Mycopathologia (2016) 181:441–444
DOI 10.1007/s11046-015-9980-4

Mating Type Gene (*MAT*) and Itraconazole Susceptibility of *Trichophyton tonsurans* Strains Isolated in Japan

Junichiro Hiruma · Miki Okubo · Rui Kano · Mai Kumagawa · Masataro Hiruma · Atsuhiko Hasegawa · Hiroshi Kamata · Ryoji Tsuboi

Received: 9 September 2015 / Accepted: 20 December 2015 / Published online: 13 January 2016
© Springer Science+Business Media Dordrecht 2016

Abstract Infection by *Trichophyton tonsurans* is an emerging fungal epidemic in Japan. Itraconazole (ITZ) and terbinafine have been used for the treatment of this infection for 15 years. However, patients with *T. tonsurans* infections have been shown to remain uncured or to become reinfected, suggesting that subclinical infection or polyphyletic strains and/or antifungal drug-resistant strains might be occurring in Japan. In this study, PCR analysis was performed to confirm the presence of the mating type locus *MAT* in genomic DNA from 60 Japanese clinical isolates of *T. tonsurans*, and to assess the previously postulated clonal origin of clinical isolates of this species. Antifungal susceptibility testing on isolates also was performed to confirm the absence of strains resistant to ITZ. PCR analysis proved that all 60 strains contained the *MAT1-1* allele, while none contained the *MAT1-2* allele. As determined by E-test, the mean MIC of ITZ in the 60 strains was 0.023 mg/L (range 0.002–0.125 mg/L). All strains of *T. tonsurans* isolated in Japan were clonal and were not resistant to ITZ. Therefore, dermatophytosis due to *T. tonsurans* is expected to respond to ITZ, since clinical isolates of *T. tonsurans* tested to date have been susceptible to this antifungal. This infection is proliferating as a subclinical infection in Japan.

Keywords Dermatophytosis · Mating type gene · *MAT1-1* · PCR · *Trichophyton tonsurans*

J. Hiruma · R. Tsuboi
Department of Dermatology, Tokyo Medical University, 6-1-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan

M. Okubo · R. Kano · M. Kumagawa · H. Kamata
Department of Pathobiology, Nihon University School of Veterinary Medicine, 1866, Fujisawa, Kanagawa 252-0880, Japan

M. Hiruma
Ochanomizu Institute for Medical Mycology and Allergology, Nakamura Bldg. 2F, 2-12-4 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

A. Hasegawa
Teikyo University Institute of Medical Mycology, 359 Otsuka, Hachioji, Tokyo 192-0395, Japan

R. Kano (✉)
Department of Veterinary Pathobiology, Nihon University College of Bioresource Sciences, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan
e-mail: kano@brs.nihon-u.ac.jp

Introduction

Trichophyton tonsurans is an anthropophilic species that is frequently reported as a source of human dermatophytosis throughout the world [9]. Since approximately 2000, cases of this dermatophytosis in Japan have increased significantly among contact sports participants [5], indicating that this dermatophytosis represents an emerging fungal infectious epidemic in this country [5]. The control of this

dermatophytosis has not been successful, and the infection has become a "hidden problem" due to the increase in asymptomatic carriers in Japan [5]. Itraconazole (ITZ) and terbinafine have been used in the treatment of this infection for 15 years. Using sequential passage in the presence of ITZ, Hryncewicz-Gwóźdź et al. [6] demonstrated in vitro selection of ITZ-resistant *T. rubrum*. These authors warned that *T. rubrum* can easily develop resistance to ITZ, a process with important implications for the clinical management for dermatophytoses [6]. Indeed, patients with *T. tonsurans* infections have been shown to remain uncured or to become reinfected [5], suggesting that antifungal drug-resistant strains might be emerging in Japan.

In a previous study, we investigated mating type (*MAT*) gene structure in anthropophilic dermatophytes. These organisms typically encode one or more transcription factors with structural motifs such as an alpha-box (in *MAT1-1*) or a high-mobility-group (HMG) DNA-binding domain (in *MAT1-2*) [7, 8]. Our PCR analysis detected the presence of *MAT1-1* in all *T. rubrum* strains; in contrast, *MAT1-2* was detected in all *T. interdigitale* strains [7, 8]. These results suggested that clinical isolates of a given species of anthropophilic dermatophytes would be genetically close (clonal) to each other due to the lack of mating [7, 8].

In the present study, PCR analysis was performed to confirm the presence of *MAT1-1* and *MAT1-2* in genomic DNA from clinical isolates of *T. tonsurans*; this analysis was expected to demonstrate the clonal origin of isolates of this species. Antifungal susceptibility testing also was performed on isolates from Japanese patients, thereby assessing the ITZ susceptibility of these strains.

Materials and Methods

Strains

The clinical isolates of *T. tonsurans* examined in this study are listed in Table 1. These isolates were obtained from 60 human cases of tinea capitis from judo athletes belonging to 17 clubs at the high school and university level in Tochigi and Tokyo.

These strains were subcultured on Sabouraud's dextrose agar (SDA) and then morphologically characterized as *T. tonsurans*. Molecular characteristics of the strains also were identified by sequence analysis of the internal transcribed spacer (ITS) region [4]. Results of comparative sequence analyses [by nucleotide BLAST analysis on the National Center for Biotechnology Information (NCBI) Web site] showed that the sequences amplified from the isolates were 99–100 % conserved among all strains, including the reference strain of *T. tonsurans*, and matched the *T. tonsurans*-defining ITS sequence deposited in the database (GenBank Accession Nos. AF170476, KP132859, and AB220038). The sequences determined in this study have been deposited into the DNA Data Bank of Japan (DDBJ) under accession numbers LC035393–LC035452 (strains LC035393–LC035452).

PCR Analysis of MAT1-1 and MAT1-2 Genes

Genomic DNA samples were derived as reported in the previous report [7]. Primers TmMATa1S and TmMATa1R amplified a 471-bp fragment of the *Arthroderma vanbreuseghemii MAT1-1* gene [7]. Primers TmHMG1S and TmHMG1R amplified a 524-bp fragment of the *A. vanbreuseghemii MAT1-2* [7].

Genomic DNA samples (100 ng/strain) from the clinical strains were amplified by PCR in a volume of 30 μL, using a reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001 % gelatin, 200 mM each deoxynucleoside triphosphate, 1.0 unit of *Taq* polymerase (Takara Bio), and 0.5 μg of a pair of primers. Amplification was carried out over 35 cycles consisting of template denaturation (1 min, 94 °C), primer annealing (1 min, 57 °C), and polymerization (2 min, 72 °C). PCR products were detected by electrophoresis on 2 % agarose gel followed by staining with ethidium bromide and visualization under UV light.

Susceptibilities of Strains to Itraconazole (ITZ)

Susceptibilities of clinical strains to ITZ were examined using E-test gradient strips of ITZ obtained from AB Biodisk (Solna, Sweden). Stock inoculum suspensions were prepared as described in the previous report [1]. For quality control, the strain *Candida parapsilosis* ATCC 22019 was used in each experiment to check the accuracy of drug dilution [1]. MIC values were determined after 4–7 days of incubation at 28 °C.

Table 1 *Trichophyton tonsurans* strains used in this study

Species	Cases[a]	Isolates	Locality	*MAT1-1*[b]	*MAT1-2*[a]
T. tonsurans	8	8	A high school in Tochigi	8	0
T. tonsurans	1	1	B University in Tokyo	1	0
T. tonsurans	3	3	C University in Tokyo	3	0
T. tonsurans	5	5	D University in Tokyo	5	0
T. tonsurans	3	3	E University in Tokyo	3	0
T. tonsurans	2	2	F University in Tokyo	2	0
T. tonsurans	2	2	G University in Tokyo	2	0
T. tonsurans	2	2	H University in Tokyo	2	0
T. tonsurans	1	1	I University in Tokyo	1	0
T. tonsurans	6	6	J University in Tokyo	6	0
T. tonsurans	5	5	K University in Tokyo	5	0
T. tonsurans	3	3	L University in Tokyo	3	0
T. tonsurans	3	3	M University in Tokyo	3	0
T. tonsurans	12	12	N University in Tokyo	12	0
T. tonsurans	2	2	O University in Tokyo	2	0
T. tonsurans	1	1	P University in Tokyo	1	0
T. tonsurans	1	1	Q University in Tokyo	1	0
Total	60	60		60	0

[a] Positive for *MAT1-2* gene

[b] Positive for *MAT1-1* gene

Results

PCR analysis proved that all 60 strains contained the *MAT1-1* allele; the *MAT1-2* allele was not detected in any of these strains (Table 1). The size of each *MAT1-1* amplicon was 470 bp, and the length matched in all the PCR products detected.

E-test determinations revealed that the mean MIC of ITZ in the 60 strains was 0.023 mg/L (range 0.002–0.125 mg/L).

Discussion

The nucleotide sequence identity of the ITS regions in all isolates of *T. tonsurans* was 99–100 % conserved among the strains examined in the present work, including the reference strain. These data indicated that these strains are genetically closely related. Sugita et al. [10] analyzed a variable internal repeat (VIR) region of the rRNA gene among 101 isolates of *T. tonsurans* collected from Japanese Judo practitioners in 2004 and reported that this species was strictly clonal.

In this study, 60 strains of *T. tonsurans* isolated in Japan were surveyed for the presence of the *MAT1-1* allele. All examined isolates harbored *MAT1-1* alone, suggesting that all of these strains should behave as the (−) mating type.

Hayashi and Takashio performed mating experiments between 11 isolates of *T. tonsurans* and tester strains of *A. simii* [3]. Those authors identified 9 *T. tonsurans* isolates with the (−) mating type, whereas 2 *T. tonsurans* isolates from Kenya exhibited the (+) mating type [3]. The results of this molecular analysis showed that *T. tonsurans* strains in Japan possessed the (−) mating type, while those of distinct geographic origin possessed the (+) mating type.

Susceptibility testing in the present report revealed that the range of MICs of ITZ (0.002–0.125 mg/L) in our strains did not apparently differ from the range (0.001–0.13 mg/L) determined in a previous study of isolates obtained in Japan from 2006 to 2010 [2]. Therefore, strains of *T. tonsurans* isolated in Japan were clonal and were not resistant to ITZ. We expect that dermatophytosis due to *T. tonsurans* should be amenable to treatment with azole, since these clinical isolates of *T. tonsurans* all are susceptible to ITZ. Therefore, this infection is proliferating as a subclinical infection in Japan. Dermatologists should be cautious about the prevalence of subclinical infection and carriage among Japanese sports participants.

Acknowledgments This study was partly funded by Health, Labor and Welfare Sciences Research Grants for Research on Measures for Intractable Diseases (H25 shinko-ippan 006) from the Japanese Government.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

1. AB BIODISK. Etest technical guide 10. Antifungal susceptibility testing of moulds. Solna: AB BIODISK; 2004.
2. Anzawa K, Mochizuki T, Nishibu A, Ishizaki H, Kamei K, Takahashi Y, Fujihiro M, Shinoda H. Molecular epidemiology of *Trichophyton tonsurans* strains isolated in Japan between 2006 and 2010 and their susceptibility to oral antimycotics. Jpn J Infect Dis. 2011;64:458–62.
3. Hayashi N, Takashio M. Mating type of *Trichophyton tonsurans*. Mycoses. 1984;27:377–9.
4. Hinrikson HP, Hurst SF, Lott TJ, Warnock DW, Morrison CJ. Assessment of ribosomal large-subunit D1-D2, internal transcribed spacer 1, and internal transcribed spacer 2 regions as target for molecular identification of medically important *Aspergillus* species. J Clin Microbiol. 2005;43:2092–103.
5. Hiruma J, Ogawa Y, Hiruma M. *Trichophyton tonsurans* infection in Japan: epidemiology, clinical features, diagnosis and infection control. J Dermatol. 2015;42:245–9.
6. Hryncewicz-Gwóźdź A, Kalinowska K, Plomer-Niezgoda E, Bielecki J, Jagielski T. Increase in resistance to fluconazole and itraconazole in *Trichophyton rubrum* clinical isolates by sequential passages in vitro under drug pressure. Mycopathologia. 2013;176:49–55.
7. Kano R, Kawasaki M, Mochizuki T, Hiruma M, Hasegawa A. Mating genes of the *Trichophyton mentagrophytes* complex. Mycopathologia. 2012;173:103–12.
8. Kano R, Isizuka M, Hiruma M, Mochizuki T, Kamata H, Hasegawa A. Mating type gene (*MAT1-1*) in Japanese isolates of *Trichophyton rubrum*. Mycopathologia. 2013;175:171–3.
9. Reiss E, Shadomy HJ, Lyon III GM. Dermatophytosis. In: Fundamental medical mycology. NJ: Wiley-Blackwell; 2012. p. 527–566.
10. Sugita T, Shiraki Y, Hiruma M. Genotype analysis of the variable internal repeat region in the rRNA gene of *Trichophyton tonsurans* isolated from Japanese Judo practitioners. Microbiol Immunol. 2006;50:57–60.