

TRICHOPHYTON TONSURANS IN CHILE: GENOTYPING IN SEARCH OF AN ORIGIN

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ABSTRACT

Trichophyton tonsurans is a cosmopolitan dermatophyte, highly prevalent in Africa, South America, and endemic in North America, where it is mostly related to cases of tinea capitis. Recently, it was also reported in Chile for the first time in two outbreaks occurred in Santiago. In the present study we sequenced the variable internal repeat (VIR) region of a Chilean isolate and compared its genotype with those of several global *T. tonsurans* isolates. The results showed that the Chilean isolate presented a new genotype which was strictly correlated with isolates from Europe, USA and Mexico.

LAY SUMMARY

Trichophyton tonsurans is a fungus able to cause superficial mycosis infecting skin and hair. It is an emerging pathogen worldwide and some cases were recently reported also in Chile for the first time. We investigated by molecular techniques which geographical area could be the source of infections.

ORIGINAL UNEDITED MANUSCRIPT

BRIEF REPORT

Dermatophytes are a group of keratinophilic fungi that are taxonomically classified into seven genera: *Trichophyton*, *Microsporum*, *Epidermophyton*, *Nannizzia*, *Paraphyton*, *Lophophyton*, and *Arthroderma*. They are important as pathogens in both humans and animals.^{1,2}

Data from a global systematic review on tinea capitis, that analyzed the last 20 years, showed that *Microsporum canis* is the most common zoophilic agent and that, over time, *Trichophyton violaceum* and *T. tonsurans* have increased in frequency, becoming the species with the highest frequency in anthropophilic transmission cases. This type of transmission and the observed epidemiological changes can be constantly influenced by migration and intercontinental travel, globalization, as well as environmental, climatic and ecological changes.³

Trichophyton tonsurans is a cosmopolitan dermatophyte, highly prevalent in Africa, South America and endemic in North America.⁴ It is mostly related to cases of tinea capitis and occasionally to tinea corporis and is increasingly found in urban centers. In highly endemic regions, patients can be infected by multiple strains and the species has caused community outbreaks of dermatophytosis.^{1,4}

In Chile, its appearance was reported in 2012. Subsequently, a study that included 155 cases of tinea capitis, found a predominance in the male gender, an average age of 5 years, with 85.2% of the cases in the preschool and school population. The most frequent isolated agent was *M. canis* in 89.8% of the cases, followed by *T. tonsurans* in 7.1%. In addition, it was confirmed that 81.8% of the cases of *T. tonsurans* were of foreign origin, mainly from Haiti and Peru.⁵ In another study, from Valparaíso region, an outbreak of *T. tonsurans* was also associated with school-age children, the vast majority of whom were Chilean.⁶ These antecedents show that *T. tonsurans* is an emerging agent in our country.

Molecularly, *T. tonsurans* and *T. equinum* are closely related. Some authors suggest that these species could have evolved from a common sexual zoophilic or geophilic ancestor, with a lineage

from one mating type becoming adapted to humans and one from the opposite mating type becoming adapted to horses.^{7,8}

Although the conventional identification of dermatophytes is based on morphological characteristics, recent molecular studies have become an important complementary diagnostic tool for mycologists working with this species, since *T. tonsurans* presents high morphophysiological diversity between strains without necessarily coinciding with its genetic variability.⁴ Nucleotide sequences are currently available in databases allowing the phylogenetic comparison of closely related species and the differentiation of intra-species variations.

Abdel-Rahman and colleagues carried out several investigations focused on demonstrating the genetic variability of *T. tonsurans* at the rRNA locus.⁹ Through multilocus genotyping analysis, they described the presence of infections with more than one strain in individual isolates.¹⁰ Subsequently, based on phylogenetic studies of the rDNA and ALP1 locus, they reported a relationship between variations in nucleotide sequences and the severity of infections.¹¹ More recently, using the same multilocus analysis strategies, they studied the degree of genetic diversity between isolates from 17 different countries where they found 47 different genotypes of *T. tonsurans*.¹²

These molecular studies focused on looking for biological and pathogenic differences, which allow establishing phylogenetic and geographical relationships between different genotypes, continue to be an area of interest little explored in our country, therefore, the objective of this study is to molecularly characterize an isolate from a child with tinea capitis and thus contribute to the epidemiology of this species.

The *T. tonsurans* strain investigated in the present study was isolated from a ringworm outbreak occurred among children attending an elementary school in Valparaiso, Chile.⁶ Species was identified by macroscopic and microscopic observation. The strain was then sent to Medical

Mycology Laboratory of Università degli Studi di Milano, Italy, for further molecular investigations.

Genotyping of the isolate was performed by sequencing the variable internal repeat (VIR) region of the non-transcribed spacer (NTS) region of rDNA. Genomic DNA was first extracted starting from a suspension of spores and hypha obtained by washing the surface of a one-week culture on Sabouraud dextrose agar. Centrifuged pellet was then added with PrepMan Ultra solution (Applied Biosystems) following manufacturer's instructions. VIR region was amplified using NTS13 and NTS21 primers as described elsewhere¹³ and then both forward and reverse strands were sequenced using the same primers. The two DNA strands were aligned with Clustal Omega software (www.ebi.ac.uk) to obtain a consensus sequence. Structure of the repeated regions inside the VIR region were identified using reference sequences from GenBank (accession numbers: AB220046-AB220051).

VIR region structure of the Chilean isolate was compared with those of further 193 *T. tonsurans* global isolates found in the literature.^{9, 10, 12-14} Isolates were from Japan, USA, Canada, Mexico, Europe, China, and India. VIR structure was codified assigning a letter to each module of the structure to obtain a final code which was then used for phylogenetic analysis. The VIR code of the Chilean isolate and those of the other global isolates were loaded in the Phyloviz software (www.phyloviz.net) and a minimum spanning tree was created by GoeBurst algorithm.

The VIR region structure (accession number: OK485139) of the Chilean isolate is shown in Figure 1. It contains five modules: a region of 104 bp (A module), repeated three times, which is present in the structure of all global isolates; a 140-bp region (B module), including a 54-bp deletion, found in other three genotypes; and a 142-bp region (G module), including a 52-bp deletion, which was not found in any of the investigated global isolates.

Comparison of the VIR genotype of the Chilean isolates with the 12 genotypes of the other global isolates showed that all genotypes were strictly correlated differing in one or two modules each to

the other (Figure 2). In agreement with results obtained by other authors¹², genotype ACA seems to be the most probable ancestor of all *T. tonsurans* genotypes here investigated including isolates from USA, Mexico, and Europe. Deletions in the C module have then generated genotypes ABA, ADA, AEA and further duplication events generated genotypes ABABA, ACACA, ACACACA, ADADA, and AEAEA. Two genetic events seem to occur more frequently in VIR region of *T. tonsurans*: the deletion of C module leading to B module, and the duplication of C module. The B module was identified in 81 isolates representing 42% of the investigated isolates, whereas C module is present both as duplicate and triplicate in 17% (33/193) of isolates.

The genotype of Chilean isolate (AGABA) generated as a new variant of the ACACA genotype by a double deletion in the C modules and probably from an intermediate genotype ACABA not yet discovered. The ACACA genotype includes isolates from Europe, USA, and Mexico which therefore are the potential geographical area from where *T. tonsurans* was imported in Chile. Although USA and Mexico seem to be the most probable origin as most of tinea capitis cases in these countries are due to *T. tonsurans* infections³, origin from European continent should not be excluded as *T. tonsurans* prevalence is increasingly reported in France, Germany, Spain and other countries.¹⁵⁻¹⁸ Since both the hypothesis of North American and European origin are possible, it is crucial to further investigate *T. tonsurans* genotypes in search of new variants more strictly correlated to AGABA, such as ACABA, which could elucidate the origin *T. tonsurans* genotype identified in Chile.

Declaration of interest

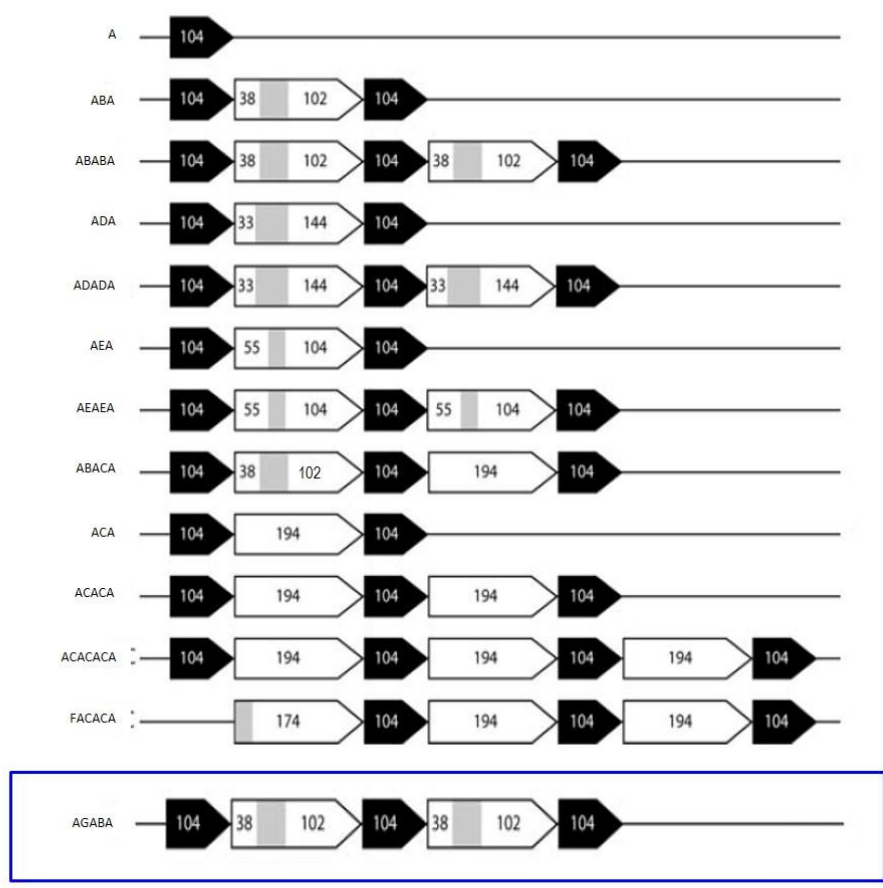
No conflict of interest is declared

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A

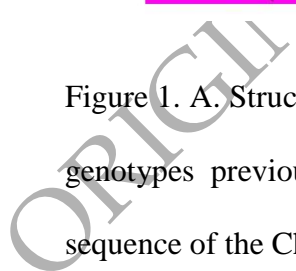


B

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TCCGGGGTCGATCCAGGCGCGCTCTACGGTCGATTTGGAGGCGATCGGTGTATGTACCTCGCCGCC
GGCGGACCCCTATGGACCCTCCAAAATCGAGTGGTCCCAGGGGGGCCGTTTCTAGAAATGGACCC
TCTGGCCAAACGGACCCCT.....52bp.....deletion.....GGCCATTTCCACAAAATGGACCCTCTGGCC
SAGGGGGCCACGGCCCTTAGGGGGCCATTTGGGGCCGAAAAAAAAAAGTCTCCAAATCGCTCC
GGGGTTCGATCCAGGCGCGCTCTACGGTCGATTTGGAGGCGATCGGTGTATGTACCTCGCCGCCGGC
GGACCCTATGGACCCTCCAAAATCGAGTGGTCCCAGGGGGGCCGTTTCTAGAAATGGACCCTCT
GGCCAAA.....54bp.....deletion.....GACCCTCTGGGCCATTTCCAAAATGGACCCTCTGGCCGAGGG
GCCACGGCCCCCTAGGGGGGCCATTTGGGGCCGAAAAAAAAAAGTCTCCAAATCGCTCCGGGGT
CGATCCAGGCGCGCTCTACGGTCGATTTGGAGGCGATCGGTGTATGTACCTCGCCGCCGGCGGACC
CCTATGGACCCTCCAAAATCGAGTGGTCCC
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- A MODULE (104 bp)
- S MODULE (142 bp)
- B MODULE (140 bp)

Figure 1. A. Structure of the variable internal repeat (VIR) region of the 12 *Trichophyton tonsurans* genotypes previously identified and the Chilean isolate (blue box). B. VIR region nucleotide sequence of the Chilean isolate. Different modules of the structure are marked with different colors.



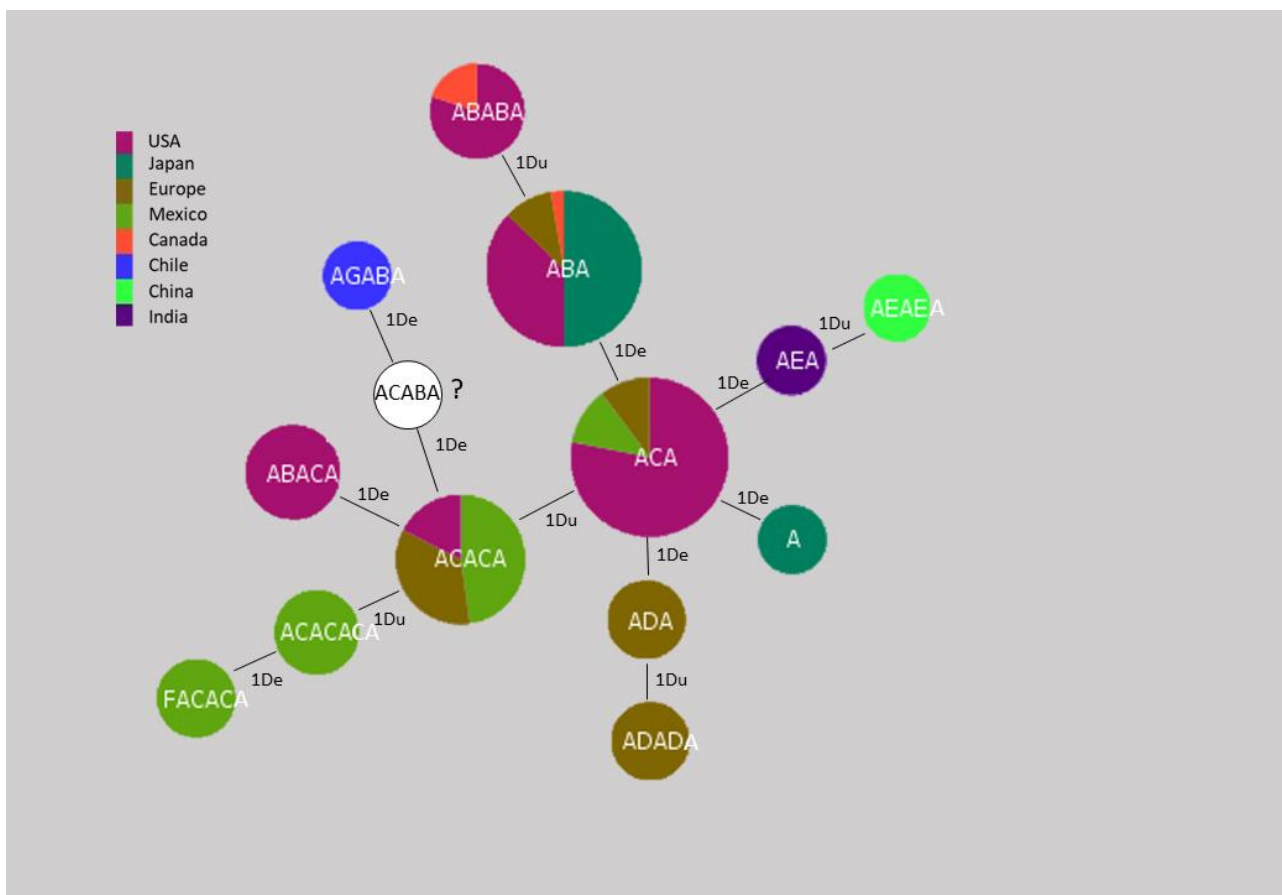


Figure 2. Minimum spanning tree comparing the 13 VIR genotypes of *Trichophyton tonsurans* and their geographical origin. Circles size is proportional to the number of isolates belonging to the same genotype. Genotype ACABA is supposed to be an intermediate genotype not yet identified. De = number of deletions, Du = Number of duplications.

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