Crusted Scabies: A Molecular Analysis of *Sarcoptes scabiei* Variety *hominis*

Populations from Patients with Repeated Infestations

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Crusted scabies is a severe debilitating disease due to hyperinfestation with the ectoparasite *Sarcoptes scabiei*. Treatment protocols include oral ivermectin and topical scabicides. After single-dose ivermectin, there may be early recrudescence, whereas after 3 doses at 14-day intervals, there is an apparent cure. However, such patients often present again after 6–12 months. To clarify the biology of recurrence, we studied genetic markers in sequential populations of *S. scabiei* mites from treated patients with multiple episodes of crusted scabies. Individual mites were genotyped at hypervariable microsatellite loci by a fluorescence-based polymerase chain reaction. Results indicated that sequential populations of mites were genetically more similar to each other than to mites from other patients. Although the majority of recurrent scabies is probably due to reinfection from inadequately treated contacts, there was evidence that in very severe crusted scabies, treatment with even 3 doses of ivermectin 14 days apart may be inadequate and relapse may occur.

Scabies is an ectoparasitic disease of the skin caused by the itch mite *Sarcoptes scabiei* [1]. The spectrum of the disease ranges from the usual ordinary scabies, with an average infestation of 10–15 mites per person [2], to the rarer crusted scabies, with up to millions of mites and associated patient debility. The predominant feature of crusted scabies is hyperkeratosis of the skin. Because of the extreme burden of mites, crusted scabies is much more infectious than ordinary scabies, with infectivity that persists for longer because of the difficulty in eradicating mites from heavily crusted skin. Local epidemics of ordinary scabies can originate from single cases of crusted scabies [3–5]. Scabies is endemic in many remote Aboriginal communities in the Northern Territory of Australia [6]. At the Royal Darwin Hospital, in the tropical north of the Northern Territory, up to 10 patients per year are diagnosed with crusted scabies, often having been flown for treatment from remote communities to Darwin. Recurrences of the disease are of significant concern for both the individual and their communities, where they may be core transmitters of scabies.

Progression from ordinary scabies to crusted scabies is uncommon. Susceptibility to crusted scabies has been linked to immunosuppression and to human immunodeficiency virus (HIV) infection [7]. In Darwin, crusted scabies has been seen in previously treated leprosy patients and has also been associated with substance abuse and systemic lupus erythematosus. At Alice Springs Hospital, in central Australia, 9 Aboriginal patients were simultaneously infected with crusted scabies and human T lymphotropic virus type 1 (HTLV-1) [8]. HTLV-1 is not endemic in the north and has not been associated with any crusted scabies cases at the Royal Darwin Hospital, where most current patients have no evident predisposing illness and are overtly immunocompetent [9].

There are increasing reports of variability in response to topical treatment for ordinary scabies, together with evidence of emerging tolerance [10, 11]. Recent treatment protocols for crusted scabies have used ivermectin in combination with repeated topical 5% permethrin and keratolytic therapy [9, 12, 13]. Oral ivermectin has recently been successfully used instead of topical therapy in nursing homes and in community scabies control programs [14, 15]. Experience with HIV-associated crusted scabies indicates that ivermectin is efficacious in treating patients not responsive to topical therapy [13]. However, there is evidence that multiple doses may be required to treat severe crusted scabies [9, 12, 13]. Mortality of patients with crusted scabies in northern Australia, mostly from secondary sepsis, is up to 50% over 5 years [12]. Data from 20 patients with crusted scabies treated with varying ivermectin doses showed treatment failure or early recrudescence after a single ivermectin dose of.
200 μg/kg, whereas 3 doses 14 days apart resulted in a “cure.” However, half of these latter cases had recurrent crusted scabies 6–12 months later, suggesting either recrudescence or reinfection [9].

To investigate the biology of recurrence after ivermectin treatment, we have applied a fluorescence-based DNA fingerprinting system to type the hypervariable microsatellite markers of S. scabiei [16]. Individual mites were typed from patients who had recurrences of crusted scabies, despite repeated doses of ivermectin together with topical 5% permethrin cream and topical keratolytic therapy, over a period of 4 years.

Materials and Methods

Collection of mites and preparation of genomic DNA. Mites assumed to be S. scabiei variety hominis were obtained from skin scrapings and crusts of patients with crusted scabies. Hyperkeratotic patients shed skin that was easily collected from bed sheets. Up to 4700 mites were found per gram of skin [17]. Mites were extracted from skin, as described elsewhere [18]. Genomic DNA was obtained from each mite for polymerase chain reaction (PCR) analysis, as outlined elsewhere [16] and more recently by use of the alkaline lysis method [19].

Fluorescence-based PCR analysis. The S. scabiei–specific microsatellite markers surveyed are as reported elsewhere [16]. Fluorescent-labeled primers were synthesized by Geneworks (S. A., Australia) and nonfluorescent primers by Beckman Coulter (NSW, Australia). Fluorescent PCR amplification products were analyzed by use of the Applied Biosystems (Perkin-Elmer Cetus, Norwalk, CT) format [18].

According to Fregeau and Fourney [20], the level of precision obtained for real-time allele size determination was observed to be ±0.2 to ±0.5 bp (intrigel) and ±0.5 to ±1.5 bp (intergel). Consequently, a conservative approach to allele size determination was developed on the basis of arbitrarily defined fixed bins of ±1.0 bp per designated allele.

Statistical evaluations. Statistical assessment was as described elsewhere [18]. Because of the small sample size, high polymorphism, and null alleles, a nonparametric exact test was used to compare genotype frequencies of different mite populations [21]. Additionally, a multilocus analysis was used to assess relationships between mite populations by means of a genetic distance based on the proportion of shared alleles [22].

Results

Analysis of sequential S. scabiei populations obtained from patients with crusted scabies. S. scabiei mites of all life stages were collected over a 4-year study period from 2 unrelated Aboriginal men (patients 1 and 2) and 2 Aboriginal sisters (patients 3 and 4) with repeated episodes of crusted scabies. All patients were HIV- and HTLV-1–negative, and patients 3 and 4 had no known risk factors. However, patient 1 had a history of intermittent alcohol excess and patient 2 had been previously treated for borderline lepromatous leprosy. Over a 2-year period, patient 1 received a total of 20 doses of ivermectin, patient 2 received 4 doses of ivermectin, and patient 3 received 12 doses. Microscopic analysis of skin scrapings from patient 1 and in vitro plate sensitivity testing (unpublished data) during this period indicated that mites remained sensitive to both ivermectin and permethrin. Live mites from the patient were still present 19 days after the start of one treatment course, despite 18-mg doses of ivermectin on days 1 and 15 [12].

In total, 1057 mites were collected from patient 1 over 2 years, sampling at ~6-month intervals. One hundred seventy-four mites were genotyped; 23 mites failed to amplify with all 3

Figure 1. Multilocus clustering based on proportion of shared alleles of Sarcopes scabiei populations collected from crusted scabies patients with sequential infestations [22]. A. Assumes all single alleles are homozygous. B. Assumes all single alleles are heterozygous nulls.
markers and were excluded. The following analyses were therefore based on 151 mites, specifically 23 from time C1, 36 from C1.1, 41 from C1.2, 24 from C1.3, and 27 from C1.4. In total, 12 alleles were observed for Sarcoptes microsatellite 1 (Sarms 1), 22 alleles for Sarms 15, and 13 alleles for Sarms 20. Genotypic disequilibrium analysis at the 3 loci revealed no significant deviations from random association of alleles, as observed in all other analyses (not shown). Multilocus analysis showed that sequential samples from patient 1 were more similar to each other than to samples from other patients (figure 1). Nevertheless, there was significant genotypic differentiation between sequential subpopulations for patient 1 at all 3 loci (table 1). This result suggests that there has been differential survival of different genotypes, or reinfection from a slightly different parent population, or both.

A similar study was undertaken for 2 episodes (1 year apart) of crusted scabies in patient 2, a 63-year-old itinerant Aboriginal man living in Darwin. One hundred fifty-three mites were genotyped from a total of 1090 collected, with 19 discarded because of nonamplification. A total of 10 Sarms 1 alleles, 13 Sarms 15 alleles, and 11 Sarms 20 alleles were observed in the 134 mites. Multilocus analysis showed that the sequential populations of mites from patient 2 also clustered together (figure 1), though there were significant genotypic differences between them (table 1). However, these differences were far less than those observed between populations of mites from unrelated persons, such as patient 2 and patient 3 (table 1; figure 2).

Mites from patient 3, a woman living in a remote Aboriginal community, were collected and analyzed from 3 sequential episodes of crusted scabies over a period of 3 years. Sixty-seven mites were genotyped from a total of 89 mites collected, with only 29 amplifying with ≥1 markers. The reason for the high PCR failure in this group of mites is unknown but may relate to delays in transport of skin scrapings to Darwin. Genotypic comparison therefore involved much smaller sample numbers, specifically 11 mites from time point C3, 4 mites from C3.1, and 16 mites from C3.2. Nevertheless, these sequential subpopulations had significantly different genotypes (table 1).

Patient 3 also has a sister with crusted scabies, patient 4. Although they live in separate households in the same community, the sisters spend a lot of time together. It is of particular interest that 2 of the subpopulations of mites from patient 3 (C3.1 and C3.2) were genetically closer to mites from population C4 than to the C3 subpopulations collected before treatment (figure 1A). This raises the possibility that after treatment, patient 3 was reinfested with mites acquired from her sister, patient 4, or another contact infested with similar genotypes.

Multilocus analysis confirming host-associated clustering of mite populations. Population multilocus analysis using the distance measure based on the proportion of shared alleles [22] showed that subpopulations of mites from the same patient were generally more similar over time than were populations from different hosts (figure 1). These results imply that current treatment may not always be totally effective in clearing all live mites from a patient with crusted scabies. The apparent diversity observed with the exact test of population differentiation among successive subpopulations collected from patients 1, 2, and 3 (table 1) may reflect changes between mite generations due to stochastic effects or the selective effects of immune pressure or treatment. An alternative explanation is that patients are in fact cleared of all live mites but are reinfested with related but somewhat different S. scabiei genotypes on return to households and communities in which scabies is endemic. The observation of similar genotypes in mites collected from patients 3 and 4 supports this possibility.

Recrudescent or reinfection? To try and distinguish between recrudescence and reinfection, 12 family members of patient 1 with clinical scabies had skin scrapings taken; unfortunately, no mites were recovered. Despite a total of 13 doses of ivermectin, patient 1 was subsequently readmitted with recurrent crusted scabies. After treatment with 3 further doses of ivermectin, he chose not to return to his remote community but remained near Darwin for follow-up. As with previous admissions, his crusted scabies steadily improved over a 2- to 4-week period once ivermectin treatment commenced, though residual scarring and depigmentation were extensive. Sensitivity studies on mites collected at this time showed unequivocal sensitivity to ivermectin; nevertheless, a single live mite was observed 47 days after the first ivermectin dose (19 days after the third dose). Approximately 6 months later, patient 1 was readmitted to the hospital with a relapse of crusted scabies. Genotypic analysis between this sample of mites (C1.4) and that collected 6 months previously (C1.3) showed considerable similarity (figure 1). Because he had been isolated from his family during this 6-month period, reinfection with mites derived from a similar gene pool was considered unlikely, and we suggest that the relapse was due to recrudescence after incomplete eradication of the earlier infestation. The residual diversity observed (table 1) is most likely due to the selective effects of the scabicide treatment but may also result from stochastic effects or immune pressure.
Table 1. *P* values associated with genotypic differentiation between sequential populations of *Sarcoptes scabiei* collected from patients with crusted scabies.a

<table>
<thead>
<tr>
<th>Patient(s) (sequential populations of <em>S. scabiei</em>)</th>
<th>Allele</th>
<th>Sarms 1</th>
<th>Sarms 15</th>
<th>Sarms 20</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (C1, C1.1, C1.2, C1.3, C1.4)</td>
<td></td>
<td>.0074</td>
<td>.0224</td>
<td>.0194</td>
<td>.0003</td>
</tr>
<tr>
<td>2 (C2.1, C2.2)</td>
<td></td>
<td>.0364</td>
<td>.0110</td>
<td>.1429</td>
<td>.0033</td>
</tr>
<tr>
<td>3 (C3, C3.1, C3.2)</td>
<td></td>
<td>.0076</td>
<td>.0939</td>
<td>.0204</td>
<td>.0011</td>
</tr>
<tr>
<td>3 and 4 (C3, C3.1, C3.2, C4)</td>
<td></td>
<td>.0659</td>
<td>.0998</td>
<td>.0053</td>
<td>.0022</td>
</tr>
<tr>
<td>1 (C1.3, C1.4)</td>
<td></td>
<td>.2460</td>
<td>.5858</td>
<td>.0023</td>
<td>.0138</td>
</tr>
<tr>
<td>2 and 3 (C2.1, C3, C3.1, C3.2)</td>
<td></td>
<td>.0001</td>
<td>.0001</td>
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</tr>
</tbody>
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NOTE. Sarms, Sarcoptes microsatellite.

a *Ho*: genotype distribution is identical across all populations [21].

These results suggest that in very severe crusted scabies, even multiple ivermectin doses can fail to eradicate the infestation.

Discussion

Previous genotyping studies of individual mites suggested that there were no significant differences among samples of mites collected from different body sites on an individual host, even when samples were collected over a period of 5 years from long-term experimentally infested hosts. Furthermore, populations of mites collected from different people revealed highly significant genotypic differences [18]. This study of successive populations of mites collected from subjects with sequential infestations, patients 1–3, making use of the same nonparametric exact tests, showed that sequential episodes of infestations were genetically closer to each other than to mites from other patients (figures 1 and 2; table 1). This suggests that some of the mites had survived treatment or that reinfestation had been from a similar source. Mites from patient 3 showed less population diversity between episodes. The analysis of the mite subpopulations obtained from the 2 sisters, patients 3 and 4, showed more heterogeneity, with the suggestion that patient 3 may have been reinfested from patient 4 or another person with similar genotypes (figure 1A).

Current protocols in northern Australia for treating patients with crusted scabies include environmental measures (e.g., house insecticide bombs, washing of bed linen), as well as treatment of all family or household members. Such procedures should reduce considerably the risk of reinfection for the patient. Therefore, the study of sequential crusted scabies in patient 1 is very informative. Patient 1 has, to our knowledge, received more doses of ivermectin than documented for any other person, yet the mites collected following each relapse after treatment showed relatively minor genotypic changes. In vitro sensitivity testing showed that the mites were not resistant to ivermectin after relapse (unpublished data). However, the similarity of mites collected at 2 successive recurrences (C1.3 and C1.4) while patient 1 remained isolated from his family cannot be explained by reinfestation from other members of the family. These results from patient 1 suggest that even multiple doses of ivermectin may be insufficient to eradicate the mite from severe cases of crusted scabies, possibly because eggs are more refractory to treatment than are adult mites. We have therefore increased our ivermectin dosing schedule for crusted scabies from 3 to 5 doses (each 200 μg/kg) given at days 1 and 2, days 15 and 16, and day 29, on the basis of the protocol for strongyloidiasis complicating AIDS [23]. For further recurrences in patients 1 and 3, we have tried monthly “suppressive” doses of 200 μg/kg with limited success (data not shown). Higher doses of ivermectin (e.g., 400 μg/kg) are another option for further studies.

Nevertheless, in the hyperendemic scabies situation currently occurring in remote Australian Aboriginal communities, reinfestation from inadequately treated contacts is probably an important cause of recurrent scabies. Because of the continuing cycles of transmission of scabies in these communities and the reinfestation of those susceptible to developing crusted scabies, who in turn act as core transmitters, coordinated community-based treatment programs are essential for control of scabies [24]. Recently, a successful program that used 5% permethrin cream in a coordinated whole-community treatment was documented from northern Australia [25]. There may well be a wider role for ivermectin in such programs once its use and safety in treating children with scabies is documented adequately for drug regulatory authorities. Concerns about possible emergence of ivermectin resistance and drug toxicity [12, 15] necessitate the continued surveillance of patient responses and mite sensitivity.

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References