likelihood-based ESTIAGE method was used to estimate the age of the most recent common ancestor (MRCA). The general principle of ESTIAGE is to estimate the number of generations that separates the sampled families from their MRCA through the length of the haplotypes shared by patients.[21] The approach can be understood as a survival analysis problem in which the starting point is the disease locus, the discrete time scale is the genetic distance, and the event of interest is the occurrence of a recombination. Recombination rates and haplotype frequencies were provided by the HapMap Project.[21-23]

3. Supplementary results

Impact of *CARD9* mutations on protein expression levels

We evaluated the consequences of the mutations identified on protein expression levels, by carrying out an immunoblot analysis for CARD9 on whole-cell extracts of HEK-293T cells transfected with a pcDNA3.1 V5 (C-terminal tagged) plasmid, empty or carrying the *WT* or one of the two mutant alleles of *CARD9* (pcDNA3.1 V5 CARD9 WT, pcDNA3.1 V5 CARD9 R101C or pcDNA3.1 V5 CARD9 Q289X). Cells transfected with the CARD9 R101C allele had CARD9 protein levels and a MW of this protein similar to those in cells transfected with the WT allele, whereas cells transfected with the CARD9 Q289X allele had normal levels of a truncated protein of about 25 kDa (Figure S5).

Author contributions:

FL, LL, MM, SP performed experiments. FL, CPr, and SP performed genetic analysis. QBV and LA analyzed the genetic data for funder effects. FL, LT, AAK, OBS, BG, FJ, JCG, KS, VdM, LB, MD, ML, HB, LM, GL, MB and OL, provided and gathered the clinical data for the patients. FL, OL and RH have done the assessment of deep dermatophytosis. SF, FJ, and GJ analyzed histological analysis. CP performed immunological explorations.

FL, SP, QBC, LL, CPr, SC, LL, RH, GJ, FC, MEB, LA, OL, JLC, CP, BG and AP analyzed the data and vouches for the data and the analysis.

JLC, CP, BG and AP designed and coordinated the study. FL, OL, LA, JLC, CP, BG and AP wrote the manuscript and decided to publish the paper. All authors discussed the results and commented on the manuscript.

4. Supplementary Figures

Figure S1: Identification of missense and nonsense mutations in *CARD9*

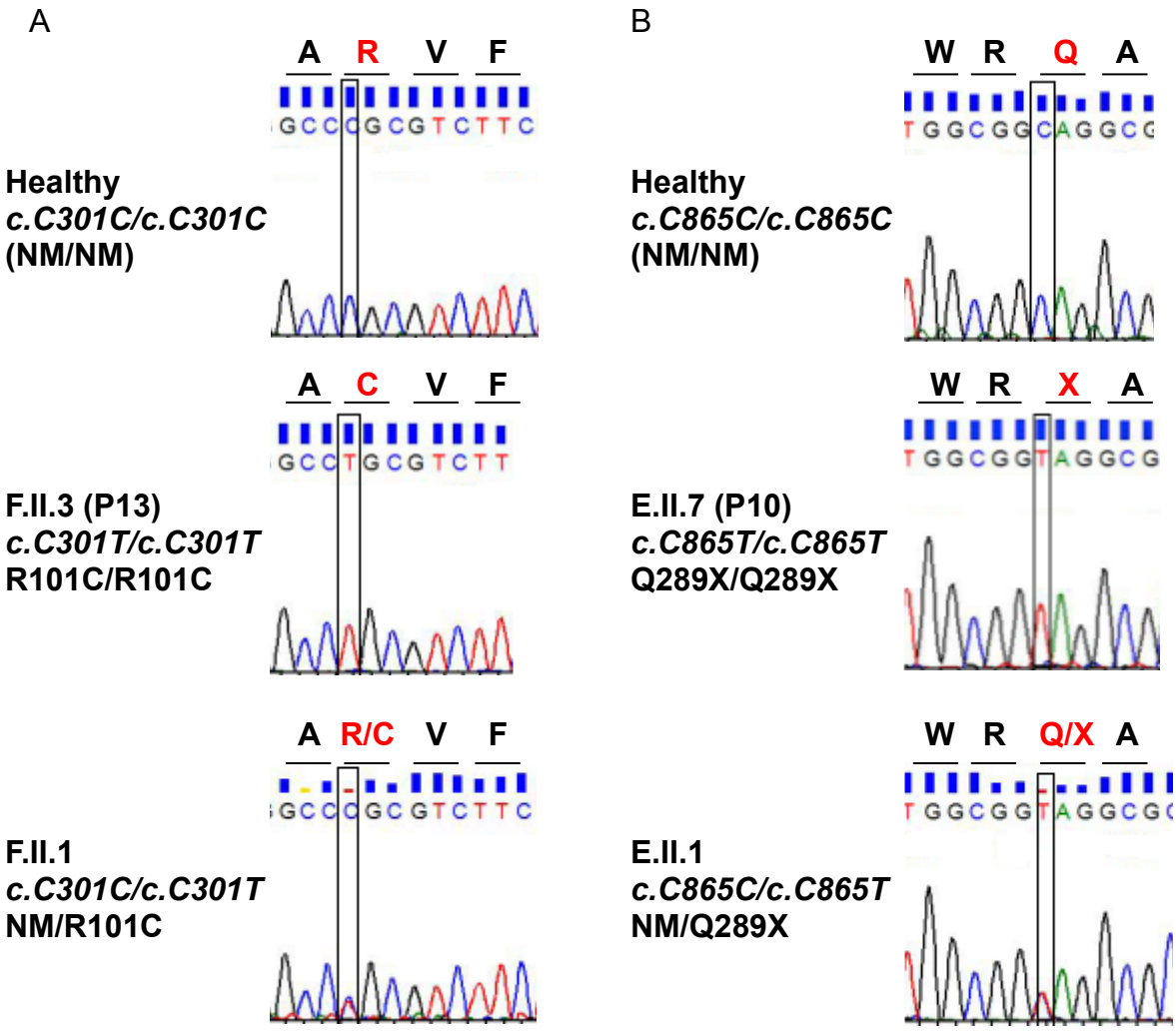


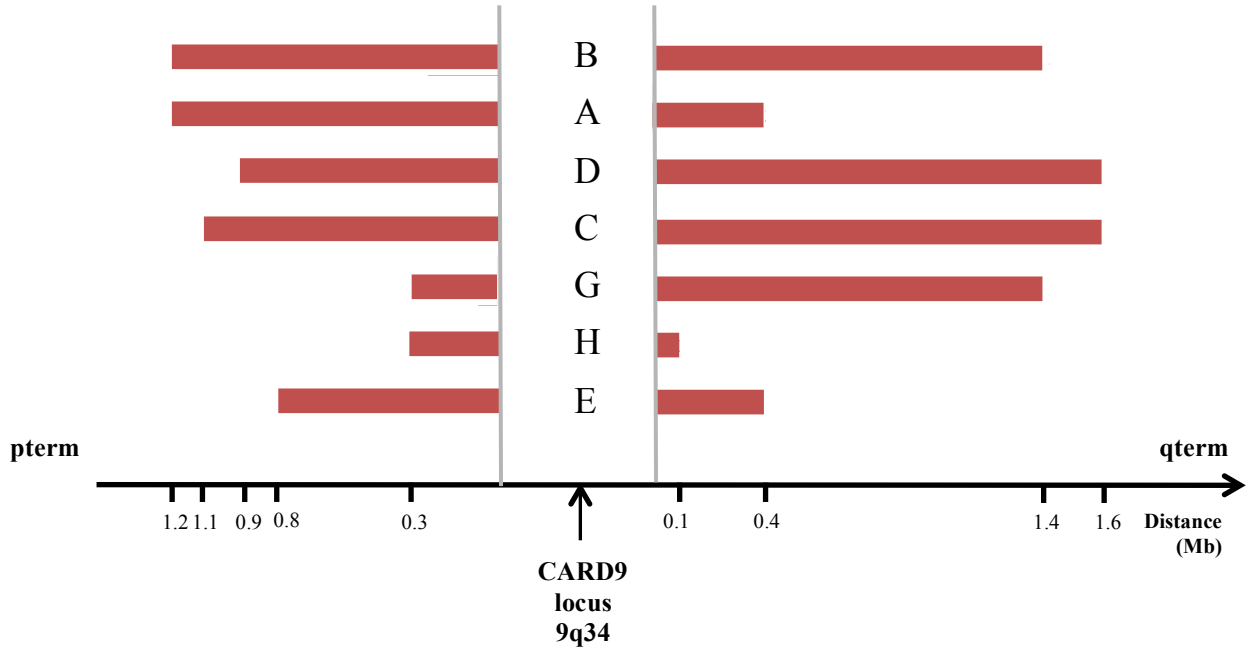
Figure S1.A. Sanger sequencing reads for a homozygous nonmutated NM/NM healthy individual (top), for P13, with a homozygous missense mutation in exon 3 of *CARD9* (*c.C301T/c.C301T*) resulting in the R101C/R101C amino-acid substitution (middle panel), and for the healthy individual F.II.1, with a heterozygous NM/R101C mutation (bottom panel).

Figure S1.B. Sanger sequencing reads for a homozygous nonmutated NM/NM healthy individual (top), for P10, with a homozygous nonsense mutation in exon 6 of *CARD9* (*c.C865T/c.C865T*) leading to a premature stop codon Q289X/Q289X (middle), and for the healthy individual E.II.1, with a heterozygous mutation (NM/Q289X).

Figure S2. Schematic representation of the human CARD9 (isoform 1) and mutations

Coding exons are numbered with roman numerals. The regions corresponding to the caspase activation and recruitment domain (CARD) and the coiled coil (CC) domain are indicated. Mutations associated with deep dermatophytosis are indicated in red.

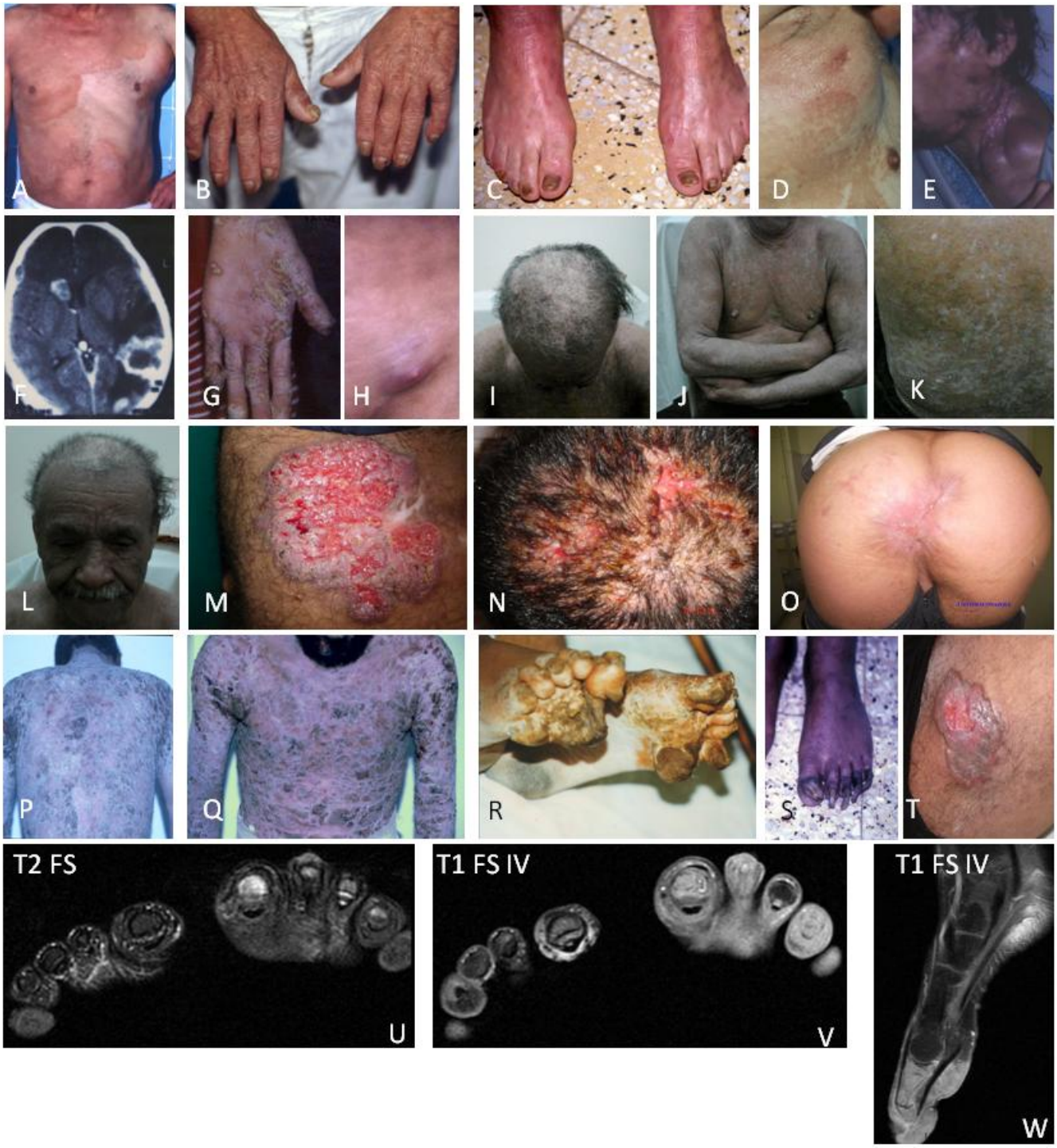
Figure S3. Haplotype common to the seven unrelated patients carrying the homozygous Q289X mutation of the *CARD9* gene.



The analysis of 250K SNP array data showed that the patients homozygous for the Q289X mutation had a common haplotype around the *CARD9* locus (five from Algeria and two from Tunisia).

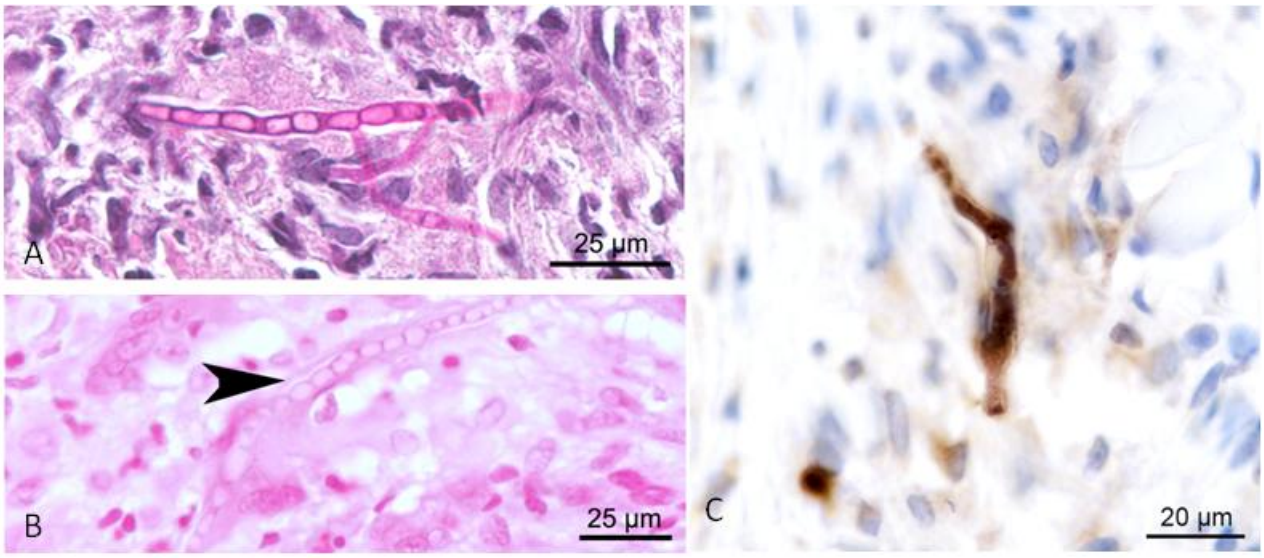
Figure S4. Clinical, radiological and histological features of CARD9 deficient patients.

Figure S4.1. Clinical and radiological features of CARD9 deficient patients.



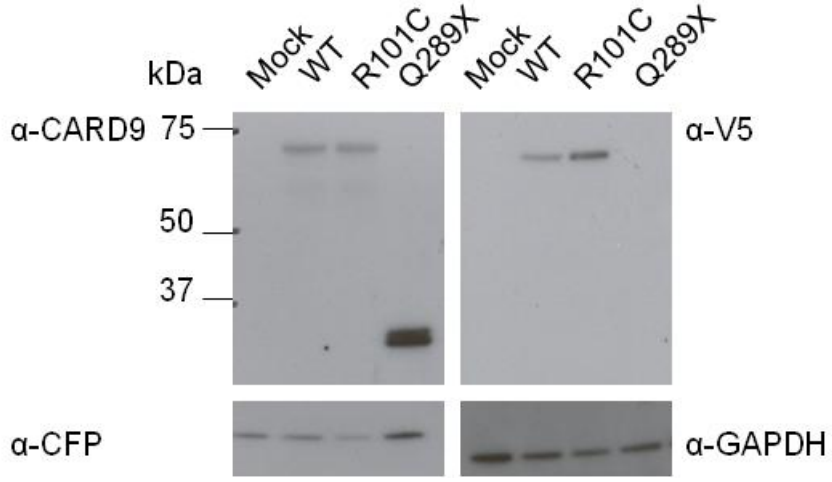
S4.1. Clinical phenotype of patients: P1 (A-D), P2 (E-F), P3 (G-H), P4 (I-L), P7 (M-N), P8 (O), P10 (P-S), P12 (T). Foot MRI fat sat: hypertrophy of toe soft tissues and toe osteomyelitis from patient P12 (U-W).

Figure S4.2. Histological features of CARD9 deficient patients.



S4.2. Histology of skin biopsy specimen from the patient P12. Irregularly branched septate hyphae (arrowhead), in the center of a granuloma containing multinucleated giant cells (*); Periodic Acid-Schiff staining (PAS) (A); Negative Fontana Masson staining (B); positive labeling using anti-Dermatophyte monoclonal antibody (XCMA01-1000, dilution 1:100, Xceltis GmbH) (C).

Figure S5. Impact of *CARD9* mutations on CARD9 protein expression levels in transfected HEK-293 T cells

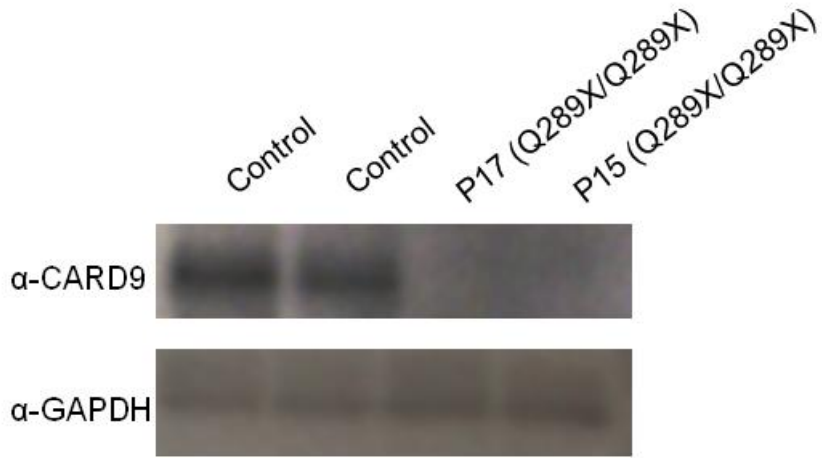


S.5. Immunoblot analysis of CARD9 in whole-cell extracts of HEK-293T cells transfected with the pcDNA3.1-V5 (C-terminal tagged) plasmid, either empty (mock), or carrying the wild-type (pcDNA3.1 V5 CARD9 WT) or mutant (pcDNA3.1 V5 CARD9 R101C and

pcDNA3.1 V5 CARD9 Q289X) CARD9 alleles, using an anti-CARD9 (left) or an anti-V5 (right) antibody. A transfection efficiency control (anti-CFP, left panel) and a protein loading control (anti-GAPDH, right panel) were used.

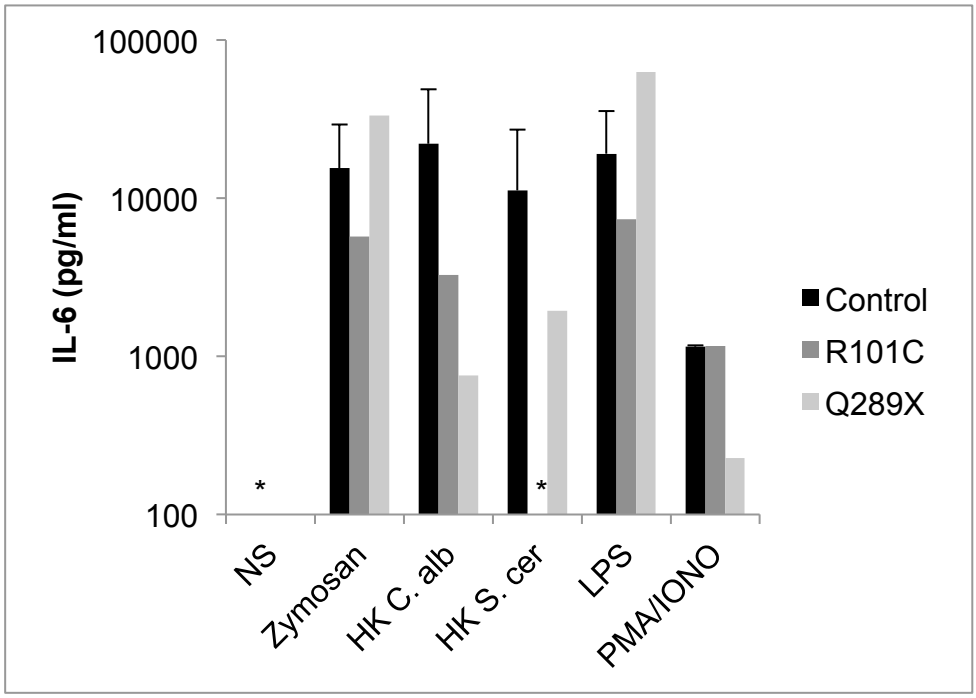
Figure S6. Impact of *CARD9* mutations on CARD9 protein expression and function

Figure S6.1 Impact of *CARD9* mutations on CARD9 protein expression



S6.1. shows CARD9 expression in monocyte-derived dendritic cells (MDDCs) of patients (Patients 17 and 15) and controls by Western blotting analysis using anti-CARD9 (H-90) antibody. Total protein amount loading was evaluated using anti-GAPDH antibody.

Figure S6.2 Impact of *CARD9* mutations on CARD9 protein function



S6.2. shows IL-6 production by whole blood cells, as measured by ELISA, from Patient 12 (CARD9 R101C/R101C) and Patients 15 and 17 (CARD9 Q289X/Q289X) after 24 hours of stimulation with zymosan, heat-killed *Candida albicans* (HK *C. alb*), heat-killed *Saccharomyces cerevisiae* (HK *S. cer*), lipopolysaccharide (LPS) and PMA plus ionomycin (PMA/IONO).

5. References

1. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the 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 (EORTC/MSG) Consensus Group. Clin Infect Dis 2008;46(12):1813-21.
2. Hironaga M, Okazaki N, Saito K, Watanabe S. *Trichophyton mentagrophytes* granulomas. Unique systemic dissemination to lymph nodes, testes, vertebrae, and brain. Arch Dermatol 1983;119(6):482-90.
3. Araviysky AN, Araviysky RA, Eschkov GA. Deep generalized trichophytosis. (endothrix in tissues of different origin). Mycopathologia 1975;56(1):47-65.
4. Beirana L, Novales J. Tina universal y granulomatosa por *T. tonsurans*. Rev Mex Derm 1959;3:4-16.
5. Blank F, Schopflocher P, Poirier P, Riopelle JL. Extensive Trichophyton infections of about fifty years' duration in two sisters. Dermatologica 1957;115(1):40-51.
6. Cheikhrouhou F, Makni F, Masmoudi A, Sellami A, Turki H, Ayadi A. A fatal case of dermatomycoses with retropharyngeal abscess. Ann Dermatol Venereol 2010;137(3):208-11.
7. Destombes P, Liautaud B, Marill FG. Histopathological study on the course of a dermatophytic disease. Bull Soc Pathol Exot Filiales 1975;68(5):443-9.
8. Hadida E, Schousboe A. Dermatophytic disease aspects. Algérie médicale 1959;63:303-36.
9. ~~Liautaud B. Defense mechanisms of dermatophytosis. Alger: Alger;Algeria, 1977.~~
10. Liautaud B, Marill FG. Dermatophytic disease. Recent Algerian observations. Bull Soc Pathol Exot Filiales 1984;77(5):637-48.
11. Cheikhrouhou F, Makni F, Ayadi A. Dermatophytic disease: litterature review. J Myc Med 2010;2010(20):61-9.
12. Seebacher C, Bouchara JP, Mignon B. Updates on the epidemiology of dermatophyte infections. Mycopathologia 2008;166(5-6):335-52.
13. Boudghene-Stambouli O, Merad-Boudia A. Dermatophytic disease in Algeria: new case and litterature review. Ann Dermatol Venereol 1991;118:17-21.
14. Boudghene-Stambouli O, ~~M-BA~~. Antifungal therapeutics in the dermatophytic diease. Failure of griseofulvine, of ketaconazole and of itraconazole. Bull Soc Path Ex 1990;83:170-6.
15. Boudghene-Stambouli O, Merad-Boudia A, Allal M. Cerebral injury in the dermatophytic disease. J Mycol Med 1992;2:106-8.
16. Boudghene-Stambouli O, Merad-Boudia A. *Trichophyton rubrum* dermatophytic disease. A new case. Ann Dermatol Venereol 1989;116(10):725-7.
17. Pruszkowski A, Bourgault Villada I, Cremer G, Ammar-Khodja A, Emilie D, Revuz J. Dermatophytic disease: role of type TC2 CD8 lymphocytes. Ann Dermatol Venereol 1995;122(Sup 1):55.
18. Boudghene-Stambouli O, Merad-Boudia A. Dermatophytic disease: ~~giant~~ cutaneous horns. Ann Dermatol Venereol 1998;125:705-7.
19. White T, Burns T, Lee S, Taylor T. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: In: Innis DH SJ, and White TJ, eds. San Diego: Academic Press, ed. A guide to methods and applications 1990:315-22.
20. Cann HM, de Toma C, Cazes L, et al. A human genome diversity cell line panel. Science 2002;296(5566):261-2.
21. Genin E, Tullio-Pelet A, Begeot F, Lyonnet S, Abel L. Estimating the age of rare disease mutations: the example of Triple-A syndrome. J Med Genet 2004;41(6):445-9.
22. Frazer KA, Ballinger DG, Cox DR, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449(7164):851-61.

23. Sologuren I, Boisson-Dupuis S, Pestano J, et al. Partial recessive IFN-gammaR1 deficiency: genetic, immunological and clinical features of 14 patients from 11 kindreds. Hum Mol Genet 2011;20(8):1509-23.