

Molecular Markers Useful for Intraspecies Subtyping and Strain Differentiation of Dermatophytes

Takashi Mochizuki · Kiminobu Takeda ·
Kazushi Anzawa

Received: 12 March 2016 / Accepted: 7 July 2016 / Published online: 25 July 2016
© Springer Science+Business Media Dordrecht 2016

Abstract Dermatophytosis is a very common skin disorder and the most frequent infection encountered by practicing dermatologists. The identification, pathogenicity, biology, and epidemiology of dermatophytes, the causative agents of dermatophytosis, are of interest for both dermatologists and medical mycologists. Recent advances in molecular methods have provided new techniques for identifying dermatophytes, including intraspecies variations. Intraspecies subtyping and strain differentiation have made possible the tracking of infections, the identification of common sources of infections, recurrence or reinfection after treatment, and analysis of strain virulence and drug resistance. This review describes molecular methods of intraspecies subtyping and strain differentiation, including analyses of mitochondrial DNA and non-transcribed spacer regions of ribosomal RNA genes, random amplification of polymorphic DNA, and microsatellite markers, along with their advantages and limitations.

Keywords *Trichophyton* · Epidemiology · Dermatophytosis · Molecular marker · Molecular epidemiology

Introduction

Dermatophytosis is a very common skin disorder and the most frequent infection encountered by practicing dermatologists. The actual prevalence of dermatophytosis has not yet been determined, but a randomized epidemiologic survey of outpatients who visited Japanese dermatologists in May 2006 showed the presence of fungal infections on the feet of 3848 (49.4 %) of 7783 patients, with or without foot disorders [1]. The causative agents of dermatophytosis include species of the anamorphic genera *Trichophyton*, *Microsporum*, and *Epidermophyton*, and the teleomorphic genus *Arthroderma*. The identification, pathogenicity, biology, and epidemiology of these causative agents are of interest for both dermatologists and medical mycologists. The dermatophyte strains isolated from tinea lesions have been identified by conventional culture-based methods, including gross colony morphology and slide culture, with identification confirmed by methods such as urease tests, mating tests, and several molecular techniques.

Since DNA–DNA hybridization methods were first used to assess the taxonomy of dermatophytes [2], many molecular techniques have been found useful for understanding the phylogenetic relationships among dermatophyte taxa. These include restriction fragment length polymorphism (RFLP) analysis of mitochondrial (mt) DNA [3–5], random amplification of polymorphic DNA (RAPD) using arbitrary primers [6, 7], and sequence analysis of genes with specific

T. Mochizuki (✉) · K. Takeda · K. Anzawa
Department of Dermatology, Kanazawa Medical
University, Daigaku 1-1, Uchinada, Ishikawa 920-0293,
Japan
e-mail: mocizuki@kanazawa-med.ac.jp

functions such as ribosomal RNA (rDNA) genes [8, 9] and genes encoding chitin synthase I [10], DNA topoisomerase II [11], and beta-tubulin [12, 13]. This resulted in new classifications of dermatophytes, including phylogenetic reconstructions based on nucleotide sequence of internal transcribed spacers (ITS) of rDNA [14]. These classifications were found to generally agree with the clinical and ecological traits of these strains [15]. Thus, progress in molecular methods has resulted in new techniques for identifying dermatophytes; comprehensive review articles have described these molecular approaches in detail [15–17].

These studies have also provided evidence of intraspecies variations. Development of more sensitive methods of detecting intraspecies subtypes and further strain level differentiation may make it easier to track infections, determine common sources of infections and recurrence or reinfection after treatment, and analyze their virulence and drug resistance [16]. This review describes methods for intraspecies subtyping and strain differentiation of dermatophytes using molecular methods such as random amplification of polymorphic DNA and analyses of mtDNA, non-transcribed spacer regions of ribosomal RNA genes and microsatellite markers, along with the advantages and limitations of each method.

Molecular Methods for Detection of Intraspecies Subtypes and Strain Differentiation

Mitochondrial DNA

Because of their small size and the availability of numerous copies [17], total mtDNA was used to subtype dermatophyte strains prior to the development of PCR-based technology. RFLP analysis of mtDNA was found to be a powerful tool for species-level identification [3–5]. In addition, several early studies revealed intraspecies polymorphisms, including differences between *Arthroderma (Nannizzia) otae* and *Microsporium canis* [4] and detection of two *Trichophyton rubrum* subtypes [3, 5]. However, one of these *T. rubrum* subtypes was later found to be *Trichophyton mentagrophytes* [18, 19], suggesting that this method was much less sensitive in distinguishing among subspecies of many dermatophyte species, including *T. rubrum*. A recent intensive

sequencing study of mtDNA [20] revealed the precise structure of this molecule and found that differences in genome sizes among various dermatophyte species were due to the sizes of intergenic regions and introns of highly conserved protein-encoding genes and RNAs. For example, the sequence between the apocytochrome *b (cob)* and NADH dehydrogenase subunit 3 (*nad3*) genes is highly variable, suggesting that these intergenic regions may serve as potential genetic markers for strain identification [20].

RAPD and Related Techniques

RAPD is a PCR-based technique that uses short primers (10 nt) of arbitrary sequence to generate PCR-amplified sequences of unknown location and function throughout the genome. The amplified DNA fragments are subsequently separated by electrophoresis on agarose or polyacrylamide gels and their fingerprint-like profiles used as molecular markers [21], with polyacrylamide gels used for high resolution of low molecular weight products. Arbitrarily primed PCR and DNA amplification fingerprinting are essentially the same, but the former uses longer primers and an amplification program that starts at low stringency and progresses to high stringency, whereas the latter uses shorter primers at higher concentration, enabling the generation of highly complex banding patterns [22]. The discussion focuses on the RAPD technique, because it is the most widely used variant of arbitrarily primed PCR.

An early RAPD analysis of five strains of *T. mentagrophytes* var. *interdigitale* isolated from humans, amplified using the primer R28 (5'-ATG-GATCCGC-3'), showed two subtypes [6]. In contrast to the uniform band patterns of isolates of *T. mentagrophytes* from humans, amplification with the primer OPAO-15 (5'-GAAGGCTCCC-3') of *T. mentagrophytes* isolated from animals showed diverse band patterns [7]. In addition, amplification of 67 clinical isolates of *T. rubrum* using the primers 1 (5'-GTGCGGGAA-3') and 6 (5'-CCCGTCAGCA-3') yielded 12 and 11 profiles, respectively [23]. A second study also showed that primers 1 and 6 were useful in distinguishing between *T. rubrum* isolates [24].

The method is suitable for obtaining results promptly and from several isolates at the same time. However, many experimental parameters, such as primer and template concentrations, annealing temperatures, the

concentration of magnesium ion in reaction solutions, kinds of polymerase used, and electrophoresis conditions [6], may alter fingerprinting profiles. Optimization of conditions is therefore essential for each study. The poor reproducibility of the profiles obtained and the difficulty of intralaboratory comparisons of banding profiles have reduced interest in this method. In addition, although many primers have been used for species-level discrimination of dermatophytes [6, 25], primers usable for intraspecies discrimination may be limited.

Internal Transcribed Spacer (ITS) Regions of rDNA

Internal transcribed spacer regions of rDNA are located between the 18S and 5.8S (ITS1) and the 5.8S and 26S (ITS2) rDNAs. The variable spacer regions are flanked by conserved sequences, and the high number of copies per cell enables simple and rapid analysis by PCR-based technology. As reported previously, the variable ITS regions have proven useful in resolving relationships among close taxonomic relatives [8] and are now widely used for species-level identification of many pathogenic fungi, including dermatophytes [26]. Several intraspecies polymorphisms have also been reported. For example, sequence variations in ITS1 (0.5–0.8 %, 1 or 2 bases per ca. 210–250 bp) and ITS2 (0.8–0.4 %, 1 or 2 bases per ca. 220–240 bp) have been reported for *Microsporum fulvum* and *T. mentagrophytes* isolates [27], five molecular types have been found among 46 strains of *T. interdigitale* [28], and two molecular types have been observed among 26 strains of *Trichophyton tonsurans* isolated in Japan [29]. The number of polymorphic sites in 16 dermatophyte species has been summarized [26]; however, the variations found in the ITS regions are very slight, suggesting that these differences are not useful markers for strain identification [16].

Non-transcribed Spacer (NTS) Regions of rDNA

Non-transcribed spacer regions, also referred to as intragenic spacers (IGS), of rDNA accumulate high degrees of sequence variations, and detection of these variations may be the method most frequently used in dermatophyte subspecies typing and strain identification. Sequence variations in NTS regions are due to the

numbers of repeat elements arranged tandemly in a variable internal repeat (VIR) [30, 31], as well as scattered single nucleotide polymorphisms (SNPs), deletions and insertions [32]. Polymorphisms were first detected by Southern blot hybridization-based RFLP analysis of *T. rubrum* [30], *T. mentagrophytes* var. *interdigitale* (*T. interdigitale*) [33], and *Arthroderma benhamiae*, a teleomorphic species of the *T. mentagrophytes* complex [34]. This method was able to identify a patient with a laboratory-based *A. benhamiae* infection [35]. Species-specific PCR primers targeting tandemly repetitive subelements in NTS of *T. rubrum*, named TRS-1 and TRS-2 [31], were synthesized to detect interspecies variations. This PCR-based technique identified polymorphisms in 101 clinical isolates of *T. rubrum*, enabling their categorization into 23 separate PCR types [31]. Moreover, these markers later indicated that two or more *T. rubrum* strain types were present in six of ten nail specimens from patients with onychomycosis [36]. These findings enabled the discovery of strain type switching in onychomycosis, especially in patients who failed treatment [37]. Polymorphisms have also been identified among isolates from patients with multiple tinea lesions and have shown inconsistent typing of strains isolated from tinea pedis and other parts of tinea (Fig. 1) [38]. Species-specific PCR primers for *T. mentagrophytes* var. *interdigitale* (*T. interdigitale*), combined with length variations in three loci, named TmiS0, TmiS1, and TmiS2, have identified 20 subtypes among 41 isolates [39]. Use of this system for molecular epidemiology enabled identification of 15 molecular types among 65 isolates in our university hospital [40]. Polymorphisms among *A. benhamiae* isolates are now easily detected by species-specific PCR primers, followed by RFLP analysis using *Mva*I digestion [41]. This NTS-targeted PCR-RFLP analysis revealed 11 molecular types among 46 isolates of *A. benhamiae*, including four types among 22 Japanese strains, with three of the latter also detected outside Japan (Fig. 2).

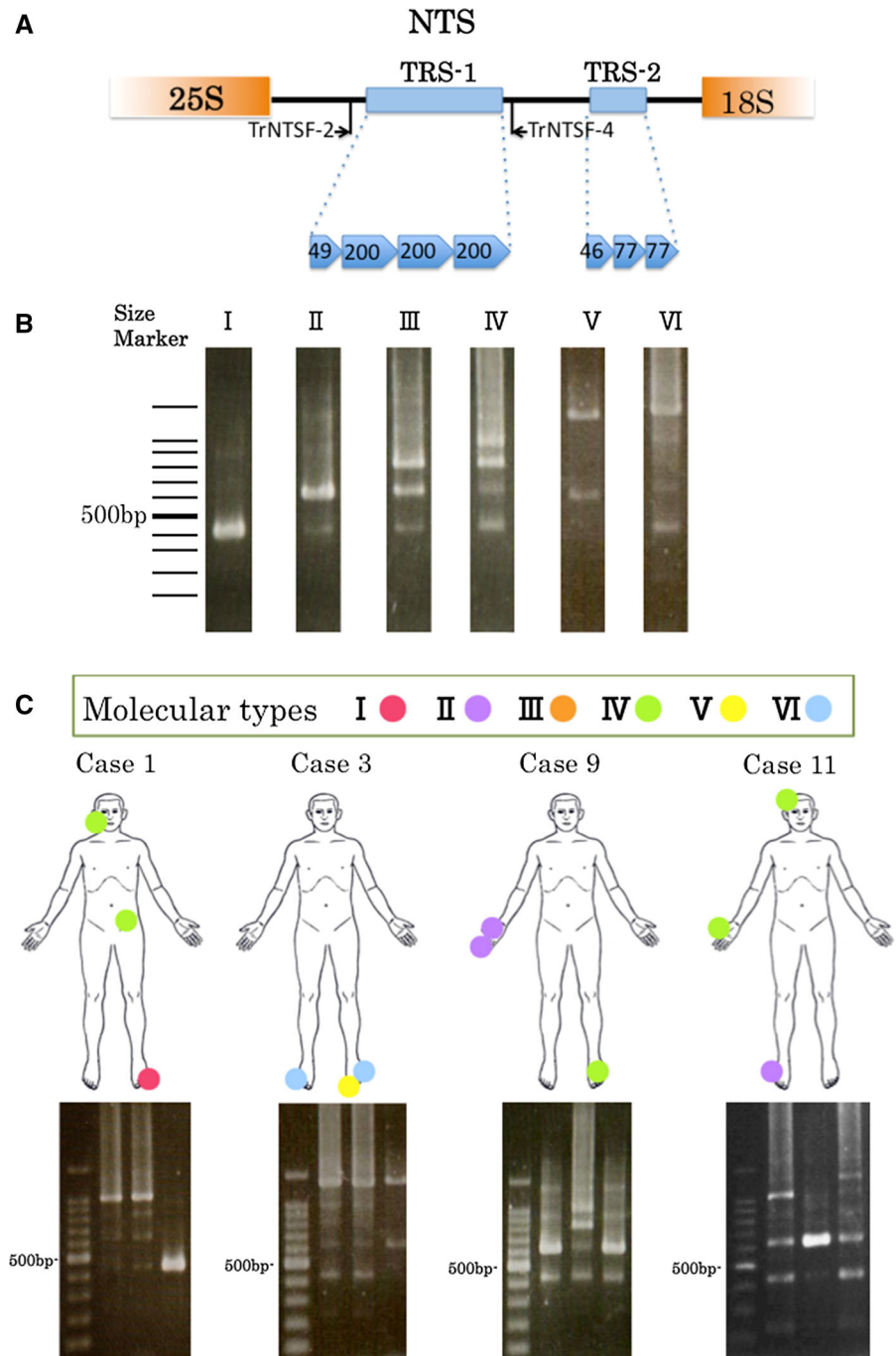
Another important pathogen of dermatophytosis is *T. tonsurans*, a major causative agent of tinea capitis among children and of tinea gladiatorum among players of contact sports such as wrestling and Judo. Outbreaks of *T. tonsurans* have led to interest in the molecular epidemiology of the isolates, enhancing understanding of the transmission of this fungus and its spread inside and outside the community. Molecular polymorphisms

Fig. 1 Strain differentiation in *T. rubrum*.

a Strain typing system of *T. rubrum*, based on length polymorphisms of non-transcribed spacer region of the ribosomal DNA detected using two sets of PCR primers, TrNTSF-2 and TrNTSR-4 for TRS-1, and TrNTSC-1 and TrNTSR-1 for TRS-2 (schematic representation reproduced from Jackson et al. [31]). TRS-1, more variable than TRS-2, was used for strain typing in this series.

b Molecular types detected for 31 *T. rubrum* isolates collected from 12 Japanese cases of tinea with multiple lesions. Isolates were classified into six molecular types although none was found in TRS-2 (K. Takeda, unpublished data).

c Multiple strains of *T. rubrum* were isolated from four of 12 tinea patients with multiple tinea lesions (K. Takeda, unpublished data)



were first identified by length variations of amplicons targeting the VIR region and insertions and deletions in the NTS combined with SNPs [16, 32]. Using this method, 92 isolates from the USA could be divided into 12 molecular types, whereas no variations were

observed within the ITS [32]. PCR-RFLP analysis using different primers targeting the NTS identified four molecular types among 19 isolates from Brazil, Italy, and China [42]. Another independent PCR-RFLP analysis of the VIR region identified six molecular

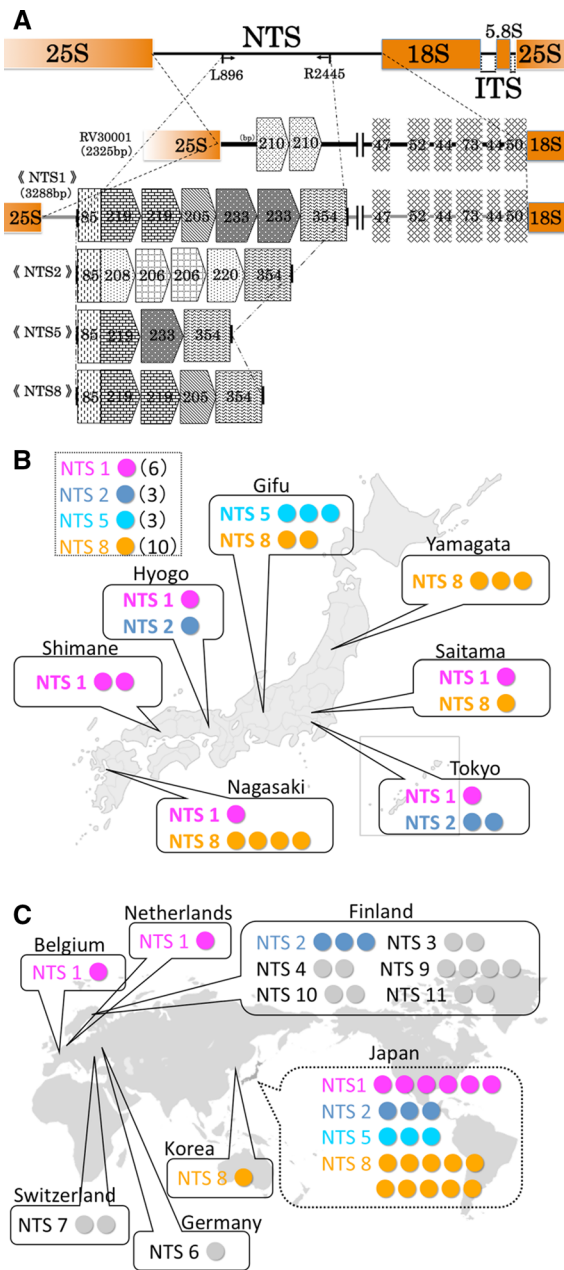


Fig. 2 Strain differentiation in *A. benhamiae*. **a** Structure of the non-transcribed spacer (NTS) region of the ribosomal DNA of *A. benhamiae*. Variations in length of the NTS were due to a variable number of repetitive units, consisting of relatively similar sequences, 205–233 bp in length, at the 5′-end of the NTS. In contrast, sequences at the 3′-end were very similar. **b** Molecular polymorphisms found among Japanese isolates of *A. benhamiae*. The 22 Japanese isolates were composed of four molecular types, i.e., NTS 1, 2, 5, and 8. The species was not found in Japan before 1980. **c** Global distribution of NTS subtypes of *A. benhamiae*. Strains showing NTS 1, 2, and 8 were found outside of Japan, suggesting this species recently came to Japan with imported animals on several occasions (reproduced from Takeda et al. [41])

types, NTS I and NTS II. In addition, all strains isolated from sumo wrestlers were classified as NTS I, suggesting that infections among sumo wrestlers originated from infections among judo athletes [45]. This study also assessed the minimum inhibitory concentrations (MICs) of terbinafine, itraconazole, fluconazole, and griseofulvin, but found no differences in MIC among these molecular types. Multilocus genotyping studies have assessed variations in NTS combined with sequence variations in enzyme coding regions for alkaline proteinase 1, carboxypeptidase Y, and metalloproteinase 5 [46] or subtilisin-like proteinases 2, 3, and 5 [47]. A wide degree of genetic variation was observed among North American isolates, whereas Australian and Japanese isolates were more clonal in nature. These findings suggest a relatively long relationship between this species and its host in North America [47], whereas outbreaks among Japanese contact sports players were caused by strains introduced from other global regions [47]. Using a similar method, large-scale population-based investigations were performed in a child care center in the USA, resulting in three types of carrier states, exclusive, predominant, and random carriers [16, 48].

Microsatellite DNA

Microsatellite DNAs, also called simple sequence repeats (SSRs) or minisatellites, are short, tandem repeating DNA sequences comprised of di-, tri-, tetra-, and penta-nucleotides, such as (AT)_n, (GT)_n, (GA)_n, (GATA)_n, and (GACA)_n. These repeats are ubiquitous in eukaryotic genomes and have been used for genetic fingerprinting of many higher organisms. These sequences are polymorphic in populations due

types among 232 strains isolated from judo athletes, wrestlers, sumo wrestlers, and several other sporadically infected individuals in Japan [43]. This result did not conflict with a report that all 101 strains isolated from Japanese judo athletes had the same profile [44], as, of the six molecular types found in the 232 isolates, one type, NTS I, was predominant, being present in 160 of 164 isolates from judo athletes [43]. However, isolates from wrestlers were of two major molecular

to the propensity for insertion/deletion mutations of multiples of these repeating units to be introduced during replication [49]. Two different approaches using microsatellite DNAs are available for molecular study of dermatophytes. One is the detection of variations in the number of repeating units at a genetic locus, as determined by PCR amplification of alleles using unique primers flanking the repeating sequence, followed by resolution of the PCR products on denaturing gels. More recently, primers labeled with fluorescent dyes have been used and the PCR products loaded onto a genetic analyzer, with the results observed as colored peaks, of sizes calculated by alignment with initial size standards [49] (Fig. 3). Multilocus microsatellite typing (MLMT) using several markers was able to detect variations among strains of *T. rubrum* [50] and was later applied to

additional isolates of this species to better understand its pathogenesis [51]. Using 22 microsatellite markers, 55 genotypes could be identified among 233 isolates, suggesting that populations of *T. rubrum* are geographically separated [51]. However, no diagnostic correlation was observed between genotypes and any phenotypic characteristics of the isolates [51]. The MLMT method was used to assess variations in *Microsporium persicolor*, a zoophilic fungus from rodents [52], and *M. canis*, a zoophilic dermatophyte pathogenic in dogs, cats, and humans [53]. The imbalance in the prevalence of MLMT genotypes among human and animal isolates of *M. canis* suggested that population differentiation was due to the emergence of a virulent genotype with a high potential to infect human hosts [53]. Using eight of 38 microsatellite markers, 22 genotypes were found

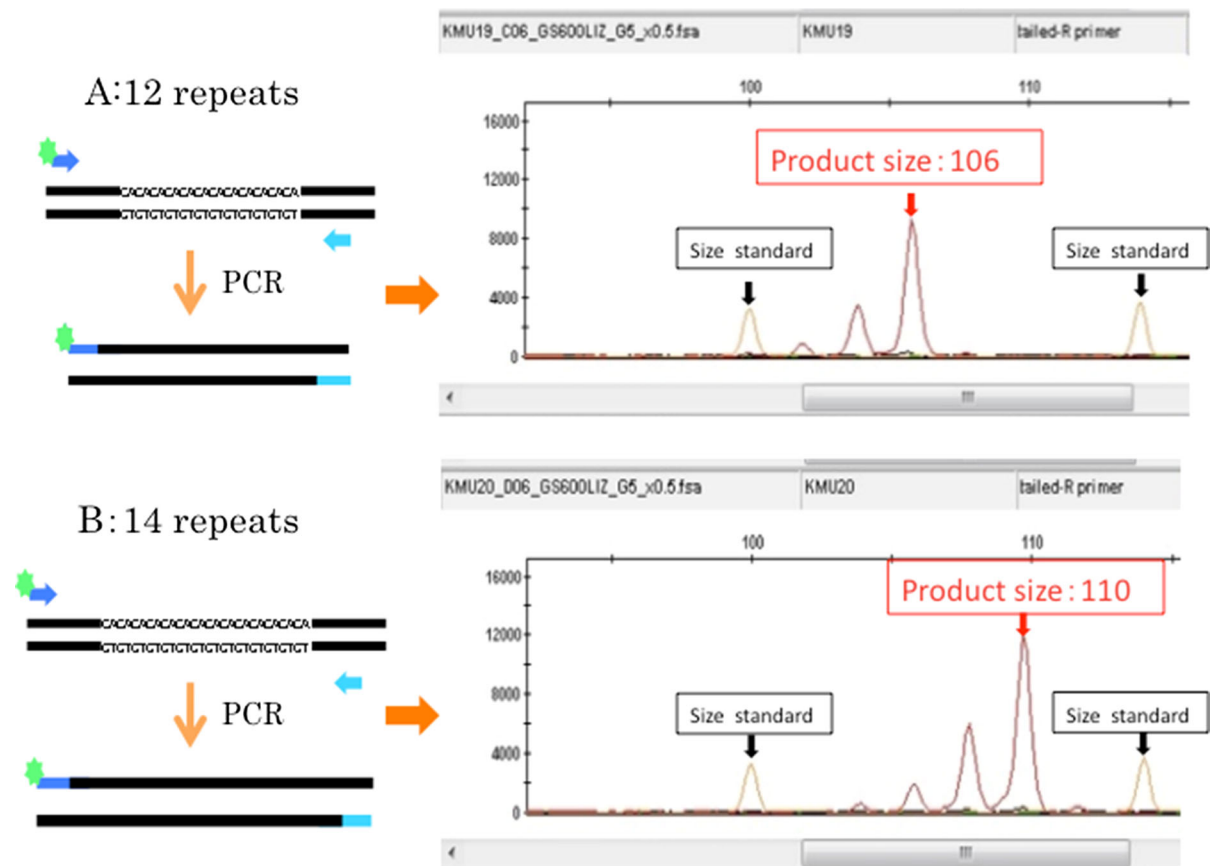


Fig. 3 Representative image of polymorphisms observed between *Microsporium canis* isolates. Microsatellite regions, polymorphic in length because of the variation in the number of repeating units (CA in this case), are first amplified by PCR

using primers flanking the repeating sequence (blue arrows). One primer is labeled with fluorescent dye (asterisk). Polymorphisms are then detected by a genetic analyzer (J. Watanabe, unpublished data)

among 26 *M. canis* strains isolated from 13 countries [49]. Using two microsatellite markers, 102 strains isolated in Brazil, including 19 from human patients, yielded a total of 14 genotypes, which could be assorted into six large populations [54]. Taken together, these findings indicated that MLMT is a reliable method for the differentiation of *M. canis* strains [49, 54], although screening for optimal probe/species combinations is laborious.

The other molecular methods using microsatellite DNA are based on a different principle and are very similar to that of RAPD. Primers designed in SSRs are used in single primer amplification reactions. This method, called microsatellite-primed PCR (MSP-PCR), is expected to amplify DNA sequences between SSRs, with the products DNA fingerprinted. One MSP-PCR study using (GTG)₅ and (GACA)₄ as single primers yielded species-specific banding profiles of *T. rubrum*, *T. mentagrophytes*, and *T. interdigitale*. However, no intraspecies polymorphisms were detected among 33 isolates of *M. canis*, which were found, by RAPD analysis using the primer OPK-20 (5'-GTGTCGCGAG-3'), to comprise five molecular types [55]. MSP-PCR using (GACA)₄ and (ACA)₅ also failed to detect molecular polymorphisms among 32 clinical isolates of *M. canis* [56], suggesting that this method is relatively insensitive.

Conclusions and Future Prospects

Methods for intraspecies subtyping and strain differentiation of dermatophytes are a new aspect in studying fungi. The knowledge obtained by these methods is accumulating, although improved discriminatory power is still required. Some of the methods described in this review are suitable for population-based investigations, determination of geographical distribution, precise descriptions in case reports, evaluation of epidemiology, and investigations of correlations between genotype and phenotype, such as antifungal susceptibility and pathogenicity. An ideal method must be sufficiently sensitive and easily performed on a number of strains at once, as well as yielding reproducible results. Whole-genome analysis has been performed among some dermatophyte species including *T. rubrum* [57], which suggests further intraspecies comparative analysis may reveal new candidate markers of strain differentiation.

However, the most sensitive current method available for strain differentiation is microsatellite analysis using primers flanking the microsatellite regions. Despite the previously reported absence of polymorphisms in the NTS region, this method was able to identify 22 genotypes among 26 isolates of *M. canis* [49]. Different molecular patterns among strains may be evidence that these strains are different. However, detection of the same pattern would not corroborate the hypothesis that these strains are the same, but only indicates the hypothesis has no conflict with the results. The use of multiple molecular markers at multiple loci makes a conclusion more certain.

Acknowledgments This publication and cited research is partially supported by the research program on emerging and re-emerging infectious diseases from Japan Agency for Medical Research and Development, AMED.

Compliance with Ethical Standards

Conflict of interest The authors declare there are no conflicts of interest including industrial links or affiliations.

References

1. Watanabe S, Harada T, Hiruma M, et al. Epidemiological survey of foot diseases in Japan: results of 30,000 foot checks by dermatologists. *J Dermatol.* 2010;37:397–406.
2. Davison FD, Mackenzie DWR. DNA homology studies in the taxonomy of dermatophytes. *Sabouraudia.* 1984;22:117–23.
3. de Bièvre C, Dauguet C, Nguyen VH, Ibrahim-Granet O. Polymorphism in mitochondrial DNA of several *Trichophyton rubrum* isolates from clinical specimens. *Ann Inst Pasteur Microbiol.* 1987;138:719–27.
4. Kawasaki M, Ishizaki H, Aoki M, Watanabe S. Phylogeny of *Nannizzia incurvata*, *N. gypsea*, *N. fulva* and *N. otae* by restriction enzyme analysis of mitochondrial DNA analysis. *Mycopathologia.* 1990;112:173–7.
5. Nishio K, Kawasaki M, Ishizaki H. Phylogeny of the genera *Trichophyton* using mitochondrial DNA analysis. *Mycopathologia.* 1992;117:127–32.
6. Mochizuki T, Sugie N, Uehara M. Random amplification of polymorphic DNA is useful for the differentiation of several anthropophilic dermatophytes. *Mycoses.* 1997;40:405–9.
7. Kim JA, Takahashi Y, Tanaka R, Fukushima K, Nishimura K, Miyaji M. Identification and subtyping of *Trichophyton mentagrophytes* by random amplification of polymorphic DNA. *Mycoses.* 2001;44:157–65.
8. Makimura K, Mochizuki T, Hasegawa A, Uchida H, Saito H, Yamaguchi H. Phylogenetic classification of *Trichophyton mentagrophytes* complex strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. *J Clin Microbiol.* 1998;36:2629–33.

9. Makimura K, Tamura Y, Mochizuki T, et al. Phylogenetic classification and species identification of dermatophyte strains based on DNA sequence of nuclear ribosomal internal transcribed spacer 1 regions. *J Clin Microbiol.* 1999;37:920–4.
10. Kano R, Nakamura Y, Watari T, et al. Species-specific primers of chitin synthase 1 gene for the differentiation of the *Trichophyton mentagrophytes* complex. *Mycoses.* 1999;42:71–4.
11. Kanbe T, Suzuki Y, Kamiya A, et al. Species-identification of dermatophytes *Trichophyton*, *Microsporum* and *Epidermophyton* by PCR and PCR-RFLP targeting of the DNA topoisomerase II genes. *J Dermatol Sci.* 2003;33:41–54.
12. Sun PL, Hsieh HM, Ju YM, Jee SH. Molecular characterization of dermatophytes of the *Trichophyton mentagrophytes* complex found in Taiwan with emphasis on their correlation with clinical observations. *Br J Dermatol.* 2010;163:1312–8.
13. Rezaei-Matehkolaei A, Mirhendi H, Makimura K, et al. Nucleotide sequence analysis of beta tubulin gene in a wide range of dermatophytes. *Med Mycol.* 2014;52:674–88.
14. Gräser Y, Scott J, Summerbell R. The new species concept in dermatophytes—a polyphasic approach. *Mycopathologia.* 2008;166:239–56.
15. Cafarchia C, Iatta R, Latrofa MS, Gräser Y, Otranto D. Molecular epidemiology, phylogeny and evolution of dermatophytes. *Infect Genet Evol.* 2013;20:336–51.
16. Abdel-Rahman SM. Strain differentiation of dermatophytes. *Mycopathologia.* 2008;166:319–33.
17. Kanbe T. Molecular approaches in the diagnosis of dermatophytosis. *Mycopathologia.* 2008;166:307–17.
18. Gräser Y, Kuhnisch J, Presber W. Molecular markers reveal exclusively clonal reproduction in *Trichophyton rubrum*. *J Clin Microbiol.* 1999;37:3713–7.
19. Kac G. Molecular approaches to the study of dermatophytes. *Med Mycol.* 2000;38:329–36.
20. Wu Y, Yang J, Yang F, et al. Recent dermatophyte divergence revealed by comparative and phylogenetic analysis of mitochondrial genomes. *BMC Genomics.* 2009;10:238.
21. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.* 1990;18:6531–5.
22. Weising K, Nybom H, Wolff K, Meyer W. Chapter 4 Methodology, IV. PCR based DNA fingerprinting. DNA fingerprinting in plants and fungi. Boca Raton: CRC Press; 1995. p. 112–36.
23. Baeza LC, Matsumoto MT, Almeida AMF, Mendes-Giannini MJS. Strain differentiation of *Trichophyton rubrum* by randomly amplified polymorphic DNA and analysis of rDNA nontranscribed spacer. *J Med Microbiol.* 2006; 55:429–36.
24. Santos DA, Araujo RA, Hamdan JS, Cisalpino PS. *Trichophyton rubrum* and *Trichophyton interdigitale*: genetic diversity among species and strains by random amplified polymorphic DNA method. *Mycopathologia.* 2010;169: 247–55.
25. Liu D, Coloe S, Baird R, Pederson J. Application of PCR to the identification of dermatophyte fungi. *J Med Microbiol.* 2000;49:493–7.
26. Irinyi L, Serena C, Garcia-Hermoso D, et al. International society of human and animal mycology (ISHAM)-ITS reference DNA barcoding database—the quality controlled standard tool for routine identification of human and animal pathogenic fungi. *Med Mycol.* 2015;53:313–37.
27. Cafarchia C, Otranto D, Weigl S, et al. Molecular characterization of selected dermatophytes and their identification by electrophoretic mutation scanning. *Electrophoresis.* 2009;30:3555–64.
28. Symoens F, Jousson O, Planard C, et al. Molecular analysis and mating behaviour of the *Trichophyton mentagrophytes* species complex. *Int J Med Microbiol.* 2011;301:260–6.
29. Mochizuki T, Tanabe H, Kawasaki M, Ishizaki H, Jackson CJ. Rapid identification of *Trichophyton tonsurans* by PCR-RFLP analysis of ribosomal DNA regions. *J Dermatol Sci.* 2003;32:25–32.
30. Jackson CJ, Barton RC, Evans EGV. Species identification and strain differentiation of dermatophyte fungi by analysis of ribosomal DNA spacer regions. *J Clin Microbiol.* 1999;37:931–6.
31. Jackson CJ, Barton RC, Kelly SL, Evans EGV. Strain identification of *Trichophyton rubrum* by specific amplification of subrepeat elements in the ribosomal DNA non-transcribed spacer. *J Clin Microbiol.* 2000;38:4527–34.
32. Gaedigk A, Gaedigk R, Adbel-Rahman SM. Genetic heterogeneity in the rRNA gene locus of *Trichophyton tonsurans*. *J Clin Microbiol.* 2003;41:5478–87.
33. Mochizuki T, Ishizaki H, Barton RC, et al. Restriction fragment length polymorphism analysis of ribosomal DNA intergenic regions is useful for differentiating strains of *Trichophyton mentagrophytes*. *J Clin Microbiol.* 2003;41:4583–8.
34. Mochizuki T, Kawasaki M, Ishizaki H, et al. Molecular epidemiology of *Arthroderma benhamiae*, an emerging pathogen of dermatophytoses in Japan, by polymorphisms of the non-transcribed spacer region of the ribosomal DNA. *J Dermatol Sci.* 2001;27:14–20.
35. Mochizuki T, Watanabe S, Kawasaki M, Tanabe H, Ishizaki H. A Japanese case of tinea corporis caused by *Arthroderma benhamiae*. *J Dermatol.* 2002;29:221–5.
36. Yazdanparast A, Jackson CJ, Barton RC, Evans EGV. Molecular typing of *Trichophyton rubrum* indicates multiple strain involvement in onychomycosis. *Br J Dermatol.* 2003;148:51–4.
37. Gupta AK, Nakrieko KA. *Trichophyton rubrum* DNA strain switching increases in patients with onychomycosis failing antifungal treatments. *Br J Dermatol.* 2015;172: 74–80.
38. Takeda K, Mochizuki H, Izumi K, et al. Polyclonality of *Trichophyton rubrum* isolates in a dermatophytosis patient with multiple lesions. *Med Mycol J.* 2016;57E:E17–20.
39. Jackson CJ, Mochizuki T, Barton RC. PCR fingerprinting of *Trichophyton mentagrophytes* var. *interdigitale* using polymorphic subrepeat loci in the rDNA nontranscribed spacer. *J Med Microbiol.* 2006;55:1349–55.
40. Wakasa A, Anzawa K, Kawasaki M, Mochizuki T. Molecular typing of *Trichophyton mentagrophytes* var. *interdigitale* isolated in a university hospital in Japan based on the non-transcribed spacer region of the ribosomal RNA gene. *J Dermatol.* 2010;37:431–40.
41. Takeda K, Nishibu A, Anzawa K, Mochizuki T. Molecular epidemiology of a major subgroup of *Arthroderma benhamiae* isolated in Japan by restriction fragment length

- polymorphism analysis of the non-transcribed spacer region of ribosomal RNA gene. *Jpn J Infect Dis.* 2012;65:233–9.
42. Abliz P, Takizawa K, Nishimura K, Fukushima K. Molecular typing of *Trichophyton tonsurans* by PCR–RFLP of the ribosomal DNA nontranscribed spacer region. *J Dermatol Sci.* 2004;36:125–7.
 43. Mochizuki T, Kawasaki M, Tanabe H, Anzawa K, Ishizaki H, Choi JS. Molecular epidemiology of *Trichophyton tonsurans* isolated in Japan using RFLP analysis of non-transcribed spacer regions of ribosomal RNA genes. *Jpn J Infect Dis.* 2007;60:188–92.
 44. 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.
 45. Anzawa K, Mochizuki T, Nishibu A, et al. Molecular epidemiology of *Trichophyton tonsurans* isolated in Japan between 2006 and 2010 and their susceptibility to oral antimycotics. *Jpn J Infect Dis.* 2011;64:458–62.
 46. Adbel-Rahman SM, Preuett B, Gaedigk A. Multilocus genotyping identifies infections by multiple strains of *Trichophyton tonsurans*. *J Clin Microbiol.* 2007;45:1949–53.
 47. Adbel-Rahman SM, Sugita T, Gonzalez GM, et al. Divergence among an international population of *Trichophyton tonsurans* isolates. *Mycopathologia.* 2010;169:1–13.
 48. Adbel-Rahman SM, Simon S, Wright KJ, Ndjountche L, Gaedigk A. Tracking *Trichophyton tonsurans* through a large urban child care center: defining infection prevalence and transmission patterns by molecular strain typing. *Pediatrics.* 2006;118:2365–73.
 49. Pasquetti M, Peano A, Soglià D, et al. Development and validation of microsatellite marker-based method for tracing infections by *Microsporium canis*. *J Dermatol Sci.* 2013;70:123–9.
 50. Ohst T, de Hoog GS, Presber W, Stavrakieva V, Gräser Y. Origins of microsatellite diversity in the *Trichophyton rubrum*-*T. violaceum* clade (dermatophytes). *J Clin Microbiol.* 2004;42:4444–8.
 51. Gräser Y, Fröhlich J, Presber W, de Hoog GS. Microsatellite markers reveal geographic population differentiation in *Trichophyton rubrum*. *J Med Microbiol.* 2007;56:1058–65.
 52. Sharma R, Presber W, Rajak RC, Gräser Y. Molecular detection of *Microsporium persicolor* in soil suggesting widespread dispersal in central India. *Med Mycol.* 2008;46:67–73.
 53. Sharma R, de Hoog GS, Presber W, Gräser Y. A virulent genotype of *Microsporium canis* is responsible for the majority of human infections. *J Med Microbiol.* 2007; 56:1377–85.
 54. da Costa FV, Farias MR, Bier D, et al. Genetic variability in *Microsporium canis* isolated from cats, dogs and humans in Brazil. *Mycoses.* 2013;56:582–8.
 55. Spesso MF, Nuncira CT, Burstein VL, Masih DT, Did MD, Chiapello LS. Microsatellite-primed PCR and random primer amplification polymorphic DNA for the identification and epidemiology of dermatophytes. *Eur J Clin Microbiol Infect Dis.* 2013;32:1009–15.
 56. Dobrowolska A, Debska J, Kozłowska M, Staczek P. Strains differentiation of *Microsporium canis* by RAPD analysis using (GACA)₄ and (ACA)₅ primers. *Pol J Microbiol.* 2011;60:145–8.
 57. Martinez DA, Oliver BG, Gräser Y, et al. Comparative genome analysis of *Trichophyton rubrum* and related dermatophytes reveals candidate genes involved in infection. *MBio.* 2012;3:e00259-12.