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ORIGINAL RESEARCH

A Homozygous CARD9 Mutation in a Brazilian Patient with Deep Dermatophytosis

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Abstract Deep dermatophytosis has been described in HIV and immunosuppressed patients. Recently, CARD9 (caspase recruitment domain-containing protein 9) deficiency has been reported in individuals with deep dermatophytosis previously classified as "immunocompetent". We report a 24-year-old Brazilian male patient with deep dermatophytosis born to an apparently non-consanguineous family. The symptoms started with oral candidiasis when he was 3 years old, persistent although treated. At 11 years old, well delimited, desquamative and pruriginous skin lesions appeared in the mandibular area; ketoconazole and itraconazole were introduced and maintained for 5 years. At 12 years of age, the lesions, which initially affected the face, started to spread to thoracic and back of the body (15 cm of diameter) and became ulcerative, secretive and painful. Terbinafine was introduced without any improvement. *Trichophyton mentagrophytes* was isolated from the skin lesions. A novel homozygous mutation in *CARD9* (R101L) was identified in the patient, resulting in impaired neutrophil fungal killing. Both parents, one brother (with persistent superficial but not deep dermatophytosis) and one sister were heterozygous for this mutation, while another brother was found to be homozygous for the CARD9 wild-type allele. This is the first report of CARD9 deficiency in Latin America.

Keywords Deep dermatophytosis · autosomal recessive CARD9 deficiency · primary immunodeficiency · *Trichophyton mentagrophytes*

Abbreviations

CARD9	caspase recruitment domain-containing protein 9
CADD	Combined Annotation Dependent Depletion

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Introduction

Dermatophytes are ubiquitous filamentous fungi, usually responsible for benign superficial infections, such as tinea capitis, tinea corporis and/or onychomycosis [1]. In rare cases, dermatophyte infection can lead to deep dermatophytosis with the invasion of the dermis and hypodermis by dermatophytes, sometimes affecting lymph nodes, brain, digestive tract and bones [2]. Most cases of deep dermatophytosis have been reported among HIV patients or patients on immunosuppressive therapy [3]. However, a number of cases were reported with deep dermatophytosis but otherwise healthy, mainly from North Africa, often born to consanguineous families and/or multiplex families, suggesting a genetic origin for the disease [4]. In 2013, the main genetic etiology for deep dermatophytosis was identified in 17 patients from eight unrelated North African families, bearing homozygous loss-of-function mutations in *CARD9* (caspase recruitment domain-containing protein 9). A homozygous premature stop codon mutation (Q289*) was identified in 15 patients from seven unrelated Algerian and Tunisian families while a homozygous missense mutation (R101C) was found in two Moroccan siblings [5]. More recently, an additional patient born to Egyptian parents with extensive skin and nail dermatophytosis was also identified with the CARD9 Q289* mutation [6]. CARD9 is an adaptor molecule, found mainly expressed in macrophages and myeloid dendritic cells in mice, that plays a central role in antifungal defense by receiving signals from several C-type lectin-like receptors and stimulating pro-inflammatory responses [7]. Indeed, CARD9 deficient cells showed a selective impairment of TNF-α and IL-6 production upon fungal agonist stimulation [5, 8, 9]. We herein report a Brazilian patient with deep dermatophytosis and CARD9 deficiency due to the homozygous R101L mutation.

Case Report

A 24-year-old Caucasian male, born to a non-consanguineous family of Italian origin, living in rural area, developed thrush at 3 years of age and erythematous cutaneous scaly lesions in mandibular area which have evolved to the whole body within the following years. At the same time, he lost his hair. Therapy has been introduced with nistatin for 6 years, followed by ketoconazole for 1 year (local and oral use) and the lesions were maintained under control at the beginning. At 11 years, well delimitated, scaly and pruritic skin lesions appeared which initially affected the face. Ketoconazole was thus reintroduced and maintained for 2 years with mild improvement at the beginning of the treatment. At 12 years, ulcerative, secretive and painful lesions installed in the face (lips) and spread to all the mandibular area. He was then referred to the Infectious Disease outpatient unit and itraconazole was introduced and maintained for 3 years, followed by terbinafin for the next 4 years. Transient improvement was observed after each therapeutic agent. In the following years, the lesions increased affecting his back (15 cm of diameter) and shoulders. Semi alopecia and onycodystrophy were observed (Fig. 1). Posaconazole was given for a period of 1 year (between 19 and 20 years old) and it was the best result obtained with antifungal therapy. In the last months, amphotericin B was administered with no response. Direct mycologic examination and biopsy identified hyaline septated hyphae positive for *Trichophyton mentagrophytes* after culture. Routine evaluation identified eosinophilia and high serum IgG and IgE levels. Immunophenotyping showed normal $CD4^+$ and $CD8^+$ T and $CD19^+$ lymphocyte counts and low numbers of NK ($CD16^+/CD56^+$) cells. Lymphoproliferative T cell responses showed no T cell response to Candidin but normal to phytohaemaglutinin. Delayed-type hypersensitivity skin tests were negative for Candidin and of 8 mm for Trychophitin. Neutrophil killing of *Candida* or *Staphylococcus* was evaluated according to Saresella et al., 1997, modified [10] and found to be impaired in the patient's sample for *Candida* only (Table 1). Neither his parents nor two of his siblings presented any symptom of fungal infection. However, one of his brothers had superficial dermatophytosis, usually treated with local antifungal cream; his lesions did not progress but were recurrent. We were unable to study viable cells in vitro or to perform flow cytometry for CARD9 protein from the patient and family members due to logistical difficulties. DNA samples from five family members were collected with their approval. *AIRE* was shown to be of wild-type sequence in the index case. *CARD9* was amplified with specific primers. The patient was found to be homozygous for a novel *c.302G>T* variation in the exon 3 of the *CARD9* gene (R101L) (CADD score at 9.927 with a cutoff of 6.612); his parents, his brother with superficial dermatophytosis and his sister were found to be heterozygous for this mutation (Fig. 2), and one brother was found to be

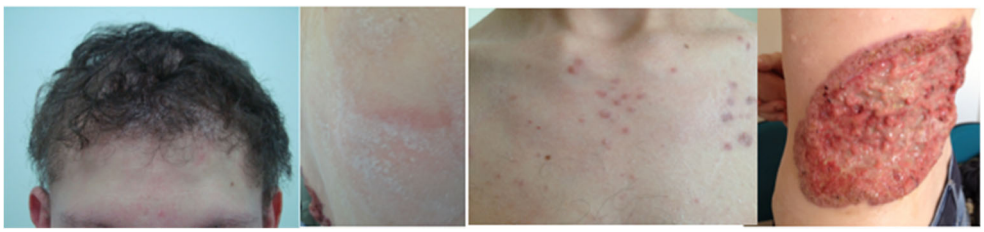

Fig. 1 Sequence of dermal lesions due to *T. Mentagrophytes*: hair, descamative skin, initial papular followed by ulcerative lesions

Table 1 Immunological evaluation of the CARD9 deficient patient

Immunological parameter	Patient	Normal range
IgG (mg/dL)	2120	770–1510
IgA (mg/dL)	101	100–490
IgM (mg/dL)	65	50–320
IgE (UI/L)	>2000	<90
Leukocytes (cells/mm^3) (15 years old)	8050	5000–10,000
Eosinophils (cells/mm^3) (%)	1368 (17)	<400 (<4)
Leukocytes (cells/mm^3)	15,200	5000–10,000
Lymphocytes (cells/mm^3)	5180 (34 %)	
CD3+ (cells/mm^3)	3739	1500–2300
CD3+ CD4+ (cells/mm^3)	1854	880–1500
CD3+ CD8+ (cells/mm^3)	1353	570–1010
CD19+ (cells/mm^3)	448	230–950
CD16+ CD56+ (cells/mm^3)	20	82–760
Proliferative response of lymphocytes to PHA (s.i.)	186	>10
Proliferative response of lymphocytes to Candidin (s.i.)	0.855	>3
Late Cutaneous Hypersensitivity Response to Candidin	Negative	>5 mm
Dihidrorodamine	100	>80
Fungal killing (*Candida*)	43 % alive; 21 % dead	Day control *: l6 % alive; 68 % dead
Bacterial killing (*S.aureus*)	10 % alive; 90 % dead	Day control *: l9 % alive; 81 % dead
HIV serology	Negative	Negative

s.i. stimulation index; * sample from a healthy volunteer collected at the same time

homozygous wild-type. No homozygous or heterozygous individuals with that variation were reported in any of the various public databases (Human Gene Mutation Database, Ensembl, NHLBI GO Exome Sequencing Project [ESP], 1000 Genomes Project, and the Exome Aggregation Consortium [ExAC]) or in our in-house WES database (>2000 exomes), suggesting that this variant is extremely rare, possibly private to this kindred, and defines a novel AR deep dermatophytosis-causing allele. In addition, the Arginine at position 101 is highly conserved among species, and finally R101L is predicted to be deleterious by Sift (with a score of 0), and probably damaging by Polyphen 2 (with a score of 1.0) (Fig. 3).

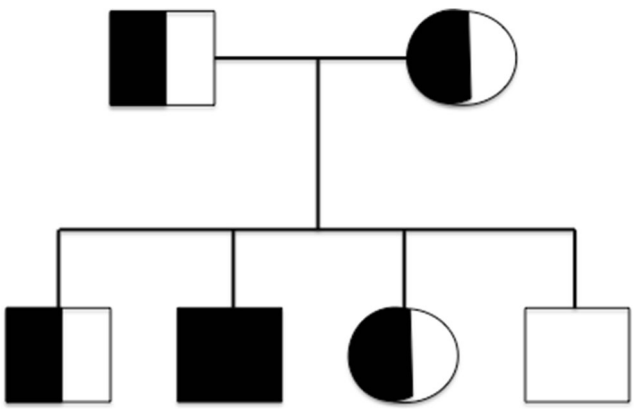

Fig. 2 Genealogy of *CARD9* deficient family

Discussion

CARD9 is a key transducer of Dectin-1, Dectin-2 and Mincle signalling. CARD9 couples to BCL10 and regulates BCL10-MALT1-mediated NF-κB activation induced by various fungal ligands such as β-glucans, (such as curdlan, a selective Dectin-1 agonist) [11]. In humans, autosomal recessive CARD9 deficiency is associated with susceptibility to fungal infections, caused by *Candida* spp, dematiaceous fungi (*Exophiala sp., Phialophora verrucosa*) or *Tricophyton spp,* while these patients displayed normal immunity to common bacteria, intracellular bacteria, or viruses [4–6, 8, 9, 12–15]. The first clinical manifestation of the patient described here was oral candidiasis that started at 3 years of age and lasted until 11 years of age despite the use of several antifungal therapies. His symptoms worsened early in the adolescence, as previously reported for most CARD9 deficient patients with deep dermatophytosis [5]. Although our patient had dermatophytosis with profound and extended cutaneous involvement, he did not develop disseminated disease, as already reported for few patients [5, 6]. However, most patients

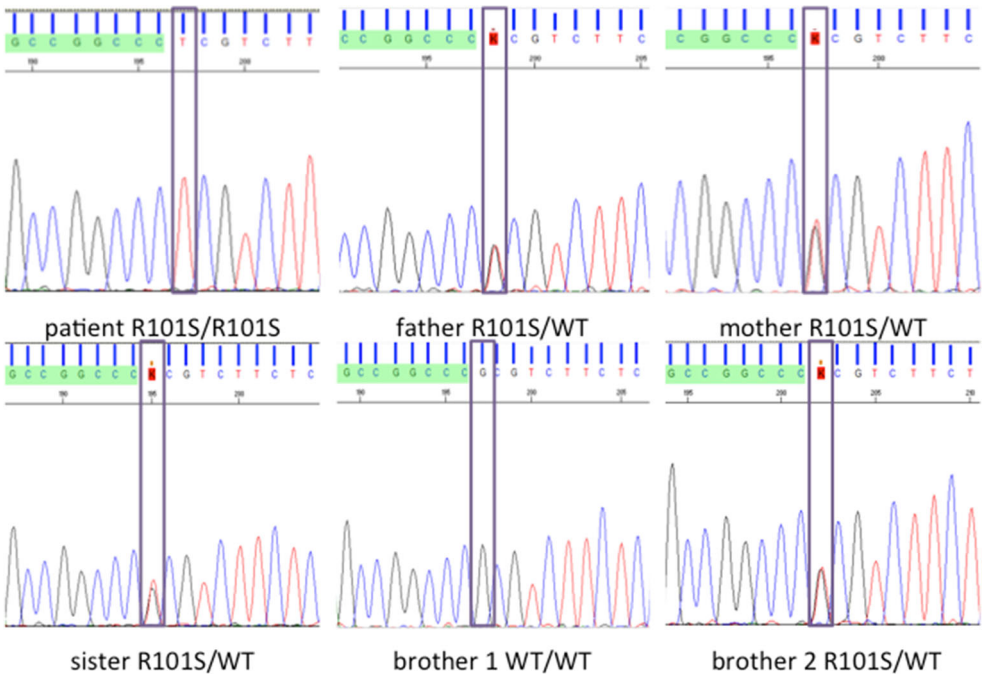

Fig. 3 Family evaluation of *CARD9* Exon 3

with deep dermatophytosis and AR CARD9 deficiency developed invasive infections with bone involvement in one patient, brain involvement in another patient, and lymph node reaction in most patients [5]. Brain infections, mainly with *Candida* spp, but also *Exophiala* dermatitidis have been reported for several CARD9 deficient patients [5, 8, 9, 12, 13, 15].

The patient was found to be homozygous for the R101L CARD9 allele, even though the family did not report any consanguinity. However, the patient was born in a town with 15,000 inhabitants (Roncador, Parana State) suggesting cryptic consanguinity. Both parents were found heterozygous for the R101L CARD9 mutation and asymptomatic. This mutation has probably a high impact according to CADD, Polyphen 2 and Sift scores. Two of the patient's siblings were also found heterozygous for the R101L CARD9 allele with one of them presenting persistent cutaneous dermatophytosis. Whether this clinical manifestation is incidental or caused by the heterozygous R101L mutation is unknown. To date, none of the heterozygous carriers reported displayed fungal infections [5, 8, 9, 12, 14, 15]. The R101L allele could affect CARD9 protein expression and/or interaction with its partners BCL-10/MALT1, as the mutation is located just after the CARD interacting domain. Unfortunately we were unable to retrieve blood in good condition to test these hypotheses. The patient displayed eosinophilia and high serum IgE levels, with normal T and B lymphocyte counts as previously reported [4–6, 12, 15], but with low numbers of $CD16^{+}/CD56^{+}$ NK cells. Furthermore, we found a negative delayed-type hypersensitivity skin test for Candidin but not Trychophitin. The patient presented a normal DHR response to PMA (phorbol mirystate acetate) suggestive of a normal NADPH oxidase activity. However, while *Staphylococcus aureus* killing by neutrophils was similar to a healthy non-related control tested in parallel, neutrophil *Candida* killing was impaired, as already reported [13]. This defect could contribute to the invasive nature of the fungal infection. Low levels of IL-6 and TNF-α by whole blood cells or monocyte-derived dendritic cells and low percentages of IL-17A-producing T cells were also reported in some CARD9 deficient patients, and may further contributing to the impaired defense against fungal microorganisms [4].

Conclusion

In summary, we describe a novel mutation related to CARD9 deficiency and deep dermatophytosis. This is the first CARD9 mutation reported in Latin American continent. Heterozygous individuals might be asymptomatic or present with mild infection. Neutrophil evaluation showed specific impaired fungal killing probably related to the severity of the disease.

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Conflict of Interest The authors declare that they have no conflict of interest.

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