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Sulfa or Sulfone Prophylaxis and Geographic Region Predict Mutations in the *Pneumocystis carinii* Dihydropteroate Synthase Gene

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To determine factors associated with mutations in the *Pneumocystis carinii* dihydropteroate synthase (DHPS) gene, a prospective study of human immunodeficiency virus (HIV)–infected patients with confirmed *P. carinii* pneumonia was conducted in Atlanta, Seattle, and San Francisco. Clinical information was obtained from patient interview and chart abstraction. DHPS genotype was determined from DNA sequencing. Overall, 76 (68.5%) of 111 patients had a mutant DHPS genotype, including 22 (81.5%) of 27 patients from San Francisco. In multivariate analysis, sulfa or sulfone prophylaxis and study site were independent predictors of a mutant genotype. Fourteen (53.8%) of 26 patients who were newly diagnosed with HIV infection and had never taken prophylaxis had a mutant genotype. The significance of geographic location as a risk factor for mutant genotype and the high proportion of mutant genotypes among persons never prescribed prophylaxis, including those newly diagnosed with HIV infection, provide indirect evidence that these mutations are transmitted from person to person either directly or through a common environmental source.

In 1981, the first reports of *Pneumocystis carinii* pneumonia (PCP), a previously rare disease, heralded the onset of the AIDS epidemic [1, 2]. Throughout much of the epidemic, PCP has remained the most common AIDS-defining opportunistic infection in the United States and a significant cause of pneumonia in human immunodeficiency virus (HIV)–infected persons [3–7]. Although the incidence of PCP has declined dramatically as a result of *P. carinii* prophylaxis and, more recently, combinations of antiretroviral therapies, the number of HIV-infected persons potentially at risk for the disease remains substantial [8].

Since 1989, *P. carinii* prophylaxis has been recommended for HIV-infected persons with a CD4 cell count <200 cells/μL, with oral candidiasis regardless of CD4 cell count, and after an episode of PCP [9]. Current guidelines recommend trimethoprim-sulfamethoxazole (TMP-SMZ) as first-line prophylaxis against *P. carinii* [10]. Dapsone is an alternative prophylaxis choice for persons who are allergic to or intolerant of TMP-SMZ [10].

The widespread use of TMP-SMZ for *P. carinii* prophylaxis has been implicated in the increases in TMP-SMZ–resistant bacteria that have been reported in HIV-infected patients [11]. Chronic use of prophylaxis has also raised concerns about the possible selection of drug resistant *P. carinii.* The lack of a standardized culture system for human *P. carinii* has, however, limited our ability to confirm drug resistance in *P. carinii.* In animals, the antipneumocystis activity of TMP-SMZ is almost entirely attributable to SMZ, an inhibitor of the *P. carinii* dihydropteroate synthase (DHPS) gene, and not to TMP, an inhibitor of the dihydrofolate reductase (DHFR) gene [12–16]. Dapsone, a sulfone, is also a DHPS inhibitor. In several microorganisms, sulfa or sulfone resistance has been demonstrated to result from specific point mutations in that organism's DHPS gene [17–24]. Several recent studies have demonstrated similar point mutations in the *P. carinii* DHPS gene and have found an association between the use of sulfa or sulfone drugs for *P. carinii* prophylaxis and DHPS mutations [25–27]. Another recent study found mutations in the DHPS gene but not in the DHFR gene in patients using sulfa or sulfone drugs [28]. These studies, however, are limited, because the number of HIV-infected patients prescribed sulfa or sulfone prophylaxis was small, ranging from 2 to 29 patients. Consequently, detailed statistical analysis to determine whether

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Informed consent was obtained from all patients. Human experimentation guidelines of the US Department of Health and Human Services and those of the participating institutions were followed in the conduct of this clinical research. Study protocols and patient consent forms were approved by each site's institutional review board (IRB) and by the IRB of the Centers for Disease Control and Prevention.

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factors other than prophylaxis also predict mutations in the *P. carinii* DHPS gene was not performed. In addition, these studies used *P. carinii* specimens from single institutions or from institutions from the same geographic region. We have shown elsewhere that geographic location appears to be an important determinant of DHPS genotype [29].

Therefore, the objective of the present study was to determine the factors that are associated with mutations in the *P. carinii* DHPS gene, by use of multivariate analytical techniques, from a large prospective study of HIV-infected patients with confirmed PCP from geographically distinct regions.

Patients and Methods

Patients. Patients were HIV-infected adults (≥18 years old) with confirmed PCP hospitalized at 1 of 9 institutions in 3 geographically distinct cities (Grady Memorial Hospital and Atlanta Veterans Affairs Medical Center, Atlanta; University of Washington Medical Center, Harborview Medical Center, Swedish Hospital Medical Center, Providence Medical Center, Virginia Mason Medical Center, and Group Health Cooperative Central and Eastside Hospitals, Seattle; San Francisco General Hospital, San Francisco). Patients were consecutive patients at each institution who consented to a patient interview conducted at the time of hospitalization and a medical chart abstraction performed ≥6 weeks after the date of discharge.

Patient interview. Trained study personnel conducted patient interviews, using a standardized questionnaire. The patient interview obtained specific information on demographic and clinical data and included details on the patient-reported use of sulfa or sulfone drugs for *P. carinii* prophylaxis in the 3 months preceding PCP diagnosis. The questionnaires used in Atlanta and San Francisco were identical and contained a specific question that asked whether the patient had ever taken TMP-SMZ for *P. carinii* prophylaxis. The questionnaire used in Seattle was similar to those in Atlanta and San Francisco but did not include the specific question that asked whether the patient had ever taken TMP-SMZ.

Medical chart abstraction. Trained study personnel performed medical chart abstraction, using a standardized abstraction form. Similar to the patient interview, the chart abstraction obtained specific information on demographic and clinical data. It also documented the date of the first positive HIV test result, prior episode(s) of PCP, and the date and result of the most recent CD4 cell count. In addition, the abstraction contained details on the provider prescription of sulfa or sulfone drugs for *P. carinii* prophylaxis in the 3 months preceding PCP diagnosis. Neither the patient interview nor the chart abstraction obtained details concerning the duration of sulfa or sulfone prophylaxis.

Sulfa or sulfone prophylaxis. For the univariate and multivariate analyses, criteria for sulfa or sulfone prophylaxis included patient report of having taken TMP-SMZ or dapsone for prophylaxis at any time, patient report of taking TMP-SMZ or dapsone in the 3 months before PCP diagnosis, or chart abstraction noting TMP-SMZ or dapsone prophylaxis prescription in the 3 months before PCP diagnosis. Additional analyses examined prophylaxis according to the source of information (patient interview or chart abstraction) and the timing of prophylaxis (ever or in the past 3 months).

P. carinii *specimens.* *P. carinii* specimens were obtained as part of routine diagnostic procedures. The procedure for specimen processing, DNA purification, polymerase chain reaction (PCR) amplification, and DNA sequencing have been described in detail elsewhere [29]. After clinical use, the remaining portion of the specimen was preserved with an equal volume of absolute ethanol and stored at 4°C for DNA extraction. Specimens were shipped to the Centers for Disease Control and Prevention for analysis, including DNA extraction that was performed using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). PCR amplification was performed at the DHPS locus by use of published primers [25, 30]. DNA sequencing was performed using dye terminator chemistry (ABI Prism™ Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit; PE Applied Biosystems, Foster City, CA). Analysis of the sequenced DNA was performed using Sequence Navigator (version 1.0.1, PE Applied Biosystems) and the Wisconsin Package (version 10.1; Genetics Computer Group, Madison, WI).

P. carinii *DHPS genotype.* For the univariate and multivariate analyses, the wild-type *P. carinii* DHPS genotype was defined as the DNA sequence observed in *P. carinii* specimens from other mammalian species and from humans before 1995 [25–27, 30]. A mutant *P. carinii* DHPS genotype was defined as any DNA sequence that differed from the wild-type sequence. The observed DHPS mutations were all nonsynonymous changes that resulted in amino acid substitutions at either amino acid positions 55 or 57 and were identical to the mutations reported elsewhere [25]. Patient specimens with multiple DHPS genotypes that included ≥1 mutant genotype were classified as mutant DHPS genotypes in the analyses.

Statistical analysis. Statistical analysis was performed using SAS (version 6.12; SAS Institute, Cary, NC). Univariate analyses were performed using the χ^2 or Fisher's exact (2-tailed) tests. Odds ratios and 95% confidence intervals were calculated to assess the univariate risk for having a mutant *P. carinii* DHPS genotype. Clinical variables significant at the $P < .10$ level were included in multivariate logistic regression analyses. $P < .05$ was used to define statistical significance.

Results

Patients. From January 1996 to March 1999, 118 HIV-infected patients were diagnosed with PCP and consented to a patient interview and medical chart abstraction. Of these 118 patients, 111 (94.1%) had the *P. carinii* DHPS locus successfully sequenced and were included in this analysis (table 1). Forty-eight patients were diagnosed with PCP in Atlanta, 36 in Seattle, and 27 in San Francisco. Patients had a mean age of 39.0 years. Most of the patients were male. All major racial, ethnic, and HIV risk groups were represented.

The date of the first positive HIV test was documented in the chart for 82 patients. These patients were known to be HIV infected for a median of 17 months (mean, 38 months) before the PCP diagnosis that was examined in this analysis. The duration of known HIV infection varied greatly (range, 0–150 months) and included 26 persons who were newly diagnosed

Table 1. Demographic and clinical characteristics of 111 human immunodeficiency virus (HIV)–infected patients diagnosed with *Pneumocystis carinii* pneumonia (PCP).

Characteristic	HIV-infected patients with PCP
Study site	
Atlanta	48 (43.2)
Seattle	36 (32.4)
San Francisco	27 (24.3)
Age, years	
Median (range)	38.0 (22.8–63.4)
Mean (±SE)	39.0 (0.7)
Sex	
Male	99 (89.2)
Female	12 (10.8)
Race/ethnicity	
Black, non-Hispanic	55 (49.5)
White, non-Hispanic	40 (36.0)
Hispanic	9 (8.1)
Asian/Pacific Islander	3 (2.7)
Other	4 (3.6)
HIV risk factor	
MSM[a]	66 (59.5)
Heterosexual contact	31 (27.9)
IDU	13 (11.7)
Other	1 (0.9)
Duration of known HIV infection, months (n = 82)	
Median (range)	17 (0–150)
Mean (±SE)	38 (5)
CD4 cell count, cells/μL (n = 106)	
Median (range)	23 (0–328)
Mean (±SE)	58 (11)
Prior PCP	
No	78 (70.3)
Yes	33 (29.7)
Sulfa or sulfone prophylaxis	
Yes	71 (64.0)
No	40 (36.0)

NOTE. Data are no. (%) unless otherwise indicated. MSM, men who have sex with men; IDU, injection drug use.

[a] MSM includes persons who report both men who have sex with men and IDU.

with HIV infection and 5 patients who were known to be HIV infected for more than a decade.

CD4 cell counts were documented in the chart for 106 patients. The median CD4 cell count was 23 cells/μL (mean, 58 cells/μL). Eight patients had a CD4 cell count >200 cells/μL (range, 203–328 cells/μL), obtained a median of 98 days (range 2–442 days) before diagnosis of PCP. Thirty-three patients (29.7%) reported a prior episode of PCP. Overall, 71 patients (64.0%) had sulfa or sulfone prophylaxis use.

P. carinii *DHPS genotype.* Most of the *P. carinii* DHPS genotypes were mutant (table 2). Overall, only 35 of the PCP specimens (31.5%) had the wild-type genotype as the sole genotype. The remaining 76 specimens (68.5%) contained ⩾1 mutant genotype, either as the sole genotype (n = 63) or as part of a mixed infection (n = 13). The most frequent genotype observed was a double mutant, with an alanine→threonine substitution at amino acid position 55 and a serine→proline substitution at amino acid position 57. This genotype was seen in 60 of the specimens (54.1%); in 55 specimens, it was the sole genotype, and in 5 specimens, it was part of a mixed infection. The second most frequent genotype observed was the wild type. Combined, these 2 genotypes were seen alone or as part of a mixed infection in >92% of the PCP specimens examined.

Overall, the proportion of mutant *P. carinii* DHPS genotypes remained stable over the study period. In the first half of the study (1996–97), 38 (67.9%) of 56 of the specimens had a mutant genotype, whereas, in the second half of the study (1998 to March 1999), 38 (69.1%) of 55 of the specimens had a mutant genotype (P = .95).

Association between clinical characteristics and P. carinii *DHPS genotype.* In univariate analysis, sulfa or sulfone prophylaxis, prior PCP, and study site were all associated with an increased risk for having a mutant *P. carinii* DHPS genotype (table 3). Patients who had sulfa or sulfone prophylaxis use were more likely to have a mutant genotype than were patients who did not. Similarly, patients with a prior episode of PCP were more likely to have a mutant genotype than were patients without prior PCP. Interestingly, patients who were diagnosed with PCP in either San Francisco or Seattle were also more likely to have a mutant *P. carinii* genotype than were patients who were diagnosed with PCP in Atlanta.

In multivariate logistic regression analyses, sulfa or sulfone prophylaxis and study site were independent risk factors for having a mutant *P. carinii* DHPS genotype (table 4). Because prophylaxis and prior PCP were highly correlated, we examined the impact of prior PCP on sulfa or sulfone prophylaxis in models that excluded and included prior PCP. In both models, patients who had sulfa or sulfone prophylaxis use were more likely to have a mutant genotype than were patients who did not. In addition, patients who were diagnosed with PCP in either San Francisco or Seattle were also more likely to have a mutant genotype than were patients who were diagnosed with PCP in Atlanta in both models. Separate analyses that examined different definitions for sulfa or sulfone prophylaxis, that excluded mixed genotypes, and that compared the wild type with the double mutant genotype (the 2 most frequent genotypes observed) yielded similar results to those presented.

Table 2. *Pneumocystis carinii* dihydropteroate synthase (DHPS) genotype in 111 human immunodeficiency virus–infected patients diagnosed with *P. carinii* pneumonia (PCP).

DHPS genotype	Amino acids	HIV-infected patients with PCP, no. (%)
Wild type		
1	Thr 55, Pro 57	35 (31.5)
Mutation(s)		
2	Ala 55, Pro 57	6 (5.4)
3	Thr 55, Ser 57	2 (1.8)
4	Ala 55, Ser 57	55 (49.5)
Mixed		
5	Thr 55, Pro 57/Ser 57	4 (3.6)
6	Thr 55/Ala 55, Pro 57/Ser 57	3 (2.7)
7	Thr 55/Ala 55, Pro 57	4 (3.6)
8	Ala 55, Pro 57/Ser 57	2 (1.8)

Table 3. Univariate analysis of demographic and clinical factors associated with mutant *Pneumocystis carinii* dihydropteroate synthase (DHPS) genotype in 111 human immunodeficiency virus (HIV)–infected patients diagnosed with *P. carinii* pneumonia (PCP).

Factor	Mutant *P. carinii* DHPS genotype			
	No./total no. (%) of patients	Odds ratio	95% confidence interval	*P*
Study site				
San Francisco	22/27 (81.5)	3.7	1.2–11.5	.02
Seattle	28/36 (77.8)	3.0	1.1–7.8	
Atlanta	26/48 (54.2)	1.0 (Reference)		
Age, per 10 years		0.7	0.4–1.2	.19
Sex				
Male	67/99 (67.7)	0.7	0.2–2.8	.75
Female	9/12 (75.0)	1.0 (Reference)		
Race/ethnicity				
Black, non-Hispanic	35/55 (63.6)	0.8	0.2–2.6	.50
White, non-Hispanic	30/40 (75.0)	1.4	0.4–4.9	
Other	11/16 (68.8)	1.0 (Reference)		
HIV risk factor				
Men who have sex with men	50/66 (75.8)	2.4	1.0–6.0	.13
Injection drug use	8/13 (61.5)	1.2	0.3–4.6	
Other	18/32 (56.2)	1.0 (Reference)		
HIV infection, per 6 months		1.01	1.0–1.2	.10
CD4 cell count, per 50 cells/μL		0.9	0.7–1.3	.72
Prior PCP				
Yes	28/33 (84.8)	3.5	1.2–10.1	.02
No	48/78 (61.5)	1.0 (Reference)		
Sulfa or sulfone prophylaxis				
Yes	57/71 (80.3)	4.5	1.9–10.6	<.001
No	19/40 (47.5)	1.0 (Reference)		

Sulfa or sulfone prophylaxis according to study site. When stratified by study site, the association between sulfa or sulfone prophylaxis and *P. carinii* DHPS genotype was lost for patients residing in San Francisco (figure 1). Similar to Atlanta (70.0%) and Seattle (88.0%), patients diagnosed with PCP in San Francisco who had sulfa or sulfone prophylaxis use had a high proportion of mutant genotypes (87.5%); however, patients in San Francisco who denied sulfa or sulfone prophylaxis use and whose charts failed to document sulfa or sulfone prophylaxis prescription also had a surprisingly high proportion of mutant genotypes (72.7%). The greater proportion of mutant DHPS genotypes in these patients accounted for the observation that sulfa or sulfone prophylaxis was no longer significantly associated with *P. carinii* DHPS genotype among patients residing in San Francisco.

P. carinii *DHPS genotype in patients newly diagnosed with HIV infection.* Twenty-six of 82 patients for whom a precise date for the first positive HIV test was available were newly diagnosed with HIV infection at the same time as or immediately before the diagnosis of PCP. Importantly, none of the 26 patients reported use of any *P. carinii* prophylaxis (including sulfa or sulfone drugs) or a prior episode of PCP during the patient interview. Despite the absence of sulfa or sulfone prophylaxis use, 14 of the 26 patients (53.8%) had a mutant *P. carinii* DHPS genotype (table 5). Among the study sites, the proportion of patients with newly diagnosed HIV infection and a mutant genotype was lowest in Atlanta (38.5%) and highest in San Francisco (70.0%), paralleling the proportion of mutant genotype in the overall cohort (Atlanta, 54.2%; San Francisco, 81.5%; table 3). At each study site, as the known duration of HIV infection increased, the proportion of mutant genotypes also increased. The only exception was among the 5 patients who were known to be HIV infected for >10 years; of these 5 patients (all residing in Atlanta), only 2 (40.0%) had a mutant genotype.

Discussion

In our prospective study conducted in 3 geographically distinct cities, we found in multivariate analyses that sulfa or sulfone prophylaxis and study site were independent risk factors for having a mutant *P. carinii* DHPS genotype. Interestingly, we found that residence in San Francisco or Seattle was a strong predictor for having a mutant genotype and, when stratified by study site, sulfa or sulfone prophylaxis was no longer significantly associated with mutant genotype for patients residing in San Francisco. We also found that more than half the patients who were newly diagnosed with HIV infection—none of whom reported prior use of any *P. carinii* prophylaxis—had a mutant genotype.

Sulfa or sulfone prophylaxis was an independent risk factor for having a mutant *P. carinii* DHPS genotype. Overall, 57 (80.3%) of 71 patients who received sulfa or sulfone prophylaxis had a mutant genotype. These results are consistent with previously published reports and confirm these prior observations in a multivariate analysis that examined a substantially larger group of patients with prophylaxis use [25, 27, 28]. Kazanjian

Table 4. Two multivariate models of demographic and clinical predictors associated with mutant *Pneumocystis carinii* dihydropteroate synthase (DHPS) genotype in 111 human immunodeficiency virus–infected patients diagnosed with *P. carinii* pneumonia (PCP).

	Mutant *P. carinii* DHPS genotype			
	Model 1		Model 2	
Predictor	AOR (95% CI)	*P*	AOR (95% CI)	*P*
Study site				
San Francisco	4.8 (1.4–16.2)	.01	5.0 (1.5–17.2)	.01
Seattle	3.1 (1.1–8.7)	.04	3.2 (1.1–9.2)	.03
Sulfa or sulfone prophylaxis				
Yes	5.2 (2.1–12.9)	<.01	3.9 (1.4–10.5)	<.01
Prior PCP				
Yes	—		2.2 (0.6–7.4)	.21

NOTE. AOR, adjusted odds ratio; CI, confidence interval.

et al. [25] found DHPS mutations in 5 (71%) of 7 HIV-infected patients whose medical and pharmacy records documented sulfa or sulfone prophylaxis use for ⩾1 month during the 4 months preceding PCP diagnosis. Helweg-Larsen et al. [27] found mutations in 18 (62%) of 29 patients who were exposed to ⩾1 week of a sulfa drug at any time after the diagnosis of HIV infection. Finally, Ma et al. [28] found mutations in 11 (84.6%) of 13 patients with prior sulfa or sulfone prophylaxis use. Our results, combined with these studies, make clear the association between sulfa or sulfone prophylaxis and DHPS mutations. What is unclear, however, is which specific feature(s) of sulfa or sulfone prophylaxis are most crucial to the selection of DHPS mutations. Unfortunately, each of these studies used a different definition of sulfa or sulfone prophylaxis, which makes it impossible for direct comparisons to be made. In addition, none of the studies, including the present one, examined the impact of potentially important features, such as the duration of sulfa or sulfone prophylaxis, on the risk of DHPS mutations. Consequently, which specific feature of sulfa or sulfone prophylaxis is associated with risk of DHPS mutations is an important but unanswered question.

The clinical significance of DHPS mutations and, specifically, whether their presence implies the existence of resistant *P. carinii* and resistance to TMP-SMZ– or dapsone-containing PCP treatment regimens is unclear at present. Helweg-Larsen et al. [27] found a significantly lower 3-month survival among patients with DHPS mutations in a multivariate analysis that controlled for patient age, CD4 cell count, and PaO2. Whether this finding establishes the presence of TMP-SMZ– or dapsone-resistant *P. carinii* could not be answered in their analysis and is currently being investigated in our cohort.

Study site was also an independent risk factor for having a mutant *P. carinii* DHPS genotype. Patients diagnosed with PCP in either San Francisco or Seattle were more likely to have a mutant genotype than were persons diagnosed with PCP in Atlanta, even after controlling for prior PCP and sulfa or sulfone prophylaxis. This result confirms our earlier report that demonstrated a significant association between DHPS genotype and study city, an association that was stronger between DHPS genotype and city of PCP diagnosis than city/place of birth [29]. Importantly, the current analysis extends our earlier one by controlling for important predictors of mutant genotypes, such as prior PCP and sulfa or sulfone prophylaxis.

Residence in San Francisco appeared to be a strong predictor for having a mutant *P. carinii* DHPS genotype. Overall, 81.5% of the patients residing in San Francisco had a mutant genotype. Consequently, sulfa or sulfone prophylaxis was no longer significantly associated with mutant genotype for patients residing in San Francisco. We believe that this result is valid for several reasons. First, the proportion of mutant genotypes from San Francisco in this analysis is similar to our earlier analysis that examined DHPS genotypes without clinical information in which 54 (81.8%) of the 66 patient specimens from San Francisco had a mutant genotype [29]. Thus, the high proportion of mutant genotypes in San Francisco is unlikely to be the result of chance due to a small sample size. Second, the proportion of patients who had a mutant DHPS genotype despite being classified as not on prophylaxis was consistent using different instruments and different time points. In San Francisco, one study nurse performed all patient interviews, and a different study nurse conducted all chart abstractions; each was blinded to the results obtained by the other. Thus, we believe that the high proportion of mutant genotypes in persons classified as not on prophylaxis is unlikely to be the result of misclassification of these patients. In fact, 10 of the 11 San Francisco

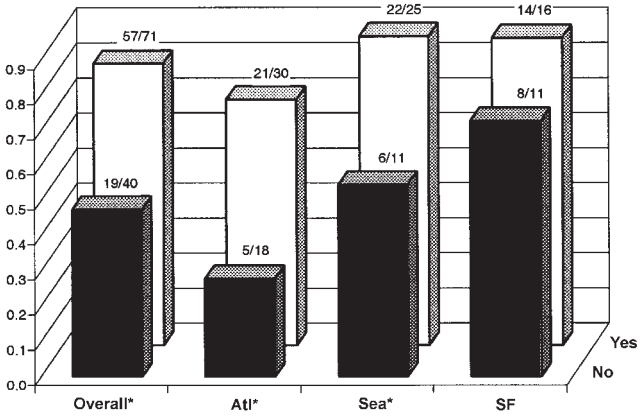

Figure 1. Proportion of mutant *Pneumocystis carinii* dihydropteroate synthase (DHPS) genotypes according to sulfa or sulfone prophylaxis and site of *P. carinii* pneumonia (PCP) diagnosis in the 111 human immunodeficiency virus–infected patients diagnosed with PCP. *White bars,* Persons who reported sulfa or sulfone prophylaxis use or persons whose chart documented sulfa or sulfone prophylaxis prescription. *Black bars,* Persons who denied sulfa or sulfone prophylaxis use and whose chart failed to document sulfa or sulfone prophylaxis prescription. Overall, all 3 cities combined; Atl, Atlanta; Sea, Seattle; SF, San Francisco. $*P < .05$ for comparison of proportions.

Table 5. Proportion of mutant *Pneumocystis carinii* dihydropteroate synthase (DHPS) genotype, according to known duration of human immunodeficiency virus (HIV) infection and site of *P. carinii* pneumonia (PCP) diagnosis, in 82 HIV-infected patients diagnosed with PCP whose date of first positive HIV test was known.

Known duration of HIV	Site of PCP diagnosis			
	Atlanta	Seattle	San Francisco	Overall
Newly diagnosed	5/13 (38.5)	2/3 (66.7)	7/10 (70.0)	14/26 (53.8)
<5 Years	9/17 (52.9)	6/6 (100)	8/10 (80.0)	23/33 (69.7)
5–10 Years	6/6 (100)	5/5 (100)	7/7 (100)	18/18 (100)
>10 Years	2/5 (40.0)	NA	NA	2/5 (40.0)
Overall	22/41 (53.7)	13/14 (92.9)	22/27 (81.5)	57/82 (69.5)

NOTE Data are no. of mutant DHPS genotypes/total no. of DHPS genotypes (%). NA, not applicable.

patients who denied ever using TMP-SMZ were newly HIV diagnosed, and 7 of these 10 had a mutant genotype.

More than half the patients (14 of 26) who were newly diagnosed with HIV infection, none of whom reported prior use of any *P. carinii* prophylaxis (including sulfa or sulfone prophylaxis) had a mutant genotype. Potentially, these people could have received a sulfa drug previously for reasons unrelated to HIV disease. Unfortunately, our patient interview and chart abstraction were not structured to detect this possibility; however, Kazanjian et al. [25], Helweg-Larsen et al. [27], and Ma et al. [28] also detected mutant DHPS genotypes in persons never exposed to sulfa or sulfone drugs. In these studies, 2 (28.6%) of 7 patients, 13 (10.3%) of 123 patients, and 3 (20.0%) of 15 patients, respectively, who were never exposed to sulfa or sulfone drugs had a mutant DHPS genotype, which are lower percentages than those observed in this study. Because the natural reservoir for human *P. carinii* remains unknown at present, the potential impact of sulfa or sulfone use on this reservoir cannot be determined.

The findings that geographic region predicts mutant *P. carinii* DHPS genotypes, that mutant genotypes are seen in persons who deny prophylaxis use, and especially that mutant genotypes are seen in more than half the patients who were newly diagnosed as HIV-infected raise interesting questions regarding the epidemiology and transmission of human *P. carinii.* Whether PCP in humans is a result of reactivation of latent infection or recent acquisition is unclear [31, 32]. Keely et al. [33] examined *P. carinii* specimens from 10 patients who each had 2 episodes of PCP $\geq$3 months apart and sequenced the large subunit of mtrRNA. In half the cases, the mtrRNA sequences observed in the second episode differed from that observed in the first, which suggests that the second episode of PCP in these individuals was the result of a new, recent infection.

Whether the reservoir of human *P. carinii* resides solely in humans or also in the environment remains unclear. Recently, a case-control study found that HIV-infected patients hospitalized with PCP were significantly more likely to have gardened, camped, or hiked in the 6 months before PCP diagnosis, compared with the age- and CD4 cell count–matched HIV-infected control patients hospitalized for a reason other than pneumonia, which suggests that recent soil exposure may be a potential source of *P. carinii* [34]. Our observations from this study are consistent with the possibility of recent acquisition and transmission of these mutant *P. carinii* DHPS genotypes, either from person-to-person or from one person to another through a common environmental source. In either case, the presence of mutant DHPS genotypes in persons never prescribed sulfa or sulfone prophylaxis argues strongly for the importance of humans in the transmission of this disease.

In summary, this prospective study found that sulfa or sulfone prophylaxis and city of PCP diagnosis were independent risk factors for having a mutant *P. carinii* DHPS genotype. The significance of city of PCP diagnosis and the finding of mutant genotypes in more than half the patients who were newly diagnosed with HIV infection, none of whom report having ever taken any prophylaxis, raises interesting questions concerning the epidemiology and transmission of human *P. carinii* that warrant further investigation.

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