Major Article

Variation in Clinical Phenotype of Human Infection Among Genetic Groups of *Blastomyces dermatitidis*

Jennifer K. Meece,1 Jennifer L. Anderson,1 Sarah Gruszka,1,a Brian L. Sloss,2 Bradley Sullivan,1 and Kurt D. Reed3

1Marshfield Clinic Research Foundation, Marshfield, Wisconsin; 2U.S. Geological Survey, Wisconsin Cooperative Fishery Research Unit, College of Natural Resources, University of Wisconsin-Stevens Point, Stevens Point; and 3Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison

**Background.** *Blastomyces dermatitidis*, the etiologic agent of blastomycosis, has 2 genetic groups and shows varied clinical presentation, ranging from silent infections to fulminant respiratory disease and dissemination. The objective of this study was to determine whether clinical phenotype and outcomes vary based on the infecting organism’s genetic group.

**Methods.** We used microsatellites to genotype 227 clinical isolates of *B. dermatitidis* from Wisconsin patients. For each isolate, corresponding clinical disease characteristics and patient demographic information were abstracted from electronic health records and Wisconsin Division of Health reportable disease forms and questionnaires.

**Results.** In univariate analysis, group 1 isolates were more likely to be associated with pulmonary-only infections (*P* < .0001) and constitutional symptoms such as fever (*P* < .0001). In contrast, group 2 isolates were more likely to be associated with disseminated disease (*P* < .0001), older patient age (*P* < .0001), and comorbidities (*P* = .0019). In multivariate analysis, disease onset to diagnosis of >1 month (*P* < .0001), older age at diagnosis (*P* < .0001), and current smoking status (*P* = .0001) remained predictors for group 2 infections.

**Conclusions.** This study identified previously unknown associations between clinical phenotype of human infection and genetic groups of *B. dermatitidis* and provides a framework for further investigations of the genetic basis for virulence in *B. dermatitidis*.

**Keywords.** *Blastomyces dermatitidis*; blastomycosis; virulence; population genetics; clinical phenotype; genetic groups.

Blastomycosis is an infection caused by the thermally dimorphic fungus *Blastomyces dermatitidis*. The disease occurs most commonly in North America along the Ohio and Mississippi River valleys, in southeastern states, and around the Great Lakes [1]. Sporadic cases have been reported in Central and South America, Europe, India, the Middle East, and Africa [2–4].

Blastomycosis is a significant public health problem in Wisconsin, where it is a reportable disease in humans [5–9]. Data collected by the Wisconsin Division of Health and Family Services from 2000 to 2006 indicate that incidences of infection as high as 10.4–41.9/100 000 have been observed in several north–central counties [10].

The symptoms of blastomycosis range from asymptomatic infection to fulminant respiratory failure with acute respiratory distress syndrome and dissemination of infection to bone, skin, central nervous system, and other organs. Clinicians treating this disease often cannot predict which patients will have favorable outcomes and those who will experience severe manifestations, including death. Although host factors, comorbidities, amount of inoculum, and other variables can influence presentation and outcome for many infectious diseases, it is well established that...
genetic variation among strains of an infecting agent can modulate virulence and have a profound influence on outcome [11–14]. Thus, genetic variability of a pathogen could be an important predictor of clinical outcome and potentially inform treatment options for individual patients.

We recently used microsatellite typing to examine the population-level genetic structure of a large group of clinical and environmental isolates of *B. dermatitidis*. Two predominant genetic groups were observed: a highly monomorphic group (group 1) and a highly polymorphic group (group 2) [15]. Given the variation of symptoms clinically observed in blastomycosis patients and the genetic variability of the organism shown previously [15], we aimed to determine whether clinical phenotype and outcomes vary based on genetic group of the infecting organism. In addition, a subgroup of the isolates was genotyped and assigned to genetic groups using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and compared to the group assignments generated by microsatellite typing.

**METHODS**

**Isolate Sources**
Marshfield Labs serves more than 500 healthcare facilities and 3000 veterinary practices located in endemic areas for blastomycosis in northern Wisconsin. All isolates of *B. dermatitidis* used in this study were recovered in the mycology section of Marshfield Labs from 1999 to 2009 and stored frozen (−20°C) at the Marshfield Clinic Research Foundation. All isolates were obtained as part of clinical diagnosis and identified as *B. dermatitidis* using standard methods, which included culture of the mold form on brain–heart infusion agar with blood at 25°C and conversion to the characteristic yeast form when incubated in Middlebrook 7H9 broth at 35°C.

**DNA Extraction, Microsatellite Typing, and Cluster Analysis**
DNA was extracted from frozen isolates using a QIAamp DNA mini kit and tissue protocol (QIAGEN), with modifications according to Meece et al [16]. For genetic typing of each isolate, 27 polymorphic microsatellite loci were evaluated as previously described [15]. Briefly, for each locus, 2 μL of extracted DNA was amplified by PCR with a HotStar Taq master mix kit (QIAGEN), according to the manufacturer’s recommendations, with a 0.2-μM final concentration of primers. Amplified products were sized on an ABI 3130xl genetic analyzer (Applied Biosystems) using GeneScan-500 ROX size standard (Applied Biosystems) in Hi-Di formamide (Applied Biosystems). DNA fragment sizes were manually grouped into their appropriate alleles using the fixed-bin method [17].

The following 3 methods were used to predict the most likely number of genetic groups present in the microsatellite data and to assign membership of each isolate: genetic distance–neighbor joining clustering, Bayesian admixture, and principle coordinate analysis (PCoA). Haplotypes were ascertained by identifying matching samples using Genetic Analysis in Excel v6.41 [18]. To minimize the effects of including multiple representatives of the same genotype (ie, haplotypes) in genetic clustering efforts, all genetically identical isolates were subsumed to a single unique haplotype. Isolates differing at 1 or more loci were considered unique. Following haplotype construction, genetic structure among the samples was first estimated by constructing an unrooted neighbor-joining tree of unique haplotypes based on an allele sharing distance in PowerMarker v3 [19–22]. Confidence in the resolved topology was based on 1000 bootstrap pseudoreplicates across loci. Next, the Bayesian approach of the program STRUCTURE [23] was used to predict the minimum number of genetic units or clusters within the composite data. Analysis settings included: K (the putative number of genetic groups) ranging from 1 to 12, the use of the admixture model, correlated allele frequencies between populations, and lambda = 1. The degree of admixture (alpha) was inferred from the data as advised in the software’s manual. Five iterations of each K were performed, each consisting of a burn-in of 100000 cycles, followed by 100000 repetitions. The method of Evanno et al [24], as estimated using Structure Harvester [25], was used to assess the most likely K given the data in conjunction with the mean and variance of the ln probability of K. The last analysis of genetic structure was a PCoA of the standardized covariance of the haploptic genetic distance, as performed in Genetic Analysis in Excel v6.41. The first and second principle coordinates were plotted to graphically illustrate clusters of haplotypes.

**PCR–RFLP Typing and Method Comparison**
Twenty-five percent of the isolates in this study (n = 56) were genotyped using PCR–RFLP, as described by McCullough et al [26] with modifications by Meece et al [16]. The genotyping group assignments from PCR–RFLP and microsatellite typing were then compared.

**Medical Record Data Abstraction**
For each *B. dermatitidis* isolate, patient demographic information and clinical disease characteristics were abstracted from the corresponding patient’s medical record according to a Marshfield Clinic Research Foundation Institutional Review Board–approved protocol. Medical record abstractions were conducted independently from genotyping at a separate phase of the study. For Marshfield Clinic patients, data were abstracted from each patient’s electronic health record. For patients not part of the Marshfield Clinic system, similar data were obtained from state-reportable disease forms and an extensive, standardized questionnaire administered by the Wisconsin
Division of Public Health. The clinical disease characteristics collected for each patient included the following: cough, hemoptysis, poor appetite, weight loss, joint pain, headache, back pain, chest pain, bone pain, fractures, fever, chills, night sweats, fatigue, muscle pain, location of infection, dissemination site, time from disease onset to diagnosis, hospitalization/duration, antifungal therapy/dosage/duration of treatment, and death. Patient demographic information collected included the following: age at diagnosis, race, gender, smoking status/packs per day, and comorbid conditions.

Association Analysis
Upon completion of phylogenetic analysis and genetic group assignment, this information was combined with medical record abstraction data for univariate and multivariate analysis. Univariate analyses, odds ratios, and corresponding 95% confidence intervals were calculated for the association between each potential risk factor and genetic types using unconditional logistic regression modeling with \( \alpha = 0.05 \). In addition, multivariate stepwise logistic regression modeling using the risk factors that showed statistically significant results by univariate analysis was used to identify sets of important predictors for the status of genetic type. All data analyses were carried out using SAS v9.2 (SAS).

RESULTS

Microsatellite Typing and Cluster Analysis
A total of 227 clinical \( \textit{B. dermatitidis} \) isolates, consisting of 132 unique haplotypes, were analyzed for phylogenetic relationships. Assembly of an unrooted neighbor-joining tree showed 2 main genetic groups separated by a deep node bootstrapped at 99% (Figure 1). STRUCTURE analysis of the unique haplotypes supported 2 genetic units in the data \((K = 2)\) based on the method of Evanno et al [24] and the linearity and variance of lnP(D). The individual ancestry of each haplotype based on \( K = 2 \) showed 129 haplotypes with a majority \( q \) value >80% (Figure 2). Principal coordinate analysis (Figure 3) showed clustering of the haplotypes into 2 primary groups. The first primary axis (coordinate 1) explained a majority of the variance (51%) and differentiated group 1 from group 2. The secondary axis (coordinate 2) explained another 18% of variance. Group membership assignment for each isolate was 100% concordant across all 3 analysis methods, with group 1 containing 34 haplotypes representing 128 isolates (56%) and group 2 containing 98 haplotypes representing 99 isolates (44%). Three isolates (monotypic haplotypes H47, H70, and H126) had a majority assignment in group 2 by STRUCTURE at <80% genetic membership threshold \((q \text{ value})\). The evidence from all 3 analysis methods showed consistent support for membership of these 3 isolates (haplotypes H47, H70, and H126 identified in Figures 1–3) in group 2, and the 3 were subsequently placed in that group for the purposes of this study.

PCR–RFLP Typing and Method Comparison
Of the 56 isolates genotyped using PCR–RFLP, 34 were classified as group A, 21 were group B, and 1 group C. Comparison of PCR–RFLP groups with microsatellite groups for these 56 isolates revealed that all 34 PCR–RFLP group A isolates were group 1 isolates by microsatellite typing. In contrast, all isolates in PCR–RFLP groups B and C belonged to group 2 by microsatellite analysis.

Patient Demographics and Clinical Disease Characteristics
The Marshfield Clinic electronic health record was the source of information for 123 cases, public health reportable disease worksheets and questionnaires for 50 cases, and a combination of both for 54 cases. The age range of patients was 3–91 years (mean 43.4), with 28 (12%) patients being age 18 years or younger at diagnosis. Gender, race/ethnicity, smoking status, and comorbidities of patients are shown in Table 1. Ninety-two patients (41%) were identified as having 1 or more comorbid condition(s). Clinical disease characteristics and patient antifungal treatments are shown in Table 2. Disseminated disease was reported in 43/227 (19%) of cases, with 9 patients (4% of total cases, or 21% of disseminated disease cases) having multiple sites of dissemination (Table 2). The time from onset of symptoms to diagnosis for each case was based on patient self-reporting of initiation of blastomycosis symptoms and ranged from <1 to 48 months. The majority of patients \((n = 154, 68\%)\) were diagnosed with blastomycosis within 1 month of onset of symptoms. One hundred and thirty-four cases (59%) required hospitalization. Duration of hospitalization ranged from 1 to 134 days. Twenty-one cases (9%) resulted in death, of which 15 (71%) were pulmonary-only disease, 5 disseminated (24%), and 1 unspecified. Locations of infection in the 5 cases of disseminated disease that resulted in death were as follows: deep tissue abscess \((n = 1)\), skin \((n = 1)\), and both skin and bone \((n = 3)\). The age range for fatal cases was 8–91 years (mean 61). Thirteen (62%) of the fatal cases were male, and 17 (81%) had an underlying medical condition.

Association Analysis
Univariate analysis demonstrated 10 clinical disease characteristics and 6 patient demographics (including comorbidity conditions) as having statistically significant associations with a particular genetic group of \( \textit{B. dermatitidis} \) (Table 3). In summary, infection caused by group 1 isolates was more likely to be associated with pulmonary-only infections and constitutional symptoms such as fever, night sweats, cough, fatigue, poor appetite, chills, and chest pain. In contrast, genetic group 2 strains were more likely to disseminate to other tissues,
especially skin. Patients infected with isolates from group 2 were more likely to have a comorbid condition, be current smokers, and be older in age.

In multivariate analysis, 3 of the 16 clinical characteristics and patient demographics identified as important in the univariate analysis maintained significant predictors for infection with genetic group 2 organisms (Table 4).

DISCUSSION

To our knowledge, the results of this study provide the first evidence that genetic variation in B. dermatitidis may be associated with differences in patient demographics and clinical disease characteristics of infection. Although our cohort of patients was roughly divided between those infected with group 1 (n = 128) and group 2 (n = 99) strains, there were significant differences in important clinical variables between the 2 groups. Patients infected with group 1 strains were more likely to report constitutional symptoms such as fever, night sweats, and chest pain and be diagnosed 6 weeks or less from onset of symptoms. In contrast, patients infected with group 2 isolates were more likely to be diagnosed later, exhibit disseminated disease, have comorbid conditions, and be current smokers. Hospitalization (P = .0565) and mean hospital duration, in days (P = .1460), were not associated with a particular genetic group. Our study cohort had a relatively small number of deaths (n = 21, 9%), which were almost equally caused by group 1 (n = 11) and group 2 (n = 10) infections (P = .8180). Notably, 15 of the 21 deaths (71%) resulted from a pulmonary-only infection. A larger study would be necessary to achieve sufficient statistical power to determine whether or not mortality rates differ significantly between the 2 groups of organisms.

Previous studies have shown that certain ethnic groups such as Asians (especially Pacific Islander and Filipino), Hispanics, and African Americans are more likely to experience
disseminated disease with the closely related dimorphic fungus, Coccidioides immitis/pasadensis, [27–30]. In our study, white patients, compared to nonwhite patients, were significantly more likely to be infected with group 2 strains of B. dermatitidis, which are associated with disseminated disease, (Table 3). Although we could not evaluate nonwhite minority groups separately due to small sample sizes, we did find that 11 of 13 (85%) strains infecting American Indian patients, 7 of 9 (78%) infecting Asian patients, and 3 of 4 (75%) infecting Hispanic patients were group 1 organisms. In addition, 25 of 27 (93%) blastomycosis cases in nonwhites manifested as pulmonary-only infections. This suggests that patient genetic background may influence host susceptibility and immune response to different B. dermatitidis strains and warrants further investigation.

A significant observation was the apparent association of group 2 infections with comorbid conditions and increased rate of dissemination compared with group 1 infections. For example, of 41 patients in group 1 with comorbid conditions, 3 (7%) had disseminated disease, whereas of the 51 patients in group 2 with comorbid conditions, 14 (27%) had disseminated disease. Although it is possible that the increased rate of dissemination observed with group 2 strains was due, at least in part, to impaired host immunity associated with comorbidities, our findings suggest that genetic group of the infecting strain also plays some role in modulating dissemination. A practical limitation of our study was that because of small numbers, all comorbidities were combined in order to obtain statistical power for evaluating association with a specific genetic group. Therefore, conditions such as human immunodeficiency
virus infection and steroid therapy, which more profoundly affect host immune function, were not considered separately or weighted as a more serious comorbid condition. Although mathematically we have shown an association between group 2 strains of *B. dermatitidis* and disseminated disease, causality has not yet been established.

Comparison of phenotypic traits between group 1 and group 2 strains could provide insight into the epidemiology of blastomycosis. For example, genetic analysis of clinical isolates associated with 2 recent Wisconsin outbreaks (Lincoln County, 2006, of which 9 samples are included in this current study [6], and Marathon County, 2009–2010, unpublished data) revealed that both outbreaks were due to group 1 strains. Additionally, retrospective analysis of clinical and environmental isolates obtained from earlier outbreaks in Wisconsin near Eagle River (Vilas County), the Tomorrow River (Portage County) [31], and the Crystal River (Waupaca County) [32] again showed only group 1 strains. This apparent overrepresentation of group 1 strains with outbreaks could be due to phenotypic traits associated with increased transmission of infections to humans. These could include, but are not limited to, increased spore formation by certain strains of *B. dermatitidis* and the ability to occupy specific ecologic niches that overlap with human activity.

The molecular basis for differences in clinical phenotype observed between the 2 genetic groups is not known. One possibility is that significant differences in the presence and/or expression of virulence factors may exist between the 2 genetic groups. Previous studies have documented the central role that...
BAD1 plays as a potent virulence factor in *B. dermatitidis* infections. BAD1 is an adhesin expressed on the surface of the yeast form of *B. dermatitidis*; however, it is absent in the mold form. It promotes lung infection by fixing yeast cells to pulmonary tissue, including macrophages [33]. Additionally, BAD1 expression is associated with other pathogenic mechanisms that alter host immunity, including suppression of tumor necrosis factor-α, induction of transforming growth factor-β, and siderophore-like action in binding calcium [34–36]. Further investigation of differences between BAD1 and other

**Table 3. Statistically Significant Associations Between Clinical Disease Characteristics, Patient Demographics, Comorbidities, and Blastomyces dermatitidis Genetic Group**

<table>
<thead>
<tr>
<th>Characteristic Location of infection</th>
<th>Genetic Group 1</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>P</em> Value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disseminated</strong></td>
<td>10 (8)</td>
<td>5.99</td>
<td>2.78–12.93</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><strong>Pulmonary-only</strong></td>
<td>118 (92)</td>
<td>1.00 (ref)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Onset to diagnosis (months)</th>
<th>Genetic Group 1</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>P</em> Value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1</td>
<td>21 (16)</td>
<td>5.20</td>
<td>2.82–9.58</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>≤1</td>
<td>107 (84)</td>
<td>1.00 (ref)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1.5</td>
<td>13 (10)</td>
<td>6.52</td>
<td>3.24–13.11</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>≤1.5</td>
<td>115 (90)</td>
<td>1.00 (ref)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Genetic Group 1</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>P</em> Value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>103 (82)</td>
<td>0.23</td>
<td>.13–.42</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Skin dissemination</td>
<td>7 (5)</td>
<td>6.57</td>
<td>2.72–15.87</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Night sweats</td>
<td>79 (68)</td>
<td>0.42</td>
<td>.25–.76</td>
<td>.0034</td>
</tr>
<tr>
<td>Cough</td>
<td>117 (91)</td>
<td>0.32</td>
<td>.15–.70</td>
<td>.0042</td>
</tr>
<tr>
<td>Fatigue</td>
<td>100 (85)</td>
<td>0.38</td>
<td>.19–.74</td>
<td>.0049</td>
</tr>
<tr>
<td>Poor appetite</td>
<td>76 (67)</td>
<td>0.49</td>
<td>.27–.86</td>
<td>.0139</td>
</tr>
<tr>
<td>Chills</td>
<td>75 (62)</td>
<td>0.54</td>
<td>.31–.93</td>
<td>.0268</td>
</tr>
<tr>
<td>Chest pain</td>
<td>83 (69)</td>
<td>0.55</td>
<td>.32–.97</td>
<td>.0381</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comorbid conditions</th>
<th>Genetic Group 1</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>P</em> Value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>All comorbid conditions&lt;sup&gt;e&lt;/sup&gt;</td>
<td>41 (32)</td>
<td>2.38</td>
<td>1.38–4.11</td>
<td>.0019</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>5 (4)</td>
<td>3.82</td>
<td>1.31–11.12</td>
<td>.0139</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient demographics</th>
<th>Genetic Group 1</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>P</em> Value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoker&lt;sup&gt;f&lt;/sup&gt;</td>
<td>27 (21)</td>
<td>5.26</td>
<td>2.82–9.84</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>≥1 (packs/day)</td>
<td>17 (14)</td>
<td>4.09</td>
<td>2.12–7.87</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>&lt;1 (packs/day)</td>
<td>102 (82)</td>
<td>1.00 (ref)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past smoker&lt;sup&gt;f&lt;/sup&gt;</td>
<td>19 (15)</td>
<td>2.45</td>
<td>1.13–5.32</td>
<td>.0240</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race/ethnicity</th>
<th>Genetic Group 1</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>P</em> Value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>97 (82)</td>
<td>3.67</td>
<td>1.33–10.14</td>
<td>.0120</td>
</tr>
<tr>
<td>Other&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22 (18)</td>
<td>1.00 (ref)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age at diagnosis (year)</th>
<th>Genetic Group 1</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>P</em> Value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Mean SD</td>
<td>128 37.2 19.5</td>
<td>99 51.5 16.7</td>
<td>1.04</td>
<td>1.03–1.06</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; OR, odds ratio.

<sup>a</sup> Genetic group 1 is the referent group.

<sup>b</sup> CI = 95%.

<sup>c</sup> *P* value was derived from unconditional logistic regression modeling.

<sup>d</sup> Referent group is shown when it is not obvious and/or conveys important data.

<sup>e</sup> Comorbid conditions included: asthma, coronary artery disease, congestive heart failure, chronic obstructive pulmonary disease, cancer, emphysema, diabetes, human immunodeficiency virus/AIDS, hypertension, hypercholesterolemia/hyperlipidemia, hypothyroidism, steroid therapy, pregnancy. Referent group did not report any comorbid conditions.

<sup>f</sup> Referent group never smoked.

<sup>g</sup> Referent group were all other combined race/ethnicities including: Asian, Black or African American, Native American, and Hispanic.

<sup>h</sup> OR per year as analyzed in 1-year increments.
virulence factor expression between the genetic groups is warranted and could be facilitated by a comparative genomics approach that uses next-generation sequencing technology.

Recent investigations of population genetic diversity in Blastomyces dermatitidis have incorporated several genotyping techniques. In order to reconcile nomenclature derived from microsatellite genotyping with PCR–RFLP groups, we selected a subgroup of isolates for genetic typing by both methods [26]. Comparison of isolate grouping by both methodologies showed that all PCR–RFLP group A isolates belonged to microsatellite group 1. In contrast, isolates in groups B and C by PCR–RFLP analysis all clustered in microsatellite genetic group 2. These results are consistent with our previous observation that group 1 strains have low allelic diversity, while group 2 strains, as a group, are more genetically diverse.

Our study has several limitations that must be considered. First, the design was retrospective in nature and collection of clinical information relied, in part, on self-reported data. We identified self-reported features that differed significantly between group 1 and group 2 infections. Some clinical difference between the 2 groups may be due, in part, to systematic differences in disease progression by group, which created a longer interval from onset to diagnosis in group 2, thereby introducing possible recall bias. However, because patients in both groups were sufficiently ill to seek treatment for their infection, differences in recall may be minimal. Second, the Blastomyces dermatitidis isolates used for analysis were limited to Wisconsin and do not represent the entire geographic range of the organism. It is possible that additional groups of Blastomyces with unique genetic and virulence characteristics are present in other geographic locations. Finally, each case-associated isolate was assigned to genetic group 1 or 2 based on a combination of the following 3 independent genetic analysis approaches: genetic distance-neighbor joining clustering, Bayesian admixture, and PCoA. The use of these 3 methods produced phylogenetic results consistent with a previous study [15] that used different isolates, thereby demonstrating the reproducibility of this approach. The results of group assignment were consistent across all 3 methods; group assignment for an individual isolate did not vary based on the genetic analysis approach that was used. We concluded that the concordance and statistical support of the 3 analysis methods justified our grouping approach for the association analysis and acknowledge that genetic substructure in the groups, especially group 2, was not taken into account.

Despite these limitations, our results provide a framework with which to further explore the relationship between the genetic diversity in Blastomyces dermatitidis and clinical disease in humans and animals. Future research will focus on phenotypic comparison between strains. In addition, clinical implications of polymorphisms in BAD1 will be explored along with whole-genome comparisons to look for additional virulence factors that may vary between the 2 genetic groups.

Notes

Acknowledgments. We thank John Archer (Wisconsin Division of Public Health) for providing access to 4151 communicable disease reporting data and corresponding blastomycosis worksheets from which clinical disease characteristics and patient demographics, including comorbid conditions, were abstracted. We also thank Po-Huang Chyoun, PhD, Carla Rotscheit, and Cathy Schneider for assistance with statistical analysis and Bruce Klein, MD, Caitlin Pepperell, MD, and Stephanie Irving, MPH, for critical review of this manuscript. The use of trade names in this manuscript does not imply endorsement by the U.S. government.

Financial support. Financial support for this work was provided by a Marshfield Clinic Research Foundation internal grant, SP code SUL10209 and the Department of Pathology, Feinberg School of Medicine, Northwestern University, Evanston, Illinois.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


