Molecular Analysis of Dermatophytes Suggests Spread of Infection Among Household Members

Mahmoud A. Ghannoum, PhD; Pranab K. Mukherjee, PhD; Erin M. Warshaw, MD; Scott Evans, PhD; Neil J. Korman, MD, PhD; Amir Tavakkol, PhD, DipBact

Practice Points

- When a patient presents with tinea pedis or onychomycosis, inquire if other household members also have the infection, investigate if they have a history of concomitant tinea pedis and onychomycosis, and examine for plantar scaling and/or nail discoloration.
- If the variables above are observed, think about spread of infection and treatment options.

Drs. Ghannoum, Mukherjee, and Korman are from University Hospitals Case Medical Center, Cleveland, Ohio. Dr. Warshaw is from the University of Minnesota, Minneapolis, and Minneapolis Veterans Affairs Medical Center. Dr. Evans is from the Harvard School of Public Health, Boston, Massachusetts. Dr. Tavakkol was from Novartis Pharmaceuticals Corporation, East Hanover, New Jersey, and currently is from Topica Pharmaceuticals, Inc, Los Altos, California.
This article was supported by a grant from Novartis Pharmaceuticals Corporation. Dr. Ghannoum has served as a consultant and/or speaker for and has received grants and contracts from Merck & Co, Inc; Novartis Pharmaceuticals Corporation; Pfizer Inc; and Stiefel, a GSK company. Dr. Mukherjee has served as a consultant and/or speaker for and has received grants and contracts from Astellas Pharma Inc; Gebauer Company; Great Lakes Pharmaceuticals, Inc; and Saval Pharmaceuticals. Dr. Warshaw has served as a consultant and/or has conducted clinical trials for Clay-Park Labs Inc; Dermik Laboratories, Inc; Fujisawa Pharmaceutical Company, Ltd; Glenmark; Novartis Pharmaceuticals Corporation; Shire; Teva Pharmaceutical Industries Ltd; and Tolmar Inc. Dr. Evans reports no conflict of interest. Dr. Korman serves as a consultant and speaker for and/or receives grant support from Abbott Laboratories; Amgen Inc; Astellas Pharma Inc; Celgene Corporation; Centocor Ortho Biotech Inc; Genentech, Inc; Genmab; Novartis Pharmaceuticals Corporation; Peplin Ltd; and Watson Pharma Company. Dr. Tavakkol was an employee of Novartis Pharmaceuticals Corporation.
Correspondence: Mahmoud A. Ghannoum, PhD, Center for Medical Mycology, University Hospitals of Cleveland and Case Western Reserve University, 11100 Euclid Ave, Cleveland, OH 44106 (Mahmoud.Ghannoum@case.edu).

Dermatophyte infection from the same strains may be an important route for transmission of dermatophytoses within a household. In this study, we used molecular methods to identify dermatophytes in members of dermatophyte-infected households and evaluated variables associated with the spread of infection. Fungal species were identified by polymerase chain reaction (PCR) using primers targeting the internal transcribed spacer (ITS) regions (ITS1 and ITS4). For strain differentiation, fungal DNA was probed with a ribosomal DNA–specific probe (containing ITS1, 5.8S ribosomal DNA, and ITS2) to detect restriction fragment length polymorphisms (RFLPs). Associations between the spread of a dermatophyte infection and fungal/host variables were determined using χ^2 *and logistic regression analyses. Among the 50 households enrolled in this study, 18 included multiple infected members (MIMs).* Trichophyton rubrum *was the most commonly isolated dermatophyte species, followed by* Trichophyton mentagrophytes *and* Epidermophyton floccosum*. Sixteen* T rubrum *strains (TR-A to TR-P) were identified, with spread of infection detected in 8 MIM households. Factors that were significantly* ($P<.05$)

associated with the spread of infection included the presence of strains TR-B or TR-D, a history of concomitant tinea pedis and onychomycosis, and plantar scaling and/or nail discoloration. This study is unique in that it used molecular evidence to demonstrate the association of certain strains with the spread of dermatophyte infection among members of the same household.

Cutis. 2013;91:237-245.

Dermatophytes are fungi that can infect keratinous tissue, including the hair, skin, and nails, resulting in cutaneous mycoses called dermatophytoses, such as tinea or ringworm infections. Fungal infection of the nails is called onychomycosis and infection of the feet is referred to as tinea pedis. Onychomycosis is most often caused by dermatophytes,[1] namely *Trichophyton rubrum* (responsible for approximately 80% of nail infections), *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*. Although onychomycosis can present in fingernails, it most often affects the toenails.[2,3] Tinea pedis occurs in nearly 50% of patients with onychomycosis[4]; in susceptible patients, many cases of toenail fungus initially begin as tinea pedis.[1,5] Tinea pedis and onychomycosis are widespread in developed countries, with nearly 10% of the population being infected at any given time.[3,6]

Tinea pedis and onychomycosis are known to be transmitted through direct or indirect contact with infected skin lesions or a contaminated environment.[7,8] Although attempts have been made to investigate the spread of dermatophyte infections among members of the same household,[9] earlier analysis precluded unequivocal demonstration of the spread of infection due to a single strain because molecular techniques were not available. In this cross-sectional phase 4 clinical trial, we isolated dermatophytes from infected members of enrolled households, typed the isolated strains using polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLPs), and determined the association of spread of infection with fungal- and disease-specific variables.

Materials and Methods

Study Design—Households with at least 2 residents and 1 individual (index person [IP]) with tinea pedis and/or onychomycosis were identified by screening. Infection was confirmed clinically and mycologically (potassium hydroxide [KOH] and culture positive) in the IP; then other members within the same household were examined for tinea pedis and/or onychomycosis using the same criteria. All participants with confirmed fungal infections were referred to their primary care physicians for appropriate treatment.

Household members were screened at 2 dermatology centers (University Hospitals Case Medical Center, Cleveland, Ohio [site 1], and University of Minnesota, Minneapolis [site 2]) and 2 podiatry centers (Blair Medical Associates, Altoona, Pennsylvania [site 3] and Joppa Foot Care, Parkville, Maryland [site 4]). Participants were reimbursed for costs associated with their participation in the study.

Enrolled participants met the following inclusion criteria: at least 2 members living in the same household, older than 14 years, and at least 1 member with current tinea pedis and/or onychomycosis as identified by clinical signs and positive KOH and culture of dermatophyte. Exclusion criteria included treatment with over-the-counter or home remedies within 4 weeks of the screening visit and use of prescription oral or topical antifungal medications within 4 months of the screening visit.

Study Duration and Evaluation Criteria—The study was approved by an institutional review board at all 4 study sites, and all participants completed an approved consent form prior to enrollment. The date of the first participant's visit was May 11, 2005, and the date of the last participant's visit was January 9, 2006. The following evaluation criteria were used for enrolling participants: clinical signs of tinea pedis and/or onychomycosis as well as positive mycologic KOH and culture.

The study consisted of 1 clinic visit (visit 1) during which all IPs were evaluated by a dermatologist or podiatrist for clinical signs of skin and/or toenail fungal infection. Samples were then taken from IPs with clinical signs of onychomycosis and/or tinea pedis; if a positive culture and KOH was reported for the IP, household members were contacted for study enrollment. For each IP, personal and family history of tinea pedis and/or onychomycosis was recorded, clinical examination of whole-body skin was conducted, and samples were collected from the feet and/or toenails for mycologic culture and KOH. The IP was notified of the results of the mycologic culture and KOH by telephone and letter. Following isolation of a dermatophyte from the IP, additional household members were enrolled and evaluated using the same evaluation criteria. Samples were collected from household members within 4 weeks of reporting the IP's culture positivity. Any use of over-the-counter treatments, home remedies, or prescription oral or topical antifungal medications by non-IP participants was documented.

The clinical signs of tinea pedis included itching, burning, redness, scaling, blisters, and tissue maceration of toe webs. Clinical signs of onychomycosis

included discoloration, onycholysis (lifting of the nail from the nail bed), and hyperkeratosis (crumbling subungual debris). Clinical signs were graded by severity (0=absent; 1=mild; 2=moderate; 3=severe). Composite scores were used in statistical programs to analyze spread of infection among household members.

Mycologic Identification—Clinical specimens (skin and nail samples) were plated onto general (potato dextrose agar) and inhibitory media (Mycosel agar) (both Becton, Dickinson and Company) and incubated at 35°C for 1 to 4 weeks. Plates were observed weekly, and colonies of interest were examined microscopically and/or were biochemically tested for genus and species identification.[10-12] All mycologic and molecular identification procedures were conducted at the Center for Medical Mycology, Cleveland, Ohio.

Polymerase Chain Reaction–Based Species Identification—Genomic DNA was isolated from collected isolates using MasterPure Yeast DNA Purification Kit (Epicentre), and the internal transcribed spacer (ITS) regions ITS1, 5.8S, and ITS2 were PCR amplified as previously described.[13] Amplification reactions were carried out with volumes of 100 μL containing reaction buffer (50 mM potassium chloride; 10 mM tris hydrochloride [pH 9.0]; 0.1% Triton X-100; 1.5 mM magnesium chloride; deoxynucleotide triphosphate mix [0.2 mM each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, deoxythymosine triphosphate]); 30 pmol each of primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′); 5 U of *Taq* DNA polymerase; and approximately 10 ng of template DNA. The PCR cycling conditions were 35 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes, followed by an extension step of 72°C for 10 minutes. Polymerase chain reaction products were purified using a QIAquick PCR Purification Kit (Qiagen), digested with *Mva*I, and analyzed by agarose gel electrophoresis.

Restriction Fragment Length Polymorphism–Based Strain Differentiation—An RFLP-based approach was used to identify dermatophytes to the strain level as previously described.[14] Each DNA sample (10 μg) was digested for 18 hours with 18 U of *Eco*RI in a total volume of 20 μL. Samples were separated via agarose gel electrophoresis and were transferred to nylon membrane using standard Southern blotting protocols.[15,16] A ribosomal DNA probe (3′-18S-ITS1-ITS2) was amplified (using same PCR conditions as above) from template DNA of ATCC isolates of *T rubrum* and *T mentagrophytes* using universal primers NS5 (5′-AACTTAAAGGAATTGACGGAAG-3′) and ITS4.[14] Hybridization signal was detected by chemiluminescence. Isolates with the same band pattern were considered identical. Spread of infection was concluded when 1 or more identical strains were isolated from members of the same household. Strain-type designations were arbitrarily assigned and were nonidentical across the 3 dermatophyte species.

Statistical Analysis—An association between the spread of infection and each variable was determined using Pearson's χ^2 test or logistic regression analysis (forced entry method) at a significance threshold of $P<.05$. Risk estimates (odds ratio [OR]) and 95% confidence intervals (CIs) were compared for variables exhibiting statistically significant association with spread of infection. The goodness of fit of different models was compared using the Hosmer-Lemeshow test. All statistical analyses were performed using SPSS (version 16).

Results

Baseline Characteristics—Of 107 households screened, dermatophyte infections were identified in 50 households (32 single infected member [SIM] households and 18 multiple infected member [MIM] households). The mean age (standard deviation [SD]) of the study participants was 57.73 (17.61) years for SIM households and 50.33 (20.80) years for MIM households (Table 1).

Distribution of Dermatophyte Species—Internally transcribed, spacer-based PCR amplification generated an approximately 640-bp amplicon from all isolates; digestion of this amplicon with *Mva*I resulted in species-specific band patterns. *Trichophyton rubrum* isolates consistently exhibited a set of 3 bands (approximately 380, 430, and 550 bp), while a fourth band of 700 bp also was detected in some isolates, likely representing undigested amplicon. In contrast, *T mentagrophytes* isolates exhibited bands of 220 bp, a doublet band set around 400 bp, and a band around 700 bp. The *Mva*I-digested band patterns for *T rubrum* (n=8) and *T mentagrophytes* (n=1) isolates obtained from participants enrolled in this study were similar to those observed for the respective reference ATCC strains of these species. Such species-specific patterns also were obtained for the clinical and the ATCC *E floccosum* isolates tested. Polymerase chain reaction–based identification of the 3 dermatophyte species was in agreement with culture-based identification.

Our PCR analysis showed that 47% (50/107) of households had dermatophytes known to cause onychomycosis and/or tinea pedis. A total of 107 dermatophyte isolates were obtained from members of households with dermatophyte infection (Figure 1). Among these 107 isolates, 42 were obtained

Table 1.

Select Baseline Characteristics of Enrolled Households

Variable	SIM Households	MIM Households
Mean age (SD), y	57.73 (17.61)	50.33 (20.80)
<25	23.71 (0.76)	22.10 (1.85)
25–49	44.62 (4.34)	41.20 (6.84)
50–74	62.26 (5.14)	65.69 (8.17)
≥75	79.80 (3.29)	78.62 (4.24)
Study Site, n		
Site 1[a]	6	2
Site 2[b]	25	14
Site 4[c]	1	2
Gender of individuals,[d] n		
Male	4	12
Female	27	23

Abbreviations: SIM, single infected member; MIM, multiple infected member; SD, standard deviation.
[a]University Hospitals Case Medical Center, Cleveland, Ohio.
[b]University of Minnesota, Minneapolis.
[c]Joppa Foot Care, Parkville, Maryland.
[d]Gender information was not provided for 1 SIM household and 3 individuals in 1 MIM household.

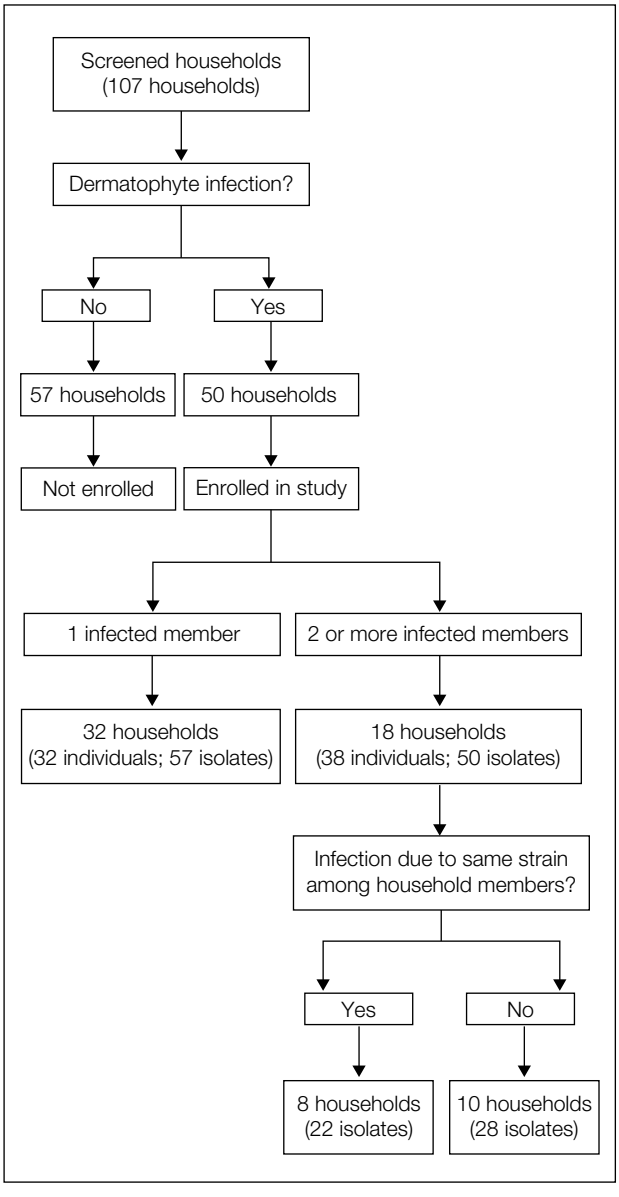

Figure 1. Schema outlining the study design.

from skin samples, 39 originated from toenail specimens, and the remaining 26 were present in both skin and toenails. Among the 50 households in which dermatophytes were isolated, 32 were SIM households (57 isolates obtained from 32 participants), while 18 were MIM households (50 isolates obtained from 38 participants). Among the 50 isolates obtained from MIM households, *T rubrum* was the most common (74% [37/50]), followed by *T mentagrophytes* (24% [12/50]) and *E floccosum* (2% [1/50]).

Distribution of Dermatophyte Strain Types—Southern blot analysis and RFLP evaluation of isolates obtained from all enrolled households identified 16 *T rubrum* strain types (TR-A to TR-P), 4 *T mentagrophytes* strain types (TM-A to TM-D), and 2 *E floccosum* strain types (EF-A and EF-B) (Figure 2). Isolates obtained from MIM households consisted of 37 *T rubrum* strains, with the most common strain types being type D (35.1% [13/37]), type B (24.3% [9/37]), and type G (13.5% [5/37]) (Figure 3). Isolates from these households also included *T mentagrophytes* isolates, with strain type TM-D being the most common. Among the 57 isolates obtained from SIM households,

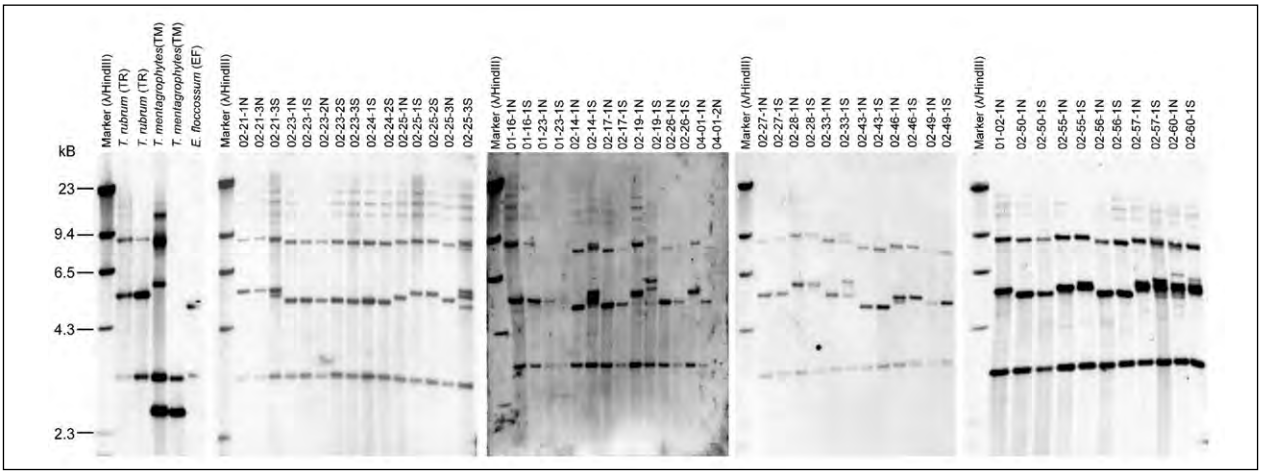

Figure 2. Representative Southern blotting images showing presence of different strains of dermatophytes in infected nail (N) or skin (S) samples. Molecular weight markers (λ/HindIII) are indicated on the left side of the images. Nomenclature of clinical samples includes Study Site-Family ID-Individual and Organism Source (ie, 02-25-1N indicates Study Site 02, Family ID 25, 1 [index person] and nail sample).

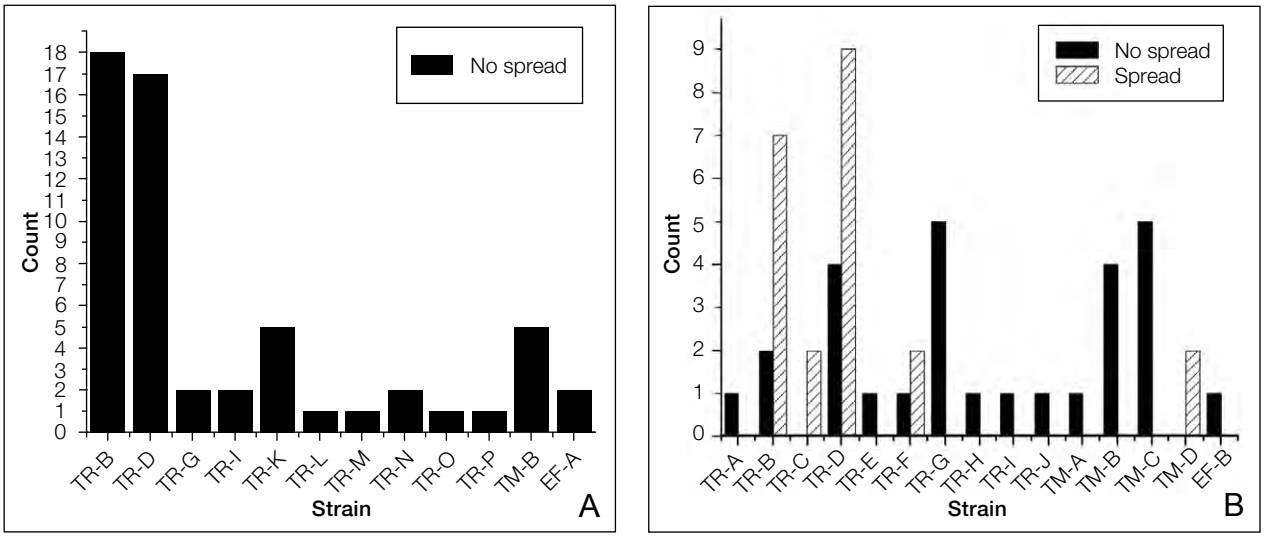

Figure 3. Distribution of dermatophyte species (*Trichophyton rubrum* [TR], *Trichophyton mentagrophytes* [TM], and *Epidermophyton floccosum* [EF]) and strains in households with a single infected member (A) or multiple infected members (B). Spread of infection due to the same strain in a household was determined based on restriction fragment length polymorphisms.

50 isolates belonging to 10 strain types of *T rubrum* were isolated, with the most common strains belonging to types TR-B and TR-D (36% [18/50] and 34% [17/50], respectively)(Figure 3). Furthermore, 5 *T mentagrophytes* isolates (all strain type TM-B) and 2 *E floccosum* isolates (both strain type EF-A) were isolated from individuals in SIM households.

Association of Spread of Infection With Fungal Species or Strains—To determine if dermatophyte infection in 1 household member could spread to other members, we analyzed the Southern blot and RFLP band patterns for different clinical isolates obtained from members residing in the same MIM households. Our study showed spread of infection was associated with specific strain types in 44% (8/18) of the MIM households, with presence of *T rubrum* in members of 7 households and with *T mentagrophytes* in members of 1 household. No spread of infection was noted in the remaining 10 MIM households. Next, we determined the trends of spread of infection among the 50 individual isolates obtained from members residing in the 18 MIM

households. Our analysis showed that 44% (22/50) of the strains were associated with spread of infection among individuals living in these households; of these strains, 20 were *T rubrum* isolates and 2 were *T mentagrophytes* isolates. Among the 20 *T rubrum* isolates involved in spread of infection, only strain types TR-B, TR-C, TR-D, and TR-F were detected. Strain type TR-D was involved in 45% (9/20) of cases, followed by TR-B, which was isolated in 35% (7/20) of cases. The 2 *T mentagrophytes* isolates involved in spread of infection belonged to type TM-D. Furthermore, χ^2 analyses revealed an OR of 6.47 for *T rubrum*, indicating that the odds of this strain being associated with spread of infection was 6 times higher than the other strain types.

Logistic regression analysis showed that *T rubrum* (as the infecting species) and strain types TR-D or TR-B were significantly associated with spread of infection due to isolates with similar genotypes ($P \leq .04$ for all comparisons).

Association of Spread of Infection With Clinical Variables—The relationship between various host-associated factors and spread of infection among individuals residing in the same households was conducted. Our analysis showed that there was no association between the spread of infection and history of tinea pedis or onychomycosis ($P > .05$). However, when comparing the spread of infection with dual history of both tinea pedis and onychomycosis, we found a significant association (OR, 3.98; 95% CI, 1.02-15.51; $P = .046$), suggesting that members residing in MIM households with history of both tinea pedis and onychomycosis were 3 times more likely to have concurrent infection than those without such history.

We also tested if symptoms localized to the feet (eg, lateral, plantar, toe web scaling) could be associated with the spread of infection. Our analysis showed a statistically significant ($P = .045$) association of only 1 such variable—plantar scaling—with spread of infection (OR, 4.4; 95% CI, 1.03-18.74), indicating that participants with plantar scaling were 4.4 times more likely to have concurrent infection than those with no such scaling. Our analyses also revealed that nail discoloration was significantly associated with the spread of infection (OR, 9.41; 95% CI, 1.07-83.02; $P \leq .04$). Although fitness centers are considered as the environment where dermatophyte infections may be likely to spread, none of the participants who exhibited concurrent infection reported that they had visited such centers.

Furthermore, among the 8 households where spread of infection was concluded, 4 had secondary infected members that genetically were related to the IP, with 1 having infected cousins (household 01-21) and 3 having an infected child and a parent (households 02-23, 02-25, and 02-29)(Table 2). Two households (02-23, 02-25) had both spouse and child infected with strains infecting the IP.

Comment

Our study used a molecular approach to investigate the spread of dermatophyte infection within a household. Our PCR analyses showed that *T rubrum* was the most common species isolated, followed by *T mentagrophytes* and *E floccosum*. This trend was similar to other reports.[12,17-20] Additionally, we were able to differentiate between various strains using RFLP analyses, which revealed the presence of 16 *T rubrum* strains (TR-A to TR-P), 4 *T mentagrophytes* strains (TM-A to TM-D), and 2 *E floccosum* strains (EF-A and EF-B). These results are consistent with the findings of Jackson et al[14] who reported 14 individual RFLP patterns (TR-A to TR-N) among 50 clinical isolates of *T rubrum* tested.

The proportion of households with infection that spread among members in our study (8 of 18 households [44%]) resembles an earlier epidemiologic study by English,[9] which reported spread of infection in 9 of 19 (47%) households with at least 1 infected member. Among MIM households, we found that only strain types TR-A through TR-J were isolated. Logistic regression analysis revealed significant association with *T rubrum* isolates (TR-D strain) and spread of infection among household members ($P = .009$). Regression analysis further showed that history of tinea pedis plus 3 clinical variables—onychomycosis, plantar scaling (a clinical sign of tinea pedis), and nail discoloration (a clinical sign of onychomycosis and generally indicative of severe nail infection)[3,21]—were statistically associated with spread of infection in MIM households ($P \leq .044$). These results are in agreement with earlier studies, which also investigated the relationship between prior disease or nail infection with the spread of dermatophyte infections. English[9] revealed that clinical signs of disease occurred in members of households that were exposed for a period of 1 to 15 years. The effect of prior exposure on carriage within a household also was investigated by Pomeranz et al[22] who showed dermatophytes may persist asymptomatically among members of an infected individual's household for up to 2 months in some cases. These authors suggest that progression of disease in the nails can be a predictor of the spread of infection among household members.[22]

The results of our study, which suggest that multiple strains were present in some samples, are in agreement with those reported by Yazdanparast et al[23]

Table 2.

Households With Spread of Dermatophyte Infection

Household No.	Participant No.	Sex	Relation to Index	Dermatophyte Species	Strain Type	Tissue Source
01-21[a]	01-21-01[b]	Male	Self	TR	F	Skin
	01-21-03	Male	Cousin	TR	D	Skin
	01-21-03	Male	Cousin	TR	F	Toenail
02-21	02-21-01[b]	Male	Self	TR	D	Toenail
	02-21-03	Female	Spouse	TR	D	Skin
02-23[a]	02-23-01[b]	Male	Self	TR	B	Toenail
	02-23-01[b]	Male	Self	TR	B	Skin
	02-23-02	Male	Child	TR	B	Toenail
	02-23-02	Male	Child	TR	B	Skin
	02-23-03	Female	Spouse	TR	B	Skin
02-24	02-24-01[b]	Male	Self	TR	B	Skin
	02-24-02	Male	Roommate	TR	B	Skin
02-25[a]	02-25-01[b]	Male	Self	TR	C	Toenail
	02-25-01[b]	Male	Self	TR	D	Skin
	02-25-02	Female	Spouse	TR	D	Skin
	02-25-03	Male	Child	TR	C	Toenail
02-29[a]	02-29-01[b]	Male	Self	TR	D	Toenail
	02-29-02	Male	Parent	TR	D	Skin
02-44	02-44-01[b]	Male	Self	TM	D	Skin
	02-44-02	Female	Spouse	TM	D	Skin
02-58	02-58-01	Male	Self	TR	D	Skin
	02-58-02	Female	Spouse	TR	D	Toenail

Abbreviations: TR, *Trichophyton rubrum*; TM, *Trichophyton mentagrophytes*.
[a]Spread of infection may be linked to a genetic disposition.
[b]Index person.

who performed culture- and PCR-based strain typing of nail specimens from 10 patients with onychomycosis due to *T rubrum*. The authors reported that 2 or more strains were present in 6 of 10 specimens.[23] In a separate study, Gupta et al[24] evaluated the variations in RFLPs among serial isolates from patients with *T rubrum* infections and reported a total of 5 RFLPs (TR-1 to TR-5, differentiated by 1 band shift) among 66 strains, with 2 major RFLP types (TR-1 and TR-3) accounting for 68% (45/66) of the samples. Similar to our findings, these investigators reported the occurrence of more than 1 RFLP type in serial samples obtained from 1 nail in a single patient.[24]

The spread of foot infections may occur in places such as shower stalls, bathrooms, or locker rooms where floor surfaces often are wet and people are barefoot.[8,25] However, we did not find an association between the spread of infection and potential risk factors such as visiting a nail salon, using a swimming pool, walking barefoot at home, wearing wet shoes, or sharing a bed; there was no association with sex, age, or number of showers taken per day. Although we found no obvious association, our findings could be limited by the relatively small number of households examined in our study. Additionally, none of the participants in our study who lived in households where a spread of the infection was observed reported visiting a fitness center; therefore, any link between visits to fitness centers and spread of infection remains to be determined using the RFLP methodology.

Our study suggests that spread of dermatophyte infection among participants likely occurred in the home environment; however, it is possible that genetic predisposition may have some role in susceptibility to the infection. Prior studies have shown a pattern of familial infection, suggesting an autosomal-dominant pattern of susceptibility and association of HLA-DR6 with reduced susceptibility to *T rubrum*.[26-29] Our results showed that 50% (4/8) of MIM households included infected members who were genetically related (eg, parent/child, cousins). However, 2 households had both spouse and child infected with the same dermatophyte strain, and both the home environment and genetic predisposition may have contributed to spread of infection. Therefore, although some household members might lack an inherent susceptibility to a given strain, they may have acquired a different strain independently. Our results indicate that although a genetic predisposition may have contributed to the spread of infection, the sample size was too small to reach a definite conclusion. Further studies need to be conducted to ascertain the contribution of genetic predisposition and home environment to spread of infection.

A limitation of the current study is that it was a cross-sectional, not longitudinal, analysis; therefore, we were unable to determine the chronology of infection, the cause-and-effect relationship between specific strain types and infection, or the household member that was infected first. The design of the study also limits our ability to distinguish between relapse or reinfection, ongoing infection, and de novo infection. In this study, the requirement for entry into enrollment for both IPs and their household members was to have both positive culture and positive KOH test for dermatophytes as well as no oral antifungal exposure at least 4 months prior to the study. Therefore, any participant who may have had antifungal exposure beyond the 4-month cutoff point with false-negative culture or KOH results was excluded from the study and considered a screen failure. However, for family members only, it is possible that residual drug from prior treatments beyond the 4 months cutoff point resulted in false exclusion from the study. If it had occurred, there would be a lower estimation of spread of infection (ie, the study actually reported fewer instances of spread than actually would have occurred, implying the spread actually may have been at a higher rate).

The source of the spread of infection also may be a common point within the household. Due to the study population size, we also were unable to evaluate the baseline rate of genetic variation of *T rubrum* dependent on geographic region. Large-scale studies are needed to overcome these limitations and to determine if there is any definitive association between the identified risk factors and spread of infection in patients with onychomycosis and/or tinea pedis and to determine if spread of infection occurs within a household. Additional studies are needed to determine the community prevalence of the identified strain types and compare it with prevalence of the molecular types in households.

Conclusion

Our findings may aid in the design of further large-scale studies and the development of important benchmarks to guide therapy for dermatophyte infections, especially in preventing the spread of infection among household members.

Acknowledgments—The authors would like to thank Maria Robinson for her consultation regarding the study, as well as Dan Isham; Nancy Isham, BA; Veronica Catalano, MLT; and Jona Matevish, MS, for their technical assistance. We also thank Farid Kianifard, PhD, from Biometrics, Novartis Pharmaceuticals Corporation for his critical review of the manuscript, as well as Brian Arnold, MS, and Helen Shapiro, BS,

both from Novartis Pharmaceuticals Corporation, for their technical support.

REFERENCES

1. Hainer BL. Dermatophyte infections. *Am Fam Physician*. 2003;67:101-108.
2. Bell-Syer SE, Hart R, Crawford F, et al. Oral treatments for fungal infections of the skin of the foot [update published in *Cochrane Database Syst Rev*. 2012;10:CD003584]. *Cochrane Database Syst Rev*. 2002:CD003584.
3. Elewski BE. Onychomycosis. treatment, quality of life, and economic issues. *Am J Clin Dermatol*. 2000;1:19-26.
4. Jennings MB, Pollak R, Harkless LB, et al. Treatment of toenail onychomycosis with oral terbinafine plus aggressive debridement: IRON-CLAD, a large, randomized, open-label, multicenter trial. *J Am Podiatr Med Assoc*. 2006;96:465-473.
5. Rupke SJ. Fungal skin disorders. *Prim Care*. 2000;27:407-421.
6. Hay R. Literature review. onychomycosis. *J Eur Acad Dermatol Venereol*. 2005;19(suppl 1):1-7.
7. Borgers M, Degreef H, Cauwenbergh G. Fungal infections of the skin: infection process and antimycotic therapy. *Curr Drug Targets*. 2005;6:849-862.
8. Seebacher C, Bouchara JP, Mignon B. Updates on the epidemiology of dermatophyte infections [published online ahead of print May 14, 2008]. *Mycopathologia*. 2008;166:335-352.
9. English MP. *Trichophyton rubrum* infection in families. *Br Med J*. 1957;1:744-746.
10. Weitzman I, Kane J. Dermatophytes and agents of superficial mycoses. In: Balows A, Hausler WJ Jr, Herrmann KL, et al, eds. *Manual of Clinical Microbiology*. 5th ed. Washington, DC: American Society for Microbiology; 1991:601-616.
11. Weitzman I, Summerbell RC. The dermatophytes. *Clin Microbiol Rev*. 1995;8:240-259.
12. Ghannoum MA, Hajjeh RA, Scher R, et al. A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. *J Am Acad Dermatol*. 2000;43:641-648.
13. Shehata AS, Mukherjee PK, Aboulatta HN, et al. Single-step PCR using (GACA)4 primer: utility for rapid identification of dermatophyte species and strains [published online ahead of print June 25, 2008]. *J Clin Microbiol*. 2008;46:2641-2645.
14. Jackson CJ, Barton RC, Evans EG. Species identification and strain differentiation of dermatophyte fungi by analysis of ribosomal-DNA intergenic spacer regions. *J Clin Microbiol*. 1999;37:931-936.
15. Leidich SD, Ibrahim AS, Fu Y, et al. Cloning and disruption of ca*PLB1*, a phospholipase B gene involved in the pathogenicity of *Candida albicans*. *J Biol Chem*. 1998;273:26078-26086.
16. Mukherjee PK, Seshan KR, Leidich SD, et al. Reintroduction of the *PLB1* gene into *Candida albicans* restores virulence in vivo. *Microbiology*. 2001;147(pt 9):2585-2597.
17. Kemna ME, Elewski BE. A U.S. epidemiologic survey of superficial fungal diseases. *J Am Acad Dermatol*. 1996;35:539-542.
18. Perea S, Ramos MJ, Garau M, et al. Prevalence and risk factors of tinea unguium and tinea pedis in the general population in Spain. *J Clin Microbiol*. 2000;38:3226-3230.
19. Aly R, Hay RJ, Del Palacio A, et al. Epidemiology of tinea capitis. *Med Mycol*. 2000;38(suppl 1):183-188.
20. Foster KW, Ghannoum MA, Elewski BE. Epidemiologic surveillance of cutaneous fungal infection in the United States from 1999 to 2002. *J Am Acad Dermatol*. 2004;50:748-752.
21. Elewski BE. Tinea capitis: a current perspective. *J Am Acad Dermatol*. 2000;42(1, pt 1):1-20; quiz 21-24.
22. Pomeranz AJ, Sabnis SS, McGrath GJ, et al. Asymptomatic dermatophyte carriers in the households of children with tinea capitis. *Arch Pediatr Adolesc Med*. 1999;153:483-486.
23. Yazdanparast A, Jackson CJ, Barton RC, et al. Molecular strain typing of *Trichophyton rubrum* indicates multiple strain involvement in onychomycosis. *Br J Dermatol*. 2003;148:51-54.
24. Gupta AK, Kohli Y, Summerbell RC. Variation in restriction fragment length polymorphisms among serial isolates from patients with *Trichophyton rubrum* infection. *J Clin Microbiol*. 2001;39:3260-3266.
25. Roberts DT, Evans EG. Subungual dermatophytoma complicating dermatophyte onychomycosis. *Br J Dermatol*. 1998;138:189-190.
26. Many H, Derbes VJ, Friedman L. *Trichophyton rubrum*: exposure and infection within household groups. *Arch Dermatol*. 1960;82:226-229.
27. Asz-Sigall D, López-García L, Vega-Memije ME, et al. HLA-DR6 association confers increased resistance to *T. rubrum* onychomycosis in Mexican Mestizos. *Int J Dermatol*. 2010;49:1406-1409.
28. Zaias N, Tosti A, Rebell G, et al. Autosomal dominant pattern of distal subungual onychomycosis caused by *Trichophyton rubrum*. *J Am Acad Dermatol*. 1996;34 (2, pt 1):302-304.
29. Faergemann J, Correia O, Nowicki R, et al. Genetic predisposition—understanding underlying mechanisms of onychomycosis. *J Eur Acad Dermatol Venereol*. 2005;19 (suppl 1):17-19.